34th Annual International Conference of the IEEE EMBS
San Diego, California USA, 28 August - 1 September, 2012

CMOS biosensor system for on-chip cell culture with read-out circuitry and microfluidic packaging

David Welch[1], Jennifer Blain Christen[2]
School of Biological and Health Systems Engineering[1] and School of Electrical, Computer and Energy Engineering[2]
Arizona State University, Tempe, Arizona 85287
Email: jennifer.blainchristen@asu.edu

***Abstract*—A 1.5 mm × 3 mm CMOS chip with sensors for monitoring on-chip cell cultures has been designed. The chip is designed in a 0.5 μm CMOS process which has 3 metal layers and 2 poly layers and is a 5 volt process. The chip contains ion sensitive field effect transistors (ISFETs), as well as ISFETs with read-out circuitry, for monitoring the pH of solutions placed on top of the chip. Interdigitated electrode structures (IDESs) are made using the top metal of the process to be used for sensing cellular attachment and proliferation via impendence. IDES read-out circuits and IDES test structures are included. The chip also contains test amplifiers, bandgap reference test structures, and connections for post-processing. We designed the chip to accommodate packaging into an environment where it will be directly exposed to a cell culture environment. Specifically we designed the chip to have the incorporated sensors near the center of the chip allowing for connections made around the edge of the chip to be sealed off using an epoxy or similar material to prevent shorting. Preliminary electrical characterization results for our amplifier indicate a gain of 48 dB, a bandwidth of 1.65 kHz, and a common mode rejection ratio (CMRR) of 72 dB. We also present a packaging technique using a flexible pcb substrate.**

I. Introduction

Current cell culture practices have a number of methods that attempt to control the environment but most are focused at macroscale control. However, the cell microenvironment has been shown to be an equal or even more important factor. The microenvironment has a distinct physiological character defined by the physicochemical properties such as pH, oxygen tension, temperature, and osmolality [1] as well as the physical properties [2]. Recent research has shown that small variations in these factors affect cell behavior [3]–[8].

The described work aims to control the microenvironment by creating a cell culture platform with integrated sensors, made using a commercial CMOS (complementary metal-oxide-semiconductor) process. Sensors with size on the same scale as the cells they are monitoring can be placed in small compartments which would not be accessible using benchtop analysis probes [9]. This also allows for multiple sensors to be placed in a small area to gain spatial resolution. The fabrication of sensing elements in the same process as read out circuitry also decreases system complexity by reducing post processing and assembly steps. Another advantage is the proximity of the transducer to the circuitry. Keeping connections short, especially avoiding transmitting the signal off of the chip, decreases noise allowing low level signal detection. The main issues with using CMOS sensors for cell culture or any biological monitoring is designing and packaging the electronics so they can function in an environment with liquids. An additional challenge is maintaining control over the liquids in the sensed area to form a complete system with feedback.

We use CMOS chips incorporated with microfluidic systems in order to integrate sensors, microelectronics, and biochemical reagents into a single device that can be controlled in a feedback configuration using a computer. Feedback systems for temperature control of cell culture systems have already been successfully demonstrated [10]. A system that can monitor and control more parameters provides a higher level of experimental control making it better suited as a biological test platform. We describe our work towards a complete microenvironment controlled system. The design and simulation of a CMOS chip (Fig. 1) with pH and capacitance sensors that function when directly exposed to liquids is described. Preliminary amplifier characterization results are also shown. This paper will also outline the packaging methods, including how electrical connections are made and fluid interaction is controlled.

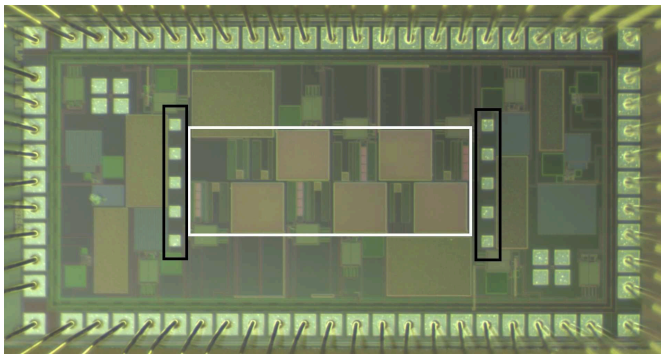

Fig. 1. A micrograph of a 1.5 mm × 3 mm CMOS chip with integrated biological sensors is shown. The white square highlights the sensing region containing both pH sensors and capacitance sensors. Black squares on each end highlight points of electrical connection for post processed electrodes. The wirebonds shown are for preliminary testing purposes and need to be insulated before the chip can be used with liquids.

