Microchip electrophoresis for UVC-induced DNA damage assessment

By

Aviva Debora Helena Tierolf

1198459

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Committee in Charge:

Prof. Dr. P. J. French, project supervisor

Dr. Y. K. Lee, co-advisor

Prof. Dr. Ir. P. M. Sarro, member

Dr. Ir. A. Bossche, member

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Abstract

In this thesis a design is presented which main objectives are to improve the speed, sensitivity and resolution of the commonly used gel electrophoresis method. To this end a background study is performed to look at the differences and similarities between both gel and micro-chip electrophoresis. Based on these findings some calculations are done to see if the expected design can theoretically improve in the above mentioned fields. A design is created and later fabricated in order to obtain these objectives. For the design a glass substrate is used with integrated electrodes, combined with a PMT sensor system to process the signals from the chip via a computer program. The main techniques used for the fabrication are wet etching and wafer bonding. The testing results produce a clear signal, which shows improvements in sensitivity and resolution, as well as producing results in less than three minutes.

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Abbreviations

AZ5214E – Photo resist for image reversal
Bp – Base Pairs
CCD – Charged-Coupled Device
CFU – Colony Forming Unit
CMOS – Complementary metal–oxide–semiconductor
DI water – Deionized/Distilled water
DNA – Deoxyribonucleic acid
DRIE – Deep Reactive Ion Etching
dsDNA – Double stranded DNA
E.Coli – Escherichia Coli (bacteria)
EDTA – Ethylenediaminetetraacetic acid (buffer)
EOF – Electro Osmotic Effect
ESS – Endonuclease Sensitive Site
FHD-5 – Developer for photo resist
HDMS – Hexamethyldisilazane (adhesion promoter for photo resist)
ICCD – Intensified Charge-Coupled Device
IPA – Isopropyl alcohol (cleaning)
Kbp – Kilo Base Pairs = 1000 Base Pairs
LOL2000 –Lift-Off-Layer (photo resist)
MS2001 – Metal rinsing
PCB – Printed Circuit Board
PMT – Photomultiplier tube
RNA – Ribonucleic acid
SoC – System-on-Chip
ssDNA – Single stranded DNA
TAE – Tris-acetate-EDTA
TBE – Tris-borate-EDTA
UV-C – Ultraviolet, type C
YOYO-1 –YOYO-1 iodine stain (dye)

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Introduction

Clean drinking water is one of the basic needs to survive. In many countries clean and accessible drinking water is not available. The places that do have the facilities to accomplish this, still need to research if the available techniques for the cleaning of the water are sufficient or that there is need for improvement. In order to find out research is still conducted to test the efficiency of the water plant facilities. One of these researches is about the Escherichia Coli (E. Coli) bacteria. The treatment that is used at the moment consists of radiating the water with the E. Coli bacteria with UV-C. During this radiation the E. Coli is damaged to such extent that the bacteria becomes harmless. However recent studies show that the E. Coli can be reactivated when exposed to sunlight. This reactivation means that the E. Coli starts to repair itself. Research has therefore been started to see to what extent this reactivation and repair occurs [1].

The research method that is used for the examination of the E. Coli repair is gel electrophoresis. By means of measuring the size of the DNA fragments before and after damaging and again after a period of time in which the photo reactivation can take place, a good indication of the extend of the damage that occurs and the level of repair can be found. Gel electrophoresis is however a slow process. The electrophoresis itself already takes around 12 hours to complete, not taking into account the pre and after treatment procedures. Also the sensitivity is low due to a high background noise. The lack of a high resolution makes it hard to distinguish the different DNA sizes. And therefore situation a new method is proposed. By means of designing and fabricating a device for micro-chip electrophoresis, the speed, sensitivity and resolution will be improved compared to the traditional method of gel electrophoresis.

The structure of this thesis is as follows. The first chapter describes the background research. Followed by the principals and developments of gel electrophoresis in the first section.
The second chapter describes the design and modelling that are done during the research. In the first section of this chapter the mask design and the accompanying choices that were made are explained. The second section will elaborate the chosen materials for both the fabrication and the experimental phase of the design. After this the most important technologies and the reason behind the choices that are made to use these technologies are given in the third section.
In the third chapter the fabrication process of the microchip is described. The first section describes the channel fabrication and the second section the electrode fabrication. The bonding process of the chip is described next in the third section. At last in the fourth section the packaging of the complete chip is explained.
The final chapter considers the experimental setup and the obtained results. In the first section the sample preparation is described. The second section will explain the polymer testing and the third section is explaining the experimental setup for the electrophoresis. The fourth and final section will give the test results obtained from the micro-chip electrophoresis.
Finally the conclusion and remarks on the possibilities for future work on this project are considered.

1. Background research

In this chapter the following subjects regarding background research for the eventual design will be discussed. At first the process of gel electrophoresis is discussed in light off the pre research that is done on UV-C damaged DNA with this method. Second, the general idea behind microchip electrophoresis and the developments in this field are discussed. And thirdly the most important parameters that influence these designs are discussed and some calculations are done to show the difference between microchip electrophoresis and gel electrophoresis.

1.1 Gel electrophoresis

Several methods can be used for the separation of DNA. The most common and oldest method is the gel electrophoresis. This method has also been used in previous research on UV-C damaged DNA. To obtain a better insight in the problems of this method and in which fields the improvements can be found, an introduction in the working of gel electrophoresis will be described. The first section discusses the basic principle of gel electrophoresis. The second section will then look into the recent developments in this area, concerning our research and the problems that arise when using this technique.

1.1.1 Basic principle

Gel electrophoresis is a commonly used technique that has been in use for DNA separation for over 35 years. The technique of gel electrophoresis on itself has already been used since the beginning of the twentieth century and was then mainly in use for RNA based research [2]. Agarose gel is one of the most commonly used gels for DNA electrophoresis. It is a less toxic variation on the acrylamide polymers that are also used. Agarose gel can be used for different DNA sizes depending on the concentration that is used. The gel is prepared by mixing Tris-acetate-EDTA (TAE) buffer or tris-borate-EDTA (TBE) buffer and DI water with a specific quantity of the gel. The concentration used for 3-15kbp (kilo base pair), as is the range for

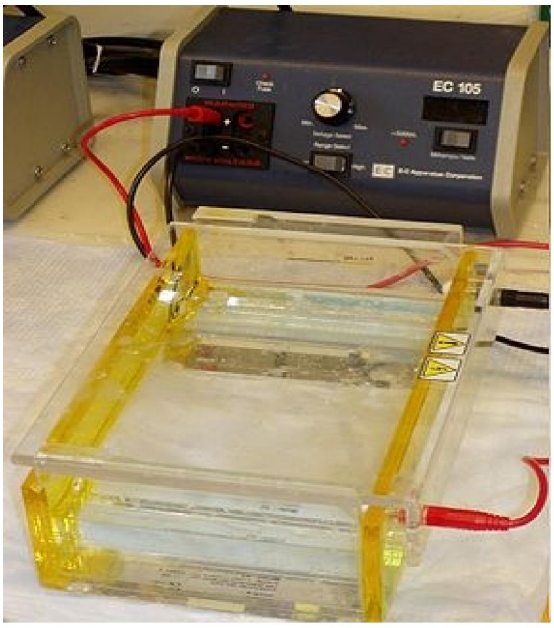

Figure 1 Gel Electrophoresis setup [3]

this research, is 0.5%. After mixing the solution is heated in the microwave until the boiling point and if needed more DI water is added. The mixture is cooled down and poured into a casting plate with a comb. The comb is present to create the holes in which the DNA sample will be inserted later on. After cooling down the gel is immerged in a selected buffer and the sample is loaded into holes after removal of the comb. When the sample is loaded a voltage is applied over the entire box. For Agarose gel electrophoresis this voltage is 24V for a box of 10cm long. This results in a relative low electric field compared to other electrophoresis researches. The low electric field is needed due to the low breakdown voltage of the gel. This is a mayor downside of the use of this gel. Lower electric field means slower movement of the DNA, which makes that a long time is needed for the electrophoresis to be completed. Normally the electrophoresis will take around 12 hours to be completed. When the DNA is finally completely separated, the gel is stained with a dye and neutralized for 2 to 3 hours. The final step is to take the gel and put this under UV-light for detection, after which the results can be interpreted and if needed processed further [2].

The process for gel electrophoresis is an easy but time consuming process. The basic steps are the preparation of the gel, the application of an electric field and the detection by means of an UV-light source. Although the basics are simple a lot of improvements are still needed to get better insight in the process. The recent developments and the problems that are encountered during this research are therefore discussed in the next section.

1.1.2 Recent developments and problems

Gel electrophoresis as a method for DNA separation has been used for around 35 years. Several different methods have been applied to optimize or change the process. A recent trend is to use gels and dyes that are less toxic than their predecessors. However these new gels and dyes do not always give better results than the ones used before. It is therefore important to optimise the process and to improve the results.

With the techniques used so far the separation still takes a long time. It is also difficult to move the gel slabs without breaking them, and to get clear pictures of the DNA. These problems arise especially if the process is not completely automated or if there is no prior experience in this field. Fully automated processes for gel electrophoresis can enhance the results [5], but still take a lot of time, therefore improvements have been investigated in the direction of microchip and nano-pillar designs. The developments in the area of microchip electrophoresis are discussed in the next section. The rest of this section is going to look at the problems that arise when using this technique for UV-C damaged DNA assessment, in order to see what kind of improvements can be made whit the use of micro chip electrophoresis.

For the research of UV-C disinfection two methods are commonly used. The first method is the Colony Forming Unit (CFU) where the number of colonies is manually counted. This is the traditional method. Another method however is Endonuclease Sensitive Site (ESS) Assay. This method works on the molecular level and uses gel electrophoresis as means to measure the resulting length of the different DNA strings [1, 4, 6].

The method for Endonuclease Sensitive Site (ESS) Assay is as follows. Dimers are induced by the UV-C disinfection in the DNA to repress the replication process of the DNA. The dimers form bonds between adjacent pyrimidines. After this the DNA is treated with Endonuclease enzymes to cut the DNA. These cuts appear at both sides of the dimmer. This ESS Assay processed DNA can now be put into the Agarose gel. The DNA pieces will now migrate according to their respective size. This gives a distribution in the DNA which can be compared to a DNA ladder. By means of this method the different sizes of the DNA can be accumulated and after exposure the size distribution gives an indication on the amount of damage inflicted on the DNA due to the UV-C radiation [1, 4, 6].

There are several issues addressed in this research. The first issue is the time that the whole process consumes. When including the DNA cutting, the electrophoresis and the staining, the whole process can take more than a whole day. The next issue is the sensitivity. The resulting pictures of the DNA have a low sensitivity with high background signals. The diffusion of the DNA can cause smears as explained later on and this makes it hard to differentiate ambiguous changes. The solutions that therefore have to be provided are as follows: a shorter separation time is required, preferably in combination with a reduction of the sample preparation time. Higher sensitivity with less background noise is needed and the diffusion of the sample has to be lowered in order to make it easier to differentiate the ambiguous changes in the DNA sample. These are the improvements that need to be accomplished by changing from gel electrophoresis to microchip electrophoresis. The following sections of this chapter shall therefore be about the basics of microchip electrophoresis and the different parameters that can be looked at before the fabrication. This is done to look at the differences between the two techniques and to see if it is theoretically possible to achieve these improvements.

1.2 Microchip electrophoresis

Another more recent method for electrophoresis, as mentioned before, is microchip electrophoresis. In this category a distinction can be made between normal microchip electrophoresis and micro-pillar array electrophoresis. Especially the last one is becoming more popular. The following sections will focus on normal microchip electrophoresis and the recent developments in this area. Also a brief explanation will be given to illustrate the difference between microchip and micro-pillar array chips.

1.2.1 Basic principle

The principle of microchip electrophoresis is similar to that of gel electrophoresis. The main differences can be found in the device feature size and the use of polymers instead of the gel used for the gel electrophoresis.

The feature size of microchip electrophoresis is in the order of one centimetre to several millimetres where gel electrophoresis is in the order of tens of centimeters. The basic principle is

again that a voltage is applied over a certain space. However for microchips the design exists of four different channels that have the form of a cross, where as gel electrophoresis has a slab of gel where the DNA is imputed in a few holes.

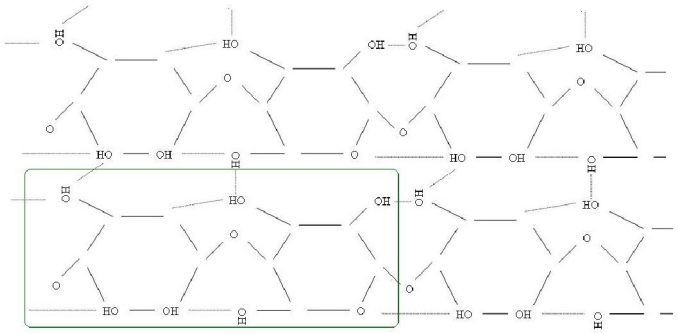

Figure 2 Chemical structure of a cellulose based polymer

One of the differences is the replacement of the gel by a polymer. This polymer works the same way as the gel, namely as a sieving matrix. A sieving matrix consists of a material with a certain molecule size. Due to the size of these molecules a raster is formed with holes in it. The size of these molecules and the gaps between them determine the way that a string of DNA can move through, as can be seen in figure 2 and 3. Figure 2 shows the structure of a cellulose based polymer. This repeating structure shows a well organized matrix, through which the DNA can migrate. Figure 3 shows the random distribution in which the DNA can migrate through the holes in the structure. Some sizes will migrate faster than others through the same polymer, which creates the sieving effect. It is therefore important to know the size of the DNA that will be tested in order to choose the right sieving matrix. The longest channel is for the separation of the DNA, the other channels are for the input of the DNA, the buffer and the sieving matrix and can be used as waste disposal channels. After preparation of the DNA sample, the sieving matrix and buffer are inserted into the channels. The sample is loaded in one of the side channels and an electric field is applied to let the sample migrate to the crossing. As the sample arrives at the crossing, the electric field is changed and the separation of the sample begins. The separation of the sample takes a few minutes and can be measured by a charge-coupled device (CCD) camera, an intensified charge-coupled device (ICCD) camera, a Photomultiplier tubes (PMT) or by manual observation through a microscope. If needed the signal can also be controlled or analyzed by computer [7, 8].

