UNIVERSITEIT TWENTE.

Bachelor's Assignment:

Self-assembled Monolayers On Nano Electrodes For Biosensing

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Contents

Abstract

This report describes the findings of a project aimed at detecting DNA hybridisation using a chip developed by NXP. The chip uses capacitive sensing of monolayer-covered nano electrodes. It has been attempted to form self-assembled monolayers - first of octadecanethiol, later from thiolated DNA probes - onto the gold electrodes. After incubation the DNA monolayer was made to hybridise with its complementary strand and this was monitored by the sensor. During the project several problems were encountered concerning cleaning the system and assembling the setup. Due to strong temperature dependant sensor values and other ambiguities the measurement data did not produce evidence of monolayers forming on the electrodes or hybridisation of DNA.

1 Introduction

In this report the results and conclusions of research on a capacitive chip for biosensing are presented. The chip has been developed by NXP. The goal with this chip is to perform DNA sequencing. The measurement principle is based on capacitive sensing of electrodes. However as opposed to a single larger electrode, many nano electrodes in an array are used to provide more data. On the gold electrodes a monolayer of the molecules under test will be incubated. To make a monolayer of DNA, it has been elongated with a 1-dodecanethiol group that can form a self-assembled monolayer (SAM) on gold or silver surfaces. The DNA will first be copied in a process called rolling circle amplification (RCA), creating long strands of copies of the beginning DNA. More information on RCA can be found in the appendix.

When the DNA has formed a monolayer, the sequencing can start. One base pair at a time, the DNA will bind to its complementary part. Since this changes the properties of the molecules, this should also change the monolayer capacitance which is measured. To overcome the problem that the electrolyte above the chip forms a big part of the measured impedance, a very high frequency of around 50 MHz is used. At this frequency the measured impedance is believed to be purely capacitive.

This research has committed to investigate the usability of the developed sensor. First experiments will be done with octadecanethiol (ODT) to form a SAM on the electrode surfaces. When these monolayers form, experiments with DNA monolayers and hybridisation can be done.

Research goals

The goals for this project were described in the research plan, and are stated there as follows:

- Attaching an ODT monolayer on the nano electrode surfaces
- Attaching a monolayer of thiolated DNA-probes to the electrodes
- Measuring the existence of these monolayers with the NXP sensor
- Hybridising the DNA-probes with their complements and measuring this with the sensor

Structure

To understand the working order of the chip and the principles behind self-assembled monolayers (SAMs), some theory has been described in the second chapter. This also concerns information about the cleaning processes for the chip and the rest of the system. In the experiments chapter, the followed process guidelines are described. The order of

actions and used concentrations are described here to ensure good reproducability. The results from the described experiments are presented and discussed in chapter four. The conclusions based on this data are given in the next chapter. Apart from the conclusions, some recommendations and possibilities for further research are given.

A timetable for this project is found in Appendix A. An explanation of rolling circle amplification is included in Appendix B.

2 Theory

2.1 The system

The core of the system is a biosensor consisting of 256 x 256 nano electrodes. The sensor and the measurement system around it are developed by NXP. The goal for this sensor is to be used for DNA sequencing. For this, a layer of DNA must be adsorbed onto the surface of every electrode. This should cause the electrode capacitance to change, which can be measured and read out to provide data on the DNA in question.

Sensor design

The development of this sensor is described in [8]. A view on the top of the sensor surface is given in figure 1. The electrodes are around 155 nm in diameter, this results in roughly 19 fm^2 for each electrode surface.

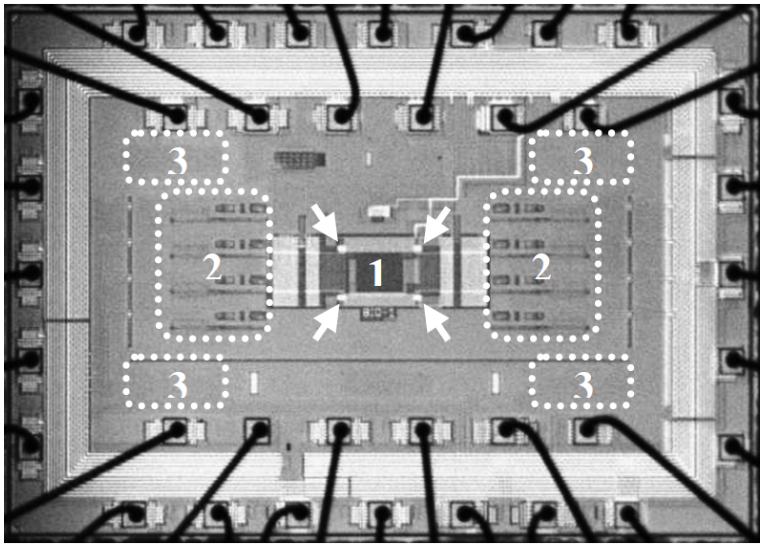

Figure 1: A top view of the sensor chip: 256x256 electrodes (1), 4 temperature sensors (arrows), 8 A/D converters (2) and the on-chip digital data accumulation (3).

Measurement principle

The measuring circuit is shown in figure 2.

The nano electrode and the connected DNA form the capacitance C. By switching Φ_T and Φ_D, the capacitance can be charged and discharged. This will be done on a frequency of around 50 MHz. The capacitance that is measured is that of an electrical double layer on the interface of the electrodes and the monolayer. This double layer is formed by the

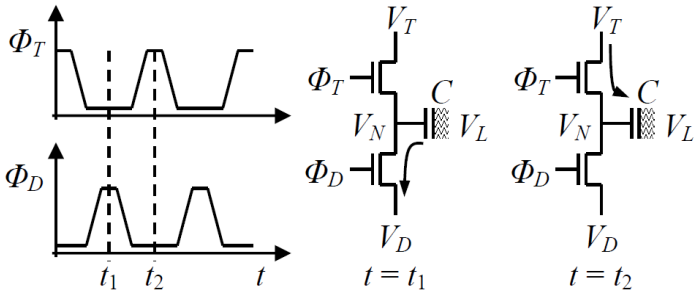

Figure 2: Measurement circuit with CMOS-transistors and the capacitance C

gold surface and the ions in the solution that are kept at a distance by the monolayer. A typical dielectric layer model describes the capacitance of the double layer something like:

$$C_m = \frac{\epsilon_m \epsilon_0 A}{d} \quad [F] \tag{1}$$

This equation is no longer correct in the case of SAM-covered electrodes. Research has shown that for alkanethiol SAMs the capacitance and series resistance change with an applied potential. Also 1 does not hold for varying temperatures and it does not include relaxation effects [3]. A newer model for a double layer capacitance is proposed by Gouy-Chapman-Stern, which divides the capacitance into the first double layer (the Helmholz capacitance) and the rest of the electrolyte (the diffuse layer capacitanc). The Helmholz capacitance complies with the previous C_m, whereas the C_D accounts for the effects of diffuse layers and relaxation. The proposed model is:

$$\frac{1}{C_{dl}} = \frac{1}{C_H} + \frac{1}{C_D} \quad [F] \tag{2}$$

This model is not yet very useful for describing de capacitance of a monolayer interface. Góes et al. modified this equation to suit SAM research. They described the total capacitance in terms of the monolayer capacitance and the previous double layer capacitance:

$$\frac{1}{C_m} = \frac{1}{C_b} + \frac{1}{C_{dl}} \quad [F] \tag{3}$$

With C_m the new modeled capacitance and C_b the bulk capacitance of the monolayer (the expected layer capacitance without ionic ingress). Now, if $C_{dl} > C_b$ then the measured capacitance approaches $C_m \approx C_b$.

