

# Design of a Device to Cyclically Stretch Isolated Cells 

Colleen Brinkmann<br>Anthony Corbo<br>Amy Morin<br>Gregory Trumbull

Advisors: Professor Kristen Billiar
Professor Domhnull Granquist-Fraser

## Table of Contents

Contents
Table of Contents ..... 1
Table of Figures and Tables ..... 3
Abstract ..... 4
Authorship Page ..... 5
1 - Introduction ..... 6
2- Background ..... 8
2.1- Mechanobiology ..... 8
2.1.1 External Mechanical Forces ..... 10
2.1.2 Internal Mechanical Forces ..... 10
2.1.3 Mechanobiological Responses ..... 11
2.1.4 Cellular Chemical Signaling (Mechanotransduction) ..... 14
2.2 Cell Stretch Devices ..... 14
2.2.1 Pressure to Achieve Stretch ..... 14
2.2.2 Vacuum to Achieve Stretch ..... 15
2.2.3 Motor Driven Stretch Devices ..... 20
3 - Design Approach ..... 22
3.1 - Design Alternatives ..... 22
3.1.1 - Four Motors System ..... 23
3.1.2 - Two Motors Systems ..... 24
3.1.3 - Vacuum (Flexcell) ..... 28
3.1.4 - One motor system ..... 28
3.2 - Design Process ..... 30
3.3- Final Design ..... 32
3.4 - Device Description ..... 33
3.4.1 - Motor Selection ..... 34
3.4.2 - Gear and Pulley System ..... 36
3.4.3 - Linear Guide System to Directly Stretch Strex Well ..... 37
3.4 - Microscopy Methods Currently Used ..... 42
Objectives. ..... 42
Constraints ..... 43
Best-Fit Microscope ..... 43
3.5 - Incubation Methods Currently Used ..... 44
4 - Device Validation ..... 46
4.1 Test Trials - High Density Mapping ..... 46
4.2 Device Validation ..... 49
5 - Conclusions and Recommendations ..... 54
5.1 Conclusions ..... 54
5.2 Recommendations ..... 54
References ..... 58
6.1 - Appendix A - Pairwise Comparison Chart ..... 61
6.2 - Appendix B - Functions Means Tree ..... 62
6.3 - Appendix C- Budget Breakdown ..... 63
6.4 - Appendix D- Strain Parameter Examples ..... 64
6.5 - Appendix E- General Use Manual. ..... 65
6.6 - Appendix F- Design Drawings. ..... 73
6.7 - Appendix G- Ordered Parts ..... 83
6.8 - Appendix H- Custom Parts ..... 104
6.9 - Appendix I- HDM Matlab Compile Function ..... 122
6.9 - Appendix J - HDM Displacement in the X Direction Raw Data ..... 127
6.11 - Appendix K- HDM Displacement in the Y Direction Raw Data ..... 133
6.12 - Appendix L- HDM Strain in the X Direction Raw Data ..... 136
6.13 - Appendix M- HDM Strain in the Y Direction Raw Data ..... 139
6.14 - Appendix N- HDM Shear Strain Raw Data ..... 142
6.15 - Appendix O- Instron Data of Strex Well Stretch ..... 145
6.16 - Appendix P-Cells imaged on the stretch device ..... 146

## Table of Figures and Tables

Figure 2.1: Schematic illustration of the "mechanical nature" of cellular mechanotransductionmechanisms (Taylor and Francis, 2008).13
Figure 2.6: Strex Cell Stretch Device (B-Bridge International, Inc. 2009). ..... 21
Figure 3.1 3-D Model Drawing of Strex design (top) and view of Strex device (bottom) ..... 25
Figure 3.2: Linear Bearings One Motor design (equibiaxial set up) ..... 29
Table 3.1 Comparison of Strex and One motor design ..... 30
Figure 3.2: Design in equibiaxial (top) and uniaxial (bottom)CAD ..... 32
Figure 3.3: Our device on the Zeiss microscope (top) and close up with cells seeded on the well ..... 33
Figure 3.4 -Exploded View of Crank Shaft. ..... 37
Figure 3.5 -Igus Linear Rail and Carriage System ..... 39
Figure 3.6 - Four Stretch Arms ..... 40
Figure 3.7 - Exploded View of X and Y Guided System ..... 41
Figure 3.8 - Exploded View of Corner Assembly. ..... 42
Figure 4.1: Hand stretched Strex well to 20\% ..... 47
Figure 4.2: HDM results in x axis (top), y axis (middle) and shear strain (bottom) ..... 48
Figure 4.3: Stretch Device on Zeiss Microscope ..... 50
Figure 4.4: Rat Aorta Cells at 40X magnification with Hoescht and Phase Views. ..... 51
Table 4.1: Heat Test Results ..... 52
Figure 4.4: Graphical Results of Heat Test Over Time ..... 53
Figure 5.1: Heim Joints (Lunsford, 2010). ..... 56
Figure 5.2: Igus plain linear bearing (left) and Igus enclosed linear bearing (right) (Igus, 2010). ..... 57


#### Abstract

Cells in the musculoskeletal system are constantly subjected to numerous mechanical forces in vivo. Years of research in the field of mechanobiology has shown mechanical forces, including tension and compression, significantly impact various cellular functions such as gene expression, cell proliferation and differentiation, and secretion of matrix proteins. Mechanical signals are also converted into a cascade of cellular and molecular events, initialized by mechanotransduction mechanisms. Multiple commercially produced and custom-built devices are available to study cellular responses to substrate strain. However, real-time data analysis of dynamic cellular responses is not available or easily feasible. WPI has both commercially manufactured and custom built stretch devices available and these devices are currently being used by Professor Billiar. However, they unfortunately do not allow for real-time analysis of cellular response to mechanical stimuli. We have developed a novel stretch device optimized for live-cell imaging. The device assembles on a standard inverted Zeiss microscope and can apply constant cyclic stretch for extended periods of time on the silicone STREX $4 \mathrm{~cm}^{2} \mathrm{X}$ - Y culture wells (B-Bridge International). The magnitude and rate of stretch are variable, with maximum stretch of $20 \%$, and a stretch frequency of 1.9 Hz (114 rpm). Interchangeable arms, and a movable linear guide allow for equibiaxial and strip biaxial (pure uniaxial) stretch profiles. Strain analysis achieved by HDM validates and shows reproducible applications of stretch. This device will be used in the future to study molecular dynamics, and cell response to mechanotransduction. Post interpretations verify the stretch device is a novel tool and is compatible with live-cell microscopy for studying dynamic structural remodeling and cellular responses under mechanical strain.


## Authorship Page

Key: All-Colleen Brinkmann, Anthony Corbo, Amy Morin and Gregory Trumbull, CB-Colleen Brinkmann, AC-Anthony Corbo, AM- Amy Morin, GT- Gregory Trumbull

## Sections

Abstract
Introduction
Background
Mechanobiology
Cell Stretch Devices
Microscopy
Incubation
Design Approach
Design Options
Design Process
Final Design
Results
Design Validation
Conclusions
Recommendations
References
User Manual

## Author/Editor

## AC

All
All
AC
All/AM
GT/AC
GT/AC
AM, GT/AC
AM, GT/AC
GT
AM, GT/AC
AC, CB/AM, GT
AC, CB/AM, GT
AM/AC
AC, CB/AM
All
AC/GT, AM

## 1 - Introduction

The human body is a dynamic example of constant changes. On a macroscopic level, people grow, age, and mature. On a microscopic level, cells are growing, dying, proliferating, and constantly changing. These cells are responding to various mechanical forces, and the study of mechanobiology suggests that these forces are crucial to the creation of specific tissue types in the body.

Bone cells for instance need to undergo a compressive force in order to create cortical bone that can withstand the normal forces bones are exposed to on a regular basis. Muscle cells also need an external force constantly acting upon them in order to maintain mechanical strength. In order to ensure these tissues are able to function under the same conditions present in the body, the mechanical properties need to be tested. It is important to see how the individual cells respond to this external force because tissues are not isotropic so the force will not be distributed evenly amongst the tissue (Mizutani et. al, 2006).

Ideally, these different mechanical forces created in the body could be replicated in a laboratory setting; however, no machine can function exactly like the body. For quite some time, devices to stretch cells have been created that attempt to mimic the in vivo conditions that would be present in the body. Stresses can be applied to test the mechanical properties in tension, compression, and shear situations. Cells can be stretched in many different directions so that they can experience similar stresses and forces that would be present in the body. The device allows those properties to be tested, and see how much stress or strain in any loading situation the cells can handle. Most devices focus on one specific type of stretch such as uniaxial, equibiaxial or strip biaxial, and some contain viewing platforms to see the individual cell responses to this
stretch while others do not. By stretching the cells various ways, we will be able to view how the cells respond to that stretch and how they change in orientation and other pathways because of that stretch.

In order to keep cells alive during testing, tissue stretching devices have been equipped with various mechanisms to maintain cell homeostasis so a realistic cell response can be seen from the different stretches the cells are subject to. Each individual cell responds to the forces acted on it during a stretch, so it is important to see not only the collective movements of a tissue membrane, but also the movements of each individual cell. This method will help to see if there are any trends in the movement of cells or if some cells experience more stress or strain than others and causes them to respond to stimuli in a different manner than the collective tissue. In order to see these cell responses, the cells need to be examined on the microscopic level while it is experiencing the stretch.

The goal of this project is to design, build, and test a cell stretching device that can stretch cells biaxially from equibiaxial to strip biaxial strain. The device must allow observation of individual cell responses to the stretch applied to the membrane before, during (through the use of cameras), and after the stretch to see how they reorient over that period of time. The machine should stretch along two axes in order to assure an area with uniform strain to see the true movement of the cells to the stretch. This project will be accomplished this with our budget of $\$ 1000$ and our time constraint of completion of the project of April 2011.

## 2- Background

## 2.1- Mechanobiology

Mechanical stimuli have been found to play a crucial role in the development of many tissues, and these mechanical stimuli effect tissues at the cellular level (Wang D. et. al, 2010). The interactions at the cellular level can cause tissues to develop differently if they were somehow changed. Different microenvironments have the potential to initiate different signal pathways in cells, which can effect gene expression.

In vitro model systems are commonly used to explore the effects of mechanical stimulus on cells. Generally, deformable materials with smooth culture surfaces are used to apply cyclic mechanical stretching to cells (Wang, J. et. al, 2005). Cyclic stretching model systems have shown mechanical stimulus induces various responses, including cell reorientation, actin cytoskeletal remodeling, and altered cell proliferation, gene expression, and protein synthesis (Wang D. et. al, 2010).

These model systems have some limitations, the most significant limitation regarding the smooth deformable material. Previous cell stretch studies on smooth substrates have shown cells randomly orient initially, and then reorient in a direction with minimal deformation after stretching (Wang, H. et. al, 1995). This induces potential for heterogeneous strain on the cells, which changes depending on their orientation to the stretching direction. To avoid heterogeneous strain on the cells equibiaxial stretch systems have been developed. These systems offer isotropic strain gradients on smooth culture substrates, allowing the cells to experience the same strain despite their orientation (Wang, J. et. al, 2005). However, equibiaxial stretching does not physiologically mimic what many cells experience. For example, tendon and ligament fibroblasts are subjected to uniaxial stretching in vivo (Wang, J. et. al, 2005). Another limitation of these systems is the variation in organization and shape. Cells in vivo are well organized and have
defined shapes, whereas cells in vitro follow a random trend. Considering cell organization and cell shape influence cell function and phenotype, it is quite possible to recognize variation in data (Chen et. al, 1997). Currently to avoid these issues culture systems are being developed which promote/control cell organization, shape and mechanical conditions more precisely.

Cells in the musculoskeletal system are subjected to various mechanical forces in vivo. Years of research have shown that these mechanical forces, including tension and compression, greatly influence various cellular functions such as gene expression, cell proliferation and differentiation, and secretion of matrix proteins. Cells also use mechanotransduction mechanisms to convert mechanical signals into a cascade of cellular and molecular events (Wang, J. et. al, 2010).

Mechanical forces act on humans at different levels, from the body as a whole to individual organs, tissues, and cells. It is well known that appropriate mechanical loads are beneficial to bone and muscle by enhancing their mass and strength. On the other hand, excessive mechanical forces can also be detrimental; for example, excessive mechanical loading of tendons plays a major role in the development of tendinopathy (Fredberg et. al, 2008)(Wang, JH. et. al, 2006). Thus, mechanical forces have a profound effect on tissue homeostasis and pathophysiology. The central players in the human body's response to mechanical forces are various types of mechano-sensitive cells. Examples of such cells include tenocytes in tendons, fibroblasts in ligaments and skin, osteocytes in bone, chondrocytes in articular cartilage, and endothelial cells in blood vessels. Mechanical forces induce a wide range of cellular events, including proliferation, differentiation, and gene and protein expression by both adult differentiated and stem cells (Wang, JH et. al, 2008).

### 2.1.1 External Mechanical Forces

Two basic ways to apply tensile mechanical forces to cells a substrate such as silicon include uniaxial or biaxial. Uniaxial stretching is appropriate for application of mechanical forces to cells originating from tendons and ligaments; these cells are aligned with the long axis of the tissue and are primarily subjected to uniaxial stretching in vivo (Chen et. al, 1997). On the other hand, biaxial stretching is applied to cells that are subjected to tensile forces in all directions in vivo, such as dermal fibroblasts. Several biaxial stretching systems have been devised, which typically use circular elastic membranes to produce isotropic strains independent of stretching direction (Wang, J. et. al, 2010). In addition to tensile forces, compressive forces can also be applied to cells that are subjected to compression in vivo. A common means of applying compressive forces is through applications of hydrostatic pressure (Wang, J. et. al, 2010). Other techniques use direct platen abutment to apply compressive forces to cells. These types of loading systems include unconfined compression, in which constant or low-cycle intermittent loads are delivered by manually applying weights (Wang, J. et. al, 2010). These compressive loading systems can be used to investigate mechanobiological responses of cells in tissues primarily subjected to compression in vivo, such as articular cartilage.

### 2.1.2 Internal Mechanical Forces

In the body, cells generate mechanical forces themselves; these forces are commonly referred to as intracellular tension. However, in non-muscle cells, intracellular tension is produced by the cross-bridging of actomyosin. This process is caused by ATP hydrolysis (Wang, J. et. al, 2010). These tensile forces are then transmitted to the ECM via focal adhesions, and the
forces acting on ECM are called cell traction forces (CTFs). CTFs play a critical function in cell mechanobiology, as they direct ECM assembly, control cell shape, permit cell movement, and uphold cellular tensional homeostasis (Balaban et. al, 2001). CTFs also deform the ECM network and cause stress and strain in the network, which in turn modulate cellular functions such as gene expression and protein secretion. Hence, CTFs are critical in many fundamental biological processes such as embryogenesis, angiogenesis, and wound healing (Wang, J. et. al, 2010).

However, mechanobiology studies rely on cell-substrate adhesions which transmit external mechanical forces to cells. This is because external forces acting on cells can alter their internal forces, thus affecting cellular mechanobiological responses. Interestingly, factors such as substrate stiffness also have a significant influence on cell behavior. For example substrate stiffness only can direct specific differentiation of human mesenchymal stem cells (hMSCs); soft substrates ( $0.1-1 \mathrm{kPa}$ ) mimicking brain tissues are neurogenic, whereas stiffer substrates (8-17 kPa ) mimicking muscle are myogenic. Finally, even stiffer substrates ( $25-40 \mathrm{kPa}$ ) resembling osteoid matrix can induce hMSCs to undergo osteogenic differentiation" (Wang, J. et. al, 2010).

### 2.1.3 Mechanobiological Responses

Depending cell type and loading conditions, various mechanical forces on cells promotes a variety of cellular responses/functions, including cell proliferation, differentiation, gene expression and synthesis of ECM proteins, production of cytokines and growth factors (Wang, J. et. al, 2010). In a study conducted by Rehfeldt et al, human tendon fibroblasts were stretched at different magnitudes and showed increase in proliferation as well as gene expression and protein production of type I collagen (Rehfeldt et. al, 2007). Another study, conducted by Yang et al, cyclically stretched human tendon fibroblasts at a magnitude of $5 \%$ and a frequency of 1 Hz for

24 hours, resulted showed significant increases in cell proliferation. However, when the cells were stretched for 48 hours cell proliferations was inhibited, this of course indicating stretchinginduced proliferation of tendon fibroblasts depends on stretching duration (Yang, G. et. al, 2004). Another test in human periodontal ligament fibroblasts, conducted at $10 \%$ cyclic equibiaxial compression test which decreased type I collagen mRNA expression and reduced synthesis of fibronectin as well as the amount of total protein; however, the same level of cyclic stretching increased type I collagen mRNA levels and total protein levels (He et. al, 2004).

These studies illustrate how tensile and compressive forces with the same magnitude induce differential cellular mechanobiological responses. Other than affecting cell proliferation and protein expression, mechanical forces also promote the expression and production of inflammatory mediators, including COX-2, PGE2, and LTB4, in a stretching magnitudedependent fashion (Wang, J. et. al, 2010). These results suggest that when tissues such as tendons are injured, appropriate levels of exercise could be beneficial as it may reduce the inflammatory response. On the other hand, excessive loading of injured tendons, which may worsen tissue inflammation, could be detrimental. These various mechanical forces have noteworthy effects on cells, understanding these forces and their affects can eventually lead to a better understanding of cell function, homeostasis and equilibrium. Understanding these can lead to future success in various biomedical applications such as: tissue-engineering, wound healing, cancer treatment, gene therapy and tissue remodeling.


Figure 2.1: Schematic illustration of the "mechanical nature" of cellular mechanotransduction mechanisms (Taylor and Francis, 2008).

Mechanical forces (MF) can induce mechanotransduction by directly altering conformation of an extracellular matrix (ECM) protein and integrin configuration and transmitting forces to the cytoskeleton and nucleus, thus eventually affecting transcription and translation. Also, mechanical forces can unfold a domain of the extracellular protein (M) and expose a cryptic site that may serve as an activating ligand for a cell surface receptor, resulting in a series of signaling events. Also, when mechanical forces are applied to "force receptors" (FR), such as integrins and G proteins, they initiate signal transduction, resulting in transcription followed by translation. As a result, soluble factors are secreted into the ECM, which act on the receptor ( $\mathbf{R}$ ) and then initiate a cascade of signaling events. Note that double arrows indicate intracellular tensions in the actin filaments (Modified with permission from Wang and Thampatty, Taylor \& Francis. 2008).

### 2.1.4 Cellular Chemical Signaling (Mechanotransduction)

For cells to respond to mechanical forces, these forces must be transformed into chemical signals inside the cell to induce a cascade of cellular and molecular responses. The cellular process of converting mechanical stimulus into chemical signals is called cellular mechanotransduction. Figure 2.1 illustrates and describes this process above. The mechanism of cellular mechanotransduction is still not entirely clear, however, it is generally established that external mechanical forces are transferred to a cell through integrin-mediated adhesions in the ECM (Juliano et. al, 1993). Integrins hold large ECM domains responsible for binding substrates to cytoplasmic domains, and are the primary adhesive receptors and mechanotransducers which link the cytoskeleton to the ECM. Consequently this ECM-integrin-cytoskeleton network plays a key role in mechano-signaling processes. Presence of mechanical stress to integrins is capable of altering the cytoskeleton and activated gene expression in a stress-dependent manner.

### 2.2 Cell Stretch Devices

In order to determine the various means to stretch cells, an extensive literature review of devices currently in existence was conducted. Each device has its own specific function to obtain specific stretching of cells, and its own specific way to obtain those desired results. The main methods of stretching cells can be broken down into systems using motors, vacuums, and a pressure force.

### 2.2.1 Pressure to Achieve Stretch

The direct platen abutment approach of compressing the cells uses a manual application of a force directly downward on the membrane to stretch (Brown, 2000). It has been used widely
in collagen applications because it mimics in vivo conditions closely. A device that uses this method has been developed by Tanaka to target specifically the low strain regime in bone. This device uses an actuator to drive the force of the platen to compress the membrane, which in this case is a 3-D bone matrix (Tanaka, 1999). This device runs at a frequency of 100 Hz and can produce cyclic loading and normal sinusoidal wave loading, but also non-typical loading (Tanaka, 1999).

Another device design uses pneumatics to compress the cells (Torzilli et al, 1997). Advantages of these devices are the ability to have cyclic loading which produces sinusoidal compression of the membrane or non-sinusoidal loading of the membrane. The forms of stretch it can attain include unconfined compression with no support of the materials from the sides as well as confined compression with support on the sides. This has been shown to be an effective form of stretching cartilage cells to determine metabolic responses (Torzilli et. al, 1996). This study involved stretching of cartilage cells with stresses ranging from 0.5-24MPa were loaded at 1 Hz sinusoidal for a 2-24 hour period of time. It is also able to obtain homogeneous compression as in the hydrostatic pressurization mechanism. Disadvantages would be its ability to attain strip biaxial strain, as well as keep the cells alive during the stretch (Brown, 2000).

### 2.2.2 Vacuum to Achieve Stretch

A widely used means of placing cells under compression is through the use of hydrostatic pressure (Brown, 2000). The pressure can use vacuum pressure which is negative or positive pressure to compress cells, and thus stretch them. There are several devices which have been designed using this basic method, and are useful for their given applications. Some specific advantages of this device are the ability to obtain homogeneous compression, which allows for
equibiaxial strain to be easily attainable. Also, the pressure does not prevent the cells from obtaining any nutrients from the cell media which allows them to remain alive during the stretch (Brown, 2000).

Some disadvantages of this device also come with the homogeneous compression in that it becomes that much more difficult for the cells to attain strip biaxial strain. Modifications to the compression chamber would have to be made for the stretch device to be able to attain strip and equibiaxial strain. Other disadvantages are the use of either pressurized $\mathrm{O}_{2}$ or $\mathrm{CO}_{2}$ can prove to be harmful to the cells under strain. In order to keep the cells alive, modifications need to be made to the cell media to compensate for that pressurized environment. Another disadvantage of hydrostatic pressurization as a cell compression method is that cells experience forces regularly that are different than just hydrostatic in nature, which therefore does not serve as a totally useful in vitro model (Brown, 2000).

This form of applying a mechanical load to cells includes circular substrates that have loads applied to them, and subsequently produce the strain on cells. There are four main ways to attain this strain on the cells. A curved platen may be used to provide the uniform strain on the cells (Williams et al, 1992). This study did show that surface strains due to bending, however, are not negligible and result in large strains near the clamped regions of the membrane (Williams et al, 1992). Another way to apply this load is through prong displacement, in which a collagen substrate is loaded and unloaded with a prong being moved by a stepper motor control (Vandenburgh, 1988). The third means of displacing the membrane would be though a fluid movement. The final option for movement of the membrane would be through vacuum displacement, in which the pressure is applied to the underside of the well and the substrate is
then stretched from the pressure (Brown, 2000). These four methods can be seen below in Figure
2.2.


