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A Method for Determining the Effect of Stiffness and Stretch on Cell Phenotype

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Abstract

Cells have the ability to sense their mechanical environment and respond by altering their adhesion, proliferation, locomotion, and morphology. In order to elucidate the interrelated effects of different mechanical stimuli on cell phenotype *in vitro*, we have developed a method for culturing mammalian cells in a two-dimensional environment at various levels of substrate stiffness and equibiaxial stretch. Polydimethylsiloxane (PDMS) was polymerized on flexible silicone membranes at different ratios of elastomer base to crosslinker in order to modulate stiffness. The membranes were then stretched using a commercially available vacuum-driven system. Image analysis was used to verify the uniformity of the strain field applied to the cells. Microscopic analysis of stretched and static samples demonstrated cell attachment and cell viability. Future research will further investigate the combined effects of substrate stiffness and stretch on valvular interstitial cell phenotype, as the phenotype of these cells influences the function of heart valves.

1 Introduction

Approximately 100,000 heart valve procedures are performed every year in the United States.[1] A common treatment involves replacing the diseased tissue with a mechanical or chemically fixed biological valve. This procedure is capable of extending patient life for many years; however, structural deterioration and thromboembolism of replacement valves continue to cause concern.[39] In order to circumvent these problems and develop improved replacement options, normal and pathological heart valves must be better understood at the cellular level.

Valvular interstitial cells (VICs) perform a vital role in the physiology of heart valves.[10, 30, 33] Healthy heart valves maintain a balance between quiescent VICs, which exhibit characteristics of fibroblasts, and their differentiated phenotype, the highly contractile and pro-fibrotic myofibroblast. Although a population of myofibroblasts is necessary for wound healing and structural integrity of the valve, an excessive population has been implicated with pathological fibrosis, scarring, and fibrocontractile disease.[10] The aim of much research has been to determine factors which cause the excessive differentiation and population of myofibroblasts in heart valves. Researchers have examined the biological response of VICs to chemical stimuli in detail;[50] however, the effects of the mechanical environment on cell phenotype are poorly understood.

Past research has demonstrated that substrate stiffness and stretch have independently caused cells to differentiate into a more contractile phenotype.[36, 45] A common method for studying the effects of stiffness on cell phenotype is to culture cells on polymer substrates that can be polymerized to different levels of stiffness. The polymer polyacrylamide (PA) has been widely used for this purpose as it can be polymerized to a range of stiffness that matches that of soft tissue.[40, 52] Polydimethylsiloxane (PDMS) can also be polymerized at a wide range of stiffness levels;[6] however, it is not as commonly used for the culture of cells. To stretch the membrane on which cells are cultured, researchers have utilized both commercially available and custom made devices.[19, 48]

Although researchers have grown cells on substrates polymerized to different levels of stiffness and have stretched cells using various devices, no research has examined the interrelated contributions of these factors on cell differentiation. It was therefore the **goal of this project** to design and validate a method to study the effects of stiffness and stretch on cell phenotype. By utilizing the design process and conducting pilot experiments, we selected a Flexcell™ device to apply equibiaxial stretch to PDMS substrates. Validation experiments were conducted to ensure that the stiffness of the PDMS substrates and the stretch induced by the Flexcell™ device were in the desired range. Fibroblasts were cultured on PDMS substrates polymerized to different stiffness levels, and microscopic analysis confirmed cell attachment and proliferation. After obtaining results from these experiments, the data were analyzed to assess the overall success of the proposed methodology, and recommendations and conclusions were made. Results from the experiments demonstrate the success of the method. Future testing will focus on determining the synergistic effects of stiffness and stretch on heart valve cell phenotype.

2 Background

Over 70 million Americans suffer from cardiac disease as a result of congenital defects, aging, infection, disease, and trauma. Valvular disease comprises a subset of this group and is responsible for the 93,000 heart valve procedures that are performed every year in the United States.[1] A common treatment involves replacing the diseased tissue with a mechanical or natural biological valve. This procedure is capable of sustaining patient life for many years; however, structural deterioration and thromboembolism of replacement valves continue to cause concern.[39] In order to circumvent these problems and develop improved replacement valve options, normal and pathological heart valves must be better understood at the cellular level. It would also be useful to determine how these cells respond to external stimuli. Biological responses to chemical stimuli have been studied in detail, but the effects of changes in the mechanical environment have not been fully established.[50]

2.1 Anatomy and Physiology of Heart Valves

The heart contains four valves which open and close in a specific order to keep the blood moving in the proper direction. Deoxygenated blood enters the right atrium of the heart through the vena cava and is pumped through the tricuspid valve and into the right ventricle. The right ventricle then pumps blood through the pulmonary valve and into the pulmonary artery to the lungs. Oxygen-rich blood from the lungs enters the left atrium, which pumps the blood through the mitral valve to the left ventricle. The left ventricle proceeds to pump blood through the aortic valve into the aorta, which distributes blood to the rest of the body. When the ventricles contract, pressurizing the blood to approximately 120 mmHg, the mitral and tricuspid valves quickly close to prevent the backflow of blood, while the aortic and pulmonary valves open. Conversely, when the ventricles relax, the blood pressure drops to approximately 10 mmHg in the ventricles and 80 mmHg in the aorta causing the opening of the mitral and tricuspid valves and the closure of the aortic and pulmonary valves. As a result of the opening and closing of the valves, leaflets are subjected to cyclic mechanical stretch.

Each valve has a set of leaflets which has a relatively low stiffness (~7 kPa) in a healthy heart.[27, 31, 40] This soft tissue is capable of withstanding large variations in stress and strain because of its fibrous, anisotropic structure. The ventricularis and fibrosa contain collagen and elastin fibers which maintain the physical structure of the valve through cyclic loading (see Figure 2-1). The spongiosa, the central portion of the valve, is less fibrous, allowing for greater flexibility.[44]

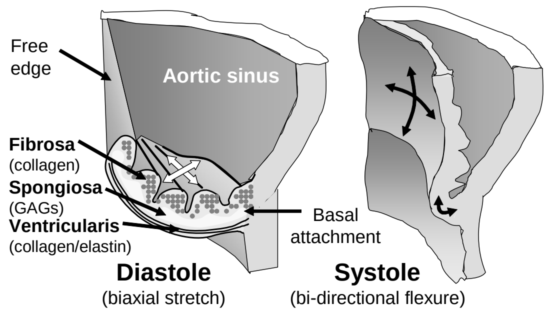

Figure 2-1: Schematic of valve leaflet*

Stiffening of these leaflets can lead to fibrosis, scarring, stenosis, and regurgitation. Researchers believe that this stiffening is related to the phenotype of the most prominent cell of the heart valve leaflet, valvular interstitial cells (VICs).[50]

2.2 Valvular Interstitial Cells

VICs perform a key role in the physiology of the heart valve. In a healthy heart valve, the majority of VICs are in an inactivated state and display characteristics of normal fibroblasts.[38] These quiescent VICs are believed to be the precursor of a highly contractile myofibroblast phenotype, which densely populates heavily loaded areas of heart valve leaflets, and expresses alpha smooth muscle actin (αSMA). Myofibroblasts not only play a crucial role in matrix remodeling through the secretion of extracellular matrix components (ECM), but also in wound healing through the increased generation of cellular contractile force.[14] When the ECM is damaged, quiescent VICs differentiate into the myofibroblast phenotype. After remodeling and healing takes place, the myofibroblast is eliminated by apoptosis.[14] In a healthy heart valve, the process of VIC

* adapted from Carew et al.[17]

differentiation and myofibroblast death is heavily regulated by cytokines that control differentiation, proliferation, contraction, and ECM component secretion.[50] Researchers postulate that dysregulation of this process results in excessive force and ECM component production which has been implicated with pathological fibrosis, scarring, and fibrocontractile disease.[50,14]

2.3 Factors Influencing Cell Differentiation in VICs

In past experiments, increased concentrations of transforming growth factor-β (TGF-β), increased stiffness, and increased mechanical stretch have independently caused cells to differentiate into a more contractile phenotype. In many of these experiments, these factors independently caused an increase in αSMA expression, which is a reliable marker for the myofibroblast phenotype.[50] Although a correlation has been established between these factors and cell differentiation, no quantitative data has been collected to elucidate the interrelated effects of substrate stiffness and stretch on VIC phenotype.

2.3.1 Stiffness

The mechanical properties of the material which cells are seeded on have been found to have an influential role on many cell behaviors. Therefore, it is essential that the *in vivo* stiffness conditions are replicated *in vitro* to obtain pertinent data. Effective methods of modulating substrate stiffness include altering material composition and/or crosslinking the material. In this section, the complex cell behaviors associated with substrate stiffness and methods of modulating stiffness will be discussed.

2.3.1.1 Methods of Modulating Stiffness

In the past, researchers have experimented with biomaterials capable of culturing cells at various levels of stiffness.[6] Commonly used substrate materials for this application are polyacrylamide (PA) and polydimethylsiloxane (PDMS). Each of these materials has distinct advantages and limitations for culturing different cell populations.

Polyacrylamide substrates of different levels of stiffness can be created by polymerizing an acrylamide base and a bis-acrylamide curing agent at different concentrations. These common materials produce a porous, bioinert substrate which has been utilized for stiffness research for many years.[36] Polyacrylamide substrates are commonly prepared at stiffness levels between 1 and 100 kPa.[25,36,51,55] In most experiments, collagen or fibronectin is bound to the substrate to promote cell adhesion.[36,51,3,25,13]

PDMS is a biocompatible, elastic polymer that can be easily and affordably manufactured. The stiffness can be modulated by adjusting the ratio of the silicone elastomer base to the curing agent, which promotes crosslinking. With this polymer, substrates have been created with a stiffness ranging from 50 kPa to 4 MPa, which is necessary for physiological applications where higher stiffness is required.[6,20] Other advantages of PDMS include its low water absorption, thermal stability, and low electrical conductivity. Unfortunately, cells do not naturally adhere to this material; therefore, a surface treatment is required. Previously employed treatment methods include exposing the substrate to an adhesive ligand solution or applying a polyelectrolyte multilayer to the surface of the substrate.[6,20] Using these techniques, cells have attached and grown for 5-10 days before detaching which suggests an unfavorable interaction between the cells and substrate, solution, or other cells.[6,20]

2.3.1.2 Effects of Stiffness

Cells have the ability to sense their mechanical environment and respond by altering adhesion, proliferation, locomotion, and morphology. A summary of experiments supporting this concept can be found in Table 2-1.

Table 2-1: Substrate Stiffness References

Reference	Cell Type	Substrate Type	Range of Stiffness	Cellular Response
Pelham, R.J., et al. (1997)	Rat kidney epithelial and 3T3 fibroblasts	PA	~15-70 kPa	Cells seeded on more flexible substrates demonstrate reduced spreading and increased rates of motility.
Lo, C.-M. , et al. (2000)	3T3 fibroblasts	PA	14-30 kPa	3T3 cells migrate toward stiff substrates and generate stronger traction forces on stiff substrates.
Wang, H.-B., et al. (2000)	3T3 fibroblasts	PA	4.7-14 kPa	Substrate stiffness affects cells growth and apoptosis of normal but not transformed cells.
Brown, X.Q., et al. (2004)	Bovine vascular smooth muscle cells	PDMS	48-1783 kPa	Less stiff substrates result in increased cell proliferation.
Engler, A.J., L. Bacakova, et al. (2004)	Rat aortic-derived smooth muscle cells	PA, collagen gels, glass	1-66 kPa	Substrate stiffness affects cell spreading, morphology
Engler, A.J., M.A. Griffin, et al. (2004)	Myoblasts and human dermal fibroblasts	PA	1-23 kPa	Myoblasts sense substrate stiffness and differentiate accordingly. Adhesion increases with substrate stiffness.
Engler, A.J., L. Richert, et al. (2004)	Aortic smooth muscle cells	PA	1-35 kPa	Cell adhesion is correlated with substrate stiffness and ligand density.
Lee, H.N., et al. (2004)	3T3 fibroblasts, osteoblasts, epithelial, and human umbilical artery endothelial cells	PDMS	200-3700 kPa	Surface chemistry and stiffness of the substrate may adversely influence cell attachment and growth of certain types of cells.
Walker,G.A., et al. (2004)	VICs from porcine aortic valve	Free floating and stressed collagen matrices	Not determined	Fibrotic tissue expresses more my fibroblasts and mechanical stretch is a factor in the expression of α-smooth muscle actin
Yeung, T., et al. (2005)	3T3 fibroblasts	PA	0.1-50 kPa	Mechanical stimuli affect different cell types in different ways.

Noteworthy patterns have emerged in these studies: cells seem to attach and proliferate preferentially on stiffer substrates, cells often migrate from soft to hard surfaces, and cells exhibit a broader, flatter morphology on harder surfaces.[36,51,25,13] Lee et al.[20] observed that cell growth and attachment characteristics are not only unique for different cell populations cultured in the same environment, but also that not all cell types are influenced by substrate stiffness. Brown et al.[6] found that proliferation rates decreased as the stiffness of the substrate was increased. These researchers postulate that their data may conflict with previous findings because they were using a different substrate material, PDMS, which has a higher range of stiffness.[6] These data further emphasizes the importance of substrate selection.

There are strong indications that stiffness may also affect cell differentiation. Engler et al.[13] supported this theory by demonstrating that myotubes differentiate according to the stiffness of their environment. With respect to the activation of quiescent fibroblasts to myofibroblasts in heart valves, Walker and colleagues[50] found that fibrotic tissue expresses a significantly higher percentage of myofibroblasts than compliant, healthy tissue. They also determined that the intrinsic stress of the substrate acts synergistically with TGF-β to increase the expression of αSMA.

It is apparent that stiffness influences cell response, but these responses are not fully understood, as the cell response to stiffness was not quantified in many previous experiments. To further complicate issues, the role of stiffness may be affected by other external stimuli, such as stretch.

2.3.2 Stretch

The mechanical stretch that a cell is experiencing is important in determining cellular functions, such as cell differentiation and synthesis. There are many aspects that need to be considered when assessing mechanical stretch. Some of these factors include the type of stretch that will be applied to the cell, the method of controlling stretch, and the effect of stretch on cellular behavior.

2.3.2.1 Methods of Stretching Cells in Culture

Researchers have studied the effects of several types of stretch on cell function.[5] Some of the types of stretch utilized include uniaxial, biaxial, and equibiaxial stretch. Ideally, the type of stretch modeled *in vitro* should match the mechanical stimuli that are found *in vivo*. While uniaxial stretch is appropriate for studying ligaments and tendons, it is not ideal for investigating the effects of mechanical stretch on planar tissues such as skin or heart valve leaflets. *In vivo*, heart valve leaflets experience mechanical stretch in multiple directions, making multiaxial stretch the appropriate stimulus to use in heart valve leaflet research. Moreover, Lee et al.[19] have shown that the cellular response of adult rat cardiac fibroblasts differs depending on the type of stretch (uniaxial, equibiaxial, and biaxial). They attribute differences in cellular response to the orientation of the cells. Equibiaxial stretch is beneficial because it eliminates the variable of cell orientation, and creates a homogenous strain field. However, equibiaxial stretch may present limitations in this study because it does not exactly mimic the anisotropic strain that is experienced by VICs *in vivo*.

Different methods have been utilized to equibiaxially stretch cells.[5] The device most commonly used by researchers for the application of equibiaxial stretch is the Flexcell™ device developed by Flexcell™ International.[49,29,54,8,47,24] With this device, cells are seeded on 35 mm flexible silicone membranes. Vacuum pressure is then used to pull the central portion of the membrane over a 25 mm cylindrical loading post. As the unsupported edges of the membrane are pulled down, a homogeneous strain field is created over the loading post. Figure 2-2 displays a schematic of the Flexcell™ membrane and loading system. In this system, the magnitude and frequency of the stretch are modulated by the magnitude and frequency of the vacuum pressure, respectively.

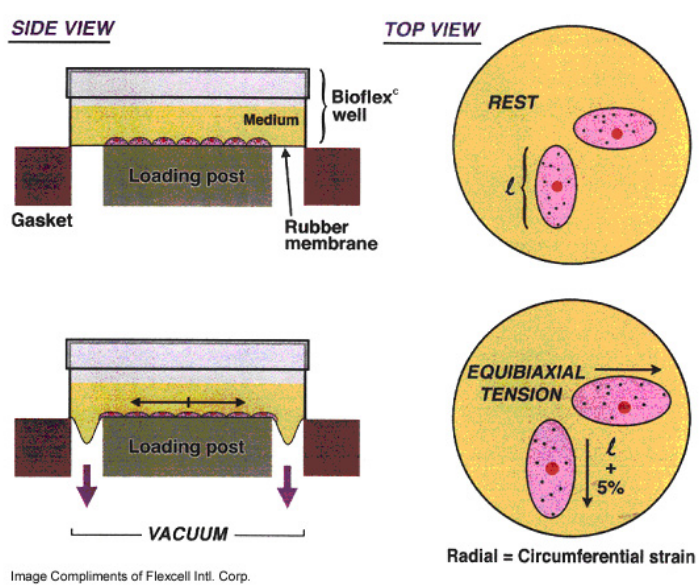

Figure 2-2: Schematic of Flexcell™ Membrane and Loading Post

Other researchers, such as Tschumperlin and Margulies,[48] and Lee and her colleagues,[19] custom designed devices to generate equibiaxial stretch. With Tschumperlin and Margulies' device, cells are seeded on a deformable silicone membrane. Equibiaxial stretch is achieved by sliding an annular indentor that contacts the bottom of the silicone membrane near the periphery of the cell culture surface. Vertical displacement of the indentor causes stretch of the membrane in the plane transverse to the direction of motion of the indentor. A DC motor provides a variable speed for the indentor, and by varying the speed of the motor, the frequency and strain rate are modulated. Figure 2-3, is a schematic of the device designed by Tschumperlin and Marguiles.

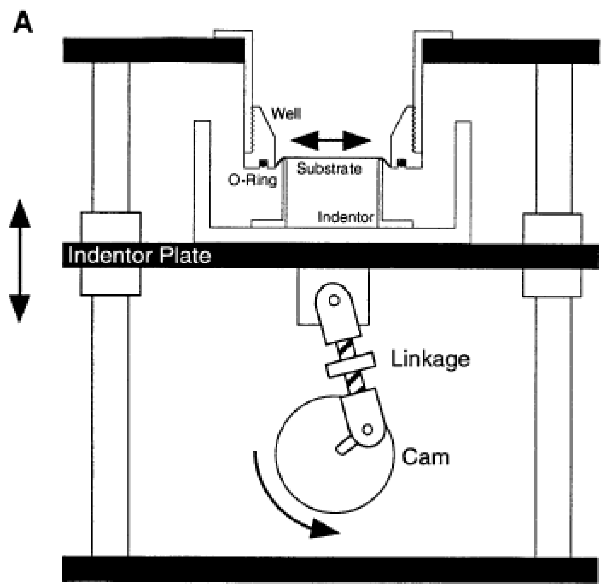

Figure 2-3: Schematic of Equibiaxial Device

The device designed by Lee and her colleagues[19] uses the same principles of the aforementioned device. This device consists of three concentric polycarbonate cylinders (an inner indenter ring, a membrane holder, and an outer screw top) and a transparent elastic membrane. The membrane forms the bottom of the cell culture chamber and the inner indenter ring forms the wall. When the screw top is turned, a flange at its top pushes down the indentor ring. Indentation of the ring against the membrane results in a homogeneous planar equibiaxial stretch of the membrane. The magnitude of strain in this device is controlled by the amount of degrees the screw top is turned. Figure 2-4 is a schematic of the device designed by Lee et al.

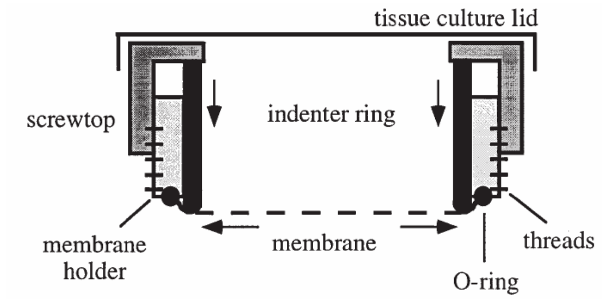

Figure 2-4: Equibiaxial Device for Stretch†

Each of the aforementioned devices have associated advantages and limitations. Lee's device is small, simple, inexpensive to make, and can induce a wide range of stretch. However, this device is not ideal because it does not allow for multiple samples, and the device does not deliver cyclic stretch. The Flexcell^{TM} device has the advantage of being able stretch up to 48 samples. This device includes custom software which controls the percentage, duration, and frequency of stretch. Moreover, this device is a self regulating system which corrects its induced pressure to produce the proper amount of stretch. The only disadvantage of this device is that its range of stretch is limited from 2-20%. Tchumperlin's device can deliver a much larger range of stretch (10-50%) and can accommodate up to nine samples. The main drawback of this device is that it is not automated, meaning it does not have a built in correction mechanism.

2.3.2.2 Effects of Stretch

Research has demonstrated, with various cell types, that mechanical stretch can modulate cell phenotype both *in vivo* and *in vitro* (see Table 2-2). Stretch has been shown to result in cells that exhibit more αSMA and produce more ECM component. These factors render a correlation between stretch and the differentiation of inactivated cells to a highly synthetic and contractile phenotype.

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Table 2-2: Stretch References

Reference	Cell Type	Type of Stretch	Cellular Response
Squier (1981)	Dermal rat fibroblasts	Uniaxial (in vivo)	Cells differentiate to the myofibroblast phenotype when stretched
Carver et al. (1991)	Cardiac fibroblasts	Uniaxial (in vitro)	Mechanical stretch causes cardiac fibroblasts to produce more collagen
Della Rocca (2000)	VICs	Multiaxial (in vivo)	Cell phenotype correlates to the presence of multiaxial strain
Prajapati et al. (2000)	Human dermal fibroblasts	Uniaxial (in vitro)	Stretch induced an increase in matrix metalloproteinase production
Kessler et al. (2001)	Human dermal fibroblasts	Biaxial (in vitro)	Stretched cells differentiate into myofibroblast phenotype and synthesize more ECM components and MMPs
Park et al., (2002)	Human mesenchymal stem cells	Uniaxial (in vitro)	Cells differentiated into vascular smooth muscle cells exhibiting αSMA when stretched
Wang et al. (2003)	Cardiac fibroblasts	Static forces (in vitro)	Cells differentiate to the myofibroblast phenotype and an increase expression of αSMA and mRNA
Schenke-Layland et al. (2004)	VICs	Multiaxial (in vivo)	Cells experiencing dynamically changing cyclic forces exhibited αSMA and were identified as myofibroblasts

Schenke-Layland et al.[42] determined a relationship between cell phenotype and location by characterizing native heart valve tissue. In their characterization, they identified two cell phenotypes in the heart valve leaflet. Cells distributed evenly throughout the heart valve leaflet were characterized as fibroblasts, whereas cells experiencing dynamically changing cyclic forces exhibited αSMA and were identified as myofibroblasts. Similarly, Della Rocca[11] suggested a possible relationship between cell phenotype and the presence of multiaxial strain. Cells experiencing stretch were found to express αSMA. Squier[45] demonstrated that normal fibroblasts in rat skin undergoing mechanical stretch differentiate to the myofibroblast phenotype. Park et al.[35] found that human mesenchymal stem cells differentiated into vascular smooth muscle cells exhibiting αSMA when subjected to uniaxial stretch. Moreover, it was verified by Wang and his colleagues[52] that static forces contribute to the differentiation of cardiac fibroblasts to the myofibroblast phenotype, which was marked by an increased expression of αSMA.

The application of mechanical stretch has been shown to contribute to increased production of ECM components. Carver et al.[7] have demonstrated that applying mechanical stretch to cardiac fibroblasts elevates the production of collagen, indicating the possible presence of a more synthetic cell phenotype. Likewise Prajapati et al.[37] determined that mechanically loading fibroblasts in collagen gels leads to an increase in protease production.

2.4 Problem Statement

Although researchers have observed the general effects of substrate stiffness and stretch on cell phenotype, the interrelated contributions of these factors have not been quantified, as there is no means to simultaneously alter both. The goal of this project is to design and validate a method to culture mammalian cells in a two-dimensional environment at various levels of stiffness and equibiaxial stretch. As previously mentioned, the methods of altering stiffness and stretch must be carefully selected to encourage cell adhesion and growth; however, the environmental conditions should also simulate that of a functioning heart valve in order to obtain relevant data.

The success of this project will rely heavily on proper substrate material selection. Previous studies have used PA or PDMS to study the effects of substrate stiffness on cell response, but each of these substrates has limitations. It could be difficult to attach a PA substrate to the silicone membrane of a stretching device. PDMS would easily polymerize to a silicone membrane because it has similar chemical properties; however, it could be more difficult to adhere cells to this substrate. It may be necessary to investigate an alternate substrate material, or develop a new method of stretching the substrate that does not involve a silicone membrane. The next chapter will detail the specific aims and assumptions associated with this project.

3 Project Approach

This chapter reviews the main objective of this project and describes the general approach employed by the design team. Any assumptions concerning how the project was approached are identified, because these assumptions could later be proven incorrect or impact data interpretation. Specific aims are also defined, which will later be used to benchmark the progress and completion of the project.

3.1 Objectives

The goal of this project was to create a method for determining the interrelated effects of stiffness and stretch on cell phenotype. The first step of this project involved selecting a stretching mechanism and substrate material. After substrate stiffness and stretch were validated, cells were seeded on the substrates so that cell adhesion and viability properties could be measured.

3.2 Assumptions

The success of this project was contingent upon certain assumptions. First, it was assumed that the substrate to which the cells were attached was much less stiff than the silicone Flexcell™ membrane. This assumption insures that the membrane will continue to stretch uniformly and the substrate will experience the same stretch of the membrane. Second, it was assumed that the cells were experiencing the same amount of stretch as the substrate. The amount of stretch experienced by the cells was not specifically measured, but assumed through measurements of substrate strain. Finally, it was assumed that the substrates used in this experiment were flat, linear-elastic, isotropic, homogenous materials. These assumptions made it possible to calculate the substrate stiffness levels.

3.3 Tasks

The following tasks were used to benchmark the progress of our project:

1. Determine a method of stretching mammalian cells as little as 5% and as much as 15%
2. Validate that the applied stretch levels are accurate

3. Determine a method of modulating the stiffness of the substrate in a range comparable to PA substrates used in literature[3, 36, 51]
4. Validate the stiffness of the substrate
5. Design a method that will ensure that the number of cells attached to stretched and static substrates are comparable
6. Validate cell adhesion for at least two days

The conclusions section of this report will address the findings pertaining to each of these tasks. These tasks will not only ensure completion of this project, but also highlight our accomplishments and assess the performance of our method.

4 Design

At the beginning of this project, we were presented with the following initial problem statement:

"Design and validate a method for determining the combined effects of stiffness and stretch on cell phenotype."

In order to clarify the problem statement, it was necessary to establish the project's objectives, functions, and constraints. Successful completion of this task required input from all parties involved in the design effort. We identified the major stakeholders in the project to be the clients: the advisors Professors Kristen Billiar and George Pins; the user: graduate advisor Angela Throm; and the design team: ourselves.

We acquired design information through background research in scientific journals, brainstorming sessions with clients, and informal interviews with both clients and experts. Transcripts from these interviews can be found in Appendix A. After establishing lists of project objectives, functions, and constraints, we organized and prioritized the objectives with objective trees, pairwise comparison charts, and a weighted objective tree. In this chapter, we will discuss the information used to clarify the design goals and develop a revised problem statement. We will also describe potential design alternatives and their elimination based upon functions and constraints. Finally, we will explain how we chose a final design using pilot experiments.

4.1 Clarification of Design Goals

The first step in further defining the problem was to generate a list of overall project goals. Through background research, brainstorming sessions, and informal interviews we generated the list of project goals displayed in Table 4-1.

Table 4-1: Project Goals

Project goals:

1. Method should modulate stretch
2. Method should accommodate a range of stiffness levels
3. Method should encourage cell adhesion
4. Method should permit culture of many samples
5. Method should allow for culture of VICs and other fibroblasts
6. Method should be easy to use
7. Method should be sterile
8. Method should be bioinert
9. Method should be assay compatible
10. Method should be user independent
11. Method should be inexpensive
12. Method should be time efficient
13. Design should be durable
14. Design should be a convenient size

These goals were then formed into objectives, functions, and constraints and used in conjunction with other design tools to construct a revised problem statement.

4.1.1 Objectives, Functions, and Constraints

We determined how the overall project goals applied to what the design should achieve (objectives), what the design should do (functions), and any design constraints or limitations. At this time, we added more detailed information, which resulted in the objectives, functions, and constrains displayed in Table 4-2, Table 4-3, and Table 4-4, respectively.

Table 4-2: Project Objectives

Project Objectives:

1. Easy to use
    1.1. Portable
    1.2. Easy to Sterilize
    1.3. Assay Compatible
    1.4. Quick to setup
2. Inexpensive
3. Quick to make
4. Durable
5. Effective
    5.1. Cell adhesion
        5.1.1. Duration of attachment
        5.1.2. Initial attachment
    5.2. Assay compatible
        5.2.1. Immunocytochemistry
        5.2.2. Immunoblots
        5.2.3. Measure of cell contraction
    5.3. Range of stretch
        5.3.1. Accurate
        5.3.2. Precise
    5.4. Range of stiffness
        5.4.1. Accurate
        5.4.2. Precise
    5.5. Sterile substrate
6. User independent

Table 4-3: Project Functions

Project Functions

1. Stretching device should apply 5-15% equibiaxial stretch to the membranes
2. Stretching device should control the rate and magnitude of stretch applied to the membranes
3. Substrate should have a range of stiffness comparable to PA with assumed values of 7 and 75 kPa (See Appendix D)
4. Substrate should encourage cell adhesion
5. Method should allow the samples to culture in an incubator
6. Method should support the culture of VICs and other fibroblasts
7. Method should allow for the culture of many samples
8. Method should allow for the desired assays and microscopy (immunocytochemistry, immunoblots, and traction force microscopy)

Table 4-4: Project Constraints

Project Constraints

1. Apparatus must cost less than 2,000 dollars
2. Substrate must be able to stretch as little as 5% and as much as 15%
3. Stiffness of the substrate must reach a level as low as "7 kPa" and as high as "75 kPa"
4. Number of cells attached to the stretched and static substrates must be comparable
5. Experiments must run for at least 2 days
6. Method must measure at least 1 of the 3 following phenotypic markers:
    a. Cell contractility force (traction force microscopy)
    b. Immunoblots
    c. Immunocytochemistry
7. Sterilization process for the device must be available on campus
8. Size of the device is limited to 2 shelves in a standard incubator
9. Stretching device must allow for multiple samples
10. Project must be completed in 6 months
11. Apparatus must require less than 3 hours for set-up
12. Entire preparation process must require less than 3 days
13. Cells must not remodel the substrate

From the list of objectives, pairwise comparison charts were created to determine the relative importance of each objective. The objectives were weighted in these charts by the clients, Professor Billiar, Professor Pins, Angela Throm, and the design team. This method helped us to quantitatively determine how the clients prioritized the objectives.

The six sets of completed pairwise comparison charts can be found in Appendix B. The results of the charts were used to generate the weighted objective tree found in Table 4-5. This table displays that effectiveness is the most important objective and within this category cell adhesion, range of stretch, and range of stiffness are rated the highest. These results were taken into consideration when developing and evaluating design alternatives. The constraints were also used to evaluate design alternatives and select a final design, as described in the following sections.

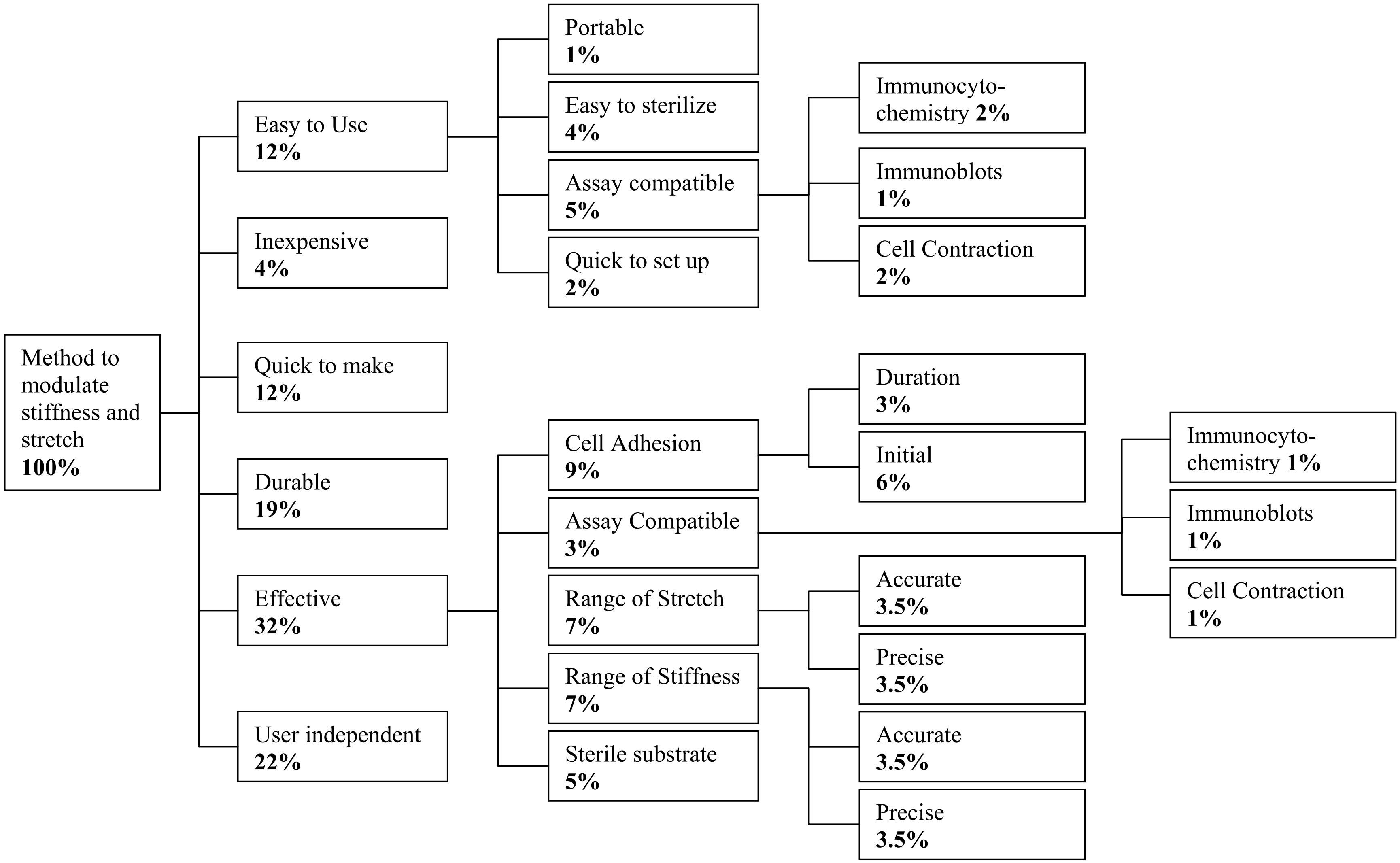
4.1.2 Revised Problem Statement

After establishing and prioritizing the objectives, functions, and constraints of our design, we were able to clarify the initial problem statement. Taking the outcomes of the pairwise comparison charts into consideration, we developed the following revised problem statement:

> *"Design and validate a method for determining the combined effects of stiffness and stretch on cell phenotype. The method should allow for a range of stretch (5-15%) and stiffness levels ("7-75 kPa")*[27,31,40] *which are comparable to normal and diseased heart valve tissue. Furthermore, the number of cells attached to the stretched and static samples should be comparable."*

This problem statement highlights the most important objectives and provides valuable information for final design selection. After these criteria had been established, we needed to develop an extensive list of design alternatives.

Table 4-5: Weighted Objective Tree



4.2 Design Alternatives

After the three main objectives (stretch, stiffness, cell adhesion) were defined, we brainstormed possible means to satisfy their parallel functions. The preliminary lists, which were formed with client input, are displayed in Table 4-6.

Table 4-6: Function Means Table

Function	Means
Apply and Control Stretch	• Vacuum • Screwtop • Plunger
Modulate Stiffness	• Polyacrylamide (PA) • Polyethylene oxide (PEO)/ Polyethylene glycol (PEG) • Hylauronic acid (HA) • Polydimethylsiloxane (PDMS) • Collagen gel • Polylactic acid and polyglycolic acid (PLA/PGA) • Polyvinyl alcohol (PVA) • Polyglycolic acid (PGA) • Fibrin gel • Gelatin • Chitosan • Agarose • Alginate
Encourage Cell Adhesion	• Fibronectin • Collagen solution • Arginine-glycine-aspartic acid (RGD)

In order to quantitatively evaluate each design alternative, we created a decision matrix for each function. These matrices first test that the design alternatives meet relevant project constraints. Once an alternative has satisfied this requirement, it is given a numerical score for each first tier objective. Only the first tier objectives are weighted, representing the full set of objectives. The score is then multiplied by the weight of the objective, which was previously determined using pairwise comparison charts, to calculate a weighted score. The decision matrices can be found in Table 4-7, Table 4-8, and Table 4-9, and the metrics used to score each objective can be found in Appendix C.

4.2.1 Stretching Devices

The mechanics of the three proposed stretching devices have been described in detail in section 2.4.2.1. All of these devices use silicone as a deformable membrane on which equibiaxial stretch can be implemented; however, there are some differences. Lee's device was eliminated because it did not meet the design constraint that allowed for multiple samples. The Flexcell™ device and Tschumperlin's device met all of the design constraints, so they were evaluated with weighted scores (see Table 4-7 and Appendix C). The Flexcell™ device was given a score of 93, only losing points in the "Effective" objective for its limited range of stretch. Tschumperlin's device received a weighted score of 72.44. This device received a lower score because it is not self-regulating, it is more costly to make, and it takes longer to assemble. Based on the scores in the stretching device decision matrix, the Flexcell™ device is the most favorable device for this experiment.

4.2.2 Polymers to Modulate Stiffness

After we established that the Flexcell™ device was the best stretching mechanism, we needed to determine a method to modulate stiffness that would satisfy relevant design constraints. In order to use this stretching device and modulate the stiffness of the substrate on which the cells were seeded, a substrate would have to be attached to the silicone membrane. As seen in Table 4-8, many of the substrate alternates were eliminated, because if seeded with cells, the cells would remodel the substrate. PVA was eliminated as a substrate alternative due to its limited ability to stretch, and HA was eliminated because it takes more than three days to prepare. PEO/PEG did not satisfy the desired range of stiffness, and PLA/PGA did not achieve the desire range of stretch. Alginate was eliminated because alginate's elastic modulus changes when exposed to saline.

The two substrate materials which were not eliminated based on constraints were PA and PDMS. PA was given a nearly perfect score, only losing 0.8 points for its expense. PDMS substrates are less expensive to produce, but the process takes longer, and the substrates may not be compatible with the chosen immunoassays. In addition, we hypothesize that PDMS substrates are less user independent, because it is difficult to accurately measure small quantities of the sticky components by mass.

PA or PDMS would be acceptable substrate materials for this experiment given that they meet all design constraints. Pilot tests were conducted to determine which substrate material would be more compatible with the Flexcell™ device.

4.2.3 Treatments to Enhance Cell Adhesion

All of the cell adhesion treatments met the design constraints, but the collagen solution earned the highest weighted score. The collagen solution treatment only lost points because of the time required for the solution to dry on the substrate. Fibronectin scored second highest, losing points for the time and expense associated with the treatment. Covalently attaching RGD peptides to the surface of the substrate could also enhance cell adhesion, but this treatment is expensive and degrades during stretching. A collagen solution was chosen as the preferred method to enhance cell adhesion because collagen was readily available in our laboratory and relatively cheap when compared to other options.

Table 4-7: Stretching Device Decision Matrix

Stretching Device Constraints		**Flexcell™ device**	**Lee's device**	**Tschumperlin's device**
C: Must cost less than $2000		Y		Y
C: Must be able to stretch as little as 5% and as much as 15%		Y		Y
C: Must run for at least 2 days		Y		Y
C: Must be easy to sterilize on campus		Y		Y
C: Must fit on two shelves of a standard incubator		Y		Y
C: Must allow for multiple samples		Y	N[19]	Y
C: Must require less than 3 hours for set-up		Y		Y

Stretching Device Objectives	**Weight (%)**	**Flexcell™ device**		**Tschumperlin's device**	
		Score	Weighted Score	Score	Weighted Score
O: Easy to Use	12	1.0	12	0.66	7.92
O: Inexpensive	4	1.0	4	0.25	1
O: Quick to Make	12	1.0	12	0.5	6
O: Durable	19	1.0	19	1.0	19
O: Effective	32	0.75	24	0.75	24
O: User Independent	22	1.0	22	0.66	14.52
TOTAL	**100**		**93**		**72.44**

Table 4-8: Substrate Decision Matrix

Substrate Constraints		**PA**	**PEO/PEG**	**HA**	**PDMS**	**Collagen gel**	**PLA/PGA**	**PVA**	**Fibrin gel**	**Gelatin**	**Chitosan**	**Agarose**	**Alginate**
C: Must be able to stretch 5%-15%		Y			Y		N[28]	N[17]				N[32]	N[22]
C: Must reach a stiffness as low as "7 kPa and as high as 75 kPa"		Y	N[41]		Y								
C: Must allow for execution of 1 desired assay		Y			Y								
C: Must have sterilization technique on campus		Y			Y								
C: Must take less than 3 days to prepare		Y		N[43]	Y								
C: Cells must not be able to remodel substrate		Y			Y	N[15]			N[4]	N[9]	N[46]		N[21]

Substrate Objectives	**Weight (%)**	**PA**		**PDMS**	
		Score	Weighted Score	Score	Weighted Score
O: Easy to Use	12	1.0	12	0.75	9
O: Inexpensive	4	0.8	3	1.0	4
O: Quick to Make	12	1.0	12	0.7	8.4
O: Durable	19	1.0	19	1.0	19
O: Effective	32	1.0	32	0.75	24
O: User Independent	22	1.0	22	0.66	14.52
TOTAL	**100**		**100**		**78.92**

Table 4-9: Cell Adhesion Treatment Decision Matrix

Cell Adhesion Constraints		Fibronectin	Collagen	RGD
C: Must cost less than $2000		Y	Y	Y
C: Must last for at least 2 days		Y	Y	Y
C: Must require less than 3 days to prepare		Y	Y	Y

Cell Adhesion Objectives	Weight (%)	Fibronectin		Collagen		RGD	
		Score	Weighted Score	Score	Weighted Score	Score	Weighted Score
O: Easy to Use	12	1.0	12	1.0	12	1.0	12
O: Inexpensive	4	0.9	3.6	1.0	4	0.7	2.8
O: Quick to Make	12	0.7	8.4	0.7	8.4	1.0	12
O: Durable	19	1.0	19	1.0	19	0.66	12.5
O: Effective	32	1.0	32	1.0	32	1.0	32
O: User Independent	22	1.0	22	1.0	22	1.0	22
TOTAL	**100**		**97**		**97.4**		**92.3**

4.3 Pilot Experiments

PA and PDMS were not eliminated as substrate materials based on design constraints; however, both of these substrates have associated limitations. PA can be polymerized to the desired range of stiffness, and proteins can be adhered to the surface to improve cellular adhesion properties. However, PA is an extremely hydrophilic material[34], which makes it is very difficult to attach it to the hydrophobic silicone membrane of the Flexcell™ device[16]. On the other hand, the properties of PDMS are similar to that of silicone[16], so it is easy to attach this material to the Flexcell™ membrane. Unfortunately, previous research has not demonstrated that the stiffness of PDMS can be tailored to go as low as that of PA ("7 kPa"). To establish if we could overcome these limitations we conducted two series of pilot tests. The first series was a group of experiments in which we chemically and physically modified the surface of the Flexcell™ silicone membrane to make it more suitable for the attachment of PA. The second series was a set of experiments to determine if the stiffness of PDMS could be modified to values lower than that found in literature and if cell life could be maintained at these new stiffness levels.

4.3.1 Polyacrylamide Pilot Experiments

The first series of pilot experiments were aimed at determining if the silicone membrane could be physically or chemically modified to encourage the adhesion of PA to this membrane. We first attempted to polymerize the PA on an untreated silicone membrane, and as expected the PA did not attach to the membrane. The next set of experiments involved using a surface treatment on the silicone membrane, physically roughening the surface, or putting an intermediate layer between the silicone and the PA. We also attempted to polymerize PA in a foam anchored silicone well. The tests which we conducted are displayed in Table 4-10.

Table 4-10: Surface Modifications for the Adhesion of PA on the Silicone Membrane

1. Chemically treating the surface
    a. H_2SO_4
    b. Diethylenetriamine (DETA)
    c. 3-aminopropyltrimethoxysilane
    d. Collagen solution
2. Physically roughening the surface with sandpaper
3. Creating an intermediate layer
    a. Collagen gel
    b. PDMS
        i. Treated with 3-aminopropyltrimethoxysilane
        ii. Treated with H_2SO_4
        iii. With sand
    c. PA with sand
4. Using foam anchored well

Each of these tests was unsuccessful because the PA failed to remain adhered to the silicone membrane. The most common reason for failure was the PA adhering to the coverslip instead of the silicone membrane (the protocol which was used to prepare PA substrates can be found in Appendix D).

To overcome the limitations of the glass coverslip, we brainstormed additional techniques to polymerize the PA substrate. A test was conducted to determine if a thick layer of PA (without a coverslip) could attach to a silicone membrane treated with 3-aminopropyltrimethoxysilane or a collagen solution. The result of this test revealed that PA can initially attach to the membrane treated with either method, and can remain attached while undergoing stretch. However, when we flooded the surface of the substrate with PBS (for storage purposes) the gel expanded (PA is a hydrogel), causing the substrate to delaminate from the silicone membrane.

The results from this test revealed that a smaller and thinner substrate is necessary to allow for expansion. To create a smaller and thinner substrate, we attempted to identify a mechanism to flatten the substrate. Our previous experiments used a glass coverslip to create thin substrates; however, as previously mentioned, in all of these tests the substrate attached to the coverslip instead of the silicone membrane. We attempted using a laminated metal coverslip and a glass coverslip coated with PDMS. Unfortunately, the coverslips prevented the PA from polymerizing.

4.3.2 PDMS Pilot Experiments

The stiffness of PDMS can be altered by changing the ratio of its two components, a base and a crosslinking agent. Brown et al.[6] determined that PDMS can attain a range of stiffness from 48-1783 kPa by changing the ratio of base to crosslinking agent from 10:1 to 50:1, respectively. Brown attempted to create a larger range of stiffness; however, at ratios lower than 10:1, the stiffness PDMS was found to plateau, and at ratios higher than 50:1, the PDMS was too difficult to work with. In order for us to use PDMS, it would have to reach a stiffness as low as 7 kPa. We hypothesized that base to crosslinker ratios higher than 50:1 were too difficult to handle when Brown used a tensile test to determine elastic modulus. This problem could easily be circumvented by employing a different method for measuring the elastic modulus. We synthesized PDMS in ratios of 65:1 and 90:1 and measured the stiffness of the polymer using a parallel plate rheometer (see section 5.2). The average stiffness of the 65:1 PDMS was 4.9 kPa and the average stiffness of the 90:1 PDMS was 1.3 kPa.

