A Hand-Held Point-of-Care Biosensor Device for Detection of Multiple Cancer and Cardiac Disease Biomarkers using Interdigitated Capacitive Arrays

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***Abstract*—This paper presents a hand-held point-of-care device that incorporates a lab-on-a-chip module with interdigitated capacitive biosensors for label-free detection of multiple cancer and cardiovascular disease biomarkers. The developed prototype is comprised of a cartridge incorporating capacitive bio-detection sensors, a sensitive capacitive readout electronics enclosed in a hand-held unit and data analysis software calculating the concentration of biomarkers using previously stored reference database. The capacitive bio-detection sensors are made of interdigitated circular electrodes, which are pre-activated with single (for detecting one biomarker) or multiple specific antibodies (for detecting multiple disease biomarkers). Detection principle of capacitive biosensor is based on measuring the level of capacitance change between interdigitated electrode pairs induced by the change in dielectric constant due to affinity-based electron exchange in between antibodies/antigens and electrodes. The more antibody-antigens binding occurs, the more capacitance change is measured due to the change in dielectric constant of the capacitance media. The device uses pre-activated ready-to-use cartridges embedded with capacitive biosensors with shelf-life of 3 months under optimal conditions, and is capable of on-site diagnosis and can report the result in less than 30 minutes. The device is verified with real patient blood samples for 6 different disease biomarkers.**

***Index Terms*—point-of-care (PoC) device, lab-on-a-chip (LoC) module, detection of cancer and cardiovascular risk markers, label-free interdigitated capacitive biosensor**

I. Introduction

POINT -of-Care (PoC) devices are getting more attention due to their rapid, sensitive, low-cost and user-friendly analysis in non-laboratory environment that does not require sophisticated equipment and professional operators [1]. Recent developments in microfluidics, nanotechnology, smart soft materials, data analytics and smartphone related technologies lead to production of efficient PoC diagnostic devices [2]. A PoC device can operate with small sample volumes, reduce medical costs, mislabeling, mishandling and deliver results faster, which enable more effective treatment of progressive diseases [3]. With these promising features, PoC diagnostic devices can play an important role in management and early detection of outbreaks [4]. For example, an easy-to-use PoC assay is developed to replicate all steps of ELISA (the enzyme-linked immunosorbent assay) and this PoC device is tested with many real human samples in Rwanda. The device showed acceptable performance in simultaneous detection of HIV (the human immunodeficiency virus) and syphilis [5]. There are also successful PoC devices used for the detection of Ebola [6] and Zika [7] which raised serious concerns with recent outbreaks and proved the necessity of low-cost, portable and robust PoC platforms especially in resource limited settings [3]. All these PoC devices rely on different technologies, but many of them are integrated with smartphones. Since smartphones are widely available and have some parts such as LED (light emitting diode) flash as an illumination source, CMOS (complementary metal oxide semiconductor) imaging sensor as a detector, powerful CPU (central processing unit) and memory as computation resources, they are coupled with different lenses, gratings, filters and can be used for colorimetric [8], fluorescence [9], luminescence [10], surface plasmon resonance [11], spectroscopy [12] and electrochemical [13] based sensing applications. Other than smartphone-integrated PoC devices, there are also paper-based [14] and polymer-based [15] PoC devices for resource-limited settings. Microfluidics and lab-on-a-chip technologies have also been extensively applied in PoC platforms due to advantages of integrability, sensitivity and practicality [16]. Microfluidic-based platforms are mostly developed for cell studies [17], molecular diagnostic [18] and infectious diseases [19]. Although most of the PoC diagnostic devices focus on a single disease, there are also multiple disease detection systems on the same platform. Detecting multiple diseases on the same platform is a cost-effective and quick solution in resource-limited and developing world settings. Instead of scanning people for different biomolecules, infections or diseases multiple times with large-volume samples, a small volume of blood sample will be enough on a multiple-sensing platform and total scanning time will be drastically reduced. Simultaneous sensing of multiple biomarkers can be achieved through either multi-label or multi-electrode approaches [20]. In multi-label systems, single electrode is used for the detection of the various biomarkers. On the other hand, multiple sensing areas are used to detect multiple biomarkers in multi-electrode systems. Although there is an extensive research carried out

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on various types of PoC devices and increased number of devices are becoming available day by day, the number of commercially available devices are still limited. Most of the commercially available PoC devices are based on lateral flow assays including pregnancy test strips [21], HIV detection [22] and analysis of protein markers in blood. Of course, one of the most popular PoC devices is self-test glucose measurement systems which dominate the PoC device market together with pregnancy test strips. It is reported in 2014 that PoC device market was $15 billion in 2011 and reached to about $18 billion in 2016 with 4% annual growth. This includes infectious diseases, cardiac markers, lipids, coagulation and hematology together with glucose monitoring and pregnancy testing. The fastest growing market within the PoC device was molecular testing and was worth $4 billion in 2011 and reached to $7 billion in 2016 [23].

Among all these alternatives, here we focus on the detection of cardiovascular diseases (CVD) and cancer with the multi-electrode approach based PoC device, since the incidence of CVD and cancer claims highest mortality rates in the US and Europe, especially in elderly population [24], [25]. Early detection of cancer and CVD is very important to increase the survival rate of patients in critical conditions. There are several clinically important biomarkers present in the serum that serve as signatures for CVD and cancer. Biomarkers are closely related to multiplicity of the large number of existing proteins which are indicative of CVD and cancer [26], it is necessary to have a reliable detection tool for cardiac and cancer markers which could probe a combination of biomarkers towards a highly accurate diagnosis and provide a multi-parametric detection system.

