

A SILICON NEURAL PROBE MONOLITHICALLY INTEGRATED WITH 20 μ M-PITCHED DUAL-COLOR MICRO-LED ARRAYS

Dacheng Mao^{1*}, Feng Sun^{1*}, Bradley Driscoll^{2*}, Zhihao Li¹, and Guangyu Xu^{1,3}

¹Department of Electrical and Computer Engineering, University of Massachusetts, Amherst, USA

²Department of Psychological and Brain Sciences, University of Massachusetts, Amherst, USA

³Department of Biomedical Engineering, University of Massachusetts, Amherst, USA

(* These authors contributed equally)

ABSTRACT

Optogenetics is a powerful neuromodulation strategy that features temporal precision, cell-type specificity, and bi-directionality (i.e. the capability to excite and inhibit the same neurons via different colors of light). Limited by feasible integration methods, neural probes integrated with arrays of dual-color light sources remain underdeveloped, hindering bidirectional optogenetic control of brain activity with high spatial resolutions. Here we report, based on heterogeneously stacked III-V films, the first silicon neural probe monolithically integrated with 20- μ m pitched dual-color micro-LED arrays, which enabled depth-dependent bi-directional *in vivo* optogenetic electrophysiology for the first time.

KEYWORDS

optoelectronics, neuroengineering, optogenetics, brain science, electrophysiology.

INTRODUCTION

With the emergence of optogenetics, optical control over cell activity has been noted for its combined advantages of temporal precision, cell-type specificity, and bi-directionality over other ways of cell modulation. In this method, targeted cell types (e.g. neurons) expressed with light sensitive proteins can excite or inhibit their activities (e.g. spiking) when they are exposed to light of different colours at millisecond precision [1]-[3]. The capability of bi-directional neuromodulation not only offers an alternative approach to dissect heterogeneous brain circuit but also holds promise to develop therapeutic interventions for neurological disorders [4].

To make full impact of optogenetics, researchers have developed implantable neural probes integrated with active [5] or passive [6] light sources and microelectrodes (used for neural recording) to interrogate brain circuits or conduct behavioral neuroscience studies in a variety of settings [7] [8]. Among them, LED-based probes have been recognized for their high scalability, localized light illumination, and low power-consumption [7]-[10]. Yet, limited by feasible integration methods, probes integrated with high-density dual-color LED arrays are still lacking, making high-resolution bi-directional optogenetic manipulation of brain activity technically challenging.

Different from previous works (Table 1), here we present an unprecedented dual-color optoelectronic neural probe aimed for high-resolution bi-directional *in vivo* optogenetic electrophysiology, where close-packed blue and red micro-LEDs were monolithically formed on the shank based on heterogeneously stacked III-V epitaxial layers (i.e. epilayers). The resulting device demonstrates its

capability in conducting bi-directional *in vivo* optogenetic electrophysiology at different depths (i.e. cortical layers) for the first time.

FABRICATION OF THE NEURAL PROBE

We first formed an 8-by-2 cross-barred blue LED array on the GaN-on-Si wafer using a similar approach as we reported before [11]. Next, to monolithically integrate red LEDs, we chose to heterogeneously stack the epilayers of an AlGaInP-on-GaAs wafer on top of the formed blue LEDs (Figure 1). We achieved this by transferring these epilayers (released from the GaAs substrate via wet etching) onto a soft-baked SU8 adhesion layer (*ca.* 5 μ m) coated on top of the blue LEDs. Afterwards, the resulting device sequentially went through a *ca.* 20 min drying step in a desiccator, an UV exposure step to cross-link the SU8, and a 30 mins hard-baking step for permanent bonding of the epilayers. Importantly, a slow temperature ramping process (*ca.* 10 $^{\circ}$ C/min) was applied in the hard-baking step to prevent the radical flow of SU8 underneath the epilayers. Consequently, these physically transferred and bonded epilayers allowed us to pattern a red LED array in the same way as the blue LED array, forming closely packed red and blue LED pairs. The resulting dual-color LEDs were then passivated by another SU8 layer (*ca.* 5 μ m) before the integration of microelectrode arrays (MEA) on top.

We next patterned an Au-based MEA and passivated it with a stack of sputtered SiO₂ (*ca.* 10 nm) and SU8 layers (*ca.* 5 μ m). Similar to the approach used to form silicon neural probes [5], we applied deep reactive ionized etching (DRIE) steps from both the front and back sides of the device to define the horizontal dimension and the thickness of a shank structure, respectively. Thereafter, we wire-bonded the as-made shank onto a printed circuit board (PCB) and encapsulated it with UV-curable epoxy.

