A Microfabricated Electronic Microplate Platform for Low-cost Repeatable Biosensing Applications

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Abstract

This work presents a disposable 'electronic microplate' (e-microplate) platform that enables the reusability of the corresponding CMOS biosensor thereby reducing cost and increasing throughput relative to non-disposable systems. The e-microplate utilizes mechanically-flexible interconnects (MFIs) and through-silicon-vias (TSVs) to electrically interconnect the electrodes on the CMOS biosensor to the electrodes on the e-microplate while maintaining a physical separation between the aforementioned substrate tiers. Measurements performed show that the incorporation of the e-microplate does not degrade the sensing amplifier's gain, 3dB bandwidth, or the input referred noise, and thus ensures the accurate sensing of weak signals from living cells under test. Impedance mapping for saline and Dulbecco's Phosphate Buffered Saline (DPBS) solutions performed through the e-microplate confirm functional accuracy of the assembled system.

Introduction

CMOS biosensors are increasingly being used for sensing electrical, magnetic, and optical properties of cellular and molecular samples. Using living cells for drug or pathogen detection links the analyte effects with corresponding changes in cellular properties that can be subsequently detected using integrated CMOS biosensors [1, 2]. However, this technique necessitates the growth of cells onto the CMOS biosensors' surface. As the CMOS biosensor needs to undergo rigorous cleaning to avoid contamination, this makes the biosensor reuse difficult and adds additional processing time and cost. To address this challenge, this work presents a disposable e-microplate platform allowing the reuse of the CMOS biosensor and thereby reducing cost and increasing throughput relative to systems that are non-disposable. The platform utilizes mechanically-flexible interconnects (MFIs) and through-silicon-vias (TSVs) to electrically interface the sensing electrodes on the CMOS biosensor to the sensing electrodes on the e-microplate while maintaining a physical separation of the biosensors from the cellular samples. A cell seeding experiment shows successful cell attachment and growth on the e-microplate. The characterization results show that the e-microplate does not degrade the performance of the underlying CMOS biosensor, as seen via the consistency of the internal amplifier gain and the input referred noise. Additionally, the e-microplate system is shown to perform successful impedance mapping on saline and DPBS solutions. The average measured resistance of the TSV-MFI link is 163 mΩ while the measured 3dB bandwidth and the integrated input referred noise with the e-microplate included is 0.5-400 Hz and 4.96 μV_{rms}, respectively. This low input noise ensures high SNR for sensing minute biological signals, which is critical for reliable analysis.

System Overview

Figure 1 shows the envisioned system. The e-microplate serves as a disposable interface separating the CMOS biosensor from the cultured cells. Mechanical self-alignment structures and pyramid pits with sub-micron alignment accuracy [3] (Fig. 2), developed for silicon photonic packaging applications, have been assimilated into this platform to enable low-cost and high-accuracy alignment between the e-microplate and the CMOS biosensor. Electrical interconnections from the sensing electrodes on the e-microplate to the CMOS biosensor are enabled using through-silicon-vias and mechanically flexible interconnect integration. Figure 3 shows the fabrication process of the TSV-MFI integration. The sensing electrodes were fabricated on the front side of the e-microplate by the deposition of Ti/Cu/Au using an evaporation process, followed by a subsequent lift-off process. After the formation of the sensing electrodes, gold passivated NiW MFIs, described in [4], were fabricated on the bottom side; the choice of NiW material allows MFIs to undergo larger deformation within the elastic region owing to its higher yield strength relative to copper [4]. The gold passivation using electro-less plating ensures reliable gold-to-gold contact between the MFI and the sensing electrode on the CMOS biosensor. Key dimensions of the TSVs and MFIs are summarized in Table 1. The layout of the e-microplate was designed to complement the sensor pixel layout on the CMOS biosensor, as described in [2]. Figure 4 shows an X-ray image of the TSV-MFI integration. The fabricated TSVs are free of any voids enabling reliable interconnections, which are critical for the accurate sensing of cellular activity. Figure 5 shows an SEM image of the fabricated pixel group MFIs on the bottom side of the e-microplate (facing the CMOS biosensor); each pixel group consists of 16 MFIs contacting the corresponding

electrodes on the CMOS biosensor (Fig. 6 [2]). The measured compliance data for the fabricated NiW MFIs, obtained from a nano-indenter, is shown in Fig. 7. The 30 µm tall MFIs regain original height after up to 10 µm of vertical displacement ensuring a good electrical interconnection to the electrodes on the CMOS biosensor while compensating for any surface variation on the biosensor surface.

