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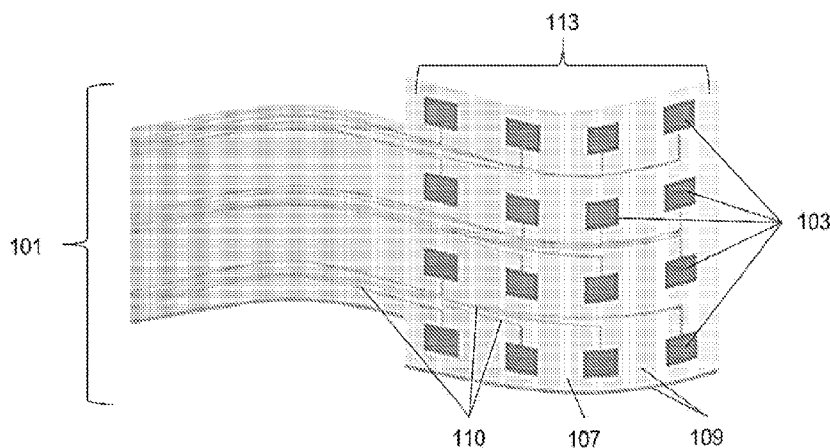


FIG. 5

(57) Abstract: A device with one or more probe(s) having one or more electrode(s) and at least one carbon-coated electrode, which may be operably configured to be connected to a controller and operably configured to measure electrical signals and/or chemical signals. A method of collecting electrical data and/or chemical data from a tissue by contacting a carbon-coated electrode with the tissue, measuring an electrical signal, measuring a chemical signal, and optionally communicating the electrical and/or chemical signal to a controller, where the electrical and/or chemical data is collected. The electrical signal may be measured by electrophysiology and the chemical signal may be measured by fast scan cyclic voltammetry. A method of making a carbon-coated probe by electrochemically depositing graphitic carbon onto a probe. The carbon-coated probe may be annealed from about 100°C to about 400°C.

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CARBON COATED ELECTRODES FOR NEUROCHEMICAL SENSING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/436,246, filed December 30, 2022; the contents of the above-identified application are hereby fully
5 incorporated herein by reference in their entirety.

BACKGROUND OF THE DISCLOSURE

[0002] Neurons in the brain communicate with one another through electrochemical signaling, such as action potentials and neurotransmitters. These two categories of signals form multi-scale temporally dynamic and spatially organized signal patterns, providing high-level
10 contextual control of cognition and behavior. Simultaneous monitoring of electrical spikes and neurotransmitters with high spatiotemporal resolution helps decode the relationships between the complex signal patterns and the functional connectivity, at the same time, facilitates the exploration of novel strategies for preventing, diagnosing, and treating a wide range of brain diseases. To achieve this, it would be enabling to develop a single platform that is able to
15 precisely localize recording sites relative to anatomical features across a wide range of spatial scales and record rapid neurochemical and electrophysiological events, but such a platform has not been possible due to formidable technical challenges.

[0003] Microelectrode-array (MEA)-based neural probes have been the gold standard and the workhorse of neuronal spike detection. If the same MEA platform could also detect chemical
20 neurotransmission, then it would provide an immediate solution to address the unmet need for the Ephys-neurochemical dual modality and readily enable numerous studies. Compared to practices such as concurrent fiber photometry-based optical sensing, a single platform minimizes the implant needs and system overhead, and the electrical approach is unique for translation (Table 1 and Table 2). However, conventional neuroelectrodes are not readily employable for
25 neurochemical sensing. Currently, a promising electroanalytical method of neurochemical measurements is fast-scan cyclic voltammetry (FSCV), due to its unique combination of temporal resolution, sensitivity, analyte selectivity, and its ability to detect and differentiate a variety of neurochemicals, including dopamine (DA), serotonin (5-HT), epinephrine (Epi), and adenosine (Ado). Compared to microdialysis (Table 2), which provides the best measurement for
30 baseline analyte levels, FSCV possesses temporal resolution (sub-second vs. minutes) and spatial

resolution (single neuron size vs. several hundred micrometers) to report the subcellular organization of neurochemical transmission and to detect fast transients. For neurotransmitters, FSCV is most popularly performed with carbon fiber microelectrodes (CFM) due to (i) being free of redox peaks in the FSCV voltage window (typically from -0.4V to 1.3V) and (ii) a great affinity to neurochemicals from its surface oxide functional groups, resulting in high electrode stability and sensitivity. Strides have been made to leverage CFMs or develop new carbon microelectrodes; however, there is still a big gap toward a truly dual functional MEA capable of parallel Ephys and real-time neurochemical measurements.

[0004] FSCV has been used in humans to measure sub-second DA fluctuations in nanomole levels. Carbon is currently the most popular material for FSCV sensing due to its superior electrochemical stability, sensitivity, and high biocompatibility. CFM has been a mainstay of neurotransmitter sensing for decades, but it's challenging to scale up CFM or integrate it with other neural interfacing devices. In addition, the silica-encapsulated CFM is often rigid and brittle, which might lead to early device failure. There has been increased interest in producing more microfabrication-friendly carbon electrodes and creating novel neurochemical probes. The carbonization strategies so far have included pyrolysis of polymer, laser-induced graphene (LIG), and chemical reduction of graphene oxide (Table 1). Pyrolysis of polymer can provide a glassy-carbon-like surface; thus, the carbon microelectrodes exhibit similar behavior to CFMs for electrochemical detection of neurotransmitters. But the carbonization process usually is performed at a temperature $\geq 1000\text{ }^{\circ}\text{C}$, making it hard to be compatible with many fabrication processes. LIG enables maskless and low-cost preparation, however, the most recently reported resolution of the laser technology is $100\text{ }\mu\text{m}$, which should be improved to achieve cellular resolution devices. Diamond electrodes are also great candidates for FSCV due to their wide potential window, low background current, and good biocompatibility, but are generally limited in their integrability and sensitivity. CVD boron-doped diamond is also grown at high temperatures, usually at $900\text{ }^{\circ}\text{C}$. Recent vitamin-C-reduced graphene oxide has integrated into standard microelectrode fabrication processes but has not demonstrated FSCV compatibility nor nM DA detection limit.

[0005] Kipke *et al.* have also demonstrated MEAs for concurrent neurochemical and Ephys recordings, but the neurochemical sensing was not done through FSCV but amperometry (with Pt microelectrodes), which has limited selectivity and temporal resolution.

[0006] Table 1: Comparison of FSCV-compatible sp²-carbon electrodes

	LOD (DA)	Demonstrated selectivity	Remarks, if any
Carbon fiber microelectrode [1]	6 nM	DA, 5-HT, Epi, Ado, NO, Glu, G, UA	Limited scalability and integratability
Pyrolysis of polymer [2, 3]	10 nM	DA, 5-HT	High processing temp. (>1000 °C)
Laser-induced graphitization [4]	5.6 nM	DA, 5-HT, Epi, Ado	Limited feature size and resolution
Our approach	5 nM	DA, 5-HT, Epi, Ado	

LOD: limit of detection. DA: dopamine. 5-HT: serotonin. Epi: epinephrine. Ado: adenosine. NO: nitric oxide. G: guanine. UA: uric acid.

Refs: [Puthongkham et al Electroanalysis 2018; 30(6): 1073-1081. [2] Nimbalkar et al Sci Rep 2018;8:6958. [3]

Cuniberto et al Sci Rep 2020; 10:9444. [4] Li et al Nature 2022;606, 94-101. References are a representative but not exhaustive list.

[0007] Table 2: Comparisons of state-of-the-art measurement methods of neurotransmitters.

<i>Methods</i>	<i>Key advantages</i>	<i>Main limitations currently</i>
FSCV	nM sensitivity, high selectivity, ms level temporal resolution	Scaling up; integration with parallel Ephys; durability
Fiber photometry	Optical level spatial resolution, cell-type specificity	Availability of fluorescent sensors; multiple micro-domain studies; translation
Microdialysis	pM sensitivity, multiple analytes of most molecules	Temporal resolution (minutes); spatial resolution (several 100s of μ m)

Bolded: advantages of the present disclosure

[0008] Great strides have been made to leverage carbon fiber electrodes or develop new carbon microelectrodes; however, there is still a big gap toward a truly dual functional neural probe capable of simultaneous spike recording and real-time neurochemical measurements.

SUMMARY OF THE DISCLOSURE

[0009] In an aspect, the present disclosure provides a device comprising one or more probe(s), each probe having a substrate and one or more electrode(s) disposed on the substrate, each electrode configured to be connected to a controller, where at least one of the electrode(s) has a graphitic carbon layer disposed on at least a portion of a surface of the electrode, and where the electrode is operably configured to measure electrical signals and chemical signals. The graphitic carbon layer may have about 12 atomic percent oxygen or less.

[0010] The graphitic carbon layer may be about 1 nm to about 10 μ m thick, including all 0.1 nm values and ranges therebetween. The graphitic carbon layer may be about 100 nm thick. The graphitic carbon layer may be polycrystalline. The graphitic carbon layer may have an atomic

ratio of amorphous carbon to graphitic carbon of about 1.26 or less. At least a portion of the carbon atoms in the graphitic carbon layer may be sp^2 -hybridized carbon atoms.

[0011] Each of the probe(s) may have an upper shaft and a lower shaft having the electrode with the graphitic carbon layer, where the width of the upper shaft is greater than the width of the lower shaft. The length of the probe may be about 1 mm or longer. The width of the upper shaft may be about 10 μm or greater. The electrode(s) may comprise an array.

[0012] The substrate of the probe may include polyimide, paralyene-C, polydimethylsiloxane, SU-8, liquid crystal polymer, silicon, glass, or the like, or any combination thereof. The electrode may include a metal, such as, for example, Au, Pt, or the like, or any combination thereof.

[0013] In an aspect, the present disclosure provides a method of collecting electrical and/or chemical data from a tissue of an individual, comprising contacting one or more electrode(s) with the graphitic carbon layer with the tissue of the individual; measuring an electrical signal; measuring a chemical signal; and communicating the electrical signal and chemical signal from the electrode(s) to a controller, where the electrical and/or chemical data is collected.

