Abstract—An integrated amperometric instrumentation system with on-chip electrodes for biosensor arrays is presented. The mixed-signal integrated circuit supports a variety of electrochemical measurement techniques including linear sweep, constant potential, cyclic and pulse voltammetry. Implemented in 0.5μm CMOS, the 3x3 mm$^2$ chip dissipates 22.5mW for a 200 kHz clock. The highly programmable chip provides a wide range of user-controlled rate and amplitude parameters with a maximum scan range of 2V and scan rate ranging between 1mV/sec to 400V/sec. The amperometric readout circuit provides ±0.5pA linear resolution and supports inputs up to 100pA. A 2x2 array gold electrode electrochemical cells was fabricated on the surface of the CMOS instrumentation chip. An all-parylene packaging scheme was developed for compatibility with liquid test environments as well as harsh piranha electrode cleaning processes. The chip was tested using cyclic voltammetry of 0.1M potassium ferrocyanide, and results were comparable to measurements using commercial instruments.

I. INTRODUCTION

Analyte detection and quantification in bio/chemical solutions plays a major role in a variety of applications, especially in health care, environmental monitoring, industrial quality control and clinical investigation [1]. Because of their required sensitivity and critical importance in daily lives, these applications require robust, easy to use, efficient, and highly accurate instrumentation systems. With recent advances in CMOS technology, there is a great opportunity to develop lab-on-chip solutions to replace large bulky lab instruments with simple low power portable systems. The compatibility between many bio/chemical sensor materials and CMOS technology makes Si-based on-chip solutions very good candidates [2].

Many techniques have been developed to measure analyte concentration in solutions including electrochemical methods, optical imaging, thermal detection, and spectrometry [3]. Electrochemical methods are attractive because they can readily be adapted to CMOS instrumentation. The two techniques most commonly used to acquire qualitative information in electrochemical sensors are voltammetry and impedance spectroscopy. We have previously reported developments in CMOS voltammetry [4, 5] and impedance spectroscopy [6, 7] circuits.

The chip-scale integration of biosensors and electrochemical circuitry requires post-CMOS microfabrication including microelectrode array fabrication and packaging for liquid environments. The packaging should provide electrical insulation of the chip and wire bonds, resistance to processing chemicals, and biological compatibility. This paper describes a new system that combines circuits for voltammetry techniques with an integrated microelectrode array to enable on-chip biosensing using, for example, enzymatic protein sensors. In voltammetry, a voltage is applied to the electrochemical cell resulting in an output current between the counter electrode (CE) and the WE measured using an amperometric readout circuit [8]. Although numerous CMOS potentiostats have been introduced over the past decade, to the best of our knowledge, a complete and autonomous on-chip electrochemical instrumentation system has not been reported.

The on-chip amperometric instrumentation system reported in this paper was implemented in 0.5μm CMOS with a 3.3V supply. The chip includes a digitally programmable voltage waveform generator capable of generating multiple waveforms including triangular, saw tooth, constant potential and square pulse, which are used for cyclic, linear sweep, constant potential and pulse voltametry techniques, respectively. There are a 2x2 array of single-ended potentiostats and amperometric readout circuits to measure response currents. The on-chip system is completed by a 2x2 gold electrode array fabricated on the chip’s surface.

Regarding chip packaging, several approaches to permit the use of CMOS circuitry within liquid test environments have been reported [9-11]. These methods utilize epoxy adhesives or PDMS to seal the electrical wires and to create microfluidic structures. However, these materials cannot survive extreme cleaning procedures, e.g. piranha cleaning, which is often required to clean electrode surfaces before biosensor interface formation. Furthermore, epoxy encapsulation has a long term reliability issue due to poor adhesion to the chip substrate and lacks of accurate alignment [11]. This paper reports a new chip packaging approach using parylene because of its excellent biocompatibility, strong insulation capability, and resistance to chemicals including piranha solution.

