**BEST****IST-2001-39266**Biomolecular rEcognition by integrated
Smart-sensor Technology

Final Report

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1. EXECUTIVE SUMMARY

The main objective of this assessment project has been the demonstration that a standard CMOS integrated electronic device is suitable for the detection of biomolecular recognition processes on a sensing surface where analysis takes place. Such device should, in the near future, substitute DNA microarrays and/or protein chips and thus drastically reduce time, costs and increasing common availability. A device based on this result is composed of a sensor array and proper circuits able to transduce a signal between biomolecular compounds on the array into an electronic output. Thanks to the foreseen reduction of costs, the application domain of this system is, besides the obvious lab use, the medical self-test for many DNA- and protein-related diseases (e.g., to detect the different pathogenic bacteria from various sources and to use it as a simple, fast and cost effective device in medical, veterinary or army laboratories). This will pave the way to a new generation of products in form of disposable kits available in few years.

The expected result has been obtained by:

1. the study, design, simulation (with proper 3-D structural simulator) and realization of a device of this kind
2. the selection of proper bio-chemical species for testing of the device on DNA and proteins
3. the study of an interface between aluminium and DNA strands that preserves hybridisation properties of DNA
4. the study of a proper electronic test setup, with the realization of custom board and control PC software
5. the use of different pH on a 3-APTS functionalized sensor to test the sensitivity to electric charge
6. the test of the chip on DNA strands

These results will be exploited by setting-up a new research proposal oriented to the realization of a disposable kit for self-test of DNA and protein related diseases.

The study of an interface between aluminium and DNA strands that preserves hybridisation properties of DNA can be further exploited to construct new electronic bio-chip devices.

The BEST consortium is composed of:

- **UNICA**- Dept of Electrical and Electronic Eng. – University of Cagliari – Cagliari – ITALY – EOLAB - Microelectronics
- **IMB-SAS** - Institute of Molecular Biology - Slovak Academy of Sciences - Bratislava - Slovak Republic
- **INSM-S3** - INFM Natl. Center - S³ c/o Dept. of Physics, University of Modena - Modena, Italy

In order to reach the goals of the project, the BEST consortium covers the wide scientific range from biology to electronics through physics.

Expertise, in particular, is present on:

- biological materials and analysis techniques (IMB-SAS),
- organic-inorganic interface (INFM-S3),
- physics of electronic device, electronic device design, measurements and lattice computation (UNICA).

Knowledge overlap between partners allowed a better understanding and cooperation in the group.

2. PROJECT OBJECTIVES

The main objective of the research in which this assessment project is framed, is the design, realization and testing of a standard CMOS integrated electronic device suitable for the detection of biomolecular recognition processes on a sensing surface where analysis takes place. Such device should substitute protein tests and DNA microarrays, drastically reducing time/costs and increasing common availability.

With the foreseen reduction of costs (due to the use of a standard low cost technology), beyond the obvious use in lab activity, the application domain of the system proposed is the medical self-test for many diseases related to DNA (e.g. tracking the activity of thousands of genes at once with a matrix of such devices would enable to monitor how patterns of gene expression change in diseases such as cancer or detection of many microbial and viral pathogens from different sources) and protein (e.g. monitoring the expression of nuclear proteins with specific alterations could bring to prompt diagnostic assessments). Ideally, the final product could be a disposable kit with very simple use suggestions.

To this aim, the exploitation of microelectronic processes for realizing structures capable to reveal molecular recognition based on non-optical methods seems to be the most promising.

The sensing surface consist of an array of “smart” sensors, each made by a sensing area where the biochemical signal is transduced into an electrical quantity with side circuits devoted to amplification and elaboration of the electrical signal produced in the sensing area. The basic working principle of such system consists in the functionalization of the sensing areas with target molecules, linked by means of biochemical functionalization methods. Receptor molecules, dissolved in solution, will link to the corresponding targets and where the molecular recognition occurs, an electrical signal is produced. The functionalization must give rise to specific sensing bio-surfaces, as it must be possible to detect a particular molecule even among very similar competitors.

The use of simple field-effect devices leads, as required by the application domain, to low-cost and small area devices since standard CMOS technologies can be used.

The main objective of this assessment phase was to verify if such field effect devices are able to detect electrical parameter variations that can be referred to a process of specific molecular recognition such as DNA hybridisation or protein-protein interaction as in the case of immunological reactions.

A second goal of the assessment phase was to understand what kind of post-elaboration is required according to the electrical effects measured. It is important to determine if the output of the sensors should be interpreted statically or dynamically, and if interactions of many sensors (exploiting the specific knowledge presents in the consortium) should be considered to obtain a stronger, clear and reliable recognition.

3. METHODOLOGIES

The approach used in the application domain can be subdivided in optical passive techniques and active techniques.

Passive optical techniques

DNA microarrays are an example of such techniques. Each array consists of a reproducible pattern of thousands of different DNAs (primarily PCR products or oligonucleotides) attached to a solid support, usually glass. Fluorescently labeled RNA or DNA prepared from messenger RNA is hybridized to complementary DNA on the array and then detected by laser scanning. Fluorescence gives a measure of the hybridization but requires expensive ancillary optical equipments and molecular tagging which is usually affected by high background level and photobleaching.

Active techniques

The use of sensors based on electrical rather than optical recognition has been proposed for example in several original papers presented at ESSDERC 2002, such as a sensor based on capacitance measurements (“A Biosensor for Direct Detection of DNA Sequences Based on Capacitance Measurements”). In the same conference an active sensor, which makes use of electrodes and several process steps, was presented (“Array-Based Electrical Detector of Integrated DNA Identification System for Genetic Chip Applications”).

The proposed approach

Our approach can be classified in the active techniques, but with a further constraint: the use of standard CMOS technology to reduce the cost. Such limit

is not usually considered in available scientific literature, because it is difficult to fulfil. However it represents the gateway to the computational power of analog and digital devices available on CMOS technology. In such a way they can be easily integrated on the same die of the sensor. Company producing DNA-arrays claims that optical techniques are more reliable than active techniques. The capability of integrating sensing and processing on the same surface can reduce noise, implement multi-step test and at least evaluate the reliability of the measure.

Given the standard CMOS constraints, the methodology employed takes advantage of standard design tools (Cadence for layout design) The main design effort is then in the sizing of the structures and translation of the structure in terms of standard CMOS layout masks (e.g. it is not usual have a window in the passivation layer outside a connection pad). For structural simulation a 3-D MEM simulatore has to be used.

4. PROJECT RESULTS AND ACHIEVEMENTS

Comparison to the original project objectives

Results are in line on what we expected. The main result of the project is the demonstration that a standard CMOS integrated electronic device is suitable for the detection of biomolecular recognition processes on a sensing surface where such analysis take place. This is the crucial result we expected and we put it in the success criteria. This will be exploited by setting-up a new research proposal oriented to the realization of a disposable kit for self-test of DNA and protein related diseases with industrial presence. A second result is the implementation of an interface between aluminium and DNA strands that preserves hybridisation properties of DNA. This could be exploited also outside the specific topic of the project.

