Optogenetic Neural Probes: Fiberless, High-Density, Artifact-Free Neuromodulation

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***Abstract*—**Evolution of "Michigan Probes" has enabled unraveling the connectivity of neurons at cellular resolution and their dynamic interaction within and across brain areas that underlie behavioral and cognitive functions. Recently, micro-LEDs were monolithically integrated for optogenetic neuromodulation at scale without requiring external optical fiber connection. The 'hectoSTAR' micro-LED optoelectrode features 256 recording electrodes and 128 stimulation micro-LEDs in four silicon micro-needle shanks, covering a large volume with 1.3-mm × 0.9-mm cross-sectional area located as deep as 6 mm inside the brain. Technical challenges to overcome in scaling of optogenetic probes will be discussed, followed by future direction.

I. Introduction

For the past years, optogenetics has transformed neural circuit analysis by introducing photosensitive proteins (opsins) into specific cell types, so that these cells can respond to an optical stimulus with well-defined action potential patterns [1]. Using appropriate wavelengths to target a particular opsin, cell-type specificity can be achieved with well-controlled temporal resolution. For example, when channelr-hodopsin-2 (ChR2) is expressed in the specific cells, the excitation of target neurons can be induced using illuminating blue (~473 nm) light. This combinatorial cell-specific targeting allows sophisticated manipulations of neural activity.

Early work relied on manual assembly of metal electrodes or passive high-density probes with optical fibers, which are bulky and may suffer from misalignment errors. More recently, various engineer efforts have been made for miniaturization and scaling of optogenetic probes to overcome the challenges such as stimulation artifact and tethering problem of optical fibers in freely-moving animal studies. This paper presents the advancement of Michigan probes toward high-density integration of optical light sources that can be precisely deliver light to the target region of brain, while simultaneously recording neural activities in behaving animals. Suppression of stimulation artifact is discussed in the implementation of fiberless optical stimulation at large scale.

II. Fiberless Approaches: Waveguide vs. LEDs

Initial optogenetic studies were conducted by using an optical fiber implanted into a rodent brain (Fig. 1) [1,2]. Light was illuminated from an external light source; therefore, illumination intensity can be easily controlled and wavelengths can be instantly altered. However, the number of illumination sites is not scalable and rigid fibers cause tethering problems in chronic behaving animal experiments. Two approaches were introduced to attain fiberless optical stimulation. One is bringing light to the distal end of optical waveguides built on a probe shank and the other is directly integrating micro-LEDs close to the recording sites.

A. Waveguide Optoelectrodes

Monolithic integration of optical waveguides on a silicon probe shank can precisely position the illumination sites with respect to the recording sites [3,4]. Still many approaches need an optical fiber to bring light from the external source. Fiberless waveguide optoeletrodes can be realized by incorporating laser diodes (LD) in the backend of a headstage and coupling light into the waveguides integrated on the probe. A four-shank dual-color optoelectrode was demonstrated with eight LDs (four red and four blue LDs) coupling to dielectric optical mixer waveguide vis gradient-index (GRIN) lenses [5]. By selectively turning on the LDs, dual-color optical stimulation was demonstrated to achieve excitation and inhibition of pyramidal neurons in the hippocampus of awake mice (Fig. 2 and 3). Scaling is limited by the miniaturization of waveguides that can be integrated in a narrow shank.

B. Micro-LED Optoelectrodes

The most scalable method would be monolithically integrating microscopic light sources directly on a probe shank. Micro-LEDs in a neuron size (~ 10 μm) were fabricated from GaN-on-silicon wafers along with recording electrodes, followed by micromachining of silicon substrate to form a think narrow probe shank. This approach is highly scalable and a multi-shank optogenetic probe was demonstrated to provide spatially confined optical stimulation of simultaneously monitored neurons in behaving animals (Fig. 4) [6]. A four-shank probe has a total of 12 micro-LEDs and 32 recording

electrodes, all monolithically integrated on the probe tips to cover a 200 μm vertical span (Fig. 5). The electrodes have a vertical pitch of 20 μm, arranged in a high-density cluster designed to identify single units from a highly populated brain structure. At the center of each octo-electrode cluster, a linear array of three micro-LEDs with a 60-μm pitch is integrated. Each micro-LED has an emission area of 150 μm^2 (10 μm x 15 μm). The micro-LED is less than 0.5 μm thick, which is at least an order of magnitude thinner than optical fibers or integrated waveguides for reduced insertion damage.

