# Disposable Paper-on-CMOS Platform for Real-Time Simultaneous Detection of Metabolites

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***Abstract*—*Objective:*** **Early stage diagnosis of sepsis without overburdening health services is essential to improving patient outcomes.** ***Methods:*** **A fast and simple-to-use platform that combines an integrated circuit with paper microfluidics for simultaneous detection of multiple-metabolites appropriate for diagnostics was presented. Paper based sensors are a primary candidate for widespread deployment of diagnostic or test devices. However, the majority of devices today use a simple paper strip to detect a single marker using the reflectance of light. However, for many diseases such as sepsis, one biomarker is not sufficient to make a unique diagnosis. In this work multiple measurements are made on patterned paper simultaneously. Using laser ablation to fabricate microfluidic channels on paper provides a flexible and direct approach for mass manufacture of disposable paper strips. A reusable photodiode array on a complementary metal oxide semiconductor chip is used as the transducer.** ***Results:*** **The system measures changes in optical absorbance in the paper to achieve a cost-effective and easily implemented system that is capable of multiple simultaneous assays. Potential sepsis metabolite biomarkers glucose and lactate have been studied and quantified with the platform, achieving sensitivity within the physiological range in human serum.** ***Conclusion:*** **We have detailed a disposable paper-based CMOS photodiode sensor platform for real-time simultaneous detection of metabolites for diseases such as sepsis.** ***Significance:*** **A combination of a low-cost paper strip with microfluidic channels and a sensitive CMOS photodiode sensor array makes our platform a robust portable and inexpensive biosensing device for multiple diagnostic tests in many different applications.**

***Index Terms*—Simultaneous detection, CMOS-based sensor system, sepsis, disposable paper, fast diagnostics.**

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## I. Introduction

A SENSITIVE, robust, portable, and inexpensive biosensing platform capable of combining multiple diagnostic tests into a single device is of significant interest in clinical applications for diagnosis and treatment monitoring at point-of-care (POC). Fig. 1 illustrates how a sophisticated multi-assay technology could be used in a range of settings including: home-health and care-home monitoring; patient diagnostic testing at a pharmacy; fast diagnosis and triage in an ambulance; rapid monitoring in hospital; diagnosis and monitoring at remote places. The challenge therefore is to devise a system or platform that can be readily adapted for many different use cases. Paper-based sensors are an excellent technology for fabricating simple, low-cost, portable and disposable analytical devices for many application areas including health monitoring and rapid diagnosis [1]–[5], food and water quality control [6]–[10], and environmental monitoring [11]–[14]. There are several main advantages of using paper as a sensing platform including: passive liquid transportation, flexibility for modification, cost-effective and compatibility with many chemicals.

Since the first paper device for enzymatic detection of glucose in urine was demonstrated by Comer [2], paper based sensors have been employed successfully in many medical applications. Immunochromatographic paper test strips (also known as lateral flow immunoassays (LFIA)) use paper-based platforms for the detection and quantification of analytes in complex mixtures, where the sample is placed on a test device and the results are displayed within a few minutes [15]. Paper based tests can be performed without sample pre-treatment or the need for trained personnel and a laboratory. The user simply requires a drop of the sample and the process is driven via passive capillary force for fluid transport and delivery. LFIA is therefore considered to be one of the most commercially feasible analytical tools for POC testing.

These immunoassays consist of a strip of paper with a sample pad (for introduction of the sample), reagent pad (containing antibodies conjugated to a signal indicator which are specific to the target antigen) and a test line (capture antibodies immobilized on the surface). When the sample is introduced at the sample