II. SYSTEM ARCHITECTURE

The chip-scale miniaturization and integration of electrochemical sensors and their instrumentation electronics has many advantages. Through miniaturization of electrodes, the limits of detection can be extended by improving the signal to noise ratio. The direct, on-chip, electrical connection of electrodes to the instrumentation circuit eliminates external wiring and provides immunity from environmental interference. The minimization of noise permits highly sensitive circuits to measure the responses of miniature biosensors, allowing a high density sensor array within the small platform of a CMOS chip.

Fig. 1 illustrates the protein-based electrochemical biosensor array microsystem that serves as the conceptual model for the work described in this paper. A silicon chip containing CMOS electrochemical instrumentation circuitry serves as the substrate of the microsystem. An array gold electrodes fabricated post-CMOS on the surface of the chip forms a link between the CMOS electronics underneath and
biosensors assembled on the electrodes. A top passivation layers insulate CMOS surface metal routing, defines size-adjustable openings over individual electrodes, and provides an interface to a variety of possible fluid handling schemes, including microfluidics.

For an autonomous microsystem, the CMOS chip would contain all of the necessary instrumentation electronics and a communication interface that permits user control of measurement operations and reporting of measurement results. For an amperometric electrochemical system, this requires a potentiostat to control electrode biasing, a multi-function waveform generator to produce the stimulus signals necessary for voltammetry techniques, and a highly sensitive amperometric readout circuit to measured the current resulting for the stimulus voltage. Fig. 2 illustrates this arrangement of components and serves as the system diagram for the circuits described in the next section.

III. CMOS DESIGN

A. Programmable Waveform Generator

A waveform generator is an important component of an autonomous electrochemical instrumentation system that requires different input waveforms for different voltammetry techniques. A multi-mode waveform generator is desired to produce signals of various shapes, amplitudes (scan range) and frequencies (scan rates). Programmable analog circuits could be constructed to generate continuous waveforms, but different analog circuits would be needed for each desired signal shape (triangle, pulse, etc.) with undesirable impact on power consumption and chip size. To avoid the adverse impact of multiple analog waveform generators, a digital solution was explored. A DAC with a digital controller could implement all shapes, but the output would be quantized and the complexity of the controller could be significant for the desired set of waveforms. A thorough examination of the impacts of stimulus quantization on electrochemical measurements was performed. Tests demonstrated that steps in quantized stimulus signals generated noise in the electrochemical output current, but the noise reduced as step size decreased. It was determined that a stimulus step size of 8mV or lower produced output results comparable to continuous stimulus signals. It was also determined that, with careful design, a very efficient digitally controlled waveform generator could be realized with low power and chip area. Therefore, a DAC-based multi-mode waveform generator was developed.

The digitally programmable waveform generator is shown in Fig. 3. It is comprised of a digital control block, a 10-bit comparator, a 10-bit bidirectional counter, and a 10-bit DAC. The control block generates non-overlapping clock signals and control signals to reset the waveform generator, up/down count for both positive and negative slope, and voltammetry mode selection. A single pin is used to serially shift the 22-bit configuration data into the control block. This configuration input selects the desired frequency and the waveform amplitude maximum and minimum values. Based on the comparator’s output, the 10-bit counter counts in either direction, as needed to produce a given waveform. The counter binary bits are fed into a 10-bit DAC to produce the final analog voltage. Although an 8-bit DAC is sufficient for these waveforms, to compensate for fabrication process variations a 10-bit segmented DAC was designed to generate a staircase analog signal. It uses a combination of an R-2R ladder network and a current mode thermometer decoder, with the lower six counter bits setting the R-2R ladder and the upper four bits controlling the thermometer decoder.

The DAC output spans the range of +1V to -1V relative to analog ground with a step size of 2mV. The output clock can be selected from CLK, CLK/2, CLK/4, or CLK/8, where CLK is the external input master clock. This provides a stimulus signal up to 2V pk-pk which is suitable for most electrochemical measurements.

