Transparent Artifact-free Graphene Electrodes for Compact Closed-loop Optogenetics Systems

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***Abstract*—**Graphene offers unique advantages for implantable neural systems by combining properties like optical transparency, flexibility, high conductivity and biocompatibility in a single material. Here we report the first demonstration of a graphene-based compact closed-loop optogenetics system with the capability of recording and processing of neural activity in real time to control the light stimulation. We develop a process flow for high-yield large-area fabrication of transparent graphene microelectrode arrays on clear flexible substrates. Transparent graphene electrodes completely eliminate light-induced artifacts, which hinder the development of compact closed-loop optogenetics systems with conventional metal microelectrodes. We successfully demonstrate closed-loop operation with transparent graphene electrodes for simultaneous optical stimulation and electrical recordings without light artifacts.

I. Introduction

Electrophysiology is a decades-old technique widely used for monitoring activities of individual neurons and local field potentials generated by neuron populations. The last decade has witnessed a rapid advent of optogenetics revolutionizing neuroscience research by enabling selective control of neural activity and casual manipulation of specific neural circuits. The combination of optogenetics with electrical recordings is crucial for investigations of neural circuit dynamics and for understanding functional connectivity in the brain. However, conventional metal-based microelectrodes are not suitable for that purpose because of the prominent light-induced artifacts due to the photovoltaic (Becquerel) and the photothermal effects [1-3]. Light-induced artifacts are hard to distinguish from neural activity, and hence cause false-positives during real-time closed-loop operation. Therefore, a new generation of optically transparent neural probes is required to eliminate light-induced artifacts in neural recordings. Photo-induced currents are intrinsically very weak and fast in graphene [4, 5], making it a promising electrode material for combining optical and electrical monitoring methods. In addition, flexibility, high conductivity, and biocompatibility of graphene make it further appealing for neural applications [6-8].

All closed-loop optogenetics systems demonstrated to-date use conventional metal-based microelectrodes and are usually tethered to computers since they have to employ complex signal processing steps to remove light-induced artifacts and control the light source. Physical tethers impede animal movements, limit their behavior in complex environments and are unsuitable for long-term studies investigating neural dynamics [9] and neurological disorders [10]. Therefore, a portable closed-loop optogenetics system is crucial for reliable long-term studies in awake animals.

Here, we present a compact battery-powered closed-loop optogenetics system based on a transparent graphene microelectrode array. The graphene array was fabricated on flexible polymer substrates, which are transparent for the wavelengths of interest for optogenetics. We developed a novel fabrication process, which minimizes the contamination of graphene and formation of cracks in large area arrays. We combined transparent graphene arrays with an optical stimulation module consisting of μLEDs coupled to optical fibers to stimulate deeper brain layers while monitoring the neural activity with the graphene array from the surface. We extensively investigated light-induced artifacts for graphene electrodes and the same size metal electrodes. A hardware system was designed to enable closed-loop control for optogenetic stimulation. Finally, we evaluated and confirmed the functionality of the system for artificial biological signals with different amplitude, frequency and durations.

II. Transparent Graphene Array Fabrication

For graphene array fabrication, we chose clear polyethylene terephthalate (PET) substrates with high optical transmission in the wavelength range of 400 nm to 900 nm (**Fig. 1**). In order to have high yield and low impedance for graphene microelectrodes, it is crucial to minimize the risks for crack formation and contamination. To that end, we adopted and revised the bubbling transfer method for graphene microelectrode fabrication [11]. We investigated different transfer and cleaning methods for PMMA (Polymethyl methacrylate) removal from the graphene surface. Electrochemical impedance results in **Fig. 2** show that PMMA/PC (polycarbonate) transfer combined with 80°C acetone bath cleaning yields lowest impedance for the graphene electrodes. Following optimization of graphene transfer and cleaning, we fabricated transparent graphene arrays consisting of 16 microelectrodes (100×100 μm^2) on PET substrates (**Fig. 3a & Fig. 3b**). **Fig. 4** shows a scanning electron microscopy (SEM) image of the graphene array. Complete process flow is summarized in **Fig. 5**. To keep the array flat during fabrication, silicon wafer was used as mechanical support (**Fig. 5a&c**). 10 nm Cr and 100 nm gold were sputtered onto PET film (**Fig. 5d**). Metal wires and pads were patterned with photolithography and wet-etching (**Fig. 5e**). PMMA/Graphene/Cu trilayer structure was dipped into 0.05M NaOH solution. As H_2 bubbles were generated between the cathode and anode, the PMMA/graphene bilayer was electrochemically exfoliated from the copper substrate. PMMA/graphene bilayer was properly cleaned and transferred onto PET substrate with gold wires (**Fig. 5f**). Baking at 125 ˚C