Figure 2.2: Methods of out-of-plane circular substrate distention (Brown, 2000).

There is one commercially available device that actually takes that concept of vacuum circular substrate distention, and that device is called the Flexcell® System. This system attempts to account for the various stresses experienced in the body, and mimic them as close to in vivo conditions as possible using a vacuum pump to push down on the substrate and apply strain (Flexcell International Corporation, 2004).

This device has been used extensively used in literature for various applications. In one application, it was used to test the development of tissue engineered tendons for anterior cruciate ligament replacement (Garvin et al, 2003). This used the Flexcell device to apply strain to the tendon to test the mechanical properties. With this device, the cells were alive with $5 \% \mathrm{CO}_{2}$ and $37^{\circ} \mathrm{C}$ by placing the base plate in an incubator while still applying the vacuum pressure for a period of 1.5 hours (Garvin et al, 2003). The cells are loaded in a circular six plate well with
anchors on both sides of nonwoven mesh, holes to accommodate the vacuum flow, and the rubber membrane from one side to the other. This can be seen below in Figure 2.3.


Figure 2.3: Top view of Flexcell well with substrate (Garvin et al, 2003).

The cells were placed in a six well dish with a Delrin Trough Loader insert that allows for the space beneath the culture to be entirely filled. The insert has holes that allow for air to escape from the top of the rubber membrane and push down on the loader (Garvin et al, 2003). Once the cells have been seeded properly on the gel, the trough loader is removed, leaving a space for the deformation of the substrate, which can be seen in Figure 2.3, which shows the side view of the substrate loading process.


Figure 2.4: Flexcell side view of well with substrate (Garvin et al. 2003).

This design allows for uniaxial movement of the substrate, as well as equibiaxial strain applied to the substrate (Garvin et al, 2003). The archtangle shape of the rubber (rectangle with circular ends) allows for a nice long axis of a fixed substrate, and then provides the sides that are not fixed to move downward when the vacuum force is applied (Triantafillopoulos et al, 2004). Some limitations of this device are the overall uniform strain and the anisotropy of the strain profiles because cells and tissues do not always experience uniform strain (Brown, 2000).

The Flexcell device is in Professor Billiar's lab currently, and does a very good job of obtaining strip and equibiaxial stretch on a soft substrate. The main issue with this device is the inability to see the real time realignment of the cells in reaction to the stretch.

### 2.2.3 Motor Driven Stretch Devices

Finally, this stretch can be achieved through biaxial strain which physically pulls the substrate outward in two directions perpendicular of one another (Brown, 2000). This can be seen in multiple devices. In a device that was used by Norton et al, a cross-shaped PTFE membrane was clamped to a two micrometer-driven mechanism with an accuracy of $5 \mu \mathrm{~m}$, to move the arms of the cross substrate and stretch the membrane (Norton et al, 1995). The topview of the device can be seen below in Figure 2.5.


Figure 2.5: Micrometer-driven biaxial device (Adapted from Norton et al, 1995).

Other devices driven by motors include the Strex Cell Stretching System, which is made by B-Bridge International, Inc. The system can be seen below in Figure 2.6. It seeds cells on a soft-substrate silicon membrane that is shaped with a cross structure to reduce the Poisson effect created in biaxial stretch. The device allows for the biaxial stretch with a two motor system that
drive threaded rods to move the corners of the silicon well to stretch it, and the cells seeded on top of the well (B-Bridge International, Inc., 2009).


Figure 2.6: Strex Cell Stretch Device (B-Bridge International, Inc. 2009).

The motors are controlled by a computer driven program that will dictate the desired amount to stretch the membrane. To overcome the moment created by pull on any one axis, the arms are also attached to bearing slides that will keep the membrane in perpendicular alignment with the device and keep the stretch either strip biaxial if only one motor is running or equibiaxial if the two motors are running (B-Bridge International, Inc., 2009).

Various other devices currently used in labs are driven by four motors to obtain equibiaxial and strip biaxial strain. With each motor controlling one of the four axes of the membrane being stretched, it allows for more precise control to ensure the desired percentage of stretch is obtained at any given point in time due to the Poisson effect created. Currently in Professor Billiar's lab is a four motor driven device.

## 3 - Design Approach

Our goal is to design, build, and test a cell stretching device. The main objective was for the device to be able to stretch the cells biaxially, meaning both strip and equibiaxial stretching. The client expressed desire for the device to stretch the tissue for a minimum of six hours, but with the hopes of obtaining a stretching period closer to that of ten to twelve hours. Also, the device should be able to strain the tissue sample at a uniform rate between $2-20 \%$ at 1 Hz . The cells also need to be kept alive and at homeostatic conditions during testing.

After these objectives, other less critical, yet still important features were for the device to have a suitable cell viewing area of 1 square cm , have a stationary viewing area during the stretching process, and to have the device itself be inexpensive.

## 3.1 - Design Alternatives

In order to determine the client's priorities in the design, a pairwise comparison chart was constructed so that objectives could be ranked in importance. Once a final design was determined, this was a useful chart in the event that some aspects of the machine cannot be completed because the cost or time constraints cannot be met. From here, we were able to determine the top priorities of this machine should be stretching with strip and equibiaxial strain, keeping the cells alive during the stretch through the use of incubation, fitting the device under a microscope stage for real-time viewing of the cells during stretch, outfitting the device to stretch the pre-manufactured Strex well, and to keep the cost under $\$ 1000$. Secondary objectives include being able to adjust the strain rates and strain percentages of the motor and to minimize the cost. The smaller objectives are keeping the device inexpensive and maximizing the viewing area to see the cellular response of the stretch. The filled our Pairwise Comparison Chart from our advisor can be seen in Appendix A.

From the objectives, we were able to determine a list of functions the machine needed to perform in order to successfully meet the constraints and objectives of the project. From those functions, we were able to determine means to achieve them, which led into preliminary design options for our machine. In the functions means tree in Appendix B, we started with basic functions and brainstormed possible ways to make this happen based on previously reviewed stretching machines or other methods of stretching membranes that may not necessarily be associated with cells.

### 3.1.1 - Four Motors System

A stretch system based off a clamped four motor platform has several advantages. This system can certainly achieve strip biaxial and equibiaxial strain and can do so without moving the center of the tissue sample in order to view its response to the stretch under the microscope. Each motor is responsible for moving one axis of the square well, and would be able to be controlled independently of each other. This allows for easy controlling for strip and equibiaxial strain. This type of system can also perform various strain percentages, strain rates, and duty cycles, though this is all based off the power, accuracy, and capabilities of the motor.

Another design option is a four motor system with linkages. This system is useful to create a stationary viewing area to see the cellular response to the stretch of the cells. Each motor is able to control the linkage movements and ensure strip and equibiaxial strain on the tissue. This will be easy to add or remove strain on the cells and have a quick response to create variable duty cycles in the movement of the motors with proper programming.

A concern with a four motor system is the design space that is reduced in order to have enough room for all four motors to move and be properly vented to prevent overheating and keep within the size constraint of the microscope viewing area. This could be overcome by moving the
motors to a plane different from the membrane being stretched, however, this would involve more design of parts that would allow for the motors to be on a different plane that that of the stretched membrane. Each motor will also have to be equipped with a motor controller board to run it, which is expensive and could increase the cost of the device.

### 3.1.2 - Two Motors Systems

The two motor threaded rod system is yet another design option. The two motors have an advantage over the four motor systems because they take up less room around the microscope stage; require less power than four motors do, and cost less money. Threaded rods are also an advantageous stretching mechanism because they are an easily obtainable system used in many applications. This aspect makes them easy to understand and work with because they are so commonly used. Using this method allows for both strip and equibiaxial stretching.

A commercially available version of this design is known as the Strex®. It involves two motors with threaded rods attached to guide rods that are connected to each corner of a square soft membrane. The bottom left corner remains stationary, but the other three corners move, stretching the plate in either one or two directions simultaneously. Reverse-engineering this is a design option, and a 3-D image model of it made in SolidWorks can be seen below.


Figure 3.1 3-D Model Drawing of Strex design (top) and view of Strex device (bottom)

Each corner is attached to a moving carriage that is attached to a threaded rod driven by motor control and a guide rod that keeps the movement perfectly perpendicular to the threaded rod. To overcome the moment force created there are linear bearings attached to the rails which will allow for the motion to remain in the correct direction. The motor drives threaded rods to move the housings attached to each arm away from the original location of the Strex well it is attached to, and allows for the aligned movement of the well (B-Bridge International, 2010).

There are some disadvantages to this design, though. For example, the main concern with using the threaded rods to stretch the tissue sample is that the rods may not be able to move fast enough to strain at the desired varying duty cycles because of the threaded design. Another major concern is that when the tissue it being stretched, it does not stay centered over the viewing area, making it difficult to monitor cell response throughout the entire stretching process. One last issue is that two motors may not be able to generate enough power to move the machine at the desired rate.

Adding linkages is another variation to the two motor design. Two motors being the benefits of having a larger viewing area, a cheaper cost, and do not need as much power as four motors do to run the device. Linkages are also a useful tool for tissue stretching, being able to both biaxially stretch and provide means for varying duty cycles.

A major disadvantage of the linkage system, as touched upon before, is the possibility of unwanted stresses occurring on the edges where the linkages are attached to the tissue sample, which could potentially alter the collected data. Disadvantages of the two motor system include not having enough power to move the machine itself and not keeping the tissue in a centered position.

Another potential stretching design will resemble the four motor design however; the motors will be replaced by pistons driven by compressed air. A piston driven stretching device, much like the four motor design, will be able to achieve both strip biaxial and equibiaxial strain. An advantage to pistons is that there will be no need to learn how to then write complicated programs to run them, although the air pressure would still need to be controlled. There can be multiple compressors giving out different levels of compressed air in order to achieve both types
of strain. Compressed air can be easily dispersed at different rates to perform variable duty cycles, strain rates and strain levels.

Although there are many benefits to using pneumatic driven pistons, there are some drawbacks. Compressors can be big and bulky and can take up valuable space. There is limited space to deal with around a microscope and compressors could impede. Also, though the pistons would be faster than motors, in order to power the pistons air hoses need to be run to them which could become cumbersome when attempting to run experiments. The intended design of this stretch device with two sets of opposing motors may be large and may prove difficult to fit within the given space under the scope. An exterior structure may have to be built in order to support the system instead of mounting it directly to the microscope.

Using hydraulic motors to stretch the tissues is another design option. Hydraulics are useful because there is direct control of the motor via control valves, which can be useful to ensure pure strip biaxial and equibiaxial strain on the membrane. A noteworthy advantage of this option is the ability of hydraulic motors to apply significant power with very small tubes and hoses. This reduces the amount of parts around the microscope stage which can aid in the visibility of the tissue with the microscope.

This option, however, could prove difficult in programming the motors to work in synchronization, especially if four different hydraulic pumps are used. Another drawback could be in the variable duty cycles, because the pumps can quickly add or remove fluid to add or remove the force, but the acceleration of the piston at that speed may not be quick enough or slow enough for the needs of a fast or slow recovery in the stretch mechanisms.

### 3.1.3 - Vacuum (Flexcell)

A vacuum based system works especially well for stretching cells equibiaxially. These systems are consistent, and exhibit good homogeneous stress on the cell samples. A system like the Flexcell would work very well, provide consistent data during testing, and culture plates would be easily available. The Flexcell device is existent in the lab at WPI, but its main issue is the inability to view the real time reaction to the cell stretch because of the closed top design of the vacuum chamber. Attempts have been made to modify the device however the results have not been able to view the results in real time. However, this device would not need any building or programming which can save large amounts of time in the design process. Ultimately this system would allow for more time testing samples and obtaining data.

### 3.1.4 - One motor system

This design consists of one motor which applies downward pressure to the tissue sample to stretch it over stationary posts. Stretching the tissue over stationary posts will achieve strain over the tissue. One of the advantages of this design is first and foremost its simplicity. There is only one motor which moves upwards and downwards and can be programmed to achieve variable duty cycles and strain rates. The motor pushing the tissue is the only moving part of this design which also contributes to its simplicity. This design will be a relatively small structure so it will not be difficult to fit under the microscope stage, but will be difficult to find a way to view the cells during the stretch. Also due to the one motor, both strip biaxial and equibiaxial strain is more difficult to achieve, and would most likely require some movement of parts to make sure. Like with all motors, a drawback is that programs must be written in order for them to function correctly; these complicated programs will have to be learned and executed.

Another one motor design angles the motor at $45^{\circ}$ in the upper right corner of the Strex well to obtain equibiaxial strain. Linear bearings are attached to guide rods which are then
attached to the four corners of the Strex plate. The motor attaches to an arm and flywheel and motor which then causes the movement of the upper right corner linear bearings which translate the motion to the other two corners of the membrane. Strip biaxial strain is attained by adjusting the angle at which the motor pulls to as close to zero as possible, and then accounting for some error in the Poisson effect which will be seen. This can be seen below in Figure 3.2.


Figure 3.2: Linear Bearings One Motor design (equibiaxial set up)

This device allows for no programming, and the parts are easily available off the shelf. However, the adjustment of the motor and linear bearing system will leave room for error, so the precise angles at which the device needs to be to obtain specific strains will need to be very accurate.

## 3.2 - Design Process

In order to determine what design would meet the most objectives desired by the client and fulfill all the constraints, we developed a function and means chart to determine what ways these various objectives could be obtained in our designs.

We developed this chart, shown in Appendix B, in order to help give us a better visual idea of which design would be the most efficient at completing our intended tasks. On the Yaxis, we had both our constraints and objectives listed. On the X-axis, we listed our potential design ideas. Where the design ideas meet with the individual constraints is where we assigned values. These assigned values are on a scale of 0-100 with 0 meaning the design will not be able to perform the constraint/objective and 100 meaning the design will definitely be able to complete the constraint/objective. We assigned these values based upon our personal opinions gained through research on whether or not the constraints and objectives could be achieved.

After assigning values, we tallied the total points up per design and ranked them accordingly.

The two main designs that are being considered are reverse engineering the Strex design, and the one motor linear bearing design. A comparison of the two designs can be seen below in Table 3.1.

Table 3.1 Comparison of Strex and One motor design

| Strex Design Reverse Engineer | Functions | One motor design |
| :--- | :--- | :--- |
| Capable of uniaxial stretch | -Uniaxial stretch | Capable of uniaxial stretch |
| Capable of equibiaxial stretch | -Equibiaxial stretch | Capable of equibiaxial stretch |
| The Strex based design would be <br> capable of achieving 2-20\% strain <br> with any strain \% in-between. | -Strain rates | This design would allow for variable <br> strain \%, however it would most <br> likely be capable of a particular set <br> of strain percentages: <br> $2,5,10,15,20 \%$ strain |
| Adjusting frequency will not be an | -Frequency | Adjusting frequency and varying |


| issue with this device, frequencies of <br> (.5, 1, 1.5, and 2) Hz can easily be <br> achieved. Also the frequency is not <br> limited to these numbers it can go <br> anywhere between and above. | frequency is possible, however the <br> device will most likely be limited to <br> several predetermined frequency <br> setting that would be dependent of <br> power source/ current. |  |
| :--- | :--- | :--- |
| This device will be capable of <br> varying duty cycles. | $\underline{\text {-Duty cycles }}$ | Varying duty cycles with this device <br> will require modifications; the user <br> would have to change an electrical <br> component (diode). |
| These devices will both fit on the <br> Zeiss stage this design will be <br> slightly larger. | $\underline{\text { Size }}$ | Slightly smaller/lighter than Strex <br> design. |
| Compatible with Strex silicon well. | Well type | Compatible with Strex silicon well. |
| Will require time consuming <br> programming. Motor control boards <br> or stepper motor drivers. <br> Programming is difficult to learn and <br> small mistakes in program can cause <br> un-noticed/unwanted results. | $\underline{\text { Programming }}$ | None is required. This system relies <br> completely on mechanics and <br> current for motion. |
| Will use stepper motors, however, <br> the other components would most <br> likely be custom made with some <br> being premade. The fabrication will <br> be more difficult in comparison to a <br> model with mostly premade parts. <br> Also the device will require a <br> computer with expensive motor <br> control boards to run the device. | $\underline{\text { Parts }}$ |  |


| command the device to perform <br> whichever stretch it wants and can <br> run it. | strain type, strain \%, and duty <br> cycles. The user would carefully <br> have to read the manual to ensure <br> the device was appropriately |
| :--- | :--- | :--- |
| adjusted. (If user does not pay |  |
| attention to detail it is possible for |  |
| miss-setup ) |  |

## 3.3-Final Design



Figure 3.2: Design in equibiaxial (top) and uniaxial (bottom)CAD


Figure 3.3: Our device on the Zeiss microscope (top) and close up with cells seeded on the well

## 3.4 - Device Description

Through careful deliberation, our team decided that a one-motor concept for a stretch device would give us the best opportunity to meet all objectives taken from our client statement. Having chosen the general design, we began to search for materials and parts that could be used to achieve major aspects of the device: the device must be powered by a single motor and must stretch a Strex silicone well both uniaxially and equibiaxially.

### 3.4.1 - Motor Selection

Calculations for determining the force, torque and power needed to stretch the wells were accomplished for initial considerations regarding choices of motors. For this design a synchronous motor suits the device best, having a system that limits the stretch to its preconfigured state the motor simply has to turn at the specified frequency in a single direction. This is why stepper motors were not considered; they do offer precise motion due to the fact that as the drivetrain turns it does so in steps not in a smooth/fluid motion. The material properties of the ST-190-XY well from B-Bridge were not available in the specifications, however, the well itself is made from silicone elastomer comprised mainly of polydiethylsiloxane (B-Bridge International). In order to determine certain specifications needed for our motor, we set up an experiment on an Instron machine to stretch the Strex well and find its material properties. We know from calculations prior to the test that the max distance needed in travel at maximum strain (20\%) for the well would be 6 mm or 0.006 m . We programmed the Instron machine to stretch to this distance and record all exerted forces. At the completion of the test we found that the maximum force produced was approximately 19 Newtons. Using this number, along with the maximum distance needed in stretch for at maximum strain, we calculated that needed torque would equal 0.114 Nm . However, this calculation is solely for uniaxial stretch as the Instron machine could only pull in one axis. Therefore, we multiplied our initial uniaxial torque value by two in order to account for the two axes of stretching. This new value of 0.228 Nm would be used as the value or torque necessary to achieve stretch in the equibiaxial position. These two values depict bare minimum values of torque needed to successfully complete uniaxial and equibiaxial stretching of the Strex well. We decided to increase they torque due to certain aspects of our device's potential usage. Our device may potentially undergo experiments where it is continuously running for long periods of time which would place a large strain on the motor.

Extra torque would be beneficial in ensuring the motor is pulling at a steady pace for the extended period of the test. Also concerning to our group was the fact that we are utilizing a 1:3 gear ratio in order to increase speed to meet one of our objectives. However, using a 1:3 gear ratio exhibits its $1 / 3$ fractional percentage on the motor's torque as well. To conclude the original torque of our chosen motor, when divided by 3 , must be well over the 0.228 Nm torque needed for equibiaxial stretch to achieve this goal, as well as, to provide a factor of safety for the overall effect of friction throughout the device.

Taking all of these factors into consideration, our team was able to decide upon a motor that would be efficient for achieving all specifications necessary for our device as described above. The motor chosen is a brushless 12V DC Synchronous Gear Motor manufactured by Wonder Motor. Unlike stepper motors which provide movement in choppy steps, synchronous motors provide smooth operation. This will reduce vibrations which could disturb the seeded cells on the Strex well. This particular motor has a maximum output of 38 RPM while providing 8.4 Nm of torque. As stated above, the chosen motor's torque, when divided by 3, must well exceed the 0.228 Nm needed for equibiaxial stretch. If you take 8.4 Nm and divide by 3 , you are still left with 2.8 Nm of torque. This value well exceeds 0.228 Nm which is excellent seeing as though we also need to account for all friction experienced throughout the device. Overall, the chosen motor will effectively stretch the well to our certain specifications and provide this stretch for extended periods of time without over exerting itself and failing.

To place this motor in the appropriate position on the device, it was necessary to devise a bracket system to contain the motor. The mounting bracket was designed in order to utilize the motor in an inverted position. This inversion allows us to have a direct belt drive design rather than run the belt off of numerous gears which would increase the net friction in the system.

### 3.4.2 - Gear and Pulley System

We are utilizing a 2:1 gear and pulley system to achieve desired rates of stretch as described in our project's objectives. Gears are necessary because we chose our motor based upon specifications needed for torque; rate of stretch was a secondary concern for us. The motor has a maximum speed of 38 RPM's; this equates to approximately 0.633 Hz . Referencing our objectives it is clear that we are aiming for a rate of stretch which can vary from anywhere between 1-2 Hz. In order to achieve the maximum rate of 2 Hz , we must utilize a 1:3 gear ratio. This $1: 3$ ratio essentially means that the gear coming off of the motor shaft will be three times the diameter and have three times as many gear teeth than the gear which will help directly stretch the well. So, by the time the gear on the motor completes one revolution, the gear directing stretch will have completed three revolutions which is where the increase of speed originates. The gears we are using are manufactured by W.M. Berg. Respectively on the motor and directing the stretch the diameters measure 47.24 mm with 36 gear teeth and 16.8 mm with 12 gar teeth. These are 3-d pulleys which entail they are turned by a belt with teeth that interlock with the teeth coming off of the gears. The belt being used to conjoin these two gears is a 3-d pulley also from W.M. Berg.

The gear directly causing stretch will do so by turning a crankshaft which will be placed within the inner bore of the gear. This assembly of gear and crankshaft will sit within a custom made bearing with the gear sitting above the top of the bearing to ensure it will spin freely and not be impeded. The crankshaft has a one inch diameter flat top surface with three pre-drilled holes. These holes are off center by 3mm, 4.5 mm , and 6 mm representing varying strain percents of 10,15 , and 20 percent. Depending on the type of stretch being tested, the location of the crankshaft will change. To achieve stretch, an arm will be positioned over one of these holes
and secured; the other end of the arm will be anchored to a housing which will be explained in the following section.


Figure 3.4-Exploded View of Crank Shaft

### 3.4.3 - Linear Guide System to Directly Stretch Strex Well

This stretch device must achieve both uniaxial, as well as, equibiaxial stretch on a silicone Strex well. To ensure this stretch occurs as smoothly and linearly as physically possible, a rail system had to be installed to counter the Poisson Effect from the silicone material.

