Expensive and repetitive software and firmware costs, have analog serial input/output interfaces, and have low robustness and non-universality in global application. On the other extreme, handheld portable potentiostats are very limited in customizability and applicability to a range of applications. Portable potentiostats are not noise efficient for biological applications and hence lack robustness. Specific problems with handheld potentiostats include high noise at low current and low voltage settings, low robustness for application to biosensing, and minimal operation choices for electrochemical applications.

[0005] Therefore, there remains a need for affordable, efficient, biodegradable diagnostic platforms.

SUMMARY OF THE INVENTION

[0006] The claimed invention is an apparatus and method for performing impedance spectroscopy with a handheld potentiometer.

[0007] In one aspect, the present invention refers to a conformal analyte sensor circuit comprising: a solid substrate having a top surface comprising a porous nanotextured substrate; a conductive material situated on the top surface of the solid substrate in a circuit design, thereby creating a circuit comprising a working electrode and a reference electrode. In another example, the present invention refers to a conformal analyte sensor circuit of as disclosed herein, wherein the analyte sensor circuit is assembled by a method comprising (a) providing the solid porous nanotextured substrate; and (b) transferring the analyte sensor circuit design onto the top surface of the porous nanotextured substrate using conductive material. In some ~~aspects~~examples, disclosed herein are conformal analyte sensor circuits comprising a porous nanotextured substrate and a conductive material situated on the top surface of the solid substrate in a circuit design, thereby creating a circuit comprising a working electrode and a reference electrode. The porosity of the nanotextured substrate is determined by the target analyte to be measured. In some ~~embodiments~~examples, the porous nanotextured substrate has a porosity at or between 10×10^5 and 10×10^{20} pores/cm^2. In some examples~~embodiments~~, the porous nanotextured substrate has a porosity at or between 10×10^7 and 10×10^{16} pores/cm^2. In some examples~~embodiments~~, the porous nanotextured substrate is an insulating substrate. In some examples~~embodiments~~, the porous nanotextured substrate is paper or nitrocellulose.

[0008] In one aspect, the present invention refers to a method of detecting or quantifying a target analyte in a sample using a handheld measuring device and a conformal analyte sensor circuit comprising the steps of (a) providing a conformal analyte sensor circuit having a reference electrode and a working electrode; (b) applying an alternating input electric voltage between the reference electrode and the working electrode of the conformal analyte sensor circuit; (c) varying a frequency of the

alternating input electric voltage between a minimum frequency and a maximum frequency; (d) amplifying an output current flowing between the reference electrode and the working electrode using a programmable gain amplifier; (e) calculating an impedance by comparing the input electric voltage to the output current using a programmable microcontroller; and (f) detecting a target analyte or calculating a target analyte concentration from the calculated impedance using a programmable microcontroller. In another aspect, the present invention refers to a method of detecting a target analyte comprising spotting a sample on the conformal analyte sensor circuit as described herein, wherein the sample wicks through the porous nanotextured substrate onto the working electrode and the reference electrode; attaching the conformal analyte sensor circuit to a source circuit; and detecting the target analyte in the sample with a source circuit. In yet another aspect, the present invention refers to a handheld device for measuring a target analyte comprising (a) a programmable gain amplifier configured to be operably coupled to a working electrode and a reference electrode; (b) a programmable microcontroller operably coupled to the programmable gain amplifier, the working electrode, and the reference electrode; wherein the programmable microcontroller is operable to apply an alternating input electric voltage between the working electrode and the reference electrode; wherein the programmable microcontroller is operable to vary a frequency of the alternating input electric voltage between a maximum frequency and a minimum frequency; the programmable gain amplifier is operable to amplify an alternating output current flowing between the working electrode and the reference electrode; the programmable microcontroller is operable to calculate an impedance by comparing the input electric voltage to the measured output current; and the programmable microcontroller is operable to calculate a target analyte concentration from the calculated impedance. In a further aspect, the present invention refers to a method of calibrating a handheld measuring device by testing a plurality of solutions having known target analyte concentrations comprising (a) applying an input electric voltage between a reference electrode and a working electrode for each of the plurality of solutions; (b) calculating an impedance for each of the plurality of solutions by comparing the input electric voltage to the output current using a programmable microcontroller; (c) calculating coefficients of the equation $z_i = b_1x^2 + b_2x + c$, wherein z_i is the impedance, x is the known target analyte concentrations, and b_1, b_2, and c are the coefficients. In some examples~~embodiments~~, the porous nanotextured substrate includes hydrophobic coatings. In some examples~~embodiments~~, the hydrophobic coatings include parylene, polyamide, PEG, polycation solutions, and polydimethlysiloxane. In some examples~~embodiments~~, the porous nanotextured substrate includes surface coatings. In some examples~~embodiments~~, the surface coatings include pre-formulated sprays and aerosols that may induce hydrophobicity on specific regions of the sensor substrates. In some examples~~embodiments~~, the surface coatings include a mixture of ethanol, polydimethlysiloxane, ethyl sulfate, chlorotrimethylsilane, siloxanes and silicones as well as pre-formulated block co-polymer

