High-resolution neurostimulation and optogenetic electrophysiology with PEDOT:PSS-coated graphene

F. Sun[1], Z. Xiong[1], J. Park[1], and G. Xu[1,*]
[1]Department of Electrical and Computer Engineering, University of Massachusetts at Amherst, Massachusetts, 01003, USA
[*]Email: guangyux@umass.edu

2020 IEEE International Electron Devices Meeting (IEDM) | 978-1-7281-8888-1/20/$31.00 ©2020 IEEE | DOI: 10.1109/IEDM13553.2020.9394908

***Abstract*—**Micron-sized graphene electrodes hold promise in neurointerfacing for their outstanding mechanical, electrical, and optical properties. To understand the neural heterogeneity, it will be essential to examine if graphene electrode arrays can scale down their pitch size and probe neural activity at cellular levels. Here we present a 28-μm pitched PEDOT:PSS-coated graphene microelectrode array (MEA) that achieves one order higher resolution than prior graphene MEAs in *both* neuro-stimulation *and* optogenetic electrophysiology. Our array features high yield (100 %), low-impedance (sub-100 kΩ), low light-induced artifact (sub-2 μV), and high charge-injection-capacity (> 1.31 mC/cm^2), suggesting its possible use for high-precision multi-modal neurointerfacing.

I. Introduction

Microelectrode arrays (MEA) are powerful tools in modern neuroscience to understand brain function at the levels of cells, circuits, and behaviors. To date, CMOS-based MEAs have been established to record and stimulate neural activity with high spatial resolution. However, their mechanical rigidity is likely to cause insertion damage to the brain and thus less optimal for long-lasting *in vivo* use. From this perspective, graphene MEAs fabricated on flexible substrates have emerged as promising devices in a variety of *in vivo* experiments [1-3], with combined benefits in mechanical compliance, low impedance, bio-compatibility, transparency, and low light-induced artifact (the latter two allow them to co-work with optical experiments).

To date, graphene MEAs have been engineered by, chemical doping [4], surface coating [5] and choosing the number of graphene layers [2, 3] to balance electrode impedance, light-artifact, and charge injection capacity. Nonetheless, these MEAs are typically built with 300-900 μm pitch, which limits their use for high-density neurostimulation and optogenetic electrophysiology. To enable high-resolution neurointerfacing, it is essential to scale down the pitch of graphene MEAs and examine if the resulting high-density array can probe neural activity ideally at cellular levels. To this end, here we report a 28-μm pitched PEDOT:PSS-coated graphene MEA that features high yield (100 %), low-impedance (sub-100 kΩ), low light-induced artifact (sub-2 μV), and high charge-injection-capacity (> 1.31 mC/cm^2). Importantly, our array achieved *one order higher* resolution than previous graphene MEAs in *both* neurostimulation *and* optogenetic electrophysiology, thanks to the optoelectronic properties of the PEDOT:PSS coating layer. These results shed light on the possible use of PEDOT:PSS coated graphene MEAs towards high-precision multi-modal neurointerfacing.

II. Methods

A. Device Fabrication

Our MEA was built on chemical-vapor-deposition grown graphene wafers, with a few layered (3-5) graphene one-time transferred to a Si/SiO_2 substrate by standard Cu-etching method (Fig. 1a). We patterned graphene electrodes in a 28-μm pitch by an oxygen reactive-ion-etching step, contacted them with 7/80 nm Ti/Au layers, and passivated the array by a 4 μm thick SU8 layer with 21 μm-by-10 μm sized opening at each electrode site. We then treated the SU8 layer with an O_2-plasma step (with graphene being protected by photoresist) to enhance its hydrophilicity, which was found to improve the PEDOT: PSS electroplating and neuroninterfacing steps. The resulting array was then wire-bonded to a printed-circuit board (PCB) and packaged by polydimethylsiloxane (PDMS).

B. Experimental Setup

To conduct electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and charge-injection-capacity (CIC) measurements, we immersed the MEA in 1× phosphate buffer saline solution and configured a three-electrode setup by Gamry Reference 600+ (Fig. 1b) [6]. We also used this three-electrode configuration to electroplate a PEDOT:PSS layer on graphene electrodes. This was achieved by immersing the array in de-ionized water mixed with 0.048 M PSS and 0.02 M EDOT monomer [5], and injecting 2.1 mA/cm^2 current from each graphene electrode for *ca.* 20 s to yield uniform PEDOT:PSS coating across the array (Fig. 1c).