In the measurement circuit 2, we find that the total charge in the capacitor is:

$$Q = N(V_T - V_D)(C + C_p) \quad [C] \tag{4}$$

Here, N is the amount of cycles and C_p is the parasitic capacitance of the transistors. When SAM molecules are caught on the electrode, the total capacitance C will change because of a difference in C_m. For a varying capacitance the equation becomes:

$$\delta Q = N(V_T - V_D)\delta C \quad [C] \tag{5}$$

The current flowing through the circuit is determined by the charge on the capacitance. Thus, by measuring this current we can find the capacitance of C.

2.2 Self-Assembled Monolayers

A monolayer is a single, closely packed layer of atoms, molecules or cells. To obtain a high sensitivity, a densely packed DNA monolayer with a backfilling agent will be used [5]. In order to test the working principle of the sensor in an affordable manner, first an alkylethiol monolayer will be formed.

SAM formation

Alkylethiols are much used to form self-assembled monolayers on gold surfaces [6]. The SAMs in this project will be deposited from a liquid (IPA) solution. The self-assembly involves several processes, and can be categorised into three different steps:

1. A low density phase in which molecules can randomly bind to the surface in a non-organized manner
2. A more dense phase where molecules can both stand up or lay down (on the surface), for more molecules to bind the flat laying ones must reorganise
3. The high density phase in which closely packed molecules stand up straight, normal to the electrode surface

In the first phase, binding can take place in different ways. The overall dynamics may be diffusion controlled, adsorption rate controlled or a mixture of these. In the second phase several molecules are already standing normal to the surface. Van der Waals forces between the tail groups of the molecules start to attract each other, organising the laying down molecules to stand up and allow for new molecules to adhere in the created space. When this process is completed (phase 3), the SAM is complete and now forms a single dense layer on top of the electrode surface [7]. The process is illustrated in figure 3.

SAM detection

If the sensor works correctly, the electrodes can be monitored during the process of the SAM deposition. This should yield evidence that a monolayer has formed if the formation

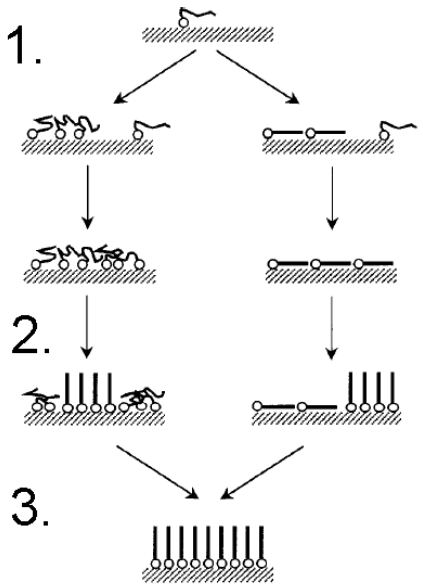

Figure 3: The formation of a self-assembled monolayer

is done correctly. However, since we need to evaluate the operation of the sensor, another method of measuring the existence of the monolayer is needed. In normal cases various options are available here. Examples are using a Quartz Crystal Microbalance, Surface Plasmon Resonance measurements or water contact angle measurement. Because of the small dimensions and the fact that verification on the actual chip is needed, these methods are no option for this project.

Another way to look at the SAM is with fluorescence microscopy. This involves adding a fluorescent tailgroup to the SAM molecules prior to the self-assembly process. Due to fluorescence quenching of the gold surface, this method will not work well with monolayers thinner than 10 nm [2]. This means that for our C18-SAM (length approx. 2,5 nm based on the bond lengths) this will not work. For the DNA probes however, this is a feasible test to find the existence of a monolayer on the electrode surfaces.

2.3 Cleaning and solvents

Since the cleanness of the electrode surface is crucial for a monolayer to form, a lot of attention is given to the cleaning process for the chip. There are several types of debris and dirt that can be expected on the chip's surface.

In the process of chip fabrication, the chips start off with hundreds or thousands on one wafer. When the individual chips are cut off the wafer, sawdust can be present in the saw's

cooling liquid and remain on the chip. This sawdust can usually be removed by simply laying the chip in IPA for a few minutes and then blowing it dry with nitrogen.

During packaging or handling, if a drop of liquid lands on the chip surface this might leave dry spots on the chip. Several salts, oils or other substances may have been dissolved in the liquid and if it evaporates, the dissolved matter can be left on the chip. To remove leftover oils and/or dry spots, chloroform can be used.

The solubility of matter in a solvent is influenced by several properties of both substances, of which the polarity is the most important. A rough measure for this is to look at the solvent's dielectric constant. The dielectric constant of chloroform is 4.8 at room temperature, which is quite low compared to IPA (18) and acetone (21) [4]. This explains partially why many salts, oils and organic substances dissolve in chloroform.

To rinse off all chloroform with the solvents, acetone can be used. Since acetone may leave dry spots, a rinse with IPA makes sure the chip can dry cleanly. A more powerful way to remove things like oils, greases and other contaminations from exposure to open air is UV-ozone cleaning. When a monolayer has formed on the chip's surface, this needs to be removed before the chip can be used again. The procedures described above should be sufficient for this step as well.

In the assembly of the measurement system, due to the construction of the chip socket there is a chance of contaminating the chip with thermal paste. Thermal pastes are often silicone-based, and may contain ethanol and other, often unspecified matter. Silicone clutter may also be coming from the PDMS seal used to enclose the flow cell on the chip. Removal of silicone using solvents is difficult, and thus mechanical procedures like ultrasone cleaning are sometimes used. To remove the silicone, UV-ozone is again a powerful method, however other contaminations may be present in the thermal paste which could leave dry spots or debris on the chip surface.

3 Experiments

3.1 Chip assembly

An overview of the system has been given in the previous chapter. A more detailed explaination of the system as shown in figure 7 will be given here. The cleaning procedures will be further specified in the following paragraphs.

