Interfacing CMOS Electronics to Biological Systems: from Single Molecules to Cellular Communities

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***Abstract*—Direct electronic interfaces between biological systems and solid-state devices offer considerable advantages over traditional optical interfaces by reducing system costs and affording increased signal levels. Integrating sensor transduction onto a complementary metal-oxide-semiconductor (CMOS) chip provides further advantages by enabling reduction of parasitics and improved sensor density. We present two sensing platforms that demonstrate the range of capabilities of CMOS-based bioelectronics. The first platform electrochemically images signaling molecules in multicellular communities, while the second focuses on single-molecule, high-bandwidth sensing using carbon nanotube field-effect transistors.**

Keywords—Biosensor; CMOS; Redox; smFET; Biofilm; Single-Molecule; Electrochemistry; Carbon Nanotube

I. Introduction

From the imaging of multicellular organisms to the study of individual molecules, the most popular experimental techniques are based on light microscopy. Light imaging can be advantageous because it is orthogonal to most biophysical processes, allowing non-invasive interrogation. By the same measure, photons do not naturally interface with most biological functions. With notable exceptions, living systems rely on chemical, mechanical, and electrical interactions rather than optical phenomena, although living systems can be genetically engineered to respond to light [1].

Non-optical pathways can offer considerable advantages in interfacing directly to biological and biomolecular systems without the need for labeling or genetic engineering. Detection based on ionic or electronic transport can yield much larger signals than those produced from typical fluorophores, allowing single-molecule levels of sensitivity to be achieved at high measurement bandwidths. Issues of isolating signals from background are often more pronounced, however, and although some electrochemical signatures can be detected directly, electronic approaches often rely on enzymes or surface-bound chemical probes to achieve specificity.

Systems designed to detect relatively large ensembles of analyte molecules generally utilize classical electrochemical techniques which directly measure charge transfer at exposed electrodes. Some of the many techniques that have been combined with CMOS microelectronics include voltammetry [2], impedance spectroscopy [3], and chronoamperometry [4]. In these techniques, each analyte molecule present typically yields fewer than one electron collected by the electronics.

Other applications rely on transducers that deliver gain, in which the presence or transport of one molecule produces many measurable electrons. One class of transducers uses molecular charge to modulate transport in active electronic channels in a solid-state device; these include exposed and buried-gate ion sensitive field effect transistors (ISFETs) and the single-molecule field-effect transistors (smFETs) [5] presented here. Other techniques employ nanoscale ion channels in which each target molecule modulates the flow of many mobile dissolved ions which are then converted to electrons at metallic electrodes [6].

The performance of each class of transducer is maximized when its receiving amplifier is properly matched to the anticipated signal. CMOS integration brings two major advantages: the ability to integrate many transducers and amplifiers in a dense array and reduction in parasitic capacitance between the transducers and amplifiers. Applications that require the highest sensitivity require gain at the transducer and/or low-noise electronics for subsequent amplification with more chip area dedicated to larger low-noise amplifiers. Recovering array density requires time-multiplexing approaches, such as those employed in CMOS imagers.

In this paper, we describe two CMOS bioelectronic sensing platforms that exemplify many of these design considerations. In Section II, we discuss a recent implementation of an arrayed platform for electrochemical analysis of redox-active signaling molecules produced by bacterial colonies [7]. This is an example of a low-gain transducer, ensemble measurement, and relatively modest requirements on the CMOS amplifier. In Section III, we describe ongoing work integrating smFETs onto a CMOS array. In this case, single-molecule levels of sensitivity at high bandwidths are feasible by combining high transducer gain with low-noise amplification. Section IV offers some conclusions and discussion of future trends.

II. Integrated Electrochemical Sensor Array for Imaging of Metabolites in Colony Biofilms

A. Background

Our representative ensemble-sensing CMOS platform is one that electrochemically images redox-active metabolites

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produced by the opportunistic pathogen *Pseudomonas aeruginosa* by directly placing a biofilm in contact with the chip surface [7]. These metabolites, called phenazines, have significant effects on community behavior in colony biofilms grown for several days on the surface of an agar plate [8-10]. Imaging phenazine production in *P. aerugionsa* biofilms lends insight into the biochemical basis for development in multicellular communities.

