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(54) Title: METABOLITE DETECTION APPARATUS AND METHOD OF DETECTING METABOLITES

FIG. 2

(57) Abstract: A CMOS-based chip having one or more sensing modalities that are able independently to detect multiple metabolites present in a biological sample. The multiple sensing modalities may be provided at different locations with respect to the chip, whereby the chip can simultaneously detect a plurality of metabolites by measuring behaviour of a test material in the different locations. The chip may utilise paper as a transport mechanism for the sample. The paper either conveys the sample to the different locations or itself provides discrete testing zones in which different metabolites can be independently detected. With this technique, multiple metabolites may be measured in real time using a small scale point-of-care device.

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Declarations under Rule 4.17:
— of inventorship (Rule 4.17(iv))

Published:
— with international search report (Art. 21(3))
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
METABOLITE DETECTION APPARATUS AND METHOD OF DETECTING

FIELD OF THE INVENTION

The present invention relates to a process and apparatus for point-of-care real time metabolite sensing. In particular, the invention relates to an apparatus and process that enables multiple metabolites to be detected simultaneously from a single sample.

BACKGROUND OF THE INVENTION

Metabolism is a vital cellular process, and its malfunction can be a major contributor to many diseases. Metabolites (i.e. substances involved in metabolism) can be good indicators of disease phenotype, and can serve as a metabolic disease biomarkers. Therefore quantification and analysis of metabolites can play a significant role in the study and early diagnosis (detection) of many diseases.

Metabolite biomarkers of different diseases are also becoming increasingly well understood which paves the way for developing new diagnostic systems. The importance of the link between metabolomics and a person's state of health is governing the need to look at both targeted and untargeted metabolites. A single metabolite can be a biomarker for several different diseases. In addition, multiple metabolites together can serve as a biomarker for a particular disease. It is therefore often necessary to detect and quantify the presence of multiple metabolites in order to accurately identify a disease. Metabolite biomarker profiling deals with the screening of a huge number of metabolites, and there are a particular panel of metabolite biomarkers which are good indicators of a person's state of health. In particular, multiplexed assaying, where multiple biomarkers are
simultaneously measured in a single run, has the capacity to provide resource-rich information for decision making and prognosis, leading to the correct diagnosis and treatment for complex disease conditions such as stroke, cancer and cardiovascular diseases where directed therapy is important.

A commonly used technique for detecting and quantifying metabolites is mass spectrometry (MS). This involves ionising a chemical species and sorting the product ions based on their mass-to-charge ratios. Separation methods such as gas chromatography and liquid chromatography are often required prior to performing a mass spectrometry measurement. Nuclear magnetic resonance (NMR) spectroscopy is another technique which is used for metabolite studies. NMR can be used to detect, identify and quantify a wide range of metabolites without having to first separate them. However, both of these techniques require bulky and expensive equipment, which confines their use to hospitals and laboratories.

As an example, elevated cholesterol levels are well known for their association with an increased risk of coronary heart disease (angina or heart attack), narrowing of the arteries (atherosclerosis), stroke, peripheral heart disease and hypertension. Such conditions are often correlated with poor diet, an excessive fat intake, lack of exercise and other lifestyle choices. Measuring or therapeutic monitoring of cholesterol level in blood serum helps to assess susceptibility of the person to develop coronary artery diseases and hence is a good indicator of the state of health of a person. One of the diagnostic methods for quantifying cholesterol concentration depends on enzyme-based assays that require a spectrophotometer to measure changes in intensity of colour products from those enzyme reactions. A general purpose spectrophotometer would incorporate a sophisticated setup of a white light source, a monochromator containing a diffraction grating and a light transducer that converts light into electrical signal such as a charge-coupled device (CCD), a
photodiode or a photomultiplier tube. The wide spectrum range of the spectrophotometer makes it bulky and power hungry which consequently confines its usefulness to laboratories and hospitals. Another method involves metabolites undergoing chemiluminescence reaction. This method requires a more specialised light detector such as a charge-coupled detector (CCD) to detect low light emission of luminol that is used for quantification of small analyte concentration.

More recently, use of a photodiode in a disposable sensing platform to measure change in colour of enzyme assay has been demonstrated as a means of detecting cholesterol by measuring intensity of transmitted light through assay solution [1]. The platform was based on a complementary metal oxide semiconductor (CMOS)-based photodiode array and an off-the-shelf light emitting diode (LED). The photodiode array is fabricated using commercial standard CMOS process, which is readily available for low-cost mass-production.

Photodiodes made in a CMOS process are generally sensitive to light in the 200 nm to 1100 nm range, owing to the bandgap of silicon (1.12 eV). This range makes them suitable for colorimetric enzyme assays that use visible light or fluorescent mediators, which often use wavelengths in the range 400 nm to 700 nm. A colour change within this range can be exploited for a range of enzyme assays, e.g. cholesterol ester hydrolase, cholesterol dehydrogenase, cholesterol esterase and cholesterol oxidase can be exploited to measure metabolites such as cholesterol. For metabolites with low concentrations, more sensitive CMOS compatible detector such as single photon avalanche diode (SPAD) can be integrated on the same chip and therefore, increase the dynamic detection range.

In other recent work, another type of CMOS-based chip fabricated with an integrated ion-sensitive field effect transistor (ISFET) array was used to measure glucose concentration in blood through the activity of hexokinase.
The action of the hexokinase on the glucose releases hydrogen ions that are detected by the ISFET [2].

Point of care diagnostics are transforming the healthcare industry, by facilitating the use of home-testing to provide an early indication of potential illness and disease. The development of low-cost, rapid, specific and high sensitivity consumable biosensors are at the forefront of the research for user-orientated testing, driven in part by the need for rapid diagnosis and monitoring without overburdening the resources of the healthcare services. For example, glucose biosensors have become widespread in their use for managing diabetes. Liquid delivery is crucial for point of care (i.e. portable) diagnostic devices. A paper strip (e.g. chromatography paper or nitrocellulose membrane) has been proven to be an effective approach for delivering liquid samples. One well known example is the pregnancy test which measures the level of human chorionic gonadotropin (HCG) in human blood or urine to give an indication of pregnancy status.

