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(54) **ELECTROCHEMICAL MEASURING OF REACTIVE OXYGEN SPECIES (ROS) LEVELS IN PERIPHERAL BLOOD TO DETECT RATIO OF LOW-DENSITY NEUTROPHILS (LDNS) TO HIGH-DENSITY NEUTROPHILS (HDNS), SUITABLE TO ALARM PRESENCE OF CANCER IN SUSPICIOUS CASES**

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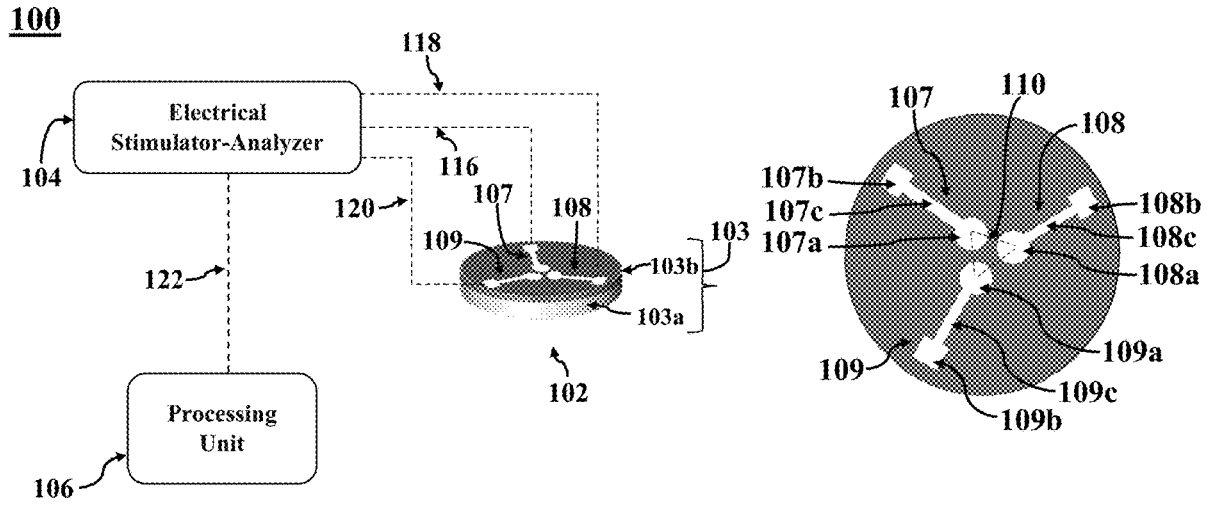
(57) **ABSTRACT**

A system for real-time detecting cancer by analyzing unprocessed blood. The system includes a sensor, an electrical stimulator-analyzer connected to the sensor, and a processing unit connected to the electrical stimulator-analyzer. The sensor includes three electrodes including a working electrode, a counter electrode, and a reference electrode configured to be put in contact with an unprocessed blood sample. The processing unit is configured to perform a method. The method includes applying a set of voltages between the reference electrode and the working electrode utilizing the stimulator-analyzer, measuring a produced set of currents between the counter electrode and the working electrode utilizing the stimulator-analyzer, measuring a level of a ratio of low-density neutrophils (LDNs) to high-density neutrophils (HDNs) in the unprocessed blood sample by measuring a maximum current of the measured set of currents, and detecting a cancer disease if the measured maximum current is less than a threshold value.



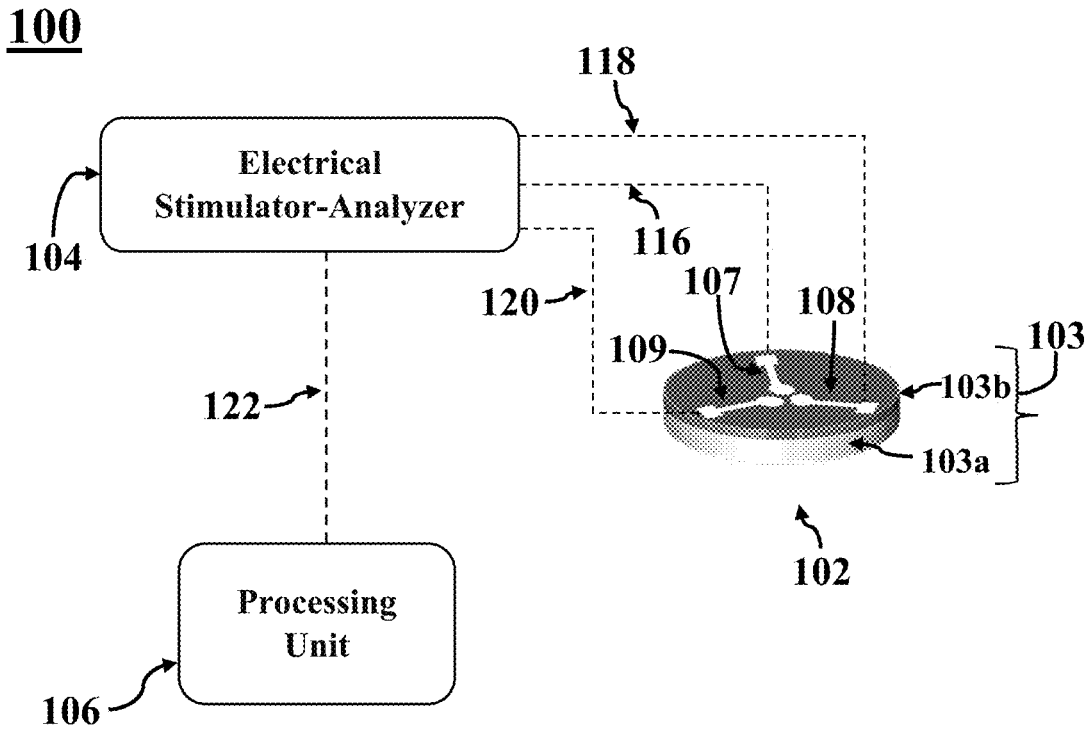

100
118
Electrical
Stimulator-Analyzer
104
116
120
107
108
109
103
103b
122
103a
102
Processing
Unit
106

FIG. 1A

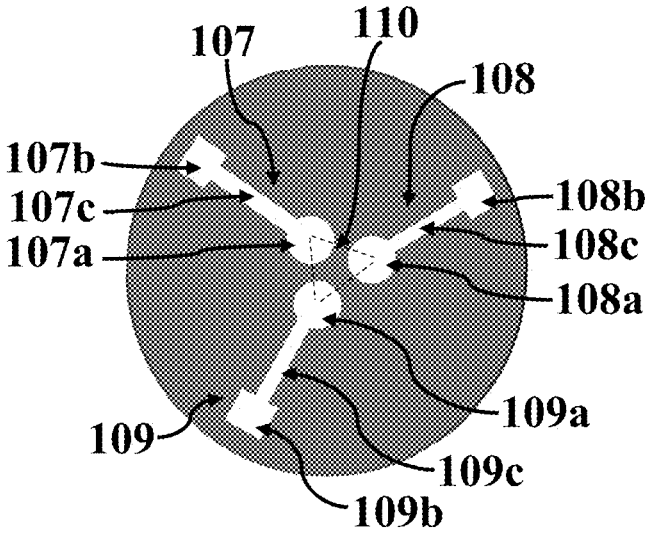


FIG. 1B

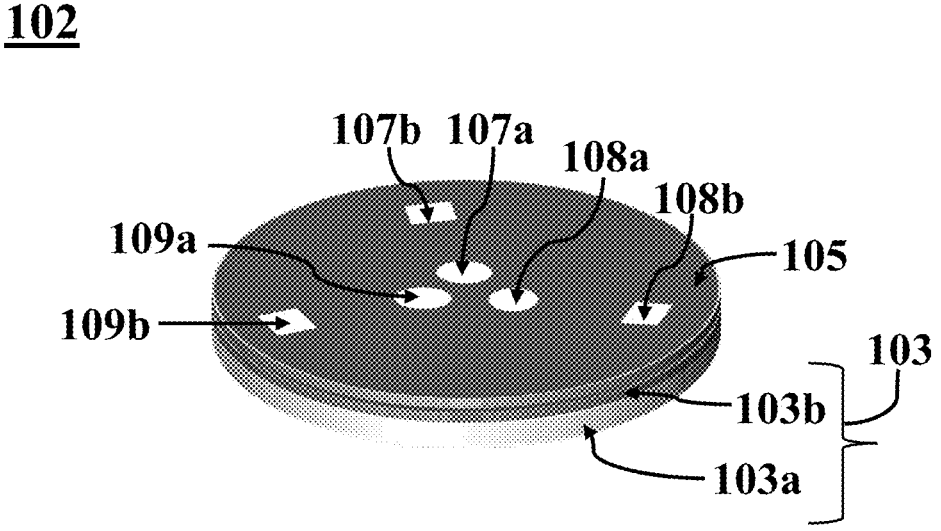


FIG. 1C

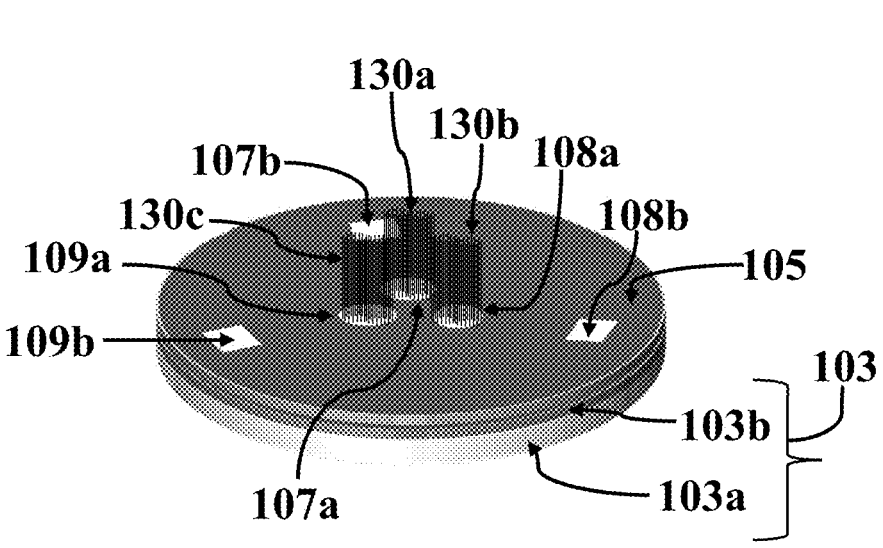

102
130a
130b
108a
107b
130c
108b
109a
105
109b
103
103b
103a
107a

FIG. 1D

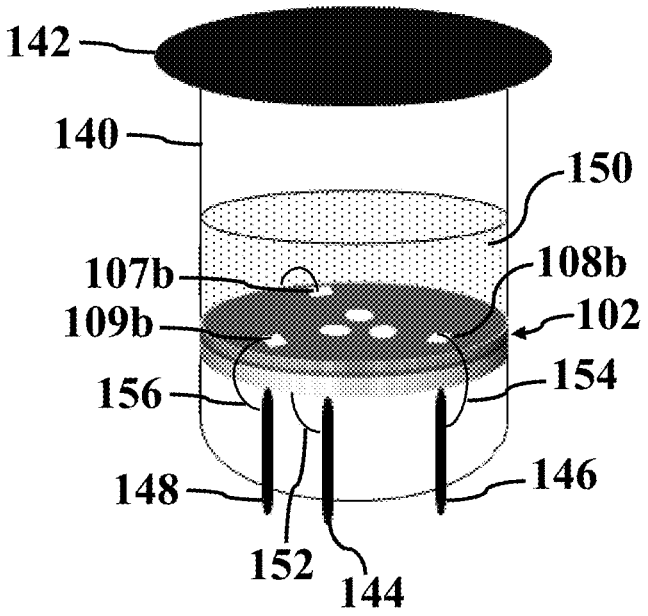


FIG. 1E

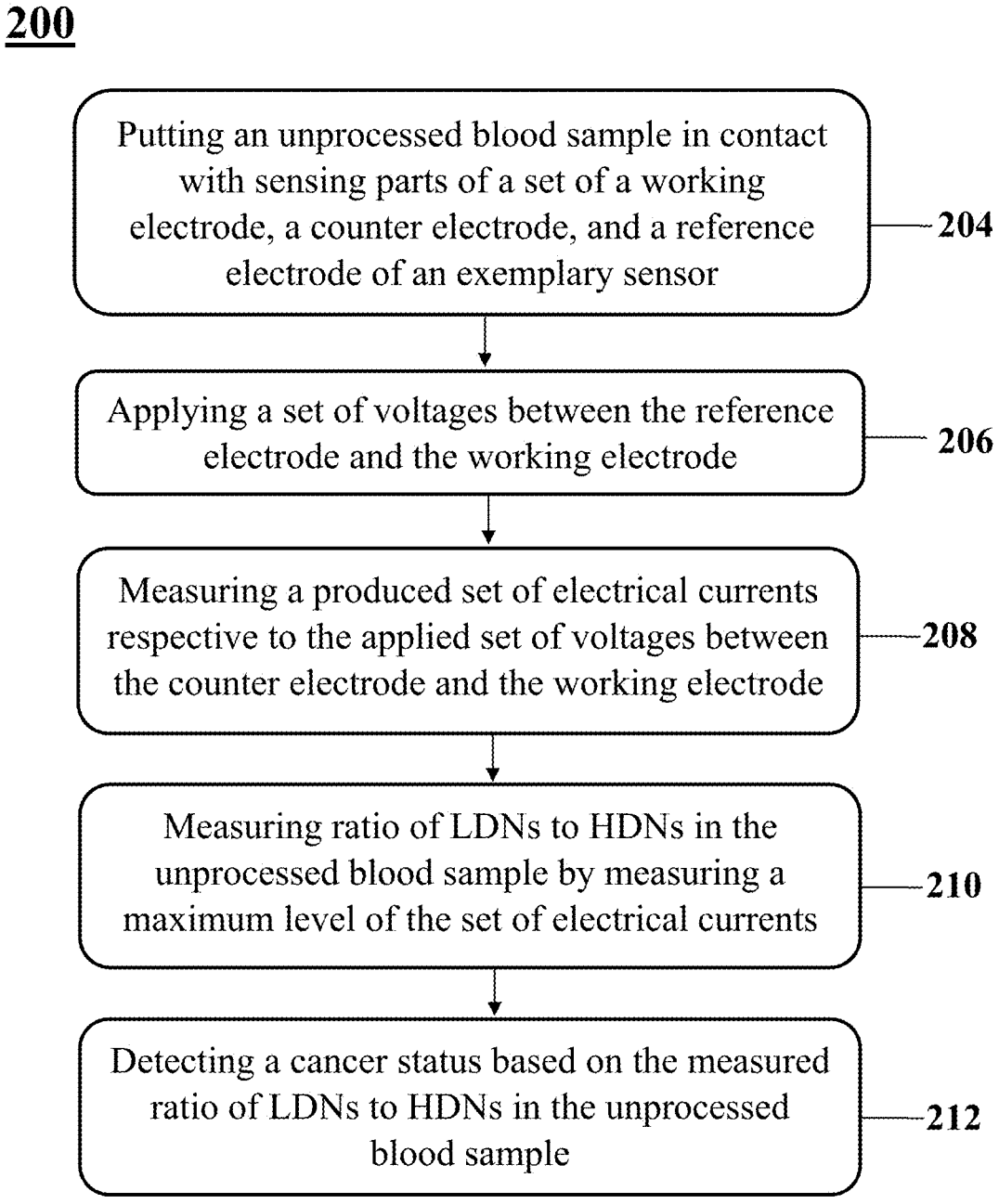

200
Putting an unprocessed blood sample in contact with sensing parts of a set of a working electrode, a counter electrode, and a reference electrode of an exemplary sensor
204
Applying a set of voltages between the reference electrode and the working electrode
206
Measuring a produced set of electrical currents respective to the applied set of voltages between the counter electrode and the working electrode
208
Measuring ratio of LDNs to HDNs in the unprocessed blood sample by measuring a maximum level of the set of electrical currents
210
Detecting a cancer status based on the measured ratio of LDNs to HDNs in the unprocessed blood sample
212

FIG. 2A

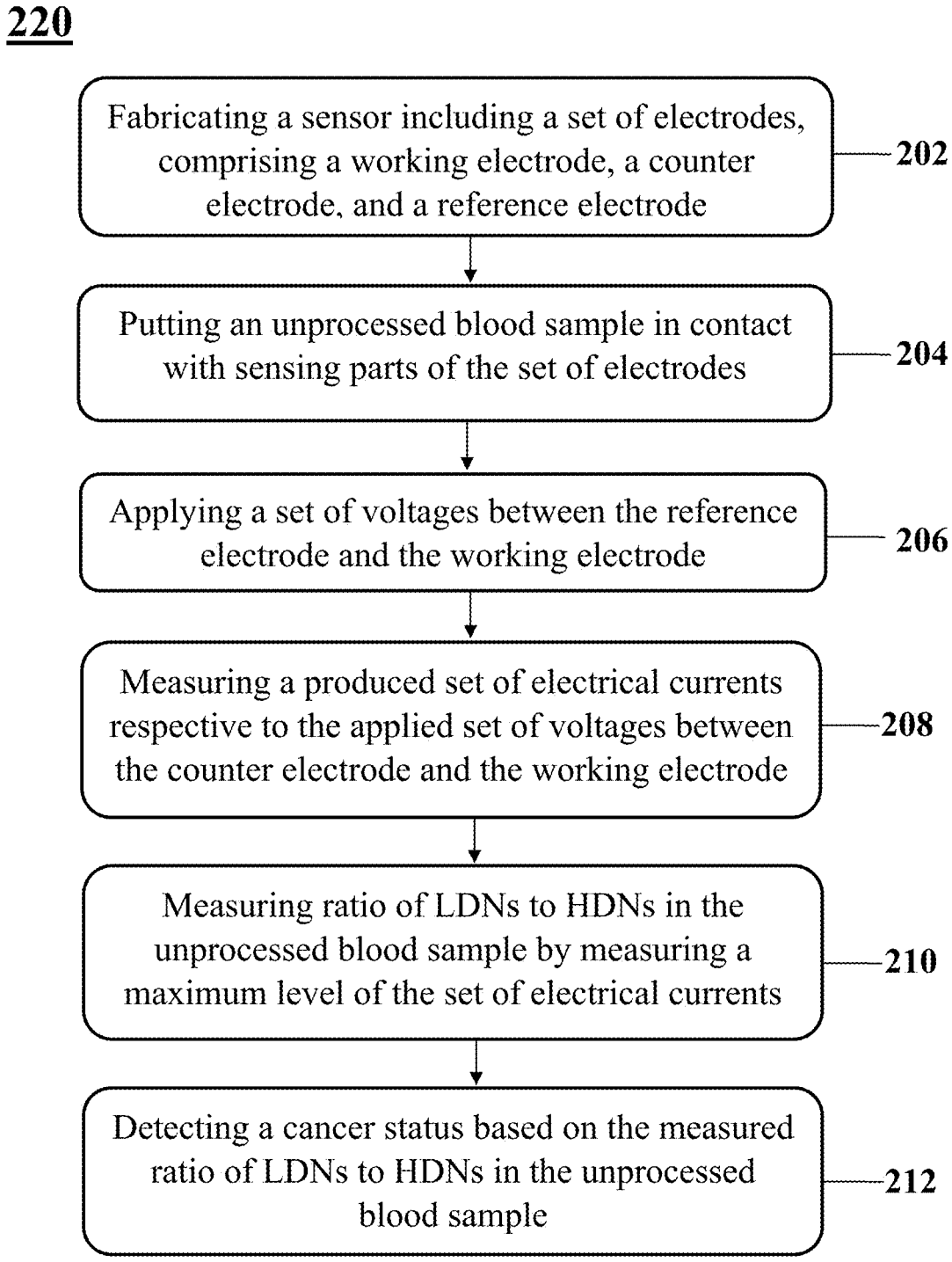

220
Fabricating a sensor including a set of electrodes, comprising a working electrode, a counter electrode, and a reference electrode
202
Putting an unprocessed blood sample in contact with sensing parts of the set of electrodes
204
Applying a set of voltages between the reference electrode and the working electrode
206
Measuring a produced set of electrical currents respective to the applied set of voltages between the counter electrode and the working electrode
208
Measuring ratio of LDNs to HDNs in the unprocessed blood sample by measuring a maximum level of the set of electrical currents
210
Detecting a cancer status based on the measured ratio of LDNs to HDNs in the unprocessed blood sample
212