A cell growth experiment was also carried out with the e-microplate to ensure cell attachment and growth over the sensing electrodes. The e-microplate was sterilized with three washes of ethanol followed by three washes using water. The surface was then coated with Matrigel (Corning) for 24 hours at 37°C prior to cell seeding. Human pluripotent stem cells were cultured in serum free media (mTeSR, StemCell Technologies) for several passages prior to seeding experiments. Cells were then dissociated to single cell suspension and seeded onto the e-microplate surface at a concentration of 200,000 cells/cm^2. The results (Fig. 8), after 48 hours of cell seeding, show good cell adhesion on all the sensing electrodes and would be expected to grow to a full confluence. Human pluripotent cells were chosen as they can be later differentiated into specialized cells (e.g. cardiac cells) for extended drug and pathogen studies.

Characterization

The electrical characterization of the e-microplate was carried out in three steps. Firstly, DC measurements were performed on the TSV-MFI link. Secondly, the effect of the incorporation of the e-microplate was tested by measuring the internal amplifier gain and input referred noise through the e-microplate's TSV-MFI interconnections. Lastly, impedance maps were generated for saline and DPBS solutions to verify functional accuracy. Figure 9 shows the assembled e-microplate system used for internal amplifier gain and noise measurements; the system includes the e-microplate, carrier, and CMOS biosensor assembly mounted onto the test board. For impedance mapping, a standard 35 mm petri-dish with a drilled out bottom was mounted onto the board and sealed using Polydimethylsiloxane (PDMS) to provide electrical isolation while maintaining biocompatibility (Fig. 10). The X-ray image in Fig. 11 confirms accurate alignment between the electrodes on the CMOS biosensor and the MFIs on the e-microplate. The schematic of the internal voltage sensing amplifier and the impedance sensing circuit is shown in Fig. 12. The in-band closed-loop gain of the amplifier, determined by the capacitance ratio of C_1/C_2, is 23.5 dB. A MOS-bipolar pseudo-resistor and capacitor C_2 provide a low cut-off frequency of 0.5 Hz. Such a low cut-off frequency is critical to monitor extracellular local field potentials from the cells [2]. To determine the resistance of the TSV-MFI link, four point resistance measurements were performed; the average resistance of the TSV-MFI links was determined to be 163 mΩ (Fig. 13). To test the effect of the e-microplate on the internal amplifier gain and input referred noise, interconnections were made by wire-bonding the sensing electrodes on the e-microplate to the test board; this allowed the test signals to traverse through the TSV-MFI link to the sensing electrode on the CMOS biosensor. Figure 14 shows the amplifier gain, measured with and without the e-microplate, along with the simulated gain. The results show that the introduction of the e-microplate does not affect the amplifier gain; also, the 3dB bandwidth remains unchanged. Similarly, the input referred noise, shown in Fig. 15, does not degrade with the incorporation of the e-microplate; the integrated input referred noise of the amplifier, from 0.5 Hz to 400 Hz, was measured to be 4.96 μV_{rms}. This low input noise ensures high SNR when measuring extremely weak biological signals from living cells (e.g. cardiac cells or neurons). Impedance mapping, using similar techniques as described in [2], was performed for two different solutions, saline and DPBS, and compared to air to verify the functional accuracy of the impedance mapping assembly. The results, shown in Fig. 16, confirm accurate impedance mapping via the sensing electrodes on the e-microplate.

Conclusion

A low-cost, disposable platform using 3D technology, capable of providing electrical interconnections between living cells and CMOS biosensors, is presented. The void free TSVs and the gold passivated NiW MFIs ensure reliable connections to the pixel array on the biosensor, which are essential for accurate sensing. The inclusion of the e-microplate does not degrade the amplifier gain or noise, hence ensuring accurate sensing of weak biological signals from living cells cultured on the e-microplate, which is critical for reliable data analysis. Impedance maps generated for air, saline solution, and DPBS confirm functional accuracy of the developed platform.

References

[1] H. Wang, "Magnetic sensors for diagnostic medicine: CMOS-based magnetic particle detectors for medical diagnosis applications," *IEEE Microwave Magazine*, vol. 14, no. 5, pp. 110-130, Jul/Aug 2013.

[2] J. S. Park, *et. al.,* "A multimodality CMOS sensor array for cell-based assay and drug screening," *IEEE ISSCC*, Feb. 2015, pp. 208-209.

[3] H. S. Yang, C. Zhang, and M. S. Bakir, "Self-Aligned Silicon Interposer Tiles And Silicon Bridges Using Positive Self-Alignment Structures and Rematable Mechanically Flexible Interconnects," *IEEE TCPMT,* vol. 4, no.11, pp. 1760-1768, Nov. 2014.

