16.1 A Nanogap Transducer Array on 32nm CMOS for Electrochemical DNA Sequencing

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Next generation sequencing (NGS) is a disruptive technology that concurrently sequences millions of DNA fragments at low cost with high throughput and impacts nearly every field of biology and medicine. The most widely used biosensing techniques today, including those for NGS, are optical in nature and thus do not benefit from the annual scaling advancements made in the semiconductor industry. ISFETs have been demonstrated as a viable approach towards all-electronic DNA sequencing [1]; however, there are technological barriers to overcome in scaling, namely the diminished SNR as the size of the sensor is reduced to fit more sensors on the same chip. Towards this end, we demonstrate an alternative high-density electrochemical biosensing technique for DNA sequencing that leverages CMOS scaling to reduce costs and enable more compact NGS systems.

The assay follows standard DNA sequencing library preparation and loading procedures for a sequencing by synthesis process [2]. The procedure is as follows (Fig. 16.1.1): 1) A unique cluster of single-stranded DNA (ssDNA) is immobilized and amplified on the surface of each transducer. 2) Custom-synthesized deoxynucleotides (dNTPs) functionalized with a cleavable redox tag (4-Aminophenol, pAP) are flooded over the entire chip, one dNTP at a time. 3) The cleavable pAP is released and its presence or absence is detected electrochemically as described below. 4) Steps 2 and 3 are sequentially repeated for each dNTP (A/G/T/C) until the entire DNA sequence on each transducer has been identified, one base at a time. The dNTPs utilize a blocking chemistry such that only one can be incorporated per cycle to improve homopolymer accuracy at the expense of lower throughput.

The "nanogap" sensor used to detect the electrochemical tags comprises two planar electrodes separated by a nanoscale gap [3]. It consists of a deposited Pt(50nm)/Cr(60nm)/Pt(120nm) structure covered with oxynitride passivation (500nm) fabricated directly on top of standard CMOS (Fig. 16.1.2). The wafer is pulled prior to top metallization and passivation to facilitate post-processing. An opening is formed in the passivation and top platinum layer to expose the sacrificial Cr layer, which is etched away leaving isolated top and bottom electrodes. By properly biasing the two electrodes, redox molecules can be repeatedly oxidized at one electrode and reduced at the other, transferring electrons with each interaction. Due to the nanoscale gap, each pAP molecule rapidly diffuses between the electrodes and generates a >50fA/molecule current until it irreversibly degrades or escapes the sensor. The sensor area can be aggressively scaled because the redox current, to first order, does not depend on the lateral electrode dimensions. We fabricated transducers as small as 4×5μm^2, limited only by prototyping lithography tools available, with efforts towards 1μm^2 transducers.

Conventionally such pA-level currents are measured with a potentiostat that fixes the voltage at the electrodes while measuring the current with a transimpedance amplifier [4] or a ΣΔ modulator [5], neither of which are easily implemented in the density required for NGS. To overcome this, we utilized a little-known electrochemical technique – the "Coulostatic Discharge" method [6]. This technique uses the intrinsic capacitance of the sensor to convert the minute redox current into an easily measured voltage. When the nanogap transducer is immersed in an ionic solution, a double layer capacitance (C_{dl}) forms at the electrode-solution interface (Fig. 16.1.3). To detect the redox molecule concentration, the bottom electrode is reset to a voltage that reduces the redox molecules and subsequently floated while the top electrode is held at an oxidizing voltage. The redox molecules discharge the capacitance of the floating bottom electrode, with the initial discharge rate proportional to the number of redox molecules in the nanogap.

Because the small currents are converted to a voltage within each pixel, a simple per-transducer readout circuit can be used. Akin to a 3T APS CMOS image sensor, each pixel contains a reset switch, a row-select switch, and a source follower in a 1μm^2 area (Fig. 16.1.3). Thick gate devices (1.8V) are used for pixel transistors due to the gate leakage of thin gate devices and to increase headroom given the source follower's drop. Thick gate devices also relax ESD constraints for the post-processing steps required to construct the sensors.