FIG. 2B

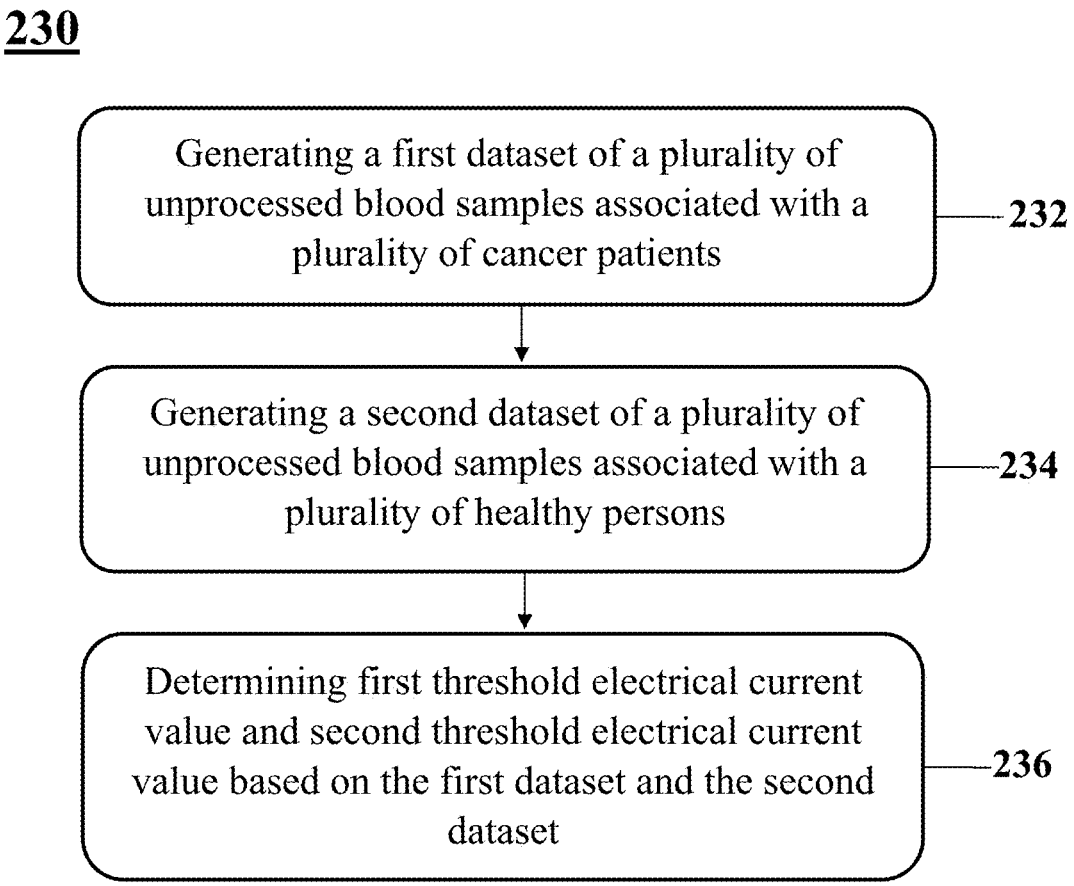

230
Generating a first dataset of a plurality of unprocessed blood samples associated with a plurality of cancer patients
232
Generating a second dataset of a plurality of unprocessed blood samples associated with a plurality of healthy persons
234
Determining first threshold electrical current value and second threshold electrical current value based on the first dataset and the second dataset
236

FIG. 2C

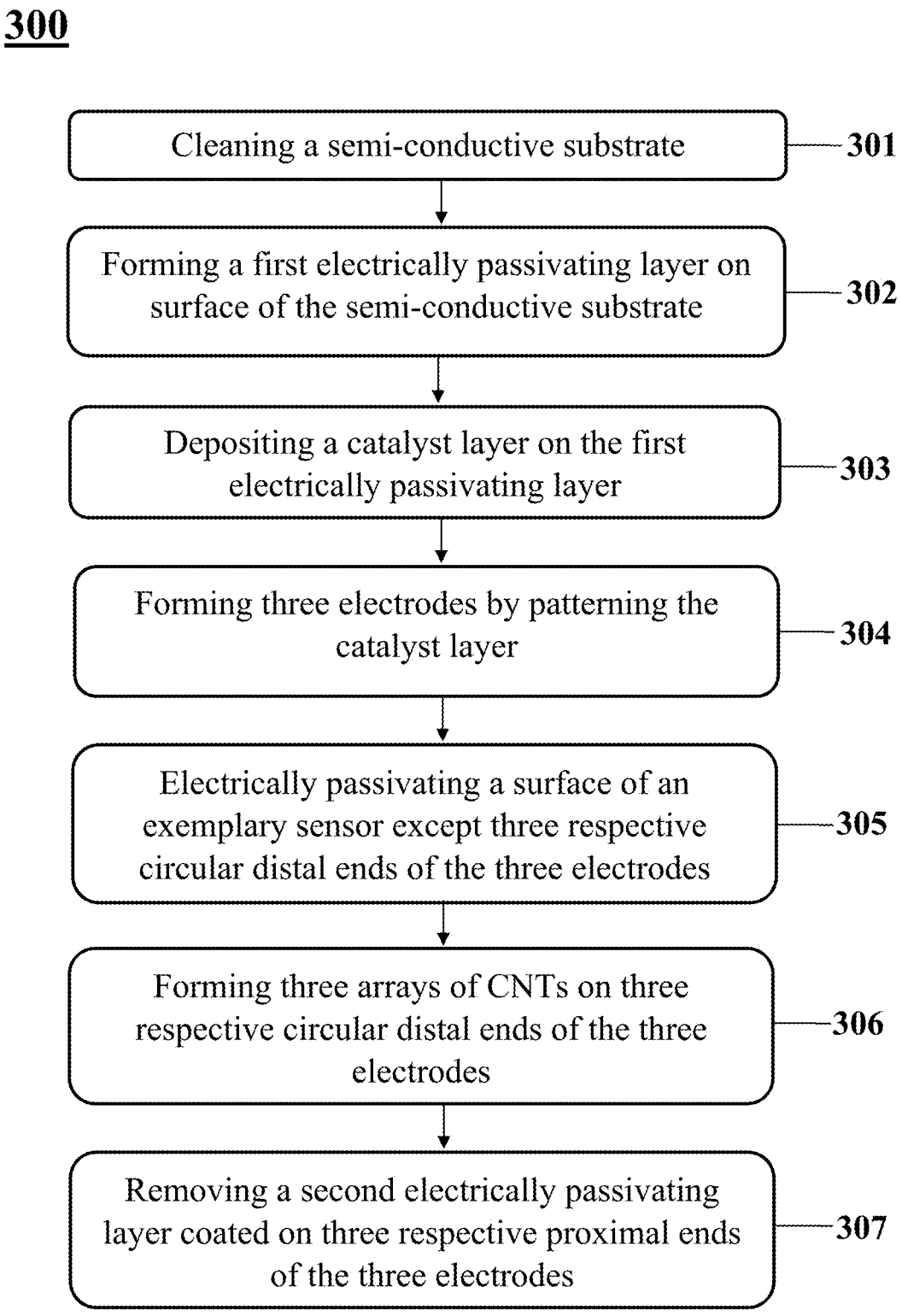

300
Cleaning a semi-conductive substrate
301
Forming a first electrically passivating layer on surface of the semi-conductive substrate
302
Depositing a catalyst layer on the first electrically passivating layer
303
Forming three electrodes by patterning the catalyst layer
304
Electrically passivating a surface of an exemplary sensor except three respective circular distal ends of the three electrodes
305
Forming three arrays of CNTs on three respective circular distal ends of the three electrodes
306
Removing a second electrically passivating layer coated on three respective proximal ends of the three electrodes
307

FIG. 3A

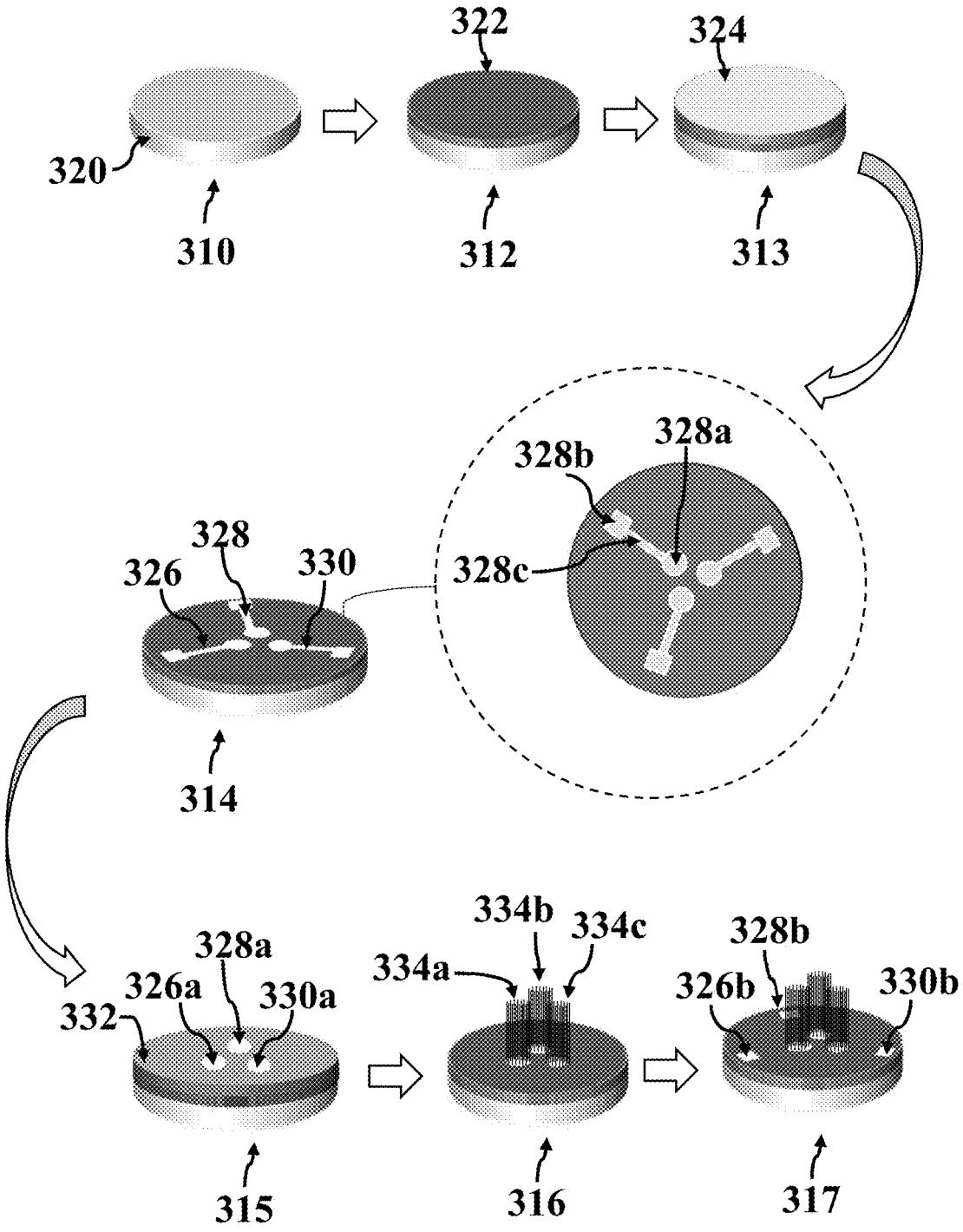

322
324
320
310
312
313
328b
328a
328
326
330
328c
314
334b
334c
328a
328b
334a
326a
330a
326b
330b
332
315
316
317

FIG. 3B

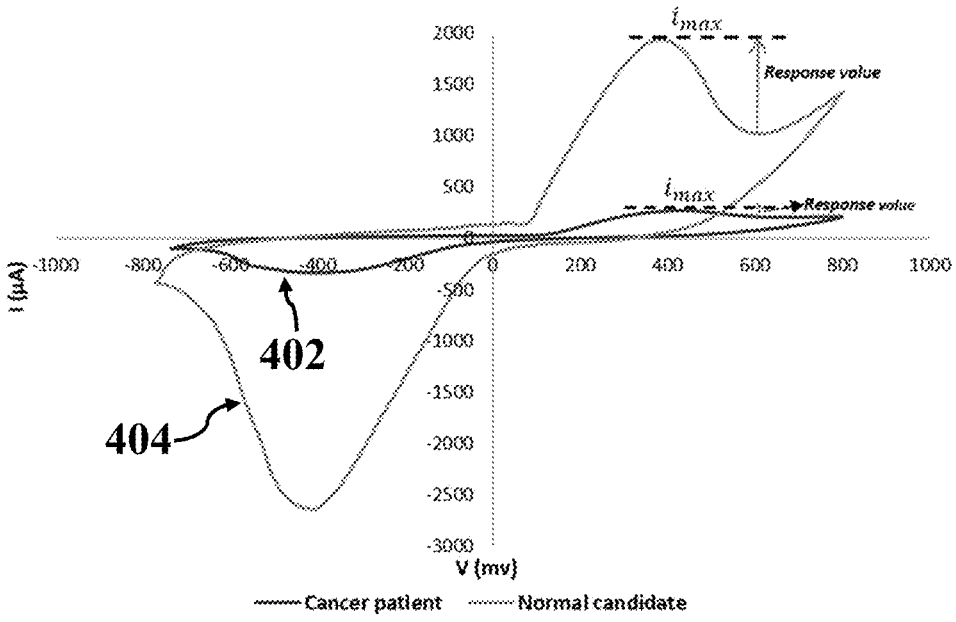

i_{max}
Response value
i_{max}
Response value
2000
1500
1000
500
-500
-1000
-1500
-2000
-2500
-3000
-1000
-800
-600
-400
-200
0
200
400
600
800
1000
I (μA)
V (mv)
402
404
Cancer patient
Normal candidate

FIG. 4

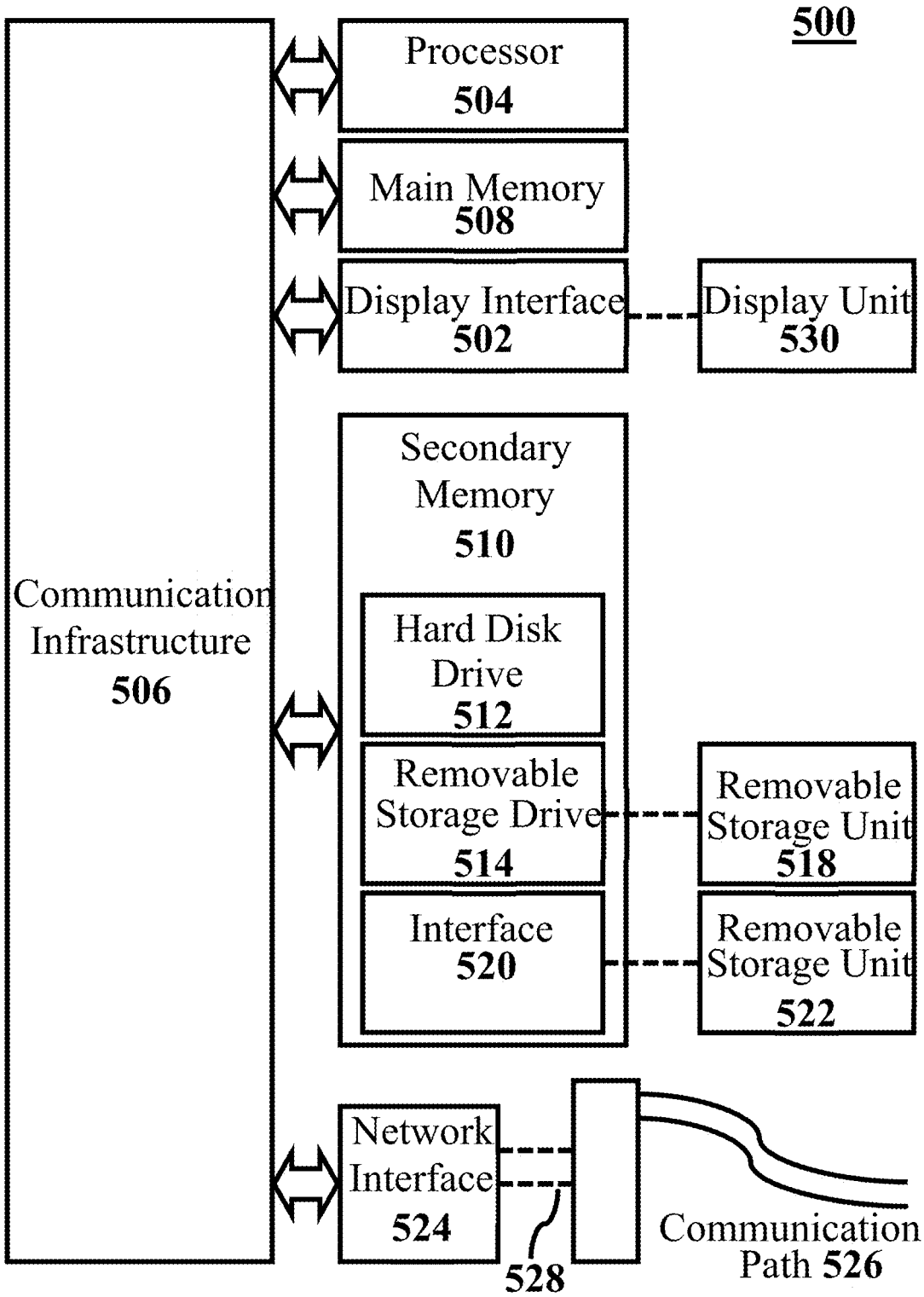

500
Processor
504
Main Memory
508
Display Interface
502
Display Unit
530
Secondary
Memory
510
Hard Disk
Drive
512
Removable
Storage Drive
514
Removable
Storage Unit
518
Interface
520
Removable
Storage Unit
522
Communication
Infrastructure
506
Network
Interface
524
528
Communication
Path 526