Several methods utilizing transducers like piezoelectric microcantilever [27], [28], quartz crystal microbalance (QCM) [29], surface plasmon resonance (SPR) [30], fluorescence [31], amperometric [32] methods have been reported for detection of CVD and cancer biomarkers. Few of these mentioned methods, such as SPR and fluorescence based detection are effective in specificity and accuracy. However, they still hold several limitations concerning portability, sensitivity and cost effectiveness [30]. Electrochemical impedance spectroscopy (EIS) has been broadly used in detection of CVD biomarkers. It is highly sensitive, low cost, reliable and provides opportunity for label-free detection with convenience to construction and miniaturization for point-of-care applications [33], [34]. Thus, EIS based biosensors have been extensively used for various biological studies [35]–[38]. In recent years, our research group has reported on detection of several cancer and CVD markers utilizing interdigitated patterned electrodes exploiting various designs and structures [39]–[42]. In this study, the previously reported interdigitated capacitive (IDC) sensors, developed by our group for the detection of multiple CVD and cancer biomarkers, has been extended to a fast, low-cost hand-held point-of-care diagnostic device. For this purpose, a novel circular IDC-based biosensor in 2x6 capacitive arrays is designed, fabricated and verified that is capable of detecting a panel of biomarkers in human serum. The IDC surface was functionalized with specific antibodies that are assigned to specific IDC element in the array. The antibody selectively binds with a corresponding biomarker protein and the IDC sensor translates the signal from antibody-biomarker binding into specific capacitance change, function of type and amount of the protein. Circular IDC chip provides sample droplet impingement and better contact and interaction with antibodies on IDCs and thus enables sensitive capacitance detection. The circular IDC sensor is coupled with a sensitive readout electronics that is enclosed in a hand-held unit. The hand-held unit is designed in a way that it enables easy use of pre-activated cartridges and ensures a reliable connection with IDC sensor. The hand-held unit also incorporates a mini computer that the software runs on it and a touch-screen to control the operation of the device and to display the results on the screen. The software calculates the new concentrations of the biomarkers in sample dropped on IDC sensor by utilizing the previously stored reference data for different concentrations of each disease biomarker. The device has the essential features of fast, on-site, low cost and accurate diagnosis and prognosis of the cancer and cardiovascular diseases.

This paper is organized as follows. Section II discusses the design and fabrication of the circular interdigitated capacitive sensor, how the circular design is selected as the sensor. Section III describes the design of hand-held PoC diagnostic device including mechanical, electronic designs and software. Section IV describes measurement protocol of the device and provides measurement result starting from reference database generation ending with real-patient blood sample tests. Finally, Section V concludes the work.

II. Design and Fabrication of the Circular Interdigitated Capacitive Sensor

The performance of the interdigitated capacitive sensor for impedance measurements is dependent upon the geometric design of the electrode pattern, number of electrode pairs, substrate thickness, electrode thickness and the gap between electrode pairs. First, the geometry of the electrode pattern was determined by running electromagnetic simulations and measurements. Fig. 1 shows three different geometries of electrode pattern. Fig. 1-a and b are both circular geometries while Fig 1-c is rectangular. Among the modeling/simulation and measurement results of all these three geometries, the second capacitive geometry (Fig. 1-b) presented the maximum resonance frequency at about 2.5 GHz. It is desirable for the capacitive biosensor to operate far from resonance frequency and at lower frequencies in the kHz ranges, for stable operation of the sensor ensuring the stability of the proteins. It is also important to have increasing relative capacitance change with increasing protein concentration until the saturation level (detection limit) reached for the specific protein. The second geometry (Fig. 1-b) is relatively more stable in the low frequency range where the capacitive sensor is operated (25 kHz). Measurement results showed that the second geometry provides increasing capacitance change with increasing protein concentrations whereas the first geometry does not for all the proteins tested. Both the first and second geometry (Fig. 1-a and b) incorporating an interdigitated capacitor consists of two interlocked and separated gold electrodes, with each having individual fingers which overlap with the other section. The first

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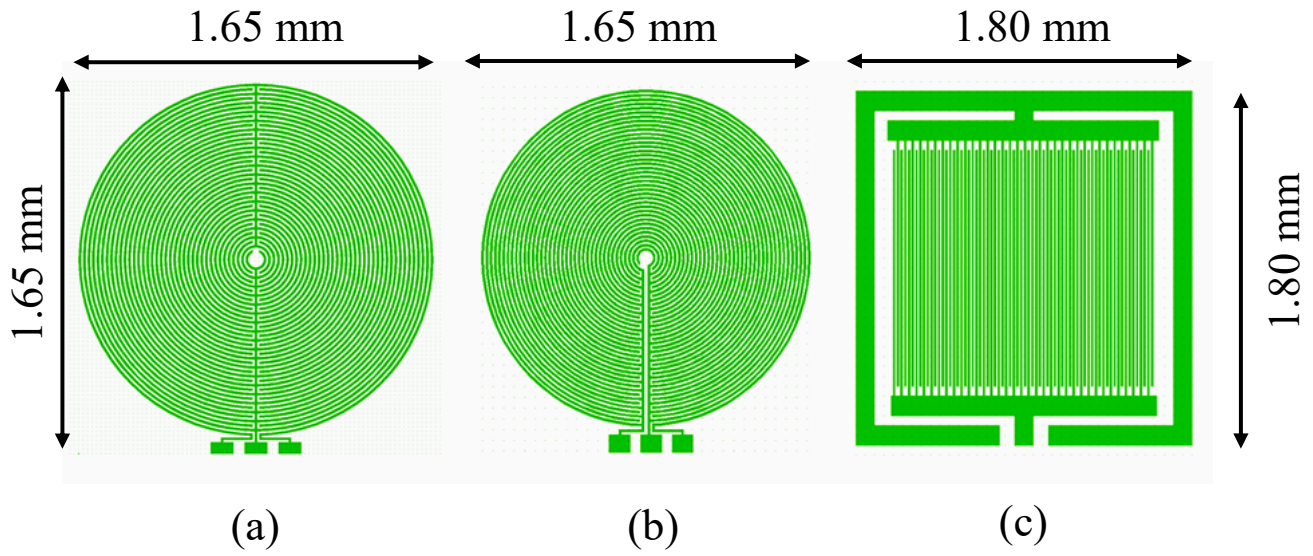

