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Electrolyte Pulse Current Measurements by CvMOS with Microsecond and Thermal Voltage Resolution

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Abstract— **Non-invasive, charge-based sensing in chemoreceptive neuron MOS (CvMOS) transistors with extended floating-gate structure has brought forth features that are beneficial to the system integration of biological sensing. This paper presents the results of fast electrolytic signal detection on silicon dioxide, which advances possible technologies for rapid DNA discrimination or external monitoring of cell action potentials.**

I. Introduction

Non-invasive, integrated electronic detection of ion transport in biological systems has many promising applications including electrophoretic molecule recognition, entropic channels, fast DNA sequencing, and recording of cell action potentials [1-5]. The basic difficulties of electrical sensing lie in the signal-to-noise ratio, memory effects, and contamination isolation in the fluidic system. In contrast to sensing through a direct DC or AC current [6] such as the stripping potential method, CvMOS uses capacitive coupling between the sensing gate and the extended floating gate for transducing, which can potentially reduce invasiveness and help fluidic isolation. For demonstration of the operational principles and achievable sensitivity, SiO_2 is used on top of the polysilicon sensing gate with the understanding that the material selection can be further optimized.

Several efforts have probed the detection limit of oxide/electrolyte responses in an FET context, including pH small-signal response, shot noise, and cellular actions [7-12]. However, detection of low-level events on the order of microseconds has not been pursued in part due to the high level of noise in microfluidic integration. Prior work has established theoretical treatment for small signal pH variations and has calculated response time constants on the order of several tens of μs for silicon nitride [7]. A study of noise in the electrolytic system discusses theoretical perturbations on the order of 0.1 sec and above for pH-generalized faradaic and nonfaradaic systems [8, 9]. Low-stimulus signals for FET-gating from reference electrode voltage pulses through a conductive, electrolytic medium have been explored for compound semiconductor substrates, with an extrapolated potentiometric response of approximately 100 μV at 100 ms for cell action potentials of cardiac tissues [10]. Other efforts have explored cell action potentials on conventional CMOS technology with extended floating gate and flip-chip processing [11, 12]. In general, though, fast response in FET oxide sensors has referred to detection on the order of milliseconds, which has been sufficient for many applications [13].

One area that requires faster electrolytic detection and which, to date, has not used integrated, nonfaradaic sensors is rapid DNA discrimination [14]. This current active area of research focuses on monitoring the electrophoretic passage of a DNA strand through a protein ion channel in the bilayer lipid membrane (BLM) by detecting changes in background electrolyte concentration or electrolytic current. To improve robustness under the electrophoretic drive, recent advances have replaced the lipid membrane with a solid-state equivalent of silicon nitride, though DC electrodes are still necessary to perform electrolytic current measurements [15]. To realize the rapid DNA discrimination system, several criteria must be met, such as the resolution of 1-10 nucleotides/μs via changes in the electrolytic current, which is on the order of a few hundreds of pA by DC electrodes, or voltage pulses on the order of 0.1V in a GΩ environment. The external verification of such a system still has major challenges, because instrumentation hits bandwidth and sensitivity limits at such low signal levels.

In a step towards realizing an integrated system with generalized fast ion-transport detection, we report the detection of 25mV electrolytic signals on the order of 1μs on silicon dioxide by chemoreceptive neuron MOS transistor (CvMOS) fabricated by a commercial foundry process. CvMOS uses an extended floating-gate structure to facilitate time-resolved sensing by taking advantage of its features of high sensitivity, large bandwidth and ease of CMOS/microfluidic integration. From the CvMOS drain current, we can monitor the ion transport via electrolytic gating on the sensing gate. With improved engineering in fluid delivery and sensing gate surfaces, our approach shows strong promise for high bandwidth DNA discrimination on standard CMOS circuits.

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II. Device Design

The CvMOS structure reduces the invasiveness of ion transport detection while maintaining the advantages of high transconductance available from FET-based sensors [16-18] (Fig. 1). The inclusion of several sensing gates coupling with a single floating gate creates a neuron-like effect, analogous to several weighted inputs connecting to a single node. The CvMOS device was fabricated through a foundry service with the AMIS 1.5µm process [19]. Tests presented in this paper are performed on a sensing gate that has a native SiO_2 over the second polysilicon layer.

