THE PACKAGING, DESIGN, AND EXPERIMENTAL EVALUATION OF A MEMS ELECTROPHORETIC SYSTEM

A Thesis
Presented to the
Faculty of
San Diego State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Mechanical Engineering

by
Steven J. Wong
Spring 2007
SAN DIEGO STATE UNIVERSITY

The Undersigned Faculty Committee Approves the

Thesis of Steven J. Wong:

The Packaging, Design, and Experimental Evaluation of a MEMS Electrophoretic System

________________________________________________________

Samuel Kassegne, Chair
Department of Mechanical Engineering

________________________________________________________

Karen May-Newman
Department of Mechanical Engineering

________________________________________________________

Paul Paolini
Department of Biology

________________________________________________________

Approval Date
Copyright © 2007

by

Steven J. Wong

All Rights Reserved
I would like to dedicate this thesis to my mom and dad. This work would not have been possible without your support, encouragement, and guidance.
ABSTRACT OF THE THESIS

The Packaging, Design, and Experimental Evaluation of a MEMS Electrophoretic System
by
Steven J. Wong
Master of Science in Mechanical Engineering
San Diego State University, 2007

This thesis investigates the behavior of carboxyl bead accumulation to pairs of positively biased electrodes on chips manufactured using Multi-User MEMS Processing (MUMPS). A testing apparatus was designed to investigate bead accumulation on a chip. Methods of attaching copper wires to the chip were explored in addition to optimization and bead migration studies. After testing of an initial chip design, an improved design was proposed, manufactured, and integrated into the bead migration set-up. Studies varying voltage conditions and switching the polarities of the electrodes were conducted on the design and demonstrated bead accumulation to the positively biased electrodes within 5 minutes at 3 Volts. Results from experimentation suggest that this device can be utilized to concentrate negatively charged particles (such as DNA) as part of a lab-on-a-chip setup with lysing and pumping technology already demonstrated in previous research.
TABLE OF CONTENTS

ABSTRACT .......................................................................................................................... vii
LIST OF TABLES ............................................................................................................. viii
LIST OF FIGURES .......................................................................................................... ix
ACKNOWLEDGEMENTS ................................................................................................. xi
CHAPTER
1 INTRODUCTION ........................................................................................................... 1
2 LITERATURE SEARCH ............................................................................................... 3
   Lab-on-a-Chip ........................................................................................................... 3
   Fabrication Technology ....................................................................................... 4
   Detection Techniques ....................................................................................... 6
   Applications ....................................................................................................... 9
Pathogen Detection .................................................................................................... 10
   PCR-Based Methods ...................................................................................... 11
   Microarray-Based Methods ............................................................................. 14
      Passive Array Technology: Affymetrix ....................................................... 14
      Active Array Technology: Nanogen .............................................................. 15
3 DESIGN ...................................................................................................................... 18
   Chip Design using Multi-User Mems Processing ........................................... 18
      Design #1 ...................................................................................................... 19
      Design #2 ...................................................................................................... 19
   Packaging ......................................................................................................... 24
4 EXPERIMENTATION .................................................................................................. 27
   Verifying the Charge Nature of the Beads: ................................................... 27
      Experiment 1: Bead Migration on a 0.5% Agarose Gel ......................... 27
      Experiment 2: Time Course of Bead Migration in a Gel Box ................. 30
   Experimental Setup on Design #1 ................................................................. 32
      Optimization of Experimentation ............................................................... 36
Bead Migration Studies on Design #1 ...............................................................40
Direct Contact Methods Explored on Design #2 ...........................................47
Bead Migration Studies on Design #2 .............................................................50
Femlab Analysis ..............................................................................................54

5 DISCUSSION AND CONCLUSIONS ...........................................................59

REFERENCES ..................................................................................................61
LIST OF TABLES

Table 1. FDA-Approved PCR-Based methods for detection of pathogens. ......................... 11
Table 2. Various conductive pastes used to adhere wires to chip..................................... 34
Table 3. Solutions proposed for various problems in the initial design............................... 36
Table 4. Optimization of voltages for design #1................................................................. 45
Table 5. Optimization of voltages for design #2............................................................... 52
Table 6. Voltage and migration velocity values ................................................................. 54
LIST OF FIGURES

PAGE

Figure 1. San Diego State research plan towards a portable lab-on-a-chip device ..................2
Figure 2. An example of a lab-on-a-chip device for nanoscale DNA analysis .....................4
Figure 3. Photolithography used to couple oligonucleotides onto an Affymetrix chip ..........6
Figure 4. (A) Capillary electrophoresis device that utilizes electrospray ionization
techniques to analyze carnitines. (B) Flow from the device to the exit port. ........8
Figure 5. An illustration of PCR. (1) denaturing. (2) annealing of primers. (3) elongation with polymerase. (4) completion of PCR cycle .........................12
Figure 6. Accumulation of DNA at the anode of a Nanochip® ..................................17
Figure 7. PolyMUMPS process file .................................................................18
Figure 8. First generation electrodes .................................................................19
Figure 9. Second generation electrodes .............................................................21
Figure 10. Cross section of optimized design ......................................................21
Figure 11. CAD file depicting castellated electrodes ...........................................22
Figure 12. First structural layer of polysilicon (yellow) is deposited onto a layer of nitride (light blue). .................................................................22
Figure 13. Second structural layer of polysilicon (brown) is deposited onto the first structural layer. .................................................................23
Figure 14. Metal layer (dark blue) is deposited onto the second polysilicon layer. ......23
Figure 15. Top and side views of the packaging apparatus and coverslip .................24
Figure 16. Copper wires threaded through holes on a coverslip .............................24
Figure 17. Solidworks schematic of the packaging apparatus (all values in millimeters) .................................................................25
Figure 18. Close-up of testing chamber in packaging apparatus .........................26
Figure 19. Electrophoresis setup of Dynabeads on a 0.5% agarose gel .....................28
Figure 20. Loading of samples in experiment 1 ...................................................28
Figure 21. Results of experiment 1 show no migration of Dynabeads ..................29
Figure 22. Pore size on high melting and low melting agarose gels set at various temperatures .................................................................30
Figure 23. Beads placed onto a gel box containing TAE buffer .............................31
Figure 24. Time course of electrophoresis of Dynabeads in TAE buffer shows migration within 10 minutes at 135 volts.

Figure 25. A high power microscope was connected to a laptop in order to view the chip under high magnification.

Figure 26. Attaching copper wires onto electrodes: Sequential steps are from left to right and top to bottom.

Figure 27. Mess associated with using conductive adhesives.

Figure 28. Results of first bead migration experiment show no concentration or accumulation of beads at the electrode interface.

Figure 29. Fogging of the coverslip decreased visibility of chip.

Figure 30. Gluing with silverpaste improved after repeated practice.

Figure 31. Bubbling problems at negative electrode interface.

Figure 32. Injection of beads near the electrode interface.

Figure 33. Bead migration studies started after diffusion became steady.

Figure 34. Accumulation of beads at the positive electrode and repulsion of beads at the negative electrode trial 1.

Figure 35. Accumulation of beads at the positive electrode and repulsion of beads at the negative electrode Trial 2.

Figure 36. Accumulation of beads at the positive electrode and repulsion of beads at the negative electrode after switching dipoles.

Figure 37. Micromanipulator.

Figure 38. Copper wires connected from the power source to the chip are folded inside the micromanipulator.

Figure 39. A magnified view of the electrodes using the Probe Station.

Figure 40. A power source was connected to the Probe Station in order for bead migration studies to occur.

Figure 41. Migration of beads to the positive dipole using a probe station.

Figure 42. Bead migration experiment using direct contact on design #2.

Figure 43. A 2D model of the electric field for design #2.

Figure 44. A Femlab graphical model of the electric field in the region between the two electrodes.

Figure 45. The surface electric field of the two electrodes using Femlab.
ACKNOWLEDGEMENTS

I am sincerely grateful for the guidance provided by my thesis advisor, Dr. Samuel Kassegne. I would also like to thank Dr. Karen May-Newman and Dr. Paul Paolini for their time serving as committee members. Lastly, I would like to thank Alex Teeter and Bao Nguyen for their invaluable input.
CHAPTER 1

INTRODUCTION

Microelectromechanical Systems-based (MEMS) approaches have been adapted in such varied areas as DNA chips (1) and lab-on-a-chip systems (2). Lab-on-a-chip technology enables sample-to-answer mechanisms for the studying of biological systems. Research at the MEMS Lab at San Diego State University has focused on a miniaturized lysing apparatus (3), a microfluidic pumping system (in progress), and a novel submicron trapping mechanism (4). With current MEMS-based approaches, it is possible to fabricate a lab-on-a-chip device by combining sample-preparation steps for applications in pathogen identification and detection (figure 1). Traditional assessment methods for pathogens need improvement, as standard culture techniques are costly and may require long time delays (5, 6). A timely and accurate assessment of pathogen infection is desired in many cases for appropriate patient triage, infection control, and treatment (5). In addition, rapid early detection and miniaturization can limit the potential threat of bioterrorism. With increasing bioterrorist activity, miniaturized, portable detection methods are desired for public safety (7).