Competing techniques have many limitations. Two-photon excitation microscopy (TPEM) has been used for detection of phenazines in homogenous cultures [11] but suffers from poor sensitivity and specificity of detection, which has prevented application of TPEM to imaging of biofilms. Phenazines, like many metabolites, are redox-active, and a few studies have employed electrochemical techniques for detecting and quantifying phenazines produced by P. aeruginosa PA14 liquid cultures [12-15]. Scanning electrochemical microscopy (SECM) has been used to study phenazines at the surface of biofilms in a spatially resolved manner [16], but due to the lack of parallel electrodes, SECM cannot use potential sweep techniques within a reasonable frame rate, and, therefore, SECM cannot quantify concentration or simultaneously detect multiple redox-active species.

The current iteration of our CMOS electrochemical imaging array is a 5 mm x 5 mm chip that has 60 electrodes connected to 5 parallel transimpedance amplifiers, while future work will use a new chip which is 1 cm x 1 cm and features more than 1800 electrodes connected to 38 parallel amplifiers. By placing a colony biofilm on the chip, potential sweep experiments at many electrodes can be performed in parallel, and the electrochemically measured concentrations of multiple phenazines at individual electrodes in the array can serve as a spatially resolved image of phenazine production by the colony. Because colony biofilms produce large phenazine concentrations (> 1 μM) in experiments with this platform, no gain is required in the transducer. Phenazines can be detected through their direct oxidation and reduction at the working electrodes using conventional analytical electrochemical methods.

Interfacing a colony with a chip is challenging because placing the chip in direct contact with the top of the colony disturbs colony morphology. One solution is to instead access phenazines from the bottom of the colony by interposing between the colony and the integrated circuit a layer of agar through which phenazines diffuse and are detected (Fig. 1). The on-chip agar layer is insufficiently thick for promoting colony growth, so colonies must first be grown on porous membranes positioned on top of >5-mm thick agar layers. These membranes have a pore size of 0.4 μm, which allows passage of nutrients and phenazines and does not interfere with colony development, while also facilitating later transfer of colonies to the on-chip agar layers. A benefit of this solution is that the on-chip agar layer permits insertion of an external platinum counter electrode and silver-silver chloride quasireference electrode. However, a major drawback of the on-chip agar layer is lateral diffusion of phenazines as they traverse the thickness of the agar from the colony to the electrodes, which degrades spatial resolution. A preferable method involves eliminating the agar layer and relying on moisture between the track-etched membrane and the electrodes to serve as the interface. In this case, the counter and reference electrodes must be integrated on the chip surface.

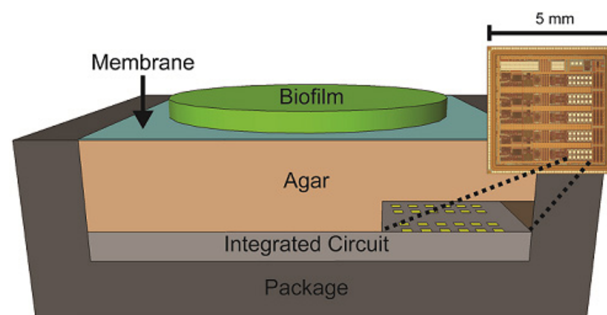

Fig. 1. Schematic of the experimental setup for electrochemical imaging of biofilms.

B. Circuit Design

The electrochemical method used in these ensemble measurements is square-wave voltammetry. In square-wave voltammetry, the frequency of the input voltage signal is typically in the 1-500 Hz range, significantly relaxing noise and bandwidth requirements in the electronics. The more important concerns in the design of the electronics include dynamic range and amplifier stability in the face of varying electrochemical interfaces. Each transimpedance amplifier (TIA) is constructed from a single-ended folded-cascode operational transconductance amplifier (OTA), a programmable compensation network, and a programmable resistor bank in the feedback loop. These feedback resistances allow the conversion electronics to handle currents in the range of a few nanoamperes to a few microamperes. The compensation network ensures stability across different feedback resistance and solution resistance values. The control amplifier is constructed from a two-stage operational amplifier consisting of a dual-input, folded-cascode first stage followed by a common-source second stage, along with a programmable RC-compensation network between the two stages.

