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Highly integrated CMOS microsystems to interface with neurons at subcellular resolution

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Abstract

CMOS high-density transducer arrays enable fundamentally new neuroscientific insights through, e.g., facilitating investigation of axonal signaling characteristics, with the “axonal” side of neuronal activity being largely inaccessible to established methods. They also enable high-throughput monitoring of potentially all action potentials in a larger neuronal network (> 1000 neurons) over extended time to see developmental effects or effects of disturbances. Applications include research in neural diseases and pharmacology.

Introduction

To understand how functions and characteristics of neuronal networks arise from the concerted interactions of the involved neurons, it is necessary to have methods that allow for interacting with neuronal functional subunits and ensembles - somas, axons, dendrites, single neurons, and entire networks - at high spatiotemporal resolution and in real time (1-3). Extracellular electrical recordings by means of microtransducer arrays complement well-established patch clamp techniques (4, 5) and optical or optogenetic techniques (6-9).

The use of CMOS technology helps to overcome the connectivity problem of how to interface thousands of tightly-spaced electrodes, while, at the same time, it improves signal-to-noise characteristics, as signal conditioning is done on chip next to where the partially very small signals (< 10 μ V) are generated (10-12). Here, we demonstrate how CMOS high-density microelectrode arrays (HD-MEAs) featuring several thousands of transducers (> 3'000 transducers per mm^2) can be used to record from or stimulate potentially any individual neuron or subcellular compartment on the CMOS chip (10-15).

CMOS HD-MEA Systems

Several different approaches relying on metal electrodes or open-gate field-effect transistors (FETs), as schematically shown in Fig. 1a, have been pursued (10-13). In the case of open-gate FET transducers, additional stimulation spots are needed and distributed between the electrodes. A sensor site and the surrounding stimulation site can be seen in Fig. 1b.

Figure 1 – CMOS realization of an open-gate field-effect transistor array. (a) Schematic cross section of a CMOS chip; the transistor gate is connected via several metal layers and vias to an electrode on top of the chip, which is covered by a dielectric material, such as high-k Ti-Zr oxide. Adapted from (13). (b) Chip surface of a fabricated CMOS-based HD-MEA based on open-gate FETs. The much larger stimulation site completely surrounds the sensor site in the center (11).

Figure 2 - Very compact integrated neural recording microsystem frontend on an index finger featuring 64 electrodes: 4 electrodes per shank and 4 chips with 4 shanks each. At the right the cable connection can be seen (16, 17).

Besides the comparably large, planar *in-vitro* systems, which are in the focus of this contribution, there are also CMOS-based needle-type probes for recording and stimulation *in vivo* that have been pioneered at the University of Michigan (3, 16-20) and adopted by other groups (21, 22). Such electrode arrays are inserted into the living brain and facilitate advances in the understanding of the nervous

system. Merged with on-chip circuitry, signal processing, microfluidics, and wireless interfaces, they are forming the basis for a family of neural prostheses for the possible treatment of disorders, such as blindness, deafness, paralysis, severe epilepsy, and Parkinson's disease (18, 22).

In the case of the transducer arrays for *in-vivo* or *in-vitro* applications, there are two conflicting key CMOS system design requirements: (i) high signal-to-noise ratio to detect partially very small signals and, at the same time, (ii) high spatial resolution. High spatial resolution requires small electrodes and small pitch and permits only very few and small circuitry elements to be realized within each electrode pixel (13, 23). Little available space for circuitry, however, translates into higher noise levels, as the noise of transistors usually scales with size, so that the signal quality is compromised by the very circuitry that enables its readout. Therefore, noise or signal-to-noise (S/N) ratio is a pivotal issue for high-density pixel-based approaches. By applying a switch matrix concept, it was possible to devise CMOS microsystems that feature (i) high S/N ratio and (ii) high spatio-temporal resolution (12, 24) at the expense of not being able to read out all electrodes or transducers simultaneously.

Figure 3 – CMOS-based bidirectional HD-MEA system. 26'400 metal electrodes (Pt) at the center of the chip ($3.85 \times 2.1 \text{ mm}^2$) are surrounded by readout, stimulation, and addressing circuitry units (scale bar: 1 mm) (12).

