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Hybrid dual mode sensor for simultaneous detection of two serum metabolites

Chunxiao Hu, Mohammed Al-Rawhani, Boon Chong Cheah, Srinivas Velugotla, and David R. S. Cumming, Fellow, IEEE

Abstract—Metabolites are the ultimate readout of disease phenotype that play a significant role in the study of human disease. Multiple metabolites sometimes serve as biomarkers for a single metabolic disease. Therefore, simultaneous detection and analysis of these metabolites facilitates early diagnostics of the disease. Conventional approaches to detect and quantify metabolites include mass spectrometry and nuclear magnetic resonance that require bulky and expensive equipment. Here we present a disposable sensing platform that is based on complementary metal oxide semiconductor process. It contains two sensors: an ion sensitive field effect transistor and photodiode that can work independently for detection of pH and colour change produced during the metabolite-enzyme reaction. Serum glucose and cholesterol have been detected and quantified simultaneously with the new platform, which shows good sensitivity within the physiological range. Low cost and easy manipulation make our device a prime candidate for personal metabolome sensing diagnostics.

Index Terms—Simultaneous detection, CMOS-based sensor system, coronary heart disease, serum metabolites, personal diagnostics

I. INTRODUCTION

RECENTLY, metabolomics studies have been proven to provide novel insights into biomarker discovery.[1-3]

Metabolism is a vital cellular process, and metabolic dysfunction can be a major contributor to many human diseases.[4] Metabolites are the ultimate readout of disease phenotype, therefore quantification and analysis of metabolites plays a significant role in the study of biochemical reaction and signaling networks. [4, 5]

One metabolite can be biomarker for different diseases but multiple metabolites can serve together as a biomarker for one disease. Neurodegenerative diseases such as Huntington’s disease,[6-8] cardiac disease includes ischemic stroke,[2, 9] and many cancers[4, 10] all have numerous metabolite biomarkers.

Mass spectrometry (MS) is the most widely used analytical technique to detect and quantify metabolites.[11, 12] It ionizes chemical species and sorts the ions based on the mass-to-charge ratio. Separation methods such as gas chromatography (GC) [13, 14] and liquid chromatography (LC) [15, 16] are usually required prior to the mass analysis. Nuclear magnetic resonance (NMR) spectroscopy is another technique that has been applied to metabolite studies.[17-20] It is a powerful tool for detecting, identifying and quantifying a wide range of metabolites without separation. However, both methods require bulky and expensive equipment. For widespread uptake of metabolite sensing in Point of Care (PoC) settings, the biosensor needs to be small, disposable and hence low cost.

The ion sensitive field effect transistor (ISFET) [21-24] and photodiode (non-UV) [25] have recently been proven to be novel and low-cost sensors for metabolite studies. An ISFET is a field-effect transistor that uses a solution as the gate electrode. It measures ion concentrations (e.g. H+) in solution and when the ion concentration changes, the current through the transistor changes accordingly.[26] Numerous metabolites (e.g. urea, acetylcholine, and glucose) produce H+ ions when they interact with specific enzymes, therefore the concentration of metabolites can be detected according to the H+ ion generated. A photodiode (PD) is a semiconductor device that absorbs light energy (photons) to generate a photo-current. If a PD is immersed in a liquid, the colour of which changes in the course of a reaction, then a change in the transmission of light through the liquid can be detected. If the light is chosen to be a wavelength that is strongly absorbed as the liquid changes colour, then quantification becomes possible. H2O2 is a common product generated during metabolite-enzyme reaction. When combined with a peroxydase and a redox colour-change indicator, the evolution of H2O2 is a reaction can then be detected by a PD. The concentration of metabolite relies on the absorption of the photons.

ISFET and photodiode can be fabricated at low cost for portable devices, providing an opportunity to make PoC platforms for metabolomics applications. Previous work has shown that both ISFETs and PDs can be used to detect single metabolites. Dual or multiple measurement might have been done but only on sole ISFET [27] or PD [28].

