Optical Control of Neural Activity with Amorphous Silicon Light Addressable Electrodes

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Abstract*:* We present an optically addressed electrical stimulation device for neuron control with single cell resolution and millisecond temporal resolution. This system allows dynamic study of interconnected neural network at single neuron level.
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1. Introductions

Cultured neurons are extensively used to study key neuronal physiological properties such as synaptic plasticity, neuronal growth and differentiation, as well as the network properties of simple circuits. Many of these processes are modulated by neuronal activity. Tools that allow simultaneous control and monitoring of neural activity are desired. Micro-fabricated electrodes have been commonly used to excite neurons; however, it is constrained by the fixed spatial position of the electrodes. Optical excitation of neurons allows true random-access of densely interconnected neurons with high spatial and temporal resolution. In this paper, we report for parallel single cell stimulation by using a thin layer of amorphous silicon functions as the light addressable electrode [1]. Unlike other optical neural excitation technique such as light-gated proteins [2] and caged compound [3], there is no need for either genetic or chemical delivery. Incorporating a digital micromirror device, virtual electrodes can be located anywhere on the culture and in any desired shape, and allow parallel control of a large number of cells. We show that single neurons are activated selectively, and action potentials can be generated with millisecond temporal precision. This method is a promising mean for remote control of neuronal activity.

2. Device Principle and Setup

The light addressable electrode consisted of 1μm thick of hydrogenated amorphous silicon (a-Si:H) deposited by PECVD on Indium Tin Oxide (ITO)-coated glass (Fig. 1). E18 hippocampal neurons were plated directly on to the a-Si:H surface after oxygen plasma cleaning and poly-L-lysine coating. The neurons were cultured for at least 7 days before experiments. The stimulation light was controlled by a Digital Micromirror Device (DMD) and focused by a 20x objective lens. Voltage pulses were applied between the ITO electrode and an AgCl electrode immersed in the media. Typical applied voltage pulses have 1ms pulse-width at 5Hz frequency.

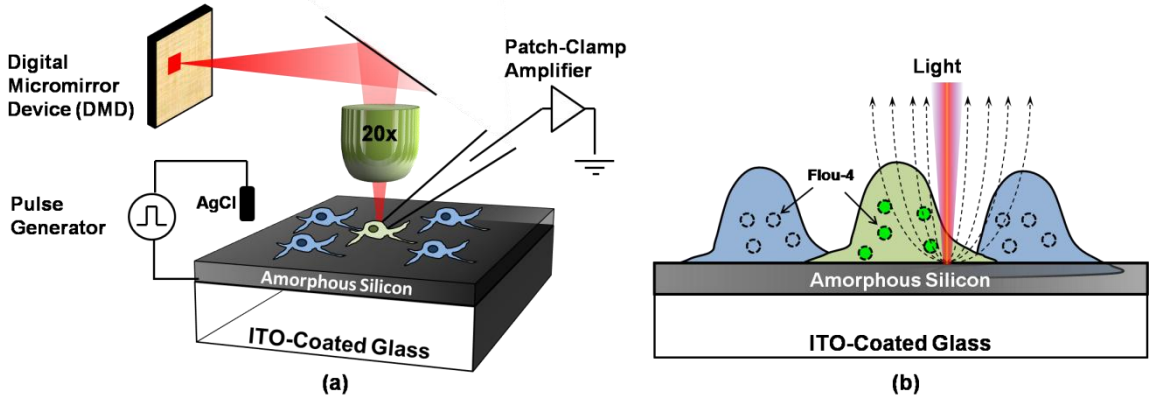

Fig 1. (a) Schematics of the optical neural stimulation setup. DMD provides dynamic light addressing capability. The open top design allow patch-clamp recording. **(b) Schematics of device operation.** Light induces local electric field, which depolarize the nearby neurons. The depolarization causes increase of Ca^{2+} in the cell, and the event can be monitored by Flou-4 fluorescent intensity increase.

Triggered neural activities were monitored by two approaches: Patch-clamp recording and calcium indicator dye: Flou-4. Patch-clamp allows direct electrical access of individual cells, and is ideal for detailed characterization of neuron response toward stimulus. However, patch-clamp is difficult to scale up to more than a few patches at a

time. On the other hand, calcium indicator dyes allow monitoring neural activity on a population basis but has a slower temporal resolution of hundreds of milliseconds. When neurons are excited, the calcium concentration inside of cell increases dramatically. Preloaded dye in the cells responds to the change and increases its fluorescent intensity (Fig. 2b). We utilized patch-clamp recording to characterize the temporal resolution of the stimulation protocol, and calcium indicator dye to demonstrate single cell control in an interconnected neural network.