**SUMMARY OF THE INVENTION**

At its most general, the present invention provides a CMOS-based chip having one or more sensing modalities that are able independently to detect multiple metabolites present in a sample. In particular, the invention relates to a scenario in which detection by the multiple sensing modalities occurs at different locations with respect to the chip, whereby the chip can simultaneously detect a plurality of metabolites by measuring behaviour of a test material in the different locations. In particular, the invention relates to the use of paper as a transport mechanism for a biological sample, wherein the paper either conveys the sample to the different locations or itself provides discrete testing zones in which different metabolites can be independently detected by one of the sensing modalities on the chip. With this technique,
multiple metabolites may be measured in real time using a small scale point-of-care device.

According to one aspect of the invention, there is provided an apparatus for detecting metabolites in a biological sample, the apparatus comprising: a sample receiving module arranged to receive the biological sample and transport it to a reaction zone for testing, wherein the reaction zone comprises a first testing region and a second testing region spatially separated from the first testing region, wherein properties of the first testing region and the second testing region are affected by the presence of metabolites to be detected; and a CMOS-based sensor unit disposed in relation to the reaction zone to detect independently the properties of the first testing region and the second testing region thereby to obtain separate signals indicative of the presence of metabolites in each of the first testing region and the second testing region. By probing different regions of a reaction zone, the invention effectively provides a multiplexed measurement system, where separate signals corresponding to different metabolites can be obtained from the same device. This is particularly useful where the first and second testing regions detected by the sensor unit are independently affected by one or more metabolites to be detected, i.e. there is substantially no cross-talk between the signals.

Herein the phrase "CMOS-based" may mean that the device is capable of fabrication using conventional semiconductor chip processes, e.g. comprising a series of depositing, masking and etching steps on a substrate. The sensor unit and its constituent components may thus be semiconductor components. This may enable the sensor unit to be mass-produced at low cost. The apparatus may thus be embodied as a compact hand-held device which is easily transportable, thus facilitating rapid point-of-care diagnostics. Compared with
current analytical methods for metabolite detection and quantification, no expensive detection equipment is required.

The sensor unit itself may resemble a semiconductor chip, and may have mounted thereon or connectable thereto means for controlling and processing the chip functions. For example, the apparatus may comprise a controller, e.g. a microprocessor or the like, arranged to send and receive signals from the sensor unit. For example, the controller may be arranged to activate the sensor unit by applying an appropriate voltage.

The properties of the first testing region and the second testing region that are detected by the sensor unit may be physical or chemical. For example, the sensor unit may be arranged to detect changes in appearance, chemical composition, mass, temperature, etc.

The reaction zone may have more than two discrete testing regions. For example, there may be three, four, five or more testing regions, as space allows. References to the first and second testing region below should be understood as being equally relevant to examples with more than two testing regions.

Each testing region may be sensitive to a different metabolite. For example, the first testing region may be sensitive to a first metabolite, and the second testing region may be sensitive to a second metabolite, whereby the separate signals are indicative of the presence of the first metabolite and second metabolite respectively. The reaction zone may include a control region that is not sensitive to the presence of metabolites to be detected, e.g. to provide a reference signal against which signals from the first testing region and second testing region can be compared.

The first testing region and the second testing region may be physically separated to prevent cross-talk therebetween. For example, a fluid flow barrier may separate the first testing region from the second testing region to
inhibit or prevent transfer of the biological sample therebetween.

In one example, the first testing region and the second testing region each comprises a respective micro-well, the micro-wells being separated from each other by a barrier portion. The barrier portion may be a raised part of the reaction zone between the micro-wells. It may be shallow, e.g. having a height relative to the base of the micro-wells in the range 1 to 200 µm, preferably 1 to 100 µm. The reaction zone may include four or more micro-wells. The micro-wells may be pre-loaded with an enzyme (and/or other reagents) in preparation to react with a substance in a sample.

The first testing region and/or the second testing region may comprise a test material arranged to support a metabolite-activated reaction upon receiving the biological sample. For example, each of the first testing region and/or the second testing region may each comprise an assay region, e.g. located within a microfluidic channel. The microfluidic channel may be pre-loaded with a test solution comprising one or more enzymes. Properties of the test solution may change due to a reaction between the enzymes and metabolites to be detected.

The biological sample is typically a liquid. In one example, the sample receiving module comprises a paper strip or other capillary structure for transporting the liquid biological sample to the reaction zone, e.g. by capillary action. Any suitable material that exhibit a wicking ability may be used for the paper strip. For example, chromatography paper or nitrocellulose membrane can be used. The paper strip may be disposed over the CMOS-based sensor unit, i.e. to carry the biological sample directly into or over the reaction zone.

In one example, the reaction zone includes microfluidic channels arranged to draw the biological sample away from the paper strip. In this case, the first testing region and the second testing region may be disposed on the CMOS-based sensor
unit. However, in other examples the first testing region and the second testing region are integrally formed in the paper strip. This may enable the sensor unit to be used for different combinations of testing regions, and may enable the sensor unit to be used repeatedly without needing to clean or re-load the reaction zone with a test solution.

The CMOS-based sensor unit may comprise an optical sensor. The optical sensor may be arranged to detect changes in the appearance of the reaction zone, e.g. by capturing an image or determining a change in optical properties thereof. The apparatus may comprise an optical source (e.g. LED or the like) for illuminating the test material with optical radiation. In one example, the optical sensor may be a spectral absorption sensor, e.g. a photodiode or an array of photodiodes or/and a single photon avalanche diode (SPAD) to increase the detection dynamic range.

