

MOUSE/RAT KNEE STATIC LOADING TEST APPARATUS

by

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TABLE OF CONTENTS

1. INTRODUCTION	1
Background and Motivation	1
Test Apparatus Development Motivations	4
2. PRELIMINARY MEASUREMENT STUDY	6
Dimensional Considerations	6
Pressure Considerations	7
3. DESIGN OF MACHINE	10
Requirements	10
Degrees of Freedom	10
Pretest Degrees of Freedom	11
Testing Degrees of Freedom	13
Design Challenges	14
Gearing System	14
Locking Upper Pot	15
Pressure Paper Thickness	16
Load Application	17
Horizontal Load Application:	17
Vertical Load Application:	18
Flexion Angle	19
Rotational Tower Alteration:	20
Assemblies	21
Tower Assembly	21
Upper Pot Mount:	23
Lower Pot Mount:	24
Loading Assembly	24
4. TESTING	26
Setup	26
Potting	26
Pressure Measurement	27
Loading	28
Operation of SLAMR	29
Calibration	30
Load Application System	30

TABLE OF CONTENTS CONTINUED

Spring Constant:.....	31
Pressure Paper	32
Pixel to Density	34
Density to Pressure Calculations	36
Operating SLAMR.....	37
Initial Test Pressure Paper Processing Methods	38
Color Mapping Images	39
Average Row Pressure	40
Applied Weight Calculation	40
Regional Pressure Measurement (RPM) Correlations	41
Force Correlation	42
5. RESULTS	44
Testing Apparatus Performance.....	44
Degrees of Freedom	44
Pre-Test DoF:.....	44
Testing Degree of Freedoms:.....	45
Flexion Angle.....	45
Initial Experiment	46
Pressure Strip Analysis	47
Color Mapping and 1-D Pressure Averaging:	47
Regional Pressure Measurement Correlations:.....	51
Force Correlation:	51
6. CONCLUSIONS.....	53
Summary	53
Future Testing	53
Recommendations.....	54
Force Feedback Loading.....	54
Leg Measurement.....	54
Electronic Pressure Sensor.....	54
REFERENCES	56
APPENDICES	58
APPENDIX A: Preliminary Calculations Measurements.....	58
APPENDIX B: Calibrations	60
APPENDIX C: Results	65

LIST OF TABLES

Table		Page
1: Average Leg Lengths for Mice and Rats		7
2: Calculated Pressure Distribution within Mouse Knee		8
3: Calculated Pressure Distribution within Rat Knee		9
4: Calculated W_A Example		41
5: Test Strip Loads vs. Load Applied to Leg		52
6: Test Strip Load vs. Load Applied to Machine.....		52

LIST OF FIGURES

Figure	Page
1. FE Model of Knee.....	2
2. Potting.....	4
3. Tibia Plateau Cartoon	7
4. Leg Geometry	11
5. Lateral Offset	12
6. Rotation of Flexion	22
7. Free Body Diagram of Test Leg	14
8. Gearing Assembly.....	15
9. Locking Pin.....	16
10. Horizontal Weight Drive System.....	18
11. Vertical Loading System.....	18
12. Original Tower Assembly.....	19
13. Rotational Tower Modifications.....	20
14. Rotational Tower Assembly	22
15. Knee Orientation in Tower	22
16. Upper Pot Mount Assembly.....	23
17. Tower Control Nobs	24
18. Loading Assembly	25

LIST OF FIGURES CONTINUED

Figure	Page
19. Knee Prepped for Testing	27
20. SLAMR Spring Calibration Chart	31
21. Spring Constant.....	32
22. Color-mapping Process Outline.....	33
23. Color Prescale Chart	34
24. Averaged Color Pixel Values.....	35
25. Averaged Color Values.....	35
26. Matlab Curve Fit Tool	36
27. Pressure to Density	37
28. Color Map Example Result.....	40
29. Regions of Measurement	42
30. Tower Assembly DoF	45
31. Flexion Range	46
32. All Test 5 Color Plots and 1D Distribution	48
33. Unloaded vs. Loaded example.....	49
34. Normal State 90 vs 135 Degree Flexion	50
35. Normal State vs. Destabilized.....	51

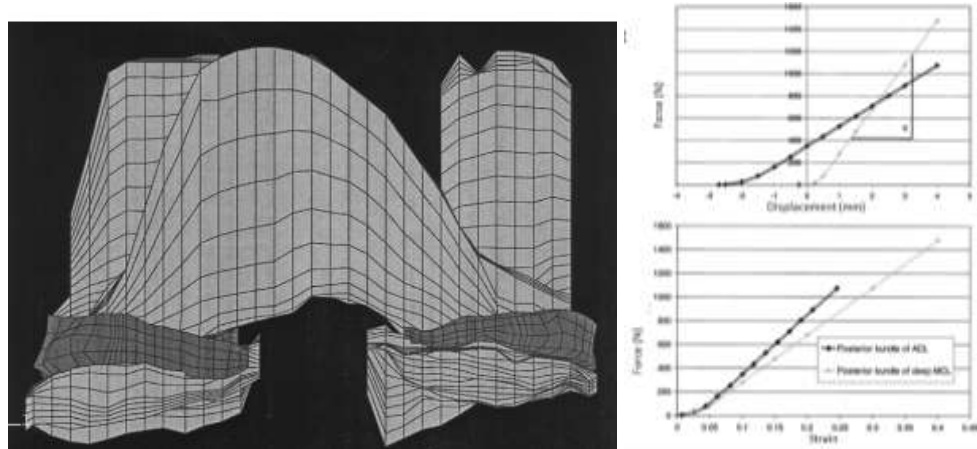
ABSTRACT

Osteoarthritis (OA) involves mechanically-related cartilage deterioration and affects millions worldwide. To date no effective treatments for OA exist and to expedite the solution process rodent models that mimic human disease are used before attempting to apply to human models. Rodent models of osteoarthritis involve mechanical destabilization of the knee joint which likely changes the contact pressure distribution. However, no methods currently exist for measuring the contact pressure distribution in mouse or rat knees. Therefore, the objective was to develop a method to measure the contact pressure distribution within a mouse knee. This research designed and tested an apparatus to apply loads to mouse knees based on measurements of young mouse knees and mature rat knees. Applied loads were used to explore measureable pressure zone shifts within the knee for varying flexion angles. Measurements of the tibia plateau were used to estimate contact area for an expected pressure range. Based on this preliminary information, a machine was designed to incorporate 6 degrees of freedom that allows the application of compressive loading while allowing the knee as natural of movement as possible. To apply the load a mechanical system was devised to both measure and apply joint loading. Several iterations of both of these systems were considered and the final product was created for testing. Several hurdles were overcome during testing, which included creating a method to interface the biological knee to the mechanical system, developing a technique to measure the pressure distribution of extremely small areas, and the requirement for accurate calibration of both the load application and measurement. It is assumed that the results will be the first pressure distribution measurements in the murine knee. Extension of these results may yield valuable insight into the mechanical environment of rodent osteoarthritis models.

INTRODUCTION

Background and Motivation

Osteoarthritis (OA) is a disease that effects people from all over the world, and has become one of the leading causes of immobility in western society. OA affects about 80% of the 75 year and up population. OA results in the degeneration of cartilage within the joint and there are currently no known effective treatments. Key to the development of possible treatments is the development of a clear understanding of the mechanical environment of the knee. An example of this research is that performed by T.L. Haut-Donahue in 2003 to map the pressure distributions inside of a human knee. The goals of this research were to one, produce material model of the meniscal tissue and two, to determine whether a transversely isotropic, linearly elastic, homogeneous material model of the meniscal tissue is necessary to achieve a normal contact pressure distribution on the tibia plateau. This research utilized a finite element model of the knee ([Figure 1), which was validated via physical testing of a single knee by utilizing the displacement of the knee and measuring the pressure loading via pressure film (3).



[Figure 1: FE Model of Knee] Model created with intention of exploring potential constitutive properties for material properties in human knee. (Tammy L. Haut Donahue, M.L. Hull, Journal of Biomechanics, Vol 36, Iss 1, Jan 2003, pg19-34)

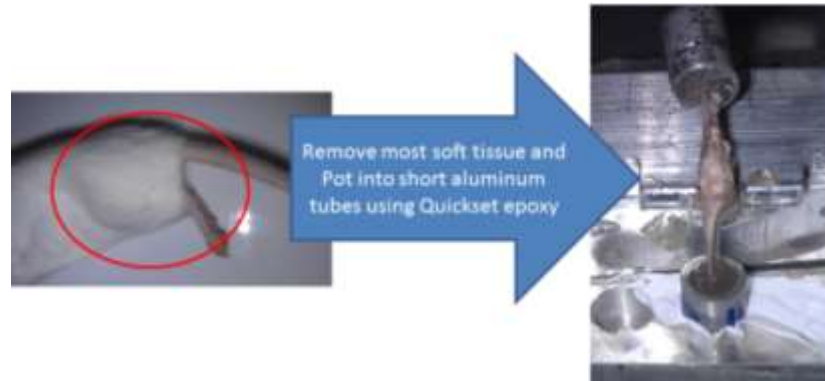
The search for an effective treatment has led to the use of a wide range of OA models in mice, including genetically modified transgenic knockout mice that develop premature cartilage degeneration. Also used are surgically induced models where the meniscus of the specimen is removed, allowing for the mechanical degeneration of the cartilage within the knee (2). Material models of the degeneration model can be developed in pursuit of a potential cure for OA.

To date there has also been no known development towards a method to test the mechanical properties within a mouse or rat knee to determine the defining characteristics of cartilage degeneration. Past research using mice knees has shown results proving that surgical procedure will in fact induce OA (2). Research involved removing the meniscus early in the animal's life, with compliance to Health Research Extension Act (HREA) and Animal Welfare Act approved methods and letting the animal actively load the joint for a predetermined period of time. The joints were then analyzed post mortem for signs of OA. Each study was conducted using a control knee otherwise known as a sham,

where the knee is left unaltered to prove that the animal did not develop OA due to natural occurrence.

Research on human knees generally has the problem of a low population of tests due to the difficulty of obtaining test specimens (3). To overcome this obstacle the smaller and more cost efficient mouse and rat knee models are being considered by many researchers (3,4,5,&6). Mouse knees have the benefit of coming at a relatively low cost and many more samples can be generated for a larger population of tests. Mice and rats have been used for biomechanical studies several times in the past for walking and degenerative studies (7), but a study of the contact mechanics of the knee has been overlooked. To make the research performed using mouse knees more viable as potential solutions for the human disease, the material properties of the mouse and rat knees will need to be defined and compared to those found in human models.

The Static Loading Apparatus for Mouse/Rat knees (SLAMR) allows for the study of mouse and rat knee mechanics, such as pressure zone movement with varying flexion angles and localization of pressure zone with removal of meniscus. Each leg is equipped with “pots” which are 8mm diameter aluminum pipes cut to a 12mm height then filled with epoxy that leg is then “potted” into ([Figure 2]).



[Figure 2: Potting] Test leg is removed from animal and fitted with 8mm diameter aluminum pipe about 12mm tall to interface with testing apparatus

Previous studies (3,4,5,&6) only utilized visual analysis of selected two dimensional sections of the knee, produced by creating slides of thin cross sections of the knee that have been died to show different cell types. However, these slides are limited by potential bias and lack of quantification. Knowledge of the pressure distributions within the knee would not only improve the understanding of the general knee mechanics, but also help to isolate expected disease locations.

Test Apparatus Development Motivations

There are several motivations that drove the development of the SLAMR testing apparatus. These included development of a means to confirm estimates for mechanical properties of the knees and to show quantifiable changes in the mechanical behavior of the knee such as (1) Measureable Pressure zone shift, and (2) measureable localization of pressure zone from surgical destabilization of knee. The ultimate goal for this research was to develop an apparatus that permits the study of effects of pressure distribution through the knee. By conducting experiments in this regard not only are the basic

mechanics of a mouse knee discovered, but the effects that OA have on the knee can be more thoroughly explored. Currently it is not known if any existing experimental apparatus capable of performing static loading tests on mouse or rat knees.

The second motivation for SLAMR was to make more precise the prediction of OA locations within the rodent's knee. Existing methods of finding rodent OA involve creating slides of the knee cross-sections discussed in the previous section and tediously searching for the desired cross-section (3,4,5,&6). With *a priori* knowledge of the high pressure areas of the knee the search range for the desired slide can be isolated.

Furthermore, the development of SLAMR is intended to enable future research to answer the question of how changes in contact pressure affect cartilage deterioration. With the development of SLAMR the motivating factors of mechanical property development, OA location method, and deterioration knowledge potential cures for the disease can be explored. Combined with the higher populations of the mouse and rat knee models answers obtained could advance researchers to a cure for OA in a shorter period of time.

PRELIMINARY MEASUREMENT STUDY

Prior to designing the machine several measurements had to be taken of the biological systems to define the size requirements of the loading apparatus. These measurements were used in both creating the size of the tower used to hold the rodent legs, as well as to help determine how much pressure would be expected inside the knee when loaded. This study also served as introductory experience to dissection of mouse and rat legs, helping to gain an understanding of the biological system and insight of the necessary tests.

Dimensional Considerations

The first step in designing an apparatus with a range of motions that encompass both a mouse and a rat knee was to establish the size range of the rodent hind limbs. Specimens of both sub 35 gram mice and full grown rats were dissected and measured using digital calipers (Mitatoyo Corperation Coolant Proof IP67). The small mice (mean weight = 28.5 grams +/- st.dev = 1.3grams with a population of 5 animals) were picked so as to design for the low end range of the testing fixture, and the full grown rats (mean weight = 340 grams, st.dev=2 grams with a population of 2 animals) would influence the maximum range of fixture. The averages of these measurements are shown in (Table 1. Raw data for measurements can be found in Preliminary Calculation Measurement section of Appendix (Appendix A, page 58).

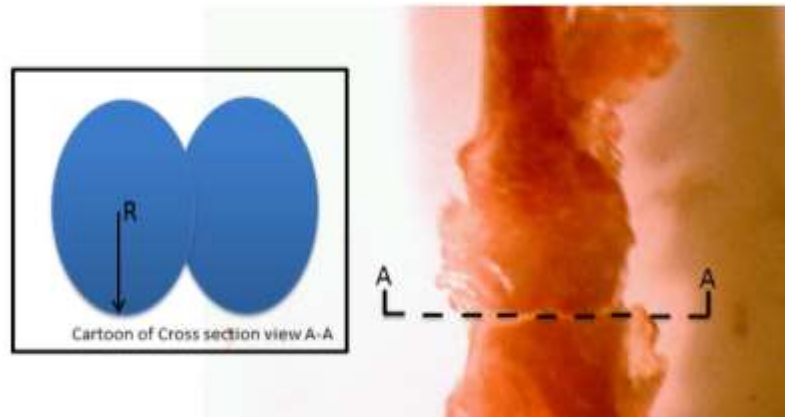
Table 1: Average leg lengths for mice and rats

Leg Length Averages	mm
Mouse Tibia	18.07
Mouse Femur	15.71667
Rat Tibia	40.71
Rat Femur	35.495

This data was used to determine the dimensions for the tower fixture discussed in the Design Chapter (CH3, page 10).

Pressure Considerations

To estimate the range of pressure measurements within the rodent knees, measurements of the general dimensions of the tibia plateau and the femoral condyles were taken.



[Figure 3: Tibia Plateau Cartoon] This diagram shows the measurements taken on the mouse and rat tibia plateaus. All measurements listed in results section of appendix

These measurements allowed for the preliminary prediction of pressure that could be expected in the knee by using Equation 1 where P is the pressure, W is the measured

weight of each animal, and R is the averaged value of the radial cross section of both sides of each knee measured in this study.

Equation 1

$$P = W / ((Percentage) * \pi * R^2)$$

“Percentage” term is used to scale the area of contact to show what the pressure would be if only that percentage of the femur condyle surface area is in contact with the tibia plateau. Pressures shown in (Table 2) and (Table 3) uses the results from equation one and show how the contact area effects the total pressure (for example 10% indicates 1/10 of the measured area). This data provide a range of possibilities for what the magnitude of the contact pressure could be, since the actual contact area within the knee is unknown.

Table 2: Calculated pressure distribution within mouse knee

Rodent Mass (gram)	Estimated Pressure (Psi)				
	10% Contact Area	25% Contact Area	50% Contact Area	75% Contact Area	95% Contact Area
15	51.6733	23.66931653	14.33465826	11.22310551	9.912978033
20	67.2311	29.89242203	17.44621102	13.29747401	11.55063738
25	82.7888	36.11552754	20.55776377	15.37184251	13.18829672
30	98.3466	42.33863305	23.66931653	17.44621102	14.82595607
35	113.904	48.56173856	26.78086928	19.52057952	16.46361541
40	129.462	54.78484407	29.89242203	21.59494802	18.10127475
45	145.02	61.00794958	33.00397479	23.66931653	19.7389341
50	160.578	67.23105509	36.11552754	25.74368503	21.37659344
55	176.135	73.45416059	39.2270803	27.81805353	23.01425279
60	191.693	79.6772661	42.33863305	29.89242203	24.65191213

Table 3: Calculated pressure distribution within rat knee

Rodent Mass (gram)	Estimated Pressure (Psi)				
	10% Contact Area	25% Contact Area	50% Contact Area	75% Contact Area	95% Contact Area
100	111.733	44.69317069	22.34658534	14.89772356	11.76136071
150	167.599	67.03975603	33.51987802	22.34658534	17.64204106
200	223.466	89.38634138	44.69317069	29.79544713	23.52272142
250	279.332	111.7329267	55.86646336	37.24430891	29.40340177
300	335.199	134.0795121	67.03975603	44.69317069	35.28408212
350	391.065	156.4260974	78.21304871	52.14203247	41.16476248
400	446.932	178.7726828	89.38634138	59.59089425	47.04544283
450	502.798	201.1192681	100.5596341	67.03975603	52.92612318
500	558.665	223.4658534	111.7329267	74.48861782	58.80680354

Results from this study provided a starting point for the design of the test fixture that would hold the test specimen as well as the expected pressure range within the knee due to the applied load to the knee. Length averages of tibia and femur will drive the constraining sizes of the testing fixture design. Pressure calculations can be used to determine loading range so that measurement devices can be considered. Also, tests performed after fabrication of machine can also be compared to the pressure calculations to determine how much contact area is truly used within the knee.

DESIGN OF MACHINE

Requirements

The overall requirements of SLAMR are to:

1. Apply a static load to a female C57 / Black 6 mouse or to an adult male Sprague-Dawley and Wistar rat hind limb and
2. Provide an operable test range of leg flexion angles from 100° to 135°.

The specific choices of mouse and rat give the smallest and largest animals that can be tested, thus defining the working dimensions of SLAMR. Motion of the hind limbs is as natural as possible so as to mimic true loading conditions that a mouse or rat would apply to its knee. SLAMR will be utilized through application of compression of mouse and rat knee joint at various flexion angles to examine the effects of knee joint destabilization on intra-articular contact pressure distributions.

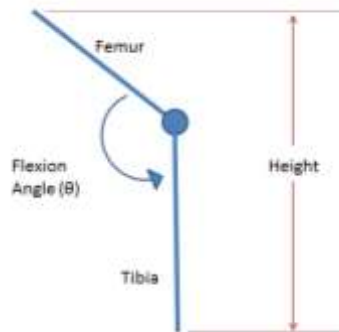
Degrees of Freedom

The degrees of freedom (DoF) for SLAMR were split into the two categories of pre-test and testing. Pre-test DoF were used to position the machine into the desired testing position; they are then being locked into place while testing is conducted. These lockable motions are all required to adjust for the varying sizes and shapes of the hind limbs of the individual animal legs. Once the setup is complete and the pre-test DoF are locked, the testing DoF are the only motions allowed with the driving requirement of mimicking the natural movement of the hind limbs. Together the pre-test and testing DoF

allow the researcher to test a variety of mouse or rat leg sizes in as natural a configuration as possible.