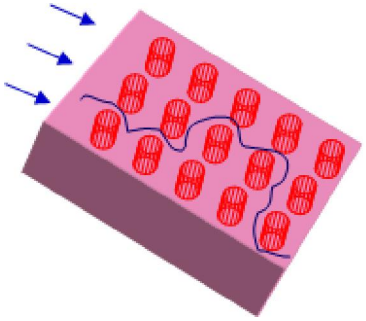

Figure 3 The effect of a sieving matrix on DNA

The main differences from gel electrophoresis as can be seen from the description are the dimensions of the device, the loading of the sample and the different detection methods that can be used. Especially the loading of the sample can make a huge difference in the performance of the whole design, where as for electrophoresis there is just one simple way of application. In the next section some recent developments will now be discussed that have influence on the performance of the chip.

1.2.2 Recent developments

Microchip electrophoresis is a field in which much research has been done the last few decades. Considerable changes have been achieved compared to the originally used methods and improvements are still possible on a large scale, making it an interesting research field [2]. Also the possibility to create integrated devices makes this an interesting field [5].

In microchip electrophoresis a distinction can be made between normal microchip electrophoresis and micro-pillar electrophoresis. The difference can be found in the sieving matrix. For normal microchip electrophoresis a sieving matrix is used by means of inserting a polymer and a buffer into the channels. For micro-pillar electrophoresis a sieving matrix is created inside the separation channel by creating micro-pillar arrays. The size and the distance between these pillars can be determined by the size of the sample that will be used for the separation. The advantage of this method is that the sieving matrix is fixed. The electro osmotic effect (EOF), as described later on, will not have any effect and there is no need for special injection systems for the polymers.

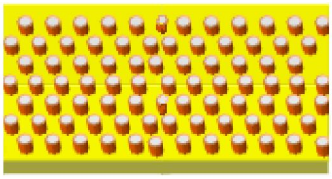

Figure 4 Micro Pillar Array Layout

When the optimal pillar array is found and implemented, high efficiencies can be obtained and repeatability will be easier due to the fact that the sieving matrix becomes a fixed parameter. The only thing about this technique is that is takes several months to years to learn how to create the right pillar size and to research the position and the size and number of pillar arrays that need to be formed. Therefore in this research the normal microchip electrophoresis is chosen. However micro-pillar array chips will most likely in the future become the most commonly used type of microchips [8, 9, 10].

Another development in recent years lies in the field of the polymer research. For the last few decades the most commonly used polymer was polyacrilamide, which was followed by linear polyacrilamide (LPA) and Poly-N,N-dimethylacrylamide (PDMA). These polymers have a high efficiency, a high toxicity and explosion level and except for PDMA a very high viscosity. To try and find less dangerous chemicals and polymers with a lower viscosity there is a search for different polymers [11, 12, 13]. Especially cellulose based polymers seem to answer to these demands. Other poly based polymers are also found to answer these criteria, however these polymers still have a certain danger level, such as carcinogenic or explosive, where cellulose based polymers can reach totally user friendly levels. Although a lot of research has already been done in this field and a lot of different polymers are usable for electrophoresis, recent studies show that there is still a long way to go. Many new chemicals are still being tested and the recently researched ones show that still a lot of work can be done to optimize the polymer choice, depending on the demands that vary with the many different designs that are in use these days for electrophoresis [14].

Smaller feature sizes are also a main issue in the last decades. Microchip designs already give a large downscale compared to gel electrophoresis tools, the question now is how much further these devices can be downscaled. The largest problems at the moment in the downscaling seem to

lie in the equipment and the manufacturing methods compared to the efficiency of the devices. The higher the separation efficiency the smaller the device can be made. By using micro-pillar arrays for sieving matrices it is possible to exactly tailor the needed feature sizes to be as small as possible. The fabrication process of these micro-pillar arrays is however difficult and the fact that the exact movement of the DNA can not be predicted makes it difficult to optimize this feature. When optimizing this feature the separation length can considerably be lowered resulting in even smaller devices. However the smallest size possible will always depend on the size of the samples used and the efficiency of the separation.

The injection system for the microchip electrophoresis is another research area. The injection system can highly influence the efficiency and accuracy of the separation. A well-defined sample plug is needed to achieve lower band broadening and a high efficiency. Different designs and methods are now researched in order to obtain such a sample plug. The most commonly used methods change the crossing of the channels to obtain different ways to hold the plug in the same place as coherent as possible before the separation starts. Ways to achieve this are the double L-injection or the double T-injection systems which are described in the next chapter.

Finally the different materials that are used for the designs are discussed. The choice of the material that is used for the chip design will depend on the interaction with the chemicals used and other parameters depending on its surrounding environment. Most designs have been made out of glass. However in recent research different materials are researched for achieve better results or to be able to integrate these devices in the future to different systems. Especially CMOS based designs look very promising when looking for circuits that can be integrated. These designs exist of both silicon and glass wafers. Another promising material is Polydimethylsiloxaan (PDMS). This material is a harmless and easy to use substitute for glass designs and is especially convenient for mass production. The different substrate materials and their advantages and disadvantages will be discussed in more detail in chapter 2 [15, 16].

As can be seen from the last section a lot of research is done on microchip electrophoresis. From complete design changes, such as the micro-pillar design and the changes in feature size and material choice and injection channel design, to the changing of the polymers. Much has changed in the last few years and many things can still be improved. This makes the microchip electrophoresis an interesting field of research in which a lot can be achieved.

1.3 Parameter considerations

The next few sections will consider the different parameters that have a major influence on the functioning of the system. These parameters are the diffusion, the viscosity, the electro osmotic effect and the electric field dependence. These parameters can be used to make an estimation of the performance and to estimate the difference in performance between microchip electrophoresis and gel electrophoresis. The first section will handle the diffusion, the second the viscosity of the polymers, the third the electro osmotic effect and the fourth section will handle the electric field dependence.

1.3.1 Diffusion

An important parameter for electrophoresis is the diffusion [17]. The diffusion of the DNA in the polymer or gel depends on several parameters and its effects can be significant. The phenomenon of diffusion is especially easy to observe in gel electrophoresis.

The dependence on the electric field and the accompanying separation time are the main reasons why the effects of diffusion on the gel electrophoresis are simple to observe. The low electric field and the very long separation time give a high diffusion of the DNA, which can be noticed by the smearing of the DNA over the whole field. In fig. 4 this can be noticed by the width of the bands. The wider the band, the more the diffusion has taken effect, thus the higher the band broadening. In extreme cases, the diffusion can even lead to a complete smearing of the band resulting in an almost uniformly distributed lane. This makes it very hard to distinguish between the different DNA bands.

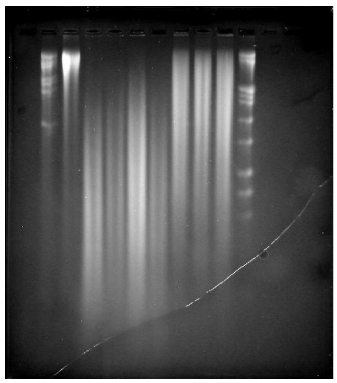

Figure 5 Gel electrophoresis results of UV-C induced damaged E. Coli

One way of determining the diffusion coefficient is through the number of theoretical plates N. This theoretical number for the distribution gives an insight on how to calculate the diffusion, when measurements are not yet possible.

$$N = \frac{L}{\left(\frac{2D_{coeff}}{\mu E} + \frac{w^2}{12L}\right)} \qquad (1)^{[17]}$$

For many different applications this number is already available and can be directly used. Here L is the column length, μ is the mobility of the DNA in the polymer/buffer, E describes the applied electric field and w is a function of the injection and detection width of the device. From this formula the diffusion coefficient can be deducted into the formula below, the complete derivation can be found in [17]:

$$D_{coeff} = \frac{k_B T}{6\pi\eta R_H} \qquad (2)^{[17]}$$

Where T is the temperature in K, η gives the viscosity of the polymer, k_B is the Boltzmann constant and R_H is the hydrodynamic radius of the DNA string. By using this formula a good estimation can be formed even before the testing to give a view of the expected band broadening which is to be calculated by the following formula:

$$H_d = 2\frac{\gamma D_{coeff}}{v} \qquad (3)^{[17]}$$

ν relates to the flow velocity and γ is a factor to take the restrictions for the diffusion into account.

Another parameter used to determine the effect of diffusion in, especially, micro-channel devices is the diffusion length L_0. This parameter is used to determine the length inside the channel at which both the injection width and the diffusion contribute equally to the width of the sample plug. This parameter is highly related to the column width L. In the case of microchip electrophoresis this parameter can be seen as the length of the part of the separation channel where the actual separation takes place. In case of $L<<L_0$, the separation will only depend on the injection width, making it possible to use very small sample plugs. Where in case of $L>>L_0$ the separation will mostly depend on the diffusion. This is the case in most conventional used methods like gel electrophoresis using relatively large structures for the separation process [17].

$$L_0 = \frac{\mu E w^2}{24 D_{coeff}} \qquad (4)^{[17]}$$

As can be seen from formula 4, L_0 is dependent on the diffusion coefficient, the mobility, the applied electrical field and the width.

The calculations given in appendix B are done by estimating the device size and by comparing parameters from previous work done in this field. They show that the diffusion coefficient, by looking at the desired polymers, is around the order of 10^{-9} cm^2/s. Since the device properties are around the order of magnitude of one centimeter at most and since the separation time will be around 3 minutes, depending on the exact applied electric field and the DNA size and mobility, the band broadening due to diffusion is so small that it can be neglected in case of microchip electrophoresis.

The main reasons why this effect is negligible in microchip electrophoresis, but not in gel electrophoresis are as follows. For one the device feature sizes are much larger for gel electrophoresis. This results in a higher w which, as can be seen from equation (1) results in a much higher diffusion constant. Also the applied electrical field is different for gel electrophoresis, as stated before. Due to the low electric breakdown voltage of the gel the electric field must be low. This results in a lower mobility of the DNA and in a far higher separation time. The difference in diffusion time and separation time between gel and microchip electrophoresis is shown in appendix C.

Band broadening is nonetheless still present in microchip electrophoresis. Even though the diffusion is negligible, the injection width of the sample is not. But since the effect of the sample injection width depends on the injection method and the width of the sample itself, this effect will be low for microchip electrophoresis. Several methods which will be mentioned later can be used to minimize this effect on the band broadening. Also microchip electrophoresis is designed to use an as small as possible plug, making this technique several factors smaller then the plugs used for gel electrophoresis and thus minimizing the effect on band broadening by itself.

1.3.2 Viscosity

Many different polymers are used for electrophoresis. The last few years a shift can be seen in the use of these polymers. Environmental issues are starting to receive a higher priority, therefore making it less attractive to use the most commonly used polymers, such as linear polyacrylamide (LPA). Performance and toxicity are now becoming equally important, creating a whole new range of polymers to be tested and improved, to reach the same level of performance with less environmental problems and better working conditions for the user.

Polymer	**Molar mass (kDa)**	**Viscosity (cP)**	**Temperature (℃)**	**Toxicity**
Linear polyacrylamide (LPA)	9000	27400	25	Carcinogenic(3), explosion danger
Poly-N,N-dimethylacrylamide(PDMA)	200	1200	30	Carcinogenic(3), explosion danger
Poly(vinyl pyrrolidine)(PVP)	1000	27	20	Carcinogenic, (3)
Polyvinyl alcohol (PVA)	17-650	50	20	Carcinogenic (3)
Poly(ethylene oxide)(PEO)	600-8000	1200	Ambient	Toxic under fire
Methylcellulose(MC)	14	4390	20	Carcinogenic (4)
Hydroxyethylcellulose(HEC)	97	5000	25	None
Hydroxypropylmethylcellulose (HPMC-50)	11.5	4390	25	Toxic to lungs

Table 1: Polymer constants [14]

In table 1 the carcinogenic level of several widely used polymers is given. As can be seen from this table, most polymers have a high toxicity level. Especially LPA and PDMA are not user friendly, however they possess the best properties for electrophoresis. Since these polymer based materials are on the market for a longer period of time compared to for example the cellulose based materials, the properties of these materials are well classified and easy to adjust to similar research in this area [14].

To make a well defined selection for the sieving matrix one must consider this balance between the performance and the toxicity. One way of looking at the performance is to view the viscosity of the sieving matrix that needs to be obtained. For a high viscosity the disadvantage is that for microchip electrophoresis it will be difficult to inject the solution into the channel, due to the small feature size. So to improve the injection into the channel the lowest possible viscosity is desired. A high viscosity can also lead to increased separation time [18]. However a low viscosity will lead to a higher electro osmotic flow (EOF) effect. This phenomena will be explained in detail

in the next section. For now let's suffice to say that the EOF will cause the sieving matrix to move due to the electric field, causing a high disturbance during the separation, which can lead an unnoticeable separation.

1.3.3 Electro osmotic flow

The electro osmotic flow (EOF) is an effect that causes a liquid to start moving due to an applied electric field. This EOF effect is caused by a double electric layer that is formed at the boundary between the wall of the micro channel and the fluid in the channel. For the silica groups which have a low PH value the following happens. The negatively charged channels attract the ions in the solution. Due to the fact that these ions are now clustered at the channel wall while they were solved throughout the whole solution they will drag the whole solution in the same direction as the migration of the ions due to the current [19, 20, 21].