II. CMOS Chip Overview

A 1.5 mm × 3 mm CMOS chip with sensors for monitoring on-chip cell cultures has been designed (Fig. 1). The chip is

designed in a 0.5 μm CMOS process which has 3 metal layers and 2 poly layers and is a 5 volt process. We designed the chip to accommodate packaging into an environment where it will be directly exposed to cultured cells. In order to accomplish this all electrical connections are sequestered from fluidic areas to prevent shorting out connections. Specifically we designed the chip to have the incorporated sensors near the middle of the chip allowing for connections made around the outside of the chip to be sealed off using an epoxy or similar material.

A. Operational Amplifier Characterization

A wide swing output transconductance amplifier (OTA) was designed largely around the design by Harrison and Charles [11] with the cascode transistors omitted (figure 2). Large transistors (W/L=800/4) were used as input transistors to decrease noise. The OTA was designed to run on a 30 μA bias current with $V_{DD} = 5$ V. Test structures of the amplifier were included on the design which allows for the amplifier to be characterized independently.

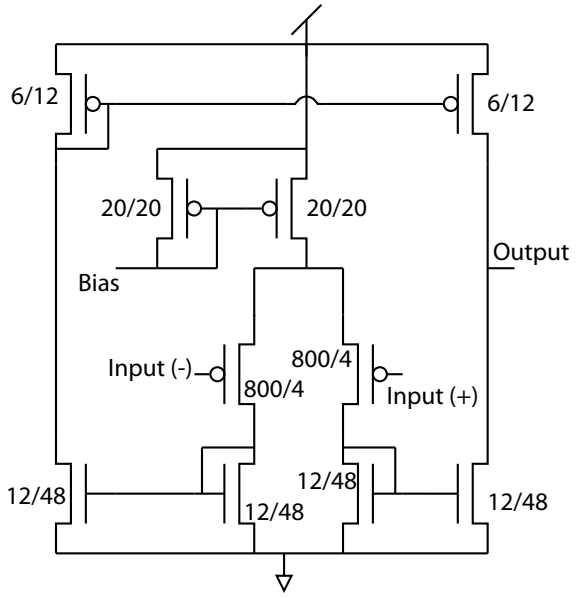

Fig. 2. Schematic of the wide swing output OTA amplifier adapted from work by Harrison [11]. Transistor W/L values are listed next to the corresponding transistor.

Testing of amplifier performance began by verifying the biasing conditions. An I_{DS} vs V_{DS} sweep was performed on the biasing PMOS and we determined that a voltage of 2.35 V provides a 30 μA bias current. We then setup the amplifier with unity gain feedback and confirmed the amplifier could act as a buffer. To see the input common-mode range we swept the input voltage from 0 V to 5 V with $V_{DD} = 5$V and $V_{SS} = 0$V. The input common-mode range of the circuit was found to extend from 800 mV to 4.2 V. We setup the amplifier in an open-loop configuration with the negative input set to 2.5 V while a sine wave with a 2.5 V DC offset was applied to the positive input. Because of the high gain of the circuit and limitations on the minimum output signal from the Keithley 3390 arbitrary waveform generator (Keithley Instruments Inc., Cleveland, OH), we used a voltage divider to attenuate the input signal. Using a TDS 2004B oscilloscope (Tektronix, Beaverton, OR) we found the peak to peak value of the input and output and calculated the gain of the amplifier to be 48 dB. While keeping the same open loop gain configuration we increased the input frequency until we found the 3 dB point at 1.65 kHz. We then set both amplifier inputs to the sinusoidal input centered at 2.5 V to find the CMRR, which we calculated to be 72 dB.