3. Results

Patch-clamp recording indicates that single action potentials were reliably generated after each applied voltage pulses without any delay (Fig. 2a). Further, action potentials were only observed with optical illumination, and no action potential was observed in the dark. The results suggest precise numbers of action potential can be optically controlled. We also investigated effect of electrical pulse duration with Ca^{+2} responses (Fig. 2b). Cell showed optimal responses to pulse width of 0.5ms and 1ms, and no response with pulse width shorter than 0.2ms.

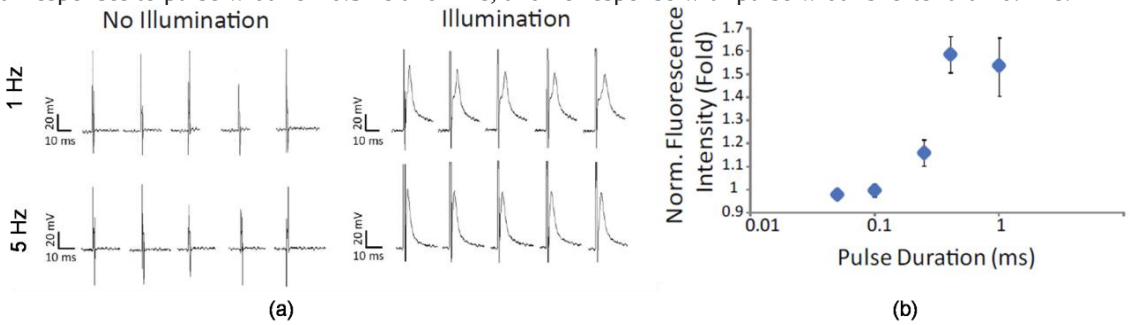

Fig. 2(a) Generation of single action potential. Electrical stimulation was applied at 1Hz (upper) and 5Hz (lower). Under illumination action potentials were reliably triggered by both 1Hz and 5Hz pulses. **(b)** Tests of pulse width showed that 0.5-1 ms were optimal for stimulation.

To demonstrate single cell control, four different spots were illuminated sequentially while the electrical bias was active (Fig. 3), while the spots location was controlled by the DMD. Each illumination spot excited only one single neuron nearby. The result shows the technique has single cell level spatial control in a dense neural cell culture.

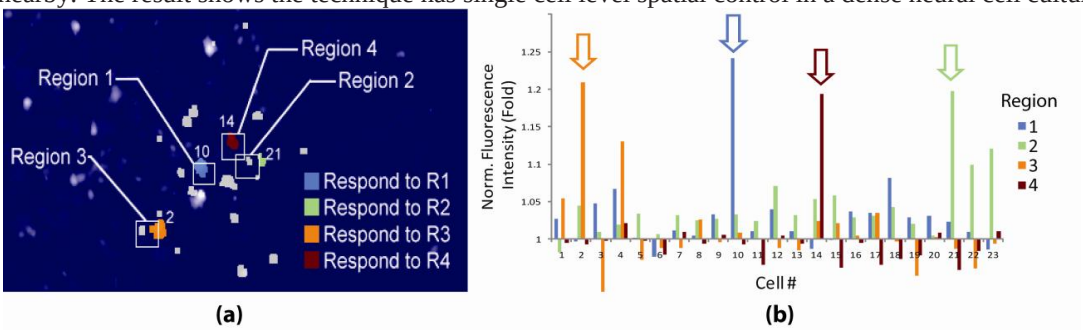

Fig. 3 Single Cell Stimulation (a) An image of Flou-4 stained neurons. Live cells are shown in grey colors. Four regions were illuminated to stimulate neurons. The cells responded to each illumination were color coded and shown on the image. Only cells near the illumination spots were stimulated. **(b)** Average Ca^{2+} intensity 10s after illumination for all cells. Cells responded to the stimulation are indicated.

4. Conclusion

We have developed a device that enables optical control of neural activity with light addressable electrodes on hydrogenated amorphous silicon. It achieves spatial control at single cell level and a temporal resolution of a millisecond. We believe that such devices add a new dimension to cultured neurons systems that facilitates the investigation of cellular neurobiology.

5. Reference

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