BioMEA™: A 256-channel MEA system with integrated electronics

G. Charvet, O. Billoint, L. Rousseau and B. Yvert

Abstract—In order to understand the dynamics of large neural networks, where information is widely distributed over thousands of cells, one of today’s challenges is to successfully record the simultaneous activities of as many neurons as possible. This is made possible by using microelectrodes arrays (MEAs) positioned in contact with the neural tissue. Thanks to microelectronics’ microfabrication technologies, it now becomes possible to build high density MEAs containing several hundreds of microelectrodes. However, increasing the number of electrodes using conventional electronics is difficult to achieve. Moreover, high density devices addressing all channels independently for simultaneous recording and stimulation are not readily available. Here, we present a 256-channel in vitro MEA system with integrated electronics allowing simultaneous recording and stimulation of neural networks. Both actions are performed independently on all channels.

I. INTRODUCTION

Multielectrode arrays (MEAs) provide an elegant way to probe the neural code distributed over large populations of neurons either in vitro [1][2][3] or in vivo[4][5]. MEAs also offer the possibility to deliver electrical stimulation to neural networks[6], making them promising technologies to build neural prosthesis[7][8][9]. The increasing number of MEA studies show that the understanding of neural network dynamics can be best achieved when the highest number of neurons can be recorded simultaneously. However, increasing the number of electrodes using conventional electronics is difficult to implement into compact device. Moreover, high density devices addressing all channels independently for simultaneous recording and stimulation are not yet available. To overcome these limitations, we designed high density multielectrode systems with integrated electronics allowing simultaneous recording and stimulation of neural networks. This paper presents a new 256-channel system named BioMEA™. Either planar or 3D electrodes arrays were realized by deep reactive ion etching techniques of a Silicon substrate and tested on whole embryonic spinal cord networks. These 256-electrode arrays are interconnected to four 64-channel ASICs dedicated to the amplification and the multiplexing of the signals and stimulation. Each ASIC includes one amplification stage and one current generator per channel, and has the capability to rapidly switch between recording and stimulation. The realization of high density MEA systems with integrated electronics offer new possibilities for both in vitro and in vivo studies of large neural networks.

II. ORGANIZATION OF THE BIOMEA SYSTEM

The BioMEA™ system comprises a high density electrode array (256 3D micro-electrodes) interconnected to four dedicated 64-channel ASICs (amplification, analog multiplexing, current stimulation) running in parallel, specific acquisition boards, and a user-friendly software.

![Fig 1: Architecture of the system BioMEA™](image)

III. 256-ELECTRODE ARRAYS

In order to be as close to the neurons as possible, the microelectrodes of in vitro systems may be shaped as 3D needles, at the tip of which the recording site is. Pioneering techniques use classic isotropic etching, (either plasma or wet etching). However, the technique’s drawback is the limitation impose on the electrode’s pitch by the electrode’s. The electrode’s pitch cannot be smaller than twice the electrode’s height: for h = 80 μm, p > 160 μm. These isotropic processes thus prevent the fabrication of dense 3D electrode arrays with large aspect ratios.
These limitations have been overcome using Deep Reactive Ion Etching (DRIE) [10] [11]. A specific process was developed in which silicon substrate is etched anisotropically in order to obtain microneedles. Our MEAs have no limitations neither in height nor diameter and pitches down to 50 μm could be achieved. This process offers the possibility to manufacture various shapes of electrodes on silicon or glass substrate. Realized electrode arrays (Fig. 2) are composed of 256 electrodes with a basis of 40μm, a height of 80 μm arranged in an 8 x 32 matrix with a pitch of 250 μm.

![Figure 2: 256 Electrode array](image)

**Fig 2 : 256 Electrode array**

IV. **THE 256-CHANNEL SYSTEM**

Interfacing neurons through MEAs using discrete electronics rapidly limits the number of channels, creating the need for highly integrated electronics to achieve sufficient spatial resolution[12][13][14]. This will be especially the case for in vivo studies in small animals (mouse).

A. **Integrated Electronic**

A dedicated ASIC named AGNES (Asic for General Neurons Electrical Study) (fig 3) has been developed in order to allow simultaneous recording and stimulation on 64 channels.

![Figure 3: ASIC AGNES](image)

**Fig 3: ASIC AGNES**

Each channel of this 64-channel CMOS chip (Fig. 4) is interfaced with neurons via a microelectrode array and includes a low noise, variable gain measurement channel. The preamplifier and the amplifier are based on the same structure which provides a unity DC gain and an AC gain of respectively 75 and 10 in the 1Hz – 3kHz bandwidth. Both can be separately switched to follower configuration. Stimulation is performed by using, for each channel, an 8-to-1 analog multiplexer (fed by 8 external input signals for the whole ASIC) and a voltage-to-current converter allowing uniform current stimulation (+/- 400μA peak max.) independently of the electrode impedance. The measured transfer function of the voltage-to-current converters shows a good linearity and a limited drift between channel 1 and channel 64. A global control signal was added to cut the residual DC current at the output of the 64 voltage-to-current converters once the stimulation patterns have been applied.