The CMOS-based sensor unit may have multiple sensing modalities. For example, it may comprise a substrate having a first sensing element and a second sensing element fabricated thereon. The first sensing element and the second sensing element may be arranged to detect simultaneously different properties of the reaction zone to enable simultaneous detection of a plurality of metabolites. The first sensing element may comprise an optical sensor (e.g. as described above). The second sensing element may detect a different property from the first sensing element. For example, the second sensing element may be a chemical sensor, e.g. arranged to determine a change in composition or chemistry within the reaction zone (or within one or more of the testing regions).

In one example, the second sensing element is a pH sensor, e.g. comprising an ion sensitive field effect transistor (ISFET) having a gate electrode in contact with the test material. The apparatus may include a reference electrode arranged to apply a voltage to the reaction zone.
The apparatus may comprise an array of CMOS-based sensor units. Respective signals can be measured from each sensor unit. Alternatively, an average signal can be measured from the array of sensor units. Measuring an average signal can greatly reduce signal noise: according to Gaussian statistics, signal noise is reduced as a function of $\sqrt{N}$, where $N$ is the number of sensor units.

Each CMOS-based sensor unit in the array may be independently addressable to obtain signals corresponding to each of the testing regions.

The biological sample may be blood serum, but the invention can be used with any biological sample capable of communicating metabolites into the reaction zone.

In another aspect, the invention may provide a method of detecting metabolites in a biological sample, the method comprising: applying, in a reaction zone of a detection apparatus, the biological sample to a test material whose properties are affected by the presence of metabolites to be detected; simultaneously measuring different properties of the test material in the reaction zone using a CMOS-based sensor unit having multiple sensing modalities, the sensor unit comprising a substrate having a first sensing element and a second sensing element fabricated thereon; and determining the presence of a plurality of metabolites based on output signals from the first sensing element and the second sensing element.

The sensor unit may comprise any of the features discussed above. For example, the first sensing element may comprise an optical sensor, wherein the method includes illuminating the reaction zone with optical radiation, and wherein the output signal from the first sensing element is indicative of absorption of the optical radiation by the test material. The second sensing element may comprise a pH sensor, wherein the output signal from the second sensing element is indicative of a pH of the test material.
In another aspect, the invention may provide a method of detecting metabolites in a biological sample, the method comprising: applying, in a sample receiving module of a detection apparatus, the biological sample to a paper strip; transporting the biological sample through the paper strip to a reaction zone comprising a first testing region and a second testing region spatially separated from the first testing region, wherein properties of the first testing region and the second testing region are affected by the presence of metabolites to be detected; simultaneously measuring properties of the first testing region and the second testing region using a CMOS-based sensor unit thereby to obtain separate signals indicative of the presence of metabolites in each of the first testing region and the second testing region; and determining the presence of a plurality of metabolites based on output signals from the CMOS-based sensor unit that correspond to the first testing region and the second testing region. The reaction zone may be located over the CMOS-based sensor unit, whereby the paper lies over the CMOS-based sensor unit.

The sensor unit may comprise any of the features discussed above. For example, the sensor unit may comprise an optical sensor, wherein the method includes illuminating the reaction zone with optical radiation, and wherein the output signals are indicative of absorption of the optical radiation at the first testing region and/or second testing region respectively. Alternatively or additionally, the sensor unit may comprise a pH sensor, wherein the output signals from the first testing region and/or second testing region are indicative of a pH.

BRIEF DESCRIPTION OF THE DRAWINGS
Embodiments of the invention will now be described by way of example with reference to the accompanying drawings, in which:

Fig. 1 shows a plan view of a CMOS chip with an array of sensors;

Fig. 2 shows a plan view of a first multiplex assay apparatus for performing multiple simultaneous measurements;

Fig. 3 shows a close up view of a CMOS chip used in the apparatus of Fig. 2;

Fig. 4 shows a close up view of a paper strip used in the apparatus of Fig. 2;

Fig. 5 shows a plan view of a second multiplex apparatus for performing multiple simultaneous measurements;

Fig. 6 shows a close up view of a paper strip used in the apparatus of Fig. 5;

Fig. 7 shows a schematic view of an ion-sensitive field-effect transistor which may be used in the present invention;

Fig. 8 shows a plan view of a third multiplex assay apparatus for performing multiple simultaneous measurements;

Fig. 9 shows a close up view of a paper strip used in the apparatus of Fig. 8;

Fig. 10 shows a schematic view of an alternative paper strip;

Fig. 11 is a set of graphs showing measurements obtained from three channels on a single paper strip;

Fig. 12 shows a schematic view of a fourth multiplex assay apparatus for performing multiple simultaneous measurements;

Fig. 13 shows a close up view of a CMOS chip used in the apparatus of Fig. 12; and

Fig. 14 is a schematic side view of an optical alignment tool for manufacturing a CMOS chip that is an embodiment of the invention.
Embodiments of the present invention provide a metabolite detection device arranged to detect simultaneously multiple metabolites from a single biological sample. The device includes a reaction zone with spatially separated testing regions that have properties that are sensitive to the presence of different metabolites. The device comprises a single CMOS-based chip having one or more sensing modalities capable of detecting the properties of the separate testing region to determine the presence of multiple metabolites in the sample.

The one or more sensing modalities are provided by components fabricated on the CMOS-based chip. In the examples discussed below, the sensing modalities include an optical sensor, e.g. for sensing optical radiation, and a pH sensor, e.g. for sensing a concentration of ionic species in a sample. However, it may be understood that the principles of the invention are applicable to any kind of sensor that can be fabricated or post processed on a CMOS chip and which is capable of detecting information indicative of the presence of a metabolite.

The sample may be a biological sample (e.g. fluid or tissue) obtained from a subject in any conventional manner. In the example discussed below the sample is blood serum, but it should be understood that the invention may encompass the use of other (or additional) sample types such as urine, sweat and swab from other body openings.

