

PHOTOELECTRIC NEURAL INTERFACE COMBINING WIRE-BONDING μ LEDS WITH IRIDIUM OXIDE MICROELECTRODES FOR OPTOGENETICS

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ABSTRACT

This paper reports the design, fabrication, electrical and thermal characterization, as well as attachment and stability of a flexible photoelectric neural interface (PENI). Upon the wire-bonding technology, three micro LED (μ LED) chips are well-connected to the polyimide substrate as an array, with their luminous surfaces over against the holes. Meanwhile, a Parylene-C-based array with four microelectrodes (200 μ m in diameter) modified by sputtering iridium oxide film is fabricated to record micro electrocorticography (μ ECoG) signals. Then, the back-to-back assembly is utilized to allow blue light through the aligned holes on both arrays with almost no attenuation. As an optogenetics tool, the PENI can be attached on the cortical surface of a mouse expressing ChR2 to realize synchronized light modulating and neural signal recording.

INTRODUCTION

Brain-computer interfaces (BCIs) stand for the frontiers of bidirectional communication pathway between brain and electronic devices in understanding the brain functions and disorders. Electrocorticography (ECoG), acquired by placing electrodes above (epidural) or below (subdural) the dura mater, has been taken seriously in research and application of functional and cognitive neuroscience recently. Compared to non-invasive recording method electroencephalography (EEG), which is acquired from the scalp, ECoG has higher spatial resolution and amplitude with broader bandwidth [1]. Besides, ECoG signals at high frequencies have been proved to carry more information about motor, cognitive and language for BCI control than EEG, and thus are far more valuable and reliable. Extracellular recording mainly applies penetrating microelectrodes into brain via invasive implantation surgery with poor signal durability. Therefore, the advantages of ECoG recording exhibit promising applications in neuroscience and clinical medicine.

Attribute to the development of MEMS technology, micro electrocorticography (μ ECoG) electrodes can record from large-area cortical surface filed potentials with high density electrodes at mesoscopic scales [2]. On the other hand, optogenetics has gained substantial interest over the last ten years, which can excite or inhibit a specific neuron

type with expression of light sensitive ion channels or pumps [3]. It provides an ideal possibility to manipulate specific neural circuits with the combination of μ ECoG electrodes [4, 5]. As the stimulation light source, μ LED is a superior choice than laser, whereas only few researches on μ ECoG are reported using μ LEDs. Thus, it is urgent to develop a novel photoelectric neural interface combining LEDs and μ ECoG electrodes for precise simultaneous optical stimulation and recording.

We first propose a novel integrated μ LED- μ ECoG neural interface using wire-bonding technology combining with iridium oxide (IrOx) microelectrodes, and this novel design concept will play as a promising optogenetics tool.

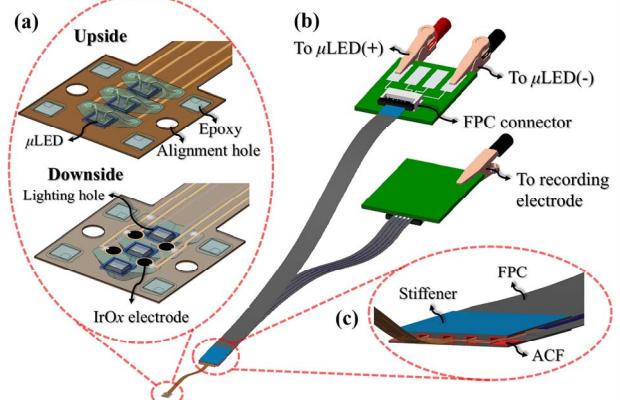


Figure 1: (a) Upside and downside views of the photoelectric neural interface (PENI) with 3 μ LEDs lighting through the aligned holes and 4 IrO_x-modified recording electrodes. (b) Overall schematic of the PENI connecting to FPC via ACF (c).