mixtures. In some examples~~embodiments~~, the porous nanotextured substrate includes track etched membranes. In some examples~~embodiments~~, the track etched membranes include nucleopore and cyclopore form factors. In some

~~embodiments~~examples, the porous nanotextured substrate includes acid etched membranes. In some examples~~embodiments~~, the acid etched membranes include silicon and alumina scaffolds. In some examples~~embodiments~~, the porous nanotextured substrate includes polymer membranes. In some examples~~embodiments~~, the polymer membranes include nylon, polyamide, nitrocellulose, and PTFE. In some examples~~embodiments~~, the porous nanotextured substrate includes electro-deposited membranes. In some examples~~embodiments~~, the electro-deposited membranes include patterned metal and hydrogel matrices. In some examples~~embodiments~~, the porous nanotextured substrate includes anodized membranes. In some examples~~embodiments~~, the porous nanotextured substrate includes ceramic membranes. In some examples~~embodiments~~, the ceramic membranes can be made conformal or flexible when they are prepared as a mixture of alumina and silica combined in a ratiometric mixture and deposited and oxidized through chemical vapor or acid etching.

[0009] The conductive material may be any appropriate material known to those of skill in the art. In some examples~~embodiments~~, the conductive material is conductive ink or semi-conductive ink. In some examples~~embodiments~~, the semi-conductive ink comprises carbon ink and additives. In some examples~~embodiments~~, the conductive ink is carbon, silver, or metal nanoparticle-infused carbon inks. In some examples~~embodiments~~, the metal nanoparticle-infused carbon ink is infused with gold, platinum, tantalum, silver, copper, tin, or grapheme. In some examples~~embodiments~~, the carbon ink is infused with 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, or more % by volume with the metal nanoparticles. In some examples~~embodiments~~, the thickness of the carbon ink ranges from 0.1 nm to 1 μm. In some examples~~embodiments~~, the thickness of the carbon ink may be controlled by the deposition method.

[0010] The circuit may be a nonlinear circuit or a non-ohmic circuit. In some examples~~embodiments~~, the circuit is further defined as a base electrode surface. In some examples~~embodiments~~, the base electrode surface is further connected to a source circuit. In some examples~~embodiments~~, the source circuit is a potentiostat. In some examples~~embodiments~~, the source circuit is a voltage source. In some examples~~embodiments~~, the source circuit is a current source. In some examples~~embodiments~~, the circuit does not contain a capture ligand or label-molecule. In some examples~~embodiments~~, the conformal analyte sensor further comprises a redox material.

[0011] In some examples~~embodiments~~, any of the conformal analyst sensor circuits disclosed herein is assembled by a method comprising (a) providing the solid porous nanotextured substrate; and (b) transferring the analyte sensor circuit design onto the top surface of the porous nanotextured substrate using conductive material. In some examples~~embodiments~~, transferring the circuit design comprises dip coating. In such examples~~embodiments~~, the feature resolution of the

circuit is up to 100 nanometers/0.1 micron. In some examplesembodiments, transferring the circuit design comprises embossing. In such examplesembodiments, the feature resolution of the circuit is up to 100 nanometers/0.1 micron. In some examplesembodiments, transferring the circuit design comprises designing the circuit on a 3D printer and embossing the circuit onto the substrate. In such examplesembodiments, the feature resolution of the circuit is up to 100 nanometers/0.1 micron. In some embodiments, transferring the circuit design comprises masking and lithography. In such examplesembodiments, the feature resolution of the circuit is 1-10 microns.

