Portable Memristive Biosensing System as Effective Point-of-Care Device for Cancer Diagnostics

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***Abstract*— Memristive biosensors have been proved as excellent candidates for ultrasensitive biosensing. In this work, a novel portable bio-detection system based on memristive biosensors is designed, developed and tested for providing a significantly fast, automatic and simultaneous sensing output of multiple memristive biosensors on a single chip. The suggested compact and independent bio-sensing prototype is achieved through the realization of an electronic board designed for addressing the specifications of the memristive biosensor signal acquisition. Memristive bio-sensing-chips are specially designed and fabricated as well. The system was tested for successfully sensing of Prostate Specific Antigen, one of the main biomarkers of prostate cancer, at fM concentrations. Overall, this novel scheme resembles to a memristive-biosensing-kit approach, paving the way for fast and ultrasensitive PoC (point-of-care) devices.**

I. Introduction

Semiconductor nanowires are considered as promising building blocks for miniaturized bioassays providing low-cost microchips for applications in the medical practice. In some cases, silicon nanowire-arrays exhibiting memristive electrical properties [1-3] are bio-functionalized with receptor molecules, such as antibodies or DNA aptamers, giving rise to the so-called *memristive biosensors*. These memristive nano-bio-sensors, have been already successfully implemented in both the diagnostics and the therapeutics field, demonstrating their immense potential for precise biosensing. Most specifically, ultrasensitive detection is achieved for cancer biomarkers, pushing the sensing performance at atto-molar concentrations [4] as well as effective screening of therapeutic compounds along with the possibility for continuous drug monitoring [5]. This ultrasensitive bio-detection paradigm is based on the hysteresis modification appearing in the electrical characteristics upon treatment of the nanodevices' surface with charged biological species [6, 7]. More specifically, the pinched hysteresis is lost upon the bio-functionalization of the nanodevice and a voltage gap is introduced in the semi-logarithmic current-to-voltage characteristics as a further memory effect to the voltage scan across the nanowire (Fig. 1).

So far, the electrical monitoring of memristive biosensors was performed by sweeping a drain-to-source voltage forward and backward using a Cascade Microtech Probe Station and a Hewlett-Packard 4165A Precision Semiconductor Parameter Analyzer while implementing tungsten needles to directly contact the NiSi pads of the nanostructures. This experimental setup resulted to an overall unpractical measuring procedure considering that the sensing could be performed only in the specific laboratory and was likewise significantly time-consuming. However, considering the fact that in medical applications minimizing the time needed for diagnostic procedures is a highly crucial aspect, there is the need to develop new acquisition platforms to provide a fast sensing output. As a first effort towards this direction, a measurement interface for memristive biosensors with a manually performed drain selection capability, by means of a mechanical switch, was previously realized [8]. Nevertheless, despite successfully reducing the measurement time, this configuration only replaced the probe station, while connection with a Source Measure Unit (SMU) was still required. This aspect determinately constrains the implementation of the ultrasensitive memristive nano-bio-sensors in the environment of a hospital or medical clinic.

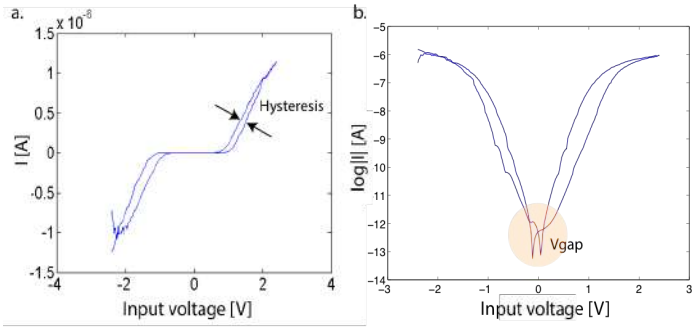

Fig.1. Electrical characteristics of a memristive biosensor acquired with the probe station and Keithley configuration. The pinched loop characterizing the memristive devices is lost upon the bio-functionalization. Hysteretic electrical properties (a) and the voltage gap (b) are the main features of interest of the memristive biosensors.

In this work, the design and realization of a completely new, portable memristive biosensing platform is developed and tested successfully, providing a fast, fully-automatized and simultaneous sensing output of multiple individual memristive biosensors. The realized system is independent from SMU configurations allowing a flexible and unrestricted sensing procedure. Moreover, single chips consisting of nanofabricated silicon wires exhibiting memristive electrical response, conjugated with metallic extension electrodes are also fabricated, allowing an integrated measurement procedure. Bio-functionalization with receptor molecules, finally provides bioassay sensing-chips based on series of memristive biosensors. The full system was successfully validated for prostate cancer biomarkers detection, namely PSA at fM concentrations (critical level of PSA 4 ng/mL ca. 133 pM), therefore demonstrating high potential for the implementation as a novel ultrasensitive medical tool for efficient bio-sensing at early stages of the disease.