Finally, we electroplated a poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) layer onto the microelectrodes as we reported before [12] to reduce their electrochemical impedance for high-fidelity neural recordings.

The resulting probe was *ca.* 200 μ m wide, 5 mm long,

Table 1. Benchmark of implantable micro-LED based neural probes.

Ref.	LED size (μm^2)	LED Pitch (μm)	LED Count	Vertical Span (μm)	Single/Dual Color	Microelectrode Integration
7	88	40	128	1330	Single	Yes
8	22500	-	2	180	Dual	No
9	399	24	1024	3140	Single	No
10	484	60	32	420	Single	Yes
This Work	49	20	32	380	Dual	Yes

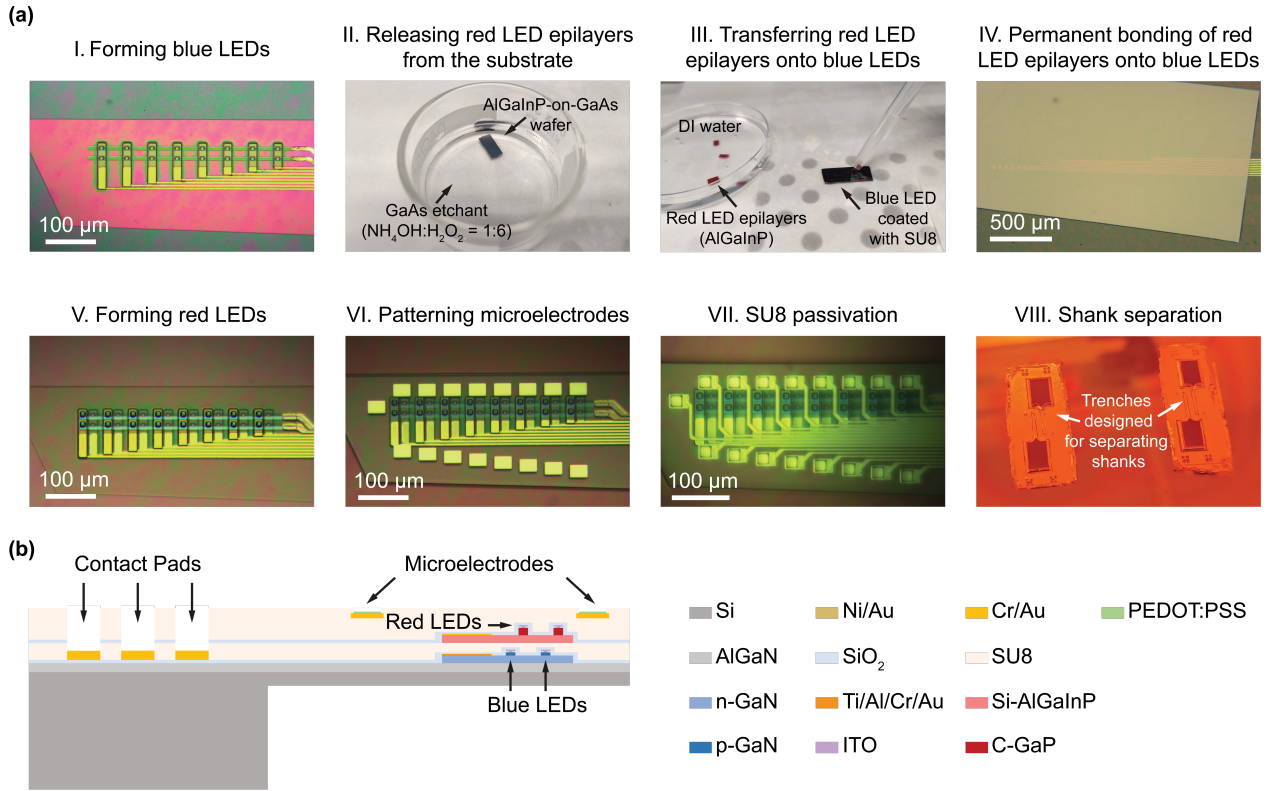


Figure 1: Fabrication of the monolithic probe. (a) Fabrication steps, including heterogeneous integration of red-LEDs, formation of microelectrodes, and shank separation. (b) Schematic cross-sectional view of the probe structure.

and 50 μm thick (Figure 2). Each probe featured 8 groups of 7 μm -by-7 μm sized blue and red LEDs with a 20/50 μm horizontal/vertical pitch, and 17 20 μm -by-20 μm sized microelectrodes next to the LEDs with a 50 μm vertical pitch, covering *ca.* 380 μm vertical span. These dimensions allow us to conduct high-resolution bi-directional *in vivo* optogenetic electrophysiology across various depths of the mouse cortex.

