Study of Disposable Microdevices for DNA Electrophoresis

by

Winston Timp

B.S., Electrical Engineering(2001), Physics(2002), Chemistry(2002), Biochemistry(2002)
University of Illinois at Urbana-Champaign

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Signature of Author .....
Department of Electrical Engineering and Computer Science
December 20, 2002

Certified by .....
Alan J. Grodzinsky
Professor of Electrical, Mechanical and Bioengineering
Thesis Supervisor

Accepted by .....
Arthur C. Smith
Chairman, Committee on Graduate Students
Department of Electrical Engineering and Computer Science

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ABSTRACT

A study was undertaken to determine if a microfluidic chip, made of economical plastic materials, is feasible. The chip was designed to perform gel electrophoresis, specifically of DNA fragments for either sequencing or identification purposes. With a disposable version of such a chip, constraints on the gel type are relaxed and lifetime issues become nonexistent.

Such a chip was created using polydimethylsiloxane(PDMS) as the plastic material, with a cast molding process. The chip was subsequently sealed against a piece of PDMS, mounted on a glass slide for structural support. Fluidic and electrical interconnects were added to the chip.

A polyacrylamide solution was injected into the chip for use in DNA separations. The chip was then placed into an apparatus designed for laser induced fluorescence(LIF) detection. Several different samples were run on the chip, including polystyrene beads, organic dye molecules, and single tandem repeat(STR) allelic ladders. The chip demonstrated its electrophoretic efficiency, evincing a low, almost negligible amount of electroosmotic flow. The separation of the dye and DNA was accomplished with good fidelity, allowing for identification of the various substitutents of the loaded sample.

The PDMS chip, though demonstrably efficient at DNA separation, needs work before it can move out of the prototype phase. Substantial work on the fluidic interconnection, as well as the basic plastic formulation is needed to move this idea forward. However, the chip is sufficient for a clear proof of the principle of disposable chips use in electrophoretic separations.

Thesis Supervisor: Alan Grodzinsky
Title: Professor of Electrical, Mechanical and Bioengineering
Lab Supervisor: Paul Matsudaira
Title: Professor of Biology and Bioengineering

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Thanks to the Whitehead maintainence staff, who, by shutting off the lights on me at 3 AM, reminded me it was time to go home.

To my family, who are always supportive. By this I mean that they attack me in every possible way to remove any observable weakness.

To my friends, whom I did not see for approximately 3 months.

Biographical Sketch

Winston Timp received a bachelor's degree in Electrical Engineering from the Univerisity of Illinois at Urbana-Champaign in 2000. He then began attending the Massachusetts Institute of Technology in fall of that year. At the same time, he finished requirements for his Chemistry, Physics, and Biochemistry BSs from UIUC in January of 2002. Winston has completed this research as part of his Master's degree requirements for Electrical Engineering at MIT, working at the Whithead Institute of Biomedical Research, under Prof. Paul Matsudaira.

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Chapter 1 Experimental Background

1.1 Introduction

Electrophoresis is defined by the dictionary as, "A method of separating substances, especially proteins, and analyzing molecular structure based on the rate of movement of each component in a colloidal suspension while under the influence of an electric field."[1] While both accurate and concise, this definition ignores many layers of complexity and potential for this single chromatographic technique. Electrophoresis is the most widely used technique for biomolecular separation, especially for nucleic acids and proteins. Its high resolution and non-destructive nature are a necessity for biochemical work.

Specifically, electrophoresis is often used for the chromatographic separation of DNA, the biomolecular method for storing and transmitting data in cellular organisms. Obviously, being able to read or interpret the data stored in this molecule would be a great advantage in the further understanding of life. To understand it, it must be analyzed, and a major method of analysis in chemistry is that of separation. Separation allows for different size pieces of DNA to be separated from one another and assayed, to compare the size of a DNA fragment from one organism to the size of another organsism's fragment.

The apparatus historically and commonly used on the bench-top for electrophoresis, and thus for DNA analysis, is clumsy and inelegant. It is slow to operate, with poor precision and requires relatively large amounts of sample. Therefore, it is proposed that the apparatus used for electrophoresis be improved. Specifically, a microfabricated apparatus is desired.

Microfabricating the electrophoretic setup, in the lab-on-a-chip format, is extremely advantageous for several reasons. First among these advantages is the reduced amount of sample needed for analysis. When operating microanalysis systems, a far more modest amount of sample is needed to obtain reliable results, several orders of magnitude less sample is needed. Second is the advantage of increased speed. Scaling down the apparatus size allows for accurate separation of DNA in ¼ the time taken by an analysis with a macroscopic apparatus. Third, is the capability for interconnection or integration; this is the most convincing reason to pursue research in microdevices for analysis. In this case, integration refers to the ability to combine several different chemical and biochemical apparatus into a single chip. This removes contamination issues, as well as greatly simplifying the analysis procedure. Sample preparation, product extraction, data analysis; all of these could be carried out on chip. This also allows for massive parallel operations, further improving analysis time.[2]

Granting that the case has been made for microdevice creation, a further point is called into question; namely, the material of choice for the chip creation. The most common materials currently used are glass and silicon. Silicon has a large disadvantage in that it is opaque to the visible and UV range of the light spectrum, making it difficult to use for systems involving optical detection. Glass is transparent, but has an amorphous molecular structure, making it tricky to create certain structures, specifically any vertical walled channels. Both glass and silicon require hazardous chemicals to etch and pattern. They also are difficult to seal shut, a necessary step in most microfluidic chip creation.[3] Finally, there is often a problem with surface charge and surface adsorption, with many biological molecules easily adhering to SiO_2.

Polymer materials are another option for fabrication, which has recently come into vogue. Polymer materials are, in general, inexpensive, both to produce and to pattern. Microchannels may be formed is polymer materials by embossing or molding rather than etching, allowing thousands of chips to be created from a single mold. Plastic chips are easily sealed, either thermally or with simple adhesive. They are unstable at high temperatures, but this is not usually a problem when dealing with bioanalysis.

The main selling point on the plastic chips is the idea of the cost per chip. The plastic chips are so inexpensive that they are, potentially, single-use. This removes risk of contamination from previous runs, a danger in reusable chips. More importantly, this allows for the use of chemical, single-run electrophoretic gels, offering separation advantages. These chemical gels are impossible to remove, once injected, which makes their use impractical for more expensive chips. However, they generally grant better separations than the physical gels currently favored in both capillary and microfabricated systems.

1.2 Theory of Electrophoresis

Passage of current through an electrolytic solution is far more complex than current movement through metals. In fact, in bears some similarities to semiconductor devices, with cations and anions replacing the solid-state electrons and holes. The major difference is the fact that, in most buffer solutions used for electrophoresis, there are several different cations and anions in each solution, with each having a different mobility, hence providing a different contribution to the overall current.

A good model for ion mobility makes it dependent on the strength of the applied force versus the counter, frictional drag forces applied. The drag forces on a molecule in liquid are described, as a spherical approximation, by Stokes' Law, which states that:

$$f = 6\pi\eta r v \tag{1}$$

with η being the viscosity of the surrounding fluid, r the radius of the molecule, and v the velocity of the molecule[4]. Although this approximation works best for elemental ions due to their spherical nature, it is also sufficient for a rough idea of more elongated molecules, such as DNA. The driving force on the molecule is :

$$f = qE \tag{2}$$

where q is the net charge of the molecule and E is the local electric field. Once the molecule reaches a constant velocity, the forces are balanced. Assuming this, a value for mobility can be calculated:

$$\mu = \frac{v}{E} = \frac{\frac{f}{6\pi\eta r}}{\frac{f}{q}} = \frac{q}{6\pi\eta r} \tag{3}$$

demonstrating that mobility is a charge to size ratio.

The problem with this model is that it is too simplistic. It is sufficient to describe the problem only in a sufficiently dilute solution. Under normal buffer conditions, the charged sample material, taken together with the surrounding electrolyte, possesses no net charge. This

is due to the assumption of electroneutrality. Electroneutrality is the assumption that the sum of all the charges in a local area of solution is 0, or:

$$\rho_e = F \sum_{i=1}^{n} z_i C_i = 0 \tag{4}$$

with z as the valence, C as concentration, F as Faraday's constant, and ? as the charge density. This assumption holds except in the near vicinity of charged surfaces.

The area in the vicinity of charged surfaces, specifically near the surface of charged macromolecules, is precisely what allows for the operation of electrophoresis. The charges present on the surface is balanced by an oppositely charged ion in the electrolytic solution, due to basic coulombic force. The relationship between the charge and electric field is defined by Gauss's law

$$\begin{gathered} \oint \varepsilon \boldsymbol{E} \cdot d\boldsymbol{A} = \int \rho_e \, dV \\ \varepsilon \nabla \cdot \boldsymbol{E} = \rho_e \end{gathered} \tag{5}$$

with e being the local permittivity, and **E** the vector representing electric field. It is often easier to work with the voltage potential, ?, defined as

$$\boldsymbol{E} = -\nabla \varphi \tag{6}$$

When assuming a constant permittivity in the liquid, this reduces to Poisson's familiar equation:

$$\nabla^2 \phi = \frac{-\rho_e}{\varepsilon} \tag{7}$$

Of course, the natural tendency of diffusion attempts to counteract this rise in concentration.

The result is an exponential decay of the net charge concentration, related to the distance from the surface. A starting point to derive the charge concentration is a Boltzman distribution of the system components:

$$C_i = C_{i\infty} e^{\frac{-z_i F \phi}{RT}} \tag{8}$$

with R as the universal gas constant, T as temperature, and C_{i8} as the concentration of the species *i* far from the surface[5]. Using this distribution, in combination with Poisson's equation and equation 4 gives the Poisson-Boltzmann equation

$$\nabla^2 \phi = \frac{-F}{\varepsilon} \sum_{i=1}^{n} z_i C_{i\infty} e^{\frac{-z_i F \phi}{RT}} \tag{9}$$

This equation is extremely difficult to solve in this form, but may be simplified greatly through use of a Taylor expansion of the exponential term, as

$$\nabla^2 \phi = \frac{-F}{\varepsilon} \sum_{i=1}^{n} z_i C_{i\infty} \left(1 - \frac{z_i F \phi}{RT} \ldots\right) \tag{10}$$

Higher order terms may be neglected, as long as the potential is small enough. The zeroth order term disappears completely, due to electroneutrality. The remaining first term yields the linearized version of the Poisson-Boltzmann equation

$$\nabla^2 \phi(r) = \phi(r) \frac{F^2}{\varepsilon RT} \sum_{i=1}^{n} z_i^2 C_i \qquad (11)$$

In this case, the only dependent variable is taken to be r, the distance from the interface. Other variables are neglected for simplicities sake. This equation is now easily solved with appropriate one dimensional boundary conditions, allowing the potential near the interface to be derived.

The first boundary condition is determined simply by setting the potential at infinity to zero. Since potential is only measured relative to another point, any reference value may be set to zero, in this case as $\phi(8) = 0$, with ϕ being a function of distance from the surface, r[6]. The other boundary condition is inferred from Gauss's Law as applied at the interface. If there is some surface charge s on the particle and the electrolyte permittivity given as e_1 and the sample material's permittivity e_2, the resultant application of Gauss's Law using an infinitely thin pillbox gives[7]:

$$\varepsilon_2 E_{r2} - \varepsilon_1 E_{r1} = \sigma \qquad (12)$$

If it is assumed that there is no electric field inside the sample material, the boundary condition for the potential at the interface becomes

$$\frac{-\zeta}{\zeta_1} = \frac{\partial \zeta}{\partial r}\Big|_{r=0} \tag{13}$$

Using these boundary conditions, a solution can easily be found for the potential. This solution gives an exponential decay, usually defined using a quantity known as the Debye length, ?:

$$\zeta(r) = \frac{\zeta}{\zeta_1} e^{-r/\kappa}$$

$$\kappa = \frac{1}{\sqrt{\frac{F^2}{\varepsilon RT}\sum_{i=1}^{n} z_i^2 C_i}} \tag{14}$$

Using this equation for the potential, the electrolyte charge density can be determined using Poisson's equation

$$\rho_e = \frac{-\varepsilon}{\kappa} e^{\frac{-r}{\kappa}} \tag{15}$$

A typical value of ? for a 0.1 M salt solution is ~1 nm[8]. It is only in extremely deionized solutions that the Debye length is on scale with the system dimensions. If this occurs, if the Debye length is on par with a charged particle, Equations (1-3) for Stokes drag balancing against coulombic force may be used. In all other, smaller Debye length systems, the electroneutrality may be assumed[9].

