A MEMS ACTUATOR AND SENSOR FOR THE STUDY OF CELL MECHANICS AND ADHESION

A DISSERTATION
SUBMITTED TO THE DEPARTMENT OF MECHANICAL ENGINEERING
AND THE COMMITTEE ON GRADUATE STUDIES
OF STANFORD UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Ehsan Sadeghipour
March 2016
I certify that I have read this dissertation and that, in my opinion, it is fully adequate in scope and quality as a dissertation for the degree of Doctor of Philosophy.

________________________________________________________________________

(Beth L. Pruitt) Principal Adviser

I certify that I have read this dissertation and that, in my opinion, it is fully adequate in scope and quality as a dissertation for the degree of Doctor of Philosophy.

________________________________________________________________________

(W. James Nelson)

I certify that I have read this dissertation and that, in my opinion, it is fully adequate in scope and quality as a dissertation for the degree of Doctor of Philosophy.

________________________________________________________________________

(Roger T. Howe)

Approved for the University Committee on Graduate Studies.
Abstract

An active area of Microelectromechanical Systems (MEMS) research over the past few years has been to develop tools that can probe the forces generated and sensed by cells. Silicon-based tools developed in this effort have the benefit of being able to apply and sense forces at a greater resolution and in a more repeatable fashion. However, they have been too small to probe more than one or a few cells at a time, and the opaqueness of silicon has prohibited inverted live microscopy, which is important for transferring the technology to biological and medical labs. In addition, while many tools exist that can apply and sense the effects of tension on cells, no devices exist that can apply shear to a sheet of epithelial cells. This is despite the fact that the ability to apply and sense shear is important in understanding how cells interact with each other mechanically, which is essential in studying cell-cell adhesions in biology and medicine.

Two types of silicon MEMS devices have been designed, fabricated, and tested, which can strain a sheet of epithelial (2D and skin-like) cells in tension and shear simultaneously. The first set of devices, which have silicon cell-adhesion pads can be used for upright bright-field and fluorescence microscopy. The second set of devices, which have silicon-nitride cell-adhesion pads can be used for both upright and inverted bright-field and fluorescence microscopy. Inverted microscopy has been made possible by spraying the bottom of 50 µm deep trenches with photoresist and exposing the area in the shape of cell-adhesion pads. In addition, the Silicon on Insulator (SOI) handle wafer has been backside etched below the cell-adhesion pads.

These devices have been integrated with a cell-delivery mechanism that can deliver 1000 cells to the cells adhesion pads. In addition, an open culture system has been implemented that allows the user to perform live cell microscopy for days without the
use of an incubator. With the combination of these devices and experimental methods we have formed single sheets of Madin-Darby Canine Kidney (MDCK) epithelial cells on these devices and observed their reorganization in response to shear and tension. Particle Image Velocimetry (PIV) shows cells in an epithelium move towards the shear plane in response to an applied shear stress. Experiments where blebbistatin, a treatment inhibiting actomyosin contractility, was applied before the application of stress showed no collective cell migration. Therefore, we showed that the collective migration due to shear is an active biological response, as opposed to a passive and purely material one.
Acknowledgements

It has taken more than six years to get to this point. The person most responsible for my ability to get here has been my advisor, Beth Pruitt. I cannot imagine having finished this PhD without her as my advisor. She has an in-depth understanding of many different fields, which has allowed her to build a lab at their intersection. In that sense, she has been the perfect mentor for a multidisciplinary project like this one. She has also been deeply kind and understanding in my moments of distress, which has been an enormous emotional asset. Finally, my wife and I consider her responsible for us meeting, and we will always appreciate her role in starting our relationship.

James Nelson has been a great mentor and teacher in biology. I have learned from him that you should have fun doing what you do. Spending time in his lab with him and his students has been one of the highlights of my PhD. I have learned enormously from Roger Howe. I have always been impressed by his humility while succeeding greatly as an academic and engineer. His tireless work for the Stanford Nanofabrication Facility (SNF) is loved appreciated by all. Alex Dunn has provided great encouragement and advice. I think of him often when considering how to apply engineering to biology. Debbie Senesky has always been generous with her time and feedback, and I have loved seeing the very cool sensors that her lab works on. Beth and Viola Vogel gave me a unique opportunity to work in Viola’s lab in Switzerland for eight months, which was a very enriching experience. I am indebted to both of them for this opportunity.

I owe many thanks to the members of the Microsystems lab. Joseph, Joey, Joe, Bex, Chelsey, Clifton, Bryan, Alex, Nahid, Sasha, Joo Yong, Gadryn, Alexandre, Eileen, Adam, Ohi, Robin, Tom, and Fred, have all been kind colleagues whom I have learned from. I especially appreciated learning from and working with Joey, Nahid, Alex, and Tom in the
SNF. Gadryn and Joo Yong have been great friends, colleagues, and travel partners. Vikram left a wonderful device behind that Ohi and I have tried to build on. Many people from the Nelson lab have been generous mentors and collaborators as well. Nicolas Borghi was an early mentor who helped me develop this project and taught me how to culture cells. Blair has provided great advice and feedback. Working with Miguel has been the most fruitful collaboration of my PhD career. I have learned enormously from his knowledge and personality.

These devices were fabricated at the SNF. Some of my highest and lowest moments have taken place in that building. Work at the SNF would not be possible without the great efforts of the staff members there. In particular I would like to thank Maureen, Mahnaz, Mary, Maurice, Uli, and Nancy who have done their best to facilitate work on research and class projects. I would also like to thank the National Science Foundation Graduate Research Fellowship Program (NSFGRFP), and Mr. and Mrs. Morgridge who provided financial support through the Gerhard Casper Stanford Graduate Fellowship (SGF).

The Persian Student Association at Stanford University has been a big part of my PhD for many years. I have made deep friendships there, and learned from my experiences. In particular, it was great working with Sahar, Reza, and Arezoo during my first time in the board of directors, and I loved working with Milad, Reza, Dorna, and Ali Reza during my second time. Hamed, Morteza, Mohammad, and Amir have been like older brothers to me. I cannot imagine having grown as much without them. During my first year, the cohort of friends that formed in graduate housing became the core of my social life, and a bright spot in an otherwise difficult year. Kosh, Jesse, Azadeh, Sarah, Max, Lilly, Jill, and Tim provided kindness and deep conversations. Azadeh later became a colleague at the SNF. Kosh and Jesse became my roommates for most of my graduate career, and important parts of my emotional and intellectual life. I cherish my friendship with them.

My family has been the greatest source of love, joy, and support throughout my life. My father, mother, sister, and I have lived in many cities, states and countries for the past three decades, and each experience has brought us together even more. If I have anything good to offer the world, it is because of them. My family has grown during this PhD. Solène has improved all aspects of my life, and I hope to one day be the kind of person that she deserves. It goes without saying that this PhD would not have been possible without her.
# Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abstract</strong></td>
<td>v</td>
</tr>
<tr>
<td><strong>Acknowledgements</strong></td>
<td>vii</td>
</tr>
<tr>
<td><strong>1 Introduction</strong></td>
<td>1</td>
</tr>
<tr>
<td>1.1 Design Criteria</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Thesis Outline</td>
<td>3</td>
</tr>
<tr>
<td><strong>2 Motivation</strong></td>
<td>5</td>
</tr>
<tr>
<td>2.1 Cellular Machinery</td>
<td>6</td>
</tr>
<tr>
<td>2.1.1 Cell-Substrate Adhesion</td>
<td>8</td>
</tr>
<tr>
<td>2.1.2 Cell-Cell Adhesion</td>
<td>8</td>
</tr>
<tr>
<td>2.2 Polymer Devices</td>
<td>10</td>
</tr>
<tr>
<td>2.2.1 Patterning</td>
<td>12</td>
</tr>
<tr>
<td>2.2.2 Measuring Traction Forces</td>
<td>12</td>
</tr>
<tr>
<td>2.2.3 Substrate Stiffness</td>
<td>15</td>
</tr>
<tr>
<td>2.2.4 Stretching</td>
<td>15</td>
</tr>
<tr>
<td>2.3 Silicon and Glass Tools</td>
<td>17</td>
</tr>
<tr>
<td>2.4 Summary</td>
<td>20</td>
</tr>
<tr>
<td><strong>3 Design of a 2D MEMS Device</strong></td>
<td>23</td>
</tr>
<tr>
<td>3.1 User Requirements</td>
<td>23</td>
</tr>
<tr>
<td>3.2 Design of Cell Adhesion Pads</td>
<td>24</td>
</tr>
<tr>
<td>3.2.1 Cell Adhesion Pad Size and Placement</td>
<td>24</td>
</tr>
</tbody>
</table>
# List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Layout of Electrical Connections for 2D MEMS Device</td>
<td>45</td>
</tr>
<tr>
<td>3.2</td>
<td>Device Stiffness by Type</td>
<td>45</td>
</tr>
<tr>
<td>4.1</td>
<td>Electrical Device Runsheet</td>
<td>48</td>
</tr>
<tr>
<td>4.2</td>
<td>Mechanical Device Runsheet</td>
<td>66</td>
</tr>
<tr>
<td>5.1</td>
<td>Process steps to prepare samples for SprayCoater</td>
<td>78</td>
</tr>
<tr>
<td>5.2</td>
<td>Nozzle Pressures and Resist Mixes Attempted</td>
<td>79</td>
</tr>
<tr>
<td>5.3</td>
<td>Arm Speeds Attempted</td>
<td>80</td>
</tr>
</tbody>
</table>
List of Figures

1.1 Drosophila Gastrulation ................................................. 2
1.2 Prior Art Comparison ................................................... 3

2.1 Cancer Metastasis ....................................................... 6
2.2 Epithelial Cell Model .................................................... 7
2.3 Adherens Junction ....................................................... 9
2.4 Chemical Patterning .................................................... 11
2.5 Mechanical Patterning .................................................. 11
2.6 Traction Force Microscopy ............................................. 14
2.7 Lung on a Chip .......................................................... 16
2.8 Cell-Cell Adhesion ...................................................... 18
2.9 Single Cell Stretch ...................................................... 21

3.1 Design of Silicon Cell Adhesion Pads ............................... 25
3.2 Design of Nitride Cell Adhesion Pads ............................... 25
3.3 Undercut of Nitride Pads During Fabrication ....................... 26
3.4 Effects of Light Diffraction on Nitride Cell Pad Size ............. 27
3.5 Fixed-Guided Beam Bending .......................................... 28
3.6 Springs in Parallel and in Series .................................... 29
3.7 Horizontal Shuttle Design ............................................. 30
3.8 Vertical Shuttle Design ................................................ 31
3.9 Model of Electrostatic Force .......................................... 32
3.10 Model of Interdigitated Electrodes .................................. 34
3.11 Expected Actuation in Air ........................................... 37
3.12 Expected Actuation in Water ................................................................. 38
3.13 Ionic Shielding of Electrode ................................................................. 39
3.14 Alternating and RMS Voltage ............................................................... 41
3.15 Alternating and RMS Voltage Squared .................................................. 42
3.16 Symmetry in Electrode Design ............................................................... 43
3.17 2D MEMS Device Design ................................................................. 44

4.1 Electrical Device Fabrication Process Flow ............................................. 49
4.2 Keyhole Formation in a Trench ............................................................... 51
4.3 Ways to Avoid Keyhole Formation ......................................................... 52
4.4 Trench Shape Affects Deposition Requirements ....................................... 53
4.5 TMAH Etch Fully Clears Nitride Windows ............................................. 54
4.6 Damage Caused by TMAH Etch to Passivation Trenches ....................... 54
4.7 SF$_6$ Gas Fully Clears Nitride Windows ............................................... 54
4.8 SF$_6$ Gas Does Not Damage Passivation Trenches .................................. 55
4.9 Passivation Trench with a Nitride Cap ................................................ 57
4.10 Nitride Pad Fabrication ................................................................. 59
4.11 Metal Evaporation and Lift-off ............................................................. 61
4.12 Wafer Dicing Path ................................................................. 63
4.13 Mechanical Device Fabrication ............................................................. 65
4.14 Electrical Devices ................................................................. 67
4.15 Mechanical Devices ................................................................. 68
4.16 Upright Image of Silicon Cell Adhesion Pads ....................................... 69
4.17 Upright Image of Nitride Cell Adhesion Pads ....................................... 70
4.18 Inverted Image of Nitride Cell Adhesion Pads ..................................... 71
4.19 Experimental Setup ................................................................. 72
4.20 Vinculin GFP MDCKs at 10X ............................................................ 72
4.21 Vinculin GFP MDCKs at 40X ............................................................ 73
4.22 Monolayer Tension Test ................................................................. 73
4.23 Monolayer Shear Test ................................................................. 74

5.1 More Viscous Resist Mixture at Various Nozzle Pressures ........................ 79
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.7</td>
<td>ESY Device Trenches Mask</td>
<td>120</td>
</tr>
<tr>
<td>B.8</td>
<td>ESY Device Nitride Mask</td>
<td>121</td>
</tr>
<tr>
<td>B.9</td>
<td>ESY Device Metal2 Mask</td>
<td>122</td>
</tr>
<tr>
<td>B.10</td>
<td>ESY Device Silicon Mask</td>
<td>123</td>
</tr>
<tr>
<td>B.11</td>
<td>ESY Device Backside Mask</td>
<td>124</td>
</tr>
<tr>
<td>B.12</td>
<td>ESY Device All Masks</td>
<td>125</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

Due to the importance of the forces and displacements generated by cells, many tools have been developed to measure these phenomena. While scientists and engineers have made important contributions in this effort, many of the design challenges remain unmet, and many questions remain unanswered. [1–6]

1.1 Design Criteria

Gastrulation is an example of a process that presents the need for additional tools to study mechanobiology. Figure 1.1 presents the gastrulation of a drosophila embryo at multiple time points. [7] In the gastrulation process, which is a feature of embryogenesis in many animals including humans, the embryo flattens and then folds on itself to form a tube. As may be seen in this series of fluorescent images, cells are stretched multiple cell lengths. What may be less apparent in this image is the fact that after the two sides of the embryo meet to form a tube, they move alongside each other in parallel to the long axis of the tube in a process known as convergent extension. In addition, all of these displacements and forces take place by the 24th minute of the experiment, which necessitates live cell imaging to observe this dynamic phenomenon. Finally, experiments such as this one often rely on mutant cell types or pharmacological treatments to perturb the system and determine signaling pathways. The need to study the mechanobiology of biological processes such as this one necessitates the invention of tools that meet the following design criteria:
CHAPTER 1. INTRODUCTION

Figure 1.1: Cells undergo tension and shear stress during Drosophila gastrulation. They may be elongated to multiple times their normal cell lengths. Reprinted with permission from [7]. Copyright 2013 Company of Biologists.

1. Live Cell Imaging in Bright-Field, Phase, and Epifluorescence

2. Inverted Imaging Capability

3. Large Displacements

4. Application and Probing of Tension

5. Application and Probing of Shear

6. Force Sensing

7. Studying Confluent Monolayers

8. Studying Mutants and Application of Pharmacological Treatments

Tools and methods from several important papers in the field have been benchmarked against these design criteria. Tambe et al. present a method of analyzing epithelial traction information in a way allows them to probe, but not apply, shear and tension. [8] In addition, they could not apply any displacements to cells, including large ones. Lam et al. were able to apply small displacements and sense shear; however, they did not probe shear or observe full monolayers. [9] Simmons et al. were successful in applying tension and studying monolayers, but their tools had shortcomings for live cell and inverted imaging. [10] Mukundan et al. were able to perform live upright imaging, but only to single cells, and without the ability to probe shear. [11] Yonemura et al. were able to probe tension using
1.2 Thesis Outline

- Chapter 2 presents the motivation for this work. It begins by describing the cellular machinery of the cell, which allows for the application and sensing of mechanical signaling. It moves on to describe many polymer devices and fabrication methods used for mechanobiology studies. This chapter ends by presenting silicon and glass
devices used for studying cell and tissue mechanics.

- **Chapter 3** presents the user requirements and principles that the design was based on. It first presents the design of the cell adhesion pads. The chapter moves on to describe the design of the folded flexures and mechanical elements. Principles of electrostatic actuation design are presented next. Finally, the fundamentals of operation in ionic media are explained.

- **Chapter 4** describes the fabrication and testing of the device. The chapter begins with an explanation of the electrical devices. The fabrication of the mechanical devices are described next. The mechanical and biological testing of the device are presented as the final components.

- **Chapter 5** deals with characterizing the SprayCoater. This chapter defines the problem that we were trying to solve. It then moves through explanations of the effects of nozzle pressure, resist mix, varying spray arm speeds, and number of passes on the conformal coverage of a wafer with photoresist. Finally, a chemical and an optical test for the conformal coverage are presented.

- **Chapter 6** discusses the methodology and results of the cell mechanics experiments. Proper conditions for the growth of cells are discussed first. The experimental setup for these tests are explained next. Finally, the principles of Particle Image Velocimetry are explained and the response of an MDCK type II G epithelium to shear stress is presented.

- **Chapter 7** concludes the thesis and presents a few directions for future work.
Chapter 2

Motivation

Mechanical communication of cells with each other and with their environment is important in a variety of biological functions and disease states. Understanding the tools and mechanisms that cells use to communicate mechanically is important in building a model of the overall process. For example, patients with metastatic cancers have much higher patient mortality rates. In fact, 90% of cancer deaths are due to cancer metastasis. To metastasize, a cancerous tumor must: i) break down the surrounding Extracellular Matrix (ECM), ii) regulate its attachment to the surrounding tissue, and iii) migrate to a new area where it can adhere and grow. All of these steps are affected by the mechanics of the environment, mechanical communication between the cells, and the ability of the cells to generate motility-inducing forces.

Gritsenko et al. present a model of breast cancer invasion. Figure 2.1 graphically presents this model. As the figure shows, the invasive carcinoma cells migrate directionally and metastasize along the aligned collagen fibers of the ECM. To do so, they must sense the topography and stiffness of the ECM. In addition, their migration as a group suggests that their cell-cell attachments are responsible for mechanical communication and group behavior of the cells. As Figure 2.1b shows, these epithelial cells attach to each other using E-cadherin proteins. They attach to the ECM using integrin molecules, as shown in Figure 2.1c. Therefore, understanding the function of E-cadherin and integrin molecules is important in understanding how cells communicate with each other and with their substrate.
2.1 Cellular Machinery

In 1980, Harris et al. reported that traction forces generated by chick fibroblasts wrinkled the thin sheet of silicone that they were plated on. This paper was the first instance in which cells were cultured in a way that enabled visualization of their traction forces. In a seminal paper in 2006, Engler et al. noted that "matrix elasticity directs stem cell lineage specification." They showed that not only did stem cells sense the stiffness of their substrates, but also this stiffness sensing led to fundamental changes in the structure and function of these cells. Ribeiro et al. showed that changing the shape and substrate stiffness of cardiomyocytes differentiated from human pluripotent stem cells (hPSC-CMs)
2.1. CELLULAR MACHINERY

Figure 2.2: Cells attach to each other using adherens junctions, and they attach to their substrate using focal adhesions. Actomyosin contractility is used in cells to generate tension. Reprinted with permission from [5]. Copyright 2013 Annual Reviews.

affects their mechanical output. [20] Taken together, these and other studies show that cells exert forces on their substrates, sense the mechanics of their substrates, and respond to this information.
CHAPTER 2. MOTIVATION

2.1.1 Cell-Substrate Adhesion

Figure 2.2 by Schoen et al. presents a model of cell-cell adhesion, cell-substrate adhesion, and several other structures believed responsible for cell mechanics. Panels 2.2d and 2.2e show how cells attach to the ECM using integrin molecules at focal adhesion complexes (FACs). FACs anchor cells to the ECM and are responsible for their ability to communicate mechanically with the substrate. FACs are composed of integrin, focal adhesion kinase (FAK), paxillin, talin, vinculin, zyxin, and vasodilator-stimulated phosphoprotein (VASP). The actomyosin cytoskeleton attaches to and is cross-linked at the FAC using actin filaments and \( \alpha \)-actinin. Figure 2.2b presents a model of Filamentous actin crosslinking and network formation due to \( \alpha \)-actinin and Filamin activity. FACs grow in size, stabilize in protein content, and mature in shape in response to increases in substrate stiffness and the continued maturation of contact.

Figure 2.2e shows how the extra-cellular domains of the \( \alpha \) integrin–\( \beta \) integrin dimer attach to the ECM. This attachment takes place through a conformational change in the dimer, which then initiates the formation of the FAC presented in Figure 2.2d. The recruitment and assembly of several proteins within the FAC have also been shown to be mechanically-activated. For example, the activity of the Myosin II motor protein, as well as the substrate stiffness, affect the recruitment of vinculin and FAK to the focal adhesion (FA). Figure 2.2a presents a model of Myosin II activity in the cell. Changes in the conformation of Talin achieved through stretching activates vinculin binding to this molecule. In a seminal paper, Grashoff et al. measured the force across vinculin in a stable FA to be approximately 2.5 pN. They also showed that FA stabilization requires both vinculin recruitment as well as force transmission across this molecule.

2.1.2 Cell-Cell Adhesion

Adherens junctions (AJs) are one of the primary structures that cells use to mechanically communicate with each other. Figure 2.2c presents a model of an Adherens junction. In Figure 2.3b, Takeichi provides more details on the cadherin–catenin core complex. The cadherin–catenin core complex is composed of p120 Catenin, \( \beta \)-Catenin, and \( \alpha \)-Catenin, which bind to the cytoplasmic domain of E-cadherin. Figure
2.1. CELLULAR MACHINERY

Figure 2.3: The intercellular domains of cadherins attach to the actomyosin cytoskeleton, and the extracellular domains attach to the cadherins of neighboring cells. Cadherins have been shown to be responsible for mechanical communication between cells. Reprinted with permission from [30]. Copyright 2014 Nature Publishing Group.