Here we report a more flexible approach for the detection of two metabolites in human serum simultaneously using a low-cost complementary metal-oxide-semiconductor (CMOS)-based device contains ISFET and PD. The ISFET and PD are able to work independently and simultaneously in the same solution. The ISFET and PD are fabricated using a standard commercial 350 nm CMOS process. Mass production reduces
the cost for a single chip so as to make it suitable for PoC applications.

Serum glucose is a vital fuel source in the human body. However elevated blood glucose can be a sign of hyperglycaemia or diabetes and may cause coronary heart disease. Serum cholesterol performs many essential duties in human body. It serves as a precursor for the biosynthesis of steroid hormones, bile acid and Vitamin D.[29] Higher levels of total cholesterol increase the risk of cardiovascular disease, particularly coronary heart disease. Both serum glucose and cholesterol have been comprehensively studied and proven to be independent risk factors for coronary heart disease.[30, 31] H. W. Cohen et al.[32] in 2004 discovered that there is an adverse synergistic interaction of fasting glucose with total cholesterol that magnifies the risk of incident coronary heart disease events associated with total cholesterol for hypertensive patients. Therefore serum glucose and cholesterol were chosen as the metabolites to be measured with our device. The glucose-hexokinase reaction produces protons and cholesterol-cholesterol oxidase reaction generates $\text{H}_2\text{O}_2$. Protons can be detected by ISFET and $\text{H}_2\text{O}_2$ can be measured by PD with the addition of redox indicator. Simultaneous detection of glucose and cholesterol from human serum was successfully performed with our CMOS-based device contains ISFET and PD. Since the two sensors work independently of each other, are detecting photons (PD) and protons (ISFET), we observe neither chemical nor electrical cross-talk in our device. This makes it suitable for detection of any other metabolites simultaneously, providing a cost-effective method for early diagnostic of disease.

II. MATERIALS AND METHODS

A. Device fabrication and structure

The chip was designed to incorporate a 16 x 16 array of sensor clusters with each cluster integrating a PD “pixel” and an ISFET “pixel” to enable simultaneous optical and ionic detection on the same surface. Each PD pixel integrates a p-n junction with a classic 3-transistor readout design. A global shutter scheme employing an integrated 4-bit row decoder and a 4-bit column decoder was used to scan through the PD array. The ISFET sensor was designed using a source follower configuration of two p-type transistors in which one gate is kept floating using a stack of three metals and exposed layer of silicon dioxide ($\text{SiO}_2$). This layer was realized by removing the two top passivation layers (polyamide and silicon nitride ($\text{Si}_3\text{N}_4$)) as illustrated in Fig. 1a. The ISFET array was scanned using two integrated decoders of 4-bits and 3-bits each, for addressing the columns and rows via an 8 to 1 analogue multiplexer outside the array active area. The entire ISFET array was designed using isolated transistors in deep n-wells to minimize crosstalk with the PD array. The chip (Fig. 1b) was fabricated using a commercially available CMOS 350 nm high voltage 4-metal process provided by austriamicrosystems. It is a ‘twin-tub’ process that gives good electrical isolation. A printed circuit board (PCB) platform was designed to interface the chip with an ARM mbed STM32 Nucleo-F334R8 board (STMicroelectronics, UK). The mbed microcontroller was programmed to provide addressing signals and to acquire the output readings from both PD and ISFET array outputs. The acquired data was then transferred by universal serial bus (USB) to a computer LabVIEW program, where it was processed and analyzed.

