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# Graphene-based neurotechnologies for advanced neural interfaces

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**Abstract**

Understanding how neuron populations transform activities of individual neurons into complex behaviors is one of the biggest challenges of neuroscience research. However, current neural monitoring and controlling technologies provide insufficient spatiotemporal resolution to unravel neural circuit functions. To this end, multifunctional neurotechnologies combining electrical, optical and chemical sensing and stimulation modalities have been proposed to overcome resolution limits. Research in multifunctional probes has fueled the demand for new materials to build minimally invasive chronic interfaces to the brain. Graphene has recently emerged as a neural interface material offering several outstanding properties, such as optical transparency, flexibility, high conductivity, functionalization and biocompatibility. The unique combination of these properties in a single material system makes graphene an attractive choice for multi-modal probing of neural activity. In this review, we discuss recent advances in graphene-based neurotechnologies, highlight different approaches and consider emerging directions inspired by unique characteristics of graphene.

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Graphene, Electrophysiology, Chemical sensing, Biocompatibility, Multimodal neural interface.

## Introduction

The complexity of neural activities has challenged both neuroscience research and clinical practice for decades. It is estimated that human brains consist of 86 billion neurons and quadrillions of synapses [1]. Understanding neuronal dynamics and information processing performed by neural populations requires advanced technologies with high-resolution sensing and stimulation capability. Clinical neuromodulation therapies widely used for neurological disorders also depend on the ability to manipulate the dynamics of neural circuits. Conventional neural interfaces offering electrical, optical, or chemical signals have greatly advanced our understanding of neural functions, however, most of these technologies are based on a single functionality. Combining multiple functionalities in a single system has recently been pursued as an integrative approach in new neurotechnology development.

Recently, graphene has drawn tremendous attention in neuroscience research owing to its flexibility, transparency, high conductivity, low noise, and biocompatibility [2–4]. This two-dimensional single-atom thick material, awarded Nobel Prize in Physics in 2010, has already shown to be promising for various neurotechnology applications including multimodal interfaces and closed-loop systems [5–10]. To-date, graphene has been used either passively in various microelectrode array configurations or actively in transistors. The primary signals detected and recorded by graphene neural interfaces are categorized as electrical, such as local field potentials, and chemical, such as neurotransmitter concentration. In Section State-of-the-art graphene-based neurotechnologies of this paper, we first review the state-of-the-art designs for graphene-based neural interfaces and evaluate their advantages for different applications. Then in Section Future Directions, we discuss future directions and potential advances for graphene-based neurotechnologies in both basic neuroscience research and medical applications.

## State-of-the-art graphene-based neurotechnologies

In this section we discuss recent work and literature on graphene-based microelectrodes, graphene field effect transistors for neural interfaces and the functionalization of graphene for chemical sensing, all of which are made possible because of the biocompatibility of graphene and its derivatives.

### Graphene-based microelectrodes

Electrophysiology has been the backbone of neuroscience research for several decades. It has led to numerous discoveries relating single-cell response to behavioral outcomes [2,11]. Although electrophysiological recordings have the unique advantage of high temporal

resolution in monitoring neural activity, the major limitation towards studying neural circuits is inability of sampling large number of neurons (~1000 cells) in neuronal populations. Last decade has witnessed rapid advancements in optical imaging methods for monitoring neural tissue. Fluorescence imaging extended the capabilities for monitoring hundreds of cells densely packed in local neuronal microcircuits. Furthermore, there has been significant progress in development of genetically encoded calcium indicators, enabling *in vivo* studies over weeks. The major drawback of cellular imaging is the poor temporal resolution due to slow kinetics of the indicators and low frame acquisition rates of microscopy systems [12,13]. Optical imaging and electrophysiological recordings can nicely complement each other as the weaknesses of one can be addressed by the strengths of the other [14]. However, conventional metal microelectrode arrays cannot be used for simultaneous optical imaging since they block the field of view, generate shadows, and cause light-induced artifacts in neural recordings [5,8]. In order to overcome this limitation, transparent flexible microelectrode arrays graphene have been proposed. The optical transparency of graphene arrays enables simultaneous electrophysiological recordings and calcium imaging [5,15].

