Passive DNA Sensor with Gold Electrodes Fabricated in a CMOS Backend Process

Franz Hofmann[1], Alexander Frey[1], Birgit Holzapfl[1], Meinrad Schienle[1], Christian Paulus[1], Petra Schindler-Bauer[1], Reiner Hintsche[2], Eric Nebling[2], Joerg Albers[2], Walter Gumbrecht[3], and Roland Thewes[1]

[1]*Infineon Technologies, Corporate Research, Otto-Hahn-Ring 6, D 81730 Munich, Germany*
[2]*Fraunhofer Gesellschaft, ISiT, Itzehoe, Germany*
[3]*Siemens AG, Erlangen, Germany*

Franz.Hofmann@Infineon.com

Abstract

A sensor for electrical detection of DNA is fabricated in a CMOS production line. A gold deposition process module is integrated in a CMOS backend process. The sensor principle is based on immobilization of single-stranded DNA probe molecules on an array consisting of interdigitated gold lines and subsequent hybridization with labeled target DNA strands. The electrical signal results from an electrochemical redox cycling process. Successful DNA detection experiments on the basis of such 'passive' chips are performed. This passive arrangement represents a test run for the extension of this principle to develop fully electronic DNA sensor arrays on active CMOS chips.

1. Introduction

Today most DNA analyses are performed with an optical technique. In this case, fluorescence light of an optical marker molecule attached to the DNA strands is detected. The required set-up utilizes CCD camera, optical filters, lenses and laser light. A simpler readout principle can be realized by detection of an electrical current in a comparatively small set-up. Thus, combination of electrical detection principles with the CMOS world is a great demand as silicon technology provides small feature sizes and integrated circuitry enables high sensitivity and robustness. A full CMOS functional read out array was presented in [1]. In this paper the integration of the bio-compatible sensor material in the backend of a CMOS process is discussed. The sensor electrodes are made of gold, a new material which has to be innovated in the standard CMOS environment.

2. Principle of the electrical DNA detection

Figure 1 shows the operation principle of the electrical detection method used [2]. First, single stranded DNA molecules (probe molecules, e.g. with about 20 bases) are immobilized on top of the gold electrodes due to gold-thiol coupling. Using a spotting machine, each sensor within a sensor array can contain individual probe molecules. In the next step, the chip is flooded with an analyte containing labeled target DNA molecules. Then hybridization takes place: chains of molecules with the specific complementary sequence compared to the immobilized probe molecules match and form double-stranded DNA. A suitable substrate is applied to the buffer solution and it is enzymatically cleaved by the label. The resulting species starts an electrochemical redox process at the electrodes. The electron current generated in the redox process is detected and translates the information 'matching DNA strands' into electrical signals.

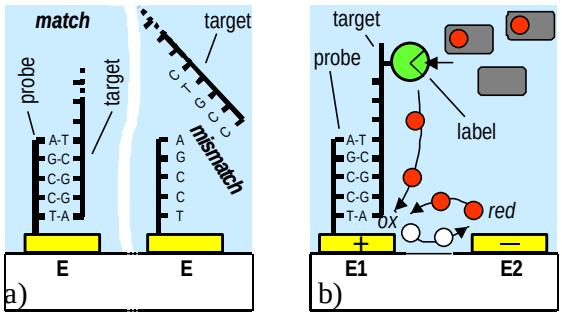
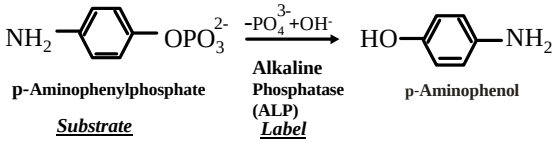


Figure 1. Principle of DNA detection
a) match and mismatch between probe and target molecules
b) redox system

3. Redox process

A suitable enzyme label, e.g. Alkaline Phosphatase (ALP), is bound to the target DNA by a biotin molecule. We use p-Aminophenolphosphate (p-APP) as a substrate to start the redox process (Figure 2a). The resulting species from the enzymatic reaction is para-Aminophenol (p-AP). It has two electrochemically active groups at the benzene structure. Para-Aminophenol is oxidized to Quinoneimine and reduced as depicted in the scheme in Figure 2b.