As one example, we show here in Fig. 3 a HD-MEA system featuring a sensing area of $3.85 \times 2.10 \text{ mm}^2$ hosting 26'400

Pt electrodes of $7 \mu\text{m}$ diameter at a center-to-center pitch of $17.5 \mu\text{m}$ (12, 14). The switch matrix allows for simultaneously routing user-configurable selections of electrodes to 1024 recording channels with 10-bit 20-kS/s A/D conversion and 32 stimulation units at the array periphery. The readout channels provide programmable bandwidth and gain up to 78.3 dB. The integrated noise voltage of the full readout chain is $2.4 \mu\text{V}_{\text{rms}}$ in the action-potential (AP) signal band (300 Hz–10 kHz). The switch matrix can be reprogrammed within 1.4 ms to adapt to the morphology of the biological sample. Possible configurations include single electrodes, sets of electrodes at points of interest, or multiple contiguous high-density blocks of up to 23×23 electrodes.

A disadvantage of CMOS chips is that silicon is not transparent to visible light in contrast to standard cell culture substrates used in biology. Additionally, the chip or its components can corrode upon operation and long-term exposure to liquids (salt water). Therefore, a good packaging solution is needed, on the one hand, to protect the chip against metabolic products and chemicals of the cell culture, and, on the other hand, to prevent the cells from being poisoned or disturbed by toxic materials released by the chip, such as the CMOS metals aluminum or copper that dissolve in saline solution.

Measurement Results

Figure 4 – Electrical signals of 3 neurons, recorded by the HD-MEA at full resolution and superimposed to a fluorescence image of the cell culture (MAP2 staining). Spike-triggered signal averages (50 trials) in green (top left neuron), red (top right) and blue (bottom right) for each electrode site (rounded white rectangles). Only signals exceeding $50 \mu\text{V}$ are displayed for clarity (14).

With this system it was possible to record subcellular-resolution data in various preparations. Figure 4 shows the electrical signals of 3 neurons, recorded by the HD-MEA at full resolution and superimposed to a fluorescence image of the cell culture (MAP2-staining). The signals of three neurons as obtained from the respective electrode sites (rounded white rectangles) are displayed in green (top left neuron), red (top right) and blue (bottom right). It is evident that many electrodes record from the same neuron and that every electrode simultaneously records activity of several neurons.

Figure 5 shows network recordings. 2000 individual single cells were identified through spike sorting of HD-MEA data (25-27), and the color-coding indicates the action potential spike amplitudes. The fluorescence image of transfected cells of the same culture shows clusters of neurons and tracks of neurite bundles.

Figure 5 – Network recordings. Top: 2000 individual single cells identified through spike sorting of HD-MEA data; circles indicate cell footprints that exceed 4.5 standard deviations of the electrode noise; color-coding indicates AP spike amplitudes. Bottom: fluorescence image of transfected cells of the same culture; clearly visible are clusters of neurons and tracks of neurite bundles (14).

It is also possible to detect small signals of action potentials traveling along thin axons (~100 nm diameter) as shown in Figure 6 (14, 28). Axonal signals across a branching point were recorded by simultaneously using 841 electrodes. The action potential waveforms that have been recorded from the electrodes marked in red in Fig. 6 are shown in Fig. 7. Typical axonal signal characteristics are observed: tri-phasic, first-positive signals (14, 28). The grey lines represent

individual signal traces, the black line the spike-triggered average signal.

Additionally, CMOS microtransducer arrays offer the capability to bi-directionally interact, also in closed loop and real time, with potentially every single neuron in a given neuronal network (14, 28, 29). Due to sophisticated post-processing procedures and packaging strategies, CMOS chips have been proven to be long-term-stable and viable in cell cultures over weeks to months (12, 14).

Figure 6 – Axonal signals across a branching point recorded by simultaneously using 841 electrodes. “Electrical image” of the axonal signals of rat cortical neurons at DIV 18 (14).

Figure 7 – Axonal signal traces recorded by simultaneously using 841 electrodes. Waveforms of the action potentials traveling along the axon as recorded from the electrodes marked in red in Figure 6. Typical axonal signal characteristics are observed: tri-phasic, first-positive signals (14, 28). The grey lines represent individual signal traces, the black line the spike-triggered average signal (14).

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