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II. System Architecture

A. Memristive biosensing board prototype

The proposed system, shown in Fig. 2, consists of the sensor module, analog-to-digital converter (ADC) and multiplexer (MUX) parts, the computing block, a data acquisition and storage scheme and a power supply. The schematic of the board is realized using Altium Designer, while the PCB layout is designed using OrCAD PCB Designer. For the sensor part, twelve independent sensing outputs, and one common excitation input are considered. This configuration corresponds to the twelve individual memristive biosensors, fabricated as discussed in section III. The realized portable (175 mm x 72 mm) printed circuit board (PCB) prototype for memristive biosensors is presented in Fig. 3. The sensor part is placed separately on one side of the board in order to avoid any electromagnetic interference from other components. For the MUX (ADG428BRZ) and the ADC (AD7785BRUZ) part the accuracy of the measurement is very high thanks to the 20-bit ADC. Furthermore, with the MUX each channel can be measured programmatically through the microcontroller. Therefore, the board can individually address and read signals from the individual sensors of the biosensing chip. For the computing part the high speed STM32 microcontroller (STM32F446RE) is used. It has a clock speed that can reach 180 MHz and 16 independent counters that can allow very accurate generation of Pulse Width Modulation (PWM) signal, with the possibility reaching a pulse with 1ms period. For the data acquisition and elaboration an on-board serial port is incorporated and can be used through wire-connection with a computer or a Raspberry Pi (RPi), allowing the data processing. In addition, a uSD memory card socket (DM3AT-SF-PEJM5, 2427719, series DM3, Micro SD) is introduced on the board, combined with a microSDXC card (64 GB, SDCA3, speed class 10) with a fast reading and writing speed of 90 MB/s and 80 MB/s, that is necessary for direct and quick signal acquisition. The main purpose of the memory card is the recording of the full current-to-voltage curves enabling the transferring of this information to a computer, for further analysis or as back-up files useful for future analysis. In addition, a flash 32 MB memory card is included for storing measurement parameters, and the possibility for a future introduction of a Wi-Fi chip that will enable wireless data transmission is also taken into consideration. Moreover, considering the very low currents obtained by these nanosensors that are in the range from some tens of pA (current minima) till some tens of μA, they are very prone to electrical noise. Therefore, the noise reduction is the key step in obtaining reliable measurement results. For this reason, suitable input bias current operational amplifies are used and a very low tolerance surface mount resistors and capacitors are chosen. In addition, a board-level shielding box (RFI SHIELD CAN 50X25X5MM, 952-2642-ND) is introduced to minimize the external noise, substituting the Faraday cage included in the measurement configuration involving the probe station and the Keithley. The noise shielding enclosure is incorporated on board with the possibility to be stabilized during the measurements through the use of on-board shield clips (952-1475-1-ND) and to be easily removed for loading/unloading of the sensing module. The humidity and temperature values can be continuously monitored through a sensor (HDC1050DMBT Texas Instruments) incorporated on the board. Finally, for the power supply a voltage regulator that can accept input voltage in the range up to 25 V is considered and can also be powered by 5V batteries.

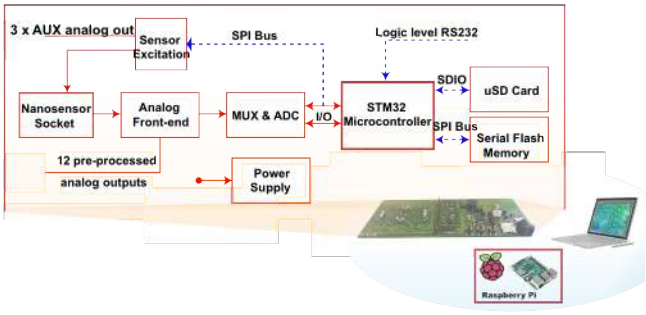

Fig.2. Overall block diagram of the portable memristive biosensors platform. The nanosensor socket with the multichannel system block interfaces with the micro-controller that drives the voltage generation circuits and receives ouput values from the readout circuits.