To analyze this behaviour one can look at the velocity of an analyte zone. This velocity is described in microchip electrophoresis as followed:

$$v_{net} = u_{net} E = \left(u_{ep} + u_{eof} \right) E \qquad (1)^{[19]}$$

In this equation v_{net} is the net velocity that arises due to the electrophoresis and the electro osmotic flow, u_{net} is the net electrophoretic mobility, E is the applied electric field strength and u_{ep} and u_{eof} are the electrophoretic mobility for the electrophoresis and the electro osmotic flow. It can be seen that the EOF has a direct impact on the total velocity. Since the EOF is normally not reproducible due to surface and chemical changes this value will vary for every experiment. Because of these changes it is almost impossible to estimate the EOF, which can be seen by looking at the following formula [19]:

$$u_{eof} = \frac{\varepsilon \zeta}{4\pi\eta} \qquad (2)^{[19]}$$

The ε stands for the relative static permittivity of the buffer that is loaded into the channel, the zeta potential, ζ, is determined by the structure of the double layer and η is the viscosity of the solution near the channel surface. By looking at these parameters on which the EOF depends it is clear to see the irreproducible aspect of the EOF. It is nearly impossible to keep these values at the same level. Even during a run the EOF can already change making it even more impossible to get similar results in consecutive runs.

Several options are possible to reduce this effect. One of these options is to coat the inside of the channels with a highly viscous polymer that will reduce the charge between the surface and the solution. It is however very difficult to insert highly viscous materials into the channels. However it is possible that clogging can occur when inserting the coating layer. If the polymer coating is not moving fast enough through the channel it can stick and cause an obstruction that makes the device inoperative. These reasons make that this solution is not very desirable.

Another way is to change the PH level of the material or of the solution. This can be done by changing the buffer concentration. By changing for example the TBE from 1x to 5x a reduction of the EOF is significant. Care has to be taken that the changing of the buffer does not affect the sample or its flow direction [19, 21].

It is also possible to use self coating polymers for the separation. These polymers have a self coating ability, although it is mostly weak, that reduces the EOF. The polymers that have this self coating effect still have a low viscosity and thereby reduce all the problems of the pre coating polymers. The overall reduction of the EOF will therefore be lower. However, research has showed that in several options it can be enough and in combination with other methods such as the changing of the buffer concentration this can give good results [9, 22, 23].

Electro osmotic flow is an important parameter which has to be taken into account when preparing the design. It is difficult to calculate the effect of the EOF on the total velocity due to its ever changing nature, however several measurements, like pre coating, using self coating polymers or changing the buffer, can be taken in advance to reduce the effect to a minimum.

1.3.4 Electric field dependence

The final parameter that will be discussed here is the electric field dependence. Many different parameters are dependent on the electric field. The mobility, number of theoretical plates and the resolution will now be reviewed to see the effect of the electric field and to see if the dependence is transverse, in which case it might be necessary to find an optimum. Since most parameters are complexly related to each other and to the electric field, simple estimations will be made to study the effect on the chosen parameters and to get a better understanding of the relationship that these parameters have with each other.

When looking at the mobility in its most simple form the following formula can be used.

$$\mu = \frac{v}{E} \qquad (1)^{[21]}$$

In this formula the mobility μ, is found by taking the velocity v and the electric field over the separation field, E. From this simple equation it can be seen that the mobility is depending on the electric field by a factor of 1/E. The next basic formula consists of the number of theoretical plates:

$$N = \frac{L^2}{\sigma_T^2} \qquad (2)^{[21]}$$

The number of theoretical plates N, is dependent on the total distance migrated by the zone, L^2, and on the total variance of the zone σ^2_T. Normally this formula depends on several factors. At the

moment we only consider the diffusion. By doing this the following formula can be derived [21]:

$$N = \frac{\mu_{app,avg}^2 E^2 t}{2D_{i,avg}} \qquad (3)^{[21]}$$

The number of theoretical plates N is now depending on the average applied mobility, the applied electric field E, the time of separation t and the average diffusion constant $D_{i,avg.}$ This formula is needed to calculate the resolution. By inserting the new formula for the number of theoretical plates into the basic equation for the resolution the formula below results [21].

$$s = \frac{\Delta\mu_{app,avg} E \sqrt{t}}{4\sqrt{2} D_{i,avg}} \qquad (4)^{[21]}$$

The resolution s can now be found by taking the applied average mobility $\Delta\mu_{app.avg}$, the electric field E, time t and the average diffusion constant. When looking at the factors that are dependent on E in this formula for the resolution, the resolution depends on the electric field by a factor of E. From this the conclusion can be drawn that the mobility of the sample and the resolution are inversely proportional to the electric field. To reach an optimal balance between these two parameters they have to be monitored during the experimental phase. Since the mobility is normally less sensitive to change in the electric field during electrophoresis than the resolution, it is easier to focus on the resolution to fine tune the electric field. From previous research it can be observed that for the range in which the electric field lies, the mobility is high enough to ensure a fast separation time. Therefore only the resolution will be taken into consideration [11, 18, 21].

2. Design and Modelling

In this chapter the choices behind the design and modelling of the microchip will be discussed. In the first section the design of the mask and the accompanying design choices will be explained. The second section will discuss the different materials that will be used in the fabrication and experimental phase and which are in need of some explanation due to the different options that are possible for these stages. Finally in the last section the techniques that are used throughout the process will be looked over and an explanation will be given for several of the important steps.

2.1 Mask design

For the design of the mask, care has to be taken of several considerations. In this section the design values and choices behind these values will be discussed.

The first step in the design is the channel length. The channel length is determined by the electric field and the separation distance that are needed. The desired electric field is around 500 V/cm for fast separation [16]. Since the high voltage supply that can be used is limited to 1000V, the channel length can have a maximal length of 0.5cm, in order to obtain a large enough electric field. On the other hand, the separation channel needs to be long enough for the DNA to separate. This parameter depends on the length of the DNA, on the sieving matrix and on the electric field that is applied. By looking at these conditions several options for the length remain. The chosen lengths that can be used for the final designs of the separation channels are 30mm and 25mm, depending on the rest of the design.

The next step is the width of the channels. Here the minimal feature size, over-etching and diffusion are of importance. When using wet etching an under etching of around 200% can occur. This has to be taken into account during the design of the mask. Therefore it is important to decide on the fabrication techniques before the designing stage. For example, when using deep reactive ion etching there is no such under etching. Another part is the minimum feature size; for the masks that are used here this is 30µm. Therefore a smaller feature size cannot be taken due to uncertainties that can arise in the mask. The last parameter for the channel width is the diffusion. Too wide a channel can cause diffusion to occur. This limit is shown to be at 100µm. By taking al these effects into consideration, two different widths are chosen for the design, namely 30µm and 40µm.

The design of the injection method is also a step which has to be carefully considered. The most commonly used methods are cross-injection, double L-injection and T-injection. Double L-injection is a technique where the DNA sample is injected in the channel by means of an extra channel as can be seen in figure 6a. The extra channel is used, together with electrokinetic manipulations, to reduce sample leakage. Another way to reduce sample leakage and to create a compact sample plug is double T-injection, as shown in figure 6b. Here the separation channel and

the upper channel are shifted compared to each other, to obtain a compact sample plug after injection. Cross-injection uses only the electric field and the mobility to minimise the sample leakage and to obtain a compact sample plug, as can be seen in figure 6c. All three methods have given good results depending on the circumstances. In order to use a sample plug as small as possible, the cross-injection design is chosen. By controlling the electric field and the mobility, sample plugs as small as the cross-section can be obtained. This technique is slightly more difficult to use than the double T-injection method, but smaller sample plugs can be formed [24, 25, 26].

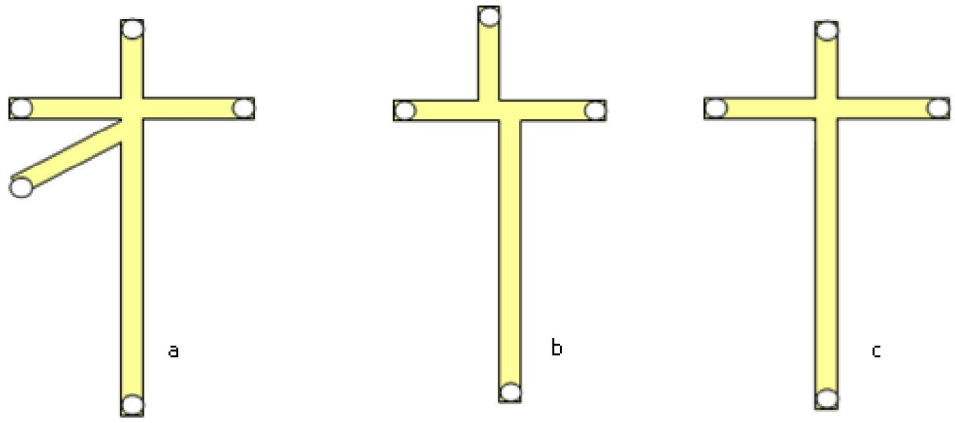

Figure 6 a) Double L-injection b) Double T-injection c) Cross-injection

The reservoirs are the next point to look at. For electrophoresis at least four reservoirs are needed at the corners. These are for injection and waste collection of the polymer and buffer. For the insertion of the sample several options are available. Some designs use one of the side reservoirs for injection, while others use an extra reservoir like in the double L-injection design or by creating an extra reservoir between the middle and one of the side reservoirs. Since a cross-injection is chosen for the injection an extra reservoir between the side and the middle channel is chosen as the injection reservoir. This way the sample can easily be controlled to reach the cross-section and to stay in that position without leakage until the start of the separation.

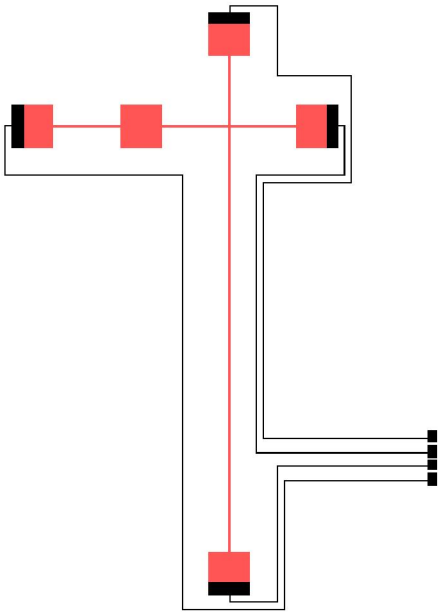

Figure 7 Layout chip design

For the final design four different designs are chosen in order to research the different parameters that can influence and therefore optimize the design. The first design has a channel width of 30µm, with a separation channel of 30mm.

The side and upper channels are 5mm long with reservoirs of 3mm in diameter. The electrode lines are 30µm and the electrode pads 500µm by 500µm. Care is taken to assure that the electrode lines are removed as far as possible from the channels to avoid electrical interference. The second design has also a channel width of 30µm but with a separation channel of 25mm. The side and upper channels are 2mm to increase the electrical field. The electrodes and reservoirs have the same dimension as for the first design. The third design has channel widths of 40µm with a separation channel length of 30mm. The other channels have a length of 5mm and the remaining parameters are the same as the first design. The wider channel is taken to see the effect of the diffusion and the mobility compared to the first design. The fourth design has a channel width of 30µm and a separation channel length of 30mm. The side and upper channels are 5mm long. The upper channel however has a channel width of 300µm. The wider upper channel is taken to decrease the effect of the electric field dependence on the length. When the upper channel is much wider than the separation channel, the electric field will neglect this part and therefore a higher electric field will be observed in comparison to a channel with equal length.

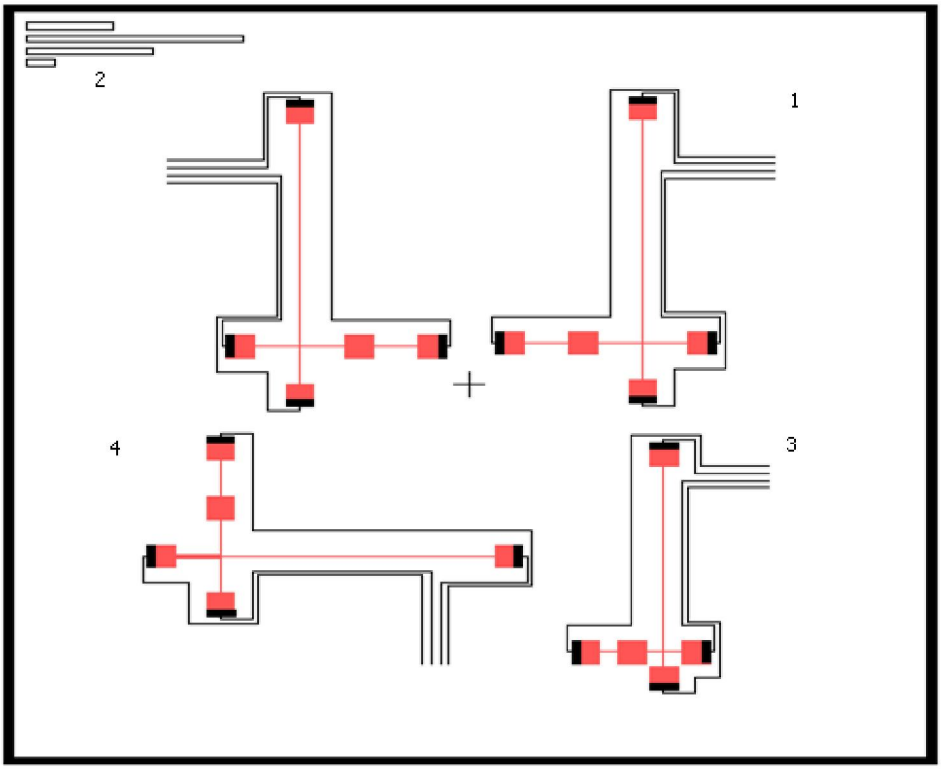

Figure 8 Mask design

The design of the mask with the four different channel designs can be seen in figure 8. The total of four designs is chosen due to the wafer size. The red channels and squares are the channels and reservoirs that are etched in the glass substrate. The black lines and patches are the electrodes and electrode patches that are attached to the second glass substrate. The numbers one to four refer to the numbers used for the description of the different designs as used in the above section.

2.2 Material choice

The following section will elaborate the choices for the most important materials of the process. First the polymer choice will be explained. The polymer choice will be explained first since the impact of this choice can greatly influence any other choices later made in the design. This is followed by the choice for glass as the substrate material for the whole chip. Many different substrates can be chosen and the choice of the substrate will also influence the techniques used and the process steps that will be taken. Finally the dye that is used for the staining of the DNA will be considered. A good dye is needed in order to have a clear view of the sample. Several dyes exist that work well with electrophoresis. Therefore one of these is chosen and the effects that this dye can have on the sample are considered.

