12-2011

Design and Fabrication of Nanofluidic Systems for Biomolecule Characterizations

Orain Ansel Hibbert
University of Arkansas, Fayetteville

Follow this and additional works at: http://scholarworks.uark.edu/etd
Part of the Biological Engineering Commons, Biophysics Commons, and the Nanotechnology Commons

Recommended Citation
http://scholarworks.uark.edu/etd/215

This Thesis is brought to you for free and open access by ScholarWorks@UARK. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of ScholarWorks@UARK. For more information, please contact scholar@uark.edu, ccmiddle@uark.edu.
DESIGN AND FABRICATION OF NANOFLOWIDIC SYSTEMS FOR BIOMOLECULE CHARACTERIZATIONS
DESIGN AND FABRICATION OF NANOFLUIDIC SYSTEMS FOR BIOMOLECULE CHARACTERIZATIONS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Microelectronics-Photonics

By

Orain Hibbert
University of Massachusetts Amherst
Bachelor of Science in Chemical Engineering, 2009

December 2011
University of Arkansas
Abstract

Nanofluidic channel systems were designed and fabricated by combining MEMS microfabrication with AFM nanolithography. In the fabrication process flow, photolithography was first utilized to pattern microfluidic channels and reservoirs on a 4” Pyrex substrate. Subsequently, atomic force microscopy (AFM) based nanolithography was used to mechanically fabricate nanochannels to connect the microreservoirs which formed the inlet and outlet of the nanofluidic system. A Tap190 Diamond-Like Carbon (DLC) AFM tip with a force constant of 48 N/m and a radius of less than 15 nm was used as the nanolithography tool. The resultant nanochannel ranges from 20 to 80 µm in length and 10 to 100 nm in depth. After AFM, the Pyrex micro- and nanochannels were sealed off by a matching silicon capping piece using anodic bonding. Fluidic connectors are then attached to the inlet and outlet openings to complete the fabrication process.

The relationship between the nanolithography parameters of the AFM and the resultant nanochannel dimensions was investigated in detail. A mostly linear trend was obtained between the AFM tip force and the nanochannel depth for a tip speed of 1 µm/s. This result was consistent with established nanotribological models and similar studies on silicon substrates. The relationship between the number of repeated scratches and the nanochannel depth was also investigated. The results indicated that the nanochannel depth increased with the number of scratches. A depth of about 20 nm was typically achieved with 25 scratches at a tip force of 25 μN. The width of the nanochannel also increased with the number of scratches. A typical nanochannel width of 120 nm was achieved for 25 scratches at 10 μN.

Two different flow tests were conducted using the nanochannel system. In the first test, a fluorescent fluid, Fluorescein, was pumped through the nanochannel to demonstrate channel
patency. To achieve this, a sequential wetting procedure was executed to modify the surface chemistry of the nanochannel system. Fluorescence microscopy confirmed the passage of fluid through a 40 μm long and 45 nm deep channel. In the second test, negatively charged nanobeads, carboxylate-modified FluoSpheres, were translocated through the nanochannel using an externally supplied DC electric field.
This thesis is approved for recommendation to the Graduate Council.

Thesis Director:

___________________________________
Dr. Steve Tung

Thesis Committee:

___________________________________
Dr. Adam Huang

___________________________________
Dr. Jin-Woo Kim

___________________________________
Dr. Russell DePriest

___________________________________
Professor Ken Vickers

The following signatories attest that all software used in this thesis was legally licensed for use by Mr. Orain Hibbert for research purposes and publication.

___________________________________
Orain Hibbert, Student

___________________________________
Dr. Steve Tung, Thesis Director

This thesis was submitted to http://www.turnitin.com for plagiarism review by the TurnItIn company’s software. The signatories have examined the report on this thesis that was returned by TurnItIn and attest that, in their opinion, the items highlighted by the software are incidental to common usage and are not plagiarized material.

___________________________________
Professor Ken Vickers, Program Director

___________________________________
Dr. Steve Tung, Thesis Director
Thesis Duplication Release

I hereby authorize the University of Arkansas Libraries to duplicate this thesis when needed for research and/or scholarship.

Agreed_______________________________

Orain Hibbert

Refused_______________________________

Orain Hibbert
Acknowledgements

First and foremost, I would like to thank Professor Ken Vickers for granting me the opportunity to join the University of Arkansas and the microEP graduate program after completing a summer research in 2008. Mr. Vickers provided continued support and motivation as a mentor and coordinator for research related activities and informed me about funding and career opportunities. The two year financial support from the microEP NSF S-STEM scholarship allowed me to travel to Canada and China. I would also like to extend my sincere gratitude to my research advisor, Dr. Steve Tung for allowing me to be a member of his research group at the Micro and Nano Systems Laboratory. I have advanced academically and professionally in the engineering community as a result of the interaction with my research advisor. One of the major highlights of graduate school career was my trip to Shenyang, China for research collaboration with graduate students at the Shenyang Institute of Automation, Chinese Academy of Sciences.

Mr. Errol Porter and Mike Glover also played a significant role in my research. I would like to thank them for giving me the opportunity to be certified to work in the clean room at the HiDEC facility. Mike Steger provided assistance vacuum pump maintenance and Dr. Mourad Benamara was extremely helpful with the SEM. Mrs. Renee Jones-Hearon was extremely helpful to me throughout my time here at the University of Arkansas especially with deadline reminders. My colleagues and research peers; Husein Rokadia, Balaji Srinivasan, Brock Schulte, Jacob Hohnbaum, Ju-Seok Lee, Benjamin Newton, Kyle Godin, and Zhuxin Dong have also assisted in the completion of this research by providing training and assistance on equipment pertinent to my research.

This work was partially supported by the National Science Foundation under No. DUE-072836. Any opinions, findings, and conclusions or recommendations expressed in this material
are those of the author and do not necessarily reflect the views of the National Science Foundation.
Table of Contents

Chapter 1: Introduction .................................................................................................................. 1
  1.1 Transport in nanofluidic channels ......................................................................................... 1
  1.2 Fabrication of Nanochannels .............................................................................................. 2
  1.3 Nanoscale Devices for DNA Analysis ................................................................................. 9
  1.4 Thesis Objectives and Organization .................................................................................. 11

Chapter 2: AFM Nanolithography ................................................................................................. 12
  2.1 Force Conversion (Force Setpoint [Volts] to Force [µN]) ............................................... 22
  2.2 Calibration using a Pyrex substrate without microchannel designs .................................... 24

Chapter 3: Materials and Methods ............................................................................................. 26
  3.1 Materials ............................................................................................................................. 26
  3.2 AFM Nanolithography Procedure .................................................................................... 29
  3.3 Wafer Processing ................................................................................................................. 33
    3.3.1 Photolithography ........................................................................................................ 33
    3.3.2 Wet Etching ................................................................................................................ 34
    3.3.3 Profilometry ............................................................................................................... 36
  3.4 Packaging ............................................................................................................................ 37
    3.4.1 Dicing, Inlet/Outlet Opening, Chip Cleaning ............................................................. 37
    3.4.2 Anodic Bonding ......................................................................................................... 37
    3.4.3 Attachment of connectors ......................................................................................... 40

Chapter 4: Results and Discussions ............................................................................................. 42
  4.1 Nanochannel Dimension Calibrations ............................................................................... 42
  4.2 Anodic Bonding .................................................................................................................. 54
  4.3 Flow Tests .......................................................................................................................... 56
    4.3.2 Fluorescence Microscopy tests using Fluorescein ..................................................... 60
  4.4 Translocation of nanobeads ............................................................................................... 62
    4.4.1 Translocation Experimental Setup .............................................................................. 62
    4.4.2 PolyDiMethylSiloxane (PDMS) Microchannel Translocation .................................... 64
    4.4.3 Nanochannel Translocation ...................................................................................... 67
  4.5 Silicon nanochannel system with embedded electrodes ...................................................... 70
List of Figures

Figure 1. (a) AR << 1: 1D nanochannel (b) AR ~ 1: 2D nanochannel

Figure 2. Surface Micromachining: Sacrificial layer process flow

Figure 3. Process flow for bulk micromachined microchannel and wafer bonding

Figure 4. Schematic showing the principles of atomic force microscopy

Figure 5. Nanopore-Based DNA Sequencing [20]

Figure 6. Components of the Atomic Force Microscope (Agilent 5500 AFM)

Figure 7. AFM nanolithography using a Pyrex substrate

Figure 8. SEM image of a new Tap 190 DLC probe

Figure 9. SEM image of an unused Tap190 DLC tip attached to a cantilever

Figure 10. Scratching Mechanism

Figure 11. Close-up view of a pristine pyramidal Tap 190 DLC tip

Figure 12. Close-up view of a damaged pyramidal Tap 190 DLC tip (after ~ 350 scratches)

Figure 13. Large scanner fitted into scanner jig before inserting AFM tip into the contact mode or AC nose cone

Figure 14. Sample topography image mapped to PICOLITH for scratching along the red arrow demonstrated in the image

Figure 15. PICOLITH control parameters enabling users to specify the length, position, and number of scratches

Figure 16. PICOLITH parameters enabling users to specify the force setpoint in volts and the cutting velocity of the tip in µm/s

Figure 17. Cantilever deflection vs. distance

Figure 18. Deflection sensitivity output from the laser deflection (V) versus the distance the tip travels (µm) attained in tapping (AC) mode
Figure 19. Average depth and width are determined based on 10 cross sections along the nanochannel length .......................................................... 25

Figure 20. Wafer design demonstrating thirteen (13) microchips containing microchannels with varying gaps (μm) ............................................................ 27

Figure 21. Schematic of Nanochannel System (Top View) ........................................ 28

Figure 22. Design of a 40 x 40 μm design chip .............................................................. 28

Figure 23. Schematic illustrating side view of nanofluidic device .................................. 29

Figure 24. Fabrication Process Flow ........................................................................... 33

Figure 25. Design profile (left) vs. Post-etch profile (right) .......................................... 35

Figure 26. Nanochannel region pre and post-etch profile ............................................. 35

Figure 27. Dektak image showing Microchannel trench depth of 3.5 μm ...................... 36

Figure 28. Dektak image showing Microchannel trench depth of 7.5 μm ................. 37

Figure 29. Anodic Bonding Experimental Setup ......................................................... 39

Figure 30. Schematic showing the anodic process ....................................................... 39

Figure 31. Schematic showing the anodic bonding mechanism .................................... 40

Figure 32. Completely Fabricated Y-shaped Microchannel Device ............................. 41

Figure 33. Completely Fabricated Straight Microchannel Device ............................... 41

Figure 34. AFM Topography image showing cross section of scratches at 5.5 V (14.24 μN). I-25 scratches, II-50 scratches, III-75 scratches, IV-100 scratches. .... 44

Figure 35. AFM cross section image showing the depth and width of fabricated nanochannels machined at 14.24 μN. I-25 scratches, II-50 scratches, III-75 scratches, IV-100 scratches. ................................................................. 44

Figure 36. Cross sectional plot demonstrating the average depth at 14.24 μN for 100 scratches ............................................................................................. 45

Figure 37. Log-log plot of Scratch depths (nm) versus applied force (μN) comparing calibration results on Pyrex to tribological scratch experiments on Si (100) . 47

Figure 38. Mean depth (nm) vs. applied force (μN) on a Pyrex 7740 substrate .......... 49
Figure 39. Mean depth (nm) versus number of scratches

Figure 40. 2D Topography image after AFM nanolithography

Figure 41. 3D Topography 3D image (45° left view) microchannels after AFM nanolithography: 40 x 40 µm chip

Figure 42. Topography 3D image (135° right view) of microchannels illustrating unsuccessful AFM nanolithography: 40 x 100 design µm chip

Figure 43. Topography 3D image (45° left view) of microchannels after AFM nanolithography: 40 x 100 µm design chip

Figure 44. Satisfactory bonding achieved at 400°C and 900 V around channels and microreservoirs. Rainbow rings are unbounded.