On the biology side, we cultured primary rat cortex neurons on a coverslip placed in well plates, and transfected them with Ca^{2+} reporters (*GCAMP7s* [7]) *or* opsins (*Chrimson* [8]) for neuro-stimulation and optogenetic electrophysiology experiments, respectively. This coverslip was then transferred to a Petri dish filled with neuroimaging medium for cell experiments (Fig. 1d). To form the array-neuron contact, we side-flipped the MEA and fixed it on a home-built lifting station as we reported before [6]. The array was then lowered, aligned to, and contacted with the neurons by fine-tuning the lifting station; an Ag/AgCl wire was immersed in the medium as the reference.

During the neurostimulation experiment, we applied 1 or 3 trains of biphasic voltage pulses (± 0.5 V) to the select graphene electrode by an A-M Model 4100 stimulator, and conduct Ca^{2+} imaging (50 ms exposure time) to examine evoked neuronal responses. The imaging data were collected by an inverted fluorescence microscopy (Leica) with a 2.21 mW/mm^2 470/40 nm excitation light pulsed at 2 frame/s, a 495 nm long-pass dichroic mirror, and a 520/40 nm emission filter.

During the optogenetic electrophysiology experiment, we applied 0.41 mW/mm^2 550/15 nm optogenetic stimulus by the same microscope to the neurons. The resulting neural response was sampled at 20 kHz (synchronized with the microscope camera) and band-pass filtered at 0.1-3 kHz by an Intan RHD2164 chip [6]. We also quantified the light artifact of our MEA under 0.41-3.24 mW/mm^2 550/15 nm stimulus.

III. Results and Discussions

We first conducted EIS, CV, and CIC measurements (Figs. 2 and 3) on individual graphene electrodes to evaluate their electrochemical properties. Our data show that the PEDOT:PSS layer effectively enhanced the electrode performance across the array. Specifically, the PEDOT:PSS electroplating step was found to decrease the 1 kHz EIS impedance of all 13 electrodes from 2.10 ± 0.80 MΩ to 54 ± 9 kΩ (Fig. 3), and increased their EIS phase in the 0.1–100 kHz range (Fig.2a). This result suggests that the PEDOT:PSS layer lowered the electrode impedance by *ca.* 38 times, and changed the electrodes to be less capacitive. Such 28 μm-pitched, sub-100 kΩ electrodes are desired to achieve high signal-to-noise ratio (SNR) in high-density electrophysiology. On the other hand, the PEDOT:PSS layer was found to :1) increase the current in CV curves by *ca.* 40 times, and 2) increase the maximum injection current of each electrode before its voltage transient goes beyond the water window of PEDOT:PSS (-0.6 V to + 0.8 V) by *ca.* 60 times. These results are likely because the PEDOT:PSS layer (~ 270 nm thick [9]) increased the effective surface area of each electrode, yielding a lowered charge transfer resistance and an increased double-layer capacitance. As a result, the PEDOT: PSS layer effectively increased CIC values from 0.02 to 1.31 mC/cm^2, which is desired for envoking neural spiking events.

To conduct the neurostimulation experiment, we contacted the PEDOT:PSS-coated MEA with neurons that are expressed with *GCaMP7s*. We chose this setup since it allows us to use Ca^{2+} imaging data (via fluorescence microscopy) to examine the neural response to the electrical stimulus provided by the MEA. Our data show that 1 or 3 trains of biphasic voltage pulses applied to a single PEDOT:PSS-coated graphene electrode can reliably trigger Ca^{2+} influx among *ca.* 30 individual neurons close to the stimulating electrode (3 typical neurons are shown in Fig. 4). This result shows that our 28-μm pitched MEA can reliably evoke neural response due to the high CIC values of PEDOT:PSS-coated electrodes. *It is noted that our array is one order denser than reported graphene MEAs that were used for neurostimulation, therefore representing a step forward towards high-resolution neurointerfacing.*