[0014] The measuring of the chemical signals may be through fast scan cyclic voltammetry. The measuring of the electrical signal may be through Ephys. The temporal gap between the measuring of the electrical signal and the measuring of the chemical signal may be about 1 s or less. The electrical data may have a spatial resolution of about 1 μm to about 10 mm, including all 0.1 μm values and ranges therebetween, and a temporal resolution of about 0.1 ms to about 1 s. The measuring of the electrical signal may have a sensitivity of about 1 μV to about 100 mV, including all 0.1 μV values and ranges therebetween. The chemical data may have a spatial resolution of about 1 μm to about 10 mm, including all 0.1 μm values and ranges therebetween, and a temporal resolution of about 1 ms to about 10 s, including all 0.1 ms values and ranges therebetween. The measuring of the chemical signal may have a sensitivity of about 1 nM to about 10 μM , including all 0.1 nM values and ranges therebetween.

[0015] In an aspect, the present disclosure provides a method of making a carbon-coated probe comprising providing one or more probe(s), each probe having one or more electrode(s); contacting the probe(s) with a mixture comprising graphene oxide; and, electrochemically depositing graphitic carbon on at least a portion of a surface of at least one of the electrode(s) of at least one of the probe(s), forming at least one carbon-coated probe. The method may further

comprise annealing the carbon-coated probe. The annealing may be performed at a temperature of about 100 °C to about 400 °C, including all 0.1 °C values and ranges therebetween. The annealing may be performed at a temperature of about 250 °C.

[0016] The mixture may be an aqueous suspension of the graphene oxide. The graphene oxide may be graphene oxide microflakes or the like.

BRIEF DESCRIPTION OF THE FIGURES

[0017] For a fuller understanding of the nature and objects of the disclosure, reference should be made to the following detailed description taken in conjunction with the accompanying figures.

[0018] FIG. 1 shows a neural-probe compatible, scalable, graphitic carbon (g-C) coating approach. a - Image of the 100-channel (100-ch) g-C coated microelectrode array (MEA) on Kapton® under optical microscope (left); SEM image of the surface morphologies of the g-C coating (middle) and the cross-section of the g-C microelectrode (right). Only one channel was not coated due to open circuit. b - EDS result of the carbon composition before and after the coating process. c - Raman spectra of the precursor and the g-C coating. d - CV curves from 99 g-C coated electrodes in a and their average. *Inset*: CV comparison from Au electrodes with and without g-C coating. CV scan rate: 100 mV/s.

[0019] FIG. 2 shows FSCV sensing performance and sensor regeneration of the g-C-coated microelectrodes. a - DA sensing result of g-C coated microelectrodes. b - Comparison of DA sensing of a CFME (Diameter 10 µm, length 100 µm) and a g-C coated microelectrode (60 µm×60 µm). c - 5-HT sensing result. d - Regeneration of g-C surface for DA sensing. The experiment was conducted in a flow cell. 100 µL of 5 µM DA was injected into the flow cell every 30 s. FSCV scan rate: 400 V/s.

[0020] FIG. 3 shows Raman spectra of the precursor, the g-C coating as deposited, and the g-C coating after annealing at 250 °C.

[0021] FIG. 4 shows that low temperature annealing improves the electrochemical stability of CCMs. a - EIS soak test results of CFMs, as-made CCMs and CCMs after annealing at 250 °C for 1 h in N₂; b - Interlayer spacing of as-made and annealed carbon coating as made by transmission electron microscopy.

[0022] FIG. 5 shows an example of a surface-contacting probe.

[0023] FIG. 6 shows an example of a penetrating probe with an electrode array.

[0024] FIG. 7 a - shows photographs of exemplary probes with both Ephys electrodes and a g-C coated microelectrode. b - shows impedance data of the Ephys electrodes depicted in 7a. c - shows an exemplary probe inserted in a transparent brain phantom material.

5 DETAILED DESCRIPTION OF THE DISCLOSURE

[0025] Although claimed subject matter will be described in terms of certain examples and embodiments, other examples and embodiments, including examples that do not provide all of the benefits and features set forth herein, are also within the scope of this disclosure. Various structural, logical, and process step changes may be made without departing from the scope of the disclosure.

10 [0026] As used herein, unless otherwise stated, “about”, “substantially”, or “the like”, when used in connection with a measurable variable (such as, for example, a parameter, an amount, a temporal duration, or the like) or a list of alternatives, is meant to encompass variations of and from the specified value including, but not limited to, those within experimental error (which can be determined by, e.g., a given data set, an art accepted standard, etc. and/or with, e.g., a given confidence interval (e.g. 90%, 95%, or more confidence interval from the mean), such as, for example, variations of +/-10% or less, +/-5% or less, +/-1% or less, and +/-0.1% or less of and from the specified value), insofar such variations in a variable and/or variations in the alternatives are appropriate to perform in the instant disclosure. As used herein, the term “about” may mean that the amount or value in question is the exact value or a value that provides equivalent results or effects as recited in the claims or taught herein. That is, it is understood that amounts, sizes, compositions, parameters, and other quantities and characteristics are not and need not be exact, but may be approximate and/or larger or smaller, as desired, reflecting tolerances, conversion factors, rounding off, measurement error, or the like, or other factors known to those of skill in the art such that equivalent results or effects are obtained. In general, an amount, size, composition, parameter, or other quantity or characteristic, or alternative is “about” or “the like,” whether or not expressly stated to be such. It is understood that where “about,” is used before a quantitative value, the parameter also includes the specific quantitative value itself, unless specifically stated otherwise.

20 [0027] Ranges of values are disclosed herein. The ranges set out a lower limit value and an upper limit value. Unless otherwise stated, the ranges include the lower limit value, the upper

limit value, and all values between the lower limit value and the upper limit value, including, but not limited to, all values to the magnitude of the smallest value (either the lower limit value or the upper limit value) of a range. It is to be understood that such a range format is used for convenience and brevity, and thus, should be interpreted in a flexible manner to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. To illustrate, a numerical range of “0.1% to 5%” should be interpreted to include not only the explicitly recited values of 0.1% to 5%, but also, unless otherwise stated, include individual values (e.g., 1%, 2%, 3%, and 4%) and the sub-ranges (e.g., 0.5% to 1.1%; 0.5% to 2.4%; 0.5% to 3.2%, and 0.5% to 4.4%, and other possible sub-ranges) within the indicated range. It is also understood (as presented above) that there are a number of values disclosed herein, and that each value is also herein disclosed as “about” that particular value in addition to the value itself. For example, if the value “10” is disclosed, then “about 10” is also disclosed. Ranges can be expressed herein as from “about” one particular value, and/or to “about” another particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about, it will be understood that the particular value forms a further disclosure. For example, if the value “about 10” is disclosed, then “10” is also disclosed.

[0028] As used herein, the terms “including,” “containing,” and “comprising” are used in their open, non-limiting sense.

[0029] As used in this disclosure, the singular forms include the plural forms and vice versa unless the context clearly indicates otherwise.

[0030] The articles “a” and “an” are used in this disclosure to refer to one or more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[0031] As used herein, unless otherwise stated or indicated, “s” refers to second(s), “min” refers to minute(s), and “h” refers to hour(s).

[0032] The present disclosure provides, *inter alia*, devices and methods for measuring electrical signals and/or chemical signals. The present disclosure also provides methods of making a carbon-coated probe.

[0033] In an aspect, the present disclosure provides devices comprising one or more probe(s) (101). In various examples, each probe comprises a substrate (102) and one or more electrode(s)

(103) disposed on (e.g., disposed on the surface of, embedded within, disposed throughout, or attached to) the substrate (102). In various examples, at least one, or a plurality, or substantially all, or all of the electrode(s) (103) comprises a graphitic carbon layer (105) disposed on at least a portion, substantially all, or all of a surface (106) of the electrode(s). In various examples, each electrode (103) is independently operably configured to be connected to a controller (104) or controllers. In various examples, each electrode comprising the graphitic carbon layer (108) is operably configured to measure electrical signals, chemical signals, or the like, or any combination thereof. In various examples, a device, or one or more, or all of the probe(s) of a device, is/are made by one or more method(s) of the present disclosure. Non-limiting examples of devices are provided herein.

[0034] In various examples, a probe is a penetrating probe. In various examples, a penetrating probe is configured to penetrate (e.g., such that the probe is in electrical and/or chemical contact with) a tissue. In various examples, a probe is surface-contacting probe. In various examples, a surface-contacting probe is configured to be disposed on (e.g., in electrical and/or chemical contact with) a tissue and not to penetrate the tissue.

[0035] In various examples, a probe (101) is an instrument where at least a portion of the instrument is configured to contact a tissue (e.g., a tissue of an individual) in vitro or in vivo. In various examples, a probe (101) is configured to be at least partially inserted into a tissue. In various examples, a probe (101) has one or more planar surface(s) (107), and at least a portion of one of the planar surface(s) is configured to contact a tissue (e.g., a portion of a planar surface is configured to be disposed on and in contact with a surface of a tissue). In various examples, the tissue comprises neural tissue, neurons, nerve cells, or the like, or any combination thereof. In various examples, the tissue is brain tissue or the like.

[0036] In various examples, the probe (101) comprises a substrate (102). A substrate (or a probe comprising the substrate) can have various shapes. In various examples, a substrate (such as, for example, a substrate of either a penetrating probe or a surface-contacting probe) is a planar substrate, such as, for example, a planar polygonal substrate (e.g., a planar rectangular substrate, a planar triangular substrate, a planar square substrate, or the like), a planar circular substrate, a planar oval substrate, or the like. In various examples, a substrate (such as, for example, a substrate of a penetrating probe) is a cylindrical substrate or a cylindrical substrate comprising a tapered (e.g., conical) end or the like.

[0037] In various examples, a substrate is a flexible substrate. In various examples the substrate (102) comprises a flexible material, such as, for example, polyimide (e.g., Kapton[®]), paralyene-C, polydimethylsiloxane, SU-8, liquid crystal polymer, or the like, or any combination thereof. In various examples, a substrate is a rigid substrate. In various examples, the substrate (102) comprises a rigid material, such as, for example silicon, glass, or the like, or any combination thereof. In various examples, the substrate (102) comprises a combination of one or more flexible material(s) and one or more rigid material(s). Other examples of flexible materials and rigid materials are known in the art.