Figure 45. Unsatisfactory bonding achieved at 350 °C and 900 V

Figure 46. Excellent bonding achieved at 450 °C and 900V

Figure 47. Syringe attached to nanofluidic device for pumping in a vacuum dessicator

Figure 48. Syringe attached to nanofluidic device for pumping in a vacuum dessicator

Figure 49. Enlarged view (10X) of microchannels before the wetting steps (dry state)

Figure 50. Result after pumping acetone for 40 minutes

Figure 51. Result after pumping acetone overnight

Figure 52. Result after pumping Methanol from the upstream connector for 40 minutes

Figure 53. Result after pumping Carboxylate-Modified 20 nm FluoSpheres for 1.5 hours

Figure 54. Fluorescent image after pumping Fluorescein for about 1 hour in a vacuum dessicator

Figure 55. Fluorescent image after pumping Fluorescein for an additional 45 minutes in a vacuum dessicator

Figure 56. Experimental setup for translocation

Figure 57. Schematic demonstrating the translocation of 20 nm carboxylate-modified Fluospheres with sewing needles inserted at the inlet and outlet of the nanofluidic device
Figure 58. Fabricated PDMS Microchannel device for flow testing with nanobeads ..... 64

Figure 59. Bright field after filling with PBS .......................................................... 65

Figure 60. Fluorescein Isothiocyanate (FITC) image after introducing beads to inlet at 8 V for 15 minutes ................................................................. 65

Figure 61. FITC image after 2 minutes after changing voltage to 10 V ..................... 66

Figure 62. FITC image at 10 V after 10 minutes (3 s exposure) ............................... 66

Figure 63. FITC image at 10 V after 15 minutes (188 ms exposure) ......................... 67

Figure 64. Schematic of 40 x 150 µm Y-shaped microchannel chip design ............... 68

Figure 65. Fluorescent image of 40 x 150 µm Y-shaped microchannel chip after translocation for 10 minutes at 10 V (10X objective) ............................ 68

Figure 66. Fluorescence Microscopy Image after pumping negatively charged fluorescent nanobeads for 1.5 hrs: 15 s exposure time ....................... 69

Figure 67. Fluorescence Microscopy Image after translocation with PBS at the outlet obtained after 15 minutes at 15 s exposure time ................................ 70

Figure 68. Design of a nanofluidic sensor on a silicon substrate with a 500 nm layer of oxide .................................................................................................. 71

Figure 69. Image showing the silicon nanochannel device with embedded electrodes and connectors attached to the inlet and outlet access holes. ..................... 71

Figure 70. SEM image illustrating two microchannels separated by a 30 µm gap with embedded electrodes that are 1 µm in width ........................................... 72

Figure 71. Close-up SEM image illustrating two microchannels separated by a 30 µm gap with 5 pairs of embedded electrodes that are 1 µm in width .................. 73

Figure 72. 3D Topography Image of Nanochannel ................................................. 74

Figure 73. Fluorescent image (20 s exposure) of DNA translocated at 10 V for 10 minutes after wetting the channels overnight .............................................. 74

Figure 74. Longitudinal electrical current signal (µA) versus time (s) through the Pyrex nanofluidic channel ................................................................. 77
List of Tables

Table 1. Examples of 1D nanochannel fabrication methods ................................................. 6
Table 2. Examples of 2D nanochannel fabrication methods .................................................. 6
Table 3. Design of experiments for AFM characterization on a Pyrex substrate ............ 42
Chapter 1: Introduction

Nanofluidic systems can potentially revolutionize various biomedical applications including drug delivery, DNA stretching and detection, single biomolecule analysis, and nanofiltration [1, 2]. The term, ‘nanofluidics’, was first coined in 1995 to differentiate it from the field of microfluidics [2]. It refers to the study of fluid flowing in a system where at least one dimension, usually the depth of a nanochannel, is in the nanometer range (1 – 100 nm based on NSF definition) [3]. The fabrication of nanochannels has gained significance due to the growing interest in the detection and manipulation of single biomolecules (DNA, viruses, and proteins) and the realization that nanochannels and most single biomolecules are comparable in size [1, 4]. Nanochannels possess a small transverse size and high degree of spatial confinement that bestows them with unique applications in sensing and nanoscale manipulation. These advantages can be leveraged into future developments of biotechnology in mass transport, chemical analysis, and other nanomedicine applications [4]. In particular, the similarity between the dimensions of nanochannels and DNA make nanofluidic devices potentially great tools for genomic analysis [4, 5, 6].

1.1 Transport in nanofluidic channels

Fluid behavior at the macroscale is frequently different from that at the micro- and nanoscale. Microscale flows are usually laminar due to a low Reynolds number [3, 7]. This is expected to be the same for nanoscale flows, although it is difficult to verify due to a lack of reliable flow visualization and measurement techniques at this scale. The characteristics of nanofluidics include: (1) an extremely high surface-to-volume ratio, (2) channel dimensions that can be close to that of single fluid molecules, (3) transport properties such as viscosity and diffusion coefficient that are different from macro- and microscale flows, (4) an interaction of
the fluid particles with the surface (hydrophilic versus hydrophobic) that directly affects flow behavior, and (5) boundary conditions at solid-liquid interfaces are not yet fully understood [8, 9]. In nanofluidics, fluids are dominated by interfacial forces and properties instead of bulk properties such as density and viscosity [2]. The characteristic length scales of interfacial forces include the Debye length, the hydrogen bonding length, and the length scales of van der Waals force [2, 9]. Almost all of these length scales are electrical in nature.

There are several different ways to transport fluids in a nanochannel. The most common method involves the combination of high inlet pressure and vacuum at the outlet. This method is somewhat counter-intuitive since conventional fluid mechanics theories indicate that an unrealistically high pressure drop is required to generate a fluid flow in a nanochannel. The other methods are electrokinetics based involving the use of electric fields. They include electroosmosis and electrophoresis. Electroosmosis refers to the movement of charged ions relative to a fixed surface in the presence of an electric field [3, 8]. Electrophoresis is the movement of a charged surface relative to a stationary liquid. The electrophoretic velocity is proportional to the strength of the applied electric field [3].

1.2 Fabrication of Nanochannels

Nanochannels can be categorized based on the aspect ratio of the cross sectional area [3]. Nanochannels can be considered one-dimensional (1D) if only one dimension (usually depth) is in the nanometer scale; they become two-dimensional (2D) if two dimensions (both depth and width) are in the nanometer scale. Figure 1 illustrates the aspect ratio (AR = height/width) of 1D and 2D nanochannels.
Multiple techniques have been utilized for nanochannel fabrication. Examples of these techniques include MEMS based surface and bulk micromachining, nanoimprinting, and direct nanolithographic methods. 1D nanochannels can be fabricated by etching shallow trenches on a substrate after standard photolithography \cite{10, 11}. Surface micromachined channels are enclosed within the substrate by the use of sacrificial layer techniques as demonstrated in Figure 2. In these channels, the channel height is defined by the thickness of the sacrificial layer. It has been demonstrated that surface nanomachined channels possess an upper limit of channel length within 3 – 5 mm \cite{10, 11}. Removing the sacrificial layer during channel fabrication can take a very long immersion time in chemical solutions. For example, a 2 mm long, 10 µm wide and 50 nm high (deep) channel can take up to 80 hours of etching time \cite{11}.
(a) Deposition and patterning of sacrificial layer material

(b) Deposition of structural material on top of sacrificial layer

(c) Patterning of inlet/outlet access holes

(d) Removal of sacrificial layer through etching to realize microchannel and access holes

Figure 2. Surface Micromachining: Sacrificial layer process flow

2D nanochannels can be achieved using direct nanolithographic techniques such as E-Beam lithography (EBL) and Focused Ion Beam (FIB) milling [11]. These nanochannel chips usually include microchannels formed using bulk micromachining before fabricating the nanochannels. The bulk micromachined microchannels can be etched by reactive ion etching (RIE) or wet etching techniques [10, 11]. After forming the microchannels, the nanochannel chips can be capped off using thermal or anodic bonding. Difficulties can arise during the
bonding process when the microchannels collapse due to their relatively large width. To prevent this failure, the ratio of the microchannel depth and width must be above a certain number [10].

Figure 3 shows the cross sectional view of a microchannel device fabricated in silicon and bonded to a matching glass chip using bulk micromachining.

![Diagram](image)

(a) Silicon wafer oxidation

(b) Channel patterning

(c) Oxide removal and wafer bonding

Figure 3. Process flow for bulk micromachined microchannel and wafer bonding

Table 1 provides the typical nanochannel height achieved through conventional photolithography. The widths of nanochannels were not reported since 1D nanochannels typically have widths in the micrometer scale. Table 2 provides the technical details for 2D nanochannel fabrication using direct write techniques such as E-Beam lithography and Focused Ion Beam milling.
Table 1. Examples of 1D nanochannel fabrication methods

<table>
<thead>
<tr>
<th>Nanochannel Pattern</th>
<th>Materials</th>
<th>Etching/Deposition</th>
<th>Height</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photolithography</td>
<td>Silicon, Glass</td>
<td>RIE for silicon, BOE for glass</td>
<td>20 nm</td>
<td>Mao, P. and Haneveld, J. Lab Chip. 2005 [14]</td>
</tr>
</tbody>
</table>

Table 2. Examples of 2D nanochannel fabrication methods

<table>
<thead>
<tr>
<th>Nanochannel Pattern</th>
<th>Materials</th>
<th>Etching/Deposition</th>
<th>Typical Dimensions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focused Ion Beam (FIB)</td>
<td>Silicon (Si$_3$N$_4$), glass, quartz, fused silica</td>
<td>FIB</td>
<td>50 nm x 50 nm</td>
<td>Hibara, A. et al. Anal. Chem. 2002 [16]</td>
</tr>
<tr>
<td>AFM</td>
<td>Silicon, glass</td>
<td>AFM</td>
<td>25 nm x 200 nm</td>
<td>Hibbert, O. et al. IMECE Proceedings. 2010 [17]</td>
</tr>
</tbody>
</table>

While effective, both FIB and EBL have major disadvantages as etching tools for nanochannels. Both techniques are etch-only tools and are not capable of measuring the etch result. Characterization of the FIB and EBL nanochannel requires a different tool such as AFM.
or SEM. Additionally, FIB and EBL etching rely on high-energy ions and electrons, respectively. There is no established direct relationship between the power of the energy sources and etch dimensions. Consequently, careful calibrations are needed to achieve the correct nanochannel dimensions when using FIB and EBL. Overall, the stated 1D and 2D nanolithography fabrication methods create nanochannels with minimum depths of less than a few hundred nanometers. However, all methods have a common deficiency in their fabrication process. These fabrication challenges can be attributed to four reasons: (1) nanochannel non-uniformity, (2) sealing of etched structures to create enclosed channels, (3) long etch times, and (4) wide nanochannels which present bonding difficulties such as channel collapse.

Atomic force microscopy (AFM) is a nanolithographic based nanochannel fabrication method that uses a sharp AFM tip, typically diamond or diamond coated, to mechanically ‘scratch’ out a nanochannel on a substrate. Unlike FIB and EBL, AFM is a multi-functional tool capable of topography scans, nanomanipulation, and surface property characterization. Figure 4 shows the basic operational principle of AFM scanning. A sharp tip at the free end of a cantilever is brought into contact with the sample surface. The AFM operated like a surface profiler since as the tip traced sample surfaces, the cantilever deflected following the topography of the surface. This deflection is measured by a reflected laser beam through a photosensitive laser detector [18]. The deflection signal is captured by a computer (control system) and the 2D topography of the sample surface is determined. The laser signal processor controls the z-position of the cantilever deflection. The scanner holds the AFM tip in contact with the surface while scanning in the x-y plane in a raster pattern for a closed loop scanner. Closed loop scanning provides a more accurate positioning by minimizing thermal drifts when compared to
open loop scanning. In a scanning operation, a low applied force ($F_N$) to the cantilever is typically applied to prevent material wear of the tip and sample surface.