The significance of this is that there is a layer of ? effective thickness surrounding any charged particle or surface. This layer, combined with the charged surface itself, is known as the electrical double layer. There are several electrokinetic phenomena associated with the electrical double layer. They all involve the movement of the diffuse charge with respect to the fixed charge in the system, and the resultant fludic affects[10].

One of these phenomena is known as electroosmosis. Electroosmosis is defined as the movement of liquid relative to a charged surface. The flow of the liquid is a result of the electric field's affect on the liquid portion of the electric double layer. To demonstrate this flow, a cylinder with uniformly charged walls will be used. An electric field, E, will be applied along the axis of the cylinder. The cylinder has a radius of L and is filled with an electrolyte of some reasonable molarity.

In order to determine the flow direction and velocity of the fluid, it is best to begin with the Navier-Stokes equation, often used to solve fluid velocity problems for Newtonian fluids[11]:

$$\rho \frac{D\mathbf{v}}{Dt} = -\nabla P + \eta \nabla^2 \mathbf{v} + \rho_e \mathbf{E} \qquad (16)$$

with **v** being the flow velocity, ? concentration, P thermodynamic pressure, and ? as the viscosity of the fluid. To solve this equation, several reasonable fluidic assumptions are made.

First, we will expect that, due to symmetry, that

$$v_\theta = 0$$
$$\left(\frac{d v_r}{d r}\right)_{r=0} = 0 \tag{17}$$

because of angular and axial symmetry, with r as the distance from the axis of the cylinder. Next, assuming that the electrolyte is incompressible, usually acceptable for liquids[12], continuity gives

$$\nabla \cdot \boldsymbol{v} = \frac{1}{r}\frac{\partial}{\partial r}(r v_r) + \frac{1}{r}\frac{\partial v_\theta}{\partial \theta} + \frac{\partial v_z}{\partial z} = 0 \tag{18}$$

A third assumption is that the flow is fully developed, that is to say, that the velocity does not vary in the direction of flow[13]. This assumption is valid if far enough from the inlet or wall; in this case it can be used for the axially directed flow, defined as the z direction in the cylinder:

$$\frac{\partial v_z}{\partial z} = 0 \tag{19}$$

Equation 9, in combination with equation 8, indicates that v_r is also 0, meaning that the flow is unidirectional in this case. Finally, we assume that the velocity is time-independent, a reasonable assumption, since none of the other variables are dependent on time. This greatly simplifies the derivation.

Returning to the Navier-Stokes equation, with these assumptions, and inertial and hydrodynamic pressure terms neglected, gives

$$0 = \frac{\mu}{r}\frac{\partial}{\partial r}\left(r\frac{\partial v_z}{\partial r}\right) + \rho_e E_z \tag{20}$$

This equation is easily solved in two steps. First, the potential must be determined. Using Poisson's equation,

$$\begin{aligned} \rho_e &= -\varepsilon\nabla^2\psi = -\varepsilon\left(\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial\psi}{\partial r}\right) + \frac{1}{r^2}\frac{\partial^2\psi}{\partial\theta^2} + \frac{\partial^2\psi}{\partial z^2}\right) \\ \rho_e &= \frac{-\varepsilon}{r}\frac{\partial}{\partial r}\left(r\frac{\partial\psi}{\partial r}\right) \end{aligned} \tag{21}$$

The second term of the Laplacian(∇^2) vanishes due to symmetry about the cylinder axis. The constant nature of the electric field in the z direction, or

$$E_z = \frac{-\partial\psi}{\partial z} = constant \tag{22}$$

ensures that the third term is also zero.

Now, ρ_e is also known from the Poisson-Boltzmann equation previously stated, as

$$\rho_e = -F\sum_{i=1}^{n} z_i C_{i0} e^{\frac{-z_i F(\phi-\phi_0)}{RT}}$$

$$\rho_e = -F\sum_{i=1}^{n} z_i C_{i0}\left(1-\frac{z_i F(\phi-\phi_0)}{RT}\right)+\ldots \qquad (23)$$

$$\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial\phi}{\partial r}\right) = -F\sum_{i=1}^{n}\left(z_i C_{i0}-\frac{z_i^2 C_{i0} F(\phi-\phi_0)}{RT}\right)$$

with $C_{i\,8}$ being the concentration and $\phi_0(z)$ the potential at the center axis. At this point, it is advantageous to introduce a dimensionless variable system, in order to simplify the further derivation:

$$\xi = \frac{r}{\lambda}$$

$$\eta = \frac{(\phi-\phi_0)F}{RT}$$

$$\frac{-RT\varepsilon}{\lambda^2 F^2}\frac{1}{\xi}\frac{\partial}{\partial\xi}\left(\xi\frac{\partial\eta}{\partial\xi}\right) = \sum_{i=1}^{n}\left(z_i C_{i0}-z_i^2 C_{i0}\eta\right) \qquad (24)$$

$$\sum_{i=1}^{n} z_i^2 C_{i0}\frac{1}{\xi}\frac{\partial}{\partial\xi}\left(\xi\frac{\partial\eta}{\partial\xi}\right) = \sum_{i=1}^{n} z_i^2 C_{i0}\left(\eta-\frac{\sum_{i=1}^{n} z_i C_{i0}}{\sum_{i=1}^{n} z_i^2 C_{i0}}\right)$$

The homogeneous solution to equations of this form is a modified Bessel function, one which is bounded at the origin, and of zeroth order, represented by I_0[14]. The full solution is represented as shown below

$$\psi = \frac{\sum_{i=1}^{n} z_i C_{i0}}{\sum_{i=1}^{n} z_i^2 C_{i0}} \left(1 - I_0\left(\frac{r}{\lambda}\right)\right) \tag{25}$$

The summation ratio is a constant which can be determined using Gauss's law boundary conditions, as in equation 13. The final equation for the potential is

$$\psi = \frac{-\zeta}{I_1\left(\frac{L}{\lambda}\right)} \left(1 - I_0\left(\frac{r}{\lambda}\right)\right) + \psi_0 \tag{26}$$

The first term is often represented as ? and termed the zeta potential, the voltage drop across the diffuse part of the double layer.

Now, using this potential, it will be possible to determine the flow velocity inside the cylinder, the second part. Taking equations 20 and 21, $?_e$ may be eliminated, leaving:

$$\left(\frac{?}{?r}\right)\left(r\frac{?v_z}{?r}\right) = \left(\frac{?}{?r}\right)\left(r\frac{?\psi}{?r}\right)E_z \tag{27}$$

Solving this equation through integration is easy, as long as boundary conditions are known. In this case, symmetry grants the boundary condition

$$\frac{?v_z}{?r} = \frac{?\psi}{?r} = 0 \tag{28}$$

The no-slip condition insures that v_z must be zero at the cylinder wall, and the potenital was previously determined. Thus, v_z becomes

$$v_z = \frac{-\varepsilon\varepsilon_0 E_z}{\eta I_1\left(\frac{L}{\kappa}\right)}\left[I_0\left(\frac{L}{\kappa}\right) - I_0\left(\frac{r}{\kappa}\right)\right] \tag{29}$$

If the radius of the cylinder, L, is far greater than the Debye length ?, then

$$\frac{I_0\left(\frac{L}{\kappa}\right) - I_0\left(\frac{r}{\kappa}\right)}{I_1\left(\frac{L}{\kappa}\right)} \approx 1 - e^{\frac{-(r-L)}{\kappa}} \tag{30}$$

which gives,

$$v_z(r) = \frac{-\varepsilon\varepsilon_0 E_z}{\eta}\left(1 - e^{\frac{-(r-L)}{\kappa}}\right) \tag{31}$$

This gives a nearly constant flow, nearly independent of r except at the very edges of the channel, where it drops rapidly to zero.

Electrophoresis is the application of the electroosmotic phenomena on a charged particle in electrolyte, rather than on a charged, fixed surface. Since the particle is free to move, the resulting force causes the particle to be thrust forward, as the surrounding diffuse portion of the double layer is thrust back. The mathematical derivation is complicated, but the resulting mobility is simple to understand[15],

$$\mu = \frac{\sigma}{\eta} \tag{32}$$

As seen, the mobility, and hence, velocity, of a charged particle is not dependent on shape and size, but only on the surface charge per unit area, s. This is going to be a problem when using electrophoresis to attempt separation of constant charge density biomolecules, such as DNA.

1.3 Methods of Electrophoresis

Performing electrophoresis in free solution, using the natural charge/size ratios to separate molecules, is called the moving boundary electrophoresis method, developed by Tiselius[16] in the late 1930s. The operation of the apparatus is relatively simple. A sample is introduced into a U-shaped tube. A buffer layer is then carefully added over the protein solution in each opening of the tube. Electrical contact is then made to the buffer solution, through the use of suitable electrodes. When voltage is applied, the generated electric field causes the charged molecules to move towards the opposite polarity electrode. Since the different species migrate at different velocities, their relative mobilities can be measured.

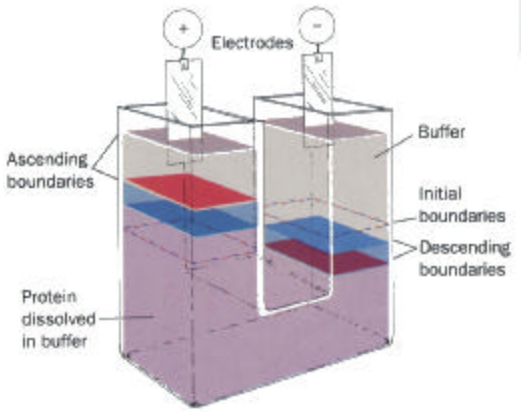


Illustration 1: Tiselius apparatus for moving boundary electrophoresis (Voet & Voet P. 90)

This apparatus, while effective, is extremely problematic to use. Convective mixing of the separating species is difficult to avoid. In order to resolve this issue, a solid support of some kind is needed. This usually consists of a porous polymeric gel of some formulation. The gel grants several important boons: it reduces dispersive effects, such as convection and diffusion, it offers mechanical stability to the separation matrix, and the gel may even affect the mechanism of separation in some way[17].

There are many types and kinds of gel which may be used for electrophoresis. There are two many categories of gel used for electrophoresis, chemical gels and physical gels. Both are made of polymer filaments, but the physical gel is not crosslinked, merely entangled. This leads to the physical gels being of a generally lower viscosity. The physical gel pore structure may be altered merely through an adjustment of the polymer concentration. This type of gel is also replaceable, as its low viscosity will allow it to be removed and replaced from its container[18].

Two classic examples of physical gels are linear polyacrylamide (LPA) and agarose. LPA is a gel solution of unbranched, uncrosslinked acrylamide polymer. The length of the polymer is measured by the molecular weight, and may be varied to alter the properties of the gel. LPA is usually used for DNA less than 2000 base pairs in length, due to the standard pore size obtained with this type of gel[19]. Agarose is used for larger fragments of DNA, able to resolve DNA of up to 20,000 base pairs in length.[20] Both these gels are merely pushed into the apparatus, with notchemical reaction taking place. Agarose must be heated in order to dissolve it at first, but there is no functional change in its conformation, no reaction taking place.

A chemical gel is crosslinked, often undergoing the polymerization reaction after filling the electrophoretic apparatus. This causes the polymer to bind to the walls of the apparatus, granting great rigidity and stability. The pore structure of these gels cannot be altered once the polymerization is complete, but altering the reagent concentration during polymerization allows for different pore sizes.

The gel often alters the separation efficiency of sample. This is due to the alteration in mobility caused by the gel, often expressed as

$$\frac{\mu}{\mu_0} = f \qquad (1)$$

where μ represents the mobility of the molecule in gel, and μ_0, the mobility in free solution. The function f may depend on the properties of the gel, as well as the properties of the charged molecule in question. Most importantly, however, it allows for separation by properties other than that of charge density with electrophoresis, granting more versatility to the process.

