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Optimization & Characterization of Interdigitated Electrodes for Microbial Growth Monitoring

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Abstract— **This study optimally designed and implemented highly sensitive microscale interdigitated electrodes (IDEs) to monitor microorganisms' growth in diverse environments. Gold interdigitated electrodes (AuIDE) with 4 mm×4 mm effective sensing area and varying microscale interdigitate gaps were designed and fabricated. The electrodes were electrically characterized voltametrically. Electrochemical impedance spectroscopy (EIS) measurements were conducted to determine the optimal geometry by observing the impedance spectra of microelectrodes through varying pH and temperature. Furthermore, the sensors sensitivity was evaluated by measuring the impedance properties of a microscale volume of microorganism concentrations in growth media solution.**

Keywords—Gold Interdigitated Electrode (AuIDE), Impedance Spectroscopy, pH, Microorganism, Temperature.

I. INTRODUCTION

Understanding the impact of global warming and human activities on ecosystems and their influences on environment and human health is one of the most critical challenges of our time. To this end, characterization, identification, and monitoring of the growth and metabolic activity of sentinel microorganisms could provide invaluable insights into the environmental changes [1]. Nowadays, biosensors play an essential role in human health care, environmental monitoring, and real-time characterization of bacteria for clinical diagnosis [2]. Electrochemical biosensors provide an effective and rapid method for microbial culture monitoring due to their accuracy, simplicity, and real-time measurement capability. They take their main advantages from miniaturization and complex integration with MEMS and microelectronics. Electrochemical microsensors are capable of monitoring microbial activities in a non-invasive, label-free, fast, highly sensitive, and real-time way. Moreover, lightweight and inexpensive microscale biosensor-based systems allow easy transportation in remote areas.

In the design of electrochemical biosensors, much attention has been paid to interface circuit design [3]. The optimal design and scaling of an electrode transducer is another critical aspect to improve the sensitivity of measurements. In microbiology applications, microelectrodes miniaturization can provide high-throughput sensing and allow for direct or indirect sensing of single-cell detection, cell counting and bacterial growth monitoring. Rectangular Interdigitated electrodes (IDEs) set in a parallel comb-shaped electrode configuration on an insulating layer are used in a wide range of biosensing applications. The main advantages of interdigitated structures are their ease of miniaturization and integration with microelectronics for lab-on-chip applications, low-cost manufacturing process, high sensitivity and high signal-to-noise ratio (SNR) [4]. IDE design must address specific requirements and follow guidelines. For instance, choosing a biocompatible material such as gold, could increase the selectivity and sensitivity of electrochemical measurements [5]. Besides, determining the appropriate gap size among electrode fingers could also increase the sensitivity of electrochemical sensors and reduce the sample volume needed.

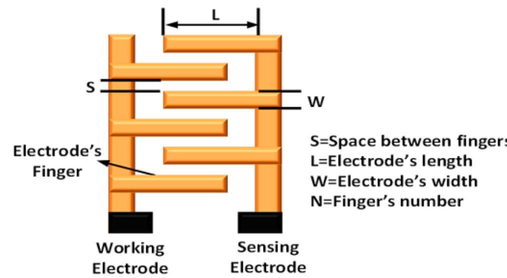

Figure 1. Structural parameters of the IDEs.

This work investigates the optimal geometry and scale of IDEs to increase the sensitivity of electrochemical sensors in microorganism growth monitoring applications. In the present study, three rectangular, comb-shaped gold IDEs (AuIDEs) with different gap sizes among the digitated fingers (i.e., to accommodate different spatial resolutions) are designed using theoretical optimization formulas seeking the best metallization ratio and fabricated on a printed circuit board (PCB). The design, fabrication and performance measurement results are presented. First, the electrode's conductivity in buffer solutions with varying pH on the sensing surface is measured. Second, the impedance magnitude is measured across pH variation in the sample solutions. Third, the temperature effect on the measured impedance of each IDEs is presented at different temperature points. Lastly, the sensitivity associated with different electrode geometries and reading frequencies is assessed by electrochemical impedance spectroscopy (EIS) of bacterial cell measurements. The

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(a) (b) (c)

Figure 2. Microscopic image of gold plated IDEs: (a) 75 µm, (b) 150 µm, (c) 200 µm gaps between the electrode's fingers.

experimental results are reported and discussed. In sum, we investigate an optimal IDE scaling to maximize the sensitivity of EIS measurements toward bacterial growth monitoring applications.