Fig. 1 shows a plan view of a complementary metal oxide semiconductor (CMOS) chip 1001 having an array of sensors 1002 across the surface of the chip. The chip 1001 is typically a silicon integrated circuit (IC), and the sensors 1002 may be photosensitive (e.g. photodiodes or/and single photon avalanche diodes (SPADs)) or chemical sensors (e.g. ion-sensitive field-effect transistors (ISFETs) or electrochemical...
electrodes) as will be explained in further detail below. Alternatively, in certain embodiments which are indicated below, each sensor 1002 may include a pH sensor in addition to a photodiode.

The present invention relates to the use of a single chip of the kind shown in Fig. 1 to make multiple simultaneous measurements on a liquid sample (e.g. blood, blood serum, urine) by dividing the array of sensors 1002 into multiple assay regions. Division of the sensors 1002 can be done by physical separation of the assay regions on the chip itself or by providing discrete treatment zones (e.g. multiple microfluidic channels) on a paper strip which is used to introduce a liquid sample to the sensors 1002. Although the chip 1001 shown is a 3 x 4 array of sensors 1002, the present invention may comprise a 16 x 16 array of sensors 1002.

Fig. 2 shows a schematic perspective view of a first multiplex assay apparatus for performing multiple simultaneous measurements on a liquid sample 105. Close up views of the chip 102 and paper strip 104 used for the assay are shown in Figs. 3 and 4, respectively. The assay apparatus comprises a chip carrier 101 for a CMOS chip 102. The surface of the chip 102 has an array of photodetectors (e.g. photodiodes or/and SPADs), in the manner shown in Fig. 1. An epoxy layer 103 is provided on the chip carrier 101 to form a channel across the surface of the chip 102 for receiving a paper strip 104. The epoxy layer 103 also protects wire bindings between the chip 102 and chip carrier 101. An LED 106 is positioned above the chip 102 to illuminate the photodiodes such that they are able to detect a change in colour during the assay, as described below.

The chip 102 is shown in more detail in Fig. 3. A series of physically discrete treatment zones are provided by microfluidic channels 110, 111, 112. In this example, there are three channels. Two of the channels are activated to respond to substances to be detected, and the third channel is
a control. The principles of the invention are not limited to this arrangement. There may be any number of channels activated in a manner to detect a plurality of different substances.

The channels are fabricated with a photoresist 107 on top of the chip 102, which is glued and wire-bound to the chip carrier 101. Microfluidic channel I 110 is coated with enzyme I 108; microfluidic channel II 111 is coated with enzyme II 109; and microfluidic channel III 112 is not coated with any enzyme so as to give a negative control channel. In this way, the photoresist physically separates assay regions on the chip 102 itself.

The paper strip 104 is shown in more detail in Fig. 4. The paper strip 104 is sized to fit the channel formed in the epoxy layer 103 across the surface of the chip 102. The paper strip 104 has a reaction zone that is arranged to fit over the chip in use. The reaction zone is modified using a hydrophobic polymer to form three microfluidic channels 113, 114, 115 which correspond respectively to the three microfluidic channels 110, 111, 112 on the surface of the chip 102. The polymer confines the sample that is transported along the paper strip within the three channels 113, 114, 115. This arrangement prevents cross-talking and cross-contamination.

To perform a multiplexed assay using the apparatus shown in Fig. 2, the paper strip 104 is inserted into the channel formed in the epoxy layer 103 until the reaction zone is in contact with the surface of the chip 102. A drop of analyte solution 105 is applied to one end of the paper strip 104. The analyte solution contains (possible among other things) substances I and II, which may be different metabolites, wherein enzyme I 108 is specific for substance I and enzyme II 109 is specific for substance II. Due to capillary force, the analyte solution 105 flows along the paper strip 104 to the top of the chip 102, where reactions takes place in the
microfluidic channels. The enzyme reactions may generate colour changes on the paper strip 104. Enzyme I 108 generates one colour change 113, and enzyme II 109 generates a different colour change 114. The negative control channel generates no colour change 115. The colour changes are detected in real time by the photodiodes on the chip 102 under the illumination of LED 106, the multiple colour changes producing multiple detections. The change in colour is detected as light absorption by the enzyme reaction products. In this way, the apparatus allows the detection of multiple metabolites in a single assay.

In the configuration depicted in Figs. 2-4, microfluidic channels 110-112 are defined on the surface of the chip 102. Each microfluidic channel is a distinct assay region which is physically separated from the other channels and which has its own photodiodes. The paper strip 104 wets the surface of the chip 102 such that the analyte solution 105 is drawn down into the microfluidic channels 110-112 on the chip surface. Optical cross-talk is limited as the colour change chemistry is in an immediately proximal channel, the channels being physically separated by the photoresist 107. Cross-talk may also occur by chemical diffusion and capillary action from the microfluidic channels on the chip into the paper, and subsequent transfer across into an adjacent channel. Such cross-talk is minimised by ensuring that the distance between adjacent microfluidic channels is large enough, for example by thickening the walls of photoresist 107.

By replacing the photosensitive sensors with chemical sensors, the apparatus described may be suitable for detecting reactions by a pH change of the solution. This is described in more detail below.

Fig. 5 shows a plan view of a second multiplex assay apparatus for performing multiple simultaneous measurements on a liquid sample 205. A close up view of the paper strip 204 is shown in Fig. 6. The assay apparatus comprises a chip carrier
201 for a CMOS chip 202. The surface of the chip 202 has an
array of photodiodes and/or single photon avalanche diodes, in
the manner shown in Fig. 1. An epoxy layer 203 is provided on
the chip carrier 201 to form a channel across the surface of
the chip 202 for receiving a paper strip 204. The epoxy layer
203 also protects wire bindings between the chip 202 and the
chip carrier 201. An LED 206 having a known, specific
wavelength is positioned above the chip 202 to illuminate the
photodiodes such that they are able to detect a change in
colour during the assay, as described below.