![Fig. 1. (a) Schematic diagram of one sensor cluster showing the internal structure of ISFET and PD (not to scale). (b) Microscope image of one CMOS chip, containing 256 individual sensor clusters (16x16). Insert is the enlarged view of a single sensor cluster, which is comprised by one ISFET and one PD sensor.](image)

B. Measurement configuration

For packaging, the chip was glued to a chip carrier and wire bonded. Biocompatible epoxy was then applied to open a sensing window on top of the chip and at the same time protect the bondwires from touching the analyte solution as illustrated in Figure 2. A custom-made plastic square base was glued onto the chip carrier, which was fitted with a custom-made rectangular cylinder. The cylinder functioned as a lid with integrated green LED (502 nm) and Ag/AgCl reference electrode. The green LED provided the light source for the PD and the reference electrode provided liquid gate voltage for the ISFET respectively. A sealed, dark and well-controlled environment for the measurement was created by the cylinder cap. A flexible heater was attached to the back of the chip carrier and its temperature was controlled by a PID temperature controller through a thermocouple. In this way, the sensor could be kept at a constant temperature of 37 °C, which is necessary for the best results to be obtained using some enzyme-based reactions.
Prior to each measurement, the entire sensing well was incubated with 3% bovine serum albumin (BSA) in 5 mM Triethanolamine buffer (see chemical preparation) for 30 minutes. It prevents molecules from attaching to the chip surface so as to minimize nonspecific binding. 10 µl silicone oil was then applied to prevent the buffer from evaporating. The reaction buffer (see chemical preparation) that contains all the enzymes and chemicals required for the reactions except the metabolites was then added to the sensing well to form a baseline signal. After 2 to 3 minutes, 10 µl 138 mg/dl human serum was added to initiate the two reactions: glucose-hexokinase and cholesterol-cholesterol oxidase. The former reaction produces protons which are detected by the ISFET and the latter generates colour change which is sensed by the PD (Fig. 2b).

![Schematic diagram](image)

**Fig. 2.** Schematic diagram of the measurement configuration. (a) All the components for the two enzyme reactions are mixed and added to the sensing window. (b) Reactions are initiated by applying the analyte: pH and colour change are sensed by ISFET and PD simultaneously.

### C. Chemical preparation

Triethanolamine hydrochloride (T9534), taurocholic acid sodium salt hydrate (T4009), sodium cholate hydrate (C1254), o-Dianisidine dihydrochloride (F5803), Adenosine 5’-triphosphate (ATP) disodium salt hydrate (A2383), Triton X-100 (T9284), silicone oil (85409), glucose (G8270), cholesterol (C3045), peroxidase from horseradish (P8250), cholesterol esterase from pseudomonas fluorescens (C9281), hexokinase from saccharomyces cerevisiae (H4502), cholesterol oxidase (VWR25108), Sodium hydroxide pellets (0583) from VMR.

The reaction buffer was prepared by mixing together the following enzymes and chemicals which are required for simultaneous glucose and cholesterol detection measurements: 46.9 µl 5mM triethanolamine HCl at pH 8, 1.7 µl 15% w/w taurocholic acid solution, 1.7 µl 15% w/w cholic acid solution, 5 µl 60U/ml peroxidase, 10 µl 500 mM NaCl, 1.7 µl 8.5 U/ml cholesterol esterase, 10 µl 100 mM MgCl2 solution, 10 µl 100 mM ATP pH8.5, 4 µl 7.89 mM o-Dianisidine, 1 µl 0.1 U/µl hexokinase, and 3 µl 10 U/ml cholesterol oxidase.

### III. Results and Discussions

#### A. Characteristics of the ISFET and PD

Prior to the actual simultaneous detection measurement with real analyte, the device was required to be characterized. The pH sensitivity of the CMOS based ISFET and the response (to the colour change) of the PD needed to be verified. The ISFET was characterized with Triethanolamine buffer that has the same salt concentration and buffering capacity as the actual measurement. Four different buffers with pH values 6, 7, 8 and 9 were made via adding 1M HCl or 1M NaOH. Prior to each measurement, the entire sensing well was incubated in 3% bovine serum albumin (BSA) in PBS to minimize nonspecific binding. After washing the chip thoroughly with deionized water, 100 µl pH 9 buffer was firstly added to the sensing well to obtain a baseline signal. The buffer was then replaced by pH 8, 7, and 6 buffer sequentially, which covered the pH range expected in actual measurements. Clear steps were observed between the buffers with different pH value (Fig. 3a). ΔV\textsubscript{ISFET} is a linear function of pH with a slope of ~35 mV/pH, which is in line with literature values for SiO\textsubscript{2} surfaces in the linear region.[33]