The apparatus for the gel electrophoresis may vary from a large rectangular slab of gel to a tiny capillary or microchannel. The basic operation is the same, no matter what the scale of the device. The advantages to scaling down the size of the device have previously been stated, speed, smaller sample size, and interconnection. However, actually loading the gel into these smaller channels can be quite a challenge. The viscosity of the gels is not insignificant, and it may require a great deal of pressure to inject the gel into a microchannel. It is just as difficult to remove a physical gel after running, and nearly impossible to remove a chemical gel, which has cross-linked into rigidity while inside the channel.

1.4 DNA

1.4.1 Molecular Structure

Deoxyribonucleic acid is the molecule which contains genetic information. All known forms of life, excepting retroviruses[21], use DNA as the method for both storing and passing information. DNA does not act on any biological processes itself, except for its own replication and transcription into ribonucleic acid(RNA). The RNA then is used to create proteins, which carry out the work of the organism. A single chromosome of human DNA can carry over 1 MB of information[22].

DNA is made up of a series of pentose sugar molecules, linked by a phosphodiester bond between the 5' carbon of one sugar and the 3' carbon of another. On the 1' carbon, a nitrogenous base is bonded to the molcule. Each individual sugar-base unit is called a nucleotide if with a phosphate group, or a nucleoside without one. In DNA, the pentose is 2'-deoxy-D-ribose, basically a ribose sugar without the hydroxyl group usually bonded to the 2' carbon[23]. It is important to note that, at biological pH levels, the phosphodiester groups which form the backbone of DNA are negatively charged. This means that for each nucleotide on the single strand of DNA, there is a unit of negative charge corresponding to it.

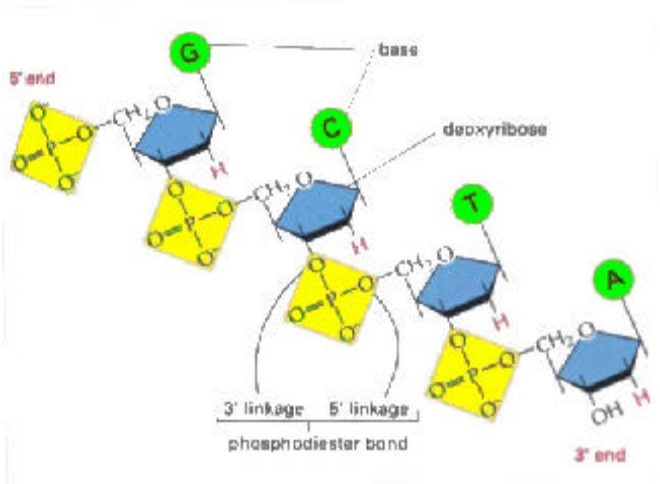


Illustration 1: DNA Backbone (Alberts)

There are four different nitrogen bases which may be part of the deoxyribonucleotide. They are derived from either purine or pyrimidine, two common aromatic cyclic compounds. Adenine and guanine are the purines, and cytosine and thymine are the pyrimidines. Usually, the entire nucleotide is represented by a single letter, the first letter of the base contained therein.

With ribonucleic acid (RNA), there is only the one strand of nucleotides, but DNA contains two strands. The strands are paired in a right-handed double helix, with 10 nucleotides per helical turn. The double helix is approximately 20 Å in diameter, with approximately 3.4 Å of rise per nucleotide. The different strands of the helix run antiparallel to one another, meaning that one strand runs in the 5' to 3' direction, and the other runs from 3' to 5'[24].

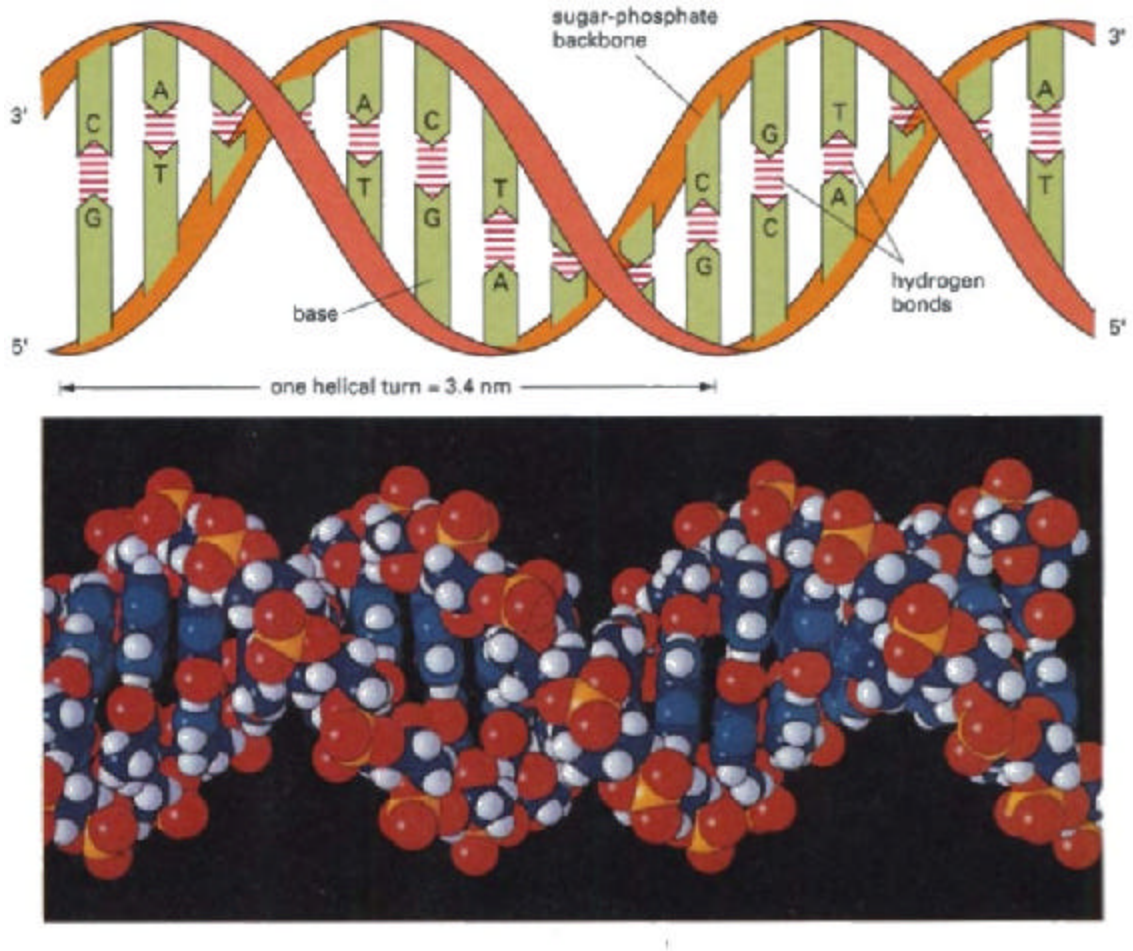


Illustration 2: DNA Double Helix

The bases of the nucleotides reside in the center of the helix, oriented nearly perpendicular to the helical axis. Each base is interlinked with its complement on the opposing strand by hydrogen bonding. The interesting thing about this is that there are only two possible base pairs, with adenine bound to thymine and guanine with cytosine. A purine base must associate with a pyrimidine base, in order to fit in the double helix formation. Anything else would be energetically unfavorable, since it would distort the stable double helix[25].

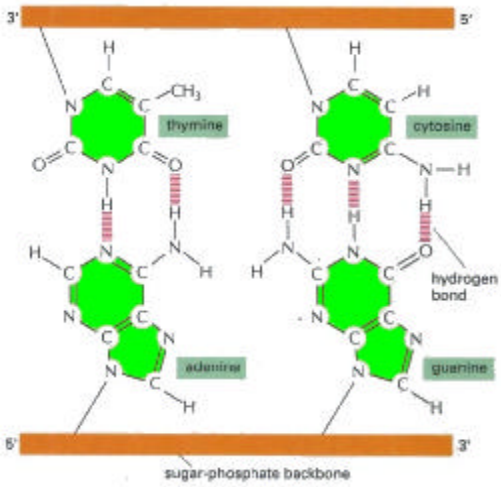


Illustration 3: Base pairing(Alberts)

The double stranded attribute of DNA has two clear benefits. First is for ease of replication, as one strand can, with the aid of catalyzing enzymes, split from the other strand. Each strand may then generate a new complement through base matching. The other, more important advantage of double stranded DNA is error checking. If bases are chemical or radiologically damaged, a process known as mutation, will no longer bind correctly to the complementary strand. If the cell notices this, the offending area is excised, and replaced based off of the complementary strand.

1.4.2 Electrophoretic Motion

DNA, having a constant charge density, must by electrophoresed in a porous polymeric support. This is due to the phosphate backbone of DNA; the charge to size ratio remains constant, rendering free solution electrophoresis ineffective at separation. Different gels are used, depending on the size of the DNA molecules being separated. It usually recommended that acrylamide gels be used for DNA less than a kilobase long, and agarose gels for DNA of

greater than 1 kb length. This is due to the different pore sizes between the polymeric gels; it is advantageous to keep the particle size on the same scale with the pore size of the gel.[26]

The first model proposed for DNA migration through a polymeric matrix was the free volume model, also known as Ogston sieving. Ogston sieving functions through the estimation of available space accessible to the molecule. The function relating the mobility in this case is described as[27]

$$\frac{\mu}{\mu_0} = f = e^{-jCS} \tag{1}$$

where C is the concentration of the polymer, j is the length of the polymer fibers per unit volume, and S the area needed for the sample particle to pass through without contacting the gel fiber, given by[28]

$$S = \pi (R + r)^2 \tag{2}$$

Here, R is the radius of the particle, and r is the radius of the gel fiber. This model was designed for use on spherical particles migrating through a random array of cylindrical fibers.

Unfortunately, DNA does not necessarily behave in the way described by the model. DNA molecules are flexible, and may not move through the gel in a twisted up, spherical form. For example, this model predicts that if the DNA molecule is larger than the pore size, then it will not be able to migrate through the gel at all. This is provably untrue.[29]

In fact, when the DNA molecule navigates through the gel, it does so by deforming to fit through the porous gel. The movement is described by the term *reptation*, refering to the

snake-like fashion of the DNA slithering through the pores in the gel. The molecule navigates its way through the gel under the influence of the electric field. This model correctly describes the mobility of the molecule as

$$\mu = \frac{\mu_0}{3N} \tag{3}$$

with N representing the number of pores taken up by the DNA molecule, given as

$$N = M / M_a \tag{4}$$

taking M as the length of the DNA molecule, and M_a as the length of a DNA molecule which takes up only one pore, also known as a "blob".[30]

There is a third method of DNA migration through gel, however, one which applies to longer DNA fragments. In essence, with the longer fragments, the DNA molecule actually aligns itself with the electric field. This orientation, the alignment, affects the mobility of the DNA, causing electric field effects to dominate the reptation motion, rather than the thermal or Brownian affects. Basically, the mobility term looks more like

$$\mu = \mu_0 \left(\frac{1}{3N} + \frac{\varepsilon^2}{27} \right) \tag{5}$$

with e given by

$$\varepsilon = \frac{q E a}{2 k T} \tag{6}$$

having q representing the charge of a blob, E the electric field, a the pore size, k as Boltzman's constant and T as the temperature.[31] When the value of N becomes sufficiently large, the mobility becomes independent of length once more.

There is a great deal more theoretical physics behind the movement of DNA in an electrophoretic gel. In fact, different models are still being proposed and developed, as this is far from a mature field. However, the outlined solutions are sufficient to have a basic grasp of the important features.

It is understood that varying the porosity of the gel, whether by the crosslinking of the polymer, or its concentration, may serve to optimize the efficiency of separation for different lengths of DNA. For the smallest lengths, Ogston sieving will apply, allowing primers and small oligos to move quickly through the gel. For the target length strands, reptation will occur, granting good separation efficiency. For longer length strands, the oriented reptation will occur, giving poor separation.

1.4.3 Preparation and Utility

In order to analyze DNA, a significant amount of it is needed. Although single molecule analysis is possible, it is extremely difficult to do. So, some method of gathering a large number of copies of the same DNA sequence is desired.