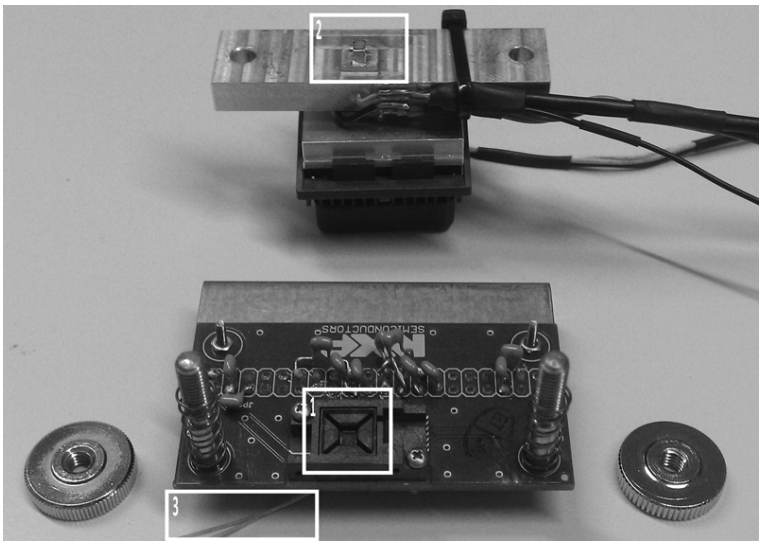

Figure 4: Main block components: the socket (1), heatsink (2) and flow tubes (3)

The sensor and temperature control block (indicated by number 2 in figure 7) needs to be assembled with cleaned parts before every measurement. The main components of the block are shown in figure 4. The liquids used in the measurements will flow through the tubes (3) and into the socket (2), where the chip will be placed. Since the chip is just a bare piece of wafer, a seal is needed to enclose the flowcell. A small rectangular piece of polydimethylsiloxane (PDMS) is used for this purpose. Once this PDMS seal has been cleaned, it is positioned in the socket. On the inside of the socket, gold pins connect the chip to the data acquisition system. The PDMS seal has to be aligned exactly between those pins, and around the input and output tubes of the flow cell. This is visible in figure 5.

The next step is to attach the chip to the aluminium heatsink. A minimal quantity of heat sink compound (DOW Corning 340) is put on top of the small rectangular chip area on the heatsink. This area is shown in figure 6. Next, the cleaned chip is placed on top of the compound with the sensor faced away from the aluminium. It is aligned to heel over equally on each side. Clean metal tweezers are used for this positioning to minimize the risk of contaminating the chip. For the same reason it is important to work reasonably

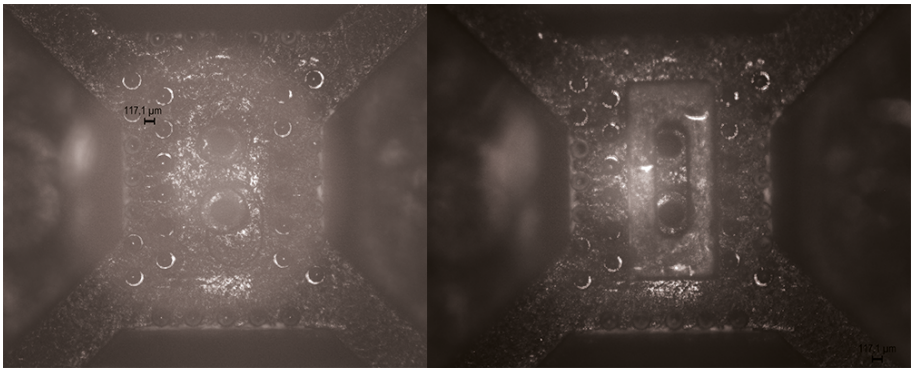

Figure 5: Socket without (left) and with the PDMS seal in place (right)

quickly once the chip is cleaned and out of its casing.

Now both the chip and seal are placed correctly, the heatsink is mounted onto the socket. Guided by the bolts on the PCB the chip and seal are placed on top of each other. The chip is pressed onto the contact pins by the nuts visible in figure 4. A distance of 5.8 mm is kept between the heatsink and PCB.

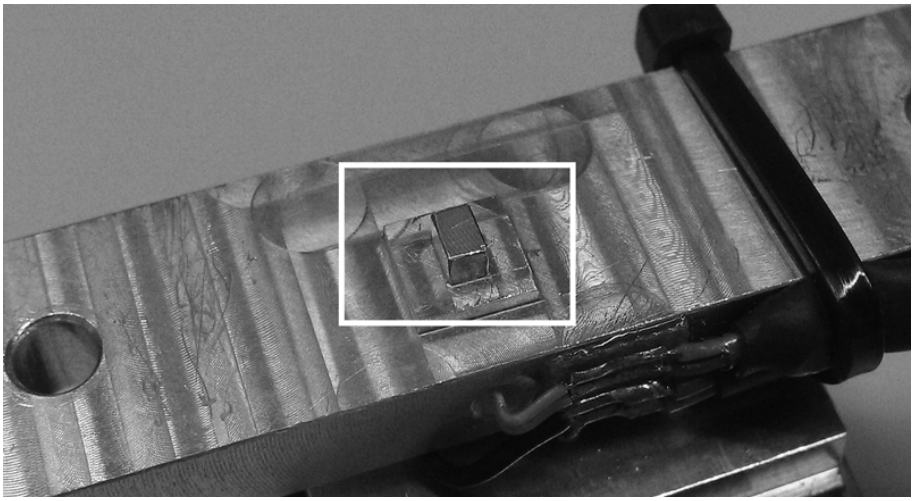

Figure 6: The aluminium heatsink with the sensor location marked

The block can now be mounted onto the data acquisition system to be used for a measurement.

3.2 PDMS cleaning procedure

Since the PDMS seals are very small (around 3x1mm) they have to be laser cut into shape. This laser cutting burns the edges of the seal. To ensure these residues don't end up on the electrodes, they have to be removed beforehand.

For this, the seal is placed in IPA (as available in the BIOS chemical lab) and is kept around 70 degrees Celsius for 3 hours. Any remaining silicone oils or burnt pieces will be extracted from the PDMS during this time. Afterwards, the seal is blown dry with nitrogen and kept in a clean container until use, preferably within a week.

3.3 Chip cleaning procedure

The cleaning of the chip is even more important. For a SAM to develop the electrode surfaces must be as pure gold as possible.

First the chip is laid into chloroform for 30 minutes. It is then taken out, and rinsed first with acetone and then with IPA (all chemicals as available in the BIOS chemical lab). Both rinsings must take place before the previous liquid has evaporated, to avoid dry spots to form. The IPA is blown dry with nitrogen and the chip is placed in a cleaned container.

Next, the chip is placed in a UV-Ozone cleaner. The used machine is the Bioforce UV/Ozone ProCleaner Plus. The machine is turned on and the chip is left to clean for 20 minutes. The chip is then taken out with clean metal tweezers and placed in a clean container, to be used directly.

3.4 Measurement procedure

For each measurement, first the cleaning procedures are followed and the sensor block is assembled. The first experiment that was conducted is an attempt to form a octadecanethiol SAM. The second one was a DNA SAM, with an extra verification experiment of fluorescence microscopy.