The PD was characterized with hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), which is a common product generated during the metabolite-enzyme interaction. Five different concentrations of H\textsubscript{2}O\textsubscript{2} from 31 µM to 375 µM were measured (Fig. 3b). The H\textsubscript{2}O\textsubscript{2} solution was prepared and diluted with Triethanolamine buffer at pH 8. A mixed solution of o-Dianisidine (7.89 mM) and peroxidase (60 U/ml) was first added to the sensing well to trigger the reaction. The H\textsubscript{2}O\textsubscript{2} solution was then added to trigger the reaction and the colour change was then detected by the PD. A linear function of PD voltage vs H\textsubscript{2}O\textsubscript{2} concentration (from 31 µM to 375 µM) was observed.
Both the ISFET and PD had been characterized using an appropriate solution and method. The characterization result demonstrates that they are sufficiently sensitive for the actual metabolite detection measurement.

### B. Chemical multiplexing and cross-talk

Two metabolite-enzyme interactions (chemical equation 1, 2, 3, 4) are carried out in one sensing well, therefore the optimization of measurement condition is important. It is well known that pH, ionic strength and temperature are crucial for the stability and activity of enzyme reactions.[34-36] Those three aspects were investigated independently.

The first condition that was checked is pH. According to the manufacturer, triethanolamine hydrochloride is useful in the range of 7.3 to 8.3. Cholesterol oxidase from streptomyces sp. is stable from pH 5 to 10 and optimum from 6.5 to 7. Hexokinase from saccharomyces cerevisiae is optimal from 7.5 to 9. Peroxidase from horseradish is stable in the range of 5 to 9 and optimal from 6 to 6.5. The interaction from glucose-hexokinase decreases pH by a value of around 1, therefore three pH values (7.5, 8 and 8.5) were investigated (Fig. 4). It turned out that 8 is the best pH value for the buffer which gives the best performance for both metabolite-enzyme interactions.

Ionic strength not only plays an important role in enzyme-based reaction, but also affects the performance of the ISFET. 50 mM was found to be an appropriate salt concentration for both requirements. In addition, buffering capacity is another aspect that matters for the measurement especially for the ISFET. When using an ISFET to detect pH change, low buffering capacity is required, because small pH variation can be screened if the buffering capacity is too high. 5 mM buffer was finally used, which is sufficient for maintaining the stability of the buffer as well as allowing the detection of small pH changes. It was unknown that if the buffering capacity was an issue for the metabolite-enzyme interaction or not. A comparison was done among the cholesterol-cholesterol oxidase measurements on PD with 5 mM, 50 mM and 500 mM buffer respectively. The result indicates that buffering capacity does not show obvious impact on the enzyme reaction performance.

As previously discussed, experiments were carried out at 37 °C to aid optimization of enzyme performance. Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised. The optimal temperature is usually around human body temperature (37 °C) for the enzymes in human cells.[37] To obtain free cholesterol from cholesterol esters in serum, 37 °C is also required. Therefore the
temperature was set to 37 °C throughout all the measurements. ISFET and PD are normally operated under room temperature. Therefore, we compared the experimental results at room temperature and at 37 °C to determine whether there is a significant difference (Fig. 5).

\[
D - \text{Glucose} + ATP \xrightarrow{\text{Hexokinase}} D - \text{Glucose} - 6 - P + ADP + H^+ \quad (1)
\]

\[
\text{Cholesterol Esters} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Oleic Acid} \quad (2)
\]

\[
\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} 4 - \text{Cholesthen} - 3 - \text{one} + H_2O_2 \quad (3)
\]

\[
H_2O_2 + o - \text{Dianisidine (reduced)} \xrightarrow{\text{Peroxidase}} H_2O + o - \text{Dianisidine (Oxidized)} \quad (4)
\]

Cross-talk is a common issue along with simultaneous detection in both electrical and optical measurements. In our study, two metabolite-enzyme interactions were carried out in one sensing window. Different enzymes and chemicals required for those two measurements were mixed together. In addition, an LED (502 nm; LUMEX) and an Ag/AgCl reference electrode were utilized for the measurement. The LED was used as light source for the PD and the reference electrode was inserted to provide gate voltage for the ISFET.

