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Author(s):

Müller, Jan; Ballini, Marco; Livi, Paolo; Chen, Yihui; Shadmani, Amir; [Frey, Urs](#) ; Jones, Ian L.; Fiscella, Michele; Radivojevic, Milos; Bakum, Douglas J.; Stettler, Alexander; Heer, Flavio; [Hierlemann, Andreas](#)

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CONFERRING FLEXIBILITY AND RECONFIGURABILITY TO A 26,400 MICROELECTRODE CMOS ARRAY FOR HIGH THROUGHPUT NEURAL RECORDINGS

J. Müller^{1,3}, M. Ballini^{1,3}, P. Livi¹, Y. Chen¹, A. Shadmani¹, U. Frey², I. L. Jones¹, M. Fiscella¹,
M. Radivojevic¹, D. J. Bakkum¹, A. Stettler¹, F. Heer¹, A. Hierlemann¹*

¹ETH Zurich, Department of Biosystems Science and Engineering, Basle, SWITZERLAND

²RIKEN Quantitative Biology Center, Kobe, JAPAN

³Both authors contributed equally.

ABSTRACT

We present a highly configurable, high-temporal-and-spatial-resolution microelectrode array (MEA) system, fabricated in CMOS technology, featuring 26'400 platinum microelectrodes ($5 \times 9 \mu\text{m}^2$) with a pitch of $17.5 \mu\text{m}$, arranged in a rectangular grid within an overall area of $3.8 \times 2.1 \text{ mm}^2$. The array constitutes a very flexible bidirectional interface to electrogenic cells. High-throughput, subcellular-resolution recording and stimulation of large networks of neurons enable studies of information processing and learning capabilities of neurons.

An exemplary recording session demonstrates the flexibility in electrode selection and shows the system's capabilities to record from widely-spaced sets of electrodes that provide a network level overview and to subsequently perform subcellular-resolution single cell recordings of selected areas of interest.

KEYWORDS

Microelectrode array (MEA), bioelectronics, CMOS, neuronal interface, switch-matrix, extracellular recordings.

INTRODUCTION

How the billions of individual neurons in our brain contribute to memory and learning is subject to studies since many decades. Fundamental operations in the brain are attributed to the activity of individual neurons, manifested in so-called action potentials (AP), which are generated through ion channels in the cell membranes. It is believed that the orchestrated activity of neurons is at the base of information processing in larger neuronal networks [1].

Using MEAs to study the dynamics and mechanisms of neuronal networks as well as those of individual neurons through extracellular field potential recordings became increasingly popular in the past few years [2]. Commercially available passive MEAs usually consist of 60 to 256 planar microelectrodes with diameters of up to $30 \mu\text{m}$ and spacings between the electrodes of 60 to $100 \mu\text{m}$. These MEAs usually can record from several neurons in parallel. However, due to their rather large electrode size and spacing, the neuronal networks featuring cells of around $10 \mu\text{m}$ diameter are usually under-sampled. This makes it difficult to investigate information processing in such networks, as the activity of large parts of the network remains unknown. Subcellular-resolution recordings would make the

assignment of AP waveforms to individual cells more accurate, thus providing a more detailed insight into the information processing of a network under study [3].

The recent advent of CMOS based HD-MEAs [4,5] offers the possibility to achieve subcellular-resolution recordings at high signal quality. By using CMOS technology, thousands of microelectrodes can be tightly packed on a chip. Amplifiers and analog-to-digital converters (ADCs) can be placed in very close proximity to the signal sources and the electrodes, which leads to high signal-to-noise ratio.

Various MEA system architectures to attain high-spatial-resolution recordings have been reviewed in the literature [6], including active pixel sensors [5], [7] or switch-matrix-based designs [8]. The system presented here inherits and further develops the switch-matrix concept, which allows a much higher electrode packing density as compared to an active pixel architecture. The recording and stimulation channels are placed outside the sensing area so that the chip area available for the readout circuitry is not constrained by the pitch of the sensor electrodes. Therefore, high-performance amplifiers with high gain and low noise levels can be implemented. Through the switch-matrix, every electrode can independently be routed to a recording channel or stimulation unit, so that recordings with nearly arbitrary configurations of subsets of selected electrodes are possible. The presented CMOS based HD-MEA microsystem can be adapted during recording sessions to meet the requirements of the actual biological sample with unprecedented flexibility. The system presented here can be used to study network features, like signal propagation and activity of cell assemblies with hundreds of coarsely distributed recording spots as well as for investigations of features of a single cell at very high resolution. The high flexibility in selecting stimulation and recording sites is particularly useful for biological experiments where the exact geometry of the sample under study is not known a priori: Instead of integrating thousands of small stimulation units with every electrode in a large area, a few tens of stimulation units, which can flexibly be connected to every electrode of the array, are enough.

