On-chip Detection of Single Vesicle Release from Neuroblastoma Cells using Monolithic CMOS Bioelectronics

Kevin A. White, Geoffrey Mulberry, Kiminobu Sugaya, and Brian N. Kim

Abstract— **Neuroblastoma cells are often used as a cell model to study Parkinson's disease, which causes reduced dopamine release in substantia nigra, the midbrain that controls movements. In this paper, we developed a 1024-ch monolithic CMOS sensor array that has the spatiotemporal resolution as well as low-noise performance to monitor single vesicle release of dopamine from neuroblastoma cells. The CMOS device integrates 1024 on-chip electrodes with an individual size of 15 μm × 15 μm and 1024 transimpedance amplifiers for each electrode, which are each capable of measuring sub-pA current. Thus, this device can be used to study the detailed molecular dynamics of dopamine secretion at single vesicle resolution.**

I. Introduction

Neurotransmitter secretion from cells is modulated by molecular manipulations from neurological disease and drugs treatments [1]. The amount of secretion can be significantly decreased for patients with Parkinson's disease [2] and can contrarily be increased with drug treatment [1]. Neurotransmitters are released in packets, which are vesicles, through the membrane fusion. The study of vesicle secretion yields rich information in the dynamics of membrane fusion [3] as well as in learning the mechanism of neurological diseases at molecular level [4]. It can also be used to reveal positive and negative side effects of drug treatments [5], [6]. Membrane fusion is a small event (typically less than 100 nm for neurons) and fast process which occurs in a few milliseconds, and thus is difficult to monitor with microscopy. Thus, an electrochemical method (amperometry) is used to monitor neurotransmitter release at high temporal resolution by oxidizing neurotransmitters at an electrode. The oxidation of molecules releases electrons into the electrode which can be measured with high-quality amplifiers. However, the amperometric measurement is a traditionally low-throughput method where each cell is measured individually, making the technology costly and time consuming to derive conclusions based on a large statistical significance.

Such limitations can be overcome by a scalable CMOS device in which the electrodes are directly integrated with the amplifiers. CMOS-based biosensors have been proposed [7], [8] to enhance the spatiotemporal resolution of various electrophysiological recordings. By monolithically integrating an on-chip electrode array, amplifier array, and peripheral circuits, it offers unprecedented capabilities, including high channel counts (100 – 10M parallel recordings) [9], low noise level [10], and high sampling rate [11]. In this paper, we present a CMOS-based electrochemical sensor array with a frame rate of 10,000 for a 1024 electrode-amplifier array, one of the fastest reported to date. The monolithic device can measure neurotransmitter release from many cells at a single-cell level, with the capability to resolve single vesicle release. The high-density electrode-amplifier array offers a great benefit to the study of the mechanism of vesicle secretion and the dynamics of neurotransmitter.

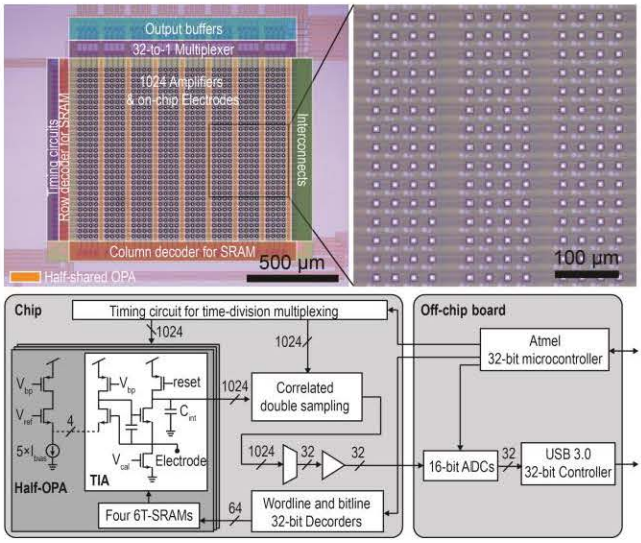

Figure 1. Photographs and architecture of CMOS die and amplifier array. The array has 1024 amplifiers and 1024 on-chip electrodes. The 32-to-1 multiplexer combines outputs by time-division multiplexing to condense the data. Row and column decoders allow for fully-addressable writing of embedded SRAMs in the array.