EXPERIMENTAL RESULTS

Optoelectronic Performance

We first examined the brightness of both blue (462/19 nm) and red (625/10 nm) LEDs by measuring their optical power density ($P_{\text{blue/red}}$) (Figure 3(a)-(d)). When biased at the injection current (I_{LED}) of 10 [22] μA , blue [red] LEDs can readily output 4 mW/mm^2 , exceeding the threshold needed for activating the bi-directional optogenetic opsin BiPOLES [13].

To characterize the MEA used for electrophysiology, we conducted electrochemical impedance spectrometry (EIS) testing for each electrode. The 1 kHz EIS impedance across all 17 electrodes was found to be effectively reduced from $3.01 \pm 0.26 \text{ M}\Omega$ to $41.29 \pm 3.53 \text{ k}\Omega$ after PEDOT:PSS coating (Figure 3(e)-(g)), likely due to the increase of their effective surface area as we reported before [12]. These low impedance values lend our MEA to high-fidelity electrical recording of neural signals with high signal-to-noise-ratios [14].

In Vivo Experiments

Since our probe has *ca.* 380 μm vertical span of closely packed LEDs and microelectrodes at the tip, we were able

to conduct *in vivo* optogenetic electrophysiology across different depth of the brain. Here we purposely inserted the probe into the somatosensory cortex of anesthetized mice, and leave the tip between cortical layers IV and V for such depth-dependent studies.

We first deflected mouse whiskers contralateral to the brain region where our probe was inserted (by pulsing a current-driven solenoid actuator, Figure. 4(a)) and recorded the local field potentials (LFPs) across the MEA;

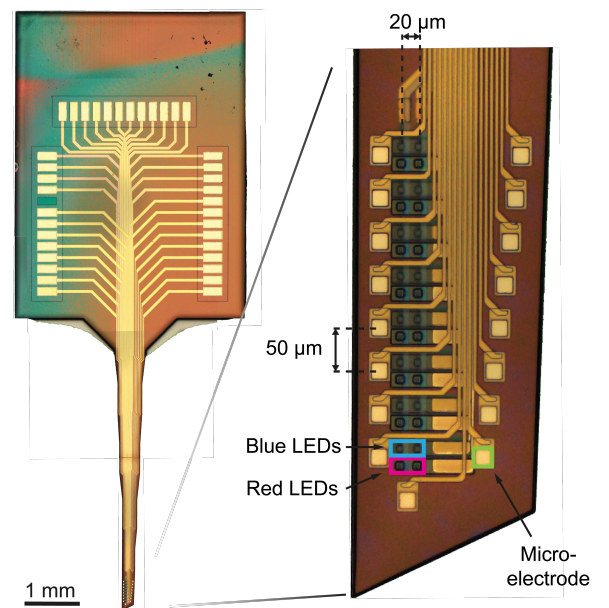


Figure 2: Dual-color optoelectronic neural probe with a zoom-in view of the probe tip.