DESIGN

The presented microsystem was fabricated in $0.35 \mu\text{m}$ CMOS technology (2P4M) and has a total die size of $7.6 \times 10.1 \text{ mm}^2$. Figure 1 shows a micrograph of the CMOS microsystem. It features a total of 26'400 platinum microelectrodes ($5 \times 9 \mu\text{m}^2$) at a pitch of $17.5 \mu\text{m}$, arranged

Figure 1: Micrograph of the CMOS-MEA system with the microelectrode array (1) in the center, surrounded by 1024 readout channels and ADCs (2,3) on the left and right side. 32 Stimulation buffers are located at the top and bottom (4,5).

on a rectangular grid of $3.8 \times 2.1 \text{ mm}^2$. The electrodes were fabricated through post-processing at wafer level. First, Si_3N_4 was deposited by means of PECVD, and the pads and electrodes were subsequently re-opened through reactive-ion etching (RIE). Next, WTi (50 nm) to promote adhesion and Pt (270 nm) as electrode were ion-beam-deposited and patterned using an ion-beam etching step. A 4-layer $1.6\text{-}\mu\text{m}$ -thick passivation stack, consisting of alternating SiO_2 and Si_3N_4 layers was deposited by PECVD, and, finally, a reopening of the platinum electrodes was achieved through an RIE step. Below the electrodes is a densely packed mesh of routing wires and $85'360$ CMOS switches, controlled by SRAM cells. The switches are used to connect an almost-arbitrary subset of the electrodes through the routing wires to the 1024 low-noise recording channels ($2.4 \text{ }\mu\text{V}_{\text{rms}}$ in the spike signal band of $300\text{Hz} - 10 \text{ kHz}$), as well as to 32 voltage and current dual-mode stimulation units. The recording channels feature high-pass filtering to remove

low-frequency drifting of the electrode potentials as well as low-pass filtering to suppress noise outside the spike signal band. The amplified signals are sampled and digitized on-chip at $20'000$ samples/s by on-chip 10-bit ADCs. The digital signals are transferred off chip at a clock frequency of 24 MHz and a total data rate of 24.6 MB/s . On the stimulation side, three digital-to-analog converters (DACs), provide near arbitrary stimulation waveforms to the 32 stimulation units for operation in either voltage ($\pm 1.5 \text{ V}$) or current mode (with a $100 \text{ }\mu\text{A}$ output range) [9]. A digital core running at 50 MHz receives configuration and stimulation commands and is used to configure all the functional units of the chip. Figure 2 shows a block diagram and an overview of the CMOS integrated microsystem.

The CMOS switches and routing wires below the electrode area are used to realize a flexible mapping of electrodes to readout/stimulation units or channels. Every electrode can potentially be routed to each and every recording channel and/or stimulation unit. By using the CMOS switches to connect suitable routing wires, each recorded signal is routed along a particular path to the corresponding recording or stimulation unit. Figure 3 shows a schematic view of the routing mesh.

The smallest unit of the array, so called pixel, consists of one platinum microelectrode, two CMOS switches with their respective SRAM cells, as well as six horizontal and six vertical routing wires. Within the microelectrode array, all signal wires are shielded with grounded wires in between to minimize crosstalk between neighboring signal lines. The switches are implemented as analog CMOS transmission gates, consisting of an n- and p-MOS transistor. The resistance of the switches depends on the operating points, i.e., the electrode potential, and can vary between $600 \text{ }\Omega$ and $1 \text{ k}\Omega$.

Custom software has been developed to find an optimal configuration of open and closed switches, which makes efficient use of all available switches and routing wires, e.g. ensuring a minimum required switches in readout and stimulation paths. An optimal configuration is then written to the SRAM cells through the on-chip digital

Figure 2: Block diagram of the integrated CMOS-MEA system. The 1024 readout channels and the 32 stimulation units can be simultaneously connected to the configurable array with its $26'400$ platinum microelectrodes. A digital core transmits the data off chip at a rate of 24.6 MB/s and receives control commands with a 50 MHz clock.