a) Process at the label



b) Redox process at the electrodes

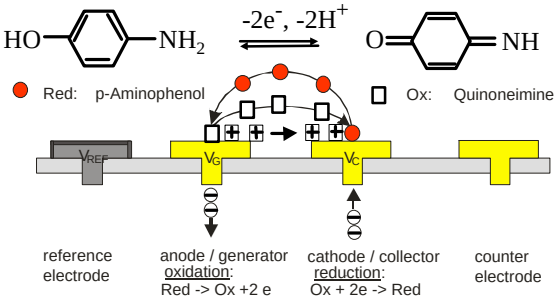


Figure 2. Schematic plot of the electrochemical process at the label (a) and redox process at the electrodes (b)

In the redox reaction 2 electrons and 2 protons are involved. The electrons can be detected in the current flow between the electrodes, the protons are exchanged within the buffer solution. For electrochemical operation, the chip is put in a fluidic cell with an output and an input flow channel, which are connected to a micro pump. Figure 3 shows a cyclic voltammogram of p-AP. This method uses a stepped sweep of the potential at the electrode and gives an oxidation potential of 260 mV and a reduction potential of 10 mV. These potentials are referred to a reference electrode. In order to get a high redox current the working electrodes should be biased a little beyond this window. Reasonable values are approximately 300 mV for the oxidation electrode and –50 mV for the reduction electrode, respectively. Maximum current outputs of such redox systems are obtained in case of interdigitated electrode arrays (IDA) [3] (Figure 4). In this configuration the diffusion areas of the redox species maximally overlap. The redox cycling process can easily be tested, even without labeled DNA, if p-AP is added to the buffer solution. Figure 5 shows a redox signal of p-AP in an IDA with an electrode width and space of 1 µm each. At the beginning, there is no current flow; no redox process occurs. After 10 µM p-AP is added to the electrolyte a redox current appears immediately.

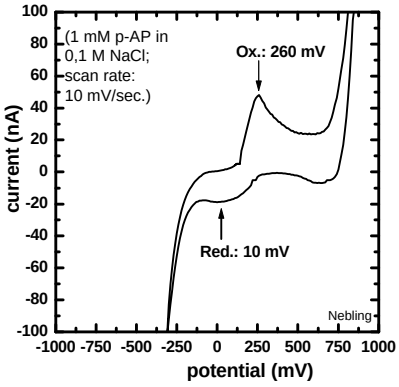


Figure 3. Cyclic voltammogram of p-Aminophenol

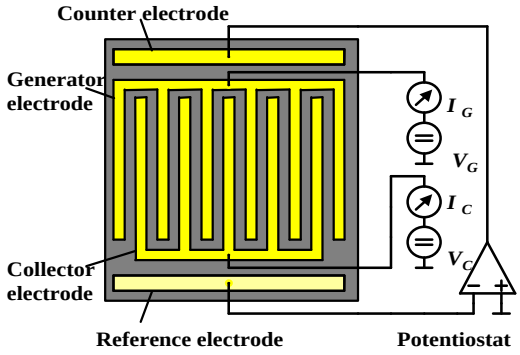


Figure 4. Schematic plot with interdigitated array of Au electrodes and potentiostat set-up

A suitable parameter to evaluate the collection efficiency (CE) is the ratio of collector and generator current CE $=I_C/I_G$. In our case we achieve CE > 0.9, which means, that nearly the whole current generated in the redox process is detected by the measurement. Consequently the diffusion length of the neutral redox species is much larger than the electrode pitch. The difference current I_G-I_C flows through the counter electrode of the potentiostat (cf. Figure 4). If the p-AP is washed out again, the current decreases at once.

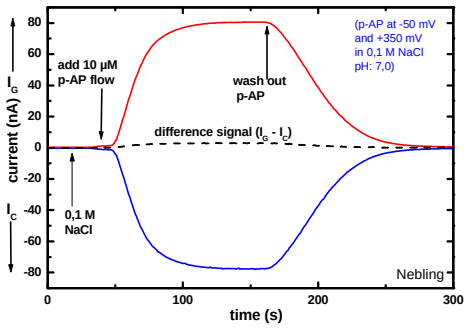


Figure 5. Redox signal of p-AP in a sensor with interdigitated Au lines

4. Process

Now the process to provide the Au-IDA in the backend of a CMOS flow is discussed. In Figure 6 the process modules are schematically depicted. Oxide is deposited on a 6"substrate wafer in order to simulate the starting point of the backend metallization process.

