A CMOS Image Sensor for DNA Microarrays

Samir Parikh, Glenn Gulak, Paul Chow
University of Toronto

Abstract- **An image sensor designed with standard 0.18µm CMOS technology is used to construct a DNA microarray scanner. The detection limit of 4590 fluorophores/µm^2 is compared with 4.49 fluorophores/µm^2 of a commercial photomultiplier-tube-based microarray scanner. The performance gap can be reduced by improving optical coupling, mechanical alignment, laser power supply noise, improved circuit noise and an increase in the conversion gain. The CMOS sensor offers multiple-pixels for reduced scan time and an integrated analog-to-digital converter.**

I. Introduction

DNA microarrays are commonly used to search for DNA sequences. A DNA microarray is a slide that consists of an array of spots, each containing multiple single-stranded DNA (ssDNA) called probes. These probe ssDNA have a specific nucleotide sequence that preferentially bonds to a target ssDNA strand containing the complementary nucleotide sequence and a fluorescing dye molecule. A solution containing the target ssDNA is introduced to the DNA microarray leading to a pairing or unpairing process at each of the spots. The DNA microarray is washed to remove the unpaired target ssDNA strands. Next the DNA microarray is optically scanned to determine the paired and unpaired spots. Each of the spots will contain a concentration of fluorescing dye molecules proportional to the number of paired ssDNA. The two main components in a microarray scanner are an excitation light source to excite the fluorescing dye molecules at each of the spots and a light detector to sense the fluorescence emissions from each spot. Through this process the concentration of a specific DNA sequence in a solution can be determined.

This paper describes the design and performance of a microarray scanner that utilises an image sensor fabricated using standard CMOS technology. Section II motivates the choice of the CMOS image sensor and distinguishes it from existing approaches. Section III elaborates on the design of the image sensor and the microarray scanner constructed in this work. Thereafter, the performance of the individual blocks and the entire system are presented in Section IV. Section V provides insight into the measured performance by comparing it against a baseline. Lastly, the conclusions of this work are summarised in Section VI.

II. Motivation

Existing microarray scanners use a Photomultiplier Tube (PMT) or a Charge-Coupled Device (CCD) to detect the emitted fluorescence photons. The PMT sensor is a very accurate light sensor, capable of detecting very low light emissions. However, PMT's are bulky, expensive devices similar to vacuum tubes; they are not suitable for portable applications. Moreover, a PMT is a single-element device, often configured with a 10µm resolution, which requires the DNA microarray to be scanned sequentially. CCD's on the other hand are not as accurate as PMT's but have multiple pixels that allow them to scan the entire DNA microarray in parallel. This ability can significantly reduce scan time. In addition, CCD's are often cooled to reduce their dark signal and thermal noise. Both these sensors are interfaced with (Analog-to-Digital Converters) ADC's and other discrete signal processing logic. In contrast, CMOS technology has the potential to create an integrated solution containing a light sensor, ADC and other processing logic in a monolithic implementation. Additionally, the use of a standard CMOS process leads to an integrated, smaller and less expensive solution that is more suited to portability.

Presented in this paper is an un-cooled image sensor fabricated in a standard CMOS process used to image DNA microarrays. The cost and power consumption due to the cooling device is eliminated. It has the advantages of a CCD sensor due its ability to have multiple pixels. Moreover, the use of a larger pixel allows for more light capture and reduces post-processing.

Some existing work in this area is presented in [1] and [2]. In contrast to [1], which uses phototransistors, our design uses photodiodes, which have better linearity. Reference [2] describes an image sensor tailored for bioluminescence using a customized CMOS process, while this work focuses on a different application: the fluorescence-based detection of DNA using standard microarrays with a sensor using a standard CMOS fabrication process.

III. Design Details

Figure 1 shows the components of the microarray scanner constructed in this work. The scanner consists of an excitation laser source, an emission filter and a CMOS chip that performs the light detection and quantification. The laser is used to excite an entire spot on a DNA microarray. Moreover, the spot is aligned directly above a pixel on the CMOS chip, which is sized to be as large as the spot. Thus, the fluorescence emissions from an entire spot can be captured at once. This eliminates the software-based spot identification that is often performed in commercial systems that scan each spot with a 10µm resolution. In this prototype each spot on the DNA microarray is individually scanned but this can be easily extended to parallel scanning with the fabrication of a multiple-pixel sensor and using a wider light source or a fast scanning laser. The emission filter is placed between the DNA microarray and the image sensor to block the excitation photons from the laser source and allow the fluorescence emissions to reach the pixel.

