Wide-range optical CMOS-based diagnostics

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***Abstract*—Colorimetric, chemiluminescence and refractive index based diagnostics are some of the most important sensing techniques in biomedical science and clinical medicine. Conventionally laboratories and medical clinics rely on bulky and dedicated equipment for each diagnostic technique independently. In this paper, we present CMOS sensor based solutions, comprising a single photon avalanche detector array and photodiode array. The CMOS platform offers low cost integration and wide range of light-based diagnostic techniques, leading to development of point-of-care devices.**

Keywords—SPAD, photodiode, colorimetric, chemiluminescence, refractive index, CMOS.

I. INTRODUCTION

Light-based sensing techniques such as colorimetric, chemiluminescence and refractive index change are well established for diagnosing diseases in laboratories and health care centers. The spectrophotometer colorimetric technique, for instance, is used in blood test for biomarker detection, while chemiluminescence is used for more sensitive assays requiring detection of low concentration biomarkers. Meanwhile, refractive index sensor can be used for label-free protein-protein or protein-antigen assessment. Each of these techniques require a dedicated instrument that is usually bulky and power hungry due to their discrete component configuration [1]–[4]. A general purpose spectrophotometer that is used for colorimetric assays usually incorporates a sophisticated setup comprising a white light source, monochromator containing a diffraction grating and a light transducer which converts light into electrical signal [5], [6]. Similarly, chemiluminescence requires an ultra-sensitive light detector such as a photomultiplier tube or charge coupled device (CCD) to sense the weak luminescence from low concentration biomarkers [2].

Complementary metal oxide semiconductor (CMOS) technology has revolutionised the microelectronics industry and made a huge impact on telecommunications and bio-sensing technologies. In this paper, we demonstrate the implementation of the aforementioned techniques using a commercially available CMOS process. For colorimetric and refractive index sensing we have used a CMOS based photodiode (PD) array while for the more sensitive detection such as chemiluminescence, we have used the CMOS based single photon avalanche detector (SPAD). Adopting CMOS technology as a platform for optically based diagnostic opens the possibility for integration of different bio-sensing techniques and microelectronics. Furthermore, it offers a low cost solution that can be mass-manufactured for use in point-of-care devices [7].

The remainder of this paper is organised as follows. In section II, a description of the PD CMOS chip and the SPAD CMOS chip is presented. This is followed in section III by a description of the implementation and practical evolution of colorimetric, chemiluminescence and refractive index sensing techniques using our designed CMOS chips.

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II. INTEGRATED CHIPS DESIGN

The PD is a p-n junction-based photosensitive detector. CMOS imagers are typically formed from an array of active pixel sensors (APS) each incorporating a PD and 3 transistors enabling data readout [8]. The PD layer structure is shown in Fig.1.a, while Fig.1.b shows the readout circuit of the PD. The incident photons on the device's active area are absorbed by the PD, which generates a photocurrent I_{ph}, leading to a change of the voltage across the diode as the device capacitance is discharged. This voltage is connected to V_{out} when V_{sel} is set to V_{dd}. The amplitude of V_{out} is inversely proportional to the intensity of the detected light.

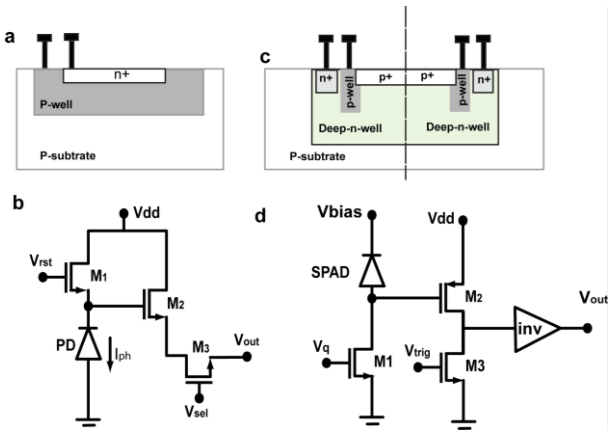

Fig. 1. a) Cross section of a rectangular shaped PD. b) Circuit diagram of a PD pixel. c) Cross section of a circular shaped SPAD. d) Circuit diagram of a SPAD readout. Both detectors were fabricated in a CMOS process.