Before an experiment can start, the cleaning procedures for the chip and PDMS are followed. Then the flowcell block is assembled. It is then placed into the setup, to complete the setup to what can be seen in figure 7. The input tube is connected to a 500 μl syringe, that will be filled with solutions throughout the experiment. The switching valve in the picture is not used in this project. The syringe is placed in a Harvard Apparatus Picoplus flow pump. The data acquisition system is connected to a PC via USB. The PC runs the software made my NXP for this sensor, PT2 Biosensor Workbench version 1.0.

3.4.1 ODT SAM experiment

This experiment is intended to form a octadecanethiol monolayer on the chip's nano electrode surfaces. The chip will conduct measurements during the incubation period to detect the formation of the monolayer.

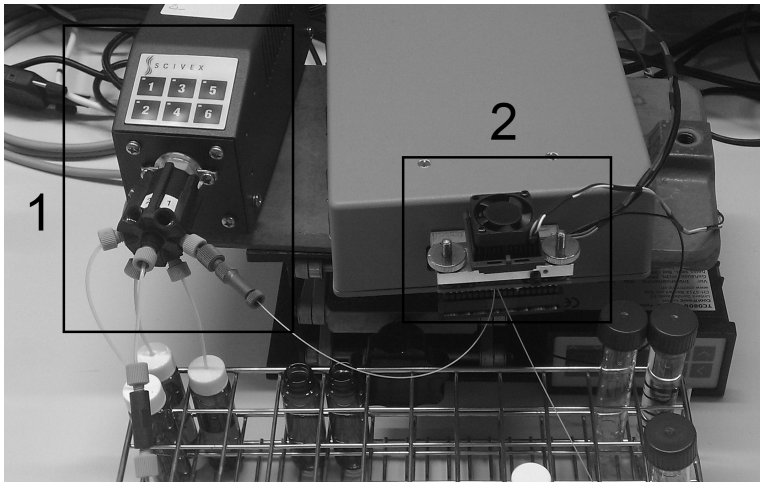

Figure 7: The experimental setup with a switching valve (1) and the sensor with temperature control (2), attached to the data readout system.

The measurement starts without any liquid in the tubes or on the chip. The syringe is filled with IPA and placed on the pump. The pump is set to 5 μl/min, and then switched on. After 10 minutes, the pump is switched off and the IPA in the syringe is removed. A solution of 1 mM octadecanethiol (11-Mercapto-1-Undecanol from Sigma Aldrich) in IPA is inserted in the syringe. The syringe is returned into the pump, and the pump is switched on again at 5 μl/min. After 5 minutes, the pump rate is reduced to 0.5 μl/min. The ODT is now left to incubate the monolayer for 4 hours. After this, the ODT solution is removed from the syringe and IPA is put in. The IPA is left to flow at 0.5 μl/min for over 10 hours to slowly wash away any remaining ODT-solution.

3.4.2 DNA SAM experiment

This experiment is intended to form a monolayer of DNA probes on the electrode surfaces. The DNA probe has been extended with a thiolated C12 spacer, which enables it to form a monolayer. The monolayer probes are then left to hybridize with their complementary target molecules. The target molecules have an added fluorescent tailgroup. These fluorescent tails provide us with an extra verification step; if the sensor doesn't show any results but a fluorescent photo shows the presence of a monolayer, something is going wrong with the measurements. The added fluorophore is Cy5. Cy5 is to be excited with light at a wavelength of 650 nm, and it then emits light at 670 nm.

Probe sequence:	CGTTTTCGATTAGCTGCCCA
	AGTCTTCAATGCATCTTACC
Target sequence:	TGAAACCCCGGTAAGATGCATTGAA
	GACTTGGGCAGCTAATCGAAAACG

To omit the possible difficulties of growing a monolayer on the chip, the SAM formation is done out of the measurement setup. The cleaned chip is placed in a clean container, and a piece of PDMS is placed on top. The PDMS has a round hole of 1.40mm diameter in it, which has to be positioned right above the chip's electrodes. The electrode area on the chip measures 150 by 200 μm. A solution of 1 μM DNA probes in phosphate buffered saline (PBS) (pH 7.4) is placed in the hole. The container is placed in a closed waterbath to stop the solution from evaporating. This is left to incubate for at least 12 hours.

The chip is then placed into the socket following the assembly procedure described above. The experiment starts without any liquids in the tubes. The pump is switched on with PBS (pH 7.4) in the syringe, at 2 μl/min. After 10 minutes, the pump is switched off, the syringe removed and emptied. It is refilled with a solution of 1 μM of the target molecules. The pump is switched on at 1 μl/min, and stays on for an hour to hybridize the probes and targets. The remaining solution is then discarded and the syringe is filled up again with PBS. With the pump on 1 μl/min it is left to flush for an hour. The computer measurements are then stopped. The chip is removed from the socket and placed directly on a fluorescence microscope to make pictures of the electrode area on the chip. The used filter on the microscope lamp is a Red Fluorescence Proteins (RFP) filter.

4 Results and discussion

4.1 Cleaning the chips

For the experiments 4 chips have been used. At first the chips came new out of the package, and were not supposed to be very dirty. Later some chips were cleaned again after some experiments.

4.1.1 New chips

When the new chips came out of their package, some debris could be seen on the surfaces. It seemed that small chunks of some metal were scattered over the surfaces, however not on the electrode surfaces. An example of this is shown in figure 8. It is not clear where this debris comes from, or what it is exactly.

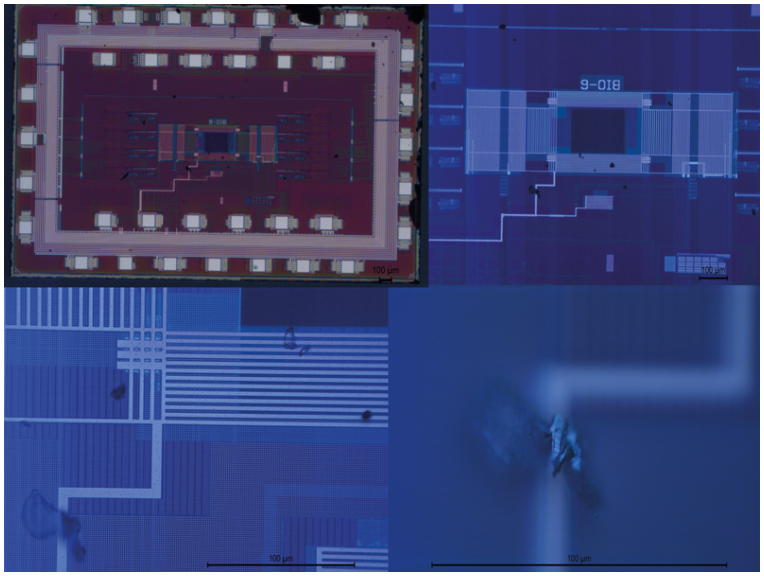

Figure 8: Debris on a new chip: whole chip (top left), electrode area (top right), zoom on debris (bottom left), zoom on debris particle (bottom right)

The chips were taken through the cleaning procedure stated in paragraph 4.3. Reviewing the chips under the microscope showed that the chunks of debris were not, or at least not all removed. Also dry spots of unknown solutes are found on the chip's surfaces. Pictures of a new chip after the cleaning process are shown in figure 9.