C. Post-processing

The post-processing required for the chip is relatively straightforward. The only necessary modification is replacement of the aluminum electrodes (the top metal of the CMOS process) with gold for electrolytic compatibility. This can be accomplished by photolithographically defining openings in photoresist above the aluminum electrodes, removing the exposed aluminum with an etchant, electron-beam evaporating gold (with a titanium adhesion layer) onto the chip, and lifting off photoresist and gold from non-electrode areas.

III. INTEGRATED CARBON NANOTUBE SENSOR ARRAY FOR SINGLE-MOLECULE STUDIES

A. Background

Many applications require moving beyond ensemble detection towards techniques with single molecule sensitivity. Such techniques build on advances in nanoscale fabrication and nanomaterials that have enabled the fabrication of solid-state transducers with dimensions rapidly approaching the size

of many molecules of interest. In particular, single-walled carbon nanotubes (CNTs) inherently possess diameters on the order of nanometers. In an exposed-channel configuration, the reduced-dimensional conducting channels in CNTs are sensitive to single electronic charges.

Typical CNT sensors are structured as four-terminal devices (Fig. 2a). Source and drain contacts provide the electric potential across the nanotube to generate a current. A backgate, often in the form of a conducting layer buried underneath the device, can be used to electrostatically modulate charge density within the CNT, providing control of the current through the device. When functioning as a sensor, the surface of the device is exposed to an electrolyte, the potential of which can be controlled by means of an Ag/AgCl reference electrode or a platinum pseudoreference electrode. The electrolyte acts as a fourth terminal, strongly coupling the solution potential to the CNT thereby providing very sensitive control of the device operating point.

Carbon nanotubes can be functionalized at a single point, confining transduction to charge fluctuations around a localized region. Using this approach, the carbon nanotube can be used as an smFET. smFET devices are capable of providing detailed information on molecular kinetics not available from ensemble platforms.

Two techniques have been used to create smFET devices. One method uses electrochemical oxidation to break a lattice bond of the CNT [17]. Disturbing the lattice structure creates a conduction barrier that localizes the carbon nanotube sensitivity. A carboxylic acid functional group can be chemically introduced at this point defect. Probe molecules can then be coupled to the carboxylic acid, thereby providing sensor specificity. This approach has been used to study DNA hybridization kinetics [5]. An alternative functionalization method utilizes a pyrene molecule. Pyrene binds to the sidewall of a CNT by means of pi-pi stacking. A functional group on the pyrene, such as a maleimide linker, may then be used to couple a molecule of interest to the pyrene. Pyrene functionalization has been used to study enzymatic activity of lysozyme variants [18] and DNA polymerase [19].

In this work we describe the integration of smFET devices onto a 5 mm x 5 mm CMOS chip (Fig. 2b). By doing so, parasitics can be minimized, allowing the sensors to operate with improved temporal resolution. Additionally, integration improves sensor density, providing the ability to create a large array of sensing sites.

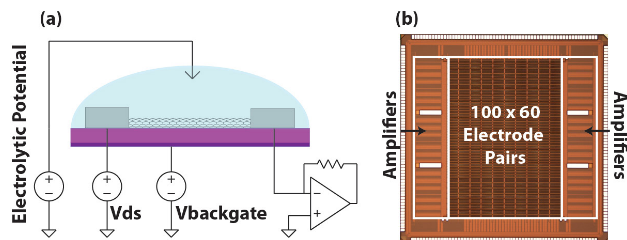

Fig. 2. (a) Schematic of smFET sensor structure. (b) smFET CMOS chip.