II. Design of Monolithic CMOS Bioelectronics

A. Integration of 1024 transimpedance amplifier array

The CMOS-based system is fabricated by designing and manufacturing a CMOS chip in 0.35-μm CMOS technology (Fig. 1). The design is ~2 mm^2, consisting of a 1024 transimpedance amplifier (TIA) and electrode array, multiplexers, output buffers, timing circuits, SRAMs, and decoders. The TIA uses an integrating capacitor (C_{int}) to integrate the electrochemical current from the electrode being measured over a period. Periodically, the voltage drop across C_{int} is sampled using correlated double sampling (CDS) and subsequently multiplexed with parallel amplifiers' output

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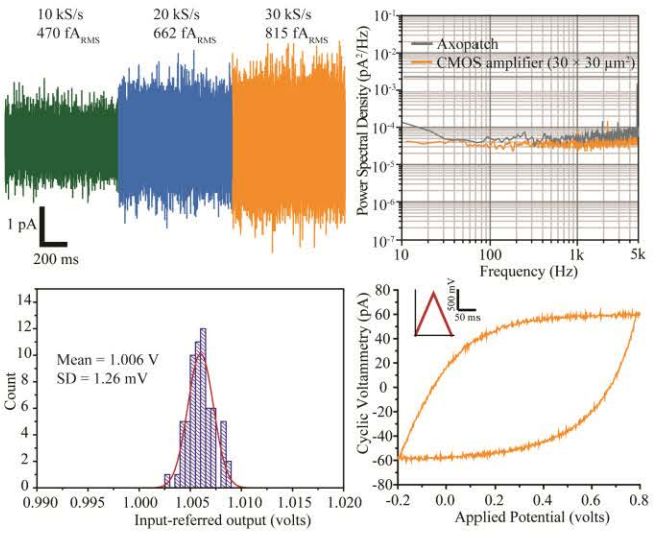

Figure 2. Characteristics and noise performance of the transimpedance amplifier array. Left (top) shows the noise level of the amplifier at 3 different sample rates. Right (top) compares the noise performance with a commercially-available amplifier. Left (bottom) shows the closely matching of input-referred outputs among amplifiers in the array. Right (bottom) shows the bipolar current recording capability.

[10]. The TIA design adapts a half-shared operational amplifier (OPA) design to minimize the power consumption and area of the array. The minimal area design allows design flexibility in determining the pitch between the electrode depending on the application. The area of each TIA is 30 × 30 μm². Every four TIA shares one non-inverting half of OPA. Each TIA can be programmed with different transimpedance gain (0.85 mV/pA and 6.98 mV/pA) and reconfigured into unity gain or testing configurations using embedded SRAMs. The set of four SRAMs in each TIA is fully-addressable and reconfigurable using row and column decoders. This allows independent control of individual TIA for simultaneous testing, recordings, and voltage stimulations. The multiplexed outputs are sampled using off-chip ADCs. Depending on the type of analysis, a National Instrument data acquisition system or off-chip 16-bit ADCs (LTC2323-16) on a custom-design PCB, USB-ADC, are used. The ADC uses a sampling rate of 4 times the rate of analog outputs from the CMOS chip. In USB-ADC, the digitized data is streamed through a USB 3.0 controller (Cypress FX3) at ~ 100 Mbytes/s. USB-ADC also has a 32-bit microcontroller (Atmel SAM E70) to generate clocks and custom waveforms to operate the CMOS chip. This allows users to control and modify the chip's performance and operational mode through simple LabVIEW commands.