Architecturally each array is arranged like an imager and the chip contains 8 arrays of 32×32 pixels for a total of 8,192 sensing sites. A row decoder selects one row of pixels to be measured with column-parallel ADCs and biasing circuits. The biasing circuitry comprises a bandgap reference and a programmable current source that is mirrored to bias each of the source followers. Control logic, memory, and serialization are implemented on-chip to facilitate readout of the acquired data. All circuits other than the pixels, including the ADCs, are implemented in thin gate devices with level translators between the row decoder and the pixels.

The per-column ADC array is implemented using a VCO-based quantizer (Fig. 16.1.4). The output of each 7-stage ring oscillator clocks a 9b up/down counter. The ring oscillator has a nominal oscillation frequency of 5.3GHz at 1.05V for a conversion time of 50ns. Every array has a reference ring oscillator which sets the conversion time to compensate for PVT variations; its input is a selectable reference voltage. A double counting technique mitigates errors such as pixel offset voltage, reset noise, incomplete charging of the nanogap, and 1/*f* noise. The first conversion occurs immediately after opening the discharge switch with the counter set to count up. A second conversion is performed after a fixed delay, this time counting down. Results are latched into memory and the next conversion occurs while the previous is read out.

Measured discharge curves at different concentrations of ferrocene carboxylic acid ranging from 0μM (buffer) to 100μM are shown in Fig 16.1.5. Buffer measurements taken before and after the serial dilution show the effectiveness of the washing procedure and demonstrate that there is minimal remnant signal. Discharge curves for a single cycle of each dNTP are also shown in Fig. 16.1.5. The incorporated dGTP shows a much faster discharge rate than the non-complementary nucleotides (14.8dB SNR). Note that while the entire discharge curve is shown for illustrative purposes, the low-noise sampling scheme only produces one point quantifying the initial discharge rate, which is proportional to the concentration of the redox molecule. This measurement can be repeated for improved SNR.

Scaling is an important requirement for massively parallel biosensing applications like NGS. Unlike ISFETs, the SNR for the nanogap detection scheme is independent of the lateral scale factor as noted in Fig. 16.1.6. Furthermore, circuit specifications are relaxed because the signal (the redox current integrated on the sensor capacitance) is relatively large and long-lasting. For a fixed SNR, this means that fewer signaling molecules are required than for ISFETs.

Circuits are implemented in a standard 32nm CMOS process, chosen based on availability, with no special devices other than thick-gate I/O transistors. Nanogap sensors are added using wafer scale post-processing. Electrical measurement data is summarized in Fig. 16.1.6. The measured leakage current at the sensing node is less than 10pA, limited by the reset switch channel length and the drain-to-body leakage. The average power consumption is 27.9mW at a nominal supply voltage of 1.05V.

In summary, we have demonstrated a highly scalable all-electronic approach towards DNA sequencing using CMOS readout electronics coupled with post-processed nanogap transducers. While this test chip demonstrated a small array of 8,192 pixels, a 25mm^2 chip could theoretically contain over 12 million pixels including I/O pads. Through careful architectural design choices and selection of a novel transduction scheme, we demonstrate that biosensing, such as DNA sequencing, can be performed on advanced CMOS process nodes.

References:

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 978-1-4673-9467-3/16/$31.00

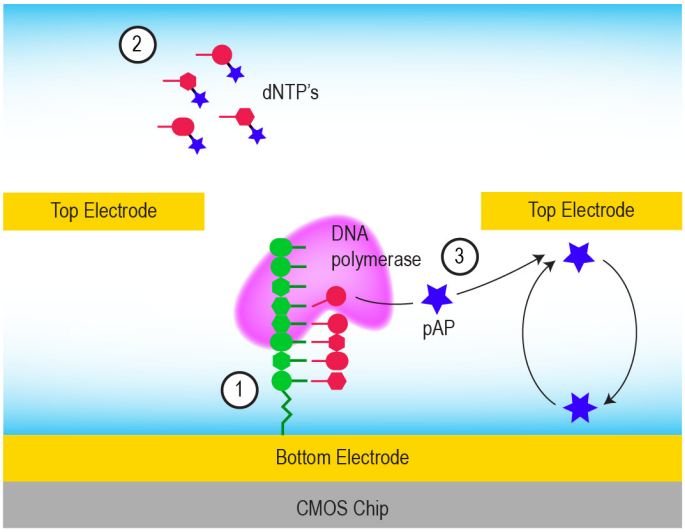


Figure 16.1.1: Overview of proposed DNA sequencing technique with nanogap sensor.