The method which is commonly used for short (<6 kb) and quick amplification is called the polymerase chain reaction, or PCR. To use this technique, all that is needed are short

pieces of DNA, known as primers, which act as bookends around the desired section of DNA. One of the primers matches to the DNA strand running in the 5'->3' direction, and the other matches the 3'->5' strand.

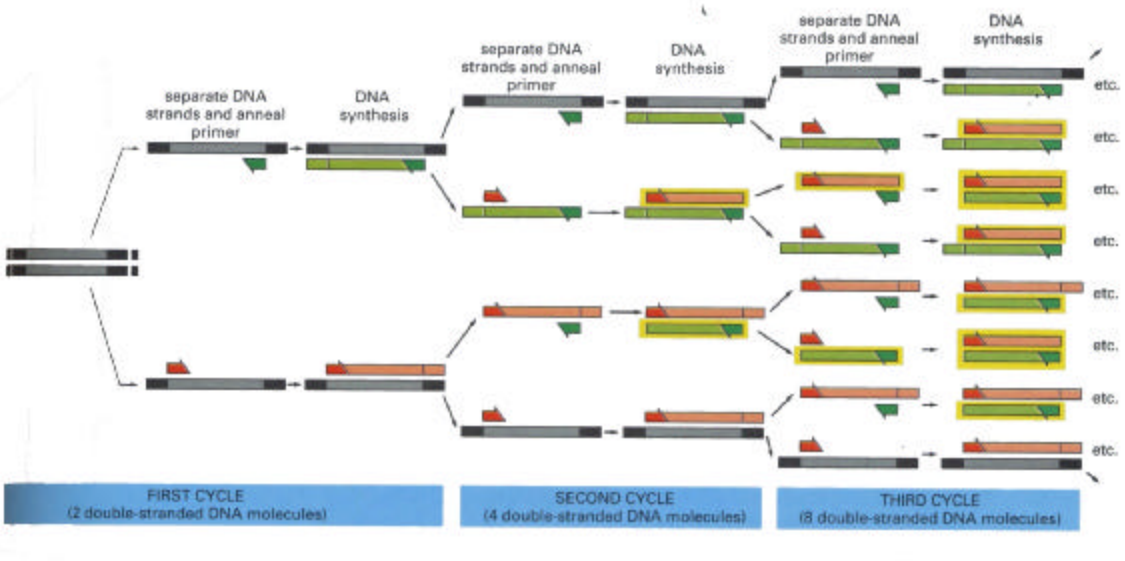


*Illustration 1: Polymerase Chain Reaction (**Cell,** Alberts)*

The primer cannot bind to the DNA sample, though, since the sample is double stranded, with no access to the inner core. In order to allow primer to bind, the orignal double stranded sample must be denatured. In order to this, the temperature of the sample is raised above the "melting point" of the DNA. This characteristic melting point varies depending on the surrounding solution of the DNA, as well as the DNA itself. The higher the percentage of G-C base pairs in the DNA strand, the higher the melting point.

After the DNA is denatured, the temperature is dropped to just below the denaturation point. Since the concentration of the primer is far greater than the concentration of the sample, it is more likely that the primer will bind than complete renaturation of the original sample.

Once the primer has bound, an enzyme is needed to complete the DNA replication. The enzyme also should be able to handle the high temperatures needed for the denaturation. There are many such DNA polymerases, with *Taq* polymerase being one of the more popular. This enzyme extends the DNA primer in the 5'->3' direction.

After sufficient time has passed for the synthesis of the new strand, the temperature is spiked again, to denature the new strands seperating them from the orignal sample. New primer anneals to both the original sample and the newly synthesized strands. Since each new strand is also available for subsequent replication, the amount of DNA is increasing exponentially, as 2^n, where n is the number of heat cycles. The only section of the sample which is amplified in this way is the section defined by the primers as bookends.[32]

Once amplified, the DNA sample must be analyzed. One method of doing this is through the selective cleaving of the DNA piece in question. Restriction enzymes are used to cut DNA for such analyses. A restriction enzyme is a protein which recognizes a specific sequence of DNA, and breaks the phosphodiester bonds at that point. The length of the cleaved fragments is then indicative of the structure of the DNA, and the cleavage site's position on the strand.

As an example, PCR with restriction enzyme analysis may be used to amplify the forensic samples gathered by law enforcement agencies. In humans, there are certain genetic markers, areas which differ from individual to individual. These areas occur on average every 200 to 500 base pairs on the genome. If one of these genetic differences occurs at a site normally cleaved by a restriction enzyme, the enzyme may not cleave, causing different length

fragments of DNA to be produced. Electrophoresis of the fragments made from the forensic DNA may then be compared to analysis of the same type of fragments created from different suspect individuals.[33]

Another common DNA analysis technique is that of sequencing. There are several different specific methods of sequencing. However, the only one which is easy to automate, and hence the only one which is feasible for sequencing large amounts of DNA, is the chain terminator method.

The chain terminator method operates with a simliar setup to PCR. There are two extremely significant differences. The first is that only one primer is used. This primer is the starting point for the sequencing reaction. The second difference is the inclusion of dideoxynucleosides. These molecules are missing the hydroxyl group on the 3' carbon, preventing any further elongation, once any one of them is integrated into a growing DNA strand. The concentration of these dideoxynucleosides is lower than that of the normal nucleosides, but still high enough to be significant.

The reaction is then heat cycled exactly as PCR would be. The primer will anneal to the sample strand, and the polymerase will begin to elongate the new DNA fragment. The new strand will grow until, through one of the dideoxynucleosides is incorporated into the strand. The fragment will stop growing. The next time the heat is cycled, a new fragment will grow, likely extending to a different length. The cycle is operates hundreds of times, as each cycle will only generate as many new fragments as there are original DNA sample molecules.

The fragments resulting from this reaction are then run on an electrophoretic gel. If different color dyes are attached to each of the dideoxynucleosides, the order of the colors eluting from the gel will illuminate the sequence of the DNA sample. The dyes are activated by some excitation beam, usually a laser of some kind, and their subsequent fluorescence is measured. Depending on the sensitivity and quantum efficiency of the dye, different amounts of sample are needed, but the results remain the same.

Chapter 2 Chip Preparation

2.1 Chip Fabrication

2.1.1 Material Choice

The first decision to make in chip fabrication is the choice of starting material. What will the chip be made of? There are a few properties that it absolutely must possess. First among these is electrical insulation. The material must not be conductive in any way, as the voltages which are applied to the chip to induce electrophoresis are in the kilovolt range. Unless the material is less conductive than the gel/electrolyte which is filling it, electrophoresis will not proceed.

Second is the optical transparency of the material. Though this is not as strict a rule, not being vital to the actual operation of the electrophoresis, it is still very important. Detection of electrophoretic products is preferentially made by fluorescent or absorbance based methods, both of which, obviously, depend on the material's transparency for success.

A third factor to consider when choosing a material is the material's chemical reactivity. It is important that the material be inert with respect to DNA, the electrolytic buffer, or, indeed, any other biological chemical which might be analyzed. It may be beneficial if the material is reactive with the polymer gel being used for separation, though this is optional. Most chemical and physical gels have sufficient viscosity to obviate the need for any bonding between the channel walls and the gel. In additon, the surface of the channel walls must be uncharged at standard buffer conditions for electrophoresis. If the walls are charged negatively, as is the case

with uncoated glass, electroosmotic flow will be induced, directed from anode to cathode. This creates a pressure gradient in the opposite direction from the DNA flow, drastically damaging the resolution of the separation.

The chemical properties are less important than that of transparency or electrical conductivity because of the ability to chemically modify the walls of the channel. If the walls are too reactive with the sample, unreactive with the gel, or contain surface charge, it is likely that chemical modification may be a simple solution to modify an otherwise useful material. It is simply a factor to consider when thinking of the economics of chip manufacture and end-to-end process time.

Considering the economics of the situation brings another factor to the forefront. The material should be cheap, cheap to obtain and cheap to micromachine. For example, glass, while easy to come by and relatively inexpensive, require significant amounts of man-hours to pattern and etch. Many plastics, while economically feasible to micromachine, are difficult to obtain or expensive to purchase.

A fourth factor which should be mentioned is that of thermal conductivity. While the microchannels are small, they still may generate substantial amounts of heat. When running the extremely high voltages needed for electrophoresis, Joule heating becomes a serious problem. The rate of heat production inside an electrophoretic channel is given by

$$\frac{dH}{dt} = \frac{k V^2}{L^2} \tag{1}$$

where dH/dt is the rate of heat generation in Joules per second per cubic meter, k the electrical conductivity of the electrolyte, V the applied voltage and L the channel length[34]. Unless the material in question can easily sink heat away from the channel, the temperature gradient throughout a long run may interfere with an effective separation.

For this project, polydimethylsiloxane was chosen as the chip material. It is a silcone polymer, with relatively long chains of $(Si(CH_3)_2O)$ repeats. It is also quite well crosslinked, leading to good mechanical stability, though the formulation used in this experiment still yields high flexibility and elasticity. PDMS was obtained from Dow Corning, under the commercial name of Sylgard 184.[35]

Electrical conductivity of PDMS is rather low, with a value of 5.6×10^{-14} $(ohm\text{-}m)^{-1}$ being measured for Sylgard 184. As previously mentioned, it is necessary that the material be insulating, and this is easily on scale with the insulating properties of glass, which has a conductivity on the order of 10^{-20} $(ohm\text{-}cm)^{-1}$. PDMS appears to fill this role nicely, with insulating properties more than sufficient for standard electrophoretic voltages, which are on the order of 200 V per cm of channel length[36].

PDMS is optically transparent in the range of 300 to 1200 nm. This allows, fluorescently tagged species may be easily detected in the channel, without concern of signal loss through absorbance. PDMS also has little to no natural fluorescence, ensuring a low signal to noise ratio for sample detection. The index of refraction of PDMS elastomer is 1.43[37], which is not far from that of common Pyrex brand glass of 1.47[38]. This indicates that standard lenses and objectives could be used for observation of a silicone chip under a microscope.

PDMS elastomers are intrinsically hydrophobic, as might be expected from its methyl side chains. As a result, it is unreactive to both sample and gel chemistry, ensures an inert surface. This can be changed, at least temporarily, by reaction in oxygen plasma, causing the surface to become oxidized, reactive, and extremely hydrophilic, with a contact angle of approximately 10^{o}.[39] Basically, the plasma oxidation causes the surface of the silicone to behave like silica, allowing for silyanization. This change also allows for irreversible bonding to a glass or silica substrate, which can be extremely useful if strong adhesion becomes necessary.

The thermal conductivity of elastomers is quite poor in comparison with previously used microfabrication materials. It's thermal conductivity of ~5 X 10^{-4} g cal-cm s^{-1} cm^{-2} ^{o}C is an order of magnitude lower than that of quartz[40]. The polymer has a reasonable working range from only around -50^{o} to 200^{o} C[41]. This low thermal conductivity may prove to be a problem, considering the substantial Joule heating which often arises from electrophoresis.

2.1.2 Mold Casting

Using PDMS as the material for the chip simplifies the creation of microchannels a great deal. Where before, with glass, a significant portion of time had to be spent in a clean room, patterning and etching each individual chip, PDMS requires only one trip to the clean room. That trip is to create the master mold.

A master mold is the topographic structure from which all of the successive iterations of silicone chips will be fabricated. Its only requisite properties are mechanical stability, chemical stability, and topographic information. The master mold is fabricated usually using standard

microfabrication techniques, as those referred to in Appendix A. In this case, the topographic information was generated through the patterning of a negative resist known as SU-8. SU-8 is a (type of resist) which, after appropriate processing, possesses great mechanical stability. Originally, the thought was that the pattern would be transferred directly into a silicon or fused silica wafer through etching, either plasma etching or wet chemical etching, but this was found unnecessary. A SU-8 patterned 6" silicon wafer has been able to produce over 100 runs of elastomer chips without pattern degradation.