Not only was it important for us to determine the stiffness of the PDMS at new ratios, but it was also important for us to determine if the new ratios created cytotoxic conditions. We seeded dermal fibroblasts at a density of 12,500 cells/cm^2 on PDMS substrates with 90:1 and 65:1 ratios of base:crosslinker. A collagen solution was dried on the substrate surface to encourage cell adhesion (see methods section for details). Cells were incubated in culture media for two days. A Hoechst stain (Molecular Probes) and epifluorescence microscope with a 10x objective were used to visualize the nuclei. Sample images are displayed in Figure 4-1 and Figure 4-2.

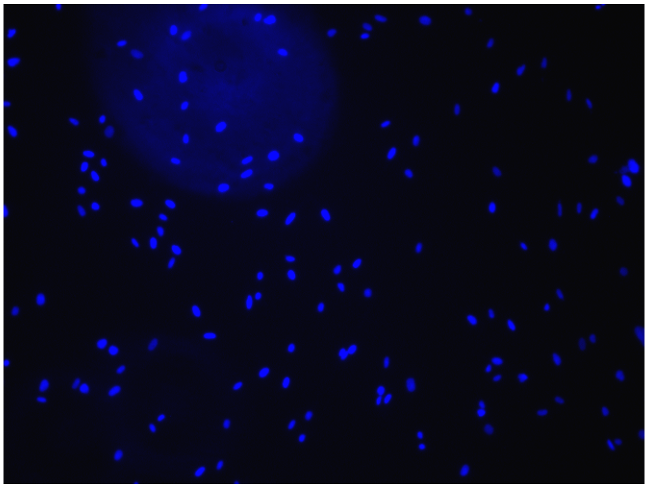


Figure 4-1: Hoechst Stain of Fibroblast Nuclei on 65:1 PDMS

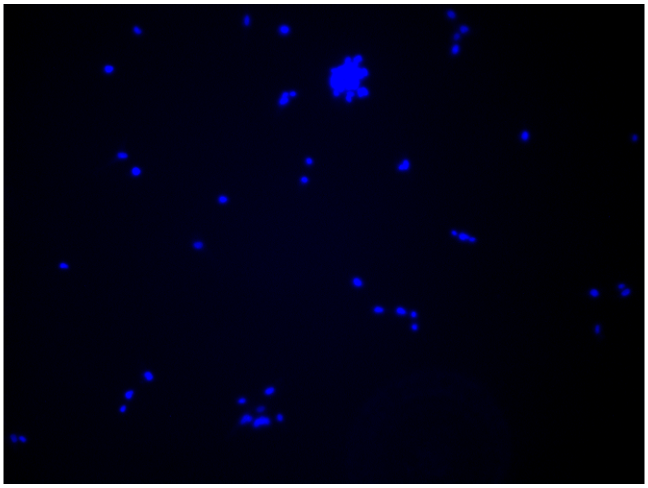


Figure 4-2: Hoechst Stain of Fibroblast Nuclei on 90:1 PDMS

Preliminary tests revealed that cells could be cultured on the PDMS substrates after they had been treated with a collagen solution. Moreover, the cells on the static substrate were confluent within four days (images unavailable).

4.4 Final Design

Based upon the project objects, functions, constraints, and pilot test results, we chose a final design. The final design consists of a Flexcell™ device used for stretch, a PDMS substrate to modulate stiffness, and a collagen solution to enhance cell adhesion.

5 Methods

This section describes materials and techniques used to conduct our experiments. All materials were purchased from VWR unless otherwise specified.

5.1 Substrate Preparation

Sylgard 184 (Ellsworth Adhesives) a two part PDMS kit consisting of a silicone elastomer base and crosslinking agent was used to prepare substrates. Different ratios of base to crosslinking agent were mixed by mass to create substrates with a range of stiffness levels. The base and crosslinking agent were mixed with a wooden stick and then degassed for 30 minutes to remove air bubbles. The prepolymer was poured inside a 3 mL syringe and 0.85 mL was added to each 3.3 cm diameter well of an untreated BioFlex® Culture Plate (Flexcell™ International) to create a 1 mm thick substrate. The tray was placed on a level countertop to allow the substrate to flatten, and then the tray was placed in a 60°C oven for 18 to 20 hours to allow the PDMS to cure.

After the PDMS had cooled to room temperature, 3 mL of an 23.54 µg/mL collagen solution was added to the surface of each substrate to achieve a collagen density most appropriate for cell culture (5-10 µg/cm^2).[2] Three mL of the collagen solution was added to each well and incubated at room temperature until the solution evaporated. Substrates were then sterilized under ultraviolet light for 10 minutes.

5.2 Validation of Stiffness

After researching and testing several methods for determining substrate stiffness, our group concluded that different testing methods produce incomparable data. The majority of previous studies have used PA substrates with assumed stiffness levels between "7 and 75 kPa" (see Table 2-1) based on Wang's protocol in Appendix D. In order to create substrates with equivalent stiffness levels, we compared substrates of PDMS to PA substrates with the assumed values of 7 and 75 kPa. PDMS was prepared as described above at 50:1, 60:1, 80:1, and 90:1 ratios. PA was polymerized between two glass plates with 8%/0.08% and 5%/0.025% ratios of acrylamide/bis. All substrate samples were equilibrated with culturing medium at 37°C for 30 minutes before they were tested.

The stiffness levels of the substrates were measured using a Bohlin Gemini stress rheometer with 20 mm smooth parallel plates. The sample was placed between the two parallel plates. The bottom plate remained stationary as the top plate was rotated. The amount of strain was gradually increased to determine the linear, viscoelastic range of the substrate and insure that the sample was not slipping. The complex modulus of the sample was measured based on the deformation and flow of the sample under stress and strain. The complex modulus was converted to elastic modulus using the equation $E = 2G(1+\nu)$ where E is the elastic modulus, G is the complex modulus, and ν is the sample's Poisson's ratio. The Poisson's ratio of PA was assumed to be 0.3[23] and the Poisson's ratio of PDMS was assumed to be 0.5[26]. Three measurements were taken for each substrate at each stiffness level. The average measurements of PA and PDMS were compared to validate similar stiffness levels.

5.3 Application of Stretch

Flexcell™® FX-4000T (Flexcell™ International, Hillsborough, NC) was used to apply uniform, equibiaxial, cyclic stretch to the substrate as described in section 2.4.2.1. Briefly, the device stretches the membrane by applying vacuum pressure which pulls the membrane over the loading posts. These vacuum pressure levels correspond to levels of stretch programmed into the Flexcell™ device. Experiments were performed with cells stretched at 5% and 15% on PDMS polymerized at 50:1 and 80:1 ratios at a frequency of 0.2 Hz for two days.

5.4 Validation of Stretch

To ensure that the PDMS substrates were actually experiencing 5% and 15% equibiaxial stretch, experiments were performed while stretching the PDMS substrates alone. High Density Mapper software (HDM) was used to measure the strain field experienced by each substrate.[18] This software was validated using the traditional triad method. An image of spots on a sheet of paper was electronically expanded to a known percentage of the original image and both an HDM and triad method analysis were performed.

Subsequent to HDM validation, the PDMS substrates were prepared for stretching. Black sand particles were added to the PDMS to create a speckle pattern. Without moving the position of the camera, raw digital images were taken while the substrates were static and stretched 5%, 10%, and 15%, using a 6 Megapixel Canon Digital Rebel XT camera with a resolution of ~0.048 pixels/µm. As displayed in Figure 5-1, the camera was attached to a vertical, thin metal slate using a screw so that it was aimed directly down at the laboratory table top. The Flexcell™ plates were placed on lubricated platens and sealed in place on a larger base plate connected to a vacuum pump and pressure reservoir. The plates were placed 16 cm below the camera, and the specific well of interest was positioned in the center of the lens's focal point. A white poster board was held curved above the camera to create an umbrella effect, and a 60 watt flood light was shined onto the poster board to create a uniform distribution of light on the substrate.

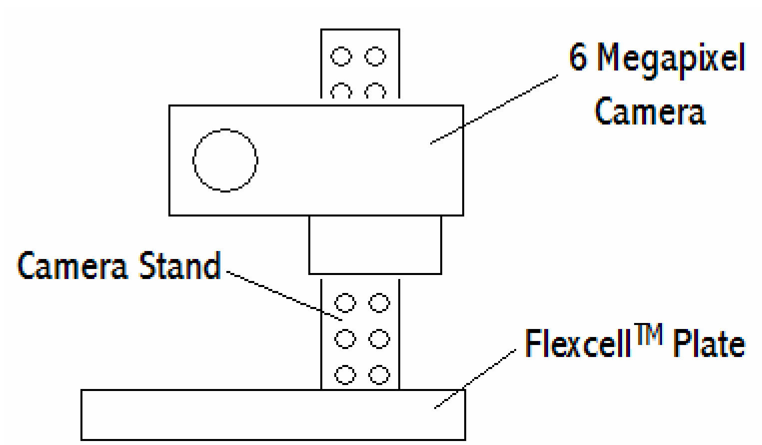


Figure 5-1: Flexcell™ Camera Setup

The images were converted to Tiff format and input into the High Density Mapper program (HDM). A square region at the center of the well, approximately 500 x 500 pixels, was selected for analysis. This area was chosen to represent the area in which the cells would experience equibiaxial stretch. The HDM data were entered into Excel, and deformations were analyzed to validate 5% and 15% strain in the longitudinal and transverse directions. Modified z-tests were used to eliminate outliers in the data. For a full HDM/Excel protocol and camera use instructions, see Appendix E and Appendix F.

5.5 Cell Culture

ATTC human foreskin fibroblasts were plated in T-150 flasks in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 100 units/mL penicillin G sodium, 100 µg/mL streptomycin sulfate, and 250 ng/mL amphotericin B. Cells were cultured in an 80-90% humid incubator at 37° C and 10% CO_2 and split when confluent. Cells were removed with 2.5% trypsin and .01% EDTA and used at passage 6.

5.6 Cell Seeding

Prior to the seeding of cells, PDMS substrates were equilibrated in 3 mL of culturing medium for 30 minutes at 37 ^{o}C. The fibroblasts were plated at 12,500 cells/cm^2 per well. The cells were cultured in DMEM supplemented with 10% fetal bovine serum and 100 units/mL penicillin G sodium, 100 µg/mL streptomycin sulfate, and 250 ng/mL amphotericin B. All plates remained static for 24 hours to allow for initial cell attachment. Media was changed every two days and the experiment resumed. A total of 36 wells were seeded with cells in order to consider the effects of stretch and substrate stiffness over a period of two days.

5.7 Validation of Cell Viability

A LIVE/DEAD® Viability/Cytotoxicity Assay Kit (Molecular Probes) was used to determine cell viability. This assay uses Calcein AM to stain the plasma membrane of live cells and Ethidium homodimer-1 to stain nucleic acids within dead cells. 20 µm of the supplied 2 mM Ethidium homodimer-1 and 5 µL of 4 mM calcein AM were added to 10 mL of sterile PBS, then vortexed to insure thorough mixing. Culturing medium was aspirated off of the cells on the Flexcell™ membranes and the cells were rinsed with sterile PBS. 1.5 mL of the LIVE/DEAD® solution was added to each Flexcell™ well and the cells were incubated for 20 minutes at 37°C. The LIVE/DEAD® solution was then removed, and the wells were stored in PBS for up to 24 hours.

An epifluorescence microscope (Nikon Eclipse E600) with FITC and Texas Red filters was used to examine the cells, and photographs were taken with Spot Digital Analysis Software. Cells were counted for each sample from multiple fields using digital image analysis software (ScionImage, Scion Corporation). A minimum of five fields were imaged from each sample with each filter. Cell adhesion to both static and stretch PDMS substrates were measured at days 0 and 2.[‡]

[‡] Day number refers to the number of days substrate has been stretched (0 and 2).

6 Results

This chapter summarizes the data collected while validating stiffness, stretch, and cell viability, using the previously described methods. Please view the appendices referenced within this chapter for complete sets of data. The results of additional experiments, which were conducted using VICs and 65:1 and 90:1 PDMS substrates, can be found in Appendix K.

6.1 Validation of Stiffness

As explained in the methodology chapter, the stiffness of PDMS samples was tested, and then compared to PA samples with assumed stiffness levels of 7 and 75 kPa. Figure 6-1 displays the average and standard deviation of the stiffness for each sample. Figure 6-2 contains an example of the complex modulus remaining linear as the strain was increased, confirming a region of linear viscoelastic response. The complete set of numerical data and complex modulus vs. strain charts can be found in Appendix G.

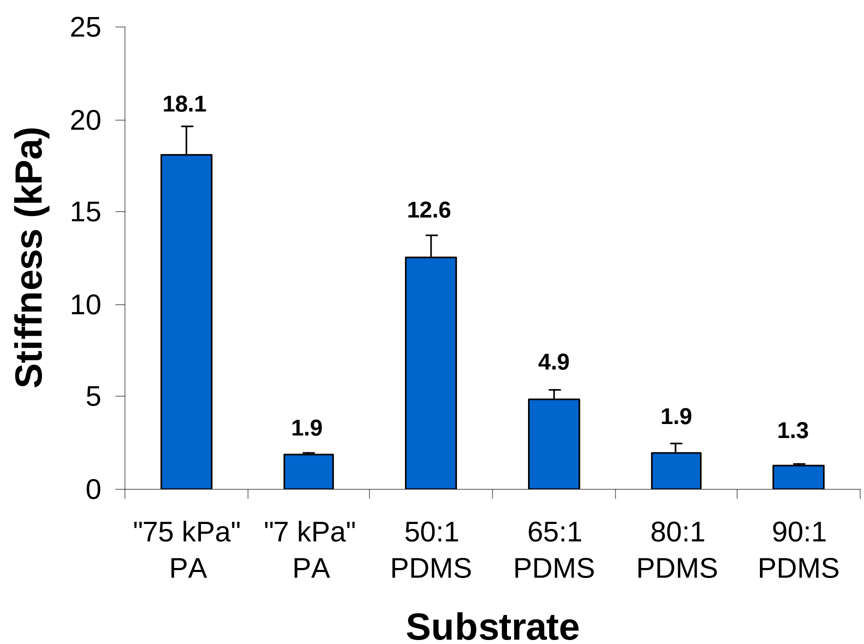


Figure 6-1: Substrate Stiffness Results

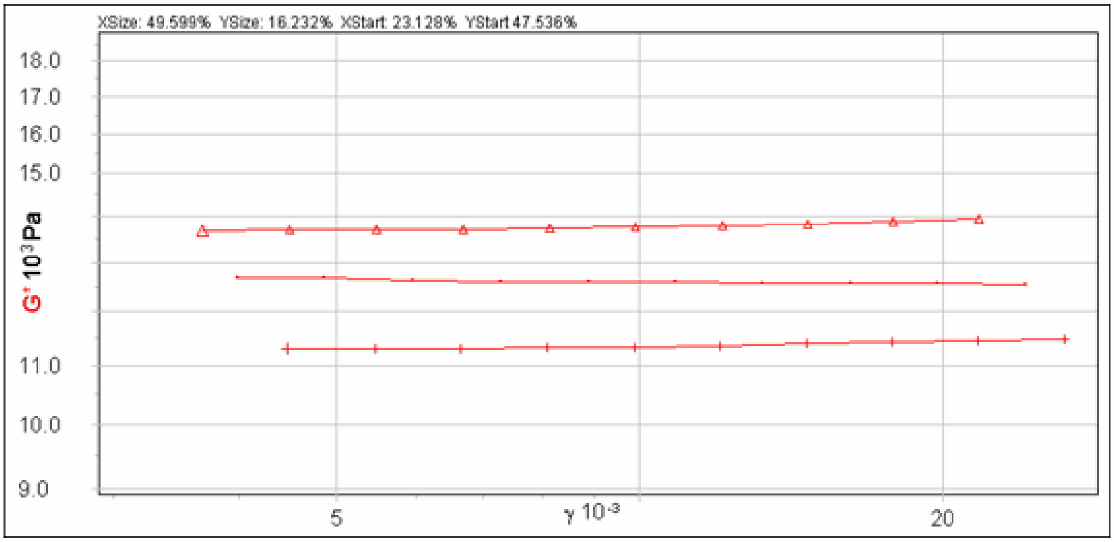


Figure 6-2: Complex Modulus vs. Strain

6.2 Validation of Stretch

To validate the vacuum pressure induced stretch produced by the Flexcell™ device, we used HDM software. Our first task was to validate the HDM software to ensure that calibration was unnecessary. We evaluated images of spots on paper and then proceeded to determine the strain fields of the 50:1 and 80:1 PDMS.

6.2.1 Spots on Paper

HDM produced displacement results of the 5% enlarged paper for the vertical, v, and horizontal, u, directions which can be found in Appendix H. These results were used to determine shear strain, ε_{xy}, and longitudinal strains, ε_{xx} and ε_{yy}, in the u and v directions, respectively. The spots on paper, stretched 5%, resulted in the strains shown in Table 6-1. A sample contour map is displayed in Figure 6-3 and a complete set of contour maps is located in Appendix H.

Table 6-1: Strains calculated from the HDM displacement results for spots on paper with 5% digital enlargement

	ε_{yy}	ε_{xx}	ε_{xy}
MAX	5.82%	5.79%	0.61%
MIN	4.25%	3.95%	-0.78%
AVG	5.00%	4.97%	0.00%
STDEV	0.29%	0.27%	0.17%

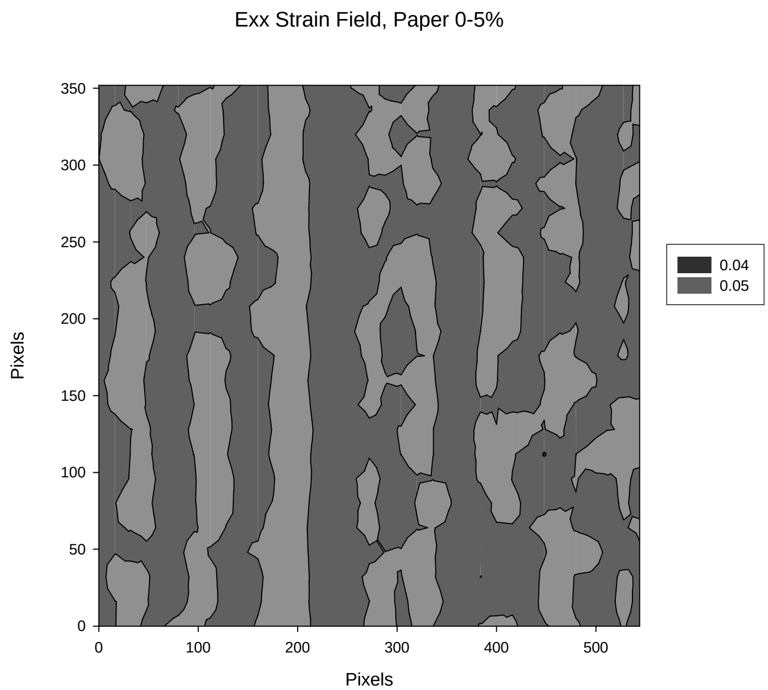


Figure 6-3: Contour Map of Eyy Strain Field for Paper with 5% Digital Enlargement

The triad method was subsequently used to validate the HDM results. The positions of three spots in the center, bottom right, and top left of the two images were measured with Image J (as seen in Appendix I), and their displacement was calculated. Table 6-2 and

Table 6-3 display the results of the Image J measurements and triad method analysis, respectively.

Table 6-2: Image J position measurements

Center	Position	
Points	u	v
1	1439.679	1028.893
2	1472.451	743.8865
3	1655.088	834.206
1'	1435.369	1028.719
2'	1469.076	730.6668
3'	1660.699	825.3424

Bottom Right	Position	
Points	u	v
1	2289.048	791.7017
2	2408.497	478.361
3	2472.659	725.735
1'	2326.807	780.05
2'	2452.268	451.1692
3'	2519.629	710.8812

Table 6-3: Triad Method Results

	ε_{yy}	ε_{xx}	ε_{xy}
Center	4.70%	4.92%	0.18%
Bottom Right	5.10%	5.14%	0.02%
Top Left	5.24%	5.16%	0.00%

6.2.2 Strain Field of 50:1 PDMS

HDM data were obtained from a 500 x 500 pixel square in the center of the well for each strain condition. The images were evaluated using a 64 pixel subimage size with a 16 pixel shift. The protocol in Appendix E was used to produce the strain results and contour maps displayed in Appendix H. The maximum, minimum, average, and standard deviation of these results can be found in Table 6-4. A sample contour plot is displayed in Figure 6-4.

Table 6-4: HDM results for 5%, 10%, and 15% strain of 50:1 PDMS

Flexcell™ Reading	**0% to 5.19%**				**5.19% to 10.3%**				**10.3% to 14.9%**		
	ε_{yy}	ε_{xx}	ε_{xy}		ε_{yy}	ε_{xx}	ε_{xy}		ε_{yy}	ε_{xx}	ε_{xy}
MAX	8.48%	8.72%	2.50%		8.94%	8.26%	1.57%		7.40%	9.08%	2.36%
MIN	2.92%	1.05%	-2.71%		3.25%	2.36%	-2.43%		2.25%	2.40%	-2.11%
AVG	5.80%	4.46%	-0.13%		5.87%	5.14%	-0.21%		4.91%	5.53%	-0.21%
STDEV	0.96%	1.31%	0.86%		0.78%	0.99%	0.69%		0.72%	1.20%	0.63%

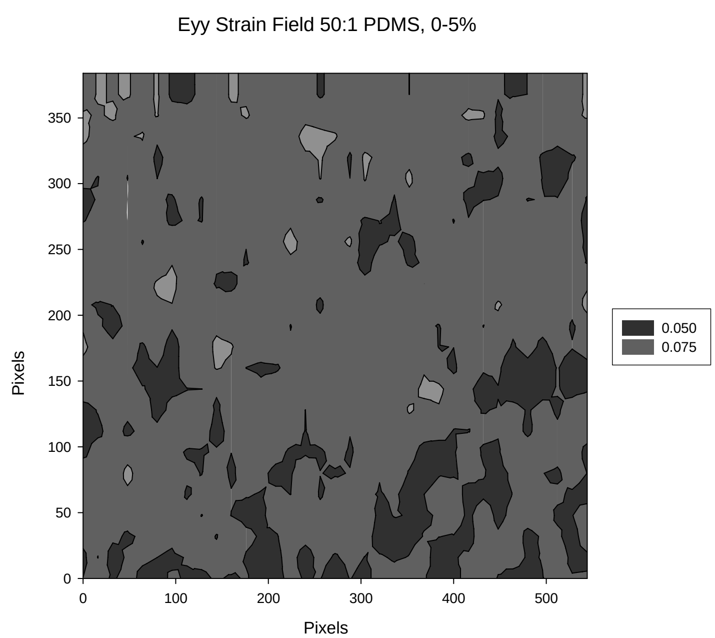


Figure 6-4: Contour Map of Eyy Strain Field for 50:1 PDMS Substrate with 5% Strain

Although the PDMS was in fact stretched from static to 5%, static to 10%, and static to 15%, results for 15% stretch were obtained by comparing the images of 0% to 5%, 5% to 10%, and 10% to 15%. Matrices were then used to determine the actual strain results for 10 % and 15% stretch (see Appendix H). The average results can be found in Table 6-5.

Table 6-5: HDM results of cumulative strain for 10%, and 15% strain of 50:1 PDMS

	ε_{yy}	ε_{xx}
0-10%	11.65%	9.54%
0-15%	16.99%	15.41%

6.2.3 Strain Field of 80:1 PDMS

HDM data were obtained from a 400 x 400 pixel square in the center of the well for each strain condition. To eliminate noise due to reflections, a smaller area was evaluated and HDM analysis was performed using a 64 pixel subimage size with a 32 pixel shift. The protocol in Appendix E was used to calculate the results displayed in Table 6-6. A sample contour map is displayed in Figure 6-5. Raw data and contour plots can be found in Appendix H.

Table 6-6: HDM results for 5%, 10%, and 15% of 80:1 PDMS

	0% to 5.26%				**5.26% to 10.4%**				**10.4% to 15.1%**		
	ε_{yy}	ε_{xx}	ε_{xy}		ε_{yy}	ε_{xx}	ε_{xy}		ε_{yy}	ε_{xx}	ε_{xy}
MAX	17.48%	17.00%	12.90%		8.85%	8.50%	5.04%		4.68%	6.78%	2.46%
MIN	-17.82%	0.22%	-15.93%		3.04%	2.51%	0.04%		0.31%	2.19%	-0.27%
AVG	8.56%	4.76%	-2.49%		5.69%	5.33%	2.99%		2.65%	4.16%	1.15%
STDEV	4.02%	2.14%	2.65%		1.38%	1.20%	1.01%		0.89%	0.85%	0.54%

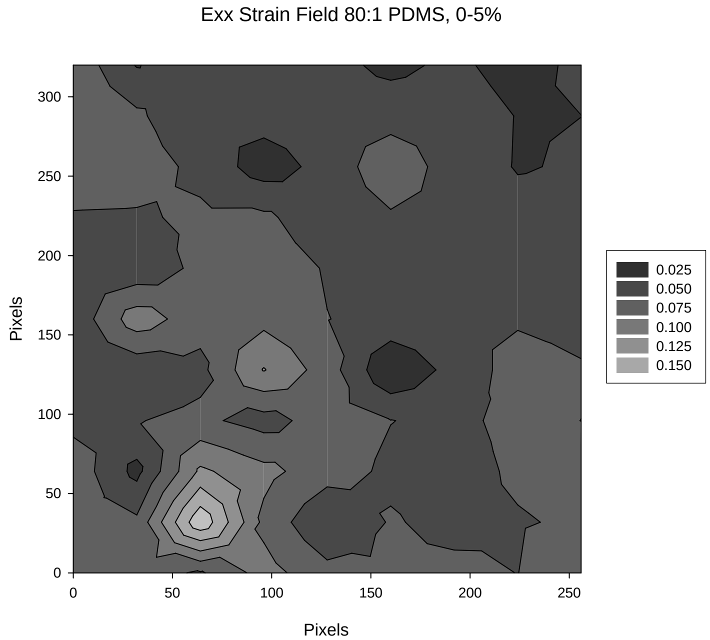


Figure 6-5: Contour Map of Eyy Strain Field for 80:1 PDMS Substrate with 5% Strain

6.3 Validation of Cell Viability

Cells were stained using the LIVE/DEAD® assay kit one day after cell seeding (Day 0), and after undergoing two days of stretch (Day 2). Five images were taken from each sample. Sample images for Day 0 of both the 5% and 15% stretch experiment are shown in Figure 6-6. Figure 6-7 displays sample images from the 5% stretch experiment, and Figure 6-8 displays sample images from the 15% stretch experiment.

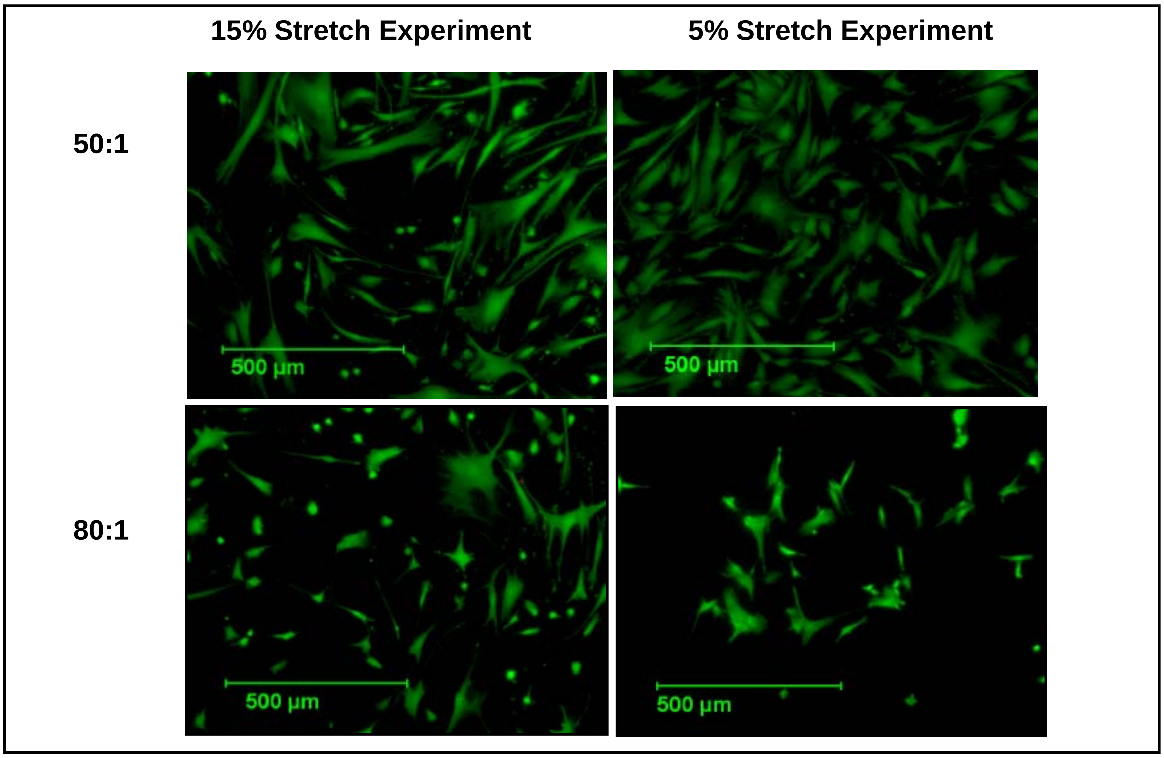


Figure 6-6: Sample Images from Day 0

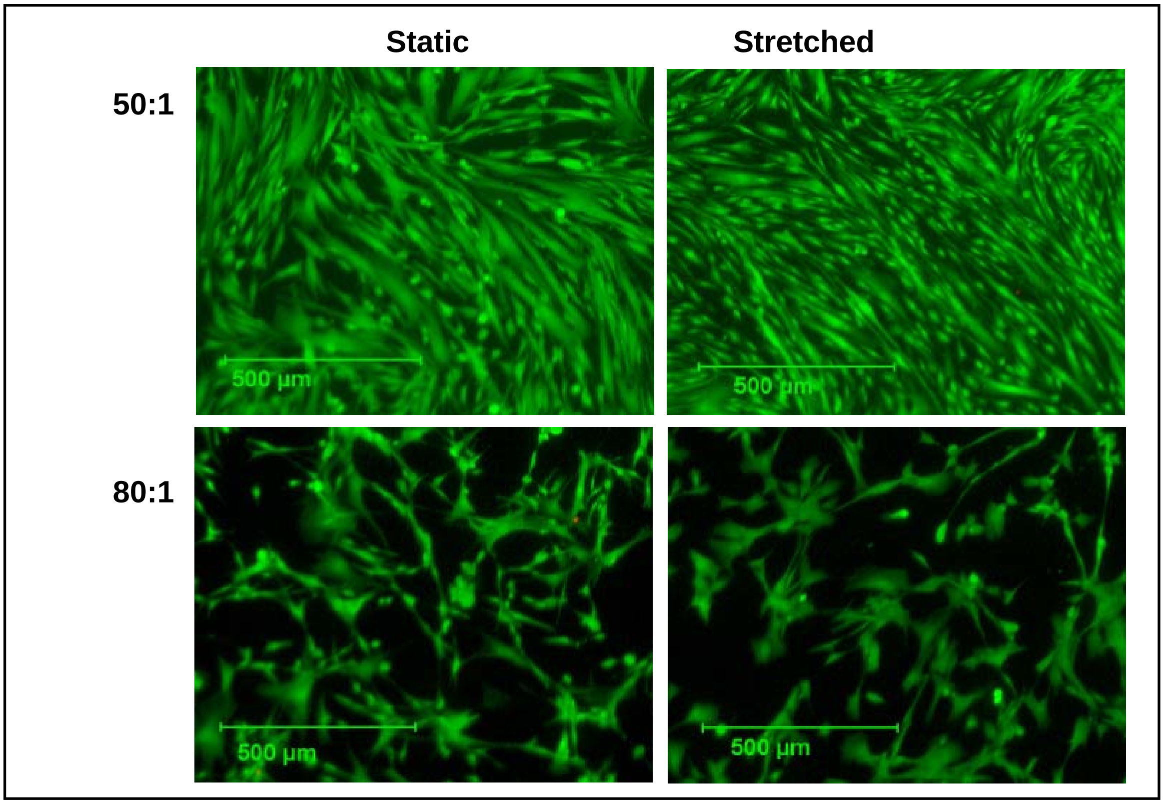


Figure 6-7: Sample Images for 5% Stretch Experiment, Day 2

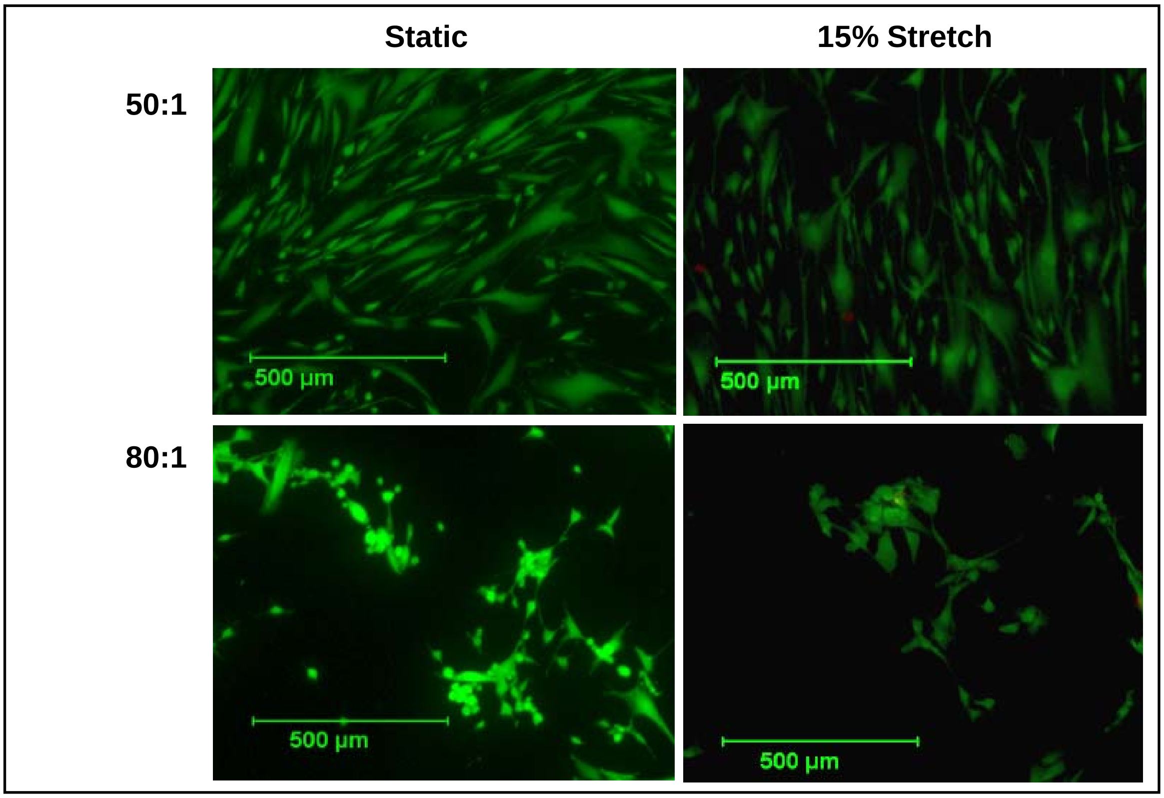


Figure 6-8: Sample Images from 15% Stretch Experiment, Day 2

Cell counts were performed to determine the average number of live and dead cells on each substrate. For raw data please refer to Appendix J. These averages were then divided by the size of the field (0.2236 cm X 0.2996 cm) and multiplied by the size of the substrate (8.55 cm^2). This calculation was performed for the three samples in each condition, and the results from the three samples were averaged. The averaged results from the cell counts for the 5% and 15% stretch experiments can be seen in Figure 6-9 and Figure 6-10. It is important to note that cells were not counted for the 50:1 samples for the 5% stretch experiments because cells became confluent on both static and stretched substrates.

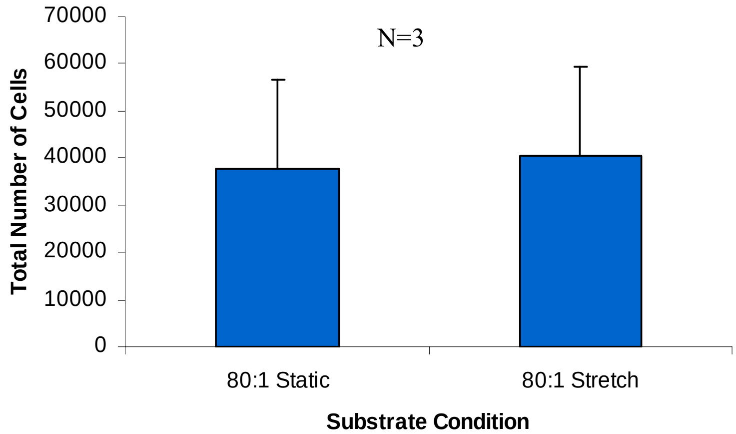


Figure 6-9: Average Total Number of Cells for 5% Stretch Experiment

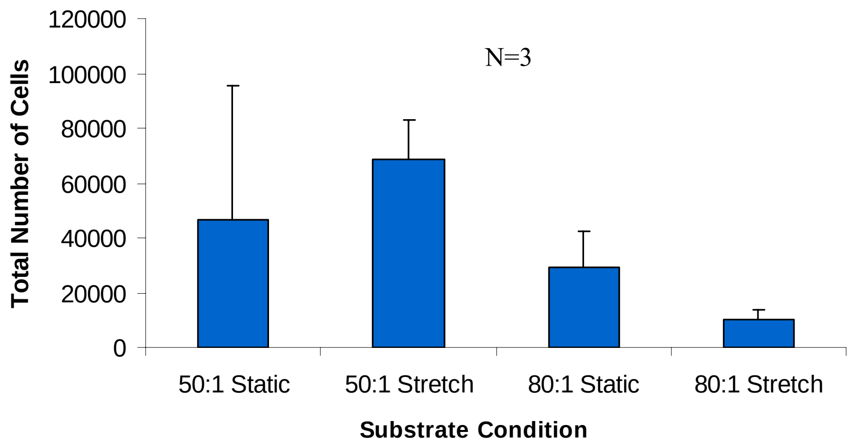


Figure 6-10: Averaged Total Number of Cells for 15% Stretch Experiment

From the Day 0 data, we were able to calculate the number of cells which initially attached (and were viable) on the 50:1 and 80:1 samples for the 15% and 5% stretch. The actual number of cells attached was divided by the number of cells that were seeded to obtain the results displayed in Table 6-7.

Table 6-7: Percentage of Initially Attached Cells

	50:1 (% of seeded cells attached)	**80:1 (% of seeded cells attached)**
5% Stretch Experiment	85	15
15% Stretch Experiment	74	30

Percent cell viability was calculated for the averaged total cell numbers by dividing the number of live cells by the total number of cells (live and dead). Cell viability percentages for the 5% and 15% experiment are displayed in Table 6-8 and Table 6-9, respectively. Cell viability could not be determined for the 50:1 Day 2 samples in the 5% stretch experiment because cells became confluent. However, it is important to note that from observation alone, cell viability appeared to be at least 90 percent.

Table 6-8: Cell Viability Percentages for 5% Experiment

	50:1 (% viability)	**80:1 (% viability)**
Day 0 Static	99	97
Day 2 Static	NA	94
Day2 Stretched	NA	98

Table 6-9: Cell Viability Percentages for 15% Experiment

	50:1 (% viability)	**80:1 (% viability)**
Day 0 Static	99	99
Day 2 Static	97	99
Day2 Stretched	99	89

More cell aggregates seemed to form on the 80:1 samples, leaving portions of the substrates without cells. Overall, the morphology of the cells on the 50:1 samples seemed to be more elongated when compared to the cells on the 80:1 samples, in which the cells seemed more “balled up”.

7 Analysis

The proposed method for culturing cells at different levels of substrate stiffness and stretch was successful. The development of this novel approach presents a means for researchers to study the combined effects of stiffness and stretch on cell phenotype. With this proposed method, we were able to stretch substrates with stiffness levels of "7 and 75 kPa", seeded with cells, at 5% and 15% stretch for at least 2 days. In this chapter, we will further discuss the implications of our results in the stiffness, stretch, and cell viability validations, and we will discuss the limitations of our design.

7.1 Validation of Stiffness

We attempted to prepare samples of PDMS with stiffness levels comparable to polyacrylamide with assumed stiffness levels of 7 and 75 kPa. Three out of the four PDMS samples had stiffness levels between the high and low stiffness of PA samples. One of the PA samples was less stiff than the compliant PA sample which proves that PDMS can be polymerized at the lowest desired stiffness level. None of the PDMS samples were as stiff as the stiffer PA sample; however, PDMS manufacturer specifications recommend polymerizing PA at a 10:1 ratio which is estimated to create a 750 kPa stiffness level[12]. Therefore, PDMS substrates can be polymerized at the same levels as polyacrylamide samples used to demonstrate cell differentiation.

7.2 Validation of Stretch

The results produced by HDM confirm that the PDMS substrate is experiencing the same stretch as the silicone membrane and that the strain field is relatively homogenous. The results obtained from enlarging the spots on paper help to determine the accuracy of the HDM software. The image was enlarged using Adobe Photoshop of which the interpolation for enlarging the image is unknown. Assuming that Adobe Acrobat enlarges the image equibiaxially, any error in the method will be attributed to the HDM software. The triad method was used to validate the HDM software. Since the results of the triad method were in the range of 4.70%-5.24%, the strain field determined by the triad method verifies a homogenous strain field.

The standard deviations of 0.29%, 0.27%, and 0.17% , for the ε_{yy}, ε_{xx}, and ε_{xy} strain fields, which were obtained from enlarging the spots on paper are considered the best accuracy that the HDM can achieve. Considering that the HDM evaluated all of the images using a subimage size of 64 x 64 pixels, the appropriate error attributed to the strain calculations is the displacement of 1 pixel out of the 64, resulting in an error of 1.56%. This error standard was used to determine the accuracy of each strain field measurement although the HDM software should be capable of sub-pixel resolution.[18] All HDM results from of the 50:1 and 80:1 PDMS, excluding 0-5% stretch of 80:1 PDMS, were under 1.56%, confirming reliable results. The contour maps exhibit the homogeneity of the strain fields because there is no systematic pattern which describes the small changes from smaller to larger strains. The images of the 80:1 PDMS substrates for 0-5% stretch had one small area of high strain which skewed the average strain value. We postulate that the unusual strain resulted from a reflection.

7.3 Validation of Cell Viability

Cell morphology, numbers, initial attachment, and viability all demonstrate the success of this new method. For all samples and for the duration of our studies, the cells were elongated and cell viability was high. These two factors are indications of cell health. Cells were confluent in multiple samples further demonstrating cell health.

Although the studies revealed that cells could be grown on these PDMS substrates, there were some limitations to the study. The first limitation to the study was our inability to count cells on the Day 2, 50:1 samples. Because cells had become confluent on these samples, we were unable to perform cell counts to determine the total number of cells and percent cell viability. However, the fact that the cells were confluent on both static and stretched samples indicates that samples were comparable and that cell counts were not necessary. The second limitation in this study was that there was more cell attachment and proliferation on the 50:1 substrates than the 80:1 substrates. However, Engler found that cells attach and proliferate preferentially to stiffer substrates.[13] Since our compliant substrate was approximately 2 kPa, it is postulated that the lower percentage of cell attachment was a result of the substrate's compliance.

Moreover, Engler also demonstrated that a cell's adhesion force is much smaller on compliant substrates than on stiffer substrates.[13]

The final limitation encountered with this method was the obstacle of uneven cell spreading. We hypothesized that uneven cell spreading resulted from the substrate surface not being perfectly flat and the collagen solution not drying evenly.

8 Recommendations

The following section contains a list of recommendations that we have generated based on difficulties that we have encountered during this project. The recommendations pertain to how the substrate is prepared and validated, how the stretch is validated, and how to encourage cell adhesion. These suggestions may or may not improve the method designed to culture mammalian cells at various levels of stiffness and stretch.

Develop an improved method of ensuring a flat substrate surface

Our first recommendation is to develop an improved method of ensuring the top of the PDMS substrate is perfectly flat. Methods of achieving a flat substrate may include spin-casting or freeze-drying. Guaranteeing a flat substrate surface could potentially improve the cell spreading and reduce the number of cell aggregates. If cell spreading was not improved, it would be clear that the surface of the substrate was not the source of the problem.

Validate the stiffness of PDMS with an additional technique

In the future, we would recommend confirming the substrate stiffness levels using a different technique. Possible techniques include atomic force microscopy and tensile tests.

Improve method of validating stretch for 80:1 PDMS substrate

Although we feel confident that the stretch in our experiments is valid, some small adjustments to the method of taking the validation images could possibly produce better results. It would be beneficial to develop a method of eliminating reflections on the substrates. The HDM software views a reflection as part of the well and measures the displacement of that reflection. We used the umbrella effect with a white poster board, but we postulate that a better method must be used to eliminate the reflections on 80:1 PDMS substrates. Because of lower stiffness of these substrates, the black sand sinks further into the substrate and creates small bumps which reflect light. Finer particles of sand may also improve results.

Connect camera to a computer when taking images to validate stretch

It is essential to keep the camera in the same position while taking all stretch validation images. The best way to accomplish this would be to connect the camera to a computer when taking images. This way, the image is captured with the camera software by clicking the mouse, rather than taking the images with the camera then downloading them onto the computer. We were unable to connect the camera to a computer in our experiments because of the distance between the Flexcell™ device and the computer.

Improve the method for adsorbing collagen to the surface of substrates

Although cell spreading was satisfactory overall, there were still some instances of uneven cell spreading. This may have been a result of the collagen solution drying unevenly. It is therefore recommended that the method for adsorbing the collagen to the substrates is improved. Some possible ways of ensuring that the collagen solution dries more evenly is to place the substrates on an orbital shaker until the solution is completely dry.

Test other protein and methods to encourage cell adhesion

Low cell attachment may have been a result of the efficacy of collagen at encouraging cell adhesion. Although, through the design process, collagen was selected as the best method (in theory) to encourage cell adhesion, it may not be the best method in practice. We would recommend trying other methods such as conjugating fibronectin to the surface of the PDMS or using a polyelectrolyte multilayer. In literature, these two methods were more often used to encourage cell adhesion, and may be more appropriate for these studies.[6, 53]

Test the method with other cell types

Although this method was tested with VICs and fibroblasts, it was designed to work with any adherent mammalian cell type. We would recommend that other cell types are tested to determine the efficacy of the method with other cell types. However, before moving to other cell types we would recommend that the method is perfected with dermal fibroblasts as these cells are relatively cheap, and easy to work with.

Develop a way of making the method compatible with all three assays

Finally, it would be useful to develop a way of making our method of culturing cells compatible with all three of the assays: immunoblots, immunocytochemistry, and traction force microscopy. Immunoblots and immunocytochemistry can easily be performed on PDMS substrates, but traction force microscopy is more difficult. This assay requires that fluorescent microbeads be embedded within the surface of the substrate. This task seems possible, but we were unable to accomplish it due to the time constraints of our project.