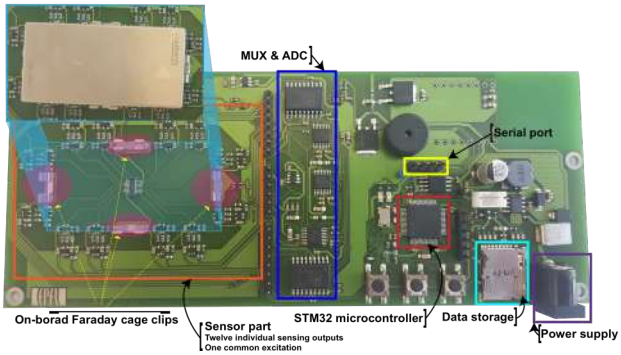

Fig.3. The memristive biosensing board prototype. The realized electronic board mainly consists of the sensor module, the ADC and MUX part, the microcontroller, a data storage scheme and a power supply.

B. Sensing Module

The sensing part in Fig. 4 is the core of the novel board platform and consists of twelve identical nanosensors, demonstrating same range and accuracy. Each nanosensor results to an independent signal that is considered through each individual drain. All nanosensors are subjected to the same excitation through the common source terminal (Fig.5). The nanosensors require input signal excitation in a form of a double source-to-drain voltage sweep following the experimental protocol established by the measurements with the Keithley. The negative biasing for the nanosensors is obtained through the control of digital-to-analog converter (DAC) (2112662, AD5686RBRUZ) with the supervision of the microcontroller. The microcontroller and DAC module are communicating through Serial Peripheral Interface (SPI) Bus. Each signal generator is realized by the microcontroller that sends an increasing or decreasing digital value to the specific DAC which converts it to an analog voltage. Overall, positive and negative potential values are applied to the nanodevices during the voltage sweep. During the voltage sweep, the measured current signals from these nanosensors are fed into the twelve-independent analog readout circuitry each consisting of two stages, current to voltage conversion stage and offset cancellation stage. The DC and low frequency gain of the 1st stage is equal to $-R_f$ that is -20 MΩ, while the gain of 2nd stage is equal to the ratio of the output voltage to the input voltage at

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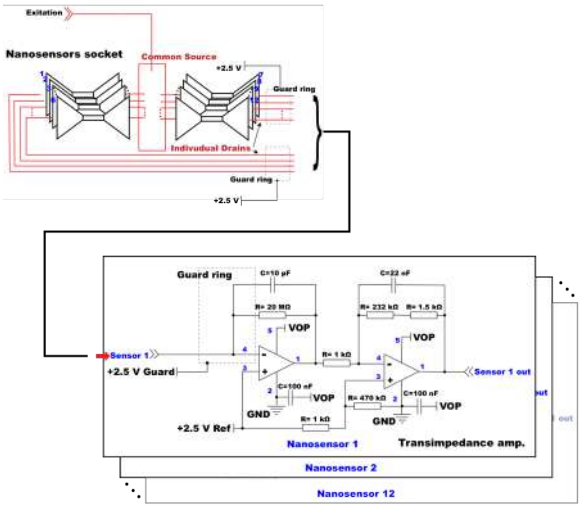

Fig.4. Sensing module detailed architecture for the nanosensor socket and low bias Transimpedance amplifiers.

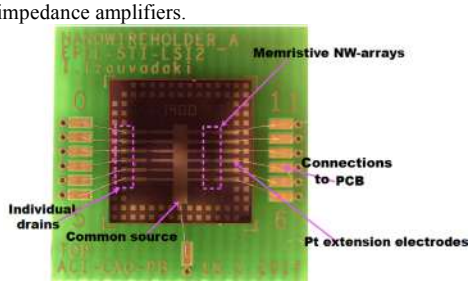

Fig.5. The realized chip-holder board including a sensing chip that consists of twelve sensors with independent outputs and common excitation. This scheme offers disposable sensing modules that can be easily connected to the main electronic board apparatus for the sensing procedures.

the inverting input, that is -233.5. Both stages are in an inverting configuration, so the gain is negative in each stage.

C. *Data-acquisition programming*

The high-precision ADC has 3 differential analog inputs, so to measure all the 12 output signals of the readout circuitry, two multiplexers are adopted. At each applied potential, the multiplexers select the output signals sequentially so that all of them can be measured and the result is stored in a 12-element array. After all output signals are measured, the array is sent through serial port of micro-controller to RPi for data processing.

III. MATERIALS AND METHODS

A. *Nano-Engineering of Memristive Biosensors*

Two-terminal, Schottky-barrier silicon nanofabricated wires arrays demonstrating memristive electrical properties are acquired through a top-down nanofabrication process using Silicon-on-Insulator (SOI) wafers. The wires are suspended and anchored between Nickel Silicide (NiSi) pads for the electrical characterization. Those pads are defined through e-beam lithography and realized with Nickel (Ni) evaporation followed by lift-off and annealing procedures.