Figure 3: Schematic view of the microelectrode array routing mesh. Marked in red is one pixel unit. Four electrode-to-readout channel routing paths are indicated with blue lines.

logic, and the respective switches are afterwards either closed or opened in order to set up all the independent paths between the selected set of electrodes and the 1024 readout channels and 32 stimulation units.

As there are sufficient routing resources of CMOS switches and wires available on the chip, the set of recording electrodes can be chosen almost arbitrarily with only a few constraints. Moreover, the full array can be easily reconfigured within 1.4 ms to accommodate various experimental paradigms.

RESULTS

In order to experimentally validate the system's capabilities, neuronal cultures derived from primary

cortical neurons isolated from E18 rat embryos were grown on top of the electrodes and cultivated for 7 days. Animal handling protocols were approved by the Basel-Stadt Veterinary office and were in accordance with Swiss federal laws on animal welfare. Experiments were conducted inside an incubator to control environmental conditions (34.5°C and 5% CO₂). For further details see [10].

After 7 days in vitro, the neurons exhibit spontaneous electrical activity. They start growing processes and start to form small networks of interconnected cells. Figure 4 shows an exemplary experimental session and is used to highlight the different possible configurations of the presented microelectrode array. First, a large 1.5×1.5 mm² patch of 900 microelectrodes, sparsely distributed with a spacing of 52 μm and covering 30% of the overall electrode area, is used to obtain a one-minute short recording. Subsequently, spiking activity is analyzed by thresholding the signal (5σ of signal noise) on each electrode. The aligned and averaged spike waveforms are shown in Figure 4a. When recording with such a sparse electrode configuration a resolution of individual neurons is hardly possible. However, the recordings provide a good overview of the biological sample under study and regions with no activity can be identified and excluded from further investigations. The respective circuitry units can instead be used for recordings from more active areas. Once an area of interest has been identified, as for example the one marked with a red dashed square in Figure 4a, a high-density configuration of electrodes (529) has been recruited in this area to perform subcellular-resolution recording on a 400×400 μm² patch (2% of sensor area). This facilitates the assignment of different APs to the respective neurons in this area through the application of spike sorting methods [11]. For the example discussed here, threshold crossings of 5σ of

Figure 4: Two electrode configurations used for recordings from cultured rat cortical neurons. a) Sparsely selected electrodes to record network activity of the cultured cells. b) 23x23 electrodes recording at highest possible spatial resolution. Spike triggered averaging reveals the spatial spread of each action potential across multiple electrodes. With the higher density electrode selection, more neurons can be discovered than in the coarse configuration. c) Close-up view of a single neuron and three highly overlapping neurons, the latter of which could be clearly separated due to spatial subcellular-resolution oversampling.

noise at each individual electrode have been identified, aligned and have been cut out together with the signals of the 8 neighboring electrodes. Principal component analysis (PCA) was performed on these cutouts and principal components were clustered with k-means to find the electrical footprint of individual neurons. It is evident that a high-resolution recording is mandatory to properly assign single cells. For example, the large amplitude spike recorded in the lower right corner of the red dashed square of Figure 4a (red arrow) seems to originate from a single neuron. However, recording this area with higher resolution reveals that this signal originates from at least three distinct neurons, as can be seen in Figure 4b and 4c. The spike waveforms that are detected on multiple nearby electrodes can be used to separate putative single neurons, so that many more neurons can be identified as compared to a recording with a more sparse configuration. In Figure 4b and 4c, the color-coded contour plots show the maximum negative signal peak of an action potential on each electrode per identified neuron.

Figure 5 shows a scanning electron microscopy (SEM) image of a cultured neuronal network. The biological sample has been fixated prior to imaging. The figure shows the size of the cells in relation to the size and pitch of the electrodes. For many cells, the electrical activity can be recorded with more than one electrode.

Figure 5: SEM of the electrode array showing a close-up of the microelectrode array and the in-house post-processed Pt electrodes with cultured rat cortical neurons on top.

CONCLUSION

The flexibility and high spatio-temporal resolution recording capabilities of the presented microsystem, which are due to its large number of recording and stimulation units and the flexible connection through a switch matrix, enable novel experimental paradigms for electrophysiological studies that include network-wide recordings and subcellular-resolution measurements in the same preparation.

The capability of the device to record from individual cells with many electrodes (up to ten) in high-resolution mode as well as to record from a large population of cells spanning over hundreds of micrometers by using a

corresponding selection of suitable electrodes was successfully demonstrated.

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CONTACT

*J. Müller, tel: +41 61 387 3178;
jan.mueller@bsse.ethz.ch