Optimization of Interdigitated Electrode (IDE) Arrays for Impedance Based Evaluation of Hs 578T Cancer Cells

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Abstract. This paper examines the effect of electrode width and spacing of interdigitated electrodes (IDEs) for impedance-based cancer detection and characterization. IDEs are desired for bioimpedance measurements because their fabrication process is simple and inexpensive, and the geometry presents a potential for improved sensitivity over other microelectrode designs. Optimizing the geometry will eliminate this problem and increase the sensitivity of these devices for bioimpedance measurement applications. This paper evaluates the effect of IDE geometry on the sensitivity of breast cancer cell impedance measurements. Equivalent circuit data analysis was conducted to quantify and characterize the cells.

1. Introduction
Increasing demand for faster and more accurate diagnostic procedures has sparked trends in the development of MEMS devices for biosensing and medical diagnostics. MEMS are desirable for use within medical diagnostics for their ability to manipulate and analyze small volumes of biological materials. Biosensors can be incorporated into portable lab-on-a-chip devices which will be able to quickly run diagnostics on samples. These techniques improve on current cancer detection techniques because they do not require surgery or chemical tags and can shorten the time to diagnosis.

Bioimpedance-based sensors offer an inexpensive, label-free solution for MEMS-based medical diagnostics and cell and tissue research which will enable non-invasive, real-time monitoring. Bioimpedance monitoring has already been employed to study cellular kinetics [1], cancer drug screening [2], and cellular adhesion [3]. One of the most important factors to be considered when designing cellular impedance sensors is electrode geometry.

Currently, interdigitated electrodes (IDEs) are implemented in various sensing devices including surface acoustic wave (SAW) sensors, chemical sensors as well as current MEMS biosensors [4]. IDEs have been optimized for a variety of sensing applications including biosensors sensors, acoustic sensors, and chemical sensors; however, optimization for cancer cell detection has yet to be reported. The output signal strength of IDEs is controlled through careful design of the active area, width, and spacing of the electrode fingers. Min et al., for example, examined the relationship between signal to noise ratio and different aspects of IDE geometry and composition for optimizing oxidation and reduction reactions of potassium ferro/ferrihexacyanide. It was found that increasing the number of electrode fingers and their finger widths yielded a proportional increase in overall signal. Also, it was found that increasing electrode thickness yielded an increase in current and overall signal with small increases in signal noise. This increase in current is thought to be due to increased surface area [5]. Another example of IDE optimization was performed by Radke et al. in [6]. Through simulations, it was found that the optimal IDE electrode finger width and spacing were 3 and 4um,
respectively, for enhanced detection of bacteria. Radke et al. demonstrated that careful design of the electrode spacing influenced the penetration depth of the electric field thus concentrating it around the captured bacteria instead of the bulk solution.

Research indicates that currently, a design model for IDEs with an application in cancer cell detection and characterization does not exist. Because design criteria for IDEs vary by application a design rule for cell monitoring and characterization must be provided in order to maximize the sensitivity of bioimpedance measurements. In order to address this need, microelectrode devices were fabricated with constant electrode finger widths and varying electrode finger spacings of 15, 30 and 60 microns. Bioimpedance measurements were then made on carcinoma cells deposited on these devices. This paper examines the impact of varying electrode spacing on the measured bioimpedance of the cancerous cellular monolayer.

2. Materials and Methods

2.1 IDE Fabrication

Gold IDEs were patterned on 4-inch glass substrates using photolithography and metal deposition techniques. Fig. 1 illustrates the fabricated interdigital electrodes. First the wafers were cleaned using acetone and methanol. Next, the interdigital electrodes and contact pads were patterned using 3000PY photoresist. A thin layer of chromium (15nm) then gold (20nm) was electron beam evaporated onto the substrate. The photoresist layer was then removed using acetone and ultrasonic agitation. Finally, a passivation layer was patterned using SU-8 photoresist. This step leaves only the electrodes and contact pads exposed. This layer of photoresist is then hard baked to create an inert polymer resin. The devices were then diced and 6mm cultivation chambers were attached and sealed using slowly heated photoresist around the outer edges of the chamber.