for 5 minutes helped graphene to bond with the PET substrate; afterwards, PMMA was removed in hot acetone bath. Graphene was patterned with photolithography and oxygen plasma etching (**Fig. 5g**). At the end, the array was encapsulated with 8 μm thick SU-8 layer leaving only active electrode areas as the openings. The graphene solution interface, shown in **Fig. 6a**, was characterized using electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) (**Fig. 6b&c**). EIS results have confirmed that our fabrication process provides very high yield (100%) and low impedance electrodes. CV measurements exhibiting no redox peaks indicate capacitive nature of the graphene/solution interface. The average impedance of the electrodes was measured as 872.85 KΩ at 1 kHz (**Fig. 6d**).

We validated the graphene microelectrode arrays in both *in vitro* and *in vivo* Calcium (Ca) experiments. In the *in vitro* experiment with hippocampal slices (**Fig. 7**), Ca responses coincide with the population spikes recorded by the graphene electrode. However, graphene electrodes can detect very fast population spikes (durations less than 5 ms), which are not detectable by Ca imaging due to limited temporal resolution [6]. In the *in vivo* wide field Ca imaging experiment over the mouse cortex (**Fig. 8**), the Ca signals and the EEG data also show strong correlations [12]. The large deflections of EEG signals often precede an increase in the Ca responses. These experiments show that laser light can penetrate through the transparent array. We have successfully performed imaging directly below the transparent graphene electrodes and recorded the electrophysiology data simultaneously without any crosstalk between the electrical and optical modalities.

III. Artifact-free Optogenetics and Electrophysiology

We have investigated light-induced artifacts for the conventional metal microelectrodes and transparent graphene electrodes. The light stimulation was generated by Thorlabs fiber-coupled LED (M470F1, 470nm, excitation wavelength for Channelrhodopsin-2) and delivered to the electrode site through a 200 μm fiber. The power intensity at the fiber tip was measured using a standard optical power meter. The electrical signals were recorded using Intan RHD2000 Evaluation System. For the Au electrode with fixed light pulse duration and varying light intensity (**Fig. 9a&b)**, the negative artifact peak grows linearly with increasing light intensity while the positive artifact peak tends to saturate. For the Au electrode with fixed light intensity and increasing light pulse duration (**Fig. 9c&d**), the negative artifact peak remains almost constant while the positive artifact peak increases for longer pulse durations. In these experiments, Au microelectrodes exhibit prominent light-induced artifacts, which can significantly interfere with biological signals.

For graphene electrodes, we have not observed detectable artifacts under the same experimental settings. **Fig. 10a&b** show the power spectrum of the recorded signals during 10 Hz light pulse (pulse duration: 20 ms) stimulations. For the Au electrode, there was an obvious 10 Hz artifact and higher order harmonics. For the graphene electrode, no significant artifact was detected within the 0 – 60 Hz range. These experiments suggest that transparent graphene electrodes can be safely used in optogenetic stimulation and electrical recording experiments, guaranteeing crosstalk-free operation.