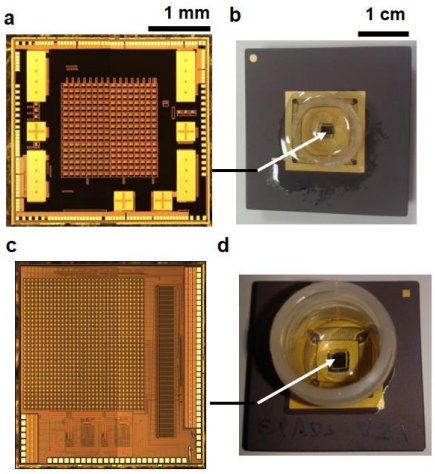

Fig. 2. a) Photograph of the 3.4 mm × 3.6 mm PD array chip. b) Packaged PD chip with epoxy and protection for wet biochemistry. c) Photograph of the 3.7 mm × 3.7 mm SPAD array chip. d) Packaged SPAD chip.

The SPAD is a pn-junction similar in structure to PD. However, unlike the PD, the SPAD is reverse-biased above its breakdown voltage to operate in Geiger mode. In order to withstand the high bias voltage, the SPAD is designed using a circular shaped layout with suitable implant spacing that allows for avalanche multiplication to take place (see Fig. 1.c) [8]. At such high bias voltage, an electric field within the active region of the junction is created. When a photon impacts on the active area, the SPAD initiates an avalanche process creating a large current pulse to be readily detected by a controlled threshold comparator (M_2/M_3). An n-channel MOSFET M_1 (Fig.1.d) is used to quench the avalanche current and thus making the device ready for the next photon to be detected [9]. The width of the pulse is determined by the bias voltage V_q which sets the discharging time of the SPAD terminal capacitance. The faster the discharge time, the lower the unwanted after pulsing and higher the detection dynamic range. The pulses rate generated by the SPAD are proportional to the number of the photons detected. An array of 32 bit digital counters is integrated on the chip to count the pulses at a given exposure time.

In order to demonstrate the capability of the two CMOS based detectors, we designed and fabricated two chips using commercially available CMOS processes. One chip was incorporated a 16×16 PDs array imager along with readout and control circuits. The PD chip (Fig. 2.a) was fabricated using a Taiwan Semiconductor Manufacturing Company (TSMC) 180 nm process. The second chip incorporated a 32×32 array of SPADs along with readout, pulse counting, addressing and control circuits. It also incorporated a variable high voltage (3-37.9 V) charge pump for reverse biasing the SPADs at 22V from a 3.3V supply. The chip (Fig. 2.c) was fabricated using Austriamicrosystems' (AMS) 350 nm high voltage process. The photodiode chip exhibited a maximum photo response at c. 600 nm whereas, the SPAD chip exhibited a maximum photon detection probability at c. 475 nm. Both chips were wire bonded and packaged using epoxy for wet biochemistry (Fig. 2.b and 2.d).

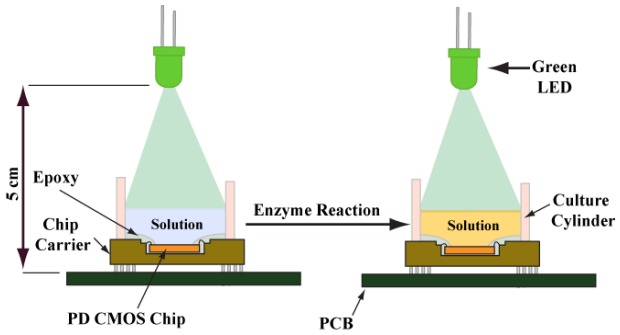

Fig. 3. A schematic illustration of the experimental mechanism of colorimetric sensing. During reaction the solution changes colour, resulting in fewer photons from the LED reaching the photodiode, changing the reading of the PD which indicates the cholesterol level.

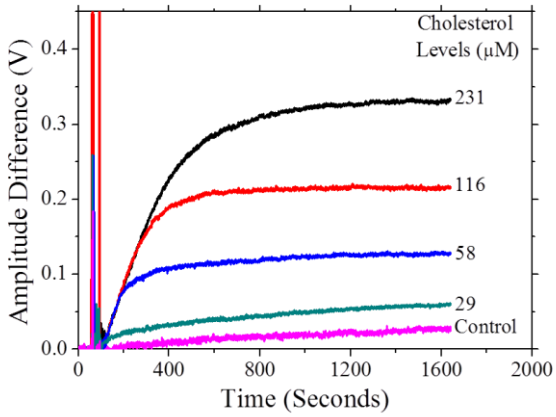

Fig. 4. Experimental results of colorimetric experiments using PD. The measurements show different cholesterol concentrations. The voltage response of the PD increases proportionally to the change in cholesterol concentration.

III. OPTICAL DIAGNOSTIC MODALITIES

In this section, biological or bio-chemical experiments are conducted to validate the use of the PD array and the SPAD array for colorimetric, chemiluminescence and refractive index sensing.