The molecular diagnostics industry has been expanding with the completion of the human genome project. Genetic sequences for humans, animals, and viruses have been used to generate PCR-based strategies to study gene expression and disease progression. Diagnostic kits are available for use and have provided clinicians with accurate assessments of disease states. In addition, microarray technology, both passive and active, has been useful in the quantification of gene expression and sequence determination. Single nucleotide polymorphisms, deletions, insertions, and DNA mismatches can all be detected using hybridization technologies present in passive and active microarrays. Passive systems utilize diffusion as a means for DNA hybridization and as a result can take longer to complete. In contrast, active microarrays offer increased transport times by utilizing the negative charge-nature of DNA as a way to concentrate DNA to a positively biased electrode within a short period of time (8).
This thesis seeks to utilize an active-microarray-based approach to concentrate negatively charged molecules to a pair of interdigitated electrodes. In particular, the packaging, set-up, and experimental evaluation of this system will be explored using carboxyl charged beads. Applications of this device will be geared towards pathogen detection as part of a lab-on-a-chip assembly.

Figure 1. San Diego State research plan towards a portable lab-on-a-chip device.
CHAPTER 2

LITERATURE SEARCH

This chapter represents a broad overview of the state and technology of lab-on-a-chip and pathogen detection systems. Emphasis will be placed on lab-on-a-chip fabrication and detection techniques as well as present DNA microarrays technologies.

LAB-ON-A-CHIP

Traditionally, chemical analysis procedures are performed in specialized laboratories that require skilled personnel and specialized equipment. The trend nowadays is to move the chemical analysis closer to the consumer and away from the laboratories. Advances in microfluidics and the MEMS field have expanded this concept so that sophisticated biological tests can occur on miniaturized platforms. Manz and coworkers first introduced the lab-on-a-chip concept in the early 1990s (9). In their paper, they described a device in which all stages of preparation and analysis can be done on a single chip. Examples of these processes can be seen in figure 2 and include sample loading, chemical and thermal reactions, gel loading, and electrophoresis (10). Since then, there has been a wealth of companies interested in developing lab-on-a-chip products, and the amount of patents generated by companies within the microfluidic field has increased significantly (11).

There are numerous advantages of miniaturization as seen on lab-on-a-chip devices. Samples processed on lab-on-a-chip are beneficial for tracking of pollutants (12), monitoring of nutrients in agriculture (13), and identifying explosives in unknown environments (14). In addition, the reduction in volume of reagents used on these devices significantly reduces the cost of biological assays. The following section describes a brief overview of lab-on-a-chip technology. Included in this overview are sections on fabrication technology, detection techniques, and lab-on-a-chip applications.
Fabrication Technology

Lab-on-a-chip devices borrow fabrication techniques generally used in the microelectronics industry. The first lab-on-a-chip devices were made of silicon or glass/quartz (15). There are a variety of fabrication processes available for silicon materials due to its extensive applications in microelectronics. In addition, many processes applied to silicon materials can also be applied to glass processing (16). Standard lithography and etching techniques, which generally require the use of flat substrates, are used to shape channels and chambers on devices in the micrometer scale for both silicon and glass substrates.

Photolithography techniques can be used as a way to couple oligonucleotides to a chip. An example of this is seen in Affymetrix’s Genechip®. The photolithographic process on a Genechip starts with coating a 5-inch by 5-inch quartz wafer with light-sensitive chemical compounds. Photolithographic masks are used to either allow or prevent transmission of light. Exposure of this compound to light allows for coupling of nucleotides.
Those areas that are not exposed are safe from coupling. Once photolithography is complete, a solution containing adenine, thymine, guanine, or cytosine couple to those regions where light is allowed to pass through. The process is then repeated indefinably to generate probes that are around 25 nucleotides. An example of this process is seen in Figure 3.

In addition to lithography, there are many etching processes that can be applied on chips. Sample reservoirs and microchannels are a few items that can be manufactured with dry or wet etching. Deep Reactive Ion Etching (DRIE), Plasma-Assisted Etching, Vapor Etching, and Wet Chemical Etching can all be used to create lab-on-a-chip products (17). These etching processes can be used alone or defined on batch fabrication. For example, in batch fabrication with MUMPS (Multi User MEMS Processing), DRIE is selectively used to etch out silicon channels. Batch fabrication is generally used when manufacturing in high volume, and offers the potential for cost reduction.

The connection of components together on a chip can be accomplished by various means depending on the materials to be bonded. High temperature fusion bonding is used to connect silicon components together on a chip, and electric field-assisted anodic bonding is used to bond silicon and glass layers. Thermal bonding is used when joining two glass layers together. Self-adhesive films, such as poly (dimethylsiloxane) has also been used to seal layers together (15).

In addition to silicon and glass, the use of polymeric materials has been increasing over the last few years. The fabrication techniques that can be used on these materials can be classified into direct fabrication and replication techniques. Laser photoablation, reactive ion etching, X-ray lithography, and mechanical milling are considered direct fabrication techniques. Injection molding, hot embossing, and polymer casting are considered to be replication methods (15).
Detection Techniques

High sensitivity is desired in lab-on-a-chip detection techniques due to the small volume of samples loaded. Detection methods for lab-on-a-chip devices include optical and electrochemical approaches as well as methods using mass spectrometry. In addition, new research in the field of laser-induced excitation (LIF) has shown that discrimination of single molecules has been achieved on lab-on-a-chip devices (19). In most microfluidic applications thus far, optical detection has been the most reliable. Work in this field aim to increase the optical detection path length, and most of the research has been done on capillary electrophoresis chips. For example, Liang et al. (20) has shown that in micromachining a U-shaped detection cell with a longitudinal path length of 120-140 μM, a 20-fold increase in the signal-to-noise ratio was observed. This effect was hypothesized to be due to a reduction in scattering. Morgensen et al. (21) has shown that in connecting waveguides to a chip, one can influence the optical path length. Crabtree et al. (22) has shown that a Shah Convolution Fourier Transform (SCOFT) method can increase the signal-to-noise ratio on the chip.

Another detection method that has been increasingly gaining interest in the past few years is the use of mass spectrometry for in-chip analysis. Advantages of using a microchip in conducting mass spectrometry studies are numerous. Flow rates generated with microfluidic devices (nl-μL/min scale) present good sensitivity. In addition, parallel analysis
for high-throughout screening can be done by integrating microfluidics with mass spectrometry. There have also been many groups that have scaled down the size of mass analyzers. However, present miniaturization technology pales in comparison with conventional sized mass spectrometers (15).

The current method of choice for on-chip detection using mass spectrometry is electrospray ionization (15). An example of a capillary electrophoresis microchip that uses this technology is seen in Figure 4. In this device, glass chips were microfabricated with channels for capillary electrophoresis. Miniaturized electrosprayers were connected to the chip’s exit via a liquid junction interface. Wells were then pressurized with nebulizing gas so as to allow for sample transport into an electrospray exit port. Mass spectrometry was then used to identify sample content. Deng et al. (23) has used this device to analyze carnitines drugs spiked in human plasma and urine. In their experiments they successfully identified three acylcarnitines and carnitine in a synthetic mixture.

Other means of detection exists and includes chemiluminescence detection and Raman spectroscopy. Chemiluminescence detection offers the advantage of not needing a light source, which makes for simplified instrument configuration. Raman spectroscopy has been used to analyze herbicides at the ppb detection level (15).
Figure 4. (A) Capillary electrophoresis device that utilizes electrospray ionization techniques to analyze carnitines. (B) Flow from the device to the exit port.²³
Applications

The majority of existing research in this field deals with the use of lab-on-a-chip for separation purposes using capillary electrophoresis. High efficiency separations of peptides, amino acids, and proteins have been carried out on glass microdevices. With this setup, however, surface passivation of glass is needed to prevent absorption of specimens onto glass (15). Detection of these specimens is usually carried out fluorescently with the aid of specialized microscopes and scanners.

