Rapid and selective detection of *Staphylococcus aureus* using insulated substrate impedance transducers

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Abstract

We introduce a new insulated substrate impedance transducer (ISIT) for fast, direct, specific and sensitive bacteria detection. The transducer is composed of interdigitated array microelectrodes (IDAM) insulated on a Si substrate by a layer of SiO_2. The Al electrodes are protected by a thin layer of Al_2O_3. It is found that the coupling of target biomolecular charges with the Si substrate free carriers can be sensed capacitively by the insulated interdigitated electrodes, which makes the ISIT more sensitive than the classical IDAM sensor. This opens a new perspective for the future designs of high sensitivity sensors. Less than 240 cells of *Staphylococcus aureus* (*S. aureus*) bacteria immobilized on the ISIT sensing area of $200\mu m \times 200\mu m$, lead to significant changes on capacitance and conductance, which can be easily and directly detected in 10 min. The present ISIT also exhibits reproducible specific detection. We investigate the dependence of the ISIT sensitivity on its configuration. The results indicate that for achieving high sensitivity, the ISIT should have a suitable ratio of the interspacing width to the finger width and an interspacing width compatible with the size of the target biomolecule. Besides featuring fast, label-free and low-cost bacteria detection, this ISIT can be used for more applications and can be easily

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integrated with complementary metal oxide semiconductor circuits.

Key words: Insulated substrate impedance transducer; interdigitated array microelectrodes; metal-oxide semiconductor structure; sensor configuration and Staphylococcus aureus.

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1. Introduction

The rapid identification of bacteria from biological samples is a major goal in the human health care, food safety and environmental monitoring. Two main identification methods are actually used: the traditional culture associated with biochemical identification and the polymerase chain reaction (PCR) (Ristaino et al., 1999; Tooley et al., 1997). The former is time consuming, whereas the later requires expansive equipment and specialized personnel training. To circumvent these disadvantages, alternative methods (Bailey et al., 2002; Gough et al., 1999; Pamela et al., 1998) are currently in development. The authors in the article (Xue et al., 2009) proposed to detect *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) cells by CdSe quantum dots coupled with bacteria based on fluorescence microscope. However, this detection method is non-specific and quantum dots smaller than 5 nm are required.

Interdigitated array microelectrodes (IDAM) (Laureyn et al., 2001; Van Gerwen et al., 1998) are generally employed as an impedance or capacitance biosensor for label-free and sensitive detections of molecules immobilized on top of the electrodes due to the following advantages: high signal-to-noise ratio, fast detection and simple testing procedure (Varshney and Li, 2009). The IDAM is composed of a set of interdigitated finger electrodes. The electrodes are most usually made of noble metal materials: gold (Au) (Radke and Alocilja, 1995), platinum (Pt) (Iwasaki and Morita, 1995), titanium (Ti), chromium (Cr) and alloy (Pt-Pb) (Bai et al., 2008). Other materials, such as carbon (C) (Fiaccabrino et al., 1996; Rishpon and Ivnitski, 1995) and indium tin oxide (ITO) (Hayashi et al., 2005) have also been used as sensitive and transparent electrodes, respectively. In this study, aluminum (Al) is chosen to fabricate the IDAM due to its low cost. Furthermore, Al is the most common electrode material used in complementary metal oxide semiconductor (CMOS) technology. However Al reacts with, and is degraded by, biological and chemical solutions with respect to the noble metal electrodes mentioned above. To solve this problem, we introduce a

thin but dense layer of alumina (Al_2O_3) on the IDAM surface to protect Al from biological and chemical attacks (Moreno-Hagelsieb et al., 2007). The addition of the Al_2O_3 layer, which features a high dielectric permittivity, enables for accurately measuring inter-finger capacitance variations caused by coupling of the target molecules at the IDAM surface.