The platform which our entire device will sit upon is a 12 "x12" piece of aluminum with a 6 mmx 6 mm square milled out in the center for viewing of cells. Because our chosen microscope came equipped with a movable $\mathrm{X}-\mathrm{Y}$ stage which added height, we also needed to install an adapter to clear this distance. The adapter plate was designed with two functional aspects in mind, one was to allow the device to be compatible to the microscope stage and second to allow the plate to clear the vertical distance between the of the movable $\mathrm{X}-\mathrm{Y}$ stage equipped to the microscope platform. The adapter plate was designed to fit snugly in the $\mathrm{X}-\mathrm{Y}$ stage, which is why no fasteners are necessary to secure the device in place. One can simply insert and remove the device with little trouble. To provide for smoother travel and to counter the moment the motor causes in the rear of the device, acrylic spacers were made. The acrylic spacers have a height of $9 / 16$ " this is because the adapter itself is $1 / 2$ " in height, though it is sitting on a $1 / 16^{\prime \prime}$ lip. The combination of both the adapter and spacers provide a level foundation for the device and allow smooth motion due to positive weight distribution. Also the acrylic can slide on the stage without scratching the coated surface of the microscope platform. Dimensions of both the adapter and the spacers can be viewed in Appendix H.

For stretch in both the X and Y axis linear rails were implemented to keep forces exerted in one direction with only negligible tolerance in the axis of stretch. A corner rail is used to apply direct strain to the upper right corner of the Strex well; stretch will be initiated from that corner. However, the type of stretch will depend on the angle of the rail. For uniaxial stretch, the rail acting upon the top right corner of the well will be parallel to the X -axis rail and be aligned so that the back edges of both the rail and Strex well line up. As the motor applies a force through the crankshaft, the Strex well will only deform in the X-axis through movement along only two rails. Conversely, if equibiaxial stretch is being tested, the rail will shift up 45 degrees towards
the Y-axis. The end of the rail will line up so it is centered with the top right corner of the Strex well. In this stretch set-up, when the motor applies force through the crankshaft, that force is applied at a 45 degree angle through the top right corner of the well. Exerting force at a 45 degree angle will evenly distribute along both the X and Y axis and be kept linear by the rigid rails. The rails used in our device are manufactured by the company Igus. They are composed of 6063-T6 Aluminum. The main reason for choosing these particular rails had to do with their extremely low profile in comparison to other similar products. They stand a mere centimeter tall which helped our team to decreases overall height of the device to fit well underneath our microscope.

Sitting directly atop the rails are cast zinc chromated carriages which will slide along the rail to provide a smooth means of stretch. Unlike most bearings, these carriages do not glide using a ball bearing system. Instead, the inner surface which interacts with the aluminum rail is coated with a wear-resistant iglide ${ }^{\circledR}$ J plastic sliding pad. This custom plastic created by Igus is self lubricating which does away with the need for ball bearings or constant application of lubrication to avoid binding. Each carriage has four screw holes for attachment to other pieces.


Figure 3.5-Igus Linear Rail and Carriage System

The next component of the linear guide system which is being placed upon each carriage is an arm that will create a bracket to hold the Strex well in place close to the objective lens. Each arm is made out of $1 / 8$ " oil hardened steel and has been cut and welded into a precise
position to hold the well perfectly. What is particularly innovative about both the X and Y axis arms is their multi-positional design. Each arm has channels and locking slots enabling the user a more simplistic means to change the stretch position between uniaxial and equibiaxial stretch. Finally, there is a stationary arm anchored to the device platform which will remain rigid to secure the bottom left corner of the Strex well.

$\qquad$

| Arm for |
| :--- |
| Top Corner |



Figure 3.6 - Four Stretch Arms

The final component of the linear guide system consists of pieces which are the same for the X and Y axis rails, but slightly modified for the rail acting upon the top right corner of the well. For the X and Y axis the last component is simply a 3 mmx 2 mm piece of $1 / 2$ " aluminum. The piece has four threaded holes which line up directly with the four holes on the carriage. The threads from this housing in combination with the carriage holes will create a very tight connection to reduce vibrations during operation. In addition to these four holes, there is also a clear hole through the 3 mm long face of the housing. The clear hole will be $1 / 8$ " in diameter and sit in the center of that face; the center of the hole be $1 / 8$ " from the top edge. However, the housing sitting atop the arm on the rail applying force to the top corner of the well is a bit
different and consists of a two part system. The first part will consist of a $2.5 \mathrm{~cm} x 2 \mathrm{~cm}$ piece of aluminum and have four screw holes that match up with those in the carriage, however, this piece will be only $1 / 4$ " thick and have one additional hole. The additional hole will be centered 7.5 mm in from the 2 cm side and 1 cm in from the 2.5 cm side. A screw will protrude through this hole from the bottom and be countersunk to maintain a level surface. The protruding screw is where the second part of this housing will be attached. The second part of the housing is a $3 \mathrm{cmx5} 5 \mathrm{~cm}$ piece of $1 / 4$ " aluminum. There will be a threaded hole 15 mm in from both the 3 cm side and 5 cm side that will attach both pieces.

In order to further increase the linearity of the forces being distributed in each axis, stainless steel rod will be inserted into the $1 / 8$ " clear holes in the sides of each of the three housings.


Figure 3.7 - Exploded View of X and Y Guided System


Figure 3.8 - Exploded View of Corner Assembly

## 3.4 - Microscopy Methods Currently Used

In order to choose a suitable microscope to use in combination with our device, our group first decided on objectives and constraints needed in a microscope for it to be considered sufficient. There are many microscopes at our team's disposal, however we needed to carefully research each one in order to find the best fit.

## Objectives

Although not quite as important to achieve as constraints, objectives (if met) will make our device much more professional and successful. The device should move fluidly around the platform of our chosen microscope to provide a smooth transition between viewing cells. To achieve that our group wants to keep the device as light as possible to keep torque down which would increase friction with the surface. Also the device was designed with protecting the microscope in mind, considerations are included in the Device Description.

## Constraints

One of our main concerns was the ability to view cells through the Strex well. We could not successfully complete our project if the cells that we are stretching could not be viewed. Due to its importance, the ability to view cells through the Strex well fell under constraints. Another concern of the group was the dimensions of available microscopes. We want to view the cells being stretched in real time; therefore our device has to fit within the dimensions of a microscope's platform. The most important dimension needed in a proper microscope is the distance been the objective and the seeded cells on the Strex device. Through trial and error, we found that a 60X objective lens would be necessary to view cell movements such as cytoskeleton realignment. With that in mind, it is key to know that as the magnification strength increases in an objective lens the closer it needs to be to what it is viewing in order to be effective.

In order to precisely move the stretched Strex well around to view individual cells, a microscope with this ability was necessary. Given our constrained supplies, the group decided a movable X-Y platform would suffice for Strex well movement. However, movable X-Y plates take away from the overall height that our device could be so the group had to take that into consideration upon designing our project.

## Best-Fit Microscope

Through careful examination of available microscopes and much deliberation, the group was able to decide upon one microscope which would be utilized for our project. That microscope is the Axiovert CFL40. This is an inverted microscope which allows us more height to design our device with. Also, because it is inverted the light source comes from above while the objective lens comes from below. This allows sufficient light for viewing and the objective lens is able to move quite close to the Strex well to compensate for the increased magnification
of the 60X lens. The dimensions of the microscope platform allow ample space for our device to be anchored upon. A movable X-Y plate is already installed on this microscope which is beneficial, however, our group will need to design around the fact that it decreases the available height between the top of our device and the light source.

## 3.5 - Incubation Methods Currently Used

Systems are needed to control isolated environments to maintain specific conditions for cells. Generally speaking these conditions are the same; maintaining appropriate temperature, $\mathrm{CO}_{2} \& \mathrm{O}_{2}$ levels, humidity and pH . These systems can be applied to microscopy by using microscope cage incubators, which have been shown to work as well as regular bench-top incubators. Most of these cage incubators use gentle streams of warm air to control the temperature. To monitor the temperature most systems use a thermocouple is inserted into a reference well to control the temperature as close as possible to the sample. Most systems aim to ensure a specimen temperature stability of $\pm 0.1^{\circ} \mathrm{C}$.

These cage incubators can be designed for virtually any inverted or upright microscope that is available. These models are generally customizable for specific needs and include various chambers and interchangeable plate adapters which allow for the use of any cell culture support (petri, glass slides, multiwell plates, and others). These cages also are compatible with manual and digital $\mathrm{CO}_{2} / \mathrm{O}_{2}$ gas controllers. pH is generally controlled with buffers however, humidity is important to ensure water from the cell bath does not evaporate out of solution increasing the concentrations of potentially harmful chemicals. All these systems generally function at similar accuracies and specifications.

The amount of time the cells need to survive is related to the type of accuracy in a system one would look for. The longer a test needs to be run, the higher accuracy one needs to maintain appropriate temperatures for their samples.

## 4 - Device Validation

In order to ensure the device could work properly, validation had to be conducted on mechanical components as well as the ability of the device to view cells under the microscope during stretch. High density mapping was used to map strain fields in the well, and other tests were performed to validate the mechanical function of the device.

### 4.1 Test Trials - High Density Mapping

Precision and validation are key components to a successful project. Although the Strex manual states that the seeded cells are subject to the same strain as the well is being pulled to (BBridge International, 2009), we wanted to personally validate this fact on our own to confirm uniform strain across the well. To give our device supporting evidence, our group decided to use high density mapping, or HDM, as a validation tool. HDM is a technique that uses pixel displacement after a sample it stretched to map its strain (Kelly D et al., 2007). Kelly, along with other researchers, developed this technique to observe a heart over the course of a heartbeat. A camera capturing the sample's movement is connected to a computer, where the data is processed. Once the test is complete, the user can review the pictures side by side to see how far each area moved between frames. This information is exported to an excel spreadsheet to calculate the exact values (Kelly D et al, 2007). This tool is very useful for mapping strain at various points on the sample, to ensure the correct strain value is being used. This is exactly what we used the program for.

To validate the strain field, the Strex well was stretched to $20 \%$ strain. This value was chosen because it is the highest strain our device will stretch the well to. The area that was
focused on was a $20 \mathrm{~mm}^{2}$ region, because this is where the cells will lie during stretching. This region is shown in the figure below.


Figure 4.1: Hand stretched Strex well to 20\%

As a reference, there are 31 pixels per mm. The HDM program was set to record 16 pixel shifts for $32 \times 32$ pixel boxes. After the high speed camera, a Photron Fastcam 1280 PCI , captured the well being pulled to this strain, the images were compiled using MATLAB. The data was also filtered using a Gaussian filter by inputting the function fspecial('gaussian', hsize, sigma). The code for the compiling function is included in Appendix I. From there, the matrices for both the u and v displacement were totaled using the function utotal=sum(udata_s, 3). The s signifies that the data has been smoothed. Once the matrices were condensed, the strain was able to be calculated using the displacement of each point.

To calculate the strain, the displacement of one point was subtracted from the following point, and the total was divided by the pixel shift used. For example, $\left(\mathrm{X}_{1,2}-\mathrm{X}_{1,1}\right) / 16$ would be the formula for the first strain point in the X direction. $\left(\mathrm{Y}_{2,1}-\mathrm{Y}_{1,1}\right) / 16$ would be the formula for the first strain point in the Y direction. $\left\{\left[\left(\mathrm{X}_{2,1}-\mathrm{X}_{1,1}\right) / 16\right]+\left[\left(\mathrm{Y}_{1,2}-\mathrm{Y}_{1,1}\right) / 16\right]\right\} / 2$ would be the formula
for the first shear strain point. These calculations produced another set of matrices. MATLAB was then used to create a contour plot. The figures below show the X axis strain, the Y axis strain, and the shear strain respectively.


Figure 4.2: HDM results in x axis (top), y axis (middle) and shear strain (bottom)

The green color represents $20 \%$ strain, the warmer colors represent a higher strain, and the cooler colors represent a lower strain. As depicted in the pictures, the strain is fairly uniform across the selected section of the well. The abnormal pockets are most likely due to the method used to stretch the well. In the future, these strain fields can be used as a template to compare our device's strain percentages. This will ensure the wells are being homogeneously stretched to the correct strain.

### 4.2 Device Validation

- Arms reached well (well fit on device)

As seen earlier, the Strex well has holes 30mm apart that allow the device to mount to a stretch device. The arms of our device were obviously designed to work within this constraint. In both Equibiaxial and Uniaxial positions the arms reached the appropriate distance to allow the standoffs to slide into place.

- Speed Change

Using a stop watch and counting rotations change in rpms were validated. The device can accurately stretch between 0 and 1.9 Hz with increments of 0.5 Hz or 30 rpm .

- Both strain types work

The device was simply tested in both equibiaxial and uniaxial setting, and in both cases the device functioned as designed. The

- Can change \% strain

By utilizing the three off-centered holes on the top face of the crankshaft, strain percentages exhibited on the well can vary between 10,15 , and 20 percent.

- Stretched well
o The device was capable of stretching the well in both settings and the steel rods or linear bearings did not bind during the stretch.
- Device compatibility with Zeiss Microscope:


Figure 4.3: Stretch Device on Zeiss Microscope

The device was outfitted with an adapter below the $1 / 8$ " plate, the adapter had dimensions based of the X-Y stage of 5.25 " x 3.5 ". The adapter was checked to ensure it would mount securely in place, then clearance was checked to ensure it cleared the back of the moving piece. After $6 \mathrm{~cm}^{2}$ viewing area was milled in the center to allow the light to come down through the well. The hole also allows the arms to come down and allows enough space for them to move.
o Motion on scope:
Motion of the device and X-Y stage was simply validated by placing the entire device on the stage and checking the weight would not exceed the $\mathrm{X}-\mathrm{Y}$ stages weight limit. Also in the rear of the device acrylic spacers were used to support the weight of the motor and avoid scratching the stage.
o Viewing cells:
The arms on the linear guides drop to ensure the cells are close enough to the objective. The device was placed on the scope and the distance to the objective was checked. The objective could come up all the way to the bottom of the well using the coarse adjustment, using the fine adjustment the cells can be viewed in focus. A picture of the seeded cells was taken below on the Zeiss microscope to validate they in fact can be seen.


Figure 4.4: Rat Aorta Cells at 40X magnification with Hoescht and Phase Views

Additional Photos can be found in Appendix P.

- Heat Test:
o The motor was ran for an extended period of time (4 hrs) at room temperature and the temperature was taken every half hour. The results of the test show the temperature never reached a level that can damage the motor or ruin any data.

Table 4.1: Heat Test Results

| Heat Test Results |  |
| :---: | :---: |
| Time (hrs) | Temperature (Celcius) |
| 0 | 25 |
| .5 | 26 |
| 1 | 25 |
| 1.5 | 27 |
| 2 | 31 |
| 2.5 | 32 |
| 3 | 33 |
| 3.5 | 33 |
| 4 | 34 |

Graphical analysis of the heat test shows a steady but slow increase in temperature. From (Figure x ) using a best fit line the change in temperature attained was 2.5 degrees/hour, therefore for every hour the temperature increased only 2.5 degrees. If the test would have ran for another 4 hours the expected temperature would have been 45 degrees. This temperature is not very high nor is it anywhere near a temperature that could damage the motor or cause complications.


Figure 4.4: Graphical Results of Heat Test Over Time

## 5 - Conclusions and Recommendations

### 5.1 Conclusions

Our completed device was able to meet our constraints of achieving strip and equibiaxial stretch, however, the corner linear bearing needs to be moved to achieve this. The device can stretch at different rates by adjusting which hole on the flywheel the arm is attached to by simply changing the distance from the corner housing screw to the crank shaft hole or changing the hole distances on the arm. This change is described in Appendix E (Device General Use).

The device can be equipped to the Zeiss Axiovert CFL40 microscope and is capable of viewing cells during stretch, while placed on the microscope stage. The device fits in an incubator that fits around the Zeiss microscope to keep the cells alive during stretch. It can run for extended periods of time and the motor does not cause a change in temperature that will compromise the cells. The device is able to stretch cells at 1.9 Hz at percent strains of ten, fifteen, and twenty percent strain.

The overall cost of the device was around $\$ 880$, including the cost of shipping of various parts. This came in well under the $\$ 1000$ budget. A large component of this cost was the $\$ 250$ spent to purchase Strex wells. The wells had to be purchased in bulk from B-Bridge, but this will be useful for many future tests. If the initial cost of the Strex wells were removed, the cost of the device would be $\$ 630$, which is significantly less than the $\$ 1000$ budget. A complete budget breakdown can be seen in Appendix C.

### 5.2 Recommendations

First, we would suggest allowing the device to include variable duty cycles. This aspect would allow the user to study how cells react to variable loading and unloading rates. A resistor
can be used to change the speed of the motor to attain these variable duty cycles. Because the body is subject to many non-uniform stretching rates, observing cell reaction during these variable cycles can be extremely useful.

We also recommend making the device more compact. Currently, our device fits well on the microscope stage and allows for motion on the X-Y stage. However, a smaller and lighter device would allow for a much more marketable device. The majority of the device's bulk is derived from the motor. Using a smaller/lighter motor would decrease the device's weight significantly. Also using different materials could significantly lower the device's mass, many strong but light plastics are available for these types of applications.

As discussed before, our device can be modified to stretch cells either uniaxially or equibiaxially. This transition between strain types requires the user to follow several steps to before successfully changing the device setting (full directions can be found in Appendix E). In the future, we suggest creating an easier and more user friendly transition. For example, creating a sliding arm mechanism with a locking pin could accomplish this. This would allow for a shorter setup time for experiments and a reduction in the chance for error in the process.

Creating an easier transition between strain percentages would also be a useful aspect. Currently, there are multiple arms used for this transition (full directions can be found in the User Manual in Appendix E). Creating one arm that can transition between strain percentages would be much simpler. This can be accomplished by using Heim joints and a turnbuckle. This mechanism is shown in the figure below. Also by being able to lengthen the arm, the device could potentially be used to compress the wells.

Figure 5.1: Heim Joints (Lunsford, 2010).

Heim joints allow for smoother stretch, because they are designed for dynamic applications. The turnbuckle has opposing threads, so it can either bring rods closer together or farther apart. These threaded rods can be marked to indicate where the correct distance for each strain percentage is located. This design would allow for a much more user friendly transition.

Linear bearings on the inside of the housing on the X and Y linear guides would be a smart design alteration. They allow for more fluid motion and reduce the amount of friction in the system. Over time, this will be a better design because friction may cause the rods to bend, bind and possibly lead to other mechanical problems. Some linear bearings available through Igus can be seen below in Figure 5.2. The linear bearings come with plastic inserts to allow them to be run without lubrication, and will remove the metal on metal contact in the design. Another potential modification to minimize friction and increase fluidity of stretch would be to ball bearings at the point of contact between the arm and the crankshaft, as well as the arm and the corner housing.


Figure 5.2: Igus plain linear bearing (left) and Igus enclosed linear bearing (right) (Igus, 2010).

Another recommendation would be to find an easier method of determining what speed the motor is running at. Currently, the only way to determine this is through counting the number of rotations experienced over the course of a period of time. The best way to improve this issue would be through a tachometer, which can measure the revolutions per minute of the crank shaft. This would be an inexpensive purchase, but would just have to fit in the design space currently available.

One final recommendation is to use stainless steel arms instead of oil hardened arms. Stainless steel rods have a higher wear resistance and are already heat treated, the oil hardened arms are much more brittle and often difficult to work with. They are also become very brittle when welded, unless allowed to anneal properly. Some of our steel arms broke due to cooling to quickly when being TIG welded.

Overall these improvements would increase the user-friendliness of our device and would reduce the time to change between strip and equibiaxial strain. This device is an innovative approach to stretching cells and with these improvements can reduce time spent in the lab setting up experiments.

## References

Ananthakrishnan R, Ehrlicher A. (2007). The forces behind cell movement. Int J Biol Sci 3(5):303-317.

B-Bridge International, Inc. (2009). Life Sciences Products by STREX. http://lifesciences.bbridge.com/products/supplier/STREX/.

Balaban NQ, Schwarz US, Riveline D, Goichberg P, Tzur G, Sabanay I, Mahalu D, Safran S, Bershadsky A, Addadi L (2001). Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. Nat Cell Biol 3(5):466-472.

Bieler, F.H.; Ott, C.E.; Thompson, M.S.; Seidel, R.; Ahrens, S; Epauri, D.R.; Wilkening, U.; Schaser, K.D.; Mundlos, S.; Duda, G.N. (2009). Biaxial cell stimulation: a mechanical validation. Journal of Biomechanics, 42(11). doi:10.1016/j.biomech.2009.04.013.

Brown, Thomas D. (2000). Techniques for mechanical stimulation of cells in vitro: a review. Journal of Biomechanics, 33(1). Doi: 10.10/16/S0021-9290(99)00177-3.

Chen, C. S., Mrksich, M., Huang, S., Whitesides, G.M., Ingber, D.E. (1997). Geometric control of cell life and death. Science 276. doi: 276:1425-1428

Flexcell International Corporation, Initials. (2004). Flexcell international corporation. Retrieved from http://www.flexcellint.com/.

Fredberg, U., Stengaard-Pedersen K. (2008). Chronic tendinopathy tissue pathology, pain mechanisms, and etiology with a special focus on inflammation. Scandinavian Journal of Medical Science Sports 18(1)doi: 18(1):3-15.

Garvin, Joanne; Qi, Jie; Maloney, Melissa; Banes, Albert, PhD. (2003). Novel system for engineering bioartificial tendons and application of mechanical load. Tissue Engineering 9(5).

Hasel, Cornelia, Dürr, Suzanne, Brüderlein, Silke, Melzner, Ingo, and Möller, Peter (2002). "A cell culture system for long-term maintenance of elevated hydrostatic pressure with the option of additional tension" Journal of Biomechanics 35 :579-584.

He Y, Macarak EJ, Korostoff JM, Howard PS. (2004). Compression and tension: differential effects on matrix accumulation by periodontal ligament fibroblasts in vitro. Connect Tissue Res 45(1):28-39.

Hsu, Hui-Ju, Chin-Fu Lee, Andrea Locke, Susan Q. Vanderzyl, and Roland Kaunas. (2010). "Stretch-Induced Stress Fiber Remodeling and the Activations of JNK and ERK Depend on Mechanical Strain Rate, but Not FAK." Plos One 5.8 Print.

Igus, (2010).Plastics for Longer Life. http://igus.com/wpck/default.aspx?PageNr=3586\&CL=USen\&scroll=367\&step=5

Juliano RL, Haskill S. (1993). Signal transduction from the extracellular matrix. Journal of Cell Biology 120(3):577-585.

Kelly DJ, Azelogu EU, Kochupura PV, Sharma GS, Gaudette GR (2010). Accuracy and reproducibility of a subpixel extended phase correlation method to determine micron level displacements in the heart. Medical Engineering and Physics 29 (2007) 154-162

Lemmon CA, Chen CS, Romer LH. (2009) Cell traction forces direct fibronectin matrix assembly. Biophys J, 96(2):729-738.

Lunsford Racing. (2010). Parts and Accessories. http://www.lunsfordracing.com/mm5/merchant.mvc.

Norton, Louis A.; Andersen, Kim L.; Arenholt-Bindslev, Dorothe; Anderson, Lis, Melsen, Birte. (1995). A methodical approach of shape changes in human oral cells perturbed by a simulated orthodontic strain in vivo. Archs oral Biology, 40(9). doi:0003-9969/95.