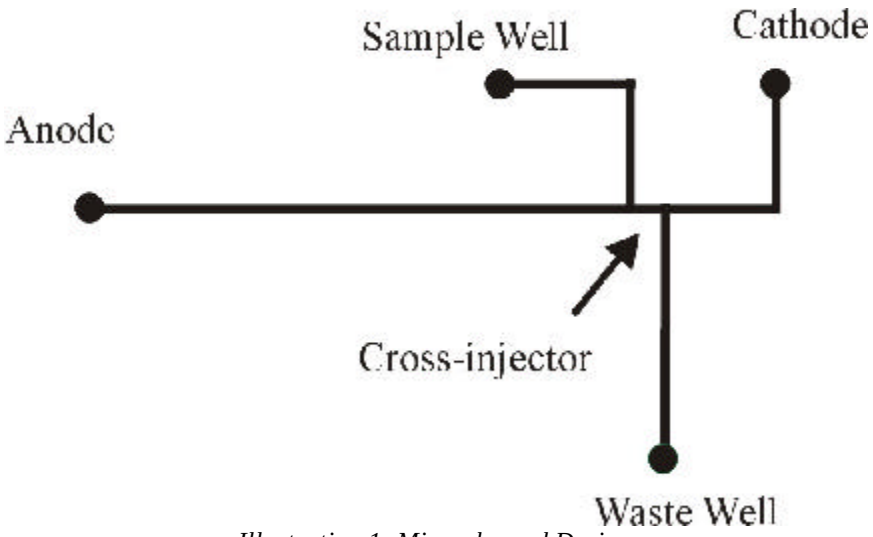


Illustration 1: Microchannel Design

The topographic pattern of the channel was taken from the standard design used for glass fabrication. Basically, it consists of a four port device, shaped in the form of a cross. At the intersection between the loading channel and run channel, there is an overlap which is known as the cross-injector. This is designed to concentrate the loaded DNA, and to keep sample from entering the run channel after the run is started.

The Sylgard 184 kit comes in two parts, a base, which is basically the long polymer chains, and cross-linkers, and a curing agent, with chemicals to catalyze the reaction. These are mixed in a 10:1 ratio. In order to cover the entire wafer, a standard batch protocol of 30mL base to 3 mL curing agent was used. This mixture was placed in a 50 mL Erlenmeyer flask, along with a teflon coated magnetic stir bar. A vacuum pump was attached to the flask, and the solution was pumped down to 25" of Hg, or about 100 millitorr. The stirrer was started at the same time as the vacuum, and the pre-polymer mixture was stirred for 10 minutes under vacuum. After 10 minutes, the stirrer is switched off, but the vacuum is allowed to stay on for 30 more minutes. At this time, examination of the mixture shows no bubbles, indicating that degassing of the solution is complete.

The mixture is then taken, along with the master mold, to a vacuum oven. The master mold is placed in a Pyrex casserole dish, and the silicone mixture is poured onto the wafer. The flask is held steady above the middle of the wafer, causing the elastomer to form a thick circular spot in the center of the mold. The casserole dish is covered with aluminum foil, and allowed to sit for 5 minutes. This causes the silicone to spread across the surface of the wafer, and allows any bubbles formed during the dispensation to vanish. At this point, the aluminum foil is removed, and the dish is placed in the vacuum oven, preheated to 100C. The oven is sealed shut, and vacuum is applied.

After 1 hour has passed, the oven is vented with N_2 and the casserole dish removed. It is covered with aluminum foil, and allowed to cool. At this point, the silicone elastomer has completed curing. Using a penknife or razor blade, the various dies are cut out of the silicone

layer, and peeled off of the wafer surface. It has been suggested that a teflon vapor coating be applied to the wafer to aid in the delamination of the silicone from the wafer, but this has proved unnecessary[42]. Once the silicone has been completely removed from the wafer surface, the wafer is ready for another run.

The chips thus obtained have the inverse topographic features of the master mold. That is to say, that in order to have channels in the PDMS chip, a tower must be created on the mater mold surface. This limits the dimensions of the channel, as it is extremely difficult to create narrow but tall features. The aspect ratio of these features usually causes collapse, whether they are made from SU-8 or even etched into silicon. For the purpose of this experiment, a 50 micron by 50 micron channel design was created.

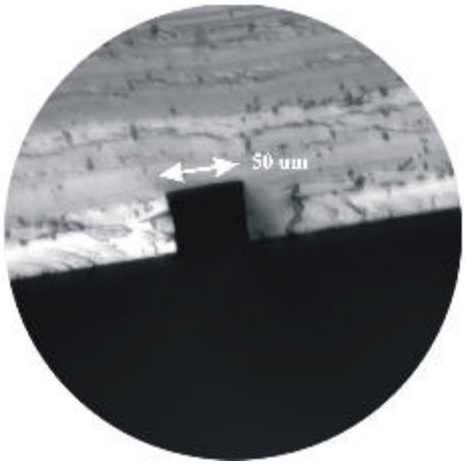


Illustration 2: Cross-sectional image of channel

2.1.3 Fluidic Inputs and Sealing

Now that the microchannels are completed, the issue of fluidic access was broached. Sample introduction and gel injection methods must be considered. It was found that having 50 micron tall, 2 mm by 2 mm reservoirs was not sufficient. The aspect ratio of these reservoirs,

combined with the flexibility of PDMS, proved disastrous, as the PDMS would buckle in the center of the reservoir. Secondly, the needles available for injection, from 30 gauge syringe needles, even down to HPLC syringes, proved difficult to inject with. For an automated system, reservoirs of a better aspect ratio may prove sufficient. However, for this human operated injection, bigger fluidic access ports were needed.

If large access ports are to be used, the reservoirs needed to contain significant amounts of liquid. The small amounts given by the reservoirs will not be enough to avoid drying out the chip at the temperatures commonly used for electrophoretic separations(50 deg C). When the reservoirs were sealed from the outside environment, as before, evaporative losses were minimal, but with large, open access ports, fluid will escape quickly. Reservoirs of at least 10 microliters should be sufficient for this task. Though again, it should be stressed that this would not prove necessary in an automated system, that small reservoirs could easily be used. This prototype system requires a few stopgap measures be undertaken to account for the human factors in the experiment.

As a result, a penknife, or razor blade, was used to cut out the PDMS above the reservoirs. By placing each chip on a glass slide, and back illuminating the slide with the aid of a drafting light box, the channels and reservoirs could be visualized unaided. The blade was then cut into the silicone at a 45 deg angle, causing something of a trapezoidal reservoir shape. This results in reservoirs which have about 1-2 microliters of capacity, since the PDMS chips are, on average, 1mm thick. The chips are then rinsed in ethanol, blown dry, and placed in a storage container.

At this point, the chips need to have the fourth wall of the channel sealed off. To accomplish this, a flat surface is needed to seal the chips against. PDMS's surface properties ensure that it will adhere well to several different surfaces, from polymethylmethacrylate(PMMA) to glass to another piece of PDMS. However, this nonspecific adhesion is too weak to allow for gel injection. Without a sufficient strong bond, the chip will delaminate during filling, and thus prove useless.

It is most desirable to use another piece of PDMS as the fourth wall sealant. Using a different material, whether it is glass, PMMA, or some other plastic, introduces another variable into the channel motion, the interaction of this new surface with the sample and gel. To this end, a glass slide was coated with PDMS, granting the mechanical rigidity of glass with the surface characteristics of silicone.

In order to improve the chip adhesion to the new PDMS layer, a partial cure was attempted. The idea is that through only partially curing the new PDMS layer, it will remain semi-liquid, allowing the chip to sink partially into the surface of the chip. This will improve the adhesion somewhat. The key is to time the partial cure such that the new layer has low enough viscosity to reflow to improve adhesion, but high enough that it will not flow in to fill the channels, which would destroy the chip. After experimentation, it was found that 1/3 of the cure time gave the best result.

So, the PDMS layer was spun onto a standard Corning glass slide at 500 rpm, using the same protocol for mixing and degassing the polymer as previously described. This was done in order to assure a thin, uniform bottom layer, since optical detection will rely on having a clear

level window. 500 rpm yields a ~150 micron thick layer. The slide was then placed in the casserole dish, and put in an oven at 100 C. After 20 minutes, the casserole dish was removed from the oven, and a previously prepared chip was gently placed on the molten PDMS surface. The dish was then placed back in the oven, and allowed to cure for the remaining 40 minutes.

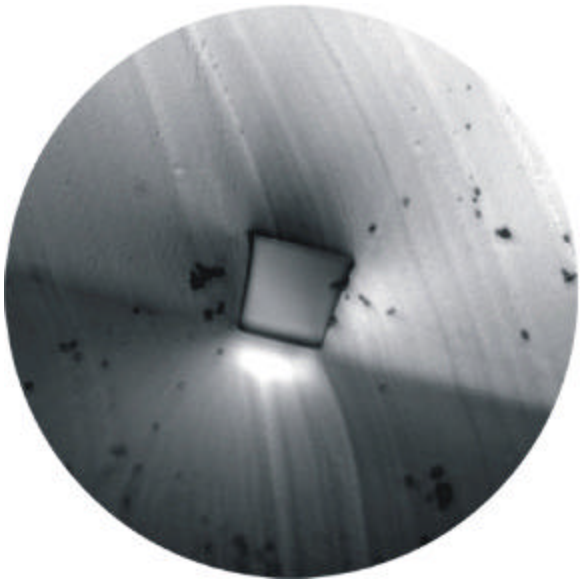


Illustration 1: Sealed Channel

This partial cure does improve the adhesion, but there is still a danger of delamination. When gel was forced into the channel, some would still seek out the chip edges and leak out. This not only disturbed the definition of the channel, but resulting in incomplete filling, since the gel would usually take this lower pressure route. In order to solve this problem, another form of silicone was used, that of caulk. Silicone caulk was spread over the edges of the sealed chip described above. This seemed to aid in halting the leakage. If too much pressure is applied, the chip still will delaminate, but it requires far more pressure, and even at that point, it does not leak.

Another problem which was solved at this point was that of evaporative fluid loss, and subsequent gel drying. To solve this, 5 and 7 mm diameter vials were caulked over the reservoirs, granting an additional volume of 40 and 150 microliters respectively. This proved more than sufficient to keep the gel well hydrated, even during extremely long runs.

2.2 Chip Filling

2.2.1 Gel Formulation

Gel formulation is an extremely key part of this experiment. Although the formulations are extremely well documented, a small error, easily made by a less experienced operator, can result in great periods of lost time. This is especially true when dealing with the small volumes of gel used in microchannel electrophoresis. A formulation error which might go unnoticed in a slab gel, or even a capillary run, would definitely result in failure for this already precarious apparatus.

In the case of this experiment, a chemical gel was decided upon. The reason for this is twofold. First, part of the entire purpose of the experiment is the demonstration of a chemical gel's utility, especially for single-use chips. The second part is the enormous difficulty in actually using physical gels with the silicone chips. The amount of pressure needed to force a 2 % LPA solution into the chip tends to destroy the chip. The chip delaminates from the surface, forming a large bubble of LPA solution in the resulting space. The viscosity of the LPA solution is too great, requiring too much pressure to load in the small channels.

The protocol for the gel is sensitive, but as long as the key steps are performed correctly, it was simply accomplished. The basic chemical formulation of the gel was obtained as a recipe from the commercial supplier of the acrylamide, Bio-Rad.[43] First, a 40 % solution of acrylamide/bis-acrylamide(19:1) was added to a 20 mL capped vial. The amount of acrylamide varied depedent on the actual percentage gel desired, but most gels made were either 4%, requiring 1.5 mL, or 7%, needing 2.625 mL. After the acrylamide, 1.5 mL of 10X TTE buffer was added to the vial. TTE, short for Tris-TAPS-EDTA, is a standard electrolytic buffer solution used in electrophoresis. It was substituted for the recipe's recommended TBE(Tris-Boric Acid-EDTA), because TTE has been shown to demonstrate better stacking of DNA. Next, 6.3 g of urea was weighed out and added to the vial. Finally, enough deionized water was added to the vial to bring the total volume to 15 mL.