DESIGN

An overall schematic of the PENI was shown in Fig 1, with a 2×2 mm² footprint. From the close-up detail of its upside and downside in Fig 1a, three bare μ LED chips (C460TR2227-0328, Cree Inc., USA), with 220×270×50 μ m³ in dimension and 460 nm in peak wavelength, were arranged in a line with luminous surface down and aligned above the holes on PI substrate to allow the light propagation without obstacle. As can be seen from the downside view, four electrode sites with a diameter of 200 μ m were modified with IrOx and distributed around three μ LEDs. To achieve high spatial resolution, the spacing of

two adjacent μ LEDs was designed as 500 μ m, as well as two neighboring μ ECoG electrode sites.

As illustrated in Fig 1b, these two subarrays were individually connected to flexible printed circuit (FPC) with anisotropic conductive film (ACF, AC2056R, HITACHI, Japan) using a pulse hot-pressing machine. To realize reliable connection with no cross interference, appropriate pressure (0.18 Mpa), temperature (240 °C), time (18 s) and direction (stiffener upwards) were applied during hot-pressing. A close view of the ACF connection is shown in Fig 1c, better than directly inserting thickened pad area into a FPC connector [6], which may cause metal exfoliation after repeated insertion and extraction. To provide power supply for μ LEDs and record signals from electrodes, the other end of the FPC was inserted into a FPC connector on PCB. Besides, three μ LEDs' anodes (+) were individually addressed, whereas their cathodes (-) were connected together through the interconnection leads.

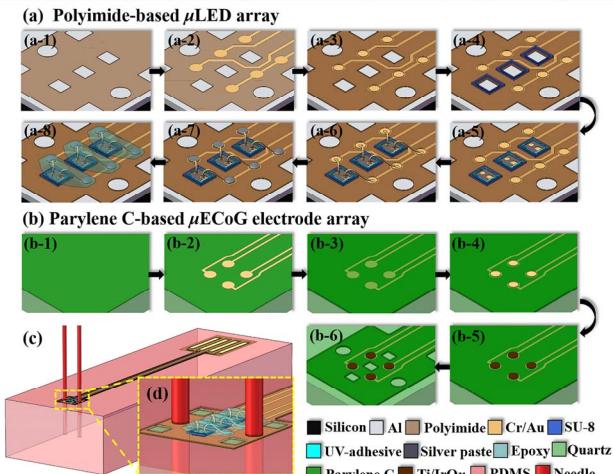


Figure 2: Independent fabrication processes of polyimide-based μ LED array (a) and parylene C-based μ ECoG electrode array (b). (c, d) Back-to-back assembly of two subarrays on PDMS with needles via the alignment holes and epoxy at corners.

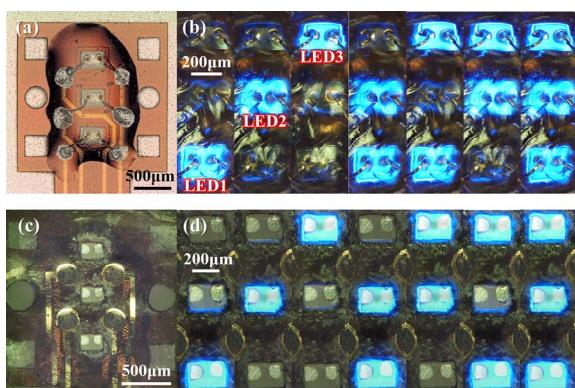


Figure 3: Micrograph of upside and downside of the PENI (a, b) with different lighting patterns (c, d).

FABRICATION AND ASSEMBLY

Wire-bonded μ LED Array

The MEMS microfabrication process was conducted as exhibited in Fig 2a. (a-1) A 500 nm thick aluminum (Al) was evaporated on a silicon wafer by physical vapor deposition as a sacrificial layer, followed by spinning