The pictures indicate that the cleaning procedure is not sufficient. There are contaminations present on the chips even when new, that are not fully removed by the steps in the guideline. The dry spots on the electrodes in figure 9 suggest that the contamination

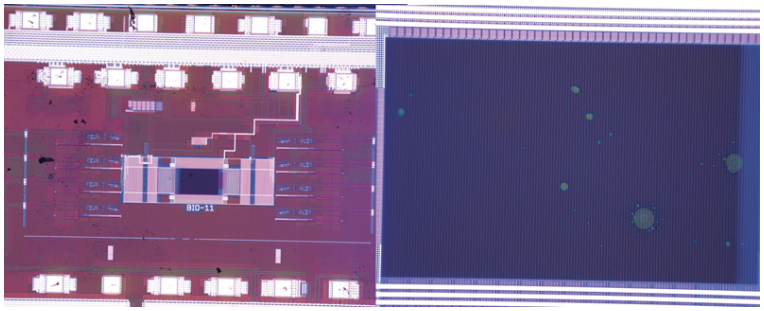

Figure 9: A new chip after cleaning: whole chip (left) and electrode area (right)

does not dissolve in chloroform. It also doesn't react to the UV-ozone cleaning step. It is not clear what the source of the filth is, as it may have accumulated from the moment the chip came out of the lithographic process. Since it is not clear what the dirt consists of, it may be difficult to find a suitable cleaning procedure to ensure a perfectly clean surface. For development purposes it will be important to resolve the source and then eliminate it.

4.1.2 Used chips

When a chip has been removed from the setup for examination after an experiment, it has to be cleaned again before another measurement can be done with it. It showed that in the process of assembling, measuring and deassembling the chip becomes quite dirty. Different sorts of filth are present on the chip after use, as can be seen in picture 10. Every of the 4 chips has been recleaned after use at least once. Figure 11 shows how the chip for the DNA experiment came out of the cleaning process after it had been used.

As with the new chips, it is clear that not all of the contaminations are removed in the cleaning process. The dirt on the right chip appears to be rests of PDMS from the seal. On the top chips in figure 10, a grease-like contamination is visible. This is probably the thermal paste that was used. Since the greasy contaminations seem to be removed after the cleaning, the process is sufficient for that.

4.2 Cleaning the PDMS seal

A total of 6 seals have been cleaned following the procedure in paragraph 4.2. To check whether any substance could leak out of the PDMS due to applied pressure, a verification experiment has been done. A cleaned chip was placed between two clean microscope slides that were pressed together by two binder clips. After 15 minutes the seal was removed and both slides were examined under a microscope. No contamination was present on either slide, suggesting that the seal did not contain any filth that could leak out during the measurements.

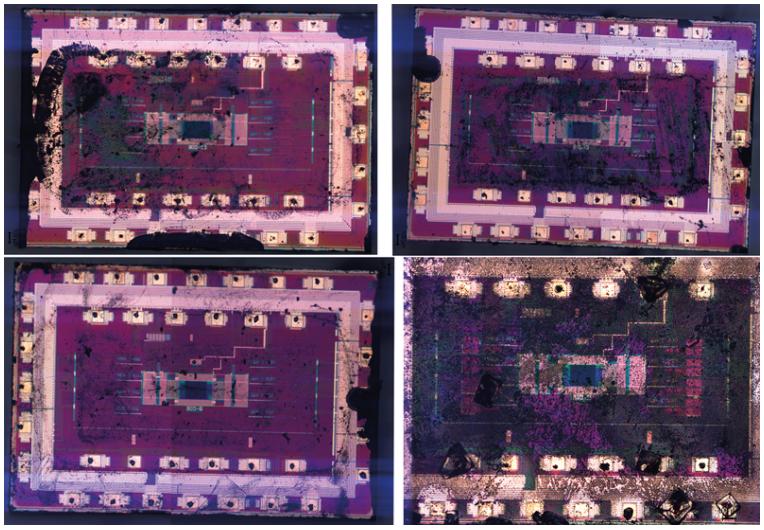

Figure 10: Four chips after experiments: after setup testing with IPA (top two and bottom left), after DNA experiment (bottom right)

4.3 Cleaning the socket

After assembling the setup a few times, it turned out that thermal paste could leak from under the chip to the socket. The paste covered the connector pins and thus came into contact with the chip when it was inserted. This resulted in the dirt that can be seen in figure 10 (above), and had to be cleaned before more measurements could be done. The DOW Corning 340 data sheet shows that it contains PDMS and metal oxides, but no solvents or cleaning agents are mentioned. After leaving the socket overnight in IPA, it was clear that the paste doesn't solve in IPA. Stronger solvents such as chloroform or acetone might damage the plastic, so were not used. The socket was then laid in IPA and put into an ultrasonic cleaner for 5 minutes. This also did not remove all the paste from around the edges. The last bit was brushed out using a very fine brush and some IPA. After this the socket was not completely clean, but the thermal paste was removed from the areas that make contact with the chips.

4.4 Assembling the setup

Assembling the setup showed some problems that had to be overcome. The first step is to align the PDMS seal perfectly into the socket. PMDS has the property that it sticks to metals, like the tweezers used to position it. Due to this the first step is not as straightforward as it might seem. It takes time and patience to align it perfectly, and the most minor deviation makes the PDMS touch the electrodes on the chip, thereby influencing the measurement.

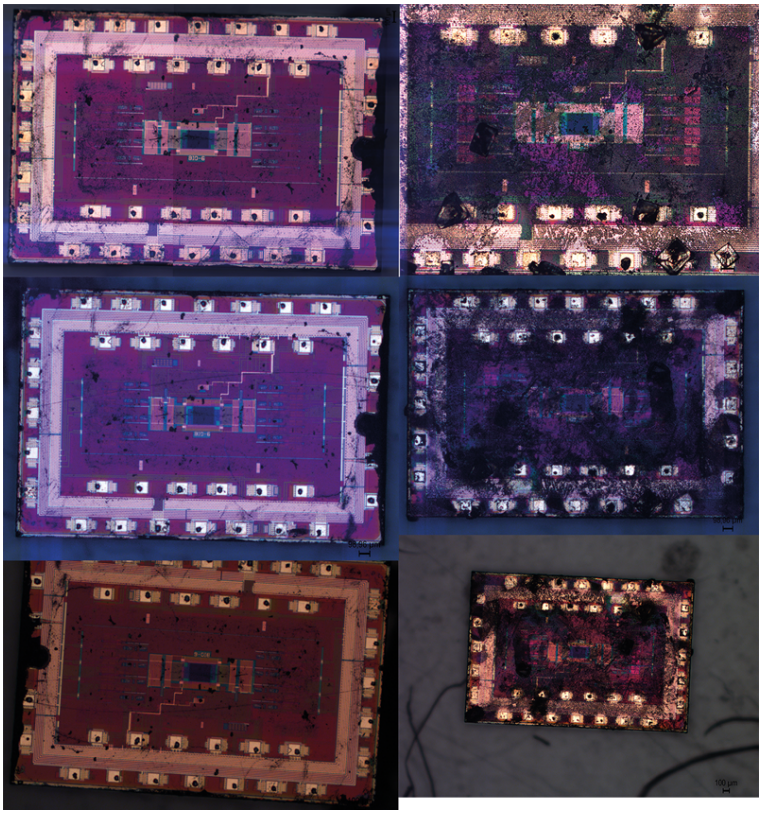

Figure 11: Chips through cleaning process: dirty chips (top), same chips after the cholo-roform step (middle), same chips after UV-ozone cleaning (bottom)