![Fig. 6](image)

Fig. 6. Measurements for the investigation on cross-talk. (a) The glucose-hexokinase reaction was performed on the PD. Compared to the buffer-buffer test, no obvious difference is observed, indicating that the pH change associated with the glucose-hexokinase reaction does not affect the performance of the PD. The spike at \( t = 200 \) s arises as a consequence of disturbing the device when reagents are added; (b) The cholesterol-cholesterol oxidase reactions were undertaken on the PD with and without the presence of glucose-hexokinase reaction. No obvious difference is seen between them, which means that the glucose-hexokinase reaction has no impact on the cholesterol-cholesterol oxidase reaction when using the PD for detection; (c) The cholesterol-cholesterol oxidase reaction was performed on the ISFET. Compared to the buffer-buffer test, no obvious difference is observed, indicating that the reaction does not affect the performance of the ISFET; (d) The glucose-hexokinase reactions were undertaken on the ISFET with and without the presence of cholesterol-cholesterol oxidase reaction. No obvious difference is seen between them, which implies that the cholesterol-cholesterol oxidase reaction has no impact on the glucose-hexokinase reaction when the ISFET is used for the measurement.
Ideally the two measurements would work independently of each other. For example, the glucose-hexokinase assay that produces pH change would have no effect on either the PD or the cholesterol-cholesterol oxidase interaction and the cholesterol-cholesterol oxidase assay that generate colour change would have no effect on either the ISFET or the glucose-hexokinase interaction. In order to evaluate the degree of cross-talk. If any, we carried out the following experiments (Fig. 6): glucose-hexokinase assay over the PD; cholesterol-cholesterol oxidase assay over the ISFET; cholesterol-cholesterol oxidase assay with and without the presence of glucose-hexokinase interaction over the PD; and glucose-hexokinase assay with and without the presence of cholesterol-cholesterol oxidase interaction over the ISFET. The result reveals that the pH drop generated from the glucose-hexokinase interaction does decrease the reaction rate of cholesterol-cholesterol oxidase interaction a little bit but not dramatically enough to affect the performance of the assay. Apart from that, no obvious cross-talk was observed.

The first measurement carried out was to perform the glucose-hexokinase assay on the PD (Fig. 6a). This is to check if the pH drop generated by the glucose-hexokinase interaction affects the performance of the PD. Only the PD sensor was adopted with the LED was switched on. The enzymes and chemicals for glucose-hexokinase were first placed on the PD to give a baseline signal and then a high concentration (3 mM) of glucose solution was added to initiate the reaction. For the comparison purpose, a buffer-buffer test was also performed. In the buffer-buffer test, Triethanolamine buffer was first placed on the PD to give a baseline signal and then the same buffer was applied to mimic the experimental procedure of metabolite-enzyme assay.

The second measurement was to perform the cholesterol-cholesterol oxidase assay only on the PD with the presence of glucose-hexokinase interaction (Fig. 6b). This is to check if the pH drop generated by the glucose-hexokinase reaction or the materials themselves affects the cholesterol-cholesterol oxidase interaction. Only the PD sensor was adopted with the LED was switched on. The enzymes and chemicals for both reactions were placed on the PD to give a baseline signal and then a solution contains 400 µM glucose and 3 mM glucose was added and mixed to trigger both reactions. For the comparison purpose, a sole cholesterol-cholesterol oxidase assay was also performed with 400 µM cholesterol.