The paper strip 204 is shown in more detail in Fig. 6. The
paper strip 204 is sized to fit the channel formed in the
epoxy layer 203 across the surface of the chip 202. The paper
strip is modified with a hydrophobic polymer 215 to form three
microfluidic channels 212, 213, 214 which extend a substantial
distance between a first end and a second end of the elongate
paper strip 204. The hydrophobic polymer 215 prevents cross
talk and cross-contamination between the three microfluidic
channels 212, 213, 214. Microfluidic channel I 212 is coated
with enzyme I 207; microfluidic channel II 213 is coated with
enzyme II 208; and microfluidic channel III 214 is not coated
with any enzyme so as to give a negative control channel.
Different assay regions are thereby defined by the different
channels on the paper strip 204.

To perform a multiplexed assay using the apparatus shown
in Fig. 5, the paper strip 204 is inserted into the channel
formed in the epoxy layer 203. To prevent cross talk and
cross-contamination the paper strip 204 should not come into
contact with the surface of the chip 202, but should instead
rest a distance away from the chip 202. A drop of analyte
solution 205 is applied to one end of the paper strip 204. The
analyte solution contains substrates I and II, which may be
different metabolites, wherein enzyme I 207 is specific for
substrate I and enzyme II 208 is specific for substrate II.
Due to capillary force, the analyte solution 205 flows along
the paper strip 204 through the microfluidic channels 212-214, where reactions with the enzymes 207, 208 take place. The enzyme reactions produce colour changes on the paper strip 204. Enzyme I 207 generates one colour change 209 in microfluidic channel I 212, and enzyme II 208 generates another colour change 210 in microfluidic channel II 213. Microfluidic channel III 214 for negative control generates no colour change 211. The colour changes are detected in real time by the photodiodes on the chip 202 under the illumination of LED 206, the multiple colour changes producing multiple detections. In this way, the apparatus allows the detection of multiple metabolites in a single assay.

In the configuration depicted in Figs. 5 and 6, microfluidic channels 212-214 are defined on the paper strip 204. Each microfluidic channel is a distinct assay region which is physically separated from the other channels. The paper strip 204 is in close proximity to the photodiodes on the surface of the chip 202, but it is not in contact. Cross talk may occur by optical scattering of large angles through the paper casting light onto adjacent sensors. The distance between the chip 202 and the paper strip 204 is made small so as to limit or eliminate such optical cross talk. In addition, channel spacing on the paper strip 204 must be large enough to keep cross talk at a low level to allow independent measurement of the colour change occurring in each microfluidic channel. This is done by varying the thickness of the microfluidic channels and the hydrophobic polymer barriers separating them.

The embodiments described above each work by detecting a colour change, using photodiodes illuminated by an LED having a known, specific wavelength. However, for embodiments where the sample is brought into contact with the chip, the invention may alternatively or additionally make use of an array of chemical sensors on a chip. For example, the chemical sensors may be ion-sensitive field-effect transistors
An ISFET is a field-effect transistor in which a solution is used as the gate electrode. A change in pH (i.e. a change in concentration of H⁺ ions) of the solution causes a current running though the ISFET to change by a measurable amount.

Fig. 7 shows a schematic view of an ISFET 300 which may be used as a chemical sensor for detecting the pH of a solution 301 in the present invention. The ISFET 300 comprises a well for receiving solution 301 formed by an epoxy 302 on the surface of the ISFET 300. The solution 301 contains a concentration of H⁺ ions to be detected. The well ensures that the solution 301 is in contact with a gate oxide layer 303. A source 304 and a drain 305 are also provided in a bulk layer 306, with both source 304 and drain 305 in contact with the gate oxide 303, on a side which is opposite the solution 301. The presence of H⁺ ions in the solution 301, which are adsorbed onto the surface of the gate oxide 303, causes migration of charge carriers to the upper surface of the bulk layer 306. Current is thereby able to flow between the source 304 and the drain 306 through the bulk layer 306. As the source/drain current is affected by the concentration of H⁺ ions, applying a known voltage to the reference electrode 307, which is at least partially immersed in the solution 301, allows the pH of the solution 301 to be determined. Alternatively, the source/drain current can be kept constant and the voltage change at the reference electrode 307 measured to determine the pH of the solution 301.

Fig. 8 shows a plan view of a third multiplex assay apparatus for performing multiple simultaneous measurements on a liquid sample 405. A close up view of the paper strip 404 used for the assay is shown in Fig. 9. The assay apparatus comprises a chip carrier 401 for a CMOS chip 402, similar to the embodiments shown above. However, in this third embodiment the surface of the chip 402 has an array of chemical sensors, in particular ISFETs, substantially as described above with
reference to Fig. 7. An epoxy layer 403 is provided on the chip carrier 401 to form a channel across the surface of the chip 402 for receiving a paper strip 404. The epoxy layer 103 also protects wire bindings between the chip 402 and chip carrier 401. An LED having a known, fixed wavelength may be provided where the sensors on the chip 402 also include a photodiode, as described herein.

The chip 402 is substantially identical to chip 102 shown in Fig. 3. However, chip 402 comprises an array of chemical sensors. Chip 402 may, however, comprise a number of microfluidic channels which are coated with enzymes, and a negative control channel which is not coated with an enzyme, in the manner of chip 102 shown in Fig. 3. The microfluidic channels provide multiple distinct assay regions.

The paper strip 404 is shown in more detail in Fig. 9. The paper strip 404 is sized to fit the channel formed in the epoxy layer 403 across the surface of the chip 402. The paper strip 404 is modified with a hydrophobic polymer to form three microfluidic channels 413, 414, 415 which correspond to microfluidic channels on the surface of chip 402. This arrangement prevents cross-talking and cross-contamination. The paper strip 404 also comprises a metallised region 406, which may be printed or impregnated with silver such that the metallised region 406 may be used as a reference electrode in the assay process as described below.