B. Potentiostat Array

The basic function of a potentiostat circuit is to control and maintain the voltage between the WE and the CE under varying current conditions. Fig. 4 shows the schematic diagram of the 3-electrode single-ended potentiostat. Output from the waveform generator (V_{DAC}) is level shifted by OP1 to match the input range of OP2, by summing it with V_{ref1}. Output from OP1, V_{out1}, is given by

\[ V_{out1} = \frac{V_{DAC} + V_{ref1}}{2} \]

The output of OP2, which is buffered through negative feedback using OP3, is connected to the CE. For microscale sensors, the solution resistance between RE and CE can be assumed to be negligible so that CE and RE are at the same potential. The input of OP3 is connected to RE and no current flows through OP3. The output current as a result of input stimulus voltage at CE can flow only between CE and WE. V_{ref2} is a DC offset control input to adjust the scan range. A 2x2 array of potentiostats was designed to support a 2x2 electrode array.
C. Amperometric Readout Array

To measure the electrochemical response current at the WE, a capacitive current readout circuit based on [5] was developed. Correlated double sampling was utilized to reduce noise and amplifier offset. To enhance sensitivity, all of the op-amps were redesigned to improve performance parameters including gain, CMRR, PSRR and power consumption. Moreover, all switches were realized with minimum size to curtail charge injection. In order to reduce clock feedthrough errors, dummy switches with precise time sequence control were used. Through these improvements, the sensitivity was increased by a factor of six compared to prior work [5].

IV. ON-CMOS ELECTRODE ARRAYS WITH PACKAGING

The amperometric instrumentation circuit described above was designed in 3M, 2P, 0.5µm CMOS and implemented on a 3×3mm² chip. The layout supports an array of electrodes formed directly on the surface of the chip using post-CMOS processing. To ensure flat surface electrodes, the top CMOS metal layer was not used for circuit routing because it is not planarized. A die photo of the chip is shown in Fig. 5.

To interface the chip with biosensors, a gold electrode array has to be fabricated on the chip’s surface and the chip needs to be suitably packaged for use in a liquid environment. The post-CMOS fabrication process begins with formation of electrodes by depositing titanium/gold (50Å/1000Å) on the CMOS chip and patterning by wet etching. Polyimide is then spin coated and patterned to insulate electrode routing and define the electrode size. The die is then wire bonded to a package, and the chip assembly is then coated with a 5μm layer of parylene. Parylene is used because of its excellent insulation property and compatibility with bio-interface packaging requirement. Most importantly, parylene is resistant to piranha solution cleaning of gold electrode, which is critical for formation of the target biosensor interfaces. Parylene can uniformly coat all surfaces, including bonding wires, which is advantageous for a chip-based system with a complicated surface profile. The parylene is subsequently patterned using reactive ion etching, exposing only the area over the on-chip electrodes. A unique step in this process is the use of acetone-soluble epoxy as a masking layer for parylene etching. A PDMS cylinder, with an attached silicon chip sized to match the to-be-exposed electrode area, is pressed on CMOS chip surface. Epoxy is then filled in to the volume surrounding the CMOS chip, and the PDMS cylinder is removed after the epoxy has cured. The parylene on the electrode area is then etched by plasma etching. Finally, the epoxy is dissolved with acetone. The cross section view of the insulation structure is illustrated in Fig. 6.

V. RESULTS

The final parylene-coated chip with electrode array is shown as Fig. 7. The four electrodes each include WE, CE and RE. This package scheme is suitable for subsequent integration with microfluidics channels mounted either to the chip or to the package. The parylene package has been verified to withstand cleaning in aggressive piranha solution, which is widely used for cleaning biosensor electrodes.

Characteristics of the on-chip waveform generator are summarized in Table I. To verify operation of the amperometric readout circuit, currents were measured across the input range of the circuit. Table II shows the control conditions (update clock frequency and gain setting) needed to for each of the five segments that span functional input range of 1pA to 100µA. To characterize the resolution of the circuit, a Keithley 6430 source meter was used to sweep the WE current from 1 to 30pA. Fig. 8 shows the difference between the measured results and the input values and demonstrates a linear resolution of ±0.5pA. The power consumption of the 4-channel instrumentation circuit could not be measured independent of test circuits but was simulated to be 22.5mW at a maximum clock frequency of 200 kHz.