2.2.1 Polymer choice

To achieve good separation it is important to carefully select the appropriate sieving matrix. There are several criteria which the sieving matrix has to fulfill, depending on the material used for the chip and on the DNA that will be used for the electrophoresis. The first criterion is the length of the DNA. Every polymer has his own constraints on which type of DNA it works best. Since the length of the DNA that will be used is already known, it is easy to set restraints on this criterion. The damaged DNA that will be used will be ranging from around 3kbp (kilo base pair) to 15kbp, as was found from the gel electrophoresis. Most research is normally done with either small, around 100bp, DNA or large, around 40kbp and higher, DNA. It is therefore essential to see whether the preferred polymer will work in both ranges. The list in table 2 gives a selection of polymers that meet this standard and can be used for the needed range.

Next it is important to look at the viscosity of the polymers. As shown in table 1, the range of the viscosity can be very large. It is important to look at the viscosity and to select a sieving matrix with an as low as possible viscosity. However to make sure that this will give no problems with the EOF effect it is important to simultaneously look at the self coating ability of the sieving matrix. The self coating ability is needed to make sure that the EOF effect will be low enough, so that the separation will be successful. If the sieving matrix does not have the ability to self coat, an extra coating layer is needed to ensure low levels of EOF. Therefore to avoid this phenomenon a self coating sieving matrix is desired [8].

Another point when choosing the sieving matrix is the toxicity of the material. A lot of commonly used sieving matrices have a very high toxicity level. In order to improve the working conditions and for environmental reasons it is best to search for methods that use less toxic materials. This leads to the conclusion that especially the polymers are not suitable to use for further improvement on this level. However these polymers give good results and thus an alternative needs to be found with lower toxicity levels and similar performance.

When looking at the above mentioned criteria the following conclusions can be drawn. The polymer needs to have a good sieving ability in the range of the DNA size used, the viscosity needs to be as low as possible, without losing the effect to withstand the EOF and it is better to find an option with low toxicity levels. By bearing these criteria in mind and by looking at table 2, the following polymers are chosen: Hydroxyethylcellulose (HEC), Poly(ethylene oxide) (PEO) and Hydroxypropylmethylcellulose (HPMC). Especially HEC holds for all the criteria and is ideal to use in this situation [27, 28, 29].

Polymers for Sieving matrices	**Advantages vs Disadvantages**
Linear polyacrylamide (LPA)	Highly hydrophilic, no self coating, viscous, lower solution better resolution larger DNA fragments., after injection long time needed for gel to return to normal structure.
Poly-N,N-dimethylacrylamide(PDMA)	Best self-coating ability, lower performance than LPA, slightly hydrophobic comp to LPA
Poly(vinyl pyrrolidine)(PVP)	Good self-coating ability, low viscosity, fast separation time,
Polyvinyl alcohol (PVA)	Self polymerization, so over time effect will decrease, self-coating
Poly(ethylene oxide)(PEO)	Self-coating, long flushing time before reuse, long treatment needed for silica surface for weakly absorbing PEO
Methylcellulose(MC)	Good for small DNA fragments, no self coating
Hydroxyethylcellulose(HEC)	Very low toxicity level, low viscosity, weak self coating
Hydroxypropyl cellulose (HPC)	Very low viscosity, no surface modification needed, relatively low current, no self coating
Hydroxypropylmethylcellulose (HPMC)	Especially useful for 1kbp to 23kbp range, weak self coating

Table 2 Disadvantages vs Advantages of selected sieving matrices [14, 27, 28, 29]

2.2.2 Glass substrate

In the following section, the substrate choice will be discussed. The chosen material is Pyrex 7740 glass. This is high quality glass. Other options for substrates where, polydimethylsiloxane (PDMS) and silicone dioxide in combination with Pyrex glass.

There are several reasons to work with glass. First of all, a lot of research done in the field of electrophoresis uses this material. This makes it easy to find references and to find specific parameters for the fabrication [19, 30]. Glass is also an easy material to work with. No mall is needed to form the device like for PDMS; common technologies like wet etching are sufficient to create the desired patterns and glass will break less easy then for example silicon, which makes it

easier to handle.

Looking at PDMS there are several aspects. The first reason to choose this material is that it is ideal for mass production. The malls that are created to form the PDMS can be used several times, increasing the production level. Also the process of forming the chips is relatively easy when the malls are complete. However, the techniques to create these malls are complex and time consuming. PDMS is also a relative new technique in electrophoresis and though the results are very promising, there is much less research done on this material, which could lead to problems with for example the coating [15, 19].

Another method is CMOS. This technique is favored due to the possibilities to integrate the electrophoresis system with other CMOS systems. Since silicon is not transparent, it is not possible to create the whole chip out of silicon. Therefore a combination of silicon and glass is used. The use of two different materials however, creates some difficulties. For one the EOF effect is much higher. Both surfaces have different electrical properties and will create a different electric field with the sieving matrix when the electric field is applied over the chip. This can cause the high EOF. To counter this extra coating of the chip can be done. However this can take a lot of time and depending on the height of the EOF it might not be enough. Another measure that can be taken is to deposit a small layer of silicon dioxide on top of the silicon to make the electrical properties of the two layers more alike. The problem here is that it seems that, although the EOF is much smaller, coating is still needed to get reliable results. Also the thickness of the silicon dioxide is of importance since this layer can have a lower electric breakdown than the other materials. The result is that lower electric fields have to be used, which will increase the separation time [10].

When looking at the different substrates that are possible several differences can be observed. The technologies used, the research that is already performed on these substrates and the handling during electrophoresis, are all different for each substrate. After looking at these factors a glass substrate is chosen for the design.

2.2.3 Dye

The dye for the staining of the DNA has a great influence on the performance of the separation, as will be explained later on. It is therefore important to check carefully if the dye is suitable for the chosen DNA and application. First it is important to see if the dye will be used for ssDNA, dsDNA or both. Most dyes have a preference for one of the two possibilities. This also has to do with the way of bonding of the dye to the DNA. There are two ways in which the dye can bind to the DNA, via intercalating or external bonding. With intercalating bonding the dye will attach itself between the two DNA strands. This can give a very high bonding strength. The other way the dye can bind, the external bonding, happens in the following way. The dye will attach itself on the outside of the DNA strands, although for most dyes this gives a weaker bonding and this method is especially needed for the staining of ssDNA. Since YOYO-1 dye will be used the effects on this dye will specifically be described, even though in most cases the same will take place for different kind of

dyes. For YOYO-1 it is the case that it can both bond by intercalating and external bonding. The intercalating bonding is here much stronger than the external bonding and to achieve external bonding a higher concentration is needed than used for intercalating bonding [31].

Apart from the staining method several phenomena can be observed that can interfere with the detection of the stained DNA. The first effect is called photo bleaching. This phenomenon causes the dye to lose its fluorescence. During the illumination the stained DNA will gradually loose its fluorescence and after a certain time the DNA can not be detected anymore and will therefore cause inconsistencies during measurement. The time that it takes for this phenomenon to occur and to become totally undetectable will vary with the dye and the amounts that are used. This phenomenon seems to occur for all dyes that are commonly used for electrophoresis. With YOYO-1 for commonly used doses of the dye bounded to the sample it occurs that a photo bleaching time of 15 minutes will cause that the sample is no longer visible [32].

Photo cleavage is another effect that can occur. Here the DNA will break into smaller pieces due to the dye. For YOYO-1 these breaks are always single stranded breaks. The double stranded breaks that can occur will always be due to earlier single stranded breaks. This photo cleavage can happen due to either illumination or oxygen and other intermediate species. Ways to delay or minimise this effect are to lower the illumination during the testing or to add Beta-mercaptoethanol. This chemical can greatly counter the effect of photo cleavage and is easy to add. A more difficult and time consuming method to lower the effect of photo cleavage is to remove the oxygen by use of a method called argon bubbling. This method uses the argon to remove the oxygen after the dye has been added to the DNA. However this can have some effect on the sample. The amount of photo cleavage also depends on the kind of DNA that is used. Super coiled DNA is for example a lot more sensitive to this effect than nicked circles and on linear DNA this phenomenon has almost no effect at all when looking at YOYO-1 dye. Since the sample that is used is linear DNA, photo cleavage will have no effect [33].

The temperature dependence of a dye can also have an impact on the fluorescence. Different dyes will have different kind of temperature dependencies. When looking at YOYO-1, research shows that this dye has a high temperature dependency. The higher the temperature is the lower the intensity. This is especially so for temperatures higher than the room temperature. Since the electrophoresis will take place around room temperature, this effect can be neglected [31].

When taking into account the sample that is used, the following of these effects have an impact on the measurements when using YOYO-1. For the binding a high concentration of dye needs to be used to ensure total binding to the ssDNA. Since the sample that is used is linear DNA, photo cleavage will have no visible effect on the whole process. Also the temperature dependence on the intensity is to be neglected due to the fact that the process will take place at room temperature. For the photo bleaching it is mostly important to make sure that there is no illumination near the sample before the start of the experiment, since the electrophoresis will take only around several minutes the effect of the photo bleaching should be minimal. A final point of attention is the spectrum of the dye. To get the best resolution it is important to check the exact spectrum of the dye and to make sure that the right filter is used when looking at the stained DNA. For YOYO-1

the maximal excitation is at 508nm and the maximal emission at 490nm [34].

2.3 Technologies

The following section will describe choice for the most important technologies that are chosen during the whole process. This begins with the main fabrication techniques, followed by the preparation techniques for the electrophoresis and finally the detection and processing methods that are used.

For the channel fabrication, both deep reactive ion etching (DRIE) and wet etching can be used. DRIE etching has a very high accuracy and a low surface roughness. Wet etching on the other hand has lower accuracy and the surface roughness can differ per treatment. However for DRIE etching it is very difficult to achieve a high depth, which is needed for the channels. With wet etching it is much easier to achieve higher depths, therefore wet etching is chosen for the channel fabrication.

The electrode fabrication uses Platinum (Pt) for the electrodes with a Titanium Tungsten (TiW) layer for extra adhesion with the substrate. The difference from most common designs is that the electrodes are integrated. Most devices use electrodes that are placed inside the reservoirs and on top of the chip, after the fabrication process. This increases the simplicity of the design and makes it easier to replace the electrodes if damage occurs. The reason to go for integrated electrodes is that it is easier to integrate the system with other devices. System on chip (SoC) designs are becoming increasingly important, so designing a system in such a way that it will be easy to adapt it to a SoC is a way to simplify future work that can be done with such a system.

Bonding of the chip will be done by water bonding. This technique is easy to use and has a lower cost due to the lack of advanced machines that are needed. Normal bonding works with adhesives. Special high temperature ovens and good clean room facilities are needed. So by choosing glass bonding by water a simple and cost effect way is used [35, 36].

For the detection of the DNA several options are possible. The easiest way is detection through microscope. This method can be used to test if the DNA is alive and whether the dyeing process is optimal. For the electrophoresis either charge-coupled device (CCD) camera's, intensified charge-coupled device (ICCD) cameras or Photomultiplier tubes (PMT) are used. The first consists of cameras, which capture the image with a certain frequency. The ICCD has a higher sensitivity than the CCD and can give a good picture of the electrophoresis process. The PMT on the other hand captures the light intensity from the chip and transforms this to a current. This technique can give highly accurate continuous signals and can be completely computer controlled. In light of the possibility to integrate this system and to computerize as much as possible a combination of CCD and PMT is used. The CCD is used here to check manually if the device has a correct working and to see if the DNA migrates in the desired direction and at the desired speed. The PMT is then used for the actual measurements.

In combination with the PMT LabView software is used to collect the signals [37]. This program is

used because since the controls are easily adjustable and the possibility is present to control several signals at the same time. This makes it possible to for example control high voltage sources together with the PMT tube to minimise switching times.

This section has given a short description on the most important technology choices that are made for the electrophoresis process. The chosen techniques are wet etching, integrated electrodes, water bonding and the detection methods that are chosen are PMT and LabView. The technologies itself will be described in more detail in the following chapters.

3. Fabrication

In this chapter the fabrication process of the chip will be described. The first section will begin with the fabrication of the micro channels in the glass substrate. The second section will describe the fabrication process of the electrodes on top of the second glass substrate wafer. The following section will explain the bonding process of the wafers and finally in the last section the packaging method that is used to finish the chip is described.

3.1 Channel fabrication

The fabrication starts with 2 Pyrex 7740 Corning glass wafers [38, 39]. These low impurity wafers are used as the basis of the microchip. The first wafer will be used to fabricate the channels of the design. The second wafer shall contain the electrodes for the electrophoresis and the outside contacts.

At first the fabrication process of the top wafer, or the channel fabrication, will be described. The wafer is first rinsed to decontaminate the surface and remove any organic and chemical residues present. To prepare the wafer for photolithography it is primed with HMDS, to ensure optimal attachment of the photo resist coating layer to the surface. The wafer is first coated with LOL2000 [40], to reach a 3000Å layer which can be used for lift off. After soft baking the wafer and a second priming of the wafer, a second layer of photo resist is coated onto the wafer. For the second coating AZ5214E [40] is used. This photo resist layer can be used as both a negative and positive photo resist layer, depending on the post treatment of the wafer. Another reason to use this photo resist is the thickness of the photo resist layer that is obtained, which is relatively thick compared to other photo resist layers after the first spin. The reason for a thick resist layer is to resist the hydrogen fluoride treatment, used in wet etching. To achieve a negative exposure of the photo resist, the wafer is exposed two times instead of once. After a second soft bake the first exposure is done on the ABM high resolution mask aligner. Exceeding is another soft bake, followed by flood exposure, to activate the negative photo resist. Care has to be taken to ensure that the exposure times are neither too short nor too long. A too short exposure can lead to patterns that are not exposed until the substrate. This will lead to non complete removal of the photo resist and can cause that the pattern is smaller or not at all transferred. Longer exposure on the other hand can lead to over exposure. These effects can be seen in fig. After the right exposure the wafer is developed in FHD-5. To insure optimal results the pattern on the wafer can be checked by infrared to see if any photo resist is still left on the pattern. When this is the case extra development can take place. This takes place by either submerging the whole wafer again or by manually removing the resist in the area concerned [40].

