Brain-Silicon Interface for High-Resolution 
\textit{In Vitro} Neural Recording

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\textbf{Abstract—} A 256-channel integrated interface for simultaneous recording of distributed neural activity from acute brain slices is presented. An array of $16 \times 16$ Au recording electrodes are fabricated directly on the die. Each channel implements differential voltage acquisition, amplification and band-pass filtering. In-channel analog memory stores an electronic image of neural activity. A $3 \text{ mm} \times 4.5 \text{ mm}$ integrated prototype fabricated in a 0.35 $\mu$m CMOS technology is experimentally validated in single-channel extracellular \textit{in vitro} recordings from the hippocampus of mice and in multi-channel simultaneous recordings in a controlled environment.

\textbf{Index Terms—} Integrated neural interfaces, Neural amplifier, On-chip microelectrodes, Acute brain slices

I. INTRODUCTION

The electrophysiology of the human brain governs a complex array of neurological functions. The human brain is a large-scale interconnected network with common behavioral properties extending across large spatial areas. To gain full understanding of how biological neural networks encode and process information, it is necessary to simultaneously record signals from many neighboring neurons.

Significant insights have been gained into ways of neural information coding through the use of micro-electrodes that record the activity of single neurons and neural populations in the brain. Recording of neural activity has been traditionally performed using bench-top biomedical instrumentation equipment. These instruments are generally stationary, bulky, limited to one or a few acquisition channels, and prone to excessive noise due to wiring. Integrated neural interfaces, fabricated on a single miniature physical substrate, lack these drawbacks. They offer a small, low-power, low-noise, and cost effective chronically implantable alternative to commercial bench-top instruments. Integrated neural interfaces perform signal acquisition, amplification, filtering, and, in some instances, quantization and neural stimulation [1]–[7]. They may also provide wireless data interface on the same chip [8].

Recording microsystems with three-dimensional (3D) electrode arrays of various configurations have been reported such as with electrodes co-planar with the die [9]. Implementations with 3D electrode arrays bonded directly to the surface of the chip have been proposed [6], [10]. Previously reported neural interfaces integrated with on-chip 3D microelectrodes have been typically limited to 100 channels [10]. Implementations with higher number of channels have been reported without electrodes and at the cost of increased circuit noise [11].

We present a CMOS brain-silicon interface for high-resolution \textit{in vitro} recording from acute brain slices. Neurophysiological studies of acute brain slices such as those of hippocampus are critical in investigating therapies for such debilitating neurological disorders as epilepsy and Alzheimer’s disease. A region of interest in the brain is extracted from an animal and sliced. The thickness of a slice is typically in the order of several hundreds of microns. As a result of slicing, acute brain slices have an outer layer of dead tissue which needs to be penetrated by recording electrodes.
Its thickness can be in the order of tens of microns. For this purpose golden 3D electrodes are post-fabricated on the surface of the die of the proposed integrated neural recording interface. The cross-section of the proposed microsystem is depicted in Figure 1 (a). Au electrodes are individually bonded directly onto the surface of the chip employing conventional die bonding equipment. This fabrication method yields low manufacturing costs, high yield, and flexibility in electrode location and shape. The size and geometry of the electrodes are chosen specifically for recording from acute brain slices of mice such as the hippocampal-entorhinal cortex slice shown in Figure 1 (b). The slice is inserted onto the recording electrodes and is placed into a fluidic chamber. The slice rests on the bases of the electrodes and is held in place by a slice anchor (or harp). This allows the tissue slice to be perfused from both above and below in order to maintain its vitality.

Each channel of the integrated neural interface contains a low-noise amplifier with up to 74dB programmable gain, a tunable anti-aliasing low-pass filter (LPF), and a high-pass filter (HPF) that removes a DC voltage offset present at the electrode-tissue interface. The brain-chip interface records action potentials in the range of tens of microvolts to hundreds of millivolts. For low-noise distributed neural potential field recording, a multi-channel integrated neural interface has been designed and prototyped.

The 256-channel integrated neural interface was fabricated in a 0.35 $\mu$m double-poly standard CMOS technology. The 3 mm x 4.5 mm die micrograph is shown in Figure 3. Each channel is connected to one on-chip data recording site and a reference recording site, for low-noise differential recording. Each recording site is comprised of a stack of several
aluminum layers with the topmost layer left unpassivated similarly to a conventional bonding pad. One on-chip reference recording pad is shared by all recording channels. An off-chip reference voltage can also be supplied from an external recording electrode.