To conduct optogenetic electrophysiology experiment, we first quantified the light-induced artifact in our MEA to decide the intensity and pulse duration of the optogenetic stimulus we should apply. Our data (Fig. 5) show that the artifact amplitude sampled at 0.1 Hz – 3 kHz increases with the stimulus intensity from 0.41 to 3.24 mW/mm^2 but weakly depends on the pulse duration from 2–60 ms. Importantly, we noted that these light-induced artifacts are largely slow varying signals and can be filtered out by applying a 100 Hz high-pass filter to the sampled voltage trace (see two red traces in Fig. 5). Consequently, we chose to sample neural activity from 100 Hz – 3 kHz under 0.41 mW/mm^2 550/15 nm optogenetic stimulus pulsed at 5 Hz (50 pulses in 10 s, 2 ms duration per pulse).

We then proceeded with the optogenetic electrophysiology experiment by contacting the PEDOT:PSS-coated MEA with neurons that are expressed with *Chrimson*. We chose this setup since it allows us to evaluate the evoked neural spiking events by the optogenetic stimulus provided by the microscope. Our data (Fig. 6) show that the optogenetic pulses we applied can reliably evoke neural spikes, whose amplitudes are significantly larger than the noise floor. Importantly, most of these recorded spikes were evoked by optogenetic stimulus since we sparsely (if not none) recorded natural neural spikes outside the 10-s stimulus window. This result shows that our 28-μm pitched MEA can reliably record optogenetically evoked spikes due to its low artifact (< 2 μV from 1000-point adjacent-averaging of the recorded trace under a 0.41 mW/mm^2 550 nm light pulse in Fig. 5a) and high SNR values (> 8) in the PEDOT:PSS-coated electrodes. *It is noted that our array is one order denser than reported graphene MEAs that were used in optogenetic electrophysiology, therefore representing another step forward towards high-resolution neurointerfacing.*

Finally, we quantified the neural spikes recorded within the 10-s optogenetic stimulus window (Figs. 7 and 8). Our data show that: 1) the peak-to-peak amplitudes of these spikes (> 35 μV) are significant compared to spike-to-spike variation (< 7 μV); 2) they settle in less than 4 ms as expected [1]-[3]; 3) all 50 optogenetic pulses succeeded in evoking 2-9 spikes; and 4) electrodes further away from the responsive neurons tend to record smaller signals. These results suggest that our PEDOT:PSS-coated array can yield high-fidelity optogenetic electrophysiology data that qualitatively match the position of optogenetically responsive neurons.

IV. Conclusion

In sum, we presented a 28 μm-pitched PEDOT:PSS-coated graphene MEA that feature high yield (100 %), low-impedance (sub-100 kΩ), low artifact (< 2 μV), and high CIC (> 1.31 mC/cm^2). As a result, our array achieved one order higher resolution than prior graphene MEAs in *both* neuro-stimulation *and* optogenetic electrophysiology experiments (Fig. 9). Our work suggests the ultimate promise of high-density graphene MEA towards high-precision multi-modal neurointerfacing.

Acknowledgment

This work was supported by National Science Foundation under ECCS-1835268.

References

[1] M. Thunemann, et al., *Nat. Commun.*, vol. 9. no. 1, p. 1-12, 2018.
[2] D. W. Park, et al., *Nat. Commun.*, vol. 5, p. 1–11, 2014.
[3] D. W. Park, et al., *ACS Nano*, vol. 12, no. 1, p. 148–157, 2018.
[4] Y. Lu, et al., *Sci. Rep.*, vol. 6, p. 33526, 2016.
[5] P. Kshirsagar, et al., *Adv. Mater. Technol.*, vol. 4, no. 1, p. 1800318, 2019.
[6] J. Park, et al., *IEEE Electron Device Lett.*, vol. 41(8): p. 1261-1264, 2020.
[7] H. Dana, et al., *Nat. Methods*, vol. 16, no. 7, p 649-657, 2019.
[8] N. C. Klapoetke, et al., *Nat. Methods*, vol. 11, no. 3, p 338-346, 2014.
[9] R. Gerwig, et al., *Front. Neuroeng.*, vol. 5, p. 8, 2012.
[10] J. Abbott, et al., *Lab on a Chip*, vol. 20, no. 17, p. 3239-3248, 2020.