IV. Closed-loop System

Based on the transparent graphene electrodes, we designed a compact battery-powered closed-loop system for electrophysiology and optogenetics. **Fig. 11a&b** show the pictures of the system. It consists of a MSP430 microcontroller, an Intan RHD2216 chip, a 16-channel graphene micro-electrode array, a micro-LED coupled to a fiber, and a 3.7V 150 mAh battery. **Fig. 11c** shows the system diagram and **Fig. 11d** is a schematic of the mouse carrying the system board. The output power at the tip of the fiber was measured as 25.72 mW/mm^2 with ~30% coupling efficiency. The system specifications are listed in **Table 1**. The standard working hour for the system with 10 kHz sampling rate is ~75 hrs. **Fig. 12** shows a picture of the working system. To test the closed-loop operation, we implemented a threshold detection algorithm. As shown in **Fig. 13**, pulse trains of two different frequencies and durations were applied to the 0.01 M saline solution and the recorded electrical signal was used to control the light source based on the threshold detection algorithm. **Fig. 13b&c** show the recording results. When the amplitude of the signal is larger than the threshold, the μLED was successfully triggered by the control circuitry. Beyond threshold detection, our system can support most of the other feedback-control algorithms based on amplitude, frequency or power detection aiming different optogenetics applications.

V. Conclusion

In this work, we fabricated low impedance transparent graphene microelectrode arrays for artifact-free electrophysiological recordings. We have demonstrated that the use of transparent graphene electrodes eliminates light-induced artifacts during the optical imaging and optogenetic stimulation. Finally, we designed a compact system incorporating both the graphene microelectrode arrays and fiber-coupled μLEDs for closed-loop optogenetics and tested it with various artificial biological signals. Crosstalk-free integration of the optical imaging, the optogenetics and electrophysiological recordings can transform spatiotemporal mapping of neural circuits and can open up unprecedented opportunities to study the functional neural connectivity.

Acknowledgments

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References

[1] J. A. Cardin, et al., *Nature Protocols*, vol. 5, p. 247-254, 2010.
[2] N. G. Laxpati, et al., *Front Neuroeng*, vol. 7, p. 40, 2014.
[3] I. Ozden, et al., *Journal of Neuroscience Methods*, vol. 219, p. 142-154, 2013.
[4] N. M. Gabor, et al., *Science*, vol. 334, p. 648-652, 2011.
[5] M. C. Lemme, et al., *Nano Letters*, vol. 11, p. 4134-4137, 2011.
[6] D. Kuzum, et al., *Nat Commun*, vol. 5, p. 5259, 2014.
[7] D. W. Park, et al., *Nature Communications*, vol. 5, 2014.
[8] Y. Lu, et al., *Sci Rep*, vol. 6, p. 33526, 2016.
[9] E. Stark, et al., *Neuron*, vol. 83, p. 467-80, 2014.
[10] E. Krook-Magnuson, et al., *Nature Communications*, vol. 4, 2013.
[11] Y. Wang, et al., *Acs Nano*, vol. 5, p. 9927-9933, 2011.
[12] H. Makino, et al., *Neuron*, vol. 94, p. 880-890.e8, 2017.

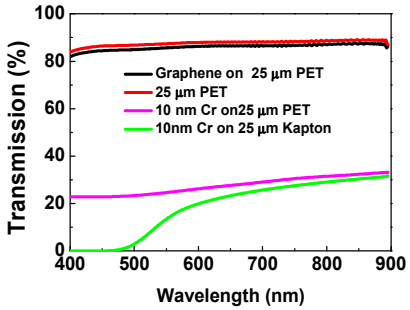

Fig. 1 Transmission spectrum of graphene on PET compared to 10nm Cr on PET or Kapton.

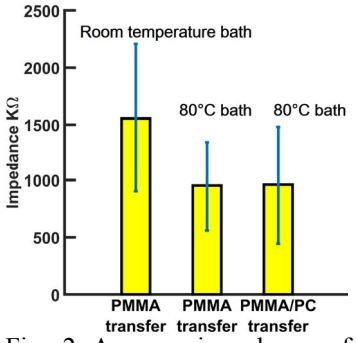

Fig. 2 Average impedance of arrays fabricated with different transfer and cleaning methods.

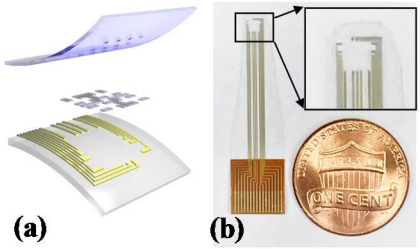

Fig. 3 (a) Array structure: graphene contacts, gold wires, a PET substrate, and encapsulation. (b) A photo of the flexible array. The inset shows the transparent recording area.