Pretest Degrees of Freedom

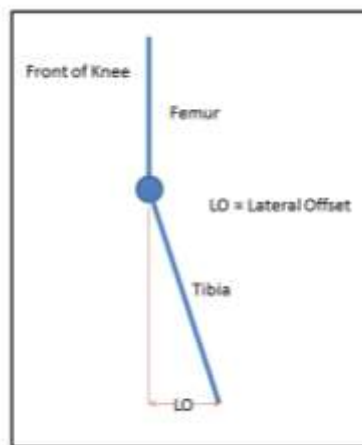
Prior to testing there are several adjustments that the testing fixture must be capable of accommodating in order to hold each leg. First, there is a significant size difference between a mouse and a rat leg, with the rat leg being over twice the size of the mouse leg (Table 1). Accommodation of both the rat and mouse legs within the fixture requires adjustment to a range with the high end of a rat leg with max flexion angle of 135° , and the low end of a mouse leg with a 90° flexion angle ([Figure 4]). Using geometric relations of the leg (Equation 2) and the values from (Table 1) the maximum and minimum leg heights that the fixture must accommodate were found to be 65.8mm and the minimum height of a mouse leg at 90° flexion was found to be 18.1mm.



[Figure 4: Leg Geometry] Illustration of how maximum and minimum leg heights are calculated for test apparatus design.

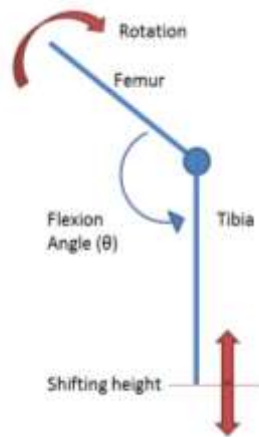
$$Height = (Tibia Length) + (Femur Length) * \sin(\theta - 90^\circ) \quad \text{Equation 2}$$

Secondly, the testing fixture must accommodate for the lateral offset of the mouse and rat knee ([Figure 5]). To allow lateral offset of any amount the length of the rat tibia was used for the distance defined as LO. After finding the high and low end ranges for both the height and the lateral offset the testing fixture was designed to a safety factor of 1.5 plus to account for any abnormally large or small legs.



[Figure 5: Lateral Offset] Illustration of the lateral offset of the knee

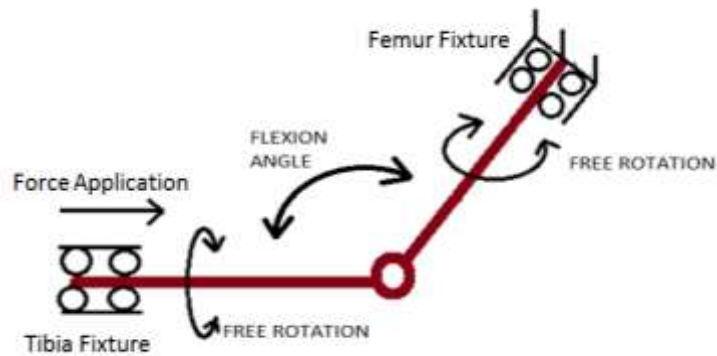
To obtain any flexion angle within the required range, one of the mounting points for the rodent knee is required to rotate in line with the knee. Using an up down motion and this rotation, the knee can then be flexed to change the height and flexion of the knee. Lastly, one mount is required to shift with the applied loading that produces a shifting height from the deformation of the material within the knee ([Figure 6]). With these pretest freedoms, the testing apparatus is capable of testing legs ranging from mice to rat size, at any flexion angles within the required range.



[Figure 6: Rotation of Flexion] Illustration of how leg will be required to rotate and translate to accommodate flexion of legs.

Testing Degrees of Freedom

To obtain a test that mimics the natural loading of a mouse or rat leg some specific DoF will be required. In order to obtain these motions several quadruped animals were observed and verified through review of past treadmill studies (8). By allowing natural motion of the leg, measured pressures in the knee are believed to be as close to the naturally occurring pressures as possible. One major obstacle for this is that while the leg needs to be allowed to move naturally it must also remain fixed at a certain flexion angle, ([Figure 7]) so that pressure distribution through the knee at a certain flexion can be measured. Given the asymmetry of a mouse or rat knee, it was determined that some rotation about the tibia fixture and femur fixture must be required for a smooth loading condition. Also motion in the direction of the force applied to the tibia will be required for the measurement of meniscus and cartilage displacement ([Figure 7]).



[Figure 7: Free Body Diagram of Test Leg] Shows the boundary conditions allowed during test of a leg tested with SLAMR

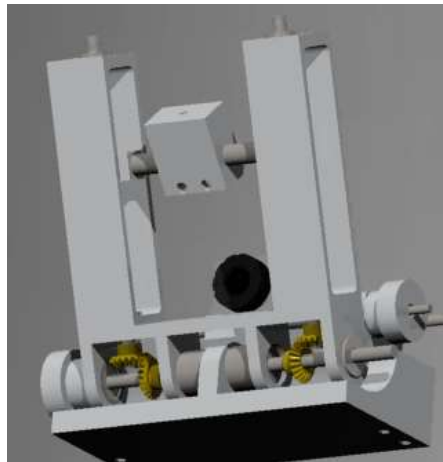
Design Challenges

During the design, manufacture, and test of SLAMR several issues arose that needed to be overcome to make a functional test apparatus. The most prominent issues included the upper pot gearing system, the locking upper pot mount, the load application, the upper pot stability, the effects of pressure paper on knee mechanics, and the rotation of the tower uprights. These challenges were all met and overcome with design alterations ultimately leading to a functional test apparatus for the Biomechanics laboratory and Montana State University.

Gearing System

A gearing system was devised to enable the upper pot to translate up and down such that SLAMR can accommodate a range of leg sizes. Given the clearance issues of the tower assembly, the gears had to be facing opposing directions. Use of opposing bevel gears as pictured in (Figure 8) would however create the problem of two threaded rods spinning in opposite directions, thus resulting in one side of the pot mount going up

while the other travels down the threaded rod. In order to use this configuration of gearing the solution of using a reverse threaded rod on one side and a standard thread on the other was devised. This allows the opposing gears to turn the threaded rods in opposite directions while having the desired effect of the pot mount sides traveling in the same direction when the nob at the base is turned. When assembling the tower it is important to get the upper pot horizontally aligned so that it travels up and down both threaded rods at the same location. If not aligned the upper pot will be off centered and the angle of flexion could be misaligned during testing.



[Figure 8: Gearing Assembly] CAD model of tower assembly illustrating the gearing system

Locking Upper Pot

As stated in the Degrees of Freedom section of this chapter (page 10), the upper pot must be locked into the pot mount linearly while still being allowed to rotate. The simple solution of giving the upper pot mount a positive tolerance hole coated in graphite

for the pot to slide then to lock the pot into the mount linearly a pin is used ([Figure 9).

The pin allows the pot to rotate while restricting it from sliding back out of the mount.



[Figure 9: Locking Pin] Pin in red circle used to keep pot from sliding back out of fixture.

Pressure Paper Thickness

Mapping the pressure of the knee will require as natural of configuration of the knee during test or else the testing will not mimic the true loading of the leg. Hindering the natural movement of the limb would result in offset data; however with a system so small the only option within the budget for the initial testing of SLAMR was to use Fuji-Film pressure paper. This film requires two sheets of chemically enhanced plastic which together are about 1.8mm thick. When pressure of the range predetermined for each paper is applied, the chemicals react from the transfer paper to the recording paper, and change the recording paper various shades of red. Testing study of pressure paper is discussed in detail within Testing chapter (CH4, page 38) and Results chapter (CH5, page 46) to determine validity of use.

Load Application

The static loading apparatus portion of SLAMR evolved from a horizontal system to a vertical system. Horizontal loading was investigated with the reasoning that the knee should be in its natural orientation while loaded. Later it was decided to switch to the vertical orientation due to the self-induced moment created by the load cell hanging in the push rod. This self-induced moment could have potentially been larger than the actual collected data resulting in large noise values. A vertical system was designed and manufactured to reduce the data collection noise from the loading system.

Horizontal Load Application: This system was designed around having a large weight pot that while unloaded sits upright on two stainless 3/4inch rods. It is held up by a horizontally mounted spring system connected to the diagonal rods. While loaded the weight pot travels down the stainless rods, pushing the diagonal rods out as shown in (Figure 10). The diagonal rods connected to the drive shaft in the middle and travel down fixed rods on either side of the apparatus. Issues with the system seizing due to off balance were one of the critical design challenges with this system. This combined with the weight of the load cell, caused the horizontal system to be abandoned in favor of the vertical system.



[Figure 10: Horizontal Weight Drive System] initial weight driven load system created, but not used due to concern of moment force from load cell suspended from shaft.

Vertical Load Application: Changing from the horizontal to the vertical system required several new parts to be fabricated, but the system is far less mechanically complex as shown in ([Figure 11]). A stand was created for the existing base by creating simple T-channels in tow pieces of aluminum. The same pillow block is used to guide the existing push rod, and a simple cylindrical pot was created to attach directly to the load cell. Lastly two channeled aluminum blocks were fabricated so that the pillow block's distance from the tower assembly could be altered with ease.



[Figure 11: Vertical Loading System] during testing the cylindrical pot attached directly to the load cell is loaded with vials of steel shot, and the compression distance of the spring is measured via the digital dial indicator at the top.

The compression distance is measured for each test while loading, so that the force absorbed by the spring can be subtracted from the total measured force. This would result in finding of actual load applied to the leg during each test which is discussed further in testing chapter (CH4 page 26) for the calculation of the spring constant and the actual applied load to the leg.

Flexion Angle

The original design of the tower, shown in ([Figure 12), allowed for the desired DoF including the rotation of the upper mount which was intended to achieve desired flexion angles.

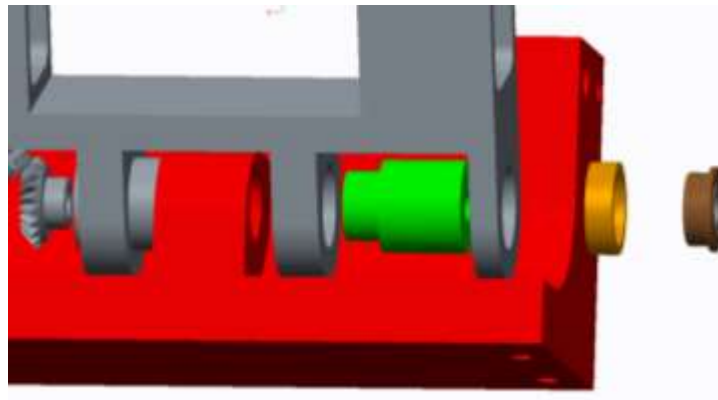


[Figure 12: Original Tower Assembly] designed to hold the leg in place while allowing desired DoF during loading.

When testing, it was observed that the flexion angles were limited to a range of about 90° to 120° of flexion in a mouse leg. Given the requirement of a range from 100° to 135° in both mice and rat legs this issue needed to be resolved. It was observed that the upper pot would require to be positioned further away from the lower pot as the leg size is

increased. To accommodate this adjustment, a pivot of the uprights was designed allowing for flexion angles through and beyond the required range.

Rotational Tower Alteration: The pivoting motion is provided by the green part in ([Figure 13]). It is made from a steel $\frac{1}{2}$ -20 threaded rod for the larger diameter portion then machined to a smaller diameter portion that fits within a second hollow $\frac{1}{2}$ -20 threaded rod that connects on the opposing side of the tab in the red base in ([Figure 13])



[Figure 13: Rotational Tower Modifications] new assembly required incorporation of pivotal mount system shown as green and orange part.

The pivot motion is provided by this pressed fit portion of the pivot because the hole in the base part (red part in [Figure 13]) will have a tolerance slightly above the dowel diameter. This fit provides a snug pivoting motion that can accommodate the tower in any position. However, for extra security a lock nut was implemented between the uprights and the base part that locks against the base part to ensure the uprights will not move during tests. The pivot has one last feature, specifically a clearance hole to allow the gear drive rod to pass through it allowing for the movement of the upper pot. In order to maintain the upper pot mount drive there are inserts (represented as the orange part in

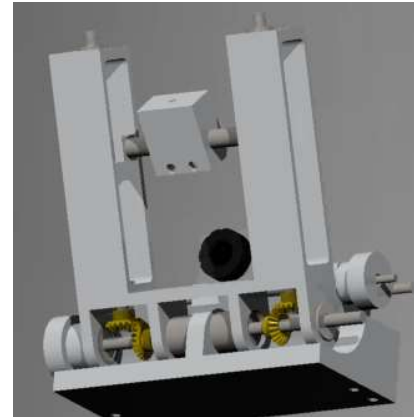
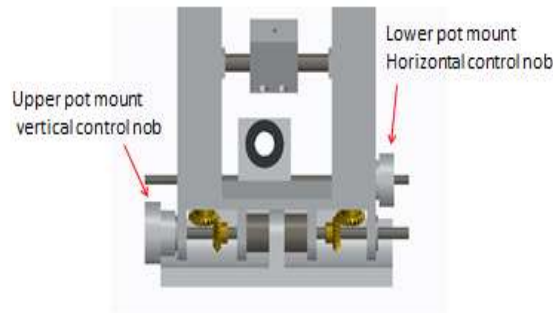
[Figure 13) that have a ½-20 outer thread and a 10mm reamed inner hole to accommodate the existing bearings (brown in [Figure 13) used in the system. This insert was created to allow for assembly of the green pivot joint to a ½-20 thread size which has the benefits of ease of machining, structural integrity, and assuring no thread interference between the outer and inner diameters for any of the parts involved in the pivot or upper pot mount drive assemblies.

Assemblies

The design of the machine was separated into two major assemblies: the tower assembly and the loading assembly. The tower assembly was then separated into further subassemblies called the upper and lower pot mount subassemblies. Each subassembly serves the purpose of housing one of the two fixtures attached to each leg using potting procedure discussed in the setup section of the Testing chapter (CH4, page 26).

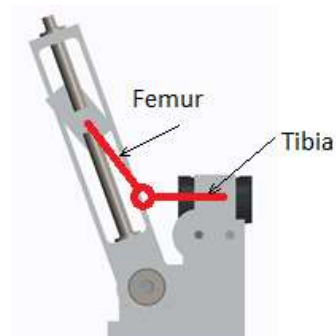
Tower Assembly

Development of a testing fixture was required to interface between the mouse or rat leg and the mechanical loading and measurement system. This system is known as the “tower” for the duration of this document and is pictured in ([Figure 14).



[Figure 14: Rotational Tower Assembly] modified from the original tower configuration shown in previous section.

This assembly consists of both an upper and lower pot mount, which are the mounts that hold the ends of the hind limb. These pot mounts are required to allow the leg to rotate freely about the femur's long axis as well as the tibia's long axis. Also, one of the pot mounts is required to allow for a linear translation so that a load can be applied to the leg, without pushing it out of the opposing pot mount.

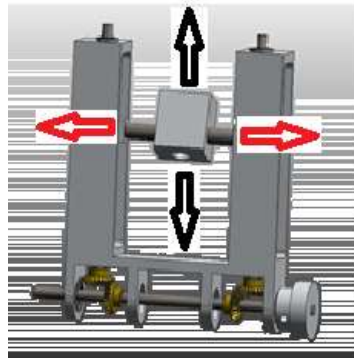


[Figure 15: Knee Orientation in Tower] Tower assembly is capable of rotating with the knee to achieve any various flexion angles desired between 80 and 180 degrees.

The mouse or rat leg is loaded into the assembly with the femur pot in the upper pot mount, and the tibia in the lower pot mount. The uprights of the tower can then rotate

with the knee to allow the desired flexion of the leg and along with the rotation of the upper pot mount about its horizontal translation axis.

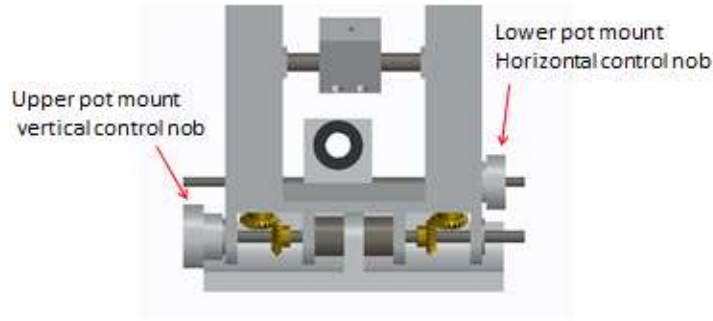
Upper Pot Mount: The upper pot mount can translate both vertically and horizontally with respect to the tower ([Figure 16]), as well as being able to rotate the mount position about the horizontal axes of the pot mount itself. The motion in the horizontal direction is needed for alignment of the legs to their natural lateral offset position. Translation in the vertical direction of the tower is for the accommodation of different leg sizes and is driven by the lower nob pictured on the right side of tower in ([Figure 16]).



[Figure 16: Upper Pot Mount Assembly] Translation in the vertical plane (black arrows) and in the horizontal plane (red arrows) also upper pot mount can rotate about the horizontal axis (in line with red arrows)

This lower pot mount control nob ([Figure 17]) is connected to a bevel gear system that rotates threaded rods which in turn rotate within the aluminum blocks supporting the upper pot. The vertical translation also allows for different leg positions to be tested when

combined with the rotational freedom of the pot mount.



[Figure 17: Tower Control Nobs] upper pot control nob (on left) and lower pot control nob (on right)

Lower Pot Mount: The lower mount translates only in the horizontal direction with respect to the tower and is controlled by the upper nob pictured at the left side of the tower in ([Figure 17]). Along with horizontal translation, the pot mount is equipped with a linear bearing where the load is applied to the bottom side of the pot that is placed within the mount. The pot inside the linear bearing is allowed to translate linearly through the bearing when a force is applied, free to rotate while the load is applied.

Loading Assembly

Loading the leg with a known weight was one of the primary requirements for SLAMR. To meet this requirement, the loading assembly was created. Simplicity of design and operation was desired for this system and several iterations of the design were made as discussed in the design challenges section of this chapter (page 14). The final design utilizes a load cell in line with a weight pot and an eight millimeter rod that is aligned with the lower pot mount's linear bearing as pictured in ([Figure 18]).



[Figure 18: Loading Assembly] Load cell with weigh pot directly mounted. Pushrod-load cell couple sits directly onto spring that compresses into pillow block. When loaded spring compresses and push rod is forced downward.

A pillow block is used to guide the push rod into the lower pot mounts linear bearing.

The pillow block is mounted to the base plate that helps to align the loading assembly with the lower pot mount subassembly of the tower. The load cell and the push rod weights are countered by a spring that is used to return the system to the unloaded state. With no spring in the system there is no recovery mechanism and the assembly will apply the full weight of the system which is greater than 2lbs, and thus far higher than the desired loading range of SLAMR.