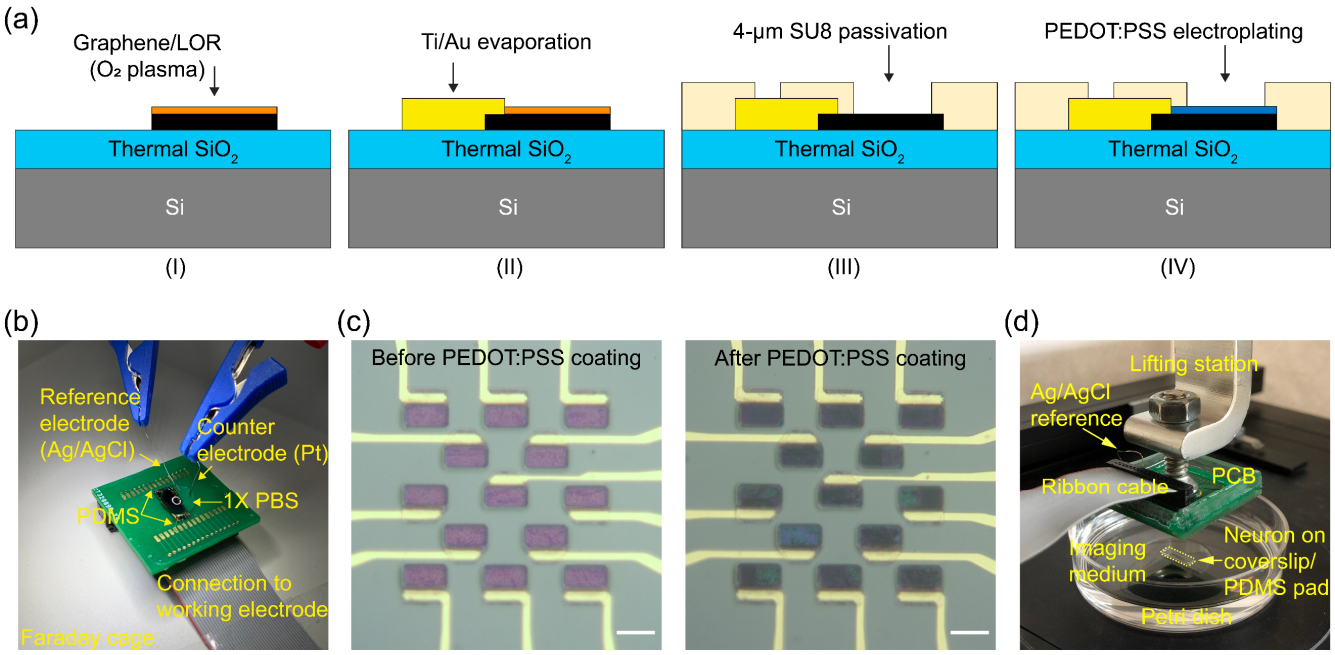

Fig. 1. Array fabrication and testing setup. (a) Fabrication flow of the PEDOT:PSS coated graphene MEA. (b) Three-electrode configuration with a Pt wire as the counter electrode and an Ag/AgCl wire as the reference electrode. The working electrode (graphene) is accessed to Gamry 600+ via a ribbon cable. The Faraday cage was connected to earth ground during the electrochemical characterizations. (c) MEA images before and after the PEDOT:PSS coating step. Scale bar, 20 μm. (d) Testing setup with the array packaged on a side-flipped PCB on a lifting station controlled by a lab jack and a positioning stage.