Since the photon emissions from a DNA microarray are faint, a pinned photodiode structure is used on the CMOS chip, which has reduced dark current. This compensates for

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the increased dark current due to the larger photodiode size. Additionally, a low reset voltage of 0.7V is used to further reduce the dark current of the photodiode. The active pixel sensor (APS), shown in Fig. 2, also contains a reset transistor and a pair of source-followers to provide a partially differential output. To maximize the light capture area, minimum size transistors are used. The transistor sizes (W/L) are (0.3µm/0.3µm), (0.35µm/0.3µm), and (0.8µm/0.35µm) for the M1a/b, M2a/b, and the M3 transistors respectively. A differential output from each pixel is required because the input to the quantization circuitry is differential. Also, a differential output is less sensitive to common-mode and power supply noise.

The differential output is connected to an on-chip 2nd order delta-sigma modulator, using the Boser-Wooley architecture, shown in Fig. 3. The Boser-Wooley architecture [3] reduces the output swing at the integrators, which allows for easier implementation. Furthermore, the use of delaying integrators allows them to settle independently of each other. The integrators are implemented with a folded-cascode op-amp that utilizes switched-capacitor common-mode feedback as shown in Fig. 4. A single-bit quantizer and feedback along with an oversampling ratio of 256 is used to provide a high-accuracy output bitstream. The single-bit quantizer and feedback are used because they have only two levels and thus, are inherently linear. This eliminates the need for any digital calibration that is often used in multi-bit quantizers. Fig. 5 shows the schematic for the regenerative feedback comparator and latch used to perform the single bit quantization. The differential implementation reduces the common-mode charge-injection while the effect of signal-dependent charge-injection is reduced through advanced clocks. Fig. 6 shows a block diagram for the testchip.

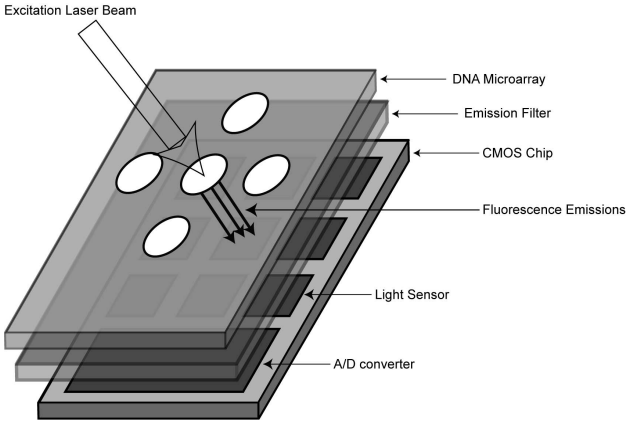

Fig. 1. Microarray Scanner Setup.

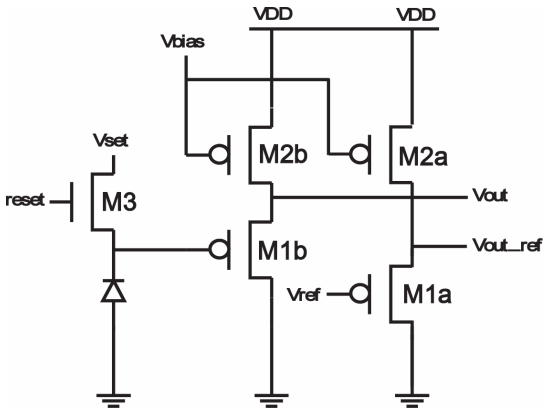

Fig. 2. APS circuit.

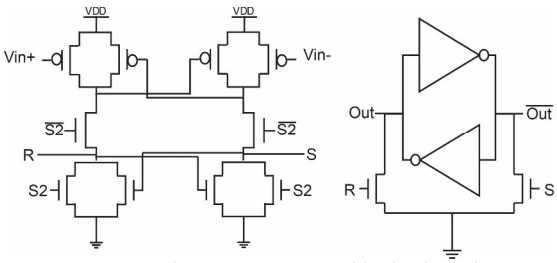

Fig. 5. Comparator and latch schematic.