To perform a multiplexed assay using the apparatus shown in Fig. 8, the paper strip 404 is inserted into the channel formed in the epoxy layer 403 until it is in contact with the surface of the chip 402. A drop of analyte solution 405 is applied to one end of the paper strip 404. The analyte solution contains multiple substrates, which may be different metabolites, wherein each enzyme in a microfluidic channel on the surface of the chip 402 is specific to a substrate, in a manner which has been described above with reference to first and second multiplex assay apparatus. Due to capillary force,
the analyte solution 405 flows along the paper strip 404 to the top of the chip 402, where reactions take place in the microfluidic channels. The enzyme reactions generate pH changes on the paper strip 404; although there is no pH change in the negative control channel. The pH changes may be detected by applying a fixed reference voltage to the metallised region 406 of the paper strip 404, and measuring the change in source to drain current through each of the ISFET chemical sensors on the surface of the chip 402. To convert these current measurements to pH values, the measurements are compared with a source to drain current obtained with a solution of known pH and the same, fixed reference voltage. In this way, the apparatus allows the detection of multiple metabolites in a single assay.

Each microfluidic channel on the surface of the chip 402 is a distinct assay region which is physically separated from the other channels and which has its own chemical sensors. The paper strip 404 wets the surface of the chip 402 such that the analyte solution 405 is drawn down into the microfluidic channels on the chip surface. Cross-talk may also occur by chemical diffusion and capillary action from the microfluidic channels on the chip into the paper, and subsequent transfer across into an adjacent channel. Such cross-talk is minimised by ensuring that the distance between adjacent microfluidic channels is large enough, for example by thickening the walls of photoresist on the surface of the chip 402.

In addition to pH sensitive detectors as described, the chip 402 may comprise an array of sensors which combine a photodiode and an ISFET. In this way, the multiplex assay apparatus of Fig. 8 may also be configured to perform assays by the light absorption method described above, using an LED having a known, specific wavelength to illuminate the paper strip 404 and the chip 402. The pH change of the reactions in the microfluidic channels may also be measured at the same
time as light absorption to carry out more in depth multiplex assays.

Fig. 10 shows a schematic view of an alternative paper strip 500 which may be used with the multiplex assay apparatus of Fig. 8. The paper strip 500 comprises a blister pack 501 for containing a solution, such as an electrolyte of pH buffer solution, which is able to aid transport of an analyte sample along the paper strip 500. The paper strip 500 also comprises a metallised region 503, which may be printed or impregnated with silver, to be connected to a reference voltage 504 such that the metallised region 503 can act as a reference electrode in a manner as described above.

In use, an analyte sample may be spotted at 502. The blister pack 501 can be squeezed or otherwise burst to release the solution within, and so aid transport of the analyte sample along the paper strip 500 for a multiplex assay.

Fig. 11 shows a set of graphs that illustrate the results of a test using a single paper strip having three channels on one single paper strip. The three channels were tested simultaneously by adding one drop of analyte from one side of the paper strip. The top image 550 in Fig. 11 is an intensity graph of the three channels (dark regions 552) on the photodiode under the illumination of an LED. The middle channel was used as control (i.e. was not printed with an enzyme). The side channels were each printed with a respective enzyme (Enzyme 1 and 2). The three lower images are graphs showing electrical signal amplitude from each of the channels with time. When the analyte reached the sensing region, there is a big step jump of the electrical signal in all channels due to the wetting of the paper. After around half minute, a decreasing signal can be seen from the side channels with enzymes. As a control, the middle channel gives nearly no signal change.

Fig. 12 shows a schematic view of a fourth multiplex apparatus 600 for performing multiple simultaneous
measurements on a liquid sample. A close up view of the chip 601 used for the assay is shown in Fig. 13. The assay apparatus comprises a chip carrier (not shown) for a CMOS chip 601. Spaced away from the chip is an annular wall 602 which defines the outer edge of four reaction chambers, or quadrants, which are separated from each other by separation walls 603. A different reaction may take place in each quadrant, as explained in more detail below. An epoxy layer 604 forms the base of the reaction chambers, and also protects wire bindings between the chip 601 and the chip carrier. However, the epoxy layer 604 does not extend over the surface of the chip 601, which ensures that the surface of the chip 601 is open to be able to detect reaction parameters, such as a colour or pH change. An LED having a known, specific wavelength may be positioned above the chip 601 if the reactions are to be detected by light absorption.

The chip 601 is positioned at the meeting point between the separation walls 603 such that the chip 601 is divided into four distinct assay regions 605a, 605b, 605c, 605d. Each assay region 605 is defined by a micro-well in the epoxy layer 604. When a liquid sample is deposited in each of the reaction chambers, the micro-wells are filled such that liquid is in contact with sensors 606 in each of the assay regions 605. The assay regions 605 are separated from each other by sidewalls 607, so that liquid cannot leak into an adjacent assay region. The sensors 606 may be photosensitive (e.g. photodiodes or single photon avalanche diodes) or chemical sensors (e.g. ISFETs or electrochemical electrodes). Alternatively, the chip 601 may comprise an array of sensors 606 which combine a photodiode and an ISFET. In this way, the multiplex assay apparatus may also be configured to perform assays by the light absorption method described above, using an LED having a known, specific wavelength to illuminate the reaction chambers and the chip 601. The pH change of the reactions in the
microfluidic channels may also be measured at the same time as light absorption to carry out more in depth multiplex assays.

In one example, the multiplex apparatus 600 is manufactured as follows. Each assay region 605 is protected by a polydimethylsiloxane (PDMS) block, which helps to shape an epoxy (preferably black epoxy), which is introduced in a succeeding step, and also protect the sensor array area of the chip 601 from damage during the manufacturing process. The use of black epoxy can prevent cross talk in a signal (either optical or electronic) from occurring between different assay regions (also referred to herein as micro-wells).