[4] C. Zhang, H. S. Yang, and M. S. Bakir, "Highly Elastic Gold Passivated Mechanically Flexible Interconnects," *IEEE TCPMT*, vol. 3, no. 10, pp. 1632-1639, Oct. 2013.

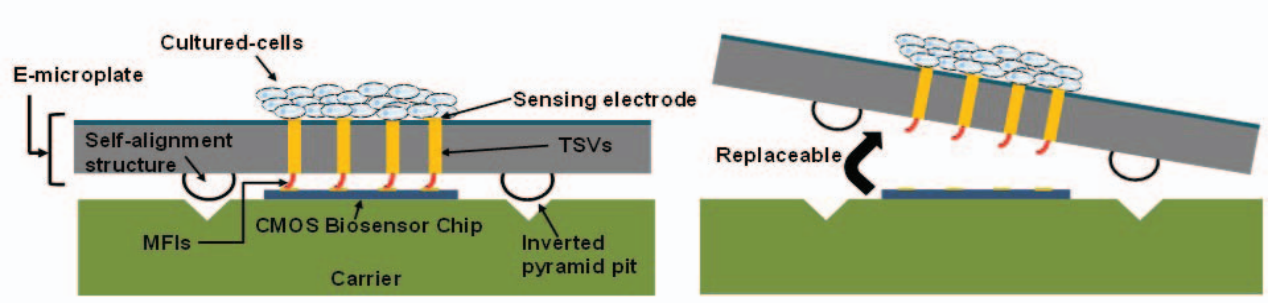


Figure 1. Envisioned micro-fabricated electronic microplate platform – the electronic microplate can be replaced and the biosensor reused

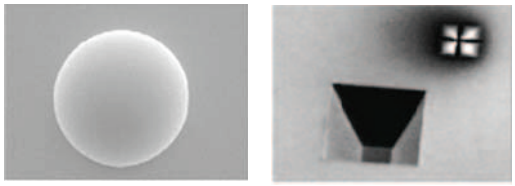


Figure 2. Self-alignment structure and pit

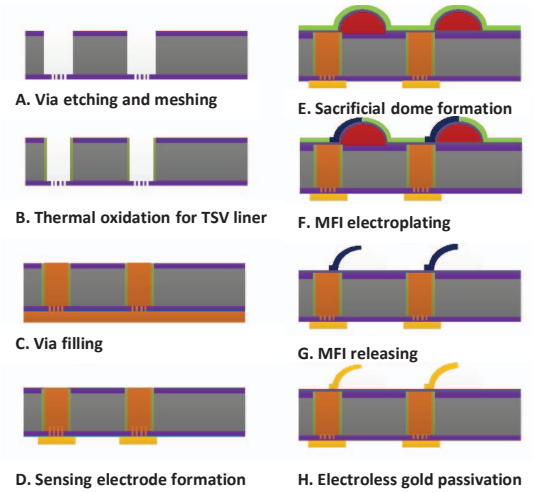


Figure 3. Fabrication of TSV-MFI link

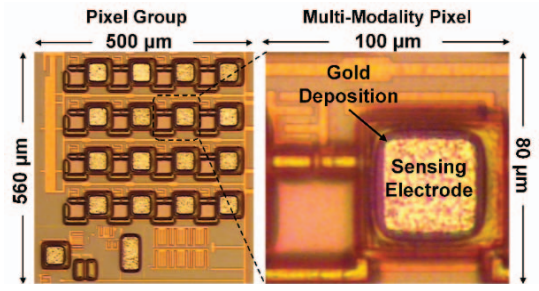


Figure 6. Pixel group layout on CMOS biosensor

	Dimension	Value (µm)
TSVs	Diameter	50
	Height	300
	Pitch	100
MFIs	Thickness	3.5
	Vertical Height	30
	Pitch	100