To verify electrochemical measurement capability, a test setup comprised of the instrumentation chip and a PC with a DAQ card running a LabVIEW user interface was prepared. A
potassium ferrocyanide. The on-chip instrumentation circuits and packaging were verified by performing cyclic voltammetry in linear resolution. The on-chip electrode array and parylene amperometric readout circuits with a 100µA range and a 1pA input current at 20Hz updating clock and gain=2 setting. The assistance of Dr. Liya Meng and the support of the MOSIS Service are gratefully acknowledged.

VI. CONCLUSION

An autonomous on-chip amperometric instrumentation system with electrode array and packaging for biosensor arrays was presented. This system can generate multiple voltage waveforms with programmable scan range and scan rate settings to support a variety of electrochemical voltammetry techniques. Scan rates of 400V/sec to 1mV/sec and a scan range of 0-2V were observed. The chip contains a 4-channel single-ended potentiostat and amperometric readout circuits with a 100µA range and a 1pA linear resolution. The on-chip electrode array and parylene packaging were verified by performing cyclic voltammetry in potassium ferrocyanide. The on-chip instrumentation circuits successfully performed similar measurements. The reported integrated microsystem enables a variety of microscale electrochemical bio-analysis tasks.

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REFERENCES


TABLE I. WAVEFORM GENERATOR CHARACTERISTICS

<table>
<thead>
<tr>
<th>Area</th>
<th>0.44mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAC Resolution</td>
<td>10 bit</td>
</tr>
<tr>
<td>Scan range</td>
<td>0-2V pk-pk, 10-bits</td>
</tr>
<tr>
<td>Scan rate</td>
<td>1mV/sec-1000V/sec</td>
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<td>Step size</td>
<td>2mV</td>
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</table>

TABLE II. CHARACTERISTICS OF THE CURRENT READOUT CIRCUIT

<table>
<thead>
<tr>
<th>Clock frequency</th>
<th>20Hz</th>
<th>50Hz</th>
<th>2KHz</th>
<th>20KHz</th>
<th>200KHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain setting</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Input current range</td>
<td>1-50pA</td>
<td>50-100pA</td>
<td>100pA-1nA</td>
<td>1nA-1µA</td>
<td>1µA-100µA</td>
</tr>
<tr>
<td>Resolution</td>
<td>1µA</td>
<td>2µA</td>
<td>10µA</td>
<td>1nA</td>
<td>10nA</td>
</tr>
</tbody>
</table>

Fig. 8. The deviation between input current and measured current for 1pA to 30µA inputs at 20Hz updating clock and gain=2 setting.

Fig. 9. CV measurement of 0.1M potassium ferrocyanide for on-chip electrode using commercial instruments. Peak separation is ~0.10V.

Fig. 10. Cyclc voltammogram of potassium ferrocyanide with oxidation and reduction peak separation of ~0.14V.

Fig. 11. Cyclic voltammetry of potassium ferrocyanide with oxidation-reduction peak separation of ~0.14V.

A typical electrolyte solution consisting of 0.1M potassium ferrocyanide and 1M potassium chloride was prepared. Measurements were performed at 25°C and a scan rate of 200mV/s with a liquid junction Ag/AgCl reference electrode. To test the on-chip electrodes and packaging, a commercial instrument was used to take a cyclic voltammetry (CV) measurement with an on-chip WE. The results in Fig. 9 show oxidation-reduction peak separation of 0.1V, which matches expectations. The chip was then connected to an external electrochemical cell composed of commercial electrodes, and a CV measurement was performed using on-chip electronics. As shown in Fig. 10, the results give a typical CV curve with oxidation-reduction peak separation of 0.14V. This compares well with measurements of the same electrochemical cell using commercial instruments, where peak separations of 0.1-0.15V were typically observed. Notice the potential sweeps across the 2V span of the on-chip waveform generator. The x-axis values for Figs. 10 and 11 are different because of internal biasing of the CMOS potentiostat.