FIG. 5

600

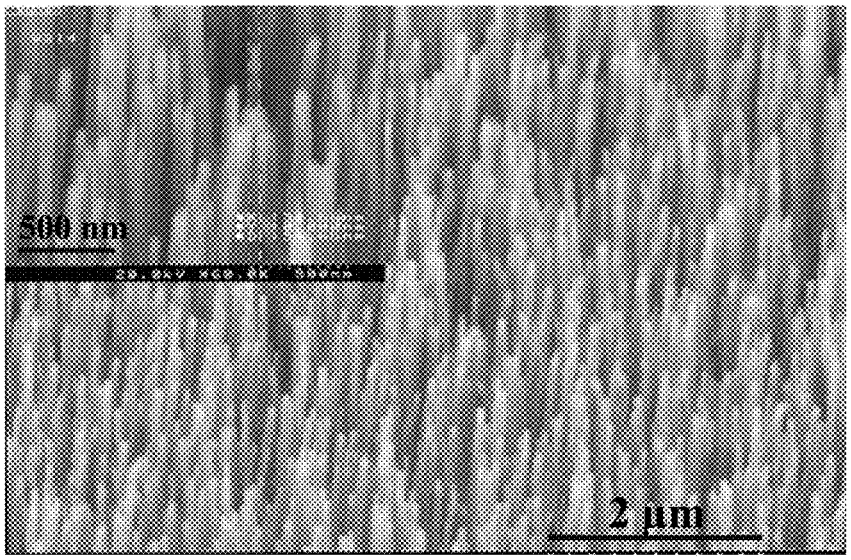


FIG. 6

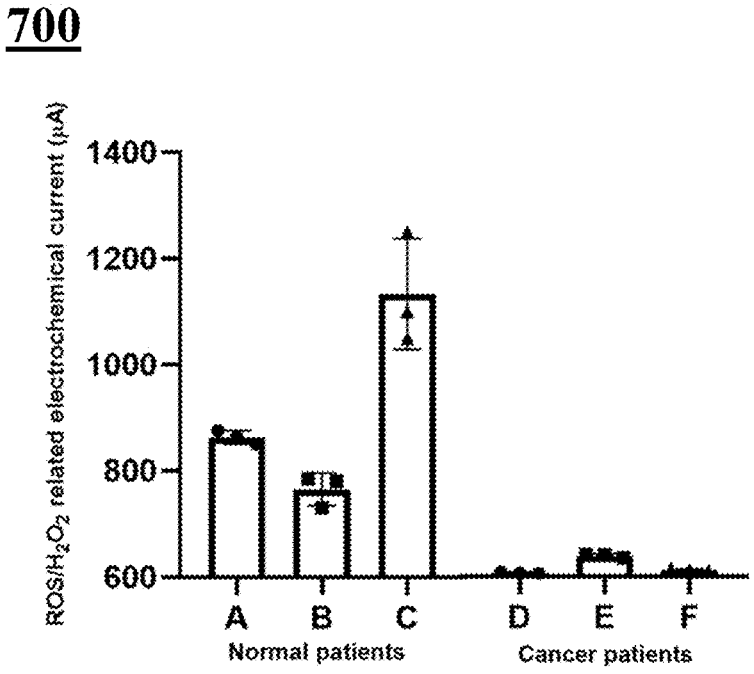

700
ROS/H_2O_2 related electrochemical current (μA)
1400
1200
1000
800
600
A
B
C
D
E
F
Normal patients
Cancer patients

FIG. 7

800

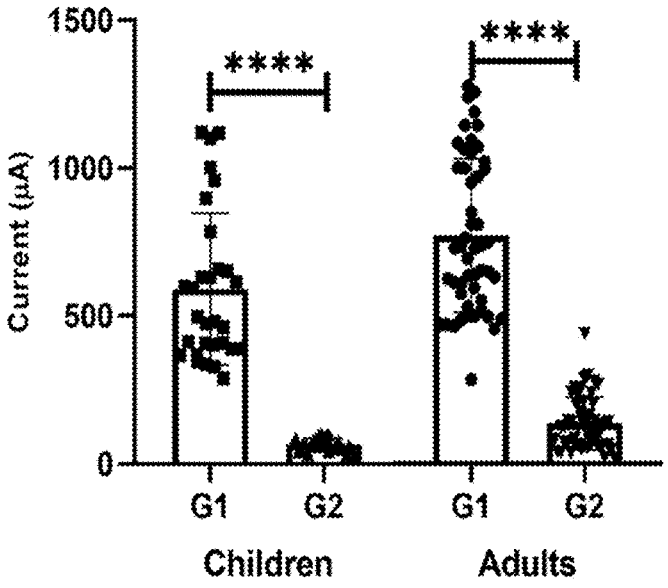


FIG. 8

900

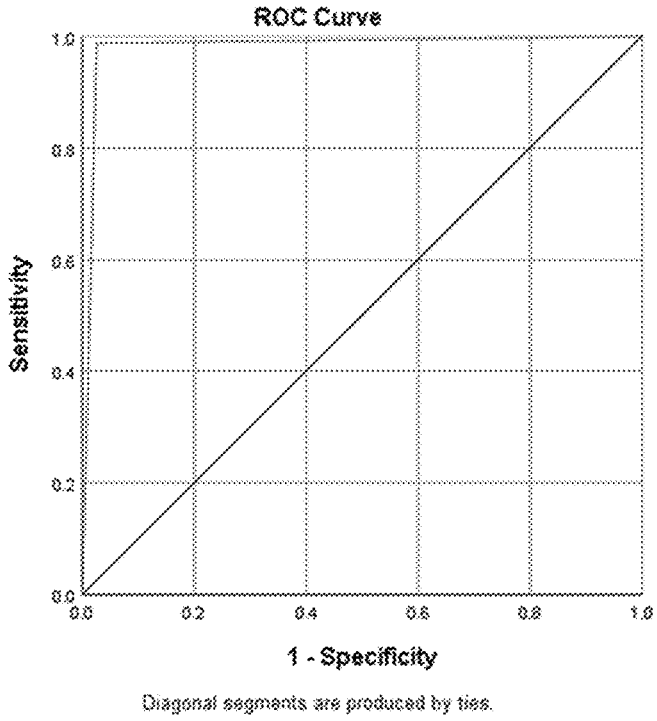

ROC Curve
Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
Diagonal segments are produced by ties.

FIG. 9

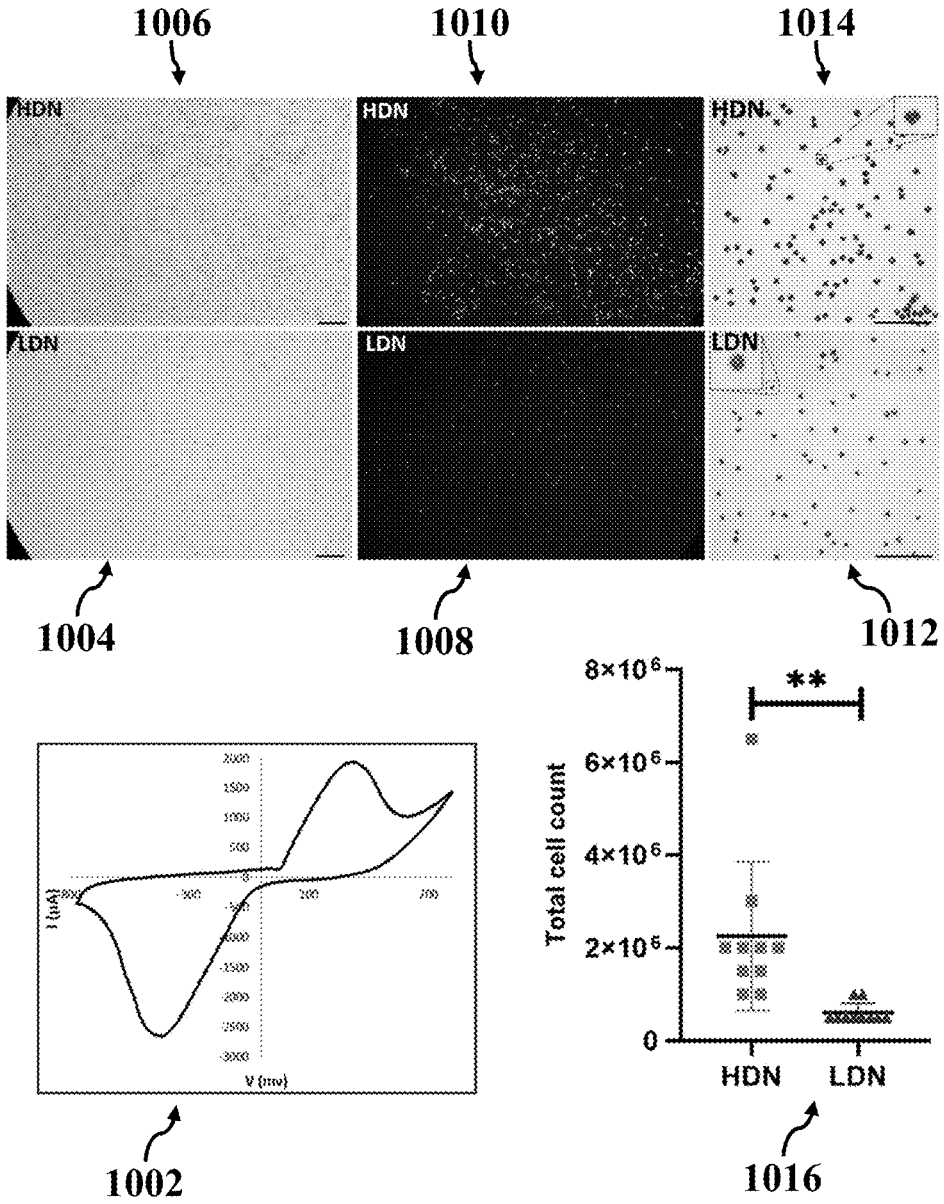

1006
1010
1014
HDN
HDN
HDN
LDN
LDN
LDN
1004
1008
1012
I (μA)
V (mv)
1002
8×10⁶
6×10⁶
4×10⁶
2×10⁶
0
Total cell count
**
HDN
LDN
1016

FIG. 10A

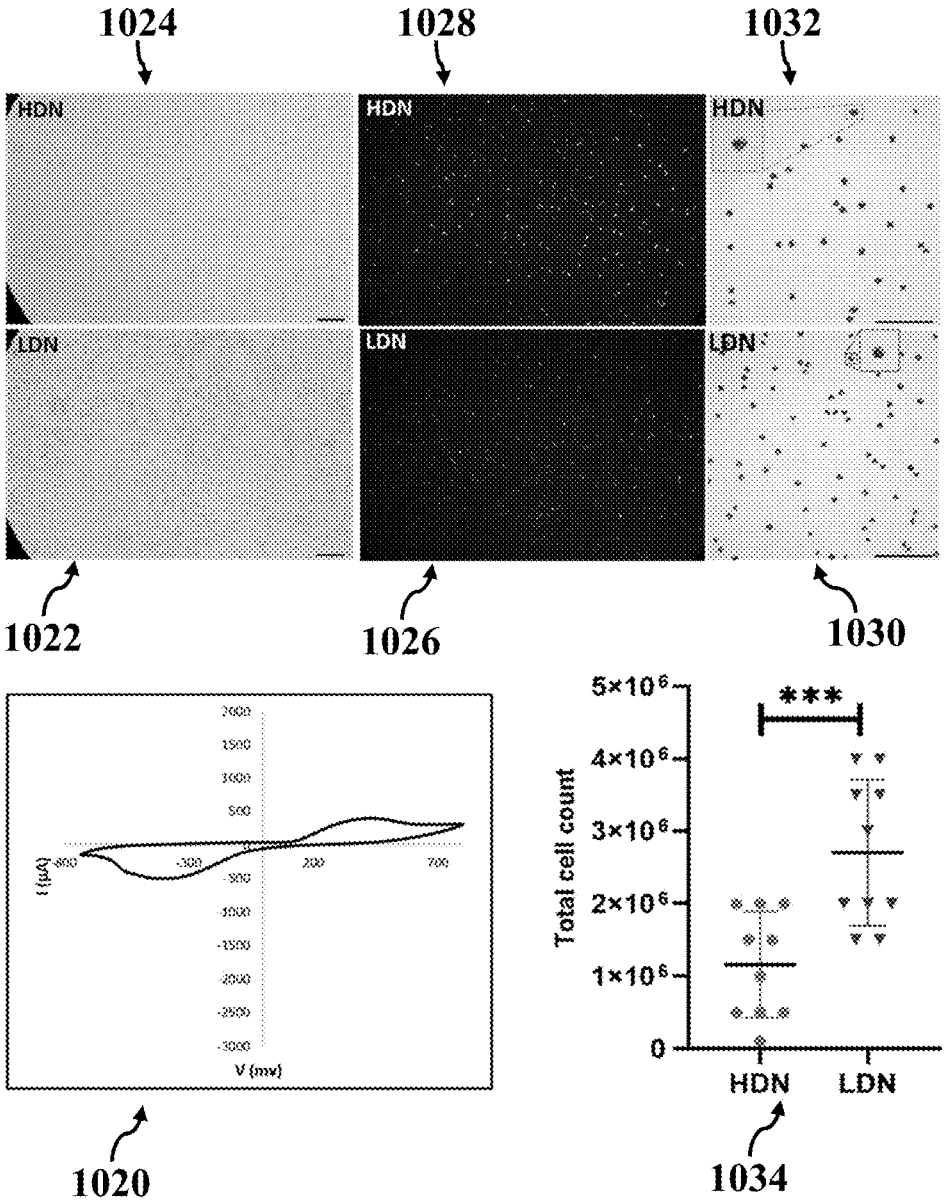

1024
1028
1032
HDN
HDN
HDN
LDN
LDN
LDN
1022
1026
1030
I (μA)
V (mv)
1020
Total cell count
5×10⁶
4×10⁶
3×10⁶
2×10⁶
1×10⁶
0
***
HDN
LDN
1034

FIG. 10B

1000

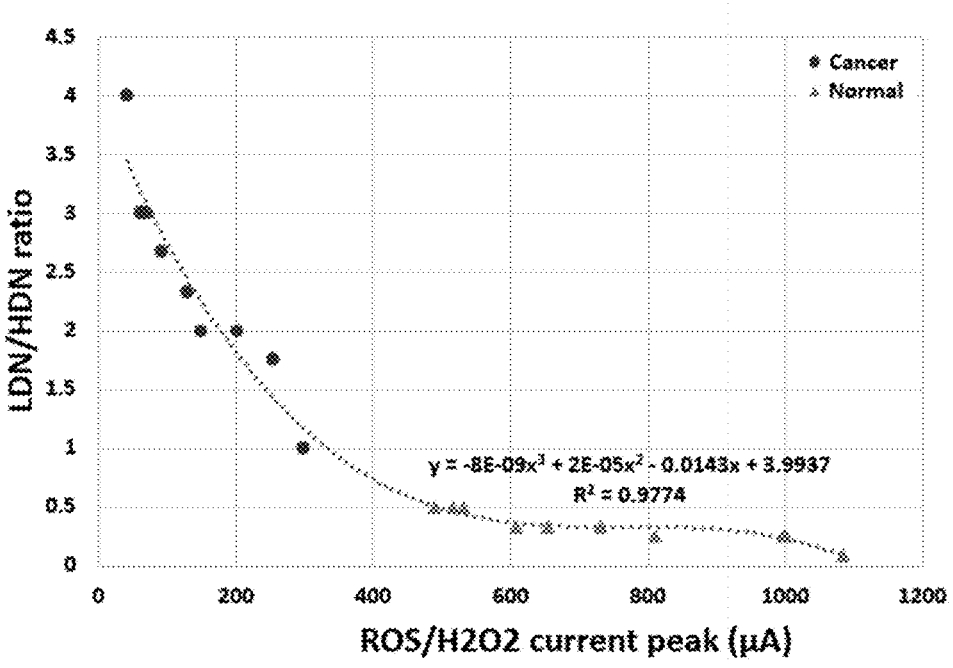

LDN/HDN ratio
ROS/H2O2 current peak (µA)
Cancer
Normal
y = -8E-09x³ + 2E-05x² - 0.0143x + 3.9937
R² = 0.9774
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
200
400
600
800
1000
1200

FIG. 10C

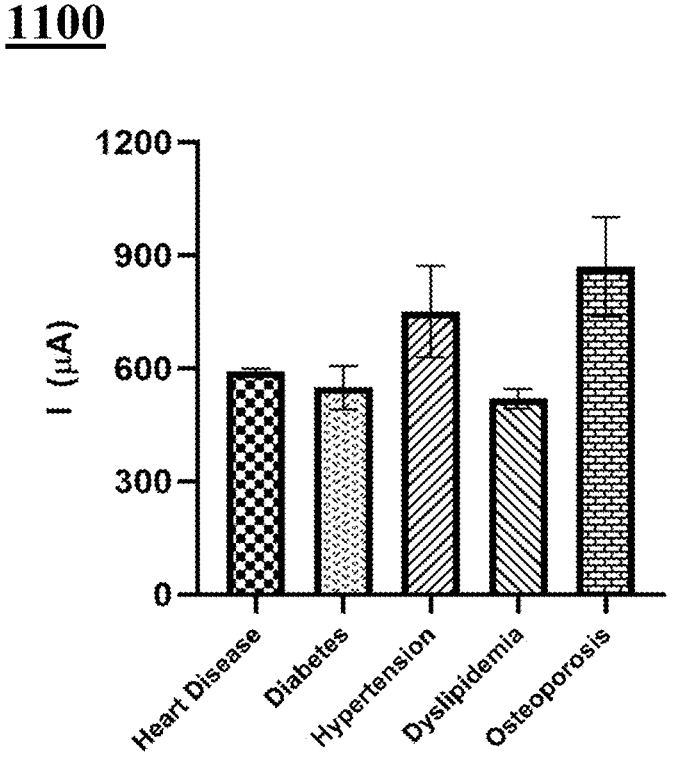

1100
1200
900
600
300
0
I (μA)
Heart Disease
Diabetes
Hypertension
Dyslipidemia
Osteoporosis

FIG. 11

ELECTROCHEMICAL MEASURING OF REACTIVE OXYGEN SPECIES (ROS) LEVELS IN PERIPHERAL BLOOD TO DETECT RATIO OF LOW-DENSITY NEUTROPHILS (LDNS) TO HIGH-DENSITY NEUTROPHILS (HDNS), SUITABLE TO ALARM PRESENCE OF CANCER IN SUSPICIOUS CASES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation-in-part of International Patent Application PCT/IB2022/051283, filed on Feb. 14, 2022, and entitled "ELECTROCHEMICAL MEASURING OF REACTIVE OXYGEN SPECIES (ROS) LEVELS IN PERIPHERAL BLOOD TO DETECT RATIO OF LOW-DENSITY NEUTROPHILS (LDNS) TO HIGH-DENSITY NEUTROPHILS (HDNS), SUITABLE TO ALARM PRESENCE OF CANCER IN SUSPICIOUS CASES", which takes priority from U.S. Provisional Patent Application Ser. No. 63/149,282, filed on Feb. 14, 2021, and entitled "ELECTROCHEMICAL MEASURING OF REACTIVE OXYGEN SPECIES LEVELS RELEASED BY LOW-DENSITY NEUTROPHILS IN THE BLOOD, AN ALARM OF CANCER IN SUSPICIOUS CASES", which are both incorporated herein by reference in their entirety.

TECHNICAL FIELD

[0002] The present disclosure generally relates to cancer diagnosis, and particularly, to a system and method for real-time cancer diagnosis by determining a ratio of low-density neutrophils (LDNs) to high-density neutrophils (HDNs) via measuring reactive oxygen species (ROS) levels in unprocessed blood samples.

BACKGROUND

[0003] Neutrophils are the most abundant (50% to 70%) type of white blood cells circulating leukocytes which are derived from hematopoietic stem cells (HSCs) in bone marrow. Neutrophils release enzymes to remodel an extracellular matrix of a tissues through which neutrophils migrate to reach a site of a wound or an infection.

[0004] Number of LDN neutrophils in blood and tumor tissues of cancer patients with solid tumors shows an association with disease progression and patient outcome. There are several cell markers for identification of immature neutrophils in human. These markers need further validation to ensure its accuracy. Hence, since neutrophils can show plasticity in response to their environment, certain markers are likely to only be proper in particular models and require efficient validation of them. So, the challenges associated with identifying and isolating populations of neutrophil maturity have limited their study and the current knowledge of their functional properties.

[0005] Hence, there is a need for a label-free method and system to determine an accurate correlation between abundancy of neutrophils, specially, LNDs in blood sample of patients with a cancer status of the patients. There is also a need for a highly precise and fast method and system for detecting a cancer status of a suspected patient to be cancerous via measuring a level of LDNs in peripheral blood without any need to process a blood sample and/or use a cell marker.

SUMMARY

[0006] This summary is intended to provide an overview of the subject matter of this patent, and is not intended to identify essential elements or key elements of the subject matter, nor is it intended to be used to determine the scope of the claimed embodiments. The proper scope of this patent may be ascertained from the claims set forth below in view of the detailed description below and the drawings.

[0007] In one general aspect, the present disclosure is directed to a method for real-time detecting cancer. The method may include putting an unprocessed blood sample in contact with sensing parts of a set of a working electrode, a counter electrode, and a reference electrode of a sensor, where the unprocessed blood sample may be drawn from a suspected person to have cancer. The method may further include applying a set of voltages in a sweeping range from −0.8 V to +0.8 V between the reference electrode and the working electrode, measuring a produced set of electrical currents between the counter electrode and the working electrode versus the applied set of voltages utilizing one or more processors, measuring a level of ratio of low-density neutrophils (LDNs) to high-density neutrophils (HDNs) in the unprocessed blood sample utilizing one or more processors, and detecting a cancer status of the suspected person based on the measured level of ratio of LDNs to HDNs in the unprocessed blood sample utilizing one or more processors.

[0008] In an exemplary embodiment, measuring the level of ratio of LDNs to HDNs in the unprocessed blood sample may include measuring a level of reactive oxygen species (ROS) in the unprocessed blood sample by measuring a maximum electrical current of the measured set of electrical currents and determining the level of ratio of LDNs to HDNs in the unprocessed blood sample based on the measured maximum electrical current of the measured set of electrical currents. In an exemplary embodiment, determining the level of ratio of LDNs to HDNs in the unprocessed blood sample based on the measured maximum electrical current of the measured set of electrical currents may include detecting the level of ratio of LDNs to HDNs in the unprocessed blood sample is more than 1 if the measured maximum electrical current of the measured set of electrical currents is less than a first threshold electrical current value or detecting the level of ratio of LDNs to HDNs in the unprocessed blood sample is less than 1 if the measured maximum electrical current of the measured set of electrical currents is more than a second threshold electrical current value. In an exemplary embodiment, detecting the cancer status of the suspected person may include one of detecting a cancer disease in the suspected person's body if the level of ratio of LDNs to HDNs in the unprocessed blood sample is more than one or detecting no cancer disease in the suspected person's body if the level of ratio of LDNs to HDNs in the unprocessed blood sample is less than one.