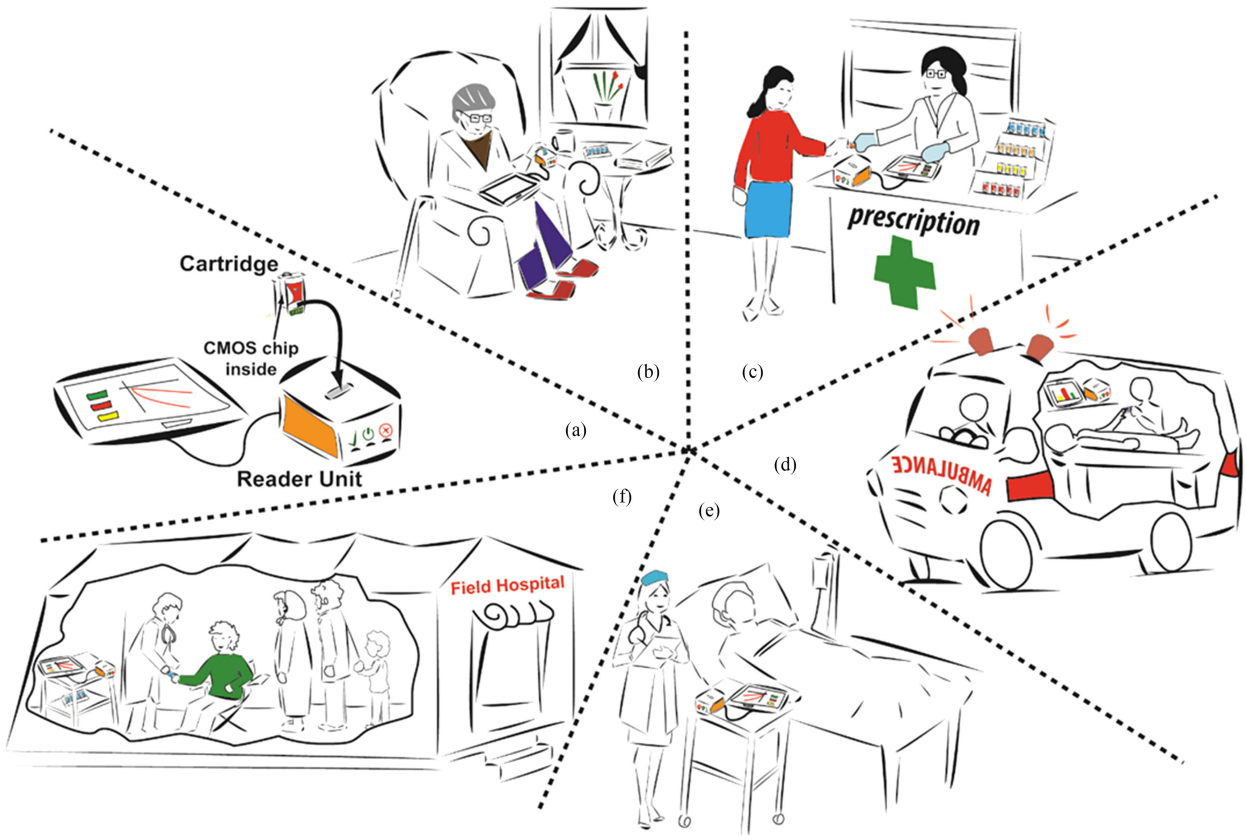


Fig. 1. Working principle of the disposable CMOS platform in different settings. (a) The system comprises disposable cartridges containing the CMOS chips and a reader connected to a tablet PC or Smart Phone. (b) The platform can be used at home for more convenient health monitoring. (c) In a pharmacy where frequent monitoring of patients requiring regular prescriptions is needed. (d) Inside an ambulance where biomarkers can be diagnosed rapidly. (e) In a hospital where rapid monitoring of a patient's condition is required. (f) In places lacking access to centralized healthcare e.g., remote areas, areas subject to natural disasters or war.

pad, it migrates along the paper strip via capillary action, where the presence of the antigen in the sample binds to the signal antibody. The formed antigen/signal antibody continues to flow along the paper strip where it is subsequently collected at the surface by the capture antibody to give a positive result. The signal indicator is typically made from coloured latex microspheres or gold nanoparticles. Although reliable, these simple and low-cost devices are generally limited to provide only a qualitative "yes/no" type of detection.

To obtain more accurate and quantitative results, Whitesides and co-workers introduced the idea of fabricating microfluidic channels on paper for multiple analyte detection; so termed microfluidic paper-based analytical devices ($\mu$PADs) [4], [16], [17]. Fabrication of $\mu$PADs is based on patterning sheets of paper into hydrophilic channels bound by hydrophobic barriers. Three main methods were used for patterning paper: photolithography using a suitable resist such as SU-8 [18]–[20], wax printing [21]–[23], and inkjet printing [24]–[26]. These methods produce micro patterns on the paper device with high resolution, but they all require additional materials to be involved in the process.

Data is read-out from these structures by measuring the light reflected from the paper's surface. To do this requires a relatively sophisticated reflectometer to be used that is not readily miniaturised into a low-cost format.

In this article, we describe a paper based platform, fabricated using laser ablation to make microfluidic channels on paper. For the first time we integrate the resulting paper microfluidic channels with a complementary metal oxide semiconductor (CMOS) integrated circuit (IC) to produce a sensor for fast simultaneous detection of multiple metabolites with relevance to sepsis. Laser ablation has recently been developed to demonstrate an effective way of producing paper channels without requiring additional materials [27]–[29]. In order to integrate paper technology effectively with CMOS we have designed and fabricated microfluidic channels on paper with micron-scale dimensions. By doing so we have demonstrated multiple parallel microfluidic tracks sharing a common pad, or reservoir, region to enable simultaneous detection of two or more metabolites. Small channels mean that the volume of the sample analyte can be reduced, saving resources. The microfluidic paper channels were functionalized using bioprinting for direct and precise enzyme patterning. In this way the reaction location was well-controlled and constrained to the detection zone. In contrast to traditional detection systems that monitor the signal of the reflected light [3], [30], the new system monitors the absorbance using a single lens transmission configuration and a CMOS Si photodiode (PD). The CMOS sensor chip can be reused and the paper strip is disposable. The configuration reduces the number of necessary

optical components, aiding miniaturisation and bringing down system cost and complexity.

Sepsis is a life-threatening condition with organ dysfunction caused by a dysregulated host response to infection [31]. The risk of death from sepsis is as high as 30%, from severe sepsis as high as 50%, and from septic shock as high as 80% [32]. Early diagnosis is necessary to properly manage sepsis, as initiation of rapid therapy is key to reducing deaths from severe sepsis [33]. Two criteria are usually used to define sepsis: one is systemic inflammatory response syndrome (SIRS) and the other one is quick sequential organ failure assessment score (qSOFA). However, there are concerns about the two criteria. SIRS can be too sensitive and not specific and qSOFA has been found to be specific but poorly sensitive [34], [35]. Metabolite biomarkers such as lactate and creatinine have been proven to have the potential to assist the diagnosis of sepsis, and ongoing work seeks panels of metabolites whose abundance changes provide increased sensitivity in diagnosis [36]–[38]. Therefore, methods to detect multiple metabolite biomarkers simultaneously in a short time will be necessary for the rapid diagnosis of sepsis, reducing the risk of death.

To illustrate the capability of the sensor platform, glucose and lactate were chosen as the analytes since they have been shown to have relevance as indicators in early diagnosis of sepsis [37], [39]–[42]. Three microfluidic channels were fabricated on a single paper strip and each channel subsequently printed with enzymes for detecting glucose and lactate. The third channel was used as a control in this study. Simultaneous detection of both metabolites was successfully performed on our platform within minutes. In this new device configuration, we use the multiplexing and sensing capabilities of CMOS to demonstrate for the first time a paper based, hence low-cost sensor exploiting a reliable absorption based measurement. A combination of a low-cost paper strip with microfluidic channels and a sensitive CMOS photodiode sensor array makes our platform a robust portable and inexpensive biosensing device for multiple diagnostic tests in many different applications.