![Figure 4: Architecture of the ASIC AGNES](image)

**Fig 4 : Architecture of the ASIC AGNES**

A Sample & Hold circuit allows snapshot style images of the 64 channels (with no time delay between channels as in a sequential reading), and can stand a maximum sampling frequency of 50kHz, which leads to a data output frequency of 3.2MHz (time-multiplexed). To get rid of the random DC offset potential existing at the electrode-electrolyte interface, the ASIC can be supplied with floating VSS and VDD. The Circuit’s size is 2.4mm x 11.2mm (0.35μm CMOS process) (Table I).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power consumption</td>
<td>125mW</td>
</tr>
<tr>
<td>Input-Refered Noise</td>
<td>4.3 μV rms</td>
</tr>
<tr>
<td>Signal Bandwidth</td>
<td>0.08Hz to 3kHz</td>
</tr>
<tr>
<td>Maximum Sampling Frequency</td>
<td>50 kHz</td>
</tr>
<tr>
<td>Maximum Multiplexing Frequency</td>
<td>3.2 Mhz</td>
</tr>
<tr>
<td>Input Impedance</td>
<td>&gt; 10^7 ohms</td>
</tr>
<tr>
<td>Variable Gain</td>
<td>1, 10, 75, 750</td>
</tr>
<tr>
<td>Maximum Stimulation Current</td>
<td>+/- 400μA</td>
</tr>
<tr>
<td>Area (in 0.35μm CMOS)</td>
<td>27mm²</td>
</tr>
</tbody>
</table>

**TABLE I  PERFORMANCE OF THE ASIC AGNES**
B. Electronic System set-up

The BioMEATM system interfaces on 256 Electrodes and performs both measurements and stimulations. This system uses four 64-Channels ASICs mounted on a mechanical support allowing electrical interconnections between four ASICs and one 256-channel array, recording and stimulation electronics capabilities, and a PC running a specific firmware and providing a user interface (fig 5).

![Image of BioMEATM system setup](image)

**Fig 5 : BioMEATM system setup**

1) Recording and stimulation electronics controls

The recording and stimulation electronics controls includes analog signal adaptation, analog-to-digital converters (ADC), digital-to-analog converters (DAC), and also a digital interface (microcontroller and FPGA) for ASIC protocol and data transmission. This hardware electronic system was designed to control up to 4 AGNES ASICs. There are four 14-bit ADCs for simultaneous conversions on the four ASICs’ analog sampled outputs. The maximum sampling frequency for each channel is 50 kHz/Channel. So, each ADC are running at 3.2 MHz (64 channels per one analog sampled signal). For stimulation, eight 14-bit DACs for simultaneous voltage-controlled patterns generation are implemented. Eight voltage-controlled patterns are stored in eight 64kB RAM blocks. A clock signal synchronizes the digital to analog conversion at a maximum sampling rate of 10MHz. The system also provides an adjustable power supply for all ASICs. Indeed, the ASIC can be supplied with floating [VSS,VDD]: from [0v,5v] to [-2.5v,2.5v]. The large data amount and the low latency require high performance communications and signal processing capabilities. To handle the large amount of output data, an FPGA running at 48 MHz and a USB 2.0 interface chip were used.

2) Acquisition software and user interface

The system is supervised by a PC for data acquisition and it provides a user interface. The software is an essential component of the system. It manages the USB data handling, the data visualization, and the stimulus generation. Low latency and high data throughput are key requirements. The software allows real time acquisition of 256 channels and data storage on all 256 channels at a time. The output data files are CED spike2 data files (SON32 data format) so that the acquired data can be read back offline using Spike2 software. Channels are displayed during acquisition and it is possible to navigate through data during recording. A user friendly interface allows the configuration of the acquisition and of the stimulation parameters.

V. Biological Tests

Developing neural networks generate spontaneous activity [15][16] that is important for the maturation of a functional circuit. We have been using multielectrode arrays to record from a whole embryonic mouse hindbrain-spinal cord preparations isolated in vitro at embryonic days E13-E16.5. We found spontaneous activity in the medulla characterized as

![Image of Recording with BioMEATM of spontaneous bulbo-cervical Local Field Potentials (LFP)](image)

**Fig 6 : Recording with BioMEATM of spontaneous bulbo-cervical Local Field Potentials (LFP)**
principally by local field potentials (LFP) recurring every 1-3 minutes (Fig. 6). This activity, which resembles sharp waves found in the cortex, could be suppressed by a pharmacological blockade of AMPA/Kainate glutamatergic receptors using CNQX (10 μM).

A more detailed spatiotemporal mapping of these spontaneous LFP could be obtained using a transparent 256-electrode array (Fig. 7). Mapping of this 256-electrode array is adapted to the shape of the spinal cord (8mm x 2mm). Maps were built using surface spline interpolation [17].

References


VI. CONCLUSION

Microelectrode arrays have been realized with a DRIE protocol allowing any aspect ratios of 3D microelectrodes. A 64-channel ASIC with recording and stimulation capabilities for each channel has been designed and produced. A complete 256-channel in vitro setup has been built. This system includes 4 ASICS, the electronics controlling the acquisition and the stimulation phases, and a user-friendly software interface. The output data files are CED spike2 data files, so that acquired data can be read back offline using Spike2 software. First validations of the full (64- and 256-channel) systems have been performed on acute spinal cord preparations.

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