2.3b shows conformational changes in αE-catenin and the recruitment of the vinculin molecule, which promote filamentous actin (FA) binding. Le Duc et al. showed that vinculin is recruited to actin-anchored sites at the AJ in a Myosin-II dependent manner, and that the presence of vinculin is important in the mechano-sensing capability of E-cadherin. [33] Yonemura et al. showed that αE-catenin, which is normally auto-inhibited, undergoes a tension-induced conformational change that reveals a vinculin binding domain. The binding of vinculin to αE-catenin promotes further filamentous actin binding and the stabilization of the AJ. [12] Benjamin et al. had already shown that the presence of αE-catenin at the membrane reduces membrane dynamics and cell motility without affecting
cell-cell adhesion. [34]

Cadherin-mediated adhesions play an important role in morphogenesis, as cells with differing adhesive properties self-assemble in structures that maximize their mutual binding. [35] Higher forces on the cells promote cadherin adhesion formation and development. [36] Liu et al. showed that Mechanical tugging force increases the size of AJs in endothelial cells. [37] One of the interesting aspects of this work was that Liu and colleagues used a bed of microneedles and fluorescently-tagged cadherins to relate cell-ECM forces to cell-cell forces. Sim et al. used similar methods and went farther by showing that tension across the cadherin molecule remained constant regardless of the spread area or shape of cell pairs. [38] Their work benefited from the work of Tseng et al. who developed ECM patterns that regulated the positioning of cell-cell junctions. [39] Huynh et al. showed that increased substrate stiffness increased cell-cell junction width, and thus the permeability of endothelial cells layers. [40] Borghi et al. demonstrated that E-cadherin at the plasma membrane of MDCK epithelial cells is under constitutive tension, even in the case of molecules not associated with the cell-cell junction. [13] The tension on the cadherin at the cell-cell junction increases if the cells are stretched.

2.2 Polymer Devices

Because of the importance of cell mechanics in biology and medicine, engineers and scientists have developed various tools to study this subject. These tools include ways to define the shape of cells, apply forces to cells, measure forces generated by cells, and ways to determine the mechanical properties of cells. These tools are often made of polymeric materials, due to the relative ease of fabrication of polymer devices, and the fact that many polymers are closer in stiffness to living cells compared with glass, silicon, or metal. [4–6, 21] These tools generally consist of ways to pattern cells, determine their traction forces, change their substrate stiffness, stretch them, or use a combination of these effects.
2.2. POLYMER DEVICES

Figure 2.4: Microcontact printing of fibronectin bowtie patterns on top of microneedles, and endothelial cell pairs confined to the ECM patterns. Adhesion to other areas has been blocked using Pluronics F127. [Cyan, fibronectin; green, β-catenin; red, nucleus; scale bar, 10 µm. Reprinted with permission from [37]. Copyright 2010 National Academy of Sciences.

Figure 2.5: Steps required for the fabrication of a culture system confining cells to the top of microneedles using the thermoresponsive polymer PNIPAAm. Reprinted with permission from [41]. Copyright 2013 Springer.
2.2.1 Patterning

Patterning of cells is generally accomplished by defining the shape of ECM patches where cells can adhere, and blocking the adhesion of cells everywhere else. This task may be accomplished chemically, using blocking agents, or mechanically, using a sacrificial layer. Figure 2.4 presents an example of chemical patterning, and Figure 2.5 presents an example of mechanical patterning. Cells attach and spread more quickly if plated on ECM. However, they can still lay down their own ECM, attach, and spread over time if no ECM is provided on the surface. Therefore, if patterning on Polydimethylsiloxane (PDMS) or glass, it is not sufficient to merely pattern the Collagen, Laminin, Fibronectin, or other ECM protein. The adhesion of cells to other areas must be actively blocked using Bovine Serum Albumin (BSA), Pluronics, poly(L-lysine)-graft-poly(ethyleneglycol) (PLL-g-PEG), or another blocking agent. Polyacrylamide (PAA) hydrogels are normally less conducive to cell attachment, and have to be activated before proper cell attachment can occur. Therefore, merely patterning the ECM and not using a blocking agent may be sufficient. Cells may also be patterned by using a lift-off or sacrificial layer that removes any cells not attached to desired positions. The thermoresponsive polymer poly(N-isopropylacrylamide) (PNIPAAm), which breaks down and may be washed away at temperatures below 32 °C is a good example of such a sacrificial layer. Finally, Gray et al. developed a unique method by positioning cells onto Fibronectin pads using dielectrophoresis, and used non-adhesive agarose barriers as an adhesion blocking mechanism.

2.2.2 Measuring Traction Forces

As mentioned earlier, Harris et al. used the deformation of silicone sheets to make inferences about the traction forces exerted by chick fibroblasts. Similarly, the most common methods of determining the traction forces exerted by cells still relies on observing how they deflect their substrates. The two main methods used for this purpose are micropost arrays and Traction Force Microscopy (TFM). Figures 2.4 and 2.5 present two different sets of micropost arrays used by Liu and Taylor. Micropost arrays are generally fabricated from PDMS negative molds. One of the benefits of these structures is that
their stiffness may be modulated by simply changing their height, while keeping constant their chemical makeup and surface area. [50] This fact allows scientists to keep all factors except substrate stiffness constant across different experiments.

Lam et al. showed how 2-3 µm-diameter posts and a stretchable substrate may be used to find the mechanical properties of single Vascular Smooth Muscle Cells (VSMCs) on a subcellular level. [9] Actuating these microposts has been a goal of other works as well. Bielawski et al. added magnetic particles to PDMS microposts to measure the viscoelasticity of biomaterials. [52] Ribeiro et al. explored how the stable and covalent attachment of the ECM to microposts is important in cell function. [53] Reliably determining the mechanical properties of these structures has proven to be a challenge as well. Sim et al. showed how these structures stiffen in response to oxidation treatment prior to functionalization. [54] Schoen et al. explored how simplified mechanics models may be inadequate in determining the mechanical properties of these structures. [55]

The other major method of determining traction forces is TFM. [56] For TFM, fiducial markers are placed in deformable substrates, and cells are allowed to adhere and spread on these surfaces. Local traction forces are determined from the displacement of the markers and the stiffness of the substrates. The substrates used for this purpose are generally PAA hydrogels, which have stiffnesses suitable for such deformations. [57] In addition, they can be made to incorporate fiducial markers, such as fluorescent beads, rather easily. [58] Maskarinec et al. showed how TFM may be extended to three dimensions to not only look at in-plane forces, but also out-of-plane forces generated by cells. [59] Delanoë-Ayari et al. add the time domain as a fourth dimension, and studied the “asymmetric cortical forces in migrating dictyostelium cells.” [60] Figure 2.6 presents how the magnitude and direction of traction forces are inferred from the displacement of fiducial markers during TFM. [51] As the figure presents, 2D methods present an incomplete accounting of the forces generated by the cell. Legant et al. used multidimensional TFM to show that Mouse Embryo Fibroblasts (MEFs) exert “out-of-plane rotational moments about focal adhesions” during migration. [61] Such platforms may also be used to understand forces generated by epithelial tissues. [8] For example, Trapat et al. used these substrates to show the importance of forces generated far from the leading edge for collective cell migration. [62]
Figure 2.6: 2D TFM is not sufficient in capturing all of the forces generated by a cell. A 3D method is required to also observe out-of-plane forces. Reprinted with permission from [51]. Copyright 2011 Nature Publishing Group.


2.2. POLYMER DEVICES

2.2.3 Substrate Stiffness

We can not only use the deformation of soft substrates as a way to study forces generated by cells, but also substrate stiffness may be used as an important input signal to change the morphology, function, and collective behavior of cells. [63] As presented earlier, Engler et al. showed that stem cells are much more likely to turn into particular tissues if they are grown on surfaces with the stiffness of that tissue. [19] Huynh et al. showed that growing endothelial cells on stiff substrates increased the permeability of their cell-cell junctions. [40] Levental et al. “found that breast tumorigenesis is accompanied by collagen crosslinking, ECM stiffening, and increased focal adhesions.” [64] Chaudhuri et al. observed that increasing the ECM stiffness of mammary epithelial cells increased their malignant phenotypes. [65] This effect disappeared when basement-membrane ligands were also increased. Ribeiro et al. showed that increasing substrate stiffness from 10 kPa to 35 kPa disrupted myofibrils and reduced the forces generated by human cardiomyocytes differentiated from pluripotent stem cells (hPSC-CMs). [20]

2.2.4 Stretching

Stretching cells (applying tension) has been another important mechanical input in mechanobiology experiments. Cells are compressed or stretched through a variety of biological functions such as transmigration, gastrulation, and growth. Some tissues, such as those in the circulatory and respiratory systems, undergo continuous and cyclic stretching. Scientists and engineers have developed many tools to apply such mechanical inputs to cells and observe the response of these biological systems. [67–70] Huh et al. developed an “organ-on-a-chip” microdevice for studying lung function. [66] Figure 2.7 presents this system. As the figure presents, this device consisted of a main biological tissue chamber, where epithelial and endothelial lung tissue was cultured, and two vacuum chambers on the sides. Applying vacuum in the vacuum chambers stretched the lung tissue and simulated normal breathing function. They used this system to show that mechanical strain promoted the uptake of toxic particles by the epithelial and endothelial tissues of the lung.

Using vacuum to stretch membranes has gained popularity across multiple devices and platforms. Lam et al. used vacuum to stretch a membrane covered with micropillars. [9]
Figure 2.7: Huh et al. used a vacuum-generated stretch to mimic breathing in lung tissue, and created a lung on a chip device. Reprinted with permission from [66]. Copyright 2010 The American Association for the Advancement of Science.

They used this system to find the stiffness of VSMCs at a subcellular level. Simmons et al. used vacuum to stretch cells at different strain levels in an integrated strain array. [10] They showed that at higher strain levels, C2C12 skeletal myoblasts and actin stress fibers realign with a circumferential preference.” Benham-Pyle et al. used the same platform to study the effects of mechanical strain on cell cycle entry. [71] They showed how the E-cadherin-independent activation of Yap1 and β-catenin is important in strain-induced cell proliferation.

Quinlan et al. used a vacuum-based stretching system and substrates of varying stiffness to study the effects of coupled mechanical strain and substrate stiffness on cell shape and size. [72] Their results led to the postulation that cells use similar internal mechanisms whether sensing substrate stiffness or strain. Moraes et al. developed a microfabricated
array for applying stretch to cells using air pressure, as opposed to vacuum. [73] Gavara et al. used a cell stretching system to study cytoskeletal tension in strained cells. [74] They noted that they actomyosin structure may be partially disrupted by mechanical strain. Krishnan et al. applied uniaxial, bi-axial, and non-homogenous stretch to cells to study their likelihood of cytoskeletal reinforcement versus fluidization. [75] Unlike other groups who had mainly focused on strain-induced cytoskeletal reinforcement, they showed that based on the type of strain applied, this structure can also fluidize.

2.3 Silicon and Glass Tools

While most devices developed for the study of mechanobiology have been made of polymers, there have been quite a few devices made of other materials. The two most common materials have been silicon and glass tools. While these materials are much stiffer than polymers, they are widely available in microfabrication and biology labs. In addition, fabrication methods for these materials have been well developed and characterized, and the fabrication methods can lead to much more repeatable feature sizes and properties. Finally, the added stiffness may be an advantage in many cases, as moving devices made of glass or silicon can avoid the large out-of-plane deflections that result from stretching polymers in-plane. This feature makes these materials more conducive to live cell microscopy while applying mechanical perturbations.

Figure 2.8 presents two cells being pulled apart by two glass micropipettes. Chu et al. developed this method and used the Johnson-Kendall-Roberts theory to determine the forces associated with cell-cell adhesion. [76] The greater the vacuum required to hold the cell at the tip of the glass pipette, the greater the adhesion between the two cells. They used this method to study the effects of E-cadherin, Rac, and Cdc42 expression levels on cell-cell adhesion, and observed the importance of these molecules in maintaining strong cell forces. [77] Rowat et al. used micropipette aspiration and confocal fluorescent microscopy to quantify the mechanics of cell nuclei. [78] They showed that cells lacking the nuclear envelope molecule, emerin, had much less stiff nuclei. Their work suggested that the "altered nuclear envelope elasticity caused by loss of emerin could contribute to increased nuclear fragility in Emery-Dreifuss muscular dystrophy patients with mutations
Figure 2.8: Chu et al. used glass pipettes to pull apart cell pairs and measure their cell-cell adhesive forces based on the vacuum required to keep the cells at the tip of the pipettes. Reprinted with permission from [76]. Copyright 2005 American Physical Society.
Atomic Force Microscopy (AFM) was developed in 1986, building on the techniques developed for Scanning Tunneling Microscopy (STM). These methods were developed as tools to visualize the topography and mechanics of surfaces at a sub-nm resolution. AFM works by measuring the deflection of a silicon cantilever as it rapidly taps on a surface. The high resolution of AFM has been used to study a variety of biological forces and displacements, ranging from the stiffness of fibroblasts on elastic substrates, to molecular binding kinetics, to even the forces required for single protein unfolding. Silicon cantilevers may be used for applying and sensing forces in other biological model systems as well. Park et al. described a force clamp system using a piezoresistive silicon cantilever, and describe how such a system may be used to study *C. elegans* body mechanics. Petzold et al. used this system to find the relationship between body mechanics, body wall muscle tone, and the sense of touch in this nematode. Eastwood et al. used this system to show the frequency-dependence of the touch response of *C. elegans*. Doll et al. used similar piezoresistive cantilevers to study the sense of hearing. Yang et al. reported a new micromachined probe for the study of cell mechanics. The tip of the probe was functionalized and adhered to a cell, the base of this tool was displaced with a piezoelectric actuator, and forces applied to the cell were inferred from the deflection of springs. The authors went on to use this tool to study the mechanics of monkey kidney fibroblasts. They found that almost all of the mechanical resistance to stretch disappeared when the actin network was disrupted using cytochalasin D. Siechen et al. used the same tool to study the mechanics-dependence of axons in the embryonic nervous system of Drosophila. They showed that axons must be under tension to ensure that the Synaptotagmin-1 vesicles were not spread diffusely within the neuron, and were instead accumulated at the pulled presynaptic terminal.

One of the earliest silicon micromachined devices for studying cell mechanics was reported by Galbraith et al. in 1997. They developed a surface covered with polysilicon cantilevers, and observed the motility of fibroblasts over this surface. They inferred the traction forces exerted by the cells using deflection data from the cantilevers. In a seminal
paper, Lin et al. developed micromachined polysilicon clamps and deflection beams to measure the contractility of single heart muscle cells. [94] They induced the cells to contract by flowing in a solution of calcium ions, and then measured forces generated by the cells by observing the deflections of the polysilicon beams. Fernandez et al. used a stiff substrate and a flexible microplate attached to a piezoelectric transducer to measure the mechanics of single fibroblasts. [95] They measured the mechanics of normal and fixed cells, and concluded that the stress stiffening of fibroblasts is a purely material response, and is unrelated to biological signaling. [96]

Micromachined electrostatic devices have also been important force transducers for biological applications. Higgs et al. reported an electrostatically actuated MEMS device for measuring the shear stiffness of hydrogels. [98] Sun et al. reported an electrostatic MEMS 2D force sensor for measuring cellular forces. [99] While both of these devices made important contributions to the effort to measure the mechanics of biological tissues, the electrostatics had to remain outside of water. Mukundan et al. developed an electrostatic sensor and actuator fully operable in aqueous ionic media. [97, 100] Figure 2.9 presents an image of the actuation side of this device, and its ability to stretch single cells. The novel actuation principles of this device will be discussed in later chapters. They used this device to measure the viscoelastic properties of living MDCK cells. [11]

### 2.4 Summary

Biological cells communicate with each other and with their environments in a variety of ways, including chemical, electrical, and mechanical methods. Mechanical communication is important in a variety of biological processes and disease states. For example, cancer metastasis depends on the ability of cells to sense the topography and stiffness of their environments, and their ability to modulate their cell-cell adhesion and motility. The machinery and structures used by cells to relate mechanically with the world has been studied for decades. The actomyosin network gives cells physical integrity, and is an important factor in their ability to sense and apply forces. Focal adhesions have been shown to be important in cell-substrate mechanical communication, and adherens junctions are important in cell-cell force transmissions. Both of these junctions are also connected with
Figure 2.9: Top: Electrostatic actuators were used by Mukundan to find the mechanical properties of single MDCK cells. Bottom: A single MDCK cell being stretched between two cell adhesion pads. Reprinted with permission from [97]. Copyright 2009 IEEE.
the actomyosin network. Engineers and scientists have built a variety of devices over the past few decades to study cell mechanics. The most common types of devices have been fabricated using biocompatible polymers. Mechanical and chemical patterning of cells and tissue has been an important tool in engineering the footprint of cells, as well as the location and length of their cell-cell adhesions. Patterning is accomplished by alternatively using ECM proteins and blocking agents in different areas. The two most common methods of measuring traction forces are using micropillar arrays and fiducial markers embedded in hydrogels, also known as TFM. In the case of micropillar arrays, forces generated by cells are quantified by measuring the deflection of pillar tips. For TFM, the displacement of fiducial markers and the mechanics of the hydrogel are used to infer the traction forces exerted by the cell. Observing the out-of-plane displacement of markers in this scheme adds and important third dimension to the force measurements.

Engineering the substrate stiffness of cells is another important parameter that can be adjusted. Cells respond to stiffer substrates by spreading more and applying greater traction forces. On softer substrates they are more likely to be round and generating smaller forces. Applying tension to cells is another important mechanical perturbation. In the case of polymer devices a common actuation technique involves using air pressure or vacuum to stretch a polymeric sheet. Polymer-based stretching devices often allow for large strains and high throughput.

Silicon and glass devices are also relatively common tools for studying cell and tissue mechanics. Micropipette aspiration has allowed scientists to study subjects ranging from cell-cell adhesion to the mechanical properties of the cell nucleus. AFM and other methods based on the deflection of silicon MEMS cantilevers have been prominent methods for the study of the physical properties of a variety of biological model systems. They have been used at a variety of length and force scales ranging from the stretch of single proteins to studying the sense of touch in small animals, like the *C. elegans* nematode. Other silicon tools relying on the deflection of calibrated beams to measure forces have been important as well. Some of the most powerful tools include on-chip electrostatic actuators and sensors. Until only a few years ago the use of these systems in aqueous ionic media, the natural environment for living cells, was not possible. The work of Mukundan et al. was an important step in allowing these devices to be operated in such environments.
Chapter 3

Design of a 2D MEMS Device

The design of this 2D MEMS chip begins with establishing the user requirements for its shape and function. There is a discussion of the shape and design features of the cell adhesion pads. A description of the folded flexure designs is used to explain the mechanical features of the device. The electrostatic actuator design is then presented to explain the electrical operation of the device. The electrostatic principles are further explained through presenting the symmetric electrode design and the signal separation methods.

3.1 User Requirements

As is the case in any design process, the first step was to determine what features the device must have in order to meet the user’s needs. The first requirement was that the device must be able to apply shear and tension to a sheet of epithelial cells. The device has been optimally designed for confluent MDCK cells, a line of epithelial mammalian cells, that has served as a model system in biology labs globally for decades. MDCK cells are a good model system because their phenotype is quite stable, and the decades of work on them has meant that they are well-studied. In addition, stable MDCK cell lines exist which have different fluorescently-tagged, up- or down-regulated, or conditionally-expressed proteins. The variety of these stable cell lines means that such a device can be useful for studying a variety of biological questions.

At confluence, these MDCK cells are 20-25 µm across. Therefore, this device had
to be able to apply up to 100 \( \mu m \) (4-5 cells lengths) of shear and up to 50 \( \mu m \) (2-3 cell lengths) of tension. In addition, cells are grown in cell culture media, which is a 150 mM aqueous solution containing salts, amino acids, and lipids. To keep the cells functioning normally and healthy, the device had to be operable in such cell culture media. The device had to allow the user to perform upright and inverted microscopy on the cells during operation. The microscopy included not only bright field imaging, but also fluorescent imaging, including Förster Resonance Energy Transfer (FRET). A final design feature was the presence of an on-chip ability to not only apply actuation in shear and tension, but also sense in these dimensions as well, which could allow for a feedback control loop.

### 3.2 Design of Cell Adhesion Pads

Two types of cell adhesion pads were designed: i) silicon pads, and ii) silicon nitride (or simply nitride) pads. The silicon pads allowed for upright bright-field and fluorescent microscopy, while the nitride pads allowed for upright and inverted bright-field and fluorescent microscopy. The transparency of the nitride pads even improved the upright fluorescent microscopy on these devices as it reduced the background noise due to light reflection.

#### 3.2.1 Cell Adhesion Pad Size and Placement

Figure 3.1 presents the mask design and a picture of the eventual fabricated device for the silicon cell adhesion pads. Each of the two pads for the silicon pad devices were 1000 \( \mu m \) \( \times \) 250 \( \mu m \). Figure 3.2 presents the design of the nitride cell adhesion pads. Each of the two pads for the nitride devices were 1060 \( \mu m \) \( \times \) 260 \( \mu m \) as designed on the mask. In each direction, 10 \( \mu m \) of this size was devoted to the expected undercut and over-etch of the pads. There will be a more detailed explanation of this effect in the next subsection. In addition, in each direction we designed a 50 \( \mu m \) overlap with the silicon element to provide structural integrity for the nitride pads. Therefore, the size of actual cell adhesion area for each pad was 1000 \( \mu m \) \( \times \) 200 \( \mu m \).
3.2. DESIGN OF CELL ADHESION PADS

Figure 3.1: a) Mask design of the two 250 µm × 1000 µm cell adhesion pads. b) Picture of the silicon pads after they have been fabricated. The distance between the large markings is 100 µm and the distance between the small markings is 20 µm.