Relations and synergies with other relevant projects

The problem of biomolecular recognition involves many aspects. The focus of our assessment project is on a smart sensor, but other approaches and many other aspects should be considered to obtain an industrially relevant device.

In IST-2001-33011-*Micro-Inductive Based Biosensor Arrays for Very High Sensitivity Detection* and IST-2001-37239-*Integrated Opto-Nanomechanical Biosensor for functional Genomic Analysis* the application domain is the same of our research (after this assessment step). The substrate they use is standard CMOS technology, in the second case followed by standard silicon micromechanical technology steps. In the first project the focus is on the realization of micro-inductive biosensor arrays, in the second the realization of arrays of microcantilevers. The physical phenomenon to be detected is different. For this fact, synergies can be viewed in comparing different approaches or in finding an integrated solution with higher reliability (limited size is a common feature of all the devices considered). IST-2000-28214-A *Bioanalytical Microsystem Based On An Optical Microchip* shows how to efficiently reduce the size of devices for DNA detection with optical techniques. The microfluidic part (probably similar) of all these systems can be object of synergies.

Outside the European Union funded projects, and also outside the Europe there are interesting projects in the field, mainly industrially funded/driven (Nanogen, Affimetrix, STMicroelectronics, HP and others).

Implications for EU policies and standards

The research can have a large impact on the European technological progress and the competitiveness of the European industry and research with respect to USA and Japan. The kind of analysis considered in this project, is usually performed with large/expensive equipment, through optical techniques. Such approach is not feasible for spread/non technical use. On the contrary the possibility of performing such test easily and at low-cost leads to the real possibility of having as final product a disposable kit for important diseases with very simple use suggestions.

Benefit to society

The social and quality of life impact of the research is obvious for its medical applications. The wide spreading of array-based diagnostic approaches would soon generate invaluable data bases mapping, for instance, the occurrence in space and time of particular diseases. This will help a lot in finding out correlations and parameters useful for more effective prevention and cure of important diseases (e.g. cancer). Same implications for protection and preservation of the natural environment.

Once assessed the proposed new type of sensors for bimolecular recognition processes, the natural exploitation could be one or more big research project with involvement of medical and industrial effort for the realization of integrated sensor systems for specific diagnostic purposes. This will lead to enhancement in the European competitiveness and support for the growth of European industry

5. DELIVERABLES AND REFERENCES

The deliverables of the project are summarized in the following table:

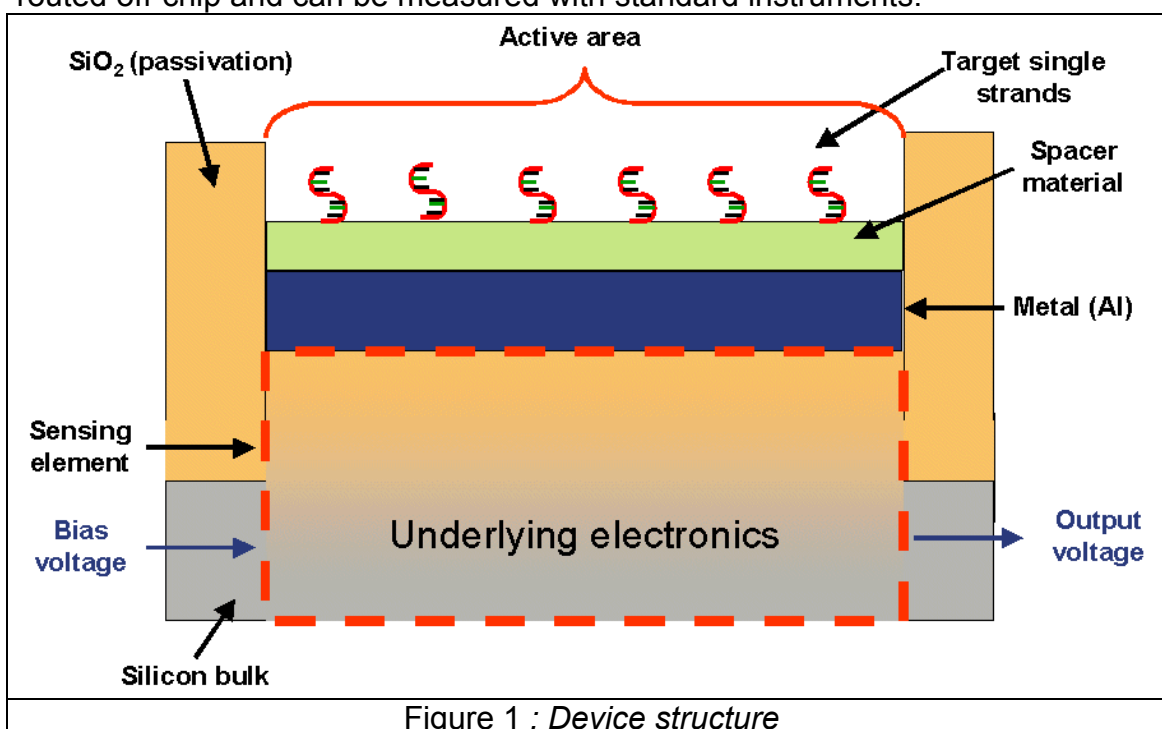
Del. No.	Title	Type	Classification	Issue Date
1	Simulation report	Report	Internal	30.04.03
2	Layout of the chip 1	Report	Internal	31.05.03
3	Smart sensor	Report	Internal	31.12.03
4	Layout of the chip 2	Report	Internal	-
5	DNA probes	Report	IST	30.04.03
6	Comparison with DNA array methods	Report	IST	31.08.03
7	Protein probes	Report	IST	30.09.03
8	Biochemical comparison	Report	IST	31.12.03
9	Functional surface 1	Report	IST	31.05.03
10	Target layers 1	Report	IST	31.07.03
11	Functional surface 2	Report	IST	31.08.03
12	Target layers 2	Report	IST	31.12.03
13	Functional test	Report	IST	31.12.03
14	Interpretation of results	Report	Internal	31.12.03
15	Project Presentation	Report	Public	31.05.03
16	Dissemination and use plan	Report	IST	30.06.03
17	Technology Implementation Plan (TIP)	Report	IST	31.12.03
18	Basic functional test	Report	Internal	31.12.03

With respect to the workplan presented in annex 1, we expanded the test activity to (i) automate the test to have constant condition and (ii) to characterize the sensor also at a lower level with respect to the final application. This activity is described in the not foreseen deliverable 18. Considering this new required effort

and the good results obtained in Deliverable 13, the realization of a second chip has been considered of limited importance for the assessment of the project and Deliverable 4 cancelled.

5.1. Deliverable 1 - Simulation report

The proposed field-effect device is shown Figure 1. The device is realized in a standard CMOS process. A hole (pad) is dug in the passivation of the chip (active area) until the underlying metal layer is reached (aluminum); this step is a standard step performed in CMOS process by the foundry so no extra-processing is needed. A spacer material is deposited on top of aluminum surface and the biomolecular target substances (e.g. DNA single strands) are linked on it. The sensing element is built underneath the active area in the silicon substrate (bulk) and is made-up of electronic solid-state circuits. A bias voltage is needed to properly set the operating point of the sensor. The output is a voltage that is routed off-chip and can be measured with standard instruments.