A. Colorimetric sensing

For colorimetric measurements, an experiment was designed to quantify cholesterol concentration in human blood serum by means of colour changes. In this experiment, the PD was used in conjunction with an off-the-shelf green LED light source and a CMOS PD array, as illustrated in Fig. 3. In order to quantify cholesterol levels in human serum, a triple enzyme assay was devised comprising two cholesterol reactions (cholesterol esterase and cholesterol oxidase), together with an oxidation step of o-Dianisidine to produce a coloured product that changes monotonically to deep orange colour via horseradish peroxidase. The coloured product is indirectly proportional to cholesterol concentration. By evaluating the colour intensity of o-Dianisidine that has an adsorption peak at

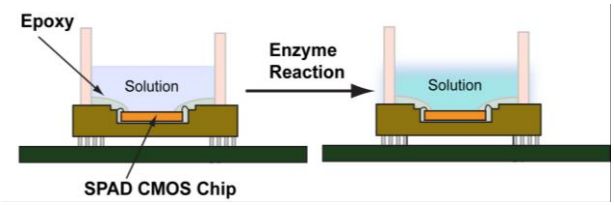

Fig. 5. A schematic illustration of the experimental mechanism of the chemiluminescence detection. The photon emission of the peroxidase conjugated antibodies reaction is detected by the SPAD.

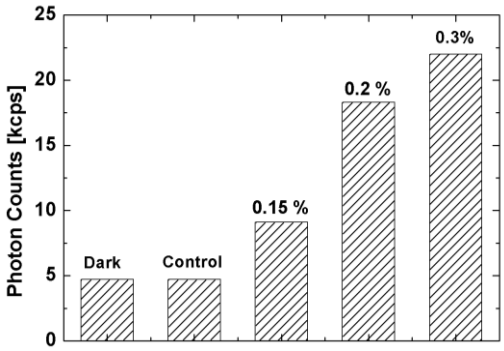

Fig. 6. Varying concentrations of hydrogen peroxide with luminol during a chemiluminescent assay are detected by the SPAD.

c. 460 nm and the PD responsivity that has a maximum peak at c. 600 nm. Therefore, the green LED was selected as a light source, with a wavelength peak around 500 nm to match the coincidence of the o-Dianisidine spectrum and PD spectrum.

In this experiment, we have used varying dilutions of human serum to produce correspondingly varying concentrations of cholesterol, with a fixed enzyme concentration. The measurement was carried out by first pretreating the serum with cholesterol esterase to release all cholesterol esters to free cholesterol, followed by transferring the cholesterol oxidase and peroxidase reactions on top of the PD array. Fig. 4 shows that the PD array is capable of resolving varying cholesterol concentrations, showing a corresponding variation in final plateau voltage. No change in voltage was observed when cholesterol was omitted to show that the enzyme assay is substrate specific. The lowest cholesterol concentration measured was 29 µM with a limit of detection of 13 µM, which is 400 times lower than physiological range, proving that it can be used for greater sensitivity applications.

B. Chemiluminescence sensing

As discussed previously, the SPAD was chosen for conducting chemiluminescence sensing. A proof of concept experiment was designed (see Fig. 5) to detect the concentration of peroxidase conjugated antibodies, which emit blue light when mixed with luminol and hydrogen peroxide. This results in a blue light emission within the visible detection range of silicon-based detectors. The data presented in Fig. 6 shows the variation in signal as the concentration of hydrogen peroxide is altered from 0.15 % to 0.3 %. It is clear that the higher concentrations of hydrogen peroxide produce more photons, which is readily detected by the SPAD array. We used this chemilunescence technique to validate the SPAD and we foresee future measurements involving immunoassays such as HIV and troponin detection [9].

The assay for chemilunescence detection was prepared as follows: First, 0.8 g potassium hydroxide was dissolved in 20 ml of purified water. Then 0.1 g luminol was added to this solution. 1 ml of the luminol solution was mixed with 1 ml of the hydrogen peroxide solution. This mixture was transferred into the culture bottle ring on top of the chip. The chip was covered with a black box and the lights were switched off to eliminate ambient light. Finally, 0.5 ml of peroxidase conjugated antibody solution was added to the liquid on top of the chip and the liquid started glowing. The light output was measured with the SPAD array. The concentration of the peroxidase conjugated antibody was varied. The antibodies were obtained from Sigma- Aldrich (A9917). 1 ml of 4-11 mg/ml concentration antibody was diluted in 200 ml of phosphate buffered saline. This stock solution (1:200) was used and further diluted in purified water for the experiments, up to 1:800. The concentration of the hydrogen peroxide solution was varied by diluting a 30% stock solution with purified water in the range of 0.15% to 0.3%.