The third measurement was done is to perform the cholesterol-cholesterol oxidase reaction on the ISFET (Fig. 6c). This is to check if the products generated from the cholesterol-cholesterol oxidase reaction affects the performance of ISFET. Only the ISFET sensor was adopted with the reference electrode was inserted and the gate voltage was applied. The enzymes and chemicals for cholesterol-cholesterol oxidase were first placed on the ISFET to give a baseline signal and then a high concentration (800 µM) of cholesterol solution was added to initiate the reaction. As a comparison, a buffer-buffer test was also performed.

The fourth measurement performed was the glucose-hexokinase assay on the ISFET with the presence of cholesterol-cholesterol oxidase interaction (Fig. 6d). This is to check if the products generated by the cholesterol-cholesterol oxidase reaction or the reaction itself affects the glucose-hexokinase reaction. Only the ISFET sensor was adopted with the reference electrode is inserted and the gate voltage is applied. The enzymes and chemicals for both reactions were placed on the ISFET to give a baseline signal and then a solution contains 400 µM glucose and 800 µM cholesterol was added and mixed to trigger both reactions. For comparison purposes, a sole glucose-hexokinase assay was also performed with 400 µM glucose.

As discussed previously, to perform measurement on the ISFET, a reference electrode is required and to undertake experiment on the PD, LED illumination is essential. Ideally the reference electrode would have no effect on the PD and the LED would have no effect on the ISFET. To evaluate the effect of LED illumination on the performance of the ISFET, a stability test was performed on the ISFET with only Triethanolamine buffer when the LED is switched on. It turns out that the ISFET is not affected by the LED no matter it is on or off (Fig. 7a). To check the effect of liquid gate voltage applied on the reference electrode on the PD, a stability test with Triethanolamine buffer was undertaken on the PD in which the electrode was inserted and gate voltage was applied. The result shows that the liquid gate voltage does not have any obvious impact on the PD (Fig. 7b).

No obvious cross-talk was observed from the above measurements. The CMOS based device demonstrates a good capability to undertake simultaneous detection of metabolites with the ISFET and PD independently.

C. Simultaneous detection of serum glucose and cholesterol

Serum glucose and cholesterol were measured simultaneously with the ISFET and PD. Human serum was purchased from Sigma-Aldrich which contains approximately 3 mM glucose and 4 mM cholesterol. It was then diluted with Triethanolamine buffer to give 4 different concentrations (45, 95, 190, and 375 µM for glucose and 50, 100, 200, and 400 µM for cholesterol). In the control measurement, instead of human serum sample, the Triethanolamine buffer was used.
Fig. 8. Simultaneous detection of serum glucose and cholesterol. (a) An extracted plot that shows the real time detection result from one high concentration of the human serum. The red dashed line indicates when the serum sample was added. The signal changes derived from the glucose-hexokinase and cholesterol-cholesterol oxidase interactions were observed and recorded immediately and simultaneously by the ISFET (blue curve) and PD (orange curve). Four concentrations of human serum were taken as the analyte for the measurement. (b) ISFET voltage vs time plot extracted from the detection of serum glucose. (c) PD voltage vs time plot extracted from the detection of serum cholesterol.