The detection process is shown in Figure 2. It is based on the electrostatic effect of the charge bound on top of the surface of the sensor (active area). When the device is operated, a bias voltage is applied and sets its operating point. The electric charge of the target biomolecules (single strand oligonucleotides Figure 2a) generates an electric field that is sensed by the underlying electronics. An output voltage is produced.

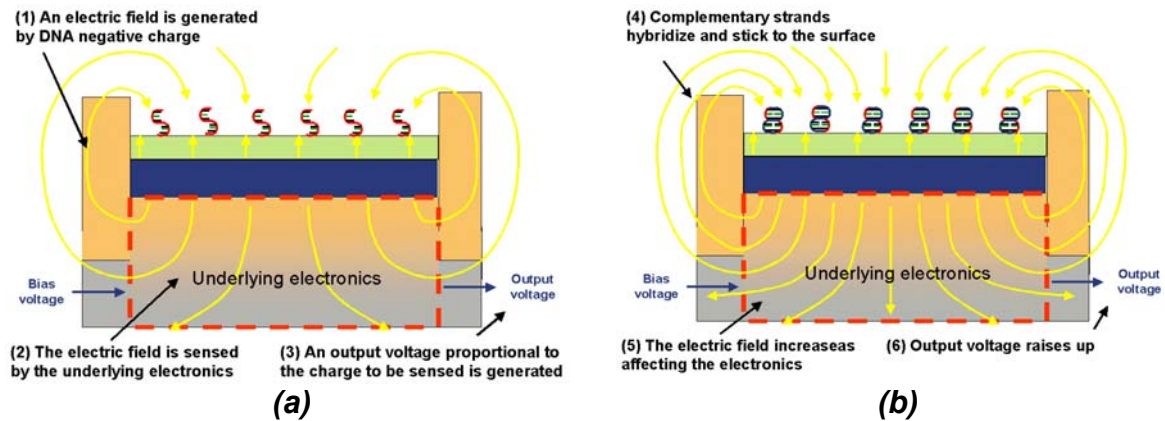


Figure 2: Hybridisation detection (a) single-strand target oligonucleotides, (b) resulting double-strand after hybridization with the probe

The output voltage depends on the device stimuli (electric charge and bias voltage), process parameters (spacer thickness, electrons mobility, passivation thickness and so on) and geometric dimensions (such as the area of the surface). In a first order model, the output voltage should be proportional both to the bias voltage and sensing charge Q_S as stated by:

$$V_{OUTPUT} = \alpha_1 V_{BIAS} + \alpha_2 Q_S \quad (1)$$

The linear coefficients α_1 and α_2 can be related to process and geometric parameters in order to predict the output voltage for a given sensing charge and bias voltage.

When a detection mechanism is triggered (Figure 2b), for example when hybridization takes place and a complementary probe strand sticks to the target strand linked on the surface, the total net charge should increase (doubling). As a consequence, the electric field will increase and affect the behavior of the underlying electronics that will produce a different output voltage (as stated by Equation 1).

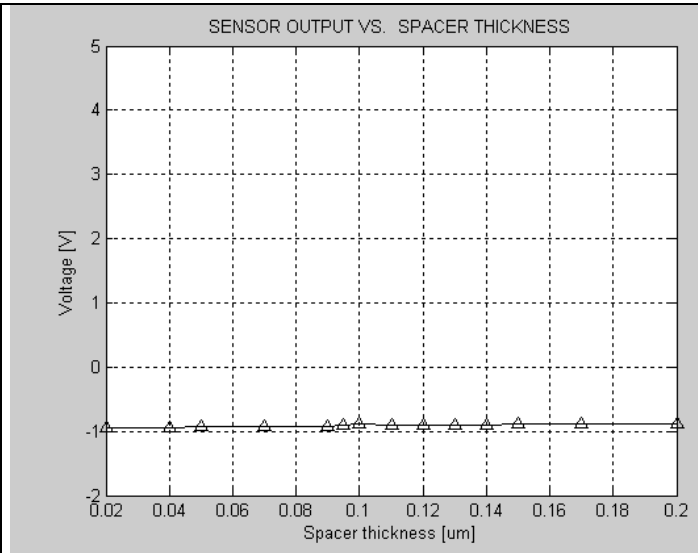
A first-order model of the device, based on knowledge of solid-state silicon structures and CMOS process, was developed. The model is expressed as a set of Equations relating the sensor output to design parameters (such as geometries and layout masks) and process parameters (such as electron mobility, field and gate oxide thickness and so on).

The Equations were validated by means of extensive simulations that checked the validity of the hypotheses that took to the formulation of the model. A 2-D version of the device could be simulated with MATLAB software, while to simulate the complete 3-D structure the use of commercial software (CoventorWare) was required.

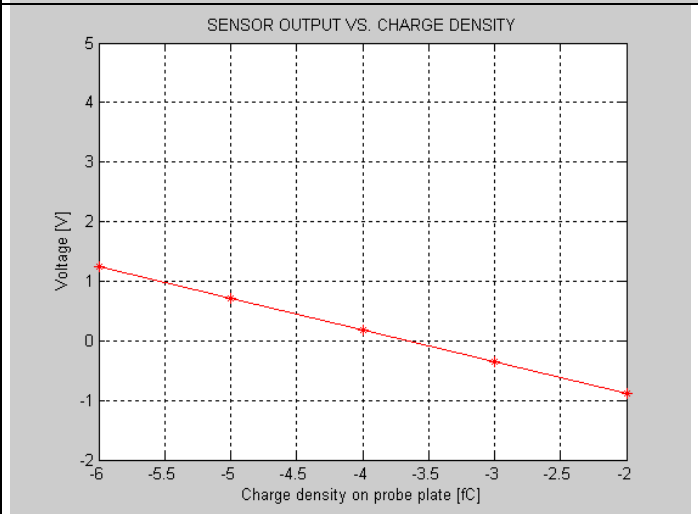
Both sets of simulations validated the Equations of the model, by assessing the correctness of the basic hypotheses and by comparing the outputs obtained from simulations with the outputs predicted applying the model with the parameters of a given CMOS process.

It has been demonstrated:

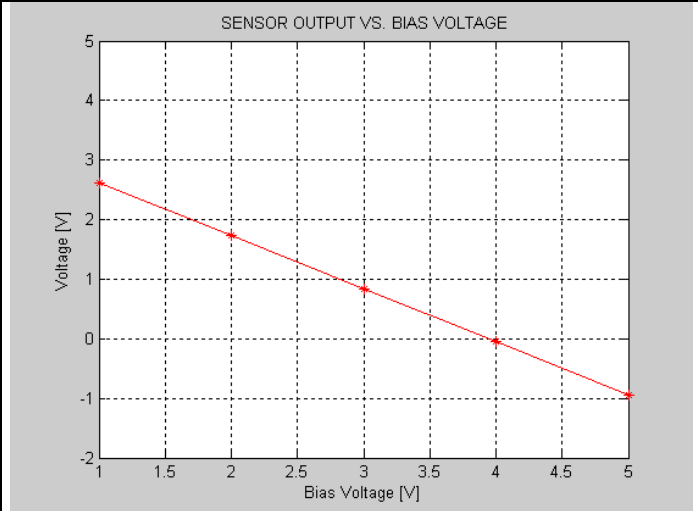
1. that spacer thickness does not influence the output voltage of the sensor, as long as it is of the same order of magnitude of silicon passivation.



