7.7 A 256×256 CMOS Microelectrode Array for Extracellular Neural Stimulation of Acute Brain Slices

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Extracellular stimulation of neurons is an important tool in investigating the function of the nervous system. Optical techniques, based on voltage- and calcium-sensitive dyes or photouncaging, along with multi-photon fluorescent microscopy have proven very successful in imaging activity in slices and *in vivo*. However, studies have been limited by the ability to stimulate different regions of tissue with enough spatial resolution and throughput. Traditional stimulation is accomplished with passive multielectrode arrays (MEAs) or bipolar electrodes. In both cases, a relatively small number of stimulation sites with coarse spatial resolution are possible. While there has been recent work on the development of CMOS chips for extracellular recordings of cultured neurons or slices on planar electrodes [1], the focus of this work is on stimulation and achieving stable electrical interfaces between acute slices and a high-density active CMOS MEA. As brain slices preserve many synaptic connections, they are ideal preparations to study neuronal microcircuits *in vitro*. Active stimulation technologies should enable detailed "reverse engineering" of neural circuitry.

The active stimulation chip was fabricated in 2.5V 0.25μm CMOS. The 4×4mm² die (Fig. 7.7.1) has a 256×256 pixel array with a total stimulation area of 3×3mm². Each square electrode has an edge length of 11.4μm and a pitch of 12.2μm. The design is aimed at high spatiotemporal resolution, with 6,724 electrodes per mm², each electrode capable of producing a unique stimulation pulse with variable amplitude ranging from 0.7V to 4.2V.

Figure 7.7.2 shows the chip architecture. The array is divided into 8 banks of electrodes with each bank containing 32 pixel rows. A 3:8 decoder uniquely selects one of the eight banks and an 8:256 column decoder selects one of the 256 columns in the pixel array. A 32b data line runs across the 32 pixel rows within each bank and is duplicated for each bank. The data lines are connected to the rows using tristate drivers with weak pull-up devices. An individual bank's architecture is shown in Fig. 7.7.3, where every 32 pixels are combined into one 32×1 block uniquely addressable by *bank_enb* and *column_enb* signals.

Each electrode is driven by a pixel cell with cell circuitry shown in Fig. 7.7.2. Each pixel contains two memory cells to allow the cell to be "loaded" while the previously loaded value is applied to the electrode. Control signals periodically switch the whole array between load and execute modes. During load, S_1 closes and S_2 opens, allowing the data line to write the first memory cell. During execute mode, S_1 opens and S_2 closes, enabling the transfer of the contents of the first memory cell into the second. The output of the second memory cell is connected through two inverters to the electrode pad. The supply voltage of the second inverter is variable, allowing independent control of the stimulation voltage amplitude.

The fabricated chip has an over-glass cut opening in the passivation layers on top of each electrode that creates a non-planar profile with the electrodes recessed. Post-processing is needed to bring the electrodes to the surface. With a photoresist mask, the residual 1.5μm thick Si_3N_4-SiO_2 stack is removed through dry etching. Figure 7.7.4 shows two images of the electrode surface before and after dry etching: only 85nm of residual profile is evident on the electrode due to partial etch of the Al in the exposed central region. A 20nm thick hafnium oxide (HfO_2) layer is then deposited by atomic-layer-deposition onto the exposed pads so that Faradaic processes at the electrode are blocked and coupling to the brain slice is purely capacitive, where voltage pulse stimuli translate into biphasic displacement currents. The currents produce gradients of extracellular potentials that elicit activities from the neurons [2]. HfO_2 was chosen for this application for its biocompatibility, high dielectric constant (16 to 18), and low leakage current (<0.1nA/μm²).

The post-processed chip is wire bonded to a ball-grid-array package. The bonding wires are encapsulated in thermally-cured epoxy with the surface of the array exposed. The packaged chip is inserted into a surface mount PCB socket with an open-center cover where the opening serves as a slice chamber. A 1mm-thick polydimethylsiloxane sheet seals the packaged chip with the socket cover to prevent electrolyte leakage. A custom-made PCB board interfaces the chip to a PC, where a software interface panel prepares the data file for signals clocked into the chip.

The conducted experiments combine simultaneous electrical stimulation from the chip and optical imaging recording, where calcium indicators are used as a measure of intracellular calcium concentration to provide an indirect measure of action potentials [3]. To promote charge-based adhesion between acute slices and the electrode surface, the chip is coated with diluted 0.1% poly-L-lysine. Thalamocortical slices are made from postnatal day 14 mice at 250μm thickness. For staining, the Ca^{2+} fluorescent indicator fura-2 AM is bulk-loaded into brain slices. Imaging is done using a 380nm excitation filter and a 510nm emission filter with a 20× objective in an upright fluorescence microscope.