[0009] In an exemplary embodiment, the method may further include generating the first threshold electrical current value and the second threshold electrical current value. In an exemplary embodiment, generating the first threshold electrical current value and the second threshold electrical current value may include generating a first dataset of a plurality of unprocessed blood samples associated with a

plurality of cancer patients, generating a second dataset of a plurality of unprocessed blood samples associated with a plurality of healthy persons, and determining the first threshold electrical current value and the second threshold electrical current value using the first dataset and the second dataset. In an exemplary embodiment, generating the first dataset of the plurality of unprocessed blood samples associated with the plurality of cancer patients may include measuring a first set of electrical current peaks of the unprocessed blood samples associated with the plurality of cancer patients, measuring a first set of ratio of LDNs to HDNs in the unprocessed blood samples associated with the plurality of cancer patients utilizing a cell counter, and assigning each measured ratio of LDNs to HDNs of the first set of ratio of LDNs to HDNs to the respective measured electrical current peak of the first set of electrical current peaks. In an exemplary embodiment, generating the second dataset of the plurality of unprocessed blood samples associated with the plurality of healthy persons may include measuring a second set of electrical current peaks of the unprocessed blood samples associated with the plurality of healthy persons, measuring a first set of ratio of LDNs to HDNs in the unprocessed blood samples associated with the plurality of healthy persons utilizing a cell counter, and assigning each measured ratio of LDNs to HDNs of the second set of ratio of LDNs to HDNs to the respective measured electrical current peak of the second set of electrical current peaks. In an exemplary embodiment, determining the first threshold electrical current value and the second threshold electrical current value may include determining the first threshold electrical current value equal to a maximum electrical current peak among the first set of electrical current peaks and determining the second threshold electrical current value equal to a minimum electrical current peak among the second set of electrical current peaks.

[0010] In an exemplary embodiment, the first threshold electrical current value is 300 μA for an adult person. In an exemplary embodiment, the first threshold electrical current value is 100 μA for a child. In an exemplary embodiment, the second threshold electrical current value is 450 μA for an adult person. In an exemplary embodiment, the second threshold electrical current value is 300 μA for a child.

[0011] In an exemplary embodiment, the method may be conducted in less than 30 seconds. In an exemplary embodiment, the method may further include acquiring the unprocessed blood sample from the suspected person to have cancer.

[0012] In an exemplary embodiment, putting the unprocessed blood sample in contact with the sensing parts of the set of the working electrode, the counter electrode, and the reference electrode of the sensor may include putting the unprocessed blood sample in contact with three respective arrays of multi-walled carbon nanotubes (VAMWCNTs) grown on the sensing parts of the set of the working electrode, the counter electrode, and the reference electrode by dropping the unprocessed blood sample on surface of the sensor.

[0013] In an exemplary embodiment, applying the set of voltages in the sweeping range from −0.8 V to +0.8 V between the reference electrode and the working electrode may include connecting respective proximal ends of the set of the working electrode, the counter electrode, and the reference electrode of the sensor to an electrical stimulator-analyzer device and applying the set of voltages in a sweeping range from −0.8 V to +0.8 V to the sensor using the electrical stimulator-analyzer device. In an exemplary embodiment, measuring the produced set of electrical currents between the counter electrode and the working electrode versus the applied set of voltages may be done utilizing the electrical stimulator-analyzer device.

[0014] In another general aspect, the present disclosure is directed to a system for real-time detecting cancer by analyzing unprocessed blood. The system may include a sensor configured to place an unprocessed blood sample drawn from a suspected person to have cancer thereon, an electrical stimulator-analyzer device electrically connected to the sensor, and a processing unit electrically connected to the electrical stimulator-analyzer device.

[0015] In an exemplary embodiment, the sensor may include a substrate and three electrodes formed on the substrate. In an exemplary embodiment, the three electrodes may include a working electrode, a counter electrode, and a reference electrode. In an exemplary embodiment, each respective electrode may include an electrically conductive layer deposited on the substrate and an array of multi-walled carbon nanotubes (VAMWCNTs). In an exemplary embodiment, the electrically conductive layer may include a proximal end and a circular distal end. In an exemplary embodiment, the array of VAMWCNTs may be grown on the circular distal end. In an exemplary embodiment, the array of VAMWCNTs may be configured to be put in contact with the unprocessed blood sample drawn from the suspected person to have cancer.

[0016] In an exemplary embodiment, the electrical stimulator-analyzer device may be electrically connected to the sensor at the respective proximal end of each respective electrode of the three electrodes. In an exemplary embodiment, the stimulator-analyzer device may be configured to apply a set of voltages in a sweeping range from −0.8 V to +0.8 V to the sensor comprising the VAMWCNTs being in contact with the unprocessed blood sample and measure a produced set of electrical currents of the sensor responsive to the applied set of voltages.

[0017] In an exemplary embodiment, the processing unit may include a memory having processor-readable instructions stored therein and a processor. In an exemplary embodiment, the processor may be configured to access the memory and execute the processor-readable instructions. In an exemplary embodiment, when the processor-readable instructions are executed by the processor, configures the processor to perform a method. In an exemplary embodiment, the method may include applying the set of voltages in the sweeping range from −0.8 V to +0.8 V between the reference electrode and the working electrode utilizing the stimulator-analyzer device, measuring the produced set of electrical currents between the counter electrode and the working electrode utilizing the stimulator-analyzer device, measuring a level of a ratio of low-density neutrophils (LDNs) to high-density neutrophils (HDNs) in the unprocessed blood sample by measuring a level of reactive oxygen species (ROS) in the unprocessed blood sample, and detecting a cancer status of the suspected person. In an exemplary embodiment, measuring the level of ROS in the unprocessed blood sample may include measuring a maximum electrical current of the measured set of electrical currents.

[0018] In an exemplary embodiment, detecting the cancer status of the suspected person may include one of detecting a cancer disease in suspected person's body if the measured ratio of LDNs to HDNs in the unprocessed blood sample is more than 1 by detecting the measured maximum electrical current of the measured set of electrical currents being less than a first threshold electrical current value or detecting no cancer disease in the suspected person's body if the measured ratio of LDNs to HDNs in the unprocessed blood sample is less than 1 by detecting the measured maximum electrical current of the measured set of electrical currents being more than a second threshold electrical current value. In an exemplary embodiment, the method may be conducted in a time period of less than 30 seconds.

[0019] In an exemplary embodiment, detecting the cancer disease in the suspected person's body may include detecting the cancer disease in the suspected person's body if the suspected person's body is an adult and if the measured maximum electrical current of the measured set of electrical currents is less than 300 μA corresponding to a ratio of LDNs to HDNs in the unprocessed blood sample being in a range of more than 1. In another exemplary embodiment, detecting the cancer disease in the suspected person's body may include detecting the cancer disease in the suspected person's body if the suspected person's body is a child and if the measured maximum electrical current of the measured set of electrical currents is less than 100 μA corresponding to a ratio of LDNs to HDNs in the unprocessed blood sample being in a range of more than 1.

[0020] In an exemplary embodiment, the method may further include detecting no cancer disease (a normal or healthy status) in the suspected person's body if the measured ratio of LDNs to HDNs in the unprocessed blood sample is less than a threshold LDNs/HDNs value of 1. In an exemplary embodiment, detecting no cancer disease (a normal or healthy status) in the suspected person's body may include detecting no cancer disease in the suspected person's body if the suspected person's body is an adult and if the measured maximum electrical current of the measured set of electrical currents is more than 450 μA corresponding to a ratio of LDNs to HDNs in the unprocessed blood sample being less than 1. In another exemplary embodiment, detecting no cancer disease (a normal or healthy status) in the suspected person's body may include detecting no cancer disease in the suspected person's body if the suspected person's body is a child and if the measured maximum electrical current of the measured set of electrical currents is more than 300 μA corresponding to a ratio of LDNs to HDNs in the unprocessed blood sample being less than 1.

[0021] In an exemplary embodiment, the method may further include determining the first threshold electrical current value and the second threshold electrical current value. In an exemplary embodiment, determining the first threshold electrical current value and the second threshold electrical current value may include generating a first dataset of a plurality of unprocessed blood samples associated with a plurality of cancer patients, generating a second dataset of a plurality of unprocessed blood samples associated with a plurality of healthy persons, and determining the first threshold electrical current value and the second threshold electrical current value.

[0022] In an exemplary embodiment, generating the first dataset of the plurality of unprocessed blood samples associated with the plurality of cancer patients may include measuring a first set of electrical current peaks of the unprocessed blood samples associated with the plurality of cancer patients, measuring a first set of ratio of LDNs to HDNs in the unprocessed blood samples associated with the plurality of cancer patients utilizing a cell counter, and assigning each measured ratio of LDNs to HDNs of the first set of ratio of LDNs to HDNs to the respective measured electrical current peak of the first set of electrical current peaks. In an exemplary embodiment, generating the second dataset of the plurality of unprocessed blood samples associated with the plurality of healthy persons may include measuring a second set of electrical current peaks of the unprocessed blood samples associated with the plurality of healthy persons, measuring a first set of ratio of LDNs to HDNs in the unprocessed blood samples associated with the plurality of healthy persons utilizing a cell counter, and assigning each measured ratio of LDNs to HDNs of the second set of ratio of LDNs to HDNs to the respective measured electrical current peak of the second set of electrical current peaks. In an exemplary embodiment, determining the first threshold electrical current value and the second threshold electrical current value may include determining the first threshold electrical current value equal to a maximum electrical current peak among the first set of electrical current peaks and determining the second threshold electrical current value equal to a minimum electrical current peak among the second set of electrical current peaks.

[0023] In an exemplary embodiment, the circular distal end has a diameter in a range of 0.5 mm to 3 mm. In an exemplary embodiment, the circular distal end has a diameter of 2 mm. In an exemplary embodiment, the three respective circular distal ends of the three electrodes may be placed apart from each other by a distance between 1 mm and 5 mm. In an exemplary embodiment, the three respective circular distal ends of the three electrodes may be placed apart from each other by a distance of 5 mm.

[0024] In an exemplary embodiment, the substrate may include a first layer of silicon dioxide deposited on a layer of silicon. In an exemplary embodiment, the sensor may further include a second layer of silicon dioxide deposited on surface of sensor except surface of the circular distal end and the proximal end of each respective electrode of the three electrodes.

[0025] In an exemplary embodiment, the electrically conductive layer may include a layer of at least one of nickel, gold, and combinations thereof. In an exemplary embodiment, the electrically conductive layer may have a thickness in a range of 5 nm to 20 nm.

[0026] In an exemplary embodiment, the array of VAMWCNTs may include VAMWCNTs with a length in a range of 2.5 μm to 5 μm. In an exemplary embodiment, the array of VAMWCNTs may include VAMWCNTs with a diameter in a range of 50 nm to 70 nm.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] The drawing figures depict one or more embodiments in accord with the present teachings, by way of example only, not by way of limitation. In the figures, like reference numerals refer to the same or similar elements.

[0028] FIG. **1**A shows an exemplary system for real-time cancer detection, consistent with one or more exemplary embodiments of the present disclosure.

[0029] FIG. **1**B shows a schematic top view of an exemplary sensor for real-time cancer detection, consistent with one or more exemplary embodiments of the present disclosure.

[0030] FIG. **1**C shows a schematic view of an exemplary sensor for real-time cancer detection, consistent with one or more exemplary embodiments of the present disclosure.

[0031] FIG. **1**D shows another schematic view of an exemplary sensor for real-time cancer detection, consistent with one or more exemplary embodiments of the present disclosure.

[0032] FIG. **1**E shows a schematic view of an exemplary sensor with an exemplary holder and an exemplary cap, consistent with one or more exemplary embodiments of the present disclosure.

[0033] FIG. **2**A shows an exemplary flowchart of an exemplary method for real-time cancer detection, consistent with one or more exemplary embodiments of the present disclosure.

[0034] FIG. **2**B shows an exemplary flowchart of another exemplary method for real-time cancer detection, consistent with one or more exemplary embodiments of the present disclosure.

[0035] FIG. **2**C shows a flowchart of an exemplary method for generating an exemplary first threshold electrical current value and an exemplary second threshold electrical current value, consistent with one or more exemplary embodiments of the present disclosure.

[0036] FIG. **3**A shows an exemplary flowchart of an exemplary method for fabricating an exemplary sensor, consistent with one or more exemplary embodiments of the present disclosure.

[0037] FIG. **3**B shows a schematic view of steps of an exemplary method for fabricating an exemplary sensor, consistent with one or more exemplary embodiments of the present disclosure.

[0038] FIG. **4** shows comparative cyclic voltammograms of an exemplary cancer patient and an exemplary healthy candidate, consistent with one or more exemplary embodiments of the present disclosure.

[0039] FIG. **5** shows an exemplary computer system in which an embodiment of the present disclosure, or portions thereof, may be implemented as computer-readable code, consistent with one or more exemplary embodiments of the present disclosure.

[0040] FIG. **6** shows an image of a Field Emission Scanning Electron Microscope (FE-SEM) of exemplary grown CNTs, consistent with one or more exemplary embodiments of the present disclosure.

[0041] FIG. **7** shows a chart representing repeatability of an exemplary fabricated sensor obtained by testing three blood samples from normal and cancer patients with a 3-times of test repeat for each blood sample, consistent with one or more exemplary embodiments of the present disclosure.

[0042] FIG. **8** shows a chart of measured peak currents associated with ROS levels in blood samples of 160 investigated patients for two groups of G1: normal candidate/patients with non-cancer diseases and G2: patients with newborns cancer, consistent with one or more exemplary embodiments of the present disclosure.

[0043] FIG. **9** shows a receiver operating characteristic (ROC) diagram for results obtained by utilizing exemplary method and fabricated sensor versus clinical diagnostics results for blood samples from 160 patients, consistent with one or more exemplary embodiments of the present disclosure.

[0044] FIG. **10**A shows representative images of ROS/H_2O_2 related electrochemical current peak, optical microscope images of LDNs and HDNs, fluorescent microscope images of LDNs and HDNs, Giemsa staining images of LDNs and HDNs, and total cell count of LDNs and HDNs isolated by magnetic, negative selection protocol neutrophils isolated from 5 ml blood of normal cases (N=10), consistent with one or more exemplary embodiments of the present disclosure.

[0045] FIG. **10**B shows representative images of ROS/H_2O_2 related electrochemical current peak, optical microscope images of LDNs and HDNs, fluorescent microscope images of LDNs and HDNs, Giemsa staining images of LDNs and HDNs, and total cell count of LDNs and HDNs isolated by magnetic, negative selection protocol neutrophils isolated from 5 ml blood of cancer patients (N=10), consistent with one or more exemplary embodiments of the present disclosure.

[0046] FIG. **10**C shows a chart of LDN/HDN ratio versus measured current peak diagram with a fitted plot for 20 cases, including normal cases (N=10) and cancer patients (N=10), consistent with one or more exemplary embodiments of the present disclosure.

[0047] FIG. **11** shows a chart of ROS/H_2O_2 assisted electrochemical current peaks of blood samples of patients with non-cancer diseases (n=15), consistent with one or more exemplary embodiments of the present disclosure.

DETAILED DESCRIPTION

[0048] In the following detailed description, numerous specific details are set forth by way of examples in order to provide a thorough understanding of the relevant teachings. However, it should be apparent that the present teachings may be practiced without such details. In other instances, well known methods, procedures, components, and/or circuitry have been described at a relatively high-level, without detail, in order to avoid unnecessarily obscuring aspects of the present teachings. The following detailed description is presented to enable a person skilled in the art to make and use the methods and devices disclosed in exemplary embodiments of the present disclosure. For purposes of explanation, specific nomenclature is set forth to provide a thorough understanding of the present disclosure. However, it will be apparent to one skilled in the art that these specific details are not required to practice the disclosed exemplary embodiments. Descriptions of specific exemplary embodiments are provided only as representative examples. Various modifications to the exemplary embodiments will be readily apparent to one skilled in the art, and the general principles defined herein may be applied to other embodiments and applications without departing from the scope of the present disclosure. The present disclosure is not intended to be limited to the embodiments shown, but is to be accorded the widest possible scope consistent with the principles and features disclosed herein.

[0049] In cancer patients with solid tumors, neutrophils can paradoxically mediate a broad range of anti- and pro-tumor activities from cancer cell killing (done by HDNs) to tumor cell proliferation, angiogenesis, metastasis, and organizing other immune responses (done by LDNs). Neutrophil density changes due to their increased granularity and varia-

tions in cell size during development. Immature neutrophils are typically found in low density (LD) fractions, whereas mature neutrophils are detected in normal/high density (N/HD) fractions. LDNs are either immature with a banded/ring nucleus or mature with segmented nuclei. All LDNs are pro-tumor that present immunosuppressive properties. HDNs (or NDNs) are mature with segmented nuclei which are antitumor. It should be noted that TGF-β could also mediate transition of HDN to LDN (displaying plasticity) in the murine model.

[0050] An increased number of immature neutrophils in peripheral blood and tissues is a consequence of cancer development in human patients. LDNs may be detectable in peripheral blood of patients with lung cancer, breast cancer, and ovarian cancer. This increase may result from promotion of immature neutrophil production and release due to increased systemic chemokines, (e.g., granulocyte colony-stimulating (G-CSF)). It should be noted that tumors produce granulocyte colony-stimulating factor (G-CSF). G-CSF reduces chemokine receptor type 4 (CXCR4) expression in human myeloid lineage cells, resulting in decreasing their response to the bone marrow retention signal stromal cell-derived factor 1 (SDF-1).

[0051] Immature neutrophils may have both anti- and pro-tumor properties. These include altered localization resulting from reduced surface chemotactic receptor expression (e.g., CXCR2) and less segmented nuclear morphology compared to mature ones which reduce their immunological functions due to their reduced ROS production and altered cell surface receptor expression. Also, immature neutrophils are unable to kill tumor cell via FcγRI receptors. These differences in properties and functions of immature neutrophils could lead to their negative effect on cancer therapy. ROS production is crucial in several neutrophil effector mechanisms including their microbicidal, phagocytic, suppressive capacity and neutrophil anti, pro-tumor functions. Reduced ROS production may be the main deficiency of immature neutrophils against cancer cells.

[0052] Herein, an exemplary real-time electrochemical diagnostic sensor, system, and method for detecting cancer by detecting a level of a ratio of LDNs/HDNs in a blood sample via detecting reactive oxygen species (ROS) levels in a small amount (i.e., about 1 cc) of peripheral blood is disclosed. An exemplary sensor may include three disks like electrodes (working electrode (WE), counter electrode (CE), and reference electrode (RE)) covered by Multi-Wall Carbon Nanotubes (MWCNTs) as a ROS detecting agent in a solution. A cancerous status of a suspected person may be determined utilizing exemplary sensor, system, and/or method in a time period of less than about 30 seconds. An exemplary method and system may be utilized for a rapid detection of cancer via a simple procedure using a small amount (i.e., about 1 cc) of an unprocessed blood sample drawn from a person suspected to have cancer.

[0053] FIG. **1**A shows an exemplary system **100** for real-time cancer detection, consistent with one or more exemplary embodiments of the present disclosure. In an exemplary embodiment, system **100** may include an exemplary sensor **102**, an exemplary electrical stimulator-analyzer device **104**, and an exemplary processing unit **106**. In an exemplary embodiment, sensor **102** may be configured to receive a biological sample thereon. In an exemplary embodiment, the biological sample may be acquired from a person suspected to have cancer. In an exemplary embodiment, the biological sample may include a blood sample drawn from a person. In an exemplary embodiment, the biological sample may include an unprocessed blood sample drawn from a person and directly placed on surface of sensor **102**. In an exemplary embodiment, one or more drops of an unprocessed blood sample may be dropped on surface of sensor **102**. In an exemplary embodiment, sensor **102** may be electrically connected to electrical stimulator-analyzer device **104** and electrical stimulator-analyzer device **104** may be electrically connected to processing unit **106**.