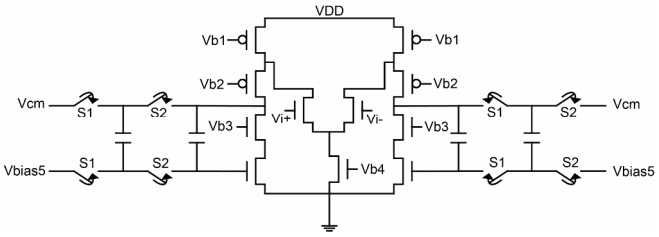

Fig. 4. Opamp and switched-capacitor common-mode feedback schematic.

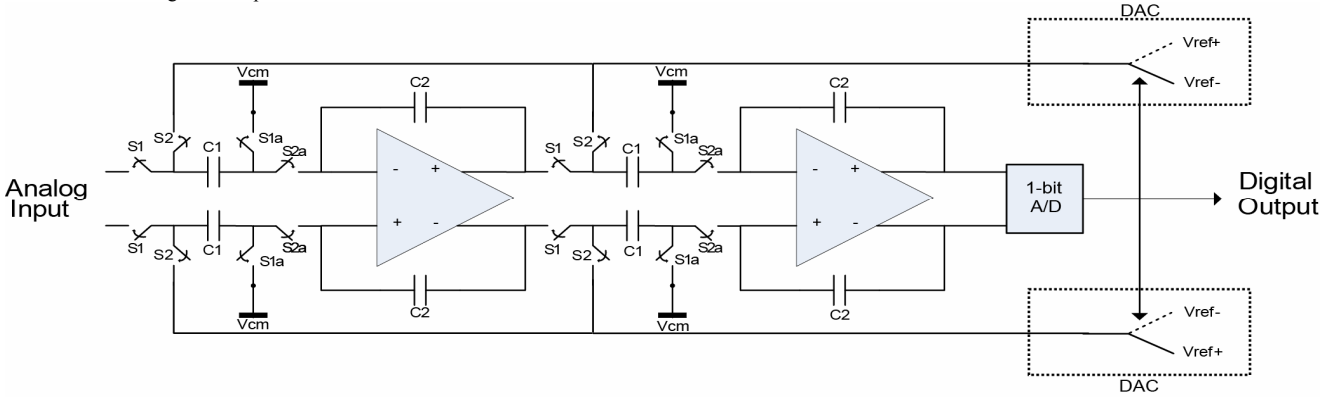

Fig. 3. Delta-Sigma Modulator Schematic

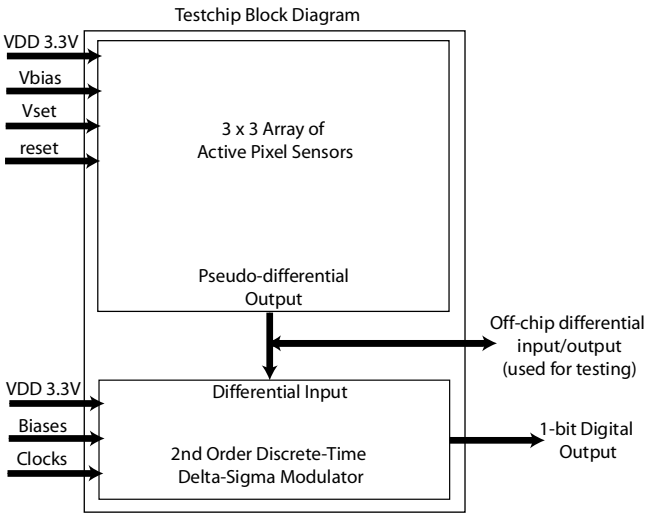

Fig. 6. Testchip block diagram.

IV. Results

The prototype image sensor is realised in a 1-poly 6-metal CMOS process with a minimum feature size of 0.18µm. The chip has a die area of 1.2×1.4 mm^2 and its micograph is shown in Fig. 7. The APS and the delta-sigma modulator blocks on the image sensor were individually characterized. The results are shown in Table I and Table II respectively. Low light sensitivity and dark current are directly a result of the pixel design and play an important role in determining the sensitivity of the microarray scanner. The accuracy of the delta-sigma modulator lies within the 12-16 bits range of commercially available microarray scanners.