2. the linear relationship between sensing charge and sensor output.



3. The linear relationship between bias voltage and sensor output.



Once proven the validity of the first-order model with 3-D electrostatic simulations, we were able to check the accuracy of predicted sensors outputs, calculated using the set of relationships tuned on a specific CMOS process. The following table shows predicted and simulated value of coefficients α_1 and α_2 which represent the linear relationship between output voltage and, respectively, bias voltage and sensing charge for different dimensions of the sensing area.

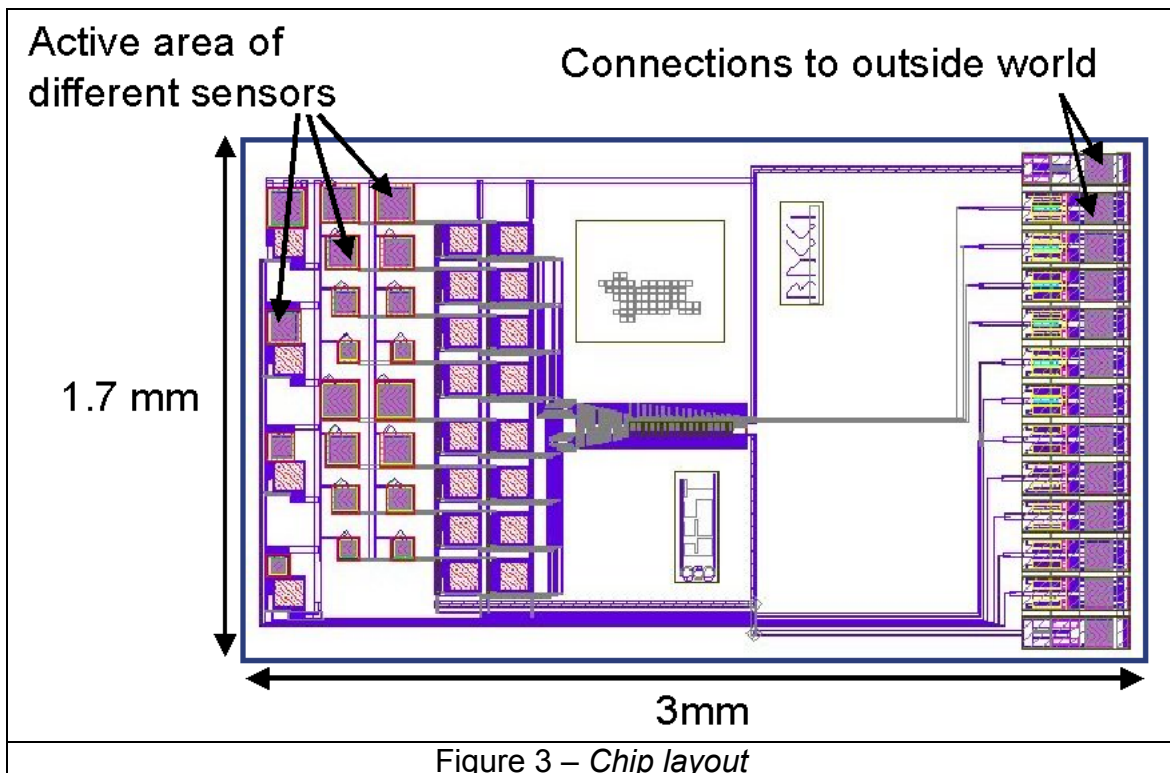
Active area	α_1 Prediction	α_1 Simulation	α_2 Prediction	α_2 Simulation
100X100	0.928	0.891	5.23 E+10	5.32E+10
80X80	0.940	0.904	5.36 E+10	5.25E+10
60X60	0.950	0.914	5.47E+10	5.44E+10
40X40	0.962	0.922	5.55E+10	5.38E+10

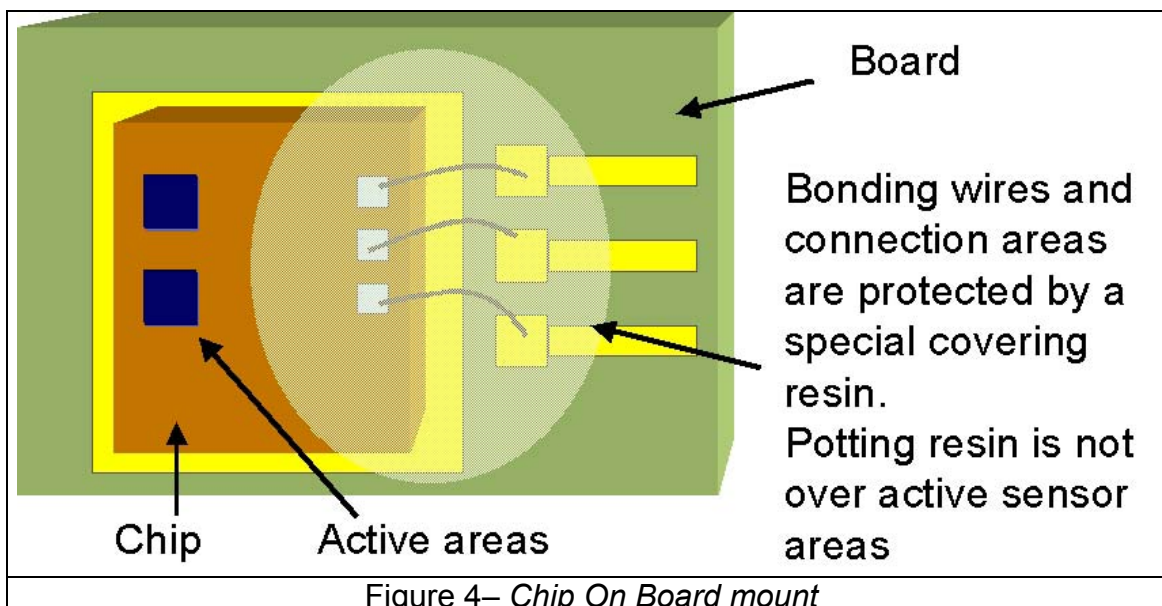
As it can be seen, the predicted values match very well the simulated ones, so the output voltage can be calculated without running time-consuming Converter or MATLAB simulations but just applying the validated relationship tuned on a particular CMOS process.