TESTING

Setup

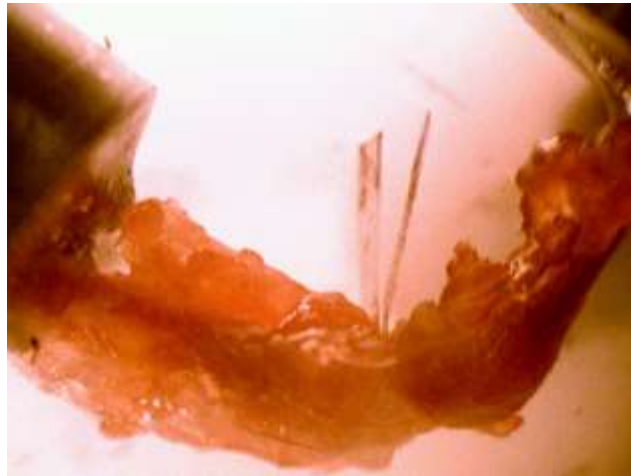
Several procedures are required before every test to insure calibrated results are created. These procedures involve weighing the animal using (Precisa 310C digital scale); this is done to ensure accurate weight loading percentages can be calculated for each test. Once the animal is weighed, the hind limbs were prepared for potting; first the skin is removed by cutting a circumference about the leg then pulling the skin gently from the leg towards the ankle. Secondly the foot is removed at the ankle joint, with care being taken not to damage the bone. The soft tissue is then removed to the bone from the outward ends of both the tibia and the femur. Once cleaned the rodent leg is severed from the carcass with care taken to not to damage the bone or puncture internals of the animal. Any residual soft tissue is then removed from the bone. Once cleaned and removed from the carcass, the hind limb is ready to be “potted” for testing.

Potting

A mechanical interface needs to be created to enable testing of the biological system of a mouse or rat leg with the mechanical loading apparatus. The mechanical interface or “pot” creates a usable means of testing that can be mounted to the mechanical testing interface outlined in the Tower Assembly section of Design Chapter (CH3, page 21). Potting is done by filling section of aluminum piping with epoxy, and placing either the free end of the tibia or the free end of the femur into the epoxy while it sets. Once set

the pot is permanently attached to the leg, and can be used as a mechanical interface to the testing apparatus.

When potting a biological system into a mechanical system there are a couple of requirements: (1) the mechanical system must fit the testing apparatus that is to be used for experimentation and (2) the epoxy must not harm the biological system while chemically hardening into a solid interface. Potting for SLAMR utilizes an 8mm aluminum pipe cut into 12mm sections. Once the leg is potted the pressure sensing mechanism is inserted into the knee before mounting the leg into SLAMR for testing ([Figure 19]).



[Figure 19 Knee Prepped for Testing] Pressure film inserted into back of knee after potting and prior to testing.

Pressure Measurement

Finding the pressure distribution within the knee requires a data collection method that is within the confining dimensions of a mouse or rat knee as discussed in the Preliminary Measurement Study Chapter (CH2, page 6). For the initial testing pressure

measurements with SLAMR, Fuji-Film© pressure paper was used. This paper was selected due to the availability and relatively low cost to three dimensional imaging equipment or a two dimensional micro electrical mechanical sensor. Several concerns of thickness and rigidity were considered, and it was decided that running a battery of experiments with this measurement system (CH4, page 38) would be the best method to conclusively determine the validity of using pressure paper as a data collection method.

To test the knees, 1mm by 5mm strips of both the transmitting paper receiving paper inserted into the mouse knee. These dimensions were determined during the initial measurements described in the Preliminary Measurements chapter (CH2, page 6). Every test requires two strips of each type of paper to be inserted from the back of the knee ([Figure 19]). For initial testing the lateral side of the knee was tested during each run of the machine. This was done because it was found through experimental analysis that inserting the pressure paper between the meniscus and the tibia plateau was only possible on the lateral side of the knee. Each set of pressure paper is scanned after testing and compared against a calibration chart provided by Sensor Products Incorporated using a custom computational program (Matlab 7.12.0 R2011a) code created by the author to generate pressure gradient readings. These gradients are mapped for each strip of receiving paper to identify the overall area of high pressure within the knee for the ranges of flexion that are tested.

Loading

After the leg has been removed from the specimen, potted, pressure measurement device inserted, and mounted into the tower assembly the testing commences. First the

data collection software is started; the load pot is loaded with the pre-calculated weight that pertains to a percentage to the weight of the individual animal. Sensor Products Incorporated Company provided instructions for use of pressure paper which indicated that best results are obtained by waiting one minute for paper to fully react. Therefore for each test the loading was allowed to sit for one minute. SLAMR is then unloaded by first removing the weight from the loading apparatus, and then the pressure paper is removed from the knee before removing the leg from the upper and lower fixtures. If further testing is to be done the leg is first sprayed with 20% Phosphate-Buffered Saline (PBS) solution which helps to maintain a constant pH level that helps maintain cellular health as well as keeping the knee from drying out between tests.

Operation of SLAMR

Testing begins by first initializing the load cell run code (LabVIEW©) with the name of the test. To test the leg the initial reading of the digital extensometer (Mitutoyo Corporation 543-611B) is recorded as a zero point, and then the load cell code is initiated. Once the code automatically zeros the load seen by the load cell the weight pot is loaded manually by gently placing plastic test tubes filled with steel shot into the pot located above the load cell. Weight of steel shot and plastic test tubes match the percent body weight loading desired for each individual test. The displaced value of extensometer after loading is recorded and a dwell period of at least one minute is provided for the loading to be captured on the pressure paper. After the dwell the load cell data collection code is stopped and the end displacement of the system recorded. Once displacement is recorded the weight system is lifted from leg by physically pushing it upwards with a

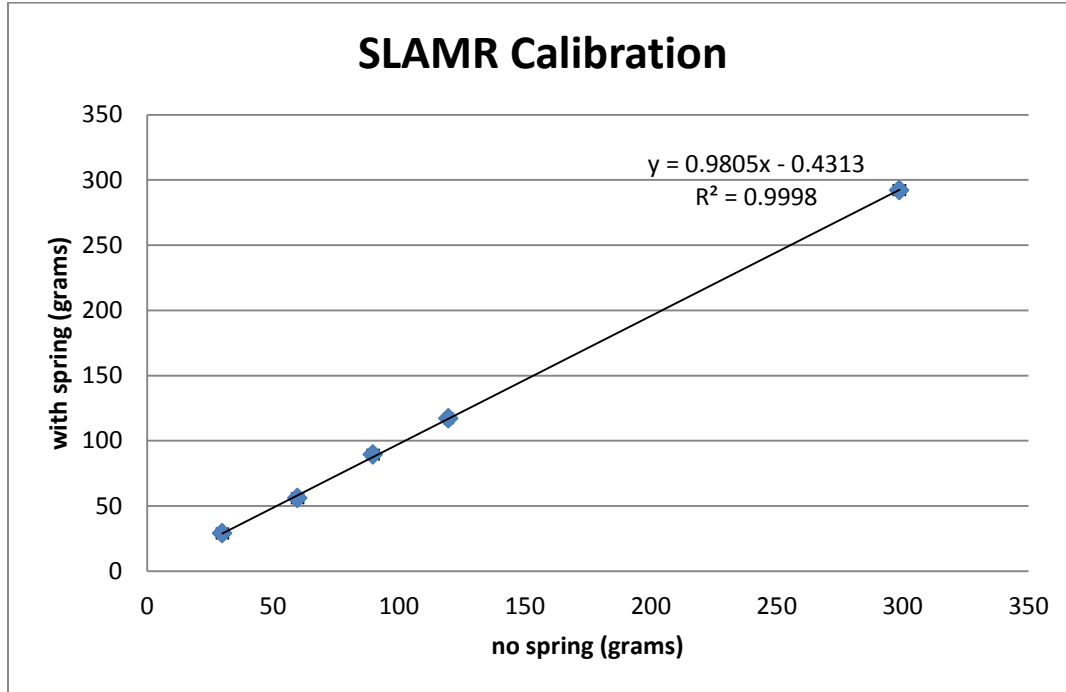
thumb. While loading is removed the pressure paper strip is pulled from the knee and attached to the worksheet in its predefined location. After all desired tests are finished for each leg the worksheet containing the pressure paper test strips is scanned as a 1200 DPI, greyscale, tiff file for processing.

Calibration

Load Application System

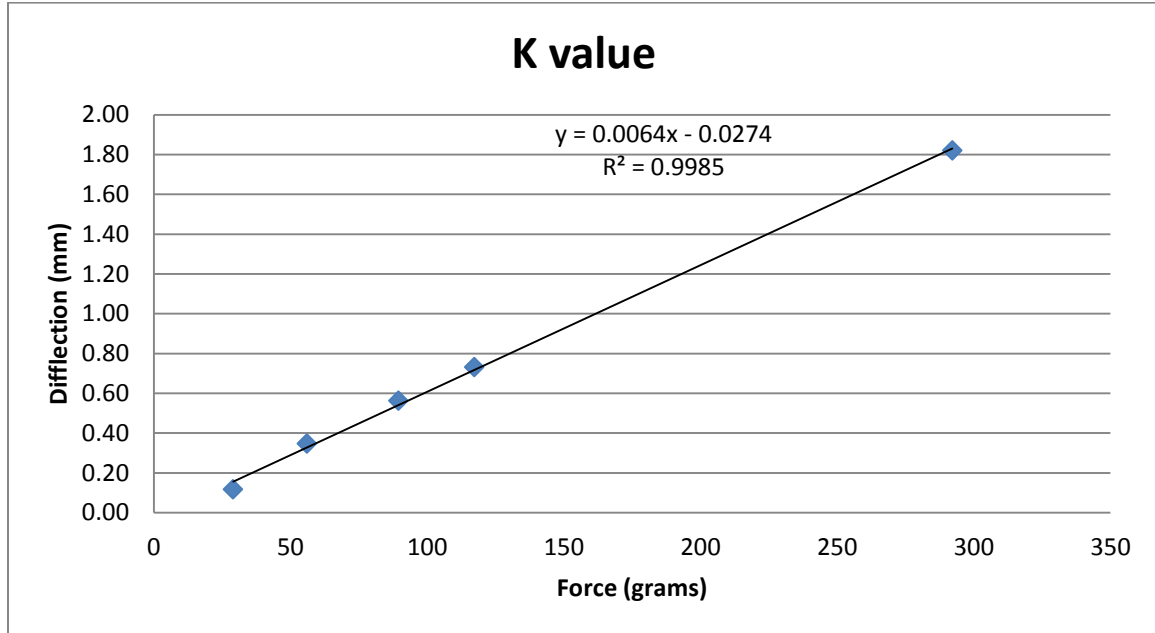
Before testing can begin the load application system must be tested to insure accuracy. These calibration tests are performed without a test specimen and used to prove accuracy as well as calculate the load that is absorbed by the spring. To do this first ten tests per increased weight value are run, with five tests utilizing a system with no spring in it and five tests utilizing a system with the spring in it. Each trial is allowed to run for 20 seconds, and the loading values are averaged over the trial time. Gold Standard calibration weights (Troemner inc. 8154F 30G S/S E/B cly. Class 1, 02-215-17A) were used at the values of 30, 60, 90, 120, and 300 grams, were each of the ten tests consisted of trials utilizing one of all five gold standard loading values. After all ten tests are conducted the load value for each test is then found by averaging all of the trial values. This data is used to develop the plot in ([Figure 20) to show the accuracy of the system with the spring in line.

The optimal outcome for this procedure is for the system “with spring” to display a linear trend when charted in comparison to the system “without spring”. As shown in ([Figure 20) the system measurements with the spring in used does indeed show a linear trend when plotted versus the system measurements without the spring.



[Figure 20:SLAMR Spring Calibration Chart] Chart showing the comparison between loading the load cell with no spring in the system (only force on load cell being the applied gold standard weight) to loading the load cell with a spring in the system (both spring and gold standard weight forces acting on the load cell).

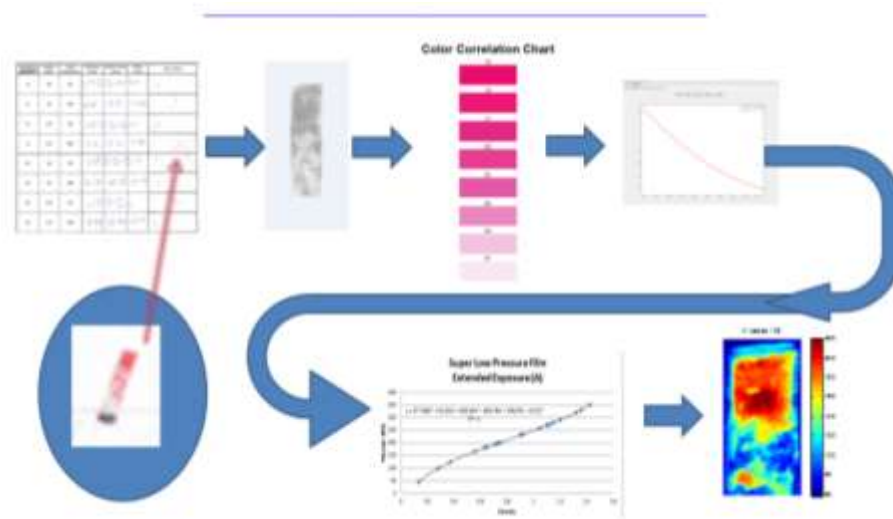
Spring Constant: While conducting the tests with the spring in line, the digital extensometer values are used to find the distance that the system compresses the spring while being loaded. These distances and the measured loading are then used with the force equation $F=kx$, where F is the force applied, x is the distance compressed and k is the spring constant to find k of the spring for the usable loading range ([Figure 21]).



[Figure 21: Spring Constant] spring used to counteract the weight of the loading system while unloaded is proved to have a linear trend within loading range.

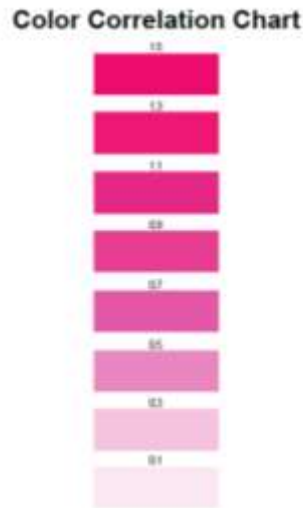
Pressure Paper

Pressure paper is used during initial testing as an attempt to find a valid method of pressure mapping within a mouse knee. For the initial testing of SLAMR this was the most cost effective method to assess the proof of concept for the apparatus, and therefore a study was deemed necessary to analyze the validity. The analysis of the pressure paper ([Figure 22]) resulted in several methods to analyze this measurement technique. If validity is proven pressure paper will continue to be used in future research beyond the scope of this report; otherwise it must be replaced by digital measurement device or a three-dimensional imagery machine.



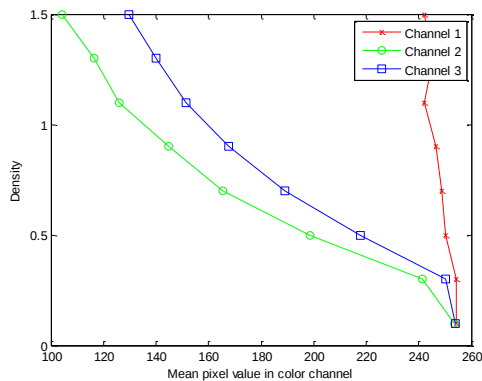
[Figure 22 Color-mapping Process Outline] Flow chart on process used to convert pressure film strips to color map images.

As background, pressure paper utilizes a chemical reaction that takes place when micro-bead chemical bubbles on a transfer sheet burst and discolor the receiving sheet to a shade of red. If more pressure is applied more bubbles will burst and create a darker shade of red which is known as a “density” measurement. This density corresponds to a max pressure that has been applied to that zone, and can be mapped using the provided color correlation chart ([Figure 23]), provided by Sensor Products Inc, that defines each shade within a scale of 0.1 to 1.5.



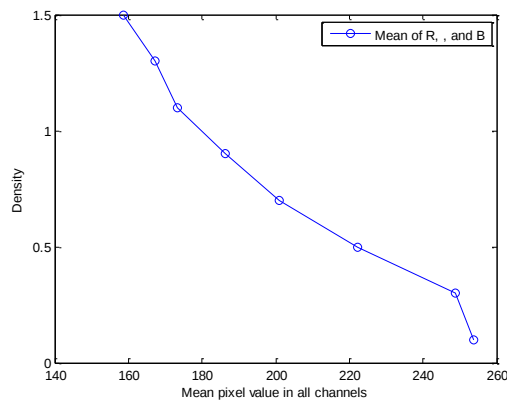
[Figure 23: Color Prescale Chart] shows the color densities that correlate to the shades of red that each test strip turns given a varied pressure.

Pixel to Density: To correlate the calibration values to the test strips a calibration study was conducted by cropping each square of color in ([Figure 23]) via computational program's (Matlab 7.12.0 R2011a) image processing toolbox, and averaging the three color pixel values (red, green, blue) using an in house generated code shown in Calibration section of Appendix (CH7, page 107). This code takes the tiff image file and converts each pixel to a numerical representative on the scale of 0 to 255. These values within the tiff code are given for each of the three channels and then each of the channels is averaged for the entire image. Each image is processed and the average result is plotted in relation to all of the other image files ([Figure 24]).



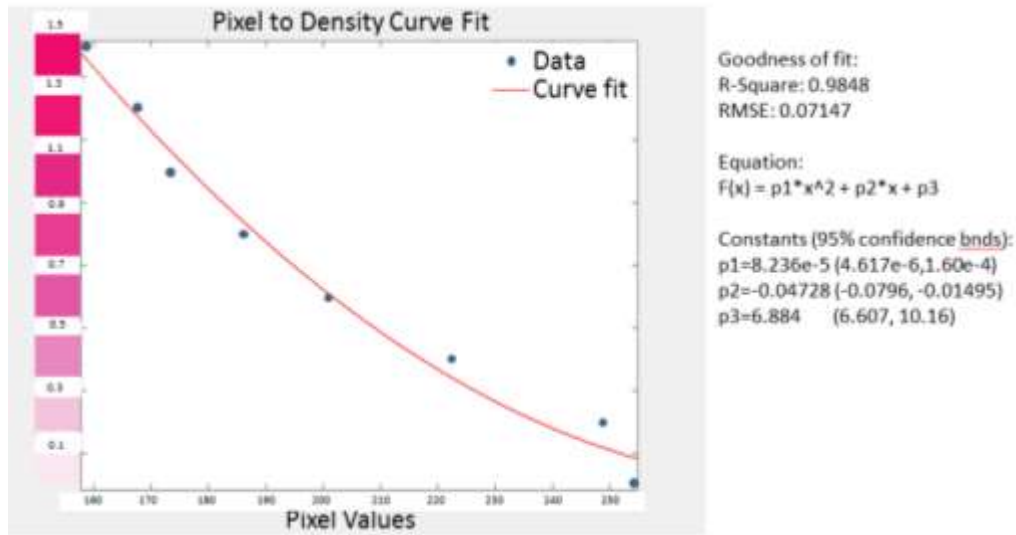
[Figure 24: Averaged Color Pixel Values] Extracted averaged value for each density square from the Color Correlation Chart plotted for each density.

The three averaged pixel values for each density block defined in the Color Correlation Chart is then averaged for each image file to find an overall pixel magnitude “density” correlation for testing ([Figure 25]).