Fig. 1: Investigated designs for interdigitated capacitive sensors

geometry has semicircular fingers distributed equally on both sides of the sensor whereas the second geometry has full-circle fingers. It is thought that capacitive coupling/interference in between two semicircular electrodes and inductive behavior of two semicircular structures at the center line of the first geometry could be the reason for unstable sensing behavior. The electrodes were designed in circular shape to provide hydrodynamic coalescence property in the sample droplet to be incubated on capacitors [43], which prompted elimination of the third geometry. Second, substrate thickness and number of electrode pairs are determined while other parameters were fixed. It is observed that substrate thickness does not affect the capacitance value especially at low frequencies. Substrate thickness was determined to be 0.75 mm for the fabrication purposes. The number of electrode pairs is determined by the following considerations. First, the sensor area should occupy a small area to accommodate as many capacitive sensors as possible for multiple detection of target biomarkers within a fixed cartridge area, which results in a less number of electrode pairs. Second, there should be enough area for the analyte to create detectable capacitance change that can be readout by the electronics, which allows more number of electrode pairs. Measurement results showed that although less number of electrode pairs result in less capacitance value, it is possible to accurately measure the capacitance change with designed readout electronics down to 19 electrode pairs. Hence, as a result of this trade-off, the number of electrode pairs were selected as 19 to have enough sample volume area, detectable capacitance change and to accommodate as many capacitive sensors as possible for multiple detection of target biomarkers within a fixed cartridge area. The electrode thickness and the gap between the electrode pairs were selected as 150 nm and 10 μm respectively for the optimum sensor performance and to fit into the allocated sensor area for each capacitive sensor. Interdigital capacitor array was fabricated using lift off process. First, photoresist was coated on clean borofloat substrate using a spin coater and patterned using standard photolithographic techniques. Then, a 30 nm adhesion layer of Ti and 150 nm Au were deposited onto the patterned photoresist by DC sputtering. The gold that was not adhered on the substrate was lifted off by soaking it in acetone. In this way, 12 capacitive sensors were designed to fit into a 30 mm x 12 mm total cartridge area. Fig. 2 shows the cartridge which consists of 12 interdigitated capacitive sensors.

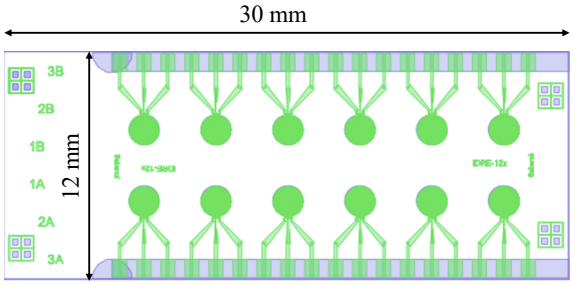

Fig. 2: Cartridge incorporating 12 interdigitated circular capacitive sensors

III. Design of the Hand-Held Device

A. Mechanical Design

Fig. 3 shows the 3D drawing of the developed point-of-care hand-held device. Design and dimensions of the device were chosen that enabled simple, easy and fast hand-held operation. The width x length x height of the device is 107 mm x 120 mm x 28 mm. The 30 mm x12 mm sized cartridge on a 47 mm x 20 mm base can be inserted into the device from front panel. Spring loaded pogo connectors were used to connect the capacitive sensors with the readout electronics for

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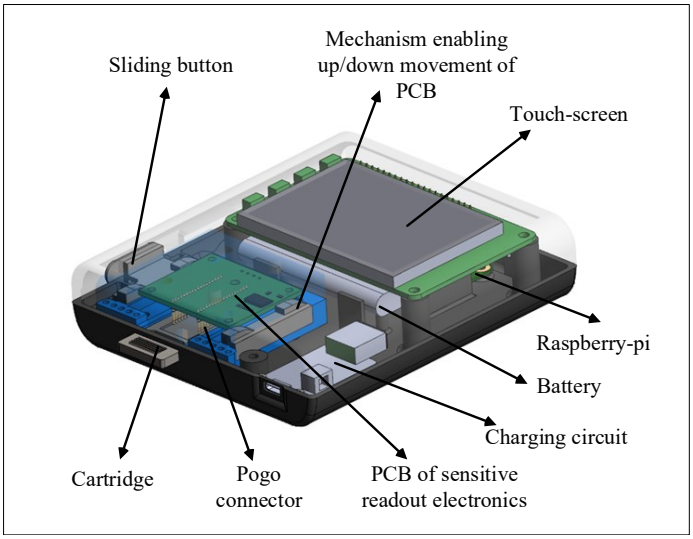

Fig. 3: Mechanical design of the point-of-care hand-held device

short, symmetric and sensitive contact. A mechanical structure, which enables up/down movement of the printed circuit board (PCB) with the help of a sliding button, prevents a possible damage to capacitive sensors due to soft touching functionality of the pogo connectors during insertion of the cartridge. By utilizing the sliding button, small magnets surrounding the mechanical structure forces the pogo connectors to contact with the electrodes. A 2.8-inch capacitive touch-screen was used to operate the device with the help of a graphical user interface. A lithium-ion battery together with a charging circuit enables the mobile on-site operation of the device.