Figure 3.2: a) Mask design of the two 200 µm × 1000 µm cell adhesion pads. The cell adhesion area of the nitride pads is 50 µm narrower than the silicon pads to allow for a 50 µm-wide silicon structural element. b) The nitride pads protrude 10 µm beyond the silicon beam to correct for undercut induced by fabrication steps. c) Cross-sectional view of nitride pads shows how they form a ≈50 µm deep well together.

3.2.2 Expected Undercut and Over-etch

Figure 3.3 presents the steps required to fabricate the nitride cell adhesion pads. The nitride pads were 3.6 µm thick, and they were defined by an isotropic wet etch process. The hard mask that was used to define them was a 1.0 µm-thick layer of Low Temperature Oxide (LTO), which was also defined by an isotropic wet etch process. Finally, the oxide hard mask was defined by a layer of photoresist, which was exposed at the bottom of a 50 µm trench. The isotropic nature of these etches meant that the pads would be ≈5 µm smaller than designed on the mask. Furthermore, we had to anticipate the over-etch each layer, which would ensure that the pads were fully separated. Uneven etch rates across the wafer and in the corners of trenches due to thermal and material transport effects meant that we had to over-etch each layer by roughly 20%. Finally, as explained in the next subsection,
the diffraction of light during the resist exposure of the nitride pads was expected to cause an additional 670 nm exposure of the pads compared to the mask design. The combination of these elements, while taking into account a small factor of safety, led to the decision to add 10 µm to the length and width of the pads on the mask. We added this additional length on the side that faced the already etched trench, and not the side that was placed on the silicon truss. Observing the pads after fabrication showed that these estimates were correct.

### 3.2.3 Diffraction of Light

The way in which the nitride pads were fabricated affected their design. The nitride pads were defined at the bottom of a trench as thick as the device layer, as opposed to all of the other structures, which were defined at the top of the device layer. This fact necessitated a design that accounted for the change in feature size caused by the diffraction of light. Figure 3.4 presents a visual model of this phenomenon. We can calculate the additional exposed area due to the diffraction of light using

\[
d \sin(\theta) = \lambda \\
\theta = \sin^{-1}\left(\frac{\lambda}{d}\right) \\
\tan(\theta) = \frac{w}{t} \\
w = t \tan(\sin^{-1}\left(\frac{\lambda}{d}\right))
\]

where \(\lambda\) is the wavelength of the light, \(d\) is the width of the opening of the slit, \(\theta\) is
the angle of incidence of the minimum intensity of light, $t$ is the thickness of the device layer (the depth at which exposure occurs), and $w$ is the additional amount exposed at the bottom of the trench due to the diffraction of light. The masks were designed for the Karlsuss contact lithography exposure tool, which uses light in the Ultra Violet (UV) range. The wavelength ($\lambda$) of UV light is 400 nm, the width ($d$) of the opening is 50 $\mu$m, and the thickness ($t$) of the device layer is 50 $\mu$m. Substituting these values into equation 3.4 results in an additional exposure ($w$) of 670 nm.

### 3.3 Folded Flexure Design

Many of the mechanical components of MEMS devices may be modeled as cantilevers. Doing so allows designers to apply beam bending theory to designing these devices.

#### 3.3.1 Beam Bending Model

Figure 3.5 presents the beam bending model applied to a fixed-guided cantilever. In this case, the force versus displacement relationship is given by
CHAPTER 3. DESIGN OF A 2D MEMS DEVICE

Figure 3.5: A fixed-guided beam has one end fixed in position and angle, and the other end movable in one or more spatial dimensions but fixed in angle.

\[ F_{\text{mech}} = kx \]  
\[ k = \frac{Etw^3}{l^3} \]  
\[ F = \frac{Etw^3}{l^3} x \]

where \( F \) is force, \( d \) is displacement, \( E \) is the material’s Young’s Modulus, and \( w, l, \) and \( t \) are the width, length, and thickness of the beam respectively. When modeled as a spring, \( k \) represents the spring stiffness. The stiffness of these springs can be tuned by changing the dimensions of the silicon beams. However, when doing so, constraints such as the length to displacement ratio \( (l/d \geq 10) \), or the thickness versus width ratio \( (t/w \geq 3) \), which are important for linearity and stability of the beams must still be met.

Another factor that can be used to tune the stiffness of these springs is to combine them in series or parallel. Figure 3.6 shows how two fixed-guided beams can be placed in parallel or in series. To find the effective stiffness of the combined structure one can find the stiffness of the springs in parallel as
3.3. FOLDED FLEXURE DESIGN

3.3.2 Horizontal Mechanics

Figure 3.7 presents the set of four folded flexures responsible for the horizontal movement of the device. Figure 3.7b gives more details on one of these horizontal flexures. Modeling the beams as springs and noting that each horizontal folded flexure is composed of three springs in series, one can develop the following expression for the overall spring stiffness of each horizontal folded flexure.

Figure 3.6: Springs in Parallel and in Series. (a) Two fixed-guided beams have been placed in parallel. (b) Two fixed-guided beams have been placed in series.

$$k_{eff} = k_1 + k_2$$ (3.8)

For springs in series the combined stiffness may be found as

$$\frac{1}{k_{eff}} = \frac{1}{k_1} + \frac{1}{k_2}$$ (3.9)

Springs can then be placed in series or in parallel to not only give a device stability, but also tune its stiffness in various dimensions. When these springs are placed in series they may also be called folded flexures.
CHAPTER 3. DESIGN OF A 2D MEMS DEVICE

Figure 3.7: The horizontal movement of the cell-adhesion pads is made possible by the horizontal shuttle (a) Four folded flexures give the horizontal shuttle stiffness and stability. (b) Details of a single folded flexure responsible for horizontal movement.

\[ \frac{1}{k_{\text{HorzFold}}} = \frac{L_{H1}^3}{Etw^3} + \frac{L_{H2}^3}{Etw^3} + \frac{L_{H3}^3}{Etw^3} \]  

(3.10)

\[ L_{H1} = L_{H3} \]  

(3.11)

\[ k_{\text{HorzFold}} = \frac{Etw^3}{2L_{H1}^3 + L_{H2}^3} \]  

(3.12)

Noting that the horizontal movement is made possible by four such folded flexures, and that these four are placed in parallel, the overall horizontal stiffness of each device may be obtained as

\[ k_{\text{Horz}} = 4 \times k_{\text{HorzFold}} \]  

(3.13)

\[ k_{\text{Horz}} = \frac{4Etw^3}{2L_{H1}^3 + L_{H2}^3} \]  

(3.14)

3.3.3 Vertical Mechanics

The same analysis may also be applied to understand the vertical stiffness of the shuttle. Figure 3.8a presents the set of four folded flexures responsible for the vertical movement of the device. Figure 3.8b gives more details on one of these vertical flexures. We can
Figure 3.8: The vertical movement of the cell-adhesion pads is made possible by the vertical shuttle (a) Four folded flexures give the vertical shuttle stiffness and stability. (b) Details of a single folded flexure responsible for vertical movement.

We use a similar analysis as the previous set of flexures to determine the stiffness of each folded flexure. In this case, the flexures responsible for vertical movement are created from four beams placed in series. The stiffness of each of these vertical folded flexures may be determined as the following

\[
\frac{1}{k_{\text{VertFold}}} = \frac{L_{V1}^3}{Etw^3} + \frac{L_{V2}^3}{Etw^3} + \frac{L_{V3}^3}{Etw^3} + \frac{L_{V4}^3}{Etw^3} \tag{3.15}
\]

\[
L_{V1} = L_{V2} = L_{V3} = L_{V4} \tag{3.16}
\]

\[
k_{\text{VertFold}} = \frac{Etw^3}{4L_{V1}^3} \tag{3.17}
\]

Once again, we note that the vertical movement is made possible by four folded flexures placed in parallel. The overall stiffness of the device in the vertical direction may be found with the following expression

\[
k_{\text{Vert}} = 4 \times k_{\text{VertFold}} \tag{3.18}
\]

\[
k_{\text{Vert}} = \frac{Etw^3}{L_{V1}^3} \tag{3.19}
\]
CHAPTER 3. DESIGN OF A 2D MEMS DEVICE

Figure 3.9: The electrical actuation of the devices is made possible by an electrostatic force, which is counteracted by a mechanical restoring force.

3.4 Actuator Design

For the electrical device, actuation and sensing are made possible electrostatically. A series of electrodes make horizontal movement possible, and another set make it possible to move the device vertically. The actuation is accomplished using voltage differences between these interdigitated electrodes.

3.4.1 Electrostatic Forces

Figure 3.9 presents a simple parallel plate capacitor, and the geometric features that are needed to calculate the capacitance and electrostatic force between the two plates. In addition, it demonstrates how the electrostatic force of the electrodes is counteracted by the mechanical force of a spring. This electrostatic force may be developed from the potential energy stored in a capacitor

\[
U = \frac{1}{2} CV^2 \tag{3.20}
\]

\[
C = \frac{\varepsilon_0 \varepsilon_r A}{d} = \frac{\varepsilon_0 \varepsilon_r tw}{d} \tag{3.21}
\]

\[
F_{elec} = \frac{\partial U}{\partial x} \tag{3.22}
\]

\[
F_{elec} = \frac{1}{2} \left( \frac{\partial C}{\partial x} \right) V^2 + CV \left( \frac{\partial V}{\partial x} \right) \tag{3.23}
\]
where $U$ is the potential energy stored in the capacitor, $C$ is the capacitance between the electrodes, $V$ is the voltage difference between the electrodes, $\varepsilon_0$ is the permittivity of free space, $\varepsilon_r$ is the relative permittivity of the material, $A$ is the area of each electrode, $t$ is the thickness of each electrode, $w$ is the width of each electrode, and $d$ is the gap between the electrodes. $x$ is the dimension in which the potential energy is changing, and the electrostatic force ($F_{elec}$) is equal to the change in the potential energy ($U$) in the ($x$) dimension. Since the voltage does not change with the movement of the electrode in the $x$ dimension, we note that

$$
\left| \frac{\partial V}{\partial x} \right| = 0 
$$

(3.24)

$$
F_{elec} = \frac{1}{2} \left| \frac{\partial C}{\partial x} \right| V^2 
$$

(3.25)

In this case, $d$ is the only component of the geometry that is in the $x$ dimension. Carrying out the partial derivative operation in the $x$ dimension we obtain

$$
\left| \frac{\partial C}{\partial x} \right| = \frac{\varepsilon_0 \varepsilon_r t w}{d^2} 
$$

(3.26)

Inserting this component back into (3.25) we obtain

$$
F_{elec} = \frac{1}{2} \frac{CV^2}{d} = \frac{\varepsilon_0 \varepsilon_r t w}{2d^2} V^2 
$$

(3.27)

As may be seen from this derivation, the electrostatic force is directly proportional to $V^2$, and not $V$. Therefore, regardless of the sign of the voltage difference, the electrostatic force will always be positive, and it will work to increase capacitance (e.g. increase $t$, increase $w$, or decrease $d$). Therefore, to counteract this always ”pulling” electrostatic force, the previously mentioned folded flexures apply a restoring mechanical force.

Overall, this method allows us to apply a voltage difference between the electrodes to cause a displacement of the device, and combine the restoring force of the springs with a
Figure 3.10: Interdigitated electrodes allow the capacitance and the electrostatic force to be directly proportional to displacement, and we have a relatively easy way to increase the number of capacitors in a small footprint.

decrease in the voltage difference to return the device to its original position. However, as equation 3.27 demonstrates, the electrostatic force is inversely proportional to the square of the gap \((d^2)\) between the electrodes. Therefore, a configuration where the gap between the electrodes changes in response to voltage difference is difficult to control because of the inherent non-linearity of the system.

### 3.4.2 Interdigitated Electrodes

Figure 3.10 presents a rendering of interdigitated electrodes. In this setup the gap between electrodes is held constant, and capacitance between them increases when the overlap between the electrodes increases. In addition, we must note that we now in fact have multiple electrodes working in parallel. In fact, another important benefit of interdigitated electrodes is that they allow us to combine multiple electrodes in a relatively small footprint to produce much larger electrostatic forces. We can add the capacitance of the electrodes easily because capacitors acting in parallel have the same overall capacitance as the sum of their individual capacitances. We can note this fact with the following relationship

\[
C_1 = C_2 = C_3 = \ldots = C_{NE} = C
\]

\[
C_1 + C_2 + C_3 + \ldots + C_{NE} = N_E \times C
\]
where $N_E$ is the number of electrodes. Each interdigitated finger forms an electrode with two other opposing fingers; therefore, the total capacitance may be written as a function of the number of fingers $N$, as

$$C = \frac{2N\varepsilon_0\varepsilon_r tw}{d}$$

(3.30)

$$\partial C / \partial x = \frac{2N\varepsilon_0\varepsilon_r t}{d}$$

(3.31)

Referring back to 3.25 and recognizing that $w$ is now the geometric component in the $x$ dimension instead of $d$, equation 3.26 changes to

$$\left| \frac{\partial C}{\partial x} \right| = \frac{2N\varepsilon_0\varepsilon_r t}{d}$$

(3.32)

Inserting this component back into 3.25 we obtain

$$F_{elec} = \frac{N\varepsilon_0\varepsilon_r t}{d} V^2$$

(3.33)

As this expression demonstrates, the electrostatic force produced by a group of interdigitated electrodes working in parallel is a constant multiplied by voltage squared, which gives us an easy way to control the electrostatic force.

### 3.4.3 Combining Mechanical and Electrostatic Forces

At steady state, the electrostatic actuation force of the electrodes equals the mechanical restoring force of the folded flexures. In this condition,

$$F_{mech} = F_{elec}$$

(3.34)

$$kx = \frac{N\varepsilon_0\varepsilon_r t}{d} V^2$$

(3.35)
We can then determine the deflection of the spring $x$ with

$$x = \frac{N\varepsilon_0\varepsilon_r t}{kd} V^2$$  \hspace{1cm} (3.36)$$

Therefore, using folded flexures and interdigitated electrodes we have developed an expression for the movement of the device that is directly proportional to the voltage squared. Substituting equation 3.14 into equation 3.36 we obtain the horizontal displacement of the device as a function of voltage

$$x_{\text{Horz}}(V) = \frac{N_{\text{Horz}}\varepsilon_0\varepsilon_r(2L_{H1}^3 + L_{H2}^3)}{4Ew^3d} V^2$$  \hspace{1cm} (3.37)$$

and substituting equation 3.19 into equation 3.36 we obtain the vertical displacement of the device as a function of voltage

$$x_{\text{Vert}}(V) = \frac{N_{\text{Vert}}\varepsilon_0\varepsilon_r L_{V1}^3}{Ew^3d} V^2$$  \hspace{1cm} (3.38)$$

One interesting aspect of this equation is the fact that displacement is not a function of thickness. Assuming that we have a wafer with a uniform device layer thickness, these terms in the electrostatic and mechanical force equations cancel each other.

Displacement is strongly influenced by the width and length of the folded flexures (both are cubic terms). Each device on the wafer has one of three possible stiffness values. These differences are achieved by using one of three possible flexure widths of 8 µm, 9 µm, or 10 µm. To calculate the displacement of the device with respect to voltage, we must also take into account the fact that as a polar molecule water has a significantly higher relative permittivity ($\varepsilon_r = 78$) than air ($\varepsilon_r = 1$). Therefore, the voltages required to achieve the full desired actuation in water will be an order of magnitude ($\sqrt{78} \approx 9$) smaller than those required in air. Figure 3.11 presents the expected actuations of the three designs in air in the horizontal and vertical directions. Figure 3.12 presents the expected actuations of the three designs in water in the horizontal and vertical directions. As may be seen in these
3.5. OPERATION IN IONIC MEDIA

Figure 3.11: Expected horizontal and vertical actuation of the devices in air ($\varepsilon_r = 1$). figures, we can achieve our full desired horizontal displacement of 100 µm in air and water at roughly 100 V and 10 V, respectively. We can also achieve our full desired vertical displacement of 50 µm in air and water at similar voltages. These are very reasonable voltages for operation in each medium.

3.5 Operation in Ionic Media

Electrostatic MEMS devices have been common mechanisms employed in many sensors and actuators. [101] Their operation has generally been limited to vacuum or air. Biological cells in science labs are cultured in cell culture media, which are aqueous solutions with concentrations of salt in the hundreds of mM. For example, the cell culture medium of Dulbecco’s Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS), which is used for culturing MDCK cells, has an ionic concentration of 150 mM. [97] Ionic shielding, which effectively blocks the electric field, is the major hindrance to electrostatic operation in such media. However, Sounart et al. and Mukundan et al. have been
able to overcome the issue of ionic shielding in ionic media through the use of high frequency Alternating Current (AC) signals for the electrodes. \cite{97, 100, 102} This setup is in contrast to the Direct Current (DC) signals generally employed for electrostatics. In addition, Mukundan et al. used differential electrode design to avoid the displacement attenuation caused by parasitic capacitances observed when operating the device at high AC frequencies. The design of this device benefited greatly from the work of Mukundan et al., and their differential electrode design.

### 3.5.1 Avoiding Ionic Shielding

After fluorine, oxygen is the most electronegative element in the periodic table. This property means that when one oxygen atom binds with two hydrogen atoms to form a water molecule, the electrons of the hydrogen atoms are strongly attracted by the oxygen atom. The water molecule is considered a polar molecule as it exhibits a polarity of electric charge: it is somewhat negative on the oxygen side, and somewhat positive on the hydrogen side. Applying an electric field within a polar liquid merely aligns the polar molecules
3.5. OPERATION IN IONIC MEDIA

Figure 3.13: a) The large electronegativity of the oxygen atom creates a strong dipole moment on the water molecule. b) These dipoles, as well as the ions in the aqueous solution, align with the electric field in a thin layer to block the overall field between the two sets of electrodes. c) The circuit model of the electrodes in ionic media. [100, 103]

with the electric field on the surface of the electrodes, and blocks the field. Reversing the direction of the field aligns the polar molecules in the other direction.

Figure 3.13a presents how the structure of the water molecule causes it to form a dipole moment. Adding charged ions to this system further diminishes the field as the electrodes will attract ions of the opposite charge. Figure 3.13b shows how these dipoles, as well as the ions present in the aqueous solution, block the electric field beyond a thin region adjacent to the electrode. This figure shows the Stern layer and the Diffuse layer, which form the Electric Double Layer (EDL). The Stern layer is composed of a layer of solvent molecules devoid of ions or charge, called the Inner Helmholtz Plane (IHP), and a layer of charged adsorbed ions, called the Outer Helmholtz Plane (OHP). The strength of the electric field within the Diffuse layer follows a Poisson-Boltzmann distribution, and
it decreases exponentially as distance from the surface increases, until the electric field vanishes. This distance is defined as the Debye length ($\lambda_d$). $\lambda_d$ is a function of the electrical properties of the medium and its ionic content. It is on the order of 1 nm for media of high ionic content, and on the order of 100 nm for media of low ionic content. A typical thickness for the Stern layer is given as $\lambda_S = 5 \, \text{Å}$. [103]

The gap between interdigitated electrodes in most MEMS devices is on the order of microns. Yet, the Debye length, after which the electric field vanishes, is 1-100 nm. Therefore, it would be impossible to operate an electrostatic device in aqueous ionic solutions using traditional methods of operation of a DC electric signal. We must recall that whereas the electrode charges essentially instantaneously, there is a finite time required for the EDL to form. Bazant and Mukundan show how we may avoid forming the EDL, and operate an electrostatic device in ionic media, by switching the direction of the electric field at a frequency higher than the characteristic frequency of the system. [100, 104] The characteristic frequency of the system is obtained from the characteristic time constant, or the time required for the formation of the EDL. The characteristic frequency is

$$f_c = \frac{2}{2\pi C_{INT} R_M}$$ (3.39)

where $C_{INT}$ is the interface capacitance, and $R_M$ is the bulk media electrical resistance, as defined in the circuit model of the electrode gap presented in Figure 3.13c. [100] $R_M$ is a material property, and $C_{INT}$ is the combined capacitance in series of the native oxide on the silicon electrodes and the Stern layer. Mukundan notes that when operating the electrostatic device under high AC frequencies, Equation 3.33 changes to

$$F_{elec} = \frac{N\varepsilon_0\varepsilon_r t}{d} f(\omega)V_{RMS}^2$$ (3.40)

where $V_{RMS}$ is the root-mean-square voltage, $f(\omega)$ is a term representing the frequency-dependent impedances of the electrode system, and $\omega$ is the angular frequency. The reader can refer to Mukundan for an in-depth treatment of this subject. [100] However, for the purposes of this design process, we must note three special conditions for $f(\omega)$:
3.5. OPERATION IN IONIC MEDIA

1. If \( \omega \to 0 \) then \( f(\omega) \to 0 \)

2. If \( \omega \to 2\pi f_c \) then \( f(\omega) \to 1/2 \)

3. If \( \omega \to \infty \) then \( f(\omega) \to 1 \)

These special conditions hold true under the assumption that \( C_{INT} \gg C_M \) in the circuit model of the system presented in Figure 3.13c. In other words, if the system is operated at a frequency significantly higher than the characteristic frequency, the frequency-dependent term drops out, and Equation 3.40 becomes

\[
F_{elec} = \frac{N\varepsilon_0\varepsilon_r A}{d} V_{RMS}^2
\]  

(3.41)

Figure 3.14 presents a sinusoidal voltage signal, the same signal with the opposite sign (180° out of phase), and the root-mean-square of these signals. As may be seen, the \( V_{RMS} \) of the sinusoidal signal is the same, regardless of the sign. Figure 3.15 presents the voltage signals of Figure 3.14 squared. In this figure, the square of the sinusoidal voltages overlap...
with each other. These figures show how the electrostatic force generated will relate to the amplitude and root-mean-square of the sinusoidal voltage in this actuation scheme.

