8.4 CMOS Electro-Chemical DNA-Detection Array with On-Chip ADC

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We report on a CMOS-based microsensor array, featuring 576 electrodes and 24 channels, for label-free electrochemical detection of DNA hybridization. Each channel comprises a potentiostatic circuit that keeps the electrode at a defined potential and measures the respective electrical current. The readout channels rely on a first-order delta-sigma architecture, where the electrode-electrolyte capacitance is used as the integrator. This results in a very compact recording circuit inclusive of A/D conversion; the circuit consists of only the measurement electrode, a comparator, a feedback capacitance and a few switches. Biological experiments with short DNA samples and DNA extracted from human immunodeficiency virus (HIV) are presented.

There is an urgent need for rapid on-the-spot diagnosis of infectious diseases like HIV. A very common diagnostic method includes the use of fluorescence-based DNA analysis on micro-arrays after preceding DNA amplification (PCR) [1]. Here we describe a label-free exclusively electronic method to detect hybridization events that obviates the need for optical setups. The measurement principle (Fig. 8.4.1) relies on cyclic voltammetry (CV). In a CV experiment, the potential on a working electrode (WE) is periodically varied with respect to a reference electrode, while the respective current is measured. This results in I-V curves, which depend on the electrochemistry at the electrode surface. The sensor relies on an electropolymer (polypyrrole), which is oxidized and reduced. The movement of the chloride counter ions is affected by the negative charges of the phosphate groups on the DNA strands, the number of which increases upon hybridization. The kinetics of the ionic travel and, consequently, the shapes of the CV curves are altered through hybridization.

A common approach to measuring electro-chemical currents includes the use of a current-conveyor to decouple the large electrolyte capacitance from the rest of the circuit [2]. The current can then be digitized using an integrating ADC. A more compact approach is to integrate the charge on the measurement electrode directly [3]. Delta-sigma converters are also suitable, since the electrochemical signals are slow, and a first-order structure provides sufficient resolution [4].

Here we use the electrode-electrolyte interface as the integrator in a delta-sigma structure (Fig. 8.4.3). In a first approximation, this interface behaves like an ideal capacitor. However, the nonlinearity of the capacitor must be considered [5] and so must the charge-transfer resistance, R_{CT}, that exists in parallel with the capacitance and causes leakage. Leaky integrators can lead to dead zones in delta-sigma architectures.

Each sensor of the 24×24 array consists of a platinum (Pt) electrode, functionalized with a polymer bilayer (Fig. 8.4.1) and an immobilized DNA probe molecule. The electrode diameter ranges between 10 and 40μm, and the electrode pitch is 100μm. A schematic of the chip architecture is shown in Fig. 8.4.2. Each row is multiplexed to one readout channel as detailed in Fig. 8.4.3. The voltage at the working electrode is sensed by the input of a comparator. The comparator is a differential amplifier with a gain of 140dB followed by a Schmitt trigger. The operation includes two phases: first, a charge of +Q or -Q is loaded onto an on-chip capacitor C depending on the output of the comparator, then the capacitor is connected to the working electrode and is discharged. In this way, a defined electrode potential is ensured, and the current magnitude can be detected at the output of the comparator.

A shift register stores the operating conditions of the chip. Furthermore, a bandgap reference for biasing and a temperature sensor are integrated on chip. An FPGA provides the chip control, digital filtering of the readout channels and communication with the PC via a USB port.

The chip was fabricated in an industrial 0.6μm 5V 3M2P CMOS process with additional post-processing steps: Ti:W (20nm) and Pt (200nm) were sputtered onto the wafer as electrode materials and patterned using a lift-off process. A 1.6μm-thick passivation layer stack (SiO_2 and Si_3N_4) was deposited for corrosion protection and opened through a reactive-ion etching (RIE) step.

Polypyrrole (PPy) has been electrochemically deposited on the Pt using Coulometry. A potential of 0.7V (vs Ag/Ag^+ reference) was applied and the current during deposition was integrated until a value of 2.5nC/μm² was reached. A linker molecule is needed to bind the DNA to the PPy, which here was p(TPTC3-PO_3H_2) deposited at 0.45nC/μm² [6]. Afterwards, the electrodes were immersed in 0.5mM $MgCl_2$ solution to arrive at a generically "activated" electrode (PPy-pTPTC3-PO_3H_2-Mg^{2+}) ready for binding to DNA probes.