Table 1. TSV-MFI specifications

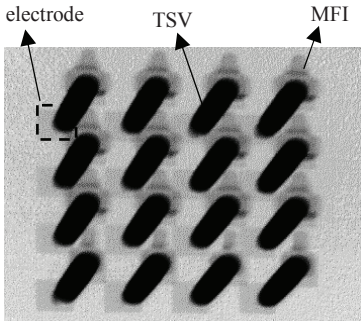


Figure 4. X-ray image of void free integrated TSV-MFI structures

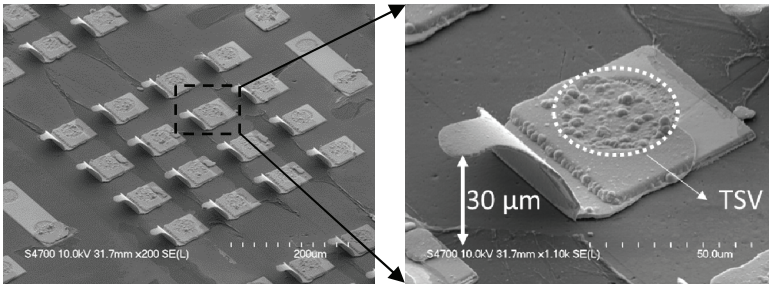


Figure 5. Pixel group with array of sixteen 30 µm tall MFIs

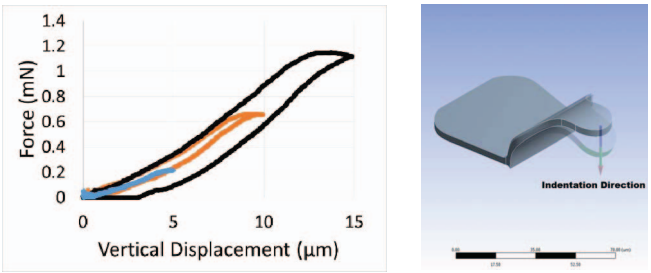


Figure 7. Compliance graph for MFIs – the MFIs recover to original height for up to 10 µm of vertical displacement

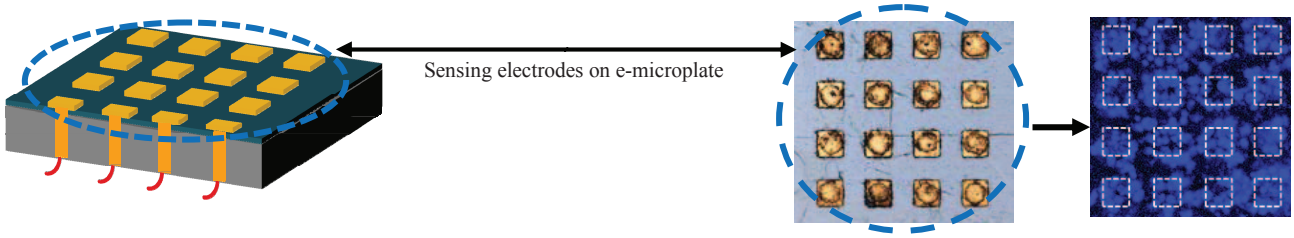


Figure 8. Cell growth on sensing electrodes shows successful attachment and growth after 48 hours

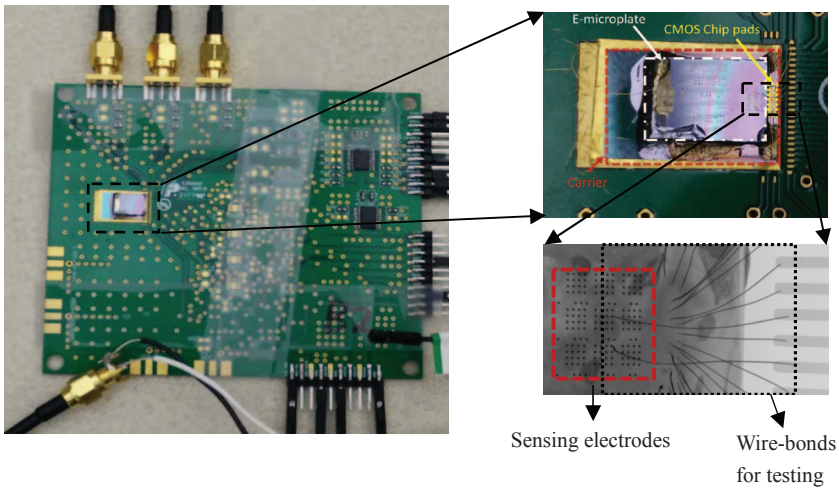


Figure 9. Test setup for gain and noise measurements – sensing electrodes were wire-bonded to test board for measurements

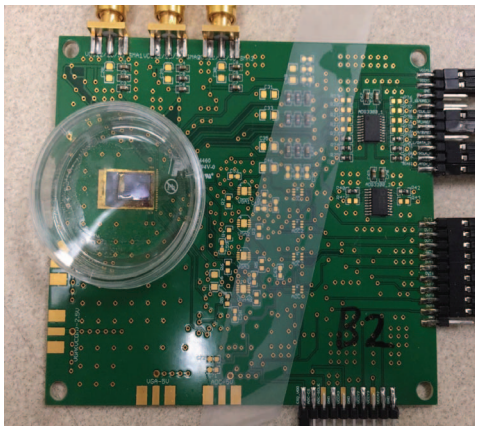


Figure 10. Test setup for impedance mapping

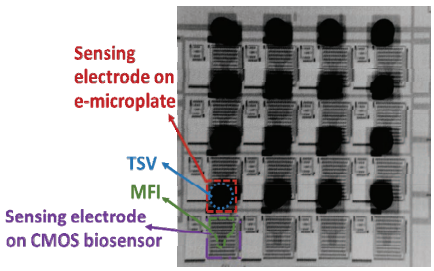


Figure 11. X-ray image showing accurate alignment between e-microplate and CMOS biosensor