**B. Microsystem Integration**

*In vitro* neural recording procedure requires preserving the vitality of a brain slice by its continuous perfusion. The perfusion fluid such as artificial cerebrospinal fluid (ACSF) is electrically conductive. The close proximity of bonding wires to the recording array necessitates their electrical insulation. To simplify the process of electrical insulation of bonding wires all wire-bonded pads are located on the two opposite sides of the die as shown in Figure 3. A margin of several hundred microns between the array and the wire-bonded pads on each of the two sides of the die relaxes the precision requirements on the bonding wires insulation process.

The microsystem electrode-silicon hybrid integration process is comprised of several fabrication steps as depicted in Figure 4. The neural recording die is packaged in an open-cavity ceramic package. To make a recording well for holding a mouse hippocampal slice, a pre-fabricated rectangular rubber molding is placed on the surface of the recording array. The molding size is approximately 4mm×12mm as needed for a

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**Fig. 4.** Fabrication steps in the the brain-silicon interface hybrid integration procedure.

**Fig. 5.** Scanning electron microscopy (SEM) photographs of golden electrodes fabricated on the surface of the chip: (a), mid-angle view; (b), low-angle view; (c), partial array view.
typical mouse hippocampal slice. With pressure applied to the surface of the molding, a bio-compatible dental molding compound is poured around the molding. The molding compound fills the package cavity flash with its surface. The resulting recording well electrically insulates and mechanically protects all bonding wires. The surface of the die is plasma-cleaned to eliminate any residual contamination. Golden electrodes are then fabricated on the surface of the partially-encapsulated die as described in more detail below. A machined bio-compatible plexiglass fluidic chamber is placed on the surface of the package with its rectangular tapered opening aligned with the fabricated recording well. A liquid gasket waterproofs the gap between the package and the fluidic chamber. Two horizontal circular openings in the fluidic chamber serve as an inlet and an outlet for the perfusion fluid.

The recording electrodes are fabricated utilizing conventional die-bonding equipment. Golden studs are manufactured on non-passivated aluminum recording pads by attaching melted gold to the pads, stretching it up, and breaking it off at a controlled height. The diameter of the base of an electrode is 80 \( \mu \text{m} \). A typical electrode has a tapered shape with the tip of several microns in diameter. The height of each electrode is approximately 100 \( \mu \text{m} \). This geometry is optimum for recording from acute hippocampal slices as it allows to penetrate the dead outer layer of an acute brain slice and perform a localized recording from within the live layer of the tissue. A set of three scanning electron microscopy (SEM) photographs of the fabricated electrodes at different angles is shown in Figure 5.

C. Recording Channel

The primary function of the acquisition channel is to amplify the weak neural signal with minimal circuit noise and non-linearities added to the output while consuming little power. Power dissipation is limited so that the surrounding tissue is not damaged by heat. Due to electrochemical effects at the tissue-electrode interface, DC voltage offsets several orders of magnitude above the actual signal level are common [13]. The recording channel requires a high pass filter (HPF) to prevent the DC component from saturating the amplifiers. Sampling of the signal requires an anti-aliasing low pass filter (LPF). Post processing of the neural recording is performed in the discrete domain by means of switched capacitor circuits.

As a high closed-loop gain is required in the recording channel, it employs a two-stage amplifier. This yields higher linearity and maintains capacitor sizes within the recording cell pitch requirement. Figure 6 shows the circuit diagram of the first stage of the recording channel. The first stage is a continuous-time difference amplifier. The channel inputs are capacitively coupled to the first stage operational transconductance amplifier (OTA) which insures DC input rejection of the amplifier. To achieve a sub-hertz HPF cut-off frequency a large resistor, in the order of gigaohms, should be employed in the feedback network. A linear resistance with such value consumes large silicon area. Therefore, the resistive element is implemented as a MOS device biased in the subthreshold region [13], [14]. The second stage is a single-ended capacitively-coupled continuous-time amplifier.

For truly simultaneous multi-channel recording, the output of the two-stage amplifier is sampled by a switched capacitor sample-and-hold circuit. The voltage is stored on a capacitor buffered by a source follower with a column-shared current source. To prevent aliasing, the cut-off frequency of the LPF is set by the bias current of the first stage OTA.

D. Low-Noise Transconductance Amplifier

An important factor in the channel design is the amount of noise added by the sensing circuits. The challenge in designing a low-noise amplifier for this application is to optimize the noise performance given a small power budget. Figure 7 shows the circuit diagram of the OTA employed in each stage of the channel.

The input pair is chosen to be a p-channel MOS with a large gate area to minimize the flicker noise contribution. According to the circuit noise analysis presented in [13] and [15], the thermal noise component of the OTA can be reduced by biasing the input pair (\( M_{1,2} \)) in week inversion...
and the mirroring transistors \( M_{3-8} \) in strong inversion. Thus, the thermal noise contribution is optimized for a given current value. The thermal noise level can be further decreased by increasing the biasing current and thus the power consumption. Table I summarizes the size and the DC operating point for each transistor.