## II. Materials and Methods

### A. Disposable Paper-Based Sensor Platform

The entire sensor platform is a handheld device comparable in size to a mobile phone (Fig. 1(a)). It is comprised of several components. A paper strip with multiple microfluidic channels was used as a substrate to store enzymes and enable sample introduction to the sensor. The CMOS chip (sensor), mounted on a chip carrier, has an array of 16 $\times$ 16 PD pixels. Each PD pixel integrates a pn-junction with a classic 3-transistor read-out design. The chip was fabricated using a commercially available CMOS 350 nm 4-metal process provided by austriaMicroSystems (AMS). A printed circuit board (PCB) 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 a PD array on the CMOS chip. The array can be used to exploit the statistical phenomenon of averaging signals from independent Gaussian noise sources, either over time or spatially, to improve the overall system sensitivity. The chip integrates addressing blocks to allow each sensor array to be controlled and operated independently or simultaneously as required, A LED mounted in a 3-D printed housing was used as a light source. The housing also doubled up as a light-proof unit to eliminate unwanted stray light from the measurements. In dark conditions, the output of the PD was 408 mV $\pm$ 22 mV. By gradually increasing the light intensity, the minimum detectable optical power density for the photodiode was measured at 50 nW/cm$^2$ and started to saturate when exposed to 4.9 $\mu$W/cm$^2$. The reaction and colour changes that occurred on the paper strip were detected and recorded by the sensor. The acquired data was transferred by universal serial bus (USB) to a computer running a LabVIEW program or to a mobile phone java program, where it was processed and analysed. The platform is interoperable with both types of computing device.

### B. Measurement Configuration

The sensor chip was attached to a chip carrier and was packaged by biocompatible epoxy to protect the bond wires from the aqueous environment [43]. A sensing window was created where the top of the chip was not coated with epoxy, as illustrated in Fig. 2(a). A green LED with a peak wavelength of 502 nm was used to provide a light source. This wavelength had been proven to produce the highest sensitivity as a consequence of the sensor's spectral sensitivity and the absorption spectrum of the dye [44]. For experimental purposes two cylinder-shaped magnet bases (Fig. 2(a)) were attached at either ends of the chip carrier to hold the paper strip in position. The paper strip was designed to have a curved shape as shown in Fig. 2(a). The shape was chosen to minimize the distance between the paper strip and the chip surface hence improve the overall sensitivity and the accuracy of registration between the paper channels and the corresponding sensors on the chip. The enzymes and chemicals required for the measurements were added by droplet printing to the specific channels and dried accordingly.

Prior to each measurement, a paper strip was first attached to the surface of the sensor chip. An LED mounted in a 3-D printed housing was then placed on top of the paper strip. Only the sample pad of the strip protruded from the housing. To start a test, a drop of sample solution containing the target metabolites was added to the sample pad by pipetting. The liquid was driven towards the absorbent pad along the paper channels by capillary force. The colour change produced by the reaction on the three paper microfluidic channels was detected individually and simultaneously by the multiplexed PD sensor array. On completion of the experiment the paper strip was discarded, and the CMOS chip was re-used without a washing step. The CMOS chips are made in a commercial foundry process. As expected the chips are all tightly inspected and performance from chip-to-chip is very repeatable. The paper strips are machine cut and all very tightly inspected. The complete measurement set-up is highly reproducible.

### C. Paper Strip Fabrication and Encapsulation

The paper was purchased from GE Healthcare Life Sciences. It was Grade 1 Chr Cellulose Chromatography Paper, which has a smooth surface (peak to valley $\sim$ 10 $\mu$m) with a thickness of