A green LED with a wavelength of 502 nm was chosen because it is the optimal wavelength for the PD and oxidized o-Dianisidine.\[25\] The gate voltage applied on the reference electrode was set to 0V, which stabilizes the liquid potential and at the same time does not interfere the enzymatic reaction. The reaction buffer which includes all the enzymes and chemicals was added to the sensing well to get baselines for both the ISFET and PD. The human serum sample was added to initiate the reactions. Signal changes occurred immediately and simultaneously on both the ISFET and PD (Fig. 8a). The metabolite-enzyme interactions were measured through the change in voltage as a function of time and the data are shown in Figure 4. The reaction buffer was initially added at time 0 and the voltage was continuously measured. Immediately after adding human serum sample to the sensing window at time 150s, a change of signal in voltage that comes from the reaction was observed from both the ISFET and PD at the same time. In total 4 concentrations of human serum and one control buffer solution were measured. The change in ISFET and PD voltage for different concentrations were plotted in the figure and the slope of the curve over the first two minutes of the reaction (marked by a red dashed square) was used to calculate the reaction rate (using Matlab). The figure of initial reaction rate and end point voltage (at 1500 second) against metabolite concentration was plotted and fitted by using Michaelis-Menten equation (Equation 1) as shown in Figure 5.
The Michaelis-Menten equation (equation 1) was also utilized to identify the Michaelis constant (Km), which is the substrate concentration at which the reaction rate is half of the maximum rate that can be achieved. It indicates how effectively a substrate interacts with a given enzyme. In addition, the Lineweaver-Burk plot was also generated (inserted in Fig. 9) to provide a graphical method for better understanding and analysis of the reaction. The Km values were calculated and found to be 428 ± 133 µM (reaction rate) and 350 ± 167 µM (end value) for glucose–hexokinase and 103 ± 39 µM (reaction rate) and 186 ± 49 µM (end value) for cholesterol–cholesterol oxidase respectively. This can be compared with the literature value of 150 µM [38] for hexokinase and 96 µM [39] for cholesterol oxidase. The Km values determined using a CMOS-based device are slightly higher than measured conventionally, this may be due to the pH and/or ionic concentration, which are not optimized for individual reaction but for the two reactions.

\[
\nu = \frac{V_{max}[S]}{K_m + [S]} \tag{1}
\]

where \(\nu\) is the reaction rate (current or threshold voltage change with time), \(V_{max}\) is the maximum rate and \(K_m\) is the Michaelis constant.

The Limit of Detection (LOD) determined from 3 x standard deviation of the blank signal is approximately 18 µM for glucose and 6 µM for cholesterol. The high sensitivity makes the method presented here is applicable to the detection of many other metabolites.

Hexokinase has the potential to detect sugars other than glucose (e.g. fructose). Therefore to demonstrate the selectivity of the dual measurement, simultaneous detection of solution glucose and cholesterol was performed in the same way as was done for human serum (above). The reaction buffer, including all the required enzymes and chemicals was firstly added to the sensing well to get baselines for both ISFET and PD. Instead of adding serum sample, solution glucose and cholesterol mixture was used to initiate the two reactions. 3 mM glucose and 800 µM cholesterol were prepared and mixed with Triethanolamine buffer to provide the highest concentration. It was then diluted with the same buffer to give in total seven different concentrations of them. The Km was found to be 244 ± 21 µM.

\[ K_m = 428 \pm 133 \mu M \quad (a) \]
\[ K_m = 350 \pm 167 \mu M \quad (b) \]
\[ K_m = 103 \pm 39 \mu M \quad (c) \]
\[ K_m = 186 \pm 49 \mu M \quad (d) \]
for the glucose and 80 ± 11 µM for the cholesterol. Data are the mean ± SEM (three measurements with two devices). The result from the solution containing glucose and cholesterol matches well with that from the human serum, indicating that among the metabolites in the human serum, only the glucose and cholesterol were detected selectively by our sensor platform.

D. Data analysis and statistics
As introduced, the chip was designed to incorporate a 16 x 16 array of clusters with each cluster integrating a PD “pixel” and an ISFET “pixel” to enable simultaneous optical and electrical detection on the same surface. Therefore the final result can be obtained by averaging the data from the 256 cluster sensors to reduce the noise level. In addition, where there are some nonfunctional pixels, results can still be acquired from the functioning pixels. A custom-written LabVIEW program was designed to acquire and average the data. (Fig. 10)

Fig. 10. Software interface for data analysis. A LabVIEW based program was written for data analysis. It contains six windows (from top left to bottom right): PD Single pixel, PD Average, Intensity Graph (PD), ISFET Single pixel, ISFET Average, and Intensity Graph (ISFET). “PD Single pixel” plots the intensity change of one PD pixel during the reaction against the time; “PD Average” gives the averaged intensity change from the entire 256 pixels; “Intensity Graph (PD)” shows the intensity layout of the 256 PD pixels. The same explanation is applied to the ISFET. As shown in the figure, the signal from a selected single pixel of PD sensor is very noisy (PD Single pixel), but after averaging the entire 256 pixels, the signal is dramatically improved (PD Average).