In order to possibly integrate the IDAM with CMOS circuits, the present IDAM is designed on a Si wafer and insulated by a layer of silicon dioxide (SiO_2). We find that charge or potential modifications due to target molecules or particles deposited on the IDAM surface can influence the space-charge region properties in the Si substrate through the SiO_2 dielectric, in similarity with the behavior of metal-oxide semiconductor (MOS) structures. Therefore, the present sensor can effectively monitor not only the IDAM capacitance change but also the Si substrate impedance change, which makes it more sensitive than the classical IDAM sensor. Hereafter, we call the present sensor as the insulated substrate impedance transducer (ISIT). Although it is believed that the ratio of the finger width to the interspacing width is a key to improve the sensitivity of IDAM sensors, quantitative studies for the size effect of the sensor on direct molecular detection have not been found in recent literature. Here we optimize the electrode configuration according to the size of target bacteria cells to reach high sensitivity. In this work, we use ISITs coated with LO-PRT (a rat monoclonal antibody anti protein A) to directly detect *S. aureus* bacteria, which is the leading cause of nosocomial infection worldwide (available from: www.cdc.gov/ncidod/dhqp/heathDis.htlm.). The results show that our sensor is able to discriminate *S. aureus* from *Staphylococcus epidermidis* (*S. epidermidis*) (Holmes et al., 1997).

2. Materials and methods

2.1. Fabrication process of ISIT

ISITs with different configurations are designed in this study. Table 1 lists finger widths and interspacing widths of three different configurations (noting them as sensors A, B and C). The three kinds of ISITs have the same detection area of $200\mu m \times 200\mu m$. A p-type Si wafer ($5 \times 10^{15} cm^{-3}$) is used as a starting substrate. After standard cleaning the wafer, a layer of SiO_2 with a thickness of 350 nm is grown at $1000°C$ by wet thermal oxidation to insulate the IDAM from the Si substrate (Pampin et al., 2008). On the insulating SiO_2 layer, the IDAM is built by resist spin-coating, photolithography, deposition of a layer of Al with a thickness of about 200 nm

and lift-off process. Then the Al electrodes are protected with a layer of Al_2O_3 by an electrochemical anodization, which is carried out using an ammonium pentaborate in ethyleneglycol based solution at a current density of 3 mA/cm^2. The Al_2O_3 layer provides not only a protection against biological and chemical erosion but also a surface suitable for the subsequent biochemical functionalization. The surface outside of the detection area is covered with a layer of resist (SU-8). A back-gate contact is formed by deposing a layer of Al on the backside of the Si substrate. Once the wafer process is completed, the wafer is saw-diced into pieces of $3mm \times 3mm$. One piece, including three different configuration ISITs and a reference device (which is a MOS structure) is finally bonded on a dual in-line package (DIP) through Al leads. In order to eliminate any interference and protect the Al leads (on-chip and off-chip) from being biochemically attacked and damaged during sensor testing, a layer of silicone is added to cover the chip extension and bonding wires. Thus only the sensing area is exposed to the detection environment. Figure 1a shows the top-view of an *as*-fabricated ISIT. The inset of the figure gives the cross-sectional scanning electron microscopy (SEM) picture for one of the fingers. The Al finger with a final thickness of about 80 nm is protected by an 80-nm thick Al_2O_3 layer.

2.2. Biological procedure

2.2.1. Coating of ISITs

The packaged ISIT is rinsed with phosphate-buffered saline (PBS) and coated with LO-PRA (a rat monoclonal antibody (MoAb) anti protein A), which is specifically expressed by *S. aureus*. The coating is performed during 12 h by incubating the ISIT with the LO-PRA diluted at 10 μg/ml in borate buffer pH 9. In such a way, the MoAb is coated on the ISIT surface and acts as specific tethers for holding target bacteria in the sequel (Yang, 2008). The correct coating of LO-PRA on the ISIT surface is confirmed by a sandwich imunodetection method using a secondary mouse anti-rat MoAb labeled with peroxidase (data not showed here). Figure 1b shows the surface morphology of one ISIT after coating the MoAb. The inset of the figure zooms on the surface of the insulating layers (Al_2O_3 and SiO_2). It is likely that there are some nano-scale defects on the surface of the insulating layers to allow small molecules such as antibodies to penetrate them, whereas the insulating layers prevent larger molecules (such as the *S. aureus* cells) from reaching the bare Al fingers.

