White Paper by Anitoa Systems, LLC

Ultra-low-light CMOS bio-optical sensor enables low cost and portable molecular test

By Zhimin Ding, Li Liu and Yuping Chung[1]

Abstract

Innovations in ultra-low-light CMOS bio-optical sensor design, combined with microfluidics technology can enable a new generation of low cost and portable molecular testing platforms. This article discusses how this complementary pair of technologies have been applied successfully in a miniaturized qPCR system, and a chemiluminescence immunoassay platform to detect a variety of human diseases based on identifying DNA/RNA and protein biomarkers. Cost reduction and miniaturization of molecular test enabled by CMOS biosensors and microfluidics will have positive impact on global battle against infectious diseases and cancer, as well as enabling new applications in food safety and environmental monitoring.

1. Introduction

The outbreak of Ebola in West Africa two year ago underscores the urgent need for globally affordable tools to help fight infectious diseases. Among these, a method to rapidly and accurately identify the infectious pathogens and their drug-resistant variants on-site is of particular importance.

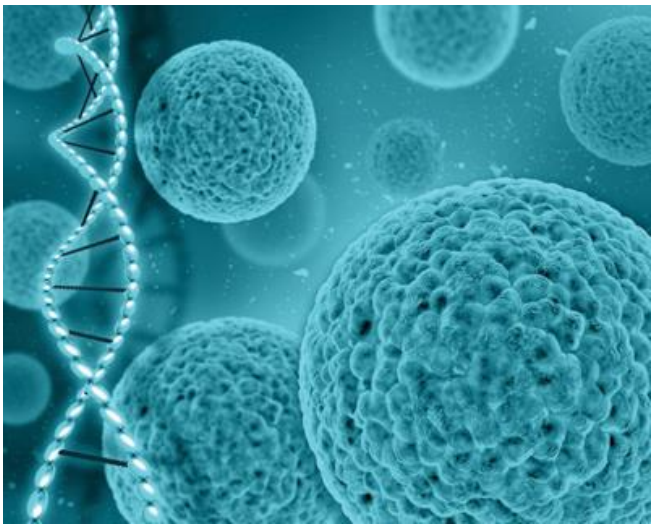

Figure 1. Molecular tests allow doctors to get precise answers about the types of virus and bacteria cells involved in infectious diseases.

[1] Author contact: zhimin.ding@anitoa.com. Anitoa Systems, LLC. 149 Commonwealth Drive Suit 1001, Menlo Park, CA 94025. +1 (408) 887-6026

The past decades have seen great advancement in molecular diagnostics technology. Nucleic acid (DNA, RNA) and protein (antibody) tests now allow doctors to get very precise information about the type of virus and bacteria cells behind each case of disease such as an infectious disease or cancer. However, much of the world's population still cannot enjoy the benefits of this technology, largely due to the cost and bulkiness of the test instruments required. At the heart of these equipment are precision optical sensing devices such as photon multiplier tubes or cooled CCDs, along with data conversion circuits and power supplies. Today's molecular diagnostics systems are designed for use in professional labs, not well suited for use in resource limited settings such as a small hospital and clinic in developing countries.

Thus, there is a push for portable systems based on the latest molecular methods that allow precise and timely testing of infectious pathogens at the point-of-care, or even in the field. To this end, much effort has been put into developing novel microfluidic “lab-on-chip” technologies [1]. Microfluidic devices enable sample and diagnostic assay to react in a small disposable chip-format device. The management of fluidic movement in microfluidic devices can be automated with better precision, while requiring smaller amount of samples and reagents. However in the end, these chips need to be "read out" by instruments such as a fluorescence microscope to obtain the final test results. Due to small reaction volume and dense reaction sites on a microfluidic chip, the type of the instruments that can perform read out are usually very bulky and expensive.

Thus microfluidic technology alone fell short of providing a complete solution for portable molecular diagnostics. What is needed is a holistic approach that includes innovations in technologies that enable compact microfluidic read out instrumentation as part of the total solution.

2. Ultra-low-light CMOS Biosensor

Engineers at Anitoa Systems, a Menlo Park, CA start up, developed a CMOS bio-optical sensor that is ultra-sensitive, highly integrated and low power. Based on fluorescence and chemiluminescence signaling principle, and combined with microfluidics technology, this CMOS biosensor can enable truly portable and affordable molecular diagnostic platforms.