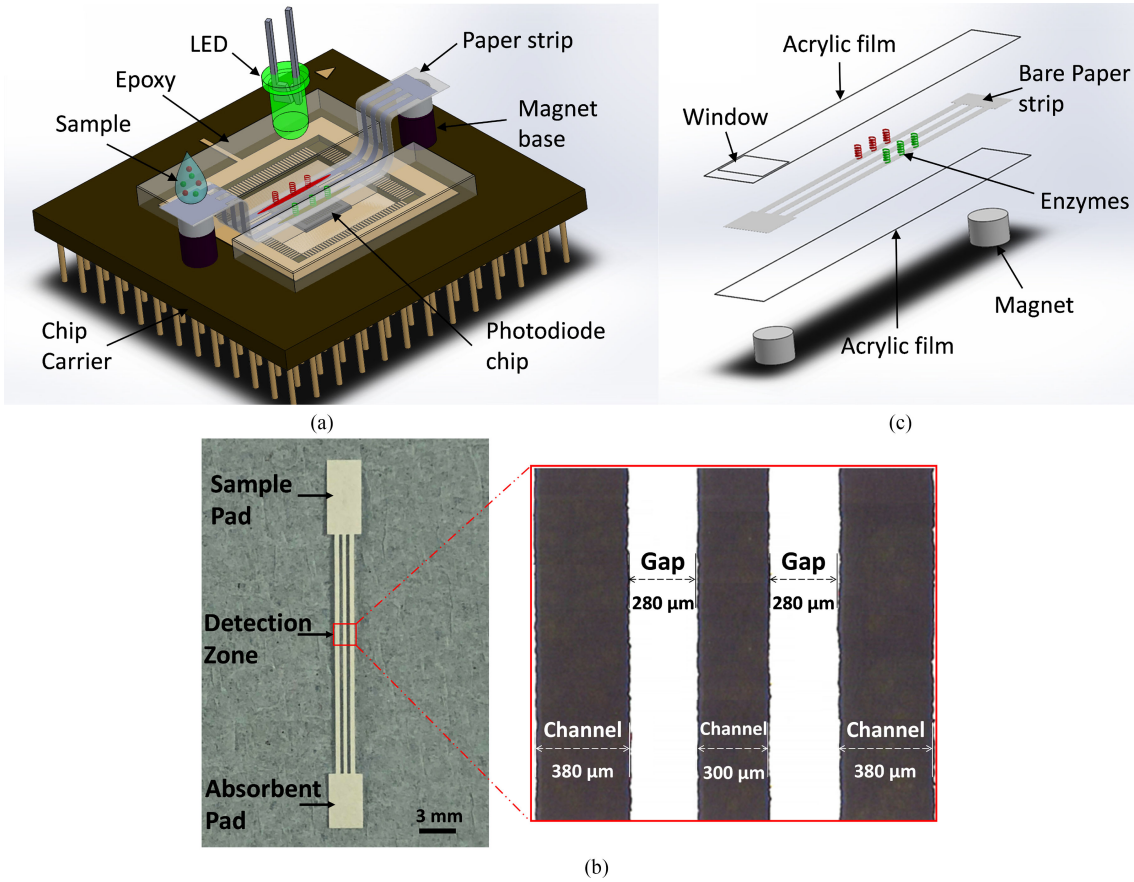


Fig. 2. Measurement configuration and paper strip. (a) Schematic diagram of the sensor system configuration. The paper strip with pre-immobilized enzymes is held in place on the sensor surface by magnetic force. (b) Schematic diagram of the paper strip. It contains three layers. One paper layer with pre-immobilized enzymes is covered by two layers of acrylic films on both sides. Two cylindrical magnets are attached to the bottom symmetrically. (c) Image of one bare paper strip with three channels. The enlarged microscope image details the size of the paper channels and the gaps between them.

0.18 mm and a specified linear water flow rate of 130 mm/30 min. This kind of paper is widely used in sensing applications and was chosen for its relatively uniform thickness and wicking properties (Martinez *et al.* 2010; Nery 2016). To define the microfluidic channels, we use a laser micromachining (Laser Micromachining Ltd.) approach. The maximum laser power of the cutter was specified to be 30 watts. We set the power level on the unit to 11%. The speed was set to half full-speed, estimated to be 60 inch/second. The frequency was 500 Hz. Laser ablation has several advantages compared to the traditional photolithographic, inkjet printing and wax-based methods. It is a single step process, it is easy to change a design, has high throughput, low cost and a cleanroom environment is not required. As shown in Fig. 2(b), the paper strip has a size of length 30 mm and width 2.8 mm that has three main parts: sample pad, detection zone, and absorbent pad. The sample pad has a length of 5 mm and is used for sample loading, the detection zone is the same size as the sensor array on the CMOS chip (2 mm $\times$ 2 mm), and the absorbent pad is 4 mm in length and is used for taking-up overflow liquid. Three microfluidic channels were fabricated for our measurements. The two outermost channels were 380 $\mu$m wide and were designed for metabolite detection and the centre channel, 300 $\mu$m wide was used for the control. There gaps between the channels were 280 $\mu$m, which we found was big enough to prevent cross-talk. 100 paper strips were fabricated in the same batch from a single sheet of paper.

As shown in Fig. 2(c), two layers of transparent acrylic film were attached to both the top and bottom of the bare paper strip to encapsulate it. The acrylic used was 3M9969 diagnostic microfluidic adhesive transfer medical tape. It is transparent, has low residual adhesive and exceptional die cutting performance, which is suitable for general medical device use. Laser ablation was also used for cutting the acrylic to the correct size (36 mm $\times$ 3.6 mm). An open window (4 mm $\times$ 2 mm) was cut out on one side of the film, directly above the sample pad. The acrylic film provides good support to the bare paper strip so that it easily conforms to the topology of the CMOS chip and chip carrier (Fig. 2(a)). The evaporation rate is also reduced dramatically due to the sealed environment. Experiments were undertaken to show that the presence of the acrylic film had no deleterious effect on the detection result. To aid experimentation two neodymium magnets (Magnet Expert Ltd.) with a diameter of 2 mm and thickness of 1 mm were glued to the bottom side of the paper strip at both ends. Due to the attractive magnetic force between the magnet on the chip carrier and those on the paper, the paper strip is immobilized at the required position (Fig. 2(a)).