2.2.2. Preparation of samples

The analyzable samples are prepared by overnight cultures of *S. aureus* or *S. epidermidis* in Luria Broth liquid medium. In this work, we prepare three groups of samples: (1) positive group, including *S. aureus* with different concentrations (1×10^9, 2×10^8 and 4×10^7 CFU/ml, CFU: Colony Forming Unit); (2) negative group, including *S. epidermidis* with a concentration of 1×10^9 CFU/ml; (3) control group, with culture medium alone.

2.2.3. Analysis of samples

A drop of the analyzable sample with a volume of 100 μl is spread over the ISIT surface for 5 min incubation to allow the specific binding of the *S. aureus* with the MoAb on the ISIT surfaces, then 3 washes with PBS and 3 washes with water are performed to eliminate the non bounded bacteria. The ISIT is next dried with nitrogen at room temperature. We use the so-called "dip-and-dry" technique (Skottrup et al., 2008), meaning that the interaction between MoAb and bacteria is performed in liquid, but the measurements are made after drying the sensor surface to avoid the parasitic capacitive signal of the liquid medium (Bratov et al., 2008). The last step is the electrical analysis. The small-signal alternating current (ac) parallel capacitance and conductance measurements are carried out by an LCR meter in the frequency range from 1 kHz to 1 MHz. This step only takes about 5 min.

Figures 1c and 1d demonstrate the surface morphology of the ISITs after the exposition to the negative and positive samples with a concentration of 1×10^9 CFU/ml, respectively. The clear surface with the negative sample indicates that *S. epidermidis* cells are not attached since they are non-specific for the MoAb and washed out. On the contrary, it is clear that *S. aureus* bacteria cells are well immobilized on the functionalized surface. The diameter of the *S. aureus* bacteria cells is in the range from 0.5 μm to 1 μm as expected.

The actual number of bacteria cells present on the ISIT surface is statistically calculated by using a image analysis software ('WCIF Image') based on SEM picture. Figure 2a shows a large-scale SEM image for an ISIT surface after immobilizing *S. aureus* bacteria with a concentration of 1×10^9 CFU/ml, while Fig. 2b is the filtered image for the actual number of the bacteria cells, which includes 1323 cells in an area of $115\mu m \times 85\mu m$. The same method is used to calculate the actual number of the bacteria cells for the positive samples with the concentrations of 2×10^8 and 4×10^7 CFU/ml. It is found that there is no linear relationship between the bacteria concentra-

tions and the actual number of *S. aureus* cells binding on the sensor surface. Specifically, the ratio of three concentrations (1×10^9, 2×10^8 and 4×10^7 CFU/ml) is 25:5:1, while the ratio of the number of the on-chip bacteria cells is here about 22:11:1. More importantly, for a given concentration, there is a difference in the actual number of *S. aureus* cells within the ISITs on the same chip.

3. Detection principle and sensitivity

3.1. Equivalent circuit and model of ISIT

For the sake of clarity, we first focus on one pair of finger electrodes. Figure 3a illustrates the schematic cross-section of an *as*-fabricated ISIT and its ac model. In the ISIT concept, an inversion layer is initially created at the SiO_2/*p*-Si substrate interface induced by positive charges existing in the oxide and/or by adequate choice of back-gate voltage (here, equal to 0 V). The global capacitance (C_{glo}) between two electrodes consists of the upper effective finger capacitance (C_{eff}) and two SiO_2 capacitances (C_{ox}) connected by the substrate electrical equivalent network. The C_{eff} is the series combination of the inter-finger capacitance through the upper medium with the protecting Al_2O_3 layer capacitance. At low frequencies, *i.e.* considering that the two C_{ox} are connected in series by the inversion layer, the global capacitance (C_{glo}) can be written as:

$$C_{glo} = C_{eff} + (\frac{1}{C_{ox}} + \frac{1}{C_{ox}})^{-1} \tag{1}$$

At high frequencies, when all the capacitances can be considered as short-circuits, the global resistance (R_{glo}) tends to R_{in}, which is the resistance of the inversion layer.