In each experiment, a large population of neurons is imaged to discern network activity in the slice to pre-programmed electrical stimuli. Figure 7.7.5 shows the artifact (captured by a unipolar AgCl electrode) produced by 14 stimuli from the chip. During each pulse (when a 3.8V amplitude is applied), approximately 3.8pC of charge is delivered to the slice per electrode. The bottom trace (a temporal profile of average brightness of all pixels in the field of view), with the first stimulus pulse applied at 700ms, illustrates a characteristic and significant change in calcium indicator dye fluorescence as the result of activation of all electrodes. The dip with fast onset and slow offset indicates induced action potential activity in neurons, a hallmark of a successful extracellular stimulation. In the same experiment, the map shown in Fig. 7.7.6 demonstrates that nearly 60% of the neurons were activated during stimulation, a percentage not easily obtained using traditional stimulation methods.

More important experiments were carried out selectively stimulating one section of the array (and slice) and imaging activity induced elsewhere. Figure 7.7.7 shows the fluorescence change over time with enhanced contrast $\Delta F/F_0=(F_1-F_0)/F_0$ images (pixel-wise subtraction of each frame from the first frame where the difference normalized to the first frame) resulted from applying stimuli to electrodes on the left half of the array and imaging cortex on the right half with a 4× objective at 66ms/frame. The time-series of frames show action potentials progressing from left to right within the cortex indicating the spatiotemporal pattern of activities in a large population of neurons. The high spatial resolution of this stimulation MEA promises the possibility for few-cell stimulation which is the focus of ongoing experiments.

Acknowledgements:
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References:
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[3] D. Smetters, A. Majewska, and R. Yuste, "Detecting Action Potentials in Neuronal Populations with Calcium Imaging," *Methods*, vol. 18, pp. 215-221, 1999.

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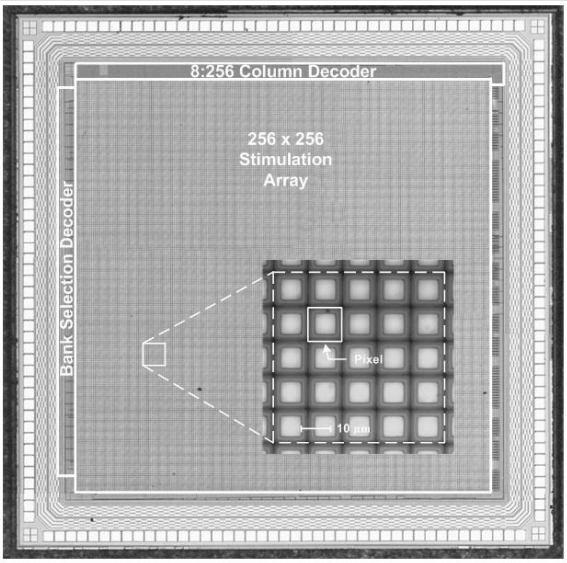

Figure 7.7.1: Chip micrograph and microscope capture of the electrodes.

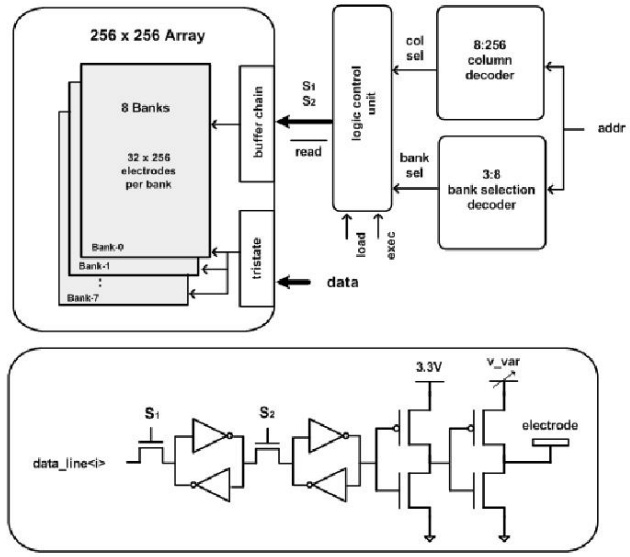

Figure 7.7.2: Chip block diagram and pixel cell schematic.

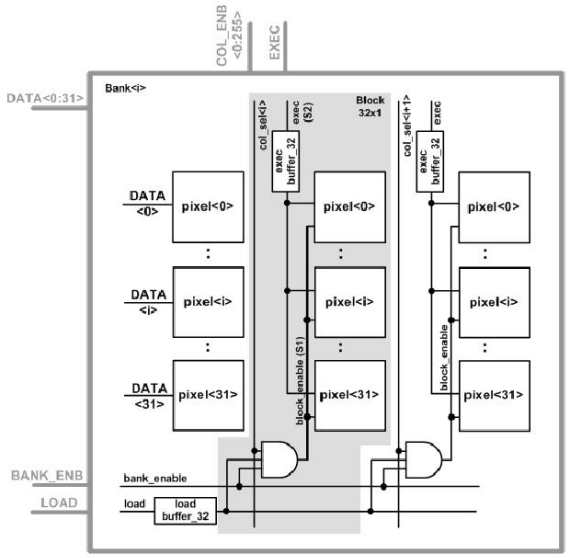

Figure 7.7.3: CMOS stimulation array individual bank architecture.