[0054] In an exemplary embodiment, sensor **102** may include an exemplary electrochemical sensor. In an exemplary embodiment, sensor **102** may include substrate **103** and three electrodes **107**, **108**, and **109** formed on substrate **103**. In an exemplary embodiment, three electrodes **107**, **108**, and **109** may include a set of electrochemical electrodes configured to be utilized for electrochemical measurements. In an exemplary embodiment, three electrodes **107**, **108**, and **109** may include a working electrode **107**, a counter electrode **108**, and a reference electrode **109**. FIG. **1**B shows a schematic top view of an exemplary sensor **102** for real-time cancer detection, consistent with one or more exemplary embodiments of the present disclosure. In an exemplary embodiment, each respective electrode of three electrodes **107**, **108**, and **109** may include a circular sensing part, a connecting pad, and a middle part between the circular sensing part and the connecting pad. In detail, referring to FIG. **1**B, an exemplary working electrode **107** may include an exemplary circular sensing part **107***a*, an exemplary connecting pad **107***b*, and an exemplary middle part **107***c*. Furthermore, an exemplary counter electrode **108** may include an exemplary circular sensing part **108***a*, an exemplary connecting pad **108***b*, and an exemplary middle part **108***c*. Moreover, an exemplary reference electrode **109** may include an exemplary circular sensing part **109***a*, an exemplary connecting pad **109***b*, and an exemplary middle part **109***c*. In an exemplary embodiment, three electrodes **107**, **108**, and **109** may be made of an electrically conductive material. In an exemplary embodiment, three electrodes **107**, **108**, and **109** may be made of a metal. In an exemplary embodiment, three electrodes **107**, **108**, and **109** may be made of at least one of nickel, gold, and combinations thereof. In an exemplary embodiment, circular sensing parts **107***a*, **108***a*, and **109***a* may include three respective circular distal ends of three respective electrodes **107**, **108**, and **109**. In an exemplary embodiment, circular sensing parts **107***a*, **108***a*, and **109***a* may be configured to be put in contact with an exemplary unprocessed blood sample dropped on surface of exemplary sensor **102**. In an exemplary embodiment, connecting pads **107***b*, **108***b*, and **109***b* may include three respective proximal ends of three respective electrodes **107**, **108**, and **109**. In an exemplary embodiment, connecting pads **107***b*, **108***b*, and **109***b* may be configured to be electrically connected to electrical stimulator-analyzer device **104** via respective electrically conductive lines **116**, **118**, and **120** as shown in FIG. **1**A.

[0055] In an exemplary embodiment, each respective electrode of three electrodes **107**, **108**, and **109** may include an electrically conductive layer deposited on substrate **103**. In an exemplary embodiment, an exemplary electrically conductive layer may have a thickness in a range of about 5 nm to about 20 nm. In an exemplary embodiment, an exemplary electrically conductive layer may include a catalyst material for a process of growing carbon nanotubes (CNTs), for

example, vertically aligned multi-walled CNTs (VAMWCNTs), thereon. In an exemplary embodiment, an exemplary electrically conductive layer may include a layer of at least one of nickel, gold, and combinations thereof. In an exemplary embodiment, an exemplary electrically conductive layer may include a circular distal end and a proximal end. In an exemplary embodiment, an exemplary circular distal end may form an exemplary circular sensing part similar to circular sensing parts **107***a*, **108***a*, and **109***a* and an exemplary proximal end may form an exemplary connecting pad similar to connecting pads **107***b*, **108***b*, and **109***b*. In an exemplary embodiment, each respective circular sensing part of circular sensing parts **107***a*, **108***a*, and **109***a* may have a diameter in a range of about 0.5 mm to about 3 mm. In an exemplary embodiment, each respective circular sensing part of circular sensing parts **107***a*, **108***a*, and **109***a* may have a diameter of about 2 mm. In an exemplary embodiment, circular sensing parts **107***a*, **108***a*, and **109***a* of three respective electrodes **107**, **108**, and **109** may be placed on substrate **103** apart from each other by a distance between about 1 mm and about 5 mm. In an exemplary embodiment, circular sensing parts **107***a*, **108***a*, and **109***a* of three respective electrodes **107**, **108**, and **109** may be placed on substrate **103** apart from each other by a triangular distance **110** (illustrated in FIG. **1**B) between of about 5 mm.

[0056] Referring back to FIG. **1**A, substrate **103** may include a semi-conductive layer **103***a* and a first electrically passivating layer **103***b* deposited on semi-conductive layer **103***a*. In an exemplary embodiment, semi-conductive layer **103***a* may include a layer of silicon. In an exemplary embodiment, first electrically passivating layer **103***b* may include a first layer of silicon dioxide (SiO_2). In an exemplary embodiment, three electrodes **107**, **108**, and **109** may include a layer of an electrically conductive material deposited and patterned on first electrically passivating layer **103***b*.

[0057] In an exemplary embodiment, sensor **102** may further include a second electrically passivating layer deposited on surface of sensor **102** except surface of circular sensing parts **107***a*, **108***a*, and **109***a* and surface of connecting pads **107***b*, **108***b*, and **109***b* of three respective electrodes **107**, **108**, and **109**. FIG. **1**C shows a schematic view of an exemplary sensor **102** for real-time cancer detection, consistent with one or more exemplary embodiments of the present disclosure. In an exemplary embodiment with reference to FIG. **1**C, exemplary sensor **102** may further include a second electrically passivating layer **105** deposited on substrate **103**. In an exemplary embodiment, second electrically passivating layer **103***b* may include a second layer of SiO_2 deposited on first electrically passivating layer **103***b* and three middle parts **107***c*, **108***c*, and **109***c* of three respective electrodes **107**, **108**, and **109**.

[0058] In an exemplary embodiment, each respective circular sensing part of circular sensing parts **107***a*, **108***a*, and **109***a* may be coated with a layer of carbon nanotubes (CNTs) deposited on the respective circular sensing part. FIG. **1**D shows another schematic view of exemplary sensor **102** for real-time cancer detection, consistent with one or more exemplary embodiments of the present disclosure. In an exemplary embodiment, three arrays of CNTs **130***a*, **130***b*, and **130***c* may be coated on respective circular sensing parts **107***a*, **108***a*, and **109***a*. In an exemplary embodiment, arrays of CNTs **130***a*, **130***b*, and **130***c* may include three respective arrays of vertically aligned multi-walled carbon nanotubes (VAMWCNTs) grown on respective circular sensing parts **107***a*, **108***a*, and **109***a*. In an exemplary embodiment, circular sensing parts **107***a*, **108***a*, and **109***a* may be configured to be put in contact with an exemplary unprocessed blood sample dropped on surface of exemplary sensor **102**. In an exemplary embodiment, three arrays of CNTs **130***a*, **130***b*, and **130***c* may be configured to be put in contact with an exemplary unprocessed blood sample dropped on surface of exemplary sensor **102**. In an exemplary embodiment, each respective array of CNTs of three arrays of CNTs **130***a*, **130***b*, and **130***c* may include a respective plurality of VAMWCNTs, where each respective VAMWCNT may have a length in a range of about 2.5 μm to about 5 μm. In an exemplary embodiment, each respective VAMWCNT of an exemplary array of VAMWCNTs may have a diameter in a range of about 50 nm to about 70 nm.

[0059] In an exemplary embodiment, system **100** may further include a holder and a cap. FIG. **1**E shows a schematic view of exemplary sensor **102** with a holder **140** and a cap **142**, consistent with one or more exemplary embodiments of the present disclosure. In an exemplary embodiment, exemplary holder **140** may include a hollow cylinder with a diameter equal or more than an outer diameter of sensor **102**. In an exemplary embodiment, exemplary holder **140** may be configured to fix sensor **102** with an exemplary unprocessed blood sample **150** therein, allowing for protecting sensor **102** and unprocessed blood sample **150** while being in contact with each other. In an exemplary embodiment, exemplary holder **140** may include three electrically conductive elements (pins) **144**, **146**, and **148**, where each respective pin may be in contact with a respective connecting pad of connecting pads **107***b*, **108***b*, and **109***b* of sensor **102**, allowing for an electrical connection between three electrodes **107**, **108**, and **109** of sensor **102** and three pins **144**, **146**, and **148**. In an exemplary embodiment, three pins **144**, **146**, and **148** may be configured to connect respective electrodes **107**, **108**, and **109** of sensor **102** to an exemplary electrical stimulator-analyzer device **104**. In an exemplary embodiment, sensor **102** may be placed inside holder **140** so that each respective pin of three pins **144**, **146**, and **148** being in contact with a respective electrode of three electrodes **107**, **108**, and **109** of sensor **102**. In an exemplary embodiment, unprocessed blood sample **150** may be dropped inside holder **140** on surface of sensor **102**.

[0060] In an exemplary embodiment, an exemplary cap **142** may include a circular element with a diameter equal or more than an outer diameter of holder **140**. In an exemplary embodiment, cap **142** may be placed on a top side of holder **140** above sensor **102** and unprocessed blood sample **150**. In an exemplary embodiment, cap **142** may be configured to protect sensor **102** and unprocessed blood sample **150** being in contact with each other away from pollutants and disturbing factors.

[0061] In an exemplary embodiment, electrical stimulator-analyzer device **104** may be electrically connected to sensor **102** at respective proximal ends of each respective electrode of three electrodes **107**, **108**, and **109** of sensor **102**. In an exemplary embodiment, electrical stimulator-analyzer device **104** may be electrically connected to sensor **102** at connecting pads **107***b*, **108***b*, and **109***b* of three electrodes **107**, **108**, and **109** utilizing three respective electrically conductive lines **116**, **118**, and **120**. In an exemplary embodiment, electrical stimulator-analyzer device **104** may include

a potentiostat device. In an exemplary embodiment, electrical stimulator-analyzer device **104** may be configured to apply a set of voltages in a sweeping range from about −3 V to about +3 V, for example, a range of −0.8 V to +0.8 V, to sensor **102** with unprocessed blood sample **150** placed thereon and measure a produced set of electrical currents of sensor **102** responsive to the applied set of voltages. In an exemplary embodiment, electrical stimulator-analyzer device **104** may be electrically connected to processing unit **106** utilizing at least one of an electrically conductive line **122**, a wireless connection (not illustrated), and combinations thereof. In an exemplary embodiment, the wireless connection may include Bluetooth devices or Bluetooth modules, which may be embedded in electrical stimulator-analyzer device **104** and processing unit **106**. In an exemplary embodiment, electrical stimulator-analyzer device **104** may be further configured to send the measured set of electrical currents to processing unit **106**.

[0062] In an exemplary embodiment, processing unit **106** may include a memory having processor-readable instructions stored therein and a processor. In an exemplary embodiment, the processor may be configured to access the memory and execute the processor-readable instructions. In an exemplary embodiment, executing the processor-readable instructions by the processor may configures the processor to perform a method. In an exemplary embodiment, the method may include an exemplary method for real-time detecting cancer described herein below.

[0063] FIG. **2**A shows a flowchart of an exemplary method **200** for real-time cancer detection, consistent with one or more exemplary embodiments of the present disclosure. In an exemplary embodiment, method **200** may include putting an unprocessed blood sample in contact with sensing parts of a set of electrodes of a sensor (step **204**), applying a set of voltages to the sensor (step **206**), measuring a produced set of electrical currents respective to the applied set of voltages (step **208**), measuring ratio of LDNs to HDNs in the unprocessed blood sample by measuring a maximum level of the set of electrical currents (step **210**), and detecting a cancer status based on the measured ratio of LDNs to HDNs in the unprocessed blood sample (step **212**).

[0064] FIG. **2**B shows a flowchart of another exemplary method **220** for real-time detecting cancer, consistent with one or more exemplary embodiments of the present disclosure. In an exemplary embodiment, exemplary method **220** may contain steps similar to exemplary method **200** while further including step **202** of fabricating an exemplary sensor. Steps of exemplary method **200** and **220** are explained below in further detail in combination with the elements of FIGS. **1**A-**1**E.

[0065] With further detail in regards to step **202**, FIG. **3**A shows a flowchart of an exemplary method **300** for fabricating an exemplary sensor (step **202**), consistent with one or more exemplary embodiments of the present disclosure. In an exemplary embodiment, exemplary method **300** of fabricating an exemplary sensor (step **202**) may include cleaning a semi-conductive substrate (step **301**), forming a first electrically passivating layer on surface of the semi-conductive substrate (step **302**), depositing a catalyst layer on the first electrically passivating layer (step **303**), forming three electrodes by patterning the catalyst layer (step **304**), electrically passivating a surface of an exemplary sensor except three respective circular distal ends of the three electrodes by coating a second electrically passivating layer on surface of an exemplary sensor except the three respective circular distal ends of the three electrodes (step **305**), forming three arrays of CNTs on three respective circular distal ends of the three electrodes (step **306**), and removing parts of the second electrically passivating layer coated on three respective proximal ends of the three electrodes (step **307**). FIG. **3**B shows a schematic view of steps of exemplary method **300** for fabricating an exemplary sensor, consistent with one or more exemplary embodiments of the present disclosure. In an exemplary embodiment, an exemplary sensor may be similar to sensor **102**, and exemplary method **300** may be described herein below in connection with FIG. **3**B and FIGS. **1**A-**1**E illustrating an exemplary structure of exemplary sensor **102**.

[0066] With further detail in regards to step **301**, an exemplary semi-conductive substrate may be cleaned. In an exemplary embodiment, an exemplary semi-conductive substrate may include semi-conductive layer **310** shown in part **310** of FIG. **3**B (similar to semi-conductive layer **103***a* of FIGS. **1**A, **1**C, and **1**D). In an exemplary embodiment, exemplary semi-conductive layer **320** may include an exemplary circular silicon (Si) wafer. In an exemplary embodiment, semi-conductive layer **320** may include a P-type Si wafer. In an exemplary embodiment, cleaning semi-conductive layer **320** (step **301**) may include cleaning semi-conductive layer **320** allowing for removing contaminations from an exemplary silicon wafer using a standard RCA method.

[0067] In further detail with respect to step **302**, step **302** may include forming a first electrically passivating layer on surface of an exemplary cleaned semi-conductive substrate. In an exemplary embodiment, an exemplary first electrically passivating layer **322** (similar to first electrically passivating layer **103***b* of FIGS. **1**A, **1**C, and **1**D) may be coated on surface of semi-conductive layer **320** in step **302** as shown in part **312** of FIG. **3**B; thereby, an exemplary substrate similar to substrate **103** may be formed. In an exemplary embodiment, step **302** may include growing a first silicon dioxide (SiO_2) layer as an example of first electrically passivating layer **322** on a cleaned silicon wafer as an example of semi-conductive layer **320** cleaned in step **301**.

[0068] In further detail with respect to step **303**, step **303** may include depositing a catalyst layer on an exemplary first electrically passivating layer as schematically shown in part **313** of FIG. **3**B. In an exemplary embodiment, step **303** may include depositing a catalyst layer **324** on exemplary first electrically passivating layer **322**. In an exemplary embodiment depositing catalyst layer **324** on an exemplary first electrically passivating layer **322** (step **303**) may include depositing a layer of a catalyst to be used for conducting a process of growing CNTs on exemplary first electrically passivating layer **322**. In an exemplary embodiment, catalyst layer **324** may include a layer of an electrically conductive catalyst for a process of growing CNTs thereon. In an exemplary embodiment, depositing exemplary catalyst layer **324** on exemplary first electrically passivating layer **322** (step **303**) may include depositing a layer of nickel (Ni) on an exemplary first silicon dioxide (SiO_2) layer as an example of exemplary first electrically passivating layer **322** formed on an exemplary cleaned silicon wafer as an example of semi-conductive layer **320** obtained via steps **301** and **302**. In an exemplary embodiment, exemplary catalyst layer **324** may have a thickness of less than about 20 nm. In an

exemplary embodiment, exemplary catalyst layer **324** may have a thickness of about 10 nm or less.

[0069] In further detail with respect to step **304**, step **304** may include forming three exemplary electrodes **326**, **328**, and **330** (similar to exemplary three exemplary electrodes **107**, **108**, and **109** of FIGS. **1**A-**1**B) by patterning exemplary catalyst layer **324** as schematically shown in part **314** of FIG. **3**B. In an exemplary embodiment, three exemplary electrodes **326**, **328**, and **330** may form a set of electrodes including an exemplary working electrode **326**, an exemplary counter electrode **328**, and an exemplary reference electrode **330**. In an exemplary embodiment, patterning exemplary catalyst layer **324** may be done via a photolithography technique. In an exemplary embodiment, patterning exemplary catalyst layer **324** may include patterning exemplary catalyst layer **324** in form of three exemplary electrodes **326**, **328**, and **330**. In an exemplary embodiment, patterning exemplary catalyst layer **324** may include patterning exemplary catalyst layer **324** in shape of three similar structures with circular heads (for example, circular head **328***a* of electrode **328**) with a diameter of about 2 mm in conformation of three electrodes **326**, **328**, and **330**, as working, counter, and reference electrodes with a triangular distance of about 5 mm from each other using a standard photolithography process. In an exemplary embodiment, patterning exemplary catalyst layer **324** may further include spin-coating a thin layer of a positive photoresist (e.g., Microchem-S1813) on surface of exemplary catalyst layer **324**, chemically developing semi-conductive layer **320** with first electrically passivating layer **322** and exemplary catalyst layer **324** thereon by illuminating with a Mask-Aligner system, etching parts of exemplary catalyst layer **324** in undesired regions using an etchant solution (e.g., a Ni-etchant solution: HNO_3:H_3PO_4:CH_3COOH, 3:3:1), and washing the photoresist layer using a solvent, for example, acetone. In an exemplary embodiment, exemplary undesired regions may include parts of exemplary catalyst layer **324** around patterned three exemplary electrodes **326**, **328**, and **330**.

[0070] In an exemplary embodiment, each exemplary electrode **328** of three exemplary electrodes **326**, **328**, and **330** may include a respective circular head **328***a* (similar to each of circular sensing parts **107***a*, **108***a*, and **109***a* illustrated in FIGS. **1**B-**1**D). Moreover, each exemplary electrode **328** of three exemplary electrodes **326**, **328**, and **330** may include a respective tail **328***b* similar to each of connecting pads **107***b*, **108***b*, and **109***b* illustrated in FIGS. **1**B-**1**D. In an exemplary embodiment, exemplary circular head **328***a* may include a respective distal end of exemplary electrode **328** of set of electrodes **326**, **328**, and **330**. In an exemplary embodiment, exemplary tail **328** may include a respective proximal end of exemplary electrode **328** of set of electrodes **326**, **328**, and **330**. In an exemplary embodiment, each respective electrode of set of electrodes **326**, **328**, and **330**, for example, exemplary electrode **328** may further include a respective middle part **328***c* between respective circular head **328***a* and respective tail **328***b*. In an exemplary embodiment, respective circular heads (e.g., circular head **328***a*) of electrodes **326**, **328**, and **330** may be configured to be put in contact with a sample (e.g., exemplary unprocessed blood sample **150**) by placing an exemplary sample thereon. In an exemplary embodiment, respective tails (e.g., tail **328***b*) of electrodes **326**, **328**, and **330** may be configured to be connected to an electrical device, for example, electrical stimulator-analyzer device **104**. In an exemplary embodiment, respective middle parts (e.g., middle part **328***c*) of electrodes **326**, **328**, and **330** may be configured to form an electrically conductive path between respective circular heads (e.g., circular head **328***a*) of electrodes **326**, **328**, and **330**, and similarly, circular sensing parts **107***a*, **108***a*, and **109***a* and respective tails (e.g., tail **328***b*) of electrodes **326**, **328**, and **330**, and similarly, connecting pads **107***b*, **108***b*, and **109***b* of electrodes **107**, **108**, and **109**.

[0071] In further detail with respect to step **305**, step **305** may include electrically passivating a surface of an exemplary sensor except three respective circular distal ends of exemplary three electrodes by coating a second electrically passivating layer on surface of an exemplary sensor except three respective circular distal ends of three exemplary electrodes. In an exemplary embodiment, step **305** may include electrically passivating a surface of exemplary sensor **102** except three respective circular heads **326***a*, **328***a*, and **330***a*) of electrodes **326**, **328**, and **330** as schematically shown in part **315** of FIG. **3**B. In an exemplary embodiment, step **305** may include coating entire surface of exemplary sensor **102** with a second electrically passivating layer **332** (similar to second electrically passivating layer **105**) and removing parts of exemplary second electrically passivating layer **332** coated on circular heads **326***a*, **328***a*, and **330***a* of respective electrodes **326**, **328**, and **330**. In an exemplary embodiment, entire surface of exemplary sensor **102** may include surface of first electrically passivating layer **322** and surface of patterned electrodes **326**, **328**, and **330**. In an exemplary embodiment, second electrically passivating layer **332** may include a second SiO_2 layer. In an exemplary embodiment, coating entire surface of exemplary sensor **102** with second electrically passivating layer **332** may include passivating exemplary sensor **102** with a 200 nm SiO_2 layer at 0.9 torr, and 20 W (deposition speed <45 nm min^{-1}). In an exemplary embodiment, removing parts of an exemplary second SiO_2 layer coated on respective circular heads **326***a*, **328***a*, and **330***a* of electrodes **326**, **328**, and **330** may be carried out by immersing exemplary sensor **102** in a buffered oxide etchant (BOE) solution to prepare circular windows for three electrodes **326**, **328**, and **330**. In an exemplary embodiment, immersing exemplary sensor **102** in the BOE solution may be done in about 7 seconds.