C. Refractive index sensing

Monolithic integration of nanophotonic sensors with the CMOS detectors such as photodiode (PD) adds another functionality to our sensing platform. The monolithic integration of nanophotonic sensors with CMOS technology not only exploits the advantages offered by nanophotonic sensors such as label free sensing and high sensitivity but also makes the use of nanophotonic sensors practical by eliminating the requirement of external laboratory based equipment used for their readout such as optical spectrum analyser (OSA). One technique used for sensing by nanophotonic sensors is refractive index sensing where a change in the refractive index of the surrounding environment caused by introduction of an analyte or by bio-functionalisation of sensor surface induces a shift in the resonant wavelength of the nanophotonic structures. In this work, by monolithically integrating nanophotonic structures with CMOS PD the wavelength shift is measured as a change in the output voltage of the PD. We used an array of gold nanodiscs as refractive index sensor due to its high sensitivity and ease of fabrication. This concept can be extended to a range of nanophotonic sensors having different designs and composed of different materials [10]. The schematic of the device is shown in Fig 7.

The resonance response of the gold nanodiscs array was optimised using commercial finite element method (FEM). software (Comsol Multiphysics). The gold nanodisc array was then fabricated on top of CMOS chip by e-beam lithography followed by a lift-off process. Prior to fabricating gold

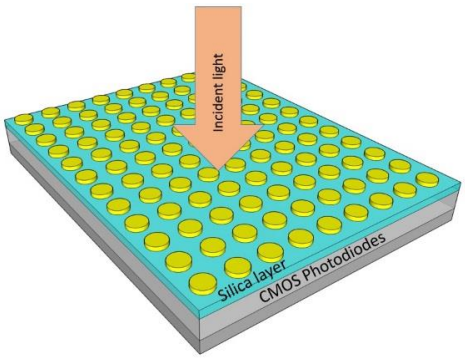

Fig. 7. Schematic of the gold nanodisc array monolithically integrated with CMOS PDs.

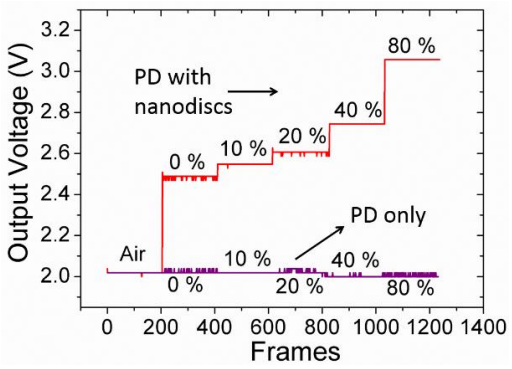

Fig. 8. Change in output voltage of the CMOS PDs with and without gold nanodiscs due to refractive index changes corresponding to different concentrations of glycerol.

nanodiscs, a 500 nm thick layer of silica was deposited on top of CMOS chip to keep the resonance of the gold nanodiscs at lower wavelengths. A monochromator was used as an incident light source to match the wavelength of the incident light with the transmission dip of the gold nanodiscs but in principle a cheap light emitting diode can be used instead. The performance of the integrated system was investigated by changing the refractive index of the resonance wavelength which was measured directly as a change in the CMOS PD output voltage as shown in Fig 8. A 10% change in glycerol concentration (refractive index change, $\Delta n \approx 0.011$) causes a wavelength shift of 2-3 nm which translates to a 0.06 V change in the output voltage of the CMOS PD. These values correspond to the optical and electrical sensitivities of 275 nm/RIU and 5.8 V/RIU respectively. While this work gives a proof of concept demonstration of working of the nanophotonic - CMOS electronic integrated sensing system by sensing changes in glycerol concentrations, future work will carry out more sensitive bio-assays by improving the sensitivity of the device.

IV. Conclusion

In this paper we described the use of two CMOS based detectors, namely PD and SPAD to interface with biology for performing colorimetric, chemiluminescence and refractive index sensing. The PD and SPAD array chip has been presented as a low cost and miniaturised version of a spectrophotometer, luminometer and conventional refractive index sensor. In this work, we demonstrated that PD, SPAD and nanophotonic sensor can be integrated onto a CMOS platform, leading to a possible integration of the three sensing techniques onto a single chip. This can be further integrated with existing microelectronics onto a single CMOS chip, paving the way for the development of point-of-care diagnostics for personalised healthcare.

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