IV. CONCLUSION
Low cost CMOS-based ISFET and photodiode sensors have been fabricated and used to detect and analyze metabolites simultaneously via pH and colour changes. An assay for simultaneous detection of risk factors for coronary heart disease (glucose and cholesterol) in human serum has been developed with pH and colorimetric readout. The assay can be performed using the CMOS-based device in one single well, unlike conventional approaches that require separate samples and different detection equipment. No obvious crosstalk was observed, indicating that this approach can be easily applied to detect other metabolites that produce pH and/or colour change during the enzyme reaction. Compared with current analytical methods for metabolite detection and quantification, no expensive detection equipment is required and the volume of the test sample is very small. An important advantage of this assay is that two metabolites were analyzed simultaneously in one sensing window in real time. This provides useful information for monitoring and diagnostic purposes. This CMOS-based ISFET and photodiode device shows potential to become a rapid point of care diagnostic system.

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Chunxiao Hu received the B.Sc. degree in automation in China in 2008, the M.Sc. degree in microsystem technology in Southampton in 2009, and the Ph.D. degree in microfluidic devices for nematodes in Southampton in 2013. His research interests include lab on chip, microfluidics, *C. elegans*, platform integration, nanowire/nanoribbon, surface functionalization, and biosensing. His research currently mainly focuses on using CMOS-based chemical sensors for multiple detection.

Mohammed A. Al-Rawhani received the B.Sc. degree in electronics and telecommunication engineering from Amman University, Amman, Jordan, in 2004, and the M.Sc. degree in electrical and electronic engineering and the Ph.D. degree in electronic integration design for biomedical applications from the University of Glasgow, Glasgow, U.K., in 2007 and 2012, respectively. His research interests include CMOS low- and highvoltage analogue/mixed-signal integrated circuits and front-ends for optical sensing biomedical applications.

Boon Chong Cheah received the B.Eng. degree in electronic and electrical engineering and the M.Sc. (Eng.) degree in nanotechnology and advanced electronic devices from the University of Leeds, Leeds, U.K., in 2010 and 2011 respectively. He is currently pursuing the Ph.D. degree in electronic and electrical engineering with the University of Glasgow, Glasgow, U.K., where he is with the Microsystem Technology Group.

Srinivas Velugotla received a BTech in Electrical & Electronic Engineering (India, 2004), his MSc in Nanotechnology & Microfabrication (Wales, 2007) and his PhD in Electrical & Electronic Engineering (Edinburgh, 2013). He worked as a Research Assistant at the University of Wales, working on dip pen nanolithography using AFM. Following completion of his PhD he worked as a Research Assistant at the University of Edinburgh for 6 months developing dielectrophoresis based device for continuous separation of cells. He is currently a Research Assistant in the Microsystems Technology Group at the University of Glasgow. His research interest include biochemical sensors, integration, microfluidics and dielectrophoresis. His research mainly focuses on developing the integration of microfluidics into a biochemical sensor and developing CMOS based chemical sensors.

David R. S. Cumming (M’97–SM’08–F’13) received the B.Eng. degree in electronic and electrical engineering from the University of Glasgow, Glasgow, U.K., in 1989, and the Ph.D. degree from the Cambridge University, Cambridge, U.K., in 1993. He currently leads the Microsystem Technology Group, School of Engineering, University of Glasgow, where he is also the Co-Founder of its spin-out, Mode Diagnostics. His research on CMOS ion sensitive array technology has also been commercialized into the ion torrent next-generation sequencing system. He was a recipient of the Royal Society Wolfson Merit Award.