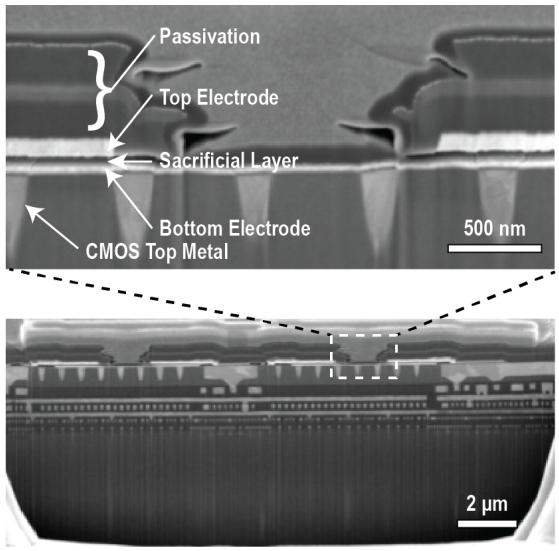


Figure 16.1.2: Cross-section of nanogap sensor integrated on CMOS.

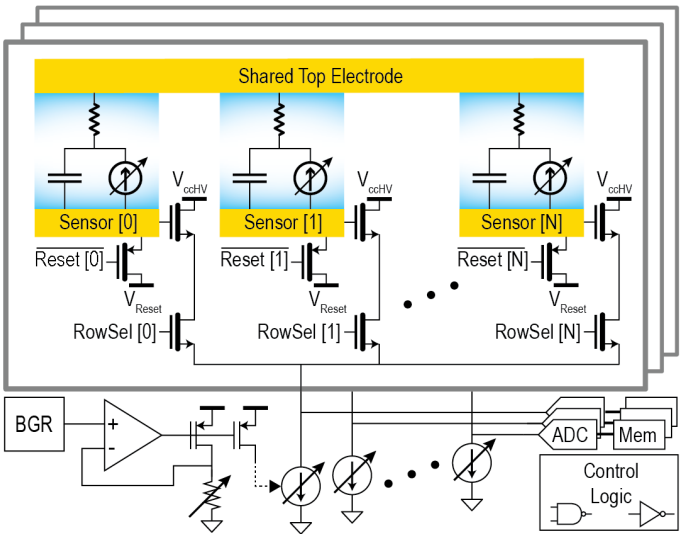


Figure 16.1.3: Chip architecture including sensor model.

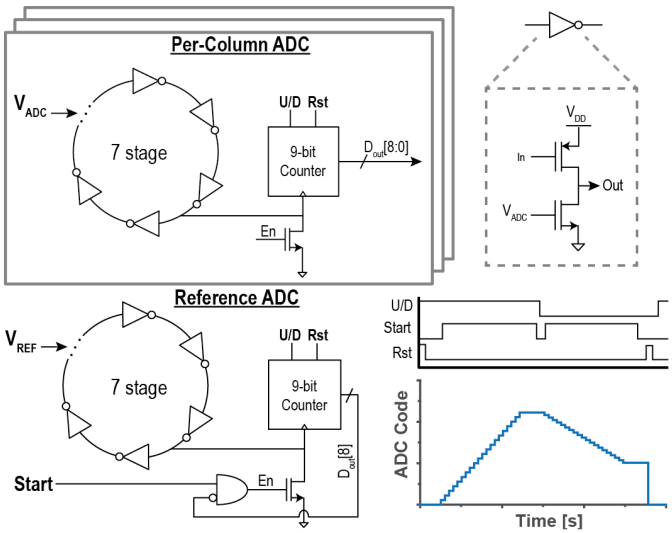


Figure 16.1.4: Schematic of VCO-based ADC and timing diagram.