B. Design of Readout Electronics

Since the aim of the work is to design a miniaturized hand-held device equipped with accurate and cost-effective readout electronics, the device demands an optimum fixed operating frequency for all selected proteins. The operating frequency of hand-held module in the current work is selected by following considerations. First, capability of capacitive readout electronics is limited by kHz range of operation. Second, since the hand-held unit is aimed to work with a number of different proteins, we have to select a fixed operation frequency in kHz range at which each of these target protein can be detected with optimum sensitivity and dynamic range of operation. Third, the operating frequency of the hand-held unit should be as low as possible to avoid any interference with other electronics in the environment and also should not raise a concern about frequency-protein interaction. Hence, 25 kHz is carefully selected for the operation of the device considering above listed criteria. The selected capacitance-to-digital-converter (CDC) IC, that operates at 25 kHz, can measure very small amount of capacitance values with up to 0.5 fF resolution [44]. It is programmable through serial I^2C protocol and can have multiple channels. The measurements performed with an impedance analyzer at earlier stage of the work, were repeated with prototype PCBs incorporating CDCs and several optimizations were carried out on PCB for an accurate measurement of capacitance values. Measurement results showed that there is small amount of capacitance difference between blank, antibody-immobilized and BSA (bovine serum albumin) blocked IDC sensors that is much less than the error margin which does not affect the accuracy of the test results. Hence, to simplify the measurement protocol, measurements were taken in duplicates, one after antibody immobilization and one after antibody-antigen (target protein) binding. Impedance analyzer is used for the verification of the prototype device. We observed consistent results as obtained with impedance analyzer using the prototype PCB design. These results verified the performance of the readout electronics of the prototype hand-held device. The PCB incorporating the readout electronics was connected to Raspberry Pi Zero W, which is equipped with bluetooth and wireless connections [45] enabling sharing measurement data with remote devices. Fig. 4 shows schematic block diagram of the PoC device and corresponding PCB layout. 4 CDC chips are used for 12 capacitive sensors, each of which is connected to 3 capacitive sensors. An I2C multiplexer chip [46] is used to select each

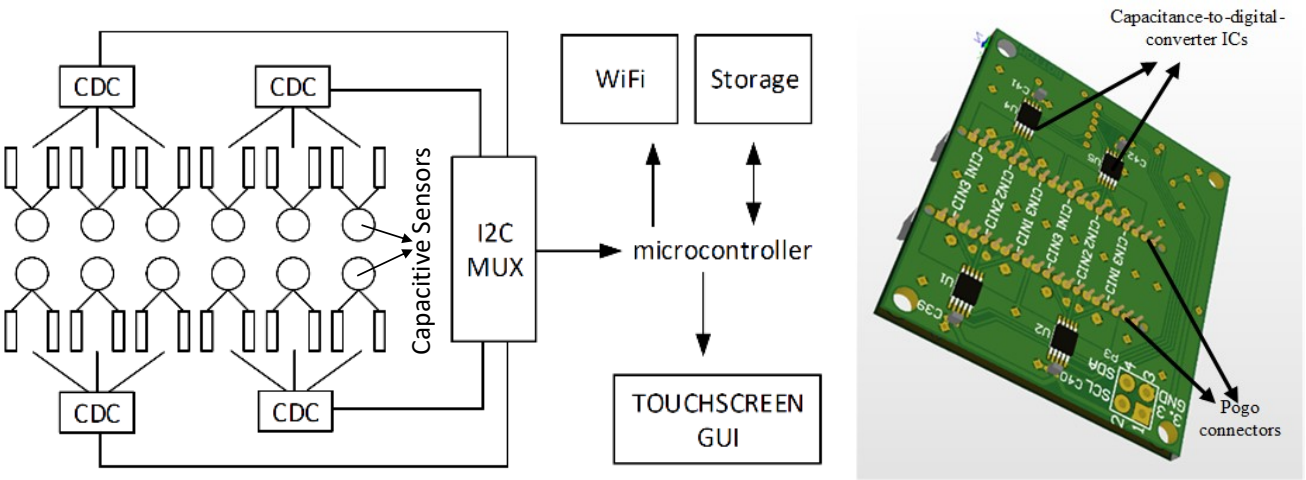

Fig. 4: Schematic block diagram of the PoC device and corresponding PCB layout

CDC chip since they share the same I2C address. During the operation of the PoC device, CDC chips are selected respectively by the I2C multiplexer and each channel of the selected CDC chip is read-out by the controller until all CDC chips are read-out. The collected data is processed and shown to the user through the touchscreen as described in the following section.