[0038] In various examples, a probe (101) comprises one or more electrode(s) (103). In various examples, an electrode (103) is disposed on (e.g., disposed on the surface of, embedded within, disposed throughout, or attached to) the substrate (102) of the probe (101). In various examples, an electrode (103) comprises a conductive material (e.g., a metal) with a surface (106), where at least a portion of the surface (106) of the electrode (103) is in electrical communication with the external environment (i.e., not conductively insulated from the external environment of the probe (101)). The external environment of the probe (101) is the physical space surrounding and in contact with an outer surface (109) of the probe (101), and anything (e.g., any chemicals) in that physical space. For example, the external environment can be neural tissue or other parts of a human or non-human animal. In various examples, the probe (101) comprises a connection (110) (e.g., wires, microwires, conductive material, or the like) that is in electrical communication with the electrode (103) and the controller (104). As an illustrative example, electrical signals can be received at the electrode (103) and then conducted through the connection (110) to a controller (104).

[0039] In various examples, the electrode (103) is operably configured to conduct electric signals from the electrode (103) to a controller (104). In various examples, the electrode (103) is operably configured to conduct an electrical signal from a surface (106) of an electrode (103) to a controller (104), where the surface (106) of the electrode (103) is in contact with a tissue, and the electrical signal is produced by the tissue.

[0040] In various examples, the probe (101) measures electrical and/or chemical signals. In various examples, an electrical signal is an electrical impulse (e.g., an electrical impulse generated by a neuron or nerve cell in a tissue). In various examples, the probe (101) measures

an electrical signal when the electrical signal contacts the surface (106) of an electrode (103) which conducts the electrical signal to a controller (104).

[0041] In various examples, a chemical signal results from and/or correlates to a change (e.g., an increase or a decrease) in concentration of a chemical or chemicals (e.g., a chemical or chemicals within a tissue), such as, for example, chemical compound(s), ion(s), biological compound(s) (e.g., protein(s), peptide(s), or the like), or the like. In various examples, a chemical signal results from and/or correlates to a change (e.g., an increase or a decrease) in pH or the like. In various examples, a chemical signal results from and/or correlates to, for example, a decrease or increase in concentration of a chemical. In various examples, a chemical signal results from and/or correlates to an increase from an unmeasurable concentration of a chemical (e.g., a concentration of about 0 or near-0) to a measurable concentration (e.g., a concentration greater than about 0), or the reverse. In various examples, a chemical signal results from and/or correlates to a change in concentration of a neurotransmitter (e.g., a neurotransmitter generated by a neuron or nerve cell in a tissue). Examples of neurotransmitters include, but are not limited to, dopamine, serotonin, epinephrine, adenosine, and the like, and any combinations thereof. In various examples, a chemical signal results from and/or correlates to a change in concentration of other chemicals of interest in neural tissue, such as, for example, glutamate, hydrogen peroxide, or the like, or any combination thereof.

[0042] In various examples, at least one of the electrode(s) (103) comprises a graphitic carbon layer (105) disposed on at least a portion of a surface (106) or surfaces of the electrode (108). In various examples, the graphitic carbon layer (105) is disposed on a portion, substantially all, or all of an outer surface (106) or surfaces of the electrode (108). In various examples, the graphitic carbon layer (105) is disposed on a portion, substantially all, or all of an outer surface (109) of the probe (101).

[0043] In various examples, the graphitic carbon layer comprises greater than about 71 atomic percent carbon. In various examples, the graphitic carbon layer comprises greater than about 90 atomic percent carbon. In various examples, the graphitic carbon layer comprises 12 atomic percent oxygen or less. In various examples, the graphitic carbon layer comprises about 12 to about 0.1 atomic percent oxygen, including all 0.05 atomic percent oxygen values and ranges therebetween (e.g., about 8 atomic percent oxygen to about 1 atomic percent oxygen). In various examples, the sum of the atomic percent oxygen and the atomic percent carbon is about

100 percent. The atomic percent of oxygen and/or carbon can be determined by methods known in the art. For example, the atomic percent of oxygen and carbon can be determined by energy-dispersive X-ray spectroscopy (EDS) or the like.

[0044] A graphitic carbon layer can have various thicknesses. In various examples, the graphitic carbon layer has a thickness of about 1 nm to about 10 μm , including all 0.1 nm values and ranges therebetween (e.g., about 100 nm).

[0045] A graphitic carbon layer can have various interlayer distances (e.g., distance between individual crystalline layers of the graphitic carbon). In various examples, the distance or average distance between individual crystalline layers of the graphitic carbon is about 0.34 nm to about 0.37 nm, including all 0.005 nm values and ranges therebetween (e.g., about 0.325 nm to about 0.340 nm, including all 0.001 nm values and ranges therebetween).

[0046] In various examples, the graphitic carbon layer comprises an atomic ratio of amorphous carbon to graphitic carbon of about 1.26 or less, or less than about 1.26. In various examples, the atomic ratio of amorphous carbon to graphitic carbon is about 1.20 to about 1.05, including all 0.001 values and ranges therebetween. In various examples, the atomic ratio of amorphous carbon to graphitic carbon is about 1.17 (FIG. 1c and FIG. 3). The atomic ratio of amorphous carbon to graphitic carbon can be determined by methods known in the art. For example, the ratio of amorphous carbon to graphitic carbon can be determined by Raman spectroscopy. In various examples, at least a portion, a portion, substantially all, or all of the carbon atoms of the graphitic carbon layer are sp^2 -hybridized carbon atoms.

[0047] In various examples, a portion of, substantially all, or all of the graphitic carbon layer is polycrystalline. In various examples, the graphitic carbon layer comprises layers of crystalline graphitic carbon flakes, such as, for example, crystalline graphitic carbon microflakes or the like.

[0048] In various examples, each of the probe(s) (101) comprises an upper shaft (111) and a lower shaft (112). In various examples, the upper shaft (111) and the lower shaft (112) are oriented along an axis corresponding to a length of the probe. In various examples, the lower shaft (112) comprises the electrode(s) (108) comprising the graphitic carbon layer (105), and a width of the upper shaft (111) is greater than a width of the lower shaft (112). In various examples, each of the probe(s) (101) individually have a length longer than or equal to about 1 mm. In various examples, the upper shaft (111) comprises a pad array (e.g., the electrode(s)

(103) are arranged in an array). In various examples, the width of the upper shaft (111) is greater than or equal to about 10 μm .

[0049] In various examples, the probe (101) comprises a substrate (102) with a planar surface (107) and an array of electrodes (103) disposed on (e.g., disposed on the surface of, embedded within, disposed throughout, or attached to) the planar surface (107). In various examples, the array of electrodes (103) is arranged in a grid (113) on the planar surface (107), such as, for an illustrative example, 25 electrodes arranged in a 5 x 5 grid, 20 electrodes arranged in a 5 x 4 grid, 16 electrodes arranged in a 4 x 4 grid, 12 electrodes arranged in a 4 x 3 grid, 9 electrodes arranged in a 3 x 3 grid, or the like.

[0050] In an aspect, the present disclosure provides a method of collecting electrical and/or chemical data about a tissue or tissues. In various examples, a method comprises contacting one or more electrode(s) (103, 108) comprising the graphitic carbon layer (105) with a tissue or tissues, measuring an electrical signal and/or measuring a chemical signal and, optionally, collecting electrical and/or chemical data corresponding to the electrical signal and/or chemical signal. In various examples, collecting electrical and/or chemical data comprises communicating the electrical signal and/or chemical signal from the one or more electrode(s) (103, 108) to a controller (104) or controllers. In various examples a method is an in vitro method or an in vivo method. Non-limiting examples of methods of collecting electrical and/or chemical data are provided herein.

[0051] In various examples, the individual is an animal. The subject may be human or non-human (e.g., mammal). Non-human animals include ungulates such as bovines. Additional non-limiting examples of non-human mammals include pigs, mice, rats, rabbits, cats, dogs, other agricultural mammals, pets, service animals, and the like. In various examples, the individual is a human. In various examples, the tissue is, for example, brain tissue or the like. In various examples, a tissue is present in an individual (e.g., in an in vivo method). In various examples, a tissue is not present in an individual (e.g., in an in vitro method).

[0052] In various examples, the measuring of the chemical signal comprises cyclic voltammetry (CV) or the like. In various examples, the measuring of the chemical signal comprises fast scan cyclic voltammetry (FSCV) or the like.

[0053] In various examples, the measuring of the electrical signal comprises electrical recording or the like. In various examples, the measuring of the electrical signal comprises electrophysiology (Ephys) or the like.

5 [0054] In various examples, the measuring of the electrical signal and the measuring of the chemical signal are substantially simultaneous (e.g., a temporal gap between the measuring of the electrical signal and the measuring of the chemical signal is about 1 s or less). In various examples, the electrical data has a spatial resolution of about 1 μm to about 10 mm, including all 0.1 μm values and ranges therebetween. In various examples, the electrical data has a temporal resolution of about 0.1 ms to about 1 s, including all 0.1 ms values and ranges therebetween. In
10 various examples, the measuring of the electrical data has a sensitivity of about 1 μV to about 100 mV, including all 0.1 μV values and ranges therebetween. In various examples, the chemical data has a spatial resolution of about 1 μm to about 10 mm, including all 0.1 μm values and ranges therebetween. In various examples, the chemical data has a temporal resolution of about 1 ms to about 10 s, including all 0.1 ms values and ranges therebetween. In various examples, the
15 measuring of the chemical data has a sensitivity of about 1 nM to about 10 μM , including all 0.1 nM values and ranges therebetween.

[0055] In an aspect, the present disclosure provides a method of making a carbon-coated probe. In various examples, a carbon-coated probe comprises one or more graphitic carbon layer(s). In various examples, a method comprises providing one or more probe(s) (101), each
20 probe (101) comprising a substrate and one or more electrode(s) (103) disposed on (e.g., disposed on the surface of, embedded within, disposed throughout, or attached to) the substrate, contacting the probe(s) (101) with a mixture comprising graphene oxide, and electrochemically depositing graphitic carbon on at least a portion of a surface (106) of at least one of the electrode(s) (103) of at least one of the probe(s) (101), forming at least one carbon-coated probe
25 (101). In various examples, a method makes a carbon-coated probe of the present disclosure. Non-limiting examples of methods of making carbon-coated probes are provided herein.

[0056] Various probe(s) can be used. Suitable probes are known in the art. In various examples, the probe(s) (101) are chosen from Ephys electrodes (e.g., Ephys microelectrodes), stereoelectroencephalography (SEEG) electrodes, electrocorticography (ECoG) electrodes, Si
30 probes, deep brain stimulation (DBS) electrodes, and the like.