Finally, the nanowires are defined by e-beam lithography and etched through Deep Reactive Ion Etching (DRIE) cycles of the Si. Metal lines are integrated on top of the already fabricated nanostructures and serve as extension electrodes to the NiSi pads of the devices (Fig. 6). A common source and separated drains

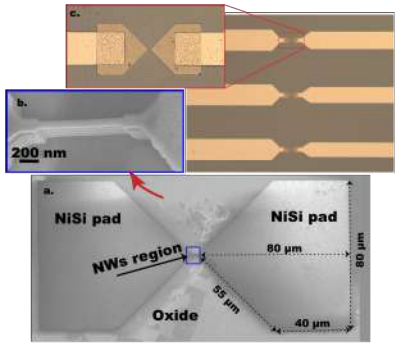

Fig. 6. SEM micrographs illustrating the nanofabricated wires anchored between the NiSi pads (a) and detail of an individual nanowire-array configuration (b). Optical microscopy image depicting the system nanowire-pads integrated with the extension Pt electrodes (c).

are considered for individual measurement of 12 integrated sensors that allow possibility for statistical analysis with average value and standard deviation. This statistical analysis is required for obtaining reliable results and overcome the variability of the sensors originating from the etching process. In addition, these multiple sensors allow the possibility for simultaneous sensing of different biomarkers. First, a photolithography process is implemented in order to define the pattern of the lines. A resist bi-layer (a lift-off resist followed by a positive photoresist) is spin-coated on the surface and the exposure of the lines design is performed through maskless direct laser writer (MLA150, Heidelberg Instruments). A 20 nm Titanium (Ti) and a 100 nm Platinum (Pt) layer are introduced through physical vapor deposition (PVD) followed by a liftoff process for the creation of the metal electrodes. The wafer is diced into single chips of 1 cm × 1 cm. Then the chips consisting of memristive nanowires conjugated with the metal lines are wire-bonded to a specially designed chip-holder (as shown in Fig.5) with connections corresponding to the main PCB and integrated through board-to-board connectors. The memristive silicon nanowires are first subjected to a soft oxygen plasma treatment for 15 min for the generation of hydroxyl terminating groups on the surface, then functionalized by exposure of the surface to antibody against Prostate Specific Antigen (PSA), i.e. anti-PSA antibody (Abcam-ab10185), solution in Phosphate Buffered Saline (PBS) (pH 7.4 Sigma-Aldrich) for 4 h at room temperature, thoroughly rinsed with the same buffer and gently dried with N_2 flow. The antigen uptake is performed through successive 45 min incubations of the sensing chip in solutions of PSA (Millipore Angebot R-1939458.1; 539834 purchased from Merck) in PBS.

B. *Measurement procedure with the realized board*

To carry out the voltage sweep, an applied voltage in the range of -0.4 V to +0.4 V is considered as a common excitation input. The generated current signals from the memristive bio-sensors are in range of pA, so a pre-processing is necessary before carrying out the measurements. This pre-processing stage includes: (a) current to voltage conversion and filtering (b) voltage signal amplification and voltage level correction. The memristive sensors react to the applied voltage immediately with a current peak and then the current becomes stable after 40-60 ms. Therefore, the measurement for each sensor are applied at least 40 ms later after applying the excitation voltage. The duration of the measurement for each sensor is 60 ms to go

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through the full voltage range with a step of 10 mV. The measurement data from ADC for each sensor channel is stored inside the micro-controller and after the measurement is done for all sensors, the results are sent to the RPi through a serial port and the data analysis is carried out. Namely, the connection with the serial port transmits the data in real time and is elaborated inside the RPi. The Tera-term software (Tera Term Project, Japan) is used at the beginning for receiving continuously the values for the serial port. Then a user-friendly graphical interface (GUI) is developed using C++ in Qt inside RPi and it contains a 'Save' Button that carries out the elaboration of data and finally saves the processed data in an .xlsx file.