Figure 8.4.4 shows SEMs of a bare Pt electrode and a polymer-covered electrode. The corresponding impedance measurements in TRIS (trishydroxymethylaminomethane) buffer solution are shown on the right. The impedance shows a plateau at low frequencies due to the charge-transfer resistance, R_{CT}.

The characterization results of the sensing circuits are shown in Fig. 8.4.5. For testing, an external ceramic capacitor of 5nF has been used. The input current was swept from –100 to +100nA. The on-chip feedback capacitance, C, was set to 1pF, the reference voltage, V_{Ref}, to 1V, the sampling frequency to 125kHz and the decimation filter was a 10Hz Butterworth LPF. Figure 8.4.5 shows the normalized output and the residuals from a linear fit. The linearity and SNR in this measurement were both 11 bits. Next, the experiment was repeated using a 25μm-diameter Pt electrode in TRIS buffer solution. Both the external and electrolyte capacitors showed the same results in terms of linearity and SNR.

The microchip system is aimed at direct disease detection, preferably without preceding PCR. In [7] they evidenced that the method is very sensitive (10^{-18} molar solutions) and capable to distinguish different types of hepatitis C viruses at these low concentrations. For the microsystem DNA tests, the electrodes were functionalized with a probe DNA (27 base pairs). CVs have been acquired both before and after contact with the complementary DNA of different concentrations (10 and 100nM). An example recording with 100nM is shown in Fig. 8.4.6. The area of the CV curve has been used as an indicator and was expected to shrink upon the build-up of the electrostatic barrier through hybridization. The area reduction was 10% for a 10nM and 38% for a 100nM complementary-sample solution. In a second experiment, a DNA probe (30 base pairs) that selectively binds to a sequence in the HIV-1 DNA [8] was immobilized. The chips were incubated with complementary HIV-1 DNA, with non-complementary Herpes simplex virus-1 (HSV-1) DNA and with plain buffer under identical experimental conditions. The average area change (25 electrodes) was 21% for the HIV-1, 10% for the HSV-1 and 6% for the plain buffer. These results show that the microchip system potentially provides a fast, purely electronic, label-free DNA test that does not require a sophisticated laboratory setup.

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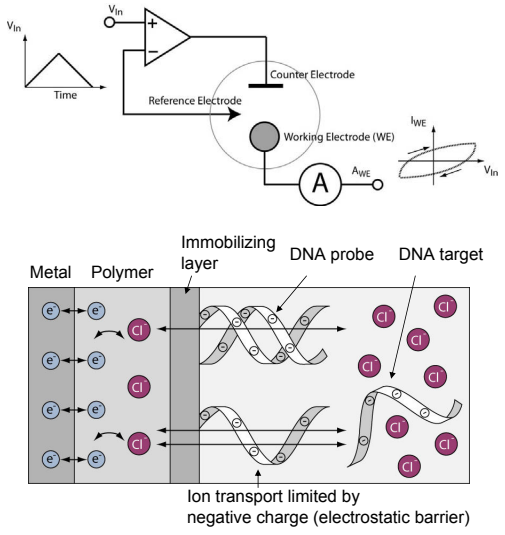


Figure 8.4.1: Illustration of a cyclic voltammetry experiment and description of the label-free electrochemical DNA hybridization detection principle.

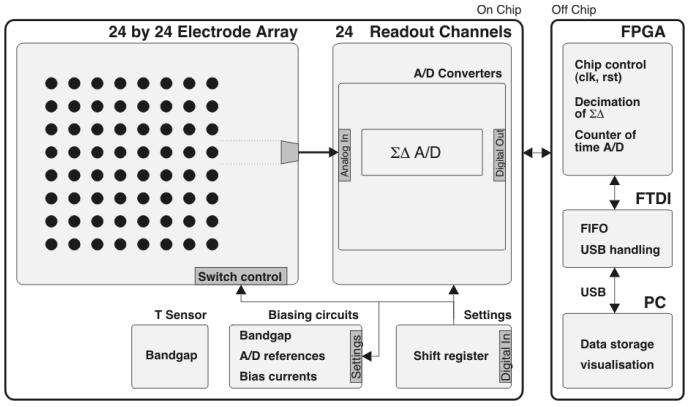
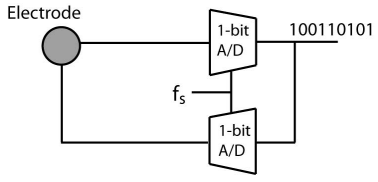


Figure 8.4.2: Chip block diagram.