[0057] In various examples, a method comprises providing one or more substrate(s), which may be probe(s) (101), each substrate comprising one or more electrode(s) (103) disposed on (e.g., disposed on the surface of, embedded within, disposed throughout, or attached to) the substrate, contacting the substrate with a mixture comprising graphene oxide, and
5 electrochemically depositing graphitic carbon on at least a portion of a surface (106) of at least one of the electrode(s) (103) of at least one of the substrate(s), forming at least one carbon-coated electrode (108).

[0058] In various examples, a method further comprises annealing the carbon-coated probe or carbon-coated electrode (108). In various examples, the annealing is performed at a
10 temperature of about 100 °C to about 400 °C, including all 0.1 °C values and ranges therebetween (e.g., about 100 °C to about 300 °C). In various examples, the annealing is performed at a temperature of about 250 °C. In various examples, the annealing is performed for a duration of about 5 min to about 5 hours, including all integer s values and ranges therebetween. In various
15 examples, the annealing is performed for a duration of about 30 min to about 3 hours, including all integer s values and ranges therebetween. In various examples, the annealing is performed for a duration of about 1 hour. In various examples, the annealing is performed in an inert atmosphere, such as, for example, in nitrogen gas or other inert gas, such as, for example, argon gas or the like, or any combination of inert gases. In various examples, the annealing is
performed at room temperature (e.g., about 15 °C to about 25 °C, including 15 °C, 16 °C, 17 °C,
20 18 °C, 19 °C, 20 °C, 21 °C, 22 °C, 23 °C, 24 °C, 25 °C, including all 0.1 °C values and ranges therebetween). In various examples, the annealing is performed at ambient pressure (e.g., from about 0.7 atm to about 1 atm, including all 0.01 atm values and ranges therebetween).

[0059] A carbon-coated probe can exhibit desirable stability. In various examples, a carbon-coated probe is resistant to failure or does not fail (e.g., the graphitic carbon layer (106) is
25 resistant to delamination or does not delaminate or the like) in electrolyte solution or tissue or the like. In various examples, a carbon-coated probe of the present disclosure does not exhibit chemical degradation of the graphitic carbon layer (106) (e.g., the graphitic carbon layer (106) does not degrade in electrolyte solution or tissue or the like). In various examples, a carbon-coated probe of the present disclosure does not fail after at least 5,000, at least 10,000, at least
30 15,000, at least 18,000, or at least 20,000 cycles of FSCV.

[0060] Without intending to be bound by any particular theory, it is considered that the annealing step decreases the interlayer distance of the graphitic carbon layer (e.g., distance between individual crystalline layers of the graphitic carbon), which increases the density of the graphitic carbon layer and improves its stability. As an illustrative example, before annealing, the distance or average distance between individual crystalline layers of the graphitic carbon is 0.35 nm, and after annealing, the distance or average distance between individual crystalline layers of the graphitic carbon is reduced to 0.33 nm.

[0061] Without intending to be bound by any particular theory, it is considered that the annealing step thermally reduces the graphitic carbon layer and lowers the atomic ratio of oxygen to carbon, where the lowered atomic percent of oxygen in the graphitic carbon layer contributes to the performance and stability of the graphitic carbon layer (e.g., improves FSCV functionality). As an illustrative example, the amount of oxygen pre-annealing is about 15 atomic percent, and after annealing is about 10 atomic percent.

[0062] Without intending to be bound by any particular theory, it is considered that the annealing step lowers the atomic ratio of amorphous carbon, which is considered to improve the performance and stability of the graphitic carbon layer (e.g., improve FSCV functionality). As an illustrative example, the atomic ratio of amorphous carbon (d-Carbon or d-C) to graphitic carbon (g-Carbon or g-C) pre-annealing is about 1.26, and after annealing is about 1.17.

[0063] In various examples, the mixture is an aqueous suspension of graphene oxide (e.g., graphene oxide flakes, few-layer graphene oxide microflakes, or the like). In various examples, contacting the probe(s) (101) with a mixture comprising graphene oxide provides a layer of graphene oxide (e.g., graphene oxide flakes, few-layer graphene oxide microflakes, or the like) disposed on at least a portion of an electrode or electrode a substrate. In various examples, a layer is monolayer of graphene oxide (e.g., graphene oxide flakes, few-layer graphene oxide microflakes, or the like) or a layer comprising a plurality of layers of graphene oxide (e.g., having a thickness of 10s of microns).

[0064] In various examples, the electrochemical depositing is performed at a potential of about -1.5 V to about -0.3 V, including all 0.01 V values and ranges therebetween, relative to an Ag/AgCl reference electrode. In various examples, the electrochemical depositing is performed for a duration of about 1 min to about 1 hour, including all integer s values and ranges therebetween. In various examples, the electrochemical depositing is performed at room

temperature (e.g., about 15 °C to about 25 °C, including 15 °C, 16 °C, 17 °C, 18 °C, 19 °C, 20 °C, 21 °C, 22 °C, 23 °C, 24 °C, 25 °C, and all 0.1 °C values and ranges therebetween). Without intending to be bound by any particular theory, it is considered that the graphene oxide in the mixture is electrochemically reduced, whereby a layer of graphitic carbon is deposited on the surface of the probe in contact with the mixture.

[0065] The steps of the method described in the various embodiments and examples disclosed herein are sufficient to carry out the methods of the present invention. Thus, in an embodiment, the method consists essentially of a combination of the steps of the methods disclosed herein. In another embodiment, the method consists of such steps.

[0066] The following Statements are not intended to be limiting in any manner.

Statement 1. A surface graphitic carbon coating method, comprising dissolving graphene oxide (GO) micro-flakes in an organic solvent to form a graphitic carbon solution; and forming a graphitic carbon coating on a surface of an electrode by: electrochemical deposition in the graphitic carbon solution; and exposing the graphitic carbon coating to a thermal treatment at greater than about 100 °C (e.g., from greater than about 100 °C to about 300 °C, such as about 250°C).

Statement 2. The method according to Statement 1, wherein the electrode is coated with the graphitic carbon coating with a thickness from about 1 nm to about 10 μm (e.g., about 100 nm).

Statement 3. The method according to any one of the preceding Statements, wherein the graphitic carbon coating has an atomic ratio percentage of carbon greater than about 71 percent.

Statement 4. The method according to any one of the preceding Statements, wherein the graphitic carbon coating is conformal.

Statement 5. The method according to any one of the preceding Statements, wherein the graphitic carbon coating is at least partially crystallized, or crystallized.

Statement 7. A probe device comprising an upper shaft comprising a pad array; and, a lower shaft comprising at least one graphitic carbon coated electrode, wherein a width of the upper shaft is greater than a width of the lower shaft.

Statement 8. The probe according to Statement 7, further comprising at least one carbon coated electrode.

Statement 9. The probe according to Statement 8, wherein the graphitic carbon coated electrode is formed using the method of Statement 1.

Statement 10. The probe according to any one of Statements 7–9, further comprising at least one electrophysiology electrode.

Statement 11. The probe according to any one of Statements 7–10, wherein the probe has a length longer than or equal to about 1 mm.

5 Statement 12. The probe according to any one of Statements 7–11, wherein the width of the upper shaft is greater than or equal to about 10 μm .

Statement 13. The probe according to any one of Statements 7–12, wherein the pad array of the upper shaft further comprises alignment holes.

10 Statement 14. A device, comprising one or more probe(s), each probe comprising a substrate and one or more electrode(s) disposed on the substrate, each electrode operably configured to be connected to a controller, wherein at least one of the electrode(s) comprises a graphitic carbon layer disposed on at least a portion of a surface of the electrode and wherein the electrode is operably configured to measure electrical signals and chemical signals, and wherein the graphitic carbon layer comprises about 12 atomic percent oxygen or less.

15 Statement 15. The device according to Statement 14, wherein the graphitic carbon layer comprises greater than about 71 atomic percent carbon.

Statement 16. The device according to Statement 14 or Statement 15, wherein the graphitic carbon layer comprises a thickness of about 1 nm to about 10 μm .

20 Statement 17. The device according to any one of Statements 14–16, wherein the graphitic carbon layer comprises a thickness of about 100 nm.

Statement 18. The device according to any one of Statements 14–17, wherein at least a portion of the graphitic carbon layer is polycrystalline.

25 Statement 19. The device according to any one of Statements 14–18, wherein the graphitic carbon layer comprises an atomic ratio of amorphous carbon to graphitic carbon of about 1.26 or less.

Statement 20. The device according to any one of Statements 14–19, wherein at least a portion of the carbon atoms of the graphitic carbon layer are sp^2 -hybridized carbon atoms.

30 Statement 21. The device according to any one of Statements 14–20, wherein each of the probe(s) comprises an upper shaft and a lower shaft comprising the electrode(s) comprising the graphitic carbon layer, wherein a width of the upper shaft is greater than a width of the lower shaft.

Statement 22. The device according to any one of Statements 14–21, wherein the probe(s) has a length longer than or equal to about 1 mm.

Statement 23. The device according to Statement 21, wherein the width of the upper shaft is greater than or equal to about 10 μm .

5 Statement 24. The device according to any one of Statements 14–23, wherein the electrode(s) comprise an array.

Statement 25. The device according to any one of Statements 14–24, wherein the substrate comprises a material chosen from polyimide, paralyene-C, polydimethylsiloxane, SU-8, liquid crystal polymer, silicon, glass, and any combination thereof.

10 Statement 26. The device according to any one of Statements 14–25, wherein the electrode(s) comprise a metal.

Statement 27. The device according to Statement 26, wherein the metal is chosen from Au and Pt.

Statement 28. A method of collecting electrical and chemical data from a tissue of an individual,
15 comprising contacting one or more electrode(s) of claim 1 comprising the graphitic carbon layer with the tissue of the individual; measuring an electrical signal; measuring a chemical signal; and, communicating the electrical signal and chemical signal from the one or more electrode(s) to a controller, wherein the electrical and chemical data is collected.

Statement 29. The method according to Statement 28, wherein the measuring of the chemical
20 signal comprises fast scan cyclic voltammetry (FSCV).

Statement 30. The method according to Statement 28 or Statement 29, wherein the measuring of the electrical signal comprises electrophysiology (Ephys).

Statement 31. The method according to any one of Statements 28–30, wherein a temporal gap
25 between the measuring of the electrical signal and the measuring of the chemical signal is about 1 s or less.

Statement 32. The method according to any one of Statements 28–31, wherein the electrical data comprises a spatial resolution of about 1 μm to about 10 mm.