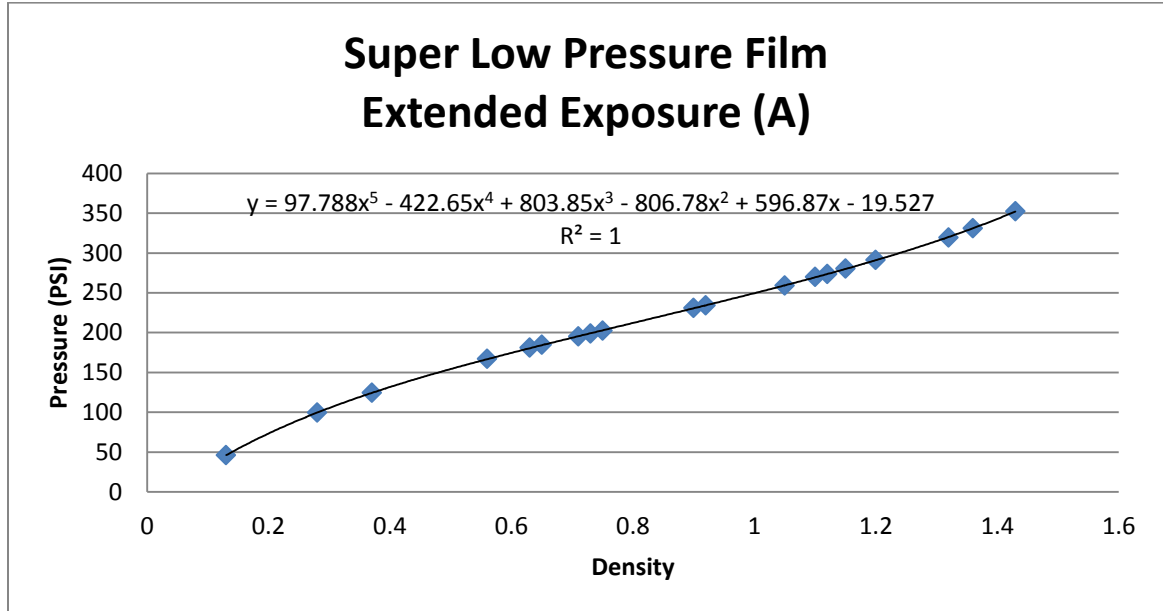
[Figure 25: Averaged Color Values] All three color averages from each density square is averaged to gain a pixel to density plot

Once the average pixel values are obtained the curve fit tool is used to generate the chart and equations shown in ([Figure 26). Using the generated equation, pixel values are translated into “density” values for each test strip.



[Figure 26: Matlab Curve Fit Tool] shows the equation obtained by applying a curve fit to the obtained pixel value to "density".

Density to Pressure Calculations: The Fuji-Film purchase package from Sensor Products Incorporated includes density to PSI calibration charts that are used to visually inspect test strips and extrapolate data. To obtain useful information, a study was conducted using known pressure values and the equation described in the Pixel to Density section of this report. The “density” values are then plotted versus the applied pressures ([Figure 27]).



[Figure 27: Pressure to Density] Chart obtained via experimental results using known pressure values to find correlating "density".

Once conversion equations were obtained, computational program (Matlab 7.12.0

R2011a) codes generated by author import the image strips and convert each pixel value from the 0 to 255 numerical pixel values to the corresponding pressure values.

Operating SLAMR

Loading SLAMR must be done while considering the biological structure of the leg, because the leg is much more fragile than the machine; the leg can be broken easily. First the femur pot is installed into the upper mount by sliding the pot into the bore-hole, then installing the retaining pin, and then snugging the pot back against the retaining pin using the screw on the backside of the pot mount. Finally the screw is unscrewed half a turn to allow rotation of the pot during test. Once leg is installed into the upper mount, the tower must be rotated towards the lower mount, the leg is positioned in front of the

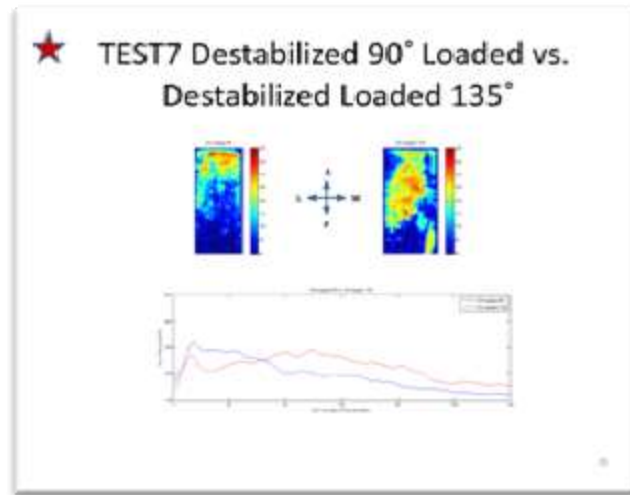
opening of the lower mounts linear bearing. Using the adjustment knobs ensure that the leg is aligned as naturally as possible, this is known when the tibia pot is able to insert into the linear bearing without any resistance. When the pot is touching the drive rod of the loading assembly it is fully installed. Then using the adjustment nobs and the rotation of the tower, the leg can be carefully positioned to the desired flexion angle using the digital goniometer as reference.

Initial Test Pressure Paper Processing Methods

For the initial testing of the apparatus it was decided to determine if pressure paper could be a viable measurement tool for mapping pressure intensities within the knee. To evaluate the results obtained, several methods were developed to quantify the data collected. These processing methods included two-dimensional color mapping of each strip with a corresponding pixel pressure value color bar, one-dimensional average row pressure charts, applied weight calculations, and a force correlation between applied loads from SLAMR and loads found by pressure strips. Some complications are the relative thickness of the pressure sensor (e.g. Fuji film pressure paper), matching the curvature of the contact surface of the mouse knee, the application of load, and the orientation of loading. With larger knees there have been studies using pressure paper resulting in an equation to define how thick the pressure paper for use with mouse knees could be in relation to the contact size (3).

Color Mapping Images

The pressure sensitive test strips are each taped to a worksheet developed for the testing to track the compressive displacement of the loading system with the file name corresponding to the loading data and test number. The worksheet is scanned with the (HP Scanjet G4010.) scanner in the Biomechanical graduate lab office as a 1200DPI, greyscale, tiff file. Images are created for each test strip by cropping the worksheet scan into individual sections using the Adobe Photoshop program. At this point each file strip is a randomly sized greyscale tiff file, and is stored in a file system used by computational program (Matlab 7.12.0 R2011a), identified in the Results section of the Appendix (CH7, 107). This program takes each greyscale file and converts the individual pixel values to density values, discussed in calibration section, and then to pressure values. Pressure values are then plotted with a colormap and color bar to show how the pressure was distributed through the lateral side of the knee for each test circumstance ([Figure 28]).



[Figure 28: Color Map Example Result] shows the end result of the color mapping and averaging processing methods.

Average Row Pressure

Once the color mapping is completed, each row of the image matrix is averaged to find the one dimensional pressure distribution of each test ([Figure 28]). These plots can be overlaid to show that the pressure zones inside of the knee indeed change with flexion angle. Also, destabilized tests are compared to nominal tests to show that the pressure is better distributed with the meniscus intact. The results from these plots are discussed more in depth in the results chapter of this report.

Applied Weight Calculation

Before each leg is dissected from the animal, the total weight is measured using the (Precisa 310C digital scale). This information is required so that the test article can be loaded with a fixed percentage of the animal's original body weight. Testing procedures include tracking the distance that the loading assembly compresses the spring during each

test. This compression distance and the known k value of the spring allows the required applied weight to be calculated using (Equation 3)

Equation 3

$$W_T - k * x = W_A$$

Where W_T is the total weight applied to the system, k is the spring constant, x is the compression distance, and W_A is the applied weight to the leg. Using W_A the percent body weight applied to each leg can be calculated and compared to the pressure found within the knee with the pressure film strips

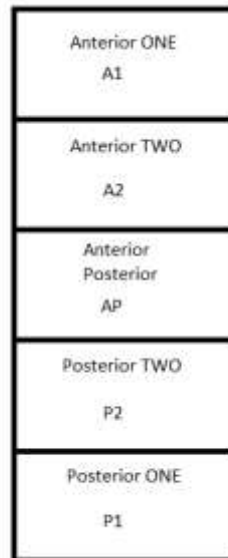
[Table 4: Calculated W_A example] weight actually applied to each of the four strips in Test one. N_90: Nominal state, loaded leg with flexion of 90°.

TEST#	TEST1	
xcel page	AP3	
Knee state	weight on leg	Percent of load
_(f.angle)	grams	%
N_90	33.67480848	80%
N_135	36.02346633	86%
DS_90	33.49161267	80%
DS_135	35.65382345	85%

The results were used to conduct a regional pressure measurement calculation battery, and results for all tests are shown in the Results section of the Appendix (CH7, page 112) and assessed in the Results Chapter (CH5, page 44).

Regional Pressure Measurement (RPM) Correlations

Using the applied loading of each trial the percent body weight loading was obtained. With this information, each like trial needed to be compared to determine any correlation of body weight to pressure distribution throughout the test strips. To do this, each test strip was split into five sections as shown in ([Figure 29).



[Figure 29: Regions of Measurement] Each trial strip was divided into 5 sections for pressure to %body weight measurement correlations.

Each section was analyzed via computational program (Matlab 7.12.0 R2011a) code shown in Results section of Appendix (CH7, page 112) and assessed further in the Results Chapter (CH5, page 44), to find the overall maximum, median, and mean pressure values. These regional pressure measurements were then compared to the same regions of other tests to find the statistical correlation between the percent body weight loading and the pressure.

Force Correlation

The final correlation attempted in order to determine if pressure paper was indeed a viable method of pressure measurement in a mouse knee was a correlation between applied loading and loading measured by the test strip. The total force applied to each strip was calculated by taking the sum of the integration of the pressure calculated for

each pixel by the area of each pixel (Equation 4). Where P_{pixel} is the pressure of each pixel, A_{pixel} is the area of each pixel, and F is the total force of the strip.

Equation 4

$$F = \int P_{\text{pixel}} * dA_{\text{pixel}}$$

Once the force applied to each strip was calculated the value was compared to the corresponding strips total applied load as well as the load calculated to be applied to the mouse leg.

RESULTS

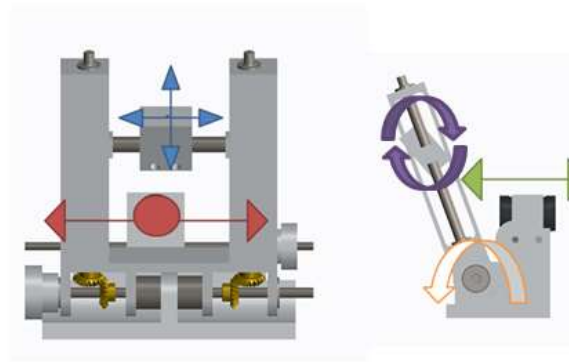
Testing Apparatus Performance

Fabrication of the testing apparatus was done primarily with hand crank mill and lathe operations, with the exception of most hardware and several external geometry features of the tower assembly which were completed via CNC operator. The tower was then tested with both small mice as well as large rat legs to the concluding result of a successful design.

Degrees of Freedom

Required DoF were reviewed in the Design of Machine chapter (CH3, pg.10). Then the design of the testing apparatus was created so as to meet these requirements. Challenges were overcome in the manufacturing process, as skills were developed with the result of an operational test fixture for the development of material properties within a mouse or rat knee.

Pre-Test DoF: The flexion of the leg is obtained through a combination of rotating the uprights of the tower, rotation of the upper pot about its axis, and translation of motion within the linear bearing in the lower pot mount assembly ([Figure 30]). Lateral offset is accommodated through the horizontal translation of both the upper and lower pot mounts. Results of the design effort were the fabrication of a testing fixture that can be used for the research and development of mouse and rat leg material properties.



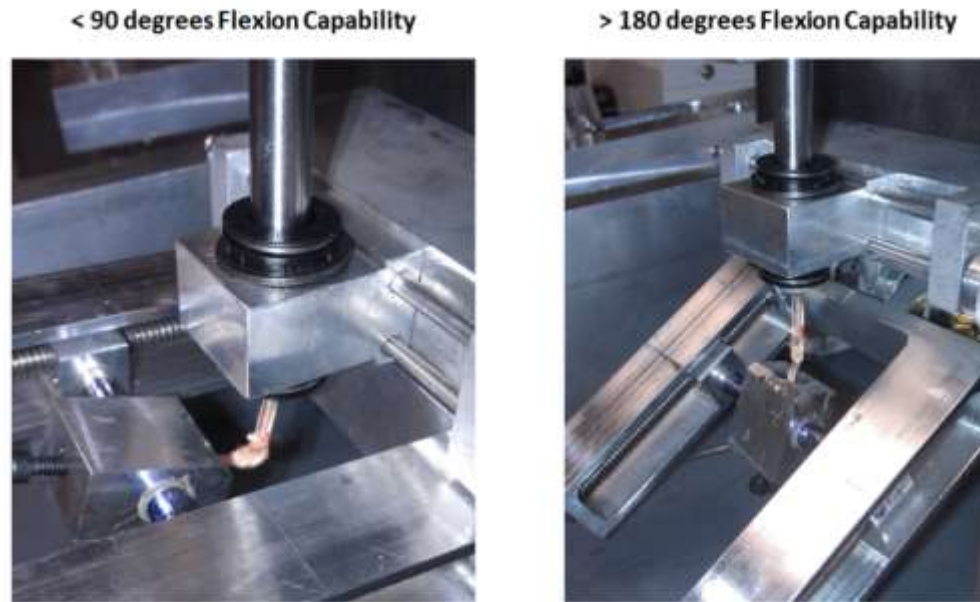
[Figure 30: Tower Assembly DoF] Blue: Vertical/Horizontal movement of upper pot assembly, Purple: rotation of upper pot mount about support, Red: Horizontal movement of lower pot assembly, Green: Loading assembly to test specimen contact point translation, Orange: rotation of uprights.

Testing Degree of Freedoms: The testing DoF's were all accommodated by allowing spin at each pot mount, and translation within the lower pot mount during loading. The upper pot mounts oversized hole with graphite powder was effective in allowing small rotations, while the linear bearing used as the lower pot mount accommodated the required large range of motion. The translation within the linear bearing required one alteration between linear bearings being the addition of small misalignments. The original solid linear bearing was destroyed due to a small offset, and it was replaced with one that could handle these offsets. After this replacement part was added to the assembly loading of the legs was smooth.

Flexion Angle

One of the primary requirements for the functionality of the testing apparatus was for it to have an operating flexion angle range for the leg between 90 and 180 degrees. With the combination of the tower uprights rotation, upper pot rotation, an upper pot

vertical motion the flexion range surpassed the required range discussed in the Requirements section of the Design chapter (CH3, page 10) to be from 100 to 135° ([Figure 31]).



[Figure 31: Flexion Range] Final assembly capable of flexion angles greater than 90 to 180 degrees.

Initial Experiment

For the inaugural testing of SLAMR, several conditions needed to be employed to test the bounding capabilities of the machines abilities. The required capabilities of SLAMR were to incorporate several DoF for both the setup of a range of leg sizes as well as to allow the leg to move as natural as possible. Also the flexion angle range was explored during several tests by comparing a 90° flexion loading to a 135° flexion loading. Maximum and minimum range flexion angles were tested on both rat and mouse knees to prove capability, however after proof of ability the initial experiments were

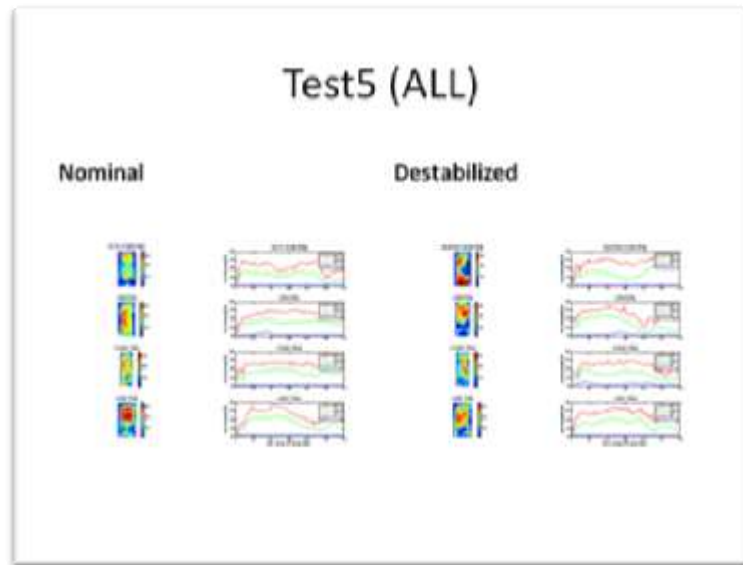
carried out with only mouse knees. These experiments were conducted with the knees both in their nominal state as well as a destabilized state at both flexion angles. Natural state tests are in reference to unaltered knee mechanics. Destabilized state tests are performed by removing the meniscus surgically from the knee, after natural state tests are performed, and then the same testing procedures are performed again. All loaded tests were performed with a control test of an unloaded condition to the same flexion angle where the leg is equipped with a pressure strip and loaded into SLAMR then the pressure strip is removed without loading the machine.

Pressure Strip Analysis

Color Mapping and 1-D Pressure Averaging: Conducting numerous trials per knee to obtain samples of pressure film for each desired setup proved difficult in that results tended to be noisy from one test to the next. While inserting the pressure film into the knee some initial resistance pressure from the knee was noted, resulting in some tests picking up undesired pressure measurements. With that said, there were some good results showing proof of concept that the pressure zone moves with flexion. Destabilized knee results also proved successful in showing that the removal of the meniscus resulted in a decreased pressure zone area and in some cases an increased overall pressure.

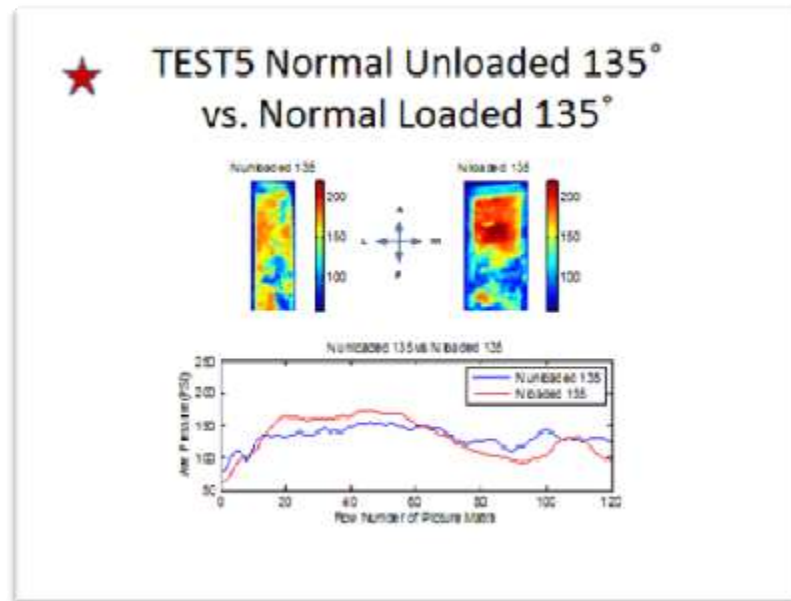
Experiments conducted on knees before destabilization, known as “Normal State”, were noted to have higher initial pressure, and thus resulted generally with higher overall test pressure readings throughout the test strip. The pressure film was plotted with an adjusted color scale to show the distribution of pressure values calculated using the method outlined in the calibration and processing sections of the Testing chapter. These

plots were compared side by side to a one dimensional pressure average plot to illustrate better where the pressure zones are localized ([Figure 32]).