B. Capacitance Monitoring

Cell impedance is indicative of a variety of cellular characteristics [12], [13]; we specifically monitor cell adhesion and growth [14]–[16]. The chip includes four designs for capacitance monitoring, each a combination of one of two sensing methods and one of two monitoring circuits. The first sensing method utilizes the top metal of the CMOS process to create an interdigitated electrode structure (IDES) for sensing. The top metal has a passivation layer over it, on top of which the cells can be cultured. Therefore, any change to capacitance is due to a change in fringe capacitance. The second capacitance sensor is also an IDES but requires post-processing. Deposition and patterning of a gold IDES structure on top of the passivation layer is similar to the methods used by Zhang et al. [17]. Unlike the other design, this sensor allows cells to culture between the capacitor "plates" to create larger, direct capacitance changes.

Two different circuit configurations are included on the chip. Both designs are based on an electrometer op amp charge amplifier circuit [18], [19]. Figure 3 shows the schematic of the circuit. One circuit design uses a resistor as shown and the other configuration utilizes a MOS-bipolar pseudoresistors to create a large resistance [11].

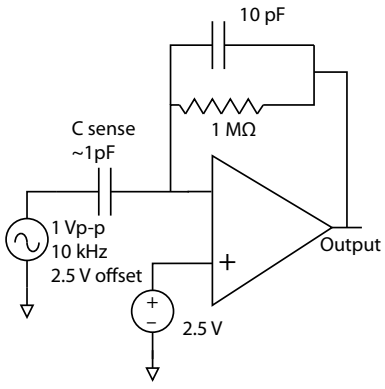

Fig. 3. The general electrometer schematic is shown with a resistor in the feedback path. The gain of this circuit is defined by the ratio of the feedback capacitor and the input capacitor. The input capacitor changes value based on changes the permittivity of the spaces within the IDES. Alternative electrometer circuits replace the feedback resistor with a pseudoresistor to create a high resistance.

C. pH Monitoring

The proposed work uses a pH sensing ISFET made in the CMOS process. CMOS ISFET designs differ from normal ISFET configurations [20] due to the large amount of oxide above the gate region as well as the need for a polysilicon gate for self-aligned source and drain regions [21]. We created a CMOS ISFET similar to the design by Bausells [21] which employs stacked levels of metal connected to the ISFET gate. The metal is left floating and simply acts as a gate connection closer to the sensing oxide surface. We have multiple ISFETs on the chip with both PMOS and NMOS configurations. We designed our ISFETs with the drain and source metal contacts far away from the floating gate metals to minimize noise [22]. We employ a circuit readout design from Morgenshtein et al. [23], the indirect complementary ISFET/MOSFET pair configuration (Fig. 4). This design eliminates body effect which can shift the threshold voltage and therefore the calculated pH value. Our chip also includes ISFETs without readout circuitry to allow for direct analysis of sensor performance.

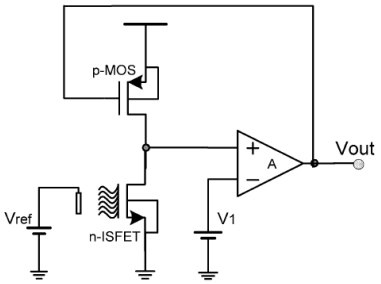

Fig. 4. Indirect complementary ISFET/MOSFET schematic from Morgenshtein et al. [23]. The circuit is well suited for ISFET readout because it eliminates errors due to the body effect. The reference electrode present in the solution can be added with post processed metal deposition on the surface or by having a Ag/AgCl wire in contact with the solution.

III. Packaging

The methods for integrating CMOS sensor chips with microfluidics have been improving in the past few years [24]–[27] but still have significant problems. We efficiently integrate microelectronics with microfluidics using a flexible printed circuit board (PCB) similar to the work by Wu et al. [28] (Fig. 5). The flexible pcb we use is made from Pyralux AC182500R obtained from Dupont (Wilmington, Delaware). The Pyralux consists of 1 mil thick polyimide covered with 0.5 oz copper. Standard photolithography techniques are used to pattern photoresist on top of the copper. We then etch the copper with ferric chloride to leave patterned copper 6. Prior to bonding, the flexible PCB is cut using a precision hole punch to allow access to the surface of the CMOS chip containing the sensing regions. The CMOS chip is flip-chip bonded to the flexible PCB copper traces terminating at the desired bond pad connections. After connections are made the area around the electrical connections is filled with an epoxy to insulate from any fluids.