Statement 33. The method according to any one of Statements 28–32, wherein the electrical data comprises a temporal resolution of about 0.1 ms to about 1 s.

30 Statement 34. The method according to any one of Statements 28–33, wherein the measuring of an electrical signal comprises a sensitivity of about 1 μV to about 100 mV.

Statement 35. The method according to any one of Statements 28–34, wherein the chemical data comprises a spatial resolution of about 1 μm to about 10 mm.

Statement 36. The method according to any one of Statements 28–35, wherein the chemical data comprises a temporal resolution of about 1 ms to about 10 s.

5 Statement 37. The method according to any one of Statements 28–36, wherein the measuring of a chemical signal comprises a sensitivity of about 1 nM to about 10 μM .

Statement 38. A method of making a carbon-coated probe, comprising providing one or more probe(s), each probe comprising one or more electrode(s); contacting the probe(s) with a mixture comprising graphene oxide; and, electrochemically depositing graphitic carbon on at least a
10 portion of a surface of at least one of the electrode(s) of at least one of the probe(s), forming at least one carbon-coated probe.

Statement 39. The method according to Statement 38, further comprising annealing the carbon-coated probe.

Statement 40. The method according to Statement 38 or Statement 39, wherein the annealing is
15 performed at a temperature of about 100 $^{\circ}\text{C}$ to about 400 $^{\circ}\text{C}$.

Statement 41. The method according to any one of Statements 38–40, wherein the annealing is performed at a temperature of about 250 $^{\circ}\text{C}$.

Statement 42. The method according to any one of Statements 38–41, wherein the mixture is an aqueous suspension of the graphene oxide.

20 Statement 43. The method according to any one of Statements 38–42, wherein the graphene oxide is graphene oxide microflakes.

[0067] The following examples are presented to illustrate the present disclosure. They are not intended to be limiting in any matter.

EXAMPLE 1

25 **[0068]** This example provides a description of a device and methods of making and using same.

[0069] A microelectrode compatible, g-C coating method (FIG. 1) is described herein. This method uses a stable aqueous suspension of few-layer graphene oxide (GO) micro-flakes as a precursor and realizes the g-C coating on microelectrodes (e.g., gold) through an electrochemical
30 reduction, followed by another thermal-reduction process at mild temperature (250 $^{\circ}\text{C}$) after the

solution processing which further improved the adhesion and performance of the g-C coated electrode. The g-C coated electrode is FSCV-compatible and can achieve a low limit of detection and high sensitivity for DA sensing (FIG. 2), which might result from the edges, defects, and oxide functional groups in the coating (FIG. 1). Moreover, the g-C microelectrodes can be easily integrated with electrophysiological electrodes on a single neural probe due to its simple solution processing and low processing temperature, which also lead to unprecedented scalability – demonstrated at the 100-ch level – of g-C electrode production.

[0070] Scalable g-C coating on neuroelectrodes. Successful g-C coating over 100-ch MEA was achieved with near unity yield. First, gold (Au) electrodes were fabricated by standard microfabrication on flexible Kapton® substrates (there is always Titanium (Ti) adhesion underneath Au unless otherwise noted). The g-C coating used an electrochemical deposition in an aqueous suspension of few-layer GO micro-flakes followed by a thermal reduction at 250 °C. The deposition process reduced the GO, which led to the oxygen ratio decreasing from 28.6% to 12.5%, and was further reduced to 6.99% by the thermal reduction step (FIG. 1b). Raman spectra shows an increasing D/G ratio, which might result from the fragmentation of the precursor flakes during electrochemical reduction (FIG. 1c). With a carbon coating around 100 nm, a wide electrochemical stability window was achieved. No Au oxidation occurred in all the 99 C-coated microelectrodes (FIG. 1d, the only one not coated was due to an open circuit).

[0071] FSCV performance on neurotransmitter sensing. The g-C coated microelectrodes have a stable background current during FSCV scanning, which enables them to achieve a good performance of DA and 5-HT sensing (FIG. 2a-c). The shapes of the oxidation peaks of DA and 5-HT are different, making it possible to distinguish DA from 5-HT. 5nM DA can be detected with a notable oxidation peak. The low limit of detection benefits from the special micro/nano-structures and chemical compositions of our carbon coating. The sensitivity of the g-C-coated microelectrode is as high as about 3 times of CFME (127.2 nA/μM vs. 33.5 nA/μM). Notably, the sensing capability can be regenerated after 30s FSCV scanning while tested in a flow cell system (FIG. 2d).

EXAMPLE 2

[0072] This example provides a description of a device and methods of making and using same.

[0073] A microelectrode-compatible carbon coating method (FIG. 1, FIG. 2, FIG. 4) was developed for parallel Ephys-FSCV. This method first employs electrochemical reduction of few-layer graphene oxide micro-flakes in a stable aqueous suspension and achieves electrochemically active carbon layers. Successful carbon coating unprecedentedly over 100-ch with near unity yield was demonstrated (FIG. 1). The 100-ch MEA initially contains standard gold (Au) microelectrodes ($20 \times 20 \mu\text{m}^2$) on Kapton[®] substrates (there is always Titanium (Ti) adhesion underneath Au unless otherwise noted). With a carbon coating of around 100 nm, a wide electrochemical window was achieved in aqueous solutions; no Au oxidation occurred in any of the carbon-coated electrodes (FIG. 1d). The present disclosure applies and optimizes carbon coating for FSCV, which demands strong coating adhesion and high electrochemical stability.

[0074] After the coating process, thermal treatment at a mild temperature (200–300 °C) under an inert environment dramatically improved the electrochemical stability of the carbon-coated microelectrode (CCM). From electrochemical impedance spectroscopy (EIS) of continuously soaked CCMs in phosphate buffered saline (PBS), the annealing had improved the stability of both resistive and capacitive components of the CCM-electrolyte interfacial equivalent circuit from a few hours to several weeks (FIG. 4a), by about two orders of magnitude. Because of that, the FSCV sensing stability of the CCM also extended by around two orders of magnitude. Importantly, the electrochemical stability of the annealed CCM has reached a similar level to the CFM (FIG. 4a). Further investigation reveals that this improvement is most likely due to the reduction of carbon interlayer spacing from annealing (FIG. 4b). After annealing, the interlayer spacing of our carbon coating has reached 0.33 nm, very close to that of CFM.

[0075] As a result, the annealed CCMs have a stable background current during FSCV scanning, which enables them to achieve a good performance of DA and 5-HT sensing (FIG. 2) and multi-analyte selectivity over DA, 5-HT, Epi, and Ado. At the same time, the annealed CCM can achieve a low limit of detection and high sensitivity for DA sensing using FSCV (FIG. 2a). 5 nM DA can be detected with a notable oxidation peak. The impressive limit of detection might result from the edges, defects, and oxide functional groups in the coating. Consequently, the sensing current of the CCM is as high as ~7 times of CFM at a similar sensing area (127.2 nA/ μM vs. 17.2 nA/ μM , FIG. 2b). Notably, the sensing capability can be regenerated by

the FSCV scan itself while tested in a flow cell system (FIG. 2d). The current waveforms of DA and 5-HT are different, making it possible to distinguish DA from 5-HT (FIG. 2c).

[0076] This carbon coating and low-temperature annealing can be integrated with Ephys electrodes on any neural probes since, typically, the thermal budget of Si or flexible MEAs is around 350 °C. For example, an Ephys-FSCV MEA (v1.0, design guided by MPI Smith), which contains 15 Ephys sites and one annealed CCM (FIG. 7a), on Kapton® substrate (3-mil thickness) was demonstrated. The MEA v1.0 is 8.3 mm in length and with a width tapered from 120 µm to 180 µm from tip to base. Ephys electrodes are sized at 15×15 µm², with a 200 µm pitch, and made of Pt-Ir-coated Ti/Au with low impedance (FIG. 7b). The CCM area is around 60 µm in diameter and has an additional Nafion coating on top to enhance its anti-biofouling. The bare 8.3 mm-long MEA v1.0 was able to insert >7mm into the brain phantom without noticeable bending (FIG. 7c). Note the cross-section of our MEA is ~100 µm×150 µm, highly miniaturized for deep probes, and is smaller than the footprint of many deep-brain stimulation (DBS) electrodes chronically used in rodents, often around or greater than 250 µm diameter.

[0077] Integrated on the same MEA, the combination of separate Ephys and FSCV microelectrodes will provide an unprecedented integration of Ephys recording and neurotransmitter measurements in highly challenging spatial and temporal domains, while with maximized design freedom as offered by the conventional MEA platform. Parallel Ephys and FSCV can be integrated through different electrodes on a single MEA from engineered carbon coating and robust FSCV-artifact prevention, and such an Ephys-FSCV MEA can be chronically durable from further improved coating and anti-biofouling. While polymer MEAs were the focus in this project, CCM concept/optimization and the integration knowledge here are generalizable to other neural devices, such as Si probes and DBS electrodes.

[0078] The Ephys-FSCV MEA can enable a wide range of novel neuroscience studies that were not possible before. In particular, these new probes can accelerate progress in two major open issues in basic neuroscience. First, understanding the interaction between DA/5-HT and spiking activity. This is a crucial link in theories of motivated behavior but has been difficult to study owing to the need to combine multiple modalities. Second, determining the outlines of how DA/5-HT release is distributed across multiple sites in animals exhibiting behaviors. Multi-site fiber photometry has been extremely technically challenging, especially for comparing different transmitters and comparing striatal microdomains. Pioneering studies have shown the release of

DA to be spatially heterogenous, but the prevalence and relevance of these patterns in a wider range of behaviors and settings are currently unknown. Additionally, these new MEAs can also enable advances in understanding and treating neurological/psychiatric disorders.

[0079] This disclosure can enable studies featuring parallel Ephys and neurochemical

5 monitoring with high spatiotemporal resolution in moving, behaving animals. This disclosure combines Ephys-FSCV MEA with an unprecedented combination of capabilities of parallel electrical recording and FSCV neurochemical sensing. The Ephys-FSCV MEA fabrication is achieved through a low-temperature carbon coating approach that is scalable, electrochemically stable, and translatable to other MEAs. The Ephys-FSCV MEA is also easily configurable to
10 achieve high density and precisely located Ephys and FSCV recording locations by conventional MEA design. Connector technology has also enabled repluggable and miniaturized connection with the monolithic Ephys-FSCV MEA v1.0 (FIG. 7a).