[0072] In further detail with respect to step **306**, step **306** may include forming three arrays of CNTs on three respective circular distal ends of three exemplary electrodes of an exemplary sensor as schematically shown in part **316** of FIG. **3**B. In an exemplary embodiment, step **306** may include forming three exemplary arrays of CNTs **334***a*, **334***b*, and **334***c* (similar to three exemplary arrays of CNTs **130***a*, **130***b*, and **130***c* of FIG. **1**D) on three respective circular heads **326***a*, **328***a*, and **330***a* of electrodes **326**, **328**, and **330**. In an exemplary embodiment, forming three arrays of CNTs **334***a*, **334***b*, and **334***c* on respective circular heads **326***a*, **328***a*, and **330***a* of electrodes **326**, **328**, and **330** may include growing a respective plurality of vertically aligned multi-walled carbon nanotubes (VAMWCNTs) on each respective circular head of circular heads **326***a*, **328***a*, and **330***a* of electrodes **326**, **328**, and **330**. In an exemplary embodiment, growing VAMWCNTs may be carried out in a direct current plasma enhance chemical vapor deposition (DC-PECVD) system in C_2H_2 and H_2 ambient at a temperature of about 680° C.

[0073] In further detail with respect to step **307**, step **307** may include removing parts of an exemplary second electrically passivating layer coated on three respective proximal ends of exemplary three electrodes of an exemplary sensor as schematically shown in part **317** of FIG. **3**B. In an exemplary embodiment, step **307** may include removing parts of second electrically passivating layer **332** from surface of three respective proximal ends (tails **326***b*, **328***b*, and **330***b*) of three electrodes **326**, **328**, and **330**. In an exemplary embodiment, step **307** may include removing parts of an exemplary coated second SiO_2 layer from surface of tails **326***b*, **328***b*, and **330***b* (similar to connecting pads **107***b*, **108***b*, and **109***b* of electrodes **107**, **108**, and **109**) using a BOE solution.

[0074] Referring back to FIGS. **2**A and **2**B, step **204** may include putting an unprocessed blood sample in contact with sensing parts of electrodes of an exemplary sensor. In an exemplary embodiment, step **204** may include placing or dropping exemplary unprocessed blood sample **150** on surface of exemplary sensor **102**. In an exemplary embodiment, step **204** may include putting exemplary unprocessed blood sample **150** in contact with circular sensing parts **107***a*, **108***a*, and **109***a* of three electrodes **107**, **108**, and **109** of sensor **102**. In an exemplary embodiment, step **204** may include dropping exemplary unprocessed blood sample **150** on respective circular sensing parts **107***a*, **108***a*, and **109***a* of electrodes **107**, **108**, and **109**. In an exemplary embodiment, exemplary unprocessed blood sample **150** may be drawn from a person suspected to have cancer. In an exemplary embodiment, exemplary unprocessed blood sample **150** may be drawn from a person who is not pregnant and/or does not infected with an inflammatory disease. In an exemplary embodiment, exemplary unprocessed blood sample **150** may be drawn from a person suspected to be involved with a cancer having a solid tumor, for example, breast cancer, colon cancer, skin cancer, etc.

[0075] In further detail with respect to step **206**, step **206** may include applying a set of voltages to sensor **102**. In an exemplary embodiment, applying the set of voltages to sensor **102** may include applying the set of voltages between exemplary reference electrode **109** and exemplary working electrode **107** of electrodes **107**, **108**, and **109** of sensor **102**. In an exemplary embodiment, applying the set of voltages to sensor **102** may be done utilizing electrical stimulator-analyzer device **104**. In an exemplary embodiment, applying the set of voltages to sensor **102** may include applying the set of voltages in a sweeping range from about −3 V to about +3 V between exemplary reference electrode **109** and exemplary working electrode **107** of electrodes **107**, **108**, and **109** of sensor **102**. In an exemplary embodiment, applying the set of voltages to sensor **102** may include applying the set of voltages in the sweeping range from about −0.8 V to about +0.8 V between exemplary reference electrode **109** and exemplary working electrode **107** of electrodes **107**, **108**, and **109** of sensor **102**.

[0076] In further detail with respect to step **208**, step **208** may include measuring a produced set of electrical currents respective to the applied set of voltages to sensor **102**. In an exemplary embodiment, a set of electrical currents may be generated between an exemplary counter electrode **108** and an exemplary working electrode **107** of the three electrodes **107**, **108**, and **109** of sensor **102** responsive to the applied set of voltages. In an exemplary embodiment, step **208** may include measuring the produced set of electrical currents between counter electrode **108** and working electrode **107** of three electrodes **107**, **108**, and **109** of sensor **102** respective to the applied set of voltages. In an exemplary embodiment, measuring the produced set of electrical currents may be carried out utilizing electrical stimulator-analyzer device **104**. In an exemplary embodiment, measuring the produced set of electrical currents may further include sending the measured produced set of electrical currents to processing unit **106** by electrical stimulator-analyzer device **104**.

[0077] In further detail with respect to step **210**, step **210** may include measuring ratio of LDNs to HDNs in exemplary unprocessed blood sample **150** by measuring a maximum level of the set of electrical currents. In an exemplary embodiment, measuring the maximum level of the set of electrical currents may be carried out by one or more processors similar, for example, processing unit **106**. In an exemplary embodiment, measuring the ratio of LDNs to HDNs in exemplary unprocessed blood sample **150** may include measuring a level of reactive oxygen species (ROS) in the unprocessed blood sample **150** by measuring a maximum electrical current of the measured set of electrical currents. In an exemplary embodiment, measuring the ratio of LDNs to HDNs in exemplary unprocessed blood sample **150** may include receiving the measured produced set of electrical currents, plotting a cyclic voltammetry (CV) diagram by plotting the measured produced set of electrical currents versus the applied set of voltages, and measuring a peak value of electrical currents of the CV diagram.

[0078] In an exemplary embodiment, measuring the ratio of LDNs to HDNs in exemplary unprocessed blood sample **150** may further include determining a level of ratio of LDNs to HDNs in the unprocessed blood sample using the measured maximum electrical current of the measured set of electrical currents. In an exemplary embodiment, determining the level of ratio of LDNs to HDNs in the unprocessed blood sample may include detecting the level of ratio of LDNs to HDNs in the unprocessed blood sample is more than 1 if the measured maximum electrical current of the measured set of electrical currents is less than a first threshold electrical current value and detecting the level of ratio of LDNs to HDNs in the unprocessed blood sample is less than 1 if the measured maximum electrical current of the measured set of electrical currents is more than a second threshold electrical current value.

[0079] In an exemplary embodiment, exemplary method **200** may further include generating the first threshold electrical current value and the second threshold electrical current value. FIG. **2**C shows a flowchart of a method **230** for generating the first threshold electrical current value and the second threshold electrical current value, consistent with one or more exemplary embodiments of the present disclosure. In an exemplary embodiment, exemplary method **230** of generating the first threshold electrical current value and the second threshold electrical current value may include generating a first dataset of a plurality of unprocessed blood samples associated with a plurality of cancer patients (step **232**), generating a second dataset of a plurality of unprocessed blood samples associated with a plurality of healthy persons (step **234**), and determining first threshold electrical current value and the second threshold electrical current value based on the first dataset and the second dataset (step **236**).

[0080] In further detail with respect to step **232**, step **232** of generating a first dataset of a plurality of unprocessed

blood samples associated with a plurality of cancer patients may include measuring a first set of electrical current peaks of the plurality of unprocessed blood samples associated with the plurality of cancer patients, measuring a first set of ratio of LDNs to HDNs in the plurality of unprocessed blood samples associated with the plurality of cancer patients utilizing a cell counter, and assigning each measured ratio of LDNs to HDNs of the first set of ratio of LDNs to HDNs to the respective measured electrical current peak of the first set of electrical current peaks.

[0081] In further detail with respect to step **234**, step **234** of generating a second dataset of a plurality of unprocessed blood samples associated with a plurality of healthy persons may include measuring a second set of electrical current peaks of the plurality of unprocessed blood samples associated with the plurality of healthy persons, measuring a second set of ratio of LDNs to HDNs in the unprocessed blood samples associated with the plurality of healthy persons utilizing a cell counter, and assigning each measured ratio of LDNs to HDNs of the second set of ratio of LDNs to HDNs to the respective measured electrical current peak of the second set of electrical current peaks.

[0082] In further detail with respect to step **236**, step **236** of determining the first threshold electrical current value and the second threshold electrical current value based on the first dataset and the second dataset may include determining the first threshold electrical current value equal to a maximum electrical current peak among the first set of electrical current peaks and determining the second threshold electrical current value equal to a minimum electrical current peak among the second set of electrical current peaks. In an exemplary embodiment, the first threshold electrical current value and the second threshold electrical current value may be equal to each other.

[0083] In an exemplary embodiment, the first threshold electrical current value and the second threshold electrical current value may depend on age of an exemplary suspected person. In an exemplary embodiment, the first threshold electrical current value may be equal to an electrical current peak of about 300 μA for an adult person. In an exemplary embodiment, the first threshold electrical current value may be equal to an electrical current peak of about 100 μA for a child. In an exemplary embodiment, the second threshold electrical current value may be equal to an electrical current peak of about 450 μA for an adult person. In an exemplary embodiment, the second threshold electrical current value may be equal to an electrical current peak of about 300 μA for a child.

[0084] Referring back to FIGS. **2**A and **2**B, step **212** may include detecting a cancer status of an exemplary suspected person based on the measured ratio of LDNs to HDNs in exemplary unprocessed blood sample **150**. FIG. **4** shows comparative cyclic voltammograms of an exemplary cancer patient (diagram **402**) and an exemplary healthy candidate (diagram **404**), consistent with one or more exemplary embodiments of the present disclosure. In an exemplary embodiment, detecting cancer status of an exemplary suspected person may include detecting a cancer disease in suspected person's body associated with exemplary unprocessed blood sample **150** if the measured ratio of LDNs to HDNs in exemplary unprocessed blood sample **150** is more than a threshold LDNs/HDNs value by detecting the measured maximum electrical current of the measured set of electrical currents being less than the first threshold electrical current value. In an exemplary embodiment, the threshold LDNs/HDNs value may equal to about 1 so that if a level of LDNs is more than HDNs in exemplary unprocessed blood sample **150**, a cancer disease may be detected for an exemplary suspected person.

[0085] In an exemplary embodiment, the first threshold electrical current value corresponding to a threshold LDNs/HDNs value of 1 may equal to 300 μA for adults and 100 μA for children. In an exemplary embodiment, "adults" as used herein may refer to people who are 18 years old or older. In an exemplary embodiment, "children or pediatrics cohort" as used herein may refer to people who are younger than 18 years, for example, children from new born to 13 years old. In an exemplary embodiment, detecting the cancer disease in the suspected person's body may include detecting the cancer disease in an adult suspected person's body if the measured maximum electrical current of the measured set of electrical currents is less than about 300 μA corresponding to a ratio of LDNs to HDNs in exemplary unprocessed blood sample **150** being more than 1. In another exemplary embodiment, detecting the cancer disease in the suspected person's body may include detecting the cancer disease in a suspected child's body if the measured maximum electrical current of the measured set of electrical currents is less than about 100 μA corresponding to a ratio of LDNs to HDNs in exemplary unprocessed blood sample **150** being more than 1.

[0086] In another exemplary embodiment, detecting cancer status of an exemplary suspected person may include detecting no cancer disease (a normal or healthy status) in the suspected person's body if the measured ratio of LDNs to HDNs in exemplary unprocessed blood sample **150** is less than the threshold LDNs/HDNs value. In an exemplary embodiment, no cancer may be detected for an exemplary suspected person if a level of LDNs is less than HDNs in exemplary unprocessed blood sample **150**. In an exemplary embodiment, no cancer may be detected for an exemplary suspected person if a level of ratio of LDNs to HDNs in exemplary unprocessed blood sample **150** is less than about 1 if the measured maximum electrical current of the measured set of electrical currents is more than the second threshold electrical current value. In an exemplary embodiment, detecting no cancer disease (a normal or healthy status) in the suspected person's body may include detecting no cancer disease in the suspected person's body if the suspected person is an adult and if the measured maximum electrical current of the measured set of electrical currents is more than 450 μA corresponding to a ratio of LDNs to HDNs in exemplary unprocessed blood sample **150** being less than 1. In another exemplary embodiment, detecting no cancer disease (a normal or healthy status) in the suspected person's body may include detecting no cancer disease in the suspected person's body if the suspected person is a child and if the measured maximum electrical current of the measured set of electrical currents is more than 300 μA corresponding to a ratio of LDNs to HDNs in exemplary unprocessed blood sample **150** being less than 1.

[0087] FIG. **5** shows an example computer system **500** in which an embodiment of the present disclosure, or portions thereof, may be implemented as computer-readable code, consistent with one or more exemplary embodiments of the present disclosure. For example, computer system **500** may include an example of processing unit **106** illustrated in FIG. **1**, and steps **206-212** of exemplary methods **200** and **220**

presented in FIGS. **2**A and **2**B in addition to steps **232**-**236** of exemplary method **230** illustrated in FIG. **2**C, may be implemented in computer system **500** using hardware, software, firmware, tangible computer readable media having instructions stored thereon, or a combination thereof and may be implemented in one or more computer systems or other processing systems. Hardware, software, or any combination of such may embody any of the modules and components in FIGS. **1**, **2**A-**2**C.

[0088] If programmable logic is used, such logic may execute on a commercially available processing platform or a special purpose device. One ordinary skill in the art may appreciate that an embodiment of the disclosed subject matter can be practiced with various computer system configurations, including multi-core multiprocessor systems, minicomputers, mainframe computers, computers linked or clustered with distributed functions, as well as pervasive or miniature computers that may be embedded into virtually any device.

[0089] For instance, a computing device having at least one processor device and a memory may be used to implement the above-described embodiments. A processor device may be a single processor, a plurality of processors, or combinations thereof. Processor devices may have one or more processor "cores."

[0090] An embodiment of the present disclosure is described in terms of this example computer system **500**. After reading this description, it will become apparent to a person skilled in the relevant art how to implement the invention using other computer systems and/or computer architectures. Although operations may be described as a sequential process, some of the operations may in fact be performed in parallel, concurrently, and/or in a distributed environment, and with program code stored locally or remotely for access by single or multiprocessor machines. In addition, in some embodiments the order of operations may be rearranged without departing from the spirit of the disclosed subject matter.

[0091] Processor device **504** may be a special purpose or a general-purpose processor device. As will be appreciated by persons skilled in the relevant art, processor device **504** may also be a single processor in a multi-core/multiprocessor system, such system operating alone, or in a cluster of computing devices operating in a cluster or server farm. Processor device **504** may be connected to a communication infrastructure **506**, for example, a bus, message queue, network, or multi-core message-passing scheme.

[0092] In an exemplary embodiment, computer system **500** may include a display interface **502**, for example a video connector, to transfer data to a display unit **530**, for example, a monitor. Computer system **500** may also include a main memory **508**, for example, random access memory (RAM), and may also include a secondary memory **510**. Secondary memory **510** may include, for example, a hard disk drive **512**, and a removable storage drive **514**. Removable storage drive **514** may include a floppy disk drive, a magnetic tape drive, an optical disk drive, a flash memory, or the like. Removable storage drive **514** may read from and/or write to a removable storage unit **518** in a well-known manner. Removable storage unit **518** may include a floppy disk, a magnetic tape, an optical disk, etc., which may be read by and written to by removable storage drive **514**. As will be appreciated by persons skilled in the relevant art, removable storage unit **518** may include a computer usable storage medium having stored therein computer software and/or data.

[0093] In alternative embodiments, secondary memory **510** may include other similar means for allowing computer programs or other instructions to be loaded into computer system **500**. Such means may include, for example, a removable storage unit **522** and an interface **520**. Examples of such means may include a program cartridge and cartridge interface (such as that found in video game devices), a removable memory chip (such as an EPROM, or PROM) and associated socket, and other removable storage units **522** and interfaces **520** which allow software and data to be transferred from removable storage unit **522** to computer system **500**.

[0094] Computer system **500** may also include a communications interface **524**. Communications interface **524** allows software and data to be transferred between computer system **500** and external devices. Communications interface **524** may include a modem, a network interface (such as an Ethernet card), a communications port, a PCMCIA slot and card, or the like. Software and data transferred via communications interface **524** may be in the form of signals, which may be electronic, electromagnetic, optical, or other signals capable of being received by communications interface **524**. These signals may be provided to communications interface **524** via a communications path **526**. Communications path **526** carries signals and may be implemented using wire or cable, fiber optics, a phone line, a cellular phone link, an RF link or other communications channels.

[0095] In this document, the terms "computer program medium" and "computer usable medium" are used to generally refer to media such as removable storage unit **518**, removable storage unit **522**, and a hard disk installed in hard disk drive **512**. Computer program medium and computer usable medium may also refer to memories, such as main memory **508** and secondary memory **510**, which may be memory semiconductors (e.g. DRAMs, etc.).

[0096] Computer programs (also called computer control logic) are stored in main memory **508** and/or secondary memory **510**. Computer programs may also be received via communications interface **524**. Such computer programs, when executed, enable computer system **500** to implement different embodiments of the present disclosure as discussed herein. In particular, the computer programs, when executed, enable processor device **504** to implement the processes of the present disclosure, such as the operations in method **200** illustrated by FIGS. **2**A-**2**C, discussed above. Accordingly, such computer programs represent controllers of computer system **500**. Where an exemplary embodiment of method **200** is implemented using software, the software may be stored in a computer program product and loaded into computer system **500** using removable storage drive **514**, interface **520**, and hard disk drive **512**, or communications interface **524**.

[0097] Embodiments of the present disclosure also may be directed to computer program products including software stored on any computer useable medium. Such software, when executed in one or more data processing device, causes a data processing device to operate as described herein. An embodiment of the present disclosure may employ any computer useable or readable medium. Examples of computer useable mediums include, but are not limited to, primary storage devices (e.g., any type of random

access memory), secondary storage devices (e.g., hard drives, floppy disks, CD ROMS, ZIP disks, tapes, magnetic storage devices, and optical storage devices, MEMS, nanotechnological storage device, etc.).

Example 1: Sensor Fabrication Process

[0098] In this example, an exemplary sensor similar to sensor **102** was fabricated via a method similar to exemplary method **300** illustrated in FIGS. **3**A and **3**B hereinabove. First, p-type Si wafer (100) was cleaned by the standard RCA method (RCA #1 method with $NH_4OH:H_2O_2:H_2O$ solution and a volume ratio of 1:1:5), rinsed in deionized (DI) water, and dried by compressed air. A thermal oxide was then grown on the wafer in a wet oxide furnace at about 1050° C. for about 2.5 hours to form a substrate. Then, a Ni catalyst layer for CNT growth with a thickness of about 9 nm was deposited on the substrate by E-beam evaporation system, at a temperature of about 120° C. and with a depositing rate of about 0.1 Angstroms/s. Afterward, The Ni layer was patterned in a shape of three circles with a diameter of about 2 mm in an exemplary conformation of three electrodes, as working, counter, and reference with a triangular distance of 5 mm from each other using standard photolithography process. For this process, a thin layer of a positive photoresist (e.g., Microchem-S1813) was spin-coated on the substrate surface. After illumination with the Mask-Aligner system, the substrate chemically developed. Then, a Ni layer in an undesired region (a region except of a region of three electrodes) was etched using a Ni-etchant solution ($HNO_3:H_3PO_4:CH_3COOH$, 3:3:1), and after that, the photoresist was washed using acetone. The entire substrate was then passivated with a 200 nm oxide layer at 0.9 torr, and 20 W (deposition speed <45 nm min^{-1}). The silicon dioxide layer deposited on the electrodes was removed by immersion of the positive PR-patterned substrate in a buffered oxide etchant (BOE) solution for about 7 s to prepare windows for the formed three-metal thin-film electrodes. Finally, the substrate with the formed electrodes thereon was located in a direct current plasma enhanced chemical vapor deposition (DC-PECVD) system to grow vertically aligned multi-walled carbon nanotubes (VAMWCNTs) on electrodes' heads. The growth of VAMWCNTs has a three-step process named annealing, graining, and growth. At first, the substrate with the formed electrodes was annealed at 680° C. in an H_2 environment with a flow rate of 20 standard cubic centimeters per minute (sccm) for 30 minutes. During the graining, the surface was plasma hydrogenated for 5 minutes with the intensity of 5.5 $W{\cdot}cm^{-2}$, which results in the catalyst graining and formation of Ni Nano-sized islands. In growth step, plasma of C_2H_2 and H_2 mixture with flow rates of 4.5 and 20 sccm was introduced to the chamber for about 20 minutes. Grown CNT arrays on electrodes were characterized by Field Emission Scanning Electron Microscope (FE-SEM). FIG. **6** shows an image **600** of a Field Emission Scanning Electron Microscope (FE-SEM) image of exemplary grown CNTs, consistent with one or more exemplary embodiments of the present disclosure. Length and diameter of nanotubes were ranged from about 2.5 μm to about 5 μm and from about 50 nm to about 70 nm, respectively. CNT arrays were used as the work, counter, and reference electrodes. Then, the passivating layer coated on connecting pads of electrodes were removed with BOE and the connecting pads of electrodes were attached to an electrical connector with three pins by conductive paste to form an exemplary final sensor. Afterwards, the electrical connector was connected to a readout system as an example of electrical stimulator-analyzer device **104** by a noiseless cable that handled all three electrodes.