When bacteria with negative charges are immobilized on the ISIT surface, as shown in Fig. 3b, the inversion region under the interspacing will be reduced by a MOS capacitor effect and a highly-resistive depletion region will eventually replace the inversion region near the top surface of the Si substrate. As a first-order approximation, the electrical behavior of the bacteria can be modeled as a resistance R_{bac} and a capacitance C_{bac} connected in parallel. In this case, the global capacitance (C_{glo}) becomes:

$$C_{glo} = C_{eff} + C_{bac} + (\frac{1}{C_{ox}} + \frac{1}{C_{ox}} + \frac{1}{C_{dep}})^{-1} \tag{2}$$

Where C_{dep} represents the depletion region capacitance in the substrate. If the C_{bac} remains small and can be neglected, *e.g.* in case of low concentration, the global capacitance can be rewritten by:

$$C_{glo} = C_{eff} + (\frac{1}{C_{ox}} + \frac{1}{C_{ox}} + \frac{1}{C_{dep}})^{-1} \tag{3}$$

Comparing equations (1) and (3), the global capacitance of the ISIT after bacteria immobilization will be smaller than that of the *as*-fabricated one. In the case of the bacteria immobilization, the global resistance (R_{glo}) at high frequencies becomes:

$$R_{glo} = (\frac{1}{R_{bac}} + \frac{1}{R_{dep}})^{-1} \tag{4}$$

Where R_{dep} represents the resistance of the depletion region. Again if the R_{bac} is very large and the term of $1/R_{bac}$ can be neglected, which implies the case in a low concentration, the global resistance equals R_{dep}. Therefore, the global resistance of the ISIT after bacteria immobilization will be larger than that of the *as*-fabricated one since $R_{dep} >> R_{in}$.

3.2. Sensitivity of ISIT

The classical IDAM sensor is commonly fabricated on an insulating substrate, such as quartz and glass. Its working principle is to register the capacitance or resistance variations before and after the immobilization of the target biomolecules through the molecules themselves. Its reliability is limited by any conduction path between two fingers and its sensitivity is deteriorated by high ion concentration. As mentioned above, the Al electrodes of the present ISIT are protected by a thin layer of Al_2O_3. Importantly, the present ISIT is fabricated on a Si substrate insulated by a layer of SiO_2. It benefits from a MOS capacitor effect and actively uses the substrate conduction as a charge detector. Indeed, if geometrical parameters, such as the finger width, interspacing width and oxide thickness are properly designed, any target biomolecule with negative charge in case of p-type substrate, no matter where it locates between the fingers, will induce a significant change on the substrate capacitance and resistance by depleting a portion of the inversion layer. Therefore, the present ISIT can register the capacitance and resistance changes, not only from the finger electrodes, but also from the Si substrate. This implies that the present ISIT potentially has a higher sensitivity compared to the classical IDAM. In other words, the sensitivity of

the present ISIT is improved by intently adding charges in the oxide layer or adequately applying a back-gate voltage to induce an initial inversion layer in the substrate.