5.2. Deliverable 2 – Layout of the chip

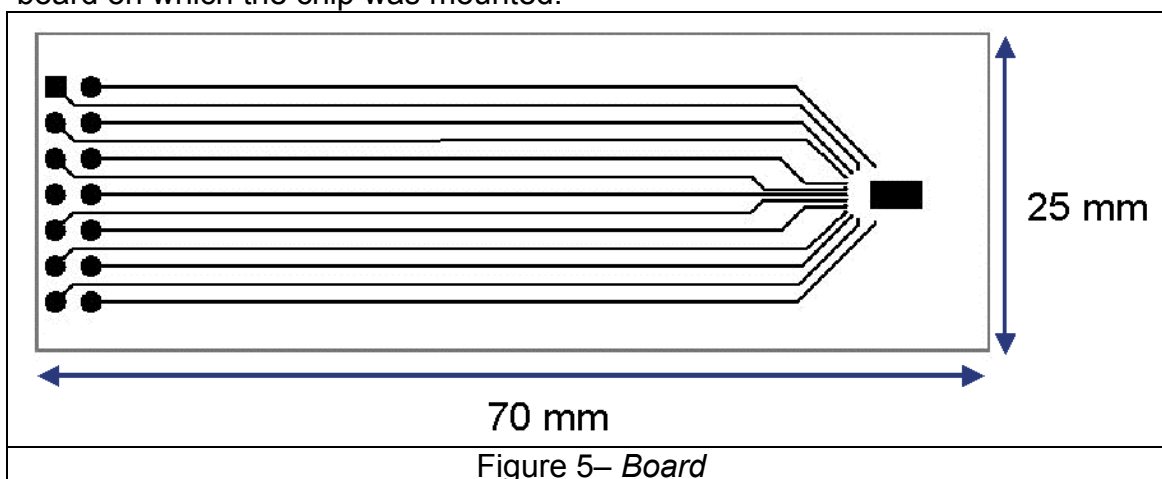
The chip was designed using Cadence software both for circuit design and simulation and for layout editing and tape-out. We were forced to reduce as much as possible the number of total pins in order to reduce the complexity of bonding. In fact, the die had to be mounted on a particular board suitable to be used in all the biochemical processing and manipulations the chip had to go through. To reduce the pins and still be able to integrate an array of 20 sensors we used random access for 16 of them while we chose to be able to independently and contemporarily access the remaining 4.

The layout of the chip is shown in Figure 3. The sensors are very close to one border of the die, all the 13 pads are on the opposite border, as far as possible from the sensors. In fact, the chip was attached directly to the board (Chip On Board mounting) and bonded without a package. A special resin was used to protect bonding wires (as shown in Figure 4) and we had to be sure this resin would not cover the active area of the sensors.





The board was designed using Protel software. We decided to use a board with the same dimensions of a laboratory glass in order to have a comfortable format for the chemical processing. The connector is placed as far as possible from the chip itself in order to preserve the wires from interferences coming from the chemical manipulation going on top of the chip surface. Figure 5 shows the board on which the chip was mounted.



5.3. Deliverable 5 – DNA Probes

We have designed different size oligonucleotides for probing with the complementary oligonucleotides and with oligonucleotides with different number of mismatches. In particular we were interested to obtain two fully not complementary pairs of oligonucleotide/complementary-probing oligonucleotide for combined testing on the same sensor surface. According to the choice of the surface functionalization, the modification of attaching oligonucleotide primer was selected as a modification at 5' end of the single strand (ss) DNA by thiol group. The attaching primer (BAC_1cs2, T0 in test results) was designed with 13-mer poly-dT spacer, followed by a specific sequence from variable region of 16S rDNA of bacterium *Bacillus subtilis* and *Francisella tularensis*, respectively. The following oligonucleotides were synthesized:

name	sequence
T0,BAC_1cs2	5' -TTTTTTTTTTTTTGGTTTCCGCCCTTAGTG-3'

P0 ,BAC_1cs2com	5' -CACTAAGGGGCGGAAACC-3'
BAC_1cs2commut	5' -CACTAAAGGTCGGAAACC-3'
BACnoncom	5' -GCTGTTTCTGCCGAACGT-3'
T1	5' -AAAAAAAAAAAAAAGTCGGTGTAAAGGCTCT-3'
P1	5' -AGAGCCTTTACACCGACT-3'

T0 and T1 are prepared to be bounded to the sensor functionalized surface, P0 and P1 are their complementary-probing oligonucleotide. BAC_1cs2commut and BACnoncom were synthesized to test hybridization with T0. The primers melting temperatures were above 50 °C and they were in 1 μM, 200nM, 40nM scale. All the material obtained has been purified and analysed by HPLC analysis.

5.4. Deliverable 9 – Functional surface 1

We demonstrated the possibility of using silanes as precursors for the formation of layers of biomolecules on an aluminium surface covered by a thin layer of native oxide. We developed both a “single step” functionalization strategy, making use of (3-Mercaptopropyl)trimethoxysilane, for immobilizing biomolecules with accessible thiol groups (SH) or disulfide bridges (S-S) and a broader “two step” strategy, making use of (3-Aminopropyl)triethoxysilane (first step) and glutaric dialdehyde (second step), for the immobilization of biomolecules with exposed amines (NH₂). The functionalized surfaces have been characterized by means of X-Ray Photoelectron Spectroscopy (XPS) and Ellipsometry.

5.5. Deliverable 10 – Target layer 1

We immobilised different kinds of proteins on oxygen exposing surfaces exploiting the two surface functionalization strategies previously developed. We concentrated on the immobilization of the protein Azurin, which bears a disulfide bridge on its outer surface and on the protein iso1-Cytochrome c from *Sacchomyces cerevisiae* which bears an accessible SH group. The obtained protein layers have been characterized by Scanning Probe Microscopy (SPM), UV-vis spectroscopy. Both kinds of proteins can be immobilized on surfaces functionalised both by 3-MPTS and by 3-APTS+GD, allowing the first functionalization method for a more uniform and specific orientation of the proteins in the layer. In fact, Azurin has only one disulfide bridge with respect to 12 surface amines (coming from lysines) uniformly distributed, and iso1-Cytochrome c from *Sacchomyces cerevisiae* has only one accessible SH group against 17 surface amines. Differences in orientation for Azurin between the two constructs have been demonstrated both by XPS, taking advantage of the signal from the Cu ion of the protein, and by linear voltammetry investigating the spreading of the formal potentials of the redox active protein layer.

5.6. Deliverable 18 - Basic functional test

We first developed a system able to give us a sufficient degree on test automation and repeatability

Figure 6 shows a diagram of the test set-up we used to measure the sensors output. We used a HP 4155 Semiconductor Parameter Analyzer to generate the stimuli (supply and bias voltages) applied to each sensor and to measure its output voltage. The HP 4155 was connected to a PC via the GPIB bus (IEEE488) in order to automatically configure the instrument and acquire and save the results. Custom software was developed in C language to handle the

GPIB connection to the HP 4155 and the generation of control signals for the multiplexer board via the parallel port.