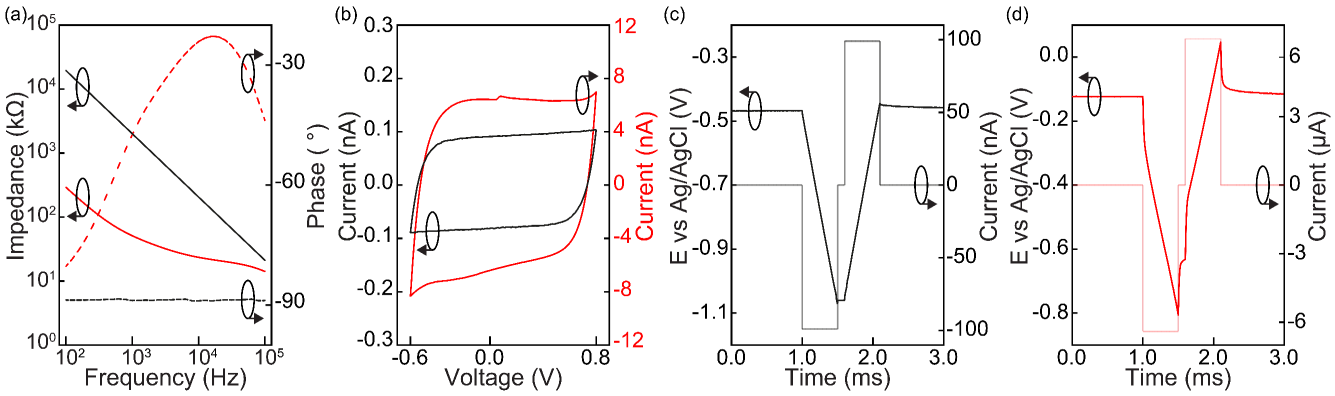

Fig. 2. Electrochemical characterization of one graphene electrode before (black) and after (red) PEDOT:PSS coating. (a) EIS impedance (solid line) and phase (dashed line). (b) CV measured in the 10th cycle and scanned at 1000 mV/s. (c) Voltage transients (dark) under a bi-phasic current pulse (light) before PEDOT:PSS coating. (d) Voltage transients (dark) under a bi-phasic current pulse (light) after PEDOT:PSS coating.

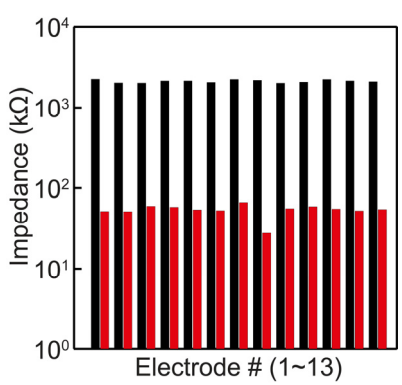

Fig. 3. EIS statistics. EIS impedance at 1 kHz of all 13 electrodes measured before (black) and after PEDOT: PSS coating (red).

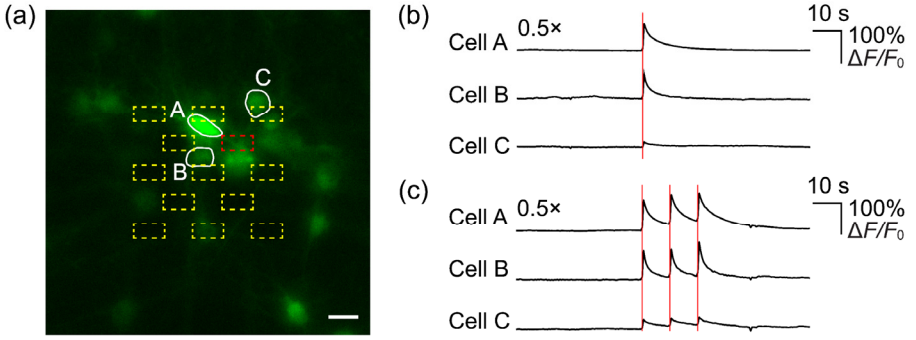

Fig.4. Neurostimulation experiment. (a) *GCaMP7s* image of neurons contacted with a graphene MEA. A total of 12 electrodes are marked in yellow; the select stimulation electrode is marked in red. Scale bar, 20 μm. (b) $\Delta F/F_0$ traces in 3 neurons (A-C) under 1 train of bi-phasic voltage pulses. (c)$\Delta F/F_0$ traces from 3 neurons (A-C) under 3 trains of bi-phasic voltage pulses. In (b) and (c), the trace of Cell A is scaled by 50 %; the red lines represent bi-phasic voltage pulses (50 cycles, ± 0.5 V amplitude, 500 μs duration per pulse with a 5 ms period).