Rehfeldt F, Engler AJ, Eckhardt A, Ahmed F, Discher DE. (2007). Cell responses to the mechanochemical microenvironment--implications for regenerative medicine and drug delivery. Adv Drug Deliv Rev 59(13):1329-1339.

Tanaka, Shiego M. (1999). A new mechanical stimulator for cultured bone cells using piezoelectric actuator. Journal of Biomechanics 32. 427-430.

Torzilli, Peter A; Grigiene, Rita; Huang, Charles; Friedman, Steven M; Doty, Stephen B; Boskey, Adele L; Lust, George. (1996). Characterization of cartilage metabolic response to static and dynamic stress using a mechanical explant test system. Journal of Biomechanics. 30 (1). Doi: S0021-9290(96)00117-0.

Triantafillopoulos, Ioannis K; Banes, Albert, MD; Bowman, Karl, PhD; Maloney, Melissa; Garrett, William E; Karas, Spero G. (2004). Nandrolone decanoate and load increase remodeling and strength in human supraspinatus bioartificial tendons. The American Journal of Sports Medicine, 32(4). doi:10.1177/0363546503261700.

Vandenburgh, Herman H. (1988). A computerized mechanical cell stimulator for tissue culture: effects on skeletal muscle organogenesis. In Vitro Cellular \& Developmental Biology 24 (7). doi: 10.1007/BF02623597.

Wang, D., Xie, Y., Yuan, B., Xu, J., Gong, P., Jiang, X. (2010). A stretching device for imaging real-time molecular dynamics of live cells adhering to elastic membranes on inverted microscopes during the entire process of the stretch. Integrative Biology 5-6 doi: 10.1039/B920644B.

Wang, H., Ip, W., Boissy, R., Grood, E.S. (1995). Cell orientation response to cyclically deformed substrates: experimental validation of a cell model. Journal of Biomechanics 28(12) doi:10.1016/0021-9290(95)00101-8.

Wang, J., Li, B. (2010). Mechanics rules cell biology. Sports Medicine, Arthroscopy, Rehabilitation, Therapy \& Technology 2(16) doi: 10.1186/1758-2555-2-16.

Wang JH, Thampatty BP (2008). Mechanobiology of adult and stem cells. Int Rev Cell Mol Biol 271:301-346.).

Wang J.H., Iosifidis, M.I., Fu, F.H. (2006). Biomechanical basis for tendinopathy. Clinical Orthopaedics and Related Research 433. doi: 10.1097/01.blo.0000195927.81845.46.

Wang, J., Yang, G., Li, Z. (2005). Controlling cell responses to cyclic mechanical stretching. Annals of Biomedical Engineering 33(3). doi: 10.1007/s10439-005-1736-8.

Williams, J.L.; Chen, J.H.; Belloli, D.M. (1992). Strain fields on cell stressing devices employing clamped circular elastic diaphragms as substrates. Journal of Biomechanical Engineering 114(3) doi: 10.1115/1.2891398.

WM Berg. (2010). MIN-E-PITCH 3D SERIES GEARS. http://www.wmberg.com/catalog/product.aspx.

Yang GG, Crawford RC, Wang JHC(2004). Proliferation and collagen production of human patellar tendon fibroblasts in response to cyclic uniaxial stretching in serum-free conditions. Journal of Biomechanics 37(10):1543-1550.

## 6.1 - Appendix A - Pairwise Comparison Chart

This was filled out by Professor Billiar (the client) in order to determine the necessities of tasks to be completed for this design.

|  | Homeostatic <br> Conditions | Biaxial <br> Stretch | Inexpensive | Culture <br> Viewing <br> Area | Duration <br> of <br> Stretch | Variable <br> Duty <br> Cycles | Total | Importance |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Homeostatic <br> Conditions | 1 | 0 | 1 | 1 | 0.5 | 0 | 2.5 | 2 |
| Biaxial <br> Stretch | 1 | 1 | 1 | 1 | 1 | 4 | 1 |  |
| Inexpensive | 0 | 0 | 0.5 |  | 0 | 0 | 0.5 | 3 |
| Culture <br> Viewing <br> Area | 0 | 0 | 0.5 | 0 | 0 | 0.5 | 3 |  |
| Duration of <br> Stretch | 0.5 | 0 | 1 | 1 |  | 0 | 2.5 | 2 |
| Variable <br> Duty Cycles | 1 | 0 | 1 | 1 | 0 |  | 4 | 1 |

## 6.2 - Appendix B - Functions Means Tree

1. Strip Biaxial stretch $2-20 \%$ at different rates of loading/unloading
a. One motor with other side anchored (spreading anchor)
b. Pull at uniform strain rate
2. Equal Biaxial stretch To perform biaxial stretch
a. Two motors pulling at equal rates
b. One motor with linkage mechanism
c. One motor modified to pull in two opposing directions
3. Homogeneous strain area
a. Pull membrane in two directions
i. 4 motor system, all moving at the same rates
ii. 2 motor system in circuits
iii. 4 motor system with linkages
b. Possibly achievable with strip biaxial stretch
4. Control temperature and pH of the media to maintain homeostasis
a. Incubator
b. Cell humidifier
c. Hot air heater
d. Acrylic polymer cage
5. See visual cell response to stimuli
a. Microscope (inverted)
b. Photography
6. Variable Duty cycles
a. Stepper motor
b. Pneumatics
c. Hydraulics
7. Usability
a. Computer program to integrate all functions to perform when needed
b. Compatible with computer system

## 6.3 - Appendix C- Budget Breakdown

| Date | Vendor | Desc. | Cost | Shipping | Total |
| :--- | :--- | :--- | ---: | ---: | ---: |
| 1/19/2011 | BBridge | Stretch Chamber | $\$ 250.00$ | $\$ 31.00$ | $\$ 281.00$ |
|  | McMaster- |  |  |  |  |
| 2/18/2011 | Carr | Steel Rods \& Sheets | $\$ 64.21$ | $\$ 9.75$ | $\$ 73.96$ |
| 2/18/2011 | IGUS | IGUBAL Rod end | $\$ 20.40$ | $\$ 5.49$ | $\$ 25.89$ |
| 2/23/2011 | IGUS | Rail \& Float | $\$ 57.71$ | $\$ 5.49$ | $\$ 63.20$ |
| 3/21/2011 | S\&S Tech | Electric motor | $\$ 129.99$ | $\$ 10.95$ | $\$ 140.94$ |
| $4 / 11 / 2011$ | WM Berg | Pulleys \& chains | $\$ 65.76$ | $\$ 50.00$ | $\$ 115.76$ |
|  |  | IGUBAL flange |  |  |  |
| $4 / 11 / 2011$ | IGUS | bearing | $\$ 7.99$ | $\$ 5.49$ | $\$ 13.48$ |
| $4 / 11 / 2011$ | Lyn-Tron | Standoffs (samples) | $\$ 0.00$ | $\$ 0.00$ | $\$ 0.00$ |
| $4 / 15 / 2011$ | Vangy Tool | Axis Arm | $\$ 155.00$ | $\$ 0.00$ | $\$ 155.00$ |
|  | Brierly |  |  |  |  |
| 4/15/2011 | Lombard | M2 tap | $\$ 14.00$ | $\$ 0.00$ | $\$ 14.00$ |
| Total |  |  |  |  | $\$ 883.23$ |
| Budget |  |  |  |  | $(\$ 624.00)$ |

## 6.4 - Appendix D- Strain Parameter Examples

## Examples of strain parameters



- 10\% Stretch \& 10\% Compression of Cells

Strain Program: 20\% stretch or compression


Use the Chamber Length Adjustment Knob to manually stretch the chamber. Use the knob to adjust for the seeding and start positions. Each $360^{\circ}$ turn of the knob changes the total chamber length by $5 \%$. or 1 mm . ST-CH-04-XY chamber length is 20 mm .

## 6.5 - Appendix E- General Use Manual

1. Strain Type Control

The arms in the X-axis \& Y-axis have slots to control the type of strain, as the corner linear guide/crank shaft move from one angle to another the arm lengths should change as well. To move from equibiaxial strain to uniaxial strain as seen below four steps need to be taken:

1. Loosening of the motor placement screw and switching from the shorter biaxial belt to the longer uniaxial belt.

- The motor should be loosened and the belt should be removed first, however, the belt being put on should be placed and secured last.

2. Removal of crank shaft screw (4)

- The crank shaft is also secured by four 4-40 screws, this must be done before the third step.

3. Loosening of $\mathrm{X} / \mathrm{Y}$-axis screws (8)

- After loosening not removing the screws securing the $\mathrm{x} \& \mathrm{y}$ arms, slide and lock arms to desired position. These are M2x. 4 screws and require a small flat screw driver.

4. Removal of linear guide screws (4)

- Four M2x. 4 screws secure the Igus linear guide in the top right corner of the device need to be removed to slide the guide to and from strain types.



## 2. Strain Percent Control

Available strain percentages include 5, 10 and 15 percent strain. In the diagram below the arm is tagged by the red arrow, the housing connecting screw is indicated by the blue arrow and the strain percent positions are indicated by the black arrow (three 256 threaded holes), and the arm/crank shaft connecting point is indicated by the green arrow. Due to the closeness of the crank shaft holes the device will require separate arms for each strain percent. Changing strain percent is simple it requires two steps:

## 1. Removal of arm

- Two nuts and washers (green \& blue positions) hold the arm down, they simply need to be removed to remove the arm.

2. Addition of new arm

- After removing the unwanted arm the new arm can be put in place (does not matter which way) then the washers and nuts should be securely re-tightened. Each arm should be labeled which strain percent it is for ( $5 \%, 10 \%$,and $15 \%$ ).


3. Motor Control

The motor being used puts out 8.4 Nm at 35 rpm for torque which is more than enough to stretch the silicone wells. However, a 1:3 gear ratio is also being used, (this reduces the torque to 2.6 Nm ) which brings the top speed from 35 rpm to 114 rpm or 1.9 Hz . The motor is capable of turning clockwise or counterclockwise, and is also capable of variable speeds.
4. Running Device

When running the device to ensure wanted results the crank shaft must start at the zero strain point, this is to ensure the well is stretched and not compressed. Therefore before securing the belt in place the crank shaft should be already adjusted for whichever strain percentage is desired.
5. Strex Well Use

Adhesiveness and strength of chamber is not guaranteed for more than 3 uses. The chambers are durable enough to withstand approximately 900,000 stretches of $20 \%$. Also the wells should not ever be stretched past $20 \%$, because strain past $20 \%$ will significantly decrease the integrity of the silicone (B-Bridge International, Inc. 2009).

## 6. Mounting Strex Well

The Strex well has 4 pin holes that are 3 mm in diameter, and these holes are used to mount the well to the stretch device. The holes are 30 mm apart from their center, and $1 / 8$ " outer diameter standoffs will be used to secure the well. The $1 / 8$ " is slightly larger than 3 mm , allowing the standoffs to provide a snug fit limiting vibration and motion in the Z-axis.

(B-Bridge International, Inc. 2009)
7. Autoclaving Strex Well

Sterilize chambers in an autoclave for 20 minutes at $121^{\circ} \mathrm{C}$. The silicone chambers can withstand temperatures up to $180^{\circ} \mathrm{C}$. Use of an autoclave is preferable. However, if an autoclave is not available, the chambers may be sterilized by
submerging them in $70 \%$ ethanol, rinsing with water, then drying in a sterile environment. Place the sterile silicone chambers in a Petri dish in preparation for coating (B-Bridge International, Inc. 2009).

## 8. Preparing Strex Well

- Protein Coatings (Strex) (B-Bridge International, Inc. 2009)


## Fibronectin Coating

Preparation of fibronectin solution:

1. Dilute human or bovine fibronectin to a final concentration of 50 to $100 \mu \mathrm{~g} / \mathrm{ml}$ in Phosphate Buffered Saline (PBS)
Coating with fibronectin solution:
2. Pour 3-6 ml of the fibronectin solution into each strain chamber
3. Incubate at 37 oC for more than 30 minutes.
4. Aspirate the fibronectin solution. If coating is successful, water will not be repelled after removing the fibronectin solution.
5. The liquid solution can be used to coat 3 or 4 chambers before discarding.

| PBS (per liter): |  |  |
| :--- | :--- | :---: |
| NaCl | 8.00 g |  |
| KCl | 0.20 g |  |
| $\mathrm{Na}_{2} \mathrm{HP}_{4}$ (anhyd.) | 1.15 g |  |
| $\mathrm{KH}_{2} \mathrm{PO}_{4}$ (anhyd.) | 0.20 g |  |
|  |  |  |

Note: Dubecco's PBS in powder form for tissue cuture applications is commercially available.
(B-Bridge International, Inc. 2009)

## $>$ Gelatin Coating

Preparation of gelatin solution:

1. Add gelatin powder to PBS at a concentration of 2\%
2. Autoclave the mixture to dissolve and sterilize

Coating with gelatin solution:
3. Pour 3-6 ml of the gelatin solution into each strain chamber
4. Incubate at $37^{\circ} \mathrm{C}$ for more than 30 minutes.
5. Aspirate the gelatin solution. If coating is successful, water will not be repelled after removing the gelatin solution.
6 . The liquid solution can be used to coat 3 or 4 chambers before
discarding.

## - Collagen Coating (Cellmatrix 1-C, P, Type 3 or 4)

Preparation of collagen solution:

1. Combine 1 part collagen to 10 parts $\mathrm{HCl}, \mathrm{pH} 3$, in a sterile tube Coating with collagen solution:
2. Coat chamber with a thin layer
3. Aspirate excess
4. Dry in biological safety cabinet at $25^{\circ} \mathrm{C}$ or below. The chamber can be stored at the same temperature.
5. Wash the chamber twice with culture medium. If coating is successful, water will not be repelled.

- Cell Culture (B-Bridge International, Inc. 2009)

Seed cells at the appropriate concentration in the freshly coated chamber. Do not over expose the cells to dissociation enzymes. Cells should be treated in the same manner (type and concentration of enzyme, temperature, and time for digestion) for all experiments.

Cells should not be cultured at a high cell density in the chambers. For example, epithelial cells often form a cell-sheet and the cell-cell adhesion seems to be stronger than a cell-surface adhesion. When this happens cells may detach from the chamber. Additionally, cultures that are grown over a week in the chambers may detach.

After overnight incubation, inspect cells with the microscope to ensure that they have adhered to the chamber.

## 9. Strex Well Issues

The PDMS (silicone) chambers are unfortunately very hydrophobic with two methyl-base on the surface. Cells adhere to surfaces coated with fibronectin or collagen through integrins. Compared to adhesion on plastic or glass wells Strex wells are slightly
different because they are not charged. If the cells on the Strex well are not attaching or are detaching during stretch, a higher concentration of either fibronectin or silicone may be necessary. These coatings of course must be applied before culturing the cells. (BBridge International, Inc. 2009)

## 10. Additional Information

- If there are wrinkles or bubbles on the bottom surface of the strain chamber when seeding cells this is because of the wells thin structure. The well is designed not to have any heterogeneous regions, though it is difficult to achieve. B-Bridge recommends using a small volume of ethanol in a Petri dish and lightly placing the chamber in the culture dish starting at one edge and moving toward the opposite edge of the chamber to remove air bubbles between the dish and chamber. The ethanol obviously must be evaporated before spreading a cell suspension on the chamber. (B-Bridge International, Inc. 2009)
- Obtaining protein or mRNA samples from the cells:

1. Proteins for Western blotting: Wash the cells once with PBS. Add SDS-PAGE sample loading dye directly into the chamber, and collect the cell extract by using a cell scraper (B-Bridge International, Inc. 2009).
2. Proteins for Immunoprecipitation: Wash the cells once with PBS. Add cell extract buffer directly into the chamber, and collect the cell extract by using a cell scraper (B-Bridge International, Inc. 2009).
3. RNA: Wash the cells once with PBS (for RNA preparation). Add RNA extraction buffer directly into the chamber, and collect the cell extract by using a cell scraper (B-Bridge International, Inc. 2009).
6.6 - Appendix F- Design Drawings


C4 Nodes: $\begin{aligned} & \text { The minimum leasth fer this ain shout } \\ & 5 \mathrm{~cm} \text {. }\end{aligned}$







.


This is the $X$ - $Y$ stage adaptor that attaches below the base in order to maintain a level device while on the microscope stage.


## 6.7 - Appendix G- Ordered Parts

| 3-D PULLEYS $\Rightarrow 3$ day lead time |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| CIR. PTTCH | BORE | strle | materials | berge - name | beit |
| 4 mm | ฮ8 | pin hue | coly | Min-E-Pitch ${ }^{\circledR}$ Three D Drive |  |



| $\begin{gathered} \text { STOCK } \\ \text { NO. } \\ \hline \end{gathered}$ | NO. OF TEETH | $\begin{aligned} & \mathrm{PITCH} \\ & \text { DIA. } \\ & \hline \end{aligned}$ | $\begin{gathered} \text { OUTSIDE } \\ \text { DIA. } \\ \hline \end{gathered}$ |  |
| :---: | :---: | :---: | :---: | :---: |
| 3TDP263A-12 | 12** | 15.22 | 16.80 | 40300 |
| 3TDP263A-13 | 13** | 16.49 | 18.07 | 4* \% < |
| 3TDP263A-14 | 14** | 17.76 | 19.34 | \% |
| 3TDP263A-15 | 15** | 19.03 | 20.61 |  |
| 3TDP263A-16 | 16. | 20.29 | 21.87 | $5 \frac{5}{5}$ |
| 3TDP263A-17 | 17 | 21.56 | 23.14 | at may |
| 3TDP263A-18 | 18 | 22.83 | 24.41 | $\cdots \mathrm{x}$ - 4 m |
| 3 3TDP263A-19 | 19 | 24.10 | 25.68 | - |
| 3TDP263A-20 | 20 | 25.37 | 26.95 |  |
| 3TDP263A-22 | 22 | 27.90 | 29.48 |  |
| 3TDP263A-24 | 24 | 30.44 | 32.02 |  |
| 3TDP263A-25 | 25 | 31.71 | 33.26 |  |
| 3TDP263A-26 | 26 | 32.98 | 34.56 |  |
| 3TDP263A-28 | 28 | 35.51 | 37.09 |  |
| 3TDP263A-30 | 30 | 38.05 | 39.63 |  |
| 3TDP263A-32 | 32 | 40.59 | 42.17 |  |
| 3TDP263A-35 | 35 | 44.39 | 45.97 |  |
| 3TDP263A-36 | 36 | 45.66 | 47.24 |  |
| 3TDP263A-40 | 40 | 50.47 | 52.32 |  |
| 3TDP263A-45 | 45 | 57.10 | 58.66 |  |
| 3TDP263A-50 | 50 | 63.42 | 65.00 |  |
| 3TDP263A-55 | 55 | 69.76 | 71.34 |  |
| 3TDP263A-60 | 60 | 76.10 | 77.68 |  |
| 3TDP263A-65 | 65 | 82.45 | 84.02 |  |
| 3TDP263A-70 | 70 | 88.79 | 90.37 |  |
| 3TDP263A-75 | 75 | 95.13 | 96.71 |  |
| 3TDP263A-80 | 80 | 101.47 | 103.05 |  |

* Sprockets with ø19.0mm P.D. and smaller are recommended for ider use only.

For 12-16 teeth, hub diameter equals 10.0
Other numbers of teeth are available on request.
Stainless Steel equivalent availabie.
Teeth could be anodized.

3-D BELT $\Rightarrow 1$ day lead time

| CIRCULAR PITCH | MATERIALS | BERG'S Q NAME | PULLEY |
| :---: | :---: | :---: | :---: |
| $\mathbf{4 m m}$ | Polyurethane (Green) <br> 8mm Di. <br> Stainless Steel Cable* | Min-E-Pitch® | Operates with 3TDP, 3TF, <br> and 3MTB series. |



| $\begin{aligned} & \text { STOCK } \\ & \text { NO. } \end{aligned}$ | No. OF PITCHES | LENGTH <br> (Ref.) |
| :---: | :---: | :---: |
| 3TDF-30-E | 30 | 120.0 |
| 3TDF-35-E | 36 | 144.0 |
| 3TDF-40-E | 40 | 160.0 |
| 3TDF-45-E | 46 | 184.0 |
| 3TDF-50-E | 50 | 200.0 |
| 3TDF-55-E | 56 | 224.0 |
| 3TDF-60-E | 60 | 240.0 |
| 3TDE-70-E | 70 | 280.0 |
| 3TDF-80-E | 80 | 320.9 V |
| 3 TOF-90-E | 90 | 360.0 |
| 3TDF-100-E | 100 | 400.0 |
| 3TDF-110-E | 110 | 440.0 |
| 3TDF-120-E | 120 | 480.0 |
| 3TDF-130-E | 130 | 520.0 |
| 3 TDF-140-E | 140 | 560.0 |
| 3TDF-150-E | 150 | 600.0 |
| 3TDF-160-E | 160 | 640.0 |
| 3TDF-170-E | 170 | 680.0 |
| 3TDF-180-E | 180 | 720.0 |
| 3TDF-190-E | 190 | 760.0 |
| 3TDF-200-E | 200 | 800.0 |
| 3TDF-210-E | 210 | 840.0 |
| 3TDF-220-E | 220 | 880.0 |
| 3 TDF-230-E | 230 | 920.0 |
| 3TDF-240-E | 240 | 960.0 |
| 3 TDF-250-E | 250 | 1000.0 |
| 3 3TDF-260-E | 260 | 1040.0 |
| 3TDF-270-E | 270 | 1080.0 |
| 3TDF-280-E | 280 | 1120.0 |
| 3TDF-290-E | 290 | 1160.0 |
| 3TDF-300-E | 300 | 1200.0 |
| 3 3TDF-310-E | 310 | 1240.0 |
| 3 TDF-320-E | 320 | 1280.0 |
| 3TDF-330-E | 330 | 1320.0 |
| 3TDF-340-E | 340 | 1360.0 |
| 3TDF-350-E | 350 | 1400.0 |
| 3 TDF-370-E | 360 | 1440.0 |
| 3 TDF-400-E | 400 | 1600.0 |
| 3 TDF-440-E | 440 | 1760.0 |






## MARHINE SEREWS


 4



WASHERS
Elat Washers - Military SpectficatiofivM alenery mation Stainiless Steel (suty worcilymi USA

- Manufactured from 18-8 stainless steel, these washers are burr-free and passivated to meet stringent military specifications.
- Conform to MS, AN or NAS chemical and physical specifications - MS washers $\dagger$ h
- "The " $L$ " in the Dash Number column refers to the light (thininer)

series of washers


F436 Structural Washers

- Made to ASTM F436 specificatioris * For structural applicatiensid

| $\begin{array}{\|l} \hline \text { Scient } \\ \text { Sze } \end{array}$ | $0 D, 1 D$ | $\begin{array}{ll}\text { Thick. (In.) } & \text { Pkg. } \\ \text { Min. } & \text { Max. } \\ \text { Oty. }\end{array}$ | $\begin{gathered} \text { Plain Steel in } \\ \text { der } \equiv \text { Price/100 } \end{gathered}$ |  | $\begin{gathered} \text { Trew Givival } \\ \text { Ordef } i \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |
| 1/ | 3/8 $\quad 1 / 22^{\circ}$ | . 051.080100 | 07812442 | \$5.15 | 7028 |
| 5/16 | $11 / 16.11 / 32$ | , 051 080 100 | ;07812217 | 5.87 | 12028 |
| 3/8 | 13/16 13/32 | -051-080-100 | 07812183 | 7.38 | 17028 |
| $1 / 2$ | 11/16 17/32 | 097, 177 | 07812134 | 14.29 | 2028418 |
| 5/8 | 15/16 11/16 | 122:177 25 | -77812209 | 22.28 | 72028 |
| $3 / 4$ | 115/32 $13 / 16$ | $122,177,20$ | 07812191 | 29.10 | \% 7228 |
| 7/8 | 13/4 15/16 | . 136.17715 | 07812225 | 48.26 | 77028421 |
| 1 | 11/8 | 136:177 10 | 07812126 | 53.09 | 17028429 |
| 11/8 | $\begin{array}{ll}21 / 4 & 11 / 4\end{array}$ | $136.177 \%^{\circ}$ | 0788159 | 91.45 | $\sqrt{2028423}$ |
| 11/4 | $21 / 2 \quad 136$ | $136.177-5$ | 07812175 | 94.19 | 102842 |
| $11 / 2$ | $3 \quad 15$ | .136.177 5 | 07812167 | 182.04 | 1028425 |

Flat Washers - Extra Thick . ose.