II. ELECTRODES MODELING

A. Theory and Design Optimization

IDEs are implemented by interdigitating multiple parallel fingers of *sensing* and *working electrodes* together. Figure 1 shows a typical rectangular IDE with its structural parameters. The geometric parameters of IDEs can considerably impact the sensitivity of biosensors; hence, the optimized design of the dimensions and geometric parameters of IDEs is essential to increase the efficiency of biosensors in different applications [6]. The optimized structural parameters of the interdigitated transducers, such as length (L), finger–finger space (S), width (W), number of fingers (N), and metallization ratio (α), are calculated and applied using the optimization rules reported in [7]. They are shown in equations below for configuration of three different sensors within a 4 mm×4 mm rectangular sensing area:

$$W = 3 \times S/2 \tag{1}$$

$$S = L/(5 \times N/2) - 1 \tag{2}$$

$$L = N \times (W + S) - S \tag{3}$$

$$\alpha = W/(S + W) \tag{4}$$

B. Design and Fabrication

Three different patterns of rectangular interdigitated microelectrodes with the same sensing area (4 mm×4 mm) and various gap sizes between the comb-like fingers are designed. The spaces between two digits are chosen on the basis of the optimized parameters mentioned above and the minimum scale limitation given by standard flexible or rigid PCB manufacturers. The gaps between the electrode's fingers are 75, 150, and 200 µm. The length and width of the electrodes are 3800 mm and 200 µm, respectively. The number of fingers varies to cover all the sensing surfaces. Photos of the fabricated AuIDEs on a flexible PCB rigidized with a stiffener layer are shown in Figure 2. Three different gold-plated IDE sensors with 4 mm×4 mm active sensing area are designed and fabricated on a two-layer standard flexible PCB with 0.2 mm thickness. The top layer of the PCB, except the sensing area, is covered using a 1.5 mm FR-4 stiffener. The utilization of a flex-PCB rigidized using a stiffener has advantages, such as inexpensive rigidity, weight and space reduction, thermal management, and reliability, over rigid PCBs.

TABLE I. IDEs GEOMETRIC PARAMETERS

Sensor	*L (µm)*	*S (µm)*	*W(µm)*	*N*	*αRatio*
(S1)	3800	75	200	14	0.72
(S2)	3800	150	200	12	0.57
(S3)	3800	200	200	10	0.50