### 3.5.2 Symmetric Electrode Design

Mukundan showed how parasitic impedances attenuated the output of the actuator at high voltage signal frequencies. Figure 3.16 presents an image of a symmetric electrode design. It must be noted that the ionic medium between the interdigitated electrodes has a small but finite conductivity (10 mS/cm for cell culture media). In this figure, if electrodes A and B produce the same high frequency voltage signal, the body of the moving shuttle acts as a current path, and the parasitic impedance of this body acts as a low pass filter. Under such circumstances high frequency signals, and the output of the actuator, will be attenuated. However, if electrodes A and B produce voltage signals 180° out of phase, the current generated by each one will be canceled by the other. In this way the parasitic impedance of the actuator shuttle will not matter.

Figure 3.17 presents the design of the 2D MEMS device, and it numbers the input
and output wire bond pads. Pads 1-4 and 13-16 represent the sensing side, and pads 5-12 represent the actuation side. Table 3.1 presents how each electrical pad should be connected. It also presents the electrode symmetry scheme required to minimize parasitic impedances. Table 3.2 presents the list of devices that have been designed. Device names that include the letter “Y” point to the existence of silicon nitride cell adhesion windows, and device names with the letter “N” point to silicon cell adhesion windows. As Figure 3.17 shows, each side of the device (actuation and sensing) is composed of a tension shuttle and a shear shuttle. These shuttles move independently of each other. The designs include three different levels of shuttle stiffness, which was achieved by employing flexure widths of 8, 9, and 10 µm. As Table 3.2 shows, the shear shuttle is significantly stiffer in the tension direction than in the shear direction, and the tension shuttle is significantly stiffer in the shear direction than in the tension direction. Therefore, we can assume that almost all of the deflection of the device in tension will be because of the tension springs, and almost all
Figure 3.17: Design of the 2D MEMS device: pads 1-4 and 13-16 represent the sensing side, and pads 5-12 represent the actuation side.
3.6. SUMMARY

of the deflection of the device in shear will be because of the shear springs.

Table 3.1: Layout of Electrical Connections for 2D MEMS Device

<table>
<thead>
<tr>
<th>Pin</th>
<th>Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+Shear</td>
</tr>
<tr>
<td>2</td>
<td>+Tension</td>
</tr>
<tr>
<td>3</td>
<td>-Tension</td>
</tr>
<tr>
<td>4</td>
<td>GND</td>
</tr>
<tr>
<td>13</td>
<td>-Shear</td>
</tr>
<tr>
<td>Sensing Side</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>+Tension</td>
</tr>
<tr>
<td>15</td>
<td>-Tension</td>
</tr>
<tr>
<td>16</td>
<td>GND</td>
</tr>
<tr>
<td>5</td>
<td>+Shear</td>
</tr>
<tr>
<td>6</td>
<td>+Tension</td>
</tr>
<tr>
<td>7</td>
<td>-Tension</td>
</tr>
<tr>
<td>8</td>
<td>GND</td>
</tr>
<tr>
<td>9</td>
<td>-Shear</td>
</tr>
<tr>
<td>Actuation Side</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>+Tension</td>
</tr>
<tr>
<td>11</td>
<td>-Tension</td>
</tr>
<tr>
<td>12</td>
<td>GND</td>
</tr>
</tbody>
</table>

Table 3.2: Device Stiffness by Type

<table>
<thead>
<tr>
<th>Device Type</th>
<th>Shear Shuttle Stiffness (N/m)</th>
<th>Tension Shuttle Stiffness (N/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESY1/ESN1</td>
<td>0.923</td>
<td>48900</td>
</tr>
<tr>
<td>ESY2/ESN2</td>
<td>1.31</td>
<td>55000</td>
</tr>
<tr>
<td>ESY3/ESN3</td>
<td>1.8</td>
<td>61100</td>
</tr>
</tbody>
</table>

3.6 Summary

A new MEMS device has been designed that allows for cell mechanics experiments on a confluent monolayer of 1000 MDCK cells. This device has the ability to apply and sense forces and displacements in shear and tension. In addition, the device allows for upright
and inverted live cell microscopy, in bright field or fluorescence. The device is operable in cell culture media. Each of the two cell adhesion pads on each chip are either 1000 µm × 250 µm for the silicon pad device, or 1000 µm × 200 µm for the silicon nitride pad device. Measurement graduations were added to the side of the cell adhesion pads at 20 µmand 100 µmintervals to assist in measuring lengths under the microscope. Designing the silicon nitride cell adhesion pads introduced fabrication challenges based on shrinkage due to undercut, over-etch, and light diffraction. These challenges were overcome by designing the pads larger than they would appear on the device.

Folded flexures allowed the ability of the devices to remain suspended, and allowed us to design a define the stiffness in different dimensions. Folded flexures follow the beam bending rules of fixed-guided beams. The stiffness of the device in each dimension was determined using beam bending theory, as well as rules regarding the combined stiffness of beams in parallel or in series. Electrostatic forces were used to provide actuation and sensing for the electrical devices. The mechanical devices used a micromanipulator for actuation, and optical sensing. Interdigitated electrodes were the particular form of electrostatics used, because of their advantages over designs where the gap between the electrodes would be changed. These devices would be stable when the electrostatic force equaled the restoring mechanical force.

These devices have been designed for operation in an ionic media (i.e. cell culture media), whereas most electrostatic devices are operated in air or vacuum. An electrostatic device cannot be operated in ionic media using a DC voltage signal, as doing so would lead to ionic shielding of the electrodes. Instead, a high frequency AC signal may be employed to take advantage of the time required for the ionic shield to form. Switching the polarity of the electrodes at higher frequencies than the time required for ionic shielding avoids this problem. At the same time, parasitic impedances may lead to high frequency roll-off of the actuation signal. A symmetric electrode design was employed to overcome this problem.
Chapter 4

Fabrication and Testing

Two sets of fabrication runs took place: one for devices with electrical actuation and sensing, and one for devices with mechanical actuation and sensing. The masks were largely the same between the two runs, although for the mechanical devices, a modified version of the Trenches mask was used, and the Metal2 mask was not used at all. Each fabrication run included devices with silicon and nitride cell adhesion pad.

4.1 Electrical Device Fabrication

The electrical devices were fabricated using a five mask process. These masks, which have been presented in Appendix B, consisted of:

1. **Trenches**: Defined the passivation trenches and cell adhesion windows
2. **Nitride**: Defined the nitride trench caps and cell adhesion pads
3. **Metal2**: Defined the metal contacts
4. **Silicon**: Defined the device structure etch
5. **Backside**: Defined the backside etch

The process flow for fabricating the electrical devices has been presented in Figure 4.1. The run sheet for fabricating the electrical devices has been presented in Table 4.1.
<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Parameters</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anneal</td>
<td>24hrs @ 1100C</td>
<td>Tylan1</td>
</tr>
<tr>
<td>2</td>
<td>Spin Resist</td>
<td>1.0 µm 3612</td>
<td>SVG Coat</td>
</tr>
<tr>
<td></td>
<td><strong>Exposé #1: Trenches</strong></td>
<td><strong>1.0 s</strong></td>
<td><strong>KarlSuss</strong></td>
</tr>
<tr>
<td></td>
<td>Trench Si Etch</td>
<td><strong>DEEP</strong></td>
<td>STS Etch</td>
</tr>
<tr>
<td>3</td>
<td>Nitride Passiv Dep</td>
<td>300 nm of NEWLSN</td>
<td>TylanNitride</td>
</tr>
<tr>
<td>4</td>
<td>Poly Dep</td>
<td>5 X 2 µm of P620POLY</td>
<td>ThermcoPoly1</td>
</tr>
<tr>
<td>5</td>
<td>Back Poly Etch</td>
<td>Isotropic SF6</td>
<td>STS Etch</td>
</tr>
<tr>
<td></td>
<td>Front Poly Etch</td>
<td>Isotropic SF6</td>
<td>STS Etch</td>
</tr>
<tr>
<td>6</td>
<td>Nitride Window Dep</td>
<td>2 X 1.8 µm of LSN2</td>
<td>ThermcoNitride</td>
</tr>
<tr>
<td>7</td>
<td>LTO Dep</td>
<td>2 X 0.75 µm of LTO400PC</td>
<td>Tylan BPSG</td>
</tr>
<tr>
<td>8</td>
<td>Spray Resist</td>
<td>20 Pass - 3.4 µm</td>
<td>SprayCoater</td>
</tr>
<tr>
<td></td>
<td>Bake</td>
<td>Bake for 300 s</td>
<td>SVG Coat</td>
</tr>
<tr>
<td></td>
<td><strong>Exposé #2: Nitride</strong></td>
<td><strong>30 X 2.0 s w/ 15 s wait</strong></td>
<td><strong>KarlSuss</strong></td>
</tr>
<tr>
<td></td>
<td>Develop Resist</td>
<td>Develop Manually 6 X 30 s</td>
<td>Headway</td>
</tr>
<tr>
<td></td>
<td>Oxide Mask Etch</td>
<td>6:1 BOE</td>
<td>WBNonmetal</td>
</tr>
<tr>
<td></td>
<td>Nitride Etch</td>
<td>30hrs of Hot Phosphoric</td>
<td>WBNitride</td>
</tr>
<tr>
<td>9</td>
<td>Spray Resist</td>
<td>60 Pass - 10 µm</td>
<td>SprayCoater</td>
</tr>
<tr>
<td></td>
<td>Polymer Descum</td>
<td><strong>10 X 1.2 s w/ 10 s wait</strong></td>
<td><strong>KarlSuss</strong></td>
</tr>
<tr>
<td></td>
<td>Native Oxide Etch</td>
<td>Descum for 30 s</td>
<td>Drytek2</td>
</tr>
<tr>
<td></td>
<td>Metal Dep</td>
<td>40s of 20:1 BOE</td>
<td>WBNonmetal</td>
</tr>
<tr>
<td></td>
<td>Lift-Off</td>
<td>Cr/Pt/Au (250A/500A/3000A)</td>
<td>Innotec</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sonicate for 5mins</td>
<td>WBSolvent</td>
</tr>
<tr>
<td>10</td>
<td>Spray Resist</td>
<td>20 Pass - 3.4 µm</td>
<td>SprayCoater</td>
</tr>
<tr>
<td></td>
<td>Bake and EBR</td>
<td>5 mm EBR + 300s Bake</td>
<td>SVGCoat</td>
</tr>
<tr>
<td></td>
<td><strong>Exposé #4: Silicon</strong></td>
<td><strong>3 X 1.1 s w/ 60 s wait</strong></td>
<td><strong>KarlSuss</strong></td>
</tr>
<tr>
<td></td>
<td>Crystal Bond</td>
<td>Crystal Bond on Backside</td>
<td>Headway</td>
</tr>
<tr>
<td></td>
<td>Device Si Etch</td>
<td>NANO</td>
<td>Deep Si Etcher</td>
</tr>
<tr>
<td></td>
<td>O2 Clean</td>
<td>O2 Plamsa</td>
<td>Drytek2</td>
</tr>
<tr>
<td></td>
<td>Resist Strip</td>
<td>Acet/Met/Isop</td>
<td>WBSolvent</td>
</tr>
<tr>
<td>11</td>
<td>LTO Dep</td>
<td>2 X 1.5 µm LTO400PC</td>
<td>ThermcoLTO</td>
</tr>
<tr>
<td></td>
<td>Spray Resist</td>
<td>20 Pass - 3.4 µm</td>
<td>SprayCoater</td>
</tr>
<tr>
<td></td>
<td>Spin Resist on Back Bake</td>
<td>7 µm 220-7</td>
<td>SVG Coat</td>
</tr>
<tr>
<td></td>
<td><strong>Exposé #5: Backside</strong></td>
<td><strong>10mins @ 90C</strong></td>
<td>Litho Oven</td>
</tr>
<tr>
<td></td>
<td>Pattern Back Oxide</td>
<td><strong>4 X 2.0s w/ 10s wait</strong></td>
<td><strong>KarlSuss</strong></td>
</tr>
<tr>
<td></td>
<td>Crystal Bond</td>
<td>6:1 BOE (15 mins)</td>
<td>WBFlexcorr</td>
</tr>
<tr>
<td></td>
<td>Back Si Etch</td>
<td>Crystal Bond on Frontside</td>
<td>Headway</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DSE FAT</td>
<td>Deep Si Etcher</td>
</tr>
<tr>
<td>12</td>
<td>Wafer Dicing</td>
<td>Diced Electrode Separations</td>
<td>DISCO Wafersaw</td>
</tr>
<tr>
<td></td>
<td>Oxide Release</td>
<td>6:1 BOE for 20hrs</td>
<td>WBGeneral</td>
</tr>
<tr>
<td></td>
<td>Critical Point Dry</td>
<td>Use IPA</td>
<td>CPD</td>
</tr>
</tbody>
</table>
4.1. ELECTRICAL DEVICE FABRICATION

4.1.1 Clean and Anneal

The fabrication process for the electrical devices began with an anneal at 1100°C for 24 hours. The wafers were cleaned in a prediffusion clean at WBClean (formerly WBDiff). The prediffusion clean was required before every high-temperature process, a process that includes the diffusion or chemical growth tubes, and was used to avoid contaminating these tools. The prediffusion clean included cleaning the wafers for 10 minutes in a 5:1:1 solution of \( H_2O:H_2O_2:NH_4OH \) at 50°C. The wafers were washed with deionized (DI) water in the dump/rinse. They were then cleaned in 50:1 HF for 30 seconds at room temperature, followed by a wash in the dump/rinse. The wafer cassette was then put through a 5:1:1 solution of \( H_2O : H_2O_2 : HCl \) at 50°C followed by the dump/rinse. Finally, the wafers were dried in the Spin-Rinse Dryer (SRD) tool.

This anneal step was included at the beginning of the fabrication process to reduce the stress between the BOX layer and the device layer of the SOI wafer. This stress is...
introduced into the wafer when the device layer is bonded to the handle and BOX, and it can lead to a lower device yield. A thin (100 Å) oxide hard mask is sometimes grown on the wafer prior to such a long anneal. This hard mask acts as a barrier against the diffusion of particles into the wafer, which can hurt the function of p-n junctions. In the case of this process, no hard mask was grown prior to the anneal, as the devices were actuated electrostatically, and did not rely on p-n junctions.

4.1.2 “Trenches” Mask Step

The next step in the fabrication process was to prepare the wafers for photo-lithography. The standard pre-litho clean process at WBNNonmetal was used for this purpose. This process began with a 20 minute cleaning process in Piranha solution (9:1 ratio of \( H_2SO_4:H_2O \)). After rinsing the wafers in DI water, the native oxide was etched for 30 seconds in 50:1 HF, and the wafers were rinsed in DI water again. The wafer cassette was then dried in the SRD tool. They were placed in the YES Oven to be singed and coated with hexamethyldisilazane (HMDS). Singeing promotes the adhesion of HMDS to the wafers, and HMDS promotes the adhesion of photoresist to them. The wafers were then coated with 1.0 \( \mu \text{m} \) of Shipley 3612 positive photoresist on the SVGCoat tracks. The Karlsuss tool was used to expose the coated wafers to the Trenches mask. The photoresist was developed on the SVGDev tool. The wafers were then etched through the device layer to the BOX in the STSEtch Deep Reactive Ion Etch (DRIE) tool. This step defined the electrical separation trenches on the devices. It also defined the cell adhesion areas for the nitride cell adhesion pads. The wafers were stripped of photoresist in \( O_2 \) plasma in the Gasonics tool, and fully cleaned of polymer residue in the Piranha bath in WBNNonmetal.

4.1.3 Passivation Nitride Deposition

The wafers were then prepared for the application of the silicon-nitride electrical passivation layer in the nitride Low Pressure Chemical Vapor Deposition (LPCVD) tube. TylanNitride was used to deposit 300 nm of low stress nitride (NEWLSN recipe). This recipe was chosen to avoid adding unnecessary stress to the devices. This thickness was chosen to avoid any pinholes in the layer. Pinholes in this layer would not only inhibit
4.1. ELECTRICAL DEVICE FABRICATION

Figure 4.2: When using an LPCVD process to deposit material in a deep trench, a keyhole may be formed at the bottom of the trench.

the function of this layer as an electrical barrier, but also they would be detrimental to the function of this layer as a silicon etch stop in subsequent fabrication steps.

4.1.4 Filling Passivation Trenches

The next fabrication step was the deposition of 10 μm of polysilicon in ThermcoPoly1. This polysilicon was intended as filler for the passivation trenches. The trenches had to be completely filled and any "keyholes" avoided when filling the trenches. Figure 4.2 presents an image of the steps in keyhole formation. When filling a trench with chemically deposited material, the filler gradually deposits on both sides of the trench and eventually closes the opening. For a trench with a high aspect ratio, the gaseous material may not have enough energy to reach and deposit at the bottom of the trench as consistently as it deposits at the top. Therefore, the top of the trench will fill and close before the bottom has filled, and an empty area will remain at the bottom. This empty area is called a keyhole. The formation of a keyhole may be reduced or avoided in three main ways:

1. Reducing the aspect ratio of the trench.

2. Increasing the energy of the carrier gas.

3. Building trenches with obtuse-angled side walls as opposed to vertical ones.

Figure 4.3 presents these three methods graphically. The first two methods were employed to reduce the likelihood of keyhole formation. As a general rule, the aspect
Figure 4.3: The likelihood of keyhole formation may be reduced by: a) reducing the trench’s aspect ratio, b) increasing the energy of the carrier gas, or c) employing obtuse-angled sidewalls, as opposed to vertical ones.

ratio of trenches must be kept below a 10:1 ratio. The aspect ratio of these trenches was held at 5:1 (50 µm thick device layer, and 10 µm wide trench). The second method suggests increasing the energy of the carrier gas, which in an LPCVD tube corresponds to increasing the temperature. P620POLY, which at a deposition temperature of 620°C is the highest temperature process possible in the ThermcoPoly tube was employed for this purpose. This high temperature, while keeping the pressure relatively low, also leads to a more conformal coverage and a more low-stress material afterwards. Finally, the third keyhole avoidance method could not be employed. This problem arose because angling the trench sidewalls using the most common methods (KOH etch) would have led to a fairly large trench footprint on the chip for such a thick device layer.

When filling a trench using an LPCVD process, the material deposits on both side walls
4.1. ELECTRICAL DEVICE FABRICATION

and the trench fills from the outside in. Therefore, for a trench with a width of 10 µm, a 5 µm deposition is required to fill the trench. However, as figure 4.4a presents, a sharp 90° turn in the trench can lead to areas that are a factor of $\sqrt{2}$ wider than the usual width (corresponding to 14.1 µm in this case). The trenches in the Trenches mask include such sharp turns. Therefore, a deposition of just over 7 µm would be required to also fill these widened areas. Finally, including a factor of safety of roughly 1.5 to ensure the trenches were fully filled led to the decision to deposit 10 µm of polysilicon in this step. Figure 4.4b shows how a circular turn may be used to avoid this increase in the trench gap. This 10 µm deposition took place as five depositions of 2 µm each, with the wafers being rotated 1/5 of a turn each time. Depositions of greater than 2 µm in one run could lead to the wafer getting stuck to the carrier boat. In addition, multiple depositions, and wafer rotations between depositions, leads to a more conformal coverage.

4.1.5 Polysilicon Clearing

The next step in the fabrication process was the clearing of polysilicon. Steps 4 and 5 in Figure 4.1 demonstrate how polysilicon was deposited and cleared. As may be seen, polysilicon had to be cleared from the top of the wafer, and quite importantly from inside
CHAPTER 4. FABRICATION AND TESTING

Figure 4.5: TMAH etch fully clears the nitride windows.

Figure 4.6: TMAH etch damages passivation trenches.

Figure 4.7: $SF_6$ gas fully clears the nitride windows.
4.1. ELECTRICAL DEVICE FABRICATION

Figure 4.8: \( \text{SF}_6 \) gas does not damage passivation trenches.

of the windows (wells) reserved for the nitride cell adhesion pads. In subsequent steps, a thicker layer of nitride was deposited and patterned in these windows, they were etched from the backside, and allowed for inverted microscopy on the chip. The first nitride passivation layer, and the subsequent thicker layer of nitride, formed the transparent cell adhesion pads. Any polysilicon not cleared in the polysilicon clearing would inhibit the ability of the user to perform inverted microscopy. However, the polysilicon in the trenches had to not only remain intact, but also it had to retain its mechanical integrity. Furthermore, we wanted to simplify the fabrication process by avoiding the use of a mask to accomplish this task.

To clear the polysilicon in selected areas without the use of a mask we benefited from the much larger opening of the cell adhesion windows compared to the passivation trenches. The windows were roughly 450 \( \mu \text{m} \times 1000 \mu \text{m} \), whereas the trenches were 10 \( \mu \text{m} \) wide and thousands of microns long. We decided to use an isotropic silicon etchant, and depended
on the fact that the etchant would clear the poly in the open areas much faster than in the narrow trenches. Furthermore, the earlier nitride passivation layer would act as a colorful etch-stop (300 nm of nitride is a yellowish-green, as opposed to the silverish-gray silicon). This feature allowed us to avoid damaging the device layer while the polysilicon cleared out of the nitride windows, and the etch was done when the wafer changed color.

An isotropic silicon etchant was needed to complete this step. The first etchant tried was Tetramethylammonium Hydroxide (TMAH). While TMAH is an anisotropic etchant of single crystal silicon, it essentially acts as an isotropic etchant of polysilicon. Other benefits included the fact that it was selective to and stopped on the passivation nitride. Furthermore, using a wet etch it allowed for batch processing, and as a clear liquid it allowed us to observe the color change of the wafers. To characterize the process a test wafer was cleared of polysilicon with this method. It was then taken through the next step of the fabrication process, which was the deposition of 3.6 µm of silicon nitride, cleaved, and then imaged using a Scanning Electron Microscope (SEM).