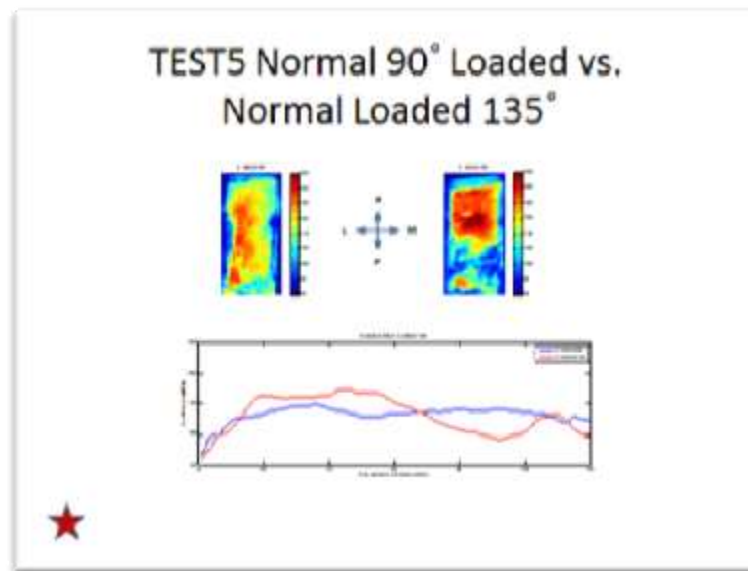
[Figure 32: All Test 5 Color Plots and 1D Distribution] Plots show how the min max and average values trace to the correlating color map of each strip for all trials of Test 5

As noted, some tests provided more favorable results than others. This was taken into consideration in the analysis of the results of this study. To determine the quality of each study the results were assessed visually by overlaying the one dimensional pressure plots of each loaded trial to the unloaded trial ([Figure 33])



[Figure 33: Unloaded vs. Loaded example] Figure shows the overlaid plot of the unloaded normal state 135 degree flexion angle to the loaded 135 flexion angle.

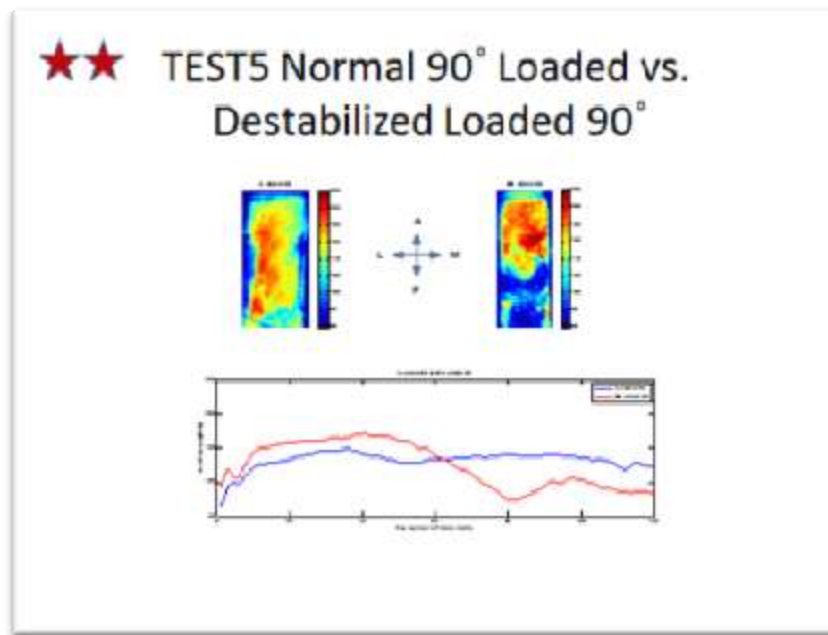
Visual comparisons were ranked high, mediocre, and low by placing a red star in the top left corner, bottom left corner, and no star respectively. This method of result interpretation was also used to assess the difference between loaded flexion angles for each nominal state test ([Figure 34).



[Figure 34: Normal State 90 vs 135 Degree Flexion] result example showing the pressure zone moving forward for the 135 degree flexion state

All results can be viewed in the Results section of the Appendix, and a good example of the pressure zones movement between flexion angles can be viewed in ([Figure 34]), where the one dimensional averaged pressure plot helps illustrate this movement as mentioned earlier.

To test this, each knee that was tested at normal state was surgically destabilized by removing the lateral meniscus. Then all tests that were conducted with the knee before destabilization were again conducted after the destabilization. The nominal state results were then compared to the destabilized results ([Figure 35]). After removal of the meniscus it was noted that inserting the pressure film into the knee became less resisted due to lower initial pressures. This was most likely due to there being less matter within the knee, resulting in a looser mechanical system.



[Figure 35: Normal State vs. Destabilized] Example of comparison between normal and destabilized state testing.

Regional Pressure Measurement Correlations: The RPM correlation results showed that the applied loading and median pressure in the AP region in destabilized knees at 135 degrees of flexion were weakly correlated ($r=0.6472$, $p = 0.0595$). Most other regional results showed less correlation between body weight and pressure distribution measurements, which may be attributed to the noise of the system. The raw data collected, and the analyzed result tables are shown in the RPM section of the Appendix.

Force Correlation: The results of the force correlation study between the load calculated to be applied to the leg and the test strip load ([Table 5]) due to the p value of all the correlations being much higher than the alpha value of 0.05, it is proven that there is no correlation between the applied load and the found test strip forces.

[Table 5: Test Strip Load vs Load Applied to Leg] Statistical correlation results for the comparison of force applied by SLAMR to leg and the test strip load.

Test Number	1	2	3	4	5	6	7	8	9
p	0.09526	0.60139	0.711433	0.415808	0.934356	0.504726	0.534168	0.219465	0.792102
R	-0.90474	-0.39861	-0.28857	0.584192	-0.06564	0.495274	-0.46583	0.780535	0.207898

Comparison of the total applied load to the found load in each strip was done in an attempt to prove correlation between the test strip and applied load with statistical results shown in ([Table 6]). The results of this correlation also proved to not be significant enough to prove correlation of the pressure strip forces and applied loading.

[Table 6: Test Strip Load vs Load Applied to Machine] Statistical correlation results for the comparison of force applied by SLAMR to leg and the test strip load.

Test Number	1	2	3	4	5	6	7	8	9
p	0.365043	0.944713	0.727325	0.367583	0.86849	0.438314	0.741887	0.142344	0.632564
r	-0.63496	0.055287	-0.27267	0.632417	0.13151	0.561686	-0.25811	0.857656	0.367436

CONCLUSIONS

Summary

The design and manufacture of SLAMR proved challenging given the need for interfacing a biological system with a mechanical testing apparatus. Six DoF were incorporated so as to accommodate legs of various sizes and three DoF were included during the actual testing of the machine, with the addition of an optional fourth. SLAMR proved to be effective at applying a load to a test specimen and will be used in the future for further study of the mouse and rat knee material properties and pressure zone mapping. Pressure film was conclusively proven ineffective as a tool for pressure mapping. All together, the creation of an operational test apparatus that meets the requirements of Applying a static load to a female C57 / Black 6 mouse or to an adult male Sprague-Dawley and Wistar rat hind limb and providing an operable test range of leg flexion angles from 100° to 135° was successful.

Future Testing

Ultimately SLAMR will be utilized to conduct mechanics studies on knees known to have OA, with the goal of obtaining the mechanical differences between healthy and degraded specimens. Material models can be explored and validated with the use of SLAMR, by measuring the force applied versus the deflection distance as was done for past human studies (3). With these findings finite element models can be developed with much more accuracy, and potential cures for OA can be explored as well as validated.

Recommendations

Force Feedback Loading

The mechanical loading system works well in applying a predetermined load to the leg, but comes with its own issues. Shock loading of the leg can occur even if loaded carefully because the loadings are so small which could affect results. Also the exact body weight percentage loaded on the leg can vary drastically and unpredictably with the current design. In the future SLAMR should be fit with a linear motor or pneumatic drive system driven by a force feedback code. This would allow the researcher more control over each experiment.

Leg Measurement

During the initial testing it was found that the displacement of each leg varied for each flexion angle, for the knee would compress different amounts and provide better mechanical resistance in higher flexion angles. This was expected, but if each leg's length of tibia and/or femur were measured prior to potting the exact mechanical loading resistance forces could be calculated per angle. Providing these forces would then tell the user how much of the displacement is mechanically driven, and how much is material displacement or yield.

Electronic Pressure Sensor

Results obtained in this study were a good proof of concept that SLAMR can be used to study the flexion and degenerative effects on mouse and rat knee mechanics. Any future studies will be done utilizing a digital pressure mapping sensor inside of the

knee. This should greatly reduce the noise produced when needing to re-insert new pressure film strips for every test. With a sensor flexion angles could be produced for a full spectrum of angles, showing more clearly the high pressure zones of the knee.

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APPENDICES

APPENDIX A

PRELIMINARY CALCULATION MEASUREMENTS

LEFT LEG													
	DOB	DOD	Weight	L Leg (Lengt	L. Femur	L (x) condyle	L (y) condyles	L Tibia (Length)	L (x) Plateau	L (y) Plateau	L side A Plateau	L side B Plateau	L GAP Plateau
Letter	-2012	(2012	(grams)	(mm)									
K	10/10/2011	4/9/2012	28.88	34.03	16.16	2.98	1.71	19.25	2.98	2.53	Measurements unknown to be needed at time of dissection		
N	10/10/2011	4/9/2012	30.26	33.76	15.74	2.95	1.74	18.3	3.01	2.98			
O	10/10/2011	4/9/2012	26.55	32.9	15.19	2.92	1.82	16.84	2.94	2.28			
954			27.49	Measurements irrelevant at time of dissection					2.74	2.74	1.1	1.07	0.57
956			29.14						3.24	3.24	1.21	1.27	0.76
RAT													
Letter	-2012	(2012	(grams)	(mm)									
George	unknown	4/24/2012	342	75.74	35.82	8.58	6.83	41.82	8.03	7.12	Measurements unknown to be needed at time of dissection		
jane	unknown	4/25/2012	338	72.92	35.79	7.85	6.29	39.51	8.04	7.01			
											0	0	0
				R. Leg (Lengt	R.Femu r	R. (x) condyle	R. (y) condyles	R.Tibia (Length)	R. (x) Plateau	R. (y) Plateau	L side A Plateau	L side B Plateau	L GAP Plateau
Mouse													
Letter				(mm)									
K				34.04	15.91	2.73	1.93	18.04	2.91	2.71	Measurements unknown to be needed at time of dissection		
N				34.05	15.9	2.79	2.45	18.44	2.96	2.75			
O				32.94	15.4	2.95	1.9	17.55	3.14	3.18			
954				Measurements irrelevant at time of dissection					3.07	3.07	1.25	1.27	0.55
956									3.05	3.05	1.14	1.34	0.57
RAT													
Letter				(mm)									
George				75.5	34.87	7.05	7.75	41.53	8.03	6.86	Measurements unknown to be needed at time of dissection		
jane				74.53	35.5	7.55	7.45	39.98	8.07	6.8			
											0	0	0

red is assumed value due to
the square nature of previous
measurements

red is assumed value due to
the square nature of previous
measurements

APPENDIX B

CALIBRATIONS

Spring Constant Measurements

Gold st (grams)	30	60	90	120	300	
nosprg(gram)	29.86811612	59.72268	89.70629	119.6443	298.7671	
stdev (grams)	4.619468674	4.54228	4.579914	3.40194	4.612833	
std error	2.309734337	2.27114	2.289957	1.70097	2.306417	
wisprg(gram)	28.98885844	56.00906	89.49024	117.2226	292.2052	
stdev (grams)	5.197803362	4.862172	4.641111	3.311222	4.616258	
std error	2.598901681	2.431086	2.320556	1.655611	2.308129	
ave. WEIGHTS	30	60	90	120	300	
nosprg(Newton)	0.292906162	0.585679	0.879718	1.173309	2.929904	
wisprg(N)	0.284283589	0.549261	0.877599	1.149561	2.865554	
ave. MEASURES	30	60	90	120	300	
no leg (mm)	0.12	0.35	0.56	0.73	1.82	
st.Dev (mm)	0.06	0.05	0.02	0.02	0.02	
Averages	30	60	90	120	300	average
k values (N/mm)	2085.057999	1601.764	1563.066	1570.264	1574.501	1678.931
st.dev	338.2902644	103.6103	37.16705	13.22906	5.536875	99.56671

Density Conversion Codes

```
function [red_mean, green_mean,
blue_mean]=Calibration_histograms_means(image_name)
% Calibration_histograms_means.m
%test image processing
%% Change file directory to match the desired test pictures
cd('F:\Thesis\Research\Calibrations\calibration_pics\ultra_super')

%Read an image
calI = imread(image_name,'TIFF');
% calI = imread('ultra_super_1p3.tif','TIFF');

%Display with colorbar
figure;
imshow(calI);colorbar

%plot each layer individuall
figure;
subplot(3,1,1);imagesc(calI(:,:,1));colorbar;title('(:,:,1)')
subplot(3,1,2);imagesc(calI(:,:,2));colorbar;title('(:,:,2)')
```

```

subplot(3,1,3);imagesc(calI(:,:,3));colorbar;title('(:,:,3)')

%Analyze histograms of pixel values
%First, convert to doubles.
rd = double(calI(:,:,1));
size_rd=size(rd);row_rd=size_rd(1);column_rd=size_rd(2);
gd = double(calI(:,:,2));
size_gd=size(gd);row_gd=size_gd(1);column_gd=size_gd(2);
bd = double(calI(:,:,3));
size_bd=size(bd);row_bd=size_bd(1);column_bd=size_bd(2);
%Now, reshape matrices into vectors (matlab
rdv = reshape(rd,row_rd*column_rd,1);
gdv = reshape(gd,row_gd*column_gd,1);
bdv = reshape(bd,row_bd*column_bd,1);
%Now, combine into one for histogram plot
pixels = [rdv gdv bdv];
%Now, finally plot histogram
figure;hist(pixels,20);
xlabel('Pixel Value')

legend('Red Channel','Green Channel','Blue
Channel','Location','NorthWest')

%For calibration, calculate mean in each channel:
red_mean = mean(rdv);
green_mean = mean(gdv);
blue_mean = mean(bdv);
end

```

```

function [red_mean, green_mean,
blue_mean]=Calibration_histograms_means(image_name)

% clear all,clc

% Calibration_histograms_means.m
%test image processing

%% Change file directory to match the desired test pictures
cd('F:\Thesis\Research\Calibrations\calibration_pics\ultra_super')

%Read an image

    calI = imread(image_name,'TIFF');
% calI = imread('ultra_super_1p3.tif','TIFF');

%Display with colorbar
figure;
imshow(calI);colorbar

%plot each layer individuall
figure;
subplot(3,1,1);imagesc(calI(:,:,1));colorbar;title('(:,:,1)')

```

```

subplot(3,1,2);imagesc(calI(:,:,2));colorbar;title('(:,:,2)')
subplot(3,1,3);imagesc(calI(:,:,3));colorbar;title('(:,:,3)')

%Analyze histograms of pixel values
%First, convert to doubles.
rd = double(calI(:,:,1));
size_rd=size(rd);row_rd=size_rd(1);column_rd=size_rd(2);
gd = double(calI(:,:,2));
size_gd=size(gd);row_gd=size_gd(1);column_gd=size_gd(2);
bd = double(calI(:,:,3));
size_bd=size(bd);row_bd=size_bd(1);column_bd=size_bd(2);
%Now, reshape matrices into vectors (matlab
rdv = reshape(rd,row_rd*column_rd,1);
gdv = reshape(gd,row_gd*column_gd,1);
bdv = reshape(bd,row_bd*column_bd,1);
%Now, combine into one for histogram plot
pixels = [rdv gdv bdv];
%Now, finally plot histogram
figure;hist(pixels,20);
xlabel('Pixel Value')

legend('Red Channel','Green Channel','Blue
Channel','Location','NorthWest')

%For calibration, calculate mean in each channel:
red_mean = mean(rdv);
green_mean = mean(gdv);
blue_mean = mean(bdv);
end

```

```

function [red_mean, green_mean,
blue_mean]=Calibration_histograms_means(image_name)

% clear all,clc

% Calibration_histograms_means.m
%test image processing

%% Change file directory to match the desired test pictures
cd('F:\Thesis\Research\Calibrations\calibration_pics\ultra_super')

%Read an image

    calI = imread(image_name,'TIFF');
% calI = imread('ultra_super_lp3.tif','TIFF');

%Display with colorbar
figure;
imshow(calI);colorbar

```

```

%plot each layer individuall
figure;
subplot(3,1,1);imagesc(calI(:,:,1));colorbar;title('(:,:,1)')
subplot(3,1,2);imagesc(calI(:,:,2));colorbar;title('(:,:,2)')
subplot(3,1,3);imagesc(calI(:,:,3));colorbar;title('(:,:,3)')

%Analyze histograms of pixel values
%First, convert to doubles.
rd = double(calI(:,:,1));
size_rd=size(rd);row_rd=size_rd(1);column_rd=size_rd(2);
gd = double(calI(:,:,2));
size_gd=size(gd);row_gd=size_gd(1);column_gd=size_gd(2);
bd = double(calI(:,:,3));
size_bd=size(bd);row_bd=size_bd(1);column_bd=size_bd(2);
%Now, reshape matrices into vectors (matlab
rdv = reshape(rd,row_rd*column_rd,1);
gdv = reshape(gd,row_gd*column_gd,1);
bdv = reshape(bd,row_bd*column_bd,1);
%Now, combine into one for histogram plot
pixels = [rdv gdv bdv];
%Now, finally plot histogram
figure;hist(pixels,20);
xlabel('Pixel Value')

legend('Red Channel','Green Channel','Blue
Channel','Location','NorthWest')

%For calibration, calculate mean in each channel:
red_mean = mean(rdv);
green_mean = mean(gdv);
blue_mean = mean(bdv);
end

```

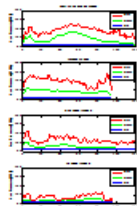
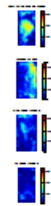
APPENDIX C

RESULTS

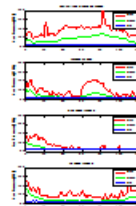
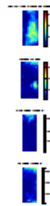
Pressure Map and Pressure Average Images

Test 1 (ALL)

Nominal

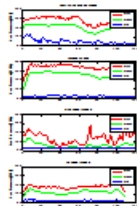
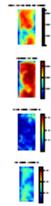


Destabilized

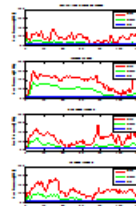
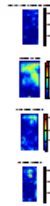


Test 2 (ALL)

Nominal



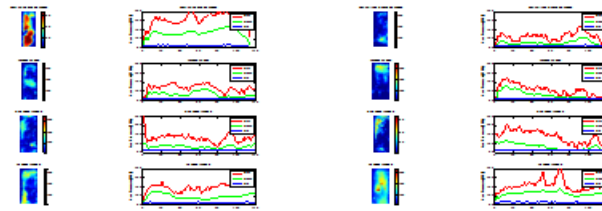
Destabilized



Test3 (ALL)

Nominal

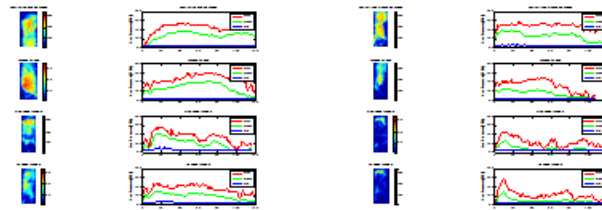
Destabilized



Test4 (ALL)

Nominal

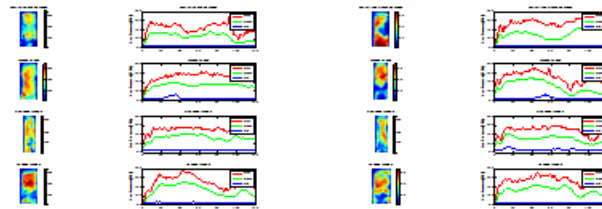
Destabilized



Test5 (ALL)