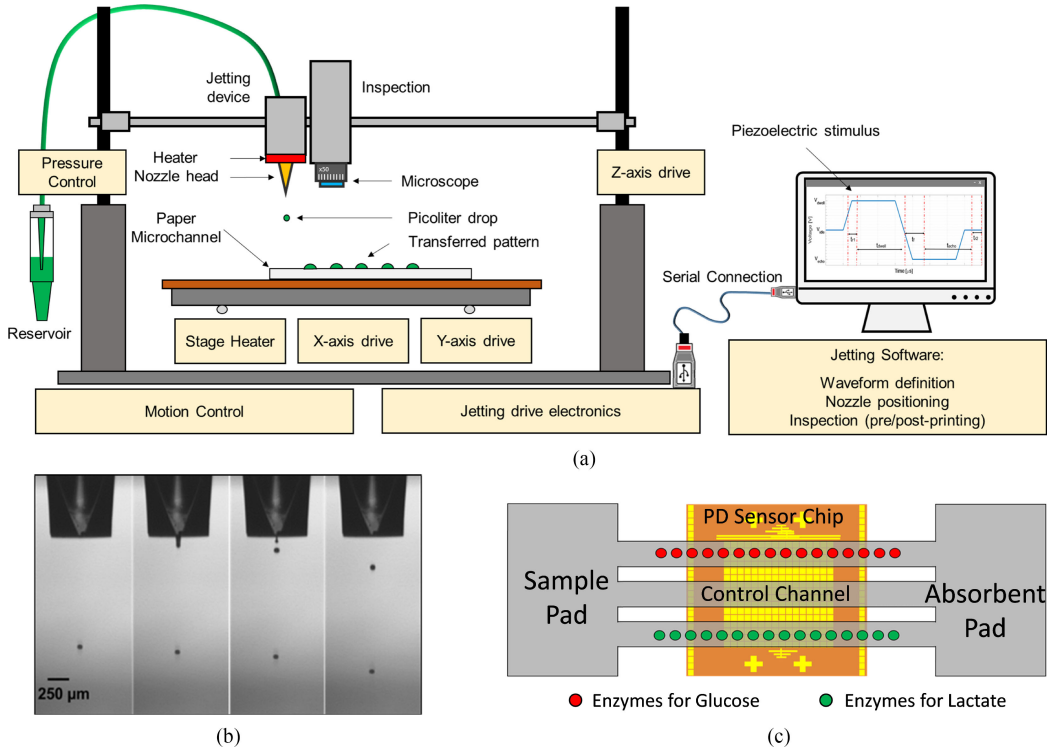


Fig. 3. Enzyme printing on paper strip. (a) Working principle of piezoelectric DoD inkjet printing. (b) Microscope images of drop ejection from a 70 $\mu$m orifice nozzle. (c) Enzymes for glucose and lactate are printed individually on the microfluidic channels.

### D. Chemical Preparation

The triethanolamine hydrochloride (T9534), o-dianisidine dihydrochloride (F5803), Sodium L-lactate (L7022), D-Glucose (G8270), peroxidase from *horseradish* (P8250), Lactate Oxidase from *Aerococcus viridans* (L9795), Glucose Oxidase from *Aspergillus niger* (G7141), Sodium acetate buffer solution (S7899) and Flavin adenine dinucleotide (FAD) disodium salt hydrate (F6625) were purchased from Sigma Aldrich. Bovine serum albumin (BSA) (11413164) was purchased from Fisherscientific. The sodium chloride (VWR27810), magnesium chloride hexahydrate (VWR25108), and sodium hydroxide pellets (0583) were bought from VMR.

Triethanolamine buffer was prepared by adding 100 mM triethanolamine hydrochloride to 100 mM sodium chloride solution. 1 M sodium hydroxide solution was then used to adjust the pH of the buffer to 8. o-dianisidine solution was prepared with deionized water saturated in oxygen and stored in a dark environment. Both glucose and lactate were prepared and diluted with Triethanolamine buffer. Glucose oxidase was prepared and diluted with sodium acetate buffer at pH 5.2 and lactate oxidase was prepared and diluted with 10 $\mu$M FAD in Triethanolamine buffer. Peroxidase enzyme solution was prepared with purified water at room temperature.

To prepare the detection zone, enzyme materials were bioprinted on to the paper as follows:

i) For glucose: 190 $\mu$l 100 mM Triethanolamine buffer at pH 8, 30 $\mu$l 600 U/ml peroxidase, 160 $\mu$l 7.89 mM o-dianisidine, and 120 $\mu$l 8 U/ml glucose oxidase.

ii) For lactate: 210 $\mu$l 100 mm Triethanolamine buffer at pH 8, 60 $\mu$l 600 U/ml peroxidase, 120 $\mu$l 7.89 mM o-dianisidine, and 100 $\mu$l 2 U/ml lactate oxidase.

### E. Enzyme Printing and Storage

A Microfab Jetlab II printer using direct write drop-on-Demand (DoD) inkjet printing was used to add the enzyme materials to the paper. With this approach, the enzyme solution could be printed on the channels with the required volume and precise position. The operating principle of piezoelectric DoD printing is shown in Fig. 3(a). The technique relies a piezoelectric transducer (Fig. 3(b)) [45] and the ideal condition for printing is the ejection of a single drop per piezo-stimulation pulse. The repetitive piezo-stimulation with a given frequency produces the ejection of a series of highly homogeneous drops. The volume of the drop ($Vd$), the deflection solid angle ($\Omega$) and its ejection speed ($v$) can be controlled by tuning the stimulating waveform.

Using DoD printing, two different inks containing respectively lactate oxidase and glucose oxidase were printed on parallel paper microfluidic channels (Fig. 3(c)). The patterns used were straight lines composed of 20 spots with 0.5 mm pitch (Fig. 3(c)). The total volume of each printed enzymatic solution was approximately 2.5 $\mu$l. The alignment accuracy has been found to be 100 $\mu$m thus repeatably dropping ink spots on the desired location.

Storage of the paper strip with printed enzymes is critical for point of care applications. A freeze drier (Lyotrap by LTE

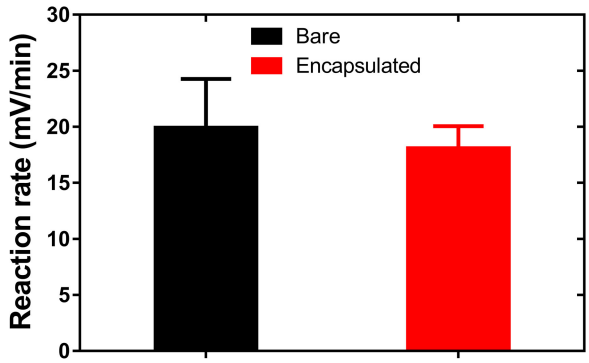


Fig. 4. Comparison of a bare and encapsulated paper strip. 2 mM Lactate was detected by both a bare and an encapsulated paper strip respectively. The reaction rates were extracted and analyzed. A significant difference was not observed (bare: 19.9 ± 2.5 mV/min and encapsulated: 18.1 ± 1.1 mV/min). Data are the mean ± SEM.

Scientific) was used to freeze dry the paper strip directly after the enzyme printing. No obvious signal drop was observed after three weeks storage at 4 °C.