4. Results and discussions

4.1. Size effect of ISIT

Figure 4 (on left column) shows the global capacitance at frequencies from 1 kHz to 1 MHz for three different configuration ISITs in the bare state and after immobilizing the positive sample with a concentration of 4×10^7 CFU/ml. For three ISITs packaged on the same chip, the global capacitances are reduced after immobilizing *S. aureus* bacteria as expected. According to the explanation in the detection principle section, the capacitance change is larger at low frequency. This is mainly attributed to the formation of the depletion region underneath the interspacing on the top surface of the Si substrate, induced by the negative charges existing in the outer surface or inside of the *S. aureus* bacteria cells (Sonohara et al., 1995; Yang and Bashir, 2008). Particularly, the largest capacitance change is observed in sensor A. This can be explained by its configuration. We can find from table 1 that sensors A, B and C have the same detection area and interspacing width, but the finger of sensor A is the narrowest. This means that among the three sensors, sensor A features the largest SiO_2 surface to be covered by bacteria cells. Therefore, its capacitance can be modified more significantly by bacteria immobilization. Furthermore, sensor A also gives the largest signal to noise ratio, owing to the combined spherical and vertical diffusion fields from the equal widths of the finger and interspacing (Stulik et al., 2000). It is worth noting that bacteria on the Al_2O_3 surface do not give any modification to the capacitance measured through the substrate because of the screening effect from the Al fingers. Our results indicate that it is important for designing the sensor with high sensitivity to have a suitable ratio of the interspacing width to the finger width and an interspacing width compatible with the size of the target biomolecule. Figure 4 (on right column) also shows the global conductance at frequencies from 1 kHz to 1 MHz for the three different configuration ISITs in the bare state and after immobilizing the positive sample with a concentration of 4×10^7 CFU/ml. It can be found that the global conductance is kept to very low values at frequencies lower than 10 kHz and is increased above 10 kHz for the all cases (we call the frequency of 10 kHz as the cut-off frequency). As discussed above, at frequencies higher

than the cut-off frequency, the global capacitance impedance becomes smaller than the resistance and only a conductance is hence needed to represent the equivalent circuit of the ISIT. For the *as*-fabricated ISIT, the conductance is mainly contributed by the inversion layer. A larger conductance change is obtained in sensor A. The reason is the same as for the capacitance explanation. Importantly, the change of the global conductance is proportional to the frequency used in the measurements. This implies that the measurement sensitivity of the ISIT might still be improved by using higher frequencies.

Based on the reference device (a MOS structure), we calculate the positive charge density in the oxide by using capacitance-voltage (C-V) technique (Colinge and Colinge, 2002). A flat-band voltage of -3.4 V is extracted from the high frequency C-V curve of the reference device. Therefore, the positive charge density in the oxide is estimated to be $1.6 \times 10^{11} cm^{-2}$. This in turn can well explain why the inversion layer in the Si substrate here is formed at zero back-gate voltage. Specifically, the presence of these positive charges is analogous to applying a negative voltage in the back-gate of the substrate, which results in an inversion layer at the interface of the oxide/p-type Si substrate. After bacteria immobilization, the conductance is governed by the depletion region and hence significantly reduced.

4.2. Background, selectivity and reproducibility of ISIT

To test the background and selectivity of the present ISIT, we spread the negative sample on the ISIT surface. Figure 5 shows the global capacitances and conductances at frequencies from 1 kHz to 1 MHz for three different configuration ISITs in the bare state and after exposition to negative sample with a concentration of 1×10^9 CFU/ml. Negligible capacitance and conductance changes are observed in the wide frequency range for all the ISITs. This is in agreement with SEM observation (seen Fig. 1c), specifically, there is no *S. epidermidis* on the ISIT surface. Moreover, the global capacitance and conductance as a function of frequency for the ISIT with MoAb only (not shown here) is nearly the same as that shown in Fig. 5. This can be explained by the fact that the size of the antibody or the number of the charge in antibody is too small (Tang et al., 2009) so that its effect on the capacitance and conductance can hardly be measured. We can conclude that the present ISIT has a high selectivity and low background. In addition, many ISITs with different configurations have been used to test the negative and positive samples with different concentrations. The capacitance and conductance changes are observed in all the ISITs with the binding of positive

samples. This implies that our sensors also have a good reproducibility.

4.3. Detection limit of ISIT

As mentioned in section 4.1, sensor C has the smallest bacterial coverage surface in all the configurations. We chose it to test the detection limit of our ISITs in the worst case. For this purpose, we immobilize the positive samples on one group of identical ISITs and perform the assay on a series of concentrations. Figure 6 shows the global conductance change (ΔG) and capacitance change (ΔC) at 1 MHz after binding positive samples with concentrations of 1×10^9, 2×10^8 and 4×10^7 CFU/ml, respectively. The ΔG and ΔC of the ISITs with the control samples and with the MoAb only are also shown in the figure. It can be observed that there is no significant ΔC difference between the ISITs with the control samples and the ISITs treated with a low concentration of 4×10^7 CFU/ml. However, an obvious ΔG difference appears which enables to clealy distinguish them in the figure. According to the equivalent circuit analysis in the ISIT model, the capacitance change at low frequencies is larger than that at high frequencies. On the contrary, the conductance change at high frequencies is larger than that at low frequencies. The experimental results confirm the theoretical prediction since the measurements are carried out at the high frequency of 1 MHz.