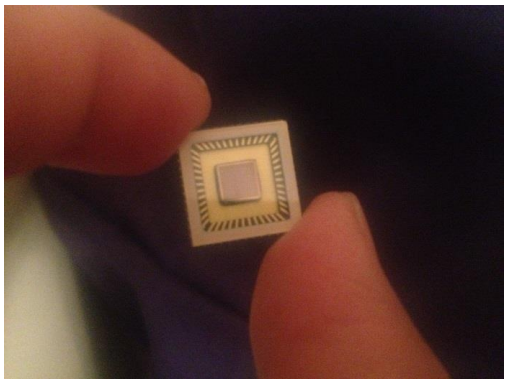

Figure 2, Anitoa's ULS24 ultra-low light CMOS Bio-optical sensor chip

Anitoa Systems has fabricated this CMOS image sensor (CIS) chip, called ULS24, on a 0.18 µm CIS technology from a leading commercial CMOS foundry. With a small die size of 4.8mm X 4.8mm, the chip has been shown to achieve 3e-6 lux detection sensitivity (characterized with narrow band light @550nm), while consuming only 30mW of power [2]. ULS24 is capable of detecting just a few molecules labelled with fluorescence reporter probes. This is sufficient to replace the much bulkier and more expensive photon multipliers (PMTs) and cooled CCDs that are commonly found in many of today's molecular test instruments.

Due to its wide adoption in consumer digital cameras and smartphone cameras space, CMOS image sensor technology have steadily improved over the years in terms of performance and cost. Still more improvements in process, circuit, and software are needed to enable CMOS to take up the jobs of PMTs and cooled-CCDs in ultra-low-light sensing in molecular testing. To meet this challenge, Anitoa uses novel manufacturing process technology and circuit design techniques to reduce the noise inherent in CMOS image sensors active pixels to achieve a high signal-to-noise ratio (SnR). The excessive noise that cannot be eliminated in the chip due to limitations of physics is further filtered through a digital signal processing algorithm, called "Intelligent Dark-current Management" algorithm.

One of the key challenge in biosensor technology is achieving good reproducibility. After all, we cannot trust a test equipment if the test results can vary wildly depending on the time of the day, or other uncontrollable factors.

The highly integrated nature of CMOS biosensors means we could add additional calibration circuitry onto the same chip to help with reproducibility. For example, we could embed junction temperature sensors onto the same CMOS chip where the active pixels operate, thus achieving accurate and dynamic monitoring of sensor operating conditions. It is known that the dark current of a CMOS image sensor may vary due to temperature. A junction temperature sensor along with built-in auto calibration circuitry can help remove this level of uncertainly.

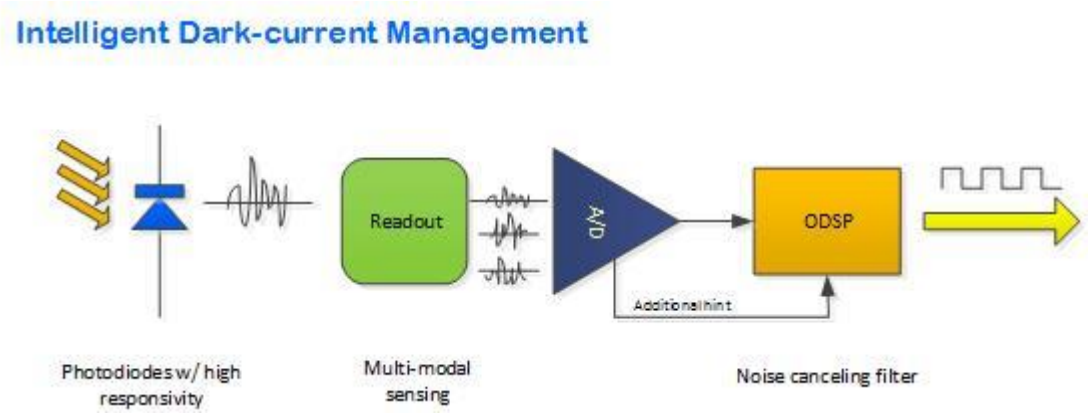

Figure 3 Dark current management

Lastly, the lower power operation of CMOS biosensor also means the sensor can operate in a cool condition without bulky heat dissipating measures. This greatly simplifies the design of small portable instrument while still achieving good data accuracy.