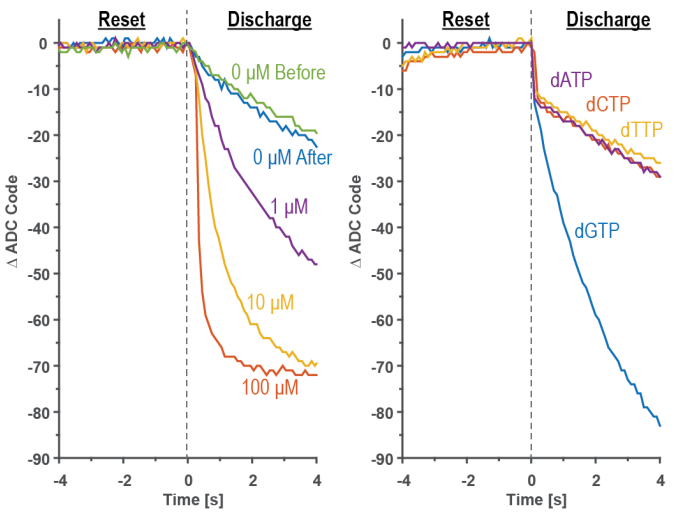
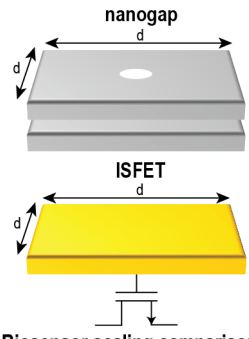


Figure 16.1.5: Measurement results for serial dilution (left) and dGTP incorporation event (right).



Biosensor scaling comparison

	ISFET	Nanogap
Frame Rate	>10 fps*	~1 fps
Noise Scaling	$1/d^2$	$1/d^2$
Signal Scaling	$1/d$	$1/d^2$
SNR	d	1

* Needed due to fast transient signal

System	
Technology	32 nm CMOS
Die size	5 mm x 5 mm
Number of pixels	8,192
Number of sensors	224
Power consumption	27.9 mW
Supply voltage	1.05 V / 1.8 V
Pixel	
Area	1 µm²
Leakage	< 10 pA
Sensor	
Area	20 µm²
Unit capacitance	~1 pF/µm²
Electrode spacing	60 nm
Signal/pAP molecule	~50 fA
ADC	
FSR	700 mV
Conversion time	50 ns
Resolution	8-bit

Figure 16.1.6: Summary of scaling comparison and chip performance.

16

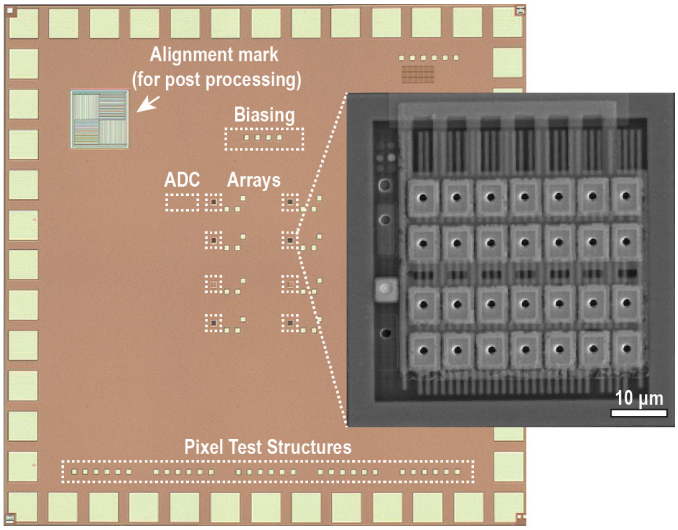


Figure 16.1.7: Die micrograph of CMOS chip with transducers on 32 nm CMOS.

978-1-4673-9467-3/16/$31.00