B. Circuit Design

smFET signals are amplified by a transimpedance amplifier, which also serves to bias the device. To improve temporal resolution, the amplifier must be optimized for low-noise performance. This generally translates into amplifiers with large input devices and high transconductance to minimize 1/f and thermal noise. However, as device size increases, the additional capacitance at the input node contributes to higher current noise. Taking into account the parasitic capacitance of the electrodes contacting the CNTs, input devices for the amplifier are sized to balance the tradeoff between lower device noise and lower input capacitance.

The chip is composed of 12 transimpedance amplifiers with digitally-programmable gain, allowing amplification of currents ranging from hundreds of picoamperes to microamperes. Bandwidth is also tunable with programmable feedback capacitors. Each of the 12 amplifiers is connected to 500 electrode pairs for a total of 6000 sensing sites in a 60 x 100 array. The electrode pairs consist of two electrodes, sized 15 μm x 15 μm each, that provide the source and drain potential to the smFET. To minimize parasitics, each electrode row in the array has a switch that can be turned off, electrically isolating inactive rows from the amplifiers. Additionally, electrodes in any one row are connected to a common net through switches that can be configured for low leakage current or low parasitic capacitance. The wiring that connects the electrodes to the amplifier is spaced far from other nets, the chip surface, and the bulk in order to keep parasitic capacitance to a minimum.

C. Post-processing

In order to create a grid of smFET sensors on the CMOS chip, carbon nanotubes must be integrated onto the die and electrically connected to electrode pairs. The ideal chip surface for this post-processing is planar with electrodes that can be contacted with minimal resistance and minimal surface area to reduce parasitic capacitances.

In a typical CMOS process, the top metal level (which will serve as the electrode) is aluminum passivated by silicon dioxide and silicon nitride. If the electrodes are large enough, they can be designed to include openings in the passivation layers to expose the metal. Alternatively, they can be left unexposed during the integrated circuit design phase in which case an etch process must be utilized later to uncover them. It may also be beneficial to remove the top metal aluminum to expose the underlying adhesion layer. Aluminum forms a natural oxide layer which could inhibit good electrical contact to the electrode and is incompatible with electrolytes often used for wet testing.

After the electrodes are exposed, pristine carbon nanotubes can be transferred to the surface using two standard methods: solution-based deposition [20] or transfer from a growth substrate [21]. CNTs cannot be grown directly on the surface due to high growth temperature requirements, typically exceeding 900 ºC. Solution deposition begins with CNTs that have been purified and suspended in a solvent. The solution is then spun or drop-casted onto the chip surface. Alternatively, transfer from a growth substrate utilizes a transfer medium that sticks to the CNTs, allowing them to be peeled off one surface

and moved to a target surface. The transfer medium is then dissolved away.

After the CNTs are transferred to the surface, a metallization step may be performed to bridge the connection between CNTs and the electrodes. An isolation step may be necessary to remove CNTs that do not fall between electrodes.

IV. DISCUSSION

Using CMOS technology to integrate direct bioelectronic transducers in a single chip offers new opportunities and capabilities for sensing platforms. The electrochemical sensing platform demonstrates how an electrode array integrated on a CMOS chip can enable spatially resolved monitoring of multicellular systems. The ability to monitor the spatiotemporal distribution of metabolites as they are produced by cellular communities during development not only reveals the biochemical processes involved but may also provide clues as to the mechanisms by which cells regulate these processes during development. Diverse metabolites and biological macromolecules, including DNA, hormones, neurotransmitters, and small signaling molecules, can be probed electrochemically, and the platform is potentially suitable for applications with both eukaryotic and prokaryotic systems.

The smFET system shows how integration of high-gain biomolecular functionalized solid-state transducers can reduce parasitics and potentially enable single molecule detection at bandwidths orders of magnitude higher than possible with fluorescence imaging. The ability to detect the presence of individual molecules translates into a sensor that does not have traditional limits of detection. The detection of a molecule is only limited by the amount of time it takes for the molecule of interest to be captured by a probe. As sensor temporal resolution improves through integration, temporal dynamics at resolutions better than 100 nanoseconds may be possible for a rich set of biomolecular systems.

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