Nominal

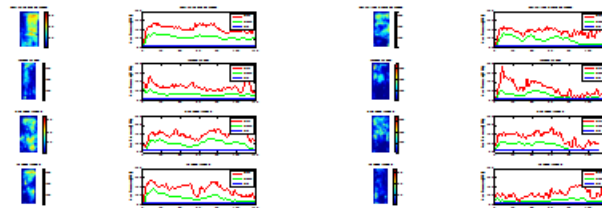
Destabilized



Test6 (ALL)

Nominal

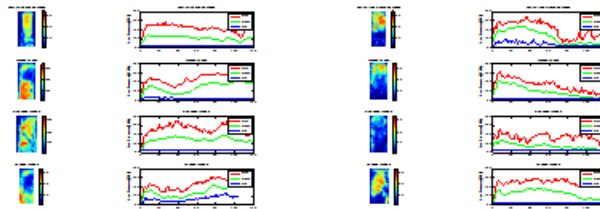
Destabilized



Test7 (ALL)

Nominal

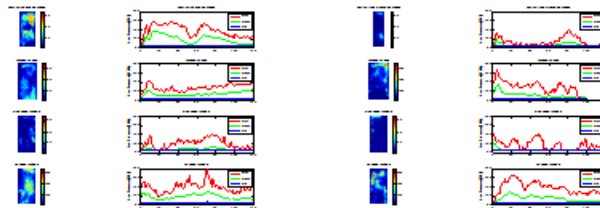
Destabilized



Test8 (ALL)

Nominal

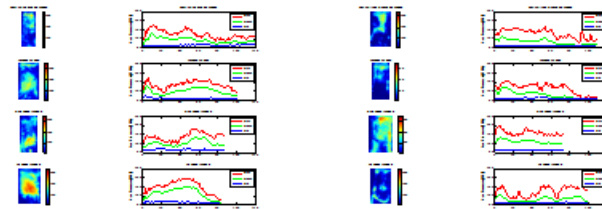
Destabilized



Test9 (ALL)

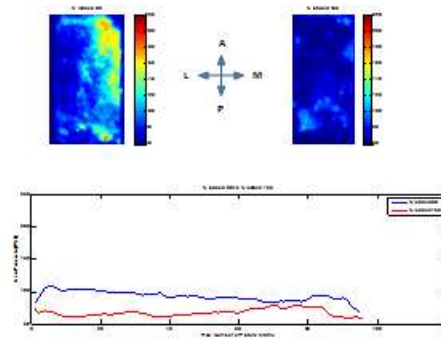
Nominal

Destabilized

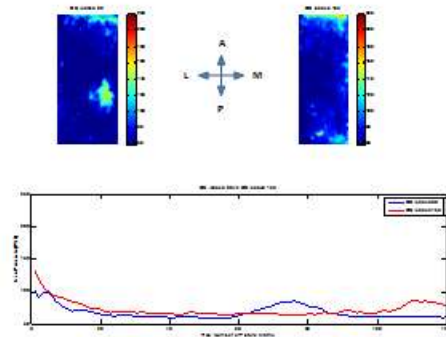


Loaded Flexion Overlays

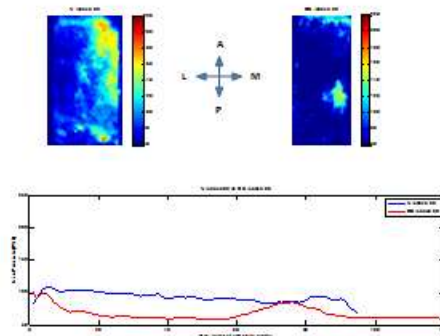
TEST1 Normal 90° Loaded vs. Normal Loaded 135°



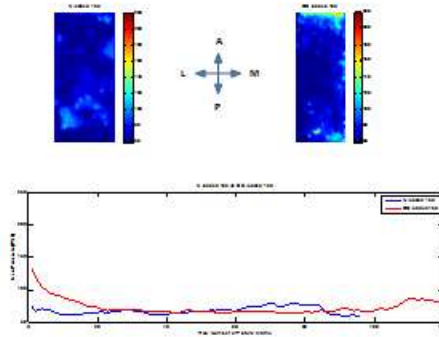
TEST1 Destabilized 90° Loaded vs. Destabilized Loaded 135°



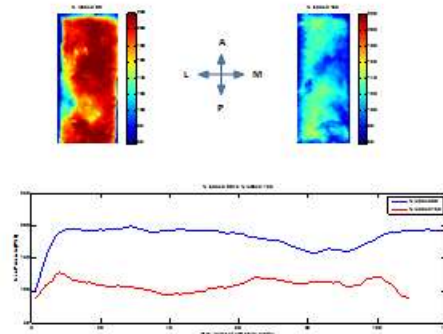
TEST1 Normal 90° Loaded vs. Destabilized Loaded 90°



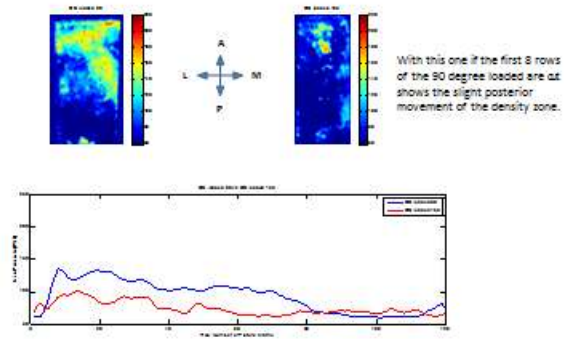
TEST1 Normal 135° Loaded vs. Destabilized Loaded 135°



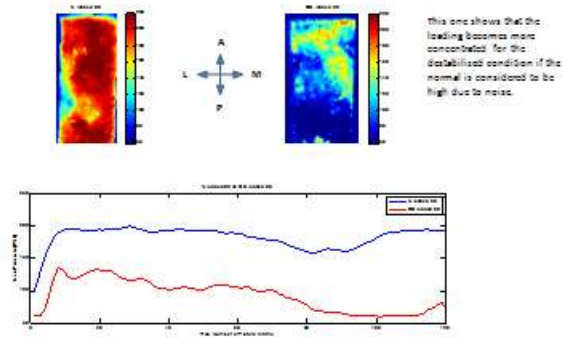
TEST2 Normal 90° Loaded vs. Normal Loaded 135°



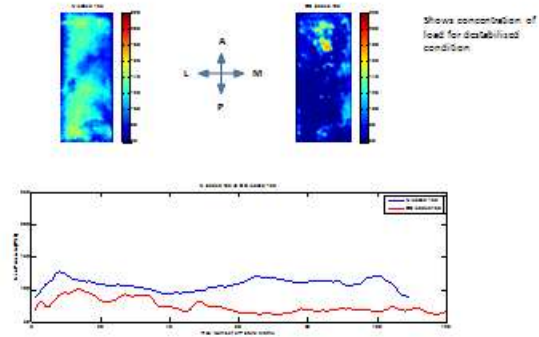
TEST2 Destabilized 90° Loaded vs. Destabilized Loaded 135°



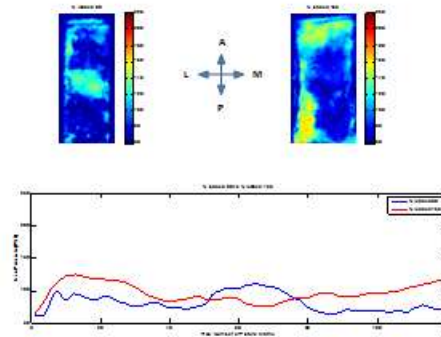
TEST2 Normal 90° Loaded vs. Destabilized Loaded 90°



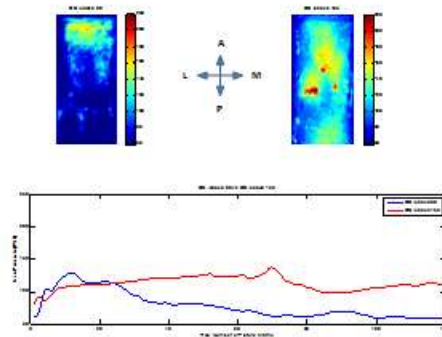
★ TEST2 Normal 135° Loaded vs.
Destabilized Loaded 135°



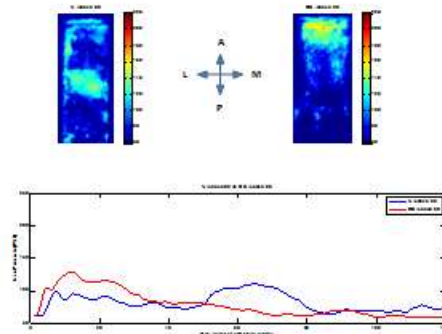
TEST3 Normal 90° Loaded vs.
Normal Loaded 135°



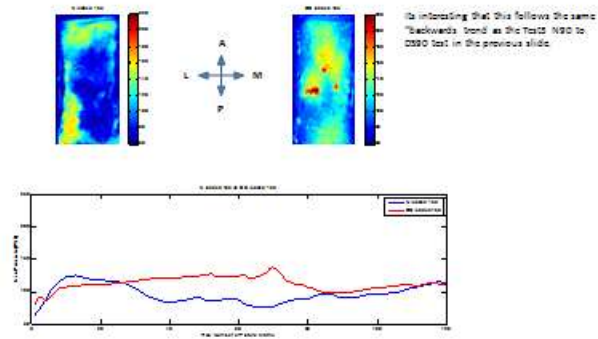
★ TEST3 Destabilized 90° Loaded vs.
Destabilized Loaded 135°



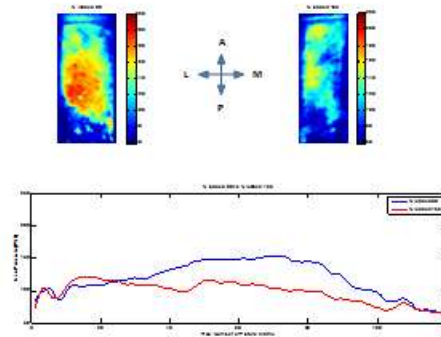
TEST3 Normal 90° Loaded vs.
Destabilized Loaded 90°



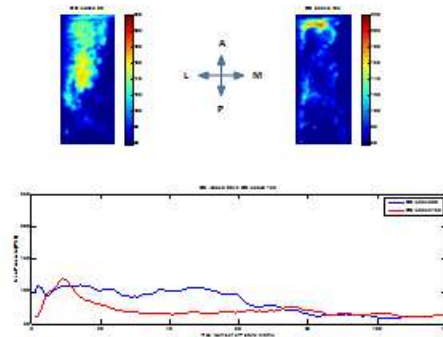
TEST3 Normal 135° Loaded vs. Destabilized Loaded 135°



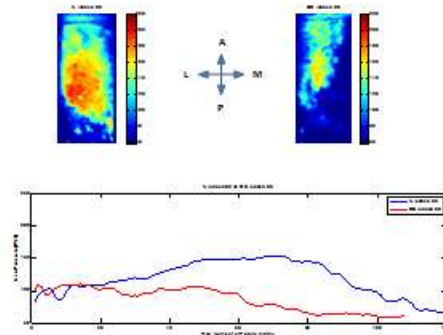
TEST4 Normal 90° Loaded vs. Normal Loaded 135°



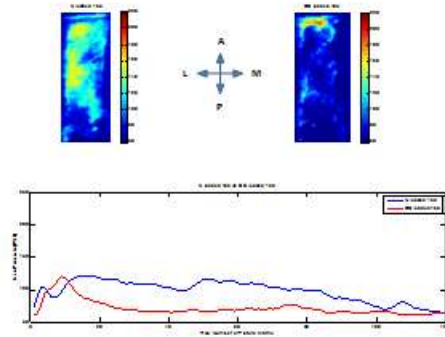
TEST4 Destabilized 90° Loaded vs. Destabilized Loaded 135°



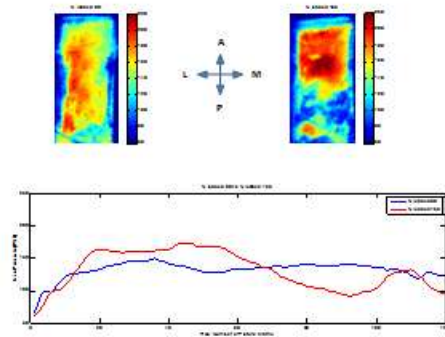
TEST4 Normal 90° Loaded vs. Destabilized Loaded 90°



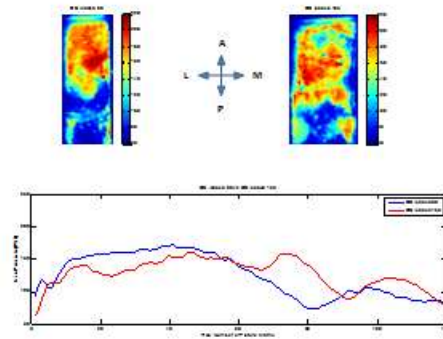
TEST4 Normal 135° Loaded vs. Destabilized Loaded 135°



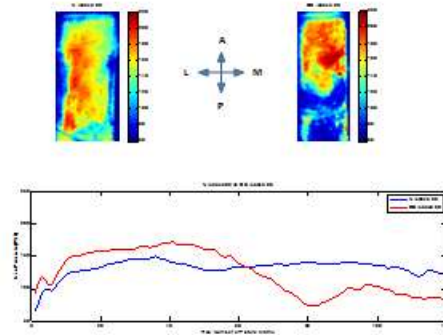
TEST5 Normal 90° Loaded vs. Normal Loaded 135°



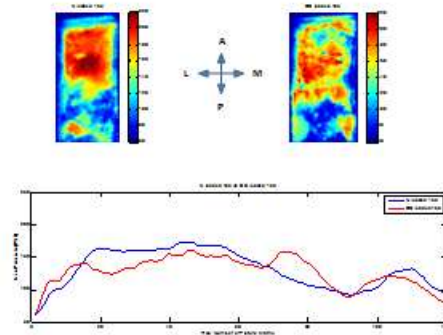
TEST5 Destabilized 90° Loaded vs. Destabilized Loaded 135°



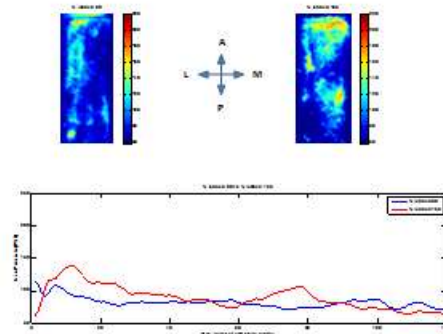
★★ TEST5 Normal 90° Loaded vs. Destabilized Loaded 90°



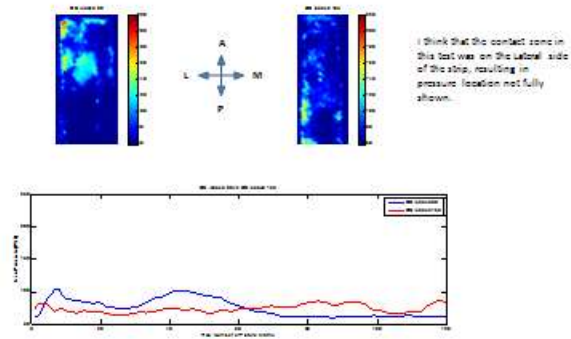
★ TEST5 Normal 135° Loaded vs.
Destabilized Loaded 135°



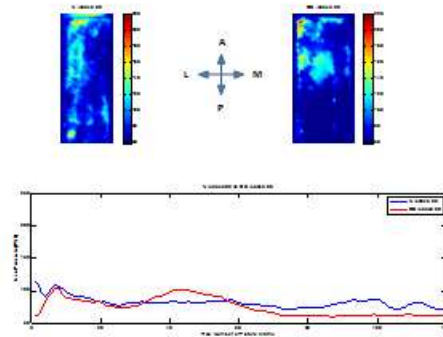
TEST6 Normal 90° Loaded vs.
Normal Loaded 135°



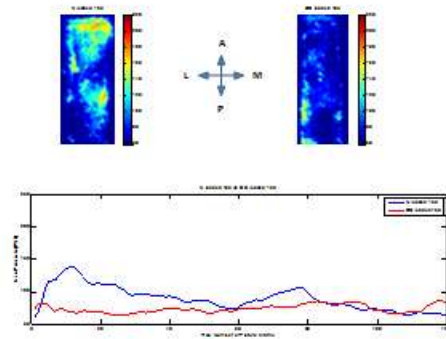
★ TEST6 Destabilized 90° Loaded vs. Destabilized Loaded 135°



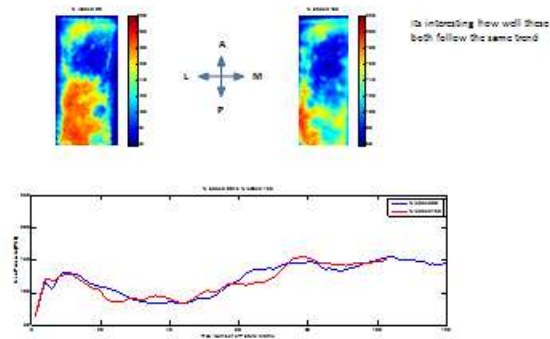
★ TEST6 Normal 90° Loaded vs. Destabilized Loaded 90°



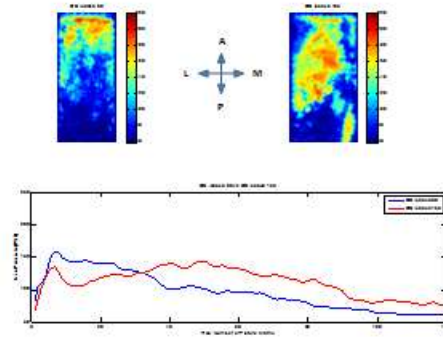
TEST6 Normal 135° Loaded vs. Destabilized Loaded 135°



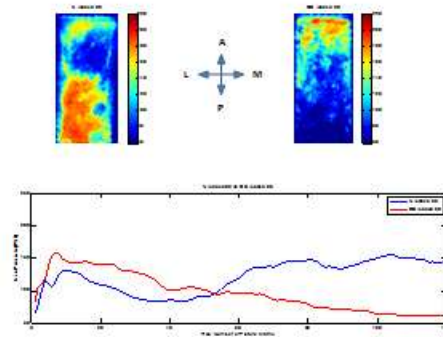
TEST7 Normal 90° Loaded vs. Normal Loaded 135°



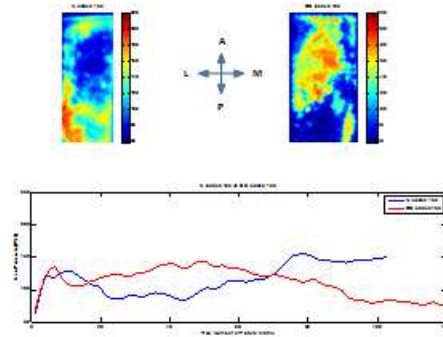
★ TEST7 Destabilized 90° Loaded vs.
Destabilized Loaded 135°



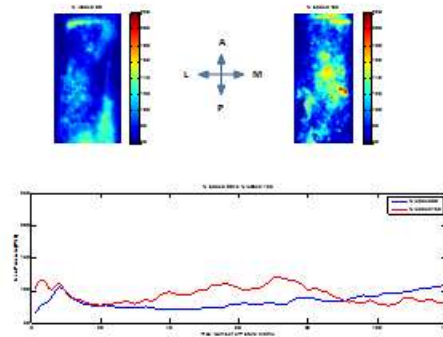
TEST7 Normal 90° Loaded vs.
Destabilized Loaded 90°