Attaching the chip to the aluminium heatsink is equally precise. A minimal amount of thermal paste has to be used to minimize the chance of problems like in paragraph 4.3. The alignment has to be perfect, or the chip will not make contact with the gold pins in the socket and no measurement can be done. Aligning the chip has to be done by eye, using metal tweezers to move it around. This takes time, which is not very good. The chip has to be perfectly clean for the experiments, yet in order to assemble the setup one is breathing over it for up to a few minutes to align it and set up the system.

4.5 Measurement results

Two measurements were done; one following the guidelines for the ODT SAM and one for the DNA SAM. The results will be presented and discussed here for both experiments. It is stated that the given chip temperatures are uncalibrated. The initial temperature is not necessarily accurate, however the variations in temperature are valid.

4.5.1 ODT SAM

This experiment has been done following the guidelines in the previous chapter. Due to problems with the software, the measurement data is split into three parts. The measurement data can be seen in figure 12.

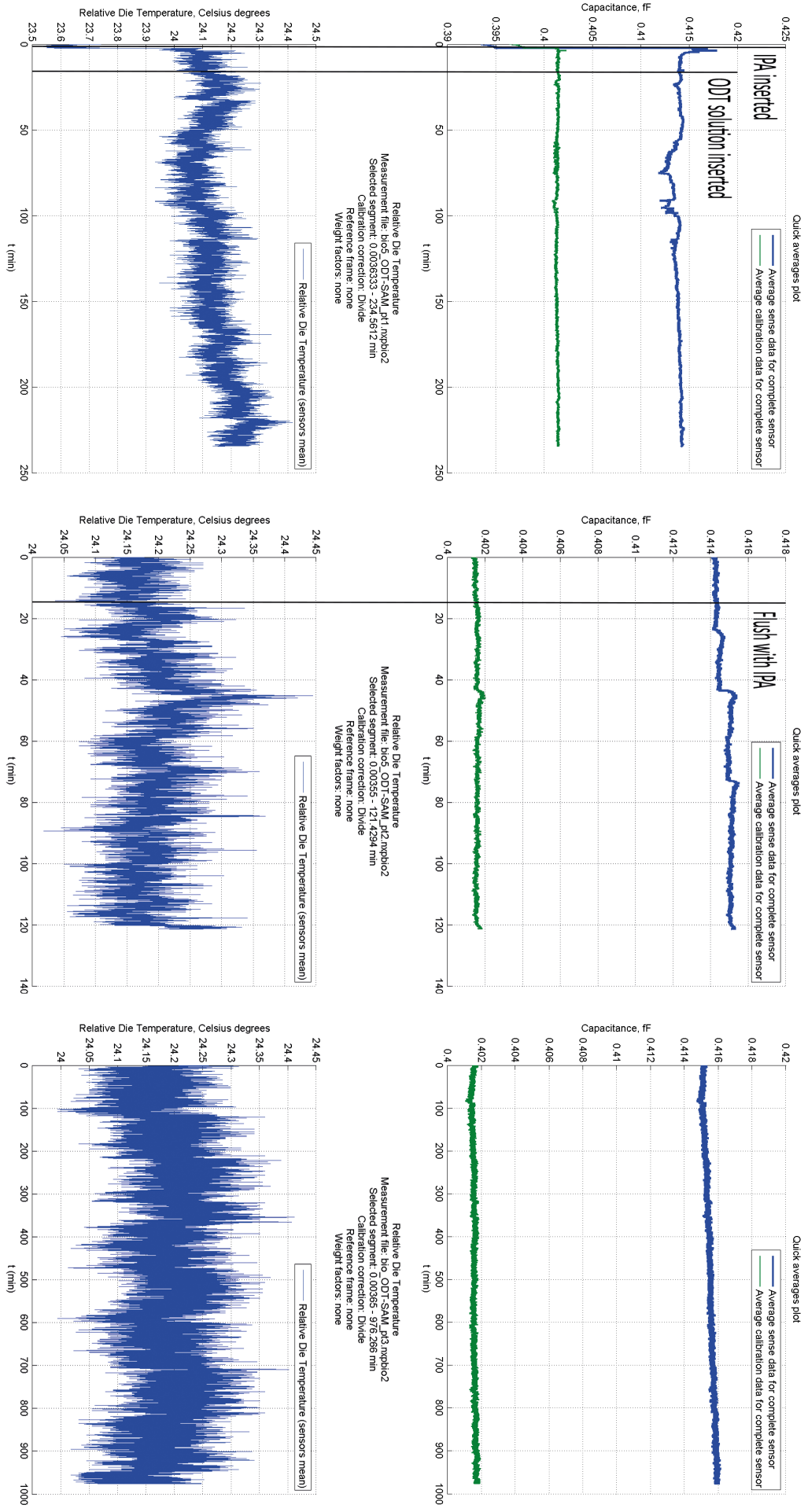

Figure 12: Measurement data for the ODT SAM experiment: average electrode capacitance (top) and uncalibrated chip temperature (bottom), divided into 3 time frames from beginning (left) to end (right)

The data from the measurement is not very conclusive. After the initial insertion of IPA, the electrode capacitance settles. Then the ODT solution is inserted, and at first not much seems to happen. Then the capacitance fluctuates both up and down, but during this time the temperature also varies in more or less the same direction. From 120 minutes (part 1) to 15 minutes (part 2) a gradual increase in both capacitance and temperature can be seen. The total rise in this period is around 1 aF. When IPA is inserted again to flush away any free flowing ODT, more inconsistensies are found. A temperature spike of 0.1 degree Celsius comes together with a capacitance increase of around 1 aF, three consecutive times. In part 3 of the measurement, the capacitance increases more gradually. The small temperature spikes seem to go less hand in hand with capacitance changes.

The fact that the temperature and capacitance sometimes change accordingly may mean two things. It is possible that the temperature coefficient in the electrode capacitance is changing the sensor output. It may also be that the reaction on the electrode surfaces that cause the capacitance to change, also change the temperature of the chip. This is not very likely, since the expected temperature changes would be more subtle in such a reaction.