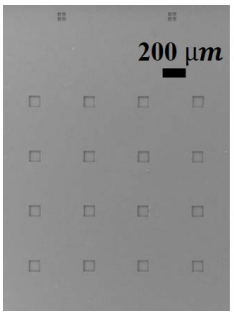

Fig. 4 SEM image of the array.

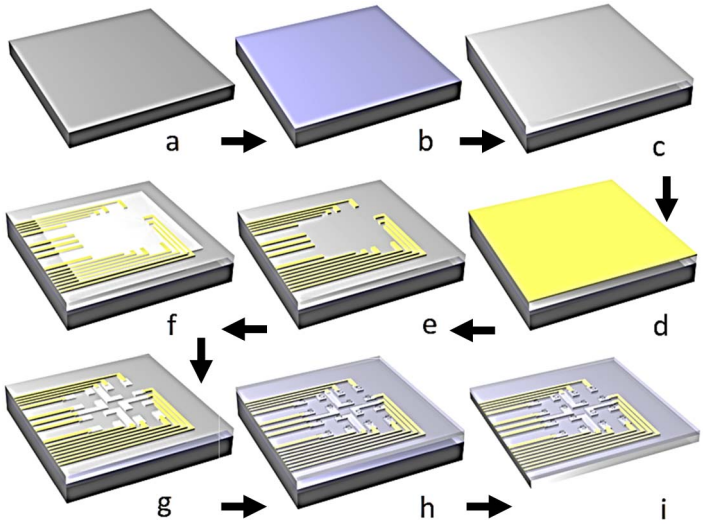

Fig. 5 Fabrication processes of the graphene microelectrode array. (a) Silicon wafer as carrier substrate; (b) PDMS adhesive layer coated on Si; (c) PET film applied on PDMS; (d) Cr/Au sputtering; (e) metal wires patterned with photolithography and wet-etching; (f) graphene transferred by bubbling-method; (g) graphene contacts patterned with photolithography and oxygen plasma etching; (h) SU8 encapsulation; (i) array peeled off from the PDMS/silicon wafer.

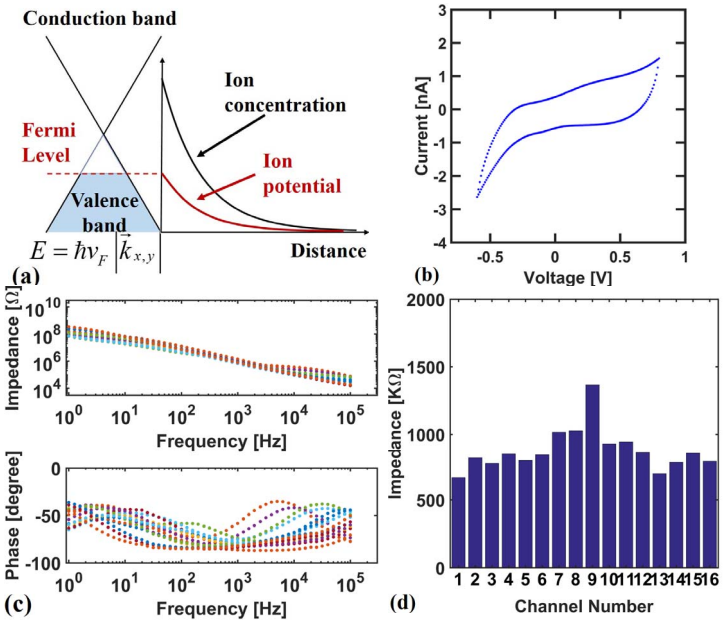

Fig. 6 (a) Graphene/solution interface (b) CV of a typical electrode of the array. (c) EIS of the 16 electrodes. (d) Impedance distribution of all 16 electrodes of the array at 1 KHz.