2.2 Cell Culturing Protocol and Sample Preparation

HS 578T breast tumor cells (ATCC) were cultured at 37°C and 5% CO₂ in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10 ng/ml epidermal growth factor (EGF), gentamicin, and 10% fetal bovine serum at final concentration. The cells were subcultured at 95% confluence and medium was changed every 2-3 days. Devices were cleaned with detergent, sterilized using 70% IPA and rinsed with Dulbecco’s Phosphate Buffer Saline (DPBS). Next, the devices were precoated with .02% gelatine solution. The solution was allowed to coat the electrodes for 20 minutes and removed prior to cellular inoculation. Cells were trypsinized using a 25% trypsin solution and harvested. Devices were then inoculated with approximately 134ul of cell suspension with a concentration of 14.67 x 10⁵ cells/ml. 200ul of medium was then added to the culture chambers. A minimum incubation period of 24 hours was allowed to ensure proper adherence of the cells to the electrodes prior to impedance measurements.

2.3 Impedance Testing Procedure

Impedance measurements were made using an Agilent 4294A impedance analyzer. A voltage of 10mV was applied to the contact pads using gold probe tips. No DC bias was applied and impedance magnitude and phase were collected over a frequency range of 100 Hz to 10 MHz. Standard calibration of the system was done using protocol from the manufacturer. Baseline measurements
were performed first using only medium, followed by cellular impedance measurements. The increase of impedance was observed as well as the phase shift.

3. Results and Discussion

Measurements were first performed with medium only in the devices. Figure 2 is a bode magnitude plot of the devices with spacings of 15, 30, and 60 µm. The slope, covering the majority of the frequency spectrum, is characteristic of the double layer capacitance at the electrode interface, caused by the absorption of ions/molecules from the medium onto the surface of the electrodes. At the high frequency end, a plateau begins to form, which is representative of the solution resistance. It can be seen from the plot that the devices with larger spacings have the greatest impedance. This is expected because the total electrode area exposed to medium is less than that of the devices with smaller spacings. Equation (1) is a simplified equation for theoretical calculation of the double layer capacitance. When the surface area increases, the capacitance increases, and the impedance decreases, since it is inversely proportional to the capacitance: \( C = \frac{\varepsilon_0 \varepsilon \cdot A}{d} \). The solution resistance also increases with increased device spacing, in accordance with the simplified equation: \( R_s = \frac{\rho l}{A} \).

As the spacing between the IDE fingers increase, the solution resistance should also increase; as demonstrated in the bode magnitude plot in Figure 2 and in Table 1. Parameters were extracted using a constant phase element (CPE) and resistor in series, representing the double layer capacitance and the solution resistance.

![Figure 2: Bode magnitude plot of medium across IDEs with different spacings](image)

**Table 1:** Extracted parameters for medium and cells, and the difference between the measured data

|      | Medium  | Cells   | Diff |
|------|---------|---------|------|
|      | Rs (Ω)  | Cdl (F) | ndl  |
| 15um spacing | 37.1 | 1.61E-07 | 0.91 |
| 30um spacing | 77.4 | 8.29E-08 | 0.90 |
| 60um spacing | 155 | 7.83E-08 | 0.90 |
|      | Rs (Ω)  | Cdl (F) | ndl  | Rs (Ω) |
| 15um spacing | 223 | 1.63E-07 | 0.91 |
| 30um spacing | 198 | 9.56E-08 | 0.88 |
| 60um spacing | 154 | 1.42E-07 | 0.83 |
With the presence of cells, the solution resistance increases, as seen in Figure 3 and Table 1. The ‘diff’ column in Table 1 shows the calculated difference between measurements with and without cells. The largest change in resistance is seen in the 15µm-spacing devices. This experimentally validates Radke’s theory [6] that smaller IDE electrode spacing (on the order of cell size) is more sensitive to the presence of cells due to the concentrated penetration depth.

4. Conclusion
It can be seen from the results that the measured solution resistance increases along with the effective area of the interdigitated electrodes. By decreasing the electrode spacing the effective electrode area is increased causing an increase in the sensitivity of the device. This increase in sensitivity can be utilized to more accurately quantify modifications within cellular culture environment due to contamination, the introduction of experimental compounds and population changes.

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