[0012] In some examplesembodiments, the handheld potentiometer comprises an LCD screen, mini-joystick, working electrode port, reference electrode port, programmable microcontroller, and programmable gain amplifier. In other examplesembodiments, the handheld potentiometer comprises a smartphone, cable, potentiostat adaptor, working electrode port, reference electrode port, programmable microcontroller, and programmable gain amplifier. In some examplesembodiments, the handheld potentiometer comprises a programmable microprocessor instead of a programmable microcontroller.

[0013] In some examplesembodiments, the handheld device for measuring a target analyte comprises (a) a programmable gain amplifier configured to be operably coupled to a working electrode and a reference electrode, (b) a programmable microcontroller operably coupled to the programmable gain amplifier, the working electrode, and the reference electrode, wherein the programmable microcontroller is operable to apply an alternating input electric voltage between the working electrode and the reference electrode; the programmable gain amplifier is operable to amplify an alternating output current flowing between the working electrode and the reference electrode; the programmable microcontroller is operable to calculate an impedance by comparing the input electric voltage to the measured output current; and the programmable microcontroller is operable to calculate a target analyte concentration from the calculated impedance.

[0014] In some examplesembodiments, the programmable microcontroller is operable to apply an input electric voltage between the working electrode and the reference electrode that has a frequency between 2 Hz and 15 kHz. In some examplesembodiments, the programmable microcontroller is operable to varying the frequencies between 50 Hz and 15 kHz in applying input electric voltages between the working electrode and reference electrode. In some examplesembodiments, the programmable microcontroller varies the frequencies in 2 Hz intervals. In some examplesembodiments, the programmable microcontroller is operable to apply an input electric voltage between the working electrode and the reference electrode that is sinusoidal. In some

examplesembodiments, the programmable microcontroller is operable to apply an input electric voltage between the working electrode and the reference electrode that is a sawtooth wave. In some examplesembodiments, the programmable microcontroller is operable to apply an input electric voltage between the working electrode and the reference electrode that is a square wave. In some examplesembodiments, the programmable microcontroller is operable to apply an input electric voltage between the working electrode and the reference electrode that is a triangle wave. In some examplesembodiments, the programmable gain amplifier has a variable gain of between 1 and 200. In some examplesembodiments, the microcontroller is operable to apply an input electric voltage of between 1 mV and 10 V. In some examplesembodiments, the handheld measuring device is operable to detect an output current of 10 pA or greater. In some examplesembodiments, the programmable microcontroller comprises an analog to digital converter and a digital to analog converter. In some examplesembodiments, the programmable microcontroller is capable of measuring a difference in phase between the input electric voltage and the output current. In some examplesembodiments, the programmable microcontroller is operable to apply a Fourier transform to the input electric voltage and output current to calculate impedance as a function of frequency. In some embodiments, the programmable microcontroller is operable to use Lissajous curves to compare the input electric voltage and output current to calculate impedance. In some examplesembodiments, the programmable microcontroller is operable to use multi-slice splitting and signal analysis to determine a frequency at which the impedance change is at a maximum or minimum. In some examplesembodiments, the device further comprises a liquid crystal display operably coupled to the programmable microcontroller; a mini-joystick operably coupled to the programmable microcontroller; wherein the mini-joystick is operable to allow users to provide input; and the liquid crystal display is capable of displaying output data. In some examplesembodiments, the device further comprises a smartphone operably coupled to the programmable microcontroller; wherein the smartphone is operable to allow users to provide input; and the smartphone is capable of displaying output data. In some examplesembodiments, the output data comprises the target analyte concentration. In some examplesembodiments, the handheld measuring device does not contain a redox probe.

[0015] In some examplesembodiments, disclosed is a kit comprising any of the conformal analyst sensor circuits disclosed herein and any of the handheld measuring devices disclosed herein.