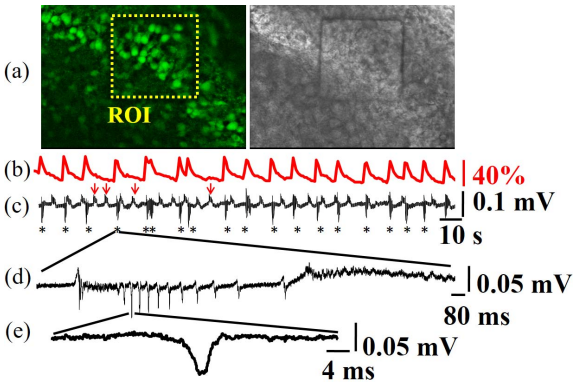

Fig. 7 *In vitro* Ca imaigng and electrical recording with hippocampal slices [6]. (a) Fluorescence image, (b) Calcium fluoresence response, and (c-e) simultaneous electrical recordings from the transparent graphene electrode at different time scales.

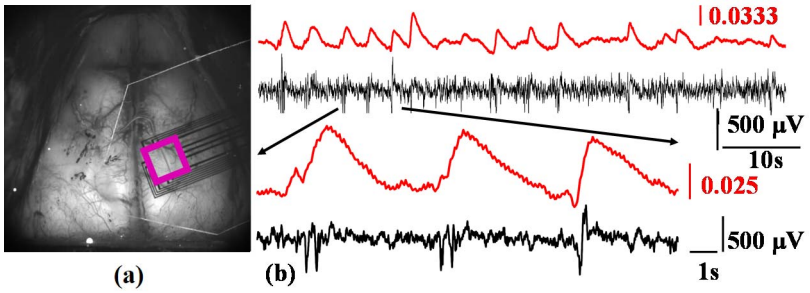

Fig. 8 Simultenous *in vivo* Ca imaging and EEG recording over the awake mouse dorsal cortex using graphene microelectrodes [12]. (a) A picture of the mouse brain with the graphene array. (b) The recorded data and its zoom-in plot showing correlations between the Ca transients (red) and the EEG signals (black).

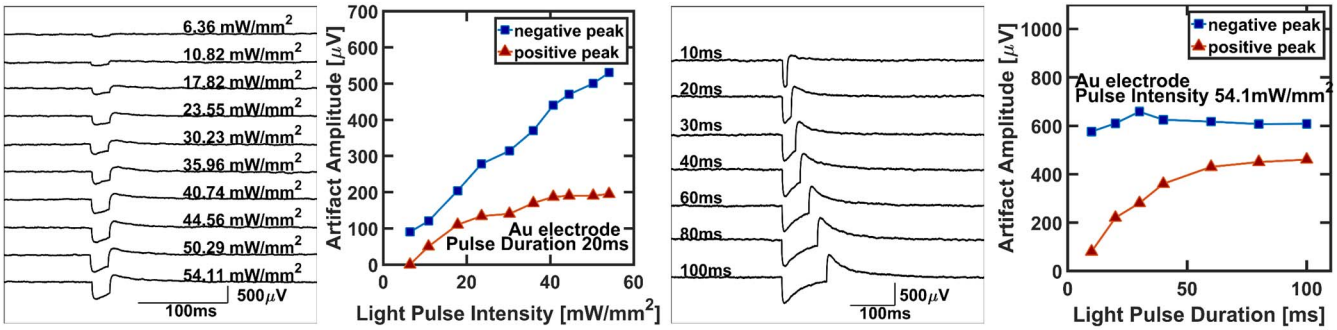

Fig. 9 (a) Au artifact time series for increasing light intensity and (b) artifact amplitude as a function of light pulse internsity for Au electrodes. (c) Au artifact time series for increasing pulse duration and (d) artifact amplitudes as a function of light pulse duration.

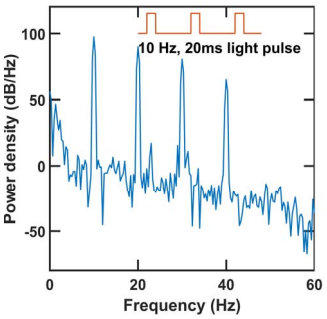

Fig. 10. Power spectrum of the artifact signal recorded by (a) Au electrode and (b) graphene electrode.