The urea is needed in order to keep any loaded DNA in its single stranded conformation. It has been well demonstrated that DNA separates better in this form, but

At this point, a teflon coated stir bar was added to the vial, and the solution stirred for approximately 5 minutes. During this time, two smaller 3 mL glass vials were prepared, one labeled TEMED and the other labeled ammonium persulfate. 1 mL of the deionized water was added to the APS vial. Once this was completed, and the stir bar removed from the acrylamide solution, these three vials were placed in a vacuum chamber, with caps off, for 2 hours. This is to remove any dissolved oxygen, to ensure full polymerization when the time comes. Oxygen is known to absorb free radicals, a vital element of the acrylamide polymerization process.[44]

After 2 hours has passed, the vacuum chamber is vented. 0.25 g of ammonium persulfate is weighed out, and added to the water in its labeled vial. This vial is vortexed until the APS has fully dissolved. 1 mL of TEMED is then added to its vial, and the vials are replaced in the vacuum chamber for an additional 30 minutes. It is vital that TEMED and APS are less than 6 months old, and stored in a dry box or dessicator, as both are hydroscopic and their activity will be greatly decreased with age[45].

After the additional 30 minutes of degassing were up, the vacuum chamber is vented. 15 microliters of TEMED is added to the acrylamide solution, then 15 microliters of APS. The acrylamide solution is then vortexed for 5 seconds, to aid in mixing. Immediately after this point, the gel begins polymerizing, so time becomes a factor. The gel should immediately be loaded into the chip at this point.

2.2.2 Gel Injection

Several methods were attempted to successfully inject the gel. The problem is to force a liquid, which is becoming more viscous with every minute after polymerization is initiated, into a 50 micron by 50 micron channel. In addition, the acrylamide must be continuous, with no bubbles or other discontinuities, and it must fill all arms of the channel, the sample arm, waste arm, cathode arm, and the run lane with the anode arm.

In order to accomplish this feat, several methods were tried. The first attempted method was through vacuum, to suck the gel through the chip. The cathode, sample and waste vials were all filled with gel, and vacuum applied to the anode. Fluid flowed through the channel

reasonably well, as demonstrated by the trials with food coloring in water. However, when attempted with acrylamide, post-polymerization examination with a microscope revealed bubbles in every channel loaded in this way. Filling was especially bad in the cross-injector region.

The method which was finally settled upon requires a slight modification to the chip design. When the silicone caulk is applied for leak sealing, an additional step is added. A PTFE/silicone septa is sealed in place on top of the 7 mm vial over the anode. Another batch of Sylgard is produced, and poured over the assembly, as well as along the seams of the chip. This ensures an airtight seal around the septa, as well as aiding the silicone caulk in the sealing of the chip.

When the acrylamide polymer has been fully prepared, it is loaded into a 5mL syringe using a 22 gauge needle. After the syringe has been filled, the 22 gauge needle is swapped out for a 30 gauge. This is to limit the speed of the gel loading, to prevent excessive pressure from being applied to the chip. With a 22 gauge needle, it is all too easy to blow the seal on the PDMS chip. So, the 30 gauge needle is then inserted through the septa The plunger of the syringe is depressed, and the anode vial begins to fill with acrylamide. As this occurs, the pressure built up in the vial forces the acrylamide through the channel out all of the other vials.

The acrylamide passing through the channel is clearly visible, as the channel becomes almost invisible once filled with the acrylamide. It was found that if the chip was placed on a sheet of white paper, that the channel could be easily visualized by eye. A shadow could be seen on the paper from the channel, a shadow which disappeared as the acrylamide filled the

chip. Each vial was individually examined as well, using a 10X eyepiece. It is important to ensure that a good sized droplet of acrylamide has emerged from each leg of the channel, that acrylamide has filled all of the legs. If this is assured, the septa is ripped off of the anode vial, to allow for easier fluidic access later.

At this point, the chip is in a delicate state. It was found to be benefecial to add more gel from the syringe to partially fill all of the vials with acrylamide. During polymerization, the gel will shrink slightly. If there is a significant reservoir of acrylamide residing over each opening to the channel, bubbles will be prevented from entering the channel. Without these reservoirs, the hydrophobic nature silicone will encourage bubbles to become trapped in the channel.

To further ensure the removal of any bubbles already present, the channel outgas technique was used.[46] This consisted of placing the chip into a vacuum chamber for approximately 10 minutes. This serves to remove any gas bubbles present in the channel, as they will migrate out through the available reservoirs. The chip was then returned to atmospheric pressure, forcing the still liquid acrylamide solution into any empty spaces in the channel.

From this point on, it was important to be careful of applying external pressure to the chip surface. The flexibility of the chip will allow any force applied directly over or near the channel to close of the channel, disturbing, or even possibly displacing the acrylamide gel filling the chip. In order to prevent this, care is taken to only handle the chip by the edges from this point forward.

Approximately 20 minutes after the polymerization reaction has began, the acrylamide remaining in the vial has visibly gelled, an indicator of successful polymerization. 2 hours after injection, after the reaction has begun, the polymerization is assumed to be complete. 1X TTE is added to fill all four of the vials, to prevent them from drying. The chip is then wrapped in Parafilm to seal the liquid in, and placed in storage until it is time to run the experiment.

2.3 Electrical Setup

2.3.1 Electrodes

In order to drive electrophoresis, an electric field must be set up in the channel. This necessitates a method of electric connection to the electrolyte of each of the four legs of the chip. The best way to accomplish this is through electrodes of either platinum or gold placed in the four reservoirs of the chip.

For this experiment, this was accomplished in two different ways. Initially, standard high voltage wires were used, with pieces of platinum wire soldered to the end. The platinum tips were then immersed in the vials, and the wires taped down to secure the chip. This method is extremely simple, but works rather well. The only problem is the danger involved with the high voltage being applied to sections of bare wire. The awkward nature of the apparatus is also an issue.

A solution which was developed and tested was through the patterning of gold electrodes on a glass surface. Gold pad electrodes 1mm by 1mm were placed on a glass slide, aligned underneath each reservoir. Interconnects were designed, running to contacts on the

edge of the slide. Wires were then soldered to the contact pads, and voltage was applied. When the current between the terminals was measured, electrophoretic current was detected, similar to that found using platinum electrodes.

These gold electrodes were patterned on a glass slide using metal liftoff techniques, as mentioned in Appendix A. The electrodes were made of a 5 nm Ti adhesion layer coupled with a 100 nm Au layer. After the glass slide was coated with PDMS and sealed to a chip, the silicone covering the electrode pads was carefully removed, granting direct electrical connection.

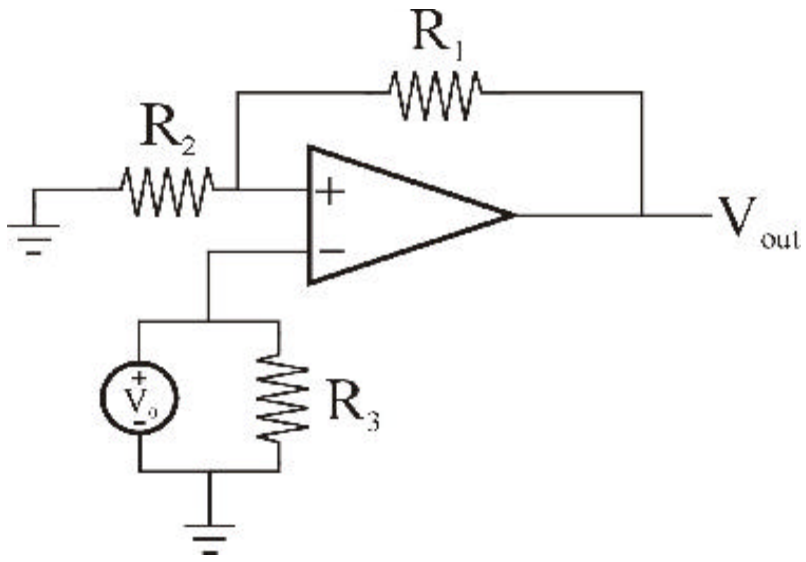
2.3.2 Circuit Design

There were two types of circuits used for this experiment. The first was an electrophoretic microchannel setup which had already been assembled, designed to apply voltage to a standard cross-injector design etched in glass. This circuit had several switches, which allowed voltage to be applied to either LOAD or RUN. LOAD indicated a driving voltage was applied between the sample and waste vials, with the cathode and anode of the chip allowed to float. The RUN mode applied the driving voltage between cathode and anode.

There was an additional switch associated with the RUN mode, known as PULLBACK. Pullback is basically a positive voltage applied to both the sample and waste vials, to ensure that after a RUN begins, that no further sample will leak into the run channel, to broaden peaks or raise the background noise of the separation.

In order to further control the voltages applied, a second power supply was designed. This power supply was controlled through a computer, with each voltage output being

independent from the others. The voltage was output from an analog ouput board (NI 6703), which had a range from 0-10 volts. This voltage was subsequently amplified by a factor of 100 through a high-power op-amp. This allows for a voltage of up to 2 kV to be applied along the run channel, generating an electric field of 500 V/cm.



In order to power the op-amp, a series of DC-DC converters were used. Although limited to 10 W each, they are able to convert from 15 V DC to 1200 V. This ability, in combination with a standard DC transformer, allows this power supply to be fed directly from an AC wall outlet.

The computer voltage output was controlled by a simple program written in Labview on a Pentium II running Windows 2000. The code allowed for any type of linear ramp or constant voltage to be applied to each of the vials individually, giving great flexibilty in voltage application.

2.4 Detection Setup

The apparatus used for detection was designed previously by members of the Ehrlich lab, to detect fluorescent particles in glass microchannel chips. This apparatus, with slight focus adjustment and intensity modification, was adequate for detection with silicone chips.

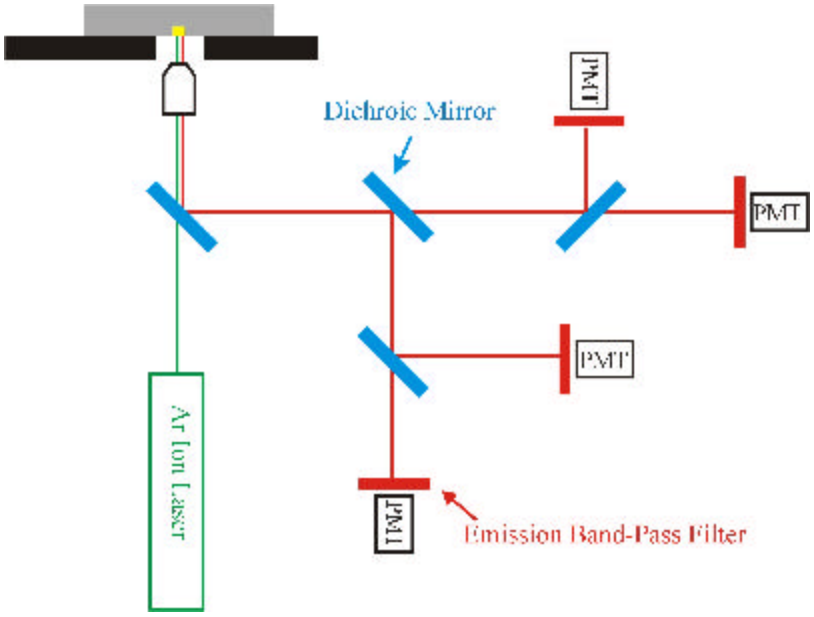


Illustration 1: Laser Induced Fluorescence Detection Apparatus

The apparatus consists of a temperature controlled plate, mounted on an optical table. This plate has an aperture for optical access, which is 2 cm in diameter. Beneath this aperture is a standard microscope objective, 50X magnification(Melles Griot). The objective is illuminated via an argon-ion laser operating at 488 nm(JDS Uniphase). The laser light illuminates the chip, causing any fluorescent material in the channel to be excited. The subsequent fluorescent light then, partially, goes back through the objective. It then reaches a series of dichroic mirrors(Omega Optical), and is split into four different parts, each representing a different part

of the spectra. This is designed to separate different color fluorescence, specifically for four-color sequencing reactions.

After the light is split, it is spectrally filtered, using a with a band-pass filter(Omega Optical), to remove extraneous light sources. The light is then sent into a photomultiplier tube (Hamamatsu), via a small pinhole, granting very sensitive detection of the fluorescent signal. The different PMTs are filtered to detect 525, 555, 580 and 605 nm wavelength light.

Chapter 3 Experiments

3.1 Electroosmotic flow test

In order to test for electroosmotic flow, a measurement of the flow rate in the channel must be performed. To do this, a colored, neutral species should be introduced into the channel. If voltage is then applied, the flow should carry the species along through simple hydrodynamic affects.