After the photolithography process an additional cleaning and descum process is executed to enhance the surface smoothness in preparation of the next step. This step consists of the sputtering of a chrome layer on top of the entire wafer. A clean wafer is essential here to make sure that the

metal layer adheres well during the etching process for the channel.

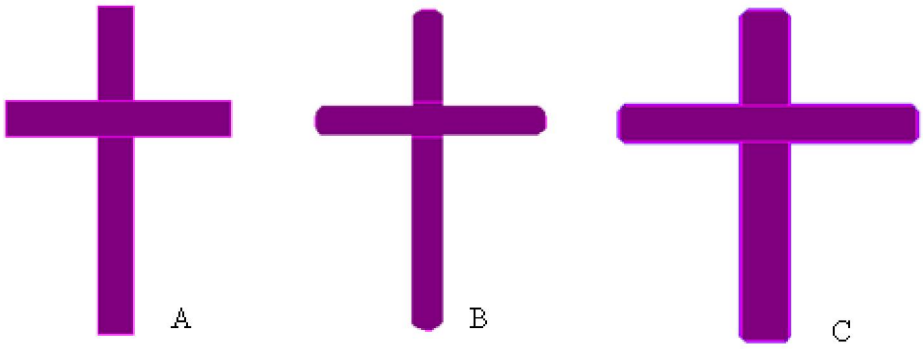

Figure 9 Pattern changing due to exposure differences A) Desired pattern B) under-exposed pattern C) over-exposed pattern

Next is the lift off process. This process consists of the removal of both photo resist layers and the excess metal. Acetone is used to remove the first layer of photo resist. Depending on the thickness of the photo resist and on the adhesion of the material it can take up to several hours to get a thorough removal of this layer. In particular the minimal feature size is of great importance for this process. The smaller the feature size, the more difficult the removal of the photo resist. After the acetone the wafer is dipped in IPA as an extra cleaning measure. Then the wafer is put into FHD-5 to remove the LOL2000. This process should afterwards be checked under the microscope to see if any residue is still left on top of the pattern. If this is the case then it would be easiest to manually swap the wafer with the above three chemicals. The manual swap is more precise and due to the slight pressure that can be applied it is easier to remove the residues. However care must be taken not to damage the photo resist of the non-exposed areas. At the end of this procedure only the chrome metal layer is left.

Initially only this metal chrome layer was used as protective mask during the wet etching with HF. However within minutes the chrome layer is pealed off, leaving the entire wafer exposed. To counter this problem an extra layer of photo resist is applied on top of the chrome layer. When using AZ5214E the protective mask lasts longer but not long enough to etch the desired depth. Therefore an even tougher photo resist was used namely HPR 204 [41]. This is a positive resist. Care has to be taken during the photolithography to ensure perfect alignment with the previous process. This can be done be applying alignment marks on the mask or by manually looking through the microscope. This second process however takes a lot of practice and time and will most likely result in some small shifts if one is not careful. When the mask is not aligned in exactly the same way, the pattern will shift and the etching of the pattern will be non-uniform due to leftover or lack of photo resist. The process of applying is the same as mentioned above until the first exposure. After the first exposure time the photo resist is immediately developed and hard baked, leaving a positive photo resist layer on top of the chrome pattern.

Next wet etching of the channels was performed by using hydrogen fluoride (HF). Caution needs

to be taken when working with this material due to the high toxicity of the HF. For the wet etching several different percentages of HF were tried to find a concentration with an as high as possible etching rate, without peel off of the protection mask. By choosing a different metal layer or by increasing the thickness of the layer higher concentrations could be used. A low metal layer is however preferred to keep the fabrication costs low. Heating the HF can greatly decrease the etching time. Although this generates toxic fumes and therefore this fabrication process was carried out at room temperature. Another point about HF is the different etching time for different materials. Since high purity glass is used, the etching time can be up to 5 times higher than for commercial glass. A stirrer was used to increase the uniformly distribution of the chemical. This can improve the etch rate, which normally will decrease over time due to settling of the chemicals at the floor of the basin. The etching is also more uniformly distributed over the wafer, to ensure that the etching depth is the same over the whole area. However the stirrer can cause damage to the wafer due to the fact that the wafer is positioned in a raster. It is therefore important to check the wafer for peeling of the photo resist and metal layer and to move the wafer several times during the etching process. Peel off of the photo resist is easy to recognize due to the change in colour of the chemical. Turning of the wafer can increase the uniformity of the etching. Because the etching rate differs for every kind of material, it is important to first research the etching rate. This can be done by taking out the wafer at specific periods of time to check under a stepper what the achieved width is before putting the wafer back in the chemical. From this method it can be seen that a fresh solution is needed for optimal speed and for slower decrease in etching rate. During the etching process the photo resist will slowly start to peel off over time. This can lead to partly covering of the pattern and can slow down the etching at that point. To decrease this effect it is advisable to descum the wafer when the height and uniformity measurements are done.

Figure 10 Wafer after channel etching

At the end of this process the remaining photo resist and chrome are removed. First the photo resist layer is removed by using acetone. Secondly the chrome layer is removed. After a final descum the channel fabrication is completed.

The above process is explained in figure 11.

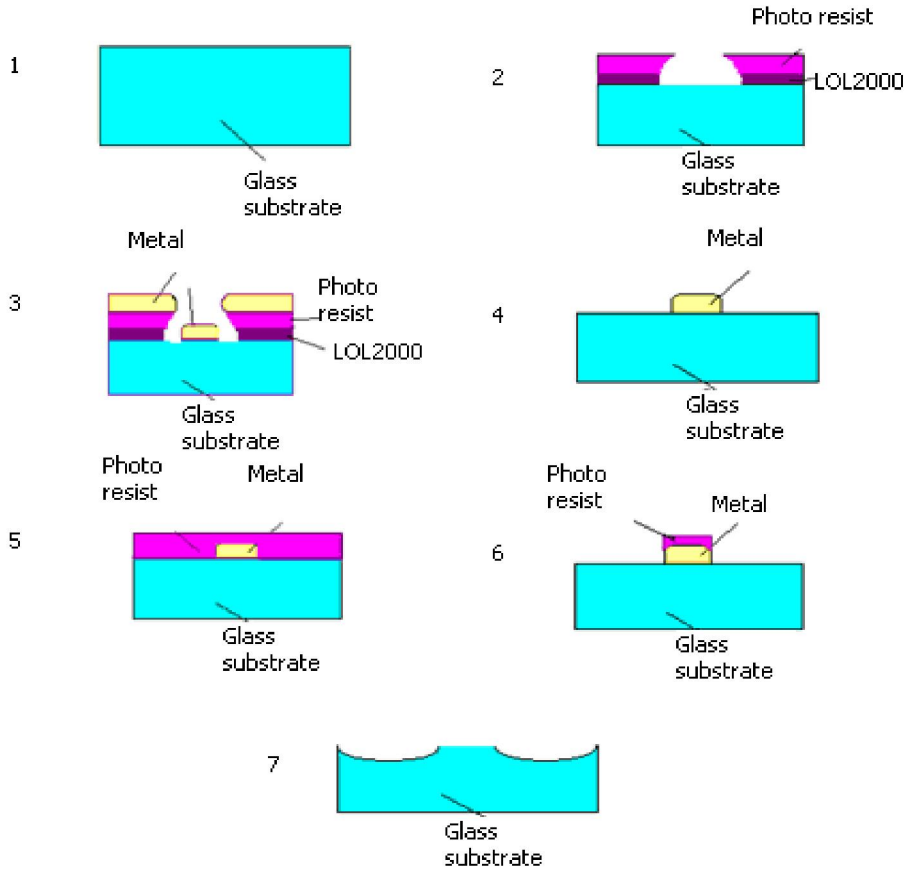

Figure 11 Channel etching process

3.2 Electrode fabrication

The next step is to fabricate the electrodes. The wafers were cleaned and rinsed. Then they were treated with primer and the first layer of LOL2000 photo resist was applied. After soft bake and another treatment with primer the second photo resist layer was deposited. Here AZ5214E was used. Photolithography is applied and since a positive resist was needed there will only be one exposure round before development and hard bake [40].

The wafers were cleaned to achieve a better adhesion with the metals. First a layer of Titanium Tungsten (TiW) was sputtered onto the surface. This is done to enhance the adhesive force. Next a layer of Platinum (Pt) was deposited right on top of the TiW layer. This gives a Pt electrode layer accompanied by a TiW layer to assure the adhesion between the surface and the Platinum.

Now that the metal layers are sputtered on the surface, the photo resist layers could be removed. This was done by lift off as described in the last section. The cleaning with, acetone, IPA, FDH-5

and descumming are the final stages of the electrode fabrication process.

This process is described in figure 12.

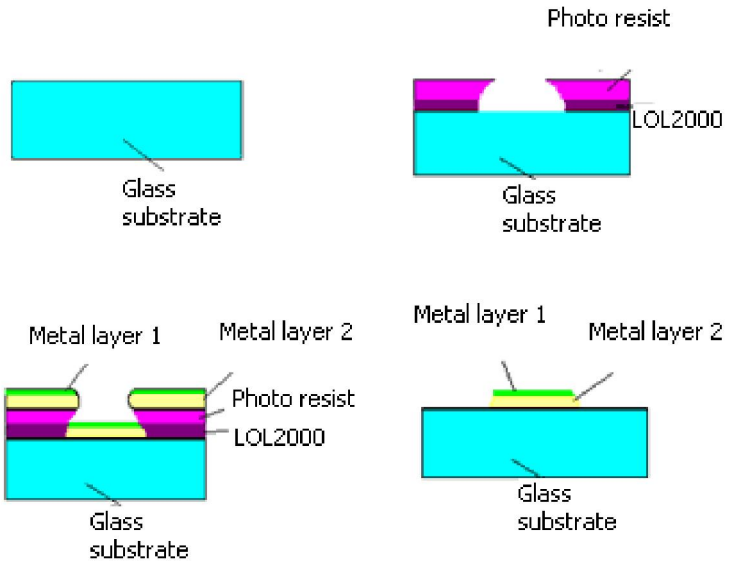

Figure 12 Electrode fabrication process

Another alternative way of depositing the electrodes on the wafer is to first etch the pattern into the glass wafer. First the pattern for the electrodes is etched in the glass substrate. The etching depth is relatively low compared to the channel etching. The protective mask can therefore consist of only LOL2000 and AZ5214E. After the double photo resist layer the wafer is put in the HF solution. After the etching the wafer is cleaned and the TiW and Pt layers were deposited on top. Lift off was executed and the electrodes were left in the small channels on the glass substrate. This technique ensures the flatness of the surface, which is needed to keep the level of complexity as low as possible for the bonding of the glass wafers. The problem with this technique is the HF etching. The surface roughness acquired with this technique is relatively high. The adhesion to the TiW layer is now lower than when deposited on the smooth glass surface. Peel off can now easily occur. To solve this problem deep reactive ion etching (DRIE) can be used. This technique gives a smoother surface and thus solves the adhesion problem. Due to lack of time this method was not used, resulting in the choice for the above mentioned direct deposit technique.

Figure 13 Wafer after electrode deposition

The differences between the depositing technique of figure 12 and the above mentioned alternative depositing technique are shown in figure 14.

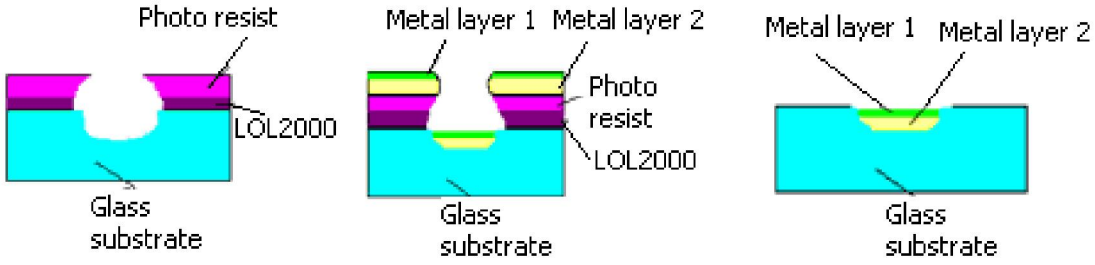

Figure 14 Optional electrode fabrication

3.3 Glass bonding

To begin the bonding process the wafer was first diced. It is possible to do this process by hand, however low accuracy and easy breaking of the wafer make it preferable to do this by machine, although the inputting of the coordinates can take al lot of time if used for only a single wafer and not for batch production.

The following process step is the bonding. The most important step here is the cleaning. Any dust particle left on the surface can lead to incomplete sealing. A particle near the channel of the device will lower the pressure that can be withstood at that point. A lower pressure means that it will be more difficult to inject the liquids in the channel and can cause leakage. Leakage can spread easily and can eventually lead to a complete breakdown of the chip. So it is important to clean the wafer thoroughly and check for any impurities before starting the bonding process.

The cleaning process begins with MS2001 cleaning in the clean room. Afterwards the wafers are removed from the clean room area. Here the wafers are washed with detergent and placed into contact under a continuous stream of DI water. The wafers are then placed in the oven at 120°C for half an hour to evaporate al the water between the wafers and establish an OH bond at the interface of the wafers. The wafer is then checked for any discontinuities at the surface. If there is any form of contamination left, there will be a discoloring of the glass and circle formed pressure rings will form. When these rings are noticeable and near the edges of the design, the wafer needs to be separated and the wafers need to undergo extra cleaning. This extra cleaning can consist of detergent or in case of high contamination, acetone and methanol cleaning. After the cleaning the wafers are brought into contact under streaming water and again placed in the oven. The process can be repeated until there are no contaminations left [35, 36].

Another reason for stress forming can be caused by the surface roughness. Especially near the electrodes, it is possible that the surface is not uniformly enough and therefore it can cause some gaps between the two wafers when put into contact with each other. When this is the case, it will be almost impossible to reach a good adhesion, no matter how clean the surface is.