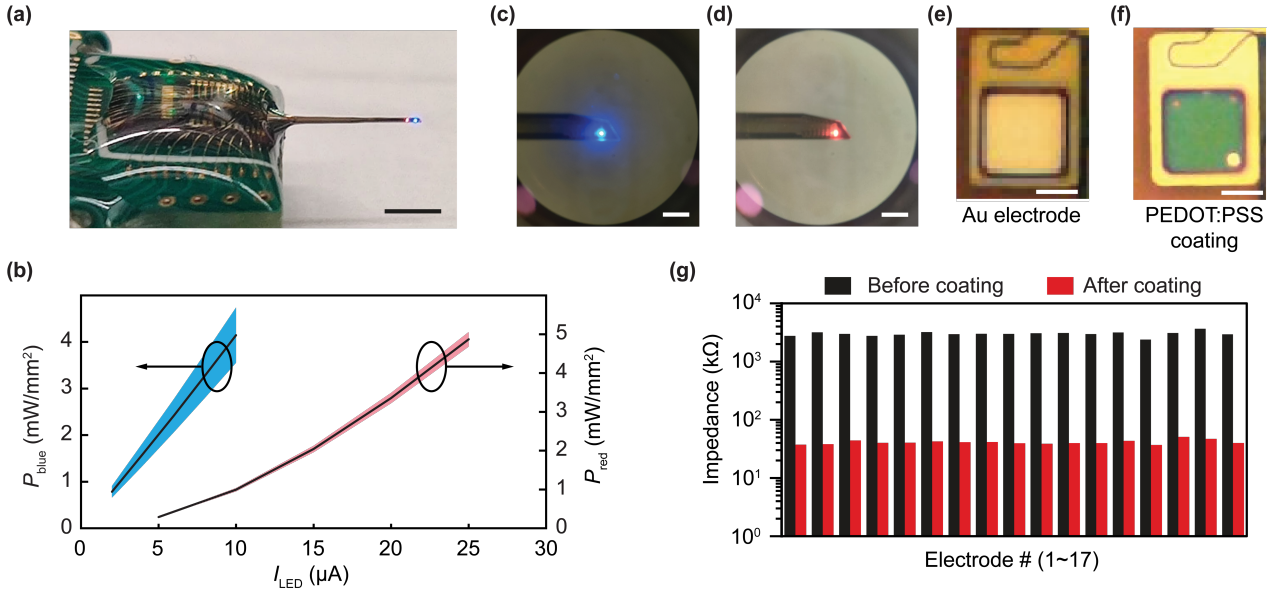


Figure 3: LED and MEA characterization. (a-d) Illuminated dual-color LEDs on the packaged probe and their optoelectronic performance. (e-g) The coated PEDOT:PSS layer effectively altered the color and the 1-kHz impedance of the microelectrodes. Scale bars: (a), 2mm; (c), (d), 200 μm; (e), (f), 10 μm.

the LFP data were applied to generate current source density (CSD) maps to analyze the propagation of neural activities across cortical layers (Figure 4(b)). Our analysis showed that whisker deflections induced a current source at electrode #5 (located in layer V) and a current sink at electrode #12 (located in layer IV), revealing the heterogeneous brain activities across these cortical layers. Notably, this CSD distribution across the MEA is consistent with the fact that cortical layers IV and V act as the input and output layers in the thalamocortical signaling pathways during whisker sensory processing [15].

Next, we used the probe itself to conduct bi-directional optogenetic electrophysiology (no whisker deflections). To achieve this goal, we inserted our probe into the BiPOLES-

expressed somatosensory cortex of another anesthetized mouse (the tip of the probe was left across layer I and II/V). Thereafter, we sequentially pulsed bottom 4 blue or 4 red LEDs (thirty 4-s period and $t_{\text{off/on/off}} = 0.5/0.5/3$ s, each 4-s period is defined as a trial here); each of these LEDs (see their locations in Figure 5) was biased to output 4 mW/mm² light pulses. To conduct artifact-free analysis of the recorded traces, we first excluded the 8-ms of data centered at the rising/falling edges of each LED pulse. The rest of the traces were bandpass filtered (250 Hz – 10kHz) to extract spikes whose negative amplitudes are >5 times the noise floor. Subsequently, we sorted the spikes using principal component analysis (PCA) and a density-based spatial clustering algorithm (DBSCAN) [16].