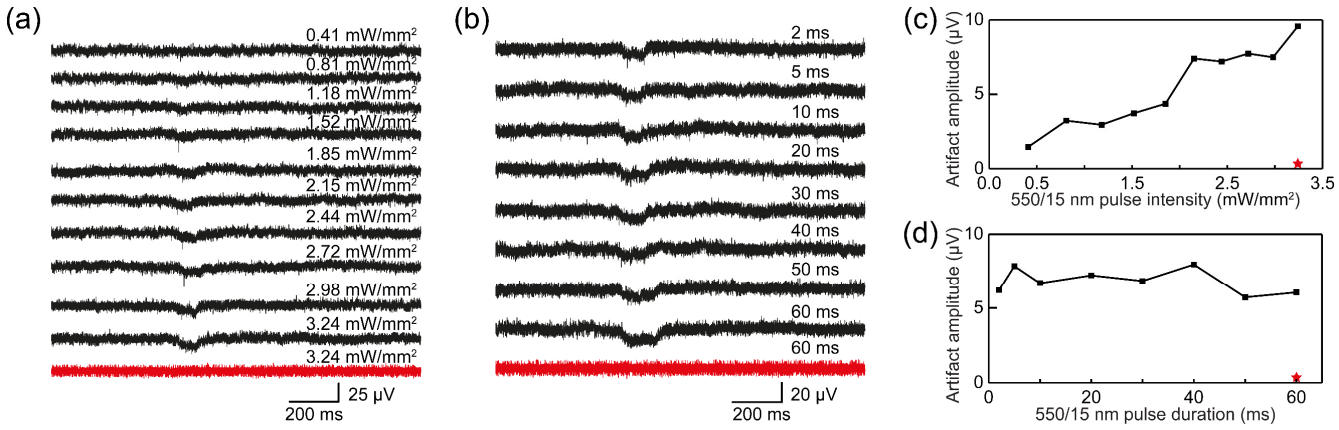

Fig. 5. Weak light-induced artifact in one PEDOT:PSS-coated graphene electrode. (a) Artifact traces (with no neurons) with a single 550/15 nm pulse (2 ms duration) at varying light intensities. (b) Artifact traces (with no neurons) with a single 550/15 nm pulse (3.24 mW/mm^2 intensity) at varying pulse durations. In (a) and (b), the red trace is recorded when one 100 Hz high pass filter is applied to the Intan chip. (c) Artifact amplitude vs. light intensity at 2 ms duration. (d) Artifact amplitude vs. light intensity at 3.24 mW/mm^2 intensity. In (c) and (d), the artifact amplitudes are defined as the absolute values of the minimum in the 1000-point adjacent-averaged traces; the red stars represent the artifact amplitude when we applied the 100 Hz high-pass filter to the Intan chip.