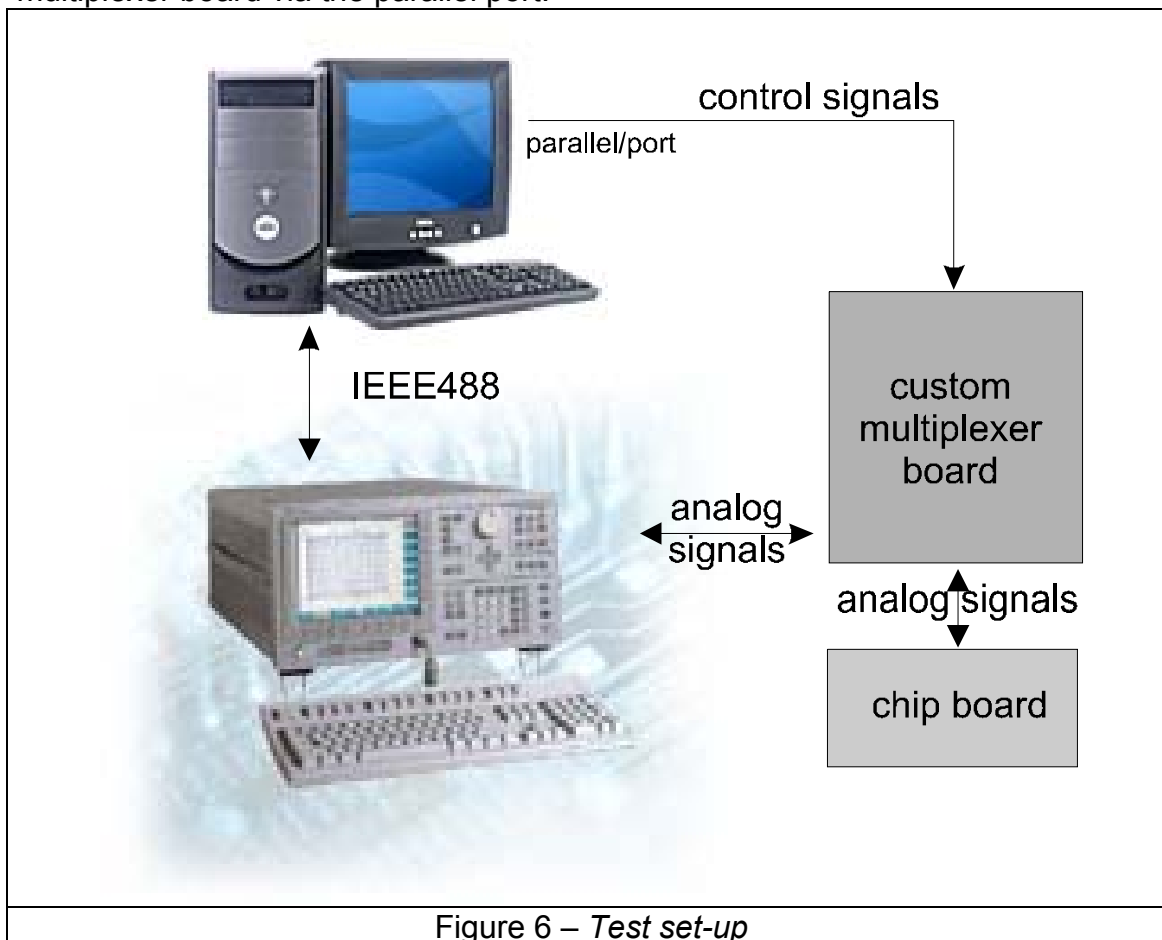
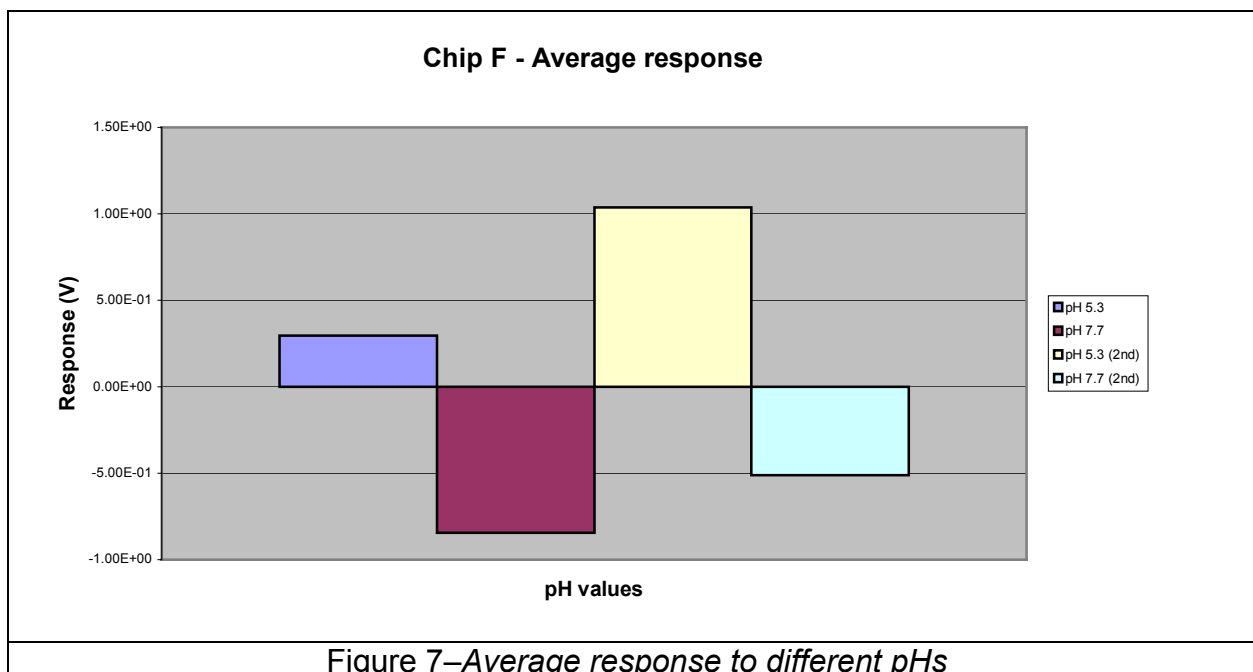


Figure 6 – Test set-up

Before testing the capability of the proposed sensor to detect complex biomolecular processes we chose to verify the working principle with a simpler approach. In fact, the recognition of a biomolecular process such as hybridization depends on a number of events (e.g. successful hybridization, biochemical set-up and so on) that could prevent the successfulness of the experiment even when the electronics perform as expected.

For this reason we performed a set of simpler experiments to validate the results of simulations and verify the capability of the realized device to detect electric charges peculiar of different chemical species. These chemical species are represented by salt solutions at different pH. We verified the ability of the sensor to react differently to different solutions.

This set of tests was performed on chip functionalised with 3-APTS, following the procedure described in Deliverable 9. The functionalised surface, exposed to a solution with a specific pH, should react differently depending on the pH of the solution itself. Particularly, an acid pH should increase protonation of amine groups exposed by the surface (increasing the ratio between protonized and free amines), while a more basic solution should decrease the number of protonized groups. In Figure 7 the results obtained on one chip show the change of response with different pH.



5.7. Deliverable 13 – Functional test

After having verified the capability of the sensor to respond to different chemical species (Deliverable 18) we set up a test procedure to verify the recognition of hybridisation of complementary strands.