C. Software

Software serves for two functions in the operation of the hand-held device. First, it has a graphical user interface to control the operation of the device and to display the measurement results. Second, it computes and predicts the target biomarker concentration using the current measurement result and previously stored reference database. Fig. 5 summarizes the operation of the hand-held PoC device. The reference database is generated with known concentrations of proteins listed in Section IV. An initial measurement is performed after surface activation and antibody immobilization for calibration purposes. Then, known concentration of target biomarker is dropped on the sensor and final measurement is performed. Using calibration measurement and final measurement data, relative capacitance change is calculated and stored in the memory. This is done with multiple samples for accurate extraction of reference database and for different concentrations of each target biomarker. Using this reference database, a polynomial interpolation function is generated for each target biomarker. When a new blood sample is dropped, same protocol is applied and relative capacitance change is calculated. Then, the relative capacitance change is inserted in the previously generated and ready-to-use function to find the corresponding new concentration. The database has enough number of previously stored measurement results for each biomarker to correctly interpolate and calculate the new concentration. The software supports following features:

- Each of 12 capacitive sensors can be set to a different target biomarker. It is possible to detect 11 different biomarkers, with the 12th capacitive sensor reserved for a reference.
- The software averages multiple measurements of the same sample to avoid errors. It is possible to set the number of measurements with an accuracy-measurement time trade-off.
- Software runs in two different modes: Basic mode and advanced mode. In basic mode, just the result is shown to the user whether the blood carries healthy or unhealthy levels of biomarkers with indicative colors. Green color represents healthy sample (levels within normal range), while red color represents unhealthy sample (risk levels). Yellow color means the measurement result is close to the boundary level of unhealthy sample. In advanced mode, calibration measurement, final measurement and the percentage change between the two are also shown together with the reference interval of healthy sample specific to the target biomarker.

IV. Measurements

A. Reagents and Bio-chemicals

Target biomarkers used for the verification of the point-of-care hand-held device, that are indicative of CVD and cancer, are BNP (B-type natriuretic peptide) (CVD) [47], SAA (serum amyloid A) (cancer) [48], IL-6 (interleukin-6) and

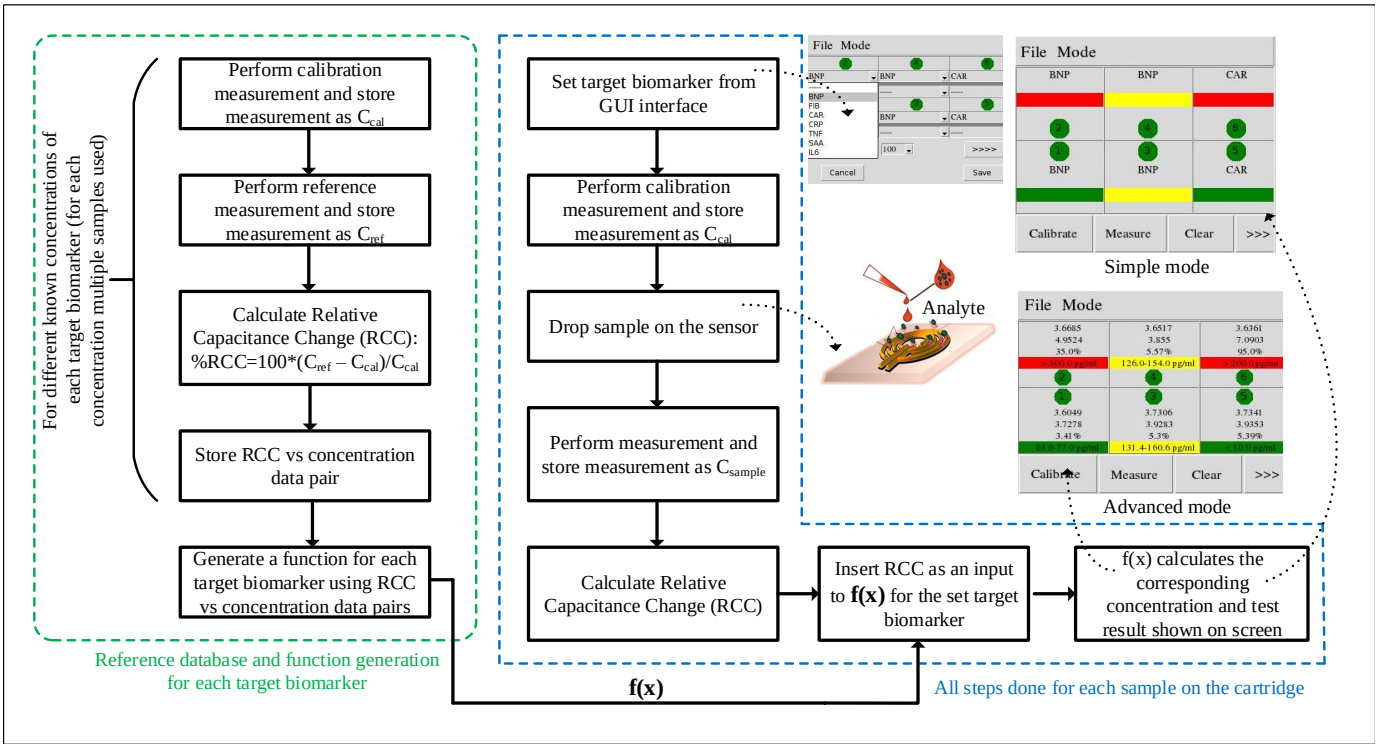

Fig. 5: Flow diagram showing the operation of the hand-held PoC diagnostic device

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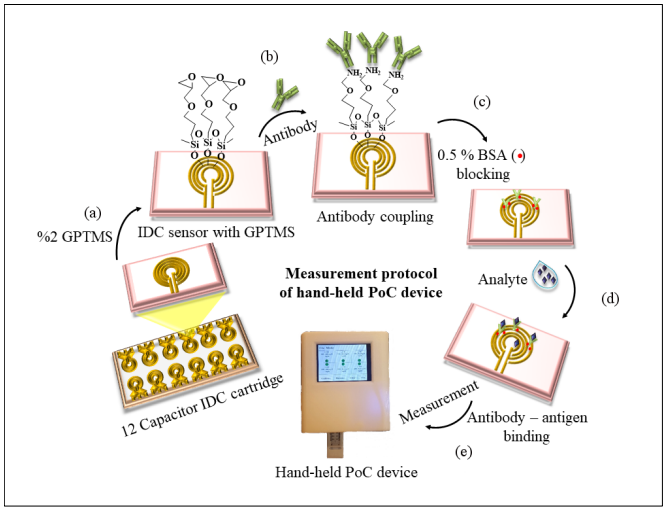