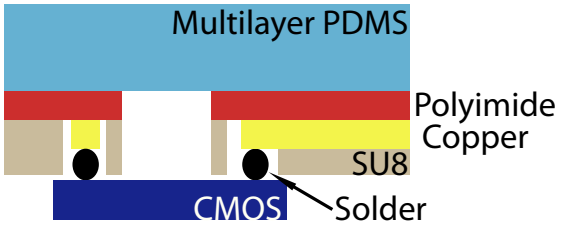

Fig. 5. Flip-chip CMOS-microfluidic system integration similar to the method employed by Wu et al. [28]. Our system uses PDMS microfluidics instead of multiple glass layers to decrease fabrication complexity.

Microfluidic structures are made using soft lithography. Polydimethylsiloxane (PDMS) is molded with lithographically patterned SU8 photoresist to create channels for fluid movement. Multiple layers of PDMS are stacked to create valves to allow for control of fluid movement [29]. An area for analysis on top of the CMOS chip will be fabricated similar to the structure used by Blain Christen et al. [10].

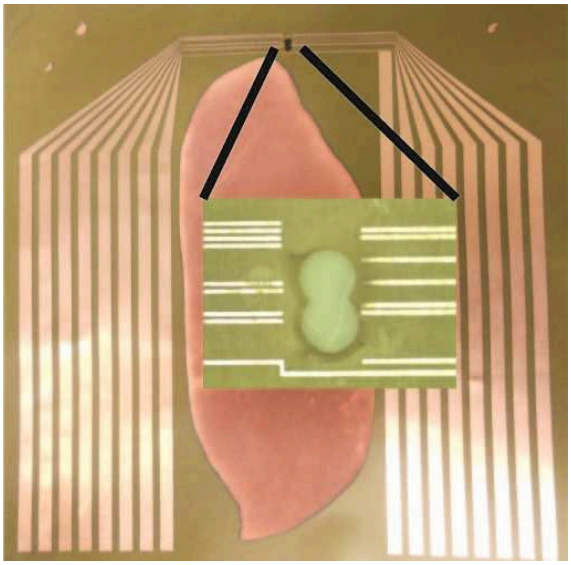

Fig. 6. Flexible pcb shown ready for flip chip bonding of a bare CMOS die. The pcb area is approximately 65 mm $\times$ 65 mm. The inset picture shows the die bonding area with a section of the polyimide removed to allow for sensor access from the opposite side where the fluidics can be attached. The termination point of each trace is the same width of a bondpad on the CMOS chip, 100 μm. The hole punched through the polyimide is approximately 1 mm $\times$ 2 mm.

IV. Conclusion and Future Work

We have presented a CMOS biosensor system for monitoring on-chip cell culture. The chip contains ISFETs and capacitive sensors as well as read-out circuits. We have characterized the amplifier used in the read-out circuitry. We measured a gain of 48 dB, a bandwidth of 1.65 kHz, and a CMRR of 72 dB. Further work is needed to fully characterize the sensors and read-out circuits.

We have also presented post-processing methods that allow the system to work in a cell culture environment. Two methods of sensing capacitance were described. The first uses the top metal of the CMOS process; the other requires post processing to deposit a direct capacitance sensing structure. The system will incorporate microfluidics integrated with the electronics using a flip-chip style assembly method. The microfluidics are created using replica molding techniques and include pneumatic actuators. The microfluidics create a culture area and allow for introduction and control of fluid interaction with the sensing region. Incorporation of PDMS microfluidics instead of multilayered glass structures as used by Wu et al. reduces processing and allows for devices to be easily replicated. The creation of a complete system for controlled cellular analysis provides precision control over the cell microenvironment making it possible to better understand cell behavior.

V. Acknowledgment

The authors would like to thank MOSIS for chip fabrication and James Laux for his assistance with Cadence and design submission.