![Diagram](image)

**Figure 4.** Schematic showing the principles of atomic force microscopy

In AFM nanolithography operation, a large normal force is applied to a sharp tip to fracture the substrate material under investigation. During the nanolithography process, the AFM is operated in contact mode where the tip makes contact with a sample surface and scratch dimensions are controlled by varying the force setpoint (volts), number of scratches, and tip speed (input control parameters) during the nanolithography process. The scratch direction can be lateral, forward, or backward and the scratch is achieved by the dragging the cantilever along a specified path. The modified input parameters are applied tip force, number of scratches, and tip speed to tailor nanochannel or scratch dimensions. The amount by which the cantilever deflects is determined by the force setpoint. The force setpoint allows a constant mechanical force between the substrate and the tip. The AFM nanolithography process is a direct write fabrication technique since a probe is used to apply a mechanical force to the surface under investigation [18]. AFM is a precise and reliable method for both the fabrication and investigation of nanostructures with scanning, characterization, and manipulation capabilities.
1.3 Nanoscale Devices for DNA Analysis

One of the first successful demonstrations of nanoscale devices for DNA analysis was the nanopore technique. Nanopores are very short (less than 10 μm) nanochannels [3]. The diameter of a nanopore is usually on the order of a few nanometers (1 – 4 nm), as shown in Figure 5. Nanopores are tailored to stretch single-stranded DNA for potential sequencing applications. Nanopore sequencing was first conceptualized in the 1990s and the first set of experimental results using biological nanopores were reported by Kasianowicz at the National Institute of Standards and Technology (NIST) [19]. The width of the pore used was 1.4 nm. An electric field was applied across the pore for translocation of the protein molecules. Later, a similar approach was applied for the translocation of single-stranded DNA (ssDNA) molecules through solid-state nanopores [6]. Figure 5 demonstrates ssDNA translocating through a 1.8 nm diameter nanopore while the blockage current of the DNA bases are measure as 50 picoamps (pA) [20].

![Figure 5. Nanopore-Based DNA Sequencing [20]](image)

In this technique, the ion flow through the pore is measured as an electrical current and because the pore diameter is on the same scale as DNA, DNA molecules are forced to pass linearly through the pore. The basic assumption is that the characteristics of DNA molecules can be determined depending on the extent and current drops [3, 6, 21]. There are two main
difficulties in using nanopores for DNA sequencing: (1) DNA molecules translocate too quickly through a nanopores and (2) the blockage current does not provide a high enough resolution for single-base reading as shown in Figure 5. Due to these shortcomings, researchers have started exploring the possibility of DNA sequencing through a nanochannel. Nanochannels offer significant advantages over nanopores since DNA has a smaller translocation speed in a nanochannel and multiple sensing spots can be located along the channel which improves read-out resolution [1, 3, 21].

Recently, a molecular dynamics based simulation was performed by Min et al [22] to demonstrate the feasibility of DNA sequencing in a nanochannel system embedded with a graphene nanoribbon. The study was based on resolving the problem associated with the stochastic motion of ssDNA through a nanopore that lacked defined interaction between the nanopore and the nucleobases. In their simulation, the graphene nanoribbon held the nucleobases firmly and prevented orientational fluctuations which were responsible for the overlapping of the current distribution between the bases [22]. An electric field was used to translocate ssDNA through the nanochannel. The use of graphene was ideal since its electrical conductivity was highly sensitive to small changes due to a high surface to volume ratio.

Xiaogon et al [3, 23] demonstrated the stretching and transporting of double-stranded DNA strands through a nanoimprint-based nanochannel. In their study, the nanochannels were 45 nm wide and 45 nm deep. Electrophoresis was used to translocate the DNA. The DNA sample was directed into the microchannel inlet using voltage a bias of 10 V [23]. Once the DNA approached the interface between the microchannel and the nanochannel, a higher voltage (50 V) was applied across the entire nanochannel to stretch the DNA into the nanochannel [23]. The
stretched DNA demonstrated a continuous motion in the nanochannel, which proved the channel continuity over the entire channel length.

1.4 Thesis Objectives and Organization

The overall goal of this thesis was to design, fabricate, and test a glass-based nanochannel system by combining MEMS microfabrication and AFM nanolithography. The specific objectives of this work were:

a) To design and fabricate microchannels and microreservoirs based on MEMS microfabrication techniques

b) To characterize the relationship between the AFM control parameters and the resultant nanochannel dimensions

c) To utilize AFM nanolithography to scratch nanochannels between microreservoirs

d) To package nanochannel systems using anodic bonding

e) To conduct flow tests to prove the patency of nanochannels

f) To evaluate nanobead translocation tests through nanochannels

g) To conduct preliminary evaluation of DNA flow through nanochannels
Chapter 2: AFM Nanolithography

AFM nanolithography techniques can be classified into two categories: (1) Force-assisted nanolithography and (2) Bias-assisted nanolithography [18, 24]. Force-assisted nanolithography includes mechanical plowing, indentation, and manipulation. On the other hand, bias-assisted nanolithography includes electrochemical deposition, electrical cutting, and electrostatic deformation [24]. In force-assisted nanolithography, the method used in this research, a large force is applied to the tip which maintains a constant mechanical force on the sample for nanopattern fabrication or scratching. Figure 6 shows the Agilent 5500 AFM used in this research.

Figure 6. Components of the Atomic Force Microscope (Agilent 5500 AFM)
Nanolithography creates nanoscale structures by controlling the force applied on the surface using an AFM tip. Nanolithography was done in contact mode using a series of applied forces to fabricate nanochannels with a Tap 190 Diamond-Like Carbon coated AFM tip. The tip had a force constant of 48 N/m which represented the ratio between the force applied at the free end of the cantilever and the cantilever deflection at that point. AFM nanolithography has been applied to many applications including forming patterns on sample surfaces (gold, oxide, nitride etc.) and the controlled manipulation of nanometer sized particles on a surface. In this research, the top-down bulk nanolithography fabrication approach has been used to generate nanochannels from a flat Pyrex glass substrate using microfabrication and force-assisted AFM nanolithography. The nanomachined channels are planar (that is, the width is in µm scale and the depth is in nm).

The AFM acts as a surface profiler and produces topography scans of many sample surfaces using a probe. The working principle behind the operation of the AFM is based on the interaction of the probe and the sample substrate. The AFM can be operated in various modes, typically: (1) contact mode, (2) tapping mode, (4) AC mode, and (5) STM [18, 24]. Contact mode is ideal for AFM nanolithography since the tip needs to be in contact with the sample surface. During AFM nanolithography, forces larger than what is required for AFM surface imaging are used to maintain a constant mechanical force ($F_N$) between the tip and sample surface. Figure 7 demonstrates the AFM nanolithography process where a force is applied to an AFM tip which deflects when contact is made with a surface and digs to the substrate to begin a scratch in the forward direction to fabricate a nanochannel.
A Tap 190 Diamond-Like Carbon (DLC) probe was used in this work for nanolithography purposes. The tip had a radius of curvature that was less than 15 nm, an average force constant of 48 N/m, and a resonant frequency of 190 kHz (± 60 kHz) [25]. Figure 8 shows an SEM image of a new Tap 190 DLC probe with the cantilever attached to the holder chip.
The diamond-like coating on the tip shown in Figure 9 was approximately 15 nm thick, which provided great resolution and reproducibility, ensures less tip wear and high durability. The cantilever is micromachined from monolithic silicon for uniformity. The cantilever was typically 225 µm in length; mean tip width was 38 µm (± 9 µm). The tip thickness was 7 µm (± 1 µm) the height was approximately 17 µm (± µm) [25].

![SEM image of an unused Tap190 DLC tip attached to a cantilever](image)

Figure 9. SEM image of an unused Tap190 DLC tip attached to a cantilever

A Tap 190 DLC probe has a lifetime of approximately 300 to 350 scratches during AFM nanolithography before pile up or unclear topography scans began to appear. After repeated nanolithography, the AFM tip will begin to wear and require the use of larger forces for the fabrication of shallower nanochannels. Since the AFM tip had a small radius of curvature ( < 15 nm), the fabricated nanochannels were much wider than the tip size due to repeated scratching as shown in Figure 10. Minimal thermal drifts allowed wider nanochannels since the AFM tip scratched in the forward direction then returned to the start point of the scratch repeatedly for a
specified number of times. In single scratching, the nanochannel width would be similar to the size of the tip as demonstrated in Figure 10.

Figure 10. Scratching Mechanism

Figures 11 and 12 compare a brand new Tap 190 DLC AFM tip to an old tip that has been used about ten times for scanning and about 350 scratches during AFM nanolithography. The wear of the Tap 190 DLC tip is clearly illustrated in the SEM image shown in Figure 12.
Figure 11. Close-up view of a pristine pyramidal Tap 190 DLC tip

Figure 12. Close-up view of a damaged pyramidal Tap 190 DLC tip (after ~ 350 scratches)
Tip geometry can be tetrahedral, pyramidal or conical. Likewise, cantilever geometry may be rectangular or triangular [25, 26]. The sharpness of the tip is defined by the radius of curvature and sidewall angles and this impacts the resolution available with the probe [27].

In the present study, AFM nanolithography was used to fabricate nanochannels between two unconnected microchannels. The Pyrex substrates had two microchannels separated by varying µm gaps to be connected with nanochannels by mechanically scratching the surface with a sharp AFM tip. Prior to scratching, AFM scanning was performed using a calibration, alignment, and imaging software, PicoView, to map the sample topography to the nanoscale positioning and manipulation, and nanolithography software, PicoLITH™. Image analysis and data manipulation is performed using the PicoImage software while the video output was displayed using CameraView software. AFM scanning was performed using a very small force setpoint (level of voltage applied to the piezoelectric scanner) of about 0.5 V to prevent surface damage. With larger force setpoints, the AFM tip fractured the sample surface while removing material and this enabled the fabrication of nanochannels.

Typically, scratching 50 times with a force setpoint of 7.5 V or 8 V and using a closed loop scanner at a tip velocity of 1 µm/s yielded nanochannels that are approximately 40 nm deep. A closed loop scanner (xyz) provided more accurate positioning and less thermal drift than the open loop scanner (xy) with four piezo plates for X and Y motion and two piezo tubes for Z motion [18]. In closed loop, ultra-precise positioning sensors measured displacement in the X/Y/Z plane or Z plane only and allows force control and accurate positioning during nanolithography. Both scanners are multipurpose because the nose cones that are ideal for different imaging modes can be inserted into the scanner. Figure 13 shows a large scanner (10 –
90 μm scan range) that had closed loop capability and was fitted into the scanner jig for assembly of a nose cone with an AFM tip.

Figure 13. Large scanner fitted into scanner jig before inserting AFM tip into the contact mode or AC nose cone.

Once nanochannels were formed, the AFM was used to characterize the average depth and width of the resultant nanochannels. AFM nanolithography is similar to the nanoindentation process [24]. The only distinction is that the tip is moved in a specified direction according to the prescribed force after the sample surface is penetrated. Figure 14 illustrates a sample topography image mapped to the PicoLITH software with a red arrow positioned to a desired location for scratching. The AFM tip started scratching from the beginning of the arrow until the end, and then it jumped back to resume scratching from the start position and repeated for the specified number of scratches.
The PicoLITH control parameters were modified before each nanolithography experiment to obtain a desired scratch depth. After mapping the sample topography to PicoLITH and drawing an etch line, the primitive properties allowed entry of the number of scratches in the “times” command from the window box shown in Figure 15. The desired length in micrometers was also input by entering a number or adjusting the line drawn. The start X and Y position controlled the positioning of the desired scratch. The color associated properties (Figure 16) were adjusted to specify the applied force of the AFM tip and the speed that the tip scratched in the forward direction repeatedly.
Figure 15. PICOLITH control parameters enabling users to specify the length, position, and number of scratches.

Figure 16. PICOLITH parameters enabling users to specify the force setpoint in volts and the cutting velocity of the tip in μm/s.

The probe moved in the forward y direction when cutting so a vertical sample orientation under the AFM was proven to be favorable for scratching with the tip moving in the forward direction. During AFM nanolithography, the force constant of the tip (usually in N/m) played a
significant role in the resultant properties of nanochannels. Z. Wang et al [28] studied the repeated nanolithography of nanochannels using large applied forces ($F_N$), less than 20 µN, to fabricate nanochannels greater than 20 nm on Si. A series of equations were used by Zhiqian et al to study the relationship between the nanochannel depth and the applied force. The experiments proved that there was no relationship between the tip speed and depth, which was also confirmed in micro- and nanotribological studies [28, 29, 30]. Experiments were run at 1 µm/s since this was the ideal cutting velocity in terms of scratching time and tip longevity as confirmed by Zhiqian et al.