## III. Results and Discussion

### A. Detection of Signal Change on Absorption

Instead of detecting the reflectance of the light as is the case with prior developments using paper as a matrix for biological sensors, the absorbance was measured directly. To achieve that, an LED was used as the light source and the wavelength was chosen according to the enzymatic assay. Cellulose chromatography paper, on which the channels were made, is permeable to visible light. To prove that the paper did not completely block the light with a wavelength of 502 nm, the transmission spectrum of the paper strip (dry and wet) was measured. The wet paper strip demonstrated higher transmission with a transmittance of 2.3%. The CMOS detector was placed directly underneath the paper strip for detecting the light going through the detection zone after absorption. The LED and CMOS detector were located at the top and bottom respectively of the paper strip; therefore, no optical lens or filters was required. Compared to the conventional reflectance detection method, the approach we adopted reduces the cost and footprint of the platform. It also increases the robustness of the system, making it extremely well suited for POC applications.

### B. Control Experiments to Evaluate Cross-Talk

The first experiment we performed was to compare the results between the bare and encapsulated paper strip (Fig. 4). This is to check if the addition of the acrylic film affects the output voltages of the photodiode sensor array. Six identical single channel paper strips were fabricated using laser ablation and printed with enzyme solution for detecting lactate as described in Section II. Only half of the strips were encapsulated with acrylic film and the other three were left as they were. The same measurement setup was used, and the six paper strips were measured sequentially by adding 2 mM lactate. The signal strength was reduced by around 8% due to the encapsulation, but no other obvious difference was observed. Besides, the standard deviation from the encapsulated paper strip is smaller than the bare paper strip, which may due to the fact that the encapsulation makes the strip stiffer and more stable. It would not move or deform during the wetting process.

The second measurement carried out was to test a high concentration of glucose and lactate mixture solution on a blank paper strip (no enzyme printed) (Fig. 5(a)). This is to check if the introduction of metabolite solution changes the colour of the paper strip and/or affects the reading of the photodiode sensor array. A solution mixed with 11.1 mM glucose and 2 mM lactate and a paper strip with three microfluidic channels were used in this experiment. No colour change was observed on the paper strip and no obvious signal change was detected by the sensor platform.

To demonstrate that the detection areas in each paper channel were specific to their intended substrate, we carried out the following tests.

The first test is to check that other substrates cannot be detected by the paper strip for glucose and lactate (Fig. 5(b)). In this experiment glutamate was tested instead of glucose and lactate as the analyte to perform the measurement on the paper strip with printed enzymes for glucose and lactate. As described in previous sections, the paper strip has three microfluidic channels. One was printed with enzymes for detecting glucose, one was for lactate and one for control. After adding the glutamate solution, no colour change was shown on the paper strip and no signal change was detected by the sensor platform.

The other test is to make sure that glucose does not interact with lactate oxidase and lactate does not react with glucose oxidase (Fig. 5(c)&(d)). In this experiment, only glucose was used as the analyte to perform the measurement on the paper strip with printed enzymes for glucose and lactate. The three microfluidic channels were treated as usual (one for glucose, one for lactate and one for control). 11.1 mM glucose was added to initiate the measurement and only the channel for glucose had a colour change. A similar experiment was conducted for lactate and no obvious cross-talk was observed.

### C. Simultaneous Detection of Glucose And Lactate

A disposable paper-based sensor platform was used for simultaneous detection of two metabolites. The device used a paper strip in conjunction with a CMOS sensor chip to form a compact sensor system. As described in the previous section, a disposable paper strip has three main parts: sample pad, detection zone, and absorbent pad. It also has three separate channels. In our measurements, the left-hand side channel was used for detection of lactate, the right-hand side one for glucose, and the middle one acted as the control (Fig. 6). Analyte solutions containing glucose and lactate were measured simultaneously with the PD array. According to the literature, the normal blood lactate concentration in the human body is around 1.26 mM [46] and the normal fasting blood glucose concentration is 5.5 mM [47]. Therefore 2 mM lactate and 11.1 mM glucose were mixed with 100 mM triethanolamine buffer to give the highest concentration for our measurements. It was then diluted by a factor of 2 and