Metric Flat Washers Corrosion Resistant

- High visibility blue color allows the user to easily distinguish the - High visibility blue color-allows the user to easily distinguish.the

| $\begin{aligned} & \text { Bolt } \\ & \text { Size } \\ & \hline \end{aligned}$ | $(\mathrm{min})=0 \mathrm{~mm})$ | Thick Pkg. Pk. $(\mathrm{mm}): O t y$ | eloordect | Pincedoo |
| :---: | :---: | :---: | :---: | :---: |
| M6 | 7.412 .0 | $3.00 \quad 100$ | 79207718 | \$28,74 |
| M8 | $9.6-17-0$ | $3.50-100$ | 79207726 | 29.8 |
| M10. | 12.0 215 | $4.50 \quad 100$ | 779207734 | 36.8 |
| M12 | 15.0 0 | $4.60 \quad 100$ | 79808028 | 59 |
| -M16 | 18:4*34.0 | 14.60-50 | ${ }^{7} 79808036$ | 99,2. |
| M20 | 1022.5 42.5 | 4.60 | 798089446 | 10. |
| -M24 | 26,5 50 | \& $4,60 \sim 25$ | $479808809^{\text {a }}$ | 129, 6 |
| M30 | 3386, 60.0 | 4,60 $\quad 20$ | 179808069 | 190\% |
| \%.e9 | if | ).1.0. |  |  |













Het Set Screws - Alloy Steel Convenience Packs ,

UMAB-to-the-source accountability




Page | 102

6.8-Appendix H- Custom Parts



Page | 105





Page | 109




Page | 112





Page | 116



Page | 118




Page | 121


## 6.9 - Appendix I- HDM Matlab Compile Function

This Matlab code was used to compile the over 100 images taken by the camera and put them into matrices.

```
function compile_data(dataset,framerate,res,loadext)
%COMPILE_DATA Compiles and stores data from the HDM Program
% Imports and reads the HDM data from the input video folder. This data
% is then stored in a .mat file as a large multidim matrix along with
```

\% several descriptive variables to identify the data.
\%\% Prepare global variables
disp('*************************************************************) ;
disp(['Compiling data for: ',dataset])
tic;
top_dir=['data_store\', dataset, '\'];
\%\% Verify input
if framerate < 1
error('framerate must be a real positive number')
end
if ~exist('loadext','var')
error('loadext input must be specified')
end
\%\% Create the output directories
disp('>>Creating output directories');
if ~exist([top_dir,'HDM_Data\'],'dir')
mkdir([top_dir,'HDM_Data\'])
end
if ~exist([top_dir, 'matlab_data\'],'dir')
mkdir([top_dir,'matlab_data\'])
end
\%\% Check for the existence of HDM data and move it to the correct folder
disp('>>Checking HDM input');
\% Check for the data files in the correct location
if $\sim e x i s t\left(\left[t o p \_d i r, ' H D M \_D a t a \backslash s t a r t 00+i m-0001 \_U . d a t '\right], ' f i l e '\right)$
\% Check for files in the images folder
if ~exist([top_dir,'images\start00+im-0001_U.dat'],'file')
\% Return an error
error('The HDM Data does not exist for this dataset');
else
\% Move the files to the correct location
movefile([top_dir,'images ${ }^{*}$.dat'], [top_dir,'HDM_Data\']);
movefile([top_dir,'images\*.txt'], [top_dir,'HDM_Data\']);
end
end
\%\% Load the correlation report file into variables
disp('>>Loading correlation report')
\%Open summary file and extract xy corner data throughout the data
\%collection process
file=fopen(strcat(top_dir,'HDM_Data\CorrelationReport.txt'));
textscan(file, '\%s\%s',1);
str='\%*s \%*s \%u \%*s \%u \%d \%d \%d \%d \%*s \%d \%*s \%d';
rawdata=textscan(file,str);
$x x y y=d o u b l e([r a w d a t a\{3\}$ rawdata\{5\} rawdata\{4\} rawdata\{6\}]);
subsize=double(rawdata\{1\}(1,1));\%\#ok<NASGU>
shift=double(rawdata\{2\}(1,1));
pixelshift=double([rawdata\{7\} rawdata\{8\}]);\%\#ok<NASGU>
fclose(file);

```
    clearvars file str rawdata
%% Prepare to loop through the dataset
    disp('>>Preparing to loop through dataset')
    % Overapproximate the width of the data matrix
    cols = round(1.1*(xxyy(1,2)-xxyy(1,1))*shift);
    % Create a format string for data processing
            format='%9f';
            for i = 2:cols
                format = [format,' %9f']; %#ok<AGROW>
            end
    % Load the first data file and determine the exact # of columns
            filename = [top_dir,'HDM_Data\start00+im-0001_U.dat'];
            file = fopen(filename);
            testdata=textscan(file,format,'multipledelimsasone',...
                1,'collectoutput',1,'emptyvalue',0);
            testdata=testdata{1};
            for i = 1:cols
            if testdata(1,i)==0
                break;
            end
            end
            cols = i-1;
            fclose(file);
    % Create the final format string for data processing
    format='%9f';
    for i = 2:cols
            format = [format,' %9f']; %#ok<AGROW>
            end
    % Determine the number of rows and columns in the data file
    file = fopen(filename);
    testdata=textscan(file,format,'multipledelimsasone',...
            1,'collectoutput',1,'emptyvalue',0);
            testdata=testdata{1};
            [rows,cols]=size(testdata);
    % Determine the number of data files to be processed
            filelist = dir([top_dir,'HDM_Data\*.dat']);
            filecount = numel(filelist)/2;
            fclose(file);
    clearvars filename file testdata filelist
%% Prepare data filter
    hsize=[res,res];
    sigma = 0.5;
    filt = fspecial('gaussian', hsize, sigma);
%% Loop through each data file
    disp('>>Beginning loop');
    % Preallocate memory to output variables
            udata_r = zeros(rows,cols,filecount);
            udata_s = zeros(rows,cols,filecount);
            vdata_r = zeros(rows,cols,filecount);
            vdata_s = zeros(rows,cols,filecount);
            errorcount = 0;
            bar= waitbar(0,'Compiling...');
    % Begin time loop
```

```
    tstart=now();
    for t = 0:filecount-1
    waitbar((t+1)/filecount,bar);
    %disp(['>>>>Compiling data at time ',num2str(t+1),' of
',num2str(filecount)]);
    %% Determine file names
    if t==0
        uf='start00+im-0001_U.dat';
        vf='start00+im-0001_V.dat';
    elseif t==(filecount-1)
        uf=['im-',numpad(t,4),'+end0000_U.dat'];
        vf=['im-',numpad(t,4),'+end0000_V.dat'];
    else
        uf=['im-',numpad(t,4),'+im-',numpad(t+1,4),'_u.dat'];
        vf=['im-',numpad(t,4),'+im-',numpad(t+1,4),'_V.dat'];
    end
    uf = [top_dir,'HDM_data\',uf]; %#ok<AGROW>
    vf = [top_dir,'HDM_data\',vf]; %#ok<AGROW>
%% Process X-Direction data
    curfile = fopen(uf);
    try
        rawdata = textscan(curfile,format,'multipledelimsasone',...
                1,'collectoutput',1,'emptyvalue',0);
    catch err
        err,
    end
    rawdata = rawdata{1};
    smoothdata = filter2(filt,rawdata);
    %errorcount = errorcount + error1;
    udata_r(:,:,t+1)=rawdata;
    udata_s(:,:,t+1)=smoothdata;
    fclose(curfile);
    %% Process Y-Direction data
    curfile = fopen(vf);
    rawdata = textscan(curfile,format,'multipledelimsasone',...
                1,'collectoutput',1,'emptyvalue',0);
    rawdata = rawdata{1};
    smoothdata = filter2(filt,rawdata);
    vdata_r(:,:,t+1)=rawdata;
    vdata_s(:,:,t+1)=smoothdata;
    fclose(curfile);
%% Calculate remaining time and update waitbar
        tleft = ((1-((t+1)/filecount))/...
        ((t+1)/filecount))*(now()-tstart);
    waitbar((t+1)/filecount,bar,...
            ['Estimated time remaining:',datestr(tleft,'HH:MM:SS')]);
end
delete(bar);
%errpercent = errorcount/(rows*cols*filecount); %#ok<NASGU>
% Clear extra variables
    clearvars uf vf curfile rawdata smoothdata error1 errorcount t
%% Mark the area of interest in the first image and save it
```

```
    disp('>> Creating overlay image');
    % Load image
        srcimg = imread([top_dir,'images\start00.tif']);
    % Create RGB image container
        outimg(:,:,1) = double(srcimg);
        outimg(:,:,2) = double(srcimg);
        outimg(:,:,3) = double(srcimg);
    % Create a polygon over the region of interest
        x = [xxyy(1,1),xxyy(1,2),xxyy(1,2),xxyy(1,1)];
        y = [xxyy(1,3),xxyy(1,3),xxyy(1,4),xxyy(1,4)];
        [h,w]=size(srcimg);
        poly = poly2mask(x,y,h,w);
    % Reduce the blue and green channels in the ROI
        poly=double(poly);
        poly(poly == 1) = 0.6;
        poly(poly == 0) = 1;
        outimg(:,:,2) = round(outimg(:,:,2).*poly);
        outimg(:,:,3) = round(outimg(:,:,3).*poly);
        outimg=uint8(outimg);imshow(outimg);
    % Save the image to the matlab data directory
        imwrite(outimg,[top_dir,'matlab_data\overlay2.tif'],'tiff');
%% Check for external data and process
    if loadext > 0
        disp('Getting external data files');
    % Check if the external data mat file exists, if not run the helper
        if ~exist([top_dir,'matlab_data\ext_data.mat'],'file')
            comp_ext_helper(dataset);
        end
        load([top_dir,'matlab_data\ext_data.mat']);
    end
%% Export data variables to compiledhdm.mat
    disp('>>Saving data file')
    clearvars bar cols filecount format h i loadext outimg poly rows...
        srcimg w x y;
    engine='HDM1'; %#ok<NASGU>
    save(strcat(top_dir,'matlab_data\compiledhdm.mat'));
    tfinal = toc;
    disp(['Compile data complete in: ',...
        datestr(datenum(0,0,0,0,0,tfinal),'HH:MM:SS')])
    clearvars
end
function num = numpad(num,len)
    if isnumeric(num)
        num = num2str(num);
    end
    if length(num) < len
        num = numpad(['0',num],len);
    end
end
```

6.9 - Appendix J - HDM Displacement in the X Direction Raw Data


| -82.17724779 | -82.22123719 | -80.3652447 | -85.63889416 | -90.08098457 | -93.369115 | -92.1982484 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| -88.81157095 | -88.62061156 | -91.39818425 | -94.75976318 | -98.43451148 | -97.36129978 | -92.96267975 |
| -87.71959477 | -91.42168378 | -93.8656273 | -92.27668136 | -92.78360105 | -96.43758753 | -96.44625323 |
| -89.31652009 | -91.92339704 | -92.59659147 | -90.31967256 | -94.22626862 | -93.74303669 | -94.313364 |
| -83.74240006 | -85.30926914 | -85.71791167 | -84.03563259 | -88.09009927 | -91.62606406 | -91.16331727 |
| -81.33184147 | -82.74908484 | -83.45654255 | -84.48432805 | -82.74449713 | -87.56414012 | -88.62337021 |
| -79.88400966 | -81.2443718 | -81.89661604 | -86.62240251 | -82.95951799 | -82.71300817 | -84.99202304 |
| -74.02867805 | -74.22834748 | -78.7272802 | -80.31395278 | -79.85331368 | -80.59690028 | -80.97284454 |
| -72.67840114 | -72.3334041 | -75.79480397 | -77.00680931 | -77.35965602 | -76.58552079 | -74.82980804 |
| -71.11325202 | -72.52858594 | $-73.09256614$ | -73.88804831 | -73.89948762 | -72.52282432 | -75.24959294 |
| -68.54392838 | -68.16623884 | -70.53393689 | -71.23414386 | -71.83166936 | -71.73829751 | -73.10867459 |
| -66.6847268 | -66.80315196 | -67.64958785 | -68.14477328 | -69.70118966 | -68.45624167 | -70.32376114 |
| -62.72187583 | -57.10637479 | -63.74009081 | -65.18942301 | -66.28937571 | -66.40464052 | -68.75642405 |
| -61.05394922 | -61.18806797 | -62.13506801 | -62.99941936 | -61.50464371 | -63.83583997 | -65.32904956 |
| -61.32188283 | -59.47855361 | -59.80407435 | -59.99190703 | -59.96319703 | -60.92500121 | -61.08213652 |
| -58.16443664 | -55.24656239 | -58.07699814 | -54.04523766 | -57.3133421 | -58.82735603 | -59.09908877 |
| -56.75615891 | -56.51487462 | -62.31146432 | -55.68738048 | -56.03706081 | -55.86464981 | -56.14373967 |
| -53.77848042 | -55.61543221 | -55.75698674 | -53.99954076 | -53.59490901 | -53.84599031 | -53.79270828 |
| -55.53293586 | -51.26584481 | -51.29850828 | -52.28600798 | -51.37358884 | -51.5139752 | -51.38793544 |
| -50.21837824 | -48.12019551 | -49.79926575 | -48.92821039 | -48.7471195 | -49.75498827 | -48.54934614 |
| -45.35513797 | -45.60975204 | -46.72116423 | -46.4366046 | -43.54528598 | -47.06341718 | -47.30137482 |
| -44.99813563 | -45.58643279 | -45.94834625 | -45.33723247 | -44.56110726 | -44.2226626 | -45.45259699 |
| -43.32699429 | -42.45725848 | -48.24416669 | -44.25547916 | -43.13982047 | -43.03867596 | -42.76841466 |
| -41.84771832 | -41.63946569 | -42.38645543 | -40.37638752 | -38.9885705 | -40.87314075 | -40.97064589 |
| -38.40044486 | -42.62001631 | -45.30009613 | -39.532965 | -38.587952 | -38.32918085 | -38.40398924 |
| -36.0012261 | -29.17935281 | -36.65125224 | -36.69500909 | -36.39073822 | -35.08057831 | -36.76690844 |
| -35.3905884 | -34.58906455 | -35.51722845 | -34.20516363 | -31.84456068 | -31.43979949 | -34.2124325 |
| -35.2081248 | -34.24569054 | -32.77682314 | -30.69073947 | -28.36930028 | -29.55200392 | -31.26279251 |
| -40.8786813 | -34.71636141 | -31.72974163 | -26.12673289 | -25.33421844 | -31.18890509 | -33.93726891 |
| -32.01451542 | -28.20476352 | -25.73050701 | -26.02377508 | -25.51602535 | -27.36470247 | -28.05968708 |


| -75.04461904 | -75.1468258 | -76.52466806 | -76.63285608 | -76.47694965 | -78.5838413 | -82.72362928 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| -81.67107689 | -77.72193717 | -83.35510625 | -84.7346017 | -85.54500519 | -86.16725094 | -88.34514364 |
| -80.61016457 | -79.47063521 | -80.94534176 | -82.23925973 | -83.92454828 | -90.27588994 | -81.55610197 |
| -79.6338258 | -78.74507363 | -74.20506181 | -76.32023148 | -77.6178813 | -83.08155306 | -81.19137741 |
| -74.99736151 | -78.54960986 | -77.99193681 | -80.07770032 | -78.85337915 | -80.51923052 | -81.81317494 |
| -76.02866838 | -81.13814121 | -77.02773388 | -76.72086173 | -78.443404 | -79.21451373 | -80.25659777 |
| -74.66385553 | -73.44988022 | -70.03522819 | -73.99013833 | -75.29149906 | -81.67773401 | -78.51251478 |
| -72.64109982 | -72.81946306 | -71.42193013 | -72.95863499 | -70.19218131 | -74.51426758 | -76.91146863 |
| -68.9625169 | -69.26703708 | -70.33283129 | -71.21676881 | -68.84893003 | -66.77041751 | -68.60373337 |
| -69.64833108 | -66.20137798 | -70.16309883 | -68.4263159 | -69.31518694 | -74.07032644 | -71.24443301 |
| -65.89129871 | -67.82840005 | -66.22651519 | -66.3704488 | -66.99911365 | -68.62689015 | -68.99018493 |
| -62.5329376 | -62.57694893 | -55.88019736 | -62.44290015 | -64.71757345 | -65.65926916 | -65.99229642 |
| -61.06079763 | -58.88072135 | -60.64708267 | -61.59170202 | -61.92251969 | -61.86392255 | -62.49575155 |
| -59.23590014 | -56.63067651 | -60.45980695 | -60.27401632 | -60.58950752 | -57.31160049 | -56.76334354 |
| -58.714123 | -57.34020279 | -59.76678295 | -58.52795707 | -58.4282286 | -58.58826991 | -65.32044278 |
| -55.95890624 | -55.12402702 | -54.53109537 | -55.98387625 | -53.4140155 | -57.15159617 | -57.56985252 |
| -55.28323086 | -50.49444637 | -53.35490546 | -54.22993943 | -52.86202051 | -48.4717116 | -47.51826418 |
| -51.46161745 | -51.81413696 | -52.9019785 | -53.28582778 | -53.14409061 | -53.39067453 | -55.41051567 |
| -49.35547082 | -50.82709626 | -50.90870167 | -55.81860073 | -54.95411087 | -59.21301725 | -52.51698673 |
| -44.88023338 | -46.63000797 | -45.99575968 | -49.7282881 | -48.56385508 | -49.30119947 | -47.8395774 |
| -44.30577218 | -45.80207414 | -44.62676679 | -49.48254487 | -44.31317255 | -45.25618197 | -36.86385603 |
| -44.29969296 | -42.69060614 | -44.24977673 | -46.45305974 | -44.62824462 | -46.15653625 | -45.48560963 |
| -42.78884257 | -43.05126228 | -42.99822287 | -43.08237923 | -41.24554552 | -42.70550025 | -42.98718745 |
| -44.46473888 | -41.32814665 | -40.95974152 | $-41.12697711$ | -40.37993079 | -40.333611 | -39.35594894 |
| -40.04459348 | -39.47010112 | -39.61452018 | -40.73618251 | -38.92628428 | -35.80970629 | -36.83189429 |
| -34.16977753 | -32.80639763 | -38.11823078 | -37.65472612 | -31.30733801 | -32.20831862 | -37.34655611 |
| -29.39608525 | -34.89202351 | -37.13819662 | -36.41599079 | -38.89019979 | -34.92571801 | -33.3745182 |
| -31.21356866 | -35.33720953 | -34.44131758 | -35.60471891 | -34.54181219 | -33.81608826 | -33.63929098 |
| -36.15988301 | -33.76168239 | -33.25100591 | -33.61837766 | -32.76022899 | -34.52812335 | -33.17388374 |
| -29.40312172 | -30.53583451 | -28.68116631 | -30.43043796 | -30.19249001 | -32.36826299 | -29.2474361 |


| -69.94467567 | -62.23924486 | -69.28608175 | -72.07740385 | -74.85158467 | -73.78731631 | -74.84226958 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| -80.77896077 | -78.01148199 | -78.61423686 | -79.60326644 | -81.14901774 | -82.27793056 | -82.94923238 |
| -76.95642254 | -71.36028887 | -77.37487852 | -78.84298168 | -79.2827836 | -80.68286926 | -81.85314124 |
| -74.76246169 | -74.95931969 | -76.41677602 | -76.93025935 | -76.3144652 | -77.89386788 | -78.70614054 |
| -73.37853647 | -74.7147925 | -74.69929925 | -74.35488012 | -75.15756711 | -75.91180167 | -76.71482157 |
| -71.37171695 | -71.86308847 | -73.24193455 | -71.75480651 | -72.59704165 | -74.49878142 | -75.58269487 |
| -69.90346617 | -70.87364636 | -71.15391404 | -66.06648931 | -71.81041733 | -73.39585749 | -74.14941529 |
| -67.71176885 | -69.2112278 | -68.87226604 | -67.96541656 | -71.55181382 | -70.14560567 | -60.93749252 |
| -66.57677124 | -66.88825264 | -67.29912075 | -66.47174836 | -68.68217839 | -68.78837065 | -68.3350783 |
| -64.93204552 | -65.52913801 | -65.41488992 | -59.69476108 | -65.39543716 | -66.83566878 | -67.37015845 |
| -63.13401554 | -63.9774429 | -68.40293473 | -63.62415854 | -64.17095996 | -65.2482643 | -65.05924958 |
| -60.60561471 | -60.77751361 | -62.30534768 | -62.5754134 | -62.28963591 | -61.29486606 | -56.25284652 |
| -58.47527435 | -58.04793871 | -59.38375926 | -56.98100929 | -56.67616617 | -53.5973143 | -59.08319041 |
| -56.50871934 | -57.1215617 | -57.46083986 | -51.53136963 | -56.45242499 | -58.93641041 | -61.17207507 |
| -55.92899351 | -54.63607067 | -54.63231064 | -54.20833511 | -55.24376066 | -57.42148268 | -61.13520261 |
| -52.83009389 | -52.50395596 | -52.57233905 | -52.29968471 | -45.9807716 | -53.43191175 | -55.42213293 |
| -50.9288863 | -51.20800602 | -51.16941309 | -52.4270149 | -50.24215707 | -51.93959852 | -57.45129621 |
| -49.33446022 | -49.22583844 | -46.30375768 | -49.6340268 | -48.11889239 | -51.7419003 | -48.38031523 |
| -47.34905439 | -47.51831459 | -47.52997499 | -49.81094712 | -49.60003453 | -49.42098601 | -48.90156794 |
| -45.20427452 | -44.96658211 | -46.97231229 | -51.43864101 | -48.31433293 | -46.84176112 | -46.24565333 |
| -43.30566427 | -42.67584984 | -43.33549872 | -44.88566403 | -47.36771191 | -45.79645688 | -40.99184724 |
| -42.78333031 | -42.61364652 | -39.76898385 | -41.9062116 | -43.61769334 | -43.48341808 | -43.51274553 |
| -40.01200054 | -40.66527618 | -41.36441069 | -41.61286575 | -42.4221199 | -37.08325005 | -41.40153287 |
| -38.94776684 | -38.14801413 | -38.90514405 | -39.77523783 | -38.53694498 | -34.69188523 | -39.1722635 |
| -37.54438446 | -36.62534402 | -37.43586912 | -38.24833153 | -37.74601754 | -37.37701216 | -38.52713374 |
| -35.39418362 | -34.59742196 | -35.22670309 | -36.17280484 | -37.10652218 | -37.22395791 | -36.84836443 |
| -34.09983933 | -34.28424984 | -35.31951823 | -35.3356109 | -40.00524869 | -38.24648242 | -33.7421198 |
| -32.27321456 | -32.87046441 | -35.21538878 | -35.58586373 | -39.05813787 | -39.15580632 | -27.76820418 |
| -31.39255866 | -31.31365489 | -32.02603002 | -32.49320024 | -32.88880759 | -33.37186744 | -28.59941643 |
| -27.18948772 | -27.15913401 | -27.79764561 | -28.00509618 | -27.3468977 | -29.29235764 | -27.5250328 |