The procedure was given by the steps:

Step 0) Functionalisation of the chip surface with 3-MPTS

Step 1) Immobilization of T0 target oligonucleotide on the active areas of a cluster of sensors

Step 2) Immobilization of T1 target oligonucleotide on the active areas of a second cluster of sensors

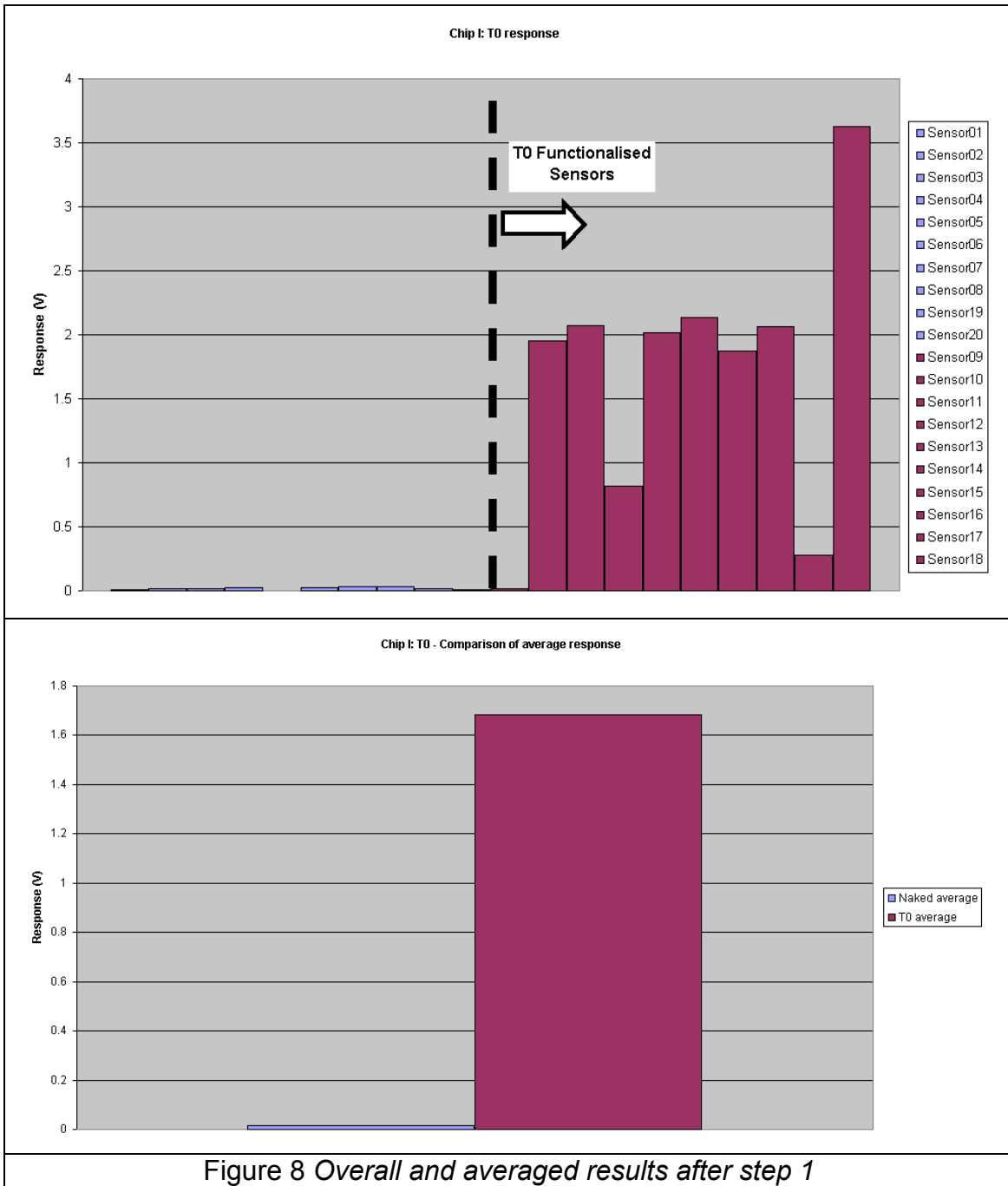
Step 3) Hybridization on the entire chip surface with P0 probe oligonucleotide (complementary to T0)

Step 4) Hybridization on the entire chip surface with P1 probe oligonucleotide (complementary to T1)

To immobilize different target oligonucleotides on different clusters of sensors we spotted a drop of each target onto a different half of the chip. During this phase we cannot use an automatic lab spotter since the chip board is too thick and could not fit into this kind of instruments. The spotting was done by hand, under an optical microscope, and not always was successful, thus a number of experiments failed for this reason.

Figure 8 shows the results on a chip after Step 1 and Figure 9 shows the same after step 3 (hybridization with P0 probe).

In red and on the right are grouped the responses of the sensors which were treated with a specific target and in blue and on the left the responses of the sensors *not* treated with that same target. The diagram is followed by a diagram with average response of the two different clusters, in blue (and left) the average response of the naked cluster and in red (and right) the average response of the cluster covered with the target. In Figure 9 the higher responses (unexpected on the blue side) correspond to the sensors on the periphery that are subject to border effect.