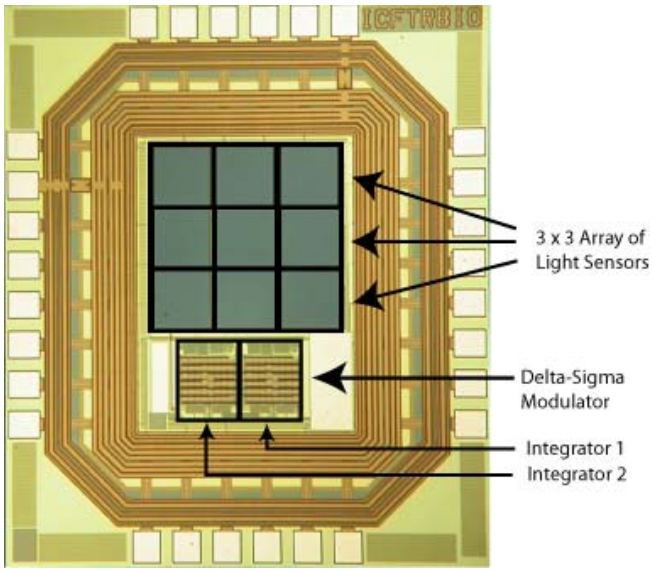

Fig. 7. Chip micrograph.

The constructed microarray scanner was also characterized with a microarray scanner calibration slide [4]. This slide consists of a dilution series of spots containing fluorescence dyes commonly used in DNA microarrays. The concentration of the dyes on these spots ranges from 1.47e+5 to 2.19e-3 fluorophores/µm^2. The microarray scanner used a 532nm laser source and captured fluorescence emissions for the Cy3 dye spots on the slide. The Cy3 dye spots were selected over the Cy5 dye spots due to their improved resistance to ozone degradation. The same setup can be used for the Cy5 dye spots with the proper ozone control equipment in the lab. The peak fluorescence emissions from the Cy3 dye occur at 575nm. However, due to the close excitation and emission peaks, an economical optical filter that strongly rejects 532nm while allowing 575nm could not be found. As a result, this microarray scanner uses a narrowband (10 nm) optical filter centered at 605nm to reject the excitation photons while allowing the fluorescing photons to pass through. A translation stage was used to align the various Cy3 spots on the calibration slide with the pixel on the testchip. Fig. 8 shows a grayscale image of the Cy3 spots on the calibration slide. The image was captured by a commercial microarray scanner. This slide is often used to evaluate the performance of a microarray scanner or to calibrate its settings.

TABLE I
APS Characteristics and Performance

Process	1P6M 0.18µm CMOS
Photodetector Type	P+/n-well/Psubstrate
Sensitivity to low light	< 2.6 x 10^{-2} lux
SNR @ 2.6 x 10^{-2} lux	16.6dB
Dark-signal (room temp.)	10mV/sec
Source-Follower non-linearity	0.12%
Photodetector Size	150µm x 150µm
Pixel Size	162.5µm x 154µm
Fill Rate	90%

TABLE II
ΔΣ Modulator Characteristics and Performance

Discrete-Time 2nd Order Single-bit ΔΣ	
Power Supply	3.3 V
Power Consumption	26.4 mW
Peak SNDR	75.9 dB
Effective Number of Bits	12 bits
Dynamic Range	74.63 dB
SFDR	85.5 dB
Sampling Rate	3.6 MHz
Nyquist Sampling Rate	14.2 kHz

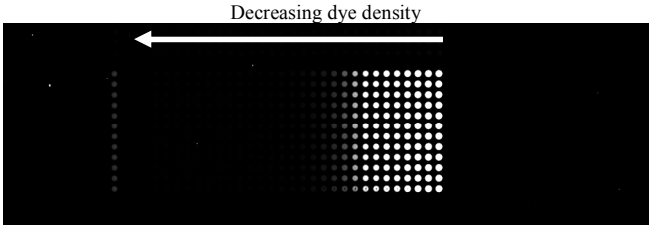

Fig. 8. Image of scanner calibration slide.