photosensitive PI (Durimide 7510, Fujifilm, Japan) with a speed of 3500 rpm and soft-baking on hotplate. After exposed, developed and rinsed, the bottom PI was cured in N₂ at 300 °C for 1 hour to form ~5 μ m in thickness. (a-2) A layer of chrome (Cr, 20 nm) and gold (Au, 300 nm) was deposited and patterned by an ion beam etching system (LJK 150, Advanced Ion Beam Ltd, China). (a-3) Another 5 μ m thick top PI layer was spun and patterned to expose electrode sites and pads, with final cure in N₂ at 350 °C for an hour. (a-4) Then, a 50 μ m thick SU-8 2025 (MicroChem Corp., USA) was spun, photoetched and developed to form a wall structure just above the aligned rectangular holes. The inner size of SU-8 wall structure was 240×290 μ m² and the wall width was 40 μ m, providing suitable settling room for the μ LED chip and enough adhesive force with PI. (a-5) An appropriate droplet of transparent UV curable adhesive was applied into the SU-8 wall, followed by picking and putting down a μ LED chip inside with two Au bond pads upwards. (a-6) After curing adhesive by UV exposure, the wire-bonding was utilized to connect the μ LED's Au bond pad (80 μ m in diameter) to the Au pad exposed on the top PI layer (160 μ m in diameter) by gold wire with a diameter of 25 μ m. Due to the ultrasonic energy absorption of the polymer substrate, the second bonding sites were generally not well-connected. (a-7) To obviate this problem, the conductive silver paste (H20E, Epoxy Technology Inc, USA) was dotted onto the Au pad. (a-8) Then, the transparent epoxy (PKM12C-1, Pattex, Germany) was applied to encapsulate the SU-8 wall, the μ LED and the gold wires. Finally, the entire device was released from Al sacrificial layer by the anodic metal dissolution approach in NaCl solution. To power the μ LEDs, ACF was contributed to well connect the device pads to the FPC.

The final encapsulated μ LED array was pictured by a digital microscope (VHX-5000, KEYENCE, Japan) in Fig 3a. Different lighting patterns from upside in Fig 3b clearly illustrated even illumination of every μ LED in the array, as well as the potential usage of different combinations in practical applications.

IrO_x-modified μ ECoG Electrode Array

Fig 2b showed the fabrication process of the sputtered iridium oxide modified microelectrodes based on parylene C [7]. Parylene C has been widely used as the structural material in biomedical devices for its outstanding properties, such as pinhole-free conformality, low water permeability, chronic biocompatibility as Class VI material, high flexibility and great mechanical strength. Accordingly, parylene was served as the structural layer in μ ECoG electrode array. (b-1) A 5 μ m thick parylene C film was deposited by the chemical vapor deposition (CVD) on a quartz wafer in PDS2010 system (Specialty Coating Systems, USA). (b-2) A 5 μ m thick positive photoresist (PR) layer was spun and patterned. After that, a Cr/Au layer with thickness of 20/200 nm was sputtered and patterned by lift-off process. (b-3) Next, a second layer of 5 μ m thick parylene C was deposited and annealed in N₂ at 200 °C for 5 hours to enhance the adhesion of two parylene layers. (b-4) Then, a 10 μ m thick PR was spun and patterned to define the microelectrode sites, and they were

exposed in the reactive ion etching (RIE) system using oxygen plasma. (b-5) A third layer of 30 μm thick PR was spun and patterned to expose the microelectrode sites again, followed by sputtering 50/300 nm Ti/IrO_x and patterning by lift-off in acetone. (b-5) The forth layer of 20 μm thick PR was patterned to define the outline, three rectangle holes for μLEDs and two round holes for alignment. At last, the device was released from substrate in a solution of 5% hydrofluoric acid. To test electrochemical properties and apply it in animal experiment, ACF bonding was utilized again. The IrO_x-modified microelectrodes were obvious in black color as demonstrated in Fig 3c.

Assembly

To assemble these two subarrays together as a whole, we proposed a back-to-back assembly method as illustrated in Fig 2c, which was less dependent on costly instruments, such as the high-precision flip-chip bonder [8]. Firstly, a μECoG electrode array was flattened on a thin and elastic Polydimethylsiloxane (PDMS) piece with microelectrode sites facing downwards. Then, a μLED array was attached onto the μECoG electrode array with light-emitting surfaces downwards, aligned them with two needles (250 μm in diameter) penetrating into PDMS via two symmetric alignment holes (300 μm in diameter). Fig 2d was the enlarged view of the assembly, where these two subarrays were bonded together with transparent epoxy right above four square holes at corners. Different lighting patterns from downside in Fig 3d showed light directly travelling through the holes, with almost no attenuation.