References

[1] E. W. K. Young and D. J. Beebe, "Fundamentals of microfluidic cell culture in controlled microenvironments," *Chem. Soc. Rev.*, vol. 39, pp. 1036–1048, 2010. [Online]. Available: http://dx.doi.org/10.1039/B909900J

[2] G. M. Walker, H. C. Zeringue, and D. J. Beebe, "Microenvironment design considerations for cellular scale studies," *Lab Chip*, vol. 4, pp. 91–97, 2004. [Online]. Available: http://dx.doi.org/10.1039/B311214D

[3] K. Kirchhof, A. Andar, H. B. Yin, N. Gadegaard, M. O. Riehle, and T. Groth, "Polyelectrolyte multilayers generated in a microfluidic device with ph gradients direct adhesion and movement of cells," *Lab Chip*, vol. 11, pp. 3326–3335, 2011. [Online]. Available: http://dx.doi.org/10.1039/C1LC20408D

[4] L. Ponsonnet, M. Boureanu, N. Jaffrezic, A. Othmane, C. Dorel, and P. Lejeune, "Local ph variation as an initial step in bacterial surface-sensing and biofilm formation," *Materials Science and Engineering: C*, vol. 28, no. 5-6, pp. 896 – 900, 2008. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S0928493107002160

[5] T. M. Keenan and A. Folch, "Biomolecular gradients in cell culture systems," *Lab Chip*, vol. 8, pp. 34–57, 2008. [Online]. Available: http://dx.doi.org/10.1039/B711887B

[6] G. B. Udy, R. P. Towers, R. G. Snell, R. J. Wilkins, S.-H. Park, P. A. Ram, D. J. Waxman, and H. W. Davey, "Requirement of stat5b for sexual dimorphism of body growth rates and liver geneexpression," *Proceedings of the National Academy of Sciences*, vol. 94, no. 14, pp. 7239–7244, 1997. [Online]. Available: http://www.pnas.org/content/94/14/7239.abstract

[7] Y. Yan, D. Yang, E. D. Zarnowska, Z. Du, B. Werbel, C. Valliere, R. A. Pearce, J. A. Thomson, and S.-C. Zhang, "Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells," *STEM CELLS*, vol. 23, no. 6, pp. 781–790, 2005. [Online]. Available: http://dx.doi.org/10.1634/stemcells.2004-0365

[8] A. Folch and M. Toner, "Microengineering of cellular interactions," *ANNUAL REVIEW OF BIOMEDICAL ENGINEERING*, vol. 2, pp. 227+, 2000.

[9] U. Guth, W. Vonau, and J. Zosel, "Recent developments in electrochemical sensor application and technologya review," *Measurement Science and Technology*, vol. 20, no. 4, p. 042002, 2009. [Online]. Available: http://stacks.iop.org/0957-0233/20/i=4/a=042002

[10] J. Christen and A. Andreou, "Design, fabrication, and testing of a hybrid cmos/pdms microsystem for cell culture and incubation," *Biomedical Circuits and Systems, IEEE Transactions on*, vol. 1, no. 1, pp. 3 –18, march 2007.

[11] R. Harrison and C. Charles, "A low-power low-noise cmos amplifier for neural recording applications," *Solid-State Circuits, IEEE Journal of*, vol. 38, no. 6, pp. 958 – 965, june 2003.

[12] I. Giaever and C. R. Keese, "Use of electric fields to monitor the dynamical aspect of cell behavior in tissue culture," *Biomedical Engineering, IEEE Transactions on*, vol. BME-33, no. 2, pp. 242 –247, feb. 1986.

[13] E. Ghafar-Zadeh and M. Sawan, *CMOS Capacitive Sensors for Lab-on-Chip Applications*. Springer, 2010.

[14] L. Ceriotti, A. Kob, S. Drechsler, J. Ponti, E. Thedinga, P. Colpo, R. Ehret, and F. Rossi, "Online monitoring of balb/3t3 metabolism and adhesion with multiparametric chip-based system," *Analytical Biochemistry*, vol. 371, no. 1, pp. 92 – 104, 2007. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S0003269707004654

[15] S. B. Prakash and P. Abshire, "On-chip capacitance sensing for cell monitoring applications," *Sensors Journal, IEEE*, vol. 7, no. 3, pp. 440 –447, march 2007.