2.1 Force Conversion (Force Setpoint [Volts] to Force [µN])

A deflection versus displacement curve was generated to investigate the relationship between the force setpoint (Volts) input parameter during nanolithography and the applied force in µN. Figure 17 shows the tip-sample interaction force curve in tapping mode. The AFM tip began its approach (red line), D, at 0.2 µm and traveled constantly (as shown on the x-axis in Figure 17) to about 0.0325µm, C, before it made contact with the sample, B. Cantilever deflection began at 0.03 µm, A, after the tip made contact with the sample surface. The deflection sensitivity (Figure 18) was obtained as a result of the force-distance plot shown in Figure 17.
The relationship between the force setpoint (V) and the applied force (µN) to the cantilever was calculated. The deflection sensitivity of the cantilever (nm/V) is measured as illustrated in Figure 18. Once this value is obtained, the cantilever deflection (nm) was calculated.
by multiplying the applied force setpoint (volts) by the deflection sensitivity. The force (µN) is then calculated using Hooke’s law as illustrated in Equation 1:

\[ F = (k)(D_s)(F_s) \]  

Equation (1)

where \( k \) represents the force constant (stiffness) of the AFM tip, \( D_s \) represents the deflection sensitivity (nm/V), and \( F_s \) represents the force setpoint (V). The vendor [25] supplied force constant of a Tap 190 DLC tip is 48 N/m with an accuracy of ± 5%. A force setpoint of 3.5 V corresponded to a force of 9.06 µN with an accuracy of ± 0.45 µN. A force setpoint of 5.5 V corresponded to a force of force of 14.24 µN with an accuracy of ± 0.72 µN. A force setpoint of 7.5 V corresponded to a force of 19.42 µN with an accuracy of ± 0.97 µN. A force setpoint of 10 V, the maximum input force setpoint, corresponded to a force of 25.89 µN with an accuracy of ± 1.29 µN.

2.2 Calibration using a Pyrex substrate without microchannel designs

AFM nanolithography was conducted using the small scanner (10 µm range) to mechanically machine nanochannels using a tip that moves up and down in the y direction to create trenches in a flat Pyrex substrate material for calibration purposes. One chip was used for the entire experiment. After the substrate stand was attached to the AFM, the scanner was inserted with the nose cone and probe in place to scan over the designated scratch area. A small scanner (9 x 9 µm scan size) was used to create at least four Nanochannels at four different force setpoints using the same tip for different number of scratches. The PicoLITH software was used for nanolithography. Each channel was approximately 4 µm long and the time taken was 3 and 4 minutes for 50 scratches respectively using a tip speed of 1 µm/s. The width and depth of the nanochannels were measured using the AFM characterization tools. Bhushan et al [29] also demonstrated that the nanochannel depth is not related to the tip speed or cutting velocity.
AFM tip wear can be encountered, so as a preventive measure a new tip was used for each force setpoint to machine nanochannels at four different numbers of scratches (25, 50, 75, and 100) and measure their corresponding depth and width as shown in Figure 19. A nanochannel fabricated at a specified number of cuts, applied force, and tip speed looked similar to the nanochannels shown in Figure 19. The average depth and width of a nanochannel was obtained by averaging 10 points along the length of the channel by measuring the arbitrary cross section. A reference point closest to 0 was selected to measure the depth as shown in the cross section to the left in Figure 19. This ensured that the true depth was measured from a flat area to the scratched depth.

Figure 19. Average depth and width are determined based on 10 cross sections along the nanochannel length.
Chapter 3: Materials and Methods

This chapter describes the materials used and the design and fabrication methodologies utilized. The focus will be on wafer selection, determination of optimum parameters for photolithography, AFM nanolithography, and anodic bonding.

3.1 Materials

A Pyrex 7740 substrate was used for AFM nanolithography and a Silicon capping piece was used for anodic bonding. The Pyrex 7740 wafers (University Wafer) used were 100 mm in diameter and 500 μm thick. The 125 mm silicon wafers used were n-type, As doped, 625 μm thick, and had a resistivity in the range of 0.001 - 0.007 Ω-cm. The Si (100) wafers were diced for capping the Pyrex chip during anodic bonding. AFM probes (Tap 190 DLC) were purchased from Budget Sensors, usually ten probes per box. Fluorescein (free acid), product name F2456-2.5G, was purchased from Sigma-Aldrich and had a 95 % dye content. Also, a 10 ml bottle of yellow-green, negatively charged, 20 nm carboxylate-modified FluoSpheres (from Invitrogen) was purchased from Invitrogen for translocation and fluorescent experiments. The FluoSpheres contained 2% solids and are transparent to light in suspensions due to their small size.

3.2. Wafer Design and chip selection

The photomask was design using AutoCAD, had microchannels separated by varying gap sizes (Figure 20) were patterned to a 100 mm Pyrex wafer and etched using a 1 : 2 : 2 solution of Buffered Oxide Etchant (BOE) : HCl : H₂O at an etch rate of 1 μm/s. The etch rate of pure BOE with HF is about 1000 Å/min at room temperature. The thickness of the wafer was 500 μm and each of the 13 Pyrex microchips had two microchannels separated by gaps ranging from 20 - 100 μm as illustrated in Figure 20. The red, hatched designs were preferred for AFM nanolithography due to
their smaller gaps (20 – 40 μm). The 40 x 40 μm, 40 x 100 μm, and 40 x 150 μm designs were reported in this thesis.

![Wafer design demonstrating thirteen (13) microchips containing microchannels with varying gaps (μm)](image)

Figure 20. Wafer design demonstrating thirteen (13) microchips containing microchannels with varying gaps (μm)

The complete nanochannel system design is shown in Figure 21 where the nanochannel region is located between microchannels. Additionally; the microchannels had two separate microreservoirs, each with a 2.50 mm radius for fluid inlet and outlet as shown in a selected design in Figure 22.
The complete nanofluidic device was 1 cm x 2 cm with inlet and outlet access holes in the micro reservoirs for fluid flow.

Figure 23 illustrates the side view of the overall structure of the nanochannel device with the silicon capping piece. Two microreservoirs are attached to microchannels and connected by a nanochannel with upstream and downstream connectors for fluid access.
Figure 23. Schematic illustrating side view of nanofluidic device

3.2 AFM Nanolithography Procedure

The Agilent 5500 AFM and scanning software, PicoView 1.4.8 were used for scanning the nanochannel regions. Nanochannels were fabricated using PicoLITH [18]. The fabricated nanochannels bridged the gap between the two microchannels. Prior to turning on the AFM machine, the microchip was placed on the substrate stand in the desired orientation. The AFM startup kits with the necessary parts were then assembled. Although the PicoView 1.4.8 software was operated in contact mode, an AC mode nose cone was selected for use in the large scanner, which allowed a 10 to 100 μm scan size. An AC mode nose cone was selected over a contact mode nose cone because when scans are performed using a high frequency tip, scan errors and unsatisfactory scans are usually achieved. With an AC nose cone, excellent topography scans can be obtained. After inserting the AC nose cone into the scanner, a diamond-like carbon (DLC) coated tip was fitted between the retaining guides on the AC nose cone.

The processor and AFM controller was then turned on and the scanner with the attached probe was flipped and fitted into its slot on the AFM stage. The scanner was tightened using the screws on the microscope. The color coded cables on the scanner were then fitted to their designated slots. The laser detector was then placed into its position before the alignment of the
laser. The high intensity illuminator was turned on to visualize laser alignment. In contact mode, the deflection can range from -0.5 V and -1.0 V and the friction should be close to 0 V to minimize drifts. A typical laser alignment deflection is -0.75 V after aligning the laser. The sample on the substrate stand was then loaded in the sample area and the laser realigned. The deflection decreased to zero when the AFM tip reached the sample surface. In PicoView 1.4.8, a specified scan size and a scan speed of 1 line per second was used for obtaining the surface topography after ensuring that the tip approached the sample surface. After the scan was finished, the scanned image was then loaded to the PicoLITH software that allowed nanolithography (scratching) by dragging the AFM tip across the surface. An arrow is drawn over the desired region on the image to be scratched and the AFM tip is then positioned close to that location. The AFM tip follows the arrow path based on the set number of scratches.

The step by step AFM nanolithography procedure was as followed.

(1) The nanolithography process began with assembling the scanner (large or small) and placing it into the scanner jig as shown in Figure 13.

(2) The desired nose cone, contact mode or AC mode, is then inserted into the scanner as shown in Figure 13.

(3) After inserting the nose cone into the scanner the AFM tip was then placed between the retaining guides on the nose cone as shown below with the tip overhanging the scanner window.

(4) The scanner with the inserted nose cone and tip was then inserted into the Agilent 5500 AFM.

(5) The machine, AFM controller, and laser were then turned on respectively. Once on, the Picoview 1.8 software was then accessed to align the laser and control AFM parameters such
as mode, closed loop (feedback)/open loop scanner, types of images to be scanned (raw
deflection, topography, friction etc.), scan size, resolution, and scan speed.

(6) The substrate was then placed on the substrate stand and then fitted under the scanner as shown in Figure 6.

(7) The laser detector was aligned using the PicoView software by adjusting the XY stage and laser detector to ensure that there was feedback between the scanner and the software. Once fitted and aligned, a manual approach was performed by pushing down the open/close button on the AFM controller. While approaching, one can gauge the distance by looking at the Scan and Motor dialog box. After approaching to a close enough distance, the Approach button was then clicked in order for the machine to sense the distance between the sample and the tip automatically.

(8) Once Approach was complete, the tip had to be withdrawn at least once by clicking Withdraw to move around and find the desired scan area on the sample. After finding the preferred scan area, an Approach was done once again to ensure that the tip was in contact with the sample surface (contact mode). Thereafter, the scan size was selected and the contrast and scale of images were modified while they were being scanned in real time. The AFM tip moved in the forward y direction so the vertical placement of samples always generated successful results when nanolithography was performed.

(9) After scanning, the scanned topography image was loaded to PicoLITH, the nanolithography and nanomanipulation software used for fabricating nanochannels. Figure 14 shows how the process works. One first has to draw a line using arrows in a desired location and the AFM tip followed this path accurately if operated using a closed looped scanner. A closed loop scanner reduced drifting and positioning inaccuracies. After drawing lines, the number of
scratches, force setpoint, and tip speed were modified to the desired settings before nanolithography began as shown in Figures 14, 15, and 16.

Figure 24 illustrates a schematic illustrating the fabrication process for microscale lithography showing the side view process flow diagrams of the micro and nanofabrication steps.

(a) 4-inch Pyrex 7740 wafer with 500 µm thickness

(b) Spin coat an 8 – 10 µm layer of AZ4620 photoresist

(c) Pattern photoresist using the photomask design shown in Figure 20 and the Karl Suss Mask Aligner

(d) Etch using a 1:2:2 ratio of BOE solution with 10% BOE: HCl: DI H₂O

(e) Strip photoresist with acetone

(f) AFM nanolithography to machine nanochannels
3.3 Wafer Processing

3.3.1 Photolithography

The Pyrex wafers were processed in the High Density Electronic Center (HiDEC) at the University of Arkansas and in Dr. Adam Huang’s laboratory at the Engineering Research Center. Prior to patterning, the wafers were cleaned with acetone to ensure that there were no contaminants on the surface. An AZ4620 positive photoresist was spun on the wafer using a spin speed of 1000 rpm for 2 mins using the Eaton Desktop Coater. Spin speeds of 1000 rpm results in a 2.5 μm resist thickness. After applying the photoresist, the wafer was soft baked on a hot plate for ten minutes at 110 °C to prevent the photoresist from sticking to the mask during exposure. The Karl-Suss MA150 Mask Aligner in HiDEC was used to align the mask to the wafer before each exposure. The intensity was recorded from the process logbook in order to
determine the exposure time needed for the wafer. To determine the exposure time, the intensity of the tool and the energy were needed. Energy was calculated using Equation 2:

\[
\text{Energy} = 35 \text{ (constant)} \times \text{resist thickness (µm)}
\]

Equation (2)

The exposure time for each wafer was calculated using equation 3:

\[
\text{Exposure time (s)} = \frac{\text{Energy needed to expose resist (mJ/cm}^2\text{)}}{\text{Intensity of the UV lamp (mW/cm}^2\text{)}}
\]

Equation (3)

After calculating the exposure time, the mask was ready for alignment and exposure. Before aligning, the touch screen settings on the Karl Suss Mask Aligner had to be modified by editing the parameters. After editing the parameters, the mask stage was centered. The wafer was then placed directly on the aligner chuck and once the wafer was aligned, the desired mask was loaded. The vacuum was turned on to ensure that the mask was fixed in place. After alignment, the wafer was ready for exposure at the calculated exposure time. The wafers were ready for developing after exposure was completed. Developing was important since it ensured that the photoresist features remained on the wafer. The develop time was determined from the resist thickness versus develop time chart. The develop time required for the wafers spun at 1000 rpm with a resist thickness of 2.5 µm was 90 s. After developing the wafers, the next step was inspection of the patterns on the wafer for defects. The Kasper Eaton Mask Aligner from Dr. Adam Huang’s lab was also used for processing wafers with an AZ4620 photoresist. After loading the mask, an exposure time of 40 s was used. The developer solution was AZ400K solution and DI water in a 1 : 3 ratio. The develop time was typically 45 to 50 s.