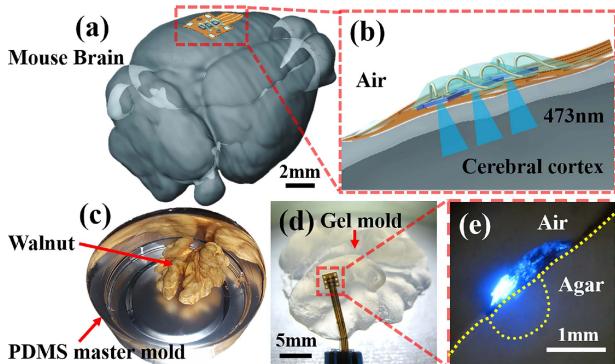


Figure 4: Illustration of the PENI attaching on the surface of mouse cerebral cortex model (a) and its enlarged side view (b). To mimic this model, a walnut (c) is used to obtain a translucent agar gel mold (d), placing the PENI on its curved surface and observing the emitting region (e).

RESULTS AND DISCUSSION

Attachment on Walnut-shaped Agar Gel

The illustration of PENI attaching on the surface of mouse cerebral cortex (model from Allen Brain Atlas) is shown in Fig 4a. From its enlarged side view in Fig 4b, the μLEDs ' light wavelength range covers 473 nm, which stands for the typical ChR2-expressing neurons' sensitive value. The light can be delivered to deep cortical layers (about 600 μm) to evoke lots of neurons at various depths.

To assess the attachment effects, a walnut-shaped agar gel mold from PDMS was applied to mimic mouse cerebral cortex as shown in Fig 4c and d. The curvature of

walnut was larger than mouse brain surface with more buckling and sulcus. The side view of the PENI well-attaching on the curved agar surface with single μLED on was photoed in Fig 4e, and the light-emitting area was indicated with yellow dashed curves.

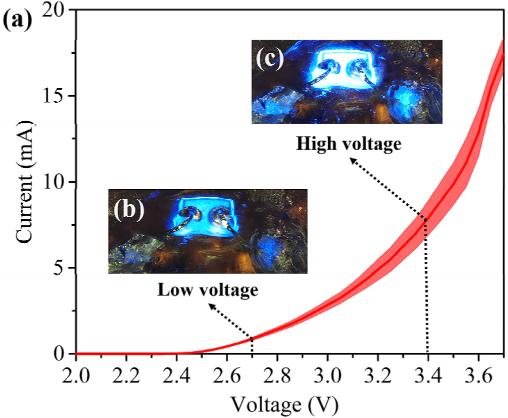


Figure 5: (a) I-V curve of 30 μLEDs integrated on the flexible substrate. (b, c) Different illuminances under low and high voltages (2.7V and 3.4V), respectively.

Electrical Characterization

The nonlinear current-voltage curve with error band was obtained after testing 30 μLEDs with a semiconductor parameter analyzer (BA1500, Agilent Technologies, USA), with voltage range from 2.0 V to 3.7 V, as shown in Fig 5. The two insets presented different illuminances of single μLED under 2.7 V and 3.4 V. The effective emission area of the μLED backside is 190 \times 240 μm^2 . To satisfy the minimum delivering light energy density of 1 mW/mm² from this area to induce neuronal action potentials, the input voltage should be no less than 2.7 V, resulting in a total power consumption about 2.4 mW, a little smaller than 3.4 mW reported by Kwon *et.al* [5].

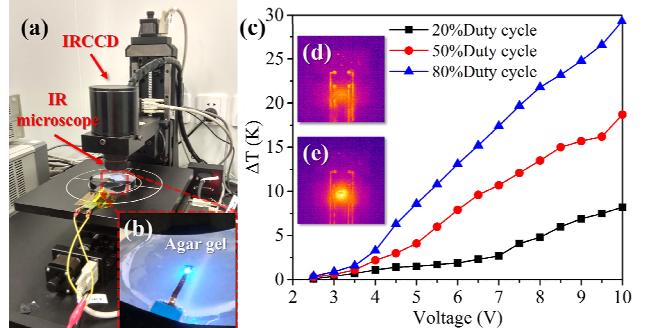


Figure 6: Thermal properties of the PENI: (a, b) Testing under the infrared microscope. (c) The temperature increases with 50Hz frequency, 20%, 50% and 80% duty cycle by driving single μLED . (d, e) Temperature distribution under low and high voltages, respectively.