2.1. CMOS-based qPCR and DNA hybridization

The combination of ULS24 ultra low-light CMOS Bio-optical sensor, with microfluidic technology, forms the basis of a miniaturized quantitative polymerase chain reaction (qPCR) system. The CMOS sensor is paired with LEDs as the optical excitation source to achieve fluorescence-based molecular sensing in a very compact platform.

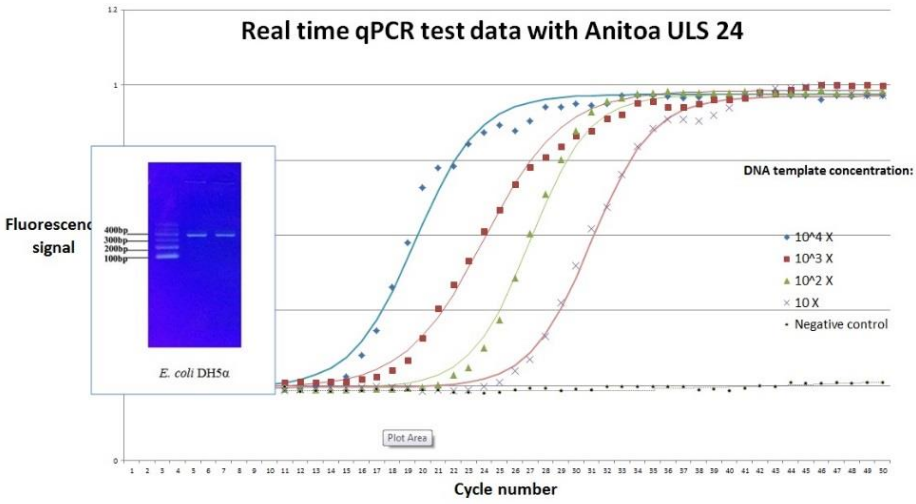

Figure 4a, Anitoa ULS24 CMOS biosensor successfully applied in qPCR to detect E Coli. (DH5a), HBV (wild type and rtM2041), and foot-and-mouth disease (EV71 and CA16), with limit of detection as low as 4 copies per reaction.

One of the important challenge with microfluidic qPCR, especially sample-to-answer type of microfluidic qPCR is the tight space to fit all functional modules around the fluidic cartridge. These include heating control, actuator to operate the pumps, and excitation and emission light paths. A compact onboard multi-spectral fluorescent camera constructed using four ULS24 chips can image multiple reaction sites on the fluidic chip in one shot. This approach eliminates the need for a multi-reaction-site scanning mechanism, reducing the total system space and complexity.

In a separate experiment (performed in collaboration with Micronit BV), the ULS24 CMOS biosensor is used in conjunction with a microfluidic chip in a lens-less configuration (Figure 4b). The CMOS sensor is configured to detect DNA hybridization. The capture DNA probes are attached to magnetic beads, and the detection probes were coupled with HRP enzyme. As the target DNA in a sample (e.g. PCR product) hybridize with the probes, they are trapped at the detection point in the microfluidic chip due to a small magnet.