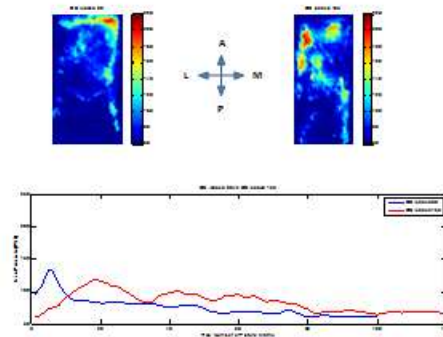
TEST7 Normal 135° Loaded vs. Destabilized Loaded 135°



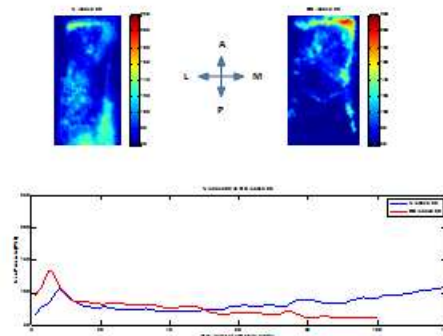
TEST8 Normal 90° Loaded vs. Normal Loaded 135°



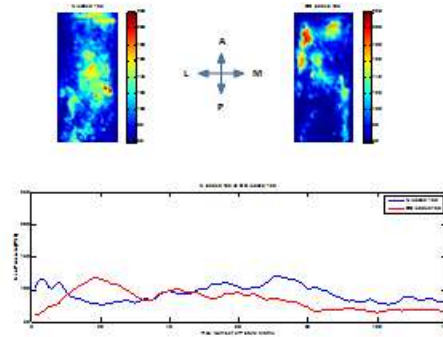
★ TEST8 Destabilized 90° Loaded vs.
Destabilized Loaded 135°



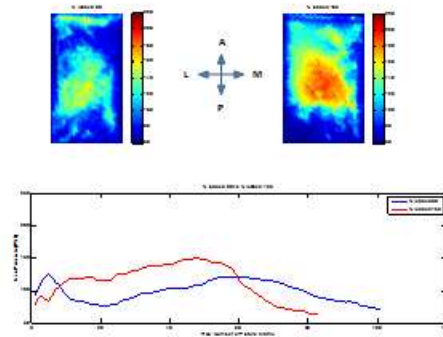
TEST8 Normal 90° Loaded vs.
Destabilized Loaded 90°



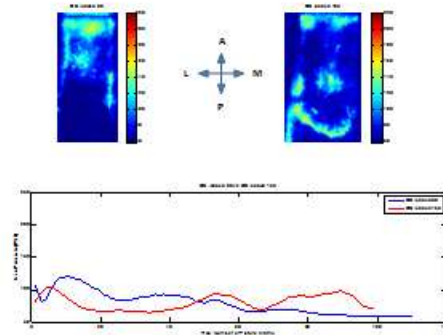
TEST8 Normal 135° Loaded vs. Destabilized Loaded 135°



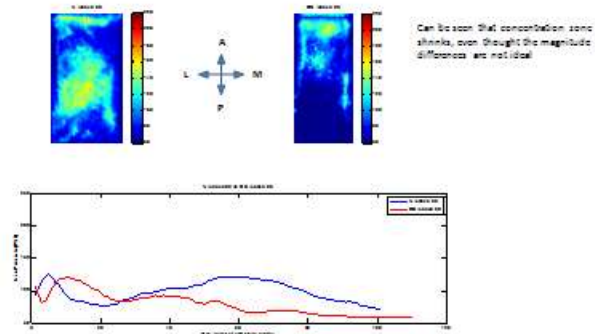
TEST9 Normal 90° Loaded vs. Normal Loaded 135°



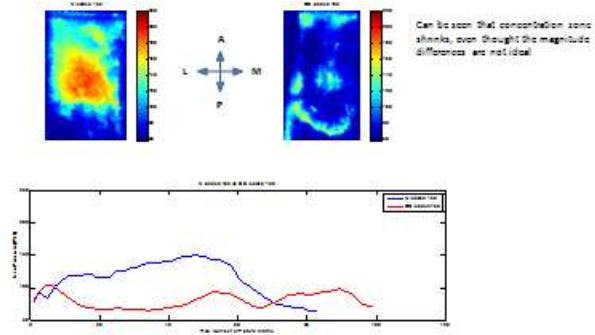
★ TEST9 Destabilized 90° Loaded vs.
Destabilized Loaded 135°



TEST9 Normal 90° Loaded vs.
Destabilized Loaded 90°

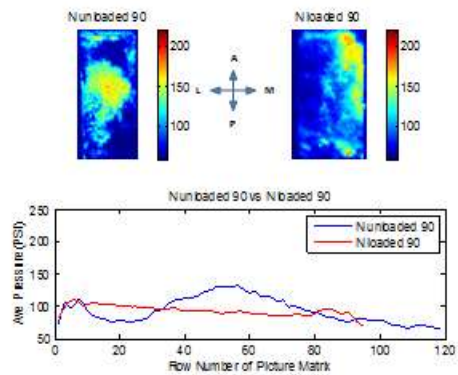


TEST9 Normal 135° Loaded vs. Destabilized Loaded 135°

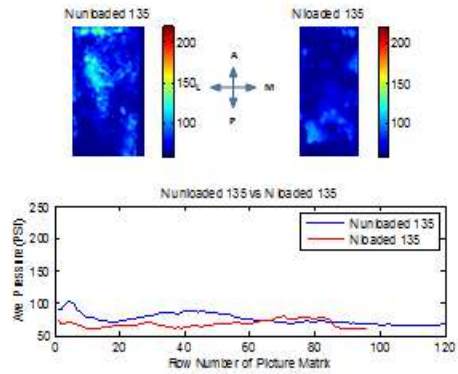


Unloaded-Loaded Overlays

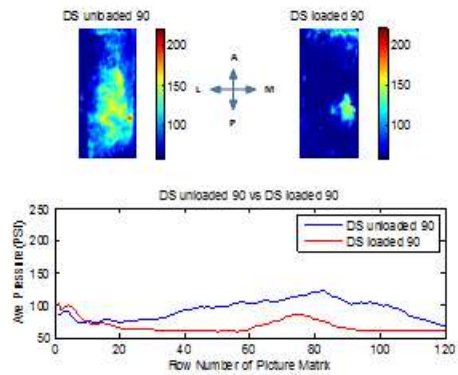
TEST1 Normal 90° Unloaded vs. Normal Loaded 90°



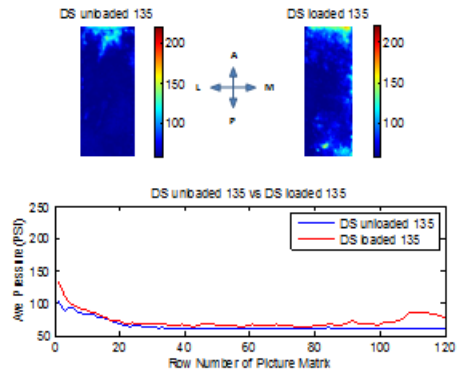
TEST1 Normal Unloaded 135° vs. Normal Loaded 135°



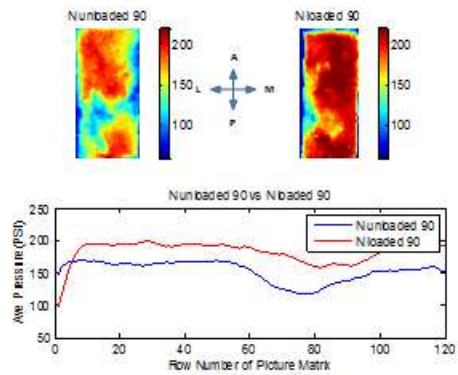
TEST1 Destabilized Unloaded 90° vs. Destabilized Loaded 90°



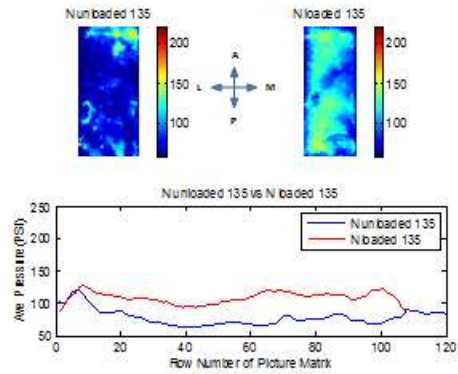
TEST1 Destabilized Unloaded 135° vs. Destabilized Loaded 135°



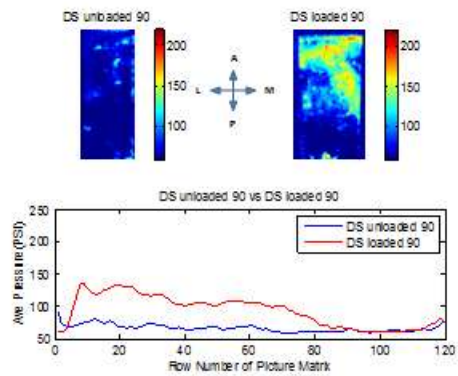
★ TEST2 Normal 90° Unloaded vs. Normal Loaded 90°



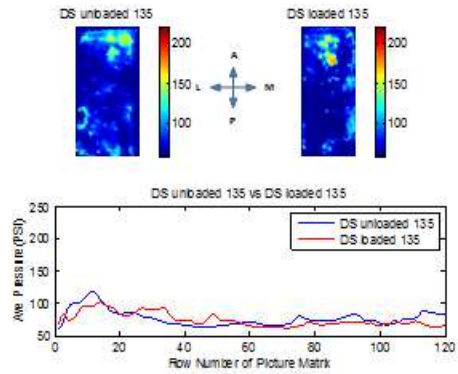
★ TEST2 Normal Unloaded 135° vs. Normal Loaded 135°



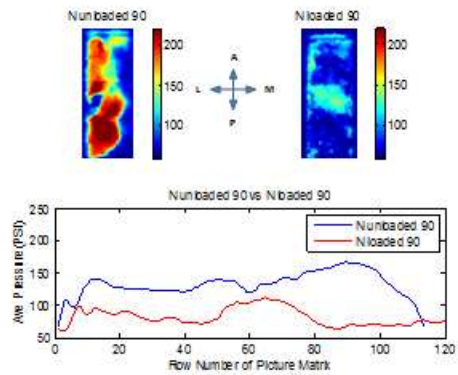
★ TEST2 Destabilized Unloaded 90° vs. Destabilized Loaded 90°



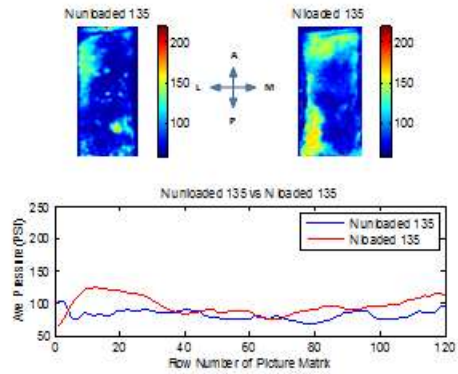
TEST2 Destabilized Unloaded 135° vs. Destabilized Loaded 135°



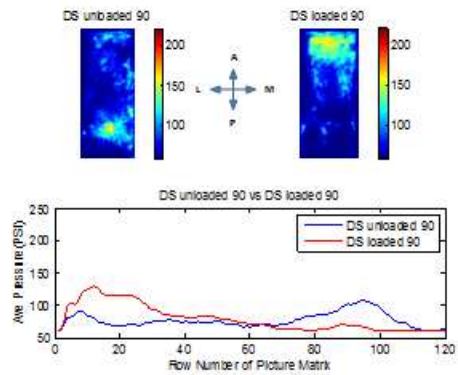
TEST3 Normal 90° Unloaded vs. Normal Loaded 90°



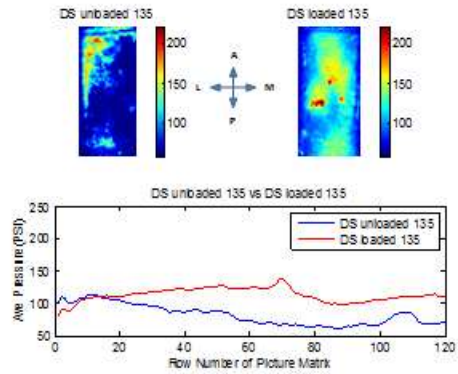
★ TEST3 Normal Unloaded 135°
vs. Normal Loaded 135°



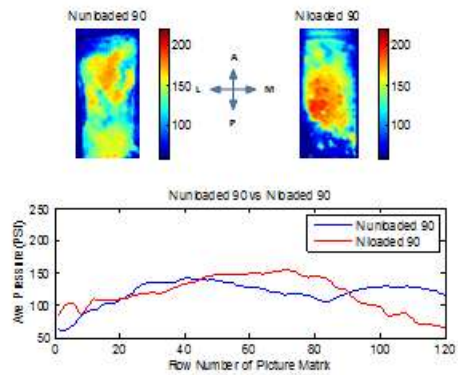
★ TEST3 Destabilized Unloaded 90° vs.
Destabilized Loaded 90°



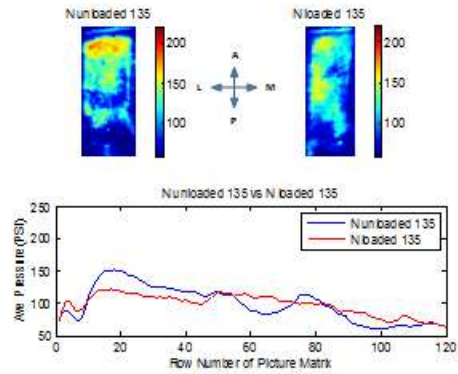
TEST3 Destabilized Unloaded 135° vs. Destabilized Loaded 135°



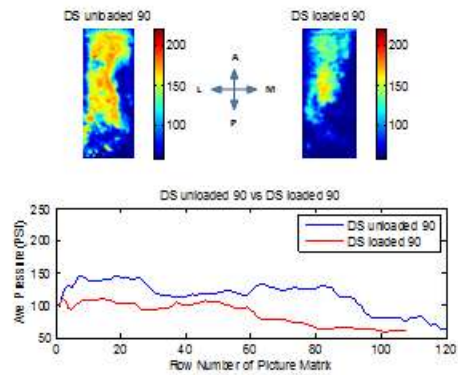
TEST4 Normal 90° Unloaded vs. Normal Loaded 90°



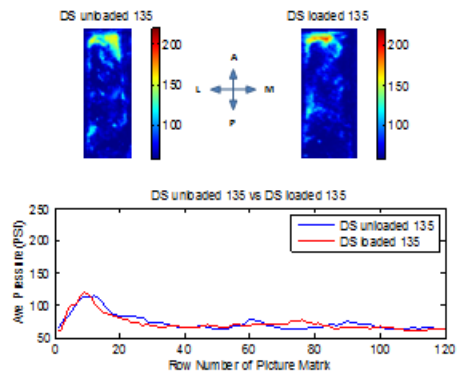
TEST4 Normal Unloaded 135° vs. Normal Loaded 135°



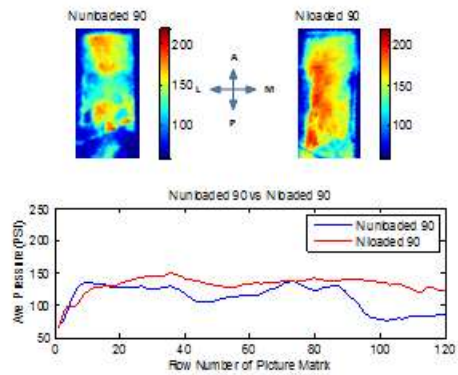
TEST4 Destabilized Unloaded 90° vs. Destabilized Loaded 90°



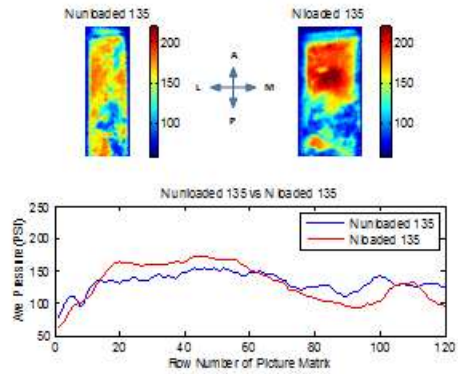
TEST4 Destabilized Unloaded 135° vs. Destabilized Loaded 135°



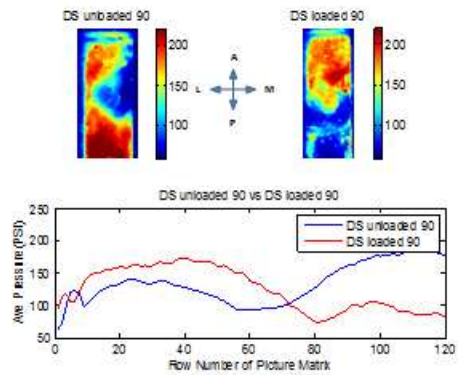
★ TEST5 Normal 90° Unloaded vs. Normal Loaded 90°



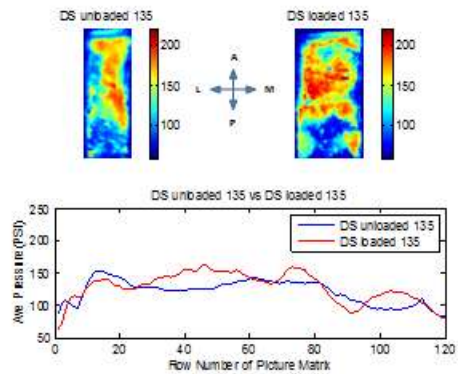
★ TEST5 Normal Unloaded 135°
vs. Normal Loaded 135°



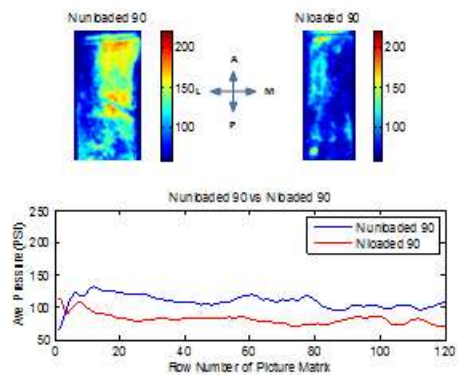
★ TEST5 Destabilized Unloaded 90° vs.
Destabilized Loaded 90°



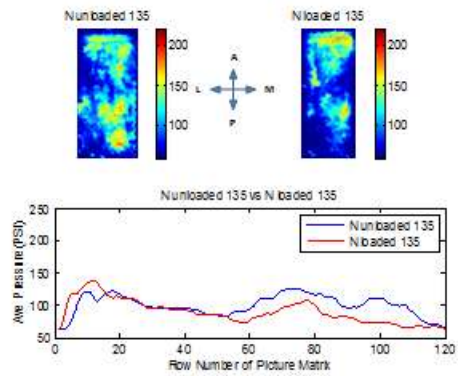
★ TEST5 Destabilized Unloaded 135° vs. Destabilized Loaded 135°



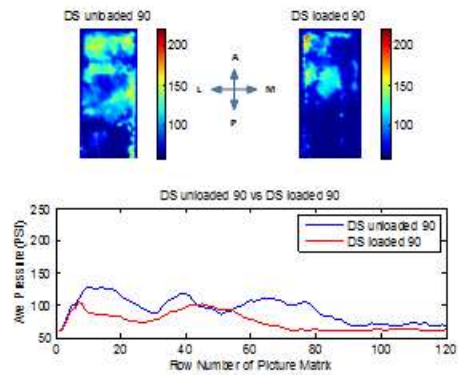
TEST6 Normal 90° Unloaded vs. Normal Loaded 90°