First-order sigma-delta converter with electrolyte integrator:



Implementation details:

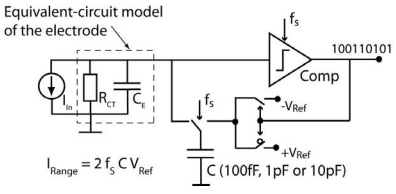


Figure 8.4.3: Schematic of a first-order delta-sigma converter with electrode-electrolyte integrator and an equivalent-circuit model of the working electrode.

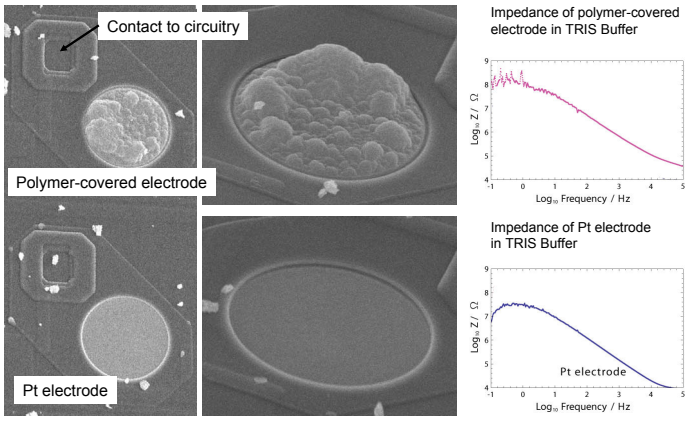


Figure 8.4.4: SEM of polypyrrole-functionalized and bare platinum electrodes and corresponding impedance measurements.

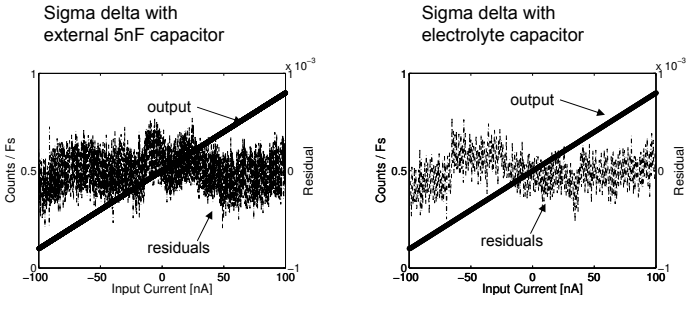


Figure 8.4.5: Measured output counts versus input current for a 5nF ceramic capacitor and the electrolyte capacitor.

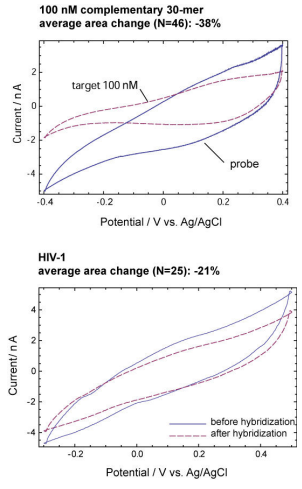


Figure 8.4.6: Top: Measurements of short DNA probes before (solid line) and after (dashed line) hybridization with a 100nM solution of complementary target strands. Bottom: Measurements of the hybridization of short probes with long DNA strands extracted from clinical HIV-1 samples.

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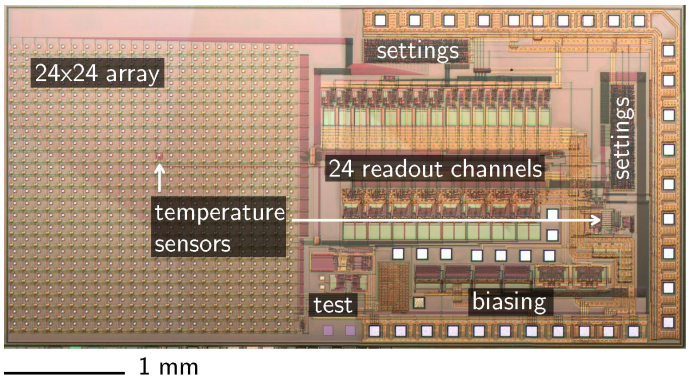

Figure 8.4.7: Chip micrograph

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