4.5.2 DNA SAM

This experiment was also subject to software problems, splitting the total data in two parts. The monolayer incubation has been done outside the measurement setup. The setup was then assembled to try to find data that shows the hybridization of target molecules to the monolayer probes. The resulting data can be seen in figure 13.

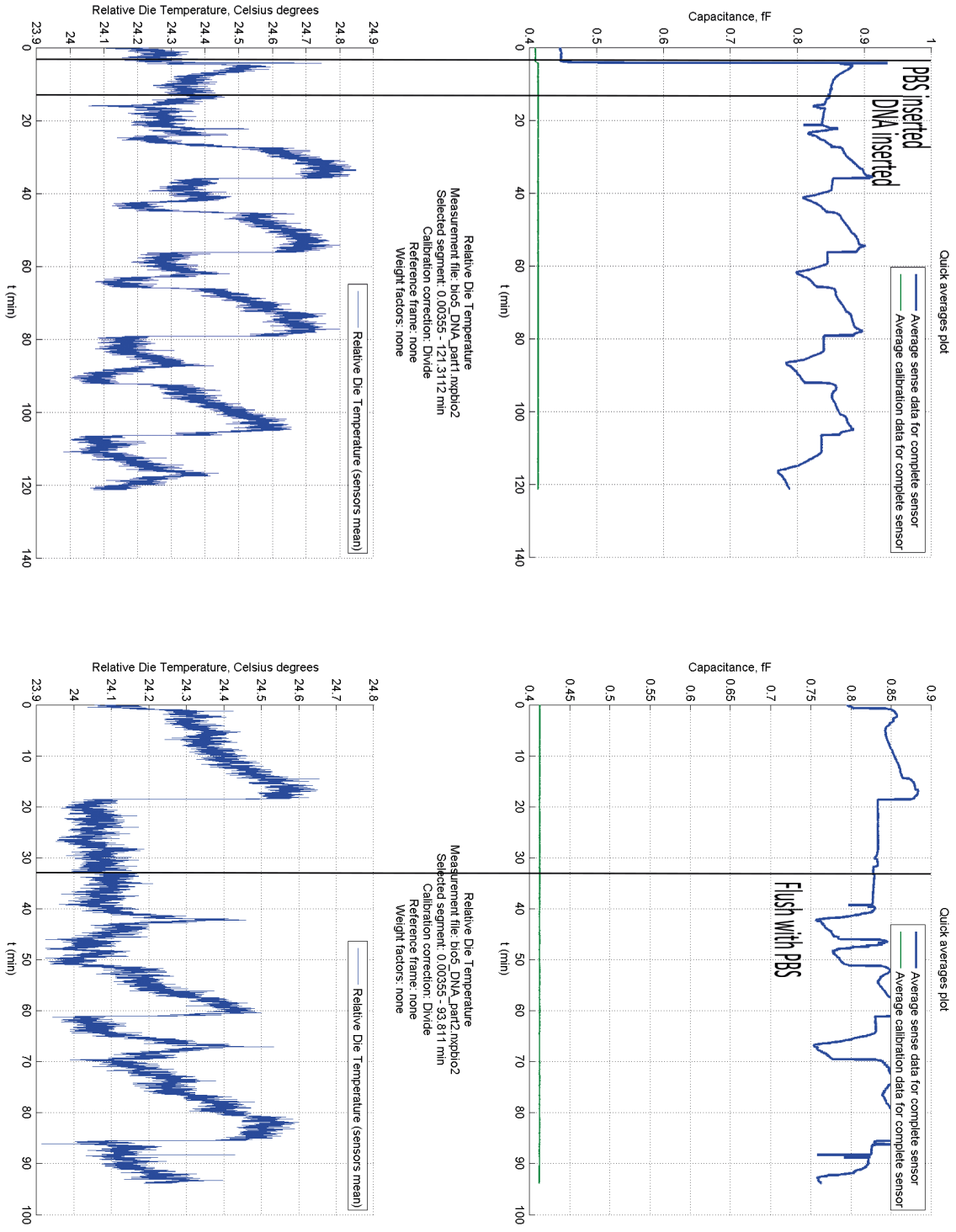

Figure 13: Measurement data for the DNA SAM experiment: average electrode capacitance (top) and uncalibrated chip temperature (bottom), divided into 2 time frames from beginning (left) to end (right)

In the shown results, again a strong correlation is present between the temperature and sensor output data. As in the ODT SAM experiment, it is most likely that the temperature differences make a big difference to the sensor output. No clear evidence of hybridization seems to be present in the data. Both during and after the hybridization time the temperature associated fluctuation is bigger than any other noticable difference. A temperature corrected data plot might show more of this, however a good model for this is not at hand.

5 Conclusions and recommendations

5.1 Conclusions

In this chapter, each research goal will be discussed with respect to the obtained results. Based on the problems found in the experimental phase of the project, several recommendations will be done.

Attaching an ODT monolayer on the nano electrode surfaces

There is not enough evidence to support the establishment of an ODT monolayer on the electrode surfaces of the chip. The results of the experiment are unclear and contain inconsistencies that make it inconclusive. A verification of the monolayer existence is hard to perform on-chip, which means that it is not clear whether the problem is in the monolayer formation or the measurement system, or even in both.

Attaching a monolayer of thiolated DNA-probes to the electrodes

There is no proof that a DNA monolayer has formed on the electrodes. The DNA monolayer incubation was done out of the measurement system to evade problems that arise when the chip is in the setup or during assembly. The monolayer formation could thus not be monitored by the sensors. To try and find useful data, the expected monolayer was made to hybridise with complementary strands with an added fluorophore while the sensor was running. This hybridisation is considered a very straightforward process, so it could be used as a verification step. No fluorescence was visible on the chip. This means that no DNA has been hybridised, or we cannot see it because there are too less fluorescent molecules and/or the molecules are too close to the gold surface. This leads to conclude that no DNA monolayer has formed.

Measuring the existence of these monolayers with the NXP sensor

It has proven to be difficult to make dependable measurements with the sensor in this system. No conclusions could be drawn from the inconsistent measurement data due to the temperature problems.

Hybridising the DNA-probes with their complements and measuring this with the sensor

An attempt at hybridising DNA has been performed without succes. No conclusive measurement data supported hybridisation, and fluorescence microscopy did not show any attached fluorophores. Since DNA-hybridisation is a straightforward process, it is most likely that this is due to the fact that no DNA monolayer was present. There may have

been DNA strands attached to the surface, but not enough or too close to the gold electrodes to show up in fluorescence microscopy.

5.2 Recommendations

To overcome some of the problems found in this research, some recommendations can be made. In this section some ideas for improvements are given, together with possible experiments to map and verify the problems and findings of this research.