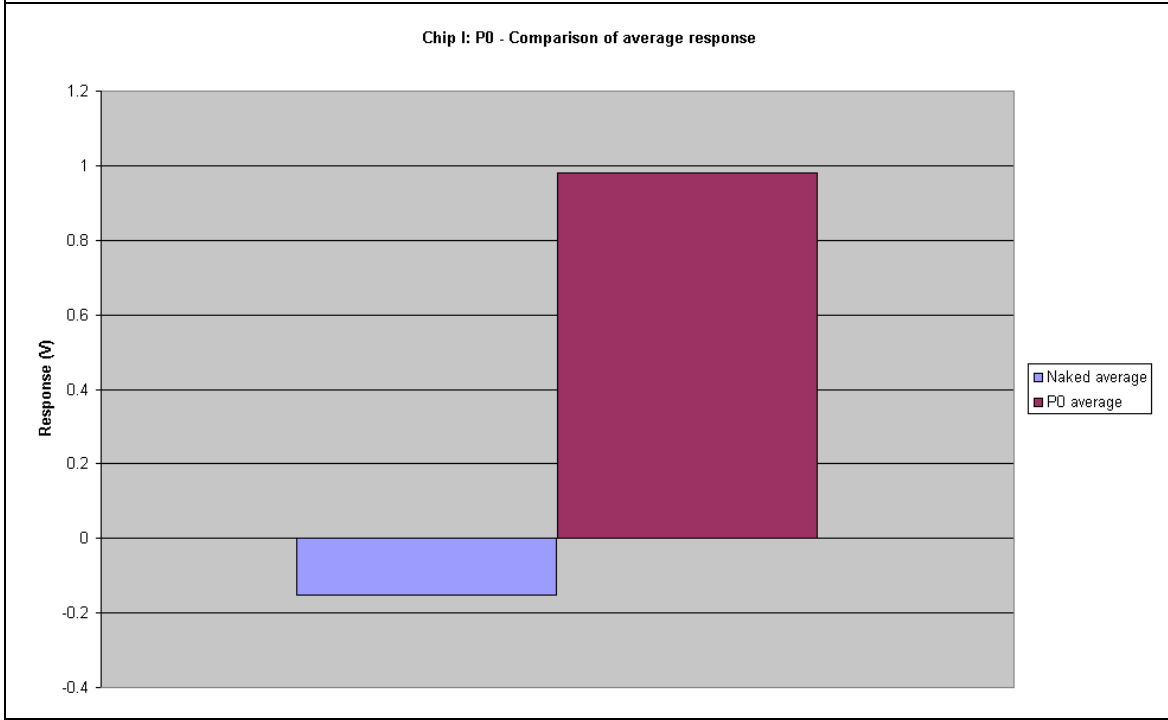
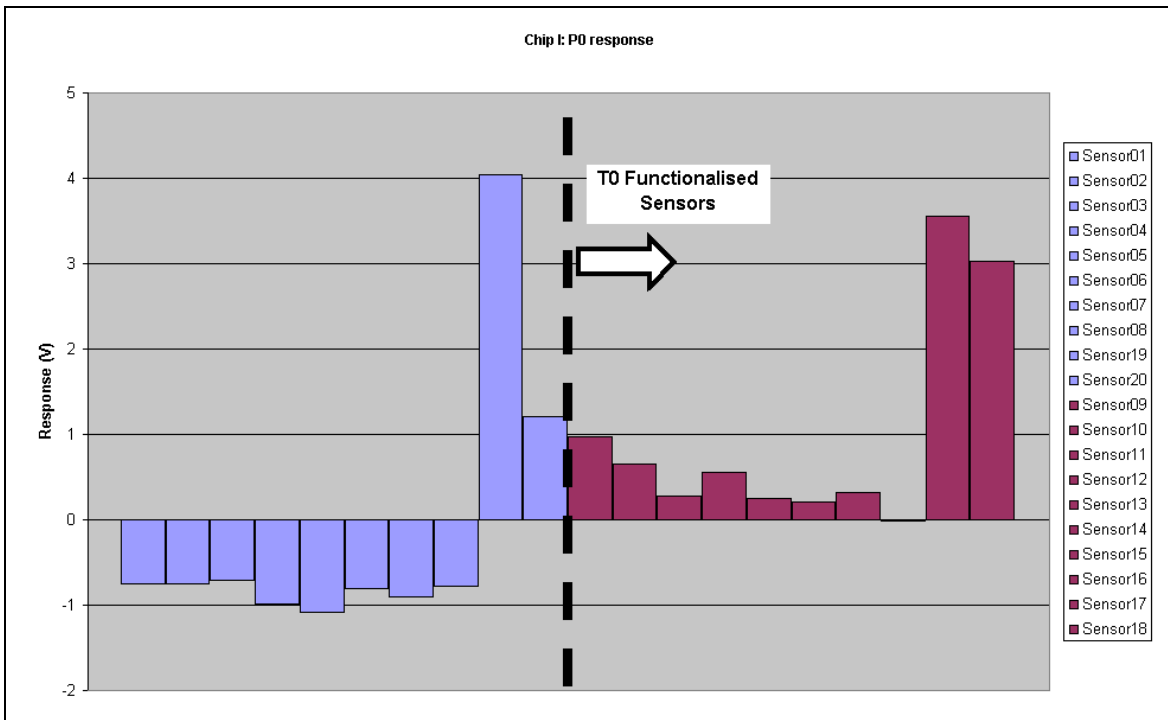


Figure 9 Overall and averaged results after step 3



Figure 10 – Drop of T0 on chip surface.

5.8. Deliverable 14 - Interpretation of results

The pH experiments described in Deliverable 18 have been interpreted.

Such test had the aim of validating the simulations of Deliverable 1 and proving the capability of the sensor to detect a chemical difference in the sample drops covering its active area. Sample drops at different values of pH (obtained solving acid or basic chemical species in bidistilled water) were used to stimulate the 20 sensors as a whole. Experiments were repeated several times to verify the reversibility of the reaction. The sensor response depends on the electric charge immobilized on its active area. The pH value of the solution covering the active area modulates the surface charge of the silane layer since it increases or decreases the number of protonized amine groups. This difference is the one we want to measure and to transduce in an electric voltage

The experiments with pH solutions proved that the realized sensor is sensitive to different chemical species on top of its active area. The response is noisy but can be easily emphasized by averaging the responses of groups of sensors. The response to different pH depends on a charge effect, because it is repetitive and reversible.

The DNA hybridization experiments aimed to obtain a detectable difference between the responses to the hybridization process by the two clusters.

It was not possible to implement the hybridization on the chip surface using standard chambers because the thickness of the test board is too high. The hybridization in open air was difficult for what concern the control of temperature, buffer and umidity. The drop of P0/P1 covering the chip had to be constantly refilled because it dried quite fast. This lead to a dilution of P0, with a consequent reduction of hybridization rate (i.e. not 100% of the target strands immobilized on the surface were hybridized).

With respect to the goal of the project, such drawback cannot be considered critical because this reduction of hybridization rate did not affect the results in such a way that it was not possible to detect the desired effect.

This problem has been solved in literature by using active hybridization i.e. creating non-uniform potential profiles on the sensor surface to attract the molecules and drastically reduce the reaction time (in the order of few minutes). To do that a new buffer (zwitterionic histidine) should be used.

We consider the active hybridization a mandatory step for the new generation of such devices.

From test results it appears that when the response is averaged on a cluster of sensors processed in the same way, the recognition is successful.

For almost all the tests a correct recognition of functionalization operation (Step 1 and Step 2) has been achieved. In all tests we were able to distinguish clusters of sensors functionalized with one target from the cluster of naked sensors. The difference in the responses of the two clusters is very large (for test in histidine buffer) and easily detectable. Some problems were encountered with the second target (T1) due to the difficulties in correct spotting of the drops and of correct labelling of periphery sensors. In most cases it is hard to assess if a sensor belongs to one cluster or to the other so the verification of correct recognition is difficult. More critical was the correct recognition of hybridization; in this case the differences between average responses were less remarkable even if still present. The problem is probably due to the reduced hybridization rate. Nevertheless we had some good results at least with two chips (chip D and chip I), which are enough to assess the capability of the sensor to detect and recognize the biomolecules.

6. FUTURE OUTLOOK

In this project we simulated, designed, realized and characterized a sensor in standard, low-cost CMOS process that shown to be able to detect biomolecular recognition processes. We set up a procedure to immobilize biomolecules on top of the active area of the sensor and tested the device in two different ways. The natural way of continuing this research is to submit a new project to FET-Open exploiting the results and the experience of this project. BEST was just a proof-of-concept project focussed on naked sensors, the new proposal will focus on:

- a more stable hybridization technique, by considering the microfluidic aspect of the problem. Probably the chip will be included in a chamber, with an alternance of washing and hybridization step. A technique similar to the one patented by Nanogen to attract the target DNA on a small spot (with improvements in scale and time) will be considered.
- the exploitation of our first experiments on proteins and their interface with the sensor.
- improvements on the chip to implement redundancy in the measure. Such redundancy will be used to improve readability and reliability of the test to have a level of reliability comparable to optical techniques. Also multistep measure will be considered.
- at a system level, microelectronic standard interfaces will be implemented to simplify the communication with standard test equipment.
- companies working in the field will be considered as part of the consortium.