Fig. 6: Measurement protocol of hand-held PoC device

Fibrinogen (CVD and cancer) [49]–[52], Troponin-I (CVD) [53] and TNF-α (tumor necrosis factor alpha) (cancer) [54]. Human serum (male; blood type, AB) was purchased from PAN^{TM} Biotech, GmbH and reconstituted by appropriate dilution in phosphate buffered saline (PBS, pH 7.4) prior to use. Here, AB-type serum was used because of its compatibility with other blood types. 3-glycidoxypropyl trimethoxysilane (GPTMS) was purchased from SigmaAldrich, Germany. BNP antibody (catalog # 10-7879), SAA antibody (catalog #: 10R-10351), IL-6 antibody (catalog #: 10R-I164A), Fibrinogen antibody (catalog #: 10R-8419), Troponin-I antibody (catalog #: 10C-CR4036M5), TNF-α antibody (catalog #: 10R-6647), BNP 32 protein (catalog #: 30-1059), SAA protein (catalog #: 30R-1380), IL6 protein (catalog #: 30R-2492), Fibrinogen protein (catalog #: 30-AF27), Troponin-I protein (catalog #: 30-1003) and TNF-α protein (Cat #: 30R-2317) were procured from Fitzgerald. All other chemicals were of analytical grades and used as received. The developed device is tested and validated with above listed biomarkers, but is not limited to the above listed biomarkers provided the sensors pre-activated with specific antibodies. With further experimental studies, it is possible to expand the list of the biomarkers that are detectable by the device.

B. Measurement Protocol

Fig. 6 summarizes the measurement protocol of the hand-held PoC device. All steps of the measurement protocol are as follows:

1) Immobilization of Antibodies: IDC arrays are throughly washed using ethanol and dried under N_2 flow. A self-assembled layer of 3-Glycidyloxypropyltrimethoxysilane (GPTMS) is formed on the IDC surface by immersing the chip in 2% GPTMS prepared in toluene for 2-4 h at room temperature (Fig. 6(a)). The surface activated chip is rinsed with toluene and dried using N_2 gas. 2 μL of 100 μg/mL of the listed antibodies in phosphate buffered saline (PBS, pH 7.4) are incubated on each IDC for 2 h at 4 °C (Fig. 6(b)). Each sensor on the cartridge can be activated by one of the listed biomarkers. In this way, only the antigens in the samples, that are specific to immobilized antibodies, bind to corresponding antibodies on activated sensor surface. If any non-specific antigens are present in the sample, these will not bind with antibodies. Hence, one sensor is pre-activated for one specific biomarker. For multiple biomarker detection, different antibodies should be immobilized on different sensor areas keeping in mind that the number of sensor areas is 12. Out of these, one of the sensor areas is reserved for a negative control, which means 11 different biomarkers can be tested at the same time with one cartridge. To remove the unbound antibodies, the chip is thoroughly washed using sterile PBS and dried under N_2. At this point, surface-activated and antibody-immobilized cartridge can be used immediately or stored for up to 3 months at 4 °C for future use.

2) Calibration Measurement: After surface activation and immobilization of antibodies, each of 12 capacitive sensors on the cartridge is measured and saved to be used for the calculation of relative capacitance change due to the antibody-antigen binding.

3) Preparation of Target Biomarkers: Since it is possible to have multiple detections at the same time, each capacitive sensor in the cartridge can be activated for different biomarkers if needed. If sensor areas are pre-activated for different biomarkers during antibody immobilization stage, the same sample can be used for each sensor area to detect multiple biomarkers simultaneously. To avoid any non-specific binding on the sensor surface, a negative control experiment is conducted before adding the unknown blood/serum sample by incubating 0.5 % BSA on each IDC for 1 h at 4°C (Fig. 6(c)). Finally, the cartridge is thoroughly washed using PBS and quickly drained the drop away from the IDCs by blowing with N_2 gas that enables removal of salts that may interfere in the signal.

4) Final Measurement: Prepared target biomarkers are spiked in 10-fold diluted real human serum and 2 μl of each concentration is incubated on their respective antibody immobilized IDC sensor surface for 30 minutes at room temperature (Fig. 6(d)). For this, a petri dish chamber is layered with a blotting paper which is sufficiently dampened by adding PBS solution. The IDC sensors are then placed on a dampened blotting paper and incubated with small volumes of sample, which provides humid environment and prevents drying out of the samples. The IDC array is then washed with sterile PBS and dried under N_2. However, use of N_2 gas is not required for drying IDC array. Therefore, the IDC sensor can be left to dry in open environment by evaporation. The capacitance of the IDC sensor is measured after antibody and target biomarker binding and the relative capacitance change is calculated. The relative capacitance change is inserted into the function generated by the reference database as explained previously. The function predicts the concentration of newly dropped sample on the sensor and the device displays the corresponding color-coded result on the screen (Fig. 6(e)).