It can be seen that ΔG and ΔC increase with the increase of the bacteria concentration. We find that for a given concentration, there is a deviation in the global conductance and capacitance changes for identical sensors. This can be explained by the differences of the actual number of bacteria cells immobilized on the sensor surface, which is caused by variations of the affinity and kinetics of the biomolecular recognition, as well as binding process. Specifically, although the same analyzable sample is spread on identical ISITs, the actual number of bacteria cells present on each ISIT surface is quite different, which is confirmed by the SEM pictures. Therefore, this makes it difficult to exactly draw a conclusion about the ISIT detection limit in terms of concentrations. It is better to express the detection performance in terms of the actual number of bacteria cells immobilized on the surface, as will be discussed in the next section. In any case, an obvious boundary line can be drawn to distinguish the ISITs with the control samples from the ISITs treated with a low concentration of 4×10^7 CFU/ml in the figure thanks to the method of the ΔG and ΔC representation. This method presents both changes (capacitance and conductance) of the ISIT in one figure. Finally, it

is important to note that the ΔG and ΔC for the sensor with the MoAb only do not show significant difference from the sensors with the control samples.

4.4. Capacitance and conductance contributions of each bacteria

Based on SEM observations of the actual number of bacteria cells present on the sensor surfaces, we calculate the capacitance and conductance contributions of each bacteria cell for the ISIT after binding positive sample with a concentration of 4×10^7 CFU/ml. For sensor A, the capacitance change (ΔC) of 6.04 pF and the conductance change (ΔG) of 1.32 μS at the measurement frequency of 1 MHz are obtained from Fig. 4, respectively. The total number (N_{tot}) of bacteria cells immobilized on the sensor surface of $200\mu m \times 200\mu m$ is counted to be 240 cells by SEM. The effective number (N_{eff}) of bacteria cells contributing to capacitance change and conductance change (*i. e.* immobilized between two adjacent fingers, not on top of them) is estimated by $N_{eff} = N_{tot}W_{spa}/(W_{spa} + W_{fin})$, here W_{spa} and W_{fin} are the widths of the interspacing and finger, respectively. With $W_{spa} = W_{fin} = 2\mu$m, only 120 cells here cover the surface of the SiO_2 layer and influence the global capacitance and conductance changes. Therefore, the capacitance contribution per bacteria ($\Delta C/N_{eff}$) is about 50 fF. The conductance contribution per bacteria ($\Delta G/N_{eff}$) is about 10 nS. This value is 3 orders of magnitude higher than that (2.5 pS) reported in the reference (Lu et al., 2008) in which the authors used IDAM for *Escherichia coli* detection. Our results show that the free charges existing in the substrate play a very important role in signal amplification and the ISIT has high potential promise for single bacteria detection. However, in this work, the detection limit is 4×10^7 CFU/ml due to the poor affinity between the bacteria and the sensor surface. This means that although a droplet *S. aureus* with a concentration of 4×10^7 CFU/ml is spread on the sensor, only small amount of bacteria is really immobilized on its surface. This problem could be solved by means of various strategies, such as developing an antibody with a higher affinity constant, using a mixture of MoAbs with different epitopes, exploring a sandwich system with coating streptavidin (a high tetravalent receptor for biotin) and labeling the bacteria with a biotinylated MoAb. However, the detection time of our sensor to obtain the final results is less than 10 min, which is at least 30 min faster than Xue's method (Xue et al., 2009) and 10-20 times faster than the conventional plate count.