The spatiotemporal precision of parallel stimulation and recording was demonstrated for cellular level analysis in deep structures in the brain of intact animals (Fig. 6). Independent control of distinct cells ~50 μm apart was achieved as well as the differential somato-dendritic compartments of single neurons. Currently, the micro-LED optogenetic probes have been disseminated to the neuroscience community under the NSF NeuroNex Hub at Michigan [7].

III. Scaling for High-Density

A. Stimulation Artifacts

Scaling of micro-LED optogenetic probes toward high-density inevitably introduces stimulation artifact. This undesired artifact mainly comes from the signal coupling between the LED driving traces to the recording traces. In order to enable precise detection of neuronal activities, the magnitude of the stimulation artifact should be reduced to lower than a threshold voltage level for neuronal activity detection. A minimal-stimulation-artifact (miniSTAR) micro-LED opto-electrodes was developed to enable effective elimination of stimulation artifact [8]. A multi-metal-layer structure was adopted to provide a shielding layer that effectively suppresses capacitive coupling of stimulation signals (Fig. 8). In addition, A heavily-boron-doped silicon substrate was used to suppress the photovoltaic effect induced from LED illumination. With transient stimulation pulse shaping, we reduced stimulation artifact on miniSTAR micro-LED optoelectrodes to below 50 μV_{pp}, much smaller than a typical spike detection threshold, at optical stimulation of > 50 mW/mm^2 irradiance. We demonstra ted high-temporal resolution (< 1 ms) optoelectrophysiology without any artifact-induced signal quality degradation during *in vivo* experiments [8,9] (Fig. 8).

B. High-Density micro-LED Optoelectrodes

A high-density optogenetic probe was developed to provide simultaneous electrical monitoring and optogenetic manipulation of deep neuronal circuits at large scales with a high spatiotemporal resolution (Fig. 9) [10]. The 'hectoSTAR' micro-LED optoelectrode features 256 recording electrodes and 128 stimulation μLEDs monolithically integrated on the surface of its four 30-μm thick silicon micro-needle shanks, covering a large volume with 1.3-mm × 0.9-mm cross-sectional area located as deep as 6 mm inside the brain (Fig. 10). The fabricated hectoSTAR probe is hybrid assembled with a custom integrated circuit in a compact headstage for fully programmable digitized signal interface (Fig. 11).

IV. Flexible High-Density Optoelectrodes

Long-term (weeks to months) recording and optogenetic stimulation present a key challenge in chronic behavioral animal studies. The penetration volume and the mechanical stiffness, which comes from the penetration of rigid silicon shanks, must be reduced to mitigate foreign-body reaction and tissue damage due to micromotion effect. Recently, ultra-flexible "Michigan type" optoelectrodes with monolithically integrated cell-sized micro-LEDs onto a polyimide substrate was developed (Fig. 12). This differs from die transfer approaches which require manual assembly of micro-LEDs on a flexible substrate. The first prototype incorporated total 12 soma-sized micro- LEDs and 32 recording electrodes. The shank substrate is polyimide with 115 μm in width and 12 μm in thickness. A fully assembled flexible micro-LED probe was implanted in a transgenic mouse. The probe was inserted into the dorsal hippocampus using a glass pipette as a mechanical shuttle. Opto-electrophysiology recordings started 2 weeks after the surgery and light-induced neuronal activity was successfully recorded at day 18 and day 26 with the same LED being stimulated.