[0080] Advantages of the present disclosure include: (1) understanding and improving carbon-coated microelectrodes and relevant anti-biofouling strategies towards chronic FSCV
15 durability *in vivo*; (2) achieving FSCV-artifact prevention strategies physically at the microelectrode level; (3) creation and miniaturization of the connector/headstage for concurrent Ephys and FSCV recordings; and (4) knowledge and knowhow of applying Ephys-FSCV MEAs in moving, behaving animals. In addition to establishing a Ephys-FSCV MEA paradigm, these innovations are also relevant to broad electrochemical-related diagnostic and interventional
20 approaches, such as point-of-care devices.

[0081] Although the present disclosure has been described with respect to one or more particular embodiments and/or examples, it will be understood that other embodiments and/or examples of the present disclosure may be made without departing from the scope of the present disclosure.

CLAIMS:

1. A device, comprising:
 - 5 one or more probe(s), each probe comprising a substrate and one or more electrode(s) disposed on the substrate, each electrode operably configured to be connected to a controller, wherein at least one of the electrode(s) comprises a graphitic carbon layer disposed on at least a portion of a surface of the electrode and wherein the electrode is operably configured to measure electrical signals and chemical signals, and wherein the graphitic carbon layer comprises about
10 12 atomic percent oxygen or less.
 2. The device of claim 1, wherein the graphitic carbon layer comprises greater than about 71 atomic percent carbon.
 - 15 3. The device of claim 1, wherein the graphitic carbon layer comprises a thickness of about 1 nm to about 10 μ m.
 4. The device of claim 1, wherein the graphitic carbon layer comprises a thickness of about
20 100 nm.
 5. The device of claim 1, wherein at least a portion of the graphitic carbon layer is polycrystalline.
 6. The device of claim 1, wherein the graphitic carbon layer comprises an atomic ratio of
25 amorphous carbon to graphitic carbon of about 1.26 or less.
 7. The device of claim 1, wherein at least a portion of the carbon atoms of the graphitic carbon layer are sp^2 -hybridized carbon atoms.

8. The device of claim 1, wherein each of the probe(s) comprises an upper shaft and a lower shaft comprising the electrode(s) comprising the graphitic carbon layer, wherein a width of the upper shaft is greater than a width of the lower shaft.

5 9. The device of claim 1, wherein the probe(s) has a length longer than or equal to about 1 mm.

10. The device of claim 8, wherein the width of the upper shaft is greater than or equal to about 10 μm .

10 11. The device of claim 1, wherein the electrode(s) comprise an array.

12. The device of claim 1, wherein the substrate comprises a material chosen from polyimide, paralyene-C, polydimethylsiloxane, SU-8, liquid crystal polymer, silicon, glass, and any combination thereof.

15

13. The device of claim 1, wherein the electrode(s) comprise a metal.

14. The device of claim 13, wherein the metal is chosen from Au and Pt.

20 15. A method of collecting electrical and chemical data from a tissue of an individual, comprising:

contacting one or more electrode(s) of claim 1 comprising the graphitic carbon layer with the tissue of the individual;

measuring an electrical signal;

25 measuring a chemical signal; and,

communicating the electrical signal and chemical signal from the one or more electrode(s) to a controller, wherein the electrical and chemical data is collected.

30 16. The method of claim 15, wherein the measuring of the chemical signal comprises fast scan cyclic voltammetry (FSCV).

17. The method of claim 15, wherein the measuring of the electrical signal comprises electrophysiology (Ephys).

18. The method of claim 15, wherein a temporal gap between the measuring of the electrical
5 signal and the measuring of the chemical signal is about 1 s or less.

19. The method of claim 15, wherein the electrical data comprises a spatial resolution of about 1 μm to about 10 mm.

20. The method of claim 15, wherein the electrical data comprises a temporal resolution of about 0.1 ms to about 1 s.

21. The method of claim 15, wherein the measuring of an electrical signal comprises a sensitivity of about 1 μV to about 100 mV.

22. The method of claim 15, wherein the chemical data comprises a spatial resolution of about 1 μm to about 10 mm.

23. The method of claim 15, wherein the chemical data comprises a temporal resolution of about
20 1 ms to about 10 s.

24. The method of claim 15, wherein the measuring of a chemical signal comprises a sensitivity of about 1 nM to about 10 μM .

25. A method of making a carbon-coated probe, comprising:
 providing one or more probe(s), each probe comprising one or more electrode(s);
 contacting the probe(s) with a mixture comprising graphene oxide; and,
 electrochemically depositing graphitic carbon on at least a portion of a surface of at least
one of the electrode(s) of at least one of the probe(s), forming at least one carbon-coated probe.

26. The method of claim 25, further comprising annealing the carbon-coated probe.

27. The method of claim 26, wherein the annealing is performed at a temperature of about 100 °C to about 400 °C.

5 28. The method of claim 26, wherein the annealing is performed at a temperature of about 250 °C.