Flowcell enclosure

As mentioned in the above chapters, it is quite difficult to position the PDMS seal. Also PDMS has the property that it adheres to some forms of dirt very well. It also absorbs several contaminations and measurement liquids. This means that when liquids flow next to it, it might release liquids that were previously absorbed. It is therefore probably not the best solution to enclose the flowcell with a PDMS seal this way. Problems with the seal have influence on the performance of the chip and on reproducability of measurement data.

There are several possibilities to improve on the design here. One option is to replace the PDMS with another material. This may be very difficult since the new material must have several properties. It has to be as chemically inert as possible. It should not absorb moisture, and should not adhere to dirt. It also has to be a little elastic to suit the form factor.

Another option is to enclose the chips in a fixed container that connects directly to an input and output tube. This would reduce the chance of accidentally demolishing the chip. Another advantage here is that the chip is not exposed to the open air while assembling the chip. The choice of the material for this container is again quite difficult. Enclosing the chip in this way means that also strong solvents will have to flow through the regular input and output tubes to clean the electrode surfaces. Also, it makes UV-ozone cleaning very difficult.

Another benefit would be the opportunity to redesign the way in which the chip is placed into the setup. Aligning the chip on the aluminium block is a big impediment in setting up the system. It allows for several inconsistencies to occur which is not good for the reproducability of measurements.

Temperature control

The temperature control system that is currently used allows the temperature on the chip to vary around 0.25 degrees up and down from a set reference. Since the temperature seems to influence the measurements quite strongly, a more precise temperature control would be useful.

Also the current temperature sensors on the chip are not calibrated, which make it difficult to draw conclusions on the basis of the temperature statistics. Before temperature dependence of the sensors can be measured, the chips have to be calibrated.

Thermal paste

In the current setup, another choice of thermal paste would be a good idea. The thermal paste currently in use does not solve well in most common solvents. If it accidentally comes into contact with the chip during the assembly, it ruins the chip which then takes a lot of time to clean again. A thermal paste that solves in (for example) IPA or ethanol makes it easier to clean both the chip and the socket after accidental spills.

Software

The current software consists of two packages; the measurement program 'pt2app' and the processing software 'pt2matlab'.

The pt2app program works quite well to accumulate data. However, during long measurements it runs extremely slow. This makes viewing the results during runtime very difficult. Also on several occurences the program crashes at seemingly random moments. This is very undesirable as important measurement data could be lost in between two different measurement files.

The pt2matlab program is a toolbox in Matlab. It features the ability to import a data file and then process it to show graphs from the sensor values. Most of the processing options are not finished yet; they cannot be selected. An example of this is temperature correction. With valid temperature data, a temperature correction could be made on the data. Other useful additions would be to be able to choose the output resolution, scaling options and editing legend information.

Experiment: Monolayer on gold plate

It is not clear why no monolayer formation has been detected. This experiment takes the monolayer formation back to a very basic form.

In principle this experiment is very much like the ODT SAM experiment described in this report. The difference is instead of the sensor chip, a glass plate with a thin gold layer is used. The incubation of an ODT SAM on a thin gold layer is almost trivial. Using the glass plate under the gold allows for example surface plasmon resonance (SPR) measurements to determine the presence of a monolayer.

A gold coated glass plate with the same dimensions as the chip is put through the cleaning stages just like the chip. It is then assembled like the chip, and the flow pump steps are done with the gold coated glass in place. After the liquid sequence is done, the glass is taken out of the setup to review.

With this experiment it can be seen if the cleaning and assembly processes make it difficult for a monolayer to form. If a monolayer clearly forms, there is reason to believe that there is something wrong with the gold surfaces on the chip.

Experiment: Temperature dependence

This experiment shows a way to determine how much the sensor output values depend on the chip's temperature. This provides useful information for temperature adjustment in data processing. It also broadens the understanding of the chips performance on this point.

A chip is cleaned and assembled as described in the ODT SAM procedure. After the setup, a measurement starts when IPA is inserted at 1.0 μl/min. The measurement runs for at least 10 hours and is then stopped. From the collected sensor and temperature data, it should be possible to see whether and how much the temperature affects a sensor value that is supposed to be stable.

Next, the experiment can be done in the same way but with IPA that has been cooled to 10 degrees Celsius, or 30 degrees Celsius. Also liquids with different heat transfer characteristics can be used.

6 Bibliography

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7 Appendices

7.1 Appendix A: Planning

Planning Bachelor's Assignment			
Week:	Hours:	Activities:	Deadlines:
Week 17	24	Reading in on the project	
Week 18	16	Reading in on monolayers and RCA	
Week 19	16	Reading in on monolayers and RCA	
Week 20	24	Start writing the research plan	
Week 21	24	Writing research plan	
Week 22	16	Finishing research plan	24-5: Research plan
Week 23	24	Starting test measurements	
Week 24	24	C18 SAM experiments	
Week 25	24	C18 and backfilling experiments	
Week 26	16	Backfilling and DNA experiments	
Week 27	32	Backfilling and DNA experiments	
Week 28	40	(If succesfull:) DNA hybridisation experiments	
Week 29	40	Finishing experiments and start writing the report	
Week 30	8	Writing the report	
Week 31	40	Writing report and preparing presentation	29-7: Ch.1 and 2 Preface and theory
			31-7: Ch.3 Experiments
Week 32	40	Writing report and preparing presentation	
			5-8: Ch.4 Results and discussion
			8-8: Ch.5 Conclusions and recommendations ;
Week 33	24	Report deadline and presentation	15-8: Finished report

7.2 Appendix B: Rolling cycle amplification

To amplify the measurement signal the DNA-strands will be elongated with copies of itself. This is done by employing Rolling Circle Amplification (RCA). For this an enzyme (DNA-Polymerase) is needed, together with a piece of DNA-primer, the DNA that is to be copied and the nucleobases needed for the copies. Before the process starts, the temperature of the DNA-strands is brought up to split them into single strands.

The actual copying is done by the enzyme DNA-Polymerase. When the primer is connected to the new single-stranded DNA, the process can start. The enzyme works from the primer's 3' end towards the DNA's 5' end and creates a loose strand of the complementary parts of all the nucleotides it goes by, in a process called the DNA-Polymerase chain reaction [1]. Usually, when the polymerase finds the end of the strand it is done. However, by fixing the 5' end of the DNA to the 3' end of the primer, we create a DNA-circle. This is illustrated in figure 14. Thus, the enzyme will not stop copying until either the process is stopped or the resources are emptied. The result is a long chain containing long strands of the complements of the primer and the DNA.

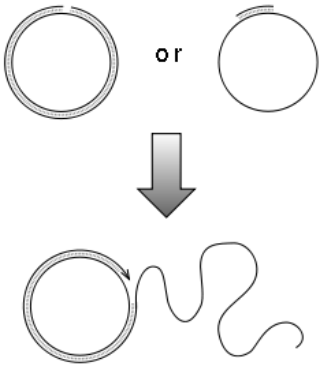

Figure 14: Illustration of the process of RCA, with the starting DNA (top) and the long replicated strain of DNA (bottom).