B. *Characteristics and Noise Performance*

The performance of individual TIA is characterized by inputting known current into the electrode node (Fig. 2). The TIA exhibits 470 fA$_{RMS}$, 662 fA fA$_{RMS}$, and 815 fA fA$_{RMS}$, at 10kS/s, 20 kS/s, and 30 kS/s, respectively. The noise spectral density of the TIA reveals a comparable noise performance to that of a high-quality electrophysiology amplifier (Axopatch 200B) (Fig. 2). In the array of amplifier, the mismatch between small transistors can cause large variations in performance and output. Because the TIA design adapts the half-shared OPAs, the inverting-half of OPA only requires two transistors, enabling a large area investment in WL. The measured variation of input-referred output across the array is 1.26 mV$_{RMS}$ (Fig. 2). The TIA can measure bipolar currents by using an offset current loaded into the electrode node. The feasibility test is performed to show the cyclic voltammetry capability by applying a triangle wave and measuring the current response from a capacitor (Fig. 2). The frequency response of the system is examined by applying sinusoidal current into the amplifier, including above the Nyquist frequency. As expected, the periodic sampling of an integrating capacitor creates a sinc function in the frequency response. Due to its intrinsic characteristic of the integrating capacitor, the noise contributed from aliasing is minimal without an additional anti-aliasing filter. Because the dominant pole of ~ 4.4 kHz is from the sinc function at the 10-kS/s sample rate, the biasing level for the OPA did not influence the TIA's frequency response while the bandwidth of the OPA is larger than the dominant pole. The lowest bias tested for each TIA is 55.2 nA.

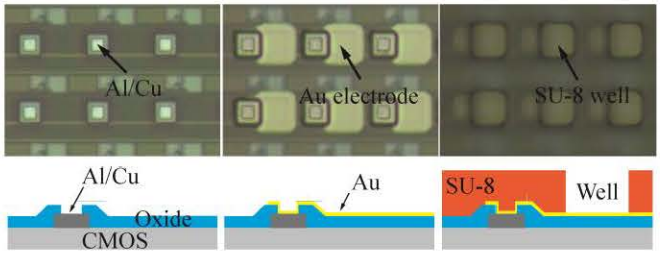

Figure 3. Post-CMOS processing for on-chip integration of electrodes and SU-8 wells. Initially, the CMOS die has Al/Cu as the top metal. Gold layer is patterned using lift-off process which is followed by the fabrication of SU-8 wells. The SU-8 wells are used to trap single cells.

III. Post-CMOS Processing

The top metal is aluminum-copper (Al/Cu) alloy in most conventional CMOS chips, which is inadequate for electrochemical sensing due to the aluminum's high reactivity to the electrolytic solutions. This causes not only high offsets in the electrochemical recording that results in a high shot noise but may also result in damaging of the chip due to water leakage [4]. Polarizable electrode materials, such as gold and platinum, are better suitable for the electrochemical recording due to low reactivity.

A. *Fabrication of on-chip electrodes and SU-8 wells*

To fabricate on-chip electrode array using gold, the CMOS die is mounted on a glass coverslip. The die is cleaned by acetone and isopropanol, followed by deionized (DI) water rinse. Negative photoresist, NR9-1500PY is used for lift-off process. After spin coating the negative photoresist, a photomask is used for photoresist exposure. After development, layers of 15 nm of titanium and 150 nm of gold are deposited using sputtering. The negative photoresist is removed by an acetone immersion and rinsing for few minutes (Fig. 3) to pattern gold electrodes. SU-8 wells are fabricated on the electrodes to trap single cell on each electrode and to prevent multiple cells secreting neurotransmitters to the same electrode. To fabricate SU-8 wells, the die is cleaned again using acetone, isopropanol, and DI water. SU-8 3010 is spin coated onto the die. After exposure using a photomask, the SU-