Thermal Characterization

Brain tissue damage caused by μLED 's heat production is a major concern during *in-vivo* application. Accordingly, the temperature variation of μLED array was investigated using an infrared microscope (IRM-640, SI Optics Ltd., China) with 0.1 °C resolution. As pictured in Fig 6a and b, the PENI was lied on the agar gel just beneath the IR microscope, with luminous surface upwards. The

infrared image was captured by an IRCCD, as exampled in Fig 6d and e under different voltages and duty cycles. With constant frequency (50 Hz), various input voltages (2.5~10.0 V) and duty cycles (20%, 50% and 80%), the maximum temperature increase was below 2 K under 3.5 V in 3 minutes self-heating, as plotted in Fig 6c. Due to the resistive subdivision, the actual voltage on the μ LED chip was not exceeding the maximum forward voltage even under 10.0V input voltage. Besides, the μ LEDs will not directly contact with the brain tissue, thanks to the back-to-back assembly with the 10 μ m thick parylene film.

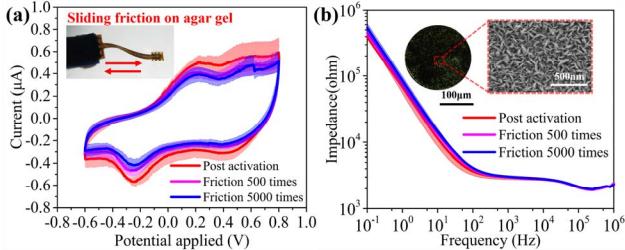


Figure 7: Cyclic voltammogram (a) and impedance (b) of 5 devices at post activation, 500 and 5000 times of friction to prove the stability of iridium oxide.

Stability of IrOx Microelectrodes

Due to the possible friction-lead damage of recording electrodes, the stability of activated iridium oxide is tested by cyclic voltammetry (CV) and impedance after 500 and 5000 times of friction in Figure 7. The PENI was slid back and forth on agar gel as shown in the inset of Fig 7a. The microelectrode site modified with IrOx was shown in the inset of Fig 7b, as well as the scanning electron micrograph (SEM) revealing a dendritic surface microstructure.

The electrochemical measurement was conducted in phosphate buffer solution (PBS) by the standard three electrodes system (PGSTAT12 Autolab, EcoChemie, Utrecht, Netherlands) with a saturated calomel electrode (SCE) as reference and a Pt-sheet as counter electrode. The averaged charge storage capacity (CSC) before and after 500 and 5000 times of friction was calculated from the CV in Fig 7a, which were 24.99 mC/cm², 24.80 mC/cm² and 22.63 mC/cm². Meanwhile, the averaged impedance at 1kHz before and after 500 and 5000 times of friction were 2.96 k Ω , 2.97k Ω and 3.03 k Ω , respectively, as shown in Fig 7b. We found little CSC decrease and unobvious impedance increase as the friction times increased, proving great stability of IrOx.

Additional work is underway to verify the usefulness of the PENI in the acute and chronic implantation in ChR2-mouse.

CONCLUSION

In summary, we presented the design, fabrication and characterization of a novel brain-computer interface named PENI, a flexible biomedical device integrating photo stimulation and recording functions for optogenetics. Three bare μ LED chips were wire-bonded to PI substrate as an array, with luminous surfaces over against the holes. On the other hand, a Parylene-C-based array with four microelectrodes (200 μ m in diameter) modified by IrOx was utilized to record μ ECoG signals. Back-to-back assembly of two subarrays allowed the light through the

aligned holes on both arrays without block. The attachment of the PENI was evaluated on the walnut-shaped agar gel. The electrical and thermal characterizations of μ LEDs array were also tested to find the proper parameters for *in-vivo* experiment. Finally, the stability of IrOx microelectrodes was verified by friction on agar gel for 500 and 5000 times, with little CSC decrease and unobvious impedance increase. The PENI can be attached on the cortical surface of a mouse expressing ChR2 to realize synchronized light modulating and neural signal recording.

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