Also the cleanliness of the equipment is of great importance. If the high temperature oven is

contaminated, particles can settle between the wafers during the drying process. Next to this it is also possible that the contamination gives a chemical reaction with the electrodes. Because it is not very clear what this contamination consists of, it will be very difficult to clean the electrodes. This can for example cause the electrodes to malfunction or to be so different of composure that soldering of the wires will not succeed. Ways on how to solve these problems will be discussed in the following section.

As soon as the wafers are clean after the heating at 120°C, the wafers are put back in the oven and are slowly heated to 550°C. This second heating process should be no faster then 4°C/min to keep the stress low enough so the wafers will not break. The wafers stay at 550°C for 1 hour, after which the wafers are cooled down at a speed lower than 1.5°C/min. A faster cooling rate can cause cracks in the glass, due to the build up stress. At 100°C the wafers can be removed from the oven, to cool down until room temperature is reached. After the high temperature bonding the bond between the wafers is so strong that they can not be removed from each other without breaking. Therefore it is important to make sure that there are absolutely no contaminations left after the pre bonding at 100°C [35, 36].

As researched, low temperature bonding should be achievable by using the previous mentioned method, without the final high temperature bonding. However it was found that the pressure that the chip could withstand was too low to be able to insert the polymers in the channels. Longer standing times as mentioned in [35] are tried out. This still gives too weak a bonding strength. Therefore the bonding is done with extra high temperature treatment.

The above used method is different from commonly used techniques, because it does not make use of any adhesives. This simplifies the process and lowers the production costs, since no expensive high temperature ovens are needed.

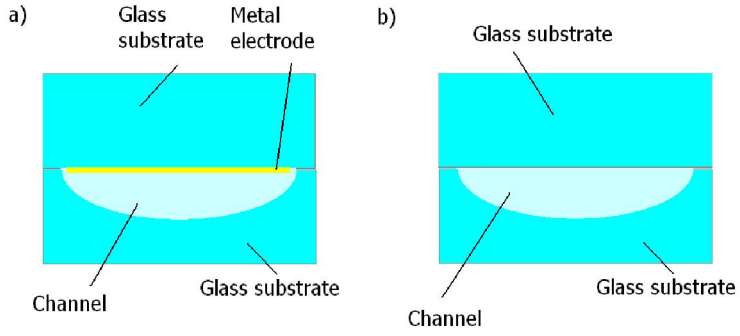

Figure 15 Cross-section of complete device a) reservoir b) separation channel

3.4 Packaging

The final step in the fabrication process is the packaging. This begins by drilling holes in the glass chips over the reservoirs. These holes will serve as inlets or outlets for the fluids that are to be injected. The holes can be drilled manually. Care has to be taken that the drilling equipment is sharp enough to avoid breaking chip. It is recommended to practice the drilling before using the

chips to avoid breakage.

The first step is to design the PCB board. Here it is important to make sure that the channels of the device stay visible. This is to ensure an optimal optical resolution when using the microscope. For this purpose a mask is designed to etch the desired pattern on the PCB board. The process for the CPB design is as follows. The pattern is printed on transparent paper and put on top of the PCB board and the whole is illuminated at 90% illumination for about one minute. The PCB board is put into NaOH 0.5% for 1 hour at 60°C. All the while the chemical is pumped through the chamber for evenly distribution and heating. After cleaning the pattern is transferred onto the PCB board surface. The pattern is now cut from the PCB board.

Now that the PCB board is finished the chip can be mounted on top of the PCB. The PCB board must have a large enough pattern to ensure that the light will not be scattered when positioned above the channels. To get an accurate reading the channels have to be in the middle of the open space in the PCB board.

The final step is the wiring. This is done by soldering. The wires are attached to the electrode patches on the chip and secured so that they can withstand enough pressure when attached to the rest of the system. Normally the soldering will be easy and the fastest way, however due to contamination from the high temperature oven it is possible that the tin will not stick to the patch. The first thing to try is to clean the surface of the electrodes. This can be done by acetone, IPA and methanol. If this does not help than the contamination is not due to organic contamination. Since it is very difficult to figure out and remove other kinds of contamination, another solution is proposed. This solution is silver paint.

The silver paint is applied on the electrode patches and is dried. This drying can be done by putting the entire chip in the oven at 120°C for 10 minutes or, if this is not possible due to melting of some components, to wait for 24 hours to let it dry at room temperature. After this process attaching the wires becomes much easier, although care has to be taken to ensure that the silver paint is dry enough to have made a good adhesion to the electrodes. It is also important to control that no interconnections are created between the wires during the process. Silver paint can also be used to mend broken lines on the chip. This can happen due to oxidation or wear from daily use. It is easy to apply if the lines are outside. At the end of the process the lines can be tested to see if there is contact with the reservoirs. If all connections look good, the chip can be used for experimenting. In figure 16 the complete chip before and after packaging is shown.

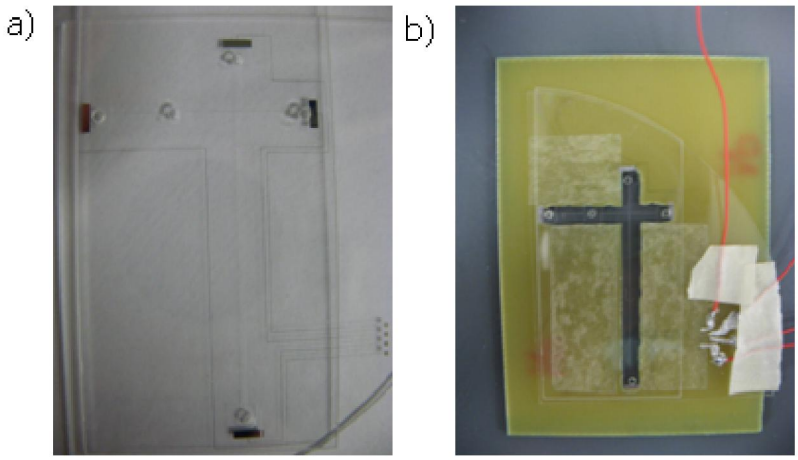

Figure 16 Complete device a) before packaging b) after packaging

4. Experimental setup and Results

In this chapter the obtained experimental results and the experimental setup will be explained. The first section will explain the DNA sample preparation and the dyeing process. The second section will handle the testing of the polymers. In the third section the experimental setup for the micro-chip electrophoresis with the fabricated devices will be explained and finally the fourth section will explain the testing results for the micro-chip electrophoresis.

4.1 DNA sample preparation and dyeing

The following section will describe the sample pretreatment of the DNA, the dyeing process for this DNA sample and the effect of photo bleaching that is measured.

The damaged and pretreated DNA samples used for the electrophoresis is acquired from the department of environmental engineering of the Hong Kong University of Science and Technology. A short description of this process is given. The DNA Escherichia coli (E. Coli) sample is irradiated under UV-light for half an hour to produce the damage. The DNA is incised at the pyrimidine dimers, which repress the replication mechanism of the DNA, at 37°C for 45 min. with an enzyme. The fragments are formed according to the dimmer distribution, due to the act that the endonucleases cut specifically on the dimmer sites. The UV-damaged DNA is then denatured with alkaline. Some of the samples undergo TiO_2 treatment before the denaturing to research the repressive effect of TiO_2 on the damaged DNA samples.

The samples are dyed with YOYO-1 iodine dye. Both enzyme treated DNA, which is ssDNA, and UV-damaged DNA without enzyme treatment (dsDNA) are tested to look for an optimal concentration of the dye. The protocol for the dyeing is established to be 100x diluted in Tris-Acetate-EDTA (TAE) buffer. The concentration of this dye for micro-channel is around ten times higher than that for gel electrophoresis. This is due to the lower sample concentrations that are used for micro-chip electrophoresis.

After the dyeing of the sample the photo bleaching time is researched. This effect has an exponential time curve. As can be seen from figure 17 the photo bleaching takes effect from the beginning. Already after 2 minutes a lower intensity is noticeable. By taking pictures with the CCD camera every two minutes with an exposure time of 1 second and a magnification of 20x, the intensity can be tracked. The electrophoresis process will approximately take 3 minutes, in which the sample will be illuminated. The photo bleaching time needs to be longer than the time that the electrophoresis will take. A longer time is preferable to be sure that, even if there is some exposure of light to the sample during the set up of the experiment, the results are still optimal. The time frame for figures 17 a to f is 10 minutes. The first picture is taken at time zero of the illumination and the next pictures are taken every 2 minutes afterwards until no visible signal can be detected. From this the photo bleaching time is found to be 10 min with a 100x dyeing concentration of the

sample. Lower concentrations of the dye give a higher photo bleaching time, up to 15 minutes. However with lower concentrations the intensity decreases. For the low amount that is needed it is tested that a 100x dye concentration is optimal in terms of intensity versus photo bleaching.

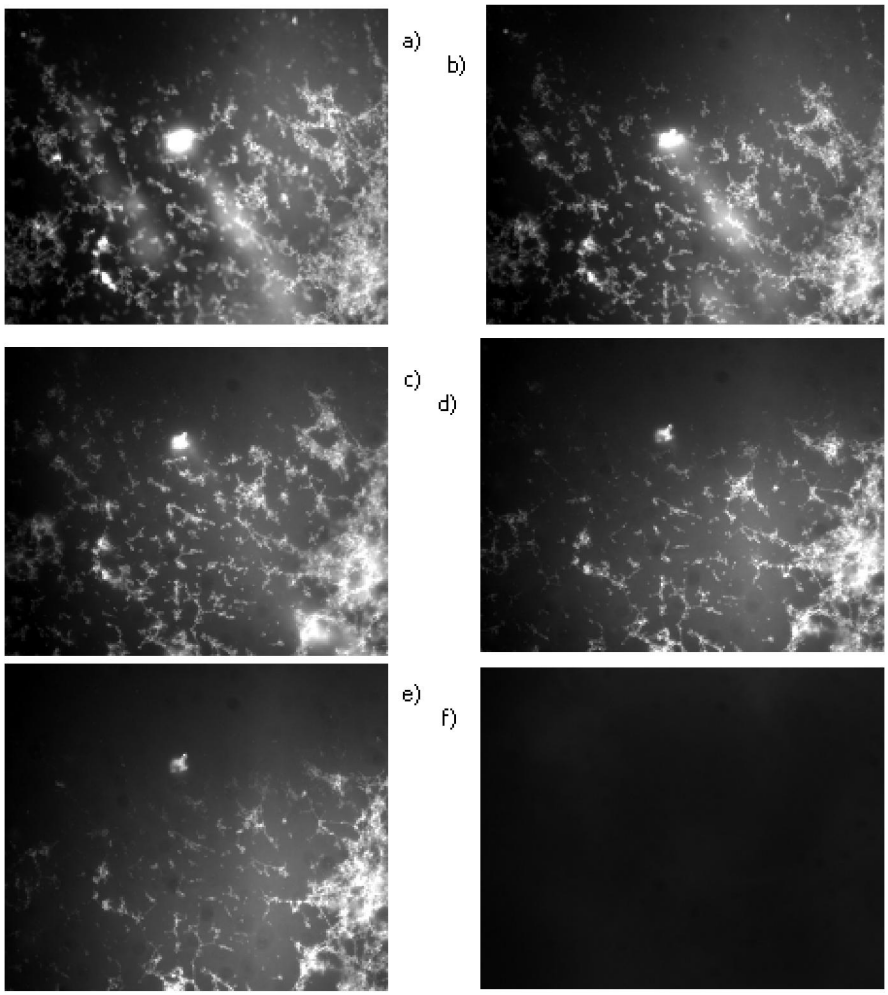

Figure 17 Photo bleaching effect of 100x YOYO-1 E. Coli DNA over time a) t=0 b) t= 2min. c) t= 4min. d) t= 6 min. e) t= 8min. f) t= 10 min.

4.2 Polymer testing

The used polymers all have a different viscosity. The first step in finding an optimal concentration is to test the highest possible percentage that can be easily injected in the micro-channel. The polymers are prepared by mixing the several concentrations at room temperature with TAE-buffer. It is important for the polymers to remain at room temperature. At temperatures above 30°C some of the polymers will already harden and are not usable for injection. For the polymers a maximal viscosity for easy injection is found to be between the 1% and 2% depending on the polymer. The DNA sample that is used for the electrophoresis has its peaks between 3kbp and 15kbp. Lower concentrations of the polymer give a better resolution for higher base pair range of the DNA. Since the used range is from middle to high base pair level, the testing will begin from 1% and

will be lowered according to the obtained results.

The next testing is about the uniformity and the depth of the micro-channel and concerns both the polymer and the sample. Uniformity of the micro-channel is needed to get an evenly distribution of the polymer. When the polymer is injected in the channels, air bubbles will almost always occur. To minimize these bubbles, which can cause acceleration or deceleration of the sample if it moves through the polymer, the channel is flushed for at least 15 minutes with the polymer. If under the microscope no more bubbles can be seen then the injection of the polymer is successful. Else the polymer can be laid to rest inside the channel for half an hour to let the air bubbles escape. If there are discontinuities in the channel design the polymer, air bubbles or the sample can get caught at these points. The next step is therefore to check the uniformity of the channel and to see if the depth of the channel is high enough near the boundaries.