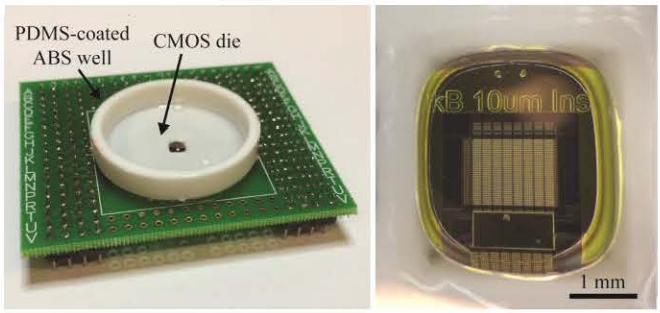

Figure 4. Biocompatible packaging of the CMOS chip by using PDMS-coated 3D-printed ABS well.1

8 layer is developed (Fig. 3). Each SU-8 well is 15 μm × 15 μm.

B. Biocompatible Packaging

The die is wire-bonded and packaged on a custom-designed PCB holder. An ABS well is 3D printed using filament-based 3D printer. The ABS well is coated with PDMS to limit contacts between living cells to ABS material. The 3D-printed well is attached to the die using PDMS to protect wire bonds/pads from electrolytic solutions (Fig. 4). In the middle of the well, the electrode array in the CMOS die is exposed to allow a direct contact of cells to electrodes (Fig. 4).

IV. Measuring Single Vesicle Secretions from Neuroblastoma Cells

SH-SY5Y cells are neuroblastoma cells and are often used as a model cell for studying Parkinson's disease because of their similarity in dopamine release to neurons in the central nervous system [12]. Neurotransmitter release from SH-SY5Y cells is measured using the monolithic CMOS bioelectronics.

A. Electrophysiology Setup

Suspended SH-SY5Y cells in the culture media are added into the ABS well and incubated at 37 °C with 5 % CO_2 for 1 hour to allow cells to settle in to the SU-8 wells. Once settled, the culture media is exchanged with 1 mL of the recording buffer. The recording buffer contains the following concentrations at a pH level of 7.3 and osmolality of 300 mmol/kg: 140 mM NaCl, 5mM KCl, 1 mM $MgCl_2$, 10 mM $CaCl_2$, and 10 mM HEPES/NaOH. Ag|AgCl reference electrode is inserted into the ABS well to make contact with the recording buffer. During recording, a stimulation is given by adding 1 mL of stimulation buffer to the cells to encourage vesicle secretions. The stimulation solution has the same pH

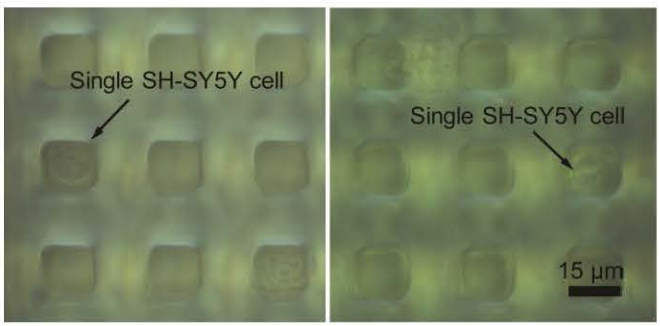

Figure 5. DIC microphotographs of SH-SY5Y cells on the monolithic CMOS bioelectronics. Single SH-SY5Y cells are trapped in SU-8 wells. The well allows only single cells to fall in to make contact with the electrode.

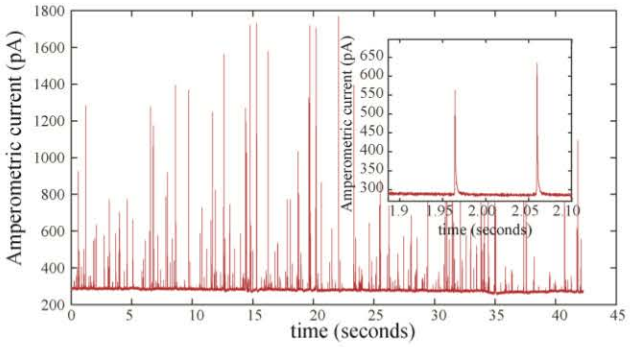

Figure 6. Vesicle secretions from a single cell measured using the monolithic CMOS bioelectronics. The 42-second amperometric recording from a SH-SY5Y cell is shown and the inset shows two representative spikes.

level and osmolality of 7.3 and 300 mmol/kg respectively with the following concentrations: 140 mM KCl, 5mM NaCl, 1 mM $MgCl_2$, 10 mM $CaCl_2$, and 10 mM HEPES/NaOH.