| -65.94223869 | -75.43752102 | -74.33406174 | -61.87332735 | -69.2560821 | -69.64848335 | -70.50226329 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| -75.03594205 | -68.72024693 | -62.48255265 | -70.85321621 | -62.13755657 | -72.78951713 | -76.89864839 |
| -76.12708911 | -65.45315027 | -62.85517628 | -71.330232 | -71.21928775 | -72.11337553 | -76.46629231 |
| -57.81637622 | -58.58948791 | -68.88194509 | -79.92368721 | -73.98733977 | -73.47201662 | -74.2718902 |
| -71.13837343 | -73.92595101 | -64.69205369 | -73.23318846 | -65.64189818 | -70.57717217 | -72.10805528 |
| -60.40837789 | -76.77566403 | -75.87699775 | -68.846105 | -66.7351857 | -69.62506849 | -70.85722716 |
| -61.80927669 | -57.07087842 | -67.46690873 | -62.92263947 | -65.33276626 | -68.10049899 | -68.54818365 |
| -60.00206229 | -59.56757201 | -62.47155602 | -62.14183275 | -65.34186376 | -64.85439323 | -66.73222733 |
| -60.41381797 | -57.83031224 | -58.78734312 | -52.56671633 | -63.60065321 | -64.78220489 | -71.07281854 |
| -57.38309794 | -58.56614892 | -59.48799111 | -58.53334151 | -60.21734361 | -62.48060789 | $-64.76075651$ |
| -58.29374046 | -58.95721373 | -57.95462527 | -58.95544209 | -59.28157927 | -59.15093116 | -61.53966857 |
| -56.06741794 | -57.35154067 | -56.23117791 | -55.31553684 | -55.97727207 | -53.31371974 | -59.17454819 |
| -57.69575721 | -57.12632097 | -54.19685887 | -53.04641249 | -54.04776521 | -57.20264405 | -57.03659061 |
| -51.68771554 | -53.2724197 | -53.26355882 | -53.82171847 | -52.97872201 | -54.89603431 | -56.39426188 |
| -48.95555924 | -52.4922446 | -51.91286847 | -53.40740774 | -51.97513318 | -52.78166864 | -54.77977027 |
| -45.37480333 | -48.39677839 | -49.78904268 | -52.29017555 | -52.21407758 | -52.09772325 | -52.12177395 |
| -44.51751753 | -48.19185816 | -49.89388142 | -49.80386 | -49,69603789 | -49.97646869 | -50.75091269 |
| -44.35126677 | -45.2337149 | -47.03326451 | -47.84451166 | -48.38344284 | -48.70017318 | -49.16779383 |
| -43.50874179 | -43.62413261 | -46.08506097 | -46.02838849 | -46.01496708 | -46.28549739 | -46.35786167 |
| -41.65899508 | -41.56229715 | -44.11544932 | -44.01964081 | -44.43166177 | -44.96643982 | -45.70295833 |
| -41.78710969 | -41.95744185 | -42.41227306 | -43.17775735 | -43.58911584 | -43.91838092 | -43.16046149 |
| -38.89962345 | -40.63886677 | -41.7568599 | -43.2882356 | -42.82380807 | -42.26305898 | -42.08615082 |
| -39.30138318 | -37.90333072 | -41.09929555 | -40.66895566 | -39.76048203 | -40.51533495 | -39.65440756 |
| -37.17432964 | -38.62902161 | -39.34564495 | -37.58025147 | -38.87803789 | -38.61077356 | -38.01532488 |
| -35.31175175 | -36.00151351 | -35.56053899 | -36.85849659 | -37.41828396 | -37.92059472 | -37.50745865 |
| -34.49069316 | -34.67157336 | -34.47546521 | -35.81109172 | -35.46305257 | -35.82363828 | -35.99181925 |
| -33.4140639 | -33.37448925 | -33.72422678 | -34.11466672 | -34.43335481 | -33.86427141 | -33.98075458 |
| -31.74125612 | -32.06239365 | -32.22852275 | -31.64497517 | -32.0024255 | -32.53366287 | -32.31942917 |
| -30.89466593 | -29.83964567 | -30.03842331 | -30.98286678 | -31.56638157 | -31.87785776 | -31.1645448 |
| -26.16684024 | -26.20709125 | -25.48127104 | -26.91583832 | -28.0297205 | -27.33146518 | -26.91460166 |



| 5.578534 | 10.11806 | 7.38725 | 5.951777 | 4.486088 | 2.203809 | 7.520539 | 3.974051 | -6.10189 | -12.8247 | -13.8686 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 6.263864 | 7.702092 | 7.255365 | 6.101267 | 3.207882 | 5.140925 | 5.512594 | 7.802004 | -8.36874 | -9.15932 | -14.7797 |
| 9.111347 | 8.225746 | 7.583588 | 5.653534 | 5.678908 | 11.51807 | 4.814772 | 4.371748 | -5.23469 | -9.94431 | -10.3125 |
| 9.310844 | 8.676324 | 11.92402 | 4.019557 | 6.042693 | 16.9828 | 5.620788 | 2.857015 | -5.14548 | -11.9733 | 81 |
| 9.032394 | 7.979337 | 7.744074 | 5.521701 | 5.061678 | 6.302239 | 6.269172 | 2.352209 | -1.99062 | -8.63036 | -9.20717 |
| 8.596685 | 5.881861 | 5.849196 | 5.229022 | 3.828929 | 3.714629 | 2.818338 | -1.518 | -5.90798 | 9.8227 | 13.9682 |
| 8.813399 | 6.24731 | 5.88053 | 2.325402 | 0.863219 | 2.847127 | 2.53394 | -0.96048 | -3.41991 | -9.35375 | 656 |
| 9.788691 | 6.758629 | 1.279457 | 3.168402 | -2.06315 | 2.179552 | 2.445272 | -0.97858 | -5.24291 | -8.08807 | -6.84603 |
| 8.259836 | 7.1185 | 2.741031 | 9.877633 | 5.474016 | 3.656819 | 2.458011 | -1.17464 | -5.22365 | -9.85813 | -8.48142 |
| 8.835851 | 7.8983 | -2.61 | 4.368 | 3.88468 | 3.5 | 1.47035 | -0.7335 | .209 | 8.10843 | 918 |
| 8.222374 | 7.594442 | 5.787688 | 3.162151 | 0.377872 | 3.566579 | 1.423761 | -0.54583 | -4.19196 | -8.78618 | 7.85879 |
| 7.710182 | 7.589897 | 5.199 | -0.52325 | 1.683839 | 3.922229 | 2.387628 | -0.32436 | -3.49292 | -6.88046 | -6.69556 |
| 8.844604 | 8.078447 | 5.80636 | 4.154233 | -0.6675 | 2.92758 | 1.76371 | 1153 | -2.974 | 5.34663 | 9695 |
| 8.574807 | 8.387685 | 10.4424 | 9.053573 | 3.687271 | 4.4276 | 2.145418 | 0.96524 | -2.6525 | -2.09776 | 5.26274 |
| 9.36437 | 7.098137 | 8.173553 | 8.623567 | 3.447759 | 3.164831 | 2.906505 | 0.362011 | -1.78926 | -3.49556 | -4.97002 |
| 8.825836 | 6.718791 | 3.333 | -2.52796 | -2.12 | 2.7 | 5.352436 | 1.930578 | -0.72026 | -3.04951 | -3.95822 |
| 8.110475 | 5.165087 | 3.25976 | -0.42701 | 3.351539 | 8.06949 | 3.416503 | 1.14255 | -0.39142 | -2.95339 | 4.1379 |
| 7.66968 | 7.560472 | 3.108751 | -4.18516 | 4.717553 | 7.703695 | 2.348376 | 0.00044 | -0.06052 | -2.40721 | -3.96013 |
| 8.548996 | 21.56106 | 7.525 | -6.42966 | 2.808106 | 3.697319 | 2.03579 | 0.773207 | 1.06709 | 1.8183 | -3.38226 |
| 8.830602 | 5.711545 | 7.029084 | 4.221363 | 5.175182 | 4.27013 | 2.978009 | 1.205572 | -1.39435 | -2.31853 | -3.18521 |
| 9.866064 | 6.38491 | 9.3458 | 5.997669 | 4.344 | 4.256 | 2.996636 | 0.657907 | 1.37382 | -0.77197 | -2.41216 |
| 8.23705 | 5.062438 | 4.290196 | 6.247692 | 6.346174 | 5.19245 | 3.503599 | 2.635939 | 1.20003 | -0.64695 | -0.73103 |
| 12.08987 | 7.451439 | 4.156875 | 2.895267 | 15.93729 | 13.61509 | 4.308516 | 2.677962 | 1.925389 | 1.120303 | -0.25312 |
| 8.495399 | 6.944301 | 6.464459 | 4.880663 | 5.791145 | 6.283296 | 4.364761 | 3.522363 | 1.853144 | 2.126958 | 0.560649 |
| 5.875299 | 7.448509 | 8.036433 | 4.448981 | -6.02642 | -0.58377 | 3.496906 | 3.28618 | 2.57713 | 2.258103 | 1.240737 |
| 10.91719 | 10.69385 | 7.725077 | 7.093413 | 3.376241 | 5.024533 | 4.447996 | 3.762169 | 3.761717 | 2.532634 | 1.175112 |
| 8.718493 | 8.92892 | 8.996005 | 7.693639 | 5.343352 | 5.295314 | 5.328058 | 4.186679 | 1.48041 | 2.023144 | 2.035925 |
| 9.106809 | 7.617126 | 7.287658 | 7.042612 | 5.623661 | 5.479816 | 6.774781 | 7.3852 | 3.072742 | 3.341913 | 5.04946 |
| 7.866667 | 6.048922 | 7.135771 | 7.569729 | 6.130933 | 5.936012 | 13.63366 | 23.38794 | 8.316503 | 3.40453 | 9.141245 |
| 5.969036 | 8.017322 | 7.943734 | 6.521759 | 4.937178 | 5.413241 | 4.170099 | 6.795436 | 4.098881 | 3.227135 | 3.64635 |


| 14.96673 | 15.95152 | 14.07651 | 13.4069 | 7.964413 | 6.79789 | 10.69978 | 11.33787 | 10.97236 | 10.10549 | 8.739087 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 15.74525 | 16.54615 | 16.86628 | 15.59899 | 13.36172 | 12.90302 | 13.29767 | 12.88423 | 12.76003 | 11.70269 | 9.128915 |
| 17.61362 | 20.29025 | 17.27907 | 15.3589 | 14.36814 | 13.73843 | 14.95553 | 13.11769 | 12.54043 | 11.24542 | 0.40626 |
| 18.13952 | 17.53072 | 16.38277 | 16.48631 | 16.5479 | 14.49423 | 13.94163 | 12.79406 | 11.40851 | 5.836011 | 10.63593 |
| 18.3158 | 17.19092 | 16.3796 | 16.41909 | 15.21547 | 13.71186 | 13.78731 | 15.93496 | 11.53291 | 9.934063 | 9.01236 |
| 18.45005 | 18.29306 | 17.41491 | 15.85 | 13.14264 | 14.0834 | 13.22 | 11.18 | 5.79216 | 10.88456 | . 900376 |
| 18.70634 | 18.10383 | 17.23569 | 18.18809 | 15.18951 | 13.936 | 10.97272 | 9.611108 | 11.37344 | 18.38571 | 0.52134 |
| 18.09425 | 17.28772 | 17.02185 | 17.55638 | 15.00123 | 13.06685 | 5.691188 | 2.460381 | 10.47237 | 14.70519 | 10.41992 |
| 17.77284 | 18.10741 | 16.70995 | 17.61221 | 16.24579 | 14.44662 | 12.18533 | 7.353419 | 10.64053 | 11.90527 | 10.79479 |
| 19.00631 | 17.45921 | 17.7572 | 23.69872 | 16.90367 | 14.07483 | 13.47522 | 14.34036 | 10.53277 | 16.30462 | 2.88716 |
| 18.88362 | 17.98158 | 17.56865 | 17.49467 | 16.02988 | 14.5382 | 13.70318 | 12.8484 | 15.50536 | 11.75053 | 10.37933 |
| 19.5389 | 18.2587 | 17.3085 | 16.5 | 15.33354 | 14.3027 | 14.37018 | 6.701585 | 12.49418 | 11.29407 | 9.998334 |
| 18.36973 | 18.70398 | 17.5832 | 16.2673 | 19.85693 | 12.3921 | 12.684 | 12.51539 | 16.97423 | 9.988273 | 0.1043 |
| 19.3995 | 18.47928 | 18.05433 | 19.0952 | 17.16986 | 12.56165 | 5.123141 | 8.742422 | -3.33939 | -0.72928 | 8.409382 |
| 18.51793 | 18.7634 | 17.70689 | 17.18838 | 14.64068 | 12.81356 | 6.395582 | 10.72321 | 9.767163 | 11.06257 | 7.242405 |
| 19.38428 | 18.47415 | 16.98999 | 15.6846 | 11.34313 | 14.1471 | 14.29 | 12.69117 | 11.21626 | 9.332639 | 8.225859 |
| 19.02918 | 17.93044 | 16.13905 | 10.40148 | 14.3927 | 13.66226 | 15.55956 | 15.12101 | 9.938681 | 9.7728 | 9.22237 |
| 18.81421 | 17.04213 | 16.08448 | 14.76285 | 14.45899 | 9.978376 | 7.993024 | 9.414287 | 10.84062 | 10.00667 | 8.148393 |
| 18.98626 | 18.33139 | 16.9719 | 16.92805 | 14.98172 | 13.1264 | 11.9694 | 10.90572 | 10.90071 | 8.722466 | 7.163153 |
| 18.94763 | 17.54145 | 16.62061 | 20.4415 | 13.96444 | 13.59492 | 13.52543 | 12.42263 | 11.18026 | 10.50673 | 8.268692 |
| 18.34216 | 16.96687 | 16.05856 | 15.8242 | 9.050978 | 13.5454 | 15.07443 | 11.00036 | 10.52692 | 10.24652 | 4.5181 |
| 17.26533 | 17.63617 | 17.57107 | 15.204 | 13.3454 | 13.41695 | 12.0259 | 9.948907 | 4.588039 | 9.550061 | 12.34754 |
| 17.34867 | 16.0646 | 14.55438 | 14.0548 | 14.70485 | 16.06165 | 12.13375 | 10.93602 | 8.740915 | 8.771812 | 10.04198 |
| 16.74735 | 15.80409 | 15.0340 | 14.61 | 13.94156 | 11.12403 | 15.02483 | 12.54713 | 9.729794 | 9.158105 | 9.792505 |
| 15.78708 | 16.23583 | 14.60837 | 13.42169 | 12.12629 | 11.03364 | 11.60751 | 11.0593 | 4.904247 | 8.553829 | 6.508197 |
| 15.70192 | 16.72368 | 14.24006 | 13.82317 | 13.01866 | 11.64888 | 11.46835 | 16.6998 | 5.259608 | 8.565219 | 9.556765 |
| 14.83898 | 14.58431 | 13.40871 | 12.65673 | 14.32185 | 16.74336 | 10.49611 | 7.987068 | 7.709056 | 8.607778 | 9.901737 |
| 15.47732 | 13.27356 | 12.12899 | 9.736348 | 18.48409 | 15.11092 | 11.2751 | 5.834519 | 4.859317 | 7.071555 | 9.351534 |
| 12.73884 | 12.36539 | 11.93936 | 10.89416 | 12.1026 | 10.15321 | 14.41306 | 10.74482 | 9.451813 | 9.196481 | 9.39791 |
| 9.941836 | 9.650677 | 9.161062 | 9.677025 | 10.38238 | 8.549698 | 8.601415 | 8.579167 | 9.605906 | 7.562437 | 2.664813 |


| 20.18337 | 30.1964 | 30.026 | 25.56962 | 20.88594 | 17.18351 | 16.18361 | 15.3137 |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 26.16414 | 37.9555 | 24.01172 | 25.3793 | 23.52698 | 27.13143 | 21.77604 | 18.72819 |
| 39.03829 | 29.28541 | 24.11309 | 21.72837 | 20.73539 | 21.53715 | 16.9967 | 18.56234 |
| 32.2487 | 34.2428 | 29.39697 | 15.65143 | 22.05154 | 23.48114 | 18.83664 | 18.46935 |
| 23.98531 | 45.15818 | 28.96579 | 17.6142 | 15.45846 | 22.77178 | 20.57837 | 19.67672 |
| 25.05095 | 37.10433 | 21.50564 | 15.61843 | 19.11754 | 21.69138 | 20.36992 | 19.05587 |
| 26.48727 | 30.44066 | 19.36048 | 15.72682 | 21.39755 | 21.83017 | 20.06689 | 19.70967 |
| 21.50392 | 28.45147 | 21.34474 | 20.98997 | 20.29209 | 19.65946 | 18.20554 | 18.06862 |
| 25.27871 | 27.06885 | 20.84194 | 21.60309 | 19.78075 | 16.42486 | 19.26816 | 12.68475 |
| 24.39493 | 24.48839 | 16.36532 | 19.88606 | 23.76523 | 28.29958 | 22.63069 | 19.58691 |
| 23.26909 | 17.92034 | 20.57471 | 21.43951 | 22.96803 | 23.25658 | 22.51254 | 20.62002 |
| 18.49334 | 23.1017 | 21.84649 | 22.78982 | 23.41327 | 23.50916 | 20.71767 | 20.54063 |
| 25.09584 | 31.96971 | 22.55582 | 22.80326 | 23.46693 | 21.82269 | 21.51797 | 21.35453 |
| 22.45407 | 24.3462 | 23.37788 | 21.31689 | 22.2943 | 21.58397 | 22.04908 | 20.47421 |
| 19.03598 | 20.622 | 21.43338 | 22.46559 | 22.20412 | 22.44789 | 21.93323 | 20.67213 |
| 23.096 | 22.26983 | 22.03812 | 22.59165 | 21.94695 | 21.05286 | 21.31887 | 21.31363 |
| 20.14205 | 21.9247 | 21.23977 | 20.82641 | 21.50866 | 20.89551 | 21.4975 | 19.59295 |
| 20.40959 | 20.59006 | 21.00316 | 20.79017 | 20.40716 | 20.69101 | 20.78094 | 19.6027 |
| 17.28652 | 18.84781 | 21.32736 | 20.41403 | 20.66515 | 20.42205 | 20.18548 | 19.77941 |
| 17.29673 | 18.98485 | 20.61573 | 19.98268 | 19.70125 | 19.7112 | 20.3513 | 19.39728 |
| 17.63051 | 20.35542 | 20.67251 | 19.03392 | 18.87612 | 19.59455 | 18.93278 | 18.47953 |
| 16.89547 | 19.10732 | 18.98508 | 19.11654 | 18.14253 | 18.90343 | 18.52353 | 17.90474 |
| 15.16398 | 18.35021 | 18.18092 | 19.10727 | 18.21503 | 18.77337 | 18.38461 | 17.74325 |
| 14.37823 | 17.77122 | 18.38124 | 18.78659 | 18.58542 | 18.37929 | 17.71849 | 15.53271 |
| 15.01206 | 17.8158 | 17.91277 | 17.45905 | 17.34003 | 17.45961 | 16.97782 | 14.91944 |
| 13.49685 | 15.62763 | 15.25792 | 15.1612 | 15.92001 | 16.78139 | 16.97369 | 13.80916 |
| 12.36746 | 15.08628 | 13.40964 | 14.23103 | 15.18396 | 14.88674 | 14.99643 | 14.59213 |
| 11.36446 | 13.67686 | 13.94206 | 13.85086 | 12.91579 | 13.29031 | 13.7754 | 14.41434 |
| 10.66007 | 12.76862 | 13.34058 | 13.4576 | 13.44472 | 13.43155 | 13.36086 | 13.55883 |
| 7.999584 | 10.11644 | 10.17968 | 10.83321 | 10.56566 | 10.59339 | 11.30213 | 11.17875 |