There has also been an increase in the use of lab-on-a-chip for drug discovery purposes (24, 25, 26). In miniaturizing a system for drug discovery, there is usually no change in the nature of molecular interactions. However, molecular diffusion and heat transfer relations in the micro realm enable the occurrence of high efficiency interactions. The characteristic of improved heat transfer on microchips has been used to synthesize chemical libraries for drug screening. In this process the concentration of reagents is reduced, in addition to improved mass and heat transfer, which enables faster synthesis of molecules. Watts et al., (27) have shown that a dipeptide synthesis procedure can be reduced from 24 hours to a period of 20 minutes when using this microchip.

Lab-on-a-chips has been vastly used for analytical applications. There have also been considerable efforts in the miniaturizing of fluorescence-activated cell-sorting devices (28, 29). Although miniaturized flow cytometers offer a maximum speed of sorting several hundred cells/second, conventional flow cytometers are still much more efficient with speeds up to thousands of cells/second (30). The advantages of sorting with a micro flow cytometers are the use of small volumes and ability of massive parallelization for drug discovery purposes. At present, DNA fragments and fluorescent bacterial cells have been sorted with a microfluidic chip at high-throughput rates (30). With this technology, the drug discovery process can become faster, more robust, and more cost efficient due to ease of parallelization.

Other applications for lab-on-a-chip include point-of-care diagnostics, DNA sequencing, and PCR amplification. In addition, blood analysis on lab-on-a-chip have been successful in analyzing biochemical content of blood, and DNA microarrays have been useful in answering questions regarding gene expression (31).
**Pathogen Detection**

Hospitals on average record well over 5 million cases of infectious disease-related illnesses annually (32). Inabilities to recognize, diagnose, and treat these illnesses can result in death depending on the severity of the disease. It has been well documented that the outcomes from infection correlate directly with time to pathogen identification (5). Currently, conventional laboratory methods of detection have several limitations including prolong assay times and the inability to culture certain pathogens. The failure of clinicians in obtaining accurate data has led some to a more conservative approach in treatment with the use of intravenous antibiotics (5). Maximum patient safety and improved outcomes have been observed with this treatment plan. However, unnecessary hospitalization, increase in antimicrobial resistance, and increase in cost can be a result of this conservative approach to treatment. There is a dire need to provide and expose clinicians to fast, affordable, and accurate methods to diagnose infection. The recent increase in bioterrorist events and the recent outbreak of Severe Acute Respiratory Syndrome (SARS) calls to attention the global importance of reliable diagnostic tests. With the myriad of data available on genetic sequences of pathogens, including SARS (33), and the H5N1 (34) strain of avian influenza, the uses of molecular diagnostics tools have been valuable in diagnosis of pathogen infection. Several companies have products that use various molecular tools that identify pathogens by targeting their genetic sequence (table 1). Unfortunately, the FDA has approved a limited number of molecular diagnostic assays, and still fewer are the preferred mode of diagnostic test for clinicians. A discussion of current diagnostic tools available for pathogen detection including PCR-based and microarray-based approaches is reviewed as follows.
Traditional approaches of pathogen identification require the observation of phenotypic characteristics of microbes. This approach offers less sensitivity than sequence-based hybridization approaches, which probe for hybridization of microbial DNA to specific sequences. Greater specificity and an elimination of excessive incubation times are some of the advantages of DNA hybridization approaches. The development of Polymerase Chain Reaction (PCR) has enabled the study of the genetic content of pathogens. PCR is an enzyme-driven process that amplifies short stands of DNA in-vitro. Amplification is accomplished through a series of annealing, elongating, and denaturing steps as seen in Figure 5.
The first step in running a PCR reaction is to design primers, which are short oligonucleotide sequences that are homologous to the beginning and end of the DNA fragment to be amplified. In regards to pathogen detection, the primers are a pair of oligonucleotides that flank the sequence that is to be detected. These oligonucleotides are typically 15-25 base pairs long and are designed on a number of considerations. GC content (which is the amount of guanine and cytosine residues in the primer), melting temperature,
and the generation of hairpin loops and primer-dimers are considered in optimizing the primer for good annealing. The process is described as follows:

1. The double-stranded DNA template is heated to temperatures between 94-98 degrees Celsius in a denaturing step.
2. The temperature is lowered to facilitate annealing of primers to single stranded DNA sequences.
3. The temperature is increased to a value appropriate for enzymatic activity of the DNA polymerase. The time of incubation in this step depends on the length of DNA to be amplified. A rule of thumb is that it generally takes one minute per kilobase of DNA for complete amplification.
4. The steps of denaturing, primer annealing, and elongation is repeated (this is typically 20 – 35 cycles).

Theoretically, billions of copies of DNA can be generated within a couple hours using PCR. This increase in sensitivity, however, does have its shortfalls including the generation of false positives and false negatives from background DNA contamination. However, various tests on the market that uses PCR as a means to probe for microbial content perform with good clinical sensitivity. Roche has several tests on the market using PCR as its primary means of detection including tests for Chlamydia trachomatis, HCV, tuberculosis, and Neisseria gonorrhoeae. Studies on these molecular diagnostic tools show good clinical sensitivity greater than 90% (table 1). However, it is unfortunate that even with good sensitivity, specificity, and speed of amplification applications of these devices in the clinical area has been moderately popular, with most clinicians still preferring the traditional approach (4).

It has been also suggested that a universal diagnostic system can be manufactured using PCR based methods. Experimental evidence shows that the 16S rRNA gene is evolutionarily conserved exclusively in bacterial species (35). Designing primers specific to this gene can allow for a universal detection system. Using this method, clinicians would be able to identify the presence of bacteria in an otherwise sterile clinical specimen (i.e. whole blood). In theory, this procedure can also be applied to a taxonomic group of pathogens by recognizing and amplifying commonly conserved regions specific to the infectious agent (5).
Other PCR methods exist for probing pathogen content and include Real-Time PCR amplification, Fluorescence Resonance Energy Transfer (FRET), Transcription-Mediated Amplification (TMA), Nucleic Acid Sequence-Based Amplification (NASBA), Ligase Chain Reaction (LCR), Strand Displacement Amplification (SDA), and Linear Linked Amplification (36).

**Microarray-Based Methods**

A microarray is a solid device, usually a chip, which contain a series of probes immobilized onto it. These probes are short oligonucleotide fragments arranged in an ordered fashion. Complementary base pair binding of tagged probes is the main method in which detection occurs. Microarrays have a high capacity for data generation, and can probe between 20,000 - 40,000 genes in a single experiment (7). This allows researchers to explore genes and pathways that are activated in an infection or a disease state without knowing anything about the genetic content of the sample. This has accelerated the understanding of complex interactions between microorganisms and the host and has brought an improvement in diagnosis and treatment of infectious diseases. In regards to pathogen applications, microarrays have been used to investigate a number of key areas including detection of mutations on genes on the human immunodeficiency virus (37) and Mycobacterium tuberculosis (38). In addition, with the recent crisis regarding transmission of avian flu from birds to humans, microarrays have been developed to identify and track mutations within its genetic makeup (CombiMatrix).

Microarrays can be classified into passive or active array technology. Affymetrix’s GeneChip® and Nanogen’s Nanochip® are examples of passive and active array technology respectively.

**Passive Array Technology:**

**Affymetrix**

Affymetrix has leveraged itself to be a dominating force within the microarray field. Based in the Silicon Valley, they manufacture a chip that allows for gene expression and sequence analysis. GeneChip® is a high-density microarray fixed with an ordered arrangement of probes. The chip is fabricated using methods developed in the microelectronics industry. Oligonucleotides of 25 bases in length are fixed onto the chip by
photolithographic means (see Figure 3). These oligonucleotides function as probes and allow for the identification of complementary DNA base pair hybridization with sample content.

The applications with GeneChip® range well beyond pathogen detection and include cancer, immunology, and cell-signaling research. Different chips, consisting of a predetermined set of oligonucleotide probes, are used for the many areas of applications identified. For example, microarrays with probes designed for infectious disease applications can be used to study host-pathogen interactions, strain characterization, transcriptional regulation, and vaccine development. On the other hand, point mutations, translocations, deletions, and altered gene expression can be studied with microarrays that have been designed for research applications.

The primary means of hybridization with the Affymetrix chip is diffusion. Typically a 16-hour incubation with the sample and the probes is necessary to allow for good hybridization reactions. Bishop et al (39) have studied the kinetics of hybridization on passive biochips. In their studies they modeled the delivery of the analyte to the surface with Fick’s law as described as $\frac{\partial C}{\partial t} = D \nabla^2 C$. In this equation, $C$ is the concentration of target in solution and $D$ is the diffusion coefficient. The affinity interaction between the probes and the target molecules can be similarly described by the equation $\frac{dB}{dt} = k_a C (R_t - B) - k_d B$. In the affinity equation, $k_a$ is the association rate constant, $k_d$ is the dissociation rate constant, $R_t$ is the initial surface concentration of probes, and $B$ is the surface concentration of bound targets.

