CMOS interface with biological molecules and cells

— Invited review paper —

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***Abstract*—CMOS technology and its Moore's Law scaling is an enormously successful technology paradigm that has continued to transform our computation and communication abilities. Outside the applications in computation and communication, CMOS technology has been increasingly applied to the life sciences, with a wealth of silicon integrated circuits developed to interface with biological molecules and cells. Concretely, large-scale arrays of active electrodes are integrated using CMOS technology for highly parallel electronic detection of biomolecular/ionic charges and cellular potentials for DNA sequencing, molecular diagnostics, and electrophysiology. Parallelism enabled by CMOS scalability is well suited to process the big data in these biotechnological applications. Here we offer a brief review on these CMOS-bio interfaces, while the corresponding presentation will focus on a sub-topic of CMOS electrophysiology with mammalian neurons.**

Keywords*—*Ion-sensitive field-effect transistor (ISFET); microelectrode array (MEA); nano-bio interface; molecular diagnostics; DNA array; DNA sequencing; electrophysiology; CMOS integrated circuit

I. CMOS Interface with Molecules and Cells

CMOS technology has been one of the most enabling technology paradigms of our time and has continued to revolutionize our computation and communication abilities over the past several decades. The Moore's Law scaling to decrease the transistor size and to pack more transistors for increased computational capacity is the central feature of the CMOS revolution. The CMOS scaling has now reached a gate length of 5 nm. This astounding advance is matched with the emerging computation and communication applications that demand ultrahigh density signal processing, such as neural network computation for deep learning and modem tasks for 5G Communication.

Outside these computation and communication applications, there has been a rising biotechnological interest to apply CMOS technology to the life sciences by directly interfacing silicon integrated circuits (ICs) with biological molecules and cells. Such an IC typically presents an array of thousands to millions of surface electrodes operated by underlying active circuits for highly parallel electronic detection of biomolecular / ionic charges or cellular potentials for applications in DNA sequencing, molecular diagnostics, and electrophysiology. Massive parallelism the CMOS scalability enables is well suited to processing the big data in these biotechnological applications.

In this paper, we provide a brief review on these CMOS-bio interfaces, while the corresponding presentation will focus in details on a sub-topic of CMOS interfaces with mammalian neurons for neuro electrophysiology, going beyond what is reviewed in this paper.

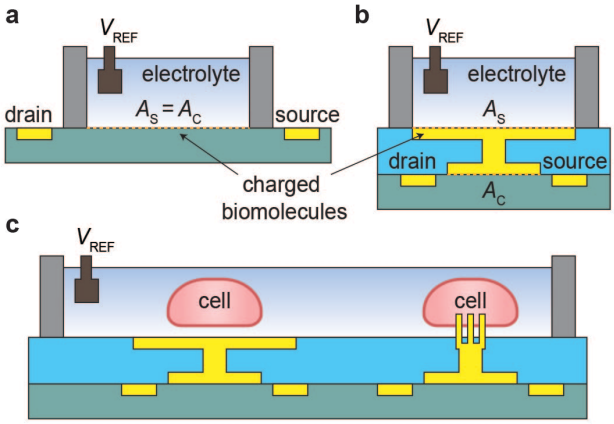

Figure 1. (a,b) Traditional ion-sensitive field-effect transistor (ISFET) and floating-gate ISFET for biomolecular / ionic charge detection. (c) Planar microelectrode (left) and vertical nanoelectrode (right) connected to underlying FETs to record membrane potentials of electroactive cells.

II. CMOS Floating-Gate ISFET Array for Biomolecular / Ionic Charge Sensing

The outposts of the CMOS IC that interface with biomolecules are metallic electrodes that act as floating gates for the underlying transistors (Fig. 1b). In this floating-gate ion-sensitive field-effect transistor (ISFET), the electrolytic solution gates the electrode, and in turn the electrode gates the transistor channel. This floating-gate ISFET is distinguished from the traditional ISFET, whose channel is directly gated by the solution (Fig. 1a) [1][2]. In the traditional ISFET, when biomolecules (*e.g.*, DNA or proteins) or ions (*e.g.*, H_3O^+) bind to the suitably treated surface of the transistor channel, their charges (*e.g.*, negative charge of DNA or protons in H_3O^+) alter the threshold voltage of the transistor. This alteration then manifests as the measurable change in the transistor channel current at a given source-drain bias. In this traditional ISFET, the sensing area, A_S, where the biomolecules or ions can adhere to, is equal to the transistor channel area, A_C.