5. Conclusions

We demonstrate a new microsensor, here named as insulated substrate impedance transducer (ISIT) for rapid, sensitive and specific *Staphylococcus aureus* bacteria detection. The ISIT consists of interdigitated array microelectrodes (IDAM) insulated on a Si substrate by a dielectric layer. The Al finger electrodes are covered by a protecting layer of Al_2O_3. The addition of the Al_2O_3 layer avoids Al deterioration by chemical and biological erosion in solution and improves the capacitance measurement sensitivity by featuring high dielectric permittivity. In the *as*-fabricated ISIT, the SiO_2/Si substrate interface is initially biased in inversion by charges existing in the SiO_2 layer or applying a back-side voltage. We provide proof-of-concept that the charges of the target biomolecules can be mirrored into the Si substrate by a MOS capacitor effect, thereby modulating the space-charge region of the Si substrate, from inversion to depletion. Therefore, the ISIT can monitor the changes of capacitance and conductance as a function of excitation frequency, not only from the IDAM but also from the Si substrate. Very significant detection signals have been obtained for about 240 bacteria immobilized on the sensor surface, with a capacitance contribution par bacteria of 50 fF and a conductance contribution par bacteria of 10 nS. This makes the present ISIT more sensitive than the classical IDAM sensor directly fabricated on a purely insulating or passive substrate as well as other previously publicated microsensors. This opens a new perspective for the future designs of high sensitivity sensors. Specifically, we can intently add charges into the SiO_2 layer or apply a bias at the backside of the Si substrate to couple charged biomolecules on the sensor surface with the substrate charges for producing more sensitive and specific detection events or signatures. Our sensor exhibits reproducible specific detection for positive samples and no false response for negative samples. The detection time of our ISIT to obtain the final results is less than 10 min. We investigate the dependence of ISIT sensitivity on its configuration. The results show that for achieving high measurement sensitivity, the ISIT should have a suitable ratio of the interspacing width to the finger width and an interspacing width compatible with the size of the target biomolecule. For example, for cells, such as the *S. aureus* bacteria with a diameter of about 0.5 μm, it is better to choose a interspacing width of about 2 μm for effectively immobilizing the biomolecules between two adjacent fingers. Moreover, for a given detection area, the narrower the finger is, the larger the signal-to-noise ratio. Besides

bacteria detection, our sensor with low cost and fast detection time is now ready for more applications.

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FIG. 1. (Color online) Morphology of ISIT surface. (a) *as*-fabricated ISIT, the inset shows the cross-section of one electrode. (b) ISIT coated with MoAb only. (c) ISIT after exposition to *S. epidermids* and (d) ISIT after immobilizing *S. aureus* cells.
FIG. 2. (Color online) Actual number of *S. aureus* cells. (a) Large-scale SEM picture of ISIT surface with immobilizition of *S. aureus* with a concentration of 1×10^9 CFU/ml and (b) the filtered image of (a).
FIG. 3. (Color online) A schematic cross-section of ISIT. (a) *as*-fabricated ISIT showing IDAM insulated on a Si substrate by a layer of SiO_2 and an inversion layer induced by charges existing in oxide layer. (b) ISIT after immobilizing *S. aureus* cells, showing a depletion region underneath the interspacing induced by the presence of bacteria cells with negative charges on ISIT surface.
FIG. 4. (Color online) Global capacitance and conductance at frequencies from 1 kHz to 1 MHz for three different configuration ISITs before and after immobilizing *S. aureus* bacteria with a concentration of 4×10^7 CFU/ml, respectively.
FIG. 5. (Color online) Global capacitance and conductance at frequencies from 1 kHz to 1 MHz for three different configuration ISITs before and after exposition to *S. epidermidis* bacteria with a concentration of 1×10^9 CFU/ml, respectively.
FIG. 6. (Color online) Global conductance and capacitance changes for the ISITs with MoAb only, with control samples and with *S. aureus* with concentrations of 1×10^9, 2×10^8, 4×10^7 CFU/ml, respectively.

Sensor name	Finger width W_{fin} (μm)	Spacing width W_{spa} (μm)	Detection area (μm^2)
Sensor A	2	2	200×200
Sensor B	5	2	200×200
Sensor C	10	2	200×200

Table 1: Configurations for three kinds of ISITs including finger width, interspacing and detection area.