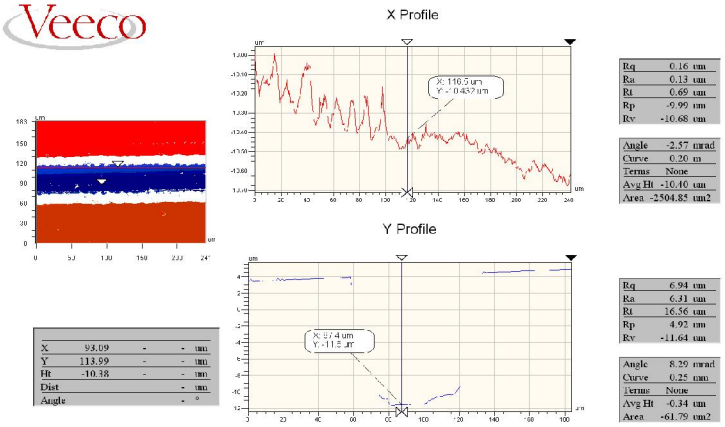

Figure 18 3-D channel cross-section profile near the edge

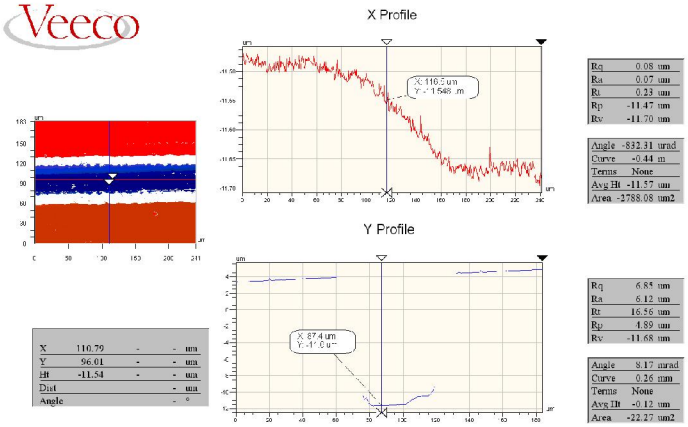

Figure 19 3-D channel cross-section profile middle section

By using an optical scanner (Wyko, NT3300, Veeco) the profile of the channels is measured. Figure 18 and 19 show a cross-section of the micro channel. This 2-D profile shows part of the channel in both the x and y direction. The different colours show the depth profile of the channel. The white areas mean that measurements cannot be performed due to the limited angle of the optical scanner. For figure 18 the measurements are done near the edge of the channel. Due to the optical properties of the scanner a complete profile cannot be made from the sidewalls, therefore a point as close as possible to the sidewall is taken. By taking measurements from several chips at several different points it can be seen that the spikes due to non-uniformity of the chip are around 0.2µm as can also be seen from the upper picture in figure 18. The decline of the line over the whole area is due to the slight tilting of the chip to achieve a better y profile as can be found in the bottom picture of figure 18. In figure 19 the same measurement is done, but now for the middle section of the channel. By looking at these profiles it can be seen that the uniformity in the center of the chip is higher then near the edges. The depth near the center of channel varies slightly due to the fabrication method that is used, however this is low enough to be negligible.

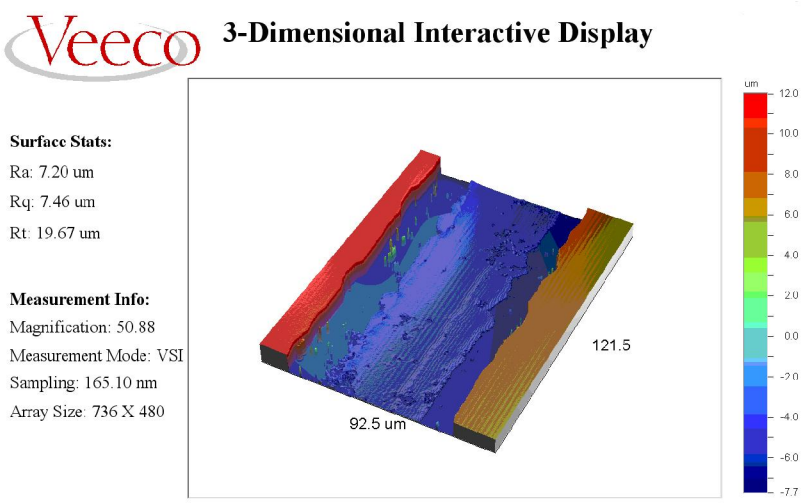

Figure 20 3-D channel cross-section

By looking at figure 20 a better impression is given. This 3-D view of the channel shows that in the area near the edge some spikes can be found. Since these are found only near the edge and since they are few in number it is not expected that these will cause any problems like clogging or delaying of the sample.

4.3 Experimental setup Electrophoresis

This section will describe the experimental setup for the electrophoresis. After the sample preparation and the polymer injection the micro-chip is connected to the circuit. This circuit is as followed. The high voltage supply source is connected to the voltage divider. This circuit will deliver the voltage to all four electrode entries on the chip. The divider is used to switch the voltage supply for the vertical and horizontal channel simultaneously. This is to prevent the

sample from leaking and to keep the sample as compact as possible in order to gain a higher resolution. The microscope is positioned above the end of the separation channel and connected to the PMT tube. A lens which can detect the wavelength of the YOYO-1 dye is chosen and the shutter is completely open for a high intensity. The PMT tube is now connect to the computer and will deliver a digital signal. The gain of the PMT tube is tuned to obtain an accurate signal without attaining saturation.

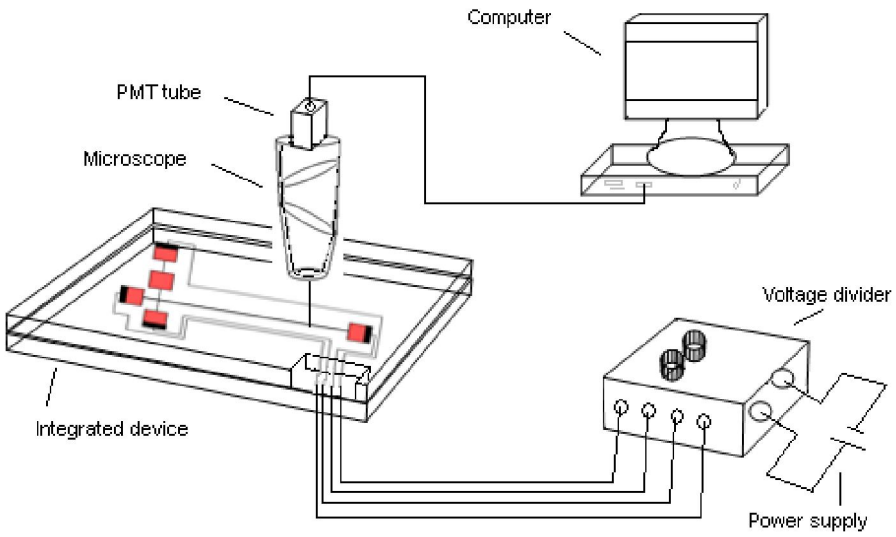

Figure 21 Experimental setup for Electrophoresis

4.4 Electrophoresis Results

This section will describe the results that are obtained from the micro-chip electrophoresis, using the created devices.

For the electrophoresis two different voltages are applied to the chip. The first voltage that is applied is for the injection of the sample. This voltage will be applied to guide the sample to the cross-section of the channels and will be applied to all electrodes to achieve a pinched injection, which will improve the compactness of the sample near the crossing. The second voltage that is applied afterwards is for the separation process. Here the voltage is applied to the two electrodes that are connected to the separation channel. The other two electrodes are left floating. Depending on the design of the micro chip a voltage is applied for the separation to obtain an electric field of 100V/cm.

The DNA samples that are used for comparison are a 48Kb DNA ladder, a 1Kb DNA ladder and UV-C damaged E. Coli DNA samples, with and without TiO pretreatment. Figure 22 and 23 respectively show electrophoresis with a 48Kb DNA ladder and electrophoresis results from UV-C damaged E. Coli DNA with TiO treatment. The 48Kb ladder in figure 22 shows the intensity measured from the PMT versus the time. The spikes that have a considerable higher intensity then

the noise level represent the different lengths of the pieces of DNA that are present in the ladder.

As can be seen in figure 23, the E. Coli DNA sample has more peaks then the DNA ladder. This is due to the fact that the DNA ladder has only specific lengths that are available, whereas the E. Coli sample can produce pieces of every size with its original size as a maximum. The DNA ladder is used in the gel electrophoresis as a marker to compare the different lengths. Since in gel electrophoresis the exact DNA bands are known for each specific concentration, the samples can be put next to this marker to determine the exact length of the DNA of the sample. However in micro-chip electrophoresis all the different lengths of the DNA marker can be detected, where in gel electrophoresis only a few are detected depending on the concentration of the gel that is used. Due to this fact the conventional DNA ladders are not suited for micro-chip electrophoresis. From the manufacturer only the detectable sizes for different gel concentration are given, therefore it is unknown which lengths exactly are present in the DNA ladder. This makes it very hard to impossible to use these DNA ladders as a marker to determine the exact length of the E. Coli samples in micro-chip electrophoresis. To get an accurate result a different DNA ladder needs to be used for the testing to be able to use this as a marker. However, because these ladders where not available during the research only an estimation can be made of the lengths on base of the lengths that are visible from the gel electrophoresis. Even though the exact length can not been given at the moment, the difference between the DNA ladder and the E. Coli sample show that the used micro-chip electrophoresis gives a repeatable result with distinct peaks, from which, in case of the use of a correct marker, the length of the DNA can be extracted.

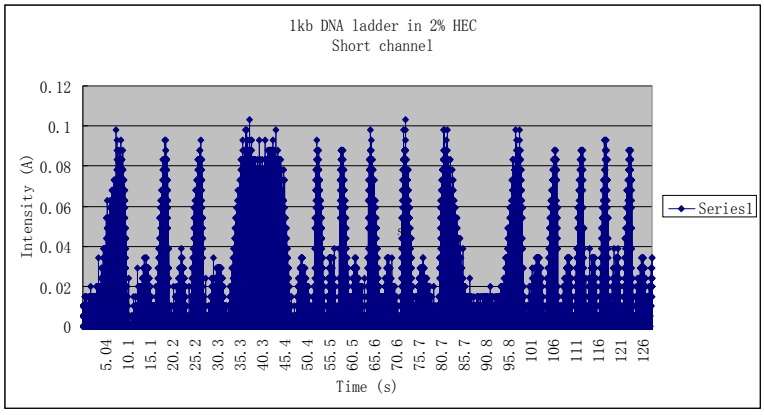

Figure 22 Micro-chip electrophoresis for a 48 Kb DNA ladder

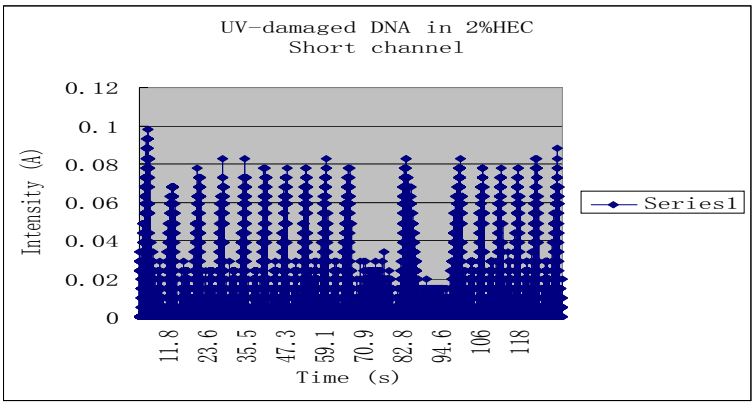

Figure 23 Micro-chip electrophoresis for UV-C damaged E. Coli DNA

In figure 24 two identical DNA ladders are shown from different runs under identical circumstances. As can be seen from these graphs the two graphs do not show the exact same peaks and the graphs look shifted compared to each other. The shifting of the graphs with respect to each other can happen due to the different starting times. The circuit is manually switched to start the measurement at the moment that the separation starts. Due to human error and reaction time, the starting times of the measurements are not simultaneous. This can cause the graphs to shift. By carefully switching this can be reduced to a minimum. To completely solve this problem the circuit can be completely automated. The on switching of the circuit can be linked to the switching of the high voltage source by means of LabView, which is already used to control the PMT tube. Another way to ensure equal switching time and exact same circumstances for multiple measurements is to design a chip with multiple channels. In this way one run will produce results the same way as gel electrophoresis is currently doing. This will increase the quality of the measurements.

The width of the peaks also varies when looking at figure 24. There are two possibilities for this phenomenon. The first possibility is that during the injection and transportation of the sample plus the sample does not stay compressed enough. Due to the spreading of the sample the DNA pieces with the same length arrive at slightly different moments, causing the peaks to broaden. Another possibility is that the sample gets caught inside the channel. This can happen due to spikes inside the channel or air bubbles that are left behind during the injection of the polymer and buffer. When the sample gets caught, even if it is for a short time, the sample will be partly delayed, also causing a broadening of the peaks. If the samples stay stuck for a longer period of time this can cause a serious error in the measurements. It is often not possible to tell whether the sample size is correct or that some peaks are due to delayed pieces.