Figure 4.5 presents a cleaved nitride cell adhesion window at an angle. As may be seen in this figure, all of the polysilicon was cleared off of the wafer as well as off of the cell adhesion pads. Even the corners of the cell adhesion windows were cleared of polysilicon. Figure 4.6 presents a cleaved passivation trench at angle. As may be seen in this figure, the TMAH entered deep into the trench, and etched away the polysilicon not only from the top, but also from the side. The subsequent thicker layer of nitride no longer acted as a cap on the trench, but acted as a poor filler. TMAH met one of the needs of polysilicon clearing, which was fully clearing the cell adhesion windows. However, it did not meet the other need of this step, which was keeping the polysilicon trench fillings intact.

We also tried an isotropic $SF_6$ silicon etch to accomplish this task. The platen generator and switching were turned off on the DEEP recipe of the STSEtch DRIE tool to transform it into an isotropic recipe. Turning off the platen generator turns off the directionality of the etch process, and turning off switching avoids the passivation step of DRIE. Using an isotropic $SF_6$ etch in STSEtch also allowed us to use the color change of the nitride etch stop, which could be observed through the etch chamber window. A test wafer was cleared of polysilicon with this method, cleaved, and then imaged using SEM.

Figure 4.7 shows the cell adhesion window cleared of polysilicon at an angle. Once
again, the etchant successfully cleared all of the polysilicon and stopped on the passivation nitride etch stop. Figure 4.8 shows two cleaved passivation trenches at an angle. As may be seen, in this case the etchant did not enter too far into the trench, and the polysilicon remained intact. The other wafers were also cleared of polysilicon using an isotropic $SF_6$ etch in STSEtch. The backside of wafers was cleared of polysilicon before clearing their frontside.

### 4.1.6 Nitride Cap and Cell Window Deposition

The next step in the fabrication process was to deposit a thicker layer of silicon nitride for the cell adhesion windows, which would also close off the top of the passivation trenches. At this point in the fabrication run TylanNitride was down and ThermcoNitride was used instead. The wafers were coated with 3.6 μm of low stress nitride (LSN2 recipe), which
was accomplished through two depositions of 1.8 µm each. The wafers were rotated 180° between depositions to obtain a more conformal deposition across the wafers. Figure 4.9 presents two cleaved passivation trenches with a nitride cap at an angle. As may be seen, the nitride cap fully closes the top of the passivation trenches. This image corresponds to step 6 of Figure 4.1. The other major purpose of this layer was to obtain a thicker nitride cell adhesion window. Figure 4.5 presents the thicker nitride window that results from the lengthier nitride deposition, albeit this window was obtained after a different polysilicon etch process.

4.1.7 Low Temperature Oxide (LTO) Hard Mask

The next task was to pattern the nitride layer, to form the nitride cell adhesion pads, and etch away everything but the nitride caps of the passivation trenches. For reasons that will be explained in future sections, we chose a hot phosphoric acid etch to pattern the nitride. Photosresist quickly melts away in hot phosphoric acid, and only a hard mask may be used. LTO gave us the best combination of speed of growth and selectivity to nitride in hot phosphoric. The LTO400PC recipe in TylanBPSG was used to deposit 1.5 µm of LTO on the wafers in two rounds of 0.75 µm each. The wafer was rotated 180° between the two depositions to ensure a more conformal coverage. Tests on the etch rates of LTO and low stress nitride in this etchant showed that low stress nitride etched at a more than 3:1 ratio with respect to this LTO. Low stress nitride is more rich in silicon and silicon dioxide than stoichiometric nitride, therefore, it has lower selectivity to an oxide hard mask. Yet, 1.5 µm of LTO provided sufficient masking, including some margin of error, for 3.9 µm of low stress nitride (both layers of nitride combined).

4.1.8 Wet Etch Nitride Windows

The steps for fabricating the nitride cell adhesion pads have been presented in Figure 4.10. As the figure shows, first the windows which host the cell adhesion pads were etched. Then the nitride of the cell adhesion pads was deposited and patterned. Finally, the silicon structures carrying the cell adhesion pads were etched. As discussed previously, an LTO hard mask was used to pattern the nitride layer. Sprayed photoresist was used to pattern the
4.1. ELECTRICAL DEVICE FABRICATION

Figure 4.10: Silicon and nitride etch steps required to form and pattern the nitride cell adhesion pads. First, the cell windows are etched, then the nitride cell adhesion pads are patterned, and finally the rest of the device layer is patterned. The left column shows the mask view, and the right column shows the cross-sectional view.

hard mask. Photoresist had to be sprayed instead of just spun, as the nitride pads were at the bottom of a 50 µm well, and spinning would lead to a smearing of the resist.

The wafers were pre-litho cleaned and coated with HMDS using the methods described
in Subsection 4.1.2. 20 passes of the SprayCoater were used to obtain a 3.4 μm-thick layer of photoresist. Much greater detail about the specifications of the SprayCoater process and of this particular recipe has been provided in Chapter 5. The wafers were baked on the hot plates on SVGCoat for 300 seconds each. The KarlSuss contact aligner was used to expose them to 30 exposures of 2.0 seconds each with 15 seconds of wait time between exposures. Wait times are used in multi-exposure processes to allow for the release of gases that are produced in the resist during the exposure process. Skipping wait times can cause bubbling in the resist. The large number of exposures was needed because unlike a step aligner, a contact aligner only focuses on the top of the wafer. Therefore, the resist that was at the bottom of cell window was out of focus and received very little energy during the exposure process.

The resist was then developed manually in MF-26A developer at the Headway station. Each wafer was dipped in developer for 30 seconds and then washed in a water bath for a few seconds. This process was repeated 6 to 8 times per wafer, and the wafers were observed under a light microscope to ensure the end of the development process. A 6:1 Buffered Oxide Etch (BOE) was used to pattern the LTO hard mask. This etchant was used because it works very quickly (290 nm of LTO per minute). However, in the future we would suggest a slower etchant like 20:1 BOE (740 nm of LTO per minute) to gain greater time resolution on the etch. After ensuring that the oxide was fully patterned, the resist was stripped in Piranha solution at WBNonmetal.

The wafers were then dipped in 50:1 HF for 20 seconds to strip the native oxide that forms on the silicon nitride in ambient air, they were gently shaken to make sure no bubbles formed on the surface. They were then placed in hot phosphoric acid at WBNitride to etch the nitride for 30 hours, which included a 20% over-etch to ensure that the etchant had reached all of the corners of the cell window. The wafers were pulled out of the etchant, washed, and observed under a microscope every few hours to monitor the state of the etch. Each time they were pulled out for observation they were again dipped in 50:1 HF to strip the new native oxide that had formed in the process.
4.1. ELECTRICAL DEVICE FABRICATION

4.1.9 Metal Contact Deposition

The wafers were cleaned using the pre-litho clean previously mentioned. They were coated with sprayed photoresist, as opposed to spun photoresist, to avoid the streaks that can occur when spinning resist on a surface with deep windows. The metal layer was to be patterned with lift-off, but there was no way to conformally coat the wafers with a lift-off layer. The SprayCoater recipe was designed for a particular mixture of positive photoresist, and would have to be designed anew for a lift-off layer. Instead, we decided to use a very thick layer of sprayed photoresist to avoid the use of a lift-off layer. The SprayCoater recipe was amended to perform 60 spray passes, as opposed to the usual 20 passes, which led to a 10 µm-thick coating of resist. Figure 4.11 presents the metal lift-off process.

The resist was exposed using the Karlsuss contact aligner for 10 exposures of 1.2 seconds each, with 10 second wait times between each exposure. The Metal2 contact mask was used for this purpose. This mask was updated from the original Metal contact mask to allow for lift-off before etching the device layer, as opposed to afterwards. Performing lift-off on an etched device layer, especially a 50 µm-thick device layer, was deemed too difficult. The wafers were then developed on SVGDev using the 7.0 µm SPR 220-7 develop recipe. They were exposed to a polymer descum using the descum recipe (O\textsubscript{2} plasma) in Drytek2. Immediately before placing them in the Innotec tool, the native oxide on the wafers was etched by placing them for 40 seconds in 20:1 BOE at WBNonmetal.

The wafers were coated with 250 Å Cr (chromium), 500 Å of Pt (platinum), and 3000 Å of Au (gold) in a single run. The chromium acted as an adhesion layer, the platinum...
as a diffusion barrier, and the gold as the main conductive layer. Gold or platinum cannot be etched very well, so they have to be patterned using lift-off instead. The coating was performed at a fairly slow rate (1.0 Å/s for all three metals) to obtain a lower stress metal layer. The wafers were gold contaminated from this point forward and were treated as such. The wafers were then taken out of Innotec and placed in acetone in the sonicator at WBSolvent. Sonicating for 5 minutes led to lift-off.

4.1.10 Device Layer DRIE

The wafers could not be cleaned using traditional pre-litho cleaning tools as they were gold contaminated. They were batch-cleaned using PRS1000, which does not attack metal, at WBFlexcorr. They were coated with 3.4 µm of photoresist using the SprayCoater. A 5 mm Edge Bead Removal (EBR) and 300 s bake was performed SVGCoat. Wafers coated with resist instead of a hard mask were generally not allowed into the Deep Silicon Etcher (DSE), because of the high energy of the DSE. Resist was considered likely to either burn, or melt and get stuck to the wafer holder. Resist could be used if the user manually stopped the etch to allow for cooling every 10 cycles, and made sure to perform a 5 mm EBR, instead of the usual 2 mm EBR. The extra room at the wafer edges made it less likely that the melted resist would reach the wafer holder.

Wafers were exposed with the Silicon mask using the Karlsuss contact aligner for 3 exposures of 1.1 s each, with 60 s wait times between the exposures. This mask defined the device layer. Anticipating the fragility of the wafers after the etch, they were bonded to carrier wafers using Crystal Bond at the Headway station. The NANO recipe of the DSE was used to etch the wafers down to the BOX layer. After the etch, an O₂ plasma clean was used to break up the hardened resist that results from a long DRIE process. The resist was then stripped and the wafers cleaned using a succession of acetone, methanol, and isopropanol at WBSolvent.

4.1.11 Handle Layer DRIE

A 3.0 µm layer of LTO was used to protect the frontside of the wafers during the backside etch. This layer was deposited in ThermcoLTO in two depositions of 1.5 µm each, and
4.1. ELECTRICAL DEVICE FABRICATION

Figure 4.12: a) The devices should be diced along the dashed yellow line to separate different electrodes and conductive paths. b) The electrodes of a diced electrical device, which have been separated at the top using the wafer saw.

The wafer was rotated 180° between the depositions. 3.4 μm of resist was sprayed on the frontside to protect it. The resist was baked for 300 s on a hot plate. 7.0 μm of SPR 220-7 photoresist was spun on the back and baked for 10 minutes at 90° in the Litho Oven (the hotplate could not be used as the frontside resist would melt and stick to it). KarlSuss was used to expose the wafers to 4 exposures of 2.0 seconds each, with 10 second wait times between the exposures. The Backside mask was used for this purpose. The resist was developed at SVGDev. The wafers were dipped in 6:1 BOE at WBFlexcorr for 15 minutes to pattern the backside LTO. They were bound on the frontside to carrier wafers using Crystal Bond at the Headway station to give them more mechanical integrity. The DSE was used to etch the wafers through the handle layer down to the BOX layer using the DSE FAT recipe. Acetone, Methanol, and Isopropanol were used in succession to strip the resist and clean the wafers.

4.1.12 Buried Oxide Release

Figure 4.12a shows a device from the Silicon mask. This image is digital data dark, and is designed for positive photoresist. As may be seen, the electrodes are electrically separated by etching trenches into the device layer. However, the electrodes may still be attached at the ends. Wafer dicing is used to not only singulate devices, but also separate the electrodes at the ends. The path for the wafer dicing tool is shown as a dashed line in Figure 4.12a. Figure 4.12b shows a device that has been singulated using a wafer saw. As may be seen, the electrodes are separated at the ends. The wafers were coated on the frontside with
photoresist, poured but not spun at the headway station, to protect it against particles. They were diced into individual devices using the DISCO wafer saw.

The devices were cleaned of particles and photoresist using acetone, methanol, and isopropanol in succession. These solvents had the added benefit of “wetting” small crevices in the devices. The devices were then washed in water and transferred to 6:1 BOE at WBFlexcorr for about 20 hours to release the BOX layer. Bubbles formed during the release process often blocked the path of the etchant after a few hours. Every five hours the devices had to be washed in water and a solvent to re-wet the areas formerly blocked by bubbles. After the devices were fully released they were washed in water and then allowed to sit in isopropanol for one hour to ensure that all of the HF had been cleared out. They were then dried in a Critical Point Dryer (CPD) tool.

4.2 Mechanical Device Fabrication

The mechanical devices were fabricated using a five mask process. The masks, which have been presented in Appendix B, were:

1. **Trenches (modified)**: Blocked trench etch, they defined the cell adhesion windows
2. **Nitride**: Defined the cell adhesion pads
3. **Silicon**: Defined the device structure etch
4. **Backside**: Defined the backside etch

The process flow for fabricating the mechanical devices has been presented in Figure 4.13. The run sheet for fabricating the electrical devices has been presented in Table 4.2. The design and fabrication process for the mechanical devices was quite similar to that of the electrical devices. The main differences were in etching and filling of the passivation trenches, and in metalizing the wafers. The Trenches mask was modified using Kapton tape to cover the areas where trenches were exposed. This mask was only used to expose the cell adhesion windows. Kapton tape is a thin adhesive, which also blocks UV light, and was deemed a good way of modifying this mask. As the trenches were not etched in
4.2. MECHANICAL DEVICE FABRICATION

Figure 4.13: Four mask steps and nine process steps are used to fabricate the mechanically-actuated devices.

this step, there was no need to fill them in future steps. Therefore, the fabrication of the mechanical devices skipped steps 3, 4, and 5 of the electrical device fabrication, which were described in Subsections 4.1.3, 4.1.4, and 4.1.5 respectively.

Depositions of the nitride window and the LTO hard mask, as well as patterning the nitride, were accomplished similar to steps 7, 8, and 9 of the electrical device fabrication process. These steps have been described in detail in Subsections 4.1.6, 4.1.7, and 4.1.8 respectively. The metal deposition step of the electrical devices was skipped. The frontside and backside of the mechanical device wafers were then patterned similar to steps 10 and 11 of the electrical device fabrication, as described in Subsections 4.1.10 and 4.1.11 respectively. At this point, the devices were not wafer diced, as they were not to be operated electrically, and there were no electrodes to be separated using a wafer dice. Instead they were released and dried as full wafers using processes described in Subsection 4.1.12. The wafers were diced and devices singulated using a laser cutter.
### Table 4.2: Runsheet Used to Fabricate the Mechanically Actuated Devices

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Parameters</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anneal</td>
<td>24hrs @ 1100C</td>
<td>Tylan1</td>
</tr>
<tr>
<td>2</td>
<td>Spin Resist</td>
<td>1.0 µm 3612</td>
<td>SVG Coat</td>
</tr>
<tr>
<td></td>
<td><strong>Expose #1: Trenches</strong></td>
<td>1.0 s</td>
<td>KarlSuss</td>
</tr>
<tr>
<td></td>
<td>Trench Si Etch</td>
<td>DEEP</td>
<td>STS Etch</td>
</tr>
<tr>
<td>3</td>
<td>Nitride Window Dep</td>
<td>2 X 1.8 µm of LSN2</td>
<td>ThermcoNitride</td>
</tr>
<tr>
<td>4</td>
<td>LTO Dep</td>
<td>2 X 0.75 µm of LTO400PC</td>
<td>Tylan BPSG</td>
</tr>
<tr>
<td>5</td>
<td>Spray Resist, Bake</td>
<td>20 Pass - 3.4 µm Bake for 300s</td>
<td>SprayCoater, SVG Coat</td>
</tr>
<tr>
<td></td>
<td><strong>Expose #2: Nitride</strong></td>
<td><strong>30 X 2.0 s w/ 15 s wait</strong></td>
<td>KarlSuss, Headway</td>
</tr>
<tr>
<td></td>
<td>Develop Resist</td>
<td>Develop Manually 6X30s</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Oxide Mask Etch, Nitride Etch</td>
<td>6:1 BOE</td>
<td>WBNonmetal, WBNitride</td>
</tr>
<tr>
<td></td>
<td>30hrs of Hot Phosphoric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Spray Resist, Bake and EBR</td>
<td>20 Pass - 3.4 µm 5mm EBR + 300s Bake</td>
<td>SprayCoater, SVG Coa</td>
</tr>
<tr>
<td></td>
<td><strong>Expose #4: Silicon</strong></td>
<td><strong>3 X 1.1 s w/ 60 s wait</strong></td>
<td>KarlSuss, Headway</td>
</tr>
<tr>
<td></td>
<td>Crystal Bond, Device Si Etch</td>
<td>Crystal Bond on Backside</td>
<td></td>
</tr>
<tr>
<td></td>
<td>O2 Clean, Resist Strip</td>
<td>NANO</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O2 Plamsa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acet/Met/Isop</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>LTO Dep</td>
<td>2 X 1.5 µm LTO400PC</td>
<td>ThermcoLTO, SprayCoater</td>
</tr>
<tr>
<td></td>
<td>Spray Resist, Spin Resist on Back Bake</td>
<td>20 Pass - 3.4 µm 7 µm 220-7 10mins @ 90C</td>
<td>SVG Coat, Litho Oven</td>
</tr>
<tr>
<td></td>
<td><strong>Expose #5: Backside</strong></td>
<td><strong>4 X 2.0s w/ 10s wait</strong></td>
<td>KarlSuss, Headway</td>
</tr>
<tr>
<td></td>
<td>Pattern Back Oxide, Crystal Bond, Back Si Etch</td>
<td>6:1 BOE (15 mins) Crystal Bond on Frontside DSE FAT</td>
<td>WBFlexcorr, Headway, Deep Si Etcher</td>
</tr>
<tr>
<td>9</td>
<td>Oxide Release, Critical Point Dry</td>
<td>6:1 BOE for 20hrs Use IPA</td>
<td>WBGGeneral, CPD</td>
</tr>
</tbody>
</table>
4.3 Testing

4.3.1 Finished Devices

Five four-inch wafers of devices with electrostatic sensors and actuators were fabricated with the aforementioned processes. Figures 4.14a and 4.14b present electrical devices with silicon and silicon nitride cell adhesion pads, respectively. The white discolorations on the surface of these devices are likely polymer residues. While the gold and platinum that constitute the metal layer are impervious to most etchants, the chrome adhesion layer below them can be damaged by many chemical processes. For example, cleaning some of the wafers with piranha solution after metalization caused their surface to chip and peel. Therefore, after metalization wafers were cleaned of polymer by solvents, mild etchants like PRS1000 and PRS3000, and using plasma polymer cleans like an \( O_2 \) descum. But none of these processes were sufficient to clean all of the polymer residue deposited during the Crystal Bond wafer adhesion or during the long DRIE etches. This problem was especially severe as the long etch processes and Crystal Bond adhesion often elevated the temperature of the wafer and burned the photoresist. Burned photoresist is notoriously difficult to remove in general, but especially so when many polymer removal processes are not available. Figure 4.14c shows a device that has been adhered to a Dual In-line Package.
(DIP) with epoxy, and wire bonded to the package. The device and DIP have been placed on a breadboard to deliver actuation signals to the chip, and receive data from the sensor.

We were not able to obtain any data on the electrostatic operation of these devices by the time of this publication. All of the electrodes were electrically shorted to each other and to the handle layer. Without the ability to create a voltage difference between the electrodes it was not possible to obtain any electrostatic actuation. At the time of these experiments, even the electrostatic devices were singulated using a laser cutter. This tool mostly cut the silicon by melting it as opposed to ablating it away. The molten silicon connecting the electrodes was deemed a cause of electrical shorting. Two of the wafers that were yet to be released were diced using a wafer saw, and then released in BOE and dried in a CPD. These devices were diced using the cutting path shown in Figure 4.12

These devices were not electrically shorted.

Six four-inch wafers of the mechanically actuated devices were fabricated as well. Figures 4.15a and 4.15b present the mechanical devices with silicon and silicon nitride cell adhesion pads, respectively. Figure 4.16 presents a typical mechanical device with silicon cell adhesion pads actuated with a micromanipulator. Figure 4.17 presents a series of bright-field images of a mechanical device with nitride cell adhesion pads using an upright microscope. In each of the Figures 4.16 and 4.17 the actuation side (lower plank) has been moved to close the built-in $50 \, \mu m$ gap between the two planks in panel a, close and apply $100 \, \mu m$ of shear displacement in panel b, open the two pads by $100 \, \mu m$ in panel c, and open the pads by $100 \, \mu m$ and apply $100 \, \mu m$ of shear displacement in panel d. These images display the full range of motion of these devices, which has been accomplished.
Figure 4.16: Upright images of the testing of mechanical device with silicon cell adhesion pads in a) closing the pads, b) 100 µm of shear, c) 100 µm of tension, d) 100 µm of shear and 100 µm of tension.

using a micromanipulator.