Passive biochips have the advantage of being simple and easy to use, however, active biochips have been shown to decrease the incubation time for hybridization by employing electrophoresis as a means of transporting and concentrating DNA.

**ACTIVE ARRAY TECHNOLOGY: NANOGEN**

Nanogen has designed active microarrays whose primary means of transport is electrophoresis of DNA molecules. The Nanochip® is a microchip that consists of electrical and microfluidic components. Each test site on the chip is 80 microns in diameter and consists of underlying platinum microelectrodes (40). These microelectrodes provide a positive biased for DNA attraction and hybridization. On top of the microarray are several microns of hydrogel impregnated with streptavidin. Electronic addressing methods allow
biotinylated oligonucleotide probes to attach to the streptavidin molecules within the hydrogel layer. This method allows the user to tailor the microarray to the unique experiment planned as opposed to using a standard microarray. In using the Nanochip®, a probe loader component allows these oligonucleotides to be loaded with DNA from a 96 well or 384 well microtiter plate as well. Currently, the MGB Eclipse probe system is used for applications involving detection and quantification of pathogens such as the Herpes Simplex Virus (HSV). Once probes have been loaded various electronic hybridization assays can be carried out with fluorescently labeled samples, and the chip can be analyzed by fluorescent detection. Fluorescent DNA samples that hybridize to the probes can be detected with the system provided by Nanogen.

Nanogen has shown that active microarrays can reduce the long assay times seen in passive hybridization approaches. It was shown that by concentrating DNA on the chip by electrophoretic means, the rate of hybridization could be shortened to minutes as opposed to hours. Theoretical models correlated well with experimental models as Kassegne et al., (41) have shown hybridization kinetics both numerically and experimentally (Figure 6). Their research compared finite element computer simulation to actual experimentation. Finite element models were based on laws of fluid flow, diffusion, and electrophoresis. Percent accumulation was modeled using analytical approaches.
Figure 6. Accumulation of DNA at the anode of a Nanochip®.

The state of active microarrays is still in its infancy. Both Sosnowski (40) and Kassegne (41) have provided useful information on optimization of electrophoretic transport, electrode size, electrode location, and electrode configuration. Active microarrays are currently insufficient in carrying out gene expression analysis on a global scale. Nanogen’s largest chip has 400 test sites and probes for single nucleotide polymorphisms, short tandem repeats, insertions, and deletions. The lack of a large number of sites makes high-throughput analysis improbable with the technology today. Future research in the field of microarrays are geared towards increasing the amount of test sites available and also incorporating microarray technology as part of a lab-on-a-chip system, where sample preparation, detection, and analysis are all done on a single chip.
CHAPTER 3

DESIGN

A packaging apparatus and pairs of interdigitated electrodes were fabricated using CNC and MEMS manufacturing technology respectively. This section describes the integration of two designs into an experimental setup proposed for the study of bead migration.

CHIP DESIGN USING MULTI-USER MEMS PROCESSING

The chips were fabricated using Multi-User Mems Processing (MUMPS). This is a standard process that allows many designs to be placed onto a silicon chip. More specifically PolyMUMPS was used to create the interdigitated electrodes. PolyMUMPS is a bulk micromachining process that utilizes three structural layers of polysilicon, two sacrificial layers, and one metal layer. Twenty-two processes consisting of Low Pressure Chemical (LPCVD) Vapor Deposition of substrates and etching by Reactive Ion Etching (RIE) are used to create the final product (Figure 7). The following sections describe two designs that were manufactured with PolyMUMPS.

<table>
<thead>
<tr>
<th>Number</th>
<th>Step Name</th>
<th>Action</th>
<th>Layer Name</th>
<th>Material Name</th>
<th>Thickness</th>
<th>Mask Name</th>
<th>Photoresist</th>
<th>Etch Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Definition Schematic</td>
<td>Substrate</td>
<td>Substrate</td>
<td>SILICON</td>
<td>300</td>
<td>GND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>LPCVD Deposition 500nm SiN&lt;sub&gt;x&lt;/sub&gt;</td>
<td>Stack Material</td>
<td>Nide</td>
<td>SiN&lt;sub&gt;x&lt;/sub&gt;_PolyMUMPs</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>LPCVD Deposition 500nm Poly</td>
<td>Stack Material</td>
<td>Poly</td>
<td>POLYSILICON_PolyMUMPs</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Etch RIE 500nm Poly</td>
<td>Straight Cut</td>
<td>Poly0</td>
<td>Poly0</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Etch RIE 500nm Poly</td>
<td>Straight Cut</td>
<td>Poly0</td>
<td>Poly0</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>LPCVD Deposition Phosphosilicate 2000nm</td>
<td>Conformal Shell</td>
<td>Oxide1</td>
<td>PSG</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Etch RIE 750nm PSG</td>
<td>Straight Cut</td>
<td>Oxide1</td>
<td>Oxide1</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Etch RIE 2000nm PSG</td>
<td>Straight Cut</td>
<td>Anchor1</td>
<td>Anchor1</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Etch RIE 2000nm PSG</td>
<td>Straight Cut</td>
<td>Anchor2</td>
<td>Anchor2</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>LPCVD Deposition 2000nm Poly</td>
<td>Conformal Shell</td>
<td>Poly1</td>
<td>POLYSILICON_PolyMUMPs</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Etch RIE 2000nm Poly</td>
<td>Straight Cut</td>
<td>Poly1</td>
<td>Poly1</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Etch RIE 2000nm Poly</td>
<td>Straight Cut</td>
<td>Anchor2</td>
<td>Anchor2</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Etch RIE 2000nm Poly</td>
<td>Straight Cut</td>
<td>Anchor2</td>
<td>Anchor2</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>LPCVD Deposition Phosphosilicate 750nm</td>
<td>Conformal Shell</td>
<td>Oxide2</td>
<td>PSG</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Etch RIE 2000nm Poly</td>
<td>Straight Cut</td>
<td>Poly1_Poly2</td>
<td>Poly1_Poly2</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Etch RIE 2000nm Poly</td>
<td>Straight Cut</td>
<td>Anchor2</td>
<td>Anchor2</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>LPCVD Deposition 1500nm Poly</td>
<td>Conformal Shell</td>
<td>Poly2</td>
<td>POLYSILICON_PolyMUMPs</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Etch RIE 1500nm Poly</td>
<td>Straight Cut</td>
<td>Poly2</td>
<td>Poly2</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Etch RIE 1500nm Poly</td>
<td>Straight Cut</td>
<td>Poly2</td>
<td>Poly2</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>LPCVD Deposition 750nm SiN&lt;sub&gt;x&lt;/sub&gt;</td>
<td>Conformal Shell</td>
<td>Metal</td>
<td>METAL_PolyMUMPs</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Etch RIE 750nm SiN&lt;sub&gt;x&lt;/sub&gt;</td>
<td>Straight Cut</td>
<td>Metal</td>
<td>Metal</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Etch RIE 750nm SiN&lt;sub&gt;x&lt;/sub&gt;</td>
<td>Straight Cut</td>
<td>Metal</td>
<td>Metal</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>HF (4%) Release Etch</td>
<td>Demise</td>
<td>PSG</td>
<td>PSG</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 7. PolyMUMPS process file.
Design #1

The initial design was proposed by Teeter (4) in his thesis. This design is a series of castellated electrodes in which one is positively biased and the other is negatively biased. These electrodes function as to concentrate oppositely charged molecules to their corresponding electrode. Negatively charged particles will migrate to the positive electrode and vice versa.

After extensive testing of Teeter’s design, it was proposed that a new design was needed to accommodate bead migration testing and to resolve unforeseen issues that occurred during manufacturing. For example, tracing elements were washed off during the manufacturing process due to its small diameter (1 micron). In addition, the dimensions of the bump pads on this design were inadequate to provide enough surface area for attachment of copper wires. The metal layer also easily flaked off this chip and was not optimally placed for strong adhesion to the structural layers. A photograph of this chip is seen in Figure 8.

Figure 8. First generation electrodes.

Design #2

Teeter’s design was optimized for bead migration experiments (Figure 9 and 10). More specifically bump pad, tracing, and electrode dimensions were increased. In the new design, the metal layer deposition was changed so that it was placed on top of the second
polysilicon structural layer (instead of onto the nitride layer) to maximize adhesion of the gold to the polysilicon.