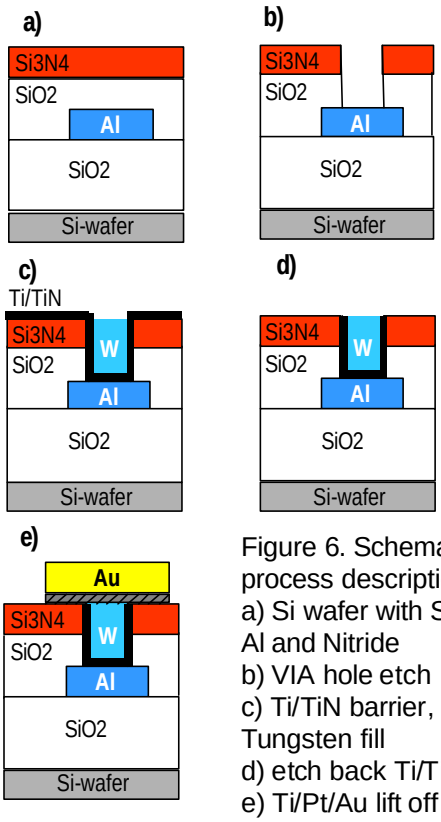


Figure 6. Schematic process description
a) Si wafer with SiO_2, Al and Nitride
b) VIA hole etch
c) Ti/TiN barrier, Tungsten fill
d) etch back Ti/TiN
e) Ti/Pt/Au lift off

Then Aluminum metal lines are processed in a standard manner with Ti/TiN barriers. This Al layer is encapsulated by Oxide, which is planarized in the following with a CMP tool. Nitride passivation is applied to the wafer to stop the humidity flow. The VIA holes are etched down to the Al layer. A Ti/TiN barrier layer is deposited and the holes are filled with Tungsten by a CVD process. In the next step Tungsten is etched back stopping at the barrier layer. Also Ti/TiN at the surface of the wafer is etched with another RIE process. The gold electrodes are now fabricated in a lift-off process, where a Ti/Pt/Au stack is evaporated.
Figure 7 shows a SEM cross section of the backend test chip. The Au electrodes are connected to the Au-bonding pads by the VIA holes and Al lines. Only the active sensor area is in contact with the analyte, so that a redox process at the supply lines does not occur. All measurements are performed using an interdigitated array of gold fingers within an area of 200 µm x 200 µm, finger width and spacing is 1 µm.

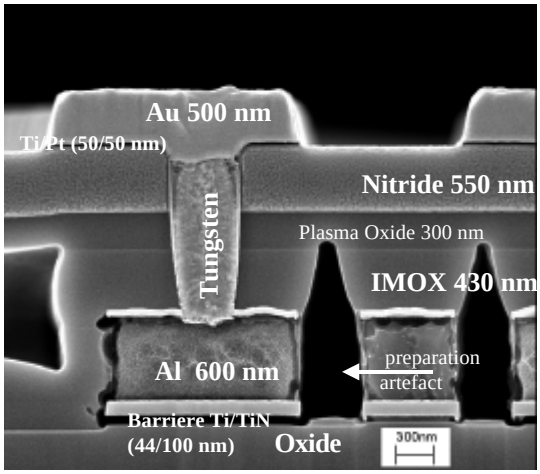


Figure 7. SEM cross section

5. Characterization of the Au electrodes

Since a new material is combined with the CMOS world, its electrical properties must be characterized. In Figure 8 a SEM picture of an IDA is given. This structure consists of very long parallel Au lines so that it can be used to monitor the yield of the IDA. As the dimensions of the Au lines are relaxed (width 1µm and space 1µm), the yield was at least more than 50% for Au lines in parallel with a length of 4.2 m. The resistance between the interdigitated finger electrodes is > 1 GOhm in the dry state. These values are sufficient for a large sensor array with more than 100 individual spots of 20 mm finger length.

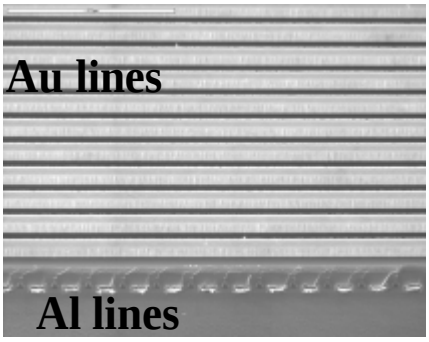


Figure 8. SEM view of interdigitated Au electrodes over buried Al lines

The stack of the Au layer consists of Ti/Pt/Au with layer thicknesses of 50 nm / 50 nm / 500 nm. Measured resistance values of Au-lines, Al-lines, and VIA holes are given in Table 1.