C. Generation of Reference Database

The prototype device is first used for the generation of the reference database. To generate the reference database, first

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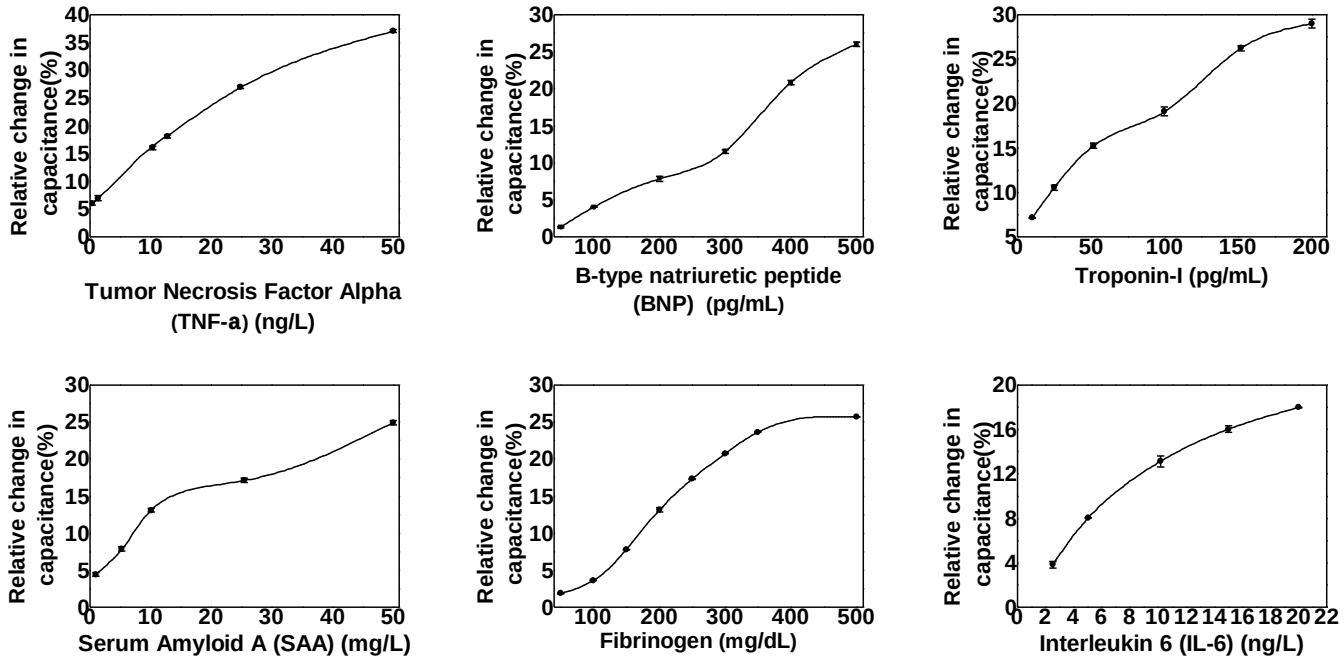

Fig. 7: Known concentrations and corresponding measured relative capacitance changes of target biomarkers for reference database

surface activation is done with GPTMS attachment and antibody immobilization. A calibration measurement is performed and stored in the memory before antibody-antigen binding. Second, antibodies are immobilized on the pre-activated surface and antibody-antigen binding occurs. Another measurement is performed, and the result is also stored in the memory. Using these two measurement results, relative capacitance change is calculated for different known concentrations of each target biomarker as summarized in Fig. 5. In this way, a reference database is generated to be used in testing for biomarkers in real blood samples. Fig. 7 represents plots showing known concentrations of each target biomarker and their corresponding relative capacitance changes in percentage. These plots are used for the generation of the polynomial interpolation functions specific to each target biomarker. The results show that relative capacitance changes increase with increasing concentrations of target biomarkers which are in-line with the measurements performed with the impedance analyzer proving that the hand-held PoC device can be used to detect the amount of target biomarker. The measurements to generate the reference database were repeated multiple times both with developed hand-held device and impedance analyzer to ensure the accuracy of the results. For BNP, Troponin-I and SAA proteins minor kinks are observed in the plots, which are attributed to the frequency behavior of the proteins at the particular concentrations. It is observed that these minor kinks do not effect the accuracy of the device as shown in Table I with real patient blood samples. The device operates in wide concentration range from pg/mL to ug/mL for different target biomarkers and can show similar relative capacitance changes for different concentrations of different target biomarkers. The reason why different target biomarkers with different concentrations give similar measurement results is due to their dielectric behaviour and physical properties of the corresponding biomolecules. All target biomarkers exhibit their specific dielectric property at the frequency of operation. Since the developed PoC device measures the capacitance change due to the change in dielectric constant, and all target biomarkers have different molecular structure with different charge distribution, different concentrations of different target biomarkers can have similar measurement results. It means that, for example, pg/mL concentration of a target biomarker could show the same amount of change in capacitance due to similar dielectric constant and physical properties with ug/mL concentration of another target biomarker. The generated functions by using the generated plots for each target biomarker accepts a newly measured relative capacitance change and outputs an estimated concentration of the target biomarker during the actual testing of a real blood sample.

D. Results

For the verification of the developed prototype device, 20 different real patient blood samples are used. Each sample is tested for all six proteins of our interest, which makes 120 test results in total with real patient blood samples. Table I shows the test results for each target biomarker. For each biomarker, 2-test results that are not close to each other to show enough concentration range for color coded results, are included in the table. The reference value shows the concentration interval of a healthy person for specific biomarkers.