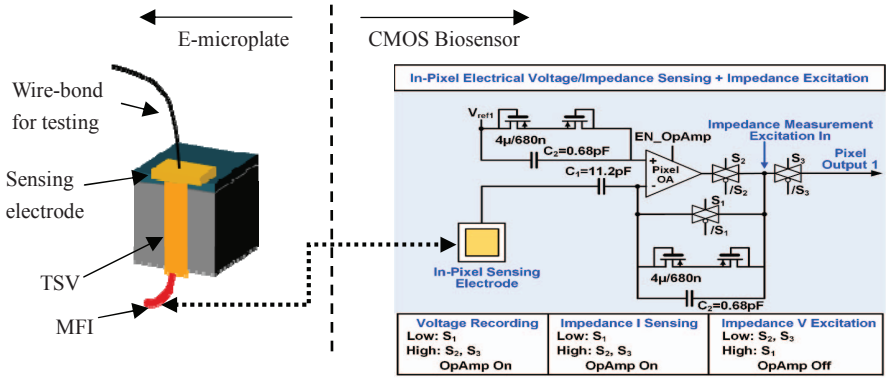


Figure 12. Schematic of internal voltage sensing amplifier and its connection to the e-microplate's pixel

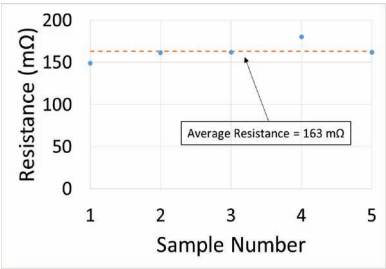


Figure 13. Four-point resistance measurements for TSV-MFI link

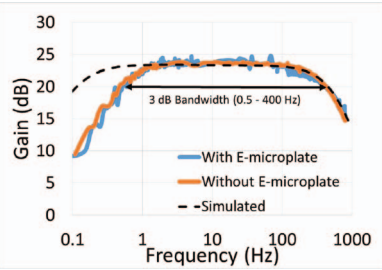


Figure 14. Amplifier gain measurements – the amplifier gain remains unchanged with the incorporation of the e-microplate

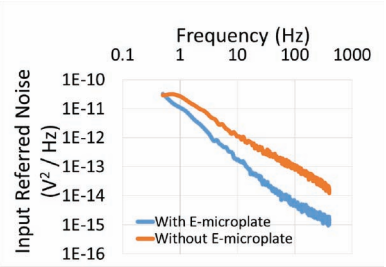
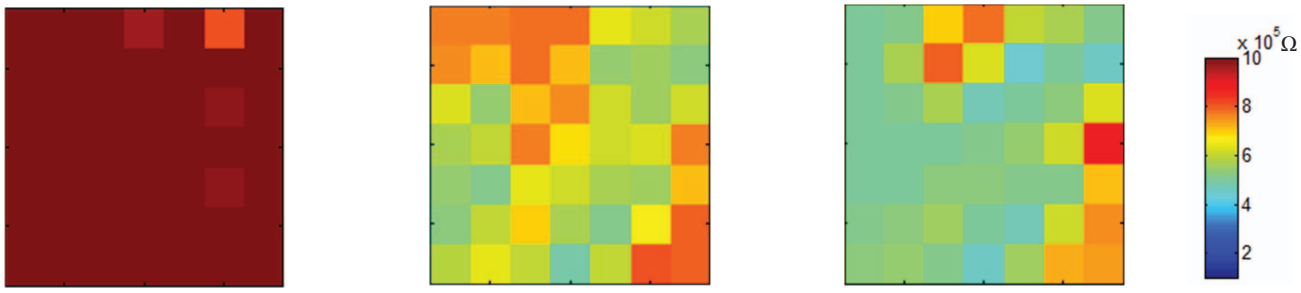


Figure 15. Input referred noise measurements – low input referred noise ensures high SNR when measuring weak signals



No Solution | Saline Solution | DPBS

Figure 16. Impedance maps for different substances measured via e-microplate's pixel group – measurements verify functional accuracy of the platform