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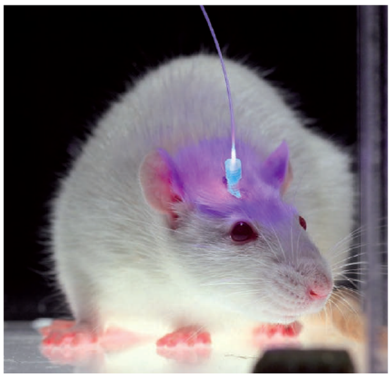

Fig. 1. Optical stimulation of neurons by light delivered from an optical fiber.

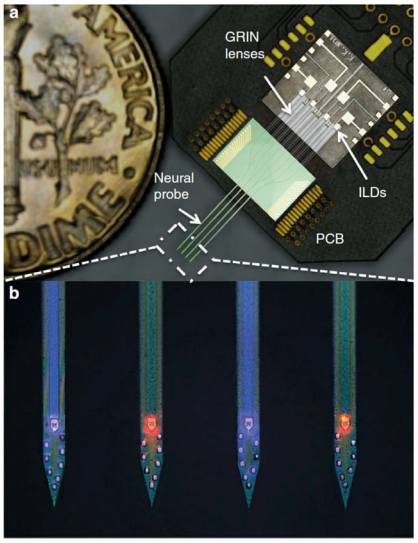

Fig. 2. Dual-color waveguide optoelectrodes. (a) Assembled device prototype and (b) Enlarged view of the probe shank tips with dual color light illumination.

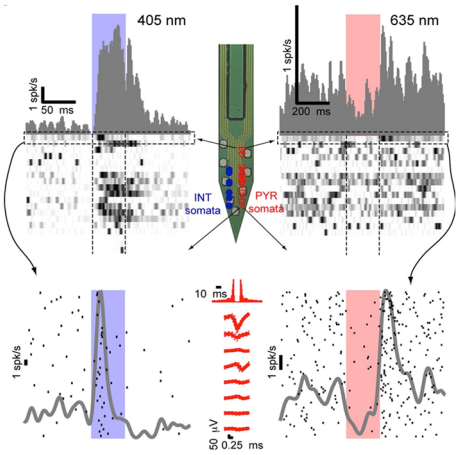

Fig. 3. Bi-directional control of pyramidal cells in the intact mouse by switching the wavelength between 430 nm (blue) and 635 nm (red). Spiking activities were increased during 405 nm light, while decreased during 635 nm light.

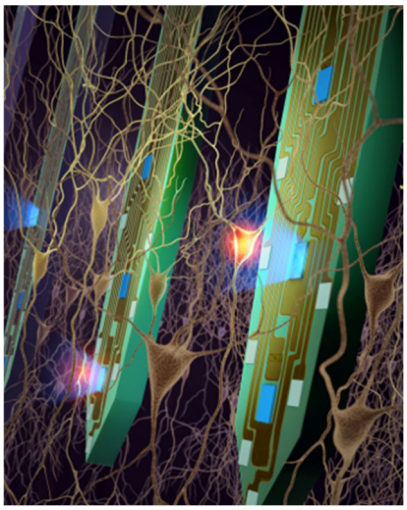

Fig. 4. Conceptual diagram of monolithically integrated micro-LEDs on silicon probe shanks.

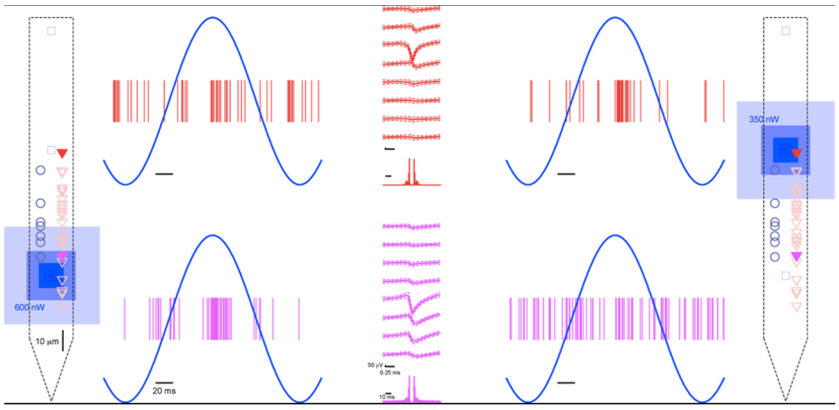

Fig. 6. Selective optical stimulation of individual neurons at sub-microwatt illumination. Two micro-LEDs in 60 μm apart can distinguishably stimulate two pyramidal neurons (red and pink) separated by 50 μm.