9 Conclusions

In the Project Approach chapter, six tasks were outlined. It was stated that these tasks would ensure the completion of the project and assess the performance of the method we developed. The first task was to determine a method for stretching mammalian cells as little as 5% and much as 15%. To complete this task we selected the Flexcell^{TM} device. This device was used to implement stretch onto prepared substrates. Using HDM software, we then validated that the Flexcell^{TM} device was stretching substrates to the desired range, which marked the completion of our second task. The third and fourth tasks were to determine a method to modulate the stiffness of substrates in a range comparable to PA used in the literature and validate the stiffness of these substrates. By polymerizing PDMS substrates with different ratios of base to crosslinker and using rheometry, we were able to complete both of these tasks. The final benchmarks of the project involved developing a method to seed cells on substrates and validating that the cells were both attached and viable. By adsorbing collagen to the surface of PDMS substrates, we were able to seed and grow cells on all PDMS substrates. The cells initially attached to the substrates and demonstrated multiple signs of cell health.

The completion of these tasks marks the development of a novel means to study the interrelated effects of stiffness and stretch on cell phenotype. Using this new method, researchers will be able to evaluate cellular responses to various mechanical environments. This information could provide critical insight into the function of healthy and pathological valves, aid the development of tissue engineered heart valve, and improve the lives of the thousands of Americans who suffer from valvular disease.

10 Glossary

Anisotropic (adj.) = possessing unlike properties in different directions

Apoptosis (n.) = programmed cell death

Bioinert (adj.) = materials which produce a minimal response from host

Cytokines (n.) = proteins which act as chemical messengers between cells

Equibiaxial (adj.) = uniform in all directions within one plane

Fibrotic (adj.) = fibrous, similar to scar tissue

Immunoblots (n.) = an assay used to analyze proteins via antigen-antibody specific reactions (e.g. quantify αSMA secreted by VICs)

Immunocytochemistry (n.) = an assay which uses immunologic methods, such as fluorescent antibodies, to identify cell constituents

Hydrophilic (adj.) = having an affinity for water

Hydrophobic (adj.) = having a low affinity for water

Mesenchymal cell (n.) = stem cell which can differentiate into many different cell types

Myofibroblast (n.) = a fibroblastic cell with some characteristics of a smooth muscle cell (e.g. contractile properties)

Pathological (adj.) = diseased or dysfunctional

Phenotype (n.) = observable traits or characteristics

Thromboembolism (n.) = obstruction of a blood vessel by a thrombus that detached from its original site of formation

Traction force microscopy (n.) = a technique used to measure cell contractility force

Valvular interstitial cell (VIC) (n.) = the most prevalent cell type in the heart valve

Viability (n.) = the ability to live and develop normally

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Appendix A: Interview Transcripts

Interview with Professor K. Billiar

Credentials: Associate Professor and Project Advisor
Date: August 31, 2005
Time: 3:00 pm
Location: Salisbury Laboratories 328
Interviewer(s): Molly Conforte, Maria Mavromatis, and Jacquelyn Youssef

Q: Could you review the objectives and limitations for this device?

A: The main objective of this project is to stretch cells on substrates with different levels of stiffness. You will probably be using VICs for the final experiments, but you can use fibroblasts to collect preliminary data.

Q: Would you like us to modify the existing Flexcell™ device or design a new creation?

A: You can use the Flexcell™ device or you can design a new stretching mechanism. It may be easier to use the Flexcell™ device, but there are problems associated with it as well. The decision will need to be part of your design selection process.

Q: Which plates / materials will we be using?

A: If you decide to use the Flexcell™ device, you will also need to select which type of culture plate you will be using. All of the Flexcell™ plates have a silicone membrane, but you can also order them with different covalently bound matrix surfaces if you want.

Q: What problems may occur with the frequency of stretch?

A: The Flexcell™ uses a vacuum/valve system. If the frequency is set too high, the pressure may not completely release in between cycles.

Q: What problems may occur with high magnitudes of stretch?

A: High magnitudes of stretch may cause the cells to pop off the substrate. You will only be collecting data of adhered cells, and if these cells are only a small percentage of the total number of cells, your data will not be accurate. So you will need to develop criteria for a certain percentage of cells which must adhere. Maybe compare the percentage of adhered, stretched cells to adhered, static cells.

Q: What range of frequencies and magnitudes of stretch should be tested?

A: I would like you to investigate two levels of stretch. The Flexcell™ is only capable of stretching between 1-25% when it is functioning optimally. Experiments have been performed at higher levels of stretch, but that wouldn't be achievable with Flexcell™.

Q: What range of stiffness should be tested?

A: I would like you to look at a high and low level of stiffness. You should ask Angie for more exact values, but 7 and 75 kPa are good estimates.

Interview with Professor S. Shivkumar

Credentials: Professor of Mechanical Engineering
Date: September 7, 2005
Time: 12:00 pm
Location: Professor Shivkumar's Office, Washburn Shops 227
Interviewer(s): Molly Conforte, Maria Mavromatis, and Jacquelyn Youssef

Q: How can we get polyacrylamide or PDMS to bond to silicone under the condition that the silicone will be experiencing up to 20% elongation?

A: It would be very difficult to attach PA to silicone. There is a problem with the interface with the two polymers because silicone is so hydrophobic. PDMS may be a better choice. PDMS can be made to adhere to silicone through solvent casting, and depending on the concentration of base and curing agent, you can change the stiffness. Maybe treat the surface with chemicals and use high temperatures. Do you know what grade of silicone you are using? Have you tried anything as simple as physically roughening the surface?

Q: What effect will stretch have on the attachment of polyacrylamide or PDMS to silicone?

A: Well, PDMS is a rubber so it should stretch fairly well if there is a good attachment to the silicone.

It might be better to find a different, more compatible polymer. You could research PGA, PEO, PVA, or HEMA. It is very easy to get these polymers to have different properties. You can buy them at different molecular weights straight from a vender. It is very easy to get different properties with HEMA, because you can crosslink it with radiation.

Q: Will cells easily adhere to these different polymers?

A: It is probably possible to get cells to adhere to these materials. You could look at Langer's textbook for information on cell adhesion. A lot of research has been done specifically with HEMA.

Interview with Professor G. Pins

Credentials: Associate Professor and Project Advisor
Date: September 9, 2005
Time: 5:00 pm
Location: Gordon Library, IT lab 3
Interviewer(s): Molly Conforte, Maria Mavromatis, and Jacquelyn Youssef

Q: What is the best treatment to use to get cells to adhere to PDMS?

A: PDMS is very hydrophobic. We would need to look into cell adhesion technology. Modifying the surface is a very nontrivial thing to do. Most people do oxygen plasma, or E beam treatment. You can get it to be passage absorbent. It is also possible to link proteins to the surface that would help adhesion.

Q: How can you get a really thin, even film of polymer?

A: Spin coating is something that is often done. This process is designed for known viscosities. If you know the viscosity of a material you can use this process to get a definite thickness. One of the other options is to let the polymer cure at room temperature instead of 60 degrees Celsius. Based on the area of the surface you can calculate how much volume you would need to create a film of a certain thickness.

Q: How can you validate that this film has a constant thickness?

A: Micromanipulator.

Q: How do we validate polymer to polymer adhesion?

A: You need to think about why this is important. You want to validate that the stretch is uniform throughout the polymer. You could validate stiffness through validation of equibiaxial stretch, strain test (we have protocol for this) or you could also do a Peel Test, which is a standard test for measuring the adhesion of two materials.

Q: How can we validate polymer to cell adhesion

A: Many people have looked at cellular adhesion. There are a lot of standard protocols for this. You could talk to many people on the third floor about this.

Q: Is it possible to use other materials such as HEMA?

A: It is close to being bioinert. It is very easy to modify its surface. It is possible to do either a matrix or a film, depending on how you process it.

Interview with Angela Throm

Credentials: Graduate student advisor
Date: September 13, 2005
Time: 3:00 pm
Location: Salisbury Laboratories 328
Interviewer(s): Molly Conforte, Maria Mavromatis, and Jacquelyn Youssef

Q: What range of stiffness is needed?

A: I have been using PA gels with 7-75 kPa stiffness. It would be nice if your experiments investigated a similar range.

Q: How long do the cells need to stay attached to the polymer? (How long will the tests run?)

A: I would like you to run the experiment for at least two days and up to a week if possible. You will need to allow 1 day for seeding and then begin stretching and counting days of stretch.

Q: If an already polymerized polymer undergoes UV sterilization, will the UV still cause crosslinking?

A: This may be something that we want to look into for polyacrylamide. UV probably won't change the cross linking, but we should look up the methods, amount of time, and wavelengths of light used in other experiments. The collagen can definitely be crosslinked with UV.

Q: Are there certain sterile methods we should be using?

A: There are a lot of antibiotics used in the media for culturing the cells, so contamination is hardly ever a problem, but I usually subject the gels to 10 minutes of UV light for sterilization (there is speculation over the usefulness of this method).

Q: What effect would autoclaving have on polyacrylamide or PDMS?

A: PDMS may be able to withstand autoclaving, but polyacrylamide may blow up or distort due to the softness and water content of the gel. It shouldn't be necessary to autoclave your substrates, so you don't have to worry about this.

Q: Is there a certain percentage of cell attachment that is considered acceptable for collecting data?

A: Most of the cells should adhere to the polymer. About 75% adhesion should be a reasonable amount, but I'm not sure how stretch will affect the adhesion numbers. We want to make sure that enough cells are initially attaching so that when we compare the stretched gels to the static gels, we can start with the same cell density and make an accurate comparison between the two.

Appendix B: Pairwise Comparison Charts

If the Goal in the row is more important than the column mark the box with a 1.
If the Goal in the column is more important than the row mark the box with a 0.

Client 1: Professor Billiar

Objectives	Easy to Use	Inexpensive	Quick to Make	Durable	Effective	User Independent
Easy to Use	*****	1	0	1	0	0
Inexpensive	0	*****	0	0	0	0
Quick to Make	1	1	*****	1	0	0
Durable	0	1	0	*****	0	0
Effective	1	1	1	1	*****	1
User Independent	1	1	1	1	0	*****

Easy to Use Objectives	Portable	Easy to Sterilize	Assay compatible	Quick to setup
Portable	*****	0	0	0
Easy to Sterilize	1	*****	0	1
Assay compatible	1	1	*****	1
Quick to setup	1	0	0	*****

Effective Objectives	Cell adhesion	Assay compatibility	Range of stretch	Range of stiffness	Sterilizable substrate
Cell adhesion	*****	0	0	0	1
Assay compatibility	1	*****	0	0	1
Range of stretch	1	1	*****	0	1
Range of stiffness	1	1	1	*****	1
Sterilizable substrate	0	0	0	0	*****

Cell Adhesion Objectives	Duration of attachment	Initial attachment
Duration of attachment	*****	0
Initial attachment	1	*****

Assay Compatible Objectives	Immunocytochemistry	Cell contraction force	Immunoblots
Immunocytochemistry	*****	1	1
Cell contraction force	0	*****	0
Immunoblots	0	1	*****

Stretch and Stiffness Objectives	Accurate	Precise
Accurate	*****	0.5
Precise	0.5	*****

Client 2: Professor Pins

Objectives	Easy to Use	Inexpensive	Quick to Make	Durable	Effective	User Independent
Easy to Use	*****	1	0.5	0	0	0
Inexpensive	0	*****	0.5	0	0	0
Quick to Make	0.5	0.5	*****	0	0	0
Durable	1	1	1	*****	0	0
Effective	1	1	1	1	*****	0.5
User Independent	1	1	1	1	0.5	*****

Easy to Use Objectives	Portable	Easy to Sterilize	Assay compatible	Quick to setup
Portable	*****	0	0	0
Easy to Sterilize	1	*****	0.5	1
Assay compatible	1	0.5	*****	1
Quick to setup	1	0	0	*****

Effective Objectives	Cell adhesion	Assay compatibility	Range of stretch	Range of stiffness	Sterilizable substrate
Cell adhesion	*****	1	1	1	0.5
Assay compatibility	0	*****	0	0	0
Range of stretch	0	1	*****	0.5	0.5
Range of stiffness	0	1	0.5	*****	0.5
Sterilizable substrate	0.5	1	0.5	0.5	*****

Cell Adhesion Objectives	Duration of attachment	Initial attachment
Duration of attachment	*****	0
Initial attachment	1	*****

Assay Compatible Objectives	Immunocytochemistry	Cell contraction force	Immunoblots
Immunocytochemistry	*****	0	1
Cell contraction force	1	*****	1
Immunoblots	0	0	*****

Stretch and Stiffness Objectives	Accurate	Precise
Accurate	*****	0.5
Precise	0.5	*****

Client 3: Angela Throm

Objectives	Easy to Use	Inexpensive	Quick to Make	Durable	Effective	User Independent
Easy to Use	*****	1	0	0	0	0
Inexpensive	0	*****	1	0	0	0
Quick to Make	1	0	*****	1	0	0
Durable	1	1	0	*****	0	0
Effective	1	1	1	1	*****	1
User Independent	1	1	1	1	0	*****

Easy to Use Objectives	Portable	Easy to Sterilize	Assay compatible	Quick to setup
Portable	*****	0	0	0
Easy to Sterilize	1	*****	0	0
Assay compatible	1	1	*****	1
Quick to setup	1	1	0	*****

Effective Objectives	Cell adhesion	Assay compatibility	Range of stretch	Range of stiffness	Sterilizable substrate
Cell adhesion	*****	1	1	1	1
Assay compatibility	0	*****	0	1	0
Range of stretch	0	1	*****	0	0
Range of stiffness	0	0	1	*****	0
Sterilizable substrate	0	1	1	1	*****

Cell Adhesion Objectives	Duration of attachment	Initial attachment
Duration of attachment	*****	0
Initial attachment	1	*****

Assay Compatible Objectives	Immunocytochemistry	Cell contraction force	Immunoblots
Immunocytochemistry	*****	0	0
Cell contraction force	1	*****	1
Immunoblots	1	0	*****

Stretch and Stiffness Objectives	Accurate	Precise
Accurate	*****	1
Precise	0	*****

Client 4: Maria Mavromatis

Objectives	Easy to Use	Inexpensive	Quick to Make	Durable	Effective	User Independent
Easy to Use	*****	1	1	0.5	0	0.5
Inexpensive	0	*****	0	0	0	0
Quick to Make	0	1	*****	0	0	0
Durable	0.5	1	1	*****	0.5	1
Effective	1	1	1	0.5	*****	1
User Independent	0.5	1	1	0	0	*****

Easy to Use Objectives	Portable	Easy to Sterilize	Assay compatible	Quick to setup
Portable	*****	0	0	0.5
Easy to Sterilize	1	*****	0.5	1
Assay compatible	1	0.5	*****	1
Quick to setup	0.5	0	0	*****

Effective Objectives	Cell adhesion	Assay compatibility	Range of stretch	Range of stiffness	Sterilizable substrate
Cell adhesion	*****	0.5	1	1	0.5
Assay compatibility	0.5	*****	0.5	0.5	0
Range of stretch	0	0.5	*****	0.5	0
Range of stiffness	0	0.5	0.5	*****	0
Sterilizable substrate	0.5	1	1	1	*****

Cell Adhesion Objectives	Duration of attachment	Initial attachment
Duration of attachment	*****	1
Initial attachment	0	*****

Assay Compatible Objectives	Immunocytochemistry	Cell contraction force	Immunoblots
Immunocytochemistry	*****	0.5	0.5
Cell contraction force	0.5	*****	0.5
Immunoblots	0.5	0.5	*****

Stretch and Stiffness Objectives	Accurate	Precise
Accurate	*****	0
Precise	1	*****

Client 5: Molly Conforte

Objectives	Easy to Use	Inexpensive	Quick to Make	Durable	Effective	User Independent
Easy to Use	*****	1	1	0	0	0
Inexpensive	0	*****	0	0	0	0
Quick to Make	0	1	*****	0	0	0
Durable	1	1	1	*****	0	0
Effective	1	1	1	1	*****	1
User Independent	1	1	1	1	0	*****

Easy to Use Objectives	Portable	Easy to Sterilize	Assay compatible	Quick to setup
Portable	*****	0.5	0.5	1
Easy to Sterilize	0.5	*****	0.5	1
Assay compatible	0.5	0.5	*****	1
Quick to setup	0	0	0	*****

Effective Objectives	Cell adhesion	Assay compatibility	Range of stretch	Range of stiffness	Sterilizable substrate
Cell adhesion	*****	1	0.5	0.5	1
Assay compatibility	0	*****	0	0	0.5
Range of stretch	0.5	1	*****	0.5	1
Range of stiffness	0.5	1	0.5	*****	1
Sterilizable substrate	0	0.5	0	0	*****

Cell Adhesion Objectives	Duration of attachment	Initial attachment
Duration of attachment	*****	0.5
Initial attachment	0.5	*****

Assay Compatible Objectives	Immunocytochemistry	Cell contraction force	Immunoblots
Immunocytochemistry	*****	0.5	0.5
Cell contraction force	0.5	*****	0.5
Immunoblots	0.5	0.5	*****

Stretch and Stiffness Objectives	Accurate	Precise
Accurate	*****	0.5
Precise	0.5	*****

Client 6: Jacquelyn Youssef

Objectives	Easy to Use	Inexpensive	Quick to Make	Durable	Effective	User Independent
Easy to Use	*****	.5	0	0	0	.5
Inexpensive	.5	*****	.5	0	0	1
Quick to Make	1	.5	*****	0	0	1
Durable	1	1	1	*****	0	1
Effective	1	1	1	1	*****	1
User Independent	.5	0	0	0	0	*****

Easy to Use Objectives	Portable	Easy to Sterilize	Assay compatible	Quick to setup
Portable	*****	0.5	0	1
Easy to Sterilize	0.5	*****	0.5	1
Assay compatible	1	0.5	*****	1
Quick to setup	0	0	0	*****

Effective Objectives	Cell adhesion	Assay compatibility	Range of stretch	Range of stiffness	Sterilizable substrate
Cell adhesion	*****	1	0.5	0.5	1
Assay compatibility	0	*****	0	0	1
Range of stretch	0.5	1	*****	0.5	1
Range of stiffness	0.5	1	0.5	*****	1
Sterilizable substrate	0	0	0	0	*****

Cell Adhesion Objectives	Duration of attachment	Initial attachment
Duration of attachment	*****	0.5
Initial attachment	0.5	*****

Assay Compatible Objectives	Immunocytochemistry	Cell contraction force	Immunoblots
Immunocytochemistry	*****	0.5	0.5
Cell contraction force	0.5	*****	0.5
Immunoblots	0.5	0.5	*****

Stretch and Stiffness Objectives	Accurate	Precise
Accurate	*****	0.5
Precise	0.5	*****

Pairwise Comparison Chart Scores

	Client #1	Client #2	Client #3	Client #4	Client #5	Client #6		Total Score	Objective Weight
Easy to Use	2	1.5	1	3	2	1		10.5	11.7
Inexpensive	0	0.5	1	0	0	2		3.5	3.9
Quick to Make	3	1	2	1	1	2.5		10.5	11.7
Durable	1	3	2	4	3	4		17	18.9
Effective	5	4.5	5	4.5	5	5		29	32.2

	Client #1	Client #2	Client #3	Client #4	Client #5	Client #6		Total Score	Objective Weight
Portable	0	0	0.5	2	1.5	0		4	11.1
Easy to sterilize	2	2.5	2.5	2	2	1		12	33.3
Assay Compatible	3	2.5	2.5	2	2.5	3		15.5	43.1
Quick to Set up	1	1	0.5	0	0	2		4.5	12.5

	Client #1	Client #2	Client #3	Client #4	Client #5	Client #6		Total Score	Objective Weight
Cell adhesion	1	3.5	3	3	3	4		17.5	29.2
Assay compatibility	2	0	1.5	0.5	1	1		6	10.0
Range of stretch	3	2	1	3	3	1		13	21.7
Range of stiffness	4	2	1	3	3	1		14	23.3
Sterilizable substrate	0	2.5	3.5	0.5	0	3		9.5	15.8

	Client #1	Client #2	Client #3	Client #4	Client #5	Client #6		Total Score	Objective Weight
Duration of attachment	0	0	1	0.5	0.5	0		2	33.3
Initial attachment	1	1	0	0.5	0.5	1		4	66.7

	Client #1	Client #2	Client #3	Client #4	Client #5	Client #6		Total Score	Objective Weight
Immunochemistry	2	1	1	1	1	0		6	33.3
Cell contraction	0	2	1	1	1	2		7	38.9
Immunoblots	1	0	1	1	1	1		5	27.8

	Client #1	Client #2	Client #3	Client #4	Client #5	Client #6		Total Score	Objective Weight
Accurate	0.5	0.5	0	0.5	0.5	1		3	50.0
Precise	0.5	0.5	1	0.5	0.5	0		3	50.0

Appendix C: Scoring Metrics

Stretching Device Scoring Metrics

<u>Easy to Use</u>
1.0 = stretching device is automated and self regulating and has computer software.
0.66 = stretching device does not satisfy one of the criteria above
0.33 = stretching device does not satisfy two or more of the criteria above

<u>Inexpensive</u>
1.0 = costs less than $50 to make
0.9 = costs less than $100 to make
0.8 = costs less than $150 to make
0.7 = costs less than $200 to make
0.6 = costs less than $250 to make
0.5 = costs less than $300 to make
0.4 = costs less than $350 to make
0.3 = costs less than $400 to make
0.2 = costs less than $450 to make
0.1 = costs less than $500 to make

<u>Quick to Make</u>
1.0 = can be assembled in less than 6 hours
0.9 = can be assembled in less than 12 hours
0.8 = can be assembled in less than 18 hours
0.7 = can be assembled in less than 24 hours
0.6 = can be assembled in less than 30 hours
0.5 = can be assembled in less than 36 hours
0.4 = can be assembled in less than 42 hours
0.3 = can be assembled in less than 48 hours
0.2 = can be assembled in less than 54 hours
0.1 = can NOT be assembled in less than 54 hours

<u>Durable</u>
1.0 = stretching device will function properly for at least two years
0.75 = stretching device will function properly for at least one and a half years
0.5 = stretching device will function properly for at least one year
0.25 = stretching device will only function properly for less than one year

<u>Effective</u>
1.0 = stretching device is self regulating, allows for a large range of stretch, has cyclic stretch capabilities, and allows for multiple samples
0.75 = stretching device does not meet one of the above criteria
0.5 = stretching device does not meet two of the above criteria
0.25 = stretching device does not meet three or more of the above criteria

User Independent
1.0 = stretching device is automated and self regulating and has computer software.
0.66 = stretching device does not satisfy one of the criteria above
0.33 = stretching device does not satisfy two or more of the criteria above

Substrate Scoring Metrics

Easy to Use
1.0 = substrate is quick to set up after it has been made, easy to sterilize, assay compatible, and a convenient size
0.75 = substrate does not satisfy one of the criteria listed above
0.5 = substrate does not satisfy two of the criteria listed above
0.25 = substrate is NOT quick to set up after it has been made, easy to sterilize, assay compatible, or a convenient size

Inexpensive
1.0 = costs less than $50 to make
0.9 = costs less than $100 to make
0.8 = costs less than $150 to make
0.7 = costs less than $200 to make
0.6 = costs less than $250 to make
0.5 = costs less than $300 to make
0.4 = costs less than $350 to make
0.3 = costs less than $400 to make
0.2 = costs less than $450 to make
0.1 = costs less than $500 to make

Quick to Make
1.0 = can be made in less than 6 hours
0.9 = can be made in less than 12 hours
0.8 = can be made in less than 18 hours
0.7 = can be made in less than 24 hours
0.6 = can be made in less than 30 hours
0.5 = can be made in less than 36 hours
0.4 = can be made in less than 42 hours
0.3 = can be made in less than 48 hours
0.2 = can be made in less than 54 hours
0.1 = can NOT be made in less than 54 hours

Durable
1.0 = substrate can withstand variations in temperature (22-37°C) and stretch (0-15%) for at least a week
0.66 = substrate will show signs of deterioration, degradation, or delamination in less than a week
0.33 = substrate will be unusable due to deterioration, degradation, or delamination in less than a week

Effective
1.0 = substrate encourages cell adhesion, is assay compatible, can tolerate the desired range of stretch, can exhibit the desired range of stiffness, and is sterile
0.75 = substrate does not satisfy one of the criteria listed above
0.5 = substrate does not satisfy two of the criteria listed above
0.25 = substrate satisfies one or less of the criteria listed above

User Independent
1.0 = substrate can be reproduced by various users following the same protocol
0.66 = user techniques (e.g. measurement techniques, aseptic technique) can alter the mechanical properties of the resulting substrate
0.33 = protocol is not detailed enough, resulting in different methods of substrate preparation

Cell Adhesion Technique Scoring Metrics

Easy to Use
1.0 = cell adhesion technique is quick to set up, easy to sterilize, and assay compatible
0.66 = technique does not satisfy one of the criteria listed above
0.33 = technique does not satisfy two of the criteria listed above

Inexpensive
1.0 = costs less than $50 to make
0.9 = costs less than $100 to make
0.8 = costs less than $150 to make
0.7 = costs less than $200 to make
0.6 = costs less than $250 to make
0.5 = costs less than $300 to make
0.4 = costs less than $350 to make
0.3 = costs less than $400 to make
0.2 = costs less than $450 to make
0.1 = costs less than $500 to make

Quick to Make
1.0 = can be made in less than 6 hours
0.9 = can be made in less than 12 hours
0.8 = can be made in less than 18 hours
0.7 = can be made in less than 24 hours
0.6 = can be made in less than 30 hours
0.5 = can be made in less than 36 hours
0.4 = can be made in less than 42 hours
0.3 = can be made in less than 48 hours
0.2 = can be made in less than 54 hours
0.1 = can NOT be made in less than 54 hours

<u>Durable</u>

1.0 = cell adhesion technique can withstand variations in temperature (22-37°C) and stretch (0-15%) for at least a week
0.66 = technique will show signs of deterioration, degradation, or delamination in less than a week
0.33 = technique will be unusable due to deterioration, degradation, or delamination in less than a week

<u>Effective</u>

1.0 = cell adhesion technique encourages cell adhesion, is assay compatible, does not affect the range of stretch, does not affect the range of stiffness, and is sterile
0.75 = technique does not satisfy one of the criteria listed above
0.5 = technique does not satisfy two of the criteria listed above
0.25 = technique satisfies one or less of the criteria listed above

<u>User Independent</u>

1.0= cell adhesion technique can be reproduced by various users following the same protocol
0.66 = user techniques (e.g. measurement techniques, aseptic technique) can alter the cell adhesion
0.33 = protocol is not detailed enough, resulting in different cell adhesion technique

Appendix D: Polyacrylamide Protocol

PREPARATION OF POLYACRYLAMIDE SUBSTRATES[53]

Materials

1. No.1 coverslip, 45x50 mm rectangular and 22 mm circular.

2. NaOH, 0.1 N, 100 ml.

3. 3-aminopropyltrimethoxy silane.

4. PBS, 500 ml.

5.glutaraldehyde, 0.5%. Mix 357 µl of 70% glutaraldehyde with 50 ml of PBS. Keep the 70 % stock tightly sealed in zip bags in a closed container at $4^{o}C$.

6. HEPES, 1 M, pH 8.5, 1 ml and 50 mM, pH 8.5, 500 ml. Use at room temperature.

7. Fluorescent latex beads, 0.2 um diameter.

8. Acrylamide (40%, Bio-Rad) and Bis (2%, Bio-Rad).

9. Ammonium persulfate (Bio-Rad) solution, 10 mg in 100ul distilled water. Prepare immediately before use in step 10.

10. TEMED (Bio-Rad).

11. sulfo-SANPAH (Pierce), 0.5 mg/ml in 50 mM HEPES pH 8.5, need 400 ul per dish. PREPARE IMMEDIATELY BEFORE USE IN STEP 15. Handle sulfo-SANPAH in the dark. Weigh the appropriate amount, add 1 ul DMSO per mg of sulfo-SANPAH. While vortexing, add 50 mM HEPES at room temperature to obtain the final concentration.

12. Protein solution for coating the substrate. Use type I collagen (10 mg/ml stock), at 0.2 mg/ml (40 ul + 2 ml PBS), or fibronectin at 10 ug/ml in a volume of 2 ml.

Procedure

1. Mark one side of a #1 coverslip with a diamond tip pen. Pass the marked side over the inner flame of a Bunsen burner.

2. Place the coverslip, flamed side up, on a test tube rack. Smear the surface with 0.1 N NaOH in the hood and allow the surface to air dry.

3. Smear the dried surface with 3-aminopropyltrimethoxy silane, wear gloves and do this in the hood. Incubate at room temperature for 5 minutes.

4. Collect the coverslips in a pan. Wash with distilled water on a shaker until the coverslip surfaces are clear.

5. Put the coverslips back on test tube rack. Pipette 0.5 % gluteraldehyde to cover the treated surface of the coverslips. Incubate for 30 minutes at room temperature in the hood. Ware gloves.

6. Collect the used glutaraldehyde in liquid waste. Wash as in step 4 and let air-dry. Activated coverslip may be stored in a dessicator for two weeks. Coverslips may be mounted onto chamber dishes before proceeding with the following steps.

7. Mix 5 ml of acrylamide solution in a small beaker according to the dilution scheme below. Beads are usually added at a volume of 50 ul. DO NOT ADD BEADS YET.

Final Acryl/Bis	**40%Acrylamide**	**2%Bis**	**1M HEPES**	**H_20+Beads**	**Young's Modulus**
8%/0.1%	1000 ul	250 ul	50 ul	3700 ul	?? kN/m2
8/0.08	1000	200	50	3750	75
8/0.06	1000	150	50	3800	30
8/0.05	1000	125	50	3825	23
8/0.04	1000	100	50	3850	17
8/0.03	1000	75	50	3875	14
8/0.02	1000	50	50	3900	10
5/0.12	625	300	50	4025	33
5/0.10	625	250	50	4075	28
5/0.08	625	200	50	4125	24
5/0.06	625	150	50	4175	15
5/0.05	625	125	50	4200	??
5/0.025	625	63	50	4262	7
3/0.10	375	250	50	4325	??

8. Degas the solution for 20 minutes to remove oxygen, which inhibits acrylamide polymerization.

9. Sonicate the fluorescent beads for 1-2 minutes in a bath sonicator.

10. Add beads, 30 ul ammonium persulfate, 20 ul TEMED. Seal the beaker with parafilm and mix gently by swirling.

11. Pipette the acrylamide mixture onto the activated coverslip. Use 15 ul for a 75 um-thick gel. Quickly place a 22 mm circular coverslip onto the acrylamide droplet and invert the chamber dish.

12. Let acrylamide polymerize for 30 minutes.

13. Flood the surface with ~2 ml of 50 mM HEPES. Remove the circular coverslip with two pairs of fine tipped tweezers.

14. Rinse the substrate well with 50 mM HEPES. The substrate may be stored at 4^{o}C for 2 weeks.

15. Remove as much liquid form the substrate as possible without drying, then layer 200 ul of the sulfo-SANPAH solution on top.

16. Place under 302 nm UV, at a distance of 2-3 inches from two 15W tubes, for 5-8 minutes. The solution will darken when activated.

17. Repeat steps 15 and 16

18. Wash with 50 mM HEPES to remove excess reagent. Do this quickly.

19. Add the protein to be coupled and incubate either 4 hours at room temperature or overnight in the cold room on a shaker.

20. Rinse with PBS and store coated substrates in the cold room for up to a week.

21. Before plating cells, expose the gel to UV for 15 minutes.

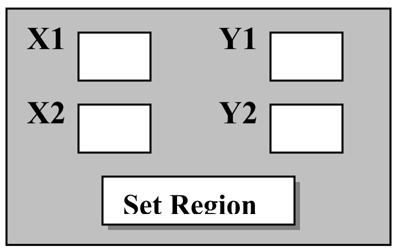
22. Replace PBS with complete culture medium. Place in incubator for 1 hour to allow equilibrium.

Appendix E: HDM/Excel Protocol

IN HDM:

From **Start** Menu: **Program -> HDM -> HDM -> File -> Open**. Select one image (i.e. unia0%.tif), hold down the control button and select 2nd image (i.e. unia5%.tif), so that both images (must be Tiff images) are highlighted **->Open.**

Using the mouse, select a rectangular region on the image to analyze. The images are superimposed on top of each other so that selecting one region sets the same region on both images. The size of the rectangle can be found in a small grey box in the upper right corner of the HDM screen. It shows:



Once an appropriate rectangle is selected click **Set Region**.

In the tool bar: **-> Correlator -> Subimage size ->** select a square size appropriate for your image (i.e. 64 X 64 pixels).

Next **-> Correlator -> Pixel shift ->** select a pixel value no greater than half of the length of a side of the subimage square (i.e. 16 pixels or a maximum of 32 pixels when using a 64 X 64 pixel subimage size).

Finally **-> Correlator -> Correlate** and allow HDM to run. In the HDM terminal the text will read **correlating…determining….shift is….finished.**

IN EXCEL:

In a new file label the first cell **u displacement (in pixels).** Select the second cell in the first column and in the tool bar **-> Data -> Import external data -> Import data**. Make sure **file of type** displaces: **All Files** and open the same folder from which you opened the images in HDM. The HDM data will be labeled ***your file name*_U.** Select **File -> Open**.

A grey box will appear on the excel screen. Select **Delimited -> Next ->** Uncheck **tab** and check **space -> next ->** check **Do not import column (skip) -> finish -> OK**.

Two cells beneath this data, still in the first column, label the cell **v displacement (in pixels).** Follow the same process for V displacement data, opening the ***your file name*_V** file.

To set a determined amount of numbers after the decimal point highlight terms, right click **->format -> numbers ->** choose number of digits to be displayed.

To reduce the standard deviation of your results it is necessary to replace outlier points by interpolating – taking the average of surrounding points and fitting into linear data progression. Before doing so, review the imported data for obvious outliers (i.e. a skip of 5 pixels from one box to an adjacent box.

Move down three cells from the bottom of the v data, still in the first column and in the first five cells along this row enter

0	16	32	48	64

if using a 16 pixel shift.

Move 3 cells over to column H and in 5 cells down enter:

0
-16
-32
-48
-64

Since the HDM program orients the axis so that the positive y direction is in the negative y direction on the standard Cartesian coordinate plane, you must enter negative numbers in order to receive true y strains.

Moving two cells down, back in the first column, label the cell **du/da**. In the first cell below this title, in the first column, input the equation **=SLOPE(A2:E2,$A$43:$E$43)** in which A2:E2 represents the first five u displacement points in the first row of data. Expand the equation (drag lower right corner of cell) to fill the correct number of cells. In this case the correct number of cells contains the same number of rows as the u displacement data and four columns less.

One cell down from the bottom of the du/da data, in the first column, label the cell **du/db**. In the cell below du/db input the equation **=SLOPE(A2:A6,$H$42:$H$46)** in which A2:A6 represents the first five u displacement points in the first column of data. Expand the equation (drag lower right corner of cell) to fill the correct number of cells. In this case the correct number of cells contains the same number of columns as the v displacement data and four rows less.

Move one cell down and label it **dv/db**. In the cell below dv/db, in the first column, input the equation **=SLOPE(A22:A26,$H$42:$H$46)** in which A22:A26 represents the first five v displacement points in the first column. Expand the equation (drag lower right corner of cell) to fill the correct number of cells. In this case the correct number of cells is the same as the du/db data.

Move once cell down, in the first column, and label it **dv/da**. In the cell below dv/da input the equation **=SLOPE(A22:E22,$A$42:$E$42)** in which A22:E22 represents the first five v displacement points in the first row v displacement data. Expand the equation (drag lower right corner of the cell) to fill the correct number of cells. The correct number of cells is the same as the du/da data.

Move one cell down and label it **Exx**. In the cell below Exx, in the first column, input the equation **=du/da+0.5*((du/da)^2+(dv/da)^2)** entering the cell numbers of the du/da and dv/da terms that you are evaluating. Again, expand the equation to fill the same number of cells as the dv/da terms.

To find the maximum Exx strain use the equation **=MAX(*all Exx terms*).** i.e. **=MAX(A123:K141*)*.** Similarly, to find the minimum Exx value, average Exx strain and standard deviation of the data input: **=MIN(A123:K141) ; =AVERAGE(A123:K141) ; =STDEV(A123:K141)**.

Move one cell down and label **Eyy**. In cell below Eyy, in the first column, input the equation **=dv/db+0.5*((du/db)^2+(dv/db)^2)** entering the cell numbers of the du/db and dv/db terms that you are evaluating (i.e. du/db=A68 and dv/db=A85 on our spreadsheet). Expand the equation to fill the same number of cells as the dv/db terms.

To find maximum Eyy strain use the equation **=MAX(*all Eyy terms*).** i.e. **=MAX(A144:K158).** Similarly, to find the minimum Eyy value, average Eyy strain and standard deviation of the data input: **=MIN(A144:K158) ; =AVERAGE(A144:K158) ; =STDEV(A144:K158)**.

Move one cell down and label it **Exy**. This is the shear strain. In the cell below Exy enter the equation **=0.5*(du/db+dv/da+(du/da*du/db+dv/da*dv/db))** entering the cell numbers of du/db, dv/da, du/da, and dv/db that you are evaluating. To find statistics: **=MAX(A161:K175) ; =MIN(A161:K175) ; =AVERAGE(A161:K175) ; =STDEV(A161:K175).**

Appendix F: Camera Preparation and Operation

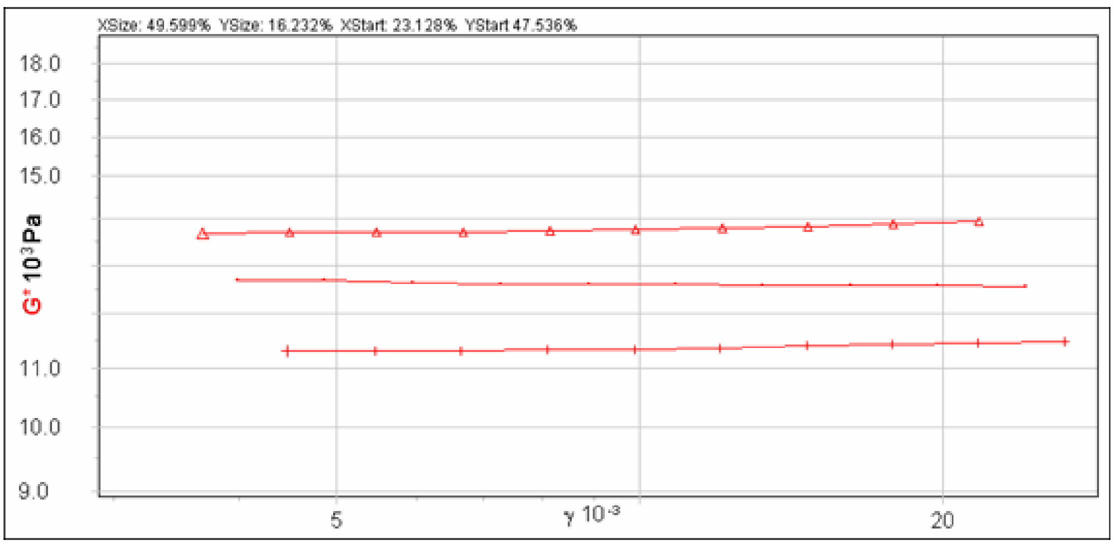
Set the camera to capture images in RAW format. For the 6 Megapixel Canon Digital Rebel XT, set the dial to **M** and select **RAW** for image capture. Using the USB cable, connect the camera to the computer. To convert the images to Tiff format, choose **Program -> Canon Utilities -> File Viewer Utility 1.3 -> File Viewer Utility.** Open the camera folders to find the appropriate images. Select multiple images by holding *control*. Go to **file -> save file -> convert and save file -> desired folder**. In **convert from RAW to other format**, select ***Tiff (8 bit).***

Appendix G: Raw Data for Substrate Stiffness Validation

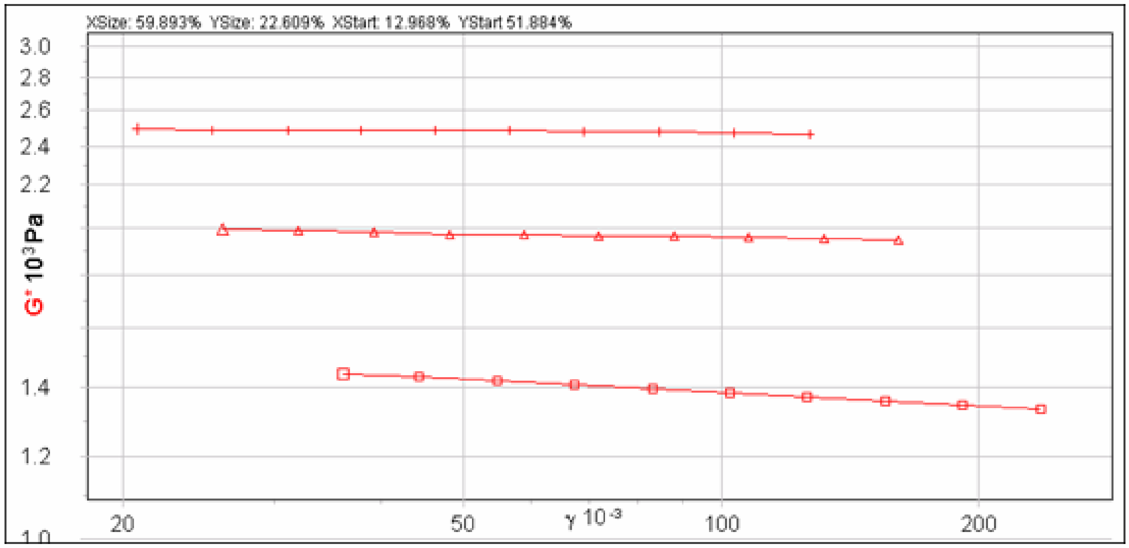
Averages and Standard Deviations

Substrate	Average Stiffness (kPa)	Standard Deviation (kPa)
50:1 PDMS	12.5	1.2
65:1 PDMS	4.9	0.5
80:1 PDMS	1.9	0.5
90:1 PDMS	1.3	0.1
“7 kPa” PA	1.9	0.1
“75 kPa” PA	18.1	1.5