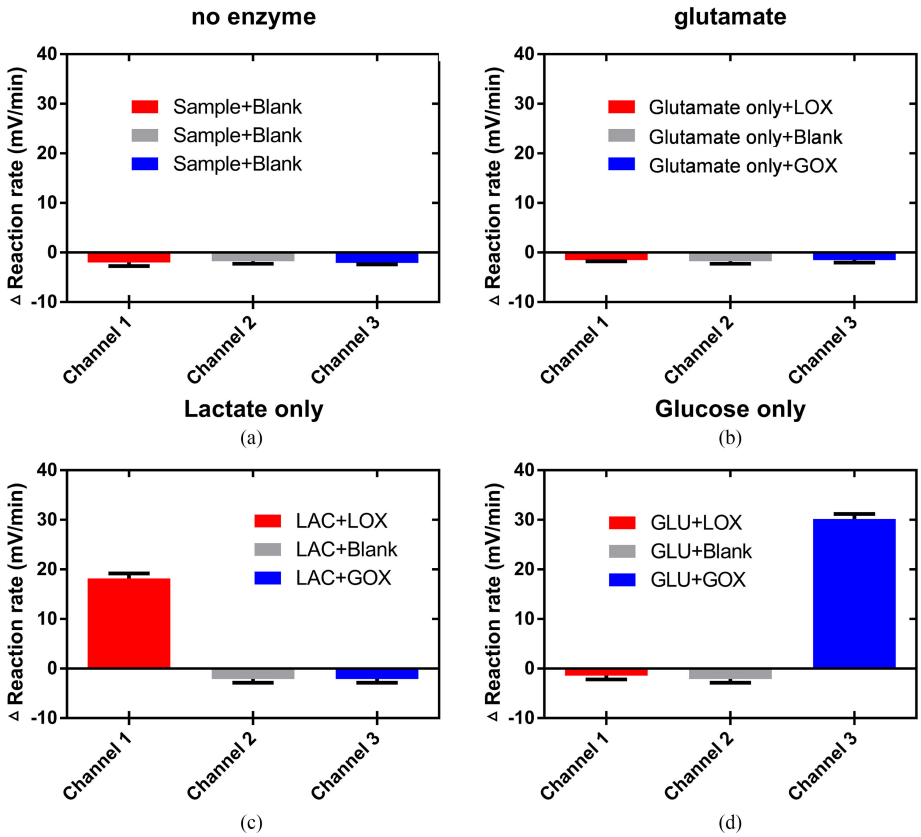


Fig. 5. Control experiments to evaluate the specificity of the paper strip. Four control experiments were undertaken to evaluate the specificity of the three paper channels. The first one is to check if the introduction of metabolite solution changes the colour of the paper strip and/or affects the reading of the photodiode sensor array. The second one is to check that other substrates cannot be detected by the paper strip specific for glucose and lactate. The last two are to make sure that glucose does not interact with lactate oxidase and lactate does not react with glucose oxidase. (a) Results from a solution containing a high concentration of glucose and lactate dropped on a blank paper strip when no enzyme is present. (b) Results obtained when the same experiment is conducted with only glutamate as the substrate. (c) Results obtained when the same experiment is conducted with only lactate as the substrate. (d) Results obtained when the same experiment is conducted with only glucose as the substrate. 'Blank' means no enzyme was printed; 'LAC' means lactate; 'GLU' means glucose; 'LOX' means lactate oxidase; 'GOX' means glucose oxidase. Glutamate was not studied in depth in this paper but used to provide a positive control experiment.

4 to cover the physiological range of the two metabolites. Both lactate-lactate oxidase and glucose-glucose oxidase reactions generate hydrogen peroxide ($H_2O_2$), which is proportional to the amount of metabolite added. The oxidization process of $H_2O_2$ with the presence of peroxidase changes the colour of o-dianisidine from clear to brownish, dependent on the amount of metabolite detected. A light source with a wavelength of 502 nm was chosen because it is the optimal wavelength for the PD and oxidized o-dianisidine [44]. As shown in Fig. 6(a), once the paper strip is placed on the chip and immobilised by the magnetic force with the LED turned on, the three paper microfluidic channels (dark areas labelled with number 1, 2, 3) can be recognized clearly on the voltage intensity map. After choosing the relevant pixel, the measurement was initiated by recording the baseline signal. Once a stable baseline signal was obtained, a drop of the sample solution containing glucose and lactate metabolites was pipetted onto the sample pad of the paper strip. The sample solution went through the microfluidic channels rapidly due to the capillary force. The volume of the solution chosen was 6 $\mu$L, which is large enough to wet the channels in a relatively short time, but not too large to cause overflow. The transition process of the paper strip from dry to wet generated a signal jump, but it did not affect the actual signal. The three microfluidic channels were wet simultaneously, which gave a good comparison of the reactions occurring on the three individual channels. As shown in Fig. 6(b), 2 mM lactate and 11.1 mM glucose were measured with one paper strip. Channel 1 measured the concentration of lactate where a signal decrease was observed. Channel 2 was used as a control channel where no signal drop was observed. Channel 3 measured the concentration of glucose where a decreasing signal was seen. The acquired data was transferred by universal serial bus (USB) to a computer running a LabVIEW program, where it was processed and analysed. Both enzymatic reactions produced a colour change from clear to brownish, which absorbed the green LED light, therefore decreasing the amount of light getting to the PD and therefore decreasing the voltage signal. Simultaneous detection of lactate and glucose was successfully undertaken in a real-time manner on a paper strip with microfluidic channels. Brownish colour changes were clearly seen on both Channel 1 and Channel 3after the measurement (Fig. 6(c)). For lactate and glucose, the dye absorbs the light hence the signal falls. For the control only a clear liquid is applied making the paper slightly more translucent, hence there is a slight signal rise.

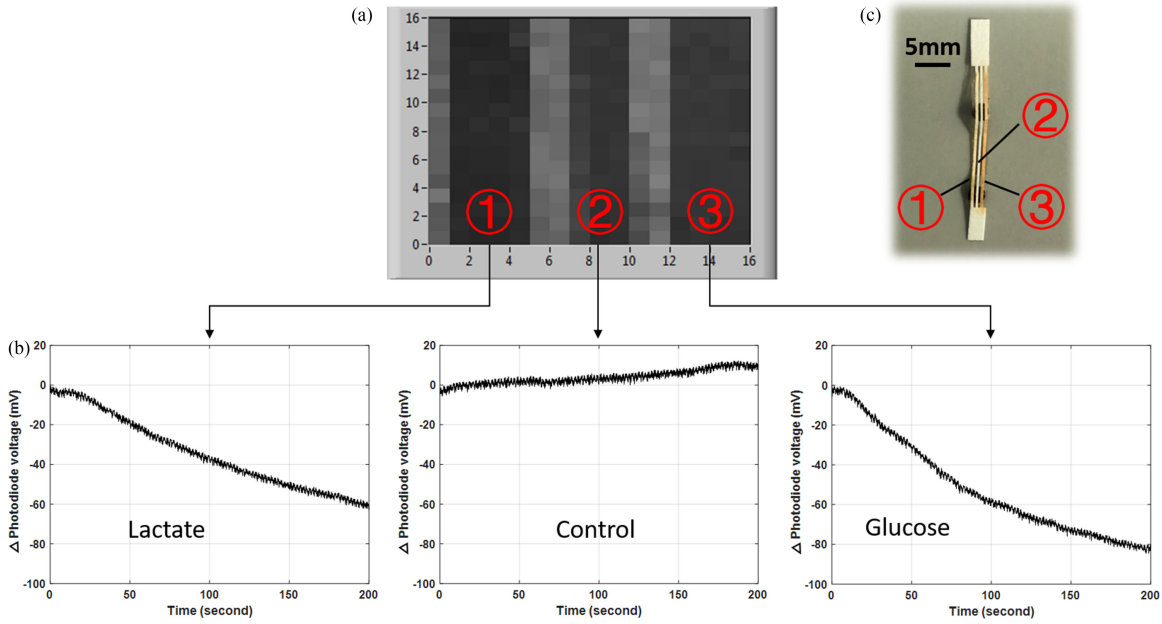


Fig. 6. Simultaneous detection of lactate and glucose. (a) Voltage intensity map of a photodiode sensor chip, showing the position of the paper channels (labelled with numbers 1, 2 and 3). Channel 1 was printed with lactate oxidase and related chemicals, channel 2 was left blank, and channel 3 was printed with glucose oxidase and related chemicals. (b) Real-time recording of the reactions detected by the three paper channels. (c) Photo of one paper strip after the measurement showing the colour change on the channels.