Fig. 9 shows the measured SNR for the various spots on the slide. Two curves are shown; one using the CMOS chip as

the light sensor and another using an optical power meter. For comparison purposes, a commercial microarray scanner was also characterized with the same calibration slide to be used as a baseline. The baseline performance (SNR vs. fluorophore density) is shown in Fig. 10. Also, shown in Figs. 9 and 10 is the SNR=3 detection floor, which is a common threshold used by biologists working in this field. The intersection of the curves with this detection floor determines the detection limit of the microarray scanner. The measured detection limit for the microarray scanner with the CMOS chip is 4590 fluorophores/μm^2 and with the optical power meter is 9190 fluorophores/μm^2. In comparison, the detection limit of the commercial microarray scanner is 4 fluorophores/μm^2. Since the commercial system is currently used to scan DNA microarrays, any new system should have the same or better DL limit to be compatible with the same DNA microarrays.

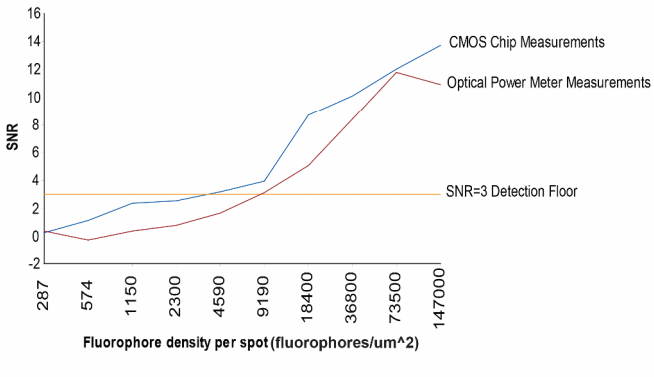

Fig. 9. SNR vs. Fluorophore density per spot, as measured by the CMOS testchip and an optical power meter.

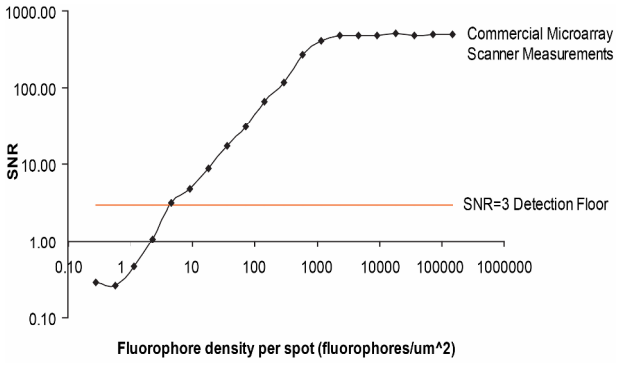

Fig. 10. SNR vs. Fluorophore density per spot, as measured by a commercial microarray scanner.

V. Discussion

As can be seen from Fig. 9 and 10, the performance of the constructed microarray scanner doesn't meet the baseline. Analysis of the microarray scanner setup shows several possibilities for improvement. Firstly, the noise and drift in the laser power introduces noise into the system level measurements. The commercial scanner uses a laser source with 0.1% noise while our scanner contains a laser with 3% noise. The use of a low-noise laser source will help to boost the SNR of each of the spots on the calibration slide. Secondly, the optical coupling between the APS and the spot can be greatly improved through the use of a lens. This will increase the amount of signal captured from the slide. Finally, improving the conversion gain of the image sensor through the use of a floating diffusion and implementing correlated double sampling to reduce the reset and flicker noise can further improve the image sensor's sensitivity. This sensitivity improvement will increase the SNR per spot and reduce the detection limit for the constructed microarray scanner.

VI. Conclusion

The CMOS Image Sensor chip shows potential to be a replacement for the PMT and CCD technologies currently used. Compared to the PMT sensor, it is a smaller and integrated detector. This, along with the elimination of cooling equipment makes it more attractive for portable applications with its presently integrated ADC and future on-board digital signal processing hardware. Moreover, a CMOS sensor is capable of performing fast DNA microarray scans due to its ability to have multiple pixels, which allows simultaneous scanning of many spots in parallel.

However, some additional work is required to reduce the detection limit of the constructed microarray scanner to be comparable to the detection limit of the commerical microarray scanner. The detection limit of the constructed microarray scanner can be improved through the use of a low noise/drift laser source, better optical coupling between the light sensor and the fluorescing spot, and a more sensitive light sensor. The sensitivity of the light sensor can be improved by reducing read and reset noise, as well as increasing the conversion gain.

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