On the other hand, in the floating-gate ISFET (Fig. 1b), the biomolecular or ionic charges at the top surface of the floating gate facing the solution create image charges at the bottom surface of the floating gate facing the transistor channel. These

image charges at the floating gate's bottom are equal in magnitude to the biomolecular/ionic charges at the floating gate's top due to charge conservation. Therefore, if the sensing area A_S (the area of the top surface of the floating gate) is made larger than the transistor channel area A_C (identical to the area of the bottom surface of the floating gate), a higher charge density can be created in the transistor channel for a given biomolecule/ionic charge density at the electrolyte interface, resulting in a higher sensitivity for charge detection.

The floating-gate ISFET array integrated in a CMOS chip can be used as an all-electronic DNA (or protein) microarray [3], detecting the inherent charges of these biomolecules in the solution in a highly parallel manner. Concretely, the ISFET DNA microarray operates by detecting the charges of the target single-stranded DNA (ssDNA) hybridized to ssDNA of complementary sequence immobilized to the floating gate of an individual ISFET in the array. In comparison to the optical microscopy that is currently the dominant technological basis for commercial DNA microarray, this all-electronic ISFET DNA microarray has the advantage of real-time operation and label-free sensing.

The floating-gate ISFET can be also used to measure pH by quantitatively detecting the amount of H_3O^+ (proton) charges in the solution [4] (the traditional ISFET was originally invented to measure pH [1][2]). The start-up Ion Torrent—now part of Thermo-Fisher—commercialized the CMOS floating-gate ISFET array as an all-electronic DNA sequencer (not to be confused with the DNA microarray), and this remarkable advance is indeed based on the ability of the floating-gate ISFET to measure pH [5]. Concretely, the Ion Torrent's CMOS chip contains over 1 million ISFETs, with each ISFET lying in a post-fabricated microfluidic well. The DNA to be sequenced is fragmented and converted to ssDNA, fixed on micrometer scale beads, and loaded into the wells. For the sequencing, the 4 nucleotides A, G, T, and C are repeatedly added to the solution, and if complementary to the next available location on the ssDNA, they bind, releasing a proton that causes a change of the pH in the well which is measured by the ISFET. In this way, the Ion Torrent's floating-gate ISFET array offers an all-electronic chip-scale method for DNA sequencing.

III. CMOS Electrode Array for Electrophysiology

The interface to measure the membrane potentials of electroactive cells such as cardiomyocytes and neurons in a CMOS chip is again a surface metallic electrode gating an underlying transistor (Fig. 1c). The left of Fig. 1c shows a planar microelectrode, which measures the change in the membrane potential from outside a cell. The sensitivity of this extracellular recording is limited, because the signal attenuates coming through the cell membrane (Fig. 1c, left). While the low signal-to-noise ratio (SNR) is a disadvantage, the extracellular recording has the advantage of not being invasive to the cell being recorded, and so it can sustain the recording for many days. A great variety of such extracellular microelectrode arrays (MEAs) have been developed on CMOS ICs, now containing as many as tens of thousands of microelectrodes with thousands of underlying active

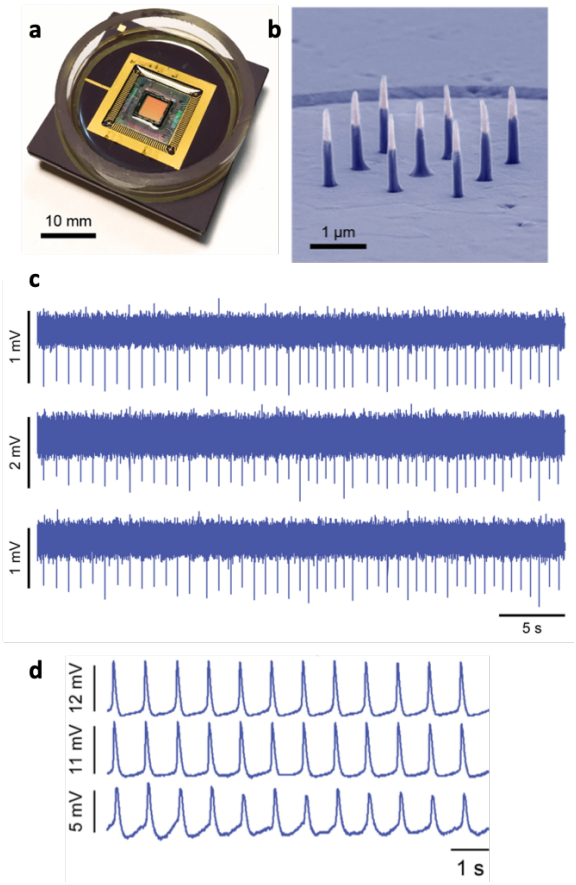

Figure 2. (a) Packaged CMOS vertical nanoelectrode array with ~1,000 recording sites [14]. (b) Each site presents 9 post-fabricated vertical nanoelectrodes. (Pt). Each electrode, coated by SiO_2 except tip, has a tip diameter of ~100 nm and a height of 3 µm. (c) Extracellular recording of cardiomyocytes from 3 example sites before membrane permeabilization: action potentials are distorted and plagued with noise. (d) Intracellular recording of cardiomyocytes from 3 example sites after membrane permeabilization. SNR improves significnatly and signal is not distorted. Intracellular recording from up to 235 cardiomyocytes in parallel was achieved.

channels to record action potentials from a large number of neurons and cardiomyocytes [6]-[13].