IV. System Testing

A. Analytical Performance of the realized board

The memristive biosensing board prototype is tested to verify the successful signal acquisition of memristive biosensors and for preserving their particular electrical characteristics. The realized board allows successful simultaneous acquisition of the signal from all the individual sensors included in the chip, and it is confirmed that the obtained electrical characteristics are in very good agreement with the measurements for memristive biosensors performed using the probe station as shown in Fig. 1. Indicative raw data from current-to-voltage measurements acquired is presented in Fig. 7. The crucial aspect of the hysteresis is fulfilled, and the electrical response express the characteristic hysteresis expected for a memristive biosensor, proving that the board successfully records the particular electrical signal of the nanosensors (Fig. 7a). Most importantly, the voltage gap that consists the main bio-detection parameter is also successfully obtained with the board, and presented in figure (Fig. 7b). It is worth highlighting that a very important benefit is the significant decrease of the output acquisition time. The same measurement procedure that with the probe station configuration requires approximately 40 min now with the implementation of the realized board it only requires some seconds and the sensing result can be available immediately. Namely, the whole measurement procedure takes up to 80 seconds to complete and the sensing parameter i.e. voltage gap is calculated automatically for each sensor finally resulting to a calibration curve that indicates the relationship between voltage gap and concentration of cancer molecules. It is important to highlight that even-though the measurement procedure is fast, the memristive hysteresis is not affected. The exact measurement time depends on the chosen measurement parameters, and the hysteresis maintenance is ensured by

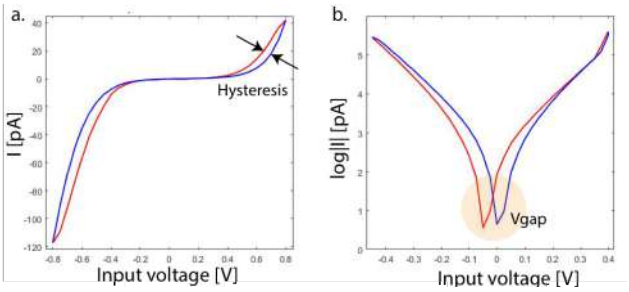

Fig.7. Indicative electrical characteristics aquired with the memristive biosensing board prototype demonsrating the characteristic hysteresis (a) and the voltage gap (b) that consits the main bio-detection parameter.

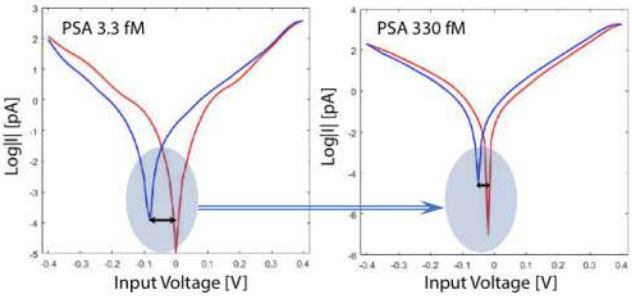

Fig.8. Sensing output for PSA as acquired with the proposed memristive biosensing platform.

accordingly adjusting the sampling parameters. More specifically, the excitation signal waveform requires four parameters from the graphical user interface: (a) the initial voltage (b) the final voltage, (c) the voltage step and (d) the sweep time. The waveform starts from the initial voltage and keeps the voltage level for a duration equal to the sweep time, then increases by the voltage step value and again keeps the voltage level for the duration of the sweep time and then increases till it reaches the final voltage level. Following that, it starts to decrease by voltage step, keeps the duration of sweep time and repeats the steps till it reaches the initial voltage. At this stage, one complete measurement period is accomplished.

B. Sensing application of prostate cancer biomarkers

Antigen coupling and sensing events are detected by measuring the current-to-voltage characteristics following incubations of the sensing chip in increasing PSA concentrations in the range of fM. Increasing concentration of antigens results to a decreasing trend of the voltage gap [7]. This trend is also successfully acquired with the electronic board prototype. Indicative results for the uptake of a low and a high PSA concentration on the same sensor are demonstrated in Fig. 8 as a-proof-of concept. Overall the obtained findings further validate the realized platform for detection of extremely small traces of cancer markers along with the high potential for efficient future sensing applications with memristive biosensors.

V. Conclusions

In this work, the design, realization and validation of a novel and portable, ultrasensitive sensing system based on memristive effect is presented. The suggested bio-sensing platform achieved a significantly fast, automatized and simultaneous sensing of multiple memristive nano-bio-sensors on a single chip. In addition, the realized system paves the way for the actual implementation of such sensors in medical environment and as point-of-care devices, thanks to the platform compactness and portability along with a user-friendly interface. The system successfully achieved detection of PSA, the main biomarker of prostate cancer, at concentrations below the critical level of the biomarker qualifying its suitability for early stage diagnostics. The suggested paradigm may further be implemented for bio-detection involving even more complex schemes (i.e. cells, tumor extracts) holding great promise for fast and highly sensitive sensing in clinical diagnostics and therapeutics.

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