[0099] Repeatability of the fabricated sensor was examined by testing three blood samples from normal and cancer patients with a 3-times of test repeat on each of them. FIG. **7** shows a chart **700** representing repeatability of an exemplary fabricated sensor obtained by testing three blood samples from normal and cancer patients with a 3-times of test repeat for each blood sample, consistent with one or more exemplary embodiments of the present disclosure.

Example 2: Detecting Cancer by Electrochemical Measuring of Reactive Oxygen Species (ROS) Levels in Blood

[0100] In this example, one hundred sixty patients of kids (2 months-13 years of age) and adults (20-73 years old) in two groups of newborn cancer with histologically confirmed cancer (n=60) and normal candidates (n=100) were tested here to investigate a correlation between ROS electrochemical peak results and abundancy ratio of HDNs to LDNs in their unprocessed blood samples. All cancer patients had advanced diseases (stages III-IV) in accordance with the American Joint Committee on Cancer criteria. Additionally, some treated patients with chemo/radiotherapy were investigated (n=20). Clinical, laboratory, and radiological characteristics and diagnosis and outcomes data were collected from all patients. Fresh blood samples prepared from 180 candidates were recorded. Also, patients' symptoms and therapeutic steps of cancer patients were recorded. For each patient, about 1 cc blood was used to measure released ROS/H_2O_2 from HDNs and LDNs in peripheral blood utilizing an exemplary system similar to system **100** including an exemplary sensor fabricated in EXAMPLE 1 hereinabove via an exemplary method similar to method **200**. Furthermore, LDNs and HDNs were isolated from whole blood with magnetic isolation and Percoll gradient. Then, LDN and HDN cells were counted by cell counter. Also, LDN and HDN cells were stained with Giemsa following thin-layer cell preparation and ROS fluorescent assay of LDNs and HDNs was carried out using a fluorescent microscopy system.

[0101] Neutrophil Isolation from Whole Blood and Quantification:

[0102] Total circulating neutrophils were isolated from 5 ml cancer patients' blood by negative selection using a Whole Blood Neutrophil Isolation Kit for human. Then, LDN and HDNs were isolated by discontinuous Percoll density gradient method. 9 ml Percoll was added to 1 ml 10×PBS (Stem cell) to create a 100% solution. Then, 2.5 ml of 78% Percoll diluted in 1×PBS was added carefully to a 15 ml falcon tube followed by a 2 ml layer of 66% Percoll and, so total neutrophils resuspended in 2 ml 54% Percoll. Centrifugation at 1545×g without brake was performed for 30 minutes at room temperature. HDNs were recovered from the 78%/66% interface and LDNs from 66%/54% interface. Cell counts were performed using a cell counter. Afterward, HDNs and LDNs cells were fixed by dipping the glass slides in 70% ethanol for 2 min and allowed to dry at room temperature before staining. HDNs and LDNs cells were stained 50 min in Giemsa solution, and washed in Tap water. Finally, glass slides were air-dried shortly and the dried slides were inspected under a light microscope.

[0103] Ros Assay:

[0104] ROS generation was analyzed with a 5-chloromethyl-2'-7'-dichlorodihydrofluorescein diacetate (CM-

H2DCFDA) assay. This probe is changed to 2'-7'-dichlorofluorescein (DCF) with a green fluorescent property by esterase enzymes in the cells' cytosol. After culturing fibroblast and MCF-7 cells overnight, the cells were washed with PBS, and then 500 μL of CM-H2DCFDA solution was added with a concentration of 20 μM. After 30 min incubation at room temperature in dark ambient, cells were again washed and then imaged with a fluorescent microscope. The cells were incubated with 10 mM of NAC as a ROS scavenger and then treated with CM-H2DCFDA. For positive control, cells were incubated with 100 μM H_2O_2 and treated by CM-H2DCFDA. Afterwards, samples were imaged with a fluorescent microscopy system.

[0105] An exemplary sensor fabricated in EXAMPLE 1 hereinabove was used to detect ROS levels in about 1 cc of an unprocessed blood sample from cancer patients and normal cases. The unprocessed blood sample was a residue of conventionally received blood from volunteers due to their checkup by informing them and their satisfaction. Intensity of released ROS levels in peripheral blood was recorded by a cyclic voltammetry procedure via a method similar to method **200** described hereinabove. Coherent results were achieved after categorizing the measured ROS current peaks and comparing them with candidates' clinical diagnostic results and abundancy of LDNs versus HDNs in their blood samples. Table 1 shows baseline characteristics of 160 patients investigated herein, including group 1 (G1) of normal candidate/patients with non-cancer diseases and group 2 (G2) of patients with newborns cancer.

[0106] Also, a calibration pattern was provided between responses measured and obtained by exemplary fabricated sensor and results of pathological state of the patients with tumors. FIG. **8** shows a chart **800** of measured peak currents associated with ROS levels in blood samples of 160 investigated patients for two groups of G1: normal candidate/patients with non-cancer diseases and G2: patients with newborns cancer, consistent with one or more exemplary embodiments of the present disclosure. As may be seen in FIG. **8**, 100% of blood samples in all children with advanced cancers (stages III-IV) showed peak currents lower than about 100 μA, while normal and non-cancerous candidates showed current peaks higher than about 300 μA. These ROS/H_2O_2 assisted current peaks for 90% of adults intended in this study with breast cancer tumors were lower than about 300 μA. In comparison, 98% of normal candidates with no complaint cases (including healthy people or diagnosed benign tumors) showed peak currents higher than about 450 μA. As a result, the ROS/H_2O_2 assisted electrochemical current peaks of the blood sample in patients with cancer tumors shows significant differences to blood current peaks of the normal/none cancer patients. This gap in pediatrics cohort is about 200 μA so that a ROS current peak for normal cases is more than about 300 μA, whereas a ROS current peak for cancer patients is less than about 100 μA. A gap for adults is about 150 μA so that a ROS current peak for normal cases is more than about 450 μA, whereas a ROS current peak for cancer patients is less than about 300 μA. So, the cut-off between normal/non-cancer disease and can-

TABLE 1

Baseline characteristics of 160 investigated patients (G1: Normal candidate/patients with non-cancer diseases, G2: Patients with newborns cancer).

	Characteristic				State of patients	
Candidates	Age	Sex		Race	Normal/Non cancer diseases (n = 80)	Malignant tumor (n = 80)
Children	2 months-13 years old	Female (n = 21)	Male (n = 39)	White	Appendicitis (n = 5) Abdominal edema (n = 6) Abdominal hernia (n = 2) Left kidney stone (n = 1) Liver Hydatid cyst (n = 3) Hypertrophic pyloric stenosis (n = 2) Osteocyte foot bone (n = 2) Vesicostomy (n = 3) Normal (n = 6)	Ewing sarcoma/neck tumor mass (n = 3) Rhabdomyosarcoma pelvis (n = 6) Ewing sarcoma/abdomen tumor mass (n = 4) Brain tumor in the left ventricle (n = 2) Lung cancer (n = 3) Wilms tumor (n = 7) Hepatoblastoma (n = 2) Neuroblastoma (n = 2) Osteosarcoma (n = 1)
Adults	20-73	Female (n = 100)	Male (n = 0)	White	Fibrocystic changes/ Microcalcification (n = 20) Fibroadenoma/ Cystic mass (n = 10) Florid ductal hyperplasia (n = 20) Normal (n = 50)	Phyllodes (cancerous) (n = 5) IDC nuclear grade 2 (n = 60) IDC nuclear grade 3 (n = 25) high grade DCIS (n = 7) Low/inter mediate grade DCIS (n = 2) ILC (n = 1)

cer cases is well detectable. It should be mentioned that adult cancer cohorts only were selected from breast cancer patients due to available clinical research center's facility.

[0107] Furthermore, receiver operating characteristic (ROC) curve analysis was done to compare between peak level responses associated with neutrophil ROS production in blood measured by exemplary fabricated sensor and results of clinical diagnostics (gold standard). The clinical diagnostics, including pathological and radiological evaluation, was considered as the gold standard test for diagnosis cancerous specimens. FIG. **9** shows a ROC diagram **900** for results obtained by utilizing exemplary method and fabricated sensor versus clinical diagnostics results for blood samples from 160 patients, consistent with one or more exemplary embodiments of the present disclosure. The result showed that an area under ROC curve (AUC) value for exemplary method **200** or method **220** of the present disclosure utilizing exemplary system **100** and sensor **102** was 0.981 (with P-value of less than about 0.0001 and CI99% 0.949-1) as represented in FIG. **9** and Table 2. Therefore, the obtained AUC value within a range of 0.9 to 1.0 may be an indicator of very high accuracy of exemplary method, system, and fabricated sensor which shows that exemplary method, system, and fabricated sensor is a reliable diagnostic tool and has a good balance of sensitivity and specificity.

[0108] Moreover, specificity, sensitivity, accuracy, precision, and selectivity was calculated for exemplary method and fabricated sensor as presented in Table 3. Also, true and false positive and negative data are shown in detail in Table 3. Accordingly, exemplary method, system, and sensor described herein can be used as an accurate diagnostic tool for detecting cancer in suspicious cases by real-time testing of peripheral blood. Exemplary fabricated sensor showed selectivity of about 98% and specificity of about 96% for the presence of cancer in patients with a breast mass and no signs of other inflammatory diseases (which may activate cytokine storm). In conclusion, exemplary fabricated sensor has proper sensitivity and specificity and can be used as a diagnostic tool for detecting suspicious cases by real-time testing of peripheral blood.

TABLE 2

AUC for exemplary fabricated sensor and method versus clinical diagnostics results for blood samples from 160 patients

Area Under the Curve

Test Result Variable(s) for exemplary fabricated sensor

		Asymptotic	Asymptotic 99% Confidence Interval	
Area	Std. Error[a]	Sig.[b]	Lower Bound	Upper Bound
0.981	0.012	0.000	0.949	1.000

The test result variable(s): Exemplary fabricated sensor has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

[a]Under the nonparametric assumption

[b]Null hypothesis: true area = 0.5

TABLE 3

Cross tabulation results for exemplary fabricated sensor, method, and system versus clinical diagnostics as a gold standard for blood samples from 160 patients

Exemplary system/method versus Clinical diagnostics Crosstabulation

			Clinical diagnostics		
			Negative	Positive	Total
Exemplary system/method	Negative	Count	77	1	78
		% within exemplary system/method	98.7%	1.3%	100.0%
		% within Clinical diagnostics	97.5%	1.2%	48.8%
	Positive	Count	2	80	82
		% within exemplary system/method	2.4%	97.6%	100.0%
		% within Clinical diagnostics	2.5%	98.8%	51.2%
Total		Count	79	81	160
		% within exemplary system/method	49.4%	50.6%	100.0%
		% within Clinical diagnostics	100.0%	100.0%	100.0%

Sensitivity = 98.80%,
Specificity = 97.50%,
Accuracy = 98.70%,
Precision = 97.60%,
Selectivity = 96.33%

[0109] In addition, isolation and characterization of high-density and low-density Neutrophils in comparison with ROS florescent assay was done to check validity of exemplary method described and conducted herein based on electrochemical measurement of ROS levels in blood samples utilizing exemplary fabricated probe hereinabove. Isolation and counting a ratio of LDNs versus HDNs in patients' blood may reveal better a relation between sensor scores and pathological states of blood volunteers. Mature neutrophils are characterized by a polymorphonuclear nucleus, which is in general large with 2-5 lobes 'segmented neutrophils', whereas immature neutrophils are shown by a one-lobed curved or ring-shaped nucleus. LDNs (immature and some mature neutrophils) are more in blood of cancer patients.

[0110] FIG. **10**A shows representative images of ROS/H_2O_2 related electrochemical current peak **1002**, optical microscope images of LDNs **1004** and HDNs **1006**, fluorescent microscope images of LDNs **1008** and HDNs **1010**, Giemsa staining images of LDNs **1012** and HDNs **1014**, and total cell count **1016** of LDNs and HDNs isolated by magnetic, negative selection protocol neutrophils isolated from 5 ml blood of normal cases (N=10), consistent with one or more exemplary embodiments of the present disclosure. Furthermore, FIG. **10**B shows representative images of ROS/H_2O_2 related electrochemical current peak **1020**, optical microscope images of LDNs **1022** and HDNs **1024**, fluorescent microscope images of LDNs **1026** and HDNs **1028**, Giemsa staining images of LDNs **1030** and HDNs **1032**, and total cell count **1034** of LDNs and HDNs isolated by magnetic, negative selection protocol neutrophils isolated from 5 ml blood of cancer patients (N=10), consistent with

one or more exemplary embodiments of the present disclosure. A P-value less than 0.05 was considered significant. Data shows means±SEM error bars. The bar in optical microscope images and Giemsa staining images represents 100 μm. As shown in FIGS. **10**A and **10**B, ROS peak levels recorded by exemplary method and system described herein are correlated well with an elevated ratio of LDNs/HDNs in normal and cancer patients as presented in detail in Table 4.

[0111] Fluorescent ROS assay may reveal a correlation between ROS/Hypoxic functions of cultured LDNs and HDNs and their recorded current peaks. As shown in FIGS. **10**A and **10**B, ROS fluorescent intensity showed weaker expression of florescent tagged cells in patients' blood with invasive and malignant cancer versus normal candidates or patients with non-cancer diseases. A direct correlation between down-regulation of ROS fluorescent images and electrochemical measured peaks was observed in all cancer blood samples.

TABLE 4

Detailed information of HDN and LDN counts and measured current peaks in 20 normal cases (N = 10) and cancer patients (N = 10)

Patient ID	State of patients	HDN cell counts in 5 ml of blood	LDN cell counts in 5 ml of blood	LDN/HDN ratio	current peak (μA) for 1 ml of blood
1	Normal	$2*10^6$	$0.5*10^6$	0.25	1000
2	Normal	$1.5*10^6$	$0.5*10^6$	0.33	730
3	Normal	$2*10^6$	$0.5*10^6$	0.25	810
4	Normal	$2*10^6$	$1*10^6$	0.5	531
5	Cancer (IDC nuclear grade 2)	$2*10^6$	$3.5*10^6$	1.75	255
6	Cancer (IDC nuclear grade 2)	$1.5*10^6$	$4*10^6$	2.67	92
7	Cancer (IDC nuclear grade 2)	$1*10^6$	$2*10^6$	2	149
8	Cancer (IDC nuclear grade 3)	$0.5*10^6$	$1.5*10^6$	3	61
9	Cancer (IDC nuclear grade 3)	$0.5*10^6$	$1.5*10^6$	3	71
10	Normal	$1.5*10^6$	$0.5*10^6$	0.33	609
11	Normal	$1*10^6$	$0.5*10^6$	0.5	489
12	Normal	$6.5*10^6$	$0.5*10^6$	0.08	1085
13	Cancer (IDC nuclear grade 3)	$2*10^6$	$2*10^6$	1	298
14	Cancer (IDC nuclear grade 2)	$1.5*10^6$	$3.5*10^6$	2.33	129
15	Cancer (IDC nuclear grade 2)	$2*10^6$	$4*10^6$	2	202
16	Normal	$2*10^6$	$0.5*10^6$	0.25	997
17	Cancer (IDC nuclear grade 3)	$1*10^6$	$3*10^6$	3	65
18	Normal	$3*10^6$	$1*10^6$	0.33	654
19	Normal	$1*10^6$	$0.5*10^6$	0.5	515
20	Cancer (IDC nuclear grade 3)	$0.5*10^6$	$2*10^6$	4	41

[0112] FIG. **10**C shows a chart **1000** of LDN/HDN ratio versus measured current peak diagram with a fitted plot for 20 cases, including normal cases (N=10) and cancer patients (N=10), consistent with one or more exemplary embodiments of the present disclosure. It may be observed that a level of LDN/HDN ratio may be precisely determined by real-time measuring electrochemical current peak of a 1 cc blood sample drawn from a person utilizing exemplary method, system, and fabricated sensor described herein.

[0113] Percentage Changes of ROS Level and LDNs/HDNs Ratio:

[0114] Herein, percentage of changes of ROS level and LDNs/HDNs ratio for cases investigated hereinabove (reported in Table 4) was calculated according to Equations (1) and (2) herein below.

Equation (1)

$$\textit{ROS level changes in cancer patient' blood (\%)} = \frac{-(\text{average blood current peaks of cancer patients} - \text{average blood current peaks of Normal cadidiates})}{\text{average blood current peaks of Normal candidates}} \times 100$$

[0115] ROS level changes in cancer patient' blood (%) according to data summarized in Table 4 using Equation (1) is calculated as follow:

Equation (2)

$$\textit{ROS level changes in cancer patient' blood (\%)} = \frac{-(136.3 - 742)}{742} \times 100 = 82\%$$

-continued

$$\textit{LDNs/HDNs ratio changes in cancer patients' blood (\%)} = \frac{-\left(\text{average } \frac{LDNs}{HDNs} \text{ ratio of cancer patients} - \text{average } \frac{LDNs}{HDNs} \text{ ratio of Normal cadidates}\right)}{\text{average } LDNs/HDNs \text{ ratio of Normal candidates}} \times 100$$

[0116] LDNs/HDNs ratio changes in cancer patients' blood (%) according to data summarized in Table 4 using Equation (2) is calculated as follow:

$$\textit{LDNs/HDNs ratio chanes in cancer patients' blood (\%)} = \frac{(2.475 - 0.332)}{0.332} \times 100 = 65\%$$

[0117] A significantly reduced ROS (82%) level in peripheral blood of cancer patients measured by an exemplary fabricated electrochemical sensor, which showed a great correlation with an increased ratio of LDNs/HDNs (65%) in such patients was observed.

Example 3: Effect of Chemo/Radiotherapy on Patients' Blood and Sensor Response

[0118] Table 5 shows detailed information of 20 patients with chemo/radiotherapy and measured electrochemical current peaks utilizing exemplary system, method, and fabricated sensor described hereinabove. As may be observed in Table 5, blood samples of non-treated patients with invasive tumors showed significantly lower levels of CV peaks than their blood after complete response (due to an associated oncologist's opinion) to chemo/radiotherapy. It revealed that exemplary system, method, and fabricated sensor has a capability of monitoring therapeutic effects on cancer patients by testing a 1 cc blood sample drawn from a patient. As COVID-19 may also increase Neutrophils' levels in blood due to cytokine storm, which may perturb cancer-associated response of exemplary sensor and method here, RT-PCR test and clinical evaluation was done on patients to be ensured from non-COVID involvement in them.

TABLE 5

Detailed information of 20 patients with chemo/radiotherapy and associated measured electrochemical current peaks.

Patient ID	Cancer type	Therapy (Surgery, Chemo/ radiotherapy (session))	current peak (μA) for 1 ml of blood
161	IDC nuclear grade 3	Chemo (8) and Surgery (mastectomy)	1090
162	IDC nuclear grade 2	Chemo (5)	550
163	IDC nuclear grade 2/3	Chemo (6)/Radio (30) and surgery (lumpectomy)	939
164	IDC nuclear grade 3	Chemo (6)/Radio (28)	731
165	IDC nuclear grade 2/DCIS component (20%)	Chemo (8)	469
166	IDC nuclear grade 3	Chemo (6)/Radio (0)	393
167	IDC nuclear grade 2/3	Chemo (4)/Radio (0)	321
168	IDC nuclear grade 2	Chemo (5) and Surgery (mastectomy)	498
169	IDC nuclear grade 2	Chemo (8)	681
170	IDC nuclear grade 2/3	Chemo (6)/Radio (20)	537
171	IDC nuclear grade 3	Chemo (6) and Surgery (mastectomy)	786
172	IDC nuclear grade 2/DCIS	Chemo (6)	606
173	IDC nuclear grade 3	Chemo (8)	815
174	IDC nuclear grade 2	Surgery (lumpectomy) and radio (0)	495
175	IDC nuclear grade 2	Surgery (lumpectomy)	562
176	IDC nuclear grade 2	Surgery (lumpectomy)	510
177	IDC nuclear grade 3	Chemo (8)	840
178	IDC nuclear grade 3	Chemo (4)	325
179	IDC nuclear grade 2	Chemo (6) and Surgery (mastectomy)	595
180	IDC nuclear grade 2/3	Chemo (8) and Surgery (mastectomy)	700

Example 4: Effect of Other Diseases on Sensor Response

[0119] Different kinds of pediatrics sickness with non-cancer diseases and normal candidates were studied as presented in Table 1. For adult investigation, electrochemical ROS detection was also performed on non-breast cancer diseases. Moreover, blood ROS levels were recorded in some patients with hypertension, heart disease, dyslipidemia, osteoporosis, and diabetes who had no tumoral diseases. FIG. **11** shows a chart **1100** of ROS/H_2O_2 assisted electrochemical current peaks of blood samples of patients with non-cancer diseases (n=15), consistent with one or more exemplary embodiments of the present disclosure. As shown in FIG. **11**, results showed peak currents higher than about 450 μA in all cases. This means that the ROS/H_2O_2 assisted electrochemical current peaks of the blood sample in patients with non-cancer diseases are significantly different from blood current peaks of the cancer patients. ROS/H_2O_2 assisted electrochemical current peaks in normal and non-cancer diseases are more than about 450 μA while in cancer diseases are less than about 300 μA.