Figure 8 depicts the experimentally measured frequency response of a single channel configured for a nominal gain of 1000 (60dB). The solid line represents the measurements done with a spectrum analyzer. Due to limitations of the available measurement equipment, the high-pass corner frequency is estimated by applying a step signal at the amplifier input and observing the amplifier transient response time constant.

Figure 9 shows the experimentally measured input referred noise of one channel. The measurement is obtained by recording the noise spectrum at the output of the amplifier and referring it back to the input. The total RMS noise is 13\( \mu V \) over the 10Hz-10kHz bandwidth.

For experimental recordings the neural recording interface prototype is placed in a custom-manufactured fluidic chamber. The fluidic chamber is positioned on the surface of the chip package and attached to the top of a protective plexiglass box to form a hydraulic seal as shown in Figure 10. The testing printed circuit board generates necessary analog and digital signals, quantizes recorded neural data and sends the data to a personal computer through a high-speed digital interface. The recorded data are buffered and displayed in Matlab.

Figure 11 depicts an extracellular neural activity recording from a mouse hippocampus performed on one channel of the integrated neural interface prototype. Hippocampus was obtained from male Wilstar rats (5-25 days old). Animals were anesthetized with halothane and decapitated in accordance with the Canadian Animal Care Guidelines. The brains were

<table>
<thead>
<tr>
<th>Transistor</th>
<th>W/L (( \mu m ))</th>
<th>( I_D (nA) )</th>
<th>( g_m (\mu A/V) )</th>
<th>( g_m/I_D (V^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( M_{1,2} )</td>
<td>400/15</td>
<td>125</td>
<td>3.14</td>
<td>25.17</td>
</tr>
<tr>
<td>( M_{3-6} )</td>
<td>10/400</td>
<td>125</td>
<td>0.83</td>
<td>6.64</td>
</tr>
<tr>
<td>( M_{7,8} )</td>
<td>10/200</td>
<td>125</td>
<td>0.65</td>
<td>5.2</td>
</tr>
<tr>
<td>( M_9 )</td>
<td>45/45</td>
<td>250</td>
<td>3.79</td>
<td>15.16</td>
</tr>
<tr>
<td>( M_{cascN} )</td>
<td>18/8</td>
<td>125</td>
<td>3.1</td>
<td>24.8</td>
</tr>
<tr>
<td>( M_{cascP} )</td>
<td>25/8</td>
<td>125</td>
<td>2.65</td>
<td>21.2</td>
</tr>
</tbody>
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dissected and maintained in oxygenated ice-cold artificial cerebrospinal fluid (ACSF). The recording represents an epileptic seizure-like activity induced \textit{in vitro} in the presence of low $Mg^{2+}$ artificial cerebrospinal fluid (ACSF).

E. Frame Buffer

Accurate distributed multi-site sensing requires maintaining a high degree of correlation in time between all channels. Multi-site recording time-multiplexed architectures do not preserve cross-channel correlation unless the sampling frequency is much higher than the neural signal bandwidth. This necessitates a memory buffer in each recording cell to store the sampled signal. Frames of samples across the whole array are captured simultaneously. This eliminates the rolling delay during serial read-out. The local memory cell also allows for delaying high-noise on-chip digital switching until after a recording has been completed. Low-noise signal acquisition is time-multiplexed with high-noise peripheral switch capacitor signal processing and read-out. This ensures no high-amplitude switching activity during the signal acquisition phase and thus prevents substrate noise from coupling into the low-amplitude signal being acquired.

In order to validate the two-dimensional recording functionality of the array the following experiment was conducted. A drop of distilled water was placed on the surface of the 16 $\times$ 16 electrode array similarly to the one shown in Figure 12 (a) and driven by a 2mV peak-to-peak sinusoidal voltage. The stimulus signal was recorded at 5kHz sampling rate and displayed in real time as an ‘electronic video’ stream. Figure 12 (b) shows a two dimensional intensity map of a recording frame corresponding to a particular instantaneous value of the input sinusoid.

The experimentally measured characteristics are summarized in Table II. The measured core power dissipation of 6mW on the $3 \times 4.5 \, \text{mm}^2$ die area falls within the limits of power density considered safe for brain tissue [16], [17].

III. Conclusions

We have presented the architecture and VLSI implementation of an integrated neural interface for simultaneous recording of distributed neural activity. A $3 \, \text{mm} \times 4.5 \, \text{mm}$ integrated prototype was fabricated in a 0.35 $\mu$m CMOS technology. 256 100 $\mu$m low-cost Au electrodes were fabricated directly on the surface of the chip for high-resolution electronic imaging of neural activity in acute brain slices. The microsystem was validated in extracellular \textit{in vitro} recordings from a mouse hippocampus.

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References


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