Figure 4.18 presents bright-field images of the same device using an inverted microscope. The images have been obtained through the backside etch of the SOI wafer. As may be seen, the transparent silicon nitride pads, as well as the backside etch allow for inverted microscopy on these devices. Panels a through d of Figure 4.18 show how a micromanipulator may be used to operate this device. In Figures 4.16, 4.17, and 4.18, while a 200 µm scale bar has been added to facilitate understanding, length scales may also be observed from the indentations on the sides of the cell adhesion pads and the reference islands. The distance between the major indentations is 100 µm, and the distance between the minor indentations is 20 µm. These shapes were an important design feature of these devices, and they allowed for rapid assessments of size and distance while performing microscopy and operating the devices.
CHAPTER 4. FABRICATION AND TESTING

Figure 4.17: Upright images of the testing of mechanical device with nitride cell adhesion pads in a) closing the pads, b) 100 µm of shear, c) 100 µm of tension, d) 100 µm of shear and 100 µm of tension.

4.3.2 Biological Testing

After ensuring that the mechanical performance of the devices was as expected, we began to test their biological performance. Figure 4.19 presents how this biological testing took place. A monolayer of MDCK cells was formed on and between the two planks, and then we applied tension and shear to the epithelium to observe the behavior of the cells. The actuation was accomplished using a micromanipulator, and the movement of the sensing arm was observed to measure epithelial forces based on its deflection. The colored dashed lines at the cell edges represent the ability of these devices to perform fluorescent microscopy on living cells. The shape and setup of the device allow the user to observe the distribution of proteins within the monolayer in response to applied tension and shear. More details on the experimental setup have been presented in Section 6.2.

Figure 4.20a presents a bright-field image of an MDCK monolayer that has been formed on a device with nitride cell adhesion pads. The Vinculin molecule in these cells was tagged with a Green Fluorescent Protein (GFP), and Figure 4.20b shows the same monolayer
Figure 4.18: Inverted images of the testing of mechanical device with nitride cell adhesion pads in a) 50 μm of tension, b) closing the pads, c) 100 μm of shear, d) 100 μm of shear and 50 μm of tension.

observed through the GFP channel. These images were obtained using a 10X objective on an upright microscope. As these figures show, cells were successfully delivered to the cell adhesion pads, they adhered to the pads, and grew into a monolayer. The devices were conducive to growing an MDCK epithelium. Furthermore, we were able to perform live cell and fluorescent imaging while operating the devices. Figure 4.21a presents the same monolayer observed through a 40X water immersion objective, and using the fluorescent light source. The fluorescent light source was used because it was difficult to see the outline of cells with the regular light source. Figure 4.21b shows the same view observed through the GFP channel. We saw that the device and the setup were conducive to high magnification imaging using a water immersion objective. In addition, the fluorescent tags were clearly visible with respect to the background, which suggested that the silicon nitride surface was not autofluorescent. The silicon cell adhesion pads were not as conducive to fluorescent imaging as they reflected much of the excitation light.

Figure 4.22 presents an MDCK monolayer adhered to a mechanical device undergoing
CHAPTER 4. FABRICATION AND TESTING

Figure 4.19: A cartoon model of shear and tension applied to an epithelial monolayer. The cell-cell junctions may be tagged with fluorescent proteins to stress propagation within the sheet.

Figure 4.20: Vinculin GFP MDCKs imaged in a) bright-field and b) GFP at 10X magnification.

tensile stress. Figure 4.22a shows the two nitride cell adhesion planks closed with the initial position of the actuator, $y_{Ai}$, and the initial position of the sensor, $y_{Si}$. In Figure 4.22b, the actuator pad has been moved with the final actuator position of $y_{Af}$, and the final sensing pad position of $y_{Sf}$. In this process, the sensing pad moved by 5.28 $\mu$m. The displacement of the actuator minus the displacement of the sensor showed that we applied a 76.0 $\mu$m tensile displacement to the epithelium. As an ESY3 device, the tensile stiffness of the
Figure 4.21: Vinculin GFP MDCKs imaged in a) bright-field and b) GFP at 40X magnification using a water immersion objective.

Figure 4.22: An MDCK monolayer a) before and b) after the application of tension, and c) the tensile stress plane.

sensing arm (and the actuator arm for that matter) was 2.05 N/m. The tensile force applied to the monolayer may be calculated as 10.8 µN. This force was applied over the red plane presented in Figure 4.22c. The following equations present these calculations:
**Chapter 4. Fabrication and Testing**

Figure 4.23: An MDCK monolayer a) before and b) after the application of shear, and c) the shear stress plane.

\[
ESY3: k_{\text{Tension}} = 2.05 \frac{N}{m} \quad (4.1)
\]

\[
\text{SensorDisplacement} : y_S = y_{Sf} - y_{Si} = 5.28 \mu m \quad (4.2)
\]

\[
\text{TensileDisplacement} : y_{Af} - y_{Ai} - y_S = 76.0 \mu m \quad (4.3)
\]

\[
F_{\text{Tension}} = k_{\text{Tension}} \times y_S = 10.8 \mu N \quad (4.4)
\]

Figure 4.23 presents an MDCK epithelium undergoing shear stress using a mechanical device. Figure 4.23a shows the two planks closed with the initial position of the actuator plank, \( x_{Ai} \), and the initial position of the sensing plank, \( x_{Si} \). The final position of the actuating plank, \( x_{Af} \), and the final position of the sensing plank, \( x_{Sf} \), are shown in Figure 4.23b. The actuation plank was moved in the shear direction by 100.8 \( \mu m \), which led to a movement of the sensing plank in the shear direction of 7.70 \( \mu m \). Finding the difference between these two values allowed us to calculate that the monolayer experienced a shear displacement of 93.1 \( \mu m \). With a shear stiffness of 0.923 N/m for the ESN1 device, this sensor displacement led to a shear force of 7.11 \( \mu N \). Figure 4.23c presents the shear stress
plane in red. The equations for obtaining this value have been presented below:

\[ ESN1 : k_{Shear} = 0.923 \frac{N}{m} \]

\[ \text{SensorDisplacement} : x_s = x_{sf} - x_{si} = 7.70 \mu m \]

\[ \text{ShearDisplacement} : x_{Af} - x_{Ai} - x_s = 93.1 \mu m \]

\[ F_{Shear} = k_{Shear} \times x_s = 7.11 \mu N \]

4.4 Summary

Two series of chips have been fabricated. A series of electrical devices have been fabricated, which use electrostatic actuation and sensing. These devices require a three-layer metal coating of Cr/Pt/Au to improve conductivity, and a series of passivation trenches to separate various actuation and sensing signals in different directions. In addition, a series of mechanical devices where fabricated that used a micromanipulator for actuation, and optical sensing. The devices were fabricated using largely the same processes and masks as the electrical devices. However, they required fewer process steps, and could be built in a shorter time period.

The fabrication of the electrical devices began by the clean and anneal of highly doped SOI wafers. Passivation trenches and cell adhesion windows were patterned and etched using DRIE. A 300 nm layer of low stress nitride was deposited using LPCVD as electrical passivation and as an etch stop. The passivation trenches were filled with undoped polysilicon. The polysilicon was cleared using an isotropic SF₆ etch. A 3.6 \( \mu \)m layer of low stress nitride was deposited using LPCVD to cap the passivation trenches and to form the nitride cell adhesion windows. A 1.0 \( \mu \)m layer of LTO was deposited to act as a hard mask. The nitride windows were patterned using a hot phosphoric acid wet etch. The metal contacts were deposited and patterned using lift-off. The device layer and then the backside were patterned using DRIE. Finally, the device was released by etching the BOX layer in 6:1 BOE. The mechanical devices were fabricated using largely the same process and masks, with the exception of the trench etch, passivation, and filling, as well as the
metalization step.

Both series of devices were successfully fabricated and presented. We are still obtaining data on the electrical devices. Results for the mechanical devices show that they can move in all expected directions. The silicon devices were integrated with an upright microscope, and the silicon nitride devices were integrated with both upright and inverted microscopes. Cells adhere, spread, and form monolayers on these devices, and they can be observed using bright field and fluorescent microscopy. Experiments showed that the devices can apply high resolution forces and displacements to cell monolayers, and that these forces and displacements can be measured using the sensing side.
Chapter 5

SprayCoater

5.1 Problem Definition

The target in this project has been the conformal coverage of wafers with 30 µm and 50 µm deep features. Conformal coverage has been defined as 3 +/- 0.5 µm of resist. The variables studied in this project were:

1. Resist Mix
2. Nozzle Pressure
3. Number of Passes
4. Velocity Profile

Table 5.1 presents the process steps used to prepare samples for each SprayCoater test.

5.2 Nozzle Pressure and Resist Mix

The two variables studied for the first test were nozzle pressure and the components of the resist mixture. Two resist mixtures of SPR220-7 resist were diluted using Methyl Ethyl Ketone (MEK) and Ethyl Lactate (EL). Each was allowed to rest for 24 hours to allow for the solvents to fully dissolve the resist. The first resist mixture had a ratio of 11.4:60.6:28
Table 5.1: Process steps to prepare samples for SprayCoater

<table>
<thead>
<tr>
<th>Description</th>
<th>Parameters</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-litho Clean</td>
<td>Piranha Clean</td>
<td>WBNonmetal</td>
</tr>
<tr>
<td>Surface Treatment</td>
<td>HMDS</td>
<td>Yes Oven</td>
</tr>
<tr>
<td>Spin Resist</td>
<td>1.0 µm 3612</td>
<td>SVG Coat</td>
</tr>
<tr>
<td>Expose: Trenches</td>
<td>1.0 s</td>
<td>KarlSuss</td>
</tr>
<tr>
<td>Trench Si Etch</td>
<td>DEEP</td>
<td>STS Etch</td>
</tr>
<tr>
<td>Cleave into Pieces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-litho Clean</td>
<td>Piranha Clean</td>
<td>WBGeneral</td>
</tr>
<tr>
<td>Surface Treatment</td>
<td>HMDS</td>
<td>Yes Oven</td>
</tr>
<tr>
<td>Mount on Carrier Wafer with Kapton Tape</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Spray Resist</strong></td>
<td><strong>Test Parameters</strong></td>
<td><strong>SprayCoater</strong></td>
</tr>
<tr>
<td>Bake</td>
<td>2 mins @150C</td>
<td>Hot Plate</td>
</tr>
<tr>
<td>Metal Dep</td>
<td>15-20 nm of Au/Pd</td>
<td>Hummer</td>
</tr>
<tr>
<td>Cleave Pieces in Middle of Trenches</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Image Trenches Using SEM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(SPR220-7:MEK:EL), and due to its higher resist-to-solvent ratio it was considered the more viscous resist. The second mixture had a ratio of 8:57:35 (SPR220-7:MEK:EL), and with its lower resist-to-solvent ratio it was considered the less viscous resist. Each of these resists was sprayed at 300 mbar, 450 mbar, and 600 mbar over 30 µm and 50 µm trenches.

Table 5.2 presents a summary of the efforts in the first test. The results for the more viscous resist at various nozzle pressures and trench depths have been presented in Figure 5.1. The results for the less viscous resist at various nozzle pressures and trench depths have been presented in Figure 5.2. All images in Figure 5.1 and Figure 5.2 have been taken at a 45 degree angle to the chip surface.

The clearly visible trend for both trench depths is that wall and corner coverage improve with increasing nozzle pressures, where a nozzle pressure of 600 mbar seems to produce the most conformal step coverage. Furthermore, the less viscous resist mixture produces better results than the more viscous resist mixture. Therefore, for all future tests the less viscous resist and a nozzle pressure of 600 mbar were used.
5.3 VARYING ARM SPEEDS

Table 5.2: Nozzle Pressures and Resist Mixes Attempted

<table>
<thead>
<tr>
<th>Nozzle Pressure (mbar)</th>
<th>SPR220-7:MEK:EL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.4:60.6:28</td>
</tr>
<tr>
<td>300</td>
<td>30 &amp; 50 µm pieces</td>
</tr>
<tr>
<td>450</td>
<td>30 &amp; 50 µm pieces</td>
</tr>
<tr>
<td>600</td>
<td>30 &amp; 50 µm pieces</td>
</tr>
</tbody>
</table>

Figure 5.1: More viscous resist mixture at various nozzle pressures (Top: 30 µm Trench Bottom: 50 µm Trench / Left: 300 mbar Center: 450 mbar Right: 600 mbar)

5.3 Varying Arm Speeds

The next test conducted was one to determine the effects of swivel arm speed on resist coverage. It must be pointed out that swivel arm speed is an important determinant of how much resist is deposited per pass. Deposition rate is recorded as volume of resist per time; therefore, if the arm moves faster thus completing an entire pass in a shorter period of time a smaller volume of resist will be deposited. The deposition rate in this study was held constant between the different experiments, which led to a smaller per-pass deposition rate for the higher arm speeds.

The samples were sprayed at the original speed presented in Table 1, as well as half, twice, and triple that speed. A summary of these tests has been presented in Table 5.3.
Figure 5.2: Less viscous resist mixture at various nozzle pressures (Top: 30 µm Trench Bottom: 50 µm Trench / Left: 300 mbar Center: 450 mbar Right: 600 mbar)

Figure 5.3 presents a graphical representation of the arm speeds attempted over the position index of the arm. Figure 5.4, Figure 5.5, Figure 5.6, and Figure 5.7 present the results of the sprays at half, same, double, and triple the original speed. These figures clearly point to the fact that wall and corner coverage significantly improve with increasing arm speeds. Recognizing this fact, all subsequent tests were conducted using an arm speed triple the original speed.

<table>
<thead>
<tr>
<th>Relative Arm Speed</th>
<th>8:57:35 (SPR220-7:MEK:EL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half Original Speed</td>
<td>30 &amp; 50 µm</td>
</tr>
<tr>
<td>Original Speed</td>
<td>30 &amp; 50 µm</td>
</tr>
<tr>
<td>Double Original Speed</td>
<td>30 &amp; 50 µm</td>
</tr>
<tr>
<td>Triple Original Speed</td>
<td>30 &amp; 50 µm</td>
</tr>
</tbody>
</table>
5.3. VARYING ARM SPEEDS

Figure 5.3: Arm Speeds Attempted

Figure 5.4: Half Arm Speed (Top: 30 µm Trench  Bottom: 50 µm Trench)
5.4 Number of Passes

The fifth set of experiments showed that an arm speed set to triple the original arm speed resulted in the best coverage of the corners and walls. However, the higher speed also led to thinner resist coverage. Therefore, the sixth set of experiments was devoted to spraying the samples at the same high speed, but with more passes to ensure complete coverage of the
wafer. Instead of the original five passes, ten, fifteen, and twenty passes were attempted in this test. Table 5 presents a summary of the number of passes attempted in this experiment. Figure 5.7, Figure 5.8, and Figure 5.9 present the result of spraying the samples with ten, fifteen, and twenty passes. As expected, and as may be seen in these figures, higher number of passes corresponds with thicker resist coverage. In addition, Figure 15 shows that complete chip coverage is achieved with twenty spray passes.
5.5 Conformal Coverage and XeF$_2$ Etch Test

The variable speed profile of the swivel arm, as well as the general physical principles involved in the spraying process may lead to non-uniform thickness of resist across the wafer. For example, the spray arm is raised by 400,000 units between index positions 6 and 10 to avoid a puddle of resist in the center of the wafer, which would result from spraying
too close to the wafer surface. Therefore, it would be worthwhile to examine the thickness of resist across the wafer. A silicon wafer was sprayed with the best combination of factors as determined through the previous tests, and the resist thickness was measured using the nanospec tool. The resist thickness across the wafer had an average of 3.84 $\mu$m, with a standard deviation of 0.11 $\mu$m. This resist thickness is a very reasonable value for most applications, and it does not interfere with the function of the ASML. Furthermore, a 0.11 $\mu$m standard deviation across the wafer is an acceptable value as well.

A visual inspection of Figure 5.10 suggests that the convex corners of the trench and its floor have been completely covered with resist, and will thus protect the silicon below if placed in an etchant. However, a more definitive verification method was required to ensure the lack of pinholes in the resist. A XeF$_2$ etch test was used to verify the lack of pinholes in the resist. Samples were covered with the best combination of resist mixture, nozzle pressure, swivel arm speed, and number of passes. They were then subjected to ten cycles of XeF$_2$ etchant at thirty seconds per cycle. The results of this test have been presented in Figure 5.11. As may be seen in this figure the entire chip has been conserved, and no damage has occurred even at the corners. This coverage, therefore, is an effective combination of factors to protect the chip.

5.6 Quantifiable Analysis

The series of experiments presented so far relied on qualitative trends in the data to draw conclusions about the effects of each variable on photo-resist coverage. However, a more quantitative approach may be even more powerful in not only determining trends, but also finding the magnitude of these trends. A data digitization program such as Engauge may be used to translate the qualitative data into actual values. Figure 5.12 presents an image that has been imported and analyzed in Engauge. As may be seen, data points may be matched to the edges of the resist and the silicon chip. The digitization program then reports the position of these data points based on the scale bar in the image. Figure 5.13 presents a MATLAB plot of these data points. In other words, after exporting the data from Engauge, a further quantitative analysis of the data may be performed in a program of ones choosing.
Figure 5.11: The XeF$_2$ etch test of 30 µm (top) and 50 µm (bottom) trenches showed that none of the silicon was etched. Therefore, the wafer was fully protected by the resist and the coverage was without pinholes.

5.7 Summary

A variety of factors are important in the behavior of the EVG SprayCoater. The factors studied in these series of experiments were resist mixture, nozzle pressure, swivel arm speed, number of passes of the swivel arm, and surface pretreatment. It was shown that for a silicon surface photo-resist adhesion is not significantly improved by including an HF acid dip of the samples after cleaning them with Piranha solution. The combination of factors that produced the best corner and wall coverage was deemed to be a 8:57:35 (SPR220-7:MEK:EL) resist mixture, 600 mbar nozzle pressure, a swivel arm speed three times the speeds presented in Table 1, and 20 passes of spray. Such a recipe leads to an average resist thickness of 3.8365 µm, and a standard deviation of 0.1090 µm across the
5.7. SUMMARY

Figure 5.12: Data Points as Chosen in the Engauge Computer Program

Figure 5.13: A MATLAB plot of the Resist and Silicon Edges
wafer. In addition, spraying deep trenches with this recipe leads to no pinholes as proven by a XeF$_2$ etch test. While the qualitative methods used in this study may be very effective, an even more powerful study would include quantities measurements of the resist thickens in the trench. Such measurements may be obtained using the data digitization program Engauge, and the data may be processed in a variety of programs including MATLAB.
Chapter 6

Cell Mechanics

6.1 Introduction

Before starting any cell mechanics experiments, we had to create conditions conducive for the growth and health of our biological model system, MDCK cells. MDCK cells were grown in cell culture media of DMEM, with 10% FBS, and 1% each of Penicillin, Streptomycin, and Kanamycin (PSK). This epithelial cell line forms a two-dimensional tissue, where at confluence the cells have an area of \( \approx 400 \, \mu \text{m}^2 \) \( (20 \, \mu \text{m} \times 20 \, \mu \text{m}) \). The cells grow at 37 °C, in cell culture media with a salt content of 150 mM, and a pH of 7.4. Deviations from these values can cause the cells to change phenotype, become unhealthy, or even die. Cell respiration can cause changes in the \( \text{CO}_2 \) concentration of the cell culture media, which can in turn affect the pH level of the media. Cells must therefore be grown in an incubator with 5% \( \text{CO}_2 \) and at 37 °C. In the absence of a gaseous source of \( \text{CO}_2 \), HEPES buffer can be added to the media at a 25 mM concentration to keep the pH level constant. In addition, the incubator air must be relatively humid to avoid excessive evaporation of water from the cell culture media, which can cause the salinity of the media to increase.

For the purpose of controlling the environment of the cells, we built an incubator around the upright microscope used for these experiments. Figure 6.1 presents this incubator and microscope. The incubator was laser cut from 0.25-inch black acrylic to avoid exposing the biological samples to undesired light sources. It also had doors on the front that allowed access to the micromanipulator, sample, and stage x-y controller, as well as a door at
CHAPTER 6. CELL MECHANICS

Figure 6.1: An incubator made of black acrylic was built around the microscope to control the light and temperature level of the experiment.

The incubator had openings in the top right that allowed a cell delivery pipette to enter. The incubator had openings in the back to allow the heater to warm the air inside, and for a humidifier to humidify the environment. The water particles generated by the humidifier were deemed to be blocking the light path; therefore, we used a layer of mineral oil on top of the cell culture media to avoid evaporation. We were not able to implement a $CO_2$ feed into the chamber, so we used HEPES buffer in the cell culture media instead.

Figure 6.2 presents the setup that was used for these experiments. The micromanipulator needle, which was adjustable in three dimension, and was used to actuate the device, is on the left of this image. The micromanipulator stage has been attached to the microscope stage to avoid the introduction of extra mechanical noise due to mismatch between the two stages. The sample could be observed using bright-field or fluorescent upright microscopy with the objective at the center of this picture. The microcapillary attached to the pipette tip on the right of the image delivered the cells to the cell adhesion pads. The pipette
Figure 6.2: A needle attached to a three-axis micromanipulator was used to actuate the mechanical devices, and a pipette attached to a three-axis micromanipulator was used to deliver cells to the device. This was accomplished underneath the objective of an upright microscope.

was attached to a micromanipulator movable in three dimensions to provide fine spatial resolution of cell delivery. Further details about the experimental setup will be provided in Section 6.2.

When deposited on a clean surface under the aforementioned conditions, MDCK cells begin as spheres and gradually attach to and begin to spread on the surface. In order to spread on the surface these cells produce and lay down a layer of ECM. ECM consists of various proteins like collagen, fibronectin, and laminin, which act as a mechanical scaffold and tethering mechanism and provide biochemical cues for the cells. To speed up the adhesion and spreading of cells, one can also coat the surface with these ECM proteins. After spreading, the cells also begin to adhere to each other through tight junctions, desmosomal junctions, and adherens junctions. Desmosomal junctions and adherens junctions have been shown to be involved in mechanical signaling between cells. However, the function of these junctions is dependent on the presence of calcium ions in the cell culture media. To avoid or delay the formation of cell-cell junctions, cells can be
kept or plated in low-calcium media (LCM) for short time periods (1-2 hours). Prolonged exposure to LCM can cause the cells to become unhealthy or even die.