3.3.2 Wet Etching

The patterned Pyrex wafers were submerged in a 10:1 buffered oxide etchant (BOE) solution with HCl and H₂O in a 1:2:2 ratio using a large plastic beaker for 10 minutes at an etch
rate of 1 µm/min. After isotropic BOE etching, undercutting shrank the nanochannel gap region between the microchannels to approximately the design gap value minus two times the etch depth as shown in Figures 25 and 26. After etching, the microchannels walls were curved as shown in Figure 25 creating contour shape for nanochannel region. The AFM tip followed that contour to connect the two microchannels. Figure 26 shows the contour shape of the nanochannel region before (L_M) and after etching (L_N). The etch depth, d, directly affected the nanochannel region. The approximate length of the nanochannel region can be calculated using Equation 4.

\[ L_N = L_M - 2d \]  

Equation (4)

where \( L_N \) = nanochannel length after isotropic BOE etch and \( L_M \) = distance between microchannels (design value) as shown in Figures 20 and 22.

Figure 25. Design profile (left) vs. Post-etch profile (right)

Figure 26. Nanochannel region pre and post-etch profile
3.3.3 Profilometry

The height and depth of the microchannels were measured in microns after the Buffered Oxide Etch (BOE) using the Sloan Dektak 3030 profilometer at the High Density Electronics Center (HiDEC). Also, a laser source connected to a voltmeter and DC supply was used to determine the depth of microchannel trenches after etching. Using a reference point, the laser detected the change in height of the sample surface. A reading of 1 mV on the voltmeter corresponded to a depth of 1 µm. Figures 27 and 28 show the depth of microchannel trenches after etching in a 1:2:2 BOE:HCl:H₂O solution for ten minutes. Figure 27 show the result of etching using a more concentrated BOE solution (7:1) while Figure 28 is the result of a 10:1 BOE solution. For AFM nanolithography and bonding purposes, microchannel etch depths were kept under 15 µm to simplify the nanolithography process and prevent the AFM tip from breaking.

![Dektak image showing Microchannel trench depth of 3.5 µm](image)

Figure 27. Dektak image showing Microchannel trench depth of 3.5 µm
3.4 Packaging

3.4.1 Dicing, Inlet/Outlet Opening, Chip Cleaning

Pyrex wafers were diced using the Micro Dicing Saw (Model 1100) with a ceramic blade at the HiDEC facility. Silicon wafers were using a diamond blade. After dicing the wafer into 13 individual chips, a Dremel tool (Drill Press) was used to drill holes in the inlet and outlet of the chips for fluid access. The diameter of access holes is 2.5 mm. For anodic bonding, substrate cleanliness is critical, therefore the Pyrex and silicon chips were cleaned with a Piranha solution (3:1 mixture of H₂SO₄ and 30 % H₂O₂) at 50 °C followed by blow drying with nitrogen.

3.4.2 Anodic Bonding

The microchip was ready for bonding once the desired scratch results were obtained. After scratching the nanochannel, anodic bonding was conducted to cap the Pyrex chip with a
silicon chip. Prior to bonding, holes were drilled at the inlet and outlet reservoirs to provide access to fluids. Anodic bonding was performed at 400 – 450 °C and 900 V on a hot plate. Figure 29 demonstrates the anodic bonding setup. The silicon was first placed on the brass chuck on top of the hot plate. The Pyrex chip was then aligned on top of the silicon before the top metal electrode was pushed down to initiate contact as demonstrated in Figure 30. Bonding began once the DC supply was turned on. The bonding process forms a layer of SiO₂ which seals the Pyrex and silicon substrates together.

A SiO₂ intermediate layer provides a great advantage for biomedical applications due to the transparent optical properties for fluorescence detection [31]. The fabricated nanochannel had a very smooth inner surface due to the property of the amorphous silicon [31, 32]. Likewise, the nanochannel exhibited hydrophobic surface properties, which is not favorable for fluid flow along the nanochannel [31]. The anodic bonding process took approximately four to fifteen minutes to complete depending on the temperature used. Complete bonding was confirmed visually as shown in Figure 31. Bonding time increases with a negative polarity configuration (Pyrex to silicon) [31,32]. However, a negative polarity configuration produced better bonding quality as opposed to a positive polarity configuration (silicon to glass).
When electric field was applied during anodic bonding, the ions present in the substrates became mobile. Pyrex wafers are rich in sodium (Na⁺) so Na⁺ ions migrated towards the anode (−) to become neutralized as shown in Figure 31. The red circle shows a black spot where bonding began after contact between the electrodes was initiated.

Figure 30. Schematic showing the anodic process
The remaining oxygen molecules at the Pyrex interface interacted with the positive silicon atom to form a layer of SiO$_2$ which yielded a permanent, irreversible electrostatic bond.

![Diagram of anodic bonding mechanism](image)

Figure 31. Schematic showing the anodic bonding mechanism

### 3.4.3 Attachment of connectors

After anodic bonding was completed, plastic connectors were attached to the inlet (s) and outlet of the device for fluid access by mixing five (5) minute epoxy. The complete device, with an inlet and outlet connector is shown in Figure 32 (Y-shaped design nanochannel system) and Figure 33 (straight microchannel design). The chip dimensions were roughly 1 cm x 2 cm.
Figure 32. Completely Fabricated Y-shaped Microchannel Device

Figure 33. Completely Fabricated Straight Microchannel Device
Chapter 4: Results and Discussions

In all nanochannel systems, the radius of the reservoirs was 2.50 mm and each chip was 1 cm x 2 cm as shown in Figures 32 and 33. The distance between the microchannels varied for each design but smaller gaps (20 – 40 μm) were preferable for AFM nanolithography due to the scan size limitation of the large AFM scanner and the time taken to scan and machine channels over larger gaps.

4.1 Nanochannel Dimension Calibrations

This section reports the results obtained from nanochannel calibration on a flat Pyrex substrate without microchannel designs to determine the relationship between AFM input parameters and the resultant nanochannel dimensions. The results obtained from these calibrations were used as estimates to tailor the resultant depth and width of nanochannels machined to connect the microchannels.

The calibration experiments were performed at different force setpoints (V) and number of scratches. The force setpoint is later converted to applied force (μN), which is the constant force applied to the cantilever. Table 3 shows the design of experiments utilized for the calibration experiments.

<table>
<thead>
<tr>
<th>Factors (Input Parameters)</th>
<th>Treatment Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force Setpoint</td>
<td>3.5 V, 5.5 V, 7.5 V, 10 V</td>
</tr>
<tr>
<td>Number of scratches</td>
<td>25, 50, 75, 100</td>
</tr>
<tr>
<td>Tip Speed</td>
<td>1 μm/s</td>
</tr>
</tbody>
</table>
Experiments were run at a tip speed of 1 \( \mu \text{m/s} \) since previous studies by Zhiqian et al.\cite{28} determined that the cutting velocity did not have a significant effect on the depth or width of resultant nanochannels. Figure 34 shows a topography image after AFM nanolithography was performed using four specified numbers of scratches. Nanochannels that were 4 \( \mu \text{m} \) long were fabricated on a Pyrex glass substrate at a tip speed of 1\( \mu \text{m/s} \) at force setpoints of 3.5 V, 5.5 V, 7.5 V, and 10 V for 25 scratches, 50 scratches, 75 scratches, and 100 scratches respectively. Figures 34 and 35 visually show that the channel width became wider for a higher number of scratches. However, a relationship was not established between the width, number of scratches, and force setpoint. For all experiments, however, nanochannels were wider at 100 scratches but there were instances where nanochannels were of similar widths at 50 and 75 scratches. Figure 35 shows the cross section surface profile of the scratches illustrated in Figure 34. The cross section image shows that the depth profile increased for higher number of scratches. The same tip was used to scratch all four nanochannels at different scratch cycles. In Figures 34 and 35, (I) represents 25 scratches, (II) represents 50 scratches, (III) represents 75 scratches, and (IV) represents 100 scratches using the same applied load of 14.24 \( \mu \text{N} \). The unnumbered scratches had shallow profiled and were a result of initial scratch attempts with a broken tip. The AFM tip was withdrawn from the sample surface after scratching then an approach had to be done to engage the tip with the surface after scanning to reveal the scratch obtained from nanolithography.
Figure 34. AFM Topography image showing cross section of scratches at 5.5 V (14.24 µN). I-25 scratches, II-50 scratches, III-75 scratches, IV-100 scratches.

Figure 35. AFM cross section image showing the depth and width of fabricated nanochannels machined at 14.24 µN. I-25 scratches, II-50 scratches, III-75 scratches, IV-100 scratches.

Figure 36 shows a close-up cross-sectional image of a nanochannel obtained at 14.24 µN at 100 scratches. Minimal pile-up (less than 5 nm) after nanolithography is ideal for anodic bonding. Sonication was used to improve surface smoothness for anodic bonding after nanolithography but the reliability of this method was not investigated. Anodic bonding results
showed that pile up prevents perfect bonding. Scratching on brittle materials such as glass does not typically produce pile up since the loose debris is pushed away by the AFM tip. On the other hand, ductile materials such as polymer and gold more often than not produce buildup of material after nanolithography. The cross section profile images resemble the geometry of the AFM tip.

![Figure 36](image)

Figure 36. Cross sectional plot demonstrating the average depth at 14.24 μN for 100 scratches

As shown in Figure 36, the cross section of the nanochannel resembles the shape of the AFM tip. The sharper the AFM tip the narrower the base of the channel’s cross section which closely matches the apex of the pyramidal shaped tip [26, 27]. During calibration, the time taken to complete a 4 μm long cut was independent of the force setpoint but increased for the number of scratches. As the number of scratches increased, the scratch time also increased. It took about 7 minutes to fabricate nanochannels at 100 scratches for all applied forces (μN).
Characterization experiments performed in many tribological studies mostly focused on silicon based substrates. Several tribological studies were explored in detail but there were no models found that correlated the numbers of scratches and applied force consistently to the resultant nanochannel width. However, tribological scratch experiments were performed by Bhushan et al [29] to investigate the scratch depth versus applied force for ten scratch cycles on undoped Si (100). Tseng et al [30] also performed a scratch depth versus applied AFM tip force for a p-type silicon substrate for a single scratch. A comparison was made to the scratch depth versus normal load study.

A microtip fabricated from single-crystal natural diamond and ground to a three-sided pyramidal shape was used for the scratch and wear tests on undoped Si (100) for ten scratch cycles with loads ranging from 20 to 100 µN [20] was used in Bhushan’s study [29]. The tip apex had an angle of 60° and the tip radius was approximately 70 nm, attached to a stainless-steel platinum coated cantilever with a spring constant (stiffness) of 50 N/m. The results indicated that scratch depth increased with the applied load (Figure 25). The results were compared to the calibration results using a Pyrex 7740 substrate with the diamond-like carbon coated tip (Tap 190 DLC) whose apex had an angle of 10° with a radius of less than 15 nm and spring constant of 48 N/m.