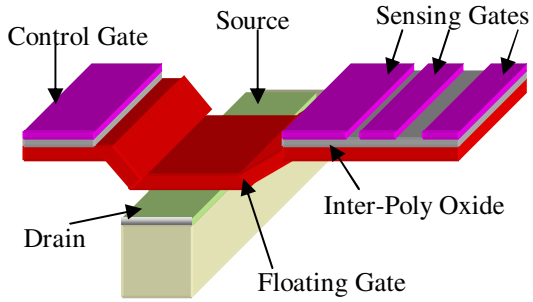

Figure 1. Schematic of a CvMOS transistor. The MOS area has similar fabrication to commercial Flash memory cells. Fluids are delivered to the sensing gates through superimposed microfluidics. Fabrication uses standard CMOS foundry processing and a post-processing overglass etch.

Notice that, with this device design, we can bias our sensing FET from the control gate independently of the sensing interface conditions. This represents a marked improvement over conventional ISFET-style sensing in which the device must be held in the saturation region via a fluid bulk potential [13].

The microfluidic delivery chamber (Fig. 2) involves a four-mask process [16]. A silicon-rich nitride membrane separates two silicon wafers bonded by PDMS. The nitride membrane houses the inlet and outlet pores, which deliver the fluid to the analyte chamber.

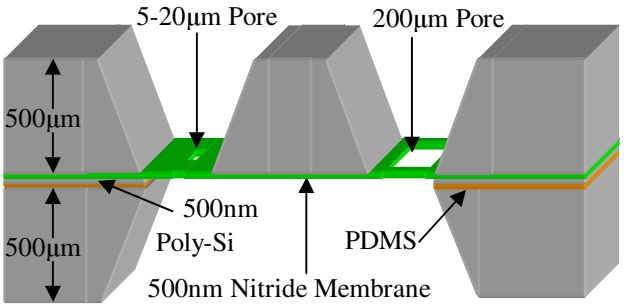

Figure 2. Physical design of a microfluidic delivery chamber. The design uses LPCVD, PDMS bonding, and wet/dry etching.

III. Experiment/ Procedures

Sensor surface treatment and microfluidic attachment was performed in a clean room. First, the entire MOSIS chip was exposed to a 10 min plasma clean at 29.6W in a Harrick Plasma Cleaner to remove organic contaminants. Then, the microfluidic chamber was sealed to the MOSIS chip. Contacts to the inlet and outlet chambers where made by sealing Teflon tubing directly to the chamber with PDMS.

A Pt electrode was inserted into the tubing approximately 1cm from the inlet chamber. The Teflon tubing from the exit chamber was connected with a waste beaker approximately 10cm away which held an Ag/AgCl electrode. All Pt voltages are measured against the Ag/AgCl electrode. All experiments were conducted in a PBS medium (phosphate-buffered saline). Resistance between the Ag/AgCl electrode and Pt electrode was measured at less than 1MΩ±0.2MΩ. The sensing surface sits along this PBS microfluidic path.

To create the electrolytic perturbations, square voltage pulses of varying duration from a function generator (HP 8011A) were applied to either the Pt or Ag/AgCl electrode. DC device characteristics were monitored and sourced by an HP4155 semiconductor parameter analyzer. Electrolyte and drain current signatures were alternately monitored by a Transimpedance Amplifier (Stanford Research 570), which performs I-V conversion. Voltage pulses and transduced electrolytic or drain current responses were measured by an oscilloscope (Tektronix TDS 340A) sampling at 2Msamples/sec. In the graphs below, all phase data has been preserved. Occasionally, the 64-sample averaging function of the oscilloscope was employed to pull a signal out of the inherent noise, but this will be clearly noted. Consequently we have bandwidth limitations from the TIA at 1MHz with minimum current levels on the order of 100s of nA. This limits reliable detection to only 1µs, but nevertheless our results show clear signals at that current level. Additionally, we reach the lower end of the source capabilities for the function generator. In the future, better measurement instrumentation may increase the sensitivity of these tests, and the TIA can be directly implemented on the CMOS circuits together with CvMOS.

The CvMOS sensors were biased in deep saturation in all cases (V_S=GND, V_G=2.5V, V_D=5V). Values for drain current were captured with a conversion factor of 1µA/V. Electrolytic currents also use the same conversion factor. All pulses use a 200µs period, with pulse widths varying from 100 µs to 1 µs at 250mV amplitude. The Pt electrode sits much closer to the sensing area than the Ag/AgCl electrode. When a voltage is applied, it is dropped across the length of the path. For a voltage applied from the Ag/AgCl electrode, the magnitude has dropped by approximately one order of magnitude by the time it reaches the sensor. Because the current integrated design has the bulk tied to the source, we initially observed some signal degradation due to substrate loss. A configuration was adapted in which a pull-up resistor was placed between the bulk and source. Future designs will allow for independent

substrate bias.