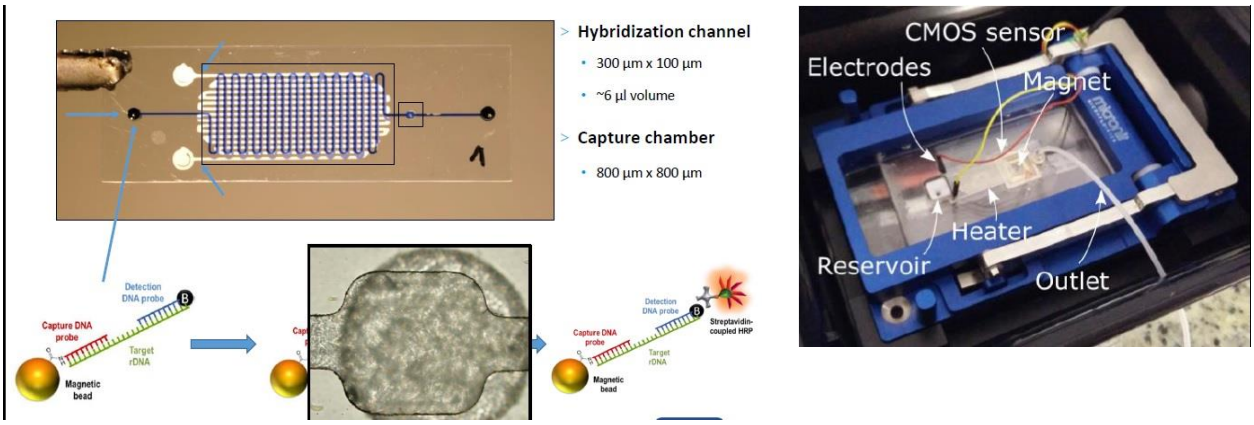

Figure 4b Microfluidic and beads based DNA hybridization set up, where the ULS24 CMOS biosensor is tightly coupled to the microfluidic chip in a lens-less configuration to detect chemiluminescence

To detect the hybridized target DNA, a luminol substrate is passed through the channel. As the HRP-conjugated probes react with the substrate, chemiluminescence emission is generated and detected (Figure 4c).

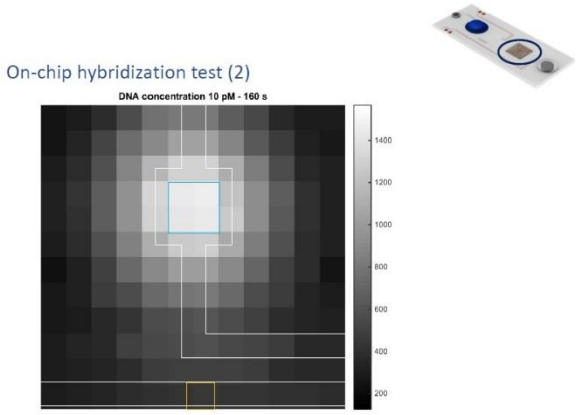

Figure 4c Chemiluminescence image captured by ULS24 CMOS Biosensor

2.2. CMOS-enabled chemiluminescence immunoassay

In a different configuration, the combination of Ultra low-light CMOS bio-optical sensor have been demonstrated to enable microfluidic-based chemiluminescence immunoassay. Immunoassay (ELISA) is a widely used method for detecting the presence and quantifying a macromolecule (typically a protein molecule) in a solution, through the use of an antibody molecule. Chemiluminescent immunoassays are variations of the standard immunoassay. An enzyme converts a substrate to a reaction product that emits photons of light instead of developing a visible color.

Chemiluminescent assays have been shown to achieve better sensitivity and wider dynamic range, while running faster and consume less sample and reagent [3]. Chemiluminescent assays have very low background noise. This leads to their much lower limit-of-detection (LOD), and as a result wide applications not only in identification of infectious pathogens, but also cardiac and cancer markers.

We have demonstrated chemiluminescence readout in microfluidic immunoassay using CMOS bio-optical sensor in a much more compact platform than that of a PMT-based system. Since chemiluminescence readout do not require filters and excitation light source, the optics design can be further simplified. For example, we tested the method to directly couple the sensing surface of CMOS bio-optical sensor to fluidic channels on the microfluidic chip in a lens free configuration. This further reduces the size and cost of the total system, while achieving lower than 100pg/ML detection limit.

2.3. CMOS biosensor in environmental testing and food safety monitoring

The low cost and portability benefits of CMOS-based molecular testing opens up opportunities in new applications in food safety and environmental monitoring. In terms of improving quality of human lives, many of these applications are just as important as their medical counter parts.