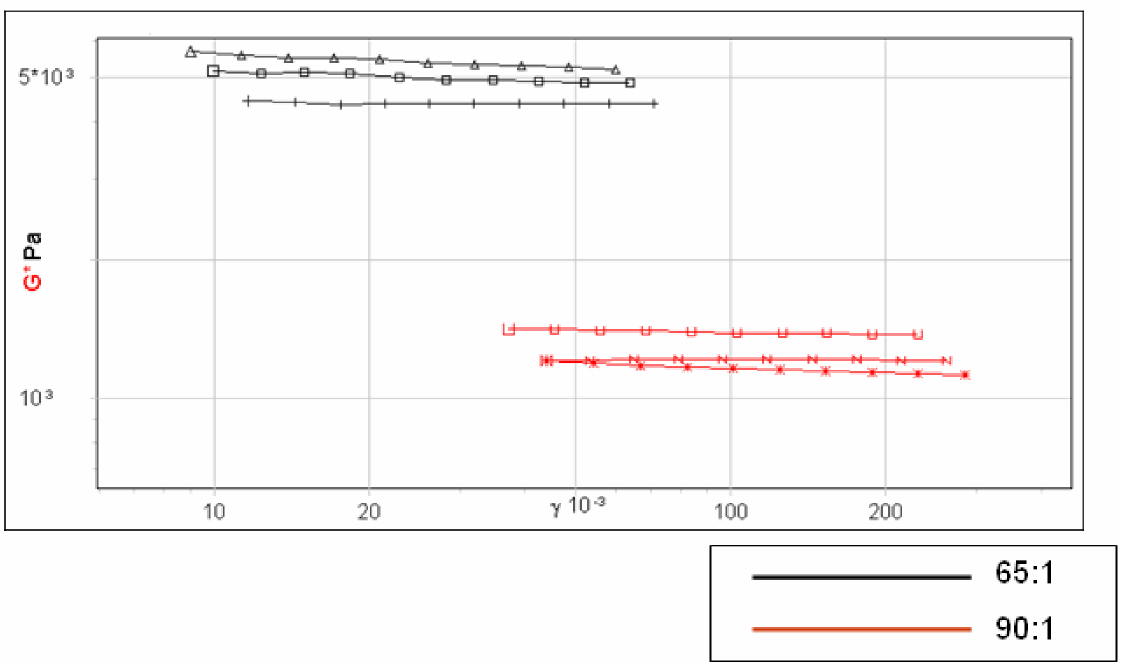
Strain Sweep for 50:1



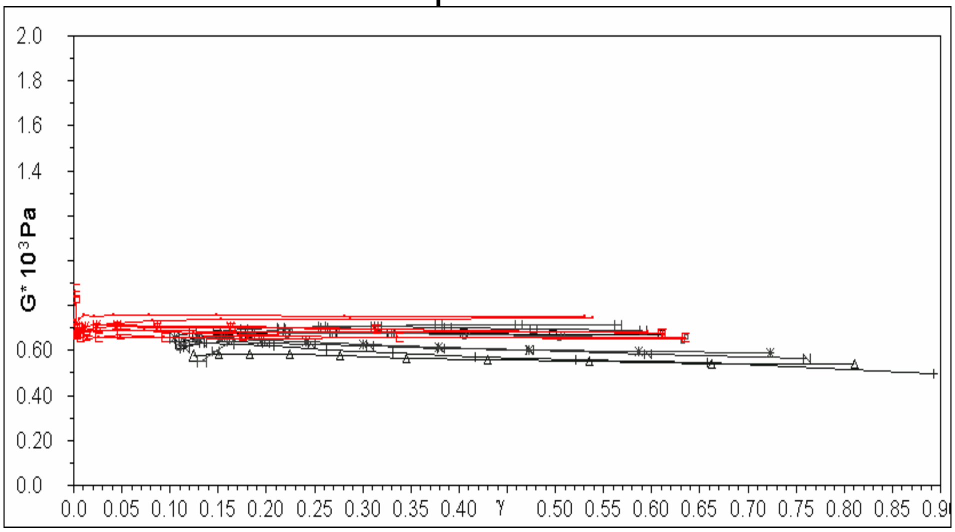
Strain Sweep for 80:1



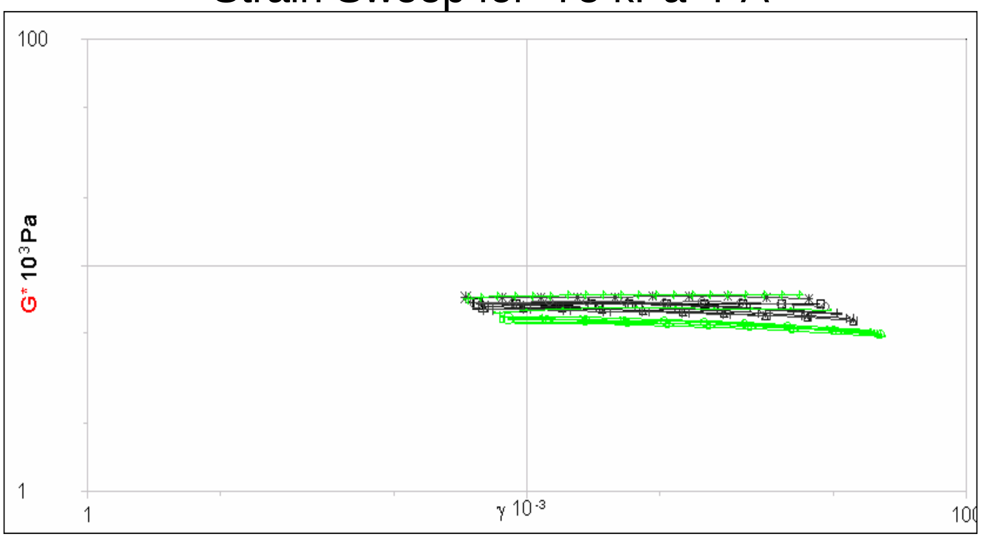
Strain Sweep for 65:1 and 90:1



Stain Sweep for “7 kPa” PA

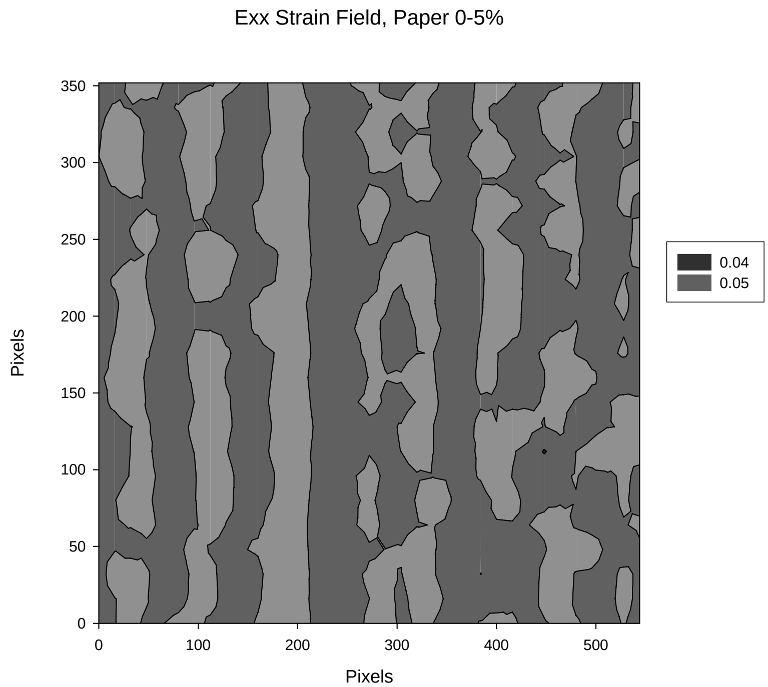


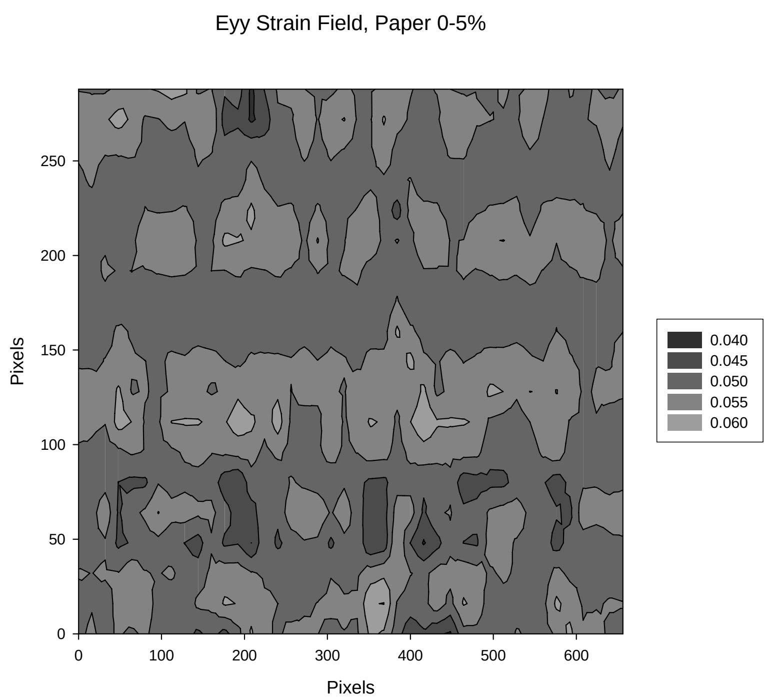
Strain Sweep for “75 kPa” PA

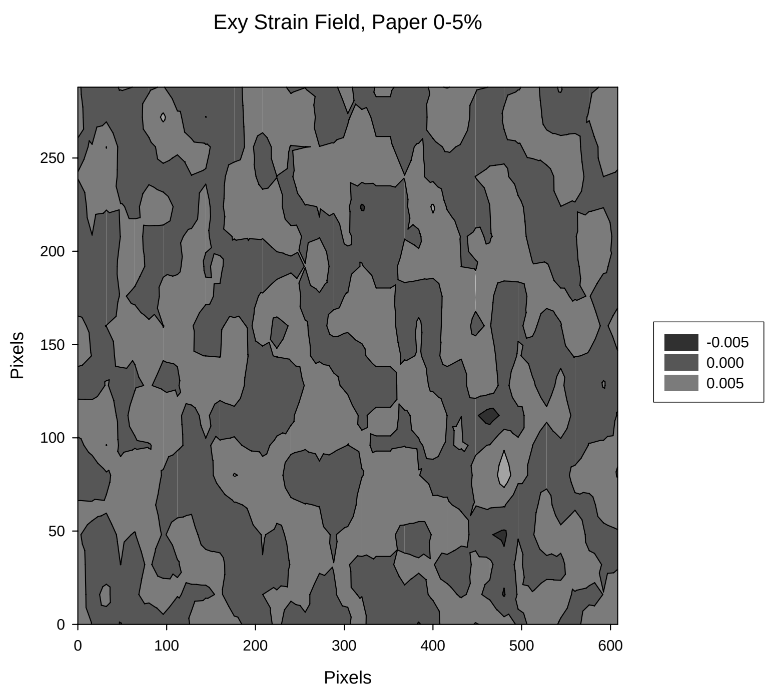


Appendix H: Contour Plots, Spreadsheets, and Matrices for Validation of Stretch

Results for spots on paper: 0% and 5% comparison






Exy Strain Field, Paper 0-5%
Pixels
0
50
100
150
200
250
0
100
200
300
400
500
600
Pixels
-0.005
0.000
0.005

u displacement																						
-12.609	-11.801	-11.1	-10.317	-9.498	-8.703	-7.94	-7.229	-6.345	-5.482	-4.679	-4.29	-3.353	-2.479	-1.604	-0.763	0.251	0.654	1.258	2.314	3.215	3.639	4.735
-12.559	-11.799	-10.802	-10.357	-9.478	-8.564	-7.707	-7.318	-6.399	-5.469	-4.605	-4.045	-3.501	-2.465	-1.589	-0.719	0.223	0.614	1.33	2.293	3.197	3.583	4.737
-12.564	-11.757	-11.19	-10.318	-9.565	-8.437	-7.731	-7.286	-6.419	-5.505	-4.555	-4.122	-3.478	-2.448	-1.665	-0.737	0.245	0.578	1.329	2.364	3.175	3.625	4.666
-12.677	-11.686	-10.863	-10.352	-9.491	-8.485	-8.163	-7.209	-6.419	-5.631	-4.691	-4.228	-3.341	-2.437	-1.614	-0.715	0.186	0.617	1.339	2.338	2.843	3.712	4.561
-12.557	-11.75	-10.835	-10.322	-9.5	-8.569	-7.774	-7.159	-6.397	-5.584	-4.722	-3.882	-3.353	-2.402	-1.64	-0.666	0.204	0.612	1.32	2.375	3.191	3.72	4.479
-12.628	-11.675	-11.162	-10.33	-9.483	-8.629	-7.781	-7.213	-6.41	-5.58	-4.73	-3.777	-3.333	-2.468	-1.562	-0.744	0.213	0.625	1.375	2.382	3.202	3.663	4.556
-12.668	-11.662	-10.81	-10.297	-9.548	-8.658	-7.692	-7.209	-6.458	-5.585	-4.652	-3.738	-3.31	-2.528	-1.57	-0.726	0.219	0.631	1.38	2.374	3.224	3.752	4.531
-12.637	-11.682	-10.823	-10.377	-9.459	-8.665	-7.768	-7.225	-6.468	-5.503	-4.719	-3.818	-3.381	-2.534	-1.62	-0.714	0.226	0.549	1.437	2.279	2.827	3.731	4.511
-12.547	-11.72	-10.87	-10.295	-9.546	-8.647	-7.793	-7.234	-6.448	-5.511	-4.692	-3.824	-3.286	-2.487	-1.658	-0.7	0.238	0.658	1.345	2.345	2.82	3.697	4.481
-12.538	-11.763	-11.221	-10.265	-9.522	-8.604	-7.798	-7.247	-6.419	-5.567	-4.731	-3.826	-3.328	-2.511	-1.623	-0.7	0.272	0.568	1.338	2.361	3.217	3.683	4.535
-12.578	-11.755	-11.207	-10.218	-9.475	-8.648	-7.786	-7.224	-6.329	-5.554	-4.676	-3.817	-3.321	-2.447	-1.63	-0.723	0.266	0.564	1.298	2.35	2.848	3.728	4.528
-12.541	-11.769	-11.198	-10.392	-9.482	-8.664	-7.79	-7.31	-6.37	-5.535	-4.712	-3.783	-3.295	-2.45	-1.652	-0.707	0.287	0.61	1.331	2.332	3.187	3.727	4.506
-12.603	-11.736	-11.126	-10.365	-9.532	-8.643	-7.769	-7.245	-6.407	-5.596	-4.701	-4.227	-3.302	-2.493	-1.593	-0.739	0.213	0.582	1.345	2.363	3.216	3.682	4.529
-12.608	-11.707	-11.13	-10.321	-9.477	-8.712	-7.767	-7.248	-6.395	-5.579	-4.709	-4.221	-3.306	-2.431	-1.636	-0.681	0.243	0.55	1.32	2.385	3.161	3.58	4.675
-12.568	-11.724	-11.179	-10.346	-9.52	-8.59	-7.799	-7.312	-6.404	-5.514	-4.704	-3.77	-3.303	-2.513	-1.584	-0.778	0.254	0.638	1.274	2.356	2.773	3.631	4.638
-12.553	-11.699	-10.816	-10.411	-9.564	-8.66	-7.858	-7.345	-6.423	-5.553	-4.713	-3.723	-3.24	-2.448	-1.655	-0.778	0.3	0.573	1.393	2.344	2.746	3.678	4.611
-12.552	-11.707	-10.79	-10.41	-9.582	-8.677	-7.733	-7.229	-6.452	-5.514	-4.708	-4.194	-3.346	-2.445	-1.654	-0.697	0.28	0.562	1.376	2.309	3.137	3.776	4.496
-12.586	-11.732	-10.793	-10.251	-9.498	-8.66	-7.811	-7.225	-6.365	-5.597	-4.685	-4.173	-3.342	-2.474	-1.572	-0.765	0.235	0.608	1.294	2.333	3.214	3.73	4.564
-12.55	-11.769	-11.167	-10.333	-9.446	-8.672	-7.813	-7.274	-6.363	-5.655	-4.529	-4.167	-3.377	-2.54	-1.662	-0.683	0.224	0.522	1.346	2.393	2.827	3.665	4.568
-12.594	-11.795	-11.262	-10.203	-9.486	-8.659	-7.845	-7.23	-6.436	-5.482	-4.534	-4.163	-3.415	-2.32	-1.686	-0.721	0.191	0.538	1.3	2.288	2.795	3.731	4.57
-12.621	-11.788	-11.315	-10.22	-9.53	-8.593	-7.827	-7.25	-6.44	-5.566	-4.588	-3.802	-3.484	-2.244	-1.798	-0.66	0.213	0.542	1.322	2.289	3.21	3.734	4.558
-12.59	-11.742	-11.169	-10.306	-9.497	-8.639	-8.121	-7.27	-6.369	-5.579	-4.708	-3.85	-3.421	-2.473	-1.696	-0.706	0.177	0.571	1.351	2.307	2.802	3.735	4.618
-12.598	-11.66	-10.864	-10.352	-9.434	-8.637	-7.746	-6.855	-6.414	-5.62	-4.666	-3.863	-3.289	-2.414	-1.671	-0.647	0.147	0.479	1.406	2.309	3.215	3.649	4.546
-12.572	-11.706	-11.158	-10.359	-9.302	-8.764	-8.301	-6.662	-6.279	-5.718	-4.701	-4.222	-3.314	-2.476	-1.612	-0.755	0.267	0.622	1.271	2.378	3.185	3.715	4.522

v displacement																						
1.258	1.318	1.229	1.217	1.336	1.37	1.253	1.27	1.218	0.802	1.172	0.782	1.273	1.299	1.292	1.21	1.298	1.287	1.253	1.23	1.234	1.233	1.294
0.394	0.401	0.399	0.469	0.416	0.423	0.329	0.262	0.4	0.562	0.58	0.389	0.358	0.419	0.422	0.438	0.397	0.451	0.493	0.43	0.417	0.432	0.524
-0.453	-0.356	-0.406	-0.42	-0.352	-0.425	-0.346	-0.39	-0.589	-0.49	-0.487	-0.402	-0.42	-0.346	-0.358	-0.393	-0.453	-0.406	-0.434	-0.387	-0.33	-0.365	-0.373
-1.175	-1.264	-1.239	-1.306	-1.156	-1.311	-1.305	-1.193	-1.225	-1.292	-0.86	-1.233	-1.158	-1.239	-1.276	-1.244	-1.367	-1.331	-1.293	-1.233	-1.281	-1.266	-1.19
-1.823	-1.792	-1.78	-1.839	-1.71	-1.739	-1.65	-1.647	-1.622	-1.728	-1.759	-1.802	-1.748	-2.197	-1.871	-1.782	-1.856	-1.803	-1.763	-1.777	-1.819	-1.775	-2.209
-2.756	-2.769	-2.694	-2.816	-3.11	-3.118	-2.723	-2.723	-2.736	-2.803	-2.793	-3.241	-3.25	-2.814	-2.767	-2.772	-2.599	-2.699	-2.756	-2.846	-2.759	-2.814	-3.2
-3.584	-3.505	-3.619	-3.583	-3.566	-3.443	-3.515	-3.608	-3.515	-3.437	-3.516	-3.524	-3.495	-3.491	-3.479	-3.497	-3.576	-3.481	-3.42	-3.472	-3.46	-3.436	-3.453
-4.098	-4.243	-4.177	-3.869	-3.784	-4.184	-4.169	-3.824	-3.794	-3.787	-3.835	-3.82	-3.78	-3.73	-4.272	-3.828	-4.209	-4.366	-4.308	-3.828	-4.254	-4.241	-3.724
-4.747	-4.812	-5.219	-4.79	-5.17	-5.179	-5.228	-5.149	-5.185	-5.206	-5.243	-5.198	-4.82	-5.111	-4.868	-4.874	-5.206	-5.166	-5.135	-5.156	-5.212	-4.758	-5.236
-5.83	-5.797	-5.657	-5.727	-5.742	-5.701	-5.751	-5.69	-5.717	-5.713	-5.686	-5.696	-5.73	-5.726	-5.681	-5.7	-5.728	-5.69	-5.665	-5.675	-5.73	-5.772	-5.75
-6.532	-6.556	-6.6	-6.482	-6.533	-6.569	-6.491	-6.513	-6.501	-6.484	-6.508	-6.544	-6.545	-6.568	-6.575	-6.557	-6.57	-6.533	-6.581	-6.581	-6.561	-6.584	-6.529
-7.323	-7.353	-7.406	-7.467	-7.381	-7.364	-7.397	-7.44	-7.462	-7.466	-7.405	-7.396	-7.43	-7.382	-7.457	-7.466	-7.379	-7.41	-7.372	-7.394	-7.433	-7.419	-7.401
-8.245	-8.217	-8.249	-8.282	-8.233	-8.27	-8.262	-8.199	-8.273	-8.309	-8.252	-8.296	-8.179	-8.278	-8.215	-8.206	-8.276	-8.365	-8.292	-8.251	-8.234	-8.176	-8.368
-8.775	-8.775	-8.753	-8.788	-8.829	-8.758	-8.728	-8.793	-8.761	-8.814	-8.807	-8.727	-8.768	-8.819	-8.84	-8.836	-8.809	-8.831	-8.724	-8.834	-8.835	-8.763	-8.865
-9.607	-9.677	-9.715	-9.779	-9.69	-9.661	-9.684	-9.662	-9.652	-9.612	-9.572	-9.66	-9.642	-9.652	-9.59	-9.623	-9.558	-9.564	-9.565	-9.669	-9.584	-9.649	-9.515
-10.471	-10.406	-10.491	-10.389	-10.509	-10.462	-10.391	-10.438	-10.492	-10.461	-10.539	-10.486	-10.454	-10.531	-10.504	-10.46	-10.563	-10.524	-10.51	-10.462	-10.459	-10.474	-10.403
-11.261	-11.197	-11.329	-11.37	-11.3	-11.302	-11.357	-11.325	-11.344	-11.359	-11.291	-11.316	-11.278	-11.286	-11.277	-11.348	-11.264	-11.297	-11.316	-11.28	-11.364	-11.324	-11.31
-11.777	-11.914	-11.804	-11.67	-11.817	-12.138	-12.163	-12.159	-12.175	-11.782	-11.791	-12.27	-12.26	-12.25	-12.231	-12.153	-12.234	-11.78	-12.166	-11.838	-11.832	-12.152	-12.193
-12.717	-12.678	-12.706	-12.668	-12.716	-12.736	-12.671	-12.693	-12.744	-12.729	-12.682	-12.775	-12.844	-13.143	-12.789	-12.708	-12.68	-12.651	-12.687	-12.735	-12.73	-12.727	-12.662
-13.582	-13.618	-13.511	-13.542	-13.56	-13.594	-13.553	-13.56	-13.507	-13.628	-13.581	-13.476	-13.345	-13.679	-13.588	-13.516	-13.592	-13.624	-13.576	-13.544	-13.485	-13.571	-13.566
-14.316	-14.362	-14.418	-14.367	-14.384	-14.407	-14.356	-14.354	-14.417	-14.407	-14.323	-14.382	-14.515	-14.369	-14.341	-14.387	-14.345	-14.356	-14.402	-14.386	-14.43	-14.369	-14.496
-15.262	-15.195	-15.175	-15.257	-15.205	-15.156	-15.212	-15.336	-15.258	-15.217	-15.195	-14.823	-14.846	-14.711	-14.742	-15.154	-15.264	-15.152	-15.19	-15.209	-15.285	-15.209	-15.154
-15.758	-15.747	-15.73	-15.785	-16.176	-16.215	-16.156	-16.229	-16.228	-15.773	-15.798	-15.676	-15.808	-15.687	-15.749	-15.846	-15.819	-15.795	-15.723	-15.722	-15.822	-15.741	-15.751
-16.64	-16.669	-16.715	-16.638	-16.579	-16.602	-16.554	-16.53	-16.614	-16.604	-16.658	-16.607	-16.588	-16.652	-16.639	-16.63	-16.506	-16.648	-16.595	-16.726	-16.652	-16.621	-16.62

du/da																						
0.04816	0.04874	0.04959	0.04834	0.04863	0.05023	0.05168	0.04715	0.04485	0.04583	0.04976	0.05502	0.05578	0.05076	0.04463	0.04476	0.04743	0.04954	0.05174	0.04768	0.04931	0.04898	0.04368
0.04753	0.04871	0.04989	0.04906	0.04628	0.04686	0.05033	0.05213	0.04513	0.04445	0.04758	0.05353	0.05746	0.04981	0.04482	0.04457	0.04767	0.04878	0.05065	0.0476	0.05045	0.05439	0.04583
0.04648	0.05166	0.05499	0.04936	0.04652	0.04485	0.05083	0.0512	0.04541	0.04494	0.04659	0.05364	0.05723	0.04976	0.04564	0.04554	0.04779	0.04963	0.04959	0.04859	0.05124	0.04985	0.04337
0.04816	0.04859	0.04542	0.04759	0.04638	0.04658	0.05326	0.04806	0.04724	0.04836	0.04966	0.05471	0.05485	0.04943	0.04524	0.04537	0.04397	0.04809	0.04886	0.04938	0.05447	0.04946	0.04619
0.04714	0.04811	0.04922	0.05033	0.0476	0.04592	0.04799	0.05143	0.04869	0.04833	0.04778	0.05091	0.05531	0.0492	0.04499	0.04499	0.04836	0.05054	0.04789	0.04574	0.0484	0.04901	0.04776
0.04772	0.04857	0.05289	0.0496	0.04726	0.04668	0.04834	0.05345	0.04973	0.04763	0.04778	0.04898	0.0551	0.04976	0.04527	0.04634	0.04834	0.04939	0.04777	0.0461	0.04921	0.05379	0.04918
0.04753	0.04544	0.04922	0.0502	0.04768	0.04613	0.04815	0.05468	0.05089	0.0466	0.04609	0.04853	0.05538	0.05067	0.04536	0.04601	0.04846	0.05054	0.048	0.04576	0.04813	0.04857	0.04816
0.04788	0.04624	0.04889	0.04997	0.04639	0.04765	0.04888	0.05352	0.04912	0.04548	0.04676	0.04981	0.05646	0.05008	0.04611	0.04498	0.04333	0.04846	0.0475	0.04885	0.05294	0.04885	0.04806
0.04642	0.04669	0.04876	0.04922	0.04756	0.04761	0.04953	0.0536	0.05007	0.04659	0.04628	0.04923	0.05522	0.05116	0.04603	0.04498	0.04282	0.04721	0.04765	0.04918	0.05311	0.04885	0.0474
0.04706	0.05011	0.05317	0.0485	0.04727	0.04658	0.04884	0.05331	0.04952	0.04697	0.04707	0.04973	0.05632	0.05033	0.04494	0.04493	0.04802	0.05068	0.04823	0.04633	0.04908	0.0498	0.0469
0.04839	0.04966	0.05258	0.04798	0.04823	0.04778	0.04931	0.05292	0.04846	0.04731	0.04664	0.04924	0.05561	0.04949	0.04464	0.04486	0.04344	0.04924	0.04899	0.0494	0.05285	0.04919	0.04678
0.04684	0.04954	0.0534	0.0491	0.04736	0.04799	0.04957	0.05445	0.04939	0.04742	0.04658	0.04872	0.05567	0.05037	0.04552	0.04451	0.04701	0.05056	0.04841	0.04674	0.04873	0.04889	0.04689
0.04696	0.04863	0.05273	0.05002	0.0478	0.0466	0.04866	0.04839	0.04737	0.04753	0.04969	0.05428	0.0549	0.04973	0.04498	0.04585	0.04867	0.05044	0.04804	0.04603	0.04906	0.05019	0.04748
0.0478	0.04777	0.05209	0.0491	0.04768	0.04774	0.04866	0.04838	0.0471	0.04812	0.0496	0.05469	0.0553	0.04901	0.04464	0.04506	0.04794	0.04938	0.04941	0.04678	0.05101	0.04946	0.04463
0.04671	0.04954	0.05323	0.04868	0.04694	0.04717	0.04993	0.0549	0.04966	0.04627	0.04686	0.04814	0.05531	0.05088	0.04458	0.04555	0.04223	0.04678	0.05002	0.04934	0.05664	0.04998	0.04423
0.04541	0.04581	0.04792	0.04899	0.04748	0.04781	0.05051	0.05596	0.05123	0.04802	0.04619	0.04672	0.05469	0.04998	0.04654	0.04586	0.04164	0.04727	0.04856	0.05123	0.05639	0.04845	0.04394
0.04523	0.04543	0.04904	0.05132	0.04818	0.04754	0.04853	0.04884	0.04708	0.04688	0.04911	0.05429	0.05625	0.04968	0.04574	0.04443	0.04663	0.05118	0.04817	0.04699	0.04844	0.04758	0.04756
0.04786	0.04649	0.04722	0.04837	0.04813	0.04733	0.04925	0.04865	0.04669	0.04743	0.04953	0.05366	0.05539	0.04982	0.04441	0.04534	0.04802	0.05103	0.04961	0.04711	0.04801	0.04818	0.04658
0.04778	0.04947	0.05231	0.04844	0.04728	0.04678	0.05117	0.0503	0.04663	0.04614	0.04601	0.05427	0.05662	0.05006	0.04513	0.04546	0.04423	0.04854	0.04823	0.04802	0.05371	0.04974	0.04803
0.0488	0.0503	0.05236	0.04742	0.04706	0.04852	0.05231	0.05023	0.04601	0.04652	0.04712	0.05383	0.05507	0.04746	0.04519	0.04454	0.04349	0.04926	0.04989	0.04907	0.05351	0.04826	0.04731
0.04844	0.05109	0.05377	0.04777	0.04702	0.04651	0.05101	0.05468	0.04798	0.04843	0.04461	0.04981	0.05611	0.04739	0.04651	0.04379	0.04838	0.0517	0.04948	0.04651	0.04863	0.04851	0.04731
0.04764	0.04924	0.04852	0.04655	0.04766	0.0492	0.05323	0.05313	0.04766	0.04687	0.04626	0.05008	0.05602	0.04976	0.04607	0.045	0.04366	0.04862	0.04976	0.0495	0.05258	0.04761	0.04725
0.04773	0.04673	0.04969	0.05426	0.04889	0.04604	0.04622	0.04833	0.05004	0.04868	0.04649	0.05031	0.05399	0.04753	0.0455	0.04482	0.04979	0.05093	0.04763	0.04699	0.04898	0.0497	0.04746
0.04929	0.04838	0.04568	0.05247	0.05093	0.05071	0.0509	0.04036	0.04641	0.04919	0.04953	0.05398	0.05552	0.05047	0.04464	0.04544	0.04745	0.05063	0.04899	0.04633	0.04858	0.04874	0.04689

du/db																						
8.8E-05	-0.00134	-0.00293	3.1E-05	0.00011	-0.00217	0.00077	-0.00156	0.00078	0.00229	0.00108	-0.00396	-0.001	-0.00114	0.00061	-0.00124	0.00082	0.00051	-0.00083	-0.00104	0.00251	-0.00182	0.0043
0.00082	-0.00159	0.00228	-0.00031	-0.00034	0.00164	0.00119	-0.00211	5.6E-18	0.00188	0.00261	-0.00485	-0.00288	-0.00025	-0.00049	-0.00013	0.00038	-0.00035	-0.00051	-0.00118	-0.00016	-0.00159	0.00343
0.00099	-0.00126	-0.00288	-0.0004	-0.00026	0.00366	-0.00288	-0.00094	0.00043	0.00068	0.00146	-0.00762	-0.00215	0.00119	-0.00151	4.4E-05	0.00016	-0.00071	-0.00086	-0.0004	-0.00286	-0.00128	0.00172
0.00019	-0.0006	-0.00066	0.00016	-0.0001	0.00281	-0.00545	0.00051	0.00099	-0.00159	-8.7E-05	-0.00603	0.00023	0.002	-0.00036	0.00036	-0.00059	0.00073	-0.0016	0.00074	-6.3E-06	-0.00044	0.0003
-6.9E-05	-0.00033	-0.00168	-4.4E-05	0.00042	0.0012	0.00016	0.00101	0.001	-0.00139	-0.00044	-0.00047	-0.00054	0.00148	0.00059	0.00024	-0.00051	-0.0001	-0.0007	0.00102	0.00698	-0.00014	0.00026
-0.00188	0.00146	0.00111	-0.00082	0.00047	-0.00038	0.00084	0.00058	5E-05	-0.00062	0.00026	0.00115	-0.00021	0.00028	0.00131	-0.00071	-0.00086	0.00054	0.00068	0.00044	0.00234	9.4E-05	0.00057
-0.00174	0.00167	0.00745	-0.00169	-0.00052	-0.00051	0.00136	0.00033	-0.00192	1.3E-05	0.00037	0.00104	-0.00019	-0.00116	0.00077	-0.00013	-0.00088	0.00072	0.00164	-0.00021	0.00226	0.0006	-0.00011
-0.00101	0.00131	0.00679	-0.00029	-0.00016	-6.2E-06	0.00023	0.001	-0.00197	0.00067	-0.00019	-0.00048	-0.00086	-0.0013	0.00022	5.6E-05	-0.00094	-0.00017	0.00162	-0.00069	-0.00468	-0.00014	-0.00023
0.00072	0.00024	0.00306	0.00167	-0.00042	0.00033	-0.00035	0.00053	-0.00082	0.00086	-6.3E-06	0.00477	-6.2E-06	-0.00031	-0.00063	0.00053	0.00022	0.00069	4.4E-05	-4.4E-05	-0.00476	-8.7E-05	-0.00042
0.00103	-0.00082	-0.00164	0.00162	-0.00021	0.00132	-0.00049	0.00014	0.00019	0.00041	-0.00012	0.0075	-0.00039	-0.00071	-6.9E-05	-0.00014	0.00069	0.00011	-6.9E-05	-0.00038	-0.0016	0.00158	-0.00176
0.00029	-0.00078	-0.00078	0.00116	0.00053	-0.00042	1.9E-05	0.00071	0.00109	-0.00023	0.00033	0.00215	-0.00016	0.00071	-0.00067	0.00053	0.00043	-0.00055	0.00037	-0.00041	0.0011	0.00213	-0.00243
-6.9E-05	-0.00095	-0.00444	0.00012	0.00095	-0.00038	0.00104	0.00086	0.00064	-0.00029	3.1E-05	-0.00361	-0.00068	1E-04	-1.9E-05	0.00113	-0.00042	0.00011	-0.00033	-0.00011	0.00828	0.00093	-0.00199
-0.00098	-0.00041	-0.00616	0.00113	0.00117	1E-04	0.00012	0.00041	0.00074	-0.00119	0.00011	-0.00353	0.00014	-0.00049	0.00088	8.1E-05	-0.00119	0.00011	-0.00084	0.00093	0.00358	-0.00179	0.00081
-0.00038	0.00021	-0.00664	-0.00048	0.00065	-0.00011	0.00014	-0.00081	-7.5E-05	0.00023	-0.00027	0.00205	0.00072	0.00011	-0.00036	0.00054	-6.3E-05	-0.00025	-0.00031	0.00094	-0.00294	-0.00278	0.00227
-1.9E-05	0.00077	-0.00029	-0.00116	-0.00134	0.00103	-0.00012	-0.00123	-0.00087	0.00204	-0.00236	0.00778	0.00156	0.0005	0.00046	-0.00127	0.00078	0.00123	-0.00028	-0.00039	-0.0036	-0.00075	0.00117
0.0005	0.00159	0.00793	-0.00308	-0.00183	-4.4E-05	0.00034	-0.00116	-0.00039	-6.2E-06	-0.00336	0.00533	0.00238	-0.00101	0.00044	-0.0008	0.00171	0.00069	0.00135	0.00018	0.00133	3.1E-05	6.2E-05
0.00091	0.00141	0.00949	-0.00267	-0.00073	-0.00106	0.00139	0.00029	0.00029	-6.9E-05	-0.00244	-0.00496	0.00218	-0.00348	0.00251	-0.00074	0.00111	0.00069	0.00064	0.00053	0.00171	0.00052	-0.00081
0.00049	0.00024	0.00563	-1.9E-05	0.00051	-0.00076	0.00396	0.00041	0.00053	-0.00078	0.00066	-0.00632	0.00166	-0.00186	0.0024	-0.00088	0.00079	0.00034	-0.00056	0.00098	0.00276	-0.00049	-0.00061
0.00058	-0.00169	-0.00437	0.00088	-8.1E-05	-0.00056	0.00089	-0.00499	0.00022	0.00017	0.0028	-0.00576	-0.00106	-0.00062	0.00018	-0.00054	0.00105	0.00033	-0.00107	0.00093	-0.00489	0.00018	-2.5E-05
-0.00042	-0.00191	-0.00412	0.00278	-0.0029	0.00159	0.00519	-0.00957	-0.00213	0.00329	0.00258	0.00112	-0.00248	0.00301	-0.00172	0.00034	-0.00054	-0.00066	-0.00016	-0.00125	-0.00491	0.00073	0.00067

dv/db																						
0.04832	0.04928	0.04785	0.04929	0.0479	0.0497	0.0465	0.04556	0.04566	0.04321	0.04564	0.04244	0.04724	0.05406	0.05015	0.04791	0.05045	0.04976	0.04886	0.04798	0.04878	0.04821	0.0545
0.04794	0.0486	0.04725	0.04993	0.05256	0.05248	0.0463	0.04517	0.04566	0.0498	0.05011	0.05413	0.0534	0.05198	0.04932	0.04881	0.04622	0.04811	0.04892	0.04964	0.04901	0.04939	0.05803
0.04902	0.04877	0.04926	0.04898	0.05239	0.04902	0.04848	0.04979	0.04602	0.04628	0.04994	0.05158	0.05151	0.04916	0.04833	0.04835	0.04674	0.04699	0.04647	0.04864	0.04836	0.04806	0.05106
0.04754	0.04794	0.04822	0.04294	0.04445	0.04656	0.04746	0.04514	0.04394	0.04187	0.04817	0.0431	0.04369	0.03923	0.0475	0.04302	0.04628	0.04843	0.04804	0.04303	0.04742	0.04757	0.03945
0.04494	0.04696	0.05226	0.04347	0.04746	0.04966	0.05376	0.05066	0.05115	0.04963	0.05006	0.04607	0.04171	0.04215	0.04687	0.04525	0.05194	0.05246	0.05185	0.04838	0.05176	0.04621	0.04111
0.04569	0.04602	0.04704	0.04393	0.04293	0.04314	0.04856	0.04672	0.0477	0.04743	0.04696	0.04115	0.03928	0.04653	0.04511	0.04521	0.0493	0.04792	0.04708	0.04589	0.04809	0.04524	0.04302
0.04768	0.04785	0.04651	0.04785	0.04933	0.04856	0.04709	0.04798	0.04934	0.05013	0.04897	0.04948	0.05031	0.05094	0.04751	0.04995	0.04692	0.04643	0.04799	0.05041	0.04799	0.04892	0.05111
0.05147	0.04978	0.04899	0.05555	0.05348	0.04844	0.04824	0.05373	0.05408	0.05398	0.05253	0.05311	0.05641	0.05476	0.05048	0.05599	0.04815	0.04659	0.04734	0.05348	0.04817	0.05114	0.05404
0.05306	0.05229	0.04881	0.05453	0.04853	0.04903	0.04821	0.04906	0.04951	0.04974	0.04836	0.04935	0.05261	0.04994	0.05294	0.05269	0.04869	0.05074	0.05013	0.04943	0.04842	0.05302	0.04947
0.04752	0.04761	0.04901	0.04951	0.04921	0.04884	0.04828	0.04933	0.04913	0.05017	0.04991	0.04884	0.04819	0.04935	0.04974	0.04951	0.04918	0.05071	0.04893	0.04993	0.04927	0.04734	0.05043
0.04751	0.0479	0.04736	0.04947	0.04851	0.04736	0.04823	0.04782	0.04751	0.04753	0.04706	0.04727	0.04708	0.04753	0.04633	0.04689	0.04629	0.04677	0.04575	0.0476	0.04655	0.04671	0.04648
0.04786	0.04729	0.04773	0.04588	0.04821	0.04742	0.04631	0.04662	0.04649	0.04558	0.04743	0.04715	0.04694	0.04795	0.04668	0.04628	0.04781	0.04642	0.04718	0.04721	0.04626	0.04739	0.04469
0.0483	0.04744	0.04936	0.04861	0.04884	0.04855	0.04908	0.04936	0.04921	0.04842	0.04881	0.04874	0.04928	0.0483	0.04868	0.04943	0.04831	0.04723	0.04896	0.04804	0.04928	0.05004	0.04639
0.04786	0.04874	0.04823	0.04597	0.04741	0.05251	0.05339	0.05247	0.05325	0.04802	0.04804	0.05464	0.05388	0.0531	0.05293	0.05224	0.05348	0.04769	0.05397	0.04762	0.04859	0.05283	0.05282
0.04704	0.04694	0.04559	0.04412	0.046	0.04891	0.04841	0.04864	0.04917	0.04722	0.0467	0.05009	0.05131	0.05438	0.05078	0.04914	0.04947	0.04644	0.04938	0.04693	0.04791	0.04896	0.05053
0.04799	0.04941	0.04636	0.04753	0.04699	0.04811	0.04774	0.04758	0.04644	0.04815	0.04672	0.04649	0.04593	0.05096	0.048	0.0467	0.04671	0.04721	0.04689	0.04762	0.04636	0.04748	0.04799
0.04947	0.05021	0.04928	0.04916	0.04944	0.04791	0.04618	0.04662	0.04674	0.04964	0.04909	0.04586	0.04724	0.04747	0.04678	0.04651	0.047	0.04976	0.04739	0.04949	0.04866	0.04693	0.04841
0.05356	0.05154	0.05284	0.05546	0.05278	0.04817	0.04864	0.05009	0.04899	0.05343	0.05281	0.04196	0.04277	0.03843	0.04109	0.04801	0.04828	0.05281	0.04852	0.05246	0.05379	0.04848	0.04848
0.04851	0.04822	0.0482	0.04968	0.05353	0.05325	0.05393	0.0553	0.05449	0.04798	0.04904	0.04468	0.04643	0.03825	0.04421	0.04946	0.04969	0.04885	0.04804	0.04774	0.0499	0.04791	0.04854
0.04724	0.04679	0.04825	0.04756	0.04894	0.0489	0.04876	0.04884	0.05016	0.04574	0.04768	0.04723	0.04862	0.0454	0.04694	0.04804	0.04564	0.04679	0.04599	0.04813	0.04829	0.0467	0.04602

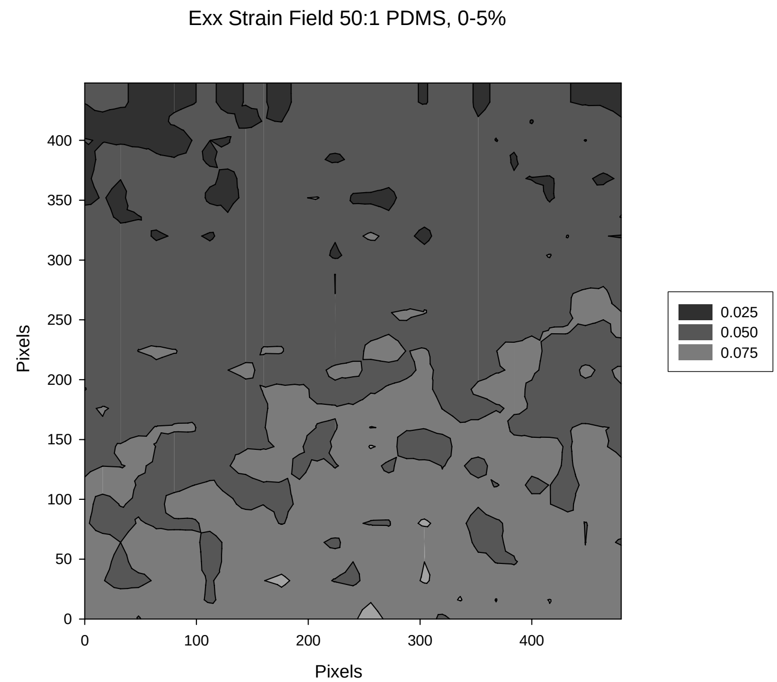
dv/da																						
0.00034	0.00132	0.00126	0.00014	-0.0021	-0.00732	-0.00394	-0.00639	0.00056	0.00684	0.00473	0.00547	-0.00024	-0.00011	-6.3E-06	-3.1E-05	-0.00116	-0.00079	0.00053	0.00043	0.00049	-0.00537	-0.0086
0.0007	0.00038	-0.00116	-0.00313	-0.00121	0.00218	0.00501	0.00271	-0.00161	-0.00318	-0.00179	0.00101	0.00061	0.00024	0.00097	0.0005	0.00012	-0.00071	0.0004	0.00164	-7.5E-05	-0.00218	-0.00464
0.00086	-0.00053	0.00072	0.00041	-0.00274	-0.00233	-0.00239	0.00049	0.00266	0.00222	0.00196	0.0005	-0.00071	-0.00134	-0.00103	0.00019	0.00166	0.00116	0.0009	0.00079	0.00022	0.00042	0.001
-2.5E-05	-6.9E-05	-0.00086	0.00048	-0.00013	0.00074	0.00494	0.00178	0.00121	-0.0012	-0.00524	-0.00087	-0.00264	-0.00172	-0.00076	0.0006	0.00169	0.00089	0.00108	0.00166	0.00708	0.00277	-0.00465
0.00112	0.0011	0.00225	0.00278	0.00168	0.00031	-0.00187	-0.00279	-0.00204	-0.00579	-0.00387	-0.00052	0.00124	0.00502	0.00122	0.00064	0.00063	0	-0.00556	-0.00735	-0.00259	0.00391	0.00861
-0.00472	-0.00696	-0.00225	0.00358	0.00714	0.00386	-0.00138	-0.00683	-0.00916	-0.00299	0.00299	0.00888	0.0084	0.00249	0.00059	-0.00191	-0.00292	-0.00146	-0.00535	-0.00759	-0.00267	0.00352	0.00813
-0.00026	0.00111	0.00218	6.2E-06	-0.00039	7.5E-05	0.00106	0.00104	-0.00029	-0.00054	0.00067	0.00044	-0.00105	-0.00048	0.00084	0.00129	0.00151	0.00031	-0.00019	0.00013	-0.00107	-0.00149	-0.00017
0.00626	0.00319	-0.00187	-0.00184	0.00213	0.00731	0.00441	-0.00021	-3.1E-05	0.00106	-0.0049	-0.00318	-0.00598	-0.00756	-0.00381	-0.00062	0.0028	0.0019	0.00472	0.00409	0.00353	0.00207	-0.00108
-0.00515	-0.00428	-0.00254	-0.00485	5.6E-18	-6.9E-05	-0.00054	-0.00098	0.00461	0.00383	0.00523	0.00375	-0.00334	-0.0028	-0.00516	-0.00308	-1.2E-05	0.00462	0.00123	-0.00146	-0.00281	-0.00506	0.00658
0.00154	0.00067	-0.00101	0.00041	0.00038	6.2E-05	0.00067	0.00012	-5.6E-05	-0.00044	-0.00013	0.00026	0.00019	0.00016	0.00026	0.00071	6.9E-05	-0.00143	-0.00167	-0.0011	0.00042	0.0014	0.00072
0.00045	0.00026	0.00082	-0.00012	0.00075	0.001	-3.1E-05	-0.00043	-0.00092	-0.00128	-0.00099	-0.00035	-0.00024	0.00047	7.5E-05	-0.00037	-0.00019	-0.00051	0.00063	0.00084	0.00065	6.2E-06	-0.00118
-0.00144	1.9E-05	0.00076	0.00024	-0.00149	-0.00168	-0.00026	0.00091	0.00084	0.00089	-0.00056	-0.00104	0.00011	0.00014	0.00141	0.00094	-0.00058	-0.00049	-0.00052	0.00039	0.00101	0.00022	-0.00047
-0.00026	-0.00056	-8.7E-05	0.00086	-5.6E-05	-0.00056	-0.00056	-0.00108	0.00126	0.00084	0.00058	0.0009	-0.00076	-0.00147	-0.00196	-0.00066	0.00124	0.00273	-0.00048	-0.00316	-0.00323	-0.00122	0.00214
-0.00076	-0.00026	0.0005	0.00057	0.00063	-0.00091	-0.00112	0.00054	0.00046	0.00018	-0.00099	-0.00181	-0.00062	4.4E-05	0.00148	0.00056	-0.00034	0.00016	-0.00132	0.0001	0.0003	-0.00541	-0.00254
-0.00168	0.00036	0.00113	0.0015	0.00047	0.00081	0.00171	0.00053	-0.00018	-0.00094	-0.00018	0.00079	0.00123	0.0013	0.00068	-0.00062	-0.00098	-0.00118	0.00075	0.00176	-0.00694	-0.00428	-0.00214
-0.00037	-0.00081	0.00079	0.00012	0.00036	-0.00062	-0.00199	-0.00089	0.00032	-0.00034	0.00016	1.3E-05	-0.00092	-0.00028	-0.00047	0.00031	0.00169	0.00094	0.00126	0.00074	0.00106	0.00213	3.8E-05
-0.00157	-0.00113	7.5E-05	0.00021	-0.00069	-0.00063	0.00061	0.00044	0.00109	0.00099	0.00036	-0.00039	-0.00021	-5.6E-05	-0.00017	0.00053	-0.00114	-0.00064	-0.0002	0.00026	0.00096	-1E-04	-0.00084
0.00102	-0.00288	-0.00741	-0.00828	-0.00461	0.00438	0.00701	0.00101	-0.00411	-0.00878	-0.00538	0.00164	0.00093	0.00586	0.00314	0.00436	0.00466	-0.00256	-0.0023	-0.00122	-0.00143	-0.00029	-0.00325
7.5E-05	-0.00079	1.2E-05	-3.1E-05	-8.1E-05	-0.00037	-0.00036	-0.00064	-0.00154	-0.00619	-0.00364	0.00118	0.00477	0.00683	0.00163	-0.00038	-0.00115	-0.00122	0.00036	0.00096	0.00116	0.00018	-0.00092
0.00075	-6.2E-06	-0.00085	-0.00018	0.00088	-0.00014	-0.00077	0.00059	0.00297	0.00084	-0.00136	-0.00202	-0.00207	0.00066	-0.00053	-0.00025	0.00184	0.00123	-4.4E-05	-0.00112	-0.00128	0.00041	0.00084
-0.00088	-0.00035	0.00052	0.00034	-8.1E-05	-0.00038	8.1E-05	0.00024	-0.00107	-0.00072	-0.00014	0.00103	0.00201	0.00014	-0.00057	-0.00034	-0.00125	-0.00034	-0.00107	-0.00115	-8.7E-05	-9.4E-05	0.00086
0.00033	0.0003	0.00017	-0.00103	-0.00179	-0.00105	0.00096	0.00681	0.00761	0.00851	0.00636	-0.00349	-0.00799	-0.00877	-0.00559	-0.00022	-0.00062	-0.00131	0.00045	0.00084	0.00024	-0.00083	-0.00047
-0.00546	-0.00864	-0.00801	-0.00542	-0.00074	0.00507	0.00732	0.0096	0.00586	0.00101	0.00054	-0.00176	-0.00113	-0.00179	0.00064	0.00215	0.00042	5.6E-05	-0.00047	-0.00089	-0.0001	-0.00083	0.00022
0.00096	0.00169	0.00224	0.00151	1.2E-05	-0.0004	-0.00176	-0.00124	0.00031	-0.00016	-4.4E-05	-0.00061	0.00116	0.00088	0.00044	-0.00176	-0.00231	-1.9E-05	0.00034	0.00176	0.00036	-0.00014	0.00032