The dimensions of the bump pads were increased 10 fold for optimized wire attachment. It was observed through experimentation that a large surface area for wire attachment was necessary because attachment with silver paste proved to be messy at times. In Teeter’s design the bump pads were 10 microns by 10 microns, which was hard to visualize with the microscope. As a result, wire attachment to the small bump pads proved to be difficult. The dimensions in the new design were increased to 100 microns by 100 microns to allow for ease of wire bonding onto the bump pads.

The dimensions of the tracing elements were increased 25 fold in the new design. In Teeter’s design the tracing elements washed in the manufacturing process due to its small size. An increase in diameter from 1 micron to 25 microns would cause for a more stable element as the amount of surface area is increased.

The dimensions of the electrodes were also increased to provide an optimized platform for bead migration studies. It was seen in experimentation that bead migration between small electrodes elements were visually hard to observe with the microscope. Increasing the spacing and sizing of the electrodes would hopefully allow for one to observe the migration of beads within each of the castellated fingers.

The design was created using the program Coventorware. This is a CAD-based program that allows for design of MEMS-based devices. The CAD schematic for the castellated electrodes can be seen in Figure 11. For this design, the PolyMUMPS process was used to fabricate the electrodes. The chip was made at the foundry and details of its fabrication can be seen in Figures 12-14.
Figure 9. Second generation electrodes.

Figure 10. Cross section of optimized design.
Figure 11. CAD file depicting castellated electrodes.

Figure 12. First structural layer of polysilicon (yellow) is deposited onto a layer of nitride (light blue).
Figure 13. Second structural layer of polysilicon (brown) is deposited onto the first structural layer.

Figure 14. Metal layer (dark blue) is deposited onto the second polysilicon layer.
PACKAGING

An acrylic coverslip was fabricated using machine shop tools. The coverslip functioned to keep liquid from overflowing out of the channels and injection ports located on a packaging apparatus (Figure 15). The coverslip consisted of a set of holes that were drilled to 2 mm in diameter. Two of these holes were stationed in the center of the injection port and exit ports so as to allow pipetting of fluid into and out of the packaging apparatus. Four of these holes were stationed directly above the testing chamber and functioned to minimize fogging issues, to minimize pressure buildup and to provide threading holes for copper wires (Figure 16).

Figure 15. Top and side views of the packaging apparatus and coverslip.

Figure 16. Copper wires threaded through holes on a coverslip.
The packaging apparatus was modeled using Solidworks (Figure 17) and was fabricated using Computer Numerical Control (CNC) Machining available through the SDSU Mechanical Engineering department. This platform was made out of acrylic and consisted of an inlet injection port, an exit port, a testing chamber, and two microfluidic channels. The diameter of the exit port was designed to be much bigger than the inlet injection port so as to provide sufficient space for waste accumulation. At 0.5 cm deep, the exit port was designed to hold 883 μL of fluid. Excess fluid would also be able to leave the exit port by direct pipetting methods. The testing chamber (Figure 18) was designed for chips 1 cm in width by 1 cm in length. An excess of 1 mm was allotted to the width and length dimensions of the chamber so as to provide ease of placement of chips. This also allows for interchangeability of chips to be tested on this platform (as 1 cm dimensions are standard for chips fabricated with MUMPS).

Figure 17. Solidworks schematic of the packaging apparatus (all values in millimeters).
Figure 18. Close-up of testing chamber in packaging apparatus.
CHAPTER 4

EXPERIMENTATION

Experiments were carried out to verify the charge nature of the carboxyl-modified beads, to optimize the experimental setup, and to perform bead migration studies.

VERIFYING THE CHARGE NATURE OF THE BEADS:

Carboxyl-charged Dynabeads (Invitrogen) were obtained for bead migration studies. The beads are 0.8 microns in diameter and were carboxyl modified so as to provide a negative charge. The beads came as a stock at a concentration of 30 mg/mL and were diluted to various concentrations in a 50 mM Histidine buffer. Two experiments were performed to verify the electrically negative nature of the beads.

Experiment 1: Bead Migration on a 0.5% Agarose Gel

In the first experiment, a gel electrophoresis setup was constructed as seen in Figure (19). Briefly, a 0.5% high-melt agarose gel was made at room temperature and placed in a small gel box. A power supply was connected to the gel box and TAE buffer was poured in the box until the gel was completely immersed in buffer. The samples were loaded as seen in Figure (20). 10 microliters of the following solutions were applied directly to the wells: Lanes 1 and 2 acted as controls with bromophenal blue loading dye and NEB 1 micron DNA ladder loaded respectively. Lane 3 consisted of beads diluted to 5 mg/mL in 50 mM histidine and lane 4 consisted of a 1:1 mixture of loading dye and beads. The gel was run at 135 volts for 30 minutes.
The results of this experiment are seen in Figure 21. The controls (lanes 1 and 2) migrated to the positive dipoles as expected. Bromophenol blue (lane 1) has a slight negative charge and will migrate to the positive dipole. Similarly, the DNA ladder (lane 2) also possesses a net negative charge due the phosphate backbone present in DNA molecules and will migrate to the positive dipole in an electric field. Lanes 3 and 4 showed no migration of beads through the agarose gel. In lane 4 (1:1 mixture of loading dye and beads), the dye
migrated to the positive dipole while the beads remained in the well. Upon close inspection of the lanes, the beads were seen to be strongly attracted to the positive dipole. Although not pictured, there was observed to be an accumulation of the beads to the side of the wells closer to the positive dipole. This correlated well with the nature of negatively charged beads. This experiment was repeated for beads at a lower concentration of .03 mg/mL (not shown) and this phenomenon was also observed.

The results of this experiment suggest that the beads are bigger than the pore size in a 0.5% agarose gel. The beads, at 0.8 microns in diameter, are larger in size than that of DNA as described in Narayanan et al. (42). His group showed that pore size varies directly with agarose type, agarose concentration, and setting temperature (Figure 22). In the case of a 0.5% High-Melt (HM) gel set at room temperature (22 °C), the pore size is predicted to be 0.5 uM. Although bead migration was not seen through the agarose gel, the observation that bead accumulation was greater at the side of the well closer to the positive dipole suggests a negative charge characteristic of the beads. A subsequent experiment was conducted in order to determine this charge characteristic.

Figure 21. Results of experiment 1 show no migration of Dynabeads.
Experiment 2: Time Course of Bead Migration in a Gel Box

In this experiment, an electrophoresis setup was constructed similar to Experiment 1 (see Figure 15). Briefly, a power source was connected to a small gel box. The gel box was filled with TAE buffer, and the bead sample was pipetted directly onto the box instead of into an agarose gel. The beads were observed to sink directly to the bottom of the gel box (Figure 23). After 5 minutes, the power supply was turned on and set to 135 Volts. Digital photographs were taken at 10-minute intervals.

The result of this experiment is seen in Figure 24. At all intervals, it can be seen that the beads are migrating to the positive dipole. This can be clearly seen after placing the beads in an electric field after 10 minutes. At the 20-minute and 30-minute time points, beads were observed to migrate in a smear-like fashion. The non-uniformity of the migration can possibly be attributed to having varying bead sizes and/or charges present. Beads with more carboxyl elements will possess a stronger negative charge and will most likely be more attracted to the positive dipole. A negative control, in which beads were applied to another gel box, was also performed in parallel (not shown). In this case no electric field was applied.
and digital photographs were also taken. It was seen that there was no migration in the negative control sample at all time points. This allowed for further evidence that the migration was due to the presence of the electric field rather than by simple diffusion. After these experiments were performed it was concluded that the carboxyl charged beads possessed a net negative charge.

Figure 23. Beads placed onto a gel box containing TAE buffer.
Figure 24. Time course of electrophoresis of Dynabeads in TAE buffer shows migration within 10 minutes at 135 volts.

**Experimental Setup on Design #1**

An experimental setup was configured using the packaging apparatus and the MEMS chip with the goal of conducting bead migration studies on the interdigitated pairs of electrodes seen in design #1. Various methods of connecting 30 gauge copper wires to the electrodes were explored. The first method involved using silverpaste as a conductive adhesive that would bind the copper wire to the chip. In this method, an experimental setup was configured as seen in Figure 25. A high-resolution camera was connected to a laptop via a USB cable, and was used to view the chip-wire interface under high magnification. Alligator clips were placed onto 30 gauge copper wires, which functioned as fine adjustments in order to place the wires to the appropriate spot on the electrode. Once the wires were placed, a 26 gauge copper wire was then dipped in a silverpaste solution, so that silverpaste was applied to the chip by means of this wire. After silverpaste application, the 30 gauge
wires and the chip were left to dry at room temperature for 4 hours. A step-by-step outline of the setup can be seen in Figure 26.