Researchers of Ben-Gurion University of Israel [4] have tried to use a CMOS biosensor (the ULS24, also provided by Anitoa Systems) to detect water toxicity in a bio-threat detection applications. A pen-size portable biosensor with wireless connectivity for water quality monitoring was developed, and tested and compared to a commercially PMT-based bench top luminometer. The biosensor is built from two parts, a non-disposable CMOS photodetector, and a one-time replaceable pad consists of bioluminescence bacteria immobilized into calcium alginate component. Both the CMOS and PMT-based devices were exposed to spiked common environmental toxicants. Interestingly, the CMOS biosensor showed a ten times more sensitive response (i.e. 1e-8 mol/L) than the commercial PMT device (i.e. 1e-6 mol/L). The increased sensitivity originates from the possibility of placing the pads directly onto the CMOS endface enabling a greater amount of collected photons. In addition to its sensitivity, the CMOS-based biosensor is shown to be more user friendly, portable, has a lower detection limit and requires smaller amounts of sample volume. These advantages make this application attractive for use in many ground-based applications, as well as, including on-site water-quality analysis in underdeveloped regions of the world.

3. Portable diagnostic systems connected to bioinformatics cloud

CMOS based biosensor technology lends itself well to seamless connectivity with bio-informatics Internet cloud through popular protocols such as Bluetooth. We envision in the near future, small and portable molecular diagnostics devices would be deployed at point-of-care, enabling rapid diagnostics of infectious disease on-the-site, so that doctors can respond quickly with life-saving drugs and treatment. The diagnostics device will be internet enabled, and the diagnostic results will be transmitted to a central bioinformatics database in the cloud, allowing doctors, drug companies and policy makers to make coordinated decisions on global epidemic control [5].

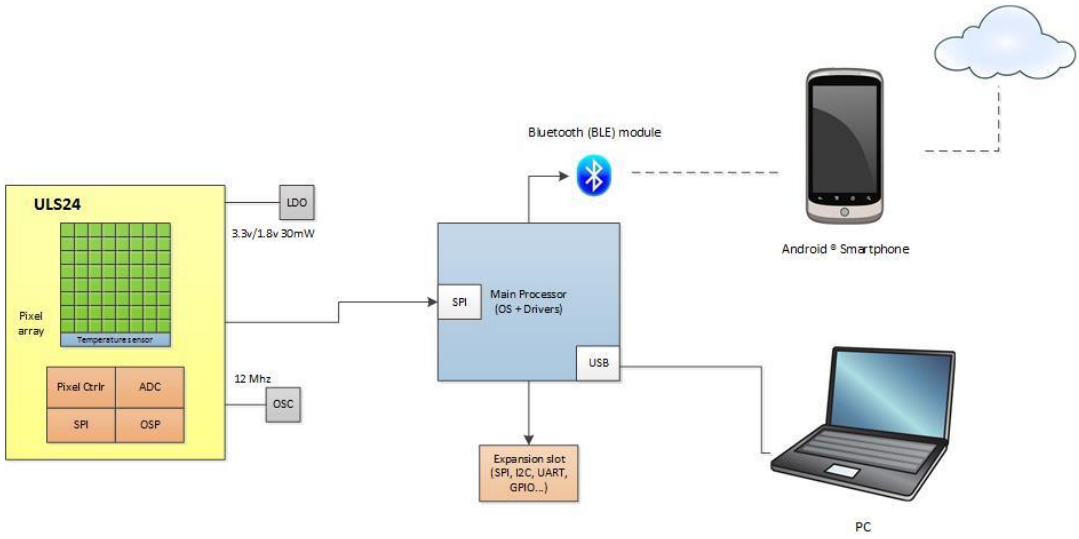

Figure 5. Anitoa ULS24 CMOS Biosensor Eco System

References

[1] Julie Clayton. Go with the microflow. Nature Methods - 2, 621 - 627 (2005)

[2] ULS24 Preliminary Datasheet. Anitoa Systems, LLC. http://www.anitoa.com/docs/uls24_brief.pdf

[3] WANG Chen, WU Jie, ZONG Chen, XU Jie, JU Huang-Xian. Chemiluminescent Immunoassay and Its Applications Chin J Anal Chem, 2012, 40(1), 3–10.

[4] Tim Axelrod, Evgeni Eltzov, Robert S. Marks Bioluminescent bioreporter pad biosensor for monitoring water toxicity. Science Direct (Talanta) 149 (2016) 290–297, Elsevier Publishing Company

[5] West Suhanic1, Ian Crandall1, and Peter Pennefather. An informatics model for guiding assembly of telemicrobiology workstations for malaria collaborative diagnostics using commodity products and open-source software Malaria Journal 2009, p. 8-164, BioMed Central.