Recently reporting in the journal *Nature Nanotechnology* in 2017 [14], on a CMOS chip, we modified the electrode morphology into a vertical shape with the electrode diameter at the nanometer scale (Fig. 1c, right; Fig. 2b; see also Ref. [15]). We demonstrated there that this vertical nanoelectrode operated by the underlying active circuits in the CMOS chip is capable of attaining intracellular access to cardiac cells [14]. The cell membrane wraps around the vertical electrodes forming a tight seal. Then, a voltage applied through the nanoelectrode locally permeabilizes the cellular membrane, allowing the intracellular solution to make a contact with the electrode, without leakage to the extracellular solution due to the tight seal. In this way, the vertical nanoelectrode achieves intracellular access, improving the recording sensitivity by orders of magnitude as compared to the extracellular recording. The vertical nanoelectrodes are post-fabricated on the CMOS chip using the standard top-down process.

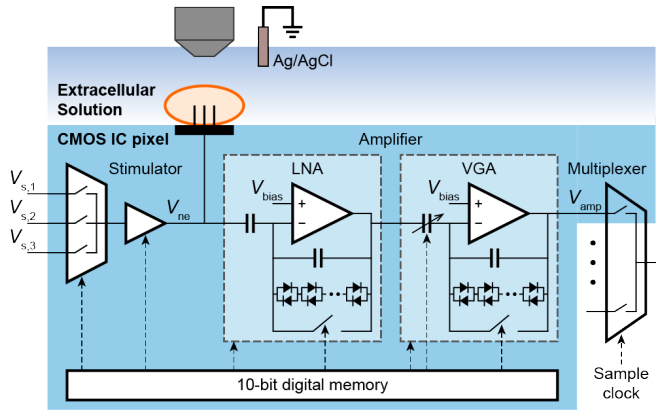

Figure 3. Circuit schematic for each array site of the CMOS vertical nanoelectrode array chip of Fig. 2 [14].

Figure 2a shows our packaged CMOS chip that implements the vertical nanoelectrodes into an array [14]. It contains 1,000+ recording sites, with each site including an amplifier for recording, a stimulator for the membrane permeabilization, and a 10-bit digital memory (Fig. 3). At each site, the amplifier and stimulator are both connected to a metallic pad right above on the IC surface. 9 vertical nanoelectrodes are post fabricated in the center region of each pad (Fig. 2b), and are addressed together electrically. The platform successfully performed extracellular and intracellular recording of cardiomyocytes (Figs. 2c and 2d), cultured *in vitro* on the chip. As we permeabilize the cellular membrane, the recording transitions from extracellular (Fig. 2c) to intracellular (Fig. 2d). The change in the action potential shape and the improvement in the SNR is evident. Importantly, this CMOS vertical nanoelectrode array was able to intracellularly record from up to 235 cardiomyocytes in parallel. Such parallelization of intracellular recording was greatly sought after but unprecedented before this work. Although not successful for neurons, at least for cardiomyocytes, we were able to make this advance of parallelization of intracellular recording by marrying the nanometer scale vertical nanoelectrodes and the scalability of CMOS technology. This network-level intracellular recording was then used to study with high precision the network dynamics of the cardiomyocyte tissue and its modulation with pharmaceutical drugs.

IV. Circuit Design

The integrated circuits underlying the front-end floating gates or electrodes are also critical in these electronic interfaces with biomolecules and cells for a number of reasons. First of all, with the large array size typically ranging from 10^3 to 10^6, it is virtually impossible to wire all electrodes or floating gates separately to an off-chip interface [6]. On-site multiplexing with the underlying CMOS circuits solves this issue. With the multiplexing, the array can be read block by block with a fast enough clock, thus lowering the total number of input/output interconnects by orders of magnitude.