Tseng et al [30] used a triangular pyramidal AFM tip with a 100 nm thick diamond coating for a low applied force range (1 to 9 µN). The tip radius was approximately 120 nm and the angle at the apex was 10°. Scratching experiments were performed on a p-type Si (100) substrate with an AFM tip attached to a silicon cantilever with a spring constant of 42 N/m. The results comparing the two studies to the AFM scratch experiments on Pyrex are illustrated in Figure 37.
Although the tip used by Bhushan et al [29] was wider and stiffer, and Tseng’s tip [30] more durable coating of diamond, the results obtained using the Pyrex substrate were comparable to both studies and a linear relationship was established as shown in the log-log plot in Figure 37.
At a load of 20 µN, Figure 37 illustrates that the scratch depth increases with the number of scratch cycles (25, 50, 75, and 100) for Pyrex. The scratch depth for undoped Si at 10 scratches was similar to the depth obtained on Pyrex for 50 scratch cycles. This may be due to the fact that the microtip used in the tribological study had a slightly higher spring constant and a radius that was almost five times larger than the Tap 190 DLC tip used on Pyrex.

As the applied force increased, the resultant nanochannel depth increased. This was consistent with tribological scratch experiments done by Bhushan et al [29] and Tseng et al [30]. The maximum force setpoint that the piezotube scanner could maintain was 10 V, which corresponded to an applied force of about 25.89 µN with the Tap 190 DLC tip. The error bars in Figure 38 indicate that there was variation in the data collected. There was a wider data range in higher loads and more scratches, which could be possibly due to fluctuations in the applied force and minimal thermal drifts. The error bars shown in Figures 38 and 39 represent indicate the maximum and minimum values of 10 data points for the average nanochannel depth. The error bars also indicate that the relationship between the applied force and the resultant nanochannel depth is mostly linear.
Figure 38. Mean depth (nm) vs. applied force (µN) on a Pyrex 7740 substrate

The number of scratches versus average depth plot (Figure 39) shows that as the number of scratches increased, the depth of nanochannels also increased. Since the scanner was actuated by piezoelectric tubes and the force setpoint went to a maximum of 10 V (applied force of 25.89 µN) as mentioned earlier, the data achieved at 10 V were not as reliable as data obtained with lower force setpoints.
Nanolithography experiments were conducted to fabricate nanochannels for the realization of channel patency. A $40 \times 40 \, \mu m$ design chip with an average depth of 45 nm and a width of approximately 175 nm was used for fluorescence microscopy experiments with Fluorescein. Figures 40 and 41 show 2D and 3D topography perspectives of a fabricated nanochannel previously described. Figure 40 and 41 demonstrated that one continuous, through channel was fabricated with other visible scratches that were discontinuous and too shallow to realize flow.
Figure 40. 2D Topography image after AFM nanolithography

Figure 41. 3D Topography 3D image (45° left view) microchannels after AFM nanolithography: 40 x 40 µm chip
Multiple scratch attempts were made on a 40 x 100 μm design chip before obtaining a channel that was deeper than 20 nm for flow testing with 20 nm nanobeads. It was desired to have a nanochannel depth greater than 20 nm to accommodate the beads and avoid flow complications. The resultant nanochannel depth was approximately 98 nm and the width was approximately 623 nm after sonication. The 3D images in Figures 42 and 43 demonstrate the before and after surface topography images.

![3D image of microchannels illustrating unsuccessful AFM nanolithography](image)

**Figure 42.** Topography 3D image (135° right view) of microchannels illustrating unsuccessful AFM nanolithography: 40 x 100 design μm chip
Figure 43. Topography 3D image (45° left view) of microchannels after AFM nanolithography: 
40 x 100 µm design chip

After nanolithography, the cross section profiles of the nanochannel were measured to determine the resultant depth and width. The average depth achieved was approximately 98 nm and a width of 623 nm with an all diamond AFM tip (DNISP, 40 nm radius, stainless steel cantilever, 150 N/m spring constant). When the AFM tip is sharper than the sample surface, the surface topography image was truly represented. However, when the sample surface was sharper than the AFM tip the feature was dominated by the geometry of the tip. Tip convolutions are evident in cross section images that show a rounded bottom for depth profiles instead of a conical shape [26, 27]. The average surface roughness of the Pyrex substrate was provided from
manufacturers (University wafer) as less than 1.5 nm Ra (15 Å) and the flatness was better than 5 μm.

4.2 Anodic Bonding

Anodic bonding is important for sealing the AFM-nanomachined chip with a matching silicon piece. The Pyrex cover (500 μm thick) with predrilled holes was placed on top of the silicon chip (625 μm thick) before the DC voltage supply was turned on. Placing the gold brass electrode in the center of the hot plate and measuring the temperature using a Multimeter ensures that there was uniform temperature distribution in the silicon chip to ensure good bonding [31, 32]. The only way to determine good bonding besides doing a bond strength test is to visually look at the Pyrex substrate on top or by attempting to pry the sealed device open. As bonding initiated, a black ring propagated from the radius of the anode in contact with Pyrex cover on top. Bond time typically took four to fifteen minutes depending on the temperature and voltage used. For a high temperature of 450 °C at 900 V, the bond time was approximately 4.5 minutes. At 400 °C and 900 V, the bond time was approximately 7 minutes while at 350 °C with the same voltage, the bond increased to 14 minutes. Figures 44 to 46 show images of nanofluidic devices after anodic bonding was completed using different process parameters. Figure 44 below shows that bonding is perfect around the channels but the rainbow rings on the outer perimeter of the chip represents the areas that have not been bonded properly or at all.
Figure 44. Satisfactory bonding achieved at 400°C and 900 V around channels and microreservoirs. Rainbow rings are unbounded.

Figure 45. Unsatisfactory bonding achieved at 350 °C and 900 V

Figure 46. Excellent bonding achieved at 450 °C and 900 V
4.3 Flow Tests

Flow tests were performed in order to demonstrate that the fabricated nanochannels were continuous over their entire length. Prior to flow testing the nanochannels were treated to modify their surface chemistry (wetting) to enable channel filling. After anodic bonding, the channels become hydrophobic so surface treatment is necessary to promote channel wetting using different fluids.

4.3.1 Wetting Steps

A fluidic connector was attached to the inlet of the device for wetting in a vacuum dessicator as shown in Figure 47. Afterwards, Tygon tubing was connected to a syringe to be attached to the connector at the inlet as illustrated in Figure 48. The vacuum dessicator is attached to a vacuum pump which was necessary for surface chemistry modification.

![Figure 47. Syringe attached to nanofluidic device for pumping in a vacuum dessicator](image)

The wetting process required the use of a vacuum dessicator that was connected to a vacuum pump to help prevent air bubbles from being trapped in the channels and to enable fluid flow. Wetting in a vacuum dessicator is important for pressurizing the nanofluidic device before
executing the wetting steps. A constant pressure was applied to the inlet of the device since the cover lid touched the top of the syringe once the vacuum was turned on. The outlet of the device was left open to atmospheric pressure in the dessicator as shown in Figure 48.

![Syringe attached to nanofluidic device for pumping in a vacuum dessicator](image)

**Figure 48. Syringe attached to nanofluidic device for pumping in a vacuum dessicator**

The device was placed inside the vacuum dessicator and the syringe was filled with Methanol, Isopropanol Alcohol (IPA) and DI water for 30 to 40 minutes sequentially. Wetting times varied depending on the result obtained after microscopic inspection. The horizontal line shown in the upstream Figure 49 is an electronic artifact without any meaning. The wetting of surfaces was important for fluid flow since surface chemistry modification (hydrophobic versus hydrophilic) would affect the flow performance of a nanofluidic device [25]. The four step process improved wettability and made the channels more hydrophilic to enable fluid transport. The idea of applying constant pressure to the system upstream while leaving the downstream exposed to vacuum was required to obtain a high pressure gradient. Before wetting began, the dry state of the channels (Figure 49) was investigated using an optical microscope (Nikon).
Figure 49. Enlarged view (10X) of microchannels before the wetting steps (dry state)

Figure 50 shows the wetting result after pumping acetone from the inlet of the device initially for 40 minutes.

After pumping acetone for minutes, pumping was continued overnight until fluid filled the upstream microchannel as shown in Figure 51.
The wetting process continued by pumping methanol from the upstream microchannel (Figure 52) followed by Isopropanol Alcohol (IPA). After IPA, the nanobeads were pumped from the inlet to introduce the beads into the microchannel (Figure 53).

Figure 51. Result after pumping acetone overnight

Figure 52. Result after pumping Methanol from the upstream connector for 40 minutes
Figure 53. Result after pumping Carboxylate-Modified 20 nm FluoSpheres for 1.5 hours

4.3.2 Fluorescence Microscopy tests using Fluorescein

After the wetting steps were completed, the microchannels and nanochannel location of the nanofluidic device were examined under the microscope using the Osprey camera software. Fluorescence microscopy was then performed using Fluorescein (Sigma Aldrich). The Fluorescein used was a yellow-green dye (95% solids) with an excitation wavelength of 460 nm, an emission wavelength of 515 nm, and absorption maxima of 493.5 nm [26].

Flow experiments were performed using Fluorescein (free acid) with 95% dye content. A 25x dilution yielded fluorescence that was bright enough to illuminate the channels at a low exposure time (3 – 4 s). The procedure used to prepare a 25x dilution of Fluorescein solution is as follows:

(a) Measure 1 mg of Fluorescein and place into centrifuge tube
(b) Add 1 ml of 100 % ethanol to the tube to achieve a concentration of 1 mg/ml
(c) Use the Vortex Touch Mixer Model 232 (Fisher Scientific) to mix the solution
(d) Centrifuge for 1 min using the Sorvall Biofuge primo centrifuge at a speed of 13,000 rpm
(e) Remove supernatant from and dilute with DI H$_2$O (25x dilution)

For the experiments, a total solution of 37.5 ml was desired. In order to achieve a 25x dilution, 36 ml of DI H$_2$O was added to 1.5 ml of the Fluorescein and Ethanol mixture. Prior to preparing a 25x dilution, a 100x dilution was prepared but did not illuminate the channels when flow experiments were performed since the fluorescent intensity was so weak. The solution used for the 100x dilution was typically 0.25 µL of Fluorescein in 100% Ethanol and 24.75 ml of DI H$_2$O to make a total solution of 25 ml. Figures 54 and 55 show fluorescent images of a 40 x 40 µm design chip after pumping with Fluorescein. Figure 54 shows the fluorescent image of a bright green, filled upstream microchannel after pumping in a vacuum dessicator for about 1 hour.

![Fluorescent image](image.png)

Figure 54. Fluorescent image after pumping Fluorescein for about 1 hour in a vacuum dessicator

Figure 55 shows that after an additional 45 minutes of pumping, the Fluorescein solution migrated through the nanochannel and filled the downstream microchannel. Figure 55 verified channel patency of a 40 µm long nanochannel with an average depth of approximately 45 nm and a width of about 200 nm.
4.4 Translocation of nanobeads

The idea of translocation stemmed from electrosomostic flow which involves a charged surface in contact with fluid particles. Negatively charged carboxylate-modified nanospheres were used to investigate the movement of charged particles through the nanochannel. When an electric field is applied across the length of the device, the oppositely charged cations (counter ions) in the diffusion layer will be pushed towards the negatively charged electrode (cathode) and viscous coupling will allow a net flow of the fluid to migrate from the positively charged electrode (anode) to the cathode [33, 34]. A voltage (typically 10 V) was found to be ideal for the translocation of negatively charged nanoparticles.

4.4.1 Translocation Experimental Setup

Translocation experiments required the use of electrodes, a direct voltage supply, a source meter (Keithley 2410) for measuring the AC current in μA and a fluorescent microscope. Figure 56 shows the general setup for translocation experiments.
Initially, translocation experiments were performed by inserting two sewing needles in the inlet and outlet connectors which are then attached to a DC supply voltage after filling the inlet connector with 20 nm charged nanobeads (suspended in distilled water and 2 mM azide solution) and the outlet with Phosphate Buffer Saline (PBS). Figure 57 shows a schematic of the nanofluidic device without fluidic connectors to demonstrate the concept of the experiment with sewing needles as electrodes.
4.4.2 PolyDiMethylSiloxane (PDMS) Microchannel Translocation

A proof of concept experiment was performed using a straight PolyDiMethylSiloxane (PDMS) microchannel, PBS, and 20 nm FluoSpheres to determine if the beads were charged using a black and white inverted Fluorescent Microscope (Leica) to capture fluorescent images at the inlet of the channel. A 500 μm thick PDMS microchannel was plasma bonded to a glass slide then capped off with another glass slide as demonstrated in Figure 58.