| -0.56675 | -0.47396 | -0.35134 | -0.41465 | -0.39996 | -0.68956 | -0.57005 | -0.5221 | -0.24951 | -0.04778 | 0.004283 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0.101279 | -0.25679 | 0.424315 | 0.068249 | -0.17507 | -0.15422 | 0.155193 | 0.353182 | 0.057732 | -0.21772 | 0.3683 |
| 0.394167 | 0.449646 | 0.022795 | -0.09981 | -0.03136 | 0.079315 | 0.122313 | -0.09017 | 0.168409 | 0.133306 | -0.26677 |
| -0.07722 | 0.160145 | -0.03886 | 0.348383 | 0.413383 | 0.429917 | 0.392752 | 0.383511 | 0.132311 | 0.196878 | 0.228013 |
| 0.025623 | 0.081545 | 0.097286 | 0.15066 | 0.160012 | 0.14 | -0.02804 | 0.334 | 0.25387 | 0.158747 | 0.07778 |
| 0.196994 | -0.15395 | 0.1090 | 0.090489 | 0.09404 | 0.097 | -0.13363 | 0.013 | 0.3031 | 0.226959 | 0.261096 |
| 0.318707 | 0.44 | 0.1 | 0.365958 | 0.438502 | 0.198083 | 0.394278 | 0.19 | 0.132257 | 0.251199 | 0.272026 |
| 0.083953 | 0.483991 | 0.519233 | 0.084392 | 0.118434 | . 18328 | 0.206696 | 0.155854 | 0.250711 | 0.38394 | 0.13546 |
| -0.02914 | -0.45624 | -0.1650 | 0.097822 | -0.0122 | 0.1688 | 0.194923 | 0.216261 | 0.253919 | -0.02624 | 0.134312 |
| 0.144755 | 0.340215 | 0.14089 | 0.160583 | 0.272647 | 0.159914 | 0.165869 | 0.129239 | 0.049033 | 0.133807 | 0.191271 |
| 0.142596 | 0.185476 | 0.18736 | 0.1162 | 0.085 | 0.1 | 0.193086 | 0.13315 | 0.205128 | 0.174057 | 0.151698 |
| 0.174691 | 0.237209 | 0.218534 | 0.247678 | 0.606049 | 0.244 | 0.184709 | 0.213238 | 0.128225 | 0.097959 | 0.116115 |
| 0.083313 | 0.28452 | 0.3582 | 0.104245 | -0.255 | 0.1003 | 0.136875 | 0.2990 | 0.16055 | 0.214211 | 0.127787 |
| 0.13508 | -0.07979 | -0.5 | -0.016 | 0.1068 | 0.1456 | 0.18797 | 0.09634 | 0.181927 | 0.265432 | 0.196873 |
| 0.313388 | 0.089792 | 0.48441 | 0.19734 | 0.264499 | 0.10794 | 0.371667 | 0.165616 | 0.131103 | 0.12394 | 0.174653 |
| 0.0345 | 0.542493 | 0.62822 | 0.088017 | -0.07927 | -0.26465 | -0.10263 | 0.079768 | 0.185169 | 0.184709 | 0.172559 |
| -0.01763 | -0.30744 | -0.4932 | 0.186105 | 0.056215 | 0.40965 | 0.10549 | 0.152634 | 0.126166 | 0.146939 | 0.101028 |
| -0.11 | -0.3 | 0.180 | -0.10 | 0.27 | 0.2786 | 0.107096 | 0.138833 | 0.145 | 0.150298 | 0.124847 |
| 0.399391 | 0.619489 | 0.29233 | 0.33216 | 0.196603 | 0.093703 | 0.209862 | 0.16415 | 0.109937 | 0.177412 | 0.14349 |
| 0.265668 | 0.252814 | 0.685983 | 0.303953 | 0.15690 | 0.19238 | 0.155725 | 0.325115 | 0.168223 | 0.077998 | 0.131802 |
| -0.01969 | -0.0562 | -0.5388 | 0.022313 | 0.00145 | 0.048301 | 0.068711 | -0.06349 | 0.177547 | 0.115549 | 0.132232 |
| 0.211419 | 0.21569 | 0.156151 | 0.104446 | 0.195573 | -0.14349 | 0.06761 | 0.08883 | 0.073999 | 0.167761 | 0.13806 |
| 0.054101 | 0.148243 | 0.226952 | 0.092455 | 0.051112 | 0.366107 | 0.242443 | 0.259453 | 0.135346 | 0.112361 | 0.177508 |
| 0.090853 | 0.282744 | 0.157753 | 0.215 | -0.06128 | -0. | 0.052714 | 0.025039 | 0.158997 | 0.160416 | 0.078255 |
| 0.476184 | 0.225087 | -0.0321 | 0.149951 | 0.84004 | 0.54055 | 0.177372 | 0.137326 | 0.203038 | 0.102318 | 0.11364 |
| -0.47393 | -0.16984 | 0.248252 | 0.038165 | -0.33811 | 0.070876 | 0.155615 | 0.284136 | 0.227549 | 0.159655 | 0.087049 |
| 0.271774 | 0.069352 | -0.01655 | 0.011404 | 0.021461 | 0.171275 | 0.219652 | 0.217204 | 0.117987 | 0.184352 | -0.10762 |
| 0.111349 | -0.0445 | 0.029088 | -0.35441 | -0.02942 | 0.065443 | 0.28525 | 0.189693 | -0.10231 | -0.16715 | 0.07341 |
| 0.160484 | 0.134991 | 0.245403 | 0.55401 | 0.406975 | 0.374952 | 0.006435 | -0.01136 | 0.239013 | 0.367349 | 0.528352 |


| -0.67714 | -0.98576 | -0.58301 | -0.47037 | -0.39359 | -0.53066 | -0.50669 | -0.41415 | -0.16094 | -0.4269 | 0.50636 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0.238909 | 0.4157 | 0.07746 | 0.047518 | 0.11664 | 0.099691 | 0.068506 | 0.066307 | -0.10929 | 0.15061 | 0.155959 |
| 0.137123 | -0.22494 | 0.059881 | 0.119545 | 0.18552 | 0.174313 | 0.196688 | 0.061021 | 0.045348 | 0.421267 | 0.369939 |
| 0.086495 | 0.015283 | 0.107342 | 0.160961 | 0.072306 | 0.123879 | 0.124457 | 0.289779 | 0.012216 | -0.23668 | 0.23484 |
| 0.125426 | 0.178232 | 0.091085 | 0.162505 | 0.160033 | 0.088314 | 0.070758 | -0.06446 | -0.16178 | 0.060263 | 0.209802 |
| 0.091766 | 0.06184 | 0.130501 | 0.35552 | 0.049164 | 0.068933 | 0.08958 | 0.085301 | 0.480516 | 0.437032 | 0.17067 |
| 0.136981 | 0.103 | 0.142603 | -0.1 | 0.016163 | 0.20 | 0.8257 | 0.126422 | 0.039401 | -0.08667 | 0.064469 |
| 0.070937 | 0.145186 | 0.098322 | 0.093354 | 0.179352 | 0.084827 | -0.46235 | 0.229911 | 0.222027 | 0.068069 | 0.108867 |
| 0.102795 | 0.084945 | 0.117764 | 0.423562 | 0.205421 | 0.1220 | 0.06030 | -0.04286 | 0.191604 | 0.010608 | 403 |
| 0.112377 | 0.096981 | -0.18675 | -0.24559 | 0.07653 | 0.099213 | 0.144432 | 0.234815 | -0.10169 | 0.246036 | 0.128492 |
| 0.158025 | 0.199996 | 0.381099 | 0.0655 | 0.117583 | 0.24708 | 0.55 | 0.209898 | 0.328216 | 0.646645 | 0.245472 |
| 0.133146 | 0.170598 | 0.182599 | 0.34965 | 0.350842 | 0.481097 | -0.1769 | 0.092009 | 0.231014 | -0.29793 | 0.0532 |
| 0.12291 | 0.057899 | 0.120182 | 0.34060 | 0.013984 | -0.3336 | -0.130 | 0.114056 | 0.140628 | 0.011705 | 0.082355 |
| 0.036233 | 0.155343 | 0.176783 | -0.16731 | 0.075542 | 0.094683 | 0.002305 | 0.032611 | -0.04435 | 0.043314 | 0.109129 |
| 0.193681 | 0.133257 | 0.128748 | 0.119291 | 0.578937 | 0.249348 | 0.357067 | 0.172201 | 0.138511 | 0.32723 | 0.159005 |
| 0.118825 | 0.080997 | 0.087683 | -0.00796 | -0.26634 | 0.09327 | -0.12682 | 0.04223 | 0.289349 | 0.073512 | 0.109621 |
| 0.099652 | 0.123885 | 0.304103 | 0.174562 | 0.132704 | 0.012356 | 0.566936 | 0.238851 | -0.08248 | 0.028308 | 0.059007 |
| 0.124088 | 0.10672 | -0.07664 | -0.0 | -0.09 | 0.14505 | 0.03 | 0.13163 | 0.061 | 0.12458 | -0.1583 |
| 0.134049 | 0.159483 | 0.034854 | -0.10173 | 0.080356 | 0.161202 | 0.165995 | 0.279702 | 0.262318 | 0.307059 | 0.380645 |
| 0.118663 | 0.143171 | 0.227301 | 0.40956 | 0.059164 | 0.065332 | 0.328363 | 0.035904 | 0.051746 | 0.085562 | 0.015359 |
| 0.032646 | 0.003888 | 0.222907 | 0.186216 | 0.234376 | 0.144565 | -0.157 | 0.00038 | 0.194467 | 0.023562 | 0.189343 |
| 0.173208 | 0.121773 | -0.09971 | 0.018334 | 0.074723 | 0.400011 | 0.131951 | 0.094428 | -0.02254 | 0.078222 | 0.210668 |
| 0.066515 | 0.157329 | 0.153704 | 0.114852 | 0.242823 | 0.14946 | 0.139329 | -0.10474 | 0.107695 | 0.127405 | 0.122213 |
| 0.087711 | 0.095167 | 0.09183 | 0.095432 | 0.049433 | -0.16782 | 0.040321 | 0.276259 | 0.116128 | 0.084076 | 0.024425 |
| 0.134388 | 0.126745 | 0.138073 | 0.1297 | 0.039968 | 0.009566 | 0.104923 | 0.367176 | 0.416481 | 0.093518 | 0.192591 |
| 0.080897 | 0.019573 | -0.0058 | 0.052325 | -0.18117 | -0.06391 | 0.19414 | 0.298356 | -0.13035 | 0.061252 | 0.077421 |
| 0.114164 | 0.088362 | 0.006508 | -0.01564 | 0.059194 | -0.05683 | 0.37337 | -0.11359 | -0.02782 | 0.168555 | 0.050704 |
| 0.055041 | 0.097301 | 0.199335 | 0.193291 | 0.385583 | 0.361496 | -0.05195 | -0.30914 | 0.09847 | 0.074394 | 0.124146 |
| 0.262692 | 0.259658 | 0.264274 | 0.280507 | 0.346369 | 0.254969 | 0.067149 | 0.422298 | 0.201615 | 0.285615 | 0.199246 |


| -0.47558 | -0.56836 | 0.41983 | 0.740719 | -0.56124 | 0.444908 | -0.19631 | -0.39977 |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| -0.06537 | -0.0682 | 0.204194 | -0.02329 | -0.02981 | -0.56761 | 0.042259 | 0.027022 |
| 0.743414 | 1.14442 | 0.428979 | -0.37667 | -0.53709 | -0.173 | -0.08492 | 0.13715 |
| -0.29736 | -0.83262 | -0.95853 | 0.261868 | 0.418156 | 0.52159 | 0.180928 | 0.13524 |
| -0.16772 | 0.670625 | -0.17811 | -0.69906 | 0.274193 | -0.06833 | 0.059506 | 0.078177 |
| -0.15811 | -0.08756 | 1.231549 | 0.525631 | 0.370217 | 0.087651 | 0.095286 | 0.144315 |
| 0.76464 | 0.112951 | -0.15604 | 0.31221 | 0.0488 | -0.00057 | 0.202882 | 0.113497 |
| -0.13131 | -0.02573 | 0.108579 | 0.230263 | 0.598445 | 0.108826 | 0.004512 | -0.27129 |
| 0.202451 | 0.18942 | -0.04599 | -0.04379 | -0.37291 | 0.211457 | 0.14385 | 0.394504 |
| 0.051388 | -0.05692 | -0.02444 | 0.095835 | -0.02638 | 0.058485 | 0.208105 | 0.201318 |
| -0.30963 | 0.139145 | 0.100355 | 0.107715 | 0.227494 | 0.206519 | 0.364826 | 0.14782 |
| 0.29195 | -0.10177 | 0.014076 | 0.127145 | 0.14182 | 0.120594 | -0.24306 | 0.133622 |
| 0.255608 | 0.375503 | 0.240869 | 0.058331 | -0.04846 | 0.066815 | 0.144163 | 0.040146 |
| 0.191204 | 0.17076 | 0.048761 | 0.084418 | 0.025894 | 0.062724 | 0.132148 | 0.100906 |
| 0.030909 | 0.223797 | 0.255967 | 0.132739 | 0.069827 | -0.01493 | 0.042747 | 0.166125 |
| 0.115626 | 0.05358 | 0.012808 | -0.00655 | 0.155395 | 0.157377 | 0.132578 | 0.085679 |
| 0.044964 | 0.010391 | 0.184884 | 0.178789 | 0.122459 | 0.082037 | 0.079768 | 0.098945 |
| 0.145255 | 0.052658 | 0.100599 | 0.059263 | 0.113508 | 0.14803 | 0.150917 | 0.175621 |
| -0.04451 | 0.115609 | 0.128865 | 0.123101 | 0.125547 | 0.098957 | 0.082441 | 0.040931 |
| -0.00886 | -0.00801 | -0.0247 | 0.106449 | 0.052618 | 0.052659 | 0.065504 | 0.158906 |
| 0.10017 | 0.180468 | 0.082411 | 0.040963 | -0.0069 | 0.047832 | 0.103458 | 0.067144 |
| 0.184214 | -0.02511 | 0.170971 | 0.041098 | 0.163705 | 0.191458 | 0.109233 | 0.151984 |
| 0.076552 | 0.132941 | -0.04536 | 0.109603 | 0.193044 | 0.055153 | 0.119035 | 0.102443 |
| 0.031342 | 0.116411 | 0.164219 | 0.236569 | 0.04511 | 0.091235 | 0.043136 | 0.031742 |
| 0.105019 | 0.051316 | 0.083121 | 0.067817 | 0.065463 | 0.122202 | 0.13106 | 0.094727 |
| 0.038814 | 0.067289 | 0.081068 | 0.046952 | 0.106027 | 0.064356 | 0.12246 | 0.125692 |
| 0.137701 | 0.10455 | 0.082006 | 0.093482 | 0.154356 | 0.151933 | 0.083163 | 0.103833 |
| -0.0382 | 0.052912 | 0.138922 | 0.136881 | 0.041382 | 0.027253 | 0.040988 | 0.07218 |
| 0.295863 | 0.295489 | 0.227035 | 0.284822 | 0.254189 | 0.221041 | 0.28415 | 0.265621 |


| 0.197535 | -0.28372 | 0.170675 | 0.089717 | 0.091606 | 0.142642 | -0.3323 | 0.221656 | 0.629747 | 0.420176 | 0.065241 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0.179066 | -0.08989 | 0.02792 | 0.072131 | 0.180837 | -0.12082 | -0.02323 | -0.14309 | 1.010672 | 0.049411 | 0.351271 |
| 0.080932 | 0.05535 | 0.040135 | 0.120628 | -0.00159 | -0.36495 | 0.418956 | 0.027689 | 0.600402 | 0.294351 | 0.023013 |
| 0.082818 | 0.039657 | -0.20298 | 0.494029 | -0.1 | -0. | 0.710126 | 0.17 | 0.500156 | 39 | 7 |
| -0.00125 | 0.065816 | 0.01470 | 0.138898 | 0.028751 | -0.07754 | 0.002067 | 0.24481 | 0.271427 | 0.414983 | 0.036051 |
| 0.081481 | 0.169676 | 0.002 | 0.038761 | 0.087506 | 0.00 | 0.056018 | 0.2710 | 0.27435 | 0.24467 | 0.259095 |
| 0.106746 | 0.160381 | 0.022924 | 0.222195 | 0.091386 | -0.12399 | 0.019574 | 0.218401 | 0.153714 | 0.370865 | 0.056991 |
| 0.039452 | 0.189379 | 0.342448 | -0.11806 | 0.326972 | -0.26517 | -0.01661 | 0.213991 | 0.266521 | 0.177822 | 0.07763 |
| 0.158435 | 0.071333 | 0.273592 | -0.44604 | 0.275226 | 0.113575 | 0.074925 | 0.227041 | 0.253063 | 0.289655 | -0.08604 |
| 0.253207 | 0.058592 | 0.65 | -0.43652 | 0.0302 | 0.02020 | 0.130691 | 0.13774 | 0.279775 | 0.181156 | 0.0162 |
| 0.13481 | 0.039246 | 0.112922 | 0.164096 | 0.174017 | -0.19929 | 0.133926 | 0.123099 | 0.227883 | 0.287139 | 0.05796 |
| 0.14301 | 0.007518 | 0.149422 | 0.35765 | -0.1379 | -0.139 | 0.095913 | 0.169499 | 0.198035 | 0.211722 | -0.01156 |
| 0.078731 | 0.047885 | 0.14200 | 0.103258 | 0.30136 | -0.22 | 0.072742 | 0.1799 | 1162 | 0.148255 | 0.028145 |
| -0.01034 | 0.011695 | -0.12842 | 0.086805 | 0.33539 | -0.0462 | 0.14264 | 0.073761 | 0.226109 | -0.03467 | 0.197811 |
| -0.13262 | 0.14164 | -0.06721 | -0.02813 | 0.323488 | 0.017683 | 0.016145 | 0.159031 | 0.134454 | 0.106644 | 0.092154 |
| -0.0375 | 0.13169 | 0.2115 | 0.366328 | -0.02505 | -0.30 | -0.1613 | 0.213866 | 0.1656 | 0.145578 | 0.056795 |
| 0.069493 | 0.184087 | 0.11908 | 0.230423 | -0.23616 | -0.2948 | 0.290812 | 0.142122 | 0.095873 | 0.160123 | 0.074032 |
| 0.02992 | 0.006825 | 0.278233 | 0.455869 | -0.55642 | -0.18663 | 0.334707 | 0.146746 | 0.00381 | 0.146668 | 0.097057 |
| -0.08662 | -0.81 | 0.877243 | 0.872177 | -0.57736 | -0.05 | 0.103845 | 0.078912 | -0.01837 | 0.180337 | 0.097747 |
| -0.03512 | 0.194941 | -0.08235 | 0.175483 | -0.0596 | 0.0565 | 0.080758 | 0.110777 | 0.162495 | 0.057762 | 0.054167 |
| -0.33425 | 0.2175 | -0.1850 | 0.209259 | 0.1033 | 0.00552 | 0.078741 | 0.146171 | -0.044 | 0.134112 | 0.102512 |
| 0.256906 | 0.198413 | 0.048265 | -0.12234 | -0.00616 | 0.07210 | 0.105554 | 0.054229 | 0.089744 | 0.115436 | 0.005255 |
| -0.12799 | 0.289902 | 0.2059 | 0.078851 | -0.81513 | 0.145137 | 0.581661 | 0.10191 | 0.047036 | 0.050318 | 0.085839 |
| 0.081069 | 0.096944 | 0.0299 | 0.098 | -0.0569 | -0.0307 | 0.119908 | 0.05265 | 0.104326 | -0.01711 | 0.097894 |
| 0.039556 | -0.09833 | -0.03675 | 0.224216 | 0.654712 | -0.3401 | -0.25504 | 0.01317 | 0.044316 | 0.019939 | 0.063585 |
| -0.08503 | 0.013959 | 0.185548 | 0.039479 | 0.232323 | -0.10302 | 0.036034 | 0.042864 | $2.82 \mathrm{E}-05$ | 0.076818 | 0.084845 |
| 0.073953 | -0.01315 | -0.00419 | 0.081398 | 0.146893 | 0.003002 | -0.00205 | 0.071336 | 0.169142 | -0.03392 | -0.0008 |
| 0.015295 | 0.093105 | 0.020592 | 0.015315 | 0.088684 | 0.00899 | -0.08094 | -0.03815 | 0.269529 | -0.01682 | -0.10672 |
| 0.095703 | 0.113609 | -0.06793 | -0.02712 | 0.089925 | 0.012183 | -0.4811 | -0.60964 | 0.941965 | 0.306998 | -0.35854 |
| -0.20651 | -0.12802 | 0.004599 | 0.088873 | 0.099036 | -0.02975 | 0.077696 | -0.16408 | 0.168535 | 0.054484 | -0.0262 |


| 0.021685 | -0.06155 | 0.117188 | 0.041851 | 0.340155 | 0.072908 | -0.24387 | -0.03988 | 0.022844 | 0.054179 | 0.0854 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0.186434 | -0.05006 | -0.02001 | 0.079206 | 0.139829 | 0.028669 | -0.02467 | 0.02584 | 0.007762 | 0.066084 | 0.160861 |
| 0.059295 | -0.16729 | 0.188199 | 0.12001 | 0.061922 | 0.039357 | -0.07607 | 0.114865 | 0.036079 | 0.080938 | 0.052448 |
| 0.020614 | 0.03805 | 0.071747 | -0.00647 | -0.00385 | 0.128354 | 0.034537 | 0.071723 | 0.086597 | 0.348281 | -0.29999 |
| 0.085058 | 0.070305 | 0.050708 | -0.00247 | 0.075226 | 0.093976 | -0.00472 | -0.13423 | 0.275128 | 0.099928 | 0.057606 |
| 0.037863 | 0.009812 | 0.054884 | 0.097346 | 0.1696 | -0.0588 | 0.053475 | 0.127716 | 0.337016 | -0.31827 | 0.061511 |
| 0.062708 | 0.037657 | 0.054258 | -0.05952 | 0.187411 | 0.078344 | 0.185205 | 0.085101 | -0.11015 | -0.43827 | 0.491523 |
| -0.0016 | 0.050408 | 0.016616 | -0.03341 | 0.159697 | 0.120899 | 0.460979 | 0.201925 | -0.50075 | -0.26455 | 0.267829 |
| -0.31801 | -0.02091 | 0.087341 | -0.05639 | 0.085401 | 0.112448 | 0.141331 | 0.301994 | -0.20544 | -0.07905 | 0.069405 |
| 0.036287 | 0.096694 | -0.01862 | -0.37135 | 0.424691 | 0.176802 | 0.037476 | -0.05407 | 0.237974 | -0.36074 | 0.213591 |
| 0.108525 | 0.056377 | 0.025808 | 0.004623 | 0.09155 | 0.09323 | 0.052189 | 0.053424 | -0.16606 | 0.234676 | 0.0857 |
| 0.062608 | 0.080013 | 0.059388 | 0.049587 | 0.073848 | 0.064427 | -0.00422 | 0.479287 | -0.36204 | 0.075007 | 0.080983 |
| 0.18655 | -0.02089 | 0.070045 | 0.082242 | -0.2243 | 0.466547 | -0.01827 | 0.0105 | -0.27868 | 0.436623 | 0.00725 |
| 0.067169 | 0.057514 | 0.026559 | -0.06505 | 0.120333 | 0.288013 | 0.464907 | -0.22621 | 0.755113 | -0.16313 | -0.57117 |
| 0.134638 | -0.01534 | 0.066032 | 0.032407 | 0.159232 | 0.114195 | 0.401123 | -0.27048 | 0.059753 | -0.08096 | 0.23876 |
| 0.120585 | 0.056883 | 0.09276 | 0.081581 | 0.271347 | -0.17525 | -0.00924 | 0.100239 | 0.092182 | 0.117726 | 0.069174 |
| 0.035236 | 0.068671 | 0.111962 | 0.358599 | -0.24945 | 0.045652 | -0.11858 | 0.02741 | 0.323895 | 0.010368 | 0.034402 |
| 0.049281 | 0.110755 | 0.059854 | 0.082602 | 0.018991 | 0.280038 | 0.124085 | -0.08883 | -0.08915 | 0.052122 | 0.116142 |
| 0.049571 | 0.04093 | 0.084968 | 0.002741 | 0.121646 | 0.115957 | 0.072313 | 0.06648 | 0.000313 | 0.13614 | 0.097457 |
| 0.028103 | 0.087886 | 0.057552 | -0.23881 | 0.404816 | 0.023095 | 0.004343 | 0.068925 | 0.077649 | 0.042096 | 0.139877 |
| 0.008585 | 0.085956 | 0.056769 | 0.014647 | 0.423327 | -0.2809 | -0.09556 | 0.254629 | 0.02959 | 0.017525 | 0.358026 |
| 0.039963 | -0.02318 | 0.004068 | 0.147917 | 0.116187 | -0.00447 | 0.086941 | 0.129812 | 0.335054 | -0.31013 | -0.17484 |
| 0.024662 | 0.080254 | 0.094389 | 0.031221 | -0.04063 | -0.0848 | 0.245494 | 0.074859 | 0.137194 | -0.00193 | -0.07939 |
| -0.07591 | 0.058953 | 0.048128 | 0.026285 | 0.041996 | 0.176095 | -0.2438 | 0.154857 | 0.176083 | 0.035731 | -0.03965 |
| -0.05423 | -0.02805 | 0.101716 | 0.074168 | 0.080962 | 0.068291 | -0.03587 | 0.034263 | 0.384691 | -0.2281 | 0.127852 |
| -0.1183 | -0.06386 | 0.155226 | 0.026056 | 0.050282 | 0.085611 | 0.011283 | -0.32697 | 0.715012 | -0.2066 | -0.06197 |
| -0.01543 | 0.015917 | 0.073475 | 0.046999 | -0.10407 | -0.15134 | 0.390453 | 0.156815 | 0.017376 | -0.05617 | -0.08087 |
| -0.06644 | 0.137735 | 0.071536 | 0.14954 | -0.54673 | 0.210823 | 0.239739 | 0.340036 | 0.06095 | -0.13826 | -0.1425 |
| 0.05125 | 0.02334 | 0.026627 | 0.065325 | -0.07553 | 0.121837 | -0.26624 | 0.229265 | 0.080813 | 0.015958 | 0.01259 |
| 0.077307 | 0.018197 | 0.030601 | -0.03225 | -0.04408 | 0.114543 | -0.00323 | 0.00139 | -0.06417 | 0.127717 | 0.306102 |