Table 1. Resistance data

	Au lines [mOhm/sq.]	VIA hole (0.8*0.8 µm^2) [Ohm]	Al lines [mOhm/sq.]
no anneal	48	0.37	79
350 °C	51	0.36	76
400 °C	61	0.34	74

In a standard CMOS process a forming gas (N2, H2) anneal is applied, to reduce damage at the MOS interface in the transistors. This process step is also applied to the Au-wafers, where the anneal is done at 400 °C and 350 °C for 30 minutes. Figure 8 shows the SEM top view of Au-lines without heat treatment. There is no change to the as deposited Au-stack up to 350 °C, but the SEM view of the high temperature anneal at 400 °C (Figure 9) shows crystallization of the Au layer. Even this IDA with crystallized Au shows full functionality in the redox current detection.

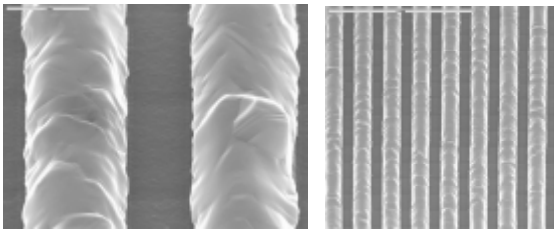


Figure 9. Au electrodes annealed at 400 °C 30 min. in forming gas

6. Electrical DNA detection

Figure 10 shows typical IDA-based measurement results obtained with DNA sequences. At one IDA sensor single-stranded DNA is immobilized which is exactly complementary to the DNA sequence in the analyte. On a neighboring sensor a non specific random sequence of DNA is immobilized. Hybridization with the target molecules in the analyte takes place only in case of the first sensor. First, a buffer is applied to wash out the labeled target molecules of the analyte which have not found a complementary immobilized probe strand. Now 4 mM p-Aminophenolphosphate is added to the electrolyte and then the redox process is started. In both sensors there is some p-APP dissociated in the solution and builds some redox species, which contribute to the measured current. In the 'match' case, the current is higher, because the p-APP is cleaved at the label at the bound target molecule and more redox pairs are actively generated. Finally, the flow of the p-APP is stopped, and no more p-APP is added to the system. At this point in time, the system begins to provide the data which must be evaluated to distinguish between the 'match' and 'mismatch' case. The 'mismatch' current remains slightly increasing from 20 nA to 30 nA. In the 'match' case, however, the current increase is by far more pronounced. In this case the p-APP is continuously cleaved by the label and the generation of redox species proceeds. Thus the current rises up to 100 nA within the time of 300 sec.

Consequently, we conclude that use of integrated CMOS circuitry in combination with such sensors enables highly sensitive electrical DNA detection on multi-sensor chips.

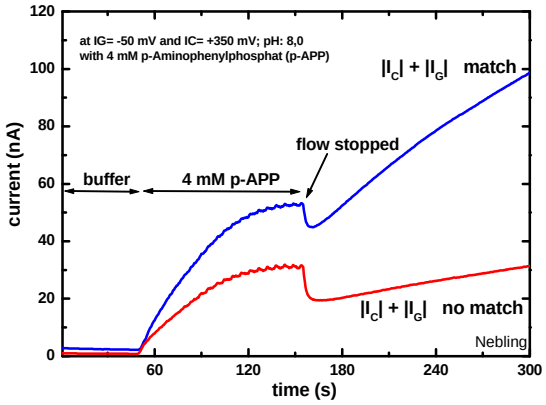


Figure 10. Redox current of a labeled DNA

7. Conclusion

We have shown, that bio compatible materials as gold can be integrated in the backend of a CMOS process. DNA detection by electrical readout can be performed very well with Au based interdigitated arrays using a redox process.

Acknowledgement

Part of this work was supported by the "Bundesministerium für Bildung und Forschung" (BMBF), reference 01M3102B

References

[1] R. Thewes et al, "Sensor Arrays for Fully Electronic DNA Detection on CMOS", *ISSCC*, Digest of Tech. Papers, 2002, pp. 350-351.

[2] R. Hintsche et al, "Microbiosensor using electrodes made in Si-technology", *Frontiers in Biosensonic*, Birkenhauser Verlag, Switzerland, 1997, pp. 267-283.

[3] M. Paeschke et al, "Voltammetric Multichannel Measurements Using Silicon Fabricated Microelectrode Arrays", *Electroanalysis*, VCH Verlagsgesellschaft, Germany, 1996, pp. 1-8.