Final Project Report for Supplementary Funding for NSF-Funded Project

Fully-Integrated CMOS biochip array for multicolor biomolecular diagnostics

Overview. The research objectives of this grant are focused on the development of biomolecular (DNA and protein) fluorescent-based sensing in complementary-metal-oxide-semiconductor (CMOS) devices. As part of this effort, we have developed two distinct fluorescence-based sensor chips, one based on conventional photodiodes and charge integration and the second based on Geiger-mode avalanche photodiodes and single-photon counting. With each of these two sensors, we are advancing two applications: DNA sensing and protein sensing. In both cases, we are developing attachment chemistries, deposition techniques, and packaging that enable direct attachment of the biomolecules to the chip surfaces.

1.1. Photodiode-based, charge-integrating chip substrate

Building on a first-generation active CMOS biosensor array [1,2,3] for fluorescent-based assays, we have tested and fabricated [4] a second-generation design. The first-generation design had quantization-noise-limited sensitivities of more than 10^8 photons/cm^2 and impulse-response time-constants of more than 1.1-ns. Impulse response was further limited by a long "tail" in the photocurrent. The second-generation design improves on our earlier efforts in several significant ways. Differential photodiodes (PD) are implemented to improve photocurrent impulse response and noise immunity. 12-bit data conversion is employed to produce reset-noise limited sensitivities, and active reset is employed to reduce reset noise.

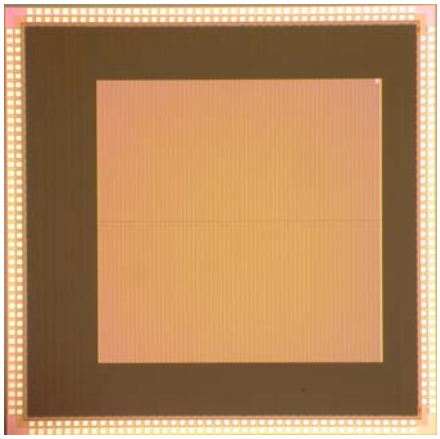

Figure 1. Die photograph of active fluorescent microarray chip.

Fig. 1 shows the die photo of the second-generation chip. The chip contains 64x64 current-mode-output pixels, with per-column transimpedance amplifiers and per-column two-step integrating analog-to-digital converters (ADC) for multiplexed conversion. The prototype 5-mm-by-5-mm chip is fabricated in a standard 0.18 μm CMOS process. The system has a reset-noise-limited sensitivity of approximately 100 e^-, which corresponds to a photon density of 2 x 10^7 photons/cm^2. N-point averaging improves this sensitivity by a factor of $\sqrt{N}$; for N=4096, sensitivity improves to 3 x 10^5 photon/cm^2. For typical ADC settings, the gain of the entire sensor path is approximately 0.5 x 10^{-7} DN/(photons/cm^2). We have used these chips this year for some preliminary microarray studies. The chips are packed in ball-grid packages which are "doughnut" epoxy encapsulated, providing access to the sensor area while protecting the bond wires. In our first efforts, the chip surface is cleaned and epoxy-derivatized with 3-glycidoxypropyltrimethoxysilane. Aminated DNA probes with a length of 21 nucleotides are immobilized on top of the detector area through contact-pin spotting. DNA hybridizations are carried out with analyte solution containing different concentrations of matching DNA (target 1)

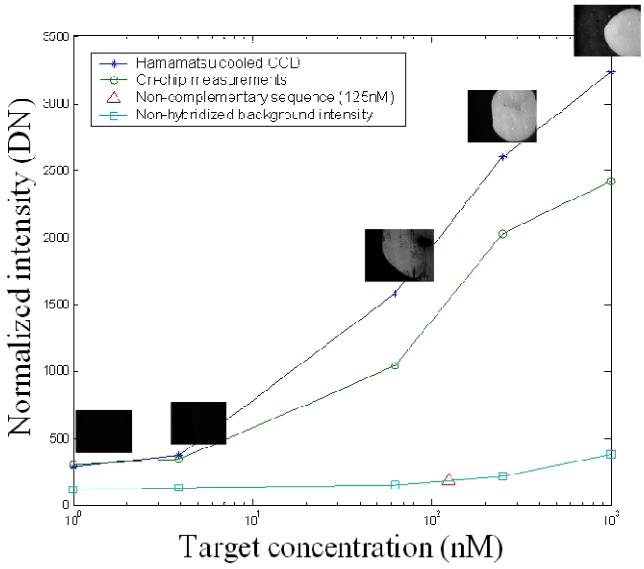

Figure 2. Measured DNA hybrization for various target concentrations.