29. The method of claim 25, wherein the mixture is an aqueous suspension of the graphene oxide.

10

30. The method of claim 25, wherein the graphene oxide is graphene oxide microflakes.

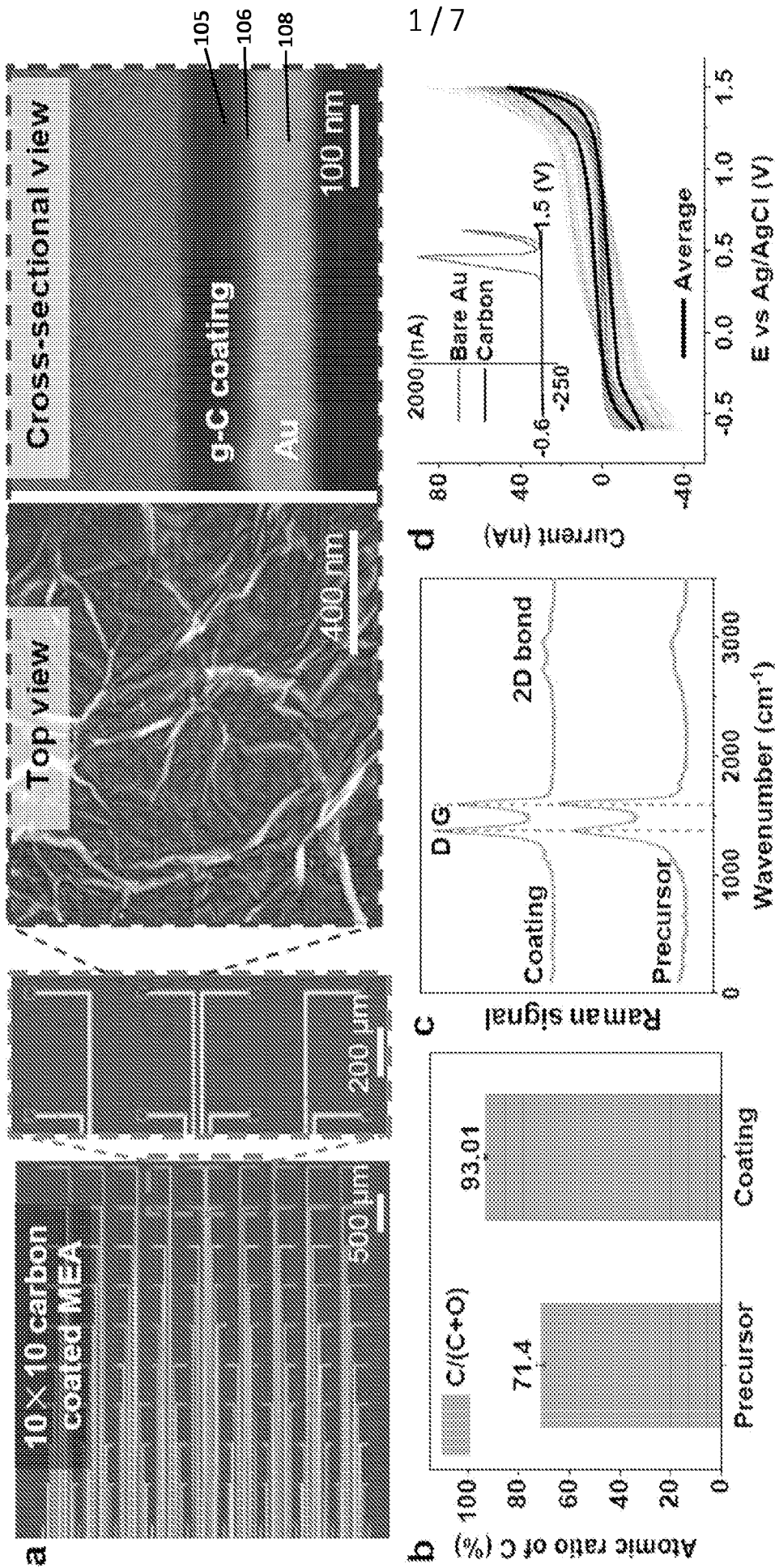


FIG. 1

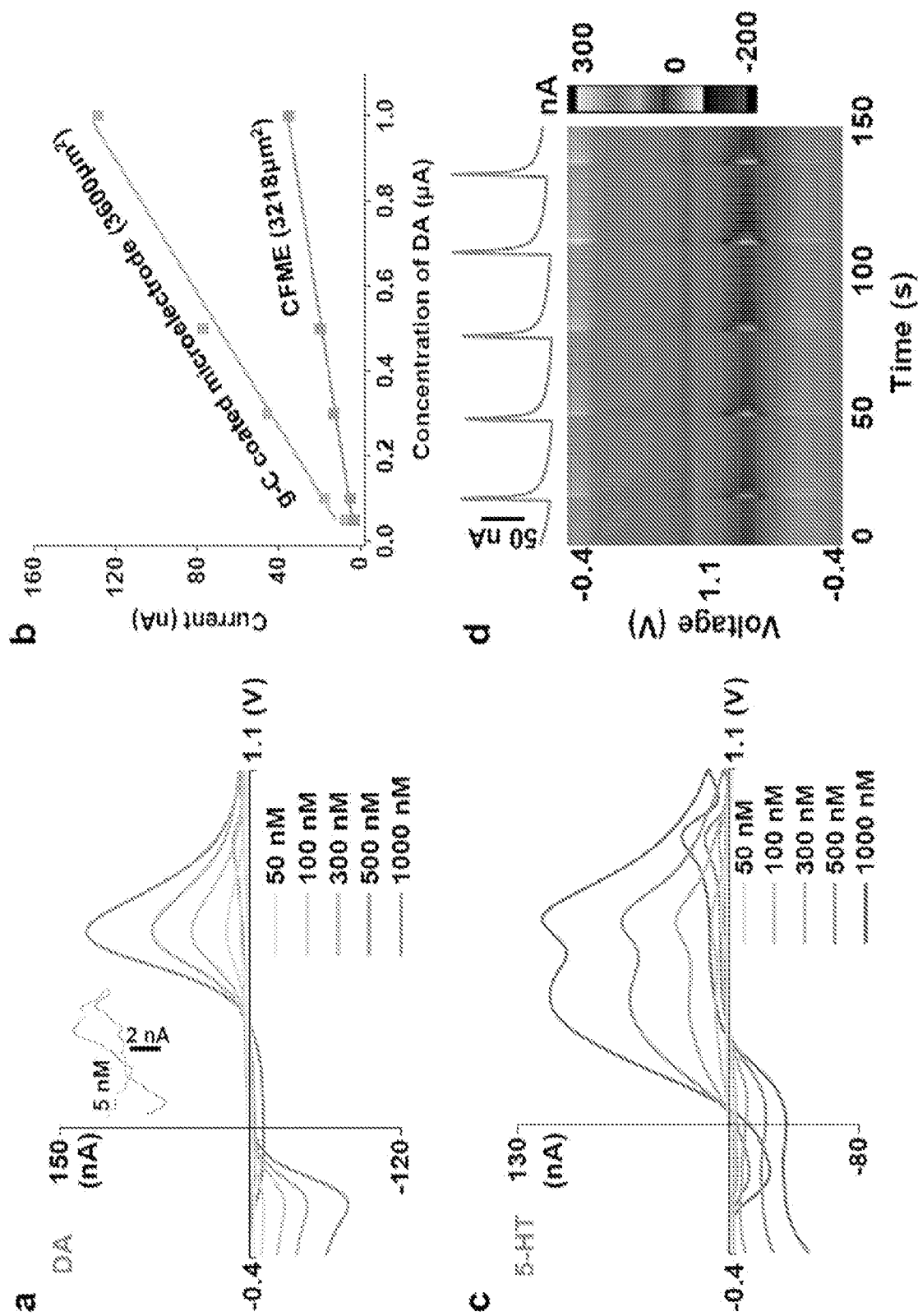


FIG. 2

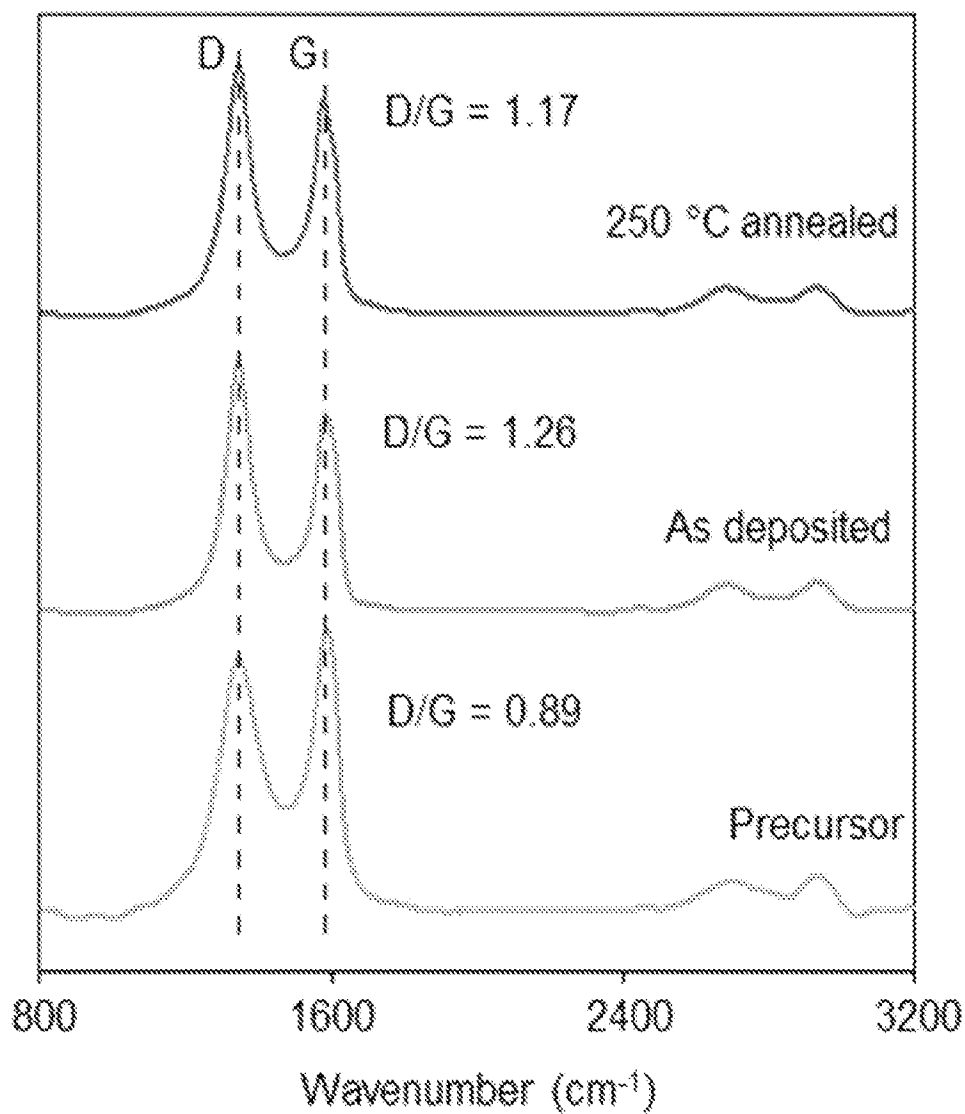


FIG. 3

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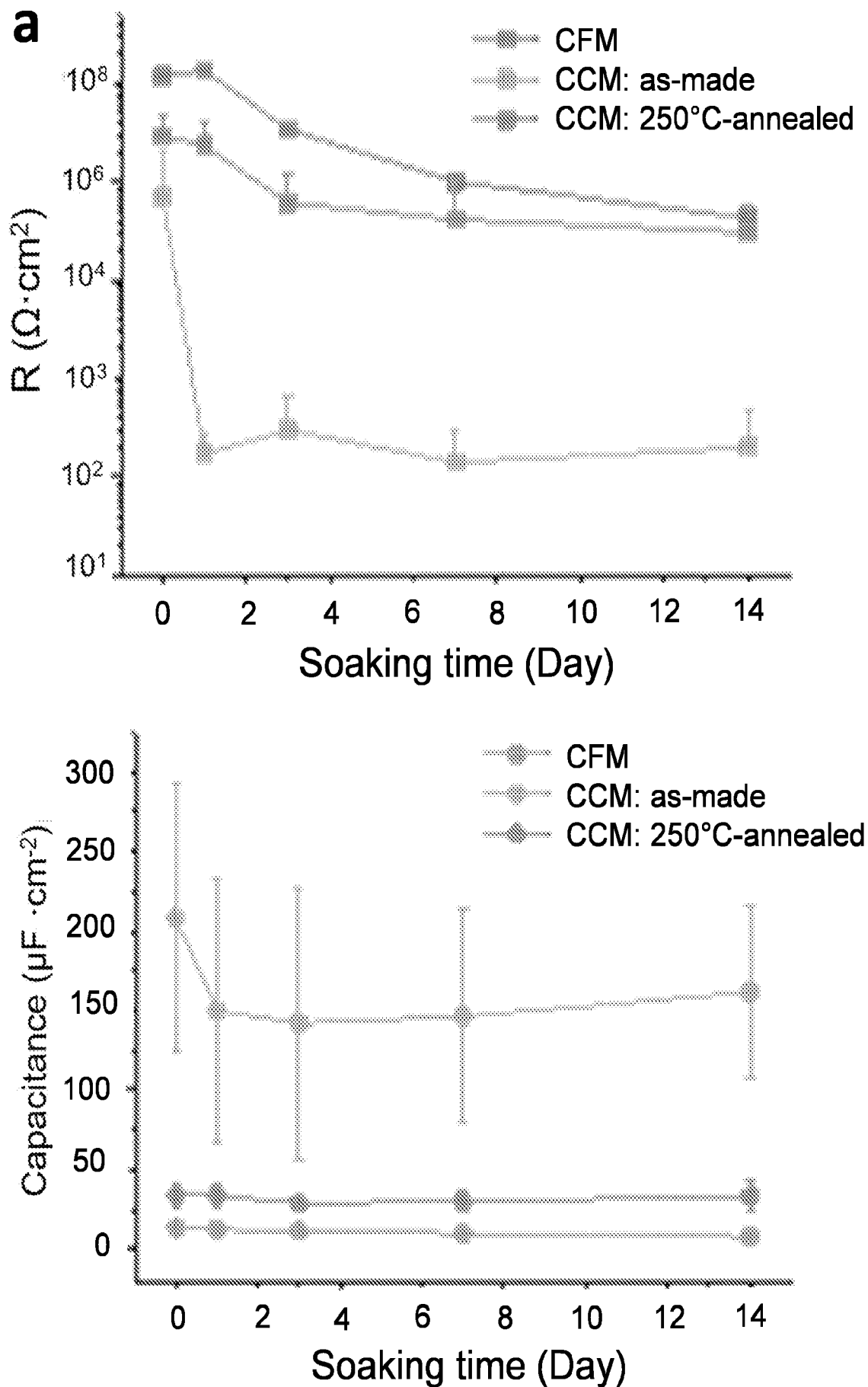