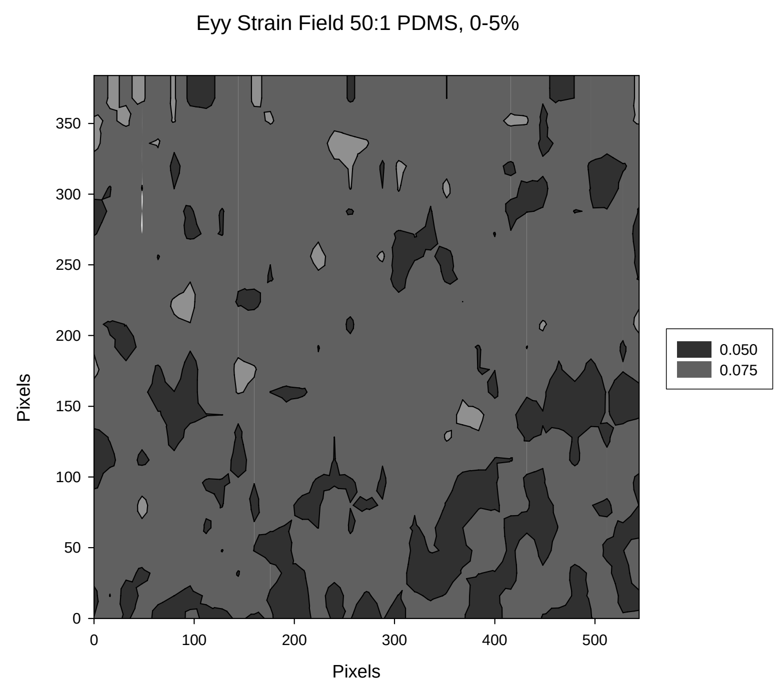
Exx	du/da+0.5*((du/da)^2+(dv/da)^2)																					
4.93%	4.99%	5.08%	4.95%	4.98%	5.15%	5.30%	4.83%	4.59%	4.69%	5.10%	5.65%	5.73%	5.20%	4.56%	4.58%	4.86%	5.08%	5.31%	4.88%	5.05%	5.02%	4.47%
4.87%	4.99%	5.11%	5.03%	4.73%	4.80%	5.16%	5.35%	4.61%	4.54%	4.87%	5.50%	5.91%	5.11%	4.58%	4.56%	4.88%	5.00%	5.19%	4.87%	5.17%	5.59%	4.69%
4.76%	5.30%	5.65%	5.06%	4.76%	4.59%	5.21%	5.25%	4.64%	4.60%	4.77%	5.51%	5.89%	5.10%	4.67%	4.66%	4.89%	5.09%	5.08%	4.98%	5.26%	5.11%	4.43%
4.93%	4.98%	4.65%	4.87%	4.75%	4.77%	5.47%	4.92%	4.84%	4.95%	5.09%	5.62%	5.64%	5.06%	4.63%	4.64%	4.49%	4.92%	5.01%	5.06%	5.60%	5.07%	4.73%
4.82%	4.93%	5.04%	5.16%	4.87%	4.70%	4.91%	5.28%	4.99%	4.95%	4.89%	5.22%	5.68%	5.04%	4.60%	4.60%	4.95%	5.18%	4.91%	4.68%	4.96%	5.02%	4.89%
4.89%	4.98%	5.43%	5.08%	4.84%	4.78%	4.95%	5.49%	5.10%	4.88%	4.89%	5.02%	5.67%	5.10%	4.63%	4.74%	4.95%	5.06%	4.89%	4.72%	5.04%	5.52%	5.04%
4.87%	4.65%	5.04%	5.15%	4.88%	4.72%	4.93%	5.62%	5.22%	4.77%	4.71%	4.97%	5.69%	5.20%	4.64%	4.71%	4.96%	5.18%	4.92%	4.68%	4.93%	4.97%	4.93%
4.90%	4.73%	5.01%	5.12%	4.75%	4.88%	5.01%	5.50%	5.03%	4.65%	4.79%	5.11%	5.81%	5.14%	4.72%	4.60%	4.43%	4.96%	4.86%	5.01%	5.43%	5.00%	4.92%
4.75%	4.78%	5.00%	5.04%	4.87%	4.87%	5.08%	5.50%	5.13%	4.77%	4.74%	5.04%	5.67%	5.25%	4.71%	4.60%	4.37%	4.83%	4.88%	5.04%	5.45%	5.01%	4.85%
4.82%	5.14%	5.46%	4.97%	4.84%	4.77%	5.00%	5.47%	5.07%	4.81%	4.82%	5.10%	5.79%	5.16%	4.59%	4.59%	4.92%	5.20%	4.94%	4.74%	5.03%	5.10%	4.80%
4.96%	5.09%	5.40%	4.91%	4.94%	4.89%	5.05%	5.43%	4.96%	4.84%	4.77%	5.05%	5.72%	5.07%	4.56%	4.59%	4.44%	5.04%	5.02%	5.06%	5.42%	5.04%	4.79%
4.79%	5.08%	5.48%	5.03%	4.85%	4.91%	5.08%	5.59%	5.06%	4.85%	4.77%	4.99%	5.72%	5.16%	4.66%	4.55%	4.81%	5.18%	4.96%	4.78%	4.99%	5.01%	4.80%
4.81%	4.98%	5.41%	5.13%	4.89%	4.77%	4.98%	4.96%	4.85%	4.87%	5.09%	5.58%	5.64%	5.10%	4.60%	4.69%	4.99%	5.17%	4.92%	4.71%	5.03%	5.14%	4.86%
4.89%	4.89%	5.35%	5.03%	4.88%	4.89%	4.98%	4.95%	4.82%	4.93%	5.08%	5.62%	5.68%	5.02%	4.56%	4.61%	4.91%	5.06%	5.06%	4.79%	5.23%	5.07%	4.56%
4.78%	5.08%	5.46%	4.99%	4.80%	4.83%	5.12%	5.64%	5.09%	4.73%	4.80%	4.93%	5.68%	5.22%	4.56%	4.66%	4.31%	4.79%	5.13%	5.06%	5.83%	5.12%	4.52%
4.64%	4.69%	4.91%	5.02%	4.86%	4.89%	5.18%	5.75%	5.25%	4.92%	4.73%	4.78%	5.62%	5.12%	4.76%	4.69%	4.25%	4.84%	4.97%	5.25%	5.80%	4.96%	4.49%
4.63%	4.65%	5.02%	5.26%	4.93%	4.87%	4.97%	5.00%	4.82%	4.80%	5.03%	5.58%	5.78%	5.09%	4.68%	4.54%	4.77%	5.25%	4.93%	4.81%	4.96%	4.87%	4.87%
4.90%	4.76%	4.84%	4.96%	4.93%	4.85%	5.05%	4.98%	4.78%	4.86%	5.08%	5.51%	5.69%	5.11%	4.54%	4.64%	4.92%	5.23%	5.08%	4.82%	4.92%	4.93%	4.77%
4.89%	5.07%	5.37%	4.96%	4.84%	4.79%	5.25%	5.16%	4.77%	4.72%	4.71%	5.57%	5.82%	5.13%	4.62%	4.65%	4.52%	4.97%	4.94%	4.92%	5.51%	5.10%	4.92%
5.00%	5.16%	5.37%	4.85%	4.82%	4.97%	5.37%	5.15%	4.71%	4.76%	4.82%	5.53%	5.66%	4.86%	4.62%	4.55%	4.44%	5.05%	5.11%	5.03%	5.49%	4.94%	4.84%
4.96%	5.24%	5.52%	4.89%	4.81%	4.76%	5.23%	5.62%	4.91%	4.96%	4.56%	5.11%	5.77%	4.85%	4.76%	4.48%	4.96%	5.30%	5.07%	4.76%	4.98%	4.97%	4.84%
4.88%	5.04%	4.97%	4.76%	4.88%	5.04%	5.46%	5.46%	4.88%	4.80%	4.73%	5.13%	5.76%	5.10%	4.71%	4.60%	4.46%	4.98%	5.10%	5.07%	5.40%	4.87%	4.84%
4.89%	4.79%	5.10%	5.57%	5.01%	4.71%	4.73%	4.95%	5.13%	4.99%	4.76%	5.16%	5.55%	4.87%	4.65%	4.58%	5.10%	5.22%	4.88%	4.81%	5.02%	5.09%	4.86%
5.05%	4.95%	4.67%	5.38%	5.22%	5.20%	5.22%	4.12%	4.75%	5.04%	5.08%	5.54%	5.71%	5.17%	4.56%	4.65%	4.86%	5.19%	5.02%	4.74%	4.98%	4.99%	4.80%
MAX	5.911%																					
MIN	3.918%																					
AVG	4.995%																					
STDEV	0.280%																					

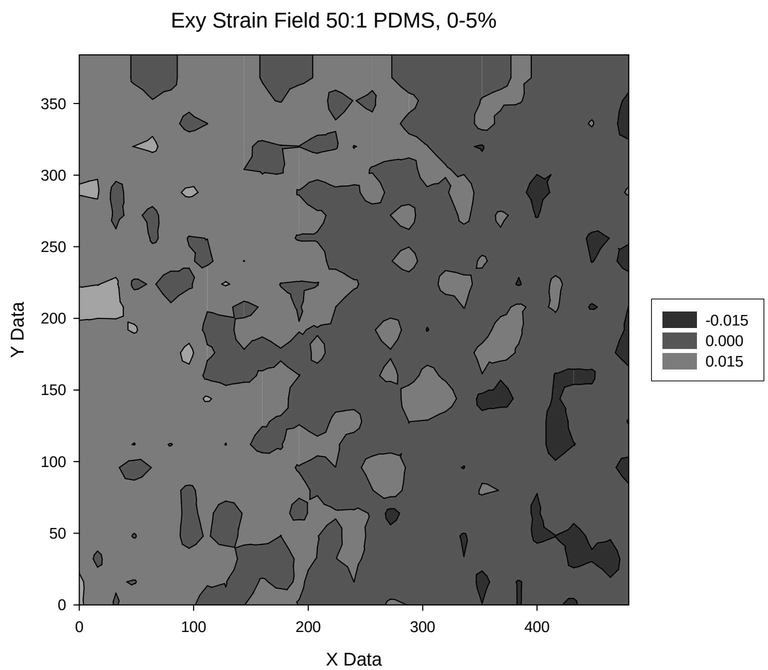
Eyy	dv/db+0.5*((du/db)^2+(dv/db)^2)																					
4.95%	5.05%	4.90%	5.05%	4.90%	5.09%	4.76%	4.66%	4.67%	4.41%	4.67%	4.33%	4.84%	5.55%	5.14%	4.91%	5.17%	5.10%	5.01%	4.91%	5.00%	4.94%	5.60%
4.91%	4.98%	4.84%	5.12%	5.39%	5.39%	4.74%	4.62%	4.67%	5.10%	5.14%	5.56%	5.48%	5.33%	5.05%	5.00%	4.73%	4.93%	5.01%	5.09%	5.02%	5.06%	5.97%
5.02%	5.00%	5.05%	5.02%	5.38%	5.02%	4.97%	5.10%	4.71%	4.74%	5.12%	5.29%	5.28%	5.04%	4.95%	4.95%	4.78%	4.81%	4.75%	4.98%	4.95%	4.92%	5.24%
4.87%	4.91%	4.94%	4.39%	4.54%	4.77%	4.86%	4.62%	4.49%	4.27%	4.93%	4.40%	4.46%	4.00%	4.86%	4.39%	4.73%	4.96%	4.92%	4.40%	4.85%	4.87%	4.02%
4.59%	4.81%	5.36%	4.44%	4.86%	5.09%	5.52%	5.19%	5.25%	5.09%	5.13%	4.71%	4.26%	4.30%	4.80%	4.63%	5.33%	5.38%	5.32%	4.95%	5.31%	4.73%	4.20%
4.67%	4.71%	4.81%	4.49%	4.38%	4.41%	4.97%	4.78%	4.88%	4.86%	4.81%	4.20%	4.01%	4.76%	4.61%	4.62%	5.05%	4.91%	4.82%	4.69%	4.92%	4.63%	4.39%
4.88%	4.90%	4.76%	4.90%	5.05%	4.97%	4.82%	4.91%	5.06%	5.14%	5.02%	5.07%	5.16%	5.22%	4.86%	5.12%	4.80%	4.75%	4.91%	5.17%	4.91%	5.01%	5.24%
5.28%	5.10%	5.02%	5.71%	5.49%	4.96%	4.94%	5.52%	5.55%	5.54%	5.39%	5.45%	5.80%	5.63%	5.18%	5.76%	4.93%	4.77%	4.85%	5.49%	4.93%	5.24%	5.55%
5.45%	5.37%	5.00%	5.60%	4.97%	5.02%	4.94%	5.03%	5.07%	5.10%	4.95%	5.06%	5.40%	5.12%	5.43%	5.41%	4.99%	5.20%	5.14%	5.07%	4.96%	5.44%	5.07%
4.86%	4.87%	5.02%	5.07%	5.04%	5.00%	4.94%	5.05%	5.03%	5.14%	5.12%	5.01%	4.93%	5.06%	5.10%	5.07%	5.04%	5.20%	5.01%	5.12%	5.05%	4.85%	5.17%
4.86%	4.90%	4.85%	5.07%	4.97%	4.85%	4.94%	4.90%	4.86%	4.87%	4.82%	4.84%	4.82%	4.87%	4.74%	4.80%	4.74%	4.79%	4.68%	4.87%	4.76%	4.78%	4.76%
4.90%	4.84%	4.89%	4.69%	4.94%	4.85%	4.74%	4.77%	4.76%	4.66%	4.85%	4.83%	4.80%	4.91%	4.78%	4.74%	4.90%	4.75%	4.83%	4.83%	4.74%	4.85%	4.57%
4.95%	4.86%	5.06%	4.98%	5.00%	4.97%	5.03%	5.06%	5.04%	4.96%	5.00%	4.99%	5.05%	4.95%	4.99%	5.06%	4.95%	4.83%	5.02%	4.92%	5.05%	5.13%	4.75%
4.90%	4.99%	4.94%	4.70%	4.85%	5.39%	5.48%	5.38%	5.47%	4.92%	4.92%	5.61%	5.53%	5.45%	5.43%	5.36%	5.49%	4.88%	5.54%	4.88%	4.98%	5.42%	5.42%
4.81%	4.80%	4.66%	4.51%	4.71%	5.01%	4.96%	4.98%	5.04%	4.83%	4.78%	5.14%	5.26%	5.59%	5.21%	5.04%	5.07%	4.75%	5.06%	4.80%	4.91%	5.02%	5.18%
4.91%	5.06%	4.75%	4.87%	4.81%	4.93%	4.89%	4.87%	4.75%	4.93%	4.78%	4.76%	4.70%	5.23%	4.92%	4.78%	4.78%	4.83%	4.80%	4.88%	4.74%	4.86%	4.91%
5.07%	5.15%	5.05%	5.04%	5.07%	4.91%	4.72%	4.77%	4.78%	5.09%	5.03%	4.69%	4.84%	4.86%	4.79%	4.76%	4.81%	5.10%	4.85%	5.07%	4.98%	4.80%	4.96%
5.50%	5.29%	5.42%	5.70%	5.42%	4.93%	4.98%	5.13%	5.02%	5.49%	5.42%	4.29%	4.37%	3.92%	4.19%	4.92%	4.94%	5.42%	4.97%	5.38%	5.52%	4.97%	4.97%
4.97%	4.94%	4.94%	5.09%	5.50%	5.47%	5.54%	5.68%	5.60%	4.91%	5.02%	4.57%	4.75%	3.90%	4.52%	5.07%	5.09%	5.00%	4.92%	4.89%	5.12%	4.91%	4.97%
4.84%	4.79%	4.94%	4.87%	5.01%	5.01%	5.00%	5.01%	5.14%	4.68%	4.88%	4.83%	4.98%	4.64%	4.80%	4.92%	4.67%	4.79%	4.71%	4.93%	4.95%	4.78%	4.71%
MAX	6.014%																					
MIN	3.898%																					
AVG	4.966%																					
STDEV	0.299%																					

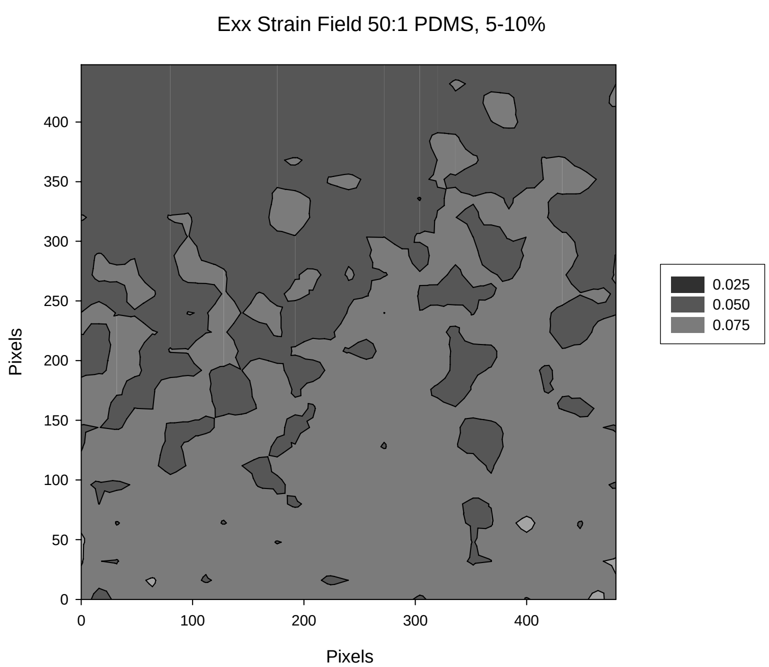
Exy	0.5*(du/db+dv/da+(du/da*du/db+dv/da*dv/db))																					
0.02%	0.00%	-0.09%	0.01%	-0.10%	-0.50%	-0.17%	-0.42%	0.07%	0.48%	0.30%	0.08%	-0.07%	-0.07%	0.03%	-0.07%	-0.02%	-0.02%	-0.02%	-0.03%	0.16%	-0.38%	-0.23%
0.08%	-0.06%	0.06%	-0.18%	-0.08%	0.20%	0.32%	0.03%	-0.08%	-0.07%	0.04%	-0.20%	-0.12%	0.00%	0.03%	0.02%	0.03%	-0.06%	-0.01%	0.02%	-0.01%	-0.20%	-0.07%
0.10%	-0.09%	-0.11%	0.00%	-0.16%	0.07%	-0.28%	-0.02%	0.16%	0.15%	0.18%	-0.38%	-0.15%	-0.01%	-0.13%	0.01%	0.09%	0.02%	0.00%	0.02%	-0.14%	-0.05%	0.14%
0.01%	-0.04%	-0.08%	0.03%	-0.01%	0.19%	-0.03%	0.12%	0.11%	-0.15%	-0.28%	-0.36%	-0.13%	0.02%	-0.06%	0.05%	0.06%	0.08%	-0.03%	0.13%	0.37%	0.12%	-0.23%
0.05%	0.04%	0.03%	0.14%	0.11%	0.08%	-0.09%	-0.09%	-0.05%	-0.38%	-0.23%	-0.05%	0.04%	0.34%	0.09%	0.05%	0.01%	-0.01%	-0.33%	-0.33%	0.23%	0.20%	0.46%
-0.35%	-0.29%	-0.06%	0.14%	0.40%	0.18%	-0.03%	-0.33%	-0.48%	-0.19%	0.17%	0.52%	0.43%	0.14%	0.10%	-0.14%	-0.20%	-0.05%	-0.24%	-0.37%	-0.02%	0.19%	0.45%
-0.11%	0.15%	0.50%	-0.09%	-0.05%	-0.02%	0.13%	0.07%	-0.12%	-0.03%	0.05%	0.08%	-0.07%	-0.09%	0.08%	0.06%	0.03%	0.05%	0.08%	0.00%	0.06%	-0.05%	-0.01%
0.28%	0.24%	0.26%	-0.11%	0.10%	0.38%	0.24%	0.04%	-0.10%	0.09%	-0.27%	-0.19%	-0.36%	-0.47%	-0.19%	-0.03%	0.10%	0.09%	0.33%	0.18%	-0.06%	0.10%	-0.07%
-0.23%	-0.21%	0.03%	-0.17%	-0.02%	0.01%	-0.05%	-0.02%	0.20%	0.25%	0.27%	0.45%	-0.18%	-0.16%	-0.30%	-0.13%	0.01%	0.28%	0.07%	-0.08%	-0.40%	-0.27%	0.32%
0.13%	-0.01%	-0.14%	0.11%	0.01%	0.07%	0.01%	0.01%	0.01%	0.00%	-0.01%	0.41%	-0.01%	-0.03%	0.01%	0.03%	0.04%	-0.07%	-0.09%	-0.08%	-0.06%	0.16%	-0.05%
0.04%	-0.03%	0.00%	0.05%	0.07%	0.03%	0.00%	0.01%	0.01%	-0.08%	-0.03%	0.09%	-0.02%	0.06%	-0.03%	0.01%	0.01%	-0.06%	0.05%	0.02%	0.09%	0.11%	-0.19%
-0.08%	-0.05%	-0.19%	0.02%	-0.03%	-0.11%	0.04%	0.09%	0.08%	0.03%	-0.03%	-0.24%	-0.03%	0.01%	0.07%	0.11%	-0.05%	-0.02%	-0.04%	0.01%	0.49%	0.06%	-0.13%
-0.06%	-0.05%	-0.33%	0.10%	0.06%	-0.02%	-0.02%	-0.04%	0.10%	-0.02%	0.04%	-0.14%	-0.03%	-0.10%	-0.06%	-0.03%	0.00%	0.15%	-0.07%	-0.12%	0.02%	-0.16%	0.15%
-0.06%	0.00%	-0.32%	0.00%	0.07%	-0.05%	-0.05%	-0.01%	0.02%	0.02%	-0.07%	0.01%	0.01%	0.01%	0.06%	0.06%	-0.02%	0.00%	-0.09%	0.05%	-0.14%	-0.43%	-0.01%
-0.09%	0.06%	0.04%	0.02%	-0.05%	0.10%	0.08%	-0.04%	-0.06%	0.06%	-0.13%	0.45%	0.15%	0.09%	0.06%	-0.10%	-0.01%	0.00%	0.02%	0.07%	-0.55%	-0.26%	-0.05%
0.01%	0.04%	0.46%	-0.16%	-0.08%	-0.03%	-0.09%	-0.11%	0.00%	-0.02%	-0.17%	0.28%	0.08%	-0.07%	0.00%	-0.03%	0.18%	0.09%	0.14%	0.05%	0.13%	0.11%	0.01%
-0.03%	0.01%	0.50%	-0.13%	-0.07%	-0.09%	0.10%	0.04%	0.07%	0.05%	-0.11%	-0.28%	0.10%	-0.19%	0.12%	-0.01%	0.00%	0.00%	0.02%	0.04%	0.14%	0.02%	-0.09%
0.08%	-0.14%	-0.10%	-0.44%	-0.22%	0.19%	0.58%	0.07%	-0.19%	-0.50%	-0.25%	-0.25%	0.14%	0.21%	0.29%	0.18%	0.29%	-0.12%	-0.15%	-0.01%	0.07%	-0.04%	-0.20%
0.03%	-0.13%	-0.23%	0.04%	-0.01%	-0.05%	0.03%	-0.30%	-0.07%	-0.32%	-0.04%	-0.24%	0.19%	0.32%	0.09%	-0.05%	-0.01%	-0.05%	-0.04%	0.10%	-0.20%	0.02%	-0.05%
0.02%	-0.10%	-0.26%	0.14%	-0.11%	0.08%	0.23%	-0.47%	0.05%	0.22%	0.06%	-0.05%	-0.24%	0.19%	-0.12%	0.00%	0.07%	0.03%	-0.01%	-0.12%	-0.33%	0.06%	0.08%
MAX	0.829%																					
MIN	-0.725%																					
AVG	-0.003%																					
STDEV	0.179%																					

Results for 50:1 PDMS

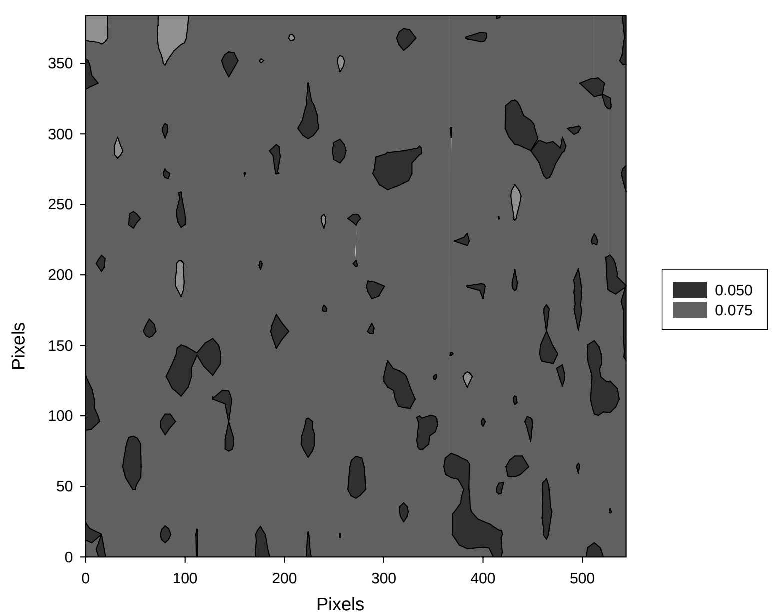




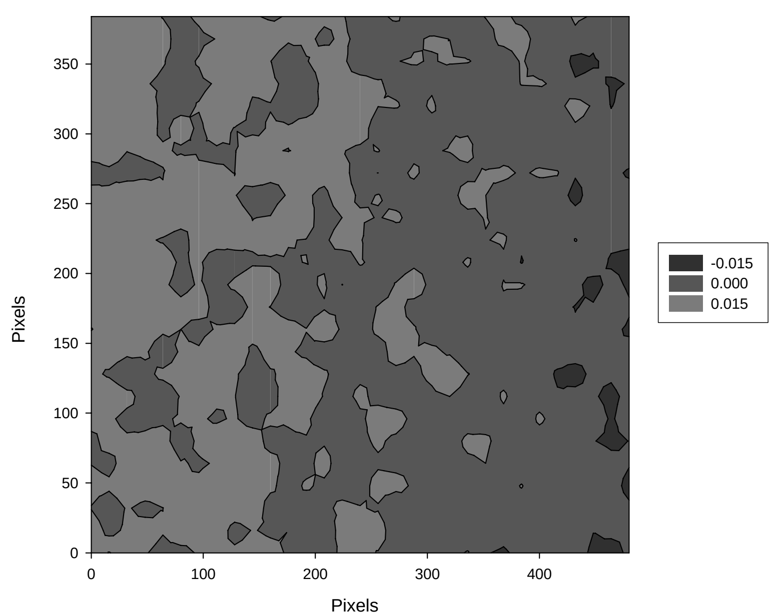

Exy Strain Field 50:1 PDMS, 0-5%
Y Data
X Data
-0.015
0.000
0.015

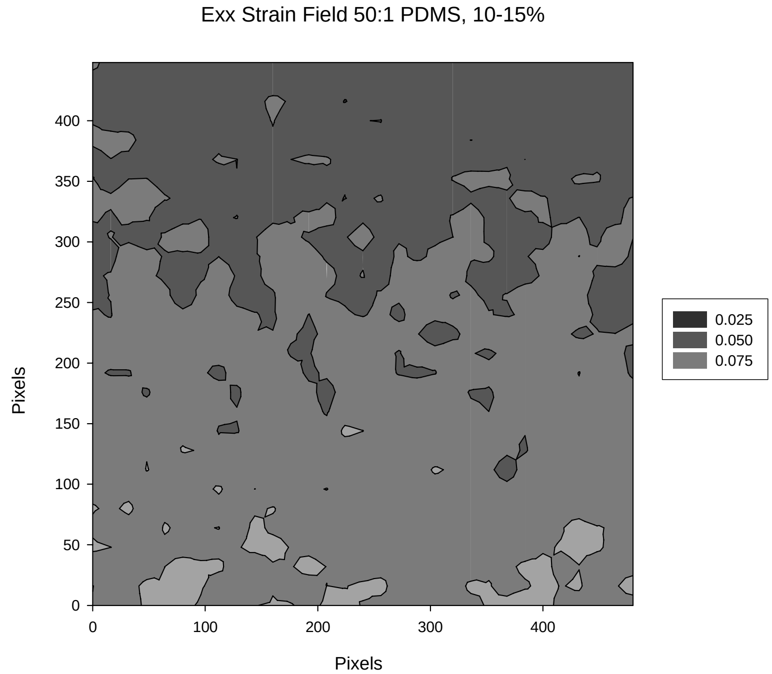

Exx Strain Field 50:1 PDMS, 5-10%
Pixels
Pixels
0.025
0.050
0.075

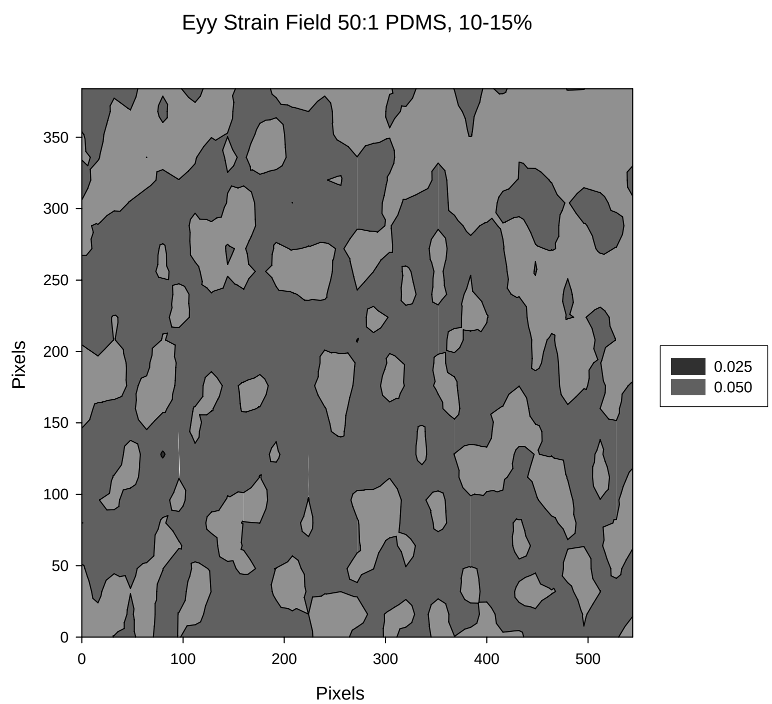
Eyy Strain Field 50:1 PDMS, 5-10%

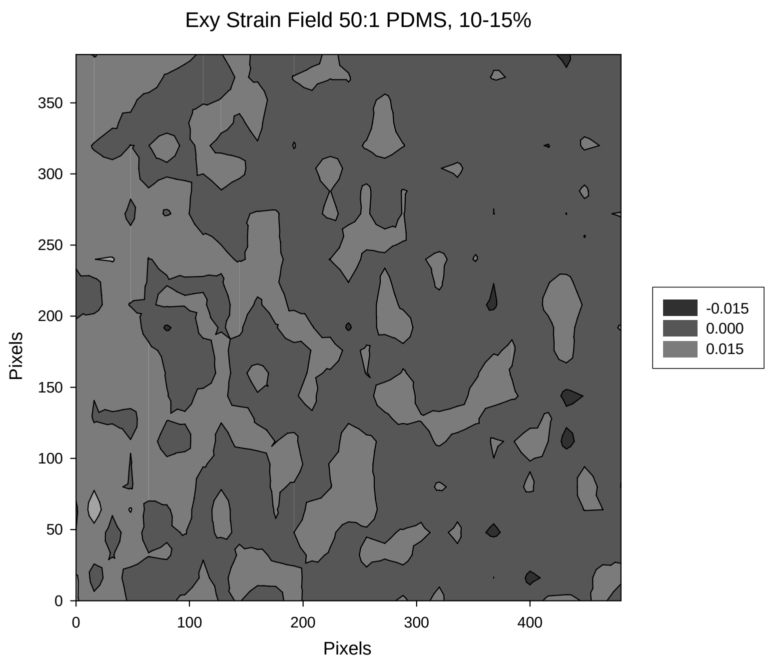


Exy Strain Field 50:1 PDMS, 5-10%




Exx Strain Field 50:1 PDMS, 10-15%
Pixels
Pixels
0.025
0.050
0.075


Eyy Strain Field 50:1 PDMS, 10-15%
Pixels
Pixels
0.025
0.050


Exy Strain Field 50:1 PDMS, 10-15%
Pixels
Pixels
0
50
100
150
200
250
300
350
0
100
200
300
400
-0.015
0.000
0.015

0 to 5.19																
u displacement																
	-32.458	-32.544	-30.287	-29.756	-28.728	-27.725	-27.568	-25.707	-25.208	-24.396	-23.677	-22.461	-21.705	-20.449	-19.629	-19.291
-32.791	-32.324	-31.594	-30.257	-29.758	-28.787	-27.672	-26.693	-25.725	-25.222	-24.279	-23.657	-22.357	-21.696	-21.344	-19.201	-19.244
-32.413	-31.740	-30.540	-29.676	-28.804	-28.661	-27.753	-26.738	-25.732	-25.321	-24.251	-23.688	-22.357	-21.389	-19.640	-18.842	-18.334
-32.537	-31.704	-30.378	-29.726	-28.622	-28.285	-27.684	-25.740	-25.579	-24.329	-23.655	-23.570	-21.647	-21.238	-20.398	-19.274	-18.282
-32.372	-31.342	-30.308	-29.656	-28.556	-27.642	-27.456	-25.649	-25.362	-24.365	-23.701	-23.251	-21.688	-21.252	-19.699	-18.708	-18.239
-31.613		-29.508	-28.909	-28.317	-27.669	-26.775	-25.705	-25.546	-24.645	-23.697	-22.280	-21.807	-20.627	-20.235	-19.235	-18.276
-31.604	-29.664	-29.801	-28.830	-27.587	-27.614	-26.461	-25.668	-25.243	-23.675	-22.740	-22.251	-21.698	-20.707	-19.644	-19.532	-18.300
-30.689	-30.247	-29.229	-28.259	-27.675	-26.567	-25.660	-25.544	-24.340	-23.780	-22.689	-22.238	-21.622	-21.081	-19.596	-19.507	-18.322
-30.248	-29.689	-28.533	-28.219	-27.380	-26.408	-25.593	-25.160	-24.313	-23.739	-23.680	-22.421	-21.169	-20.591	-19.387	-18.711	-18.649
-29.663	-29.294	-28.466	-28.278	-27.286	-26.394	-25.378	-24.521	-24.371	-23.622	-22.601	-21.739	-21.645	-20.340	-19.592	-18.743	-18.246
-29.293	-29.312	-28.216	-27.331	-26.835	-26.308	-25.659	-24.700	-23.678	-23.270	-22.326	-22.169	-21.767	-20.615	-19.276	-18.864	-18.267
-28.715	-28.589	-27.757	-27.324	-25.660	-25.674	-24.898	-24.297	-24.341	-22.824	-22.303	-22.199	-21.782	-20.228	-19.551	-18.869	-18.440
-27.622	-27.758	-27.316	-26.743	-26.269	-25.263	-24.786	-24.367	-23.613	-22.631	-22.350	-21.655	-21.331	-19.778	-19.327	-18.461	-17.609
-28.398	-27.829	-27.134	-26.406	-26.276	-25.642	-24.772	-24.614	-23.422	-23.299	-21.708	-21.630	-20.371	-19.612	-19.247	-19.306	-18.273
-27.606	-27.249	-26.780	-26.164	-25.827	-25.357	-24.712	-22.793	-22.798	-22.295	-21.637	-21.250	-20.722	-19.618	-18.476	-18.348	-18.408
-27.766	-26.734	-26.549	-26.410	-24.632	-24.574	-24.235	-23.185	-22.828	-22.599	-21.376	-21.250	-20.314	-19.684	-18.628	-18.622	-17.314
-27.560	-26.855	-26.305	-25.680	-25.689	-24.201	-24.301	-23.566	-22.432	-22.282	-21.373	-21.202	-20.364	-19.666	-19.263	-18.329	-17.653
-27.289	-26.516	-26.299	-25.320	-25.301	-24.315	-24.279	-22.750	-22.726	-21.712	-21.293	-21.119	-20.316	-19.702	-18.767	-18.237	-17.723
-26.367	-25.740	-26.182	-24.680	-24.805	-24.232	-23.804	-22.415	-22.468	-21.630	-21.287	-20.771	-20.219	-19.643	-19.537	-18.427	-17.238
-26.328	-25.719	-25.244	-25.204	-24.235	-23.843	-23.689	-22.329	-22.338	-21.651	-21.149	-20.463	-20.223	-19.659	-18.368	-18.239	-17.300
-25.692	-25.588	-24.792	-24.756	-23.119	-23.109	-23.144	-22.294	-21.790	-21.726	-21.271	-20.773	-19.845	-19.687	-18.633	-17.731	-17.685
-25.599	-25.505	-24.589	-24.328	-23.600	-23.664	-23.224	-22.655	-21.729	-21.692	-20.637	-20.426	-20.309	-19.233	-18.754	-17.659	-17.295
-25.338	-25.307	-24.343	-24.311	-24.315	-23.300	-23.349	-21.757	-21.661	-21.227	-20.707	-20.624	-20.270	-19.319	-19.228	-17.644	-17.255
-24.632	-25.240	-24.344	-23.783	-23.703	-23.219	-22.675	-22.187	-21.339	-21.302	-20.693	-20.310	-20.273	-19.181	-19.176	-18.690	-17.832
-24.762	-24.381	-24.291	-24.210	-23.571	-22.445	-22.262	-21.829	-21.698	-20.662	-20.625	-20.549	-19.418	-19.017	-18.339	-18.284	-17.726
-24.322	-23.687	-23.683	-22.781	-22.790	-22.250	-22.266	-21.720	-21.614	-21.473	-20.451	-20.360	-20.290	-18.878	-18.676	-18.280	-17.415
-23.809	-23.706	-22.781	-23.153	-22.641	-22.479	-22.340	-21.670	-21.624	-20.657	-20.389	-19.720	-19.759	-19.413	-18.564	-18.403	-17.675
-23.791	-23.698	-23.470	-22.555	-22.390	-21.678	-21.621	-21.700	-20.766	-20.426	-20.368	-19.647	-19.694	-18.732	-18.762	-18.659	-17.330

u displacement																	
-17.633	-16.444	-15.560	-13.634	-13.643	-12.298	-11.355	-11.397	-9.539	-8.476	-8.423		-5.665	-4.301	-4.195	-2.723	-1.717	-0.343
-17.411	-16.708	-15.290	-14.670	-13.966	-13.460	-11.615	-10.436	-9.627	-9.325		-6.387	-5.689	-4.671	-3.285		-1.636	-1.417
-17.715	-16.396	-16.374	-14.377	-14.186	-12.366	-11.382	-10.589	-9.609	-8.431	-7.716	-7.177	-5.706	-5.169	-4.341	-3.625	-1.725	-1.578
-17.784	-16.396	-16.312	-14.616	-14.275	-12.628	-11.536	-10.351	-9.689	-9.159	-8.292	-7.268	-6.342	-4.873	-4.336	-4.352	-1.712	-1.676
-17.829	-16.640	-14.657	-14.404	-14.313	-12.349	-11.561	-10.346	-10.379	-9.567	-8.250	-7.313	-6.296	-5.273	-4.514	-4.328	-2.673	-2.481
-16.705	-16.282	-16.180	-14.756	-13.703	-12.304	-11.311	-10.738	-10.252	-9.639	-8.293	-7.295	-6.214	-5.780	-4.600	-4.280	-2.694	-2.375
-17.256	-16.681	-15.284	-14.666	-13.726	-12.559	-11.643	-11.560	-9.594	-9.551	-7.616	-7.556	-6.356	-6.281	-4.768	-3.705	-3.609	-2.390
-17.216	-16.427	-15.750	-14.345	-13.542	-13.364	-11.305	-11.344	-9.757	-9.679	-8.371	-7.597	-7.219	-6.392	-5.573	-4.320	-3.323	-2.404
-16.695	-16.548	-15.633	-14.576	-14.268	-12.284	-12.355	-11.676	-10.385	-10.377	-8.208	-7.845	-7.410	-6.434	-5.330	-4.383	-3.204	-3.220
-18.201	-15.699	-15.383	-13.729	-13.828	-14.343	-12.333	-11.595	-10.429	-10.262	-8.406	-8.254	-7.383	-6.302	-5.750	-4.392	-4.314	-3.684
-17.251	-16.248	-15.410	-15.318	-13.723	-12.858	-12.654	-11.382	-10.434	-9.650	-8.798	-8.641	-7.293	-7.221	-5.808	-5.242	-4.362	-4.145
-16.753	-16.626	-15.566	-14.285	-13.724	-12.451	-11.664	-11.441	-10.700	-9.645	-8.781	-8.354	-7.533	-7.266	-6.205	-6.273	-5.330	-4.310
-17.162	-16.472	-16.356	-14.327	-14.196	-13.402	-12.535	-11.760	-11.644	-9.622	-9.261	-8.682	-8.453	-7.588	-6.299	-6.323	-5.536	-4.241
-16.642	-16.230	-15.764	-15.450	-13.611	-13.599	-12.608	-12.243	-11.742	-10.612	-9.604	-9.322	-8.254	-7.743	-7.206	-5.698	-5.731	-4.360
-17.521	-16.260	-16.299	-14.615	-13.753	-13.669	-12.701	-11.825	-11.779	-10.292	-9.600	-8.626	-8.402	-7.247	-7.054	-6.284	-5.665	-5.290
-17.308	-16.350	-15.698	-14.674	-14.347	-13.566	-13.446	-12.442	-11.675	-10.596	-10.498	-9.674	-8.345	-7.738	-7.518	-6.564	-5.525	-4.366
-17.505	-16.856	-15.671	-15.291	-14.440	-13.275	-12.718	-12.686	-11.680	-11.699	-10.444	-10.302	-9.461	-8.515	-7.573	-6.673	-6.256	-5.292
-16.554	-15.704	-15.463	-15.317	-14.362	-14.251	-12.420	-12.401	-12.328	-11.233	-10.702	-9.741	-9.598	-8.323	-7.674	-6.495	-6.412	-5.773
-17.174	-16.254	-15.720	-15.523	-14.349	-14.281	-13.622	-12.431	-12.302	-11.721	-10.283	-9.799	-9.675	-8.456	-8.332	-7.682	-6.620	-6.266
-17.320	-17.265	-15.614	-15.248	-14.722	-14.148	-13.510	-12.582	-12.370	-11.705	-11.232	-9.764	-9.686	-8.557	-8.478	-7.670	-7.454	-6.267
-17.170	-16.734	-14.675	-14.758	-14.613	-14.391	-13.445	-12.587	-12.253	-11.685	-11.258	-10.313	-9.761	-9.468	-7.758	-8.308	-8.386	-7.305
-16.873	-16.673	-15.431	-15.334	-15.305	-13.801	-13.638	-13.568	-12.236	-12.253	-11.374	-10.687	-10.360	-10.424	-8.550	-8.419	-8.339	-6.639
-16.361	-16.368	-16.371	-15.613	-15.270	-14.275	-13.603	-13.167	-12.356	-11.682	-11.459	-10.804	-10.355	-10.414	-9.403	-9.306	-8.339	-7.586
-17.236	-16.357	-16.582	-15.348	-15.327	-14.260	-13.263	-12.896	-12.385	-11.646	-11.506	-11.265	-10.663	-10.271	-9.611	-9.634	-9.331	-8.259
-17.132	-17.211	-16.320	-15.725	-15.194	-14.373	-14.336	-13.249	-12.752	-12.309	-12.170	-11.719	-10.416	-10.453	-9.334	-9.295	-8.303	-8.144
-17.205	-16.693	-16.302	-15.678	-15.192	-14.699	-13.755	-13.427	-13.352	-12.360	-12.338	-11.737	-11.308	-10.365	-10.358	-10.338	-8.641	-8.422
-17.614	-16.368	-16.187	-15.701	-15.467	-14.384	-13.770	-14.342	-13.222	-12.663	-12.472	-12.142	-11.573	-10.463	-10.428	-9.111	-9.535	-8.893
-17.250	-16.665	-15.753	-15.589	-15.426	-14.630	-14.374	-13.610	-13.583	-13.221	-12.518	-11.623	-11.631	-11.194	-10.721	-10.436	-10.344	

v displacement																
	38.286	39.296	39.766	40.238	40.625	41.568	41.582	42.204	42.571	42.185	42.354	42.514	43.651	43.679	43.822	43.476
36.582	38.156	37.790	39.714	39.794	39.403	40.390	40.651	41.726	42.594	42.236	42.261	42.316	42.334	43.343	42.719	43.330
36.359	37.607	37.564	38.288	38.753	39.249	39.533	40.552	40.251	40.739	41.894	41.820	41.778	41.397	42.130	42.611	42.186
35.601	36.408	36.350	37.753	38.562	38.391	38.367	39.598	40.295	40.018	39.896	40.212	40.362	41.248	41.675	41.403	41.704
34.209	34.671	36.354	36.297	37.326	37.769	38.449	38.399	39.422	39.756	39.710	39.329	40.233	40.675	40.315	40.674	40.313
34.329		35.498	36.519	36.381	36.253	37.392	37.729	37.671	38.552	38.299	39.768	39.679	39.266	39.640	40.680	39.683
32.303	33.633	33.645	35.276	35.686	35.635	35.672	35.707	36.654	37.686	37.304	37.753	38.390	38.671	38.372	38.430	38.346
31.254	31.741	33.279	33.585	33.604	35.408	35.714	36.285	35.849	36.202	37.239	37.662	37.770	37.457	37.788	37.722	38.205
30.437	31.628	32.368	32.347	33.388	33.587	33.633	35.481	35.845	35.719	36.203	36.373	37.371	37.387	37.356	36.649	37.346
30.254	30.601	31.710	31.684	32.698	32.383	33.373	33.560	34.240	34.382	35.296	35.688	35.841	36.488	36.411	36.532	36.244
29.412	29.465	30.267	31.223	31.365	32.294	32.261	33.376	33.710	34.534	34.353	34.244	34.233	34.611	35.467	35.328	35.660
28.620	29.347	29.656	30.601	30.313	31.697	30.674	32.297	32.351	33.416	33.794	33.701	33.690	33.741	33.781	34.530	34.247
28.229	28.274	28.607	28.584	29.641	30.695	30.633	31.682	31.658	32.531	32.300	32.367	32.690	33.608	33.374	33.755	33.692
25.707	26.226	27.326	28.301	29.577	29.660	30.569	30.546	31.372	31.330	32.195	31.728	32.359	32.302	32.597	32.604	32.303
25.661	26.525	26.649	28.221	28.324	29.439	29.364	29.224	29.340	29.665	29.632	31.353	31.296	31.636	31.664	31.650	31.578
23.720	25.315	26.403	25.711	27.354	27.421	28.316	28.644	28.728	28.722	29.210	29.407	29.730	29.703	30.598	31.317	30.799
23.612	24.605	25.714	25.671	25.722	26.326	27.648	27.790	27.718	28.359	28.706	28.760	29.631	29.672	30.508	29.833	29.905
22.547	23.503	23.873	25.328	25.297	25.623	25.675	26.572	26.683	27.598	27.431	27.436	28.304	28.261	28.688	28.371	29.685
21.692	21.754	23.729	23.429	24.619	24.334	24.366	25.757	25.719	26.463	27.353	27.308	27.689	28.212	28.219	28.384	27.335
20.288	21.588	22.624	22.656	23.697	23.788	24.252	24.243	24.299	25.614	25.398	26.335	26.311	25.737	26.294	26.315	27.405
19.794	20.203	21.352	21.393	22.726	22.796	23.275	24.230	24.261	24.338	25.368	25.349	25.750	25.720	25.378	25.735	26.155
19.712	19.902	20.528	21.638	21.596	21.728	22.694	23.262	23.575	23.638	23.405	24.198	24.262	24.543	24.639	25.627	25.370
18.578	18.571	19.731	19.752	20.399	21.202	21.416	21.698	22.281	22.681	22.683	23.415	23.720	23.363	23.344	24.364	24.611
16.572	18.568	18.283	19.614	19.595	20.686	20.685	20.437	20.688	21.394	22.615	22.319	22.348	22.777	22.752	22.769	22.334
16.600	16.411	18.407	18.341	18.484	19.815	19.733	20.256	20.250	20.519	20.660	21.371	21.426	22.504	21.849	21.707	21.729
14.322	16.206	16.224	16.677	16.642	18.261	18.381	18.295	19.671	19.503	20.440	20.376	20.284	20.573	20.554	20.439	20.642
13.667	14.208	14.721	16.512	16.628	16.542	17.684	18.176	18.208	18.510	18.363	18.435	19.565	19.531	20.478	20.338	20.391
13.517	13.710	14.487	14.538	15.660	16.303	18.326	18.172	17.311	17.682	17.755	18.246	18.267	19.329	19.374	18.535	19.352