IV. Results

In Figs. 3-6, electrolytic and drain current data are purposefully offset for easier viewing. In Fig. 3, a 5μs pulse from the Pt electrode is included to demonstrate that electrolytic and drain currents have sufficient clarity in real time, though averaging does improve the readout. Averaging is not realistic in real-time applications, but signal processing based on the history convolution can cancel apparent noise similar to averaging. The electrolytic current is measured over a distance of ~15cm, which heavily contributes to the large noise observed. Future designs will include a shorter distance to monitor the electrolytic current, although this is only for control experiment purposes, since the intended readout is at the CvMOS drain current.

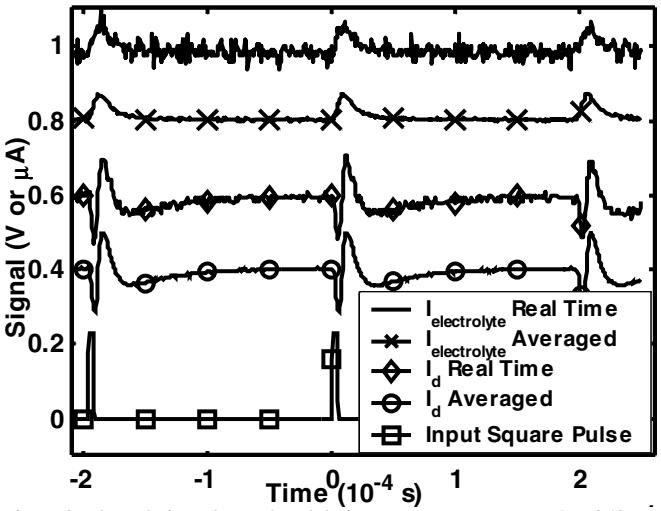

Figure 3. Electrolytic and transduced drain current responses to a 5μs, 250mV voltage pulse from the Pt electrode, which sits ~1cm from the sensing surface.

As the pulse width is shortened to 1 μs from the Pt electrode, we approach the bandwidth limitations of the measurement setup (Fig. 4). At this pulse width, noise starts to overcome the electrolytic signal, but the drain signal still has clarity in real time. When a 1 μs pulse occurs at the Ag/AgCl electrode and the electrolytic voltage has dropped by an order of magnitude at the sensing surface, and noise starts to dominate the drain current as well (Fig. 5). However, signal processing or averaging can obtain the clear signal to tens of nA in the electrolyte at approximately a thermal voltage potential (Fig. 6).

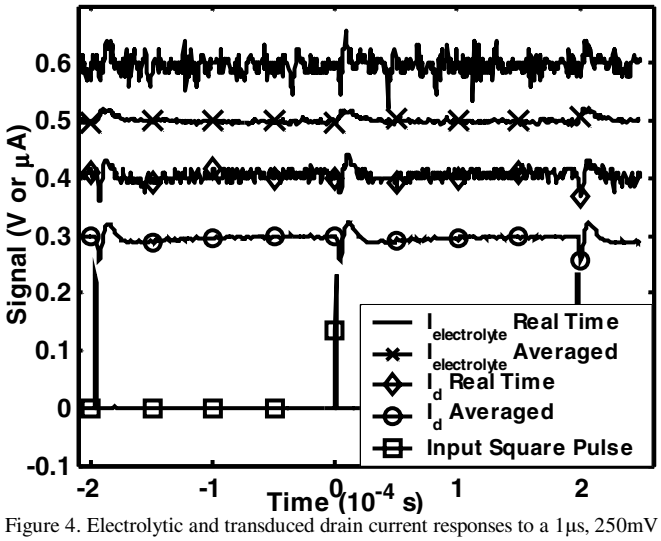

Figure 4. Electrolytic and transduced drain current responses to a 1μs, 250mV voltage pulse from the Pt electrode, which sits ~1cm from the sensing surface.