| -0.62581 | 0.01065 | 0.278524 | 0.29273 | 0.231401 | 0.062494 | 0.05437 |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| -0.73696 | 0.871486 | -0.08547 | 0.11577 | -0.22528 | 0.334712 | 0.190491 |
| 0.609555 | 0.323271 | 0.149044 | 0.062061 | -0.05011 | 0.283778 | -0.09785 |
| -0.12463 | 0.302865 | 0.859096 | -0.40001 | -0.08935 | 0.290282 | 0.022955 |
| -1.3233 | 1.012025 | 0.709474 | 0.134734 | -0.45708 | 0.137088 | 0.056353 |
| -0.75334 | 0.974918 | 0.367951 | -0.21869 | -0.16086 | 0.082591 | 0.082128 |
| -0.24709 | 0.692511 | 0.227104 | -0.35442 | -0.02704 | 0.110205 | 0.022326 |
| -0.43422 | 0.444171 | 0.022173 | 0.043617 | 0.039539 | 0.09087 | 0.008557 |
| -0.11188 | 0.389182 | -0.04757 | 0.113896 | 0.209743 | -0.17771 | 0.411463 |
| -0.00584 | 0.507692 | -0.22005 | -0.24245 | -0.2834 | 0.354306 | 0.190237 |
| 0.334297 | -0.1659 | -0.05405 | -0.09553 | -0.01803 | 0.046503 | 0.118282 |
| -0.28802 | 0.078451 | -0.05896 | -0.03897 | -0.00599 | 0.174468 | 0.011065 |
| -0.42962 | 0.588368 | -0.01547 | -0.04148 | 0.102765 | 0.019045 | 0.010215 |
| -0.11826 | 0.06052 | 0.128812 | -0.06109 | 0.044396 | -0.02907 | 0.098429 |
| -0.09913 | -0.05071 | -0.06451 | 0.016342 | -0.01524 | 0.032167 | 0.078818 |
| 0.051635 | 0.014482 | -0.0346 | 0.040294 | 0.05588 | -0.01663 | 0.000328 |
| -0.11142 | 0.042808 | 0.025835 | -0.04264 | 0.038322 | -0.03762 | 0.119034 |
| -0.01128 | -0.02582 | 0.013312 | 0.023938 | -0.01774 | -0.00562 | 0.07364 |
| -0.09758 | -0.15497 | 0.057083 | -0.0157 | 0.015194 | 0.014786 | 0.02538 |
| -0.10551 | -0.10193 | 0.039566 | 0.017589 | -0.00062 | -0.04001 | 0.059626 |
| -0.17031 | -0.01982 | 0.102412 | 0.009863 | -0.0449 | 0.041361 | 0.028328 |
| -0.13824 | 0.00764 | -0.00822 | 0.060876 | -0.04756 | 0.023744 | 0.038674 |
| -0.19914 | 0.010581 | -0.0579 | 0.055765 | -0.0349 | 0.024297 | 0.040085 |
| -0.21206 | -0.03813 | -0.02533 | 0.012573 | 0.012883 | 0.0413 | 0.136611 |
| -0.17523 | -0.00606 | 0.028357 | 0.007439 | -0.00747 | 0.030112 | 0.128649 |
| -0.13317 | 0.023107 | 0.006045 | -0.04743 | -0.05384 | -0.01202 | 0.197783 |
| -0.16993 | 0.10479 | -0.05134 | -0.05956 | 0.018576 | -0.00686 | 0.025269 |
| -0.14453 | -0.01657 | 0.0057 | 0.058441 | -0.02341 | -0.03032 | -0.03993 |
| -0.13178 | -0.03575 | -0.00731 | 0.000805 | 0.000823 | 0.004418 | -0.01237 |
| -0.1323 | -0.00395 | -0.04085 | 0.016722 | -0.00173 | -0.0443 | 0.007711 |


| 0.016545 | -0.00966 | 0.125247 | -0.0124 | -0.03857 | 0.033785 | 0.102053 | 0.258439 | 0.031915 | 0.077954 | -0.60388 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0.114309 | 0.035809 | 0.078316 | 0.000584 | 0.071252 | 0.286085 | 0.083242 | 0.00764 | 0.064401 | -0.16199 | -0.46184 |
| 0.0589 | 0.21256 | -0.13685 | 0.141547 | 0.127059 | 0.247146 | -0.02447 | -0.03149 | 0.116975 | -0.06314 | -0.5332 |
| 0.03185 | 0.148959 | -0.18969 | 0.300853 | 0.050808 | -0.31273 | -0.05089 | 0.106306 | 0.083488 | 0.12229 | -0.28452 |
| -0.05188 | -0.01349 | -0.01878 | 0.051142 | 0.010441 | -0.06809 | -0.16041 | 0.005746 | -0.01192 | -0.05172 | -0.49046 |
| 0.060602 | 0.035517 | 0.033544 | -0.05714 | -0.04839 | -0.005 | 0.023231 | -0.03694 | 0.228366 | 0.047756 | -0.10771 |
| 0.071145 | 0.215549 | -0.24 | 0.069203 | -0.0 | -0.00 | 0.14 | -0.11503 | -0.06467 | 0.110772 | -0.13362 |
| -0.13423 | 0.146311 | 0.120587 | 0.119576 | 0.241776 | 0.186756 | 0.049982 | -0.02052 | 0.023839 | -0.04357 | -0.30201 |
| -0.05599 | -0.04058 | -0.11012 | -0.04484 | -0.06045 | 0.10518 | 0.007011 | 0.024811 | -0.02376 | -0.00019 | -0.1069 |
| 0.008606 | 0.1391 | 0.174311 | -0.04179 | -0.06536 | 0.017786 | 0.023403 | 0.006223 | -0.01121 | 0.064032 | -0.20723 |
| 0.00364 | 0.050726 | -0.00704 | -0.12911 | 0.029009 | 0.085105 | 0.052002 | 0.025594 | 0.018927 | 0.102378 | -0.19932 |
| 0.106534 | 0.044695 | 0.029383 | 0.16781 | -0.06978 | -0.00463 | -0.00402 | 0.02392 | -0.02271 | 0.106292 | -0.19641 |
| 0.001907 | 0.007833 | 0.164623 | 0.160171 | -0.0394 | 0.254181 | 0.05722 | 0.099391 | 0.013666 | 0.17502 | -0.21687 |
| 0.034533 | -0.14273 | -0.0880 | 0.1206 | -0.00329 | -0.0098 | 0.050795 | -0.06556 | 0.099826 | 0.002982 | -0.18121 |
| -0.01995 | -0.00685 | 0.059122 | -0.47344 | -0.23182 | -0.00212 | 0.082305 | 0.048121 | 0.063463 | 0.01885 | -0.12445 |
| -0.10266 | 0.068246 | 0.010773 | 0.084235 | 0.080023 | 0.2540 | -0.18649 | 0.077503 | 0.057589 | 0.011495 | -0.18704 |
| -0.05652 | -0.06234 | -0.03451 | 0.171242 | 0.035148 | 0.169712 | -0.24038 | -0.02476 | 0.004953 | 0.02579 | -0.1698 |
| 0.023049 | 0.445224 | 0.201133 | -0.1211 | -0.00227 | -0.12078 | -0.06469 | 0.011504 | 0.043084 | 0.016738 | -0.13434 |
| -0.01822 | -0.36221 | -0.22475 | 0.427093 | -0.05938 | 0.018921 | 0.060304 | -0.015 | -0.07253 | -0.01957 | -0.13352 |
| -0.00403 | 0.044085 | 0.026722 | 0.129847 | -0.09152 | 0.052045 | -0.02664 | -0.02277 | 0.118001 | 0.010654 | -0.09855 |
| -0.21245 | -0.01186 | -0.42025 | 0.273166 | 0.070498 | 0.063981 | 0.00695 | -0.02854 | 0.104511 | 0.011343 | -0.09708 |
| 0.063375 | 0.122415 | -0.02513 | -0.12 | 0.318107 | 0.274517 | 0.006056 | -0.02294 | 0.012091 | 0.093662 | -0.14302 |
| -0.16973 | 0.029776 | 0.080915 | 0.072663 | -0.34425 | -0.04828 | -0.12289 | -0.00848 | -0.00542 | 0.023012 | -0.11767 |
| -0.10522 | 0.014309 | 0.018572 | 0.064378 | -0.37581 | -0.19125 | -0.08994 | -0.05075 | 0.081517 | 0.007145 | -0.15443 |
| 0.101 | 0.004024 | 0.022213 | 0.131656 | 0.425695 | 0.259012 | -0.1505 | -0.01466 | 0.028932 | 0.010917 | -0.13665 |
| -0.26707 | -0.027 | 0.200286 | -0.02328 | -0.15171 | 0.241959 | 0.028869 | 0.003757 | -0.11223 | 0.036776 | -0.11336 |
| 0.089454 | -0.16488 | -0.10186 | 0.042658 | -0.01629 | 0.034771 | 0.004208 | 0.026185 | 0.037112 | 0.127856 | -0.00978 |
| -0.07197 | -0.07169 | -0.01027 | 0.065498 | -0.01422 | -0.03165 | 0.14915 | 0.427541 | 0.200827 | 0.055419 | 0.169899 |
| -0.08612 | 0.116759 | -0.01707 | 0.208026 | -0.22988 | -0.10967 | -0.47083 | -0.54328 | 0.051158 | 0.080343 | -0.24997 |


| 0.006904 | -0.22221 | 0.307394 | 0.155732 | 0.255359 | 0.157527 | 0.114151 | 0.054647 | 0.059059 | 0.09297 | 0.015563 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0.179646 | 0.030519 | 0.031736 | 0.023404 | 0.079755 | 0.061385 | 0.072786 | -0.03265 | -0.13027 | 0.161747 | 0.083026 |
| 0.031751 | -0.26111 | 0.159947 | 0.08111 | 0.081861 | 0.067371 | 0.004887 | -0.04896 | -0.07098 | -0.12296 | 0.047612 |
| 0.020839 | -0.00447 | 0.045447 | 0.013946 | -0.06088 | 0.024907 | 0.020561 | 0.127143 | -0.02389 | -0.01381 | 0.015363 |
| 0.043898 | 0.0762 | 0.031869 | -0.02832 | -0.03969 | 0.035182 | 0.007612 | -0.20213 | -0.06839 | 0.012276 | 0.092931 |
| 0.024087 | 0.009442 | 0.037488 | 0.026362 | 0.090284 | 0.054821 | -0.0366 | -0.03523 | 0.334086 | 0.105961 | 0.009815 |
| 0.023225 | 0.004815 | 0.002076 | -0.17872 | 0.17361 | 0.022384 | -0.14 | -0.20738 | -0.06609 | -0.22172 | 0.120421 |
| 0.020567 | 0.072473 | -0.02034 | -0.02659 | 0.150968 | -0.00083 | -0.08481 | 0.518645 | 0.010829 | -0.13117 | 0.059737 |
| -0.10196 | -0.01052 | 0.045566 | 0.164348 | 0.089634 | -0.0083 | 0.026144 | 0.237949 | 0.006149 | 0.170786 | 0.09301 |
| 0.001519 | 0.034983 | -0.00946 | -0.37263 | 0.15084 | 0.059488 | 0.023827 | 0.024569 | 0.047676 | -0.01851 | -0.13264 |
| 0.070301 | 0.035017 | 0.130167 | -0.17995 | -0.0046 | 0.026306 | 0.014937 | -0.16609 | -0.03356 | -0.06432 | 0.00741 |
| 0.008184 | 0.019287 | 0.056331 | 0.000699 | 0.132425 | -0.09079 | -0.21024 | 0.377934 | 0.141377 | -0.25008 | 0.208396 |
| 0.077139 | -0.02038 | 0.056465 | 0.013283 | -0.093 | -0.09092 | -0.0648 | -0.056 | -0.70293 | -0.27972 | -0.02345 |
| -0.02397 | 0.02803 | -0.00026 | -0.24488 | 0.074746 | 0.085497 | 0.109628 | 0.001394 | 0.328167 | 0.488156 | -0.04227 |
| 0.062986 | -0.04944 | -0.02252 | -0.06024 | -0.07069 | 0.109729 | 0.36291 | -0.01416 | 0.002349 | 0.02177 | -0.00798 |
| 0.011038 | -0.02718 | -0.02445 | -0.17362 | -0.10217 | 0.217695 | 0.101712 | 0.092707 | -0.06601 | -0.00477 | 0.07654 |
| -0.00116 | -0.01904 | -0.00291 | 0.175593 | -0.06621 | -0.06208 | -0.06421 | -0.24609 | -0.12146 | 0.096698 | -0.00622 |
| 0.010585 | 0.036895 | -0.06358 | 0.171733 | -0.0310 | 0.211595 | 0.019212 | 0.142898 | 0.012894 | -0.00614 | 0.01879 |
| 0.029767 | -0.0194 | -0.01061 | 0.181076 | -0.03838 | 0.009046 | 0.032394 | 0.061588 | 0.054724 | 0.058308 | 0.187982 |
| -0.0345 | -0.02538 | 0.045115 | -0.00472 | -0.25118 | -0.04756 | 0.029778 | -0.08712 | 0.034264 | -0.02795 | 0.00056 |
| -0.02911 | 0.001234 | 0.06788 | 0.029074 | 0.211765 | -0.05312 | -0.24541 | 0.070702 | -0.13883 | -0.05849 | 0.396413 |
| 0.024391 | -0.05441 | -0.18317 | 0.030865 | 0.095967 | 0.078451 | 0.004287 | 0.055439 | 0.079493 | 0.024404 | -0.0032 |
| -0.00762 | 0.012274 | 0.036838 | 0.025222 | 0.001436 | -0.32114 | 0.225292 | 0.093701 | 0.039103 | 0.010414 | -0.00517 |
| -0.00087 | -0.0115 | 0.010358 | -0.01005 | -0.09542 | -0.12298 | 0.033221 | 0.118895 | -0.24882 | -0.0304 | -0.09741 |
| -0.00151 | -0.01347 | 0.013819 | 0.037936 | 0.012189 | 0.007695 | 0.031592 | 0.223686 | -0.00685 | 0.004869 | 0.13032 |
| -0.04564 | -0.09175 | -0.00631 | -0.00689 | 0.069903 | 0.162872 | -0.04212 | -0.35598 | 0.03394 | 0.167325 | -0.0037 |
| 0.02367 | -0.0352 | -0.00764 | -0.09076 | 0.275996 | -0.10597 | -0.11642 | -0.20308 | 0.082694 | 0.022186 | -0.03976 |
| -0.08702 | -0.00972 | 0.067353 | 0.047759 | -0.09091 | -0.15188 | -0.2578 | 0.261115 | 0.272379 | 0.038407 | 0.037806 |
| -0.08028 | -0.0873 | -0.06456 | -0.02344 | -0.04139 | -0.03501 | -0.33075 | 0.168588 | -0.07013 | -0.06702 | -0.19893 |


| 0.538465 | 0.108781 | -0.04043 | -0.30687 | 0.541583 | 0.187026 | 0.133384 |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 0.07144 | -0.1942 | -0.30902 | 0.174346 | -0.44719 | 0.183519 | 0.123228 |
| 0.498712 | -0.16844 | -0.27109 | 0.305975 | 0.057283 | 0.085438 | 0.133123 |
| 0.484396 | 0.010685 | 0.382976 | 0.139021 | -0.20768 | 0.038325 | 0.062727 |
| 0.159241 | -0.14602 | -0.35093 | 0.381257 | -0.27099 | 0.147713 | 0.028438 |
| -0.21649 | 0.444441 | -0.0247 | -0.14847 | -0.06163 | 0.080839 | 0.058936 |
| -0.10569 | -0.08607 | 0.489349 | -0.17655 | 0.007482 | 0.028325 | -0.03729 |
| 0.239109 | -0.02929 | 0.10991 | -0.02628 | -0.00108 | 0.017973 | -0.10956 |
| 0.148889 | -0.22063 | -0.02375 | -0.06988 | 0.715896 | 0.142003 | 0.412274 |
| 0.030793 | 0.168514 | 0.077353 | -0.05475 | -0.10497 | 0.067035 | 0.103539 |
| 0.452113 | 0.060477 | 0.010867 | 0.045189 | 0.018085 | -0.06017 | 0.072167 |
| 0.342932 | 0.062295 | -0.03459 | -0.02694 | -0.03202 | -0.05823 | 0.208586 |
| 0.024433 | 0.007894 | -0.13799 | -0.0726 | 0.023832 | 0.115187 | -0.0327 |
| 0.086339 | -0.01124 | 0.03562 | 0.014624 | 0.000654 | 0.056296 | 0.053005 |
| 0.264437 | 0.12942 | -0.01417 | 0.038668 | -0.08835 | 0.006006 | 0.082487 |
| 0.105713 | 0.069488 | -0.01166 | 0.064464 | -0.0073 | 0.001946 | -0.05302 |
| 0.105814 | 0.107429 | 0.052056 | -0.03724 | -0.00976 | -0.01363 | 0.024506 |
| 0.110362 | 0.037708 | 0.044482 | 0.033414 | 0.008437 | -0.00871 | 0.020135 |
| 0.215388 | -0.01863 | 0.063424 | -0.03189 | -0.02263 | 0.013636 | -0.00968 |
| 0.173875 | -0.00125 | 0.050137 | -0.02878 | 0.00923 | -0.02762 | -0.00566 |
| 0.091615 | -0.04741 | 0.016795 | 0.000997 | -0.00874 | -0.0025 | -0.04165 |
| 0.06681 | 0.029221 | 0.034648 | 0.050121 | -0.01858 | -0.02186 | -0.01057 |
| 0.177038 | -0.03743 | 0.089853 | -0.00187 | -0.0407 | 0.002773 | -0.09598 |
| 0.16833 | 0.03082 | -0.01909 | -0.09409 | 0.011816 | -0.0315 | -0.03777 |
| 0.05622 | -0.06141 | -0.08559 | -0.00381 | -0.0037 | 0.015568 | -0.04761 |
| 0.134337 | -0.05211 | -0.0352 | 0.018737 | -0.07008 | -0.05052 | 0.029724 |
| 0.092972 | 0.015401 | -0.00095 | -0.05868 | -0.03993 | -0.05594 | -0.00192 |
| 0.125208 | -0.00876 | -0.0071 | -0.00171 | 0.015584 | 0.003647 | -0.03343 |
| 0.025156 | -0.13175 | -0.0758 | -0.06046 | -0.07046 | -0.0546 | -0.09667 |

### 6.15 - Appendix 0- Instron Data of Strex Well Stretch

The mechanical properties of the Strex well were not available, and the manufacturing methods were not either. Knowing the mechanical properties of the well was necessary for determining motor specifications. This is why it was necessary to perform tensile testing on a well to determine the maximum load necessary to achieve a strain of $20 \%$. Of three separate tests the maximum force ever needed to obtain $20 \%$ stretch was 18.72 N . This value was used to determine the motor specifications.

## Instron Uniaxial Test 1

| Dimension : Length | 30 | mm |
| :--- | ---: | :--- |
| Dimension : Thickness | 1 | mm |
| Dimension : Width | 30 | mm |
| Maximum Load : Maximum Load | 16.408 | N |
| Maximum Load : Tensile extension at Maximum Load | 6.07 | mm |
| Maximum Slope (Automatic Young's) : Maximum Slope (Automatic <br> Young's) | 3.303 | $\mathrm{~N} / \mathrm{mm}$ |

Instron Uniaxial Test 2

| Dimension : Length | 30 mm |  |
| :--- | ---: | :--- |
| Dimension : Thickness | 1 mm |  |
| Dimension : Width | 30 mm |  |
| Maximum Load : Maximum Load | 17.474 | N |
| Maximum Load : Tensile extension at Maximum Load | 6.08 | mm |
| Maximum Slope (Automatic Young's) : Maximum Slope (Automatic <br> Young's) | 3.112 | $\mathrm{~N} / \mathrm{mm}$ |

Instron Uniaxial Test 3

| Dimension : Length | 30 | mm |
| :--- | ---: | :--- |
| Dimension : Thickness | 1 | mm |
| Dimension : Width | 30 | mm |
| Maximum Load : Maximum Load | 18.72 | N |
| Maximum Load : Tensile extension at Maximum Load | 6.32 | mm |
| Maximum Slope (Automatic Young's) : Maximum Slope (Automatic <br> Young's) | 3.21 | $\mathrm{~N} / \mathrm{mm}$ |

6.16 - Appendix P-Cells imaged on the stretch device