v displacement																	
43.730	43.814	43.754	43.857	43.792	43.697	42.644	42.649	42.275	42.016	41.876		40.327	39.864	39.790	39.365	38.368	37.811
42.794	43.270	42.788	42.714	42.332	42.694	42.366	41.743	41.699	41.455		39.728	39.806	39.345	38.778		37.558	36.773
42.322	42.316	42.287	41.660	41.720	41.576	41.303	41.239	40.385	40.216	40.365	39.425	39.742	38.639	37.697	37.568	36.360	36.405
41.703	41.655	42.167	40.650	41.617	40.715	40.411	40.304	40.260	39.448	39.688	38.606	38.066	37.396	37.702	36.332	35.602	35.557
41.726	40.651	40.704	40.280	39.685	39.763	39.663	39.772	39.761	38.385	38.238	37.437	37.841	37.346	36.464	36.233	35.321	34.377
39.403	39.666	39.709	39.322	39.608	39.651	38.687	39.388	38.221	38.222	37.858	36.612	36.211	36.261	35.446	34.362	34.569	34.206
38.656	38.628	38.732	39.299	39.667	38.539	38.223	37.780	37.574	37.564	36.360	36.280	35.851	35.732	34.770	34.368	33.689	32.243
38.570	37.678	37.736	37.760	37.648	37.498	37.336	37.333	36.459	36.325	35.744	35.738	34.748	33.660	33.739	33.613	32.598	31.754
37.403	37.450	37.516	37.259	36.498	37.581	36.443	36.372	35.615	36.205	35.691	34.417	34.653	33.675	33.357	32.434	32.665	32.508
36.289	36.287	35.478	36.359	36.332	36.355	36.385	36.302	35.566	34.324	34.175	34.287	32.577	32.255	32.226	31.707	30.295	30.203
35.343	35.605	35.300	35.267	35.815	34.743	35.244	35.305	34.246	33.809	33.421	33.342	31.436	31.376	30.423	30.751	29.776	29.775
34.513	34.627	34.435	34.291	33.770	34.309	33.523	33.256	33.310	33.650	32.673	32.239	31.585	31.323	30.316	30.266	29.394	28.317
33.398	33.720	33.664	33.788	33.677	32.577	32.641	32.278	32.126	32.316	32.605	31.464	30.501	30.421	29.806	29.552	28.319	27.760
33.352	32.316	32.265	32.692	32.339	32.392	31.444	31.615	31.729	30.734	31.622	31.402	30.381	30.325	29.308	28.284	27.772	27.605
31.576	32.245	31.832	31.289	31.654	31.675	30.725	31.618	31.544	29.804	29.780	29.497	29.663	28.627	28.646	27.718	27.666	26.643
30.560	30.187	30.256	30.738	30.355	30.395	29.521	29.727	29.684	29.454	28.553	28.394	28.285	27.595	27.469	26.635	25.705	24.343
29.791	30.676	29.608	29.470	29.726	29.445	29.449	29.393	28.338	28.273	28.345	27.656	27.347	27.220	26.307	25.427	25.658	24.618
28.672	28.490	28.607	28.598	28.311	28.343	27.751	27.791	28.204	27.278	27.364	26.356	26.377	26.357	25.575	24.626	24.340	22.179
27.334	28.234	28.285	28.232	27.715	27.721	27.707	27.747	28.215	26.301	26.300	25.680	25.670	24.353	24.281	24.283	23.388	22.281
27.368	26.784	27.688	26.693	26.623	27.560	26.392	26.487	25.750	26.192	25.361	24.466	24.436	24.465	23.723	22.412	22.625	21.689
25.369	25.373	27.397	26.687	26.523	26.325	25.522	25.531	24.292	24.316	24.650	23.688	23.780	23.503	22.280	21.764	21.621	21.314
25.354	25.422	25.363	25.437	25.379	24.331	24.295	24.291	24.305	24.305	23.392	22.640	22.383	22.384	21.543	21.729	20.353	19.412
23.775	23.777	24.302	23.739	24.532	24.239	23.683	23.366	24.235	23.711	23.430	22.649	22.281	21.584	21.418	20.682	19.816	19.600
23.366	23.681	23.374	23.376	23.384	22.327	23.369	22.618	21.798	22.248	22.406	21.345	20.747	20.311	20.322	19.698	19.407	18.300
21.388	22.549	22.297	22.281	22.596	22.211	21.801	21.413	21.339	21.700	20.314	20.236	20.293	19.584	19.259	19.314	18.271	17.709
20.585	21.474	21.671	21.665	20.682	20.686	20.338	20.624	20.727	20.347	20.262	20.245	19.316	18.323	17.762	18.159	16.524	16.350
20.341	20.236	20.280	20.320	20.485	20.350	20.283	19.583	19.313	18.626	18.520	19.658	17.786	17.782	17.604	16.586	16.077	14.474
19.301	19.319	19.581	19.529	19.428	19.364	19.609	18.438	18.332	18.560	18.441	17.607	18.271	17.306	16.553	15.638	16.332	
0	16	32	48	64			0										
							-16										
							-32										
							-48										
							-64										

du/da																	
0.06476875	0.06405	0.06998	0.04668	0.05786	0.05661	0.05636	0.05683	0.05014	0.05588	0.06166	0.063175	0.0526	0.058137	0.06254	0.06866	0.083669
0.05083125	0.05569	0.05821	0.05759	0.0635	0.05673	0.05161	0.04699	0.05188	0.05609	0.04894	0.062031	0.05451	0.066688	0.06914	0.06474	0.070431
0.0580125	0.04934	0.04118	0.04329	0.05042	0.05438	0.05263	0.04738	0.05239	0.06099	0.07201	0.077556	0.06621	0.054088	0.04759	0.04296	0.057844
0.0613	0.05371	0.04268	0.05569	0.05394	0.06261	0.05918	0.03915	0.05389	0.05119	0.05529	0.061506	0.05434	0.0564	0.05934	0.04881	0.055025
0.0582375	0.0572	0.04824	0.05696	0.05238	0.05405	0.05496	0.04036	0.05289	0.05149	0.06252	0.069219	0.05901	0.051913	0.04373	0.06063	0.067762
0.051926786	0.03818	0.04191	0.04969	0.04691	0.04548	0.0451	0.05437	0.06152	0.06204	0.05361	0.047887	0.05284	0.061269	0.06523	0.05065	0.047281
0.055425	0.03946	0.04935	0.04656	0.04146	0.05685	0.05897	0.05836	0.05321	0.04361	0.04835	0.046825	0.04982	0.051537	0.05126	0.06322	0.05775
0.0501	0.05571	0.05519	0.04653	0.04808	0.04309	0.04816	0.05164	0.04361	0.04041	0.04589	0.0468	0.05109	0.056275	0.05393	0.05881	0.058875
0.0450375	0.04822	0.04807	0.04941	0.04614	0.04136	0.03279	0.03819	0.04754	0.05504	0.0651	0.057513	0.04325	0.053312	0.04809	0.05161	0.05755
0.0360625	0.04363	0.05038	0.05889	0.04814	0.04094	0.04033	0.04584	0.04584	0.047	0.04636	0.050281	0.05247	0.03515	0.05205	0.05792	0.074075
0.04310625	0.04618	0.03836	0.04024	0.04951	0.05036	0.0506	0.04009	0.03077	0.03668	0.04784	0.056881	0.05469	0.048356	0.04793	0.05579	0.048369
0.04609375	0.04954	0.04605	0.0426	0.02509	0.03911	0.04164	0.03896	0.03589	0.03571	0.04672	0.055569	0.05027	0.050381	0.04979	0.05263	0.059356
0.02325625	0.03773	0.04088	0.03897	0.0388	0.04023	0.0413	0.04179	0.03463	0.04203	0.04952	0.05245	0.05476	0.043438	0.04381	0.03342	0.046063
0.03541875	0.0327	0.0343	0.0318	0.0421	0.03772	0.04652	0.04801	0.04857	0.05444	0.04337	0.036075	0.02814	0.043212	0.05436	0.05704	0.040775
0.02901875	0.02961	0.03089	0.04911	0.05389	0.05024	0.04155	0.02654	0.03248	0.03918	0.04971	0.050313	0.03686	0.026637	0.03287	0.03904	0.05505
0.0412	0.03898	0.0404	0.04279	0.03123	0.03348	0.0394	0.03326	0.03986	0.04307	0.04414	0.043388	0.04414	0.037912	0.03669	0.04258	0.043062
0.03073125	0.03702	0.03429	0.0351	0.04468	0.03567	0.04462	0.03617	0.0326	0.03901	0.03598	0.042794	0.04224	0.037075	0.03524	0.03821	0.040988
0.032325	0.03375	0.03153	0.03851	0.04197	0.04224	0.04381	0.02934	0.03383	0.03123	0.04043	0.045706	0.04157	0.045875	0.04881	0.04729	0.036894
0.02615	0.02746	0.03253	0.03457	0.04057	0.04088	0.03637	0.02793	0.03348	0.03151	0.02892	0.033562	0.04486	0.045231	0.04887	0.03999	0.030525
0.02938125	0.02976	0.02794	0.03935	0.03318	0.03584	0.03599	0.03076	0.03386	0.03069	0.03979	0.039394	0.04541	0.035912	0.01953	0.03303	0.036313
0.0373625	0.04144	0.03089	0.03062	0.02171	0.02575	0.02696	0.02226	0.03027	0.0344	0.03976	0.0456	0.03922	0.037388	0.02724	0.04414	0.052181
0.03234375	0.02919	0.02121	0.02326	0.02969	0.03399	0.03836	0.03469	0.02566	0.03279	0.03099	0.044306	0.04751	0.038619	0.03093	0.03174	0.033525
0.0190125	0.02526	0.01874	0.03796	0.04282	0.03646	0.03634	0.02012	0.02116	0.02658	0.02664	0.043763	0.04816	0.049306	0.04377	0.02146	0.020462
0.02071875	0.02927	0.02439	0.02638	0.036	0.03231	0.03031	0.0275	0.01953	0.02914	0.02602	0.027106	0.03358	0.032713	0.04432	0.03557	0.035138
0.01595625	0.0287	0.03639	0.03794	0.02726	0.02581	0.02776	0.02271	0.02921	0.02811	0.03815	0.035056	0.02573	0.027394	0.0213	0.02777	0.030088
0.0248125	0.02354	0.02103	0.01654	0.01801	0.01379	0.02423	0.02427	0.02351	0.03344	0.03145	0.036088	0.03967	0.028794	0.03151	0.02924	0.027356
0.01805625	0.01621	0.00972	0.02042	0.01777	0.02725	0.03072	0.03209	0.02917	0.01949	0.02473	0.023931	0.03236	0.028044	0.03238	0.03587	0.033594
0.02465625	0.032	0.02859	0.01549	0.02016	0.02099	0.02363	0.02815	0.01827	0.02539	0.02579	0.018175	0.03001	0.027475	0.03502	0.04048	0.031119

du/da													
0.06744	0.06381	0.060913	0.042263	0.056931	0.059125	0.054906	0.062406	0.058559	0.069425	0.070007	0.055825	0.059213	0.064963
0.0558	0.04888	0.0535	0.067619	0.073138	0.064113	0.047994	0.062723	0.067588	0.072407	0.064525	0.064525	0.063977	0.050981
0.05673	0.06405	0.074969	0.064875	0.068319	0.060269	0.059313	0.054481	0.056625	0.053337	0.054738	0.052931	0.059413	0.061238
0.05499	0.05983	0.072125	0.070431	0.071556	0.054906	0.048	0.047269	0.053656	0.065762	0.064419	0.048988	0.061131	0.056363
0.05793	0.05579	0.051544	0.067925	0.061694	0.042163	0.046256	0.051219	0.065125	0.065888	0.05945	0.04845	0.051194	0.046406
0.04706	0.06521	0.076187	0.065175	0.052925	0.039931	0.044594	0.055281	0.065125	0.061231	0.055631	0.047775	0.053375	0.054475
0.05672	0.06126	0.058681	0.051844	0.057894	0.050406	0.062894	0.062412	0.052944	0.04875	0.043569	0.058063	0.050438	0.055881
0.05894	0.05209	0.061694	0.051494	0.059938	0.055738	0.047081	0.0555	0.044738	0.048288	0.042506	0.05125	0.06165	0.063913
0.04266	0.06183	0.0553	0.048206	0.052338	0.03615	0.059956	0.061494	0.053012	0.054275	0.044794	0.056275	0.065394	0.053463
0.06698	0.02667	0.034288	0.036019	0.059662	0.062913	0.057419	0.054406	0.050625	0.055894	0.0454	0.058481	0.0503	0.0417
0.04991	0.05292	0.049825	0.055881	0.050338	0.053975	0.059025	0.044488	0.045569	0.039769	0.04625	0.051769	0.049006	0.047488
0.05249	0.0637	0.060237	0.048425	0.044113	0.0411	0.047263	0.050581	0.047656	0.037538	0.039	0.034313	0.033744	0.042419
0.05048	0.05187	0.053544	0.042469	0.042162	0.052819	0.054288	0.053369	0.045762	0.030475	0.043862	0.04295	0.044369	0.046606
0.04276	0.04634	0.051019	0.046356	0.031838	0.04275	0.047744	0.049875	0.051663	0.0443	0.039844	0.05185	0.044319	0.051506
0.05738	0.0483	0.050888	0.04145	0.0362	0.047975	0.048344	0.053606	0.052625	0.04555	0.040444	0.0377	0.040231	0.033144
0.04749	0.04324	0.035075	0.033531	0.040425	0.048194	0.048388	0.041956	0.047388	0.049181	0.04935	0.044044	0.042588	0.054606
0.04809	0.05246	0.049513	0.043325	0.038181	0.026188	0.034594	0.037525	0.036469	0.045944	0.047056	0.057163	0.051575	0.048519
0.02982	0.02504	0.0447	0.048587	0.036987	0.0383	0.028775	0.043412	0.04345	0.043275	0.046713	0.0526	0.05125	0.039762
0.03988	0.03323	0.033987	0.043194	0.03715	0.04025	0.046175	0.045519	0.04485	0.044612	0.032781	0.034856	0.043025	0.038075
0.04508	0.04454	0.033175	0.0409	0.039187	0.037663	0.033956	0.042338	0.045681	0.049013	0.041969	0.033725	0.033444	0.035025
0.04431	0.02968	0.017669	0.034438	0.040775	0.041275	0.032975	0.034644	0.039725	0.037069	0.049031	0.037581	0.024437	0.023113
0.02797	0.03669	0.031994	0.032494	0.039819	0.028112	0.036519	0.0414	0.033237	0.0292	0.036944	0.039663	0.037794	0.048631
0.01836	0.03304	0.042962	0.040994	0.04335	0.040206	0.036081	0.035144	0.0305	0.02275	0.028137	0.024675	0.032125	0.042
0.03017	0.03406	0.048287	0.04355	0.0453	0.038162	0.029775	0.025881	0.023906	0.022456	0.0299	0.026963	0.020631	0.0269
0.03351	0.04251	0.03325	0.036312	0.03755	0.0357	0.03295	0.022763	0.032887	0.034163	0.043363	0.037063	0.03365	0.035306
0.03151	0.03186	0.037956	0.037119	0.03095	0.031756	0.024381	0.027463	0.029444	0.031375	0.033325	0.023425	0.033506	0.035019
0.03101	0.0293	0.038444	0.027594	0.028325	0.024938	0.026719	0.032188	0.023869	0.033119	0.036044	0.045044	0.033925	0.025206
0.02953	0.02748	0.023231	0.031313	0.029413	0.022556	0.025631	0.031494	0.034388	0.030881	0.025144	0.020525	0.020825	0.017719

du/db																	
-0.00708125	-0.0178	-0.0356	-0.0112	-0.0221	-0.0167	-0.0033	-0.0299	-0.0052	-0.0161	-0.0126	-0.00587	-0.0141	-0.00853	-0.0153	-0.01106	-0.01916
-0.01498125	-0.0186	-0.0275	-0.017	-0.0196	-0.0203	-0.0131	-0.0192	-0.0045	-0.0132	-0.0107	-0.01994	-0.0111	-0.01422	-0.0135	-0.00041	-0.01269
-0.0158875	-0.0339	-0.0147	-0.0157	-0.0171	-0.0169	-0.0218	-0.0136	-0.0063	-0.0186	-0.0186	-0.02603	-0.0072	-0.01234	-0.001	0.00838	-0.00046
-0.0279	-0.0287	-0.0175	-0.0235	-0.0179	-0.0216	-0.0315	-0.0023	-0.0162	-0.0112	-0.0181	-0.0229	-0.0003	-0.00537	-0.0104	0.00806	0.000881
-0.032325	-0.0235	-0.0239	-0.022	-0.0187	-0.0223	-0.0303	-0.0071	-0.0207	-0.0132	-0.0066	-0.01064	-0.0076	-0.00543	-0.0079	0.00174	0.005412
-0.03285	-0.0104	-0.021	-0.0117	-0.0142	-0.0235	-0.0229	-0.018	-0.0205	-0.0124	-0.0078	-0.0057	-0.0053	-0.00431	-0.0096	-0.01128	0.001806
-0.0353	-0.0104	-0.0246	-0.0186	-0.0118	-0.0174	-0.0118	-0.0185	-0.0194	-0.006	-0.0057	-0.00414	0.00101	-0.00578	-0.0046	-0.01312	-0.00089
-0.03064375	-0.0231	-0.0204	-0.0172	-0.0286	-0.0118	-0.0091	-0.0185	-0.004	-0.0149	-0.0133	-0.00206	0.00574	-0.01051	-0.0013	-0.00702	-0.00091
-0.03875	-0.0285	-0.0196	-0.0244	-0.0241	-0.0188	-0.0131	-0.0113	-0.0089	-0.0188	-0.0185	-0.0067	0.00288	-0.01086	-0.001	-0.00234	-0.01179
-0.02625625	-0.028	-0.0223	-0.0271	-0.0162	-0.0159	-0.013	-0.0009	-0.0123	-0.008	-0.011	-0.00457	-0.0187	-0.01433	-0.004	0.00452	-0.00377
-0.02306875	-0.0305	-0.0218	-0.0203	-0.0088	-0.0121	-0.0126	-0.0219	-0.0167	-0.0092	-0.0123	-0.01504	-0.0219	-0.01631	-0.0119	-0.00372	0.000719
-0.0119625	-0.0264	-0.0185	-0.015	-0.0156	-0.0132	-0.0088	-0.0237	-0.024	-0.0049	-0.016	-0.01439	-0.0222	-0.0078	-0.0169	-0.00379	-0.00908
-0.004725	-0.0181	-0.0163	-0.0133	-0.0175	-0.02	-0.0094	-0.0189	-0.0185	-0.0087	-0.0143	-0.00804	-0.0124	-0.00095	-0.0047	-0.00593	-0.00544
-0.01415	-0.0189	-0.0134	-0.0166	-0.013	-0.0238	-0.0087	-0.0185	-0.011	-0.0199	-0.0068	-0.00669	-0.0029	0.001425	-0.0011	-0.01348	-0.01159
-0.01846875	-0.0202	-0.009	-0.0254	-0.0086	-0.0157	-0.0111	-0.0074	-0.0048	-0.0139	-0.0049	-0.00681	-0.0063	0.000425	0.01413	-0.00142	-0.01207
-0.02543125	-0.0197	-0.0171	-0.0213	-0.0105	-0.0089	-0.0099	-0.0179	-0.0059	-0.0159	-0.0034	-0.01253	-0.002	-0.00046	-0.0015	-0.00418	-0.00277
-0.02935625	-0.0208	-0.0255	-0.0123	-0.0388	-0.0166	-0.0182	-0.0185	-0.0104	-0.0073	-0.0022	-0.00946	-0.0071	-6.2E-06	-0.0104	-0.00746	-0.00224
-0.02534375	-0.0136	-0.0301	-0.0119	-0.0318	-0.0152	-0.0173	-0.0019	-0.0167	0.00035	-0.0083	-0.00865	-0.0024	-0.00559	-0.0058	-0.01158	-0.00256
-0.01741875	-0.0067	-0.0271	-0.0101	-0.0101	-0.0128	-0.0086	-0.0062	-0.0139	-0.0048	-0.0104	-0.00207	0.00118	-0.00671	-0.0015	-0.01341	0.000181
-0.0234125	-0.0077	-0.0141	-0.0205	0.00083	-0.0066	-0.0114	-0.0051	-0.0133	-0.0075	-0.0092	-0.00284	0.00328	-0.00828	0.01382	0.00509	0.003963
-0.01766875	-0.0167	-0.0078	-0.0102	0.00629	-0.0111	-0.0145	-0.0087	-0.0036	-0.0157	-0.0077	-0.00353	-0.0056	-0.0087	-0.001	0.01336	0.003869
-0.0195625	-0.0285	-0.0116	-0.02	-0.0148	-0.023	-0.0188	-0.0112	-0.0012	-0.0063	-0.0028	-0.00129	-0.0056	-0.00632	-0.0065	0.01176	0.004444
-0.02105	-0.0297	-0.0237	-0.0207	-0.0266	-0.0163	-0.0152	-0.004	0.00126	-0.0061	-0.0055	-0.01099	-0.0063	-0.00072	-0.0114	0.00693	0.002644
-0.01646875	-0.0235	-0.0204	-0.022	-0.0222	-0.0191	-0.0127	-0.0071	-0.0076	-0.011	-0.0055	-0.01347	-0.0051	-0.00314	-0.0038	0.00036	-0.00659

du/db																	
0.00478	0.0005	-0.0049	0.009287	0.010306	-0.00456	0.002081	-0.01367	0.010888	0.0126	-0.0016	0.017931	0.011969	0.013413	0.010556	0.027179	0.012425	0.0283438
-0.0081	-0.0038	0.000394	0.001244	-0.00249	-0.01456	-0.00268	0.002256	0.012625	0.011025	0.010556	0.0122	0.01025	0.014512	0.017519	0.012131	0.01915	0.0176188
-0.0125	0.00285	-0.01445	0.004487	-0.00933	0.000387	0.001856	0.014556	0.003331	0.017	-0.00124	0.004906	0.007325	0.019569	0.006988	0.00055	0.029688	0.0145188
-0.0107	0.00064	-0.00311	-0.00175	-0.01283	0.010513	-0.00238	0.02	-0.00406	0.0064	-0.00297	0.005631	0.011338	0.025288	0.01705	-0.00429	0.025988	0.0085312
-0.011	-0.0002	0.009512	-0.000419	-0.00157	0.005813	0.009887	0.020413	-0.00302	0.010375	-3.7E-05	0.008537	0.020206	0.018338	0.016281	0.000938	0.010569	0.0094188
0.01519	-0.0081	-0.00778	-0.0134	0.00495	0.023769	0.017225	0.011438	0.007156	0.01295	0.005112	0.013794	0.0212	0.007481	0.017888	0.005638	0.017719	0.02155
0.00609	-0.01	-0.00072	0.0043	0.00175	0.009856	0.019063	-0.00066	0.0147	0.004881	0.014994	0.017669	0.012738	0.011188	0.014106	0.019663	0.015606	0.0299375
-0.0023	0.00061	-0.00369	0.003887	-0.00113	-0.00783	0.006356	-0.00062	0.012094	-0.00497	0.008813	0.014438	0.003194	0.015844	0.010888	0.029781	0.032325	0.0296063
-0.0032	0.00484	0.010181	0.000362	-0.00155	0.00215	-0.00193	8.75E-05	0.017431	-0.01329	0.015506	0.011087	0.013975	0.02045	0.014956	0.036006	0.0355	0.016675
-0.02	0.00804	0.010675	0.015319	0.000244	-0.0059	0.002694	0.010463	0.023975	0.0042	0.017869	0.013606	0.018138	0.020306	0.021269	0.023081	0.02505	0.00905
0.00268	-0.0023	0.01235	-0.001506	-0.00033	0.017313	0.006487	0.01055	0.023325	0.014069	0.015169	0.005863	0.018369	0.003306	0.021831	0.009431	0.018794	0.014625
0.00918	-0.0048	0.001294	0.006663	0.005019	0.015606	0.023313	0.012919	0.013031	0.016075	0.023581	0.01615	0.009831	0.003769	0.021131	0.003394	0.003244	0.0072563
0.00845	0.00555	-0.00898	0.0072	0.00765	-0.00179	0.007525	0.012819	3.12E-05	0.025862	0.020375	0.02245	0.013169	0.011556	0.017875	0.009787	0.007713	0.013175
-0.0012	-0.0028	-0.00769	0.002563	0.013681	0.005687	-0.00224	0.007356	0.006706	0.016556	0.019	0.015713	0.023419	0.015175	0.009094	0.012394	0.012206	0.017675
-0.0091	-0.0041	-0.00871	0.015369	0.007544	0.011931	0.0051	0.007319	0.010619	0.021844	0.009813	0.015081	0.023744	0.018769	0.01695	0.017044	0.017481	0.0209938
-0.0019	0.00768	-0.00074	0.008625	0.004119	0.013562	0.00645	0.000156	0.012575	0.014	0.008169	-0.00202	0.0181	0.009869	0.016744	0.020131	0.026387	0.02985
0.0006	0.00823	-0.01151	-0.007094	0.004413	0.013306	0.0159	-0.00011	0.007425	0.002775	0.013488	0.000281	0.0043	0.013375	0.007337	0.027781	0.033138	0.02825
0.00396	0.01511	-0.00693	-0.004569	0.013438	-0.00494	0.014119	0.015562	-0.00146	0.012525	0.014494	0.015038	0.010063	0.032588	0.007362	0.027963	0.035125	0.0173188
-0.013	-0.0023	0.006994	0.001662	0.015156	-0.00224	0.000563	0.015363	-0.00016	0.002938	0.015588	0.018331	0.012712	0.036144	0.013837	0.024981	0.027019	0.018825
-0.0061	-0.0136	0.0227	0.006594	0.011669	0.000675	-0.0021	0.00755	0.000831	-0.00076	0.004681	0.021831	0.015925	0.027338	0.024444	0.030788	0.023169	0.0266563
0.00179	0.00399	0.027756	0.012175	0.0074	0.002644	0.008794	0.004075	0.007169	0.004006	0.012225	0.021188	0.010081	0.011356	0.026331	0.019931	0.005163	0.0206125
0.00897	0.00552	0.010569	0.005	-0.00189	0.011838	0.006044	-0.00125	0.016425	0.005256	0.016494	0.018844	0.012231	-0.00049	0.022169	0.023919	0.00355	0.025775
0.01547	0.0021	-0.00405	0.003163	0.001619	0.004106	0.005162	0.018006	0.016869	0.016725	0.017863	0.019675	0.019256	0.0012	0.017481	0.001963	0.010638	0.0173563
0.00319	-0.0014	-0.01119	0.002862	0.002944	0.004694	0.01035	0.015756	0.017912	0.0219	0.014538	0.007119	0.019331	0.0116	0.020713	0.008875	0.020363	0.013625

dv/db																	
0.04923125	0.05611	0.04577	0.05562	0.0441	0.04202	0.05163	0.04637	0.04372	0.05129	0.04556	0.050619	0.04073	0.043988	0.05248	0.04757	0.0497
0.0416	0.07284	0.03621	0.05238	0.05158	0.04863	0.04425	0.04998	0.05587	0.05667	0.06286	0.046731	0.04262	0.042863	0.05763	0.03759	0.057294
0.05865	0.06269	0.05431	0.04536	0.05197	0.05854	0.05436	0.07224	0.06136	0.04732	0.06736	0.053612	0.04662	0.046462	0.05969	0.05678	0.060631
0.06625	0.06483	0.05532	0.05848	0.07223	0.05063	0.05052	0.05824	0.07288	0.06064	0.04825	0.041725	0.04392	0.059912	0.06073	0.06004	0.056031
0.06636875	0.05188	0.06369	0.06771	0.06658	0.05756	0.07069	0.0455	0.0561	0.06515	0.05046	0.050113	0.04771	0.052406	0.04856	0.0688	0.046325
0.0626	0.05756	0.05533	0.07874	0.0604	0.06118	0.06298	0.05352	0.04794	0.06442	0.04442	0.059625	0.05434	0.04275	0.04671	0.06298	0.049237
0.0423875	0.05922	0.05203	0.06254	0.05968	0.06067	0.05727	0.04617	0.04686	0.05077	0.04903	0.0562	0.06402	0.056806	0.04492	0.04621	0.045831
0.03933125	0.04344	0.05842	0.04433	0.05378	0.05447	0.07158	0.06301	0.05707	0.04223	0.05463	0.062819	0.07061	0.0638	0.06189	0.04816	0.060012
0.0378125	0.04976	0.05985	0.05381	0.06174	0.04044	0.05437	0.05538	0.06414	0.04589	0.05818	0.062494	0.07196	0.064406	0.06621	0.04869	0.058156
0.06423125	0.06213	0.06518	0.05878	0.04979	0.04403	0.04522	0.04826	0.04868	0.05067	0.05159	0.061231	0.05317	0.058594	0.06076	0.05893	0.061562
0.06509375	0.05626	0.05979	0.0519	0.04261	0.04842	0.03687	0.06284	0.06074	0.0739	0.06901	0.048469	0.04503	0.046181	0.05494	0.05801	0.063175
0.0773	0.06133	0.0529	0.06339	0.04522	0.0613	0.03741	0.06103	0.05978	0.07659	0.07397	0.060013	0.05821	0.0628	0.05048	0.05332	0.056313
0.07013125	0.05156	0.04193	0.0526	0.06288	0.06861	0.05139	0.06054	0.06578	0.06845	0.06358	0.059594	0.05467	0.065444	0.04832	0.05707	0.056738
0.05230625	0.04604	0.04901	0.0531	0.06976	0.06992	0.0719	0.05864	0.06875	0.05481	0.06534	0.069856	0.06109	0.062788	0.05609	0.06427	0.043181
0.05694375	0.07096	0.05231	0.06229	0.05917	0.07505	0.07898	0.05629	0.05804	0.04705	0.03961	0.062881	0.054	0.051812	0.055	0.05924	0.06
0.0549	0.06441	0.05964	0.0522	0.05261	0.05786	0.07131	0.06772	0.06786	0.0507	0.05611	0.047475	0.05488	0.0587	0.06811	0.07158	0.058488
0.06184375	0.06699	0.06233	0.07017	0.04745	0.05559	0.06356	0.05906	0.05811	0.06266	0.05443	0.049519	0.06097	0.065175	0.07909	0.06407	0.061125
0.0473	0.05471	0.05667	0.05885	0.05809	0.0583	0.04408	0.05092	0.04796	0.06278	0.06273	0.052719	0.06264	0.06205	0.06837	0.05086	0.061312
0.042525	0.05033	0.06307	0.05232	0.06588	0.05202	0.04661	0.05687	0.0475	0.05963	0.07083	0.062019	0.06242	0.068075	0.07128	0.05455	0.046769
0.05405	0.04795	0.06439	0.04828	0.06582	0.04874	0.05621	0.0634	0.05751	0.06311	0.05157	0.062287	0.06223	0.051731	0.05699	0.05289	0.073038
0.05955	0.05574	0.05084	0.0508	0.06553	0.04378	0.05683	0.06733	0.06818	0.06176	0.06379	0.061469	0.06601	0.051238	0.05591	0.06821	0.0743
0.0797375	0.0597	0.06208	0.07083	0.07389	0.05201	0.06443	0.0711	0.06149	0.0652	0.04971	0.06055	0.06406	0.054994	0.06041	0.08146	0.077112
0.07545	0.0693	0.07549	0.05886	0.06559	0.07341	0.06105	0.05741	0.05727	0.06396	0.06759	0.074394	0.06484	0.061675	0.04956	0.06489	0.063325
0.05651875	0.07449	0.07049	0.07488	0.06079	0.07524	0.04229	0.04131	0.05498	0.05896	0.07511	0.069263	0.06264	0.061681	0.05079	0.06148	0.045638

dv/db																	
0.03187	0.04963	0.042006	0.057613	0.055806	0.061544	0.049481	0.044956	0.040419	0.057931	0.054498	0.048075	0.04195	0.043656	0.0483	0.052189	0.050313	0.050525
0.04611	0.05546	0.048381	0.051025	0.046769	0.049369	0.056238	0.038606	0.047375	0.051856	0.056069	0.051375	0.056819	0.046631	0.049356	0.060731	0.043856	0.0447625
0.0602	0.05853	0.0598	0.037813	0.038219	0.044613	0.049275	0.048962	0.047881	0.040813	0.0615	0.051775	0.060231	0.043431	0.050687	0.052313	0.039844	0.0604687
0.05835	0.06236	0.067713	0.042256	0.049725	0.047863	0.047438	0.049587	0.061181	0.044169	0.061038	0.043081	0.053912	0.056788	0.060125	0.045644	0.04775	0.060875
0.05924	0.05244	0.052181	0.047525	0.052087	0.040731	0.048694	0.055344	0.062837	0.039106	0.04505	0.043213	0.048994	0.062144	0.049506	0.052169	0.045519	0.0386875
0.04676	0.0496	0.060488	0.049787	0.060756	0.047188	0.0399	0.047375	0.045431	0.057219	0.050219	0.040706	0.052912	0.062931	0.049081	0.045275	0.059825	0.0483813
0.05567	0.04648	0.057012	0.059156	0.056375	0.054594	0.043181	0.037381	0.047181	0.059444	0.046544	0.045794	0.068756	0.063231	0.063794	0.057125	0.063306	0.0405437
0.06359	0.04967	0.055112	0.055813	0.052744	0.0576	0.055156	0.057631	0.047919	0.048413	0.052575	0.050456	0.059644	0.043581	0.061125	0.052356	0.058106	0.0600438
0.06116	0.057	0.054669	0.056312	0.051275	0.075338	0.065413	0.070213	0.057713	0.052825	0.047962	0.049713	0.0581	0.0465	0.056325	0.045031	0.059956	0.0711375
0.04887	0.06142	0.050388	0.055081	0.063275	0.063075	0.078031	0.077506	0.061213	0.054206	0.037012	0.0478	0.033294	0.030094	0.040331	0.050281	0.040644	0.0450688
0.05434	0.05644	0.056913	0.059719	0.060956	0.050331	0.069481	0.056344	0.043656	0.068288	0.052081	0.053294	0.029688	0.0406	0.028512	0.0503	0.036513	0.0436
0.0608	0.06472	0.063688	0.060031	0.055331	0.054563	0.062	0.048238	0.048963	0.06815	0.069156	0.060356	0.046487	0.057813	0.042838	0.05685	0.050194	0.0566563
0.06254	0.05136	0.063256	0.066187	0.061787	0.051631	0.051919	0.047863	0.060131	0.058537	0.072431	0.0664	0.052525	0.057075	0.055231	0.061869	0.046181	0.0596625
0.06966	0.05763	0.059625	0.062544	0.0624	0.06455	0.054137	0.061706	0.0641	0.052769	0.062194	0.074581	0.064525	0.058394	0.061281	0.060044	0.05545	0.0804813
0.06482	0.06074	0.054644	0.051588	0.062012	0.06225	0.048788	0.060488	0.050863	0.057387	0.050931	0.06045	0.061837	0.061162	0.0664	0.055494	0.062006	0.06805
0.05526	0.0578	0.040369	0.0583	0.059219	0.046212	0.05	0.050788	0.049944	0.0531	0.052681	0.06145	0.058594	0.057044	0.059488	0.059938	0.052688	0.0477812
0.06342	0.07695	0.033381	0.046694	0.050588	0.043894	0.057581	0.056425	0.065912	0.05625	0.058706	0.061413	0.056719	0.058288	0.061913	0.059625	0.061181	0.0443625
0.05376	0.05623	0.0461	0.049169	0.0441	0.058875	0.056856	0.0576	0.073256	0.049569	0.059963	0.0589	0.061738	0.054975	0.062906	0.051956	0.060881	0.0406313
0.05708	0.06423	0.064319	0.064012	0.047563	0.063706	0.063406	0.068487	0.058781	0.044169	0.048181	0.0493	0.055194	0.047619	0.049413	0.049281	0.05885	0.0477438
0.05999	0.04876	0.073269	0.059887	0.052931	0.07845	0.049281	0.061894	0.049756	0.053081	0.044563	0.045506	0.055481	0.063919	0.0479	0.040687	0.051506	0.053075
0.06219	0.04618	0.076181	0.067956	0.061556	0.06395	0.0523	0.061931	0.052581	0.045556	0.060363	0.051244	0.053812	0.061944	0.045394	0.043319	0.047788	0.0520125
0.07453	0.05702	0.058681	0.056263	0.070813	0.058237	0.061225	0.058044	0.062825	0.062044	0.0586	0.045019	0.050763	0.063263	0.060756	0.053175	0.057519	0.0500938
0.06031	0.05806	0.060919	0.053431	0.067475	0.058869	0.061444	0.05975	0.068219	0.075444	0.074775	0.044263	0.065131	0.05995	0.063675	0.060819	0.064756	0.0762625
0.05736	0.06898	0.060019	0.060344	0.062644	0.048669	0.056488	0.063688	0.055988	0.065313	0.060775	0.050338	0.046619	0.048825	0.057456	0.0678	0.05215	0.0802312

dv/da																
0.0395375	0.03513	0.03377	0.03101	0.03056	0.0283	0.01389	0.00953	0.00252	0.01556	0.02678	0.025631	0.01309	-0.00028	0.00111	0.00126	0.004913
0.0498875	0.02811	0.03056	0.01544	0.03195	0.04824	0.03522	0.02331	0.00529	-0.0028	0.01429	0.012144	0.01508	0.005669	-0.0004	0.00049	-0.00774
0.03418125	0.02796	0.03062	0.03318	0.02687	0.02311	0.03068	0.02612	0.02584	0.0075	0.00031	0.012088	0.01269	0.011912	0.00052	-0.00324	-0.00679
0.04541875	0.03861	0.0292	0.02184	0.02921	0.03239	0.02174	0.00518	0.00205	0.01829	0.02871	0.023094	0.01774	0.005869	0.00163	0.00924	-0.01027
0.049125	0.0448	0.03539	0.03329	0.03014	0.03092	0.02424	0.01343	0.00747	0.01476	0.01598	0.017325	0.00099	0.013125	0.01077	0.00249	-0.0068
0.035294643	0.01329	0.02201	0.02144	0.02535	0.03048	0.01648	0.02941	0.0327	0.01755	0.01363	0.011156	0.00889	0.001981	-0.0077	-0.01224	-0.0026
0.05255625	0.03778	0.02758	0.0053	0.01255	0.03178	0.03277	0.02964	0.02212	0.0191	0.01909	0.00835	-0.0021	-0.00035	0.00461	0.00554	0.012387
0.0409	0.04787	0.04183	0.04694	0.03354	0.01077	0.01854	0.0259	0.03314	0.01901	0.00558	0.000863	0.00709	0.016519	0.00393	-0.00312	-0.01077
0.04138125	0.03086	0.02356	0.04071	0.04255	0.04047	0.03361	0.01339	0.02316	0.02815	0.02075	0.003356	-0.0049	0.000138	0.00589	0.01149	-0.00038
0.03731875	0.02845	0.02516	0.02767	0.02663	0.03041	0.02917	0.0332	0.02818	0.02973	0.01894	0.014113	0.00531	-0.00353	-0.0031	-0.01291	-0.00363
0.0354	0.04223	0.03162	0.03251	0.03608	0.03706	0.03339	0.01487	0.00472	0.00021	0.01622	0.021263	0.02232	0.010356	0.00182	-0.00069	-0.00518
0.029	0.03348	0.01958	0.02346	0.02922	0.03197	0.04599	0.02657	0.01852	0.00341	8.7E-05	0.010931	0.01189	0.012563	0.01047	0.00119	6.25E-05
0.0195875	0.03673	0.03852	0.04493	0.03138	0.02936	0.02614	0.01257	0.01187	0.0159	0.02118	0.021625	0.01344	-0.00064	0.00209	-0.00096	0.002862
0.06134375	0.05699	0.04903	0.03426	0.02798	0.02589	0.02522	0.01992	0.01483	0.01318	0.00861	0.012437	0.00119	0.011287	0.00116	-0.00416	-0.00193
0.0438875	0.04689	0.04155	0.01904	0.01136	0.00267	0.00611	0.02844	0.035	0.03504	0.02717	0.006012	0.00361	-0.00129	0.0068	0.00644	-0.00201
0.0479	0.03227	0.0346	0.04268	0.02482	0.01884	0.01166	0.01255	0.01681	0.01551	0.0192	0.0293	0.02345	0.011969	-0.0099	-0.01709	-0.00266
0.0330375	0.02156	0.02827	0.03853	0.0341	0.02585	0.01678	0.0183	0.02642	0.02219	0.02822	0.018894	0.00443	-0.00228	0.00184	0.00201	-0.00658
0.04578125	0.0354	0.02437	0.01791	0.02326	0.03099	0.02836	0.01547	0.01925	0.01374	0.02087	0.014088	0.01795	0.011369	-0.0006	-0.00452	-0.01399
0.04705625	0.03781	0.01362	0.02752	0.02264	0.03507	0.04175	0.0296	0.02991	0.02396	0.01647	0.016763	-0.0033	-0.0165	-0.0064	0.00438	0.017156
0.0492875	0.03421	0.02742	0.02331	0.01037	0.02312	0.02289	0.03302	0.02966	0.00724	0.00746	-0.00036	0.01729	0.027331	0.01271	0.01328	-0.0069
0.0440875	0.041	0.03281	0.03889	0.02815	0.02544	0.02684	0.02091	0.02493	0.01966	0.00244	0.0025	0.00516	0.000469	-0.0023	0.01589	0.019325
0.0344	0.0295	0.02764	0.02716	0.03433	0.02938	0.01124	0.01064	0.01209	0.01667	0.01758	0.020219	0.02063	0.014706	0.00808	-0.00297	0.000894
0.03014375	0.03706	0.03012	0.03068	0.02662	0.02389	0.02198	0.02398	0.02257	0.01501	0.00794	0.009513	0.01739	0.013069	0.00173	-0.00599	-0.00761
0.044325	0.03467	0.03672	0.0171	0.01211	0.00887	0.03011	0.03557	0.02653	0.01562	0.00458	0.00815	-0.0002	0.00475	0.01534	0.01598	0.013075
0.0356125	0.04303	0.02579	0.03174	0.02483	0.01203	0.01323	0.0165	0.02003	0.0296	0.02194	0.006844	-0.0012	-0.0147	0.00676	0.0125	0.012581
0.03194375	0.0283	0.03686	0.03109	0.03807	0.02359	0.03329	0.03082	0.01312	0.0124	0.00266	0.002475	0.00364	0.0007	0.01241	0.0206	0.019575
0.0514125	0.04109	0.03723	0.0274	0.02996	0.02787	0.01058	0.00421	0.01649	0.02028	0.03329	0.029494	0.01537	0.009581	-0.003	-0.00169	-0.00127
0.0319625	0.03974	0.05902	0.06209	0.03232	0.01089	-0.0102	0.0037	0.01548	0.02379	0.02701	0.010531	0.0086	-0.00049	0.0041	0.01287	0.003963

dv/da													
0.00104	-0.0012	-0.01487	-0.022275	-0.02551	-0.02332	-0.01356	-0.01611	-0.03063	-0.03658	-0.03409	-0.0185	-0.02761	-0.03455
-0.0092	-0.01	-0.0054	-0.011925	-0.01386	-0.01966	-0.01736	-0.03266	-0.03446	-0.03213	-0.02069	-0.02069	-0.03549	-0.03977
-0.0116	-0.0128	-0.01283	-0.007869	-0.01879	-0.02274	-0.01812	-0.0228	-0.01298	-0.02361	-0.03826	-0.03599	-0.04897	-0.03628
-0.0074	-0.0152	-0.02154	-0.011863	-0.01953	-0.01678	-0.01439	-0.0248	-0.03269	-0.03579	-0.03239	-0.0307	-0.03745	-0.03611
-0.0278	-0.0175	-0.01624	-0.006488	0.001006	-0.01661	-0.02648	-0.03871	-0.02993	-0.01547	-0.02274	-0.02366	-0.03846	-0.04426
0.00041	-0.0008	-0.01072	-0.004931	-0.01898	-0.02077	-0.01765	-0.03697	-0.03519	-0.03481	-0.03234	-0.03291	-0.03239	-0.03117
0.01683	0.00473	-0.01111	-0.028013	-0.03091	-0.01624	-0.02464	-0.02634	-0.02956	-0.02608	-0.0233	-0.03066	-0.03555	-0.05037
-0.011	-0.0028	-0.00664	-0.007288	-0.01589	-0.02014	-0.0262	-0.02441	-0.02506	-0.03954	-0.03805	-0.03287	-0.02717	-0.03096
-0.0125	-0.0047	-0.0114	-0.011431	-0.01859	-0.02238	-0.01044	-0.02396	-0.0232	-0.03811	-0.03381	-0.03289	-0.03261	-0.01891
0.00099	0.00619	0.011312	-0.000381	-0.00991	-0.03051	-0.03999	-0.03388	-0.03759	-0.03585	-0.03706	-0.03444	-0.03195	-0.03772
0.00379	-0.0076	-0.00398	-0.003094	-0.0161	-0.01791	-0.03214	-0.02969	-0.03804	-0.04282	-0.04976	-0.03872	-0.02466	-0.02406
-0.0114	-0.0081	-0.01129	-0.014481	-0.01233	-0.00957	-0.00816	-0.01669	-0.03038	-0.03589	-0.03519	-0.03259	-0.03399	-0.04334
0.00391	-0.0142	-0.02036	-0.02535	-0.02126	-0.00648	-0.00021	-0.00718	-0.02564	-0.03684	-0.04151	-0.02824	-0.03271	-0.04256
-0.0103	0.00141	-0.01214	-0.019056	-0.01248	-0.01894	-0.00328	-0.00333	-0.01267	-0.01287	-0.03566	-0.04568	-0.04537	-0.0436
-0.005	-0.0082	-0.01142	-0.001694	-0.00173	-0.01827	-0.02315	-0.03754	-0.02543	-0.01544	-0.01961	-0.02859	-0.03064	-0.03093
0.00088	0.00322	-0.01133	-0.01785	-0.01256	-0.01074	-0.01381	-0.02373	-0.02411	-0.02491	-0.01854	-0.02709	-0.03825	-0.05167
-0.0083	-0.0147	-0.00214	-0.002694	-0.01767	-0.02159	-0.0208	-0.02167	-0.01624	-0.0194	-0.0282	-0.03436	-0.03232	-0.03658
-0.0038	-0.0037	-0.01229	-0.013588	-0.00479	-0.01048	-0.00804	-0.02319	-0.0286	-0.01768	-0.02236	-0.02664	-0.03628	-0.05994
0.00475	-0.01	-0.01042	-0.006113	0.006412	-0.01458	-0.02663	-0.03781	-0.03569	-0.02829	-0.03353	-0.02614	-0.02896	-0.03148
-0.0099	0.00304	-0.01078	-0.004019	-0.01762	-0.02111	-0.01473	-0.02769	-0.02721	-0.02737	-0.02048	-0.03013	-0.03547	-0.04156
0.02264	0.00644	-0.0257	-0.020706	-0.03285	-0.0328	-0.01849	-0.0208	-0.01033	-0.0156	-0.03078	-0.03343	-0.03786	-0.03148
0.00041	-0.0135	-0.02026	-0.0211	-0.01367	-0.00026	-0.0112	-0.02634	-0.03443	-0.03032	-0.02471	-0.01664	-0.02947	-0.04459
0.00923	0.00721	-0.00461	-0.009969	-0.00917	-0.00315	-0.00101	-0.01399	-0.03106	-0.03377	-0.03181	-0.02998	-0.03645	-0.03481
-0.0017	-0.0169	-0.00662	-0.009569	-0.01801	-0.01081	-0.01435	-0.01211	-0.01878	-0.03458	-0.03251	-0.02324	-0.02058	-0.03086
0.01343	-0.0024	-0.00664	-0.015819	-0.0207	-0.00927	-0.01679	-0.02112	-0.02223	-0.02658	-0.01726	-0.01799	-0.02696	-0.02961
0.00241	-0.016	-0.02278	-0.015162	0.000175	-0.00181	-0.00268	-0.00764	-0.01828	-0.03121	-0.04326	-0.03579	-0.03592	-0.0324
0.00233	0.00271	0.000225	-0.010475	-0.01944	-0.02761	-0.02802	-0.00402	-0.01264	-0.01514	-0.02318	-0.03954	-0.02884	-0.05089
0.0029	-0.0004	-0.00068	-0.012506	-0.01949	-0.01803	-0.01384	-0.00971	-0.00672	-0.01674	-0.02548	-0.03535	-0.03466	-0.02398