[16] A. Mucha, M. Schienle, and D. Schmitt-Landsiedel, "Sensing cellular adhesion with a cmos integrated impedance-to-frequency converter," in *Sensors Applications Symposium (SAS), 2011 IEEE*, feb. 2011, pp. 12 –17.

[17] J. Zhang, Y. Huang, N. Trombly, C. Yang, and A. Mason, "Electrochemical array microsystem with integrated potentiostat," in *Sensors, 2005 IEEE*, 30 2005-nov. 3 2005, p. 4 pp.

[18] R. B. Northrop, *Introduction to Instrumentation and Measurement*. CRC Press, 1997.

[19] E. Ghafar-Zadeh, M. Sawan, and D. Therriault, "A 0.18-[mu]m cmos capacitive sensor lab-on-chip," *Sensors and Actuators A: Physical*, vol. 141, no. 2, pp. 454 – 462, 2008. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S0924424707007054

[20] P. Bergveld and A. Sibbald, *Analytical and Biomedical Applications of Ion-Selective Field-Effect Transistors*, Svehla, Ed. Elsevier, 1988.

[21] J. Bausells, J. Carrabina, A. Errachid, and A. Merlos, "Ion-sensitive field-effect transistors fabricated in a commercial cmos technology," *Sensors and Actuators B: Chemical*, vol. 57, no. 1-3, pp. 56 – 62, 1999. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S0925400599001355

[22] B. Paln, F. V. Santos, J. M. Karam, B. Courtois, and M. Husk, "New isfet sensor interface circuit for biomedical applications," *Sensors and Actuators B: Chemical*, vol. 57, no. 1-3, pp. 63 – 68, 1999. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S0925400599001367

[23] A. Morgenshtein, L. Sudakov-Boreysha, U. Dinnar, C. G. Jakobson, and Y. Nemirovsky, "Cmos readout circuitry for isfet microsystems," *Sensors and Actuators B: Chemical*, vol. 97, no. 1, pp. 122 – 131, 2004. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S0925400503006919

[24] A. Hierlemann, "Integrated chemical microsensor systems in cmos-technology," in *Solid-State Sensors, Actuators and Microsystems, 2005. Digest of Technical Papers. TRANSDUCERS '05. The 13th International Conference on*, vol. 2, june 2005, pp. 1134 – 1137 Vol. 2.

[25] M. Dandin, I. D. Jung, M. Piyasena, J. Gallagher, N. Nelson, M. Urdaneta, C. Artis, P. Abshire, and E. Smela, "Post-cmos packaging methods for integrated biosensors," in *Sensors, 2009 IEEE*, oct. 2009, pp. 795 –798.

[26] E. Ghafar-Zadeh and M. Sawan, "A hybrid microfluidic/cmos capacitive sensor dedicated to lab-on-chip applications," *Biomedical Circuits and Systems, IEEE Transactions on*, vol. 1, no. 4, pp. 270 –277, dec. 2007.

[27] E. Ghafar-Zadeh, M. Sawan, and D. Therriault, "Cmos based capacitive sensor laboratory-on-chip: a multidisciplinary approach," *Analog Integrated Circuits and Signal Processing*, vol. 59, pp. 1–12, 2009, 10.1007/s10470-008-9239-9. [Online]. Available: http://dx.doi.org/10.1007/s10470-008-9239-9

[28] A. Wu, L. Wang, E. Jensen, R. Mathies, and B. Boser, "Modular integration of electronics and microfluidic systems using flexible printed circuit boards," *Lab Chip*, vol. 10, pp. 519–521, 2010. [Online]. Available: http://dx.doi.org/10.1039/B922830F

[29] T. Thorsen, S. J. Maerkl, and S. R. Quake, "Microfluidic large-scale integration," *Science*, vol. 298, no. 5593, pp. 580–584, 2002. [Online]. Available: http://www.sciencemag.org/content/298/5593/580.abstract