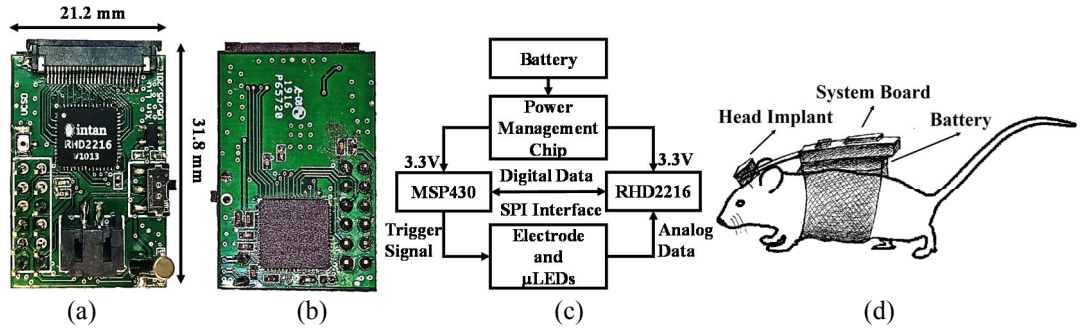

Fig. 11. The designed closed-loop electrophysiology/optogenetics system. (a) The front side picture (b) The back side picture. (c) The diagram of the closed-loop system. (d) A schematic of the mice carrying the board

Table 1 Electrical and Physical characterization of the closed-loop system

Transparent Graphene Array	Board	Power Supply
16 channels Electrode dimensions: 100 μm×100 μm Total size: 4 mm×4 mm	1kHz – 30 kHz per channel Size: 21.2 mm×31.8 mm Weight: 6.65 g	3.7 V Li Battery 600 mWh ~ 75 h operation Size: 19.75mm×26.02mm×3.8mm Weight: 4.65 g

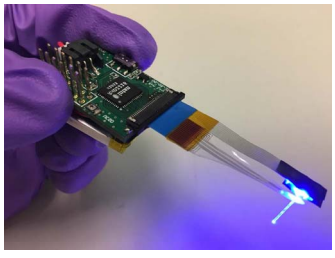

Fig. 12. A picture of the working system consisting of a transparent graphene array and fiber-coupled μLED.

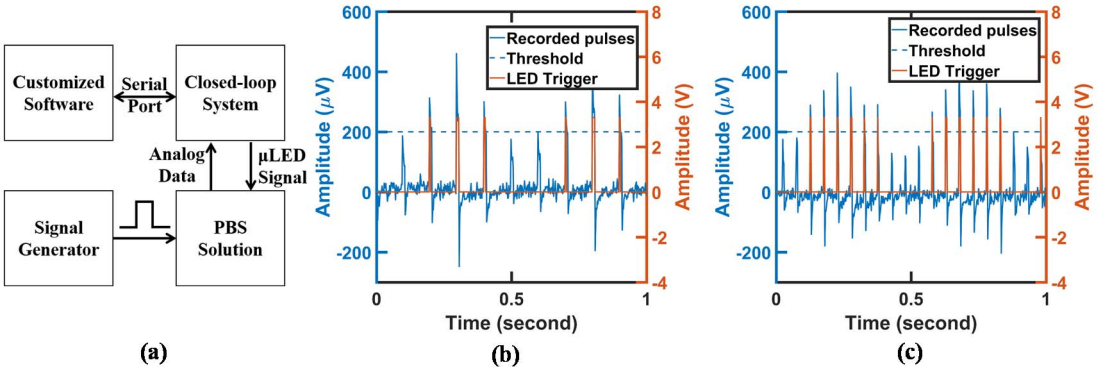

Fig. 13 (a) The test setup for the closed-loop system. (b) Typical real-time recording data for one channel. A train of 10 Hz pulses (10 ms pulse duration) modulated by a 2 Hz sine wave was applied to the saline and the threshold was set to 200μV. (c) The recorded signal on the software for a train of 20 Hz (5ms pulse duration) modulated by a 2 Hz sine wave. The threshold remains the same.