Exx	du/da+0.5*((du/da)^2+(dv/da)^2)															
6.76%	6.67%	7.30%	4.83%	6.00%	5.86%	5.80%	5.85%	5.14%	5.76%	6.39%	6.55%	5.41%	5.98%	6.45%	7.10%	8.72%
5.34%	5.76%	6.04%	5.94%	6.60%	5.95%	5.36%	4.84%	5.32%	5.77%	5.02%	6.40%	5.61%	6.89%	7.15%	6.68%	7.29%
6.03%	5.09%	4.25%	4.48%	5.21%	5.61%	5.45%	4.88%	5.41%	6.29%	7.46%	8.06%	6.85%	5.56%	4.87%	4.39%	5.95%
6.42%	5.59%	4.40%	5.75%	5.58%	6.51%	6.12%	3.99%	5.53%	5.27%	5.72%	6.37%	5.60%	5.80%	6.11%	5.00%	5.66%
6.11%	5.98%	5.00%	5.91%	5.42%	5.60%	5.68%	4.13%	5.43%	5.29%	6.46%	7.18%	6.08%	5.33%	4.47%	6.25%	7.01%
5.39%	3.90%	4.30%	5.12%	4.83%	4.70%	4.63%	5.63%	6.39%	6.41%	5.51%	4.91%	5.43%	6.31%	6.74%	5.20%	4.84%
5.83%	4.10%	5.09%	4.77%	4.24%	5.90%	6.12%	6.05%	5.49%	4.47%	4.97%	4.80%	5.11%	5.29%	5.26%	6.52%	5.95%
5.22%	5.84%	5.76%	4.87%	4.98%	4.41%	4.95%	5.33%	4.51%	4.14%	4.70%	4.79%	5.24%	5.80%	5.54%	6.05%	6.07%
4.69%	4.99%	4.95%	5.15%	4.81%	4.30%	3.39%	3.90%	4.89%	5.70%	6.74%	5.92%	4.42%	5.47%	4.93%	5.30%	5.92%
3.74%	4.50%	5.20%	6.10%	4.97%	4.22%	4.16%	4.74%	4.73%	4.85%	4.76%	5.16%	5.39%	3.58%	5.34%	5.97%	7.68%
4.47%	4.81%	3.96%	4.16%	5.14%	5.23%	5.24%	4.10%	3.13%	3.74%	4.91%	5.87%	5.64%	4.96%	4.91%	5.74%	4.96%
4.76%	5.13%	4.73%	4.38%	2.58%	4.04%	4.36%	4.01%	3.67%	3.63%	4.78%	5.72%	5.16%	5.17%	5.11%	5.40%	6.11%
2.37%	3.91%	4.25%	4.07%	4.00%	4.15%	4.25%	4.27%	3.53%	4.30%	5.10%	5.41%	5.63%	4.44%	4.48%	3.40%	4.71%
3.79%	3.49%	3.61%	3.29%	4.34%	3.88%	4.79%	4.94%	4.99%	5.60%	4.44%	3.68%	2.85%	4.42%	5.58%	5.87%	4.16%
3.04%	3.11%	3.22%	5.05%	5.54%	5.15%	4.24%	2.73%	3.36%	4.06%	5.13%	5.16%	3.75%	2.70%	3.34%	3.98%	5.66%
4.32%	4.03%	4.18%	4.46%	3.20%	3.42%	4.02%	3.39%	4.08%	4.41%	4.53%	4.48%	4.54%	3.87%	3.74%	4.36%	4.40%
3.17%	3.79%	3.53%	3.65%	4.63%	3.66%	4.58%	3.70%	3.35%	4.00%	3.70%	4.39%	4.31%	3.78%	3.59%	3.89%	4.18%
3.39%	3.49%	3.23%	3.94%	4.31%	4.36%	4.52%	2.99%	3.46%	3.18%	4.15%	4.69%	4.26%	4.70%	5.00%	4.84%	3.77%
2.76%	2.85%	3.31%	3.55%	4.16%	4.23%	3.79%	2.88%	3.45%	3.23%	2.95%	3.43%	4.59%	4.64%	5.01%	4.08%	3.11%
3.10%	3.08%	2.87%	4.04%	3.38%	3.68%	3.69%	3.18%	3.49%	3.12%	4.06%	4.02%	4.66%	3.69%	1.98%	3.37%	3.70%
3.90%	4.31%	3.19%	3.18%	2.23%	2.64%	2.77%	2.27%	3.10%	3.52%	4.06%	4.66%	4.00%	3.81%	2.76%	4.52%	5.37%
3.35%	3.01%	2.18%	2.39%	3.07%	3.50%	3.92%	3.53%	2.61%	3.35%	3.16%	4.55%	4.89%	3.95%	3.14%	3.22%	3.41%
1.96%	2.63%	1.94%	3.92%	4.41%	3.74%	3.72%	2.06%	2.16%	2.70%	2.70%	4.48%	4.95%	5.06%	4.47%	2.17%	2.07%
2.19%	3.03%	2.54%	2.69%	3.67%	3.29%	3.12%	2.85%	2.01%	2.97%	2.64%	2.75%	3.41%	3.33%	4.54%	3.63%	3.58%
1.67%	3.00%	3.74%	3.92%	2.79%	2.62%	2.82%	2.31%	2.98%	2.89%	3.91%	3.57%	2.61%	2.79%	2.15%	2.82%	3.06%
2.56%	2.42%	2.19%	1.72%	1.89%	1.42%	2.51%	2.50%	2.39%	3.41%	3.19%	3.67%	4.05%	2.92%	3.21%	2.99%	2.79%
1.95%	1.72%	1.05%	2.10%	1.84%	2.80%	3.12%	3.26%	2.97%	1.99%	2.56%	2.47%	3.30%	2.85%	3.29%	3.65%	3.42%
2.55%	3.33%	3.07%	1.75%	2.09%	2.13%	2.40%	2.86%	1.86%	2.60%	2.65%	1.84%	3.05%	2.79%	3.56%	4.14%	3.16%

Exx	du/da+0.5*((du/da)^2+(dv/da)^2)												
6.97%	6.58%	6.29%	4.34%	5.89%	6.11%	5.65%	6.45%	6.07%	7.25%	7.30%	5.76%	6.13%	6.77%
5.74%	5.01%	5.49%	7.00%	7.59%	6.64%	4.93%	6.52%	7.05%	7.55%	6.68%	6.68%	6.67%	5.31%
5.84%	6.62%	7.79%	6.70%	7.08%	6.23%	6.12%	5.62%	5.83%	5.50%	5.70%	5.50%	6.24%	6.38%
5.65%	6.17%	7.50%	7.30%	7.43%	5.66%	4.93%	4.87%	5.56%	6.86%	6.70%	5.07%	6.37%	5.86%
6.00%	5.75%	5.30%	7.03%	6.36%	4.32%	4.77%	5.33%	6.77%	6.82%	6.15%	4.99%	5.32%	4.85%
4.82%	6.73%	7.91%	6.73%	5.45%	4.09%	4.57%	5.75%	6.79%	6.37%	5.77%	4.95%	5.53%	5.64%
5.85%	6.32%	6.05%	5.36%	6.00%	5.18%	6.52%	6.47%	5.48%	5.03%	4.48%	6.02%	5.23%	5.87%
6.07%	5.34%	6.36%	5.28%	6.19%	5.75%	4.85%	5.73%	4.61%	5.02%	4.41%	5.31%	6.39%	6.64%
4.37%	6.38%	5.69%	4.94%	5.39%	3.71%	6.18%	6.37%	5.47%	5.65%	4.64%	5.84%	6.81%	5.51%
6.92%	2.70%	3.49%	3.67%	6.15%	6.54%	5.99%	5.65%	5.26%	5.81%	4.71%	6.08%	5.21%	4.33%
5.12%	5.43%	5.11%	5.74%	5.17%	5.56%	6.13%	4.59%	4.73%	4.15%	4.86%	5.39%	5.05%	4.89%
5.39%	6.58%	6.21%	4.97%	4.52%	4.20%	4.84%	5.20%	4.93%	3.89%	4.04%	3.54%	3.49%	4.43%
5.18%	5.33%	5.52%	4.37%	4.33%	5.42%	5.58%	5.48%	4.71%	3.16%	4.57%	4.43%	4.59%	4.86%
4.37%	4.74%	5.24%	4.76%	3.24%	4.38%	4.89%	5.11%	5.31%	4.54%	4.13%	5.42%	4.63%	5.38%
5.90%	4.95%	5.22%	4.23%	3.69%	4.93%	4.98%	5.57%	5.43%	4.67%	4.15%	3.88%	4.15%	3.42%
4.86%	4.42%	3.58%	3.43%	4.13%	4.94%	4.97%	4.31%	4.88%	5.07%	5.07%	4.54%	4.42%	5.74%
4.93%	5.39%	5.07%	4.43%	3.91%	2.68%	3.54%	3.85%	3.73%	4.72%	4.86%	5.94%	5.34%	5.04%
3.03%	2.54%	4.58%	4.99%	3.77%	3.91%	2.92%	4.46%	4.48%	4.44%	4.81%	5.43%	5.32%	4.23%
4.07%	3.38%	3.46%	4.41%	3.79%	4.12%	4.76%	4.73%	4.65%	4.60%	3.39%	3.58%	4.44%	3.93%
4.61%	4.55%	3.38%	4.17%	4.01%	3.86%	3.46%	4.36%	4.71%	5.06%	4.31%	3.47%	3.46%	3.65%
4.56%	3.01%	1.82%	3.52%	4.21%	4.27%	3.37%	3.55%	4.06%	3.79%	5.07%	3.88%	2.55%	2.39%
2.84%	3.75%	3.27%	3.32%	4.07%	2.85%	3.72%	4.26%	3.44%	3.01%	3.79%	4.06%	3.89%	5.08%
1.86%	3.36%	4.39%	4.19%	4.43%	4.10%	3.67%	3.59%	3.14%	2.36%	2.90%	2.54%	3.33%	4.35%
3.06%	3.48%	4.95%	4.45%	4.65%	3.89%	3.03%	2.63%	2.44%	2.33%	3.09%	2.76%	2.11%	2.77%
3.42%	4.34%	3.38%	3.71%	3.85%	3.64%	3.36%	2.32%	3.37%	3.51%	4.45%	3.79%	3.46%	3.64%
3.20%	3.25%	3.89%	3.79%	3.14%	3.23%	2.47%	2.79%	3.00%	3.24%	3.48%	2.43%	3.47%	3.62%
3.15%	2.97%	3.92%	2.80%	2.89%	2.56%	2.75%	3.27%	2.42%	3.38%	3.70%	4.68%	3.49%	2.68%
3.00%	2.79%	2.35%	3.19%	3.00%	2.30%	2.61%	3.20%	3.50%	3.15%	2.58%	2.14%	2.16%	1.82%
MAX	7.91%												
MIN	1.82%												
AVG	4.66%												
STDEV	1.29%												

Eyy	dv/db+0.5*((du/db)^2+(dv/db)^2)															
5.05%	5.78%	4.75%	5.72%	4.53%	4.30%	5.30%	4.79%	4.47%	5.27%	4.67%	5.19%	4.17%	4.50%	5.40%	4.88%	5.11%
4.26%	7.57%	3.72%	5.39%	5.31%	5.00%	4.53%	5.14%	5.74%	5.84%	6.49%	4.80%	4.36%	4.39%	5.94%	3.83%	5.90%
6.05%	6.52%	5.59%	4.65%	5.35%	6.04%	5.61%	7.49%	6.33%	4.86%	6.98%	5.54%	4.77%	4.76%	6.15%	5.84%	6.25%
6.88%	6.73%	5.70%	6.05%	7.50%	5.21%	5.23%	5.99%	7.57%	6.25%	4.96%	4.29%	4.49%	6.17%	6.26%	6.19%	5.76%
6.91%	5.35%	6.60%	7.02%	6.90%	5.95%	7.36%	4.66%	5.79%	6.74%	5.18%	5.14%	4.89%	5.38%	4.98%	7.12%	4.74%
6.51%	5.93%	5.71%	8.19%	6.23%	6.33%	6.52%	5.51%	4.93%	6.66%	4.54%	6.14%	5.58%	4.37%	4.79%	6.50%	5.05%
4.39%	6.10%	5.37%	6.47%	6.15%	6.27%	5.90%	4.74%	4.81%	5.21%	5.02%	5.78%	6.61%	5.84%	4.59%	4.74%	4.69%
4.06%	4.47%	6.03%	4.55%	5.56%	5.60%	7.42%	6.52%	5.87%	4.32%	5.62%	6.48%	7.31%	6.59%	6.38%	4.93%	6.18%
3.93%	5.14%	6.18%	5.56%	6.39%	4.14%	5.59%	5.70%	6.62%	4.71%	6.00%	6.45%	7.45%	6.65%	6.84%	4.99%	5.99%
6.66%	6.45%	6.75%	6.09%	5.12%	4.51%	4.63%	4.94%	4.99%	5.20%	5.30%	6.31%	5.48%	6.04%	6.26%	6.07%	6.35%
6.75%	5.83%	6.18%	5.35%	4.36%	4.97%	3.76%	6.51%	6.27%	7.67%	7.15%	4.98%	4.63%	4.74%	5.65%	5.97%	6.52%
8.04%	6.36%	5.45%	6.55%	4.64%	6.33%	3.81%	6.32%	6.18%	7.95%	7.68%	6.19%	6.02%	6.48%	5.19%	5.47%	5.79%
7.26%	5.30%	4.29%	5.41%	6.50%	7.12%	5.28%	6.25%	6.81%	7.08%	6.57%	6.14%	5.62%	6.76%	4.95%	5.87%	5.84%
5.38%	4.73%	5.03%	5.46%	7.23%	7.26%	7.45%	6.05%	7.12%	5.65%	6.75%	7.23%	6.30%	6.48%	5.77%	6.64%	4.42%
5.87%	7.37%	5.37%	6.46%	6.10%	7.80%	8.22%	5.79%	5.97%	4.83%	4.04%	6.49%	5.55%	5.32%	5.66%	6.10%	6.19%
5.67%	6.67%	6.16%	5.38%	5.40%	5.96%	7.39%	7.02%	7.02%	5.21%	5.77%	4.87%	5.64%	6.04%	7.04%	7.42%	6.02%
6.42%	6.95%	6.46%	7.27%	4.93%	5.73%	6.57%	6.10%	5.99%	6.47%	5.59%	5.08%	6.29%	6.73%	8.23%	6.62%	6.30%
4.87%	5.63%	5.87%	6.07%	6.03%	6.01%	4.52%	5.22%	4.93%	6.48%	6.47%	5.41%	6.46%	6.40%	7.07%	5.22%	6.32%
4.36%	5.16%	6.54%	5.37%	6.81%	5.35%	4.77%	5.85%	4.87%	6.14%	7.34%	6.39%	6.44%	7.04%	7.38%	5.61%	4.79%
5.58%	4.91%	6.66%	4.97%	6.80%	4.99%	5.79%	6.54%	5.93%	6.51%	5.29%	6.42%	6.42%	5.31%	5.87%	5.43%	7.57%
6.15%	5.74%	5.22%	5.21%	6.77%	4.48%	5.86%	6.96%	7.05%	6.38%	6.59%	6.34%	6.82%	5.26%	5.75%	7.06%	7.71%
8.31%	6.19%	6.41%	7.35%	7.67%	5.36%	6.67%	7.37%	6.34%	6.73%	5.09%	6.24%	6.61%	5.65%	6.23%	8.48%	8.01%
7.85%	7.21%	7.86%	6.08%	6.81%	7.62%	6.30%	5.91%	5.89%	6.60%	6.99%	7.72%	6.70%	6.36%	5.09%	6.70%	6.53%
5.83%	7.75%	7.32%	7.79%	6.29%	7.83%	4.33%	4.22%	5.65%	6.08%	7.79%	7.18%	6.46%	6.36%	5.21%	6.34%	4.67%
5.83%	7.75%	7.32%	7.79%	6.29%	7.83%	4.33%	4.22%	5.65%	6.08%	7.79%	7.18%	6.46%	6.36%	5.21%	6.34%	4.67%

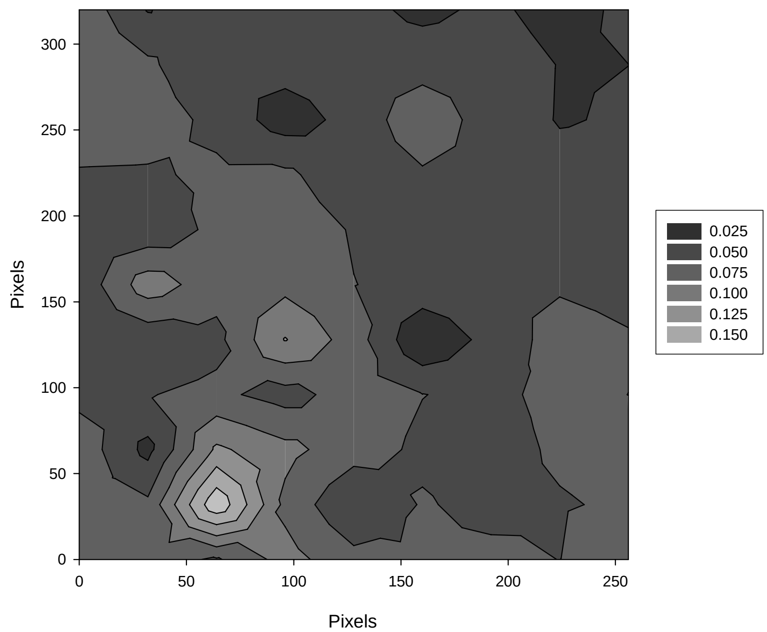
Eyy	dv/db+0.5*((du/db)^2+(dv/db)^2)																
3.24%	5.09%	4.29%	5.93%	5.74%	6.34%	5.07%	4.61%	4.13%	5.97%	5.60%	4.94%	4.29%	4.47%	4.95%	5.39%	5.17%	5.22%
4.72%	5.70%	4.96%	5.23%	4.79%	5.07%	5.78%	3.94%	4.86%	5.33%	5.77%	5.28%	5.85%	4.78%	5.07%	6.26%	4.50%	4.59%
6.21%	6.02%	6.17%	3.85%	3.90%	4.56%	5.05%	5.03%	4.90%	4.18%	6.34%	5.31%	6.21%	4.46%	5.20%	5.37%	4.11%	6.24%
6.01%	6.43%	7.00%	4.32%	5.10%	4.91%	4.86%	5.10%	6.31%	4.52%	6.29%	4.40%	5.54%	5.87%	6.21%	4.67%	4.92%	6.28%
6.11%	5.38%	5.36%	4.87%	5.34%	4.16%	4.99%	5.71%	6.48%	3.99%	4.61%	4.42%	5.04%	6.42%	5.09%	5.35%	4.66%	3.95%
4.80%	5.09%	6.23%	5.11%	6.26%	4.86%	4.08%	4.86%	4.65%	5.89%	5.15%	4.16%	5.45%	6.49%	5.04%	4.63%	6.18%	4.98%
5.72%	4.76%	5.86%	6.09%	5.80%	5.61%	4.43%	3.81%	4.84%	6.12%	4.77%	4.70%	7.12%	6.53%	6.59%	5.89%	6.54%	4.18%
6.56%	5.09%	5.66%	5.74%	5.41%	5.93%	5.67%	5.93%	4.91%	4.96%	5.40%	5.18%	6.14%	4.47%	6.31%	5.42%	6.03%	6.23%
6.30%	5.86%	5.62%	5.79%	5.26%	7.82%	6.76%	7.27%	5.95%	5.43%	4.92%	5.10%	5.99%	4.78%	5.80%	4.67%	6.24%	7.38%
5.03%	6.33%	5.17%	5.67%	6.53%	6.51%	8.11%	8.06%	6.34%	5.57%	3.79%	4.90%	3.40%	3.08%	4.14%	5.18%	4.18%	4.61%
5.58%	5.80%	5.86%	6.15%	6.28%	5.17%	7.19%	5.80%	4.49%	7.07%	5.36%	5.47%	3.03%	4.14%	2.92%	5.16%	3.74%	4.47%
6.27%	6.68%	6.57%	6.19%	5.69%	5.62%	6.42%	4.95%	5.02%	7.06%	7.18%	6.23%	4.76%	5.95%	4.40%	5.85%	5.15%	5.83%
6.45%	5.27%	6.53%	6.84%	6.37%	5.30%	5.33%	4.91%	6.19%	6.06%	7.53%	6.89%	5.40%	5.88%	5.69%	6.38%	4.73%	6.15%
7.21%	5.93%	6.14%	6.45%	6.44%	6.66%	5.56%	6.36%	6.62%	5.43%	6.43%	7.75%	6.69%	6.02%	6.32%	6.19%	5.71%	8.39%
6.70%	6.26%	5.62%	5.30%	6.40%	6.43%	5.00%	6.23%	5.22%	5.93%	5.23%	6.24%	6.40%	6.32%	6.87%	5.72%	6.41%	7.06%
5.68%	5.95%	4.12%	6.00%	6.10%	4.74%	5.13%	5.21%	5.13%	5.46%	5.41%	6.33%	6.05%	5.87%	6.14%	6.19%	5.44%	4.94%
6.54%	7.99%	3.40%	4.78%	5.19%	4.49%	5.94%	5.80%	6.81%	5.78%	6.05%	6.33%	5.83%	6.01%	6.39%	6.18%	6.36%	4.57%
5.52%	5.79%	4.72%	5.04%	4.52%	6.06%	5.86%	5.94%	7.59%	5.09%	6.19%	6.07%	6.37%	5.70%	6.49%	5.37%	6.34%	4.16%
5.88%	6.63%	6.64%	6.61%	4.88%	6.57%	6.54%	7.10%	6.05%	4.51%	4.95%	5.07%	5.68%	4.94%	5.07%	5.08%	6.09%	4.91%
6.18%	5.00%	7.62%	6.17%	5.44%	8.15%	5.05%	6.38%	5.10%	5.45%	4.56%	4.68%	5.71%	6.63%	4.93%	4.20%	5.31%	5.48%
6.41%	4.73%	7.95%	7.03%	6.35%	6.60%	5.37%	6.39%	5.40%	4.66%	6.23%	5.28%	5.53%	6.39%	4.68%	4.45%	4.89%	5.36%
7.73%	5.87%	6.05%	5.79%	7.33%	6.00%	6.31%	5.97%	6.49%	6.40%	6.05%	4.62%	5.21%	6.53%	6.28%	5.49%	5.92%	5.17%
6.22%	5.97%	6.28%	5.49%	6.98%	6.06%	6.33%	6.17%	7.07%	7.84%	7.77%	4.54%	6.74%	6.17%	6.59%	6.27%	6.69%	7.93%
5.90%	7.14%	6.19%	6.22%	6.46%	4.99%	5.81%	6.58%	5.77%	6.77%	6.27%	5.16%	4.79%	5.01%	5.93%	7.01%	5.37%	8.35%
5.90%	7.14%	6.19%	6.22%	6.46%	4.99%	5.81%	6.58%	5.77%	6.77%	6.27%	5.16%	4.79%	5.01%	5.93%	7.01%	5.37%	8.35%
MAX	8.48%																
MIN	2.92%																
AVG	5.80%																
STDEV	0.96%																

Exy	0.5*(du/db+dv/da+(du/da*du/db+dv/da*dv/db))															
1.70%	0.91%	-0.14%	1.05%	0.43%	0.59%	0.56%	-1.08%	-0.14%	-0.03%	0.73%	1.03%	-0.06%	-0.47%	-0.75%	-0.52%	-0.78%
1.81%	0.52%	0.13%	-0.09%	0.64%	1.45%	1.15%	0.22%	0.04%	-0.84%	0.20%	-0.42%	0.20%	-0.46%	-0.74%	0.00%	-1.09%
0.97%	-0.29%	0.85%	0.92%	0.51%	0.33%	0.47%	0.69%	1.04%	-0.59%	-0.98%	-0.77%	0.28%	-0.03%	-0.02%	0.27%	-0.38%
0.94%	0.54%	0.63%	-0.08%	0.62%	0.55%	-0.53%	0.15%	-0.75%	0.38%	0.55%	-0.01%	0.91%	0.03%	-0.46%	0.91%	-0.50%
0.91%	1.11%	0.63%	0.61%	0.62%	0.46%	-0.30%	0.33%	-0.69%	0.09%	0.49%	0.34%	-0.35%	0.41%	0.15%	0.23%	-0.07%
0.15%	0.16%	0.07%	0.54%	0.60%	0.39%	-0.32%	0.60%	0.63%	0.28%	0.30%	0.29%	0.19%	-0.13%	-0.91%	-1.24%	-0.04%
0.88%	1.46%	0.16%	-0.69%	0.05%	0.77%	1.11%	0.57%	0.14%	0.69%	0.70%	0.22%	-0.06%	-0.32%	0.00%	-0.41%	0.60%
0.52%	1.28%	1.14%	1.55%	0.27%	-0.05%	0.52%	0.41%	1.54%	0.22%	-0.40%	-0.06%	0.68%	0.32%	0.14%	-0.54%	-0.62%
0.12%	0.12%	0.22%	0.86%	1.00%	1.13%	1.10%	0.12%	0.76%	0.48%	0.11%	-0.18%	-0.11%	-0.56%	0.26%	0.48%	-0.64%
0.63%	0.05%	0.17%	0.03%	0.55%	0.76%	0.85%	1.69%	0.84%	1.14%	0.42%	0.51%	-0.70%	-0.93%	-0.37%	-0.44%	-0.40%
0.68%	0.63%	0.54%	0.65%	1.42%	1.31%	1.07%	-0.35%	-0.61%	-0.47%	0.22%	0.32%	0.01%	-0.31%	-0.53%	-0.23%	-0.24%
0.94%	0.39%	0.07%	0.46%	0.73%	1.01%	1.93%	0.18%	-0.26%	-0.07%	-0.83%	-0.18%	-0.53%	0.26%	-0.33%	-0.14%	-0.48%
0.81%	0.99%	1.16%	1.68%	0.76%	0.53%	0.88%	-0.32%	-0.32%	0.39%	0.38%	0.72%	0.05%	-0.08%	-0.13%	-0.36%	-0.13%
2.50%	2.01%	1.88%	0.95%	0.82%	0.15%	0.90%	0.09%	0.22%	-0.36%	0.10%	0.32%	-0.09%	0.67%	0.00%	-0.93%	-0.70%
1.37%	1.47%	1.72%	-0.32%	0.15%	-0.68%	-0.25%	1.12%	1.61%	1.11%	1.16%	-0.04%	-0.13%	-0.05%	1.09%	0.27%	-0.74%
1.20%	0.70%	0.94%	1.13%	0.77%	0.53%	0.11%	-0.25%	0.59%	-0.02%	0.84%	0.88%	1.13%	0.61%	-0.61%	-1.13%	-0.29%
0.24%	0.07%	0.18%	1.43%	-0.24%	0.50%	-0.06%	0.01%	0.86%	0.80%	1.38%	0.50%	-0.13%	-0.12%	-0.44%	-0.28%	-0.47%
1.09%	1.16%	-0.26%	0.33%	-0.43%	0.85%	0.58%	0.71%	0.15%	0.75%	0.68%	0.29%	0.83%	0.31%	-0.34%	-0.84%	-0.88%
1.56%	1.64%	-0.67%	0.93%	0.68%	1.18%	1.74%	1.25%	0.85%	1.02%	0.34%	0.78%	-0.12%	-1.23%	-0.42%	-0.47%	0.91%
1.39%	1.39%	0.74%	0.15%	0.60%	0.87%	0.62%	1.49%	0.88%	0.00%	-0.09%	-0.17%	1.09%	1.01%	1.38%	0.96%	-0.16%
1.42%	1.29%	1.32%	1.52%	1.82%	0.76%	0.68%	0.67%	1.15%	0.23%	-0.27%	-0.05%	-0.01%	-0.43%	-0.18%	1.55%	1.24%
0.85%	0.10%	0.87%	0.43%	1.08%	0.36%	-0.38%	-0.01%	0.58%	0.56%	0.78%	1.00%	0.81%	0.45%	0.09%	0.45%	0.28%
0.55%	0.46%	0.41%	0.55%	0.03%	0.44%	0.38%	1.06%	1.26%	0.49%	0.14%	-0.06%	0.60%	0.66%	-0.51%	0.03%	-0.27%
1.50%	0.65%	0.92%	-0.21%	-0.51%	-0.51%	0.92%	1.49%	1.01%	0.26%	-0.04%	-0.26%	-0.28%	0.09%	0.61%	0.87%	0.34%
1.50%	0.65%	0.92%	-0.21%	-0.51%	-0.51%	0.92%	1.49%	1.01%	0.26%	-0.04%	-0.26%	-0.28%	0.09%	0.61%	0.87%	0.34%

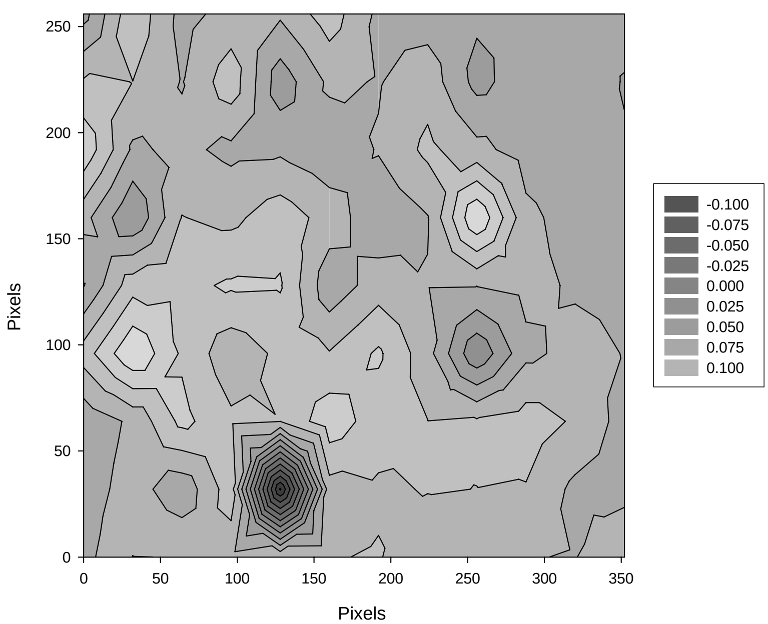
Exy	0.5*(du/db+dv/da+(du/da*du/db+dv/da*dv/db))												
0.31%	-0.04%	-1.03%	-0.69%	-0.80%	-1.48%	-0.60%	-1.57%	-1.02%	-1.26%	-1.88%	-0.02%	-0.80%	-1.09%
-0.91%	-0.73%	-0.26%	-0.56%	-0.86%	-1.81%	-1.06%	-1.58%	-1.13%	-1.10%	-0.53%	-0.44%	-1.33%	-1.32%
-1.28%	-0.53%	-1.46%	-0.17%	-1.47%	-1.17%	-0.85%	-0.43%	-0.50%	-0.33%	-2.10%	-1.63%	-2.21%	-0.85%
-0.95%	-0.77%	-1.32%	-0.71%	-1.71%	-0.32%	-0.88%	-0.25%	-1.95%	-1.53%	-1.88%	-1.31%	-1.37%	-0.57%
-2.05%	-0.93%	-0.35%	-0.36%	-0.03%	-0.56%	-0.87%	-0.97%	-1.75%	-0.25%	-1.19%	-0.79%	-0.95%	-1.39%
0.82%	-0.48%	-0.99%	-0.97%	-0.75%	0.15%	-0.02%	-1.33%	-1.46%	-1.15%	-1.43%	-0.99%	-0.59%	-1.26%
1.21%	-0.28%	-0.63%	-1.26%	-1.54%	-0.34%	-0.27%	-1.40%	-0.77%	-1.13%	-0.44%	-0.67%	-1.23%	-2.09%
-0.71%	-0.11%	-0.55%	-0.18%	-0.90%	-1.48%	-1.05%	-1.32%	-0.68%	-2.33%	-1.54%	-0.97%	-1.27%	-0.77%
-0.83%	0.01%	-0.06%	-0.58%	-1.06%	-1.09%	-0.66%	-1.28%	-0.31%	-2.71%	-0.96%	-1.14%	-0.98%	0.09%
-1.02%	0.74%	1.15%	0.77%	-0.51%	-1.94%	-2.01%	-1.27%	-0.74%	-1.67%	-0.99%	-1.08%	-0.70%	-0.89%
0.34%	-0.52%	0.44%	-0.24%	-0.87%	-0.03%	-1.38%	-1.02%	-0.77%	-1.56%	-1.82%	-1.73%	-0.31%	-1.08%
-0.12%	-0.69%	-0.53%	-0.42%	-0.39%	0.31%	0.79%	-0.20%	-0.91%	-1.08%	-0.66%	-0.89%	-1.27%	-2.10%
0.65%	-0.45%	-1.55%	-0.98%	-0.73%	-0.44%	0.39%	0.30%	-1.36%	-0.62%	-1.16%	-0.34%	-1.03%	-1.64%
-0.61%	-0.07%	-1.05%	-0.88%	0.04%	-0.71%	-0.29%	0.21%	-0.32%	0.19%	-0.91%	-1.63%	-1.19%	-1.51%
-0.74%	-0.65%	-1.06%	0.71%	0.30%	-0.35%	-0.95%	-1.60%	-0.78%	0.33%	-0.52%	-0.73%	-0.39%	-0.67%
-0.05%	0.57%	-0.63%	-0.50%	-0.45%	0.15%	-0.39%	-1.24%	-0.61%	-0.58%	-0.55%	-1.54%	-1.08%	-2.21%
-0.41%	-0.36%	-0.71%	-0.51%	-0.70%	-0.44%	-0.28%	-1.15%	-0.48%	-0.88%	-0.79%	-1.81%	-1.48%	-1.23%
0.00%	0.58%	-1.01%	-0.95%	0.45%	-0.81%	0.30%	-0.41%	-1.61%	-0.27%	-0.43%	-0.62%	-1.40%	-1.47%
-0.42%	-0.65%	-0.19%	-0.24%	1.12%	-0.89%	-1.39%	-1.22%	-1.90%	-1.32%	-0.95%	-0.42%	-0.87%	0.23%
-0.84%	-0.55%	0.59%	0.13%	-0.32%	-1.10%	-0.88%	-1.08%	-1.38%	-1.48%	-0.83%	-0.45%	-1.05%	-0.80%
1.30%	0.54%	0.03%	-0.48%	-1.36%	-1.61%	-0.52%	-0.89%	-0.17%	-0.61%	-0.99%	-0.66%	-1.48%	-1.09%
0.48%	-0.43%	-0.53%	-0.86%	-0.83%	0.59%	-0.28%	-1.46%	-0.98%	-1.34%	-0.45%	0.11%	-0.91%	-2.40%
1.28%	0.49%	-0.46%	-0.36%	-0.40%	0.05%	0.21%	0.19%	-0.79%	-0.96%	-0.79%	-0.56%	-0.95%	-1.78%
0.08%	-0.97%	-0.94%	-0.36%	-0.80%	-0.32%	-0.23%	0.16%	-0.07%	-0.72%	-0.98%	-0.86%	-0.09%	-1.02%
0.08%	-0.97%	-0.00938	-0.00358	-0.00803	-0.00323	-0.00225	0.00164	-0.00075	-0.00722	-0.00976	-0.00855	-0.00091	-0.01023
MAX	2.50%												
MIN	-2.71%												
AVG	-0.13%												
STDEV	0.86%												

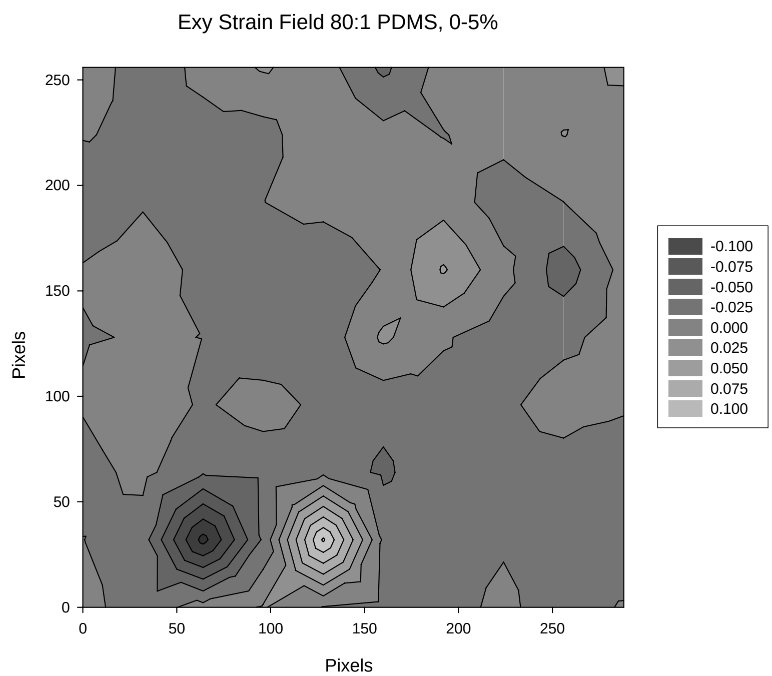
Results for 80:1 PDMS

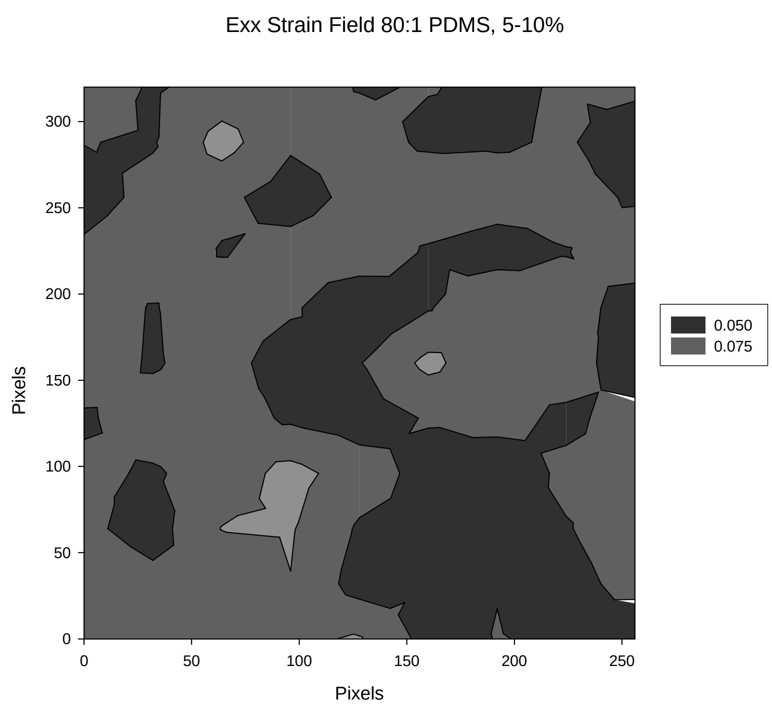
Exx Strain Field 80:1 PDMS, 0-5%

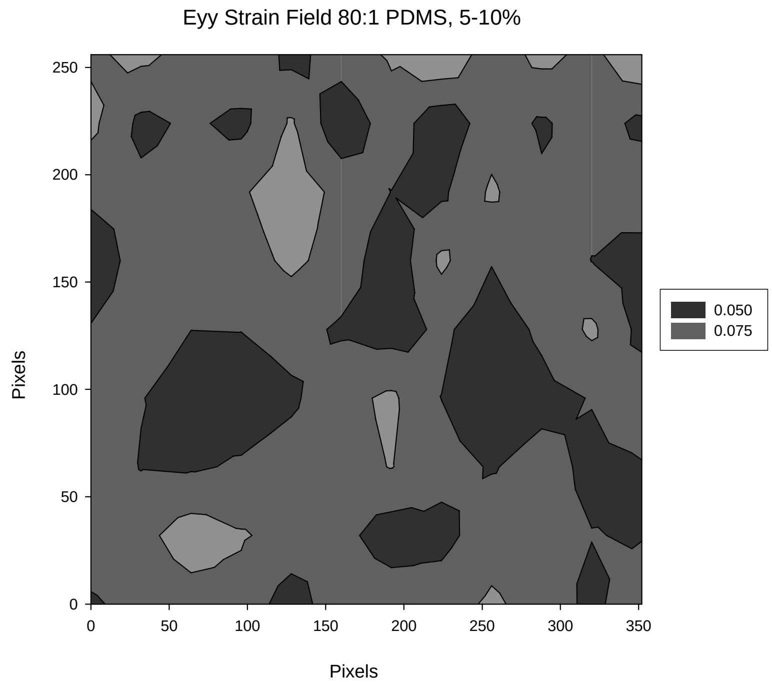


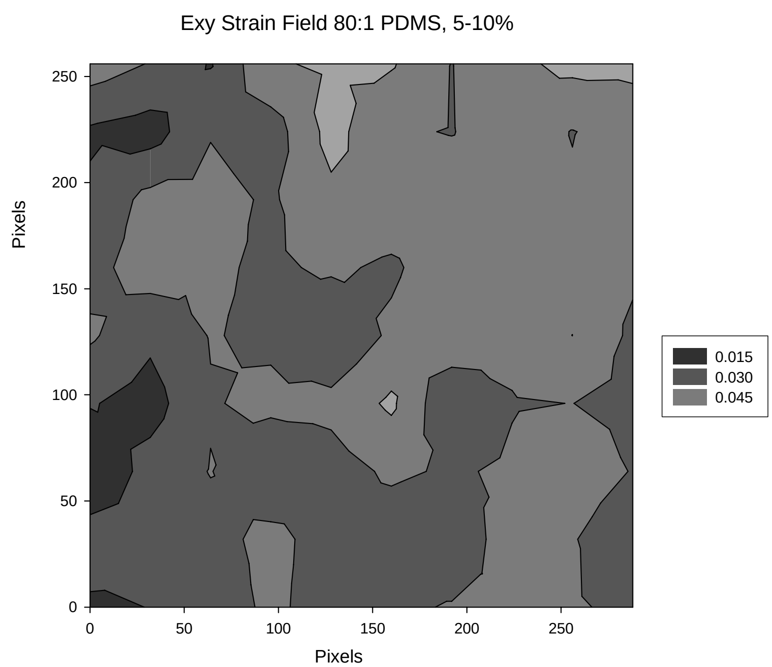
Eyy Strain Field 80:1 PDMS, 0-5%



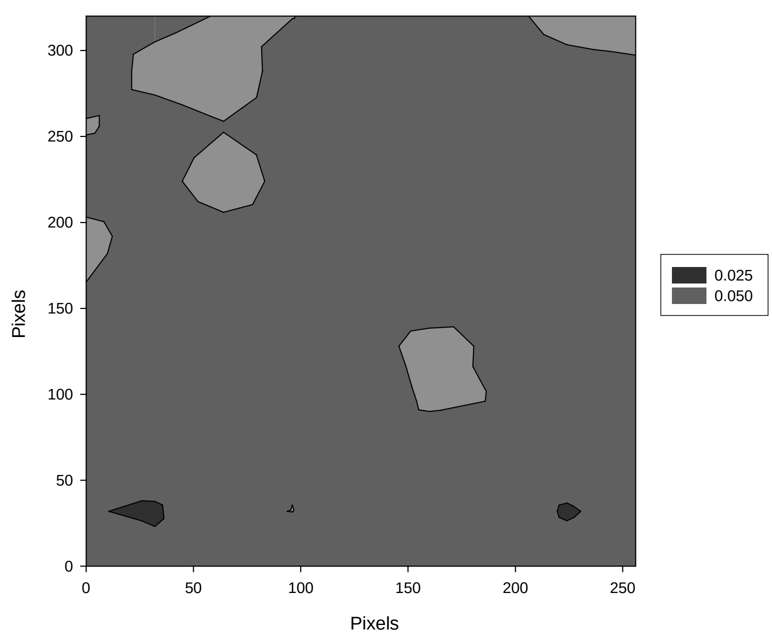

Exy Strain Field 80:1 PDMS, 0-5%
Pixels
Pixels
0
50
100
150
200
250
-0.100
-0.075
-0.050
-0.025
0.000
0.025
0.050
0.075
0.100


Exx Strain Field 80:1 PDMS, 5-10%
Pixels
Pixels
0
50
100
150
200
250
300
0.050
0.075

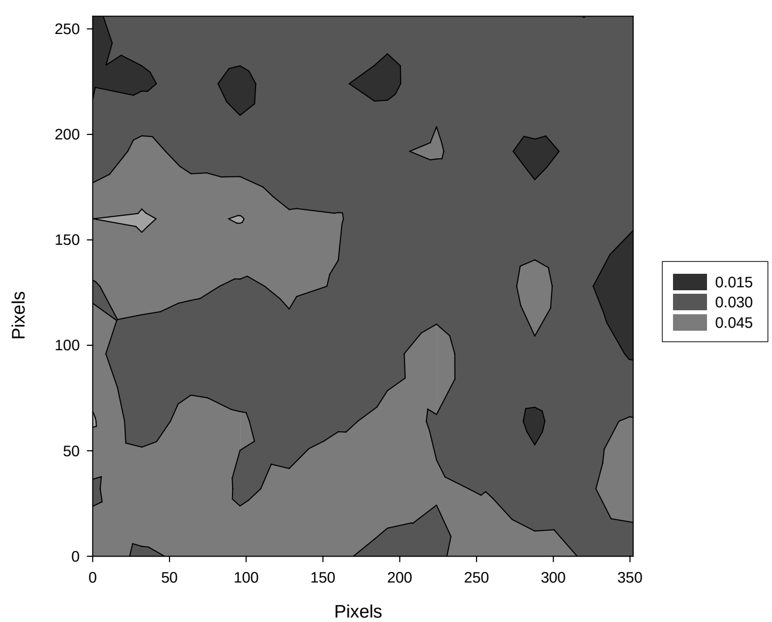

Eyy Strain Field 80:1 PDMS, 5-10%
Pixels
Pixels
0
50
100
150
200
250
0
50
100
150
200
250
300
350
0.050
0.075