Second, it is desirable to be able to control each array site individually. For example, in the case of floating-gate ISFET array to measure local pH, due to device-to-device variability, each ISFET must be calibrated individually and be biased with its own bias parameters according to that calibration. In the case of the CMOS nanoelectrode array for intracellular electrophysiology, for membrane permeabilization, different magnitudes of stimulation voltages (or currents) are required at different sites because the electrode-to-cell coupling varies from site to site. Having an *in situ* memory at each array site enables an individual site programming, allowing for versatile and real-time control of all individual sites across the array. Without such individual site memories, one would have to use global control signals from off-chip electronics shared by all sites, which results in limited functionality and controllability.

Third, the front-end signal as a response to the biomolecular or ionic charge or the cellular membrane potential can be very small, necessitating *in situ* amplification at every array site on chip. With an off-chip amplifier, the front-end signal can substantially leak through the parasitic capacitance of the long signal path before amplification. In contrast, *in situ* amplification at each array site greatly shortens the signal path, hence minimizing unnecessary attenuation. This *in-situ* amplification is already implied in Fig. 1: as the electrodes are already acting as the gates of the underlying transistors, these transistors typically serve as the input transistors to the overall site amplifiers.

Fourth, in these CMOS-bio interface applications, it is important to maintain the biological compatibility (*e.g.*, cell viability) by regulating the local temperature of the chip within a target range. To this end, the CMOS integrated circuit can contain distributed temperature sensors on chip (*e.g.*, *pn*-junction based temperature sensors) as well as heaters (*e.g.*, poly silicon resistors) in a negative feedback loop.

The design of the integrated circuits for the CMOS-bio interface presents quite a unique set of challenges. To help appreciate these design issues, we here briefly discuss the design of the site amplifier for the intracellular electrophysiology chip (Figs. 2 and 3). First, the spectral contents of the electrophysiological signals such as action potentials and sub-threshold signals typically range from ~ 1 Hz to several kHz. This is the spectral region where the MOS transistor's $1/f$ noise is substantial. Therefore, it is critical to achieve low noise in the first-stage amplifier that directly interfaces with the electrodes, for the first-stage amplifier is the most critical building block in determining the sensitivity of the overall recording chain. Second, to suppress the significant low-frequency fluctuation associated with the electrolyte yet to filter in all spectral contents of the electrophysiological signal, the amplifier should have the bandpass filter character with the low-frequency cutoff near ~1 Hz. To obtain the bandpass characteristic, the amplifier can be constructed from an op-amp with a parallel *RC* filter in the negative feedback loop, but the low-frequency cutoff around ~1 Hz mandates the resistance R to be on the order of 10^{12} Ω because the capacitance C cannot be made arbitrarily large due to such design considerations as gain and noise optimization. Since it is practically impossible to obtain such a large resistance with standard resistive materials in CMOS technology, active pseudo resistance such as reversed biased *pn*-junction diodes at zero *DC* current or MOSFET-based active resistance circuit

should be employed. Such active resistors introduce a complex design tradeoff entailing nonlinearity, which the design should take into account. Third, due to the variability with the cell-electrode intracellular coupling, different array sites need a different gain for the linearity control. To this end, each site amplifier requires its own optimized gain value.

V. Towards High-Fideltiy CMOS Neuro Electrophyiology

Our CMOS vertical nanoelectrode array (Fig. 2) [14] demonstrated highly parallel intracellular recording from hundreds of mammalian cardiomyocytes. This was the first step towards high-fidelity (intracellular) investigations of complex electrogenic cellular networks, but the chip of Fig. 2 was largely unsuccessful in coupling with mammalian neurons. One natural next step thus will be to significantly improve the electrode and CMOS circuit design to the next level to achieve parallel intracellular recording for mammalian neuronal networks, a direction we have already taken.

Such massively parallel, intracellular recording of mammalian neurons across a network has been greatly sought after in neurobiology, but has not been achieved. For example, the CMOS MEA [6]-[13] possesses the massive parallelism in neuronal recording, but it can only perform extracellular recording that has a substantially low sensitivity. The intracellular recording by the patch clamp boasts unrivaled sensitivity, but this bulky glass electrode cannot be scaled into a dense array, and thus far only ~10 parallel patch recordings have been possible. Optical methods, based on voltage-sensitive dyes/proteins, have been developed in hopes of parallelizing intracellular recording, but they have not been able to perform recording from more than ~30 neurons in parallel.

The proposed advance in the massively parallel intracellular neuronal recording, if achieved, can open up many new exciting possibilities such as functional connectome mapping, high-throughput electrophysiological screening of drugs, and copying biological neuronal network for machine intelligence.

VI. Acknowledgement

We thank Samsung Electronics (A37734), the Catalyst Foundation, Valhalla, New York, the US Army Research Office (W911NF-15-1-0565), the National Institutes of Health (1-U01-MH105960-01), the Gordon and Betty Moore Foundation, and the US Army Research Laboratory and Army Research Office (W911NF1510548) for their support.

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