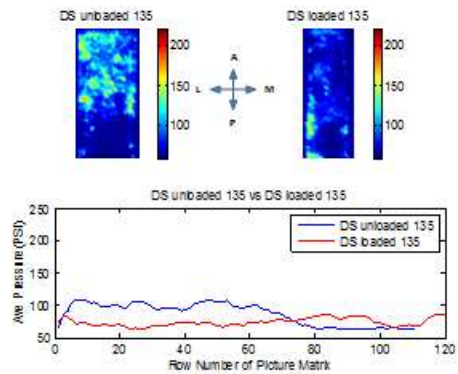
TEST6 Normal Unloaded 135° vs. Normal Loaded 135°



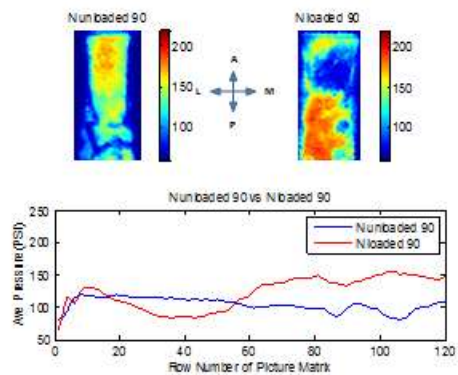
TEST6 Destabilized Unloaded 90° vs. Destabilized Loaded 90°



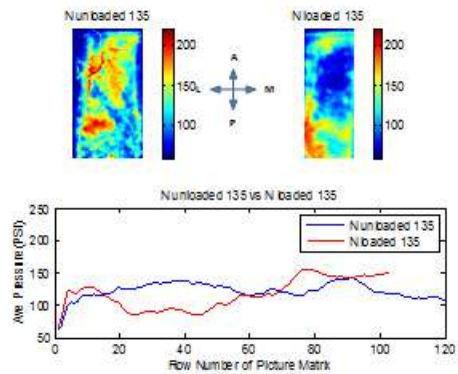
TEST6 Destabilized Unloaded 135° vs. Destabilized Loaded 135°



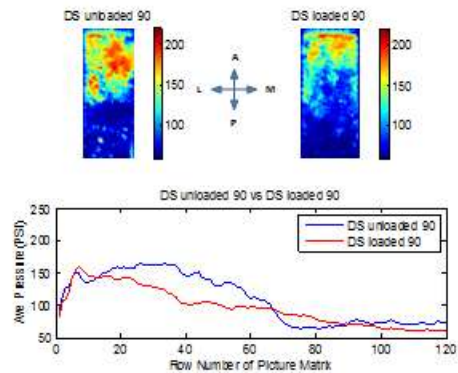
TEST7 Normal 90° Unloaded vs. Normal Loaded 90°



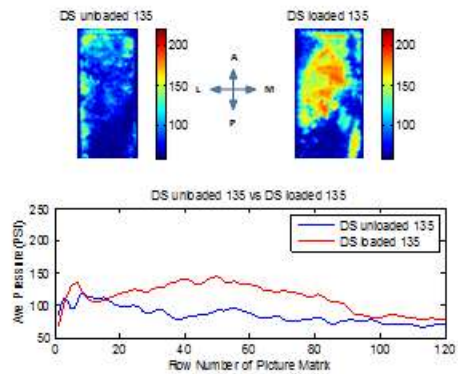
TEST7 Normal Unloaded 135° vs. Normal Loaded 135°



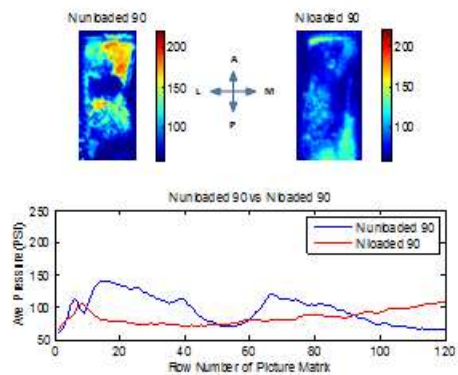
TEST7 Destabilized Unloaded 90° vs. Destabilized Loaded 90°



TEST7 Destabilized Unloaded 135° vs. Destabilized Loaded 135°

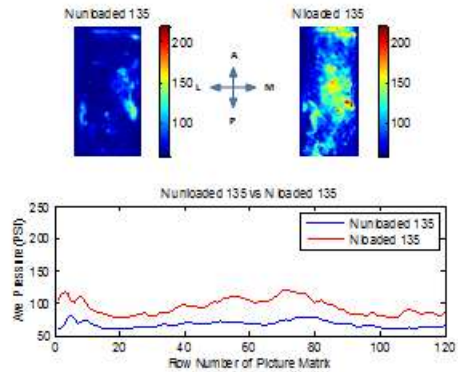


TEST8 Normal 90° Unloaded vs. Normal Loaded 90°

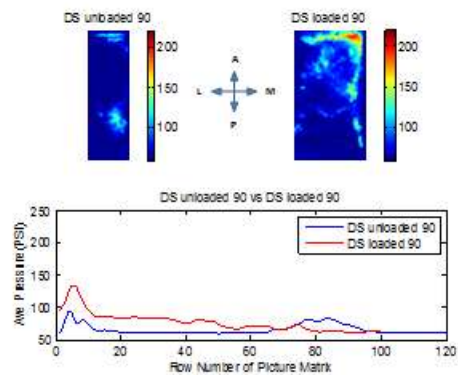




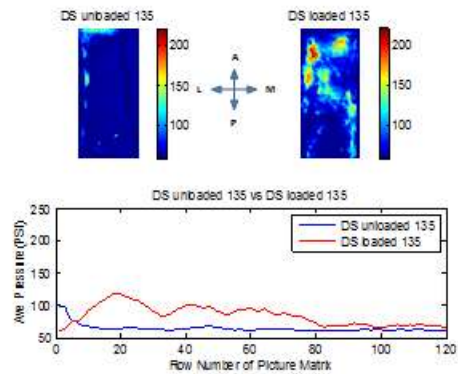
TEST8 Normal Unloaded 135° vs. Normal Loaded 135°



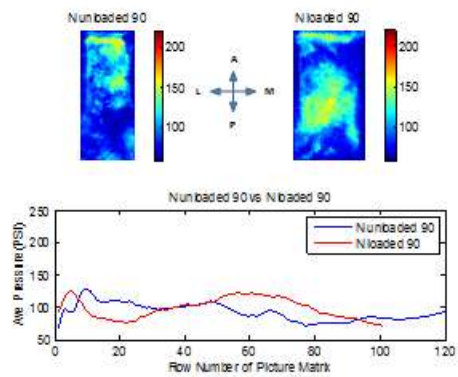
TEST8 Destabilized Unloaded 90° vs. Destabilized Loaded 90°



★ TEST8 Destabilized Unloaded 135° vs. Destabilized Loaded 135°

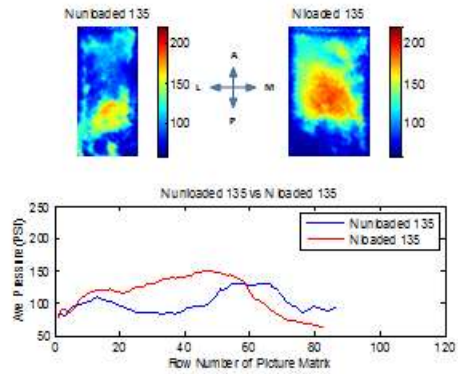


TEST9 Normal 90° Unloaded vs. Normal Loaded 90°

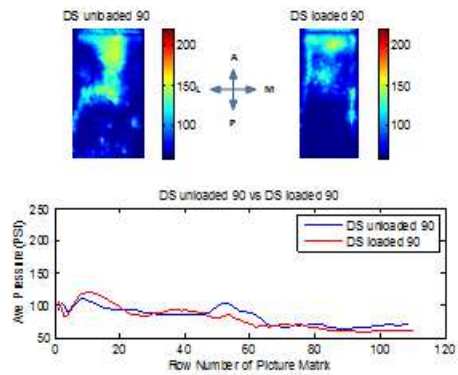




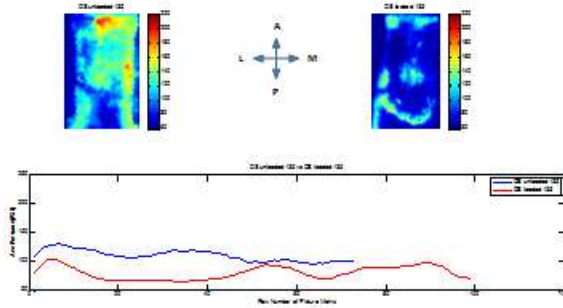
TEST9 Normal Unloaded 135° vs. Normal Loaded 135°



TEST9 Destabilized Unloaded 90° vs. Destabilized Loaded 90°



TEST9 Destabilized Unloaded 135° vs. Destabilized Loaded 135°



Pressure Map and Pressure Average Code

```
function color_int_maps_D(Directory)
% clear all;close all;clc
% Directory='G:\Thesis\Research\Data\April3\Trimmed';

% SLAMR Data color/intensity mapping
% Tom J. Rose
% 4/10/2013

%This program combines the color mapping and intensity mapping files to
%create both a color map and an averaged intensity plot for each fuji-
film
%test strip.
% Directory =
'D:\Ron_Files\Ron_BASE\Academic\Research\June_Lab\Ron_Projects\Rodent_K
neebuster\tom_small\april11\Trimmed4';
cmin = 58; %Look through all data and find minimum reasonable pressure
cmax = 220; %look through all data and find maximum reasonable pressue
cd(Directory);
fnames={'N_0per_90deg.tif';'N_90per_90deg.tif';'N_0per_135deg.tif';
        'N_90per_135deg.tif'};
titles={'Nominal Unloaded 90deg';'Loaded 90deg';'Unloaded 135deg';
        'Loaded 135deg'};

% Constants for conversion equations
p1= 0.00008337 ; p2=-0.04051 ; p3=5.083;
g1=97.788;g2=-422.65;g3=803.85;g4=-806.78;g5=596.78;g6=-19.527;

figure('Name',Directory,'NumberTitle','off')
for i=1:length(fnames)
    Images{i,:}=imread(fnames{i});
    Images{i,:}=single(Images{i,:});
```

```

temp=Images{i,:}; ends=length(temp);
if ends >= 120
    for j=1:120
        temp2(j,:)=temp(j,:);
    end
elseif ends < 120
    for j=1:ends
        temp2(j,:)=temp(j,:);
    end
end
temp=temp2;
pixel2density=p1.*temp.^2 + p2.*temp + p3;
density2pressure=g1*pixel2density.^5+g2*pixel2density.^4+...
    g3*pixel2density.^3+g4.*pixel2density.^2+...
    g5*pixel2density +g6;
row_average=mean(density2pressure.');"
row_max=max(density2pressure.');"
row_min=min(density2pressure.');"
% Color Plot
subplot(4,2,2*i-1), imshow(density2pressure,'DisplayRange',[0 350])
title(titles{i});
colormap(jet(256));colorbar;
caxis([cmin cmax]);
% Stats Plot
subplot(4,2,2*i), plot(row_max,'r');hold on
plot(row_average,'g');plot(row_min,'b');legend('show','max','mean','min',
'Location','NorthEast');
axis manual, axis([0 120 50 250]),title(titles{i});
ylabel('Ave Pressure(PSI)');
clear temp, clear temp2
end
xlabel('Row Number of Picture Matrix');

fnames={'DS_0per_90deg.tif';'DS_90per_90deg.tif';'DS_0per_135deg.tif';
'DS_90per_135deg.tif'};
titles={'Destabilized Unloaded 90deg';'Loaded 90deg';'Unloaded 135deg';
'Loaded 135deg'};

figure('Name',Directory,'NumberTitle','off')
for i=1:length(fnames)
    Images{i,:}=imread(fnames{i});
    Images{i,:}=single(Images{i,:});
    temp=Images{i,:}; ends=length(temp);
    if ends >= 120
        for j=1:120
            temp2(j,:)=temp(j,:);
        end
    elseif ends < 120
        for j=1:ends
            temp2(j,:)=temp(j,:);
        end
    end
end
temp=temp2;

```

```

pixle2density=p1.*temp.^2 + p2.*temp + p3;
density2pressure=g1*pixle2density.^5+g2*pixle2density.^4+...
    g3*pixle2density.^3+g4.*pixle2density.^2+...
    g5*pixle2density +g6;
row_average=mean(density2pressure.');"
row_max=max(density2pressure.');"
row_min=min(density2pressure.');"
% Color Plot
subplot(4,2,2*i-1), imshow(density2pressure,'DisplayRange',[0 350])
title(titles{i});
colormap(jet(256));colorbar;
caxis([cmin cmax]);
% Stats Plot
subplot(4,2,2*i), plot(row_max,'r');hold on
plot(row_average,'g');plot(row_min,'b');legend('show','max','mean','min
','Location','NorthEast');
axis manual, axis([0 120 50 250]),title(titles{i});
ylabel('Ave Pressure (PSI)');
clear temp, clear temp2
end
xlabel('Row Number of Picture Matrix');

```

Regional Pressure Measurements (RPM) Code

```

% Tom J. Rose/Ron June
% April 25, 2013

% Regional Pressure Measurements (RPM) function created to compare the
% pressure distribution of each test strip with the Percent Body
% Weight(%BW) applied to the leg via SLAMR.
% RPM function outputs 1 Figure that compares test strips regional
% average,
% median, and max values according to the %BW applied to the leg.
function data=RPM_vRon(Directory,test)
% clear all, close all, clc
% Directory='G:\Thesis\Research\Data\april10\Trimmed2';
% Directory = 'G:\Thesis\Research\Data';
cd(Directory)
%following matrix is the %BW loads applied to all strips in the 9 tests
%evaluated in the initial testing of SLAMR, and the order is tests per
%column and N_90 through DS_135 per row
BW=[80.14, 66.95, 94.57, 93.93, 100.60, 71.36, 61.15, 90.09, 84.60,
    85.73, 79.40, 86.33, 83.77, 89.22, 70.72, 69.83, 78.22, 47.66,
    79.70, 65.12, 67.53, 89.95, 96.13, 63.21, 72.14, 57.12, 82.73,
    84.85, 66.89, 71.60, 71.84, 97.12, 62.37, 70.90, 58.99, 63.25];
pBW=BW';

% where T1=Test one, TN=final test, N=Nominal, DS=Destabilized, and the
% 90/135=flexion angles that loads are applied at.

regions={'A1';'A2';'AP';'P2';'P1'}; %A1 is most anterior, and P1 is

```

```

most posterior
symbol={'^','o','s','d'};
color={'r','b','g','r'};
fnames={'N_90per_90deg.tif';
        'N_90per_135deg.tif';
        'DS_90per_90deg.tif';
        'DS_90per_135deg.tif'};
TITLE={'ave N loaded 90'; 'med N loaded 90'; 'max N loaded 90';
       'ave N loaded 135'; 'med N loaded 135'; 'max N loaded 135';
       'ave DS loaded 90'; 'med DS loaded 90'; 'max DS loaded 90';
       'ave loaded 135'; 'med loaded 135'; 'max loaded 135'};

% conversion constants
p1= 0.00008337 ; p2=-0.04051 ; p3=5.083;
g1=97.788;g2=-422.65;g3=803.85;g4=-806.78;g5=596.78;g6=-19.527;

%Master counter
ctr = 1;

%K loop for each region/section of individual strip

    cd(Directory);%figure('Name',Directory,'NumberTitle','off')
    %Opens each file (meaning strip) within the K loop
    for i=1:length(fnames);
        cd(Directory)
        X=imread(fnames{i});
        Image=single(X);

        %convert pixel values to pressure values ("super" scale fuji-
film)
        temp1=p1.*Image.^2 + p2.*Image+ p3; %pixel to density
        psi=g1*temp1.^5+g2*temp1.^4+...
            g3*temp1.^3+g4.*temp1.^2+...
            g5*temp1 +g6; %density to pressure

        %Now, separate into regions of 1/5 of image
        Lmax = size(Image,1);
        deltaL = round(Lmax/5);

        %Now, separate into regions
        A1 = psi(1:deltaL);
        A2 = psi(deltaL+1:2*deltaL);
        AP = psi(2*deltaL+1:3*deltaL);
        P2 = psi(3*deltaL+1:4*deltaL);
        P1 = psi(4*deltaL+1:end);

        %Now, calculate Regional Pressure Measurements (RPMs)

        %Max
        A1_max = max(reshape(A1,1,size(A1,1)*size(A1,2)));
        A2_max = max(reshape(A2,1,size(A2,1)*size(A2,2)));
        AP_max = max(reshape(AP,1,size(AP,1)*size(AP,2)));

```

```

P2_max = max(reshape(P2,1,size(P2,1)*size(P2,2)));
P1_max = max(reshape(P1,1,size(P1,1)*size(P1,2)));

%Mean
A1_mean = mean(reshape(A1,1,size(A1,1)*size(A1,2)));
A2_mean = mean(reshape(A2,1,size(A2,1)*size(A2,2)));
AP_mean = mean(reshape(AP,1,size(AP,1)*size(AP,2)));
P2_mean = mean(reshape(P2,1,size(P2,1)*size(P2,2)));
P1_mean = mean(reshape(P1,1,size(P1,1)*size(P1,2)));

%Median
A1_median = median(reshape(A1,1,size(A1,1)*size(A1,2)));
A2_median = median(reshape(A2,1,size(A2,1)*size(A2,2)));
AP_median = median(reshape(AP,1,size(AP,1)*size(AP,2)));
P2_median = median(reshape(P2,1,size(P2,1)*size(P2,2)));
P1_median = median(reshape(P1,1,size(P1,1)*size(P1,2)));

%Save Regional Pressure Data.
%tile a big matrix (once each strip
%CONTINUE HERE--ADD BODY WEIGHT loading
data(5*(i-1)+1:5*(i-1)+5,:) = [A1_max A1_mean A1_median
BW(i,test)
                                A2_max A2_mean A2_median BW(i,test)
                                AP_max AP_mean AP_median BW(i,test)
                                P2_max P2_mean P2_median BW(i,test)
                                P1_max P1_mean P1_median BW(i,test)];

end

```

RPM

Leg Loading Percentages

For inaugural testing SLAMR was loaded with 80% weight of a mouse leg. The distances that the weight pot dropped were then recorded, and using the load cell average data the actual weight applied to the leg could be extrapolated.

Knee Size _17.81gW	TEST1			TEST2			TEST3			TEST4			TEST5		
	AP2			AP2			AP2 K2			AP22 K1			AP22 K2		
	Weight on leg	Percent of load	%	Weight on leg	Percent of load	%	Weight on leg	Percent of load	%	Weight on leg	Percent of load	%	Weight on leg	Percent of load	%
N_30	22.87400848	80%		22.12245779	87%		22.72840271	80%		22.48800882	84%		42.27220838	100%	
N_120	22.02240888	80%		22.28400888	79%		22.17221288	80%		22.10221240	84%		27.48888120	80%	
OS_30	22.40181287	80%		27.2840884	88%		22.27488128	82%		27.7074084	80%		40.20472028	88%	
OS_120	22.88222248	88%		22.12740840	87%		22.08842124	72%		22.18221028	72%		40.00702240	87%	
Knee Size _17.81gW	TEST6			TEST7			TEST8			TEST9					
	AP21 K1			AP21 K2			AP21 K3			AP21 K4					
	Weight