The cell adhesion pads have a combined area of 400,000 \( \mu \text{m}^2 \) \((2 \times 200 \mu \text{m} \times 1000 \mu \text{m})\) for the nitride pads, and a combined area of 500,000 \( \mu \text{m}^2 \) \((2 \times 250 \mu \text{m} \times 1000 \mu \text{m})\) for the silicon pads. The device has a total area of 1.55e8 \( \mu \text{m}^2 \) \((8790 \mu \text{m} \times 17620 \mu \text{m})\). Therefore, \(\approx 1000\) cells have to be delivered and adhered to an area on the device that constitutes 0.26\% of the total area. If the cells fall in any other location they can disrupt the movement of the moving parts. This is especially true if they get caught in the gap between the device layer and the handle layer (4 \( \mu \text{m} \) gap) or the gap between the interdigitated electrodes (5 \( \mu \text{m} \) gap). Therefore, to deliver the right number of cells to the correct location and operate the device properly, we need a cell-delivery method with a volumetric resolution of \(\approx 100\) nL of cell-containing media and a spatial resolution of less than 10 \( \mu \text{m} \).

### 6.2 Experimental Setup

#### 6.2.1 Hardware Preparation

To prepare the tip we used a razor blade to cut the tip off of a 10 \( \mu \text{L} \) pipette tip. The filter was removed from the pipette tip. We broke a (6-inch) 5 \( \mu \text{L} \) capillary in half, and inserted the broken tip of the capillary tube just inside the cut end of the pipette tip. A thin strip of parafilm was wrapped around the overlap in a way that avoided any air (vacuum) leakage.

To prepare the device holder we used a 35 mm cell culture dish. A stainless steel paperclip was cut into a V-shape to act as a device holder. We used 5-minute epoxy to adhere the holder to the bottom of the cell culture dish. The epoxy was allowed to rest overnight so that it fully cured, and it would not leak any chemicals into the media afterwards. We left 70\% ethanol in dish for five minutes to disinfect. The apparatus was washed three times in PBS. We left PBS in the dish for five minutes to absorb any chemicals leaking from epoxy. The media was left in the dish for five minutes to absorb any chemicals leaking from the epoxy. We changed media in the dish to media desired for cell seeding.
6.2. EXPERIMENTAL SETUP

Figure 6.3: E-cadherin Red MDCK cells on a nitride cell adhesion pad observed using a 63X water immersion objective. The cells spread well and formed normal cell-cell junctions.

6.2.2 Device Preparation

For a new device, before preparing the device for cell delivery, the device had to first be wetted in a solvent. If this first step was not taken, the high surface tension of water would break the device. We used 70% ethanol to wet the device, as it was the most commonly available solvent in the lab. However, isopropanol, methanol, acetone, pure ethanol, or other solvents would have also worked.

Over many experiments we realized that the most common cause of cells not adhering or spreading on the cell adhesion pads of already-used devices was that the device was not fully cleaned since the previous use. Therefore, we incubated already-used devices in 2 µg/mL of collagenase for 30 minutes at 37 °C (possibly in an incubator). Collagenase is an enzyme that cleaves the collagen ECM. It will lift off any adherent cells and clear the surface of the ECM. When devices are cleaned completely, the cells adhere and spread well,
and they form a healthy monolayer. Figure 6.3 presents MDCK cells where the E-cadherin was tagged with DsRed fluorescent molecules (E-cadherin Red MDCKs). As may be seen, the epithelium looks healthy and the E-cadherin has been recruited to cell-cell junctions.

To prepare the device for cell delivery we removed organics by incubating device in sodium hypochlorite (bleach) for 48 hours at room temperature. We washed device three times in 70% ethanol. It was disinfected by incubation in 70% ethanol for 5 minutes. We washed the device three times in 0.01% acetic acid. The chip was incubated for one hour at room temperature in 50 µg/mL of type I collagen in 0.01% acetic acid. We washed it once in PBS. We placed the device under the holder in a 35 mm dish with regular cell culture media with a 25 mM concentration of HEPES buffer.

6.2.3 Cell Placement

To load cells onto the cell-delivery pipette we grew cells to 40-50% confluence in a petri dish. At this level of confluency the cells can more easily be removed from the dish and individuated into single cells. The cells were incubated in 0.05% trypsin in EDTA for five minutes to remove them from the petri dish. We made sure the cells were individuated by pipetting them with trypsin on the dish three times. LCM was added to the dish at a 1:1 media to trypsin ratio. We spun the cells down in a centrifuge to form a pellet. We resuspended the cells in LCM and used a cell counter to form a solution of one million cells/mL. A P10 micropipette was set to 5 µL, we and drew 2 µL of cell-containing media by rotating the plunger slowly. The tip was withdrawn from the cell solution and we drew 2 µL of air by rotating the plunger to move the cells further up the capillary. We washed the capillary tip in cell culture media to wash off any extra cells stuck to the capillary walls.
6.2. EXPERIMENTAL SETUP

Figure 6.5: MDCK cells spreading and forming a monolayer over five hours. From left to right the bright-field images were taken 0.5 hours, 3 hours, and 5.5 hours after seeding.

at the tip. The last two steps ensure that cells would not begin to exit the capillary as soon as the capillary tip enters the media over the device. In this way, the cells would only be ejected when the tip is placed over the cell adhesion pads and the plunger screw is turned. Figure 6.4 presents the size of the cell delivery micro-capillary and its movement with respect to the cell adhesion planks. The three-axis micromanipulator allowed us to control the position of the cell delivery to within a few microns in all spatial dimensions, and we were able to deliver volumes in the tens of nanoliters.

To place the cells onto cell adhesion pads we focused on the device under the upright microscope with a 5X objective. We brought the two (250 µm × 1000 µm) cell adhesion pads together to form a single (500 µm × 1000 µm) cell adhesion pad. The P10 micro-pipette with the specially-made micro-pipette tip was firmly attached to the cell-delivery µ-manipulator apparatus. The capillary tip was lowered through the air-media barrier and placed it just over the cell-adhesion pads. We rotated the micro-pipette plunger at a rate of roughly 0.1 µL/s to release cells onto the cell-adhesion pads. The tip was moved over the pads while releasing cells to cover the entire area with cells. We allowed the cells to rest for a few minutes before removing the tip, so that the turbulence caused by the movement of the tip did not disturb the cells. Figure 6.5 presents how individual cells adhere to the pads, spread, attach to each other, and spread over time. From left to right, these images were taken 0.5, 3, and 5.5 hours after the cell deposition. As may be seen, the environment of the microscope incubator is conducive to normal MDCK cell function.
Figure 6.6: The epithelium was sheared 100 µm. The zoomed-in region shows how cells at the boundary of the two cell adhesion pads are deformed in shear. The cartoon further highlights this deformation.

6.3 Cell Migration Experiments

The response of cells to tensile stress has been a well-studied subject. Epithelial response to shear stress has not been studied much before. Tambe et al. used TFM and a simplified mechanics model to approximate normal and shear stresses within an epithelial monolayer. Combining this information with data from the movement of cells within the monolayer they claimed that cells “migrate along orientations of minimal intercellular shear stress.” They coined the term “plithotaxis” to describe the movement of cells in the direction of maximum principal stress. Zaritsky et al. used the same data and similar analysis techniques to better understand how stress within the monolayer affects collective migration. They used a very indirect analysis to find what they termed “shear-strain events”: instances where shear stress is generated by leader cells at the boundary of a monolayer. They postulated that these “shear-strain events” coordinate cell movement deeper within the monolayer. These studies made an attempt to understand the effects of shear stress on cell migration within a monolayer, but they never had a direct way of applying and quantifying shear stress. In addition, especially in the case of Zaritsky, the claim of having observed a response due to shear strain using such methods is rather dubious. This device gives us the opportunity to apply stresses in tension and shear to an epithelial monolayer directly, and to quantify the stress.
6.3. CELL MIGRATION EXPERIMENTS

Figure 6.7: The epithelium is shown at time points before and after shear. The zoomed-in sections show the deformation of the cells at the boundary of the cell adhesion pads. The PIV analysis is generated by comparing the position of the same region between two consecutive time points.
6.3.1 Particle Image Velocimetry

We performed a series of experiments to determine the effects of shear stress on collective cell migration within a monolayer. E-cadherin Red MDCKs were used in these experiments. Cells were deposited, allowed to adhere, and grew for 18 hours prior to the start of experiments. The micromanipulator was used to move the actuation pad by 100 µm in the shear direction. Figure 6.6 presents a before and after image for this step function in actuation. The shear deformation of the cells near the stress plane is visible in these bright-field images. The cartoon in this figure more explicitly demonstrates this phenomenon. Cells were observed for one hour before and six hours after the shear stress was induced, and they were imaged using bright-field microscopy at 5 minute intervals during this time period.

The left column in Figure 6.7 presents images of the monolayer at time points before and after the application of shear displacement. As may be seen in these images there is a significant change in the morphology of the cells near the shear plane after the initiation of actuation. In addition, the cells in the monolayer seem to begin directed movement shortly after shear force is applied. The center column in Figure 6.7 shows a zoom-in on the morphology and movement of the cells near the shear plane at these time points. The shear deformation of these cells reduces over time. The right column in Figure 6.7 shows a Particle Image Velocimetry (PIV) analysis of the movements within the monolayer. Each PIV image compares the position of patches (smaller than one cell) of the monolayer from one time point to the next. The magnitude and direction of each vector in these images represents the magnitude and direction of the velocity of each patch. The PIV analysis was performed using the open source program PIVlab, which runs in MATLAB. PIVlab produced a series of PIV analysis images, where each image consisted of a field of velocity vectors. Overall, each field represented the collective migration of cells within the monolayer between two time points.
6.3. CELL MIGRATION EXPERIMENTS

Figure 6.8: The data generated by PIVlab is a vector field where the position, magnitude, and direction of each velocity vector is specified. The angles of these vectors with respect to the horizontal may be calculated from this data.

6.3.2 MDCK Response to Shear Stress

Figure 6.8 presents a cartoon of one of these velocity fields. Knowing the magnitude, direction, and position of each vector within these fields allowed us to analyze the bulk behavior of the monolayer spatially and temporally. Knowing the position of each vector allowed us to separate the data for the top and bottom planks. In addition, information about the direction of the velocity vectors allowed us to calculate the angle of each vector with respect to the horizontal axis. The angles of the velocity vectors in the top and bottom planks could then be plotted in separate rose plots for each plank. A rose plot is an angular histogram. This plot divides the 360-degree perimeter of a circle into smaller bins, where the height of each bin signifies the frequency of observation for angles in the range specified by the bin. A taller bin signifies more frequent observations in that angular range, and a shorter bin signifies less frequent observations in that range.

Figure 6.9 presents rose plots at different time ranges for an epithelium undergoing a
Figure 6.9: MDCK cells in the epithelium move towards the stress plane in response to shear stress.
6.3. CELL MIGRATION EXPERIMENTS

shear experiment. These time ranges are 15 minutes long each, and they include one 15-minute time range before the application of shear, and three 15-minute time ranges after the application of shear. The rose plots have been generated from the results of PIVlab, and they separate the results for the top and bottom planks. As may be seen, in the 15 minutes before the application of shear there is no significant difference in the direction of movement of cells in the top or bottom planks. In addition, the movement of cells in either plank does not display a collective and directed behavior. After the application of shear, within 15 to 30 minutes, the epithelium on the top plank begins to change direction downwards, and the epithelium in the bottom plank begins to shows a collective migration upwards. By 30 to 45 minutes after the application of shear, the epithelium on the top plank is decidedly moving downwards with a narrow spread in the range of downward angles. Furthermore, the cells in the epithelium on the bottom plank have begun to move upwards collectively. This collective and directed migration continues even after the 45-minute time point.

Figure 6.10 presents rose plots at different time ranges for an epithelium undergoing a shear experiment in the presence of blebbistatin. Blebbistatin is a pharmacological treatment that inhibits the action of myosin II muscle molecules, and that is how it disrupts the actomyosin contractility of the cells. The blebbistatin was added seconds before the shear displacement was applied, to achieve a concentration of 50 µM. As may be seen in Figure 6.10 the epithelium on either plank does not display any collective directed migration before the application of shear and blebbistatin. This behavior matches the trend in the earlier experiment presented in Figure 6.9. Blebbistatin begins to inhibit myosin II contractility within minutes. However, as may be seen in the time frames after shear, the epithelium on either plank does not display any directed migration even after the application of strain.

Figure 6.11 presents the force borne by the a wild type (WT) MDCK epithelium treated with blebbistatin in the minutes after the application of shear. In this experiment, the treatment was added one hour before shear, immediately before shear, and each hour after the initiation of shear force. As may be seen in this figure, the force reduces to zero within minutes of the application of shear. An ESN1 device was used for this experiment, and Equation 4.8 was used to calculate the shear force at each time point. The force relaxation
Figure 6.10: MDCK cells treated with blebbistatin do not migrate in a collective way in response to shear stress.
of the epithelium in shear shows a viscoelastic behavior.

Other than the MDCK E-cadherin Red cells used in the first two sets of experiments, a second cell line was tested in shear as well. These cells were zonula occludens-1 and -2 knockdown (ZO-1/2 KD) MDCKs. The tight junctions in this cell line have been disrupted by the knockdown. Figure 6.12 presents rose plots at different time ranges for an epithelium of ZO-1/2 KD MDCKs undergoing a shear experiment. The figure shows the behavior of the cells 15 minutes before and 45 minutes after the initiation of shear. As may be seen, the cells do not migrate in a directed way in response to stress.

The results presented in Figures 6.9, 6.10, and 6.12, which are typical of several such experiments carried out, have four important implications. First, an epithelium responds to shear stress by initiating directed cell migration. Second, this directed migration of the cells in the epithelium is towards the shear plane. Third, this response is an active biological response as opposed to a passive material one. Fourth, normally functioning tight junctions are required for this response.
Figure 6.12: ZO-1 KD MDCK cells do not migrate in a collective way in response to shear stress.
The third conclusion was drawn from differences in the behavior of the cells in the absence or presence of blebbistatin. From a purely material standpoint, cells are bundles of polymers. In fact, Fernandez et al. go through great lengths to show that the strain stiffening of fibroblasts in tension is no different than the response of any other polymer. However, unlike a piece of plastic, they have been shown to sense the mechanical properties of their environment, and to actively respond to this information. If the epithelium showed the same response whether or not blebbistatin was present, the observed movement had to be purely material. However, since the presence of the force inducing myosin II activity was required for the collective migration, we could conclude that the behavior took place as the cells sensed the shear and responded to it. The fourth conclusion was based on the behavior of the ZO-1/2 KD cells.

### 6.4 Summary

MDCK cells were grown in DMEM with 10% FBS and 1% PSK. The cell culture dishes were kept in a humid incubator at 37 °C and 5% CO₂. They were deposited on the silicon devices where they were subjected to mechanical stresses under a microscope. An incubator was built around the microscope to keep the temperature at 37 °C, and to block any undesired light from reaching the sample. HEPES buffer was added to the media to avoid pH changes due to a lack of 5% CO₂. The open culture system was covered with mineral oil to avoid water evaporation, which could lead to increased salinity of the media. A needle attached to a three-axis micromanipulator was used to actuate the device, and force sensing was accomplished by observing the displacement of the sensing plank. A separate three-axis micromanipulator was used to guide the tip of a micropipette over the cell adhesion pads, which allowed for precise spatial control of cell delivery.

A 10 µL pipette tip and a glass capillary were combined and attached to a P10 pipette to form a cell delivery mechanism with a volumetric resolution of ≈ 100 nL. When attached to the three-axis micromanipulator, we had a tool capable of delivering 1000 cells to a 1000 µm × 500 µm cell adhesion region, representing 0.26% of the total area of the chip. A mechanism to hold the device stationary in the 35 mm cell culture dish was developed by using epoxy to attach a paper clip to the dish. The device was cleaned with bleach,
disinfected with 70% ethanol, and coated with Collagen I. This process allowed the MDCK cells to adhere and spread well on the cell adhesion pads. The fine resolution of cell delivery was allowed by rotating (as opposed to squeezing) the pipette plunger. Cells were allowed to grow for 18 hours before the initiation of mechanical shear and tension experiments.

While quite a few studies have looked at the response of cells to tensile stress, very few have looked at their response in shear. We used this device to apply 100 μm of shear displacement to an epithelium and observe its response using PIV. PIV tracks the movement of different pixel patterns through sequential time points. Rose plots were used to help us better quantify the bulk behavior of the different regions of the epithelium. The PIV analysis showed that within 15-30 minutes of the application of shear, the cells in the top plank moved down towards the shear plane, and cells in the bottom plank moved up towards the shear plane. This result held across multiple experiments. We repeated this experiment in the presence of 50 μM of blebbistatin, a pharmacological treatment that inhibits myosin II activity. Under these conditions no collective migration was observed. These results showed that the cells in an epithelium migrate towards the stress plane in response to shear stress, and that this movement is an active biological response. Finally, ZO-1/2 KD cells did not exhibit a migratory response, which shows that normally functioning tight junctions are required for this behavior.
Chapter 7

Conclusions and Future Work

A new device has been designed, fabricated, and tested with the ability to apply and sense tension and shear to a monolayer of epithelial cells. This silicon MEMS device allows for upright and inverted live cell microscopy, including fluorescent imaging. The design criteria for this work were presented in Chapter 1. Chapter 2 began with a motivation for this effort. It gave a short background on the model systems that this device could be used to study. It went on to describe polymer, glass, and silicon devices that have been built in the past for mechanobiology studies. Chapter 3 described the design principles of the device. It began by discussing the design of the cell adhesion pads. The designs of the folded flexure springs, as well as the electrostatic actuators and sensors were presented next. Chapter 4 focused on the fabrication. The fabrication of the electrical devices was described first, and the fabrication process for the mechanical devices was based on the earlier description. This chapter ended with a presentation of the mechanical and biological testing results.

Chapter 5 presented the work done to characterize the performance of the SprayCoater. The SprayCoater characteristics studied ranged from the nozzle pressure and arm speed, to resist mix and number of spray passes. Chapter 6 presented the cell mechanics experiments. The chapter first covered the conditions necessary for growing epithelial cells. It then presented the experimental protocols for these tests. Finally, the shear response of epithelial monolayers in the absence or presence of blebbistatin, as well the response of ZO-1/2 KD cells, were discussed. This work encompassed a relatively wide scope in fields as diverse as silicon MEMS fabrication to mechanobiology. A device that meets these
design specification is an important addition to the tools already existing in the field. The development of a device that can apply and directly sense shear in epithelia has not been accomplished before, and it allows for many new experiments. Scientific and research work is certainly never completed. The following sections present some new directions that this work could take.

7.1 Improving Inverted Microscopy

While the version of this device with the nitride cell adhesion pads does allow for inverted microscopy, this ability is limited by two main factors. To use these devices for inverted microscopy, the light has to travel through 4 $\mu$m of silicon nitride, the 4 $\mu$m gap of the BOX layer, the 471 $\mu$m thickness of the handle layer, and then through the 175 $\mu$m-thick glass bottom of a MatTek dish. At more than 650 $\mu$m, this length is greater than the working distance of most high magnification inverted microscope objectives. In addition, while the silicon nitride layer is transparent, it is not nearly as optically conducive as a glass substrate. These features place significant hurdles in the use of these devices for high magnification inverted microscopy. Imagining the use of such devices for more sophisticated forms of microscopy such as FRET or FRAP becomes even more difficult.

The issue of the working distance limits may be significantly improved by using a thinner handle layer. A handle layer of 300 $\mu$m would be a major improvement. Such a reduction would certainly make the wafers more fragile. However, the fabrication process has already been optimized for thick device layers, and some of the mechanical integrity may be regained by using an even thicker device layer.

The shortcomings in the optical properties of silicon nitride are another matter. Silicon nitride was used for the cell adhesion pads as it provided stiffness, transparency, and selectivity to silicon dioxide during the long BOX release process. A simple solution to this problem may be to simply invert the nitride and oxide steps. More specifically, if the sacrificial layer between the device and handle layers is composed of silicon nitride, then the cell adhesion pads may be composed of silicon dioxide (glass). To release the devices then, we would have to etch the nitride through a wet process, or a dry backside etch step. If choosing to use a thinner device layer ($\leq 10 \mu$m), this layer may be in the form
of polysilicon deposited on a wafer covered with silicon nitride. If choosing to use a thicker device layer, we may have to perform wafer bonding in the lab.

7.2 Dynamic Microwells

As may be seen in the cross-sectional view of the nitride cell adhesion pads presented in Figure 3.2c, these structures form shallow wells. In this case, the depth of these wells is as thick as the device layer, or 50 µm. Microwells are tools with important bioengineering applications, including a role in the differentiation of cardiac cells from hESCs. [110–114] The devices presented in this thesis may be used as dynamic microwells. In such a use, they would ability to apply tension and shear to the cells within the microwell, and also sense forces in these dimensions. The transparent nature of these structures would have added benefits for microscopy as well. In addition, grooved surfaces have been shown to increase forces generated by cardiomyocytes. [115] Adding grooves to the silicon or nitride (using the SprayCoater) cell adhesion pads can provide a tool for exercising or testing cardiomyocytes in tension and shear on a grooved surface.