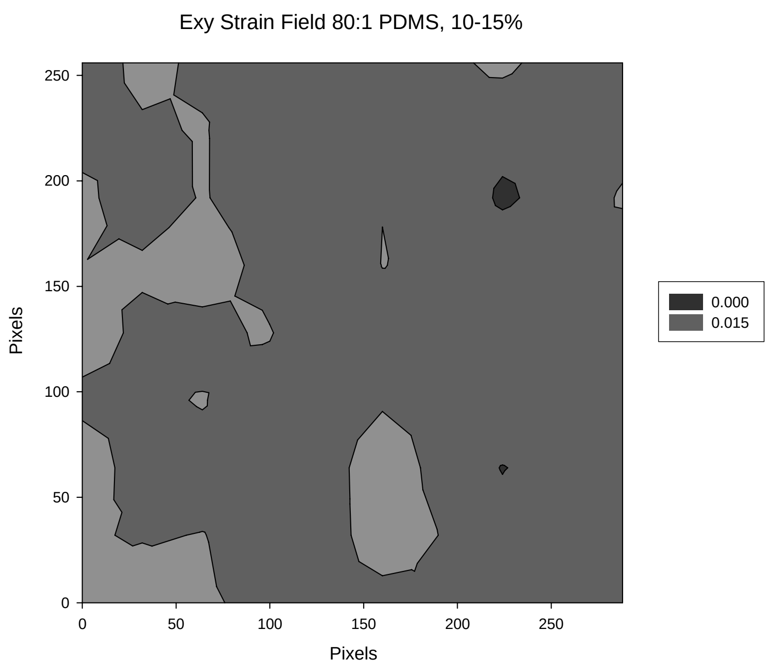

Exy Strain Field 80:1 PDMS, 5-10%
Pixels
Pixels
0
50
100
150
200
250
0
50
100
150
200
250
0.015
0.030
0.045

Exx Strain Field 80:1 PDMS, 10-15%



Eyy Strain Field 80:1 PDMS, 10-15%




Exy Strain Field 80:1 PDMS, 10-15%
Pixels
Pixels
0
50
100
150
200
250
0
50
100
150
200
250
0.000
0.015

0 to 5.26											
u displacement											
-9.766	-7.546	-5.666		-2.736	-0.13	0.859	3.179	4.292	6.245	8.689	9.611
-8.41	-7.281	-4.711	-3.84	3.083	0.347	2.639	3.565	5.269	6.542	8.543	10.264
-7.751	-5.702		-4.589	-1.421	0.467	2.313	3.275	4.842	6.6	8.458	9.617
-6.373	-3.803	-3.741	-0.792	-0.288	1.687	3.58	4.776	6.323	8.243	9.433	10.292
	-3.288	-2.305	-1.279	0.372	4.792	4.198	4.935	6.335	8.645	9.559	9.76
-4.311	-5.811	-2.343	-0.377	1.372	3.768	4.431	6.399	7.235	9.332	9.59	10.338
-3.58	-1.744	-0.81	0.218	2.699	4.236	5.699	6.679	7.697	9.639	10.376	11.141
-2.802	-1.606	0.126	1.286	3.525	4.701	5.561	7.611	8.354	9.609	10.339	11.095
-2.533	-0.605	1.618	3.35	4.271	4.151	6.386	8.244	8.711	9.74	10.419	11.664
-2.307	0.236	2.269	3.614	3.863	5.778	6.303	8.249	8.737	9.772	10.307	11.685
-1.698	2.298	2.454	3.786	5.23	6.73	7.324		9.236	9.689	11.178	
v displacement											
34.294	35.655	34.3		34.342	33.711	32.592	30.541	30.299	28.153	26.712	25.736
32.208	33.346	31.268	32.237	12.985	29.839	29.432	27.663	26.544	25.666	23.683	22.401
30.185	29.556		29.671	28.374	27.679	26.406	25.451	24.743	23.458	22.15	19.731
28.451	28.062	27.716	25.379	25.751	24.377	23.528	21.496	20.455	19.662	19.417	18.279
	24.656	23.763	23.352	22.266	19.308	19.704	19.34	18.445	16.692	16.22	15.718
21.706	17.813	20.396	20.286	19.314	18.213	15.745	16.312	20.246	15.404	14.291	13.696
20.189	17.699	16.592	15.619	14.617	15.699	14.324	14.668	13.784	11.645	11.781	11.371
16.567	16.307	14.289	14.577	12.371	12.732	12.269	11.765	9.906	10.384	10.358	9.327
11.555	13.326	11.648	11.419	10.288	12.169	9.583	8.219	8.705	7.715	8.253	8.13
10.283	10.236	9.769	7.618	10.158	7.788	7.738	6.268	7.288	6.37	6.335	6.292
	6.162	7.517	6.272	5.419	5.337	5.295		5.301	4.403	4.79	
0	32	64			0						
					-32						
					-64						
du/da											
0.0640625	0.05875	0.04578	0.08144	0.05617	0.0517	0.05364	0.04791	0.0687	0.05259		
0.057796875	0.05377	0.12178	0.06542	-0.0069	0.05028	0.04109	0.04652	0.05116	0.05816		
0.06403125	0.01739	0.099	0.079	0.05834	0.04388	0.03952	0.05195	0.0565	0.04714		
0.041125	0.04705	0.05395	0.03873	0.06044	0.04827	0.04286	0.05417	0.04859	0.03202		
0.03071875	0.03139	0.04183	0.09486	0.05978	0.00223	0.03339	0.05797	0.05038	0.01742		
0.03075	0.08491	0.05805	0.06477	0.0478	0.04111	0.04381	0.04583	0.0368	0.01572		
0.04328125	0.03066	0.05483	0.06278	0.04688	0.03817	0.03122	0.04625	0.04186	0.02347		
0.04575	0.04519	0.05311	0.05336	0.03181	0.04547	0.04364	0.03122	0.03102	0.02322		
0.064859375	0.0618	0.04145	0.01252	0.03305	0.06395	0.03633	0.02338	0.02669	0.03006		
0.0715	0.05278	0.02491	0.03381	0.03813	0.03861	0.03803	0.0238	0.02453	0.02989		
0.064875	0.02325	0.04338	0.046	0.03272	0.01856	0.02988	0.01416	0.03034	0.04653		
du/db											
-0.031484375	-0.0288	-0.0298	0.02341	-0.0205	-0.0093	-0.0227	-0.0015	-0.0086	-0.0055	0.00361	-9.4E-05
-0.031828125	-0.0543	-0.0152	-0.0476	0.05267	-0.0209	-0.0147	-0.0189	-0.0165	-0.0266	-0.0139	-0.00044
-0.0430625	-0.0377	-0.0449	-0.0517	-0.028	-0.0676	-0.0295	-0.0259	-0.0233	-0.032	-0.0172	-0.00223
-0.03221875	0.03138	-0.0218	-0.0065	-0.0259	-0.0325	-0.0133	-0.0254	-0.0143	-0.017	-0.0025	-0.00072
-0.02284375	-0.0241	-0.0234	-0.0234	-0.0364	0.00869	-0.0235	-0.0273	-0.0213	-0.0155	-0.0128	-0.02158
-0.023578125	-0.0657	-0.0386	-0.026	-0.0336	-0.0146	-0.0177	-0.0189	-0.0175	-0.0043	-0.0117	-0.01183
-0.016359375	-0.0178	-0.0379	-0.0489	-0.0246	0.00133	-0.0107	-0.0245	-0.0158	-0.0016	-0.0007	-0.00817
-0.007734375	-0.0288	-0.0335	-0.0364	-0.0053	-0.0168	-0.0116	-0.01	-0.006	-0.0025	0.0005	-0.00922
-0.013046875	-0.0454	-0.0131	-0.0068	-0.015	-0.0403	-0.0147	-0.0002	-0.0082	0.0008	-0.0119	-0.00066
dv/db											
0.064203125	0.0953	0.09475	0.08019	0.09325	0.09425	0.09666	0.07953	0.08681	0.07336	0.07128	0.093828
0.058703125	0.08256	0.0555	0.10716	-0.1995	0.08534	0.09225	0.09636	0.09514	0.09381	0.06666	0.064406
0.0541875	0.07656	0.12353	0.09873	0.09544	0.1308	0.10472	0.09548	0.09841	0.10572	0.09266	0.062703
0.105390625	0.16014	0.11438	0.07958	0.10058	0.09631	0.12161	0.081	0.00327	0.06653	0.08009	0.071609
0.04740625	0.1087	0.11205	0.12083	0.11952	0.05639	0.08406	0.073	0.07283	0.07886	0.06936	0.067922
0.080296875	0.02353	0.09542	0.0892	0.10848	0.08564	0.05431	0.07105	0.16156	0.07844	0.06145	0.068266
0.13490625	0.06833	0.07725	0.06563	0.06764	0.05516	0.07408	0.10077	0.07936	0.06141	0.05513	0.050641
0.0981875	0.09486	0.07063	0.10873	0.03458	0.07725	0.0708	0.08589	0.04091	0.06272	0.06286	0.047422
0.03975	0.11194	0.06455	0.08042	0.07608	0.10675	0.067	0.06097	0.05319	0.05175	0.05411	0.057438

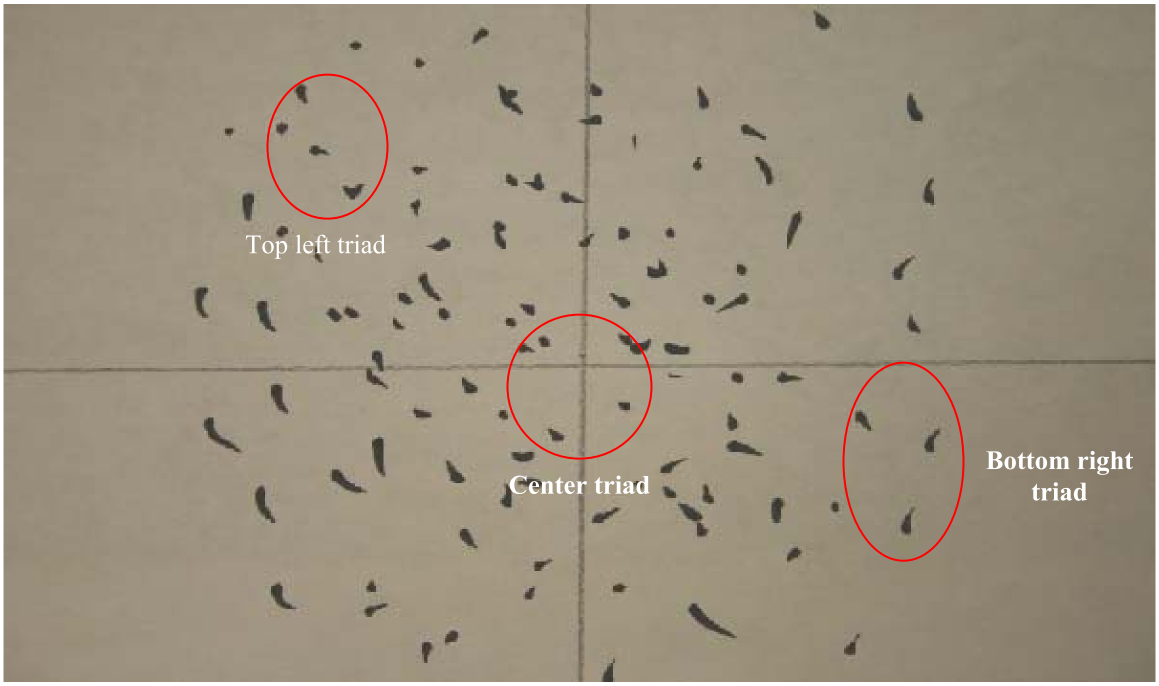
dv/da											
9.375E-05	-0.0423	0.00066	-0.0197	-0.0273	-0.0495	-0.0358	-0.0373	-0.056	-0.0378		
-0.0146875	-0.0173	-0.2857	-0.0375	0.25698	-0.034	-0.0451	-0.0312	-0.0447	-0.051		
-0.01965625	0.0018	-0.0405	-0.0311	-0.0308	-0.0348	-0.026	-0.0311	-0.0405	-0.0582		
-0.011484375	-0.0419	-0.0307	-0.0157	-0.0347	-0.045	-0.048	-0.0287	-0.0162	-0.0216		
-0.02790625	-0.0204	-0.0234	-0.0632	-0.04	0.0005	-0.0197	-0.0414	-0.0348	-0.0152		
-0.02046875	0.03864	-0.0169	-0.0324	-0.0558	-0.0297	0.07033	-0.0142	-0.093	-0.0267		
-0.056203125	-0.0325	-0.0309	0.00125	-0.0046	-0.0161	-0.0084	-0.0472	-0.0313	-0.0043		
-0.03559375	-0.027	-0.03	-0.0288	-0.0016	-0.0151	-0.0369	-0.0216	0.00706	-0.0165		
0.001453125	-0.0298	-0.0213	0.01172	-0.011	-0.0617	-0.0137	-0.0079	-0.0071	0.00648		
-0.00803125	-0.0409	0.00608	0.00266	-0.0378	-0.0238	-0.007	0.00159	-0.0149	-0.0012		
0.04234375	0.00172	-0.0328	-0.0146	-0.0019	-0.0013	9.4E-05	-0.0281	-0.008	0.01209		
Exx	**du/da+0.5*((du/da)^2+(dv/da)^2)**										
6.61%	6.14%	4.68%	8.49%	5.81%	5.43%	5.57%	4.97%	7.26%	5.47%		
5.96%	5.54%	17.00%	6.83%	2.61%	5.21%	4.30%	4.81%	5.35%	6.11%		
6.63%	1.75%	10.47%	8.26%	6.05%	4.54%	4.06%	5.38%	5.89%	4.99%		
4.20%	4.90%	5.59%	3.96%	6.29%	5.04%	4.49%	5.60%	4.99%	3.28%		
3.16%	3.21%	4.30%	10.14%	6.24%	0.22%	3.41%	6.05%	5.22%	1.77%		
3.14%	8.93%	5.99%	6.74%	5.05%	4.24%	4.72%	4.70%	4.18%	1.62%		
4.58%	3.17%	5.68%	6.48%	4.80%	3.90%	3.17%	4.84%	4.32%	2.38%		
4.74%	4.66%	5.50%	5.52%	3.23%	4.66%	4.53%	3.19%	3.15%	2.36%		
6.70%	6.42%	4.25%	1.27%	3.37%	6.79%	3.71%	2.37%	2.71%	3.05%		
7.41%	5.50%	2.52%	3.44%	3.96%	3.96%	3.88%	2.41%	2.49%	3.03%		
6.79%	2.35%	4.49%	4.72%	3.33%	1.87%	3.03%	1.47%	3.08%	4.77%		
MAX	17.00%										
MIN	0.22%										
AVG	4.76%										
STDEV	2.14%										
Eyy	**dv/db+0.5*((du/db)^2+(dv/db)^2)**										
6.68%	10.03%	9.97%	8.37%	9.78%	9.87%	10.16%	8.27%	9.06%	7.61%	7.38%	9.82%
6.09%	8.74%	5.72%	11.40%	-17.82%	8.92%	9.66%	10.12%	9.98%	9.86%	6.90%	6.65%
5.66%	8.02%	13.22%	10.49%	10.04%	14.16%	11.06%	10.04%	10.35%	11.18%	9.71%	6.47%
11.15%	17.35%	12.12%	8.28%	10.60%	10.15%	12.91%	8.46%	0.34%	6.89%	8.33%	7.42%
4.88%	11.49%	11.86%	12.84%	12.73%	5.80%	8.79%	7.60%	7.57%	8.21%	7.18%	7.05%
8.38%	2.60%	10.07%	9.35%	11.49%	8.94%	5.59%	7.38%	17.48%	8.15%	6.34%	7.07%
14.41%	7.08%	8.10%	6.90%	7.02%	5.67%	7.69%	10.61%	8.26%	6.33%	5.66%	5.20%
10.30%	9.98%	7.37%	11.53%	3.52%	8.04%	7.34%	8.96%	4.18%	6.47%	6.48%	4.86%
4.06%	11.92%	6.67%	8.37%	7.91%	11.33%	6.94%	6.28%	5.46%	5.31%	5.56%	5.91%
MAX	17.48%										
MIN	-17.82%										
AVG	8.56%										
STDEV	4.02%										
Exy	**0.5*(du/db+dv/da+(du/da*du/db+dv/da*dv/db))**										
-1.67%	-3.84%	-1.52%	0.20%	-2.58%	-3.20%	-3.16%	-2.09%	-3.50%	-2.32%		
-2.46%	-3.80%	-15.93%	-4.61%	12.90%	-2.94%	-3.23%	-2.70%	-3.31%	-4.20%		
-3.33%	-1.82%	-4.74%	-4.50%	-3.17%	-5.50%	-2.97%	-3.07%	-3.46%	-4.89%		
-2.31%	-0.79%	-2.86%	-1.18%	-3.29%	-4.17%	-3.39%	-2.89%	-1.56%	-2.03%		
-2.64%	-2.37%	-2.52%	-4.82%	-4.17%	0.46%	-2.28%	-3.66%	-2.98%	-1.61%		
-2.32%	-1.59%	-2.97%	-3.15%	-4.85%	-2.37%	2.79%	-1.75%	-6.31%	-1.66%		
-4.04%	-2.65%	-3.66%	-2.53%	-1.53%	-0.78%	-1.01%	-3.88%	-2.51%	-0.31%		
-2.36%	-2.98%	-3.37%	-3.51%	-0.35%	-1.69%	-2.58%	-1.69%	0.06%	-1.01%		
-0.62%	-4.06%	-1.81%	0.29%	-1.37%	-5.56%	-1.49%	-0.43%	-0.79%	0.38%		
MAX	12.90%										
MIN	-15.93%										
AVG	-2.49%										
STDEV	2.65%										

Matrices for Strain Accumulation

Matrix Fn =	**1+du/da**	**du/db**			
	dv/da	**1+dv/db**			
50:1 PDMS	**F1**	**0-5%**			
	1.0433	-0.0010			
	0.0012	1.0563			
	F2	**5-10%**		**F' = F2*F1**	**0-10%**
	1.0499	-0.0029		1.0954	-0.0041
	0.0008	1.0570		0.0021	1.1165
	F3	**10-15%**		**F'' = F3*F'**	**0-15%**
	1.0536	-0.0005		1.1541	-0.0049
	0.0008	1.0479		0.0031	1.1699
80:1 PDMS	**F1**	**0-5%**			
	1.0452	-0.0183			
	-0.0218	1.0787			
	F2	**5-10%**		**F' = F2*F1**	**0-10%**
	1.0514	0.0293		1.0980	0.0124
	0.0280	1.0565		0.0662	1.1391
	F3	**10-15%**		**F'' = F3*F'**	**0-15%**
	1.0406	0.0068		1.1429	0.0206
	0.0150	1.0260		0.0229	1.1689

Appendix I: Images of Spots on Paper and PDMS Substrates

Images of Spots on Paper



Original Image of Spots on Paper

Image enlarged 5%

50:1 PDMS Images

Static 50:1 PDMS

50:1 PDMS experiencing 5% strain

50:1 PDMS experiencing 10% strain

50:1 PDMS experiencing 15% strain

80:1 PDMS Images

Static 80:1 PDMS

80:1 PDMS experiencing 5% strain

80:1 PDMS experiencing 10% strain

80:1 PDMS experiencing 15% strain

Appendix J: Raw Data from Cell Counts on 50:1 and 80:1 PDMS Substrates

Cell Counts for Day 0 of 5% Stretch Experiment

	50:1		**80:1**	
	Live	**Dead**	**Live**	**Dead**
Sample 1	515	1	183	0
	887	2	67	2
	905	1	290	1
	659	5	102	6
	944	9	303	7
Sample 2	929	3	40	3
	712	5	205	8
	771	3	23	1
	767	2	37	2
	850	7	101	6
Sample 3	541	3	270	4
	678	3	46	2
	587	2	33	7
	738	3	101	3
	529	8	111	0
Averages				
1	782	3.6	189	3.2
2	805.8	4	81.2	4
3	614.6	3.8	112.2	3.2
TOTAL CELLS	95548.99146	439.8675	23093.04	390.9933
	98457.00424	488.7416	9921.455	488.7416
	75095.15364	464.3046	13709.2	390.9933
Average	89700.38311	464.3046	15574.57	423.5761

Cell Counts for Day 0 of 15% Stretch Experiment

	50:1		80:1	
	Live	**Dead**	**Live**	**Dead**
Sample 1	641	4	690	4
	524	2	393	0
	634	2	64	3
	634	1	136	8
	591	2	568	9
Sample 2	788	1	82	0
	754	1	102	1
	716	2	43	1
	515	3	101	3
	475	5	78	4
Sample 3	574	1	578	1
	726	2	128	6
	764	3	71	0
	690	1	424	0
	652	3	455	2
Averages				
1	604.8	2.2	370.2	4.8
2	649.6	2.4	81.2	1.8
3	681.2	2	331.2	1.8
TOTAL CELLS	73897.73662	268.8079	45233.04	586.49
	79371.64303	293.245	9921.455	219.9337
	83232.70202	244.3708	40467.81	219.9337
Average	78834.02722	268.8079	31874.1	342.1192

Cell Counts for Day 2 of 5% Stretch Experiment for 80:1 Samples

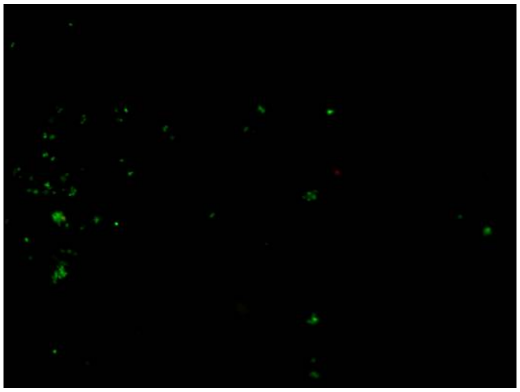
	Static		Stretch	
Sample 1	**Alive**	**Dead**	**Alive**	**Dead**
	59	14	482	7
	253	8	582	7
	248	44	56	3
	136	25	314	4
	60	16	563	5
Sample 2	490	7	148	8
	750	7	617	5
	852	80	515	13
	194	8	252	13
	323	4	859	6
Sample 3	27	17	107	5
	434	11	68	2
	369	11	35	5
	15	11	142	6
	406	17	218	6
Averages				
1	151.2	21.4	399.4	5.2
2	521.8	21.2	478.2	9
3	250.2	13.4	114	4.8
TOTAL CELLS	18474.43	2614.768	48800.85	635.3641
	63756.35	2590.331	58429.06	1099.669
	30570.79	1637.285	13929.14	586.49
Averages	37600.52	2280.794	40386.35	773.8409

Cell Counts for Day 2 of the 15% Stretch Experiment

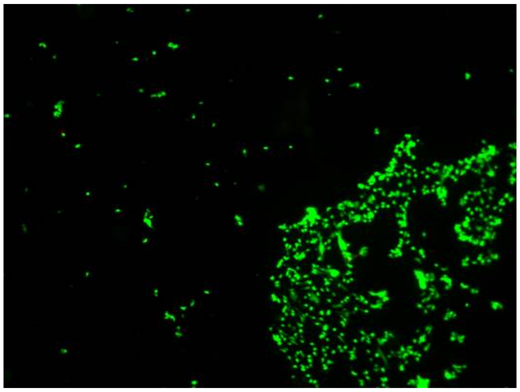
	Static		Static		Stretch		Stretch	
	50 to 1		80 to 1		50 to 1		80 to 1	
Sample 1	**Alive**	**Dead**	**Alive**	**Dead**	**Alive**	**Dead**	**Alive**	**Dead**
	548	20	108	5	316	10	13	11
	969	9	27	9	335	8	70	13
	1102	5	268	1	581	3	46	11
	1061	4	78	1	387	4	25	4
	1023	7	100	0	530	1	36	8
Sample 2	6	6	552	10	648	2	85	8
	474	2	76	0	310	2	110	9
	123	8	49	1	656	6	60	6
	85	11	165	4	718	14	137	15
	236	7	195	16	1165	0	102	10
Sample 3	41	39	93	1	291	3	85	7
	4	17	144	0	329	1	107	27
	7	17	572	1	871	2	64	6
	11	17	984	3	851	0	102	3
	5	4	145	1	474	2	179	9
Averages								
1	940.6	9	116.2	3.2	429.8	5.2	38	9.4
2	184.8	6.8	207.4	6.2	699.4	4.8	98.8	9.6
3	13.6	18.8	387.6	1.2	563.2	1.6	107.4	10.4
TOTAL CELLS	114927.6	1099.669	14197.94	390.9933	52515.29	635.3641	4643.046	1148.543
	22579.86	830.8608	25341.25	757.5495	85456.48	586.49	12071.92	1172.98
	1661.722	2297.086	47359.07	146.6225	68814.82	195.4967	13122.71	1270.728
Averages	46389.73	1409.205	28966.09	431.7218	68928.86	472.4503	9945.892	1197.417

Appendix K: LIVE/DEAD Images, Cell Counts from VICs on 65:1 and 90:1 PDMS Substrates

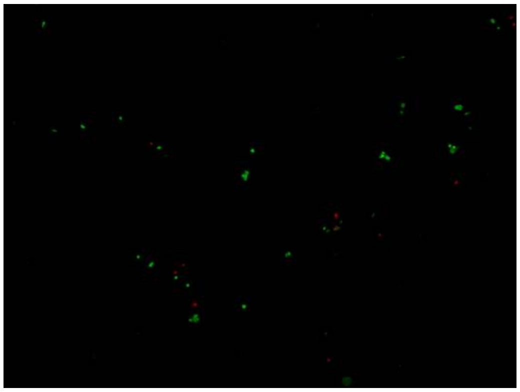
65:1 PDMS Substrates, 5% Stretch Experiment



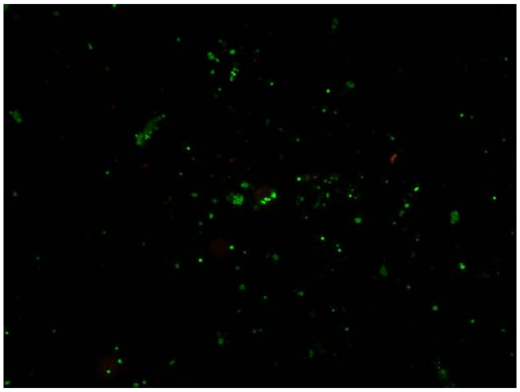
65-1, 5% experiment, day 0



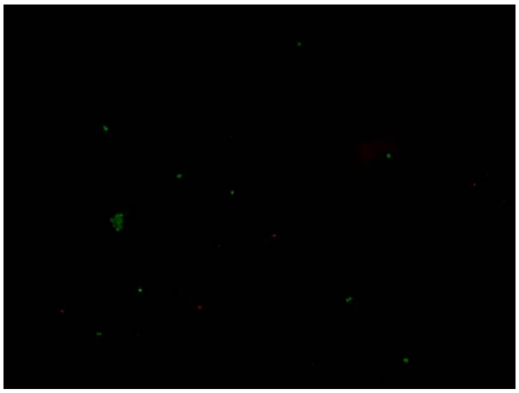
65-1, 5% experiment static, day 2



65-1, 5% experiment static, day 4

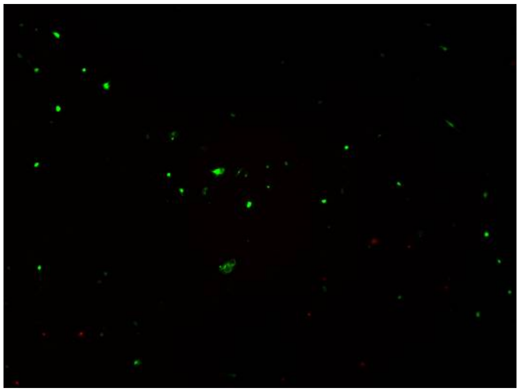


65-1, 5% stretch, day 2

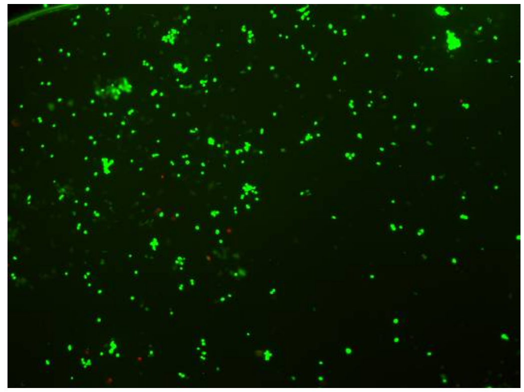


65-1, 5% stretch, day 4

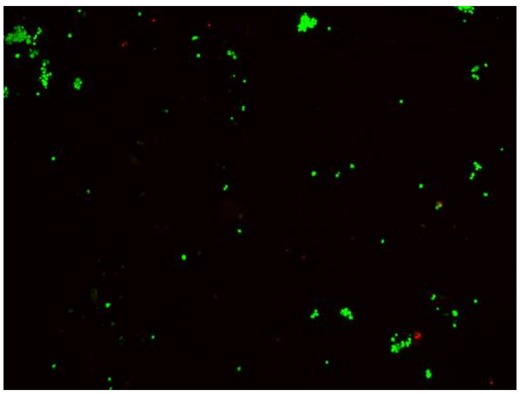
65:1 PDMS Substrates, 15% Stretch Experiment



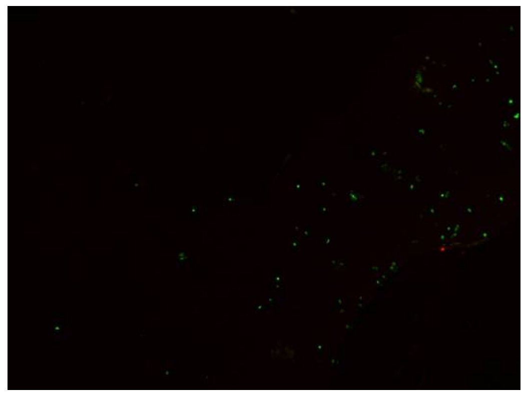
65-1, 15% experiment, day 0



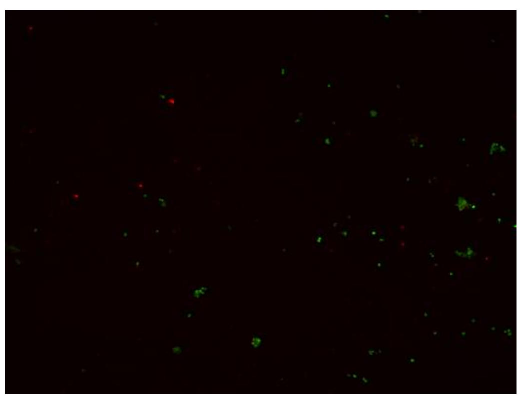
65-1, 15% experiment static, day 2



65-1, 15% experiment static, day 4

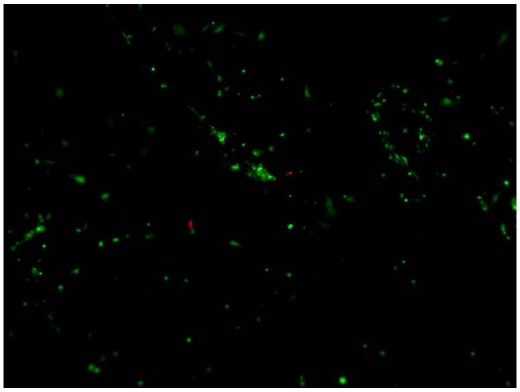


65-1, 15% stretch, day 2

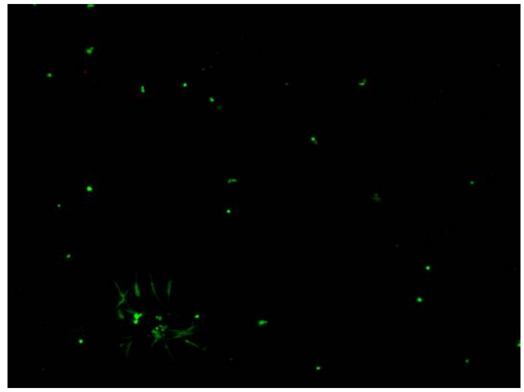


65-1, 15% stretch, day 4

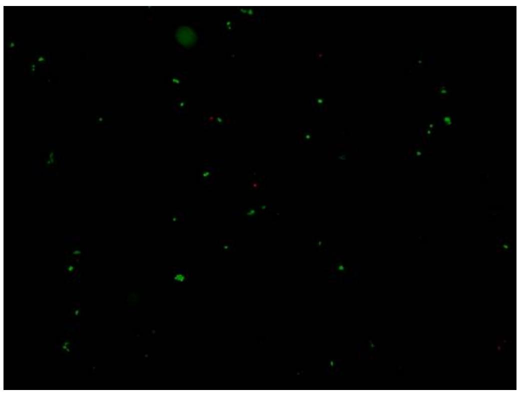
90:1 PDMS Substrates, 5% Stretch Experiment



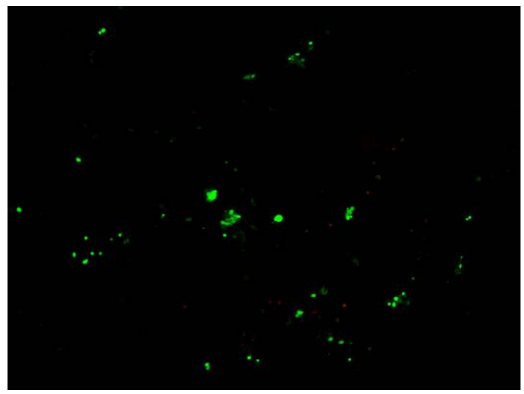
90-1, 5% experiment, day 0



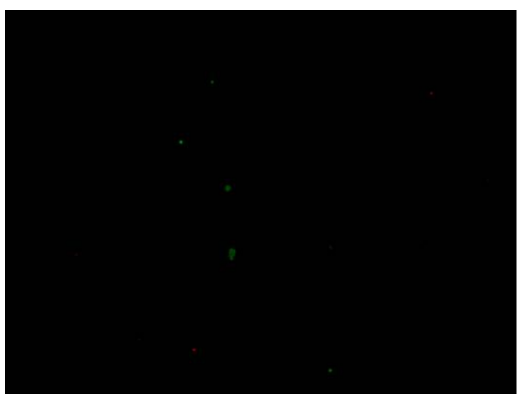
90-1, 5% experiment static, day 2



90-1, 5% experiment static, day 4

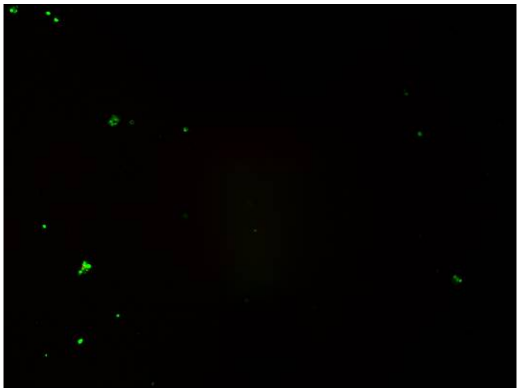


90-1, 5% stretch, day 2

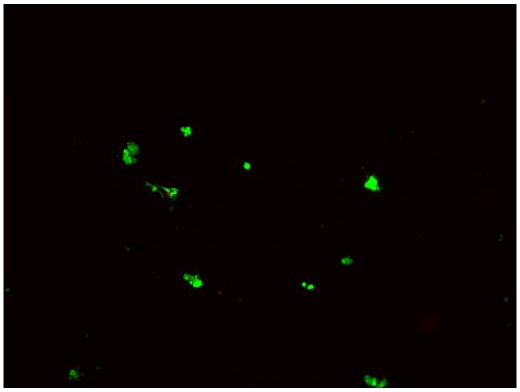


90-1, 5% stretch, day 4

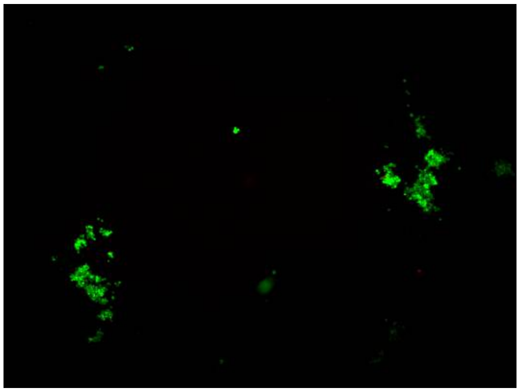
90:1 PDMS Substrates, 15% Stretch Experiment



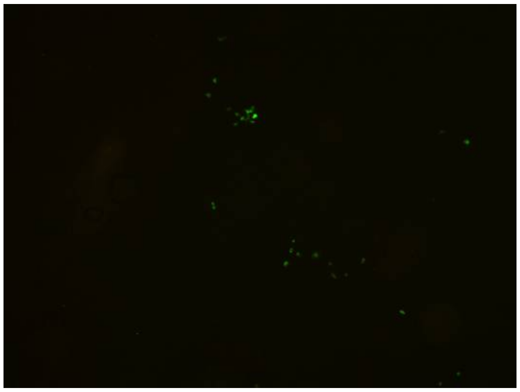
90-1, 15% experiment, day 0



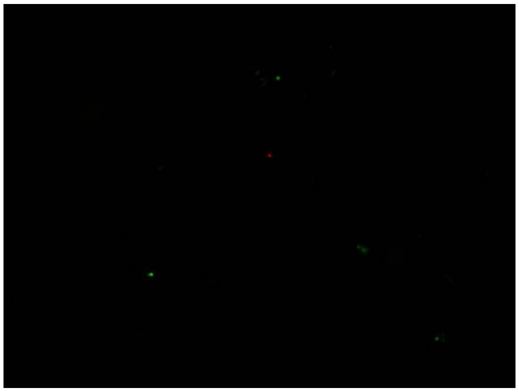
90-1, 15% experiment static, day 2



90-1, 15% experiment static, day 4



90-1, 15% stretch, day 2



90-1, 15% stretch, day 4

Table 1: 15% Stretch Experiment Day 0 Cell Counts

	65:1	65:1		90:1	90:1
	Live	**Dead**		**Live**	**Dead**
Sample 1	114	2		4	3
	73	2		17	2
	31	3		6	0
	35	10		5	3
	113	6		9	0
Sample 2	31	3		9	3
	17	3		13	0
	115	1		4	0
	0	4		10	4
	10	6		12	0
Sample 3	277	1		5	6
	90	3		14	4
	139	2		5	3
	130	2		13	13
	70	1		67	23
Averages	**live**	**dead**		**live**	**dead**
1	73.2	4.6		8.2	1.6
2	34.6	3.4		9.6	1.4
3	141.2	1.8		20.8	9.8
TOTAL CELLS	8943.97	562.05		1001.92	195.50
	4227.62	415.43		1172.98	171.06
	17252.58	219.93		2541.46	1197.42
Averages	10141.39	399.14		1572.12	521.32
% Initially Attached	0.093040267			0.014423	
% viability	0.962132921			0.750973	

Table 2: 15% Stretch Experiment Day 2 Cell Counts

	Static		Static		Stretch		Stretch	
	65:1		90:1		65: 1		90:1	
Sample 1	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**
	90	14	32	4	50	4	34	37
	348	33	31	8	38	12	4	4
	40	10	15	14	13	1	7	6
	109	26	55	1	99	5	12	6
	305	12	28	5	32	4	5	3
Sample 2	55	8	124	82	25	3	8	5
	16	11	122	29	25	5	9	2
	40	29	12	5	31	21	4	0
	55	14	18	20	9	5	3	1
	152	21	44	42	26	1	2	1
Sample 3	43	8	7	12	25	10	6	10
	60	5	9	6	36	8	17	2
	57	9	10	8	31	0	10	4
	33	7	28	12	27	15	11	8
	143	0	12	11	125	14	21	2
Averages	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**
1	178.4	19	32.2	6.4	46.4	5.2	12.4	11.2
2	63.6	16.6	64	35.6	23.2	7	5.2	1.8
3	67.2	5.8	13.2	9.8	48.8	9.4	13	5.2
TOTAL CELLS	21797.88	2321.5	3934.37	781.98	5669.40	635.36	1515.10	1368.47
	7770.99	2028.3	7819.87	4349.8	2834.70	855.30	635.36	219.93
	8210.86	708.67	1612.85	1197.4	5962.65	1148.54	1588.41	635.36
Averages	12593.24	1686.1	4455.69	2109.7	4822.25	879.74	1246.29	741.26
T-test Stat & Stretch 65:1 (p val)	0.174372							
T-test Stat & Stretch 95:1 (p val)	0.155437							
65: 1 % Static/Stretch	0.382924							
90: 1 % Static/Stretch	0.279707							
% viability	0.881917		0.67866		0.845714		0.627049	

Table 3: 15% Stretch Experiment Day 4 Cell Counts

	Static		**Static**		**Stretch**		**Stretch**	
	65:1		**90:1**		**65:1**		**90:1**	
Sample 1	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**
	97	17	21	2	48	14	8	1
	14	6	22	1	107	16	13	1
	44	6	23	8	73	39	4	4
	45	10	34	6	29	10	12	5
	110	11	20	1	46	8	4	0
Sample 2	119	16	48	5	48	1	7	8
	95	21	6	1	26	4	5	1
	50	35	144	9	86	4	47	4
	35	7	22	5	58	6	22	2
	89	10	30	7	55	2	12	2
Sample 3	35	30	20	3	30	5	20	1
	46	70	24	2	16	3	27	0
	38	24	11	2	33	8	7	4
	64	27	16	1	24	0	34	2
	116	43	14	1	18	9	11	3
Averages	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**
1	62	10	24	3.6	60.6	17.4	8.2	2.2
2	77.6	17.8	50	5.4	54.6	3.4	18.6	3.4
3	59.8	38.8	17	1.8	24.2	5	19.8	2
TOTAL CELLS	7575.50	1221.85	2932.45	439.87	7404.44	2126.03	1001.92	268.81
	9481.59	2174.9	6109.27	659.80	6671.32	415.43	2272.65	415.43
	7306.69	4740.79	2077.15	219.93	2956.89	610.93	2419.27	244.37
Averages	8121.26	2712.52	3706.29	439.87	5677.55	1050.80	1897.95	309.54
T-test Stat & Stretch 65:1 (p val)	0.187174							
T-test Stat & Stretch 95:1 (p val)	0.23855							
65: 1 % Static/Stretch	0.699097							
90: 1 % Static/Stretch	0.512088							
% viability	0.749624		0.89391		0.843826		0.859779	

Table 4: 5% Stretch Experiment Day 0 Cell Counts

	65:1			90:1	
	Live	**Dead**		**Live**	**Dead**
Sample 1	290	8		47	4
	45	1		38	3
	46	1		39	1
	29	5		175	8
	54	2		13	2
Sample 2	182	3		224	10
	433	11		49	4
	421	3		40	6
	28	4		29	13
	91	3		14	2
Sample 3	163	1		57	1
	159	3		109	6
	169	8		54	1
	216	3		40	7
	91	8		191	13
Averages	**live**	**dead**		**live**	**dead**
1	92.8	3.4		62.4	3.6
2	231	4.8		71.2	7
3	159.6	4.6		90.2	5.6
TOTAL CELLS	11338.81	415.43		7624.37	439.87
	28224.83	586.49		8699.60	855.30
	19500.79	562.05		11021.12	684.23
Averages	19688.14	521.32		9115.03	659.8012
% Initially Attached	0.180625161			0.083624	
% viability	0.97420395			0.9325	

Table 5: 5% Stretch Experiment Day 2 Cell Counts

	Static		Static		Stretch		Stretch	
	65:1		90:1		65:1		90:1	
Sample 1	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**
	117	3	340	4	52	22	66	3
	344	7	45	1	223	31	44	3
	14	4	79	8	50	24	41	5
	276	5	23	3	40	10	51	10
	25	5	475	14	137	22	71	11
Sample 2	44	2	222	7	313	10	25	9
	35	1	18	3	46	15	11	14
	83	9	70	10	230	12	11	5
	349	4	10	3	25	4	23	6
	8	4	84	6	70	8	22	8
Sample 3	103	4	302	6	54	4	290	2
	83	3	123	16	57	4	23	11
	43	3	70	0	224	8	51	6
	126	5	166	3	296	26	81	7
	474	51	42	2	554	18	46	9
Averages	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**
1	155.2	4.8	192.4	6	100.4	21.8	54.6	6.4
2	103.8	4	80.8	5.8	136.8	9.8	18.4	8.4
3	165.8	13.2	140.6	5.4	237	12	98.2	7
TOTAL CELLS	18963.18	586.49	23508.47	733.11	12267.42	2663.64	6671.32	781.99
	12682.85	488.74	9872.58	708.68	16714.96	1197.42	2248.21	1026.36
	20258.34	1612.84	17179.27	659.80	28957.94	1466.23	11998.61	855.30
Averages	17301.45	896.03	16853.44	700.53	19313.44	1775.76	6972.71	887.88
T-test Stat & Stretch 65:1 (p val)	0.733549							
T-test Stat & Stretch 95:1 (p val)	0.110983							
65: 1 % Static/ Stretch	12111.02	1.11629						
90: 1 % Static/ Stretch	11797.41	0.413726						
% viability	0.950761		0.960093		0.915798		0.887047	

Table 6: 5% Stretch Experiment Day 4 Cell Counts

	Static		Static		Stretch		Stretch	
	65:1		90:1		65:1		90:1	
Sample 1	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**
	18	3	24	1	11	4	9	3
	21	5	27	0	12	4	9	3
	47	4	27	2	17	2	11	2
	27	9	35	4	16	1	12	0
	19	5	18	1	11	3	9	8
Sample 2	10	8	45	5	24	4	16	12
	16	3	4	1	21	4	24	12
	12	4	114	8	16	2	12	7
	10	2	14	2	17	1	45	4
	6	1	35	7	14	2	69	5
Sample 3	16	5	20	3	19	1	172	7
	33	4	24	2	36	2	23	12
	38	5	13	2	7	2	33	8
	32	6	19	1	20	3	108	9
	20	2	15	2	7	2	31	3
Averages	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**
1	26.4	5.2	26.2	1.6	13.4	2.8	10	3.2
2	10.8	3.6	42.4	4.6	18.4	2.6	33.2	8
3	27.8	4.4	18.2	2	17.8	2	73.4	7.8
TOTAL CELLS	3225.69	635.36	3201.26	195.50	1637.28	342.12	1221.85	390.99
	1319.60	439.87	5180.66	562.05	2248.21	317.68	4056.56	977.48
	3396.75	537.62	2223.77	244.37	2174.90	244.37	8968.41	953.054
Averages	2647.35	537.62	3535.23	333.97	2020.13	301.39	4748.94	773.84
T-test Stat & Stretch 65:1 (p val)	0.41661							
T-test Stat & Stretch 95:1 (p val)	0.64291							
65: 1 % Static/ Stretch	0.7630769							
90: 1 % Static/ Stretch	1.343317							
% viability	0.831202046		0.913684211		0.870175439		0.859882006	