Recent *in vitro* work with hippocampal slices reported that high frequency population bursts could be detected with the graphene electrodes while visually resolving the neural network and identifying the exact location of the active neurons with calcium fluorescence microscopy [5]. Figure 1A-a shows a steady-state fluorescence image of dentate gyrus, captured through the graphene electrode (the black square). Recordings with the graphene electrode (Figure 1A-b) exhibited population bursts, while calcium transient peaks ($\Delta F/F_0$) (Figure 1A-c) showed activity levels and spatial locations of individual cells (cell 1−6) overlapping with the graphene electrode. The temporal resolution of recordings with the graphene electrode enables detection of high frequency population spikes, which cannot be resolved by the calcium fluorescence responses. In contrast, calcium imaging responses captures complex network contributions of individual neurons, which are not evident in the electrical recordings. To illustrate, Figure 1A-c shows that most of the cells (cell 1−6) contribute to population bursts. However, cell 2 and cell 3 selectively responds to some of the population bursts and the peak amplitude of their response for each event varied [5]. Similarly, the transparent graphene electrode arrays were also used in conjunction with simultaneous wide-field calcium imaging in awake mice (Figure 1A-d). Electrical recordings synchronized with wide-field calcium imaging serves as a gold standard to confirm that wide-field calcium response was a good proxy for local activities of individual cortical modules [15].

In addition to calcium imaging, optogenetic modulation was also integrated with graphene electrode recordings [10,16]. The electrode array based on 4 layers of chemical-vapor-deposited (CVD) graphene allowed electrophysiological recording of neural activities activated by blue light of 473 nm wavelength in transgenic mice expressing channelrhodopsin-2. The biological responses induced by optogenetic stimulation were clearly detected with spatial distributions after a stimulus of 3 ms duration, however, as shown in the post-mortem control data (Figure 1B), the significant light-induced artifacts were observed, possibly due to increased absorption by multiple layers of graphene or relatively high impedance [16], which can be mitigated by using monolayer graphene or reducing impedance [6]. A more recent study has shown that careful design of key steps in the fabrication process for transparent graphene electrodes can mitigate the light-induced artifact problem and that virtually artifact-free local field potential recordings can be achieved within operating light intensities for optogenetic stimulation [17]. The same study has demonstrated that transparent graphene microelectrode arrays enable crosstalk-free integration of 2-photon microscopy, optogenetic stimulation, and cortical recordings in the same in vivo experiment by eliminating light-induced artifacts.

Although previous work has demonstrated that the microelectrode arrays based on CVD graphene could record local field potentials with high signal-to-noise ratio (SNR) of 40.8 [5], the impedance of monolayer graphene is relatively high compared to other faradaic or porous electrode materials. Chemical doping of graphene with nitric acid has shown to decrease the impedance up to 50% [5,18], nevertheless the lack of faradaic reactions as indicated by cyclic voltammetry [2,5,16] limits further decrease of the impedance and impedes scaling down the electrode dimensions to the single-cell size for high spatial resolution.

Reduction in impedance is also crucial for employing monolayer graphene for neural stimulation. Neural stimulation is widely used for mapping cortical regions in clinical practice. Although monolayer graphene provides numerous desirable characteristics for neural recording and imaging, the charge injection capacity of monolayer graphene electrodes is not sufficient to evoke electrical responses. Porous graphene consisting of 3D flakes of multi-layer graphene and graphene oxide was suggested as an electrode material to fabricate low impedance and high charge injection capacity microstimulation electrodes [7]. One of the major advantages of porous graphene is the simple and scalable fabrication in large areas. 3D porous graphene layers can be formed on standard polyimide substrates using $CO_2$ laser pyrolysis [19]. Most of the coatings that are used for reducing impedance suffer from delamination problems