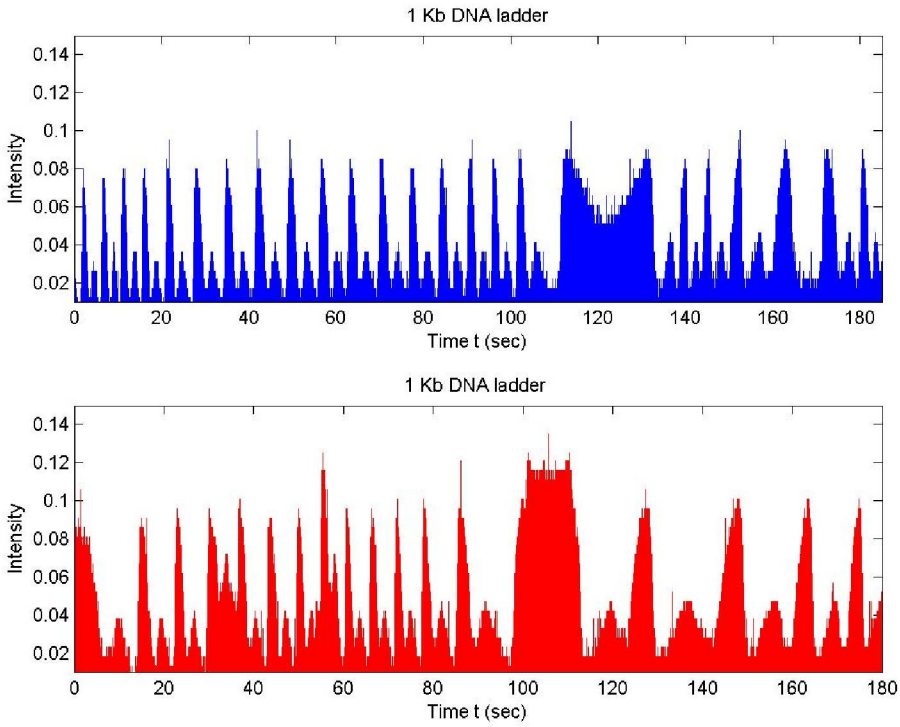

Figure 24 1Kb DNA ladders after electrophoresis under equal conditions for error comparison

During the design phase several different designs are constructed. The major difference between these designs is in the length and width of the separation channels. When testing these designs several differences can be observed between these different designs. The largest differences are found between the short and long separation channels. As can be seen in figure 25 for the same experimental conditions, the separation time of the short channel is much faster. This is due to the higher electric field compared to the long channel for the same applied voltage. When looking at the long channel designs the width of the channel influences the width of the peaks. The larger the width of the channels, the wider the peaks are. However this difference is very minimal and could be neglected for the chosen designs. For smaller widths this effect will most likely have a larger impact. The most interesting effect is apparent when looking at the design with the wider upper channel. By application of the same voltage, the samples give, instead of the measurements as for the long channel, a measurement that is almost as fast as the short channel device. When looking at the different designs, the following can therefore be concluded. The short channel design gives the fastest separation time caused by the higher electric field that can not be achieved for the long channel designs due to the limit that occurs due to the high voltage power supply. The width of the separation channel does affect the width of the peaks, but due to the small difference in the chosen designs the variation is very small. And finally a wider upper channel for the separation channel can increase the electric field and thus lower the separation time.

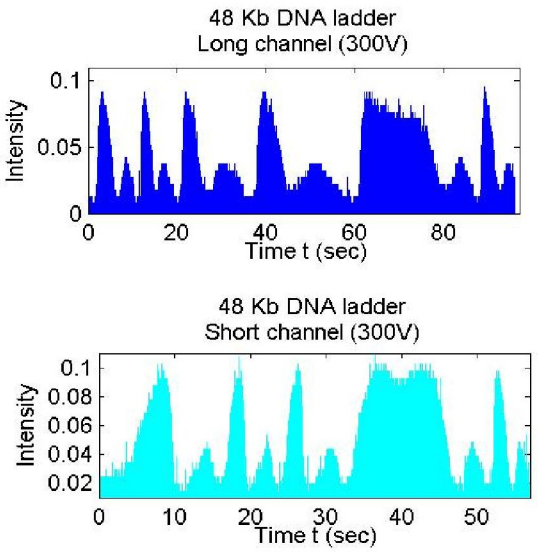

Figure 25 48Kb DNA ladders after electrophoresis for long channel design (upper) and short channel design (lower)

By comparing the different results from the different samples the following conclusions can be drawn. The designed device can be used to measure the different lengths of the DNA pieces that are present in the sample. Although the categorizing of these pieces is not yet accurately done due to the missing of an accurate marker, the differences between the used DNA ladders and the E. Coli samples are clear. The differences between identical sample plots are explained to be caused by starting time differences, sample injection errors and obstruction of the sample within the channel. The different designs that are used show a difference in separation time and peak width due to variations in channel length and width. Looking at all this, it can be seen that there is still room for improvement. The last chapter will therefore go in on the possible improvements, next to the conclusions.

Conclusion and Future work

During the background research the working behind both gel and micro-chip electrophoresis are shown. From the most important characteristics a comparison is made to show the differences between these two methods. Based on the calculations and the background it is found out that micro-chip electrophoresis should improve the sensitivity and the resolution and it should drastically reduce the time that is needed for the electrophoresis.

By looking at the desired values an optimal chip design for the E. Coli sample that will be used is created. The different techniques for the fabrication process are reviewed and judged on their method of execution, the time to master these methods and the disadvantages that arise from these design.

When reviewing the techniques and fabrication methods, methods like PDMS substrates and micro-chip arrays can seem to give better results, but they take a long time to produce and not very much is known yet about the principles. Therefore they are not chosen for this design. However, for future research these options are most likely to produce the better results.

During the fabrication process several different techniques are tested to find the best working solution. After trial and error the wet etching gives good results, although in future research different techniques like (DRIE) can most likely improve the surface roughness and the profile. New methods are used for the bonding and for the packaging and several techniques are used to improve the stability of the device and the surrounding equipment.

As can be seen from the micro-chip electrophoresis results the devices show repeatable results in which the different DNA lengths can be distinguished. The peaks are however not always the same width indicating a sample plug that is not compressed enough near the cross-section after injection or that a part of the DNA is slowed down due to air bubbles inside the polymer or spikes inside the channel. The pattern is also sometimes shifted over time. This can happen due to the manual starting of the separation and the measuring device, and can easily be remedied by automating triggering of the high voltage source and measuring the results.

To improve the quality of the channel and to improve the equal distribution of the depth and to remove the spikes that exist due to wet etching, the following options are possible. The first option is to change the wet etching to deep reactive ion (DRIE) etching. This method is more accurate and will give a more evenly distributed depth and fewer spikes. The second option is to use a different material like PDMS. This material is easier to manipulate after the creation of the mall. To resolve the timing problem a digital circuit to trigger the high voltage source and the measuring circuit will be the best solution.

When looking at the whole process it can be concluded that the objectives that are stated in the introduction are reached. The fabricated device is faster by almost twelve hours, taking roughly

only three minutes. The resolution is higher than that of the gel electrophoresis, the peaks are easy to distinguish from each other and this can be improved even further by experimenting with the polymer concentration. Furthermore the sensitivity of the signal is improved. With a background signal of around 0.01A compared to a signal of 0.1A the signal is easy to differentiate from the noise. So, even though there is room for improvements in the overall design and testing area, a good working device has been fabricated that provides an improvement in all the areas where the gel electrophoresis needed to be enhanced.

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Appendix A : Process Flow for Microchip Fabrication of Electrophoresis Glass Microchip

Glass (Pyrex 7740) substrate
Channel etching

1. Wafer preparation

1.1 Wafer selection: 4 inch wafer, Pyrex 7740

1.2 Pre-cleaning: H_2O:H_2O_2:NH_4 OH(5:1:0.02), 80C, 5 mins

1.3 Dump rinse for 4 cycles

2. Lithography

2.1 HMDS priming for 10 min.

2.2 Coat LOL2000 at 1000rpm, resulting in a thickness of 3000Å

2.3 Soft bake at 130℃ for 5 min. on hotplate

2.4 HMDS priming for 10 min.

2.5 Coat wafer with AZ 5214E at 4000rpm, resulting in 1.3 to 2μ thickness

2.6 Soft bake at 90℃ for 2 min. on hotplate

2.7 first exposure: 2.5 sec on ABM high resolution mask aligner

2.8 Post exposure bake, 110℃ for 3 min. on hotplate

2.9 Flood exposure, 10 sec. on ABM high resolution mask aligner

2.10 Develop for 60 sec. in FHD-5

3. Sputtering

3.1 Descum, 70 degrees, 0.7 min.

3.2 Sputtering of 400Å Chrome (Cr) layer

4. Lift off

4.1 Strip PR using acetone

4.2 Dip in IPA

4.3 Remove LOL2000 by using FHD-5

5. PR Mask Coating

5.1 HMDS priming, 10 min.

5.2 Coat wafer with HPR 204 at 4000rpm, resulting thickness between 1.0 to 1.8μ

5.3 Soft bake 110℃ for 60 sec.

5.4 Exposure for 5 sec. on ABM high resolution mask aligner

5.5 Develop at FHD-5 for 60 sec.

5.6 Hard bake at 120℃ for 30 min.

6. Wet etching

6.1 Descum, 70 degrees, 0.7 min.

6.2 Substrate etching in BOE + 12%HF, for 20μ (~6 hrs)

6.3 PR removal acetone

6.4 Cr removal using Cr etchant

Electrode fabrication

1. Wafer preparation

1.1Wafer selection: 4 inch wafer, Pyrex 7740

1.2 Pre-cleaning: H_2O:H_2O_2:NH_4 OH(5:1:0.02), 80C, 5 mins

1.3 Dump rinse for 4 cycles

2. Lithography

2.1 HMDS priming for 10 min.

2.2 Coat LOL2000 at 1000rpm, resulting in a thickness of 3000 Å

2.3 Soft bake at 130℃ for 5 min. on hotplate

2.4 HMDS priming for 10 min.

2.5 Coat wafer with AZ 5214E at 4000rpm, resulting in 1.3 to 2μ thickness

2.6 Soft bake at 90℃ for 2 min. on hotplate

2.7 Exposure 2.5 sec. on ABM high resolution mask aligner

2.8 Develop for 60 sec. at FHD-5

3. Sputtering

3.1 Descum, 70 degrees, 0.7 min.

3.2 Deposition of a 200Å TiW layer

3.3 Deposition of an 800Å Pt layer

4. Lift off

4.1 Strip PR using acetone

4.2 Dip in IPA

4.3 Remove LOL2000 by using FHD-5

Wafer Bonding

1. Cleaning

1.1 Pre cleaning MS2001 at 70℃ for 300 sec

1.2 Dump rinsing DI water for 4 cycles

1.3 PH neutralization by using household soap (Dettol)

1.4 Rinsing by DI water for 10 min.

2. Bonding

2.1 Bring both wafers into contact under streaming DI water

2.2 Put into oven at 120℃ for 30 min.

2.3 Heat up oven to 550℃ at 4℃/min

2.4 Keep in oven at 550℃ for 1 hr

2.5 Cool chip down to 100℃ at 1.5℃/min

Appendix B: Diffusion calculations

Diffusion coefficient for:

$$D_{coeff} = \frac{k_B T}{6\pi\eta R_H}$$

- Low Reynolds number

- Circular/cylindrical shape bacteria

D_{coeff} → diffusion constant
K_B → Boltzmann constant
T → absolute temperature
η → viscosity
R_H → hydrodynamic radius

Viscosity of chosen polymers:
- HEC η = 80-125 cP
- PEO η = 65-115 cP
- HPMC η = 6 cP

Boltzmann constant
- $1.38 * 10^{-23}$ $m^2*kg*s^{-2}K^{-1}$

Temperature
- Room temp 293 K

Hydrodynamic radius
- E Coli. R_H ~ 3-7nm

Taking the lowest and highest possible values of the viscosity for the different polymers results in the following diffusion constants:

$HEC_{\eta low}$ D_{coeff} = 5.3 x 10-9 cm^2/s
$HEC_{\eta high}$ D_{coeff} = 3.4 x 10-9 cm^2/s
$PEO_{\eta low}$ D_{coeff} = 6.6 x 10-9 cm^2/s
$PEO_{\eta high}$ D_{coeff} = 3.7 x 10-9 cm^2/s
HPMC D_{coeff} = 7.15 x 10-8 cm^2/s

Band broadening

$$H_d = 2\frac{\gamma D_{coeff}}{v}$$

γ → factor related to the restrictions of the diffusion
v → flow velocity
D_{coeff} → diffusion coefficient

Dependence of the separation length on diffusion and injection width

$$L_0 = \frac{\mu E w^2}{24 D_{coeff}}$$

L_0 → diffusion length
μ → mobility of the DNA (Taken as in water. For better results the mobility should be the mobility of the DNA in the respective polymer/buffer used during the experiment.)
E → applied electrical field
D_{coeff} → diffusion constant
w→ $(w_{inj}^2+w_{det}^2)^{1/2}$ width as a function of the injection versus detection width

$\mu \sim 2*10^{-4}$ cm^2Vs
E ~ 300V/cm
w ~ 0.005 cm

Using the diffusion constants calculated in the previous section gives the following values:

$HEC_{\eta low}$	L_0 = 118 mm
$HEC_{\eta high}$	L_0 = 183 mm
$PEO_{\eta low}$	L_0 = 94 mm
$PEO_{\eta low}$	L_0 = 169 mm
HPMC	L_0 = 8.8 mm

From the diffusion distance the estimated separation time can be found by using the following formula in case that $L=L_0$

$$t_0 = \frac{w^2}{24 D_{coeff}}$$

$HEC_{\eta low}$	t_0=196 s ≈3.2 min
$HEC_{\eta high}$	t_0=306 s ≈5.1 min
$PEO_{\eta low}$	t_0=158 s ≈2.6 min
$PEO_{\eta high}$	t_0=282 s ≈4.7 min
HPMC	t_0=146 s ≈2.4 min

Appendix C: Estimations for a comparison between Gel and Microchip electrophoresis

Number of theoretical plates

$$N = \frac{L}{\left(\frac{2D}{\mu E} + \frac{w^2}{12L}\right)}$$

Number of theoretical plates for small values of L

$$N = 12\left(\frac{L}{w}\right)^2 \frac{1}{1 + L/L_0}, \qquad L_0 = \frac{\mu E w^2}{24 D}$$

Peak resolution

$$\frac{\sqrt{N}}{t_m} = \mu E \frac{\sqrt{N}}{L} = 2\sqrt{3}\frac{\mu E}{w}\frac{1}{\sqrt{1 + L/L_0}}$$

Separation time for optimized design ($L = L_0$)

$$t_0 = \frac{L}{\mu E}$$

Constants for gel electrophoresis

- Mobility μ = 0.2~0.4 cm^2/VHrs (Viovy, 2000)
- Diffusion coefficient $D_{coeff} \sim 10^{-8}$ cm^2/sec (Pluen 1999)
- DNA length 3~15 kbp
- Electric field E = 2V/cm
- W_{inj}= 0.6cm
- L = 12 cm

Constants for microchip electrophoresis

- Diffusion constant $D = 4*10^{-8} cm^2/s$
- E= 200 V/cm
- $\mu \approx 2*10^{-4} cm^2/Vs$
- W = 0.005 cm
- L_0 = 1cm

Gel results

- Number of theoretical plates $N \approx 4.6 * 10^3$
- Separation time $t_0 \approx 15$ hrs

Microchip results

- Number of theoretical plates $N_0 = 2.6*10^5$
- Separation time t_0 =26 s