[0016] The handheld potentiostats and porous nanotextured conformal circuits disclosed herein may be used separately or in combination to detect and/or quantify a target

analyte. In some examplesembodiments, disclosed is a method of detecting a target analyte comprising spotting a sample on a disclosed conformal analyte sensor circuit, wherein the sample wicks through the porous nanotextured substrate and the circuit design, attaching the conformal analyte sensor circuit to a source circuit, and detecting the target analyte in the sample with a source circuit. In some examplesembodiments, the source circuit is a potentiostat. In some examplesembodiments, the source circuit is a voltage source. In some examplesembodiments, the source circuit is a current source. In some examplesembodiments, the sample contains 1, 2, 3, 4, 5, 6, 7 8, 9, 10, 11, 12, 13, 14, 15, or more μL of a fluid, or any amount in between. The sample may be, for example, blood, urine, sweat, saliva, lysis buffer, assay buffer, human serum, plasma, river water, stream water, and deionized water. In some examplesembodiments, the target analyte is a protein, DNA, RNA, SNP, small molecules, pathogens, heavy metal ions, or physiological ions. In some examplesembodiments, the sample is not labeled. In some examplesembodiments, the sample is labeled. In some examplesembodiments, detecting the target analyte comprises detecting an electrical change.

[0017] In some examplesembodiments, disclosed is a method of detecting or quantifying a target analyte in a sample using a handheld measuring device comprising the steps of (a) applying an input electric voltage between a reference electrode and a working electrode, (b) amplifying an output current flowing between the reference electrode and the working electrode using a programmable gain amplifier, (c) calculating an impedance by comparing the input electric voltage to the output current using a programmable microcontroller, and (d) calculating a target analyte concentration from the calculated impedance using a programmable microcontroller. In some examplesembodiments, the input electric voltage has a frequency between 2 Hz and 15 kHz. In some examplesembodiments, the input electric voltage has a frequency between 50 Hz and 15 kHz. In some examplesembodiments, the input electric voltage is sinusoidal. In some examplesembodiments, the input electric voltage is a sawtooth wave. In some examplesembodiments, the input electric voltage is a square wave. In some examplesembodiments, the input electric voltage is a triangle wave. In some examplesembodiments, the input electric voltage is between 1 mV and 10 V. In some examplesembodiments, the input electric voltage is between 1 mV and 100 mV. In some examplesembodiments, the input electric voltage is between 100 mV and 10 V. In some examplesembodiments, the output current is between 10 pA and 10 mA. In some examplesembodiments, the output current is between 10 pA and 100 nA. In some examplesembodiments, the output current is between 100 nA and 10 mA. In some examplesembodiments, the output current is amplified by a factor between 1 and 200. In some examplesembodiments, the method further

comprises calculating a difference in phase between the input electric voltage and the output current. In some examplesembodiments, the method further comprises calculating impedance as a function of frequency by applying a Fourier transform. In some examplesembodiments, the method further comprises calculating impedance using Lissajous curves. In some examplesembodiments, the method further comprises calculating impedance as a function of frequency using multi-slice splitting and signal analysis. In some examplesembodiments, the method further comprises displaying the calculated target analyte concentration. In some examplesembodiments, the method further comprises displaying an output on an LCD display. In some examplesembodiments, the method further comprises displaying an output on a smartphone. In some examplesembodiments, the method further comprises providing an input using a mini-joystick. In some examplesembodiments, the method further comprises providing an input using a smartphone. In some examplesembodiments, the measured impedance is non-faradaic.

[0018] The handheld potentiometer detects concentrations of a target analyte by applying an alternating voltage between the working and reference electrodes. The applied alternating voltage results in a current flowing between the working and reference electrodes. The resulting current is amplified by a programmable amplifier and passed onto the programmable microcontroller. The programmable microcontroller compares the applied voltage to the resulting current to calculate the impedance of the tested sample. The impedance is used to calculate the concentration of the target analyte in the tested sample. In some examplesembodiments, to perform testing of a target analyte using the handheld potentiometer, the handheld potentiometer is first calibrated by testing and calculating the impedance of samples containing known quantities of the target analyte. In some examplesembodiments, the system applies voltages of varying frequencies and determines the frequency at which the maximum impedance change occurs for a particular tested analyte. The claimed system may perform non-Faradaic electrochemical impedance spectroscopy ("EIS") by testing samples without using a redox electrode.

[0019] In some examplesembodiments, disclosed herein is a method of calibrating a handheld measuring device by testing a plurality of solutions having known target analyte concentrations comprising (a) applying an input electric voltage between a reference electrode and a working electrode for each of the plurality of solutions, (b) calculating an impedance for each of the plurality of solutions by comparing the input electric voltage to the output current using a programmable microcontroller, and (c) calculating coefficients of the