7.3 Tracking of Nuclei or Outlines

While PIV has proven to be a powerful tool in analyzing collective cell migration in this project, it may not be the ideal method to quantify this phenomenon. PIV works by correlating visual features between consecutive images. For us, this correlation serves as a proxy for cell movement. However, tracking the movement of actual cells would not only be more accurate, but also it would provide us with more detailed information about the behavior of the epithelium. Doing so may be possible by tracking individual cell nuclei or outlines. Hoechst staining of the nuclei would be helpful in nuclear tracking. The use of cell lines with fluorescent junctional proteins, like E-cadherin Red MDCK cells, could ease the tracking of cell outlines. Quantifying the deformations of cell boundaries would have the added benefit of giving us insight into some of the effects presented in Figures 6.6 and 6.7. These figures show how cells at the boundary of the cell adhesion pads deform in shear after the initiation of stress, and then relax and regain their normal shape over time.
Quantifying the changes in cell shape will give us insight into the stress relaxation of the epithelium over time.

### 7.4 Force Propagation in Epithelium

This device allows for stresses and strains to be applied to entire monolayers, instead of one or a few cells. This feature is an important capability that has not been significantly taken advantage of yet. For example, we can apply shear and tension at the junction between the two cell adhesion pads. However, we need to develop ways of determining how this stress propagates spatially and temporally within the monolayer. Observing the migration of the different regions of cells is an indirect way of studying this phenomenon, but it does not lead to quantifiable forces. Using cells with FRET-based tension sensors or adding TFM to the cell adhesion pads would be helpful steps in this process.

Grashoff and Borghi used FRET-based tension sensors placed in vinculin and E-cadherin molecules, respectively. [13, 29] Using cell lines stably transected with these units would allow us to quantify forces across the epithelium from the force measurements at focal adhesions or adherens junctions. These readings can then be related back to the applied force, or the collective migration of the cells.

Furthermore, we can increase the capabilities of this tool by combining this device with the methods of Tambe and Zaritsky. [8, 105] However, doing so would require adding a TFM component to this device. Considering the fact that such a component would have to be made of a soft polymer like a hydrogel, adding this step to a fabrication run sheet full of harsh acids and high temperatures seems impossible. Adding hydrogels to a released device without breaking it also poses significant challenges. Dip Pen Nanolithography (DPN) may be used to deposit a deformable substrate on released devices. Alternatively, it may be possible to use a heat-sensitive polymer like PNIPAAm and a micromanipulator to attach small hydrogels sheets to the cell adhesion pads. The hydrogels would have to be removed after the experiment to allow the device to be fully cleaned for the next experiment. A new hydrogel could be reattached before the next experiment.
7.5 Finding the Mechanism

Thus far we have shown the response of epithelial cells to shear stress, and the fact that this is a biological response. However, we have not been able to determine the mechanisms and signaling cascades that underlie this effect. An important first step would be to continue these experiments with available mutants such as T151 cells, which lack the extracellular domain of E-cadherin. Cell lines like this, with disruptions in the structure and function of their junctional proteins, will begin to shed light on the mechanisms involved in shear response. In the long term, to truly probe the questions of mechanism, we have to find a way to perform immunofluorescence staining, or Western blots on these epithelia. With these added tools in hand, we can begin to probe the up or downregulation of specific proteins believed to be involved in the shear sensing and response.
## Appendix A

### Glossary of Mathematical Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{INT}$</td>
<td>Interface Capacitance of Electrodes</td>
<td>F/m²</td>
</tr>
<tr>
<td>$C$</td>
<td>Capacitance</td>
<td>F</td>
</tr>
<tr>
<td>$d$</td>
<td>Gap Between Electrodes</td>
<td>m</td>
</tr>
<tr>
<td>$\varepsilon_0$</td>
<td>Permittivity of Free Space</td>
<td>F/m</td>
</tr>
<tr>
<td>$\varepsilon_r$</td>
<td>Relative Permittivity of Media</td>
<td>–</td>
</tr>
<tr>
<td>$f_c$</td>
<td>Crossover Frequency</td>
<td>Hz</td>
</tr>
<tr>
<td>$f(\omega)$</td>
<td>Captures Frequency Dependence of Impedances</td>
<td>–</td>
</tr>
<tr>
<td>$F_{elec}$</td>
<td>Electrical Force from Electrostatic Actuators</td>
<td>N</td>
</tr>
<tr>
<td>$F_{mech}$</td>
<td>Mechanical Force from Springs</td>
<td>N</td>
</tr>
<tr>
<td>$k$</td>
<td>Spring Constant</td>
<td>N/m</td>
</tr>
<tr>
<td>$L$</td>
<td>Length</td>
<td>m</td>
</tr>
<tr>
<td>$N$</td>
<td>Number of Finger Pairs</td>
<td>–</td>
</tr>
<tr>
<td>$R_M$</td>
<td>Resistance of Electrode Gap</td>
<td>$\Omega \cdot m^2$</td>
</tr>
<tr>
<td>$t$</td>
<td>Device Thickness</td>
<td>m</td>
</tr>
<tr>
<td>$V$</td>
<td>Voltage</td>
<td>V</td>
</tr>
<tr>
<td>$V_{RMS}$</td>
<td>Root-Mean-Square Voltage</td>
<td>V</td>
</tr>
<tr>
<td>$w$</td>
<td>Width</td>
<td>m</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular Frequency</td>
<td>rad/s</td>
</tr>
<tr>
<td>$x$</td>
<td>Displacement</td>
<td>m</td>
</tr>
</tbody>
</table>
Appendix B

Mask Layouts

Figures B.1, B.2, B.3, B.4, B.5 present the five masks used to fabricate the electrical devices with silicon cell adhesion pads. Figure B.6 presents how these masks overlay each other. Figures B.7, B.8, B.9, B.10, B.11 present the five masks used to fabricate the electrical devices with silicon cell adhesion pads. Figure B.12 presents how these masks overlay each other. The mechanical devices are fabricated using the same masks but with two changes. First, the trenches, but not the cell adhesion wells, in the Trenches mask are covered using Kapton tape. Second, they do not require metal, so they do not use the Metal2 mask.
Figure B.1: ESN Device Trenches Mask
Figure B.2: ESN Device Nitride Mask
Figure B.5: ESN Device Backside Mask
Figure B.6: ESN Device All Masks
Figure B.7: ESY Device Trenches Mask
Figure B.8: ESY Device Nitride Mask
Figure B.9: ESY Device Metal2 Mask
Figure B.10: ESY Device Silicon Mask
Figure B.11: ESY Device Backside Mask
Appendix C

PIVlab Workflow

The open source program PIVlab was used to generate the PIV data. This program is an application within MATLAB. It was used to process the *.bmp image files, and exported its analysis as distributions of velocity vectors in *.csv files. This output was then run through a MATLAB code for further processing and plotting.

1. Open PIVlab within MATLAB

2. Click on “Load images”
   
   (a) An image selection window will open

3. Choose “Sequencing style 1-2, 2-3, 3-4, ...”

4. Use the browser button to go to the desired directory

5. Use the *.bmp extension for your images

6. Highlight desired images

7. Click on “Add” button

8. Ensure all desired images are in the “Selected Files” bin

9. Click on “Import”
(a) The image selection window will be closed and the PIVlab home screen will be highlighted

(b) The “Image” bin shows the images that were just imported

10. Under “Analyses settings” click on “PIV Settings”

11. Under “PIV algorithm” choose “FFT”

12. Make sure the box for “Pass 2” is checked, and “Pass 3” and “Pass 4” boxes are not checked

13. In “Pass 1” use an interrogation window of 64 and step size of 32

14. In “Pass 2” use an interrogation window of 32 and step size of 16

15. For “Window deformation” use the default “linear” option

16. For “Sub-pixel” choose the default “Gauss 2x3-point” option

17. Under “Analysis” click on “Analyze!”

18. Choose “Analyze all frames”

   (a) Depending on the number and size of images, this may take some time to run

19. After run is complete, under “Post Processing” click on “Vector validation”

20. Under “Vector validation” use the default checked box of “Stdev filter Threshold” and enter a value of 7

   (a) The “Local median Threshold” remains unchecked and has a value of 5

   (b) Epsilon has a value of 0.1

21. Uncheck the box next to the “Interpolate” option

22. Click on “Apply to all frames”

   (a) This step removes vectors that are outliers
23. Under “File”, under “Save”, click on “ASCII-file (x,y,u,vort)”

24. Under “Export as ASCII chart” use the default “comma” option for “Delimit”

25. Click on “Export all frames”

   (a) A browser will open

26. Choose the correct folder

27. Under “Format” choose “All Files” as opposed to the default “*.txt” format

28. Save as your preferred file name, and make sure to include *.csv at the end of the file name

   (a) The MATLAB code used to process this data imports the *.csv file format

29. This data can now be imported and analyzed in MATLAB
Appendix D

MATLAB Code

The code used to quantify the movement of the sensing arm, which is a measure of the stress relaxation in the monolayer, has been presented in Section D.1. This code relies on the function in Section D.2. The code used to divide the monolayer into tiles, and create rose plots from files generated by PIVlab has been included in Section D.3.
D.1 StressRelaxation.m

% StressRelaxation.m
% Written by: Ehsan Sadeghipour & Miguel Garcia
% Date: 2/29/2016
% Measures the stress relaxation of a sheared monolayer
% over time by observing the movement of the sensor.
% It also outputs the movement of the actuator.
% This file calls corrFunction.m
% Benefited from the code of Vikram Mukundan from PhD Thesis

clear all; close all; clc;

% Pixel conversion (for Leica 10x, 1.0x)
pxl2mic = 870.0/1344;  %Guesstimate for 10X
                      %(1344 Pixels were ~870 um)

%Parameters

time_interval = 1/3;  %Minutes per image

% Stiffness of different device designs
% This may be used to output force instead of displacement
k1 = 0.923;  %N/m for ESN1/ESY1
k2 = 1.31;   %N/m for ESN2/ESY2
k3 = 1.80;   %N/m for ESN3/ESY3

%Obtain file path for series of images and image range
%Images must be sequential or loop will fail
Fpath=uigetdir();
n_0=input('No. of reference file?');
nl=input('No. of last file?');
Correct name of file paths that are too short

```matlab
if n_0 < 10
    file_path_a = strcat(Fpath,'/0',num2str(n_0),'.bmp');
else
    file_path_a = strcat(Fpath,'/',num2str(n_0),'.bmp');
end
```

Opens first file to let user pick upper left and lower right hand corners of regions of interest on actuator, sensor, and the stationary island: six points total.

```matlab
a = imread(file_path_a);
a = imadjust(a);
figure, imshow(a);
[x1,y1] = ginput(2);
[x2,y2] = ginput(2);
[x3,y3] = ginput(2);
```

Displacements between each image and the original image

```matlab
Disp(1:(nl-n_0),4)=0;
Corr_i(1:4) = 0;
```

```matlab
for i=n_0:nl
    [Corr_i(1), Corr_i(2), Corr_i(3), Corr_i(4)] = ... corrFunction(Fpath,n_0,i,x1,y1,x2,y2,x3,y3);
    Disp(i-n_0+1,1:4) = Corr_i(1:4);
end
```

Plotting the relaxation
mic_disp = -pxl2mic * Disp;
t = 0:time_interval:time_interval*(nl-n_0);
figure
plot(t, mic_disp(:,1), '*', t, mic_disp(:,3), '+');
xlabel('Time Point (min)');
ylabel('Displacement (\mu m)');
axis([t(1) t(end) 0 100])
grid
%CorrFunction.m
%Written by: Ehsan Sadeghipour & Miguel Garcia
%Date: 3/1/2016
%Finds x and y actuation and sensing using
%cross-correlation and adjusts for possible vibrations
%by subtracting movement of stationary items

%Corrects for length of file name
if n_0 < 10
    filepath_a=strcat(Fpath,'/0',num2str(n_0),'.bmp');
else
    filepath_a=strcat(Fpath,'/',num2str(n_0),'.bmp');
end

if n_i < 10
    filepath_b=strcat(Fpath,'/0',num2str(n_i),'.bmp');
else
    filepath_b=strcat(Fpath,'/',num2str(n_i),'.bmp');
end

%Reads reference image and image of interest
a = imread(filepath_a);
b = imread(filepath_b);

function [act_x, act_y, sens_x, sens_y] = ...
    corrFunction(Fpath, n_0, n_i, x1, y1, x2, y2, x3, y3)
%Crops to ROI
aw1 = imcrop(a,[x1(1) y1(1) x1(2)-x1(1) y1(2)-y1(1)]);
bw1 = imcrop(b,[x1(1) y1(1) x1(2)-x1(1) y1(2)-y1(1)]);
aw2 = imcrop(a,[x2(1) y2(1) x2(2)-x2(1) y2(2)-y2(1)]);
bw2 = imcrop(b,[x2(1) y2(1) x2(2)-x2(1) y2(2)-y2(1)]);
aw3 = imcrop(a,[x3(1) y3(1) x3(2)-x3(1) y3(2)-y3(1)]);
bw3 = imcrop(b,[x3(1) y3(1) x3(2)-x3(1) y3(2)-y3(1)]);

%Cross-correlation for actuator
cca1 = normxcorr2(aw1,a);
[ypeaka1, xpeaka1] = find(cca1==max(cca1(:)));
corr_offset_a1 = ...
    [(ypeaka1-size(aw1,1)) (xpeaka1-size(aw1,2))];
ccb1 = normxcorr2(bw1,a);
[ypeakb1, xpeakb1] = find(ccb1==max(ccb1(:)));
corr_offset_b1 = ...
    [(ypeakb1-size(bw1,1)) (xpeakb1-size(bw1,2))];

%Cross-correlation for sensor
cca2 = normxcorr2(aw2,a);
[ypeaka2, xpeaka2] = find(cca2==max(cca2(:)));
corr_offset_a2 = ...
    [(ypeaka2-size(aw2,1)) (xpeaka2-size(aw2,2))];
ccb2 = normxcorr2(bw2,a);
[ypeakb2, xpeakb2] = find(ccb2==max(ccb2(:)));
corr_offset_b2 = ...
    [(ypeakb2-size(bw2,1)) (xpeakb2-size(bw2,2))];

%Cross-correlation for stationary island
cca3 = normxcorr2(aw3,a);
[ypeaka3, xpeaka3] = find(cca3==max(cca3(:)));
corr_offset_a3 = ... 
   [(ypeaka3−size(aw3,1)) (xpeaka3−size(aw3,2))];
ccb3 = normxcorr2(bw3,a);
[ypeakb3, xpeakb3] = find(ccb3==max(ccb3(:)));
corr_offset_b3 = ...
   [(ypeakb3−size(bw3,1)) (xpeakb3−size(bw3,2))];

%Aadust values to correct for vibrations
x_correction = corr_offset_a3(2) − corr_offset_b3(2);
y_correction = corr_offset_a3(1) − corr_offset_b3(1);

%x&y movement of actuator and x&y movement of sensor
act_x = (corr_offset_a1(2) − corr_offset_b1(2)) − ...
x_correction;
act_y = (corr_offset_a1(1) − corr_offset_b1(1)) − ...
y_correction;
sens_x = (corr_offset_a2(2) − corr_offset_b2(2)) − ...
x_correction;
sens_y = (corr_offset_a2(1) − corr_offset_b2(1)) − ...
y_correction;

end
D.3 PlotPIVwithTiles.m

%PlotPIVwithTiles.m
%Created on: 9/23/15
%Created by: Ehsan Sadeghipour & Miguel Garcia
%This program takes a folder of *.csv files from PIVlab.
%The data, which represents an entire monolayer, is broken
%into an arbitrary number of regions (in rows and columns)
%and then plotted as rose plots.

clear all; close all; clc;

%If changing rose_rlim also manually correct axis tick marks
rose_rlim = 1000;  %Radius of rose plots
rose_bins = 20; %Number of bins for rose plots
y_strips = 2;  %Arbitrary number of horizontal strips
x_cols = 3; %Arbitrary number of vertical strips
y_position(1:y_strips+1) = 0;
x_position(1:x_cols+1) = 0;

%The directory of *.csv files is picked
scrdir=uigetdir();
cd(scrdir)
alist=dir(fullfile(scrdir,['*.csv']));
disp(length(alist))

for i = 1:length(alist)
  %Data from file are extracted
  A = importdata(alist(i).name, ',', 3);
x = A.data(:,1);
y = -A.data(:,2);
u = A.data(:,3);
v = -A.data(:,4);

% Calculating mag and dir of velocity vectors
mag = sqrt(u.^2 + v.^2);
theta = atan2(v, u);

% Determining the size of each horizontal strip
strip_size = min(y)/y_strips;
count = 2;
for j = 1:length(y)
    if count ~= y_strips + 1 && y(j) < ...
        (count - 1) * strip_size
            y_position(count) = j;
        count = count + 1;
    elseif count == y_strips + 1 && y(j) < y(j+1)
        y_position(count) = j;
        break;
    end
end

% Determining the size of each vertical strip
col_size = max(x)/x_cols;
count = 2;
for j = 1:length(x)
    if count ~= x_cols + 1 && x(j) > ...
        (count - 1) * col_size && x(j) < x(j+1)
            x_position(count) = j;
        count = count + 1;
    elseif count == x_cols + 1
        break;
    end
APPENDIX D. MATLAB CODE

end

x_position(end) = length(x);

%C r e a t i n g n e w d a t a s t r u c t u r e f o r s e g m e n t e d d a t a
x_columned(1:x_position(2),1:x_cols)=...
    NaN(x_position(2),x_cols);
y_columned(1:x_position(2),1:x_cols)=...
    NaN(x_position(2),x_cols);
u_columned(1:x_position(2),1:x_cols)=...
    NaN(x_position(2),x_cols);
v_columned(1:x_position(2),1:x_cols)=...
    NaN(x_position(2),x_cols);

%E n t e r i n g d a t a i n t o n e w d a t a s t r u c t u r e
for j = 1:x_cols
    x_columned(1:x_position(j+1)-x_position(j),j)=...
        x(x_position(j)+1:x_position(j+1));
y_columned(1:x_position(j+1)-x_position(j),j)=...
        y(x_position(j)+1:x_position(j+1));
u_columned(1:x_position(j+1)-x_position(j),j)=...
        u(x_position(j)+1:x_position(j+1));
v_columned(1:x_position(j+1)-x_position(j),j)=...
        v(x_position(j)+1:x_position(j+1));
end

%Number of data bars due to data structure
data_bars_withX = length(y_columned(:,1))/...
    y_position(end);
x_columned_striped(1:data_bars_withX*y_position(2),...
    l:x_cols,l:y_strips)=NaN(data_bars_withX*...
    y_position(2),x_cols,y_strips);
y_columned_striped(1: data_bars_withX *...
    y_position(2), 1: x_cols, 1: y_strips) = ... 
NaN(data_bars_withX*y_position(2), x_cols, y_strips);

u_columned_striped(1: data_bars_withX *...
    y_position(2), 1: x_cols, 1: y_strips) = ... 
NaN(data_bars_withX*y_position(2), x_cols, y_strips);

v_columned_striped(1: data_bars_withX *...
    y_position(2), 1: x_cols, 1: y_strips) = ... 
NaN(data_bars_withX*y_position(2), x_cols, y_strips);

%Data is fully segmented here
for f = 1: x_cols
    for j = 1: data_bars_withX
        for k = 2: y_strips+1
            x_columned_striped((j - 1)*(y_position(k) -...
                y_position(k-1))+1:j*(y_position(k) -...
                y_position(k-1)), f, k-1) = ... 
            x_columned((j - 1)*y_position(end) +...
                y_position(k-1)+1:(j -1)*...
                y_position(end)+y_position(k), f);
            y_columned_striped((j - 1)*(y_position(k) -...
                y_position(k-1))+1:j*(y_position(k) -...
                y_position(k-1)), f, k-1) = ... 
            y_columned((j - 1)*y_position(end) +...
                y_position(k-1)+1:(j -1)*...
                y_position(end)+y_position(k), f);
            u_columned_striped((j - 1)*(y_position(k) -...
                y_position(k-1))+1:j*(y_position(k) -...
                y_position(k-1)), f, k-1) = ... 
            u_columned((j - 1)*y_position(end) +...
                y_position(k-1)+1:(j -1)*...
APPENDIX D. MATLAB CODE

```matlab

% Mag and dir of velocity of each segment calculated
mag_columned_striped = sqrt(u_columned_striped.^2 + v_columned_striped.^2);
theta_columned_striped = atan2(v_columned_striped, u_columned_striped);

% This section plots one rose plot for each segment
figure
for j = 1:y_strips
    for f = 1:x_cols
        subplot(y_strips, x_cols, (f-1)+(j-1)*x_cols+1)
        P=polar(0, rose_rlim);
        set(P, 'Visible', 'off');
        hold on
        hrose = rose(theta_columned_striped(:,f,j),...
                      rose_bins);

        set(hrose, 'LineWidth', 1, 'Color', [0,0,1]);
        set(findall(gcf, 'String', '30', '-or',...
'String', '60', '−or', 'String', '120', ...
'−or', 'String', '150', '−or', 'String', '...',
'210', '−or', 'String', '240', '−or', '...',
'String', '300', '−or', 'String', '330') ...
'String', '...')

%This is where axis tick marks are removed
set(findall(gcf, 'String', '−1000', '−or', ...
    'String', '−3000'), 'String', '−300');

th = findall(gcf, 'Type', 'text');
for m = 1:length(th),
    set(th(m), 'FontSize', 16)
end
end
end

set(gcf, 'Visible', 'on');

aa = annotation('textbox', [0 0.9 1 0.1], ...
    'String', strtok(alist(i).name, '.'), ...
    'EdgeColor', 'none', 'HorizontalAlignment', 'center');
aa;
aa.FontSize = 10;

%Printing the plot as an image
print(strtok(alist(i).name, '.'), '-dpng')

clear A x y u v mag theta strip size count ...
data_bars_withX ...
x_columned y_columned u_columned v_columned ...
x_columned_striped y_columned_striped ...
v_columned_striped ...
u_columned_striped mag_columned_striped ...
theta_columned_striped;
end
Bibliography