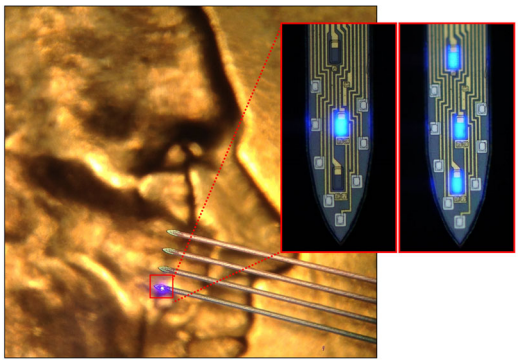

Fig. 5. Micro-LED optogenetic probes with four shanks that contain a total of 12 micro-LEDs and 32 recording electrodes. Micro-LEDs can be selectively controlled in a high spatial-temporal resolution.

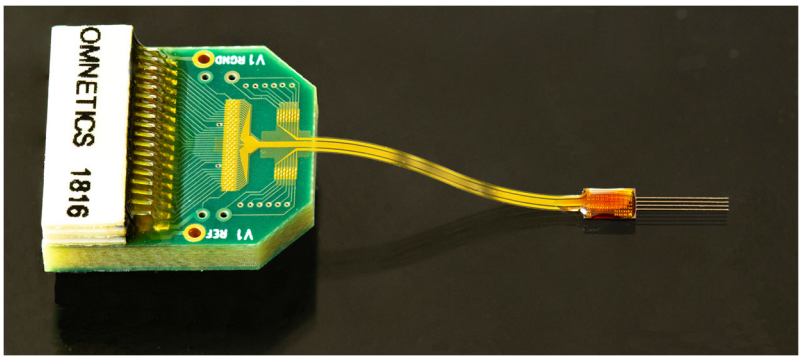

Fig. 7. Assembled micro-LED optogenetic probes for chronic animal studies.

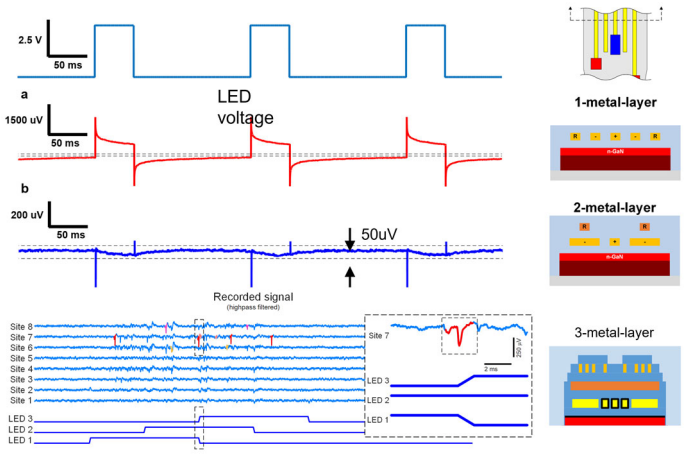

Fig. 8. Stimulation artifact reduction from metal shielding. By stacking three layers of metal, artifact was removed below signal noise level, so that the spikes firing during the LED signal transition can be monitored without compromise.