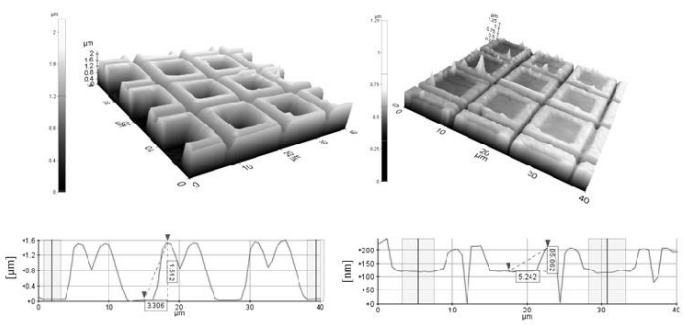

Figure 7.7.4: Atomic force microscope profilometry of the electrodes: before planarization (top left), after planarization (top right), and z-direction profiles for each case (bottom).

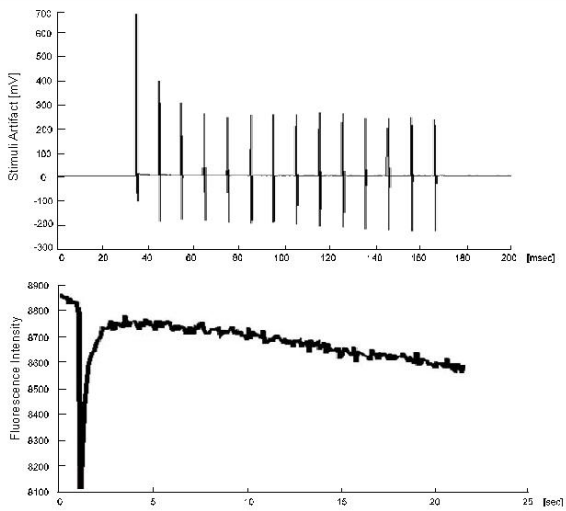

Figure 7.7.5: Stimulation artifacts measured using field electrode (top) and frame-wide average calcium indicator dye fluorescence intensity profile (bottom).

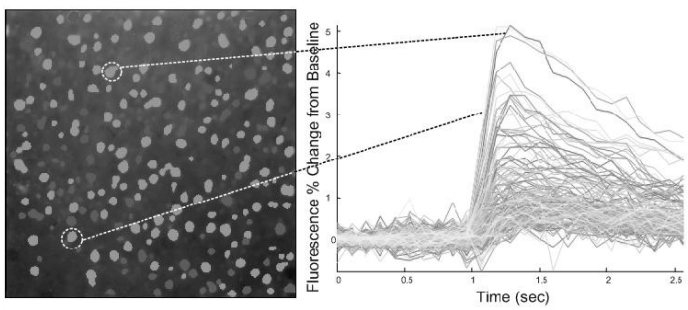

Figure 7.7.6: Map of neurons clustered into activated (bright spot) and non-activated (dark spot) groups based on individual calcium indicator intensity profiles (left). Brightness profiles of all neurons in the movie (right).

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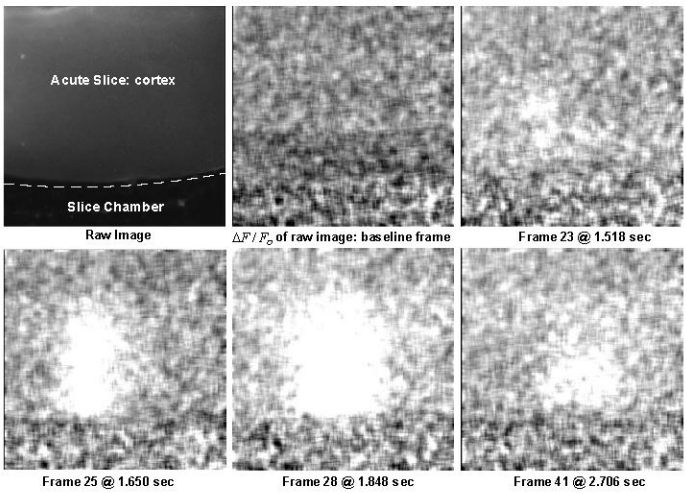

Figure 7.7.7: Time series of enhanced contrast $\Delta F/F_0$ frames from movie of acute cortical slice with MEA stimulation. White areas indicate regions with increased calcium concentrations inside neural tissues.

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