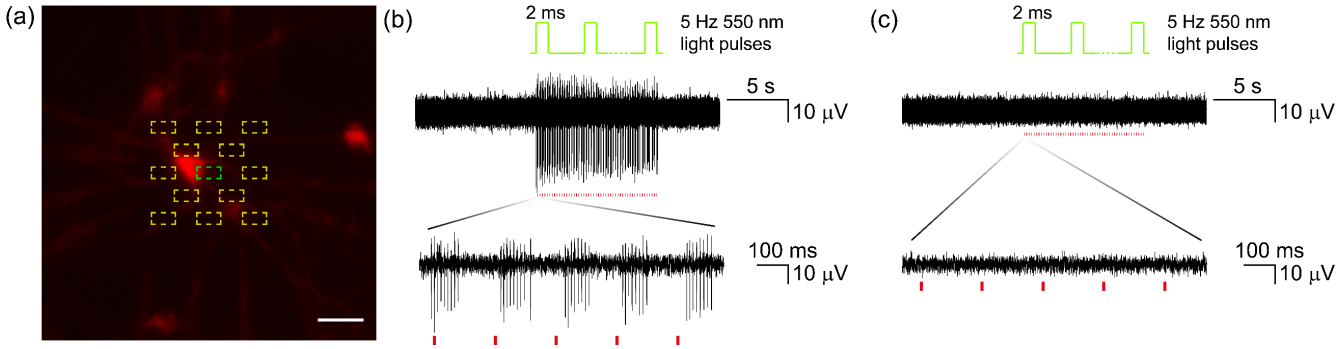

Fig. 6. Optogenetic electrophysiology experiment. (a) *Chrimson* image of neurons contacted with a graphene MEA. Electrodes are marked in dashed boxes; the central electrode (green) is selected for the following analysis. Scale bar, 40 μm. (b) 5-point adjacent-averaging trace of the central electrode under 0.41 mW/mm^2 550/15 nm optogenetic stimulus. (c) a control trace of the central electrode (measured without neurons) under 0.41 mW/mm^2 550/15 nm optogenetic stimulus. In (b) and (c), the green curve indicates the 10-s window of 550/15 nm stimulus, red dashed lines represent individual 550/15 nm pulses.

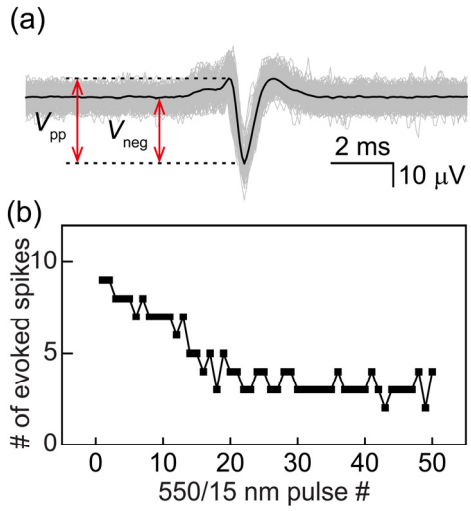

Fig. 7. Quantification of the neural spikes recorded within the 10-s optogenetic stimulus window by the central electrode (see Fig. 6a). (a) 226 recorded spikes with their average shown in black. The spikes were selected for analysis if their negative spike amplitudes (V_{neg}) are more than 5 times of the standard deviation in the background (i.e. the 10-20 s data in Fig. 6c) [10]. The peak-to-peak spike amplitude (V_{pp}) is 36.7 ± 6.5 μV. (b) The number of evoked spikes by each 550/15 nm pulse.

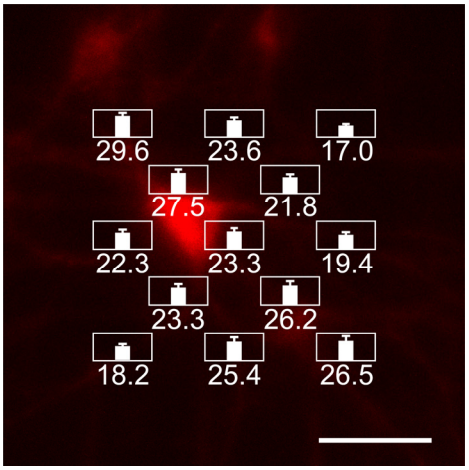

Fig. 8. Spatial mapping of V_{neg} amplitudes (averaged by 226 spikes in Fig. 7) across the MEA. A *Chrimson* image of neurons (contacted with a graphene MEA) is overlaid with 13 white boxes that indicate the electrode sites. Bar plots in each box and the numbers on top indicate the V_{neg} amplitude each electrode has recorded. The height of each white box is 40 μV. Scale bar, 40 μm.

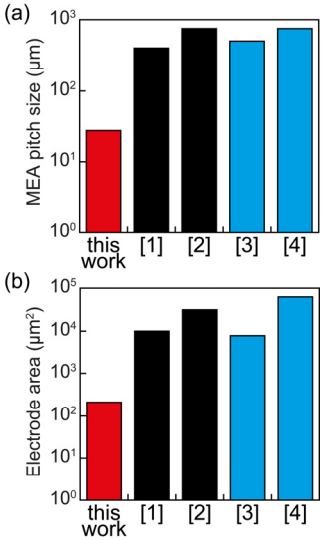

Fig.9. Spatial resolution achieved in this work and prior graphene MEA based neurostimulation (blue) and optogenetic electrophysiology (black) experiments. (a) a benchmark of the pitch size. (b) a benchmark of the electrode area.