The PDMS blocks are positioned by microscope assisted translation on top of the chip 601. The gap between them determines the separation distance between adjacent micro-wells, and the height of the blocks is an upper limit to the micro-well depth. Epoxy mixture is then carefully poured around the blocks, and over the surface of the chip carrier to form the epoxy layer 604. The epoxy is left to cure and harden for around 12 hours. The annular wall 602 is then introduced to form the outer wall of the four reaction chambers. The annular wall 602 may be a ring made of plastics material, having a height of around 8 mm. The annular wall may be held in place with epoxy. PDMS blocks are then used to define the shape of the reaction chambers and separation walls 603. They may be shaped and positioned manually, or the separation walls may be first made from a polystyrene sheets with the desired separation wall dimensions and PDMS poured into the cavities defined by the polystyrene and annular wall 602 to be cured. When the polystyrene sheets are removed, the sidewalls 603 can be properly formed from epoxy by filling the gaps between the PDMS blocks. After curing of the epoxy, the PDMS blocks may be removed, as well as the PDMS blocks defining the micro-wells 605. The micro-wells thus have pipettable access and sidewalls 603 separate the liquid into individual reaction chambers to prevent mixing. This multilevel sequential die casting
technique is compatible with CMOS processing since it can be used at room temperature. It may be expedited by raising temperature to 70°C.

In this technique, the resulting micro-wells may exhibit a step profile opening into a wider area. This allows pipettable access to the micro-wells without requiring micro-tubing or fluid management pumps for sample delivery. In turn this can save on time required for the delivery of samples.

In another example, the reaction chambers (and micro-wells discussed below) are manufactured by mounting a pre-formed cartridge over the chip carrier. The cartridge may be formed from any suitable material, e.g. plastic. It may have a form similar to the annular wall 602 and separation walls 603 discussed above. The cartridge may be affixed to the chip carrier in any conventional manner, e.g. by screws or the like.

Fig. 14 is a schematic view of an optical alignment tool 570 may be used to align the cartridge 572 with the chip carrier 574 during the mounting process and to maintain alignment during the attachment (e.g. screwing) process. The optical alignment tool comprises a reciprocating movement mechanism 571 for bringing a movable holder 573 into contact with a platform 575. The chip carrier 574 is mounted on the platform 575 via an alignment stage 576 that permits adjustment in two orthogonal linear dimensions (e.g. x- and y-dimensions) in the plane of the platform 575 and a rotation dimension (e.g. about an axis extending perpendicular to the platform 575). A pivoting lever may be used to operate the linear motion of the reciprocating movement mechanism 571.

A laser source 578 is mounted on the movable holder 573 to emit a laser beam towards the platform 575. The cartridge 572 is mounted on the holder to partially block the laser beam, whereby a pattern of the separation walls is projected on to the chip carrier 574 on the platform 575.
With this arrangement, the laser illuminates a spot on the chip with a pattern that is indicative of alignment with the cartridge.

The movable holder 573 may use suction or a magnetic retainer to hold the combination of laser source and cartridge. The alignment between the cartridge and chip carrier can be adjusted via the alignment stage 576 during linear movement of the cartridge and subsequent fixing thereof to the chip carrier.

In one example, the optical alignment tool may be fabricated as part of a microscope. Visual inspection of alignment with the chip itself can be carried out through the microscope while fixing the cartridge in place.

To perform a multiplexed assay using the apparatus shown in Fig. 12, a different reaction enzyme is pipetted into each of three micro-wells 605a, 605b, 605c, leaving one micro-well 605d without an enzyme to act as a negative control. An analyte solution may then be introduced into each of the four reaction chambers. The analyte solution may contain at least three substrates, or metabolites, wherein each enzyme is specific to a substrate, as described above with reference to other embodiments of the invention. Reactions between the enzymes and substrates take place in each of the micro-wells 605a–c, where they are detected by sensors 606. For example, the reaction may cause a colour change which may be detected by photodiodes and/or a pH change which may be detected by chemical sensors in a manner substantially as described above. In this way, the apparatus allows the detection of multiple metabolites in a single assay, using a single chip 601.

In embodiments of the invention that use the micro-well arrangement discussed above, it may be desirable to have a minimum of 3×3 pixels per micro-well. The width of the wall separating adjacent micro-wells may be around 40 µm. This ensures that the separation between micro-wells consumes no more than one pixel row or column. The walls may seal against
the sensor array area of the chip using a pressure sensitive adhesive. The adhesive may expand under applied pressure, so a thickness of the walls is set within a tolerance to ensure this expansion does not block additional pixels.

The micro-wells may have a height selected to be between an average height for a microchannel (e.g. ~150 µm) and a typical assay height (e.g. ~3 mm) for 4 micro-well chip. To facilitate rapid delivery of different analytes, the device may comprise a plurality of inlet ports for directing a fluid sample into a respective micro-well. A pitch of the inlet ports may be matched to a pitch of multi-channel micropipette to enable simultaneous delivery. To maintain the reagent fluidic volume and for easy passage of reagents by capillary forces, each micro-well may comprise an outlet. The inlet into each micro-well may include both a capillary conduit to enable liquid delivery through capillary action, as well as one or more reservoirs for reagent mixing before delivery to the micro-well.

In one example, a reference electrode for the micro-wells may be formed on or integrated with the walls that define the micro-wells. For example a 100 µm diameter Ag/AgCl electrode may be integrated into the micro-wells from the side of the micro-wells. This arrangement can provide an independent reference electrode for each micro-well, which in turn enables the ISFET function of chip to be used simultaneously in each micro-well.

If the chip is equipped with multiple sensing modalities, the number of analytes that can be assayed simultaneously can multiply by the number of micro-wells that are present. For example, in an arrangement with four micro-wells and two independent sensing modalities, one can assay eight analytes simultaneously in real time.

In one example, the delivery of fluid to the device may be controlled through a fluid management algorithm configured to effect sequential delivery of the reagents including any or
all of the steps of (i) diluting of the analyte, (ii) introducing supporting reagents for the reaction, (iii) introducing the sample (e.g. human bodily fluid such as blood, serum, urine, etc.), and (iv) introducing the enzyme to initiate the reaction.