FIG. 4

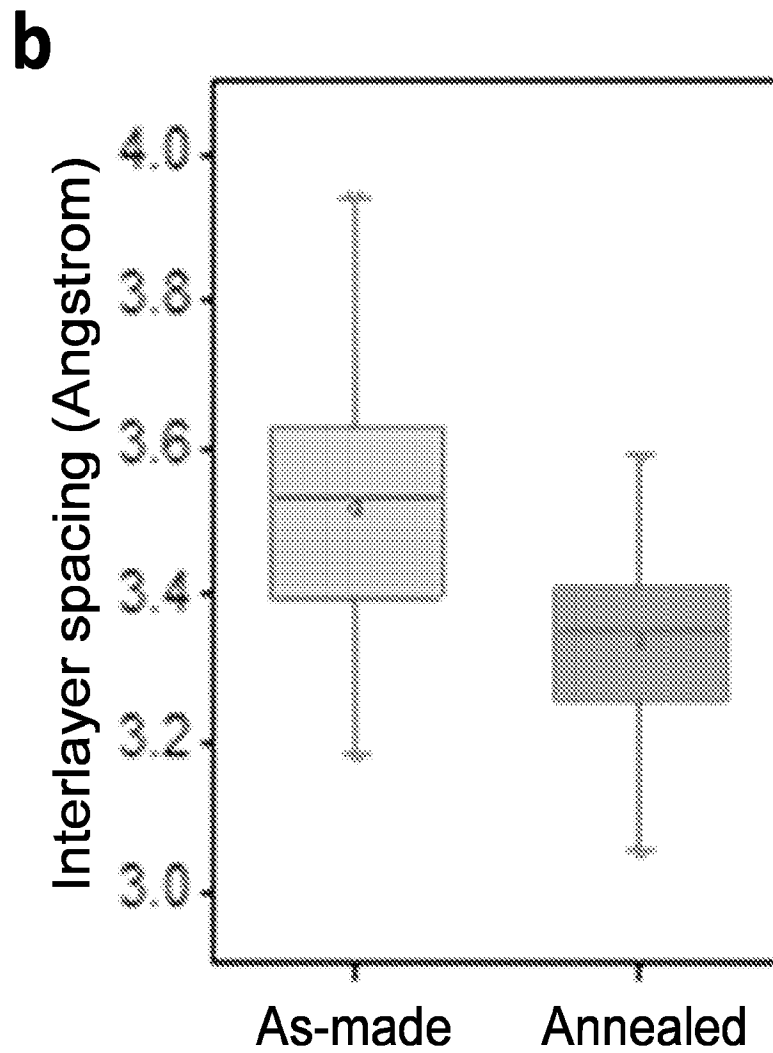


FIG. 4 (continued)

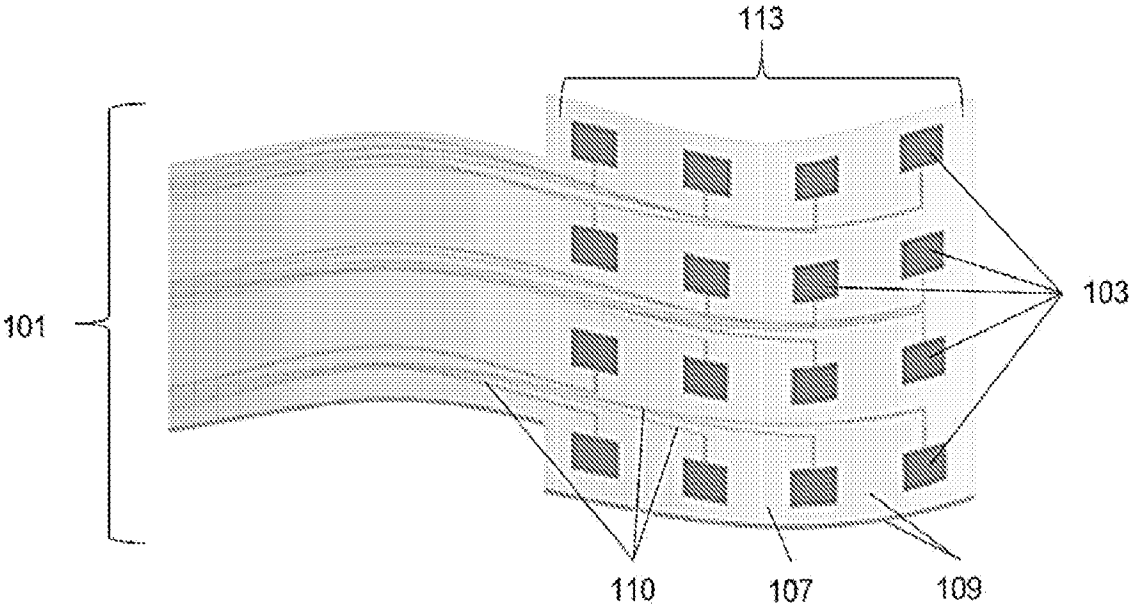


FIG. 5

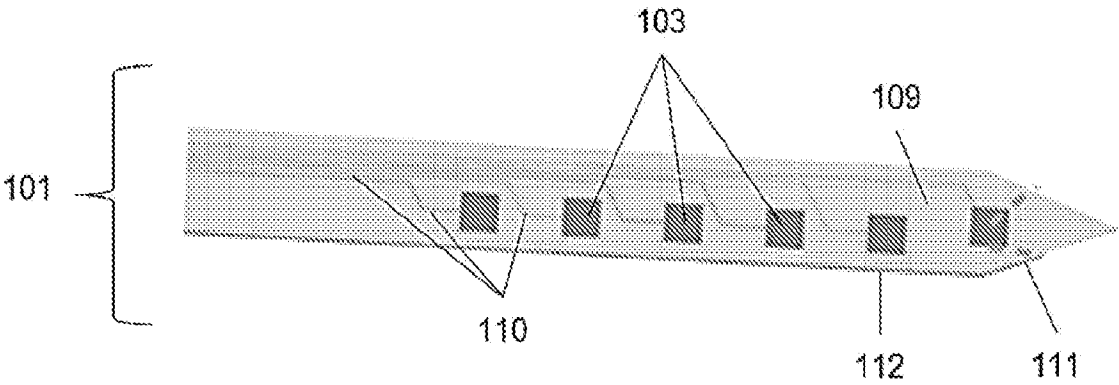


FIG. 6

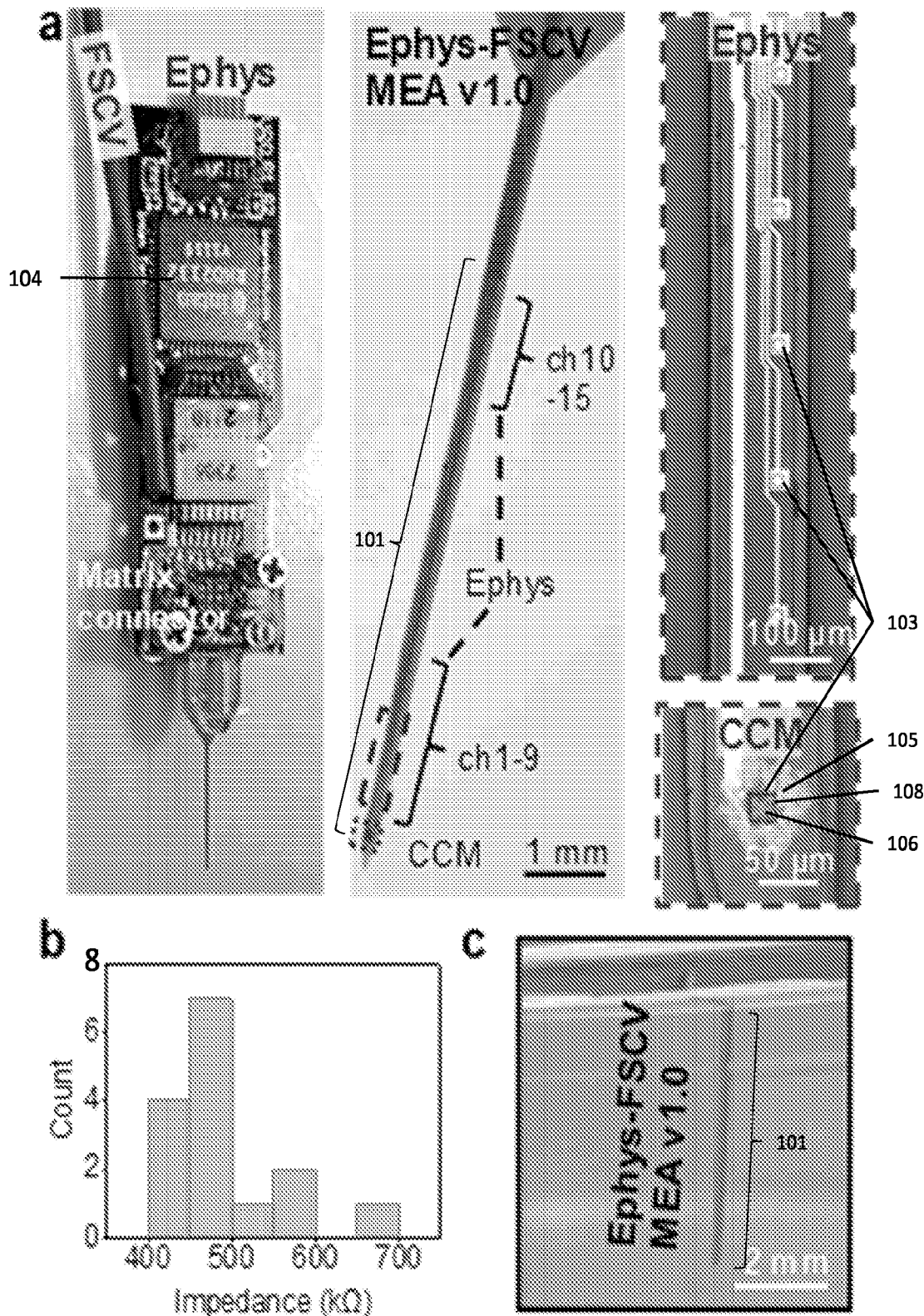


FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US24/10080

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. G01N 27/28; C25B 11/043; G01R 27/26 (2023.01)
 ADD. B82Y 30/00 (2023.01)
 CPC - INV. G01N 27/28; C01B 2204/00; C25B 11/043; G01R 27/26
 ADD. B82Y 30/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 See Search History document

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
 See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2021/0332489 A1 (IOWA STATE UNIV RESEARCH FOUNDATION INC) 28 October 2021; Abstract; Figure(s) 1; Paragraph(s) 3, 6, 56, 57, 61, 72, 94, 96, 97, 100, 103, 108, 122, 128, 132, 141, 236, 237, 240, 256, 258, 355, 360, 411	1-30
A	US 2014/0015548 A1 (NAUGHTON MICHAEL J) 16 January 2014	1-30
A	US 2021/0396708 A1 (LYTEN INC) 23 December 2021	1-30

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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Date of the actual completion of the international search

11 April 2024 (11.04.2024)

Date of mailing of the international search report

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