Figure 25. A high power microscope was connected to a laptop in order to view the chip under high magnification.

Figure 26. Attaching copper wires onto electrodes: Sequential steps are from left to right and top to bottom.
Using silver paste as the conductive adhesive for binding a 30 gauge copper wire to a MEMS chip was seen to be messy in that the silver paste would cover the majority of the electrode (Figure 27). Alternative methods of attachment were explored with the intent of increasing the strength of the chip/wire interface and minimizing the mess associated with using conductive pastes (table 2). The methods explored were using a combination of superglue and silver paste, using a conductive silver pen, and using conductive silver epoxy. In these alternative methods, the same procedure was applied as seen in the first approach with the exception of the conductive adhesive used.

![Figure 27. Mess associated with using conductive adhesives](image)

<table>
<thead>
<tr>
<th>Conductive Adhesion</th>
<th>Strength</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silverpaste</td>
<td>Weak</td>
<td>Messy</td>
</tr>
<tr>
<td>Silverpaste + Superglue</td>
<td>Weak</td>
<td>Messy</td>
</tr>
<tr>
<td>Silverpen</td>
<td>NA</td>
<td>Width of trace drawn in mm scale</td>
</tr>
<tr>
<td>Silver Epoxy</td>
<td>Strong</td>
<td>Viscous and Cumbersome</td>
</tr>
</tbody>
</table>

In using a combination of superglue and silver paste, superglue was first applied to the connection between the copper wires and the electrodes. This was done in order to form a strong bond between the copper wires and the chip. After drying, a layer of silver paste was
applied to the wire-electrode interface for conductive purposes. After testing this method, it was decided that using silverpaste without super glue was sufficient in binding the copper wire to the electrode. That is, adding superglue in this case did not strengthen the bond significantly between the wires and the chip because of the small amount applied to the 30 gauge wire.

In using a silverpen, a trace was drawn from the electrode to another region away from the electrodes. A copper wire was then positioned to the end of the trace, and silverpaste was applied to the copper wire/trace interface. This method seeks to minimize the messiness associated with using silverpaste alone. That is, if the copper wire and silverpaste interface was positioned in a region of the chip away from the electrodes then the mess associated with applying silverpaste to the electrode would not be a significant problem. After several attempts in performing this procedure it was seen that the thickness of the trace drawn by the silver pen was much greater than the dimensions of the components on the chip. For instance, the silverpen would draw traces in the mm scale whereas the components on the chip are in the micron scale. After this was observed, it was decided that this method was incompatible with the dimensions seen on the chip and another method was explored.

In using silver epoxy to bind the copper wires to the chip, equal amounts of bright silver epoxy and gray silver hardener were mixed as per the instructions listed on the product. The silver epoxy mixture was then applied to the 30 gauge wire/chip interface. The wire and the epoxy were left to dry at room temperature for 4 hours as per the product specification. After drying, it was observed that silver epoxy covered the majority of the electrode and appeared to be more viscous than silverpaste.

After exploring all 4 methods of attaching a copper wire to the chip, it was decided that silverpaste alone would be sufficient in connecting the copper wires to the chip (for design #1). The method of using silverpaste + superglue showed comparable adhesion strength to using silverpaste alone. The method of using silver epoxy was a more cumbersome approach with the pre-mix procedure and the relatively higher viscosity of the epoxy, which made it more difficult to apply. Although using silverpaste was seen to be a messy approach, it was determined that this would be sufficient for testing design #1. For testing design #2, a new approach would have to be tested that would limit the amount of mess associated with adhering wires to the chip.
After setup was complete, various migration studies were performed on this platform in order to test for migration of carboxyl charged beads to positively biased electrodes.

**Optimization of Experimentation**

A set of experiments was conducted to determine the best method of studying bead migration. The experimental apparatus and procedure were optimized through several rounds of primary bead migration experiments. Initially, a chip was placed on a testing apparatus in the testing chamber. Copper wires were attached to the electrodes on the chip using the Silverpaste method described earlier. These wires were threaded through holes in a 1 mm thick, acrylic coverslip and connected to a *Hewlet Packard* power supply. Histadine buffer was injected through the injection port until buffer flowed through to the outlet port. Afterwards, electrically charged beads at concentrations of 0.03, 0.3, 3, and 30 mg/mL were injected into the inlet injection port. The power supply was then turned on. Voltages between 1 – 10 volts were used in this study. Bead accumulation was recorded by visual observation using the microscope-laptop setup. The experiment was concluded after 10 minutes. Videos capture and digital photographs comparing before and after shots were used to determine the outcome of the experiment.

The primary goal of the initial bead migration studies was to optimize the setup and to address any problems associated with the experimental design. Although these experiments did not show bead migration (Figure 28), many lessons were learned through these initial failed bead migration studies (table 3).

<table>
<thead>
<tr>
<th>Problem</th>
<th>Effect</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fogging of Coverslip</td>
<td>Reduced visibility</td>
<td>Perform experiments without coverslip to maximize visibility.</td>
</tr>
<tr>
<td>Detachment of Copper Wires</td>
<td>Electrodes not biased properly</td>
<td>Apply more silverpaste to the electrode-wire interface for stronger support</td>
</tr>
<tr>
<td>Bubbling of buffer at negative electrode interface</td>
<td>Reduced visibility</td>
<td>Use lower voltages to minimize bubbling</td>
</tr>
</tbody>
</table>
In the first set of experiments a series of difficulties needed to be overcome. Fogging of the coverslip, detachment of the copper wires, and bubbling of buffer at the negative electrode were some of the problems faced.

Fogging of the coverslip reduced visibility of the chip under the microscope (Figure 29). In addition threading the copper wires through the coverslip posed a significant challenge. Many times the copper wires would detach from the chip when threaded due to excessive manipulation of the wires. Reconstructing the copper wire-electrode interface by reapplying silverpaste onto the electrode was initially done when the wires came off the chip. However, this was a time consuming process and proved to be inefficient. Two methods were explored to alleviate the problem of poor wire attachment namely conducting the experiment without the coverslip and applying more surface area to the wire-electrode interface.

It was decided upon that the series of experiments could be done without the coverslip. Excluding the coverslip solved two problems. Wire manipulation was reduced which resulted in fewer detachment incidences, and also it eliminated any fogging or visibility issues.
In order to address the problem of poor wire attachment more silverpaste was applied to the wire-electrode interface. This increased the surface area of the wires glued onto the chip, which made for a stronger connection. Initially the gluing was sloppy, and the silverpaste covered the whole electrode (Figure 30a). But with practice, the gluing became more precise. After much practice, the copper wire was placed with precision directly onto the electrodes and gluing with silverpaste was cleaner (Figure 30b).
Another problem faced in the initial attempts was bubbling of buffer at the negative electrode. The bubbling occurred due to hydrolysis reactions at the electrodes. This was especially seen at higher voltages (above 4 Volts). Bubbling posed a problem because it obscured visibility of bead migration under the microscope. An example of bubbling is seen in Figure (31). To reduce this effect, lower voltages were used and the time of electrophoresis was increased. This practice significantly reduced any problems associated with bubbling.

Figure 30. Gluing with silverpaste improved after repeated practice

Figure 31. Bubbling problems at negative electrode interface
Although no bead migration was recorded through the initial set of experiments, a wealth of knowledge was gained through the observations made during these trial runs. Fogging, detachment, and bubbling problems all were resolved by using various means. In addition, invaluable experience was obtained during these practice runs. With the observations made during the initial experiments, the bead migration protocol was modified in the next set of experiments so as to allow for an optimized approach of study. Because of the observations and extensive troubleshooting done in the initial stage, bead migration was successfully recorded in the next experiments.