To this end, a chip was prepared to run the test. This chip measured 4 cm from cathode to anode, with the channel filled with 1X TTE buffer, and substantial reservoirs of the buffer available in each vial. The amount of liquid in each vial was carefully dispensed, in order to avoid any hydrostatic flow. Gel was not introduced because it would only slow or impede any natural electroosmotic flow. The chip was place over the detection apparatus, and platinum electrodes were immersed in each vial.

To test operation of the chip, 1 kV voltage was applied in both the LOAD and RUN modes. Current was detected in both modes, with ~3 μA for LOAD and ~12 μA for RUN. This shows that the channel is completely filled with electrolyte, that the channel is neither pinched off nor blocked by an air bubble or other debris.

Now, fluorescein labeled beads, 1 μm in diameter, were introduced into the cathode vial, and 200 V/cm was applied, for 800 V total. Pullback was engaged as well, applying +20 V to both sample and waste vials. After 30 minutes, no evidence of the colored material was detected through the LIF apparatus. The chip was then taken to the epifluorescence

microscope, and examined throughly. No evidence of color was found in any vial except the cathode, with some of the color having diffused into the channel region near the cathode.

The experiment was then rerun with the opposite polarity, applying positive voltage to the vial previously known as the cathode, and negative to the anode. Again, no flow was detected. This result was interpreted to indicate that the silicone chip has extremely low electroosmotic flow. Therefore, the flow will not prove an impediment to the electrophoretic separation.

3.2 Charged Labeled Beads

The second experiment was run to ensure that the chip was actually working correctly, that charged particles would, in fact, enter the gel-filled chip. A 4% acrylamide solution was loaded in the chip, through the methods previously described, and the four reservoirs filled with 1X TTE buffer.

For sample, Fluoresbrite Carboxylate YG Microspheres were obtained from Polysciences, Inc. These are polystyrene spheres, 0.1 μm in average diameter, with their surface covalently modified. The surface of the spheres is bound with both carboxylate groups, negatively charged at the experimental pH, and fluoresein, a standard dye molecule for fluorescence microscopy.

These beads were loaded into the sample vial, 20 μL of beads to 20 μL of water. The load voltage was then applied, and the chip was examined in the microscope. Brightly

fluorescing beads were found in the cross-injector, and the sample and waste vials. However, no fluorescence was detected in either the cathode or anode vials.

Run voltage was then applied to the chip, of approximately 200 V/cm, with pullback being applied after 15 seconds. After 30 minutes, the chip was again examined in the microscope. Fluorescence was detected in sample, waste, and anode vials, but not in the cathode vial. This indicates that the gel is capable of passing particles of tenth micron size within a half hour's time. The motion is also in the correct direction only, removing the possibility of hydrostatic flow or even electro-osmotic flow pushing against the natural separation. This merely re-verifies the results obtained from the earlier, uncharged beads.

3.3 Dyes Run

This experiment was run to determine if separation of molecules is, in fact, occurring in the silicone chips. To determine this, gel tracking dye was used, containing organic molecules which migrate through an electrophoretic gel at different rates. The molecules should be easily detected by the LIF apparatus, granting data which qualitatively demonstrates the effectiveness of the chip.

To this end, a 4 cm chip was prepared with 7 % acrylamide solution loaded. The chip was then placed on the detection apparatus, and standard pre-electrophoresis was run. A sample containing both bromophenol blue and xylene cyanol was placed into the sample vial, diluted to a 1X concentration. This sample was loaded and run under standard conditions.

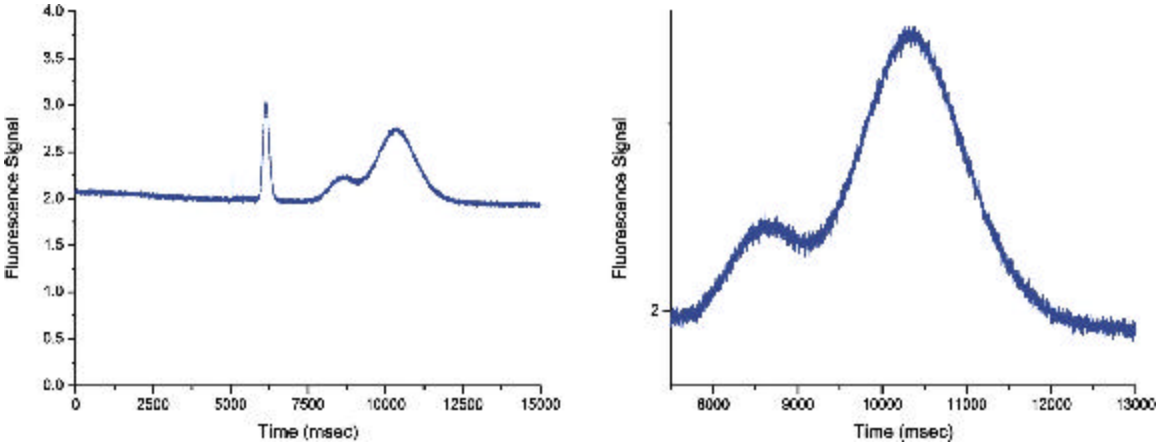


Illustration 1: Dye Run Data(left) Zoom in (right)

The result, while not as spectacular as desired, demonstrates the chips ability to actually separate different molecules. The first sharp peak observed is solely due to the shifting from LOAD to RUN voltage; this causes a spike in the output of all the photomultiplier tubes. The next peak is identified as bromophenol blue, and the last as xylene cyanol. Even after running the chip for approximately 30 minutes, no further signal was seen.

So, the two chemicals are only separated by around 2 seconds. This value is slightly low. These chemicals are used as tracking markers in DNA gels, and usually are separated a distance equivalent to 100 DNA bases. Of course, this gel may not be optimized to separate molecules of this size and type. The only important information which was gained was that the gel successfully separates chemicals. Therefore, it was decided to move on to the next, and more important experiment.

3.4 STR Allelic Ladder

For the most important experiment, a simple DNA allelic ladder was electrophoresed. The ladder chosen for this case was the HUMTH01 allelic ladder, one of the many loci used for identification purposes by forensic science. The ladder has DNA which differs in length by 4 bases, with lengths roughly found to be : 184, 188, 192, 196, 200, 203, and 208 basepairs[47] . This DNA was labeled at its 5' terminus with fluoroscein, a common dye molecule which is easily excited and has a wide fluorescence bandwidth. The sample was diluted 5 fold from the original 100 µg/µL concentration, and loaded on the chip. A standard 4 cm chip was prepared, as described from previous experiments.

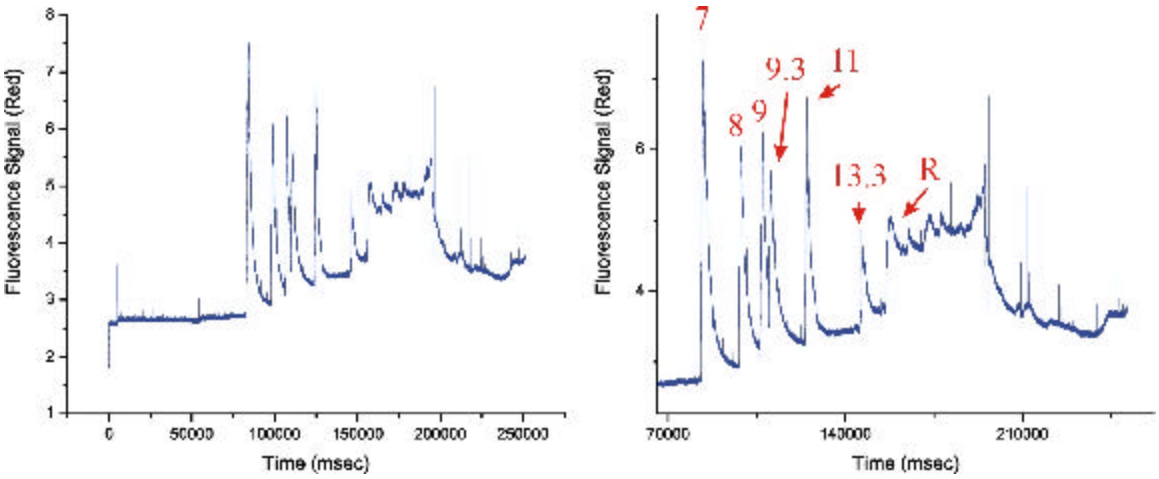


Illustration 1: HUMTH01 Ladder (left) Zoom in (right)

Although not perfect, this run demonstrates the ability of the chip to resolve DNA fragments with 4 base pairs of difference. Considering that the chip itself is merely 3 cm long, this is quite an achievement. The long shoulder is probably due to the sample being too concentrated, and to the long run time needed for this measurement.

Allele	*Location(msec)*	*Width*	*Resolution*	*Length*	*? Length*
8	83984.93	6719.33		196	
9	98792.34	6719.33	2.2	200	4
9.3	107569.24	3417.44	1.7	203	3
10	110765.59	5482.79	0.71	204	1
11	125077.49	5228.37	2.67	208	4
13.3	146322.03	5508.34	3.96	219	11
R	158117.52	5228.37			

It appears as if this 4 cm chip was able to resolve fragments only 1 base apart, between allele 9.3 and allele 10, an excellent result. However, there are several problems with the result. First, the large broad peak labeled as "R". It is believe that this is a peak representing DNA which was not efficiently separated, either due to hybridization or binding to the walls, some factor slowing the DNA motion. The second problem is the inconsistent result of the separation distance between allele 11 and allele 13.3. The distance of separation for these alleles should be approximately 33% greater than it actually is. Finally, the chip took an extremely long time to separate this DNA. The voltages used probably need a more thorough calibration.

Chapter 4 Conclusion

4.1 Summary

In conclusion, it is clear to see that the stated goal of this thesis, the creation and initial testing of a disposable electrophoretic chip, has been met. The chip was created out of silicone, a cross-linked methylated polymer which takes easily to microstructure molding. After much experimentation, the chips were sealed against a planar silicone surface, and filled with a cross-linked polyacrylamide solution. The completed chip proved to have low to nonexistant electro-osmotic flow, but capable of electrophoresis of charged beads. Dye molecules and DNA ladders were separated by the chip, despite it's short length. In sum, the chip, despite being a prototype, has good resolution and is easy and cheap to make.

However, there are many problems still remaining. First, the chip itself is needlessly bulky, due to the large reservoirs of liquid needed to avoid gel dehydration. The chip is sensitive to external pressure, due to the flexibilty of the silicone polymer. This is dangerous, allowing the channel to be deformed, creating voids or bubbles of air in the acrylamide gel. There is also a danger that with longer runs, the chip may heat up, since the silicone is not an excellent heat conductor[48].

In addition, even with the current protocol, the chips completely fail approximately 50% of the time. This is simply unacceptable, and the reasons for it are unclear. It is most probably due to problems in the gel loading section, though improvement in this area is difficult. Perhaps

the gel formulation itself needs improvement. More DNA separations should also be attempted, in order to better quantify the electrophoretic ability of the silicone chip.

4.2 Future Experiments

The first set of experiments would be designed to increase the resolution of the chip through increasing the channel length. This can be done in two ways. The first, and most obvious way is to simply lengthen the sepeartion channel, using the same design. A second method might be through the introduction of bends into the channel. By bending the channel, the available space can be used to its fullest extent. This allows the footprint of the device to remain small, while increasing the resolution.[49]

Another important experiment which may be tried is the use of a different plastic. Polydimethylsiloxane, while an excellent material in general, is problematic to use for this purpose, for reasons earlier outlined. At the very least, a survey of other likely plastics should be attempted, such as carbonates, acrylates, and perhaps even styrenes. Another option is to use a different formulation of silicone. If more cross-linking is initiated in the silicone, it may grant more rigidity to the microchannel structure. This would be advantageous, removing the problems that so often occur from accidental pressure applied to the chip.[50,51]

Another important advance, one which is necessary to realize the benefits of the small channels, is that of sample delivery. The current method of sample delivery, by pipetting into a 5 mm diameter vial, is simply unacceptable. An easily proposed method would be another layer of complexity, integrating some sort of microfluidic device to deliver the sample. The delivery

system may even be manufactured out of silicone as well, with the motive force being either electro-osmotic flow[52] or peristalic pumping.[53] This allows for great reduction of the amount of sample needed, as well as easing any future integration of the chip with other microfluidic systems. It may also aid in solving the gel loading problem, since the initial, pre-polymerizated acrylamide solution has a viscosity approaching that of water, and could easily be loaded by the same apparatus.