![Fabricated PDMS Microchannel device for flow testing with nanobeads](image)

Figure 58. Fabricated PDMS Microchannel device for flow testing with nanobeads

The channel was first flushed then filled with PBS for translocation experiments. A DC supply voltage with used with two sewing needles attached to the negative and positive voltage source. The needle with the negative supply was inserted into the inlet of the device after filling the connector with a drop of the bead solution. The other needle was connected to the positive outlet and experiments were run at 8 V but the channel did not fill at this voltage. Once the voltage was switched to 10 V the channel began to illuminate starting from the inlet. Figures 59 to 63 show the sequence of steps to track the fluorescence of nanobeads in the PDMS microchannel.
Figure 59. Bright field after filling with PBS

Figure 60. Fluorescein Isothiocyanate (FITC) image after introducing beads to inlet at 8 V for 15 minutes
Figure 61. FITC image after 2 minutes after changing voltage to 10 V

Figure 62. FITC image at 10 V after 10 minutes (3 s exposure)
4.4.3 Nanochannel Translocation

Translocation experiments were performed using a DC supply voltage and two sewing needles. The sewing needle in the inlet of the device was connected to a negative voltage while the needle in the outlet had a positive voltage. The channels were first filled using the aforementioned sequential steps and subsequently filled with PBS. After filling with PBS, two drops of the stock solution of negatively charged FluoSpheres (carboxylate-modified nanospheres), 20 nm in diameter, were introduced in the inlet to be passed through the channels. The FluoSpheres had a yellow-green color when viewed under the optical microscope. Experiments were run at 5 V and 10 V for 2 to 10 minutes and fluorescence microscopy was performed thereafter to examine the migration of the fluorescent nanobeads.

A Y-shaped microchannel chip configuration (two inlets and one outlet) as demonstrated in Figure 64 was first used to demonstrate the translocation of nanobeads. An initial voltage of 5 V was used to pull the 20 nm FluoSpheres through the nanochannel but there was no trace of fluid movement after 10 minutes. The procedure was repeated using 10 V for 10 minutes and after a fluorescent microscope inspection, the upstream (section with two inlets) was illuminated.
by a bright green fluorescence as shown in Figure 65. A third attempt was done to drag the nanobeads through the nanochannel using the same voltage for 20 mins with repeated attempts at higher voltages but the fluorescent image did not change.

Figure 64. Schematic of 40 x 150 µm Y-shaped microchannel chip design

Figure 65. Fluorescent image of 40 x 150 µm Y-shaped microchannel chip after translocation for 10 minutes at 10 V (10X objective)
After the failure of performing translocation in a Y-shaped microchannel configuration, a similar test was repeated using a straight microchannel. Flow tests were performed on a 40 x 100 µm chip design to determine if the fabricated nanochannel (40 µm long, 98 nm average depth, and 623 nm average width) shown in Figure 43 was unobstructed. The sequential wetting procedure was followed after attaching a piece of tubing on the fluidic connector; a 3 ml syringe was placed over the Tygon tubing as shown in Figure 48. The device was then placed in the vacuum dessicator and inspected 40 mins after using each fluid before proceeding to use the carboxylate-modified 20 nm FluoSpheres (Figure 48). After pumping FluoSpheres (beads) from the inlet of the device for about 1.5 hours, the fluorescent bead solution illuminated the entire upstream microchannel while passing through the nanochannel to the downstream microchannel as shown in Figure 66. After pumping the beads through the nanochannel for 1.5 hours, a positive electric field was applied using 10 V (Figure) while measuring the AC current in µA from inlet (- V) to outlet (+ V) using a source meter (Keithley 2410).

![Figure 66. Fluorescence Microscopy Image after pumping negatively charged Fluorescent nanobeads for 1.5 hrs: 15 s exposure time](image-url)
The shape of the downstream fluorescence in Figure 67 was notably different from that illustrated in Figure 66 and showed that the nanobeads were pulled through the 40 μm long, 98 nm deep, and 623 nm wide nanochannel fabricated with an all diamond AFM tip. Figure 67 shows the fluorescence microscopy image after filling the downstream with PBS and applying a negative voltage to the inlet and positive voltage to the outlet for about 15 minutes at an exposure time of 15 s.

![Fluorescence Microscopy Image](image)

Figure 67. Fluorescence Microscopy Image after translocation with PBS at the outlet obtained after 15 minutes at 15 s exposure time

4.5 Silicon nanochannel system with embedded electrodes

A preliminary DNA translocation test using a silicon nanochannel system obtained from the Shenyang Institute of Automation (SIA), Chinese Academy of Sciences, was conducted. In addition to the results obtained using the designs on Pyrex, nanolithography and flow test experiments were run at SIA on a 1.5 cm x 3 cm silicon chip that had a silicon dioxide layer of 500 nm (Figure 68). The design had 5 pairs of electrodes fabricated through deposition and lift off techniques using titanium and platinum. The platinum line was 40 nm thick and was located
between the micro reservoirs separated by a 30 µm gap. The width of each electrode was 1 µm, the height was 40 nm, the width of each bond pad was 2 mm, and the microchannels were etched to a depth of 20 µm.

Figure 68. Design of a nanofluidic sensor on a silicon substrate with a 500 nm layer of oxide

The complete silicon nanochannel device with leads attached to the bond pads is illustrated in Figure 69. The inlet connector was filled with a fluorescent DNA solution.

Figure 69. Image showing the silicon nanochannel device with embedded electrodes and connectors attached to the inlet and outlet access holes.
An SEM image was taken to visualize the structure of the silicon nanochannel chip with embedded electrodes. Figure 70 shows an SEM image with the five pairs of electrodes located between the 30 µm microchannel gap. The resultant nanochannel was 24.56 nm deep and about 500 nm wide after being fabricated using an all diamond tip with a force constant of 215 N/m.

![SEM image](image.png)

Figure 70. SEM image illustrating two microchannels separated by a 30 µm gap with embedded electrodes that are 1 µm in width.

Figure 71 shows a close-up view of the 1 µm wide electrodes located between the nanochannel gap. During nanolithography, an AFM tip will scratch to break the electrodes and connect the two microchannels to fabricate a complete system. After scratching, the conductivity of the electrodes was measured to that the tip has broken the electrode trace without causing shorts due to metal smearing. The electrodes shown were 40 nm in height and 1 µm wide at the narrowest points.
Figure 7. Close-up SEM image illustrating two microchannels separated by a 30 μm gap with 5 pairs of embedded electrodes that are 1 μm in width.

Translocation experiments were also performed at 10 V using λ-DNA (Takara Bio Inc. Japan) that was prepared using 10,000 X concentration of SYBR (Invitrogen) green, fluorescent dye solution in a centrifuge tube filled to 50 μL to demonstrate channel patency. The fabricated nanochannel demonstrated in Figure 72 was approximately 24.99 nm deep, 520 nm wide, and 30 μm long. Figure 73 illustrates that DNA was successfully translocated from the upstream microchannel to the downstream microchannel through the nanochannel using a 10 V DC supply.
Figure 72. 3D Topography Image of Nanochannel

Figure 73. Fluorescent image (20 s exposure) of DNA translocated at 10 V for 10 minutes after wetting the channels overnight.
Chapter 5: Conclusions

A glass-based nanochannel system fabricated by combining MEMS techniques and AFM nanolithography was developed. AFM nanolithography was demonstrated as an effective method for fabricating nanochannels with depths ranging from 10 to 100 nm. The channel width was in the range of 200 to 600 nm. Nanochannel dimension calibrations demonstrated that there was a linear relationship between the applied AFM tip force and the depth of resultant nanochannel. The depth also increased with increasing number of scratches.

Continuous flow through the AFM nanochannel was demonstrated both optically and electrically. Fluorescence microscopy indicated the passage of liquid flow from the inlet of the nanochannel to the outlet. Nanobead translocation through the nanochannel was also demonstrated using 20 nm carboxylate-modified FluoSpheres. Preliminary DNA translocation was performed on a silicon-based nanochannel system fabricated by SIA. The result indicated that double-stranded λ-DNA can be transported through the nanochannel using a small voltage bias. Overall, the results of the present study suggest the possibility of using nanochannels fabricated by AFM nanolithography to perform nanobead and DNA translocation studies.
Chapter 6: Future Work

The following list of tasks can potentially improve the functionality of the nanochannel system for biomolecule characterizations:

(a) The fabrication of nanoelectrodes on Pyrex glass using lift-off techniques will improve the functionality of these nanofluidic devices. Attempts were made to deposit a 40 nm thick platinum line at the gap between the microchannels using FIB milling and Electron Beam Lithography (EBL) techniques but the Pyrex glass, rich in Na\(^+\), was accumulating too much surface charge.

(b) Micro/Nano electrodes can be deposited on a silicon substrate that has an oxide layer in the 500 – 700 nm range. With a silicon substrate, EBL can be used to deposit electrode lines between microchannels.

(c) Designs with shorter gaps between microchannels (10 – 50 \(\mu\)m range) should be implemented since that would simplify the AFM nanolithography process. The smaller the distance between microchannels, the easier it is to machine a nanochannel.

(d) An improved mask design should include a longer upstream micro reservoir with a short downstream to enhance flow testing results.

The passage of FluoSpheres through a nanochannel should clearly be demonstrated by fluorescent images and a sharp increase in the current between the upstream and downstream electrodes. An attempt was made to observe the current flow through the nanochannel system. Figure 74 shows the raw longitudinal current data signal through the device. When current passed through the nanochannel region, there was a noticeable fluctuation in the current signal which could have demonstrated fluid passage through the nanochannel. A voltage of 10 V DC was supplied using the Keithley 2410 source meter. A negative voltage was introduced to the
inlet reservoir of the device to initiate the flow of FluoSpheres which could be representative of Zone A in Figure 74. In Zone B, a dramatic increase in current was observed which is inexplicable because of the short time duration illustrated in Figure 74. Theoretically, the flow of the conductive nanobeads should enhance the electrical conductivity across the nanochannel [34]. However, the inflection illustrated in Zone B increase in current signal in the longitudinal direction (- V to + V) is not yet fully understood so a future study could examine the changes in current signal when there is no fluid in the channel and when the current supply is turned on and off.

Figure 74. Longitudinal electrical current signal (μA) versus time (s) through the Pyrex nanofluidic channel
References


Appendix A: Description of Research for Popular Publication

Nanoscale chips are breaking new grounds in biotechnology

Orain Hibbert, a University of Arkansas Microelectronics-Photonics Master’s candidate working with Dr. Steve Tung in Mechanical Engineering, is doing novel research at the University of Arkansas. He is working on making a nano device that has potential applications in the biomedical field. In this field, devices with embedded electrodes can revolutionize the area of DNA detection and drug delivery by sensing DNA information using electrodes.

Mr. Hibbert stated that when he presented his research to his peers, the main questions asked were “why are you doing this?”, “what’s the purpose of these devices”, “how does this research apply to real life applications?”, and “how would these devices help in the future?”

Although nanofluidic devices are not yet commercial, most of the potential applications lie in the detection and manipulation of single biomolecules such as DNA, viruses, or proteins. These devices are small in size they can have added functionality with the integration of electrodes and are expected to be used in research to sequence DNA molecules since all of our information are piled along a linear DNA molecule.
A single DNA molecule has four base pairs, A, T, C, and G. Each base pair contains our genetic information so the device can be used to read out DNA base information and for chemical analysis. Dr. Steve Tung says “this is an exciting, new research area that can offer significant advantages in direct DNA sequencing.” According to Orain Hibbert, “nanochannel systems will be commercially viable and popular devices in the next five years due to the promising potential applications in biotechnology.”

The complete device is approximately 0.25 inches in length and 0.75 inches in width; about the same size as cell phone SIM cards. The device consists of two layers that are bonded electrically together. The bottom layer is silicon and the top layer has the micro and nanochannel patterns on Pyrex glass. The top layer also has predrilled holes in the microreservoirs to create inlet and outlet access holes for fluid flow.