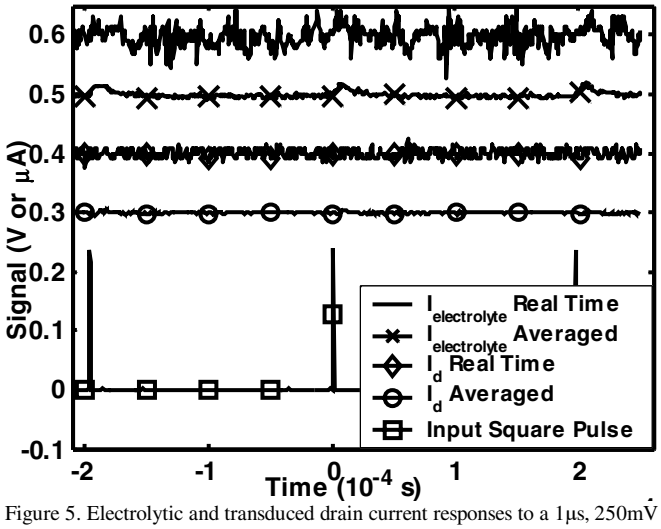

Figure 5. Electrolytic and transduced drain current responses to a 1μs, 250mV voltage pulse from the Ag/AgCl electrode, which sits ~15cm from the sensing surface. The voltage pulse magnitude has dropped by at least an order of magnitude by the time it reaches the sensing surface.

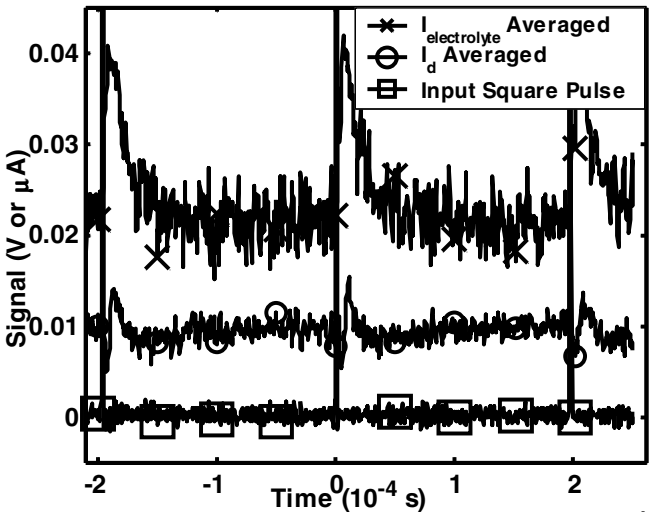

Figure 6. A magnified and normalized depiction of Figure 5. Here, we sense the response to a 1 μs signal at effectively 25mV, which corresponds to an electrolytic current on the order of tens of nA, as seen in the plot. As the signal

becomes faster and lower, we approach bandwidth limitations of the present measurement setup.

V. Discussions

In this experiment, we can ignore the rate at which an oxide surface can protonate or de-protonate according to changes in pH, since we use a pH buffered solution. However, the surface can respond with sufficient frequency at small-signal pH changes. Here we must consider the perturbation of the layers set up at the oxide surface. Corrections for adsorption do not significantly impact oxide since they are less sensitive to ionic adsorption. Therefore, a standard Gouy-Chapmen-Stern (GCS) model [20] can be built to describe the oxide/electrolyte interface, which can change from protonated to neutral to deprotonated. The GCS surface model can explain the high pH sensitivity. The charge layer by ions on oxide can respond to perturbations at roughly THz frequencies [21], since it involves ion movement within a few nanometers and not chemical reactions. The diffusive layer at the concentration of PBS used is less than 10 angstroms. When the sensing gate area is scaled further, number fluctuation of ions on oxide can cause additional noises, but this will be investigated in the future study.

The inverted pulse shape from the ionic system response, which is more clearly shown by drain current output, has been observed elsewhere in a different setup [15]. It is rather distinct from random waveforms and can be explored for further sensitivity. The pulse shape may arise from ionic waves in the system generated by impulse responses and be damped out quickly by the resistive components. Alternatively, it may result from the pull-up resistor configuration used in our measurement to separate the source and bulk terminals, though initial SPICE modeling makes this possibility questionable.

VI. Conclusions

The extended floating gate structure of CvMOS allows for fast electrolytic current sensing. In a step towards integrated rapid DNA discrimination, we have demonstrated that a non-optimized CvMOS design can detect 10s of nA current at 1µs and 25mV, at which point our present instrumentation limit was reached. System requirements for rapid DNA sequencing include 0.1µs-1µs detection for 100s of pA at 100s of mV. With optimization and further characterization, a fully CMOS-compatible DNA sequencer can be likely realized.

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