B. Single-cell Amperometry

Individual SH-SY5Y cells are trapped in SU-8 wells, allowing single-cell amperometry from each electrode (Fig. 5). The size of each SH-SY5Y cells in suspension is ~12 μm in diameter, which is marginally smaller than the well size (15 μm). Also, clumps of cells are observed but they failed to fit into the well. In this experiment, roughly ~10% of 1024 wells are covered with single cells. A measurement of neurotransmitter releases from a cell is shown in Fig. 6. The CMOS device successfully measured many amperometric spikes, each spike corresponding to the single vesicle secretion. To analyze the recorded amperometric events, the open-source program Quantal Analysis, version 8.20, is used to extract the amplitude, half-width, number of events, and quantal size [13]. Vesicle secretions as small as 9.6 pA are observed, and the largest spike measured is 1487 pA. In the 42-second measurement, 333 vesicle secretions are detected from the single cell. The average half-width of spikes is 1.26 ms and the average amplitude is 132.4 pA. The currents measured from each spike can be integrated (quantal size) to reveal the number of released neurotransmitters. The average quantal size is 0.288 pC which corresponds to ~900,000 dopamine molecules per vesicle.

V. Conclusion

We demonstrated a monolithic CMOS-based electrochemical sensor with the simultaneous current channel count of 1024, for every individual electrode in the array. All 1024 electrodes are simultaneously recording with noise levels as low as 470 fA_{RMS} at 10 kS/s. Because each electrode has a dedicated TIA, the array can operate at full 10,000 frames per second. Using embedded SRAMs, the TIA can be programmed to perform testing, recording, and stimulation. The system enables high spatiotemporal resolution in parallel electrochemical recordings of neurotransmitter release from neuroblastoma cells. A comparison to recent state-of-the art CMOS bioelectronics is presented in Table I. The monolithic CMOS bioelectronics can be used to accelerate the study of revealing the molecular effect of Parkinson's disease drugs to neurotransmitter release.

TABLE I. COMPARISON TO STATE-OF-THE-ART CMOS BIOELECTRONICS

Reference	[10] 2013	[14] 2017	[15] 2018	This Work
Technology Node	0.5 μm	0.18 μm	0.18 μm	**0.35 μm**
Die Size	3 mm × 3 mm	12 mm × 8.9 mm	5 mm × 2.65 mm	**5 mm × 5 mm**
Number of Electrodes	100	59760	200	**1024**
Electrode Size	15 μm × 15 μm	3 μm × 7.5 μm	10 μm × 10 μm	**15 μm × 15 μm**
Current Measurement Channels	100	28 (Neutransmitter Detection), 32 (Impedance Measurement)	200	**1024**
Noise performance	~ 100 fA_{rms}	200 pArms (Neurotansmitter TIA_A), 120 pArms (Neurotansmitter TIA_B), 6.4 pArms (Impedance measurement)	93 pArms (FSCV), 21.6 pArms (CV), 0.48 pArms (110 Hz BW limited Amperometry)	**470 fA_{rms}**
Bandwidth	-	16 kHz (Impedance measurement)	110 Hz - 10 kHz	**4.4 kHz**
Accumulated Sample Rate	200 kS/s	80 kS/s (Neurotransmitter Detection)	200 kS/s	**10 MS/s**
ADC	16-bit off-chip	10-bit on-chip	10-bit on-chip	**16-bit off-chip**
Total Power	-	86 mW	3.21 mW	**12.5 mW**

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