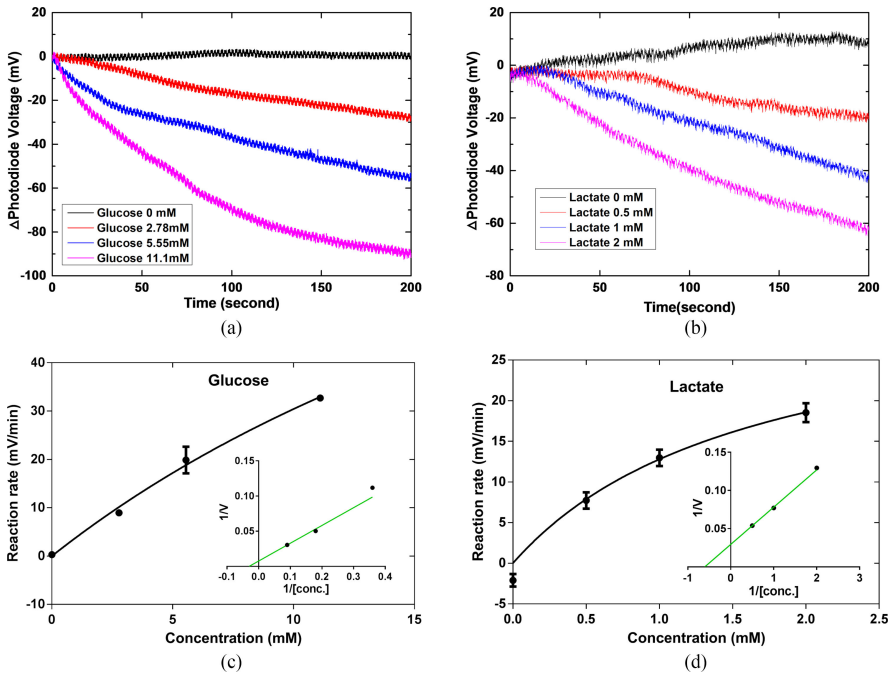


Fig. 7. Results extracted from the simultaneous detection measurement. (a), (b) Plot of the reaction curves extracted from the paper strip measurements. Four concentrations of glucose (0 to 11.1 mM) and lactate (0 to 2 mM) were sensed simultaneously and independently. (c), (d) Plot of the initial reaction rates (extracted from the first 2 minutes of the reaction) for the (c) glucose and (d) lactate reactions. The plots were fitted to the Michaelis-Menten equation. Data are the mean ± SEM. Inset shows the Lineweaver-Burk plot.

In total, three concentrations of lactate (0.5, 1, 2 mM) and glucose (2.78, 5.55, 11.1 mM) were measured (Fig. 7). In between each of the measurements, a used paper strip was simply replaced by a new one. No wash step was required due to the encapsulation of the paper strip. The change in PD voltage for different concentrations is plotted in Fig. 7 and the slope of the curve over the first two minutes of the reaction used to calculate the reaction rate (using MATLAB). The figures of initial reaction rates against concentration for both metabolites were plotted and fitted as shown in Fig. 7(c)&(d). The Michaelis constant $K_m$ values were estimated and found to be 33 ± 13 mM for glucose-glucose oxidase and 1655 ± 527 $\mu$M for lactate-lactate

oxidase respectively. This can be compared with the literature value of 26 mM [48] for glucose oxidase and 940 $\mu$M [49] for lactate oxidase. Note that the system is not intended for use as an analytical instrument, hence very accurate results were not expected. The Limit of Detection (LOD) determined from 3 x standard deviation of the blank signal is approximately 520 $\mu$M for glucose and 110 $\mu$M for lactate. Although lower limits of detection have been achieved with previous biosensors, this presents a LOD low enough for the metabolites that we studied.

## IV. Conclusion

We have detailed a disposable paper-based CMOS photodiode sensor platform for real-time simultaneous detection of metabolites for diseases such as sepsis. An assay for simultaneous detection of potential sepsis biomarkers, glucose and lactate, has been developed on a paper strip with microfluidic channels, which were defined in the cellulose paper strip by laser ablation. Besides, the enzymes and relevant chemicals were directly printed by droplet printing. Both laser ablation and bioprinting methods are precise and flexible. The number of microfluidic channels can be easily increased from three to six or even more for simultaneous detection of more metabolites, which provides the possibility for applications requiring multiple measurements from a single sample. Compared with current paper based methods for metabolite or protein detection and quantification, our platform has two main advantages: utilization of laser ablation to fabricate channels in the paper strip, which increases the flexibility and reduces the cost, and secondly is the use of absorption rather than reflection as the detection approach, thereby negating the requirement off optical lenses and filters and therefore reducing the size and cost of the system. Since the system relies on measuring the rate of change of the optical absorption, variation in the absolute absorption could be ignored. In future the alignment and encapsulation method for paper strips could be achieved in a single manufactured part with plastic snap-fasteners or similar fixtures. The number of microfluidic channels can be increased for detecting more potential biomarkers (such as glutamate, valine, and creatinine) at the same time. In addition, the paper strip can be easily modified for detecting other metabolites for different types of diseases. The strip can also be functionalized for monitoring binding events of proteins, DNA or viruses. This makes our system an excellent candidate for health monitoring and fast diagnostic applications.

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