III. EXPERIMENTAL RESULTS AND DISCUSSION

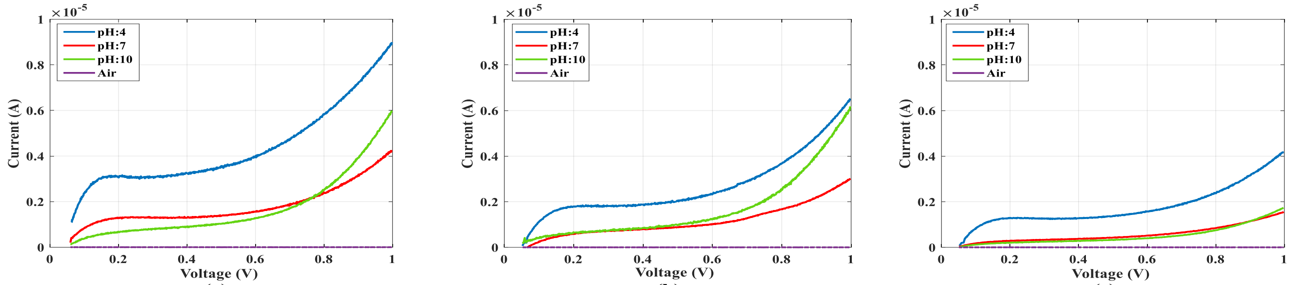
A. Cyclic Voltammetry Measurements

In this experiment, cyclic voltammetry (CV) was performed. A DC voltage was set on the electrodes, and the DC output current was measured as an indication of an ionic concentration. The measured DC current can be plotted as a function of the applied DC voltage as an I–V curve. The electrical characterization and stabilities of the three AuIDE sensors were verified by measuring the I–V curves with a Princeton Applied Research VersaSTAT3 Potentiostat from AMETEK (Scientific Instruments, UK) in a total voltage from 0 to 1 V. Voltammetric measurements were performed at room temperature after dropping 50 µl of three buffer solutions with pH values of 4 (acidic), 7 (neutral), and 10 (alkaline) on the sensing area of each IDE. First, the tests were conducted on the bare electrodes exposed to air to confirm that sensors were electrically stable and there no short circuit existed inside the IDEs. The results in Figure 3 showed only a slight current variation in the order of 10^{-6} A for all bare IDE sensors. Secondly, a voltammetry technique was applied to each sensor for solutions with various pH levels.

The experimental results shown in Figure 3 indicated that the current values correlated with the pH variation in each IDE configuration. The solutions with pH 4 and 10 had higher conductivity at 1 V than the neutral solution with pH 7 for all sensors. This behavior could be explained by the presence of the hydroxide (OH^-) and hydrogen (H^+) ions in the alkaline and acidic solutions, respectively. H^+ in acidic solutions had high ion mobilities, which caused high conductivity [5]. As shown in Figure 3, the current had a high value in electrodes with a smaller gap between the electrode fingers and a high metallization ratio (α) due to the increase in the active sensing area of IDEs. The results showed that the electrodes with smaller gap sizes (75 and 150 µm) were more sensitive to pH variation.

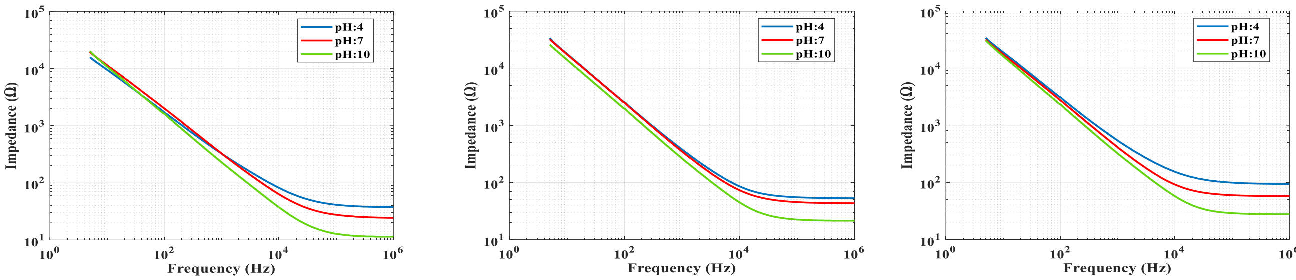
B. EIS versus pH Variation

EIS measurements were performed to assess the conductivity of the electrodes versus pH variation. The impedance measurements were carried out with an impedance analyzer (E5061B, Keysight). Three solutions with different pH values of 4, 7, and 10 were used in this experiment. All the measurements were performed in the frequency of 5 Hz to 1 MHz at 2 min intervals with 50 µl volume of a buffer solution. The impedance magnitudes of the pH buffers were obtained and plotted against frequency for all IDE configurations . The results are displayed in Figure 4. The results show that when the electrode's gap is decreased, the impedance value is



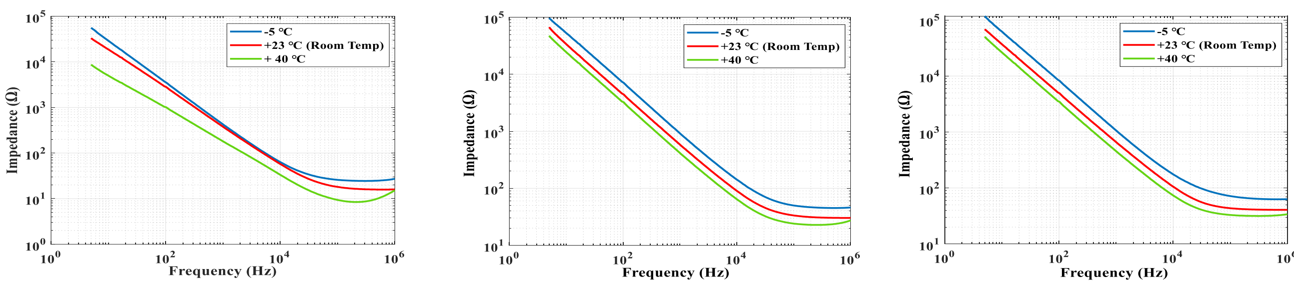
(a) (b) (c)

Figure 3. I-V measurements of IDEs at different pH values, (a) 75µm, (b) 150µm, and (c) 200µm gaps.



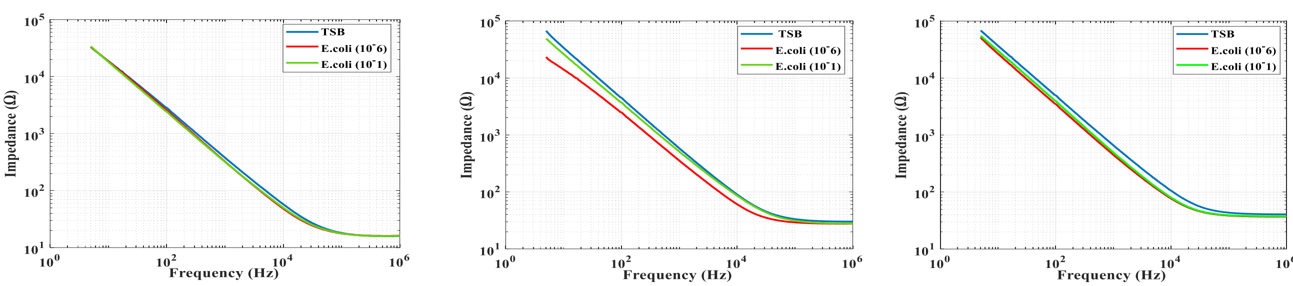
(a) (b) (c)

Figure 4. Impedance measurements for different pH buffer solutions on IDEs, (a) 75µm, (b) 150µm, and (c) 200µm gaps.



(a) (b) (c)

Figure 5. Effect of the temperature variation on impedance spectra of TSB solutions, (a) 75µm, (b) 150µm, and (c) 200µm gaps.



(a) (b) (c)

Figure 6. Impedance spectrum of *E. coli* cell suspensions at different concentrations in TSB, (a)7 5µm, (b) 150µm, and (c) 200µm gaps.

decreased in all pH buffers due to a higher α ratio. In the three IDEs configurations, the impedance magnitude of all pH solutions decreased with increasing frequency. The increase in the impedance value at low frequencies is known as the double-layer capacitance effect. This behavior is caused by biomolecules acting as dielectrics, and reducing the gap size between the electrodes and causing impedance augmentation. The measured data in Figure 4 clearly showed that the impedance values followed the behavior explained above in electrodes with 150 and 200 µm gaps in all frequency ranges. However, in electrodes with 75 µm space between the fingers, no evidence of this effect was obtained at low frequencies. Thus, at low frequencies (less than 10 kHz), electrodes with a higher spatial resolution are more sensitive to pH changes.

C. EIS versus Temperature Variation

EIS measurements were performed by dropping 50 µl of Trypticase soy broth (TSB) on the IDE surfaces. The impedance spectra was measured using a Keysight E5061B impedance analyzer over a frequency range of 5 Hz to 1 MHz. Impedance values were measured at −5 °C, 23 °C (room temperature), and +40 °C to evaluate the effect of temperature variation while the IDEs were located inside a Tenney environmental chamber, model SPX. The measured impedance spectra against temperature are shown in Figure 5. According to obtained results, the impedance magnitude increased when the space between the electrode fingers increased. Moreover, a negative correlation existed between the increase in temperature and the impedance value. As temperature increased, the impedance value decreased due to increased ionic activity. The same behavior was observed in all IDE configurations. However, in microelectrodes with a 75 µm finger pitch, the impedance variation was more severe at high temperatures (+40 °C) rather than at low temperatures, such as at −5 °C. By contrast, a minimal difference existed between the impedances at +40 °C and 23 °C. The impedance changes due to the temperature variation in the environmental chamber were almost identical for the IDEs with 150 and 200 µm interdigitated gaps. Overall, the IDEs with large gaps (e.g., 150 and 200 µm) were higher sensitive to temperature variation.

D. EIS of Bacterial Cell Suspensions

The Keysight impedance analyzer was first used to measure the impedance of TSB as the baseline value. Then the impedance values of two different concentrations of *Escherichia coli* B (HER 1024) suspension in TSB were measured. A culture of *E. coli* was grown overnight at 37 °C in TSB. The culture was serially diluted from 10^{-1} to 10^{-6}. The 10^{-1} dilution was used as a positive control, and the 10^{-6} dilution was used for tests. EIS was performed within a frequency range of 5 Hz to 1 MHz. All measurements were performed at room temperature, with 50 µl volume of sample solutions, after dropping. The measured impedances for the IDEs with different microgaps are shown in Figure 6. The sensitivity of the IDEs was derived by comparing the impedance magnitude of the IDEs with the presence of bacteria (*Zs*) in the solution with the impedance magnitude (*Zi*) of the TSB without any bacterial cells [8], by using the equation below:

$$\%Sensitivity = \frac{Z_s - Z_i}{Z_i} \times 100 \quad (5)$$

As can be seen in Table II, the sensitivity of all electrode patterns was calculated for the impedance magnitude measured for an *E. coli* dilution of 10^{-6} at four different frequencies between 100 Hz and 100 kHz. As shown in Figure 6, impedance was correlated with bacterial concentration in all three IDE configurations. The impedance of the culture with 10^{-1} dilution was higher than that of the culture with 10^{-6} dilution due to the higher bacterial concentration. Consequently, the curves in Figure 6 and Table II show that the electrodes with 75 and 200 µm gaps were minimally sensitive to bacterial concentration. The IDE configuration with 150 µm spatial resolution and 0.57 metallization ratio (ɑ), which was close to the optimum value of 0.6 reported in [9], could characterize and measure the impedance variation caused by bacterial concentration with a high measurement sensitivity.

TABLE II. SENSORS SENSITIVITY FOR 10^{-6} *E. COLI* CONCENTRATION

f (Hz)	*S1% (75µm)*	*S2% (150µm)*	*S3% (200µm)*
100	9.60	44.71	29.67
2K	15.53	37.53	31.49
10K	16.11	32.51	27.14
100K	2.22	12.34	10.85

IV. CONCLUSION

AuIDEs with different microgaps and 4 mm×4 mm sensing surfaces on PCB were designed and fabricated. The IDEs' characteristics were assessed using optimal design criteria. CV and EIS were used to test the optimized IDE configurations by measuring the current and impedance in a variety of environmental conditions, such as pH and temperature. The current measured under different pH showed that the lower microgaps were highly sensitive to pH variation. On the other hand, the impedance measured under different temperatures showed that the AuIDEs with larger microgaps were much more sensitive to temperature variation. Finally, EIS was used to test the bioimpedance of *E. coli* dilutions to find the most optimized and responsive IDE configuration for bacterial growth monitoring. The proposed IDE, with a 0.57 metallization ratio and a 150 µm gap, was found to have high reliability through critical variables, such as pH and temperature, as well as high sensitivity in different bacterial concentrations. To summarize, although reducing the gaps between the electrode's finger may improve the sensitivity of the measurements, an optimized design trade-off is necessary between miniaturized IDE's geometric parameters and choosing the precise gap size and ɑ ratio in the IDE's effective sensing area to increase EIS measurements sensitivity. A customized CMOS-integrated chip for highly sensitive EIS measurement of bacterial cell suspensions will be used in future experiments.

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