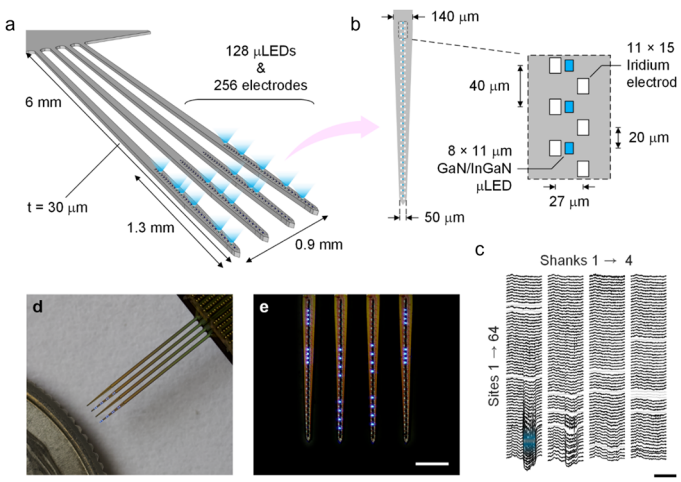

Fig. 9 HectoSTAR micro-LED optoelectrodes for high-precision, large-scale electrophysiology. It contains total 256 recording sites and 128 micro-LEDs.

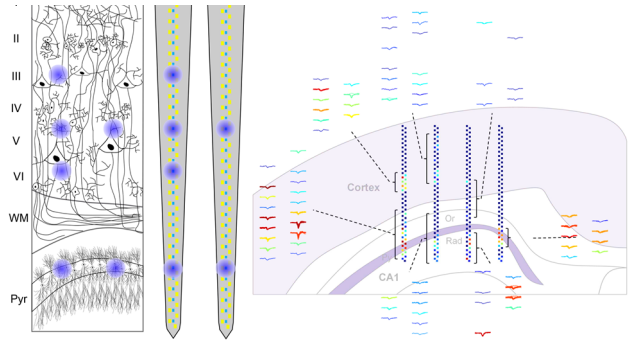

Fig. 10. Coverage of hectoSTAR micro-LED probes from Cortex to hippocampus with a 1.3-mm × 0.9-mm cross-sectional area located as deep as 6 mm inside the brain.

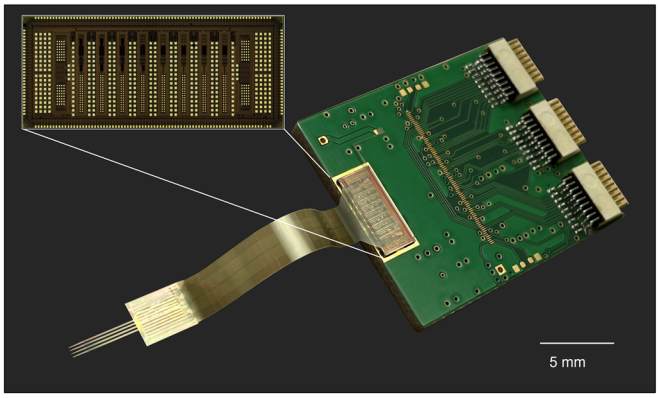

Fig. 11. Assembled hecto-STAR micro-LED optoelectrodes in a hybrid package with a custom interface circuit chip for fully programmable digitized signal interface.

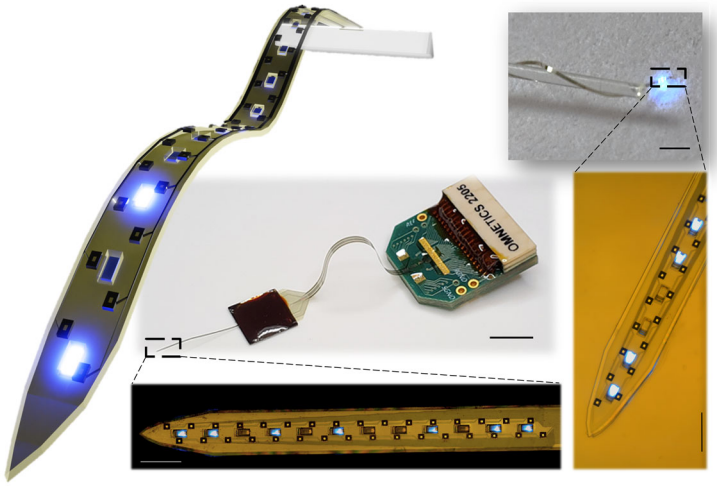

Fig. 12. Ultra-flexible micro-LED optoelectrodes with monolithically integrated cell-sized micro-LEDs onto a polyimide substrate.