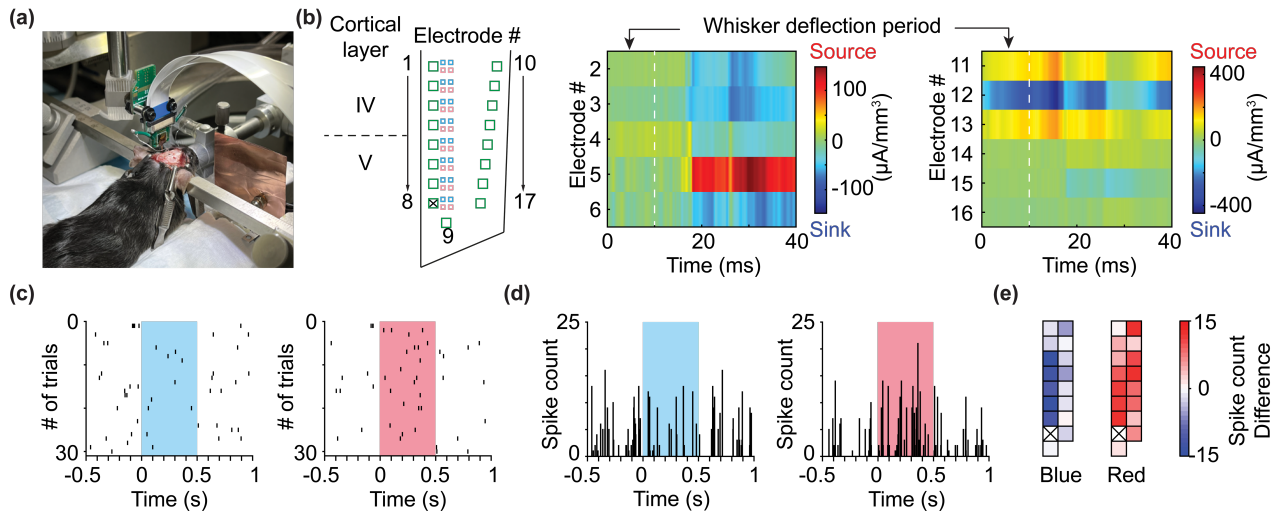


Figure 4: In vivo experiments of the probe inserted into the mouse cortex. (a) Experimental setup. (b) CSD analysis based on whisker-evoked LFPs recorded by 16 active electrodes (one was disconnected in experiments). (c-d) Raster plots and PSTH based on 30 trials of blue and red LED pulsing periods (0.5-s illumination pulses, labelled in blue and red). (e) Spike count differences across 16 active electrodes, calculated by subtracting spike counts recorded in the 0.5-s period before each illumination pulse from those recorded during the illumination pulses.

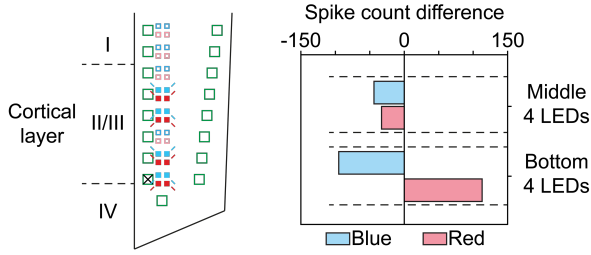


Figure 5: Spike count differences from all 16 active electrodes (defined in Figure 4(e)) caused by pulsing 4 blue/red LEDs at different depths of the cortical layers.

To quantify the optogenetic effect of pulsed LEDs, we plotted the spike timing (i.e. raster plots) during thirty 1.5-s windows, one from each 4-s trial (Figure 4(c), data were aligned by red or blue LED pulses), followed by summing spike count from 16 electrodes (one electrode failed in experiments) in these 30 windows to yield peristimulus time histograms (PSTH, Figure 4(d)). Moreover, we compared spike count recorded at each electrode within $[0, 0.5 \text{ s}]$ and $[-0.5 \text{ s}, 0]$ windows to examine LED-induced optogenetic effects across the MEA (Figures 4(e)). Our data showed that illuminating blue [red] LEDs can reliably decrease [increase] total spike counts by 49% [124%], demonstrating the bi-directional optogenetic control over neural activities *in vivo* using our dual-color micro-LEDs.

Taking one step further, we found that illuminating the middle 4 LEDs (see their locations in Figure 5) leads to the decrease of neural spikes, regardless of the LED color. This result is different from the bi-directional neuromodulation effect when illuminating the bottom 4 LEDs, which may relate to the details of local neural circuits surrounding the dual-color LEDs, which were mostly placed in layers II/III [17][18]. Nevertheless, such depth-dependent optogenetic effects reaffirm the heterogeneity of cortical circuits and the need for high-resolution neuromodulation using high-density dual-color micro-LED arrays.

CONCLUSION

We have presented a monolithic silicon neural probe integrated with close-packed dual-color micro-LEDs and microelectrodes, aiming for high-resolution bi-directional optogenetic electrophysiology. The physical transfer of the epilayers, the permanent-bonding process, and the coating of PEDOT:PSS layers are key steps to achieve high-density dual-color LED arrays as well as the low-impedance MEA. The resulting probe enabled depth-dependent bi-directional *in vivo* optogenetic electrophysiology for the first time.

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CONTACT

*Guangyu Xu, Email: guangyux@umass.edu.