In each of the multiplex assay apparatus described above, the chip and chip carrier may be mounted on a printed circuit board (PCB), where the chip is integrated with a microcontroller to provide addressing signals and to acquire output readings from the array of sensors on the chip. The readings may then be transferred wirelessly or via universal serial bus (USB) to a computer based program (e.g. LabVIEW ®) or android based program in which the data may be processed and analysed.

Where an LED is used to perform the multiplex assays, the optical characteristics of the LED and the sensors on the chip must be evaluated prior to carrying out the assay to examine their spectral relationship. The LED should preferably be selected to emit light having a wavelength which is close to the peak sensitivity of the photosensitive sensors.

While the invention has been described in conjunction with the exemplary embodiments described above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure.

Accordingly, the exemplary embodiments of the invention set forth above are considered to be illustrative and not limiting.

REFERENCES


Quantification, IEEE Transactions on Biomedical Circuits and Systems 10(3) : 721-730
1. An apparatus for detecting metabolites in a biological sample, the apparatus comprising:
   a sample receiving module arranged to receive the biological sample, the sample receiving module comprising a paper strip configured to transport the biological sample to a reaction zone for testing, wherein the reaction zone comprises a first testing region and a second testing region spatially separated from the first testing region, wherein properties of the first testing region and the second testing region are affected by the presence of metabolites to be detected; and
   a CMOS-based sensor unit disposed in relation to the reaction zone to detect independently the properties of the first testing region and the second testing region thereby to obtain separate signals indicative of the presence of metabolites in each of the first testing region and the second testing region.

2. An apparatus according to claim 1, wherein the paper strip is disposed over the CMOS-based sensor unit.

3. An apparatus according to claim 1 or 2, wherein the first testing region and the second testing region are integrally formed in the paper strip.

4. An apparatus according to claim 1 or 2, wherein the first testing region and the second testing region are disposed on the CMOS-based sensor unit.

5. An apparatus according to any preceding claim comprising a fluid flow barrier separating the first testing region from the second testing region.
6. An apparatus for detecting metabolites in a biological sample, the apparatus comprising:

a sample receiving module arranged to receive the biological sample and transport it to a reaction zone for testing, wherein the reaction zone comprises a first testing region and a second testing region spatially separated from the first testing region, wherein properties of the first testing region and the second testing region are affected by the presence of metabolites to be detected; and

a CMOS-based sensor unit disposed in relation to the reaction zone to detect independently the properties of the first testing region and the second testing region thereby to obtain separate signals indicative of the presence of metabolites in each of the first testing region and the second testing region,

wherein the first testing region and the second testing region each comprise a respective micro-well formed over a respective portion of the CMOS-based sensor unit, the micro-wells being separated from each other by a barrier portion.

7. An apparatus according to claim 6, wherein the barrier portion is a wall formed from black epoxy.

8. An apparatus according to any preceding claim, wherein the first testing region is sensitive to a first metabolite, and the second testing region is sensitive to a second metabolite, whereby the separate signals are indicative of the presence of the first metabolite and second metabolite respectively.

9. An apparatus according to any preceding claim, wherein the reaction zone includes a control region that is not sensitive to the presence of metabolites to be detected.
10. An apparatus according to any preceding claim, wherein the first testing region and/or the second testing region comprises a test material arranged to support a metabolite-activated reaction upon receiving the biological sample.

11. An apparatus according to any preceding claim, wherein the CMOS-based sensor unit comprises an optical sensor.

12. An apparatus according to claim 11 including an optical source for illuminating the reaction zone with optical radiation.

13. An apparatus according to claim 11 or 12, wherein the optical sensor is a photodiode and/or a single photon avalanche diode.

14. An apparatus according to any preceding claim, wherein the CMOS-based sensor unit has multiple sensing modalities, and comprises a substrate having a first sensing element and a second sensing element fabricated thereon.

15. An apparatus according to claim 14, wherein the first sensing element comprises an optical sensor that incorporates a photodiode and/or single photon avalanche diode.

16. An apparatus according to claim 14 or 15, wherein the second sensing element is a chemical sensor.

17. An apparatus according to any one of claims 14 to 16, wherein the second sensing element is a pH sensor.
18. An apparatus according to any one of claims 14 to 17, wherein the second sensing element comprises an ion sensitive field effect transistor (ISFET) having a gate electrode in contact with the reaction zone.

19. An apparatus according to claim 18 including a reference electrode arranged to apply a voltage to the reaction zone.

20. An apparatus according to any preceding claim comprising an array of CMOS-based sensor units.

21. An apparatus according to claim 20, wherein each CMOS-based sensor unit in the array is independently addressable.

22. An apparatus according to any preceding claim, wherein the biological sample is blood serum.

23. A method of detecting metabolites in a biological sample, the method comprising:
   applying, in a sample receiving module of a detection apparatus, the biological sample to a paper strip;
   transporting the biological sample through the paper strip or by capillary action to a reaction zone comprising a first testing region and a second testing region spatially separated from the first testing region, wherein properties of the first testing region and the second testing region are affected by the presence of metabolites to be detected;
   simultaneously measuring properties of the first testing region and the second testing region using a CMOS-based sensor unit thereby to obtain separate signals indicative of the presence of metabolites in each of the first testing region and the second testing region; and
determining the presence of a plurality of metabolites based on output signals from the CMOS-based sensor unit that correspond to the first testing region and the second testing region.

24. A method according to claim 23, wherein the sensor unit comprises an optical sensor, and wherein the method includes illuminating the reaction zone with optical radiation.

25. A method according to claim 23, wherein the sensor unit comprises an optical sensor, and wherein the method includes performing a chemiluminescence assay.
A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/49

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

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

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

EPO-Internal , WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of Box C.  
[X] See patent family annex.

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Date of the actual completion of the international search

3 October 2018

Date of mailing of the international search report

10/10/2018

Name and mailing address of the ISA/

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