TABLE I: Measurement results of PoC hand-held device with real patient blood samples

Color Code	Target Biomarker	Reported ASM[1] Hospital	Measured by PoC Device[2]	Ref. Value	Detection Range	Sensitivity	Accuracy[3]	Unit
Green	BNP	94	90-99	70-133	50-500	50	99.5%	pg/mL
Red	BNP	746	>500	70-133	50-500	50	NA	pg/mL
Green	SAA	<4	3.15-3.48	0-7	1-50	1	NA	mg/L
Red	SAA	9.7	9-10	0-7	1-50	1	98%	mg/L
Green	IL-6	<2	<2.5	0-5.9	2.5-20	2.5	NA	ng/L
Red	IL-6	18	15.4-17	0-5.9	2.5-20	2.5	90%	ng/L
Green	Fibrinogen	320.8	292-323	200-400	50-500	50	95.9%	mg/dL
Red	Fibrinogen	511.9	>500	200-400	50-500	50	NA	mg/dL
Green	Troponin-I	<10	<10	0-23	10-200	10	NA	pg/mL
Red	Troponin-I	1300	>200	0-23	10-200	10	NA	pg/mL
Yellow	TNF-α	6.8	7.3-8.1	0-8.1	0.3-50	0.3	86.8%	ng/L
Red	TNF-α	15.7	16.1-18	0-8.1	0.3-50	0.3	91.5%	ng/L

[1] Anadolu Medical Center in Affiliation with Johns Hopkins Medicine
[2] Real human blood samples in this study are used with necessary approval from ethical committees.
[3] Midpoint of the measured value by PoC device is used to calculate the accuracy. Accuracy of the test results that are out of detection range is not calculated and indicated by NA.

The test results shown in the table are from both healthy and unhealthy blood samples for each biomarker. Detection range shows the interval that the device operates. However, the device can show results indicating that the measured values are lower than the minimum detection limit or above the maximum detection limit. The detection range is selected in a way to successfully quantify concentrations close to the risk level for the corresponding target biomarker. Hence, the results incorporating concentraions that are far below the risk level or far above the risk level, are indicated as lower than minimum detection limit or higher than maximum detection limit. Sensitivity shows the minimum detectable concentration. Accuracy shows how much the measured concentration values differ from the reported concentration values from the hospital. For this, first the measurement error is calculated by dividing the difference between the measured and reported values to the reported value. Then, calculated error percentage is subtracted from 100 to find the accuracy. To check the accuracy of the prototype device, concentrations of biomarkers reported by the medical center were included in the table. Measurement results taken by the PoC device may slightly deviate from the results reported by the medical center. Since the device is not competing with bulky, costly and slow laboratory techniques in terms of accuracy, color-coded results are used to compensate the possible small deviations of the measurement results. The average accuracy by including all test results not shown in Table I except the ones that are out of detection range is calculated as 91.2%. 10% interval around the predicted concentration value of the device is a safe interval, in which all reported values by the medical center falls into. Thus, if this 10% interval intercepts with threshold level of the target biomarker, the result is shown in yellow. If this 10% interval is fully above the threshold level, the result is shown in red. Finally, if the 10% interval is fully under the threshold level, the result is shown in green. These color-coded results prevent misleading of the patient and guarantees that the patient will have correct test result and compensates almost 10% accuracy problem. If the color-coded results show yellow or red, the patient can be advised for further detailed analysis.

V. Conclusion

A prototype point-of-care hand-held diagnostic device was developed to detect cancer and CVD target biomarkers. The device incorporates interdigitated capacitive sensors in a cartridge that can be inserted into the device. The device can detect multiple biomarkers simultaneously and display the results in less than 30 minutes. The cartridges can be pre-activated with the target antibodies and can be stored 3 months under optimum conditions. The prototype device was validated for 6 different target biomarkers as cancer and CVD risk indicators with more than 20 real patient blood samples. The results are compared with the reported results of the medical center and show that the developed device is a promising device that is suitable for fast, on-site and low-cost screening of patients for cancer and CVD. The device does not compete with advanced, expensive laboratory tests in terms of accuracy. The average

TABLE II: Specifications of the prototype PoC device

Parameter	Specification
Target Biomarkers[1]	BNP, Fibrinogen, Troponin I, TNF-α, SAA, IL-6
Accuracy[2]	91.2%
Sensitivity	Given in Table I for each target biomarker
Sample Volume	2-5 μL
Detection Time (min.)	<30
Number of sensors on a cartridge[3]	12
Dilution Ratios	Yes (1/10, 1/100 and 1/1000)
Averaging	50-1500 measurements
Connectivity	Wi-Fi, bluetooth
Display	2.8 inch (320x240) capacitive touch screen
Battery(Operation time)	2500 mAh Lithum-Ion (9 hours)
Software Language	Python
Device Dimensions	116x103x27 (mm)
Cartridge Dimensions	30x12 (mm) With base: 47x20x3 (mm)
Weight	230 gr

[1] Verified with real human blood samples
[2] Accuracy is calculated as the average of all test results including the ones that are not included in Table I excluding the ones that are out of detection range.
[3] One sensor is used for negative control. Remaining 11 sensors can be used for detection of different target biomarkers.

accuracy of the developed device is 91.2%. 10% possible error in the measurement is also corrected by the color coded results ensuring the accuracy of the tests. It is intended to be used in first level health facilities, areas that have limited access or no-access to advanced laboratory tests. Although the prototype device is validated for 6 target biomarkers with real patient blood samples, it also successfully measured the concentration levels of epidermal growth factor receptor 2 (EGFR2), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and insulin-like growth factor binding protein 2 (IGFBP2). However, they are not included in the work, since they are only tested with artificial biomarkers procured from related companies, not with real patient blood samples as related patient blood samples were not available at the time of this study. Specifications of the developed hand-held PoC diagnostic device are summarized in Table II. The prototype device uses Raspberry Pi and touchscreen to run the software and to display the results. However, it is compatible for smartphone integration to have a more compact device by connecting the sensitive readout electronics with smartphone through bluetooth or wireless connection.

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