Appendix A. Microfabrication

A.1 Photolithography

Photolithography is a mature process, which is used thousands of times a day to write patterns onto substrates for microfabrication. It operates through the simple principle of exposing parts of a sample to light, while leaving other parts blocked from the light. The light then triggers a reaction within a layer of photoresist, a light-sensitive polymeric substance.

The beginning of any photolithographic process must be the photomask design. The photomask refers to a piece of material which blocks or masks certain areas of the substrate from exposure to light. The most important properties of the photomask are its optical transparency at the exposure light wavelength and a flat, highly polished surface to minimize errors from light scattering. An opaque layer must then be patterned on the surface of this mask, in order to selectively block the exposure. Photomasks may be either directly to scale of the desired features (1X) or larger (5X, 10X), with a reduction in image size accomplished during exposure[54].

Commercial masks are usually made out of fused silica, with a chromium metal layer for the opaque pattern. Masks can easily be made down to submicron resolution, allowing small features to be patterned. Another possible option for a mask is far cheaper and easier to fabricate: a simple optical transparency, created by a laser printer. Common office printers, with a resolution of 600 dpi, can create masks with features 250 μm wide. This is sufficient for

rough microfluidic development masks. Higher resolution printers, photo-quality printers of >3000 dpi can create features on the tens of micron scale[55].

All of the work described in the above thesis used a 1X mask. A 1X mask is most often used with a contact mask aligner, which places the mask in close contact with the substrate surface. The only problem with using contact masks is that the defect density is often high. Actually coming into contact with the substrate surface often introduces these defects. This is often offset by proximity printing, in which the mask is floated off of the wafer surface by a thin layer of inert gas[56].

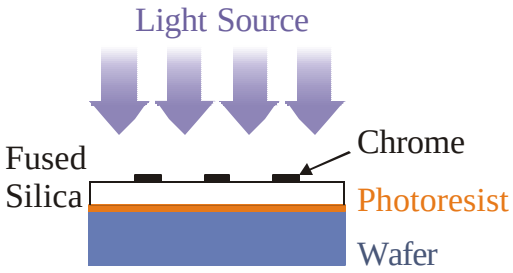


Illustration 1: Contact Mask Exposure

The photoresist is a vital, perhaps the most vital, part of the patterning process. There are two clear types of resist, determine by the its polarity. Positive polarity photoresist reacts to light by becoming soluble in developer. Negative polarity resists react to light by becoming insoluble in developer. This means that after the exposure, and subsequent processing steps, with positive resist samples, the areas exposed to light will have no protective film, whereas with negative resist, the areas which where masked will have no protective resist film.

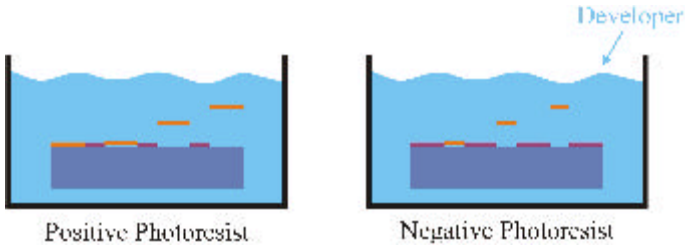


Illustration 2: Positive versus negative photoresist in developer

Photoresist processing is a series of many steps, all of which must be carefully performed to ensure correct image transferrence. To begin, a smooth, substrate must be prepared, usually a silicon or SiO_2 wafer, though glass slides are also usable. The first preparative step is a dehydration bake, to remove excess water from the surface of the substrate. This is usually done at around 150˚- 200˚C, driving off all but a single monolayer of water molecules from the surface.

The next step, which is an optional though highly recommended one, is the deposition of an adhesion promoter on the sample surface. Different types of adhesion promoters may be used, though hexamethyldisilazane (HMDS) is the most popular. This chemical treatment will aid in adhesion between the resist and the substrate later in the process. Adhesion promoters are applied either through direct application or allowing vapor from the chemical to condense on the sample surface.

The next step is the application of the photoresist. This should be done soon after the adhesion promoter/dehydration bake, to ensure that the sample surface remains clean and receptive to the resist. The sample is placed on a ciruclar hollow metal piece, called a chuck. Vacuum is applied to this piece, in order to hold the sample tightly to the chuck. The chuck is on a spindle, which can spin the sample at a rotational speed of up to 6000 rpm. Resist is then

poured onto the surface of the sample. After the resist has sat on the sample for enough time to remove any bubbles created during dispensation, the sample is spun at a set rate of speed in order to produce a uniform layer of a set thickness. The thickness of the resist layer depends on the spin speed like

$$T_r \propto \frac{1}{\sqrt{\omega}} \tag{1}$$

where T_r is the resist thickness and ? the angular velocity of the sample. Spin speed to thickness curves are often published by resist manufacturers to aid in reaching target resist thicknesses[57].

After the resist is applied and spun, the substrate undergoes a process known as a softbake. This step is designed to remove some of the solvent from the photoresist formulation. The amount of solvent in the formulation will have a great bearing on the resolution and sensitivity of the resist during exposure. Softbake times are usually determined through experimentation, and varied along with exposure times to get the best results.

After the softbake is performed, the exposure is done, as previously described. Once the exposure is complete, some resists require a post exposure bake. This step is often performed with negative resists, to aid in the crosslinking of the exposed polymer. As with the softbake, the temperatures and times are often determined empirically, through coordination with the exposure time.

Now the sample must be developed. Development refers to the process of immersion in a chemical developer, which causes parts of the resist to dissolve into the solution. As

previously stated, the area of the resist which dissolves is dependent on the polarity of the resist. After a set amount of time, the sample is removed from the developer bath and rinsed in deionized water.

Finally, the sample is treated to a hardbake, a final bake of the completed pattern. This hard bake is often above 100˚C, used to drive off the remainder of the solvent in the resist, to further harden and densify it. This allows the resist to withstand more damaging and energetic processes. This bake is relatively insensitive, with the only real danger being a cracking of the resist film.

A.2 Liftoff

Liftoff is the name for a common technique used for patterning thin layers of metal on the surface of a substrate. It allows for micron scale patterns without the use of caustic, difficult to control metal etchants. Put simply, it only allows the metal to adhere to the region where it is eventually desired.

The liftoff process relies on the poor step coverage of metal evaporation processes.[58] Step coverage refers to the process's ability to coat vertical walls, to conformally smooth over features. Sputtering and plasma sputtering have better step coverage, making them ineffective techniques for liftoff. Metal evaporation, whether by electron beam or thermal heating, is a very directional process.

To begin the liftoff process, a sacrificial layer is patterned on the surface of the sample. The sacrificial layer should be one which is easily chemically etched, usually photoresist is

suitable for this purpose. The layer is patterned in such a way that the areas where metal is desired are bare of photoresist. Before development, the sample is often soaked in chlorobenzene to create an overhang of the photoresist, which is beneficial later in the process. The chlorobenzene acts to remove solvent, making the top layer of the photoresist denser, more resistant to development[59].

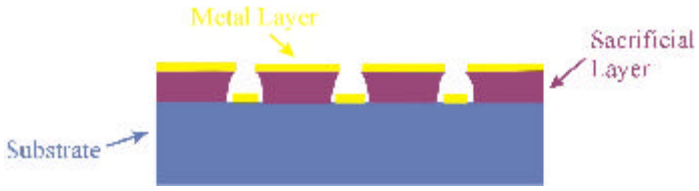


Illustration 1: Photoresist after chlorobenzene treatment and metal evaporation

After the sample is patterned, it is placed in a metal evaporation system. There are two main types of metal evaporation: thermal and electron-beam. In thermal evaporation, also termed resistive evaporation, the metal to be evaporated is heated by electrical current. The sample is placed, along with a piece of the desired metal, in a vacuum chamber. The metal is either in a tungsten or molybendum boat, or made into a filament. When current is passed through the boat or filament, it heats through a simply Joule heating mechanism. Thermal evaporation is limited by the temperatures which can be achieved; refractory metals are difficult to evaporate using this method. There is also significant substrate heating, due to radiative heating from the metal source.

The second method, electron beam evaporation, is also a thermal method. The only difference is in how the metal source is heated. An electron beam on the order of 10 keV is run across the metal source, heating the metal easily to temperatures of 2800˚C.[60] This allows for the deposition of high melting point metals such as titanium or platinum, and refractory metals

such as tungsten. E-beam deposition also grants higher deposition rates than resistive evaporation.

In order to deposit certain metals, adhesion layers are needed. For example, gold does not naturally adhere to a SiO_2 substrate. Gold is so unreactive that it will merely rest on the surface of the substrate, granting poor adhesion at best. The solution to this is the deposition of a thin, intermediate layer to promote adhesion, a layer which will react with the gold and the SiO_2. Ti is often chosen as such a layer, though Cr also works well.

After the deposition of the metal, the sample is removed from the evaporator and place in a release solution. This solution will etch or dissolve the sacrificial layer without damaging the sample or metal layers. As a result, the metal which rests on the top of the sacrificial layer will also be removed, to float away in the solution. For photoresist, acetone is often used as the release solution. Ultrasonic agitation may be useful to speed up or improve the release.

A.3 SU-8 Protocol

SU-8 is an epoxy based negative photoresist which is designed for use as a thick chemically and mechanically stable film. Film thicknesses of up to 200 microns are easily achievable, with good resolution and nearly vertical sidewalls. It is usually processed using i-line UV light (365 nm). The SU-8 process used for these experiments is outlined below.

The photoresist begins stored in a refrigerator, to minimize solvent evaporation and lengthen the resist life time. It should be removed from the refrigerator before any further processing is attempted, as it will need time to warm to room temperature. While the resist is

warming, an HMDS coating is applied to the sample wafer, using an oven designed for this purpose. The HMDS is exposed to the wafer for only 30 seconds before being pumped out again, with a temperature of BLAH. No dehydration bake was done for these wafers.

After this point, the SU-8 was applied to the wafer. An SU-8 50 formulation was used, from Microchem Inc. This formulation had extremely high viscosity, requiring quite a long time to be poured from the bottle onto the wafer. Once a sufficient amount had been dispensed, the wafer and resist was left to settle for 5 minutes. This allowed bubbles to work themselves out of the resist; this time period was known as the relaxation time.

Then the resist was spun, starting with a rate of 750 rpm for 30 seconds. This step was used in order to first spread the resist fully across the surface. Once this step was complete, the spinner accelerated to 2000 rpm, a speed it held for 60 more seconds. This spin rate yields a film of approximately 50 microns in thickness.

After this point, the wafer was placed on a hot plate, pre-heated to 65C. The hot plate was left at this temperature for 6 minutes, then ramped up to 95C for 20 more minutes. The wafer was then removed from the hot plate, with the softbake complete, and placed in a cassette to cool.

A UV exposure was done using an Electronic Visions contact mask aligner. The exposure was completed in four, 20 second intervals, with 5 seconds between each one. The time between the exposures was used to prevent excessive heating of the resist.

Next, the post exposure bake was performed. This consists of a bake, on a hot plate, of 65C for 2 minutes, and 95C for 5 minutes. It should be pointed out that the temperature

ramp of the hot plate is not instantaneous. The hot plate is pre-heated to 65, but its ramp up to 95 takes at least 2 minutes to complete. The 5 minutes of the 95C bake includes this ramp time.

After the wafer has cooled from the post bake, it is immersed in a PGMEA solution for development. The development is allowed to proceed for approximately 3 minutes, then the wafer is removed and rinsed in isopropyl alcohol. Many times white residue is seen on the surface of the wafer, indicating an incomplete development. If this is the case, the wafer is then reimmersed in the developer for an additional 3 minutes. This sequence is repeated until the wafer remains clean after the IPA rinse.

Finally, the wafer may be subjected to a 150C hardbake. This process step, while not necessary, grants additional mechanical stability to the resist, and has proved extremely useful. This hardbake is carried out for 30 minutes.

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