[0120] LDNs may be increased in blood due to cancer, infection, and inflammation. One of the inflammatory diseases is COVID-19 as a dangerous viral pandemic. It is often more severe in people over 60 years old or with health conditions like lung or heart disease, diabetes, or conditions that affect their immune system. In COVID-19 patients, a significant increase of immature neutrophil numbers may be observed in acute patients compared to healthy donors/recovered patients, while the number of mature neutrophils decreased. Also, a meaningful shift in a ratio between mature and immature neutrophils may associate with a severity of viral involvement. The presence of "low density inflammatory neutrophils" may be strongly correlated with disease severity, and IL-6 levels. In addition, immature neutrophil numbers may strongly associate with IL-6 and IP-10. IL-6 and IP-10 may be consistently upregulated during cytokine storms and may be correlated with severe acute respiratory distress syndrome (ARDS). In addition to inflammatory monocytes as a source of IL-6, immature neutrophils may also be a non-negligible source of IL-6

through COVID-19 induced cytokine storm. So, an increase in number of LDNs may occur in cytokine storm stage of COVID disease. To evaluate an effect of COVID-19 as the most frequent infectious disease currently on peripheral blood ROS, exemplary system, method, and fabricated sensor was tested on blood sample of 14 patients with COVID-19 who were hospitalized in the intensive care unit (ICU).

[0121] Table 6 shows electrochemical ROS peak currents measured for blood samples of COVID-19 infected patients with any comorbidity. ROS/H_2O_2 assisted current peaks for 87% of adults with COVID-19 infection were lower than about 300 μA, same as patients with breast cancer tumors. But it is worth noting that when a patient comes to a medical center for evaluation of her/his breast tumor, COVID-19 involvement is prechecked for her/him, and global vaccination drastically reduces a probability of COVID-19 involvement with cytokine storm in the people. Hence, herein candidates suspicious to have high-risk breast tumors with no trace of inflammatory diseases or pregnancy were evaluated.

TABLE 6

Electrochemical ROS peak currents in COVID-19 infected patients with any comorbidity.

Patient ID	Age	Gender	Past disease history	Symptoms	ESR	CRP	RT-PCR	CT-Scan	I (μA)
1	61	Male	Lung cancer/DM	Dyspnoea, Unconscious	42	24.7	Pos.	Pos.	125
2	72	Male	Colon cancer	Myalgia or fatigue, Dyspnoea, Lack of appetite	16	20.3	Pos.	Pos.	228
3	63	Male	IHD, CVA, DVT	loss of consciousness, hypoxemia	29	31.3	Pos.	Pos.	355
4	79	Male	IHD, DM, Alzheimer's	Fever, Myalgia or fatigue, loss of consciousness	24	29.5	Pos.	Pos.	149
5	73	Female	PI, HLP, DM, HTN, DLP	Fever, Myalgia or fatigue, loss of consciousness	27	21.3	Pos.	Pos.	194
6	48	Male	HLP, PMH, IHD, DM	Fever, Myalgia or fatigue, Dyspnoea, Chest pain	34	28.6	Pos.	Pos.	202
7	67	Male	DM, Parkinson	Fever, Myalgia or fatigue, Vomit, loss of consciousness	35	20.5	Pos.	Pos.	125
8	73	Male	HTN, Pneumonia	Fever, Cough, Dyspnoea, Diarrhoea	10	14	Pos.	Pos.	157
9	58	Female	DM, HTN	Fever, Cough, loss of consciousness	20	25.8	Pos.	Pos.	348
10	67	Male	DM, HTN,	Fever, Cough, Chest pain	26	22.5	Pos.	Pos.	125
11	62	Male	DM	Fever, Dyspnoea, fatigue, loss of consciousness	34	26.5	Pos.	Pos.	149
12	59	Female	IHD, HTN	Fever, fatigue, chest pain	17	20	Pos.	Pos.	251
13	70	Male	DM, Parkinson	Fever, Dyspnoea, Chest pain	15	19.3	Pos.	Pos.	205
14	73	Female	IHD, DM, Alzheimer's	Fever, Dyspnoea, loss of consciousness	32	27.3	Pos.	Pos.	188

IHD: ischemic heart disease, CVA: Cerebrovascular accident, DVT: Deep vein thrombosis, PI: primary immunodeficiency, HLP: Hyperkeratosis lenticularis perstans, DLP: Dyslipidemia, PMH: Progressive macular hypomelanosis, DM: Diabetes mellitus, and HTN: Hypertension.

[0122] Furthermore, ESR and CRP (blood tests for detecting inflammation) were investigated in breast cancer patients. ROS peak levels recorded by exemplary system described here, ESR and CRP of these patients are shown in Table 7.

TABLE 7

Electrochemical ROS peak currents in patients with breast cancer tumor

Patient ID	Age	Gender	Tumor type	ESR* (mm/hr.)	CRP** (mg/L)	I (μA)
1	39	Female	ILC	22	7	118
2	38	Female	Papillary with Atypia	40	1.9	106
3	38	Female	IDC	10	2.2	134
4	38	Female	DCIS	20	3	102
5	54	Female	IDC	18	4	96
6	40	Female	IDC	34	10	89
7	55	Female	IDC	7.3	2	97
8	46	Female	IDC	0.5	1	104
9	41	Female	IDC	5.4	5	124
10	43	Female	IDC	11	3	128
11	39	Female	IDC	14	0.5	171
12	72	Female	IDC	19	2	202
13	39	Female	IDC	21	3	123
14	50	Female	IDC	41	6	178
15	42	Female	IDC	45	8	147

*The normal range of ESR is 0-29 mm/hr for women

**The normal range of CRP is less than 10 mg/L

[0123] Hence, patients with observable mass in their breast but asymptomatic signature about other inflammatory diseases would-be a candidate for exemplary system and method described here to detect their LDNs associated ROS levels in blood in favor of their tumor disease stage. In addition, a cytokine storm occurs when a patient has symptoms. However, there is a tumor in cancer and the suspected person who is referred for diagnosis has no symptoms in favor of inflammatory disease.

[0124] While the foregoing has described what are considered to be the best mode and/or other examples, it is understood that various modifications may be made therein and that the subject matter disclosed herein may be implemented in various forms and examples, and that the teachings may be applied in numerous applications, only some of which have been described herein. It is intended by the following claims to claim any and all applications, modifications and variations that fall within the true scope of the present teachings.

[0125] Unless otherwise stated, all measurements, values, ratings, positions, magnitudes, sizes, and other specifications that are set forth in this specification, including in the claims that follow, are approximate, not exact. They are intended to have a reasonable range that is consistent with the functions to which they relate and with what is customary in the art to which they pertain.

[0126] The scope of protection is limited solely by the claims that now follow. That scope is intended and should be interpreted to be as broad as is consistent with the ordinary meaning of the language that is used in the claims when interpreted in light of this specification and the prosecution history that follows and to encompass all structural and functional equivalents. Notwithstanding, none of the claims are intended to embrace subject matter that fails to satisfy the requirement of Sections 101, 102, or 103 of the Patent Act, nor should they be interpreted in such a way. Any unintended embracement of such subject matter is hereby disclaimed.

[0127] Except as stated immediately above, nothing that has been stated or illustrated is intended or should be interpreted to cause a dedication of any component, step, feature, object, benefit, advantage, or equivalent to the public, regardless of whether it is or is not recited in the claims.

[0128] It will be understood that the terms and expressions used herein have the ordinary meaning as is accorded to such terms and expressions with respect to their corresponding respective areas of inquiry and study except where specific meanings have otherwise been set forth herein. Relational terms such as first and second and the like may be used solely to distinguish one entity or action from another without necessarily requiring or implying any actual such relationship or order between such entities or actions. The terms "comprises," "comprising," or any other variation thereof, are intended to cover a non-exclusive inclusion, such that a process, method, article, or apparatus that comprises a list of elements does not include only those elements but may include other elements not expressly listed or inherent to such process, method, article, or apparatus. An element proceeded by "a" or "an" does not, without further constraints, preclude the existence of additional identical elements in the process, method, article, or apparatus that comprises the element.

[0129] The Abstract of the Disclosure is provided to allow the reader to quickly ascertain the nature of the technical disclosure. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims. In addition, in the foregoing Detailed Description, it can be seen that various features are grouped together in various embodiments. This is for purposes of streamlining the disclosure, and is not to be interpreted as reflecting an intention that the claimed embodiments require more features than are expressly recited in each claim. Rather, as the following claims reflect, inventive subject matter lies in less than all features of a single disclosed embodiment. Thus, the following claims are hereby incorporated into the Detailed Description, with each claim standing on its own as a separately claimed subject matter.

[0130] While various embodiments have been described, the description is intended to be exemplary, rather than limiting and it will be apparent to those of ordinary skill in the art that many more embodiments and embodiments are possible that are within the scope of the embodiments. Although many possible combinations of features are shown in the accompanying figures and discussed in this detailed description, many other combinations of the disclosed features are possible. Any feature of any embodiment may be used in combination with or substituted for any other feature or element in any other embodiment unless specifically restricted. Therefore, it will be understood that any of the features shown and/or discussed in the present disclosure may be implemented together in any suitable combination. Accordingly, the embodiments are not to be restricted except in light of the attached claims and their equivalents. Also, various modifications and changes may be made within the scope of the attached claims.

What is claimed is:

1. A system for real-time detecting cancer by analyzing unprocessed blood, comprising:

a sensor, comprising:

a substrate; and

three electrodes formed on the substrate, the three electrodes comprising a working electrode, a counter electrode, and a reference electrode, each respective electrode comprising:

an electrically conductive layer deposited on the substrate, the electrically conductive layer comprising a proximal end and a circular distal end; and

an array of multi-walled carbon nanotubes (VAMWCNTs) grown on the circular distal end, the array of VAMWCNTs configured to be put in contact with an unprocessed blood sample dropped on surface of the sensor, the unprocessed blood sample being drawn from a suspected person to have cancer;

an electrical stimulator-analyzer device electrically connected to the sensor at the respective proximal end of each respective electrode of the three electrodes, the stimulator-analyzer device configured to:

apply a set of voltages in a sweeping range from −0.8 V to +0.8 V to the sensor comprising the VAMWCNTs being in contact with the unprocessed blood sample; and

measure a produced set of electrical currents of the sensor responsive to the applied set of voltages; and

a processing unit electrically connected to the electrical stimulator-analyzer device, the processing unit comprising:

a memory having processor-readable instructions stored therein; and

a processor configured to access the memory and execute the processor-readable instructions, which, when executed by the processor configures the processor to perform a method, the method comprising:

applying, utilizing the stimulator-analyzer device, the set of voltages in the sweeping range from −0.8 V to +0.8 V between the reference electrode and the working electrode;

measuring, utilizing the stimulator-analyzer device, the produced set of electrical currents between the counter electrode and the working electrode;

measuring a level of a ratio of low-density neutrophils (LDNs) to high-density neutrophils (HDNs) in the unprocessed blood sample by measuring a level of reactive oxygen species (ROS) in the unprocessed blood sample, measuring the level of ROS in the unprocessed blood sample comprising measuring a maximum electrical current of the measured set of electrical currents; and

detecting cancer status of the suspected person, comprising:

detecting a cancer disease in suspected person's body if the measured ratio of LDNs to HDNs in the unprocessed blood sample is more than 1 by detecting the measured maximum electrical current of the measured set of electrical currents being less than a first threshold electrical current value; or

detecting no cancer disease in the suspected person's body if the measured ratio of LDNs to HDNs in the unprocessed blood sample is less than 1 by detecting the measured maximum electrical current of the measured set of electrical currents being more than a second threshold electrical current value.

2. The system of claim 1, wherein detecting the cancer disease in the suspected person's body comprises one of:

detecting the cancer disease in the suspected person's body if the suspected person's body is an adult responsive to the measured maximum electrical current of the measured set of electrical currents being less than 300 μA corresponding to a ratio of LDNs to HDNs in the unprocessed blood sample being more than 1; or

detecting the cancer disease in the suspected person's body if the suspected person's body is a child responsive to the measured maximum electrical current of the measured set of electrical currents being less than 100 μA corresponding to a ratio of LDNs to HDNs in the unprocessed blood sample being more than 1.

3. The system of claim 1, wherein detecting no cancer disease in the suspected person's body comprises one of:

detecting no cancer disease in the suspected person's body if the suspected person's body is an adult responsive to the measured maximum electrical current of the measured set of electrical currents being more than 450 μA corresponding to a ratio of LDNs to HDNs in the unprocessed blood sample being less than 1; or

detecting no cancer disease in the suspected person's body if the suspected person's body is a child responsive to the measured maximum electrical current of the measured set of electrical currents being more than 300 μA corresponding to a ratio of LDNs to HDNs in the unprocessed blood sample being less than 1.

4. The system of claim 1, wherein the method further comprises determining the first threshold electrical current value and the second threshold electrical current value, comprising:

generating a first dataset of a plurality of unprocessed blood samples associated with a plurality of cancer patients, comprising:

measuring a first set of electrical current peaks of the unprocessed blood samples associated with the plurality of cancer patients;

measuring a first set of ratio of LDNs to HDNs in the unprocessed blood samples associated with the plurality of cancer patients utilizing a cell counter; and

assigning each measured ratio of LDNs to HDNs of the first set of ratio of LDNs to HDNs to the respective measured electrical current peak of the first set of electrical current peaks;

generating a second dataset of a plurality of unprocessed blood samples associated with a plurality of healthy persons, comprising:

measuring a second set of electrical current peaks of the unprocessed blood samples associated with the plurality of healthy persons;

measuring a second set of ratio of LDNs to HDNs in the unprocessed blood samples associated with the plurality of healthy persons utilizing a cell counter; and

assigning each measured ratio of LDNs to HDNs of the second set of ratio of LDNs to HDNs to the respec-

tive measured electrical current peak of the second set of electrical current peaks; and

determining the first threshold electrical current value and the second threshold electrical current value, comprising:

determining the first threshold electrical current value equal to a maximum electrical current peak among the first set of electrical current peaks; and

determining the second threshold electrical current value equal to a minimum electrical current peak among the second set of electrical current peaks.

5. The system of claim **1**, wherein the method is conducted in less than 30 seconds.

6. The system of claim **1**, wherein the circular distal end has a diameter in a range of 0.5 mm to 3 mm.

7. The system of claim **1**, wherein the three respective circular distal ends of the three electrodes are placed apart from each other by a distance between 1 mm and 5 mm.

8. The system of claim **1**, wherein the substrate comprises a first layer of silicon dioxide deposited on a layer of silicon.

9. The system of claim **8**, wherein the sensor further comprises a second layer of silicon dioxide deposited on surface of sensor except surface of the circular distal end and the proximal end of each respective electrode of the three electrodes.

10. The system of claim **1**, wherein the electrically conductive layer comprises a layer of at least one of nickel, gold, and combinations thereof.

11. The system of claim **1**, wherein the electrically conductive layer has a thickness in a range of 5 nm to 20 nm.

12. The system of claim **1**, wherein:

the array of VAMWCNTs comprises VAMWCNTs with a length in a range of 2.5 μm to 5 μm; and

the array of VAMWCNTs comprises VAMWCNTs with a diameter in a range of 50 nm to 70 nm.

13. A method for real-time detecting cancer, comprising:

putting an unprocessed blood sample in contact with sensing parts of a set of a working electrode, a counter electrode, and a reference electrode of a sensor, the unprocessed blood sample drawn from a suspected person to have cancer;

applying a set of voltages in a sweeping range from −0.8 V to +0.8 V between the reference electrode and the working electrode;

measuring, utilizing one or more processors, a produced set of electrical currents between the counter electrode and the working electrode versus the applied set of voltages;

measuring, utilizing one or more processors, a level of ratio of low-density neutrophils (LDNs) to high-density neutrophils (HDNs) in the unprocessed blood sample, comprising:

measuring a level of reactive oxygen species (ROS) in the unprocessed blood sample by measuring a maximum electrical current of the measured set of electrical currents; and

determining the level of ratio of LDNs to HDNs in the unprocessed blood sample, comprising:

detecting the level of ratio of LDNs to HDNs in the unprocessed blood sample is more than 1 if the measured maximum electrical current of the measured set of electrical currents is less than a first threshold electrical current value; and

detecting the level of ratio of LDNs to HDNs in the unprocessed blood sample is less than 1 if the measured maximum electrical current of the measured set of electrical currents is more than a second threshold electrical current value; and

detecting, utilizing one or more processors, cancer status of the suspected person, comprising one of:

detecting a cancer disease in the suspected person's body if the level of ratio of LDNs to HDNs in the unprocessed blood sample is more than one; or

detecting no cancer disease in the suspected person's body if the level of ratio of LDNs to HDNs in the unprocessed blood sample is less than one.

14. The method of claim **13**, further comprising generating the first threshold electrical current value and the second threshold electrical current value, comprising:

generating a first dataset of a plurality of unprocessed blood samples associated with a plurality of cancer patients, comprising:

measuring a first set of electrical current peaks of the unprocessed blood samples associated with the plurality of cancer patients;

measuring a first set of ratio of LDNs to HDNs in the unprocessed blood samples associated with the plurality of cancer patients utilizing a cell counter; and

assigning each measured ratio of LDNs to HDNs of the first set of ratio of LDNs to HDNs to the respective measured electrical current peak of the first set of electrical current peaks;

generating a second dataset of a plurality of unprocessed blood samples associated with a plurality of healthy persons, comprising:

measuring a second set of electrical current peaks of the unprocessed blood samples associated with the plurality of healthy persons;

measuring a second set of ratio of LDNs to HDNs in the unprocessed blood samples associated with the plurality of healthy persons utilizing a cell counter; and

assigning each measured ratio of LDNs to HDNs of the second set of ratio of LDNs to HDNs to the respective measured electrical current peak of the second set of electrical current peaks; and

determining the first threshold electrical current value and the second threshold electrical current value, comprising:

determining the first threshold electrical current value equal to a maximum electrical current peak among the first set of electrical current peaks; and

determining the second threshold electrical current value equal to a minimum electrical current peak among the second set of electrical current peaks.

15. The method of claim **13**, wherein:

the first threshold electrical current value is 300 μA for an adult person;

the first threshold electrical current value is 100 μA for a child;

the second threshold electrical current value is 450 μA for an adult person; and

the second threshold electrical current value is 300 μA for a child.

16. The method of claim **13**, wherein the method is conducted in less than 30 seconds.

17. The method of claim **13**, further comprising acquiring the unprocessed blood sample from the suspected person to have cancer.

18. The method of claim **13**, wherein putting the unprocessed blood sample in contact with the sensing parts of the set of the working electrode, the counter electrode, and the reference electrode of the sensor comprises putting the unprocessed blood sample in contact with three respective arrays of multi-walled carbon nanotubes (VAMWCNTs) grown on the sensing parts of the set of the working electrode, the counter electrode, and the reference electrode by dropping the unprocessed blood sample on surface of the sensor.

19. The method of claim **13**, wherein applying the set of voltages in the sweeping range from −0.8 V to +0.8 V between the reference electrode and the working electrode comprises:

connecting respective proximal ends of the set of the working electrode, the counter electrode, and the reference electrode of the sensor to an electrical stimulator-analyzer device; and

applying the set of voltages in a sweeping range from −0.8 V to +0.8 V to the sensor using the electrical stimulator-analyzer device.

20. The method of claim **19**, wherein measuring the produced set of electrical currents between the counter electrode and the working electrode versus the applied set of voltages is done utilizing the electrical stimulator-analyzer device.

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