![Pyrex nanochannel device]

The device, however, has to be pretreated with chemicals to enhance the wetting of channel surfaces before flowing particles or fluids. The real challenge with glass-based nanofluidic systems is integrating electrodes and nanofluidic channels. Ultimately, this device will be used to sequence our DNA and detect other single biomolecules such as protein or viruses once electrodes are integrated.
Appendix B: Executive Summary of Newly Created Intellectual Property

The following list of new intellectual property items were created in the course of this research project and should be considered from both a patent and commercialization perspective.

1. A method for performing AFM nanolithography by performing calibration experiments to determine the ideal input parameters that yielded a desired width and depth of nanochannels on Pyrex glass.

2. A quick method for filling nanoscale channels where the device is completely submerged in different fluids to modify the surface chemistry of the channels. This is performed using a sequential wetting procedure with Acetone, Methanol, and Isopropyl Alcohol for 40 minutes. The channels were either soaked with DI Water or PBS in a vacuum dessicator as an alternative to the syringe method.

3. A method to use DC voltage to perform translocation experiments using two sewing needles. A negative voltage is applied to the inlet of the device and a positive voltage was applied to the outlet after filling the downstream microchannel with PBS. A drop of 20 nm FluoSpheres solution is introduced to the inlet fluidic connector. The application of voltage created a potential for the migration of beads from the inlet to the outlet of the device.
Appendix C: Potential Patent and Commercialization Aspects of listed Intellectual Property Items

C.1 Patentability of Intellectual Property

The three items listed are considered from the perspective of whether or not each item could be patented.

1. The AFM nanolithography process on a glass substrate can be patented. Performing nanolithography on glass (Pyrex) substrates using a sharp AFM tip is novel to date.

2. Channel wetting (surface modification) methods cannot be patented. A unique approach was employed to ensure complete channel wetting. A vacuum pump attached to a dessicator forced air bubbles out of the channels and enhanced the wettability of channels. The sequential wetting steps were obtained from previous studies and improved for the nanofluidic devices used in this research. While effective, this technique was a slight modification to established channel wetting technology and would be obvious to one skilled in this area.

3. A method to use DC voltage to perform translocation experiments using two sewing needles cannot be patented. Sewing needles were inexpensive replacements for electrodes and this technique would be obvious to one skilled in this area.
C.2 Commercialization Prospects

The three items listed are considered from the perspective of whether or not the item should be patented.

1. AFM nanolithography on a Pyrex glass substrate should be patented. A patent disclosure was submitted to the Technology Licensing Office at the University of Arkansas and is still under review. An intellectual property disclosure form identified that this thesis contained an invention of commercial interest and was signed by Jeff Amerine, Technology Licensing Officer at the Innovation Center at the University of Arkansas, on September 19, 2011. The technology described is novel and defensible owing to the cross sectional profiles of the resultant nanochannels. Nanochannels fabricated on Pyrex using AFM nanolithography can be detected by reverse engineering since other nanochannel fabrication techniques have dissimilar cross sectional channel profiles.

2. Channel wetting techniques should not be patented. See prior analysis that this should not be patented (Appendix C.1).

3. A method to use DC voltage to perform translocation experiments using two sewing needles should not be patented. See prior analysis that this should not be patented (Appendix C.1).
C.3 Possible Prior Disclosure of IP

The following items were discussed in a public forum that could impact the patentability of the listed IP:

(1) AFM nanolithography on Pyrex substrates for the fabrication of nanochannel systems and flow testing techniques were discussed in details on November 18, 2010 at the ASME 2010 International Mechanical Engineering Congress & Exposition in Vancouver, British Columbia.
Appendix D: Broader Impact of Research

D.1 Applicability of Research Methods to Other Problems

Nanofluidic systems can be utilized in nanoscale diagnostics to perform single molecule analysis, detection, and separation of single biomolecules. In DNA analysis, nanochannels on the scale of the persistence length of approximately 40 nm provide a perfect environment for the spontaneous uncurling and stretching of the DNA chain. This facilitates single-base sequencing if the DNA chain can be made to pass through nanoscale sensing electrodes positioned along the channel. Nanofluidic devices can be also be utilized for chemical analysis and virus detection once electrodes are coupled to the devices.

D.2 Impact of Research Results on U.S. and Global Society

The novel fluid flow experiments will stimulate researchers and scientists studying nanofluidics and related disciplines. Nanofluidics will facilitate the understanding of transport phenomena such as slip flow and electrokinetics. This research will also accelerate the development and application of nanofluidics based lab-on-a-chip devices. Engineering and science students will realize the value and impact of BioMEMS devices in everyday applications.

D.3 Impact of Research Results on the Environment

Nanofluidic chips can serve as small, portable analyzers that are cost effective since they reduce the volume of reagents. The small size of nanofluidic devices decreases waste generation hence less fluid is required for fluid flow experiments. According to the Toxicological Sciences Journal, people will become more exposed to materials at the nanoscale since nanodevices will improve the quality of life and consumer products in the near future.
Appendix E: Microsoft Project for MS MicroEP Degree Plan
<table>
<thead>
<tr>
<th>D</th>
<th>Task Name</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Enter Grad School (Orientation, Summer Camp etc)</td>
<td>8/17</td>
<td>8/19</td>
<td>8/17</td>
</tr>
<tr>
<td>2</td>
<td>Choose Research Professor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Literature Searches for Masters Thesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Research Group Meetings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Observe Experiments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Small Group Meetings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Pick professors for Thesis Committee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Research Project</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Deposit oxide (PECVD) on glass wafers with Hussein Rakadis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Dicing Saw Training with Mike Glover</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>AFM Training in Dr. Weng's Lab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Equipment Training with Balaji Venkata AVM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Equipment Training with Kyle: Mask Aligner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Form nanochannels on Pyrex substrates using the Agilent 5500 AFM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Anodic bonding of silicon to glass using Pyrex 7740 wafers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Work with JuSeok on Fluorescence Microscopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>AFM Characterization Experiments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Factors: Number of cuts, tip speed (um/s), force setpoint (V)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Treatments (4 per factor): Number of cuts &gt; 25, 50, 75, 100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Tip speed (um/s): 0.8, 1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Force Setpoint (V): 3.5, 5.5, 7.5, 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Experimental Design = Full factorial, N(r) of runs = 7!F = 4!3 = 64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Response Variables to analyze: Depth, width, length, time taken for each experiment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Run experiments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Analysis using a 1/4 factorial design for all combinations with a 1.0 um/s tip speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Fabrication, packaging (attachment of connectors + anodic bonding) and testing of nanofluidic devices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Experimental Matrix: AFM Nanolithography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Pattern 4 inch Pyrex Wafer using Mask Aligner in Dr. Huang's Lab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.</td>
<td>Task Name</td>
<td>2009</td>
<td>2010</td>
<td>2011</td>
</tr>
<tr>
<td>----</td>
<td>---------------------------------------------------------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>33</td>
<td>Buffered Oxide Etch (BOE) the patterned wafer for 45 mins at 0.1 um/s etch rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Strip photoresist in acetone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Dice water using Ding Saw in HDEC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Dice silicon wafer into individual chips to cap off Pyrex chips during anodic bonding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Quality Control using a Microscope to determine if microchannels are properly etched</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Chip 1 (80 x 100 um) - Desired Depth (30 - 60 nm) Desired nanochannels (2 per chip)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>Factors: Number of cuts (50) Tip Speed (1.0 um/s) Force Setpoint (5.5V)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Chip 2 (40 x 150 um; 2 inlets, 1 outlet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>Factors: Number of cuts (50) Tip Speed (1.0 um/s) Force Setpoint (7.5V)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Chip 3 (40 x 40 um)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Factors: Number of cuts (50) Tip Speed (0.8 um/s) Force Setpoint (5.5V)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>Chip 4 (30 x 250 um)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>Factors: Number of cuts (50) Tip Speed (1.0 um/s) Force Setpoint (5.5V)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>Create a log of number of channels per chip, depth, length of channel achieved, and width</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>Flow testing using a vacuum desiccator for a 40 x 40 um chip</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>Clean chips using Pranax (3:1 mixture of H2SO4 to H2O2) for 20 mins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>Sonicate chips in IPA to ensure that surfaces are contaminant or particle free for 15 mins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Pump with Acetone for 30 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>Examine flow under microscope and capture images of inlet, gap, and outlet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>Pump with Methanol for 30 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>Examine flow under microscope and capture images of inlet, gap, and outlet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>Pump with isopropyl Alcohol (IPA) for 30 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>Examine flow under microscope and capture images of inlet, gap, and outlet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>Pump with DI water for 30 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>Examine flow under microscope and capture images of inlet, gap, and outlet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>Pump using Fluorescein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>Examine flow under microscope and capture images of inlet, gap, and outlet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Examine Fluorescence using an FITC Filter on the Fluorescent microscope</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>Capture images to visually determine if fluid traveled from the inlet to outlet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>Translocation to check the existence and patency of nanochannels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>Experimental Setup—Preparation of solutions and assembly of equipment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ID</td>
<td>Task Name</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>-----------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>Perform the aforementioned sequential wetting procedures on the new 40 x 150 um chip</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Fill the nanochannel inlet, and outlet by soaking in 80 ml of 0.1 M PBS solution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>Very concentration of the negatively carboxylate-modified 20 nm FluoSpheres</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>Very applied DC voltage (5V, 10V, 15V, 20V)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>Capture Fluorescent images to prove nanochannel existence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>Electrode deposition on 2 chips using the Focused Ion Beam (FIB)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>Work with Mourad on depositing a 40 nm thick platinum line between microchannel gap</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>Use conductive epoxy to create bond pads</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>Form 1 nanochannel on each chip using AFM nanolithography to break the electrodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>Microscale lithography, AFM Nanolithography, and anodic bonding for fabrication of 6 nanofluidic devices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>Flow Testing and translocation experiments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>78</td>
<td>China trip for translocation experiments with Pyrex chip and DNA detection using a Silicon nanochannel chip with electrodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>SEM imaging of old and new AFM tip and surface roughness of Pyrex with Mourad</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>AFM scan of 3 concentrations of 20 nm FluoSphere Beads</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>Thesis Writing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>Abstract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>Introduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>Experimentation/Materials and Methods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>Results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>Discussion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>Appendices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>References</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>End Dates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>Dead Day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>92</td>
<td>Last day to defend Thesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>93</td>
<td>Last day of Public Presentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94</td>
<td>Send info to MicroEP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>Graduate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix F: Identification of All Software Used in Research and Thesis Generation

Computer #1:
   Model Number: Dell Precision 390
   Serial Number: 3XD9XC1
   Location: Micro/Nano Systems Laboratory (ENRC3402)
   Owner: Dr. Steve Tung
Software #1:
   Name: Microsoft Office 2007
   Purchased by: University of Arkansas
Software #2:
   Name: Microsoft Excel 2007
   Purchased by: University of Arkansas
Software #3:
   Name: Microsoft PowerPoint 2007
   Purchased by: University of Arkansas
Software #4:
   Name: Solid Works
   Purchased by: University of Arkansas
Software #5:
   Name: AutoCAD
   Purchased by: University of Arkansas

Computer #2:
   Model Number: Dell Optiplex GX260
   Serial Number: 8GFYV21
   Location: Micro/Nano Systems Laboratory (ENRC3402)
   Owner: Dr. Steve Tung
Software #1:
   Name: Osprey SwiftCap
   Purchased by: Dr. Steve Tung

Computer #3:
   Model Number: Gateway GT5018E
   Serial Number: CCT5A81006873
   Location: Bio/Nano Technology Laboratory (ENRC3516)
   Owner: Dr. Jin-Woo Kim
Software #1:
   Name: QCapture
   Purchased by: University of Arkansas
Appendix G: All Publications Published, Submitted and Planned

There are no publications submitted or published to date. A technical abstract was submitted and presented at the International Mechanical Engineering Congress & Exposition (IMECE) in Vancouver, British Columbia on November 18, 2010. The targeted journal publications are:

- IEEE NEMS
- Sensors and Actuators