**Bead Migration Studies on Design #1**

Earlier observations revealed difficulties in conducting experiments on bead migration. These difficulties were notably fogging, wire-detachment, and visibility issues. These problems were addressed in the initial rounds of experimental optimization discussed in the previous section. However, even with these problems corrected, the initial experiments still failed to show bead migration. To address this issue, it was decided upon that the protocol of pipetting beads into the injection port would have to change. For the next experiments, a concentrated sample of beads would be injected into the buffer in the area directly above the chip (see Figure 32). This would allow for easier detection of bead accumulation because the beads would be closer to the electrodes, would not have to concentrate in the testing chamber, and would not have to travel through the channel that lead to the testing chamber. Repulsion at the negative electrode and accumulation at the positive electrode would be determined by observation through a microscope.
The revised protocol is described as follows: A microscope setup was arranged as described in the materials and methods section. Copper wires were attached to the electrodes on the chip using the silverpaste method. These wires led to a Hewlett Packard power supply, which provided a constant voltage of 3 volts for this study. The chip was placed into the testing chamber on the testing apparatus and 1 mL of 50 mM Histadine buffer was transferred directly onto the chip. 10 μL of 30 mg/mL Dynabeads was then transferred directly into the buffer in the area above the chip (see Figure 32). The experiment started after 5-10 minutes until the solution had appeared to be in steady state. That is, the experiment started after there was no more visible movement of the beads due to the initial pipetting of the beads into the buffer. Digital photographs were taken within this interval so as to record movement of the beads until steady state (see Figure 33). Afterwards, the power supply was turned on and photographs were taken again at various intervals to allow for depiction of bead migration. Bead accumulation was identified by comparing photographs within this time point to the initial photograph taken at the start of the experiment.
Figure 33. Bead migration studies started after diffusion became steady.

This experiment was conducted twice to show reproducibility of bead migration on two chips. The results can be seen in Figure 34 and 35. As one can see by comparing the photographs within trial 1 (Figure 34), there is a slight attraction of the carboxyl-modified beads towards the positive dipole. However, there was no bead accumulation seen in trial 2, and it was speculated that this was due to strong repulsion forces present within the area photographed. In both trials, repulsion was observed to be much more noticeable than attraction. This behavior can be seen in both cases within a minute of turning on the power supply to 3 volts, and becomes much clearer after 5 minutes of migration. Bubbling was slight at this condition and did not cause visibility problems. Various voltages were tested, as seen in table 4, and the conditions were optimized for minimum disturbance from bubbling.
It was observed that the 3-volt condition was optimal in this study because bubbling caused visibility problems at higher voltages. Testing at voltages lower than 3 volts, took a longer time for beads to concentrate due to the reduction in the strength of the electric field.

Figure 34. Accumulation of beads at the positive electrode and repulsion of beads at the negative electrode trial 1.
Figure 35. Accumulation of beads at the positive electrode and repulsion of beads at the negative electrode Trial 2.
Table 4. Optimization of voltages for design #1.

<table>
<thead>
<tr>
<th>Voltage (V)</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No Bubbling (slow accumulation)</td>
</tr>
<tr>
<td>2</td>
<td>No Bubbling (slow accumulation)</td>
</tr>
<tr>
<td>3</td>
<td>Slight Bubbling (Optimum)</td>
</tr>
<tr>
<td>4</td>
<td>Bubbling</td>
</tr>
<tr>
<td>5</td>
<td>Bubbling</td>
</tr>
<tr>
<td>7.5</td>
<td>Bubbling</td>
</tr>
<tr>
<td>10</td>
<td>Intense Bubbling</td>
</tr>
<tr>
<td>12.5</td>
<td>Intense Bubbling</td>
</tr>
<tr>
<td>15</td>
<td>Intense Bubbling</td>
</tr>
</tbody>
</table>

A second time course was also conducted to verify the repeatability of this protocol. Testing was done on the chip used in trial 1 but with the exception that the polarities of the electrodes were reversed. That is, the leads connecting to the power supply were switched so that the electrode that had a positive bias now had a negative bias. This experiment is depicted in detail in Figure 36. In this case the electrode on the left became the negative electrode and the electrode on the right became the positive electrode. It can be seen that throughout the time course, there is an area of bead depletion in the area where the silverpaste was applied to the negative electrode. This is seen after less than a minute after the start of the experiment. It is also seen that beads accumulated in the vicinity of the silverpaste that was applied to the positive electrode. The area of accumulation occurred on the side that was furthest away from the negative electrode, which further provided strong evidence that bead migration was due to the attraction of the carboxyl group towards the silverpaste rather than by diffusion. In addition this behavior was captured on video, and review of the video further showed bead migration away from the negative dipole.
Figure 36. Accumulation of beads at the positive electrode and repulsion of beads at the negative electrode after switching dipoles.

Although, the results of for both experiments were positive, there is a need to further improve this protocol. For instance, it can be seen in Figure 35 that the area of bead accumulation and repulsion is seen between the silverpaste glue. This accumulation and
repulsion is furthermore not observed in the area between the positive and negative electrodes. This behavior can be attributed to the large area of silverpaste as compared to the area of the electrodes. Applying silverpaste onto the chip was a challenging issue in this experiment. On the one hand, if less silverpaste was applied onto the chip, then this resulted in wire-detachment problems. If more silverpaste were applied, then the beads would be attracted more towards the silverpaste than towards the electrodes. It was decided that with the anticipation of a new design which would resolved issues dealing with the metal layer flaking, small bump pads, and tracings that washed away, testing would be done with wires that were glued onto the chip with more silverpaste rather than less. With this setup, experimentation would be easier because problems dealing with detachment of the wires would be avoided. Also, with the new design, silverpaste would be applied on a bump pad in a considerable distance away from the electrode. In this new design a trace would connect the electrode with the bump pad so that the silverpaste would be far away from the electrode.

**DIRECT CONTACT METHODS EXPLORED ON DESIGN #2**

Direct contact methods such as using micromanipulators and using a probe station were explored in order to position the copper wires precisely onto the electrodes. In the direct contact method, two micromanipulators (Figure 37) were used to precisely position the copper wires to the location of interest on the chip. One micromanipulator was used to place the copper wire from the power source to the positive electrode while the other was used to place the wire from the power source to the negative electrode. These micromanipulators had the capability of positioning the wires in all three dimensions (x, y, z) with precision. The micromanipulator provided a means of holding the wires to the bump pads by direct contact without the messiness associated with using conductive glues.

For this setup, copper wires connected the power source to the chip. The copper wire from the power source was folded inside the micromanipulator and place accordingly (Figure 38). Three knobs on the micromanipulator acted as fine adjustments in the x, y, and z directions. The chip was glued onto a coverslip, which was taped onto a petri dish. Bead migration experiments were performed by pipetting carboxyl charged beads on top of the chip. The voltage was adjusted until optimal migration occurred.
Figure 37. Micromanipulator.

Figure 38. Copper wires connected from the power source to the chip are folded inside the micromanipulator.

The second method explored involved using a probe station to position a pair of copper wires onto the bump pads present on the chip. The probe station allowed for accurate placement of wires with the use of a high-powered microscope and several micromanipulators. A computer monitor was also able to magnify chip components to allow the user to position the wires carefully onto the chip. Electrode components were easily seen on the monitor and bead migration studies could be performed (figure 39). In order for these
studies to be performed, a power source was connected to two plugs on the probe station via copper wires and alligator clips. Pairs of micromanipulators located on the probe station were attached to the corresponding plugs. 20 micron thick needles were placed into the brass fitting of the micromanipulator and were positioned to bump pads on the electrodes using the fine adjustments present on the probe station. Once setup was complete (figure 40), the power source was turned on in order to test for bead migration.

Figure 39. A magnified view of the electrodes using the Probe Station.

Figure 40. A power source was connected to the Probe Station in order for bead migration studies to occur.
**Bead Migration Studies on Design #2**

Earlier experimentation with chip design #1 revealed that the method of attaching copper wires onto the chip was a limiting factor. Attaching the wires to the chip with silverpaste showed to be cumbersome and messy. The setup using silver paste was also lengthy due to the amount of time it takes for conductive pastes to dry.

It was also discovered upon testing that design #1 was not suitable for conducting bead migration experiments. As a result, this design was optimized such that the sizings of the elements were increased. Bump pads were increased 10 fold for optimal wire attachment. Tracing elements were increased 25 fold to account for the difficulties that were encountered during in the production process. The electrodes were also increased and spaced appropriately for optimal bead migration. With the improvement in the design of the electrodes and the improvement in the method of attaching copper wires to the chip bead migration experiments were performed.

Two direct contact methods were explored. The first methods involved using a probe station and a 20 micron thick probing needle placed on the bump pads. A power source supplied a constant voltage of 3.5 volts during the bead migration studies. The results of this study can be seen in figure 41. Time elapsed photos were taken during the bead migration study using the probe station. It can be seen in the figure that bead migration occurred to the positive dipole. Bead migration occurred in the vicinity of the probing needle.
An alternative direct contact method was also used to study bead migration. This approach explored the use of a micromanipulator to position the wires directly onto the electrodes. Direct contact was the mechanism in which the wires attached to the bump pads. After placement of wires, varying voltages were applied to the electrodes in order to determine optimal bead migration with this new setup (table 5). Bead migration was recorded on the microscope using a laptop, which was connected to the microscope via a USB cable. The results for the experiment are seen in Figure 42.
Table 5. Optimization of voltages for design #2.