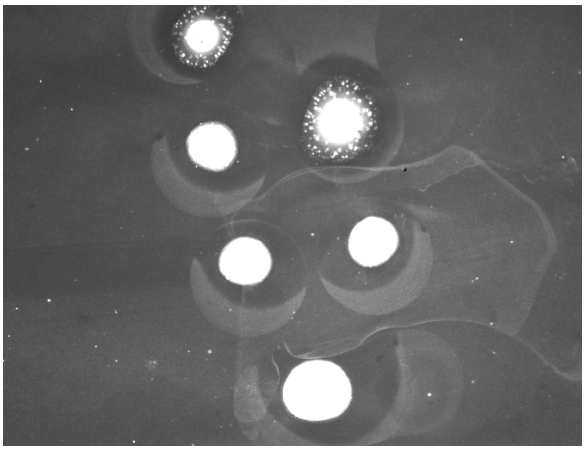

Figure 3. Typical hybridization results from array spots, 100 to 150 μm in diameter, prepared using NHS/EDC activated probes and parylene coated, uncleaned glass slides. Target concentration was 100 pM. The non-contact piezoelectric deposition, which shoots droplets of probe solution onto the surface, shows spatter for some spots. The "halos" are believed to originate from droplet contraction during probe deposition.

strands, which are end-labeled with biotin molecules. Hybridizations with non-complemetary (target 2) sequences are also carried out as a control. After hybridization, streptavidin-conjugated Qdot-655 solution is used to label and quantify the amount of hybridization and thus the concentration of matching target DNA molecules in the original analyte solution. Data from on-chip detection is verified by also imaging the chip surface in an epi-fluorescence microscope with a Hamamatsu cooled CCD imager and 500nm long-pass filter cube. The results are shown in Fig. 2 and show correlation between chip and CCD measurements. As shown from the figure, hybridization sensitivity is primarily limited by non-specific binding of either target DNA molecules or fluorescent labels to the substrate surface, rather than the sensitivity of the sensor.

Several surface chemistries were evaluated as alternatives to the epoxysilane films, including solution-deposited layers of aminosilane and aminosilane-maleimide, and chemical vapor deposited (CVD) aminoparylene films. Silane-based surface modification is standard for glass slide microarrays and relies on reaction with surface silanol groups. These methods, however, exhibited significant failure rates when used with the imager because the susceptibility of the devices to harsh chemicals interfered with surface precleaning. Therefore, a different route to surface modification was pursued in which amine groups are introduced through CVD of aminomethyl-(2,2)paracyclophane, an amine containing molecule that polymerizes to make an aminoparylene film. The CVD process uniformly coats the surface and does not require bonding to surface silanols. Carboxylic acid modified probes are then conjugated to surface amines using aqueous carbodiimide (EDC) coupling, a standard bioconjugation method.

Test assays were performed on aminoparylene films deposited on deliberately uncleaned glass slides, using biotin-labeled target analyte. Fluorescence signal was developed post-hybridization using streptavidin-coated quantum dots which bind to the target biotins. Strong signals were observed down to the lowest (100 pM) target concentration tested, shown in Fig. 3. These tests also demonstrate our first use of a Piezoarray robot (Perkin Elmer), installed in February 2008, to deposit probe arrays. The Piezoarray is a non-contact deposition tool that can also accommodate the nonconventional shape and size of the CMOS microarrays.

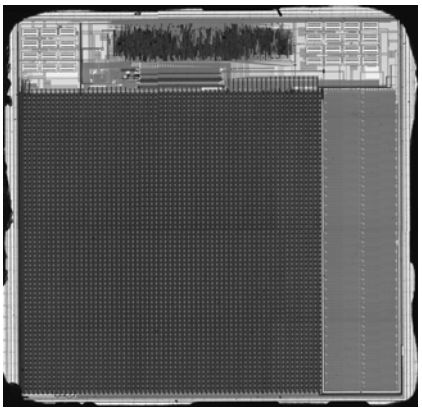

Figure 4. Die photo of SPAD array chip.

Current work involves adaptation of quantum-dot (Q-dot) labeling protocols to gene expression measurements. Once complete, we will initiate of the imager combining: (i) parylene chemistry for probe immobilization, (ii) non-contact Piezoarray deposition for array fabrication, and (iii) gene expression studies based on Q-dot fluorescence assays.

1.2. Geiger-mode APD substrate

In addition to the photodiode-based sensors, we are actively developing additional prototypes based on Geiger-mode avalanche photodiodes (APDs) in a LOCOS-based 0.35μm CMOS technology. As part of this work, we have fabricated and tested a prototype 64-by-64 active array substrate[5,6]. The chip die photo is shown in Fig. 4. As a high-gain sensor, these detectors deliver two orders of magnitude more sensitivity than the photodiode-based array.

We have used these arrays as an active oligonucleotide microarray platform for time-resolved fluorescence resonance energy transfer (TR-FRET) assays[7]. In TR-FRET assays, immobilized probe is labeled with a donor fluorophore and analyte target is labeled with a fluorescence quencher. Changes in the fluorescence decay lifetime of the donor are measured to determine the extent of hybridization. TR-FRET offers several advantages over standard fluorescence-based microarray assays, including low sensitivity to variances in probe surface density and the potential for real-time hybridization detection.

References

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[7] D. E. Schwartz, P. Gong, and K. L. Shepard, “Time-resolved fluorescence-resonance-energy-transfer DNA assay on an active CMOS microarray,” accepted to Bioelectronics and Biosensors.