Final Report to Catalyst Foundation
Project title: CMOS-assisted nano-bio array for neurotechnology
Reporting period: February 2014 – January 2019

Jeffrey Abbott[§,†,¶], Donhee Ham[¶], and Hongkun Park[§,†]
[¶]School of Engineering and Applied Sciences, Harvard University
[§]Department of Chemistry and Chemical Biology, Harvard University
[†]Department of Physics, Harvard University

Traditional electrophysiology tools are not capable of both intracellular and network-level parallel recording. The patch clamp technique can perform intracellular recording but it is not suited for parallel recording due to the lack of scalability. The microelectrode array boasts parallel recording, but because the microelectrodes are too large to penetrate into live neurons, their recording is limited to extracellular signals with much lower sensitivity. A tool that could record intracellular potentials across a large electrogenic cellular network can open up new exciting possibilities. The goal of this project is to develop an array of vertical nanoelectrodes, as first investigated by the principal investigator Park [1], [2], on the surface of a CMOS integrated circuit (IC), where the electrodes would allow intracellular access while the CMOS electronics—an array of analog amplifiers and stimulators—right underneath the electrode array would facilitate the parallel operation of the electrode array and enhance the recording sensitivity [3]. In this way, we seek to achieve network-level intracellular recording across an electrogenic cellular network.

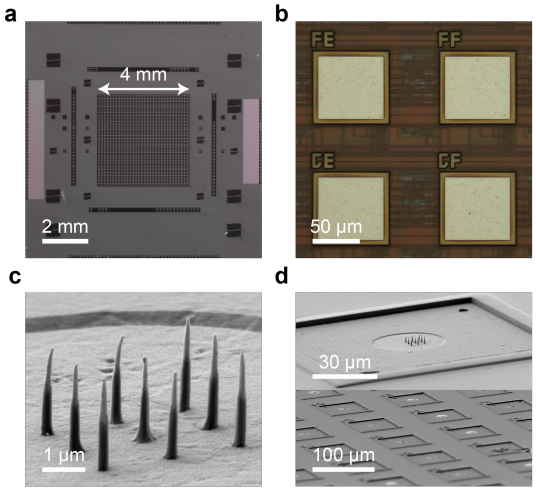

Figure 1. (a) CMOS IC in 0.35 µm technology with a 32×32 pixel array at the center. (b) 4 example pixels. (c, d) 9 vertical nanoelectrodes post fabricated per pad.

Our 1st-generation CMOS nanoelectrode array (CNEA), a major outcome of this project, demonstrated parallel intracellular recording with *in vitro* cardiomyocytes [4]. The CNEA is based upon a CMOS IC fabricated in 0.35 µm technology (Fig. 1a) which contains an array of 32×32 = 1,024 pixels with a 126-µm pitch (four pixels shown in Fig. 1b). Each pixel consists of an amplifier to record electrophysiological events, a stimulator to manipulate membrane potential, and a memory to switch the pixel

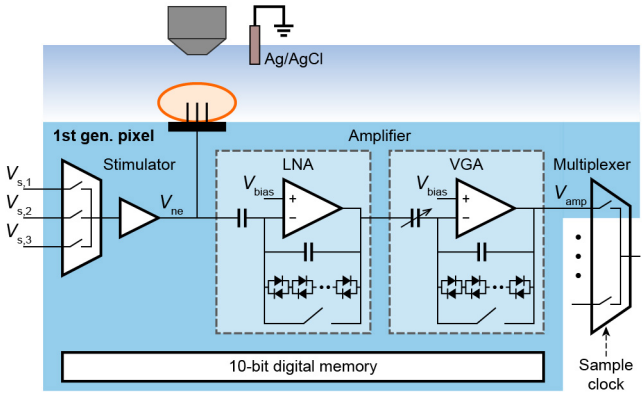

Figure 2. The critical elements of an individual pixel are an amplifier (LNA + VGA) and a stimulator.

operation between recording and stimulation (Fig. 2). The amplifier (described in [5]) and stimulator are connected to a metallic pad on the IC surface in which nine vertical nanoelectrodes are post-fabricated (Fig. 1c-d). The tips of the electrodes are Pt and are electrically connected together, and to the pad, to reduce the access impedance.

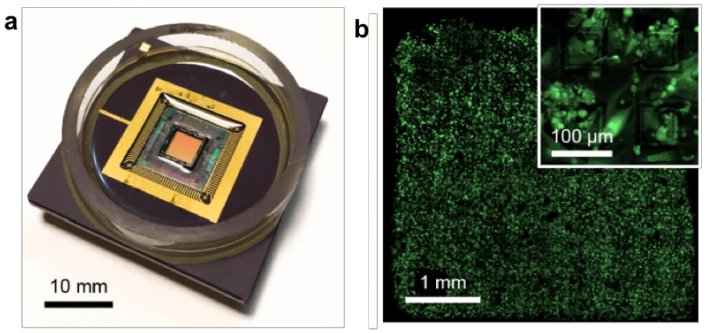

Figure 3. (a) Packaged CNEA. (b) Fluorescence image of cardiomyocytes cultured on the CNEA.