<table>
<thead>
<tr>
<th>Voltage (V)</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>No Bubbling (slow accumulation)</td>
</tr>
<tr>
<td>2.0</td>
<td>No Bubbling (slow accumulation)</td>
</tr>
<tr>
<td>3.0</td>
<td>Slight Bubbling</td>
</tr>
<tr>
<td>3.5</td>
<td>Slight Bubbling</td>
</tr>
<tr>
<td>4.0</td>
<td>Bubbling</td>
</tr>
<tr>
<td>5.0</td>
<td>Bubbling</td>
</tr>
</tbody>
</table>

In Figure 42, it can be seen that at the beads did not accumulate at the positive electrode and migrated away from them. This was speculated that the 30 gauge wire was too large for the setup described above. A 30 gauge wire corresponds to a 255 μM diameter. This is more than twice the size of the bump pad. For optimal design considerations it is speculated that a 38 gauge wire (100 μM in diameter) will yield much better results. However, using the current micromanipulator setup, it is most likely not possible to use the direct contact method with such a thin wire. A 38 gauge wire will be less stiff and difficult to manipulate using the micromanipulators. Adhesive glue would most likely be a better approach with the smaller wire. However, the process of applying adhesive glues onto a chip is much more cumbersome than using a direct contact approach as seen with the silverpaste method. Currently, the SDSU MEMS lab is working on bonding gold wires to chips using a wire bonder. It is speculated that one will be able to observe bead migration to the positive electrode if gold wires are successfully bonded to the chip and similar experimentation are performed.
Another study was also conducted to determine the velocity of the beads. In viewing various bead migration studies it was observed that the velocity of bead migration was dependant on the applied voltage. Higher voltages resulted typically in faster migration times. Lower voltage conditions resulted typically in slower bead migration or accumulation. Various studies were conducted to determine the velocity of bead migration using different voltage conditions (table 6). In these studies, the distance of migration was approximated and was determined through CAD modeling. The time of migration was captured through a digital camera with various time recording capabilities available. Through viewing and analyzing these studies, the results further validate the experimental approach. That is, a higher applied voltage would generate a stronger electric field, which would results in faster bead migration.

**Figure 42. Bead migration experiment using direct contact on design #2.**
**Table 6. Voltage and migration velocity values**

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Migration Velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Volt</td>
<td>420 μm/s</td>
</tr>
<tr>
<td>4 Volt</td>
<td>150 μm/s</td>
</tr>
<tr>
<td>8 Volt</td>
<td>90 μm/s</td>
</tr>
</tbody>
</table>

**EXCEL CHART. Voltage Vs Velocity. COMPARE WITH Known DNA VELOCITY.**

**Finite Element Simulation of Electrophoretic Chip**

An electric field model was created with FEMLAB (Comsol, MA) and shows the theoretical electric field generated in using gold wires (figure 43). In this model there were various conditions that were assumed. For example, the conductivity of gold and the 50 mM histadine buffer was approximated to be 4.56x10⁶ S/m and 0.006 S/m. An applied voltage of 3.5 volts, which simulated the optimum condition of experimentation, was applied to the left electrode. The right electrode was biased negatively to allow for an electric field gradient in the vicinity of the electrodes. All other elements in the model were assumed to be electrically insulated. In observing the FEMLAB models generated (Figure 43-45), it can be seen that the electric field is greatest in the vicinity between the two electrodes. This is seen in figure 43 by the large arrows, simulating the electric field, pointing from the positive electrode to the negative electrode. This is also seen graphically in figure 44 in which the region between the two electrodes exhibits a high electric field gradient. Through observing the models generated it can be seen that this configuration will be ideal for electrophoresis of negatively or positively charged particles. That is, charged beads will migrate to the corresponding electrodes and concentrate in the region between the two electrodes. Positively charged beads will have a tendency to migrate towards the negatively biased electrode and negatively charged beads will migrate towards the positively biased electrode. These models verify the design intent of the electrodes. Although, bead migration assays showed that migration occurred towards the electrode-wire interface, these models show theoretically how the electric field will behave under optimal conditions.
Figure 43. A 2D model of the electric field for design #2.
Figure 44. The surface electric field of the two electrodes using Femlab.
Figure 45. Electric field distribution in the region between the two electrodes along Section A-A’. Maximum electric field is 18kV/m.
Figure 46. Electric field distribution in the region between the two electrodes along Section A-A’. Maximum electric field is 18kV/m.
CHAPTER 5

DISCUSSION AND CONCLUSIONS

This thesis seeks to describe an experimental setup that tests for charged particle migration on a micron scale. This setup consists of a packaging apparatus that was designed using Solidworks and optimized in experimentation to reduce various issues such as fogging. The packaging apparatus allows for 1 cm x 1 cm chips to be evaluated for bead migration. This feature also allows for various chips to be tested interchangeably. It is also noted that this packaging apparatus can test other attributes that require inlet and outlet injection ports. That is, the packaging is not entirely specific to bead migration experimentation but other assays can be developed using the packaging system.

In addition to packaging, two electrode systems were designed and manufactured using MUMPS technology. The first design was evaluated using a bead migration setup in which it was seen that various elements on the chip were much smaller than what was needed for optimal migration. A second design was proposed and manufactured that addressed these issues by increasing bump pad, tracing, and electrode sizing. After receiving the second design back from the foundry it was seen that the design was manufacturable in that the traces and all other elements were present on the chip as intended in the design.

Once chips were received back from the foundry an experimental approach was developed to test for the ability of the chips to perform electrophoresis. First, carboxyl charged beads were evaluated by means of electrophoresis on a macro scale. After verifying the negative charge nature of the beads, bead migration experiments were performed using both designs. Numerous problems were encountered in the first bead migration trials. This includes detachment of wires from the electrodes, fogging issues, bubbling issues, and the messiness associated with using adhesive glues on the micron scale. These issues were all addressed and an optimal approach to bead migration study was developed using direct contact methods such as the use of micromanipulators and a probing station. After experimentation, it was shown that still further improvement is needed for bead migration to occur to the electrodes. Although it was seen throughout the various bead migration trials
conducted that bead migration occurred on the micro scale, it was noted that the device and setup was not sensitive enough to pick up the electrode/bead interactions. That is, it is most likely that this setup will not be sufficient to test electrodes of various geometries. Furthermore, improvements in wire bonding will need to be accomplished in order to improve the design. In order to address this issue, the MEMS lab is currently perusing attaching wires to a chip using a gold wire bonder. Currently, there are several graduate students working on evaluating this method of attachment. It is also suggested that testing beads of different charges and sizes be performed to further validate the experimental setup.

One of the intents of this thesis is to create a lab-on-a-chip subassembly. This thesis explores both the design of electrodes and the design of an experimental procedure of a micro electrophoresis system. Previous work in the SDSU MEMS lab have demonstrated the vast potential of using MEMS to create novel cell lysing and pumping mechanisms (3). With the combination of these technologies one can move forward in creating devices in which all stages of sample preparation, sample transport, and sample concentration can be done on a single platform with the use of microfluidic channels to connect these subcomponents. This technology is predicted to have high value for the field of pathogen detection. The current approach in the identification of pathogens is the use of gene chips, which uses passive diffusive mechanisms. It is predicted that using MEMS lab-on-a-chip technology along with an active approach in concentrating pathogens on a chip will greatly improve the process of pathogen detection.
REFERENCES


ABSTRACT OF THE THESIS

The Packaging, Design, and Experimental Evaluation of a MEMS Electrophoretic System
by
Steven J. Wong
Master of Science in Mechanical Engineering
San Diego State University, 2007

This thesis investigates the behavior of carboxyl bead accumulation to pairs of positively biased electrodes on chips manufactured using Multi-User MEMS Processing (MUMPS). A testing apparatus was designed to investigate bead accumulation on a chip. Methods of attaching copper wires to the chip were explored in addition to optimization and bead migration studies. After testing of an initial chip design, an improved design was proposed, manufactured, and integrated into the bead migration set-up. Studies varying voltage conditions and switching the polarities of the electrodes were conducted on the design and demonstrated bead accumulation to the positively biased electrodes within 5 minutes at 3 Volts. Results from experimentation suggest that this device can be utilized to concentrate negatively charged particles (such as DNA) as part of a lab-on-a-chip setup with lysing and pumping technology already demonstrated in previous research.