Using neonatal rat cardiomyocytes *in vitro* cultured on the packaged CNEA (Fig. 3), we experimentally confirmed the parallel intracellular recording ability of the CNEA [4]. Figure 4a, top, shows the extracellular recording readily available to the CNEA before electroporation. We then alter the CNEA operation into the intracellular mode, by applying simultaneous electroporation signals all across the pixel array. In the cardiomyocyte tissue, we initially observed a spiral pattern of action potential propagation around the periphery with a conduction velocity of 2 cm/s, but with time, we start to record the intracellular signals from cells at the central part of the tissue as well, and the number of intracellularly recorded cells simultaneously increases to 235 at 48.3 s after electroporation (Fig. 4b, right). The propagation pattern and the conduction velocity of action potentials then return to their original, pre-electroporation, values within a few minutes. A total of 364 pixels were intracellularly coupled at least once over the recording time, demonstrating 36% overall intracellular coupling efficiency. Moreover, we used intracellular membrane recordings from hundreds of connected myocytes in a connected tissue to investigate the effects of pharmaceuticals on the fine network dynamics (Fig. 4c): we measure the effects of a drug application (ATX-II) to the cardiac tissue in which local polarization dynamics in different regions of the tissue were observed to induce an

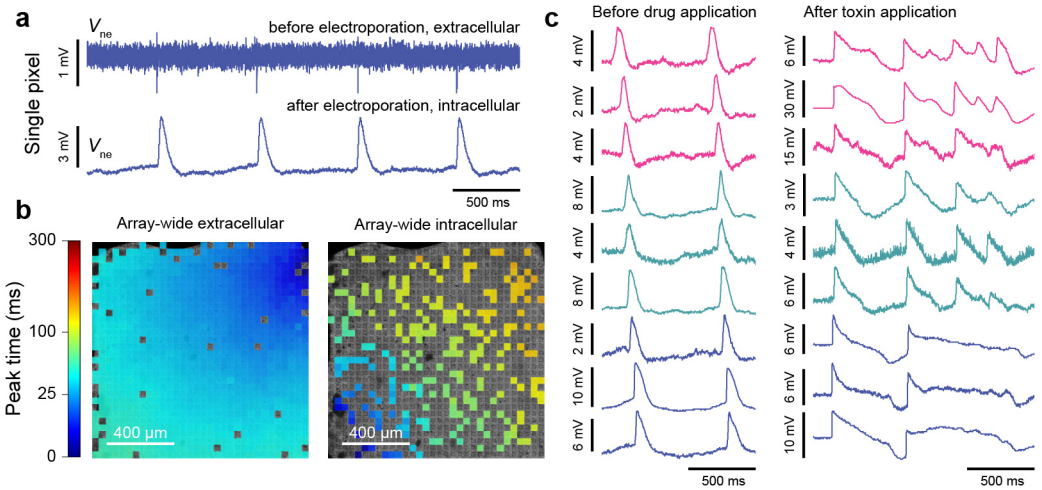

Figure 4. (a) Extracellular and intracellular recordings of cardiac action potentials from a cardiomyocyte before and after electroporation. (b) Tissue-wide intracellular recordings can be performed across the array; over 300 pixels (~30% coupling rate) record intracellular signals. (c) The intracellular and network effects of drugs on the cardiac tissue can be measured: after application of a sodium ion channel toxin, ATX-II, significant AP widening and subthreshold early afterdepolarizations (EADs) are observed in some regions of the array, inducing a tissue arrhythmia.

arrhythmia. This network-level intracellular access could enable new opportunities in tissue-based pharmacological screening for cardiac diseases as well as fundamental studies of electrogenic cells and their networks.

There is much room for advancement beyond this 1st generation device. In fact, we have been developing the 2nd generation device for higher sensitivity and spatial resolution, and a range of electrophysiological investigations are underway [6].

References

[1] J. T. Robinson, M. Jorgolli, A. K. Shalek, M.-H. Yoon, R. S. Gertner, and H. Park, "Vertical nanowire electrode arrays as a scalable platform for intracellular interfacing to neuronal circuits," Nature Nanotech., vol. 7, no. 3, pp. 180–184, 2012.

[2] J. T. Robinson, M. Jorgolli, and H. Park, "Nanowire electrodes for high-density stimulation and measurement of neural circuits," Front. Neural Circuits, vol. 7, no. 38, 2013.

[3] J. Abbott, T. Ye, D. Ham, and H. Park, "Optimizing Nanoelectrode Arrays for Scalable Intracellular Electrophysiology," Acc. Chem. Res., vol. 51, no. 3, pp. 600–608, Feb. 2018.

[4] J. Abbott, T. Ye, L. Qin, M. Jorgolli, R. S. Gertner, D. Ham, and H. Park, "CMOS nanoelectrode array for all-electrical intracellular electrophysiological imaging," Nature Nanotech., vol. 12, no. 5, pp. 460–466, Feb. 2017.

[5] J. Abbott, L. Qin, T. Ye, M. Jorgolli, R. S. R. S. Gertner, H. Park, and D. Ham, "CMOS electronics probe inside a cellular network - Invited review paper," in 2018 IEEE Custom Integrated Circuits Conference, CICC 2018, 2018, pp. 1–7.

[6] J. Abbott, T. Ye, K. Krenek, R. Gertner, S. Ban, Y. Kim, L. Qin, W. Wu, H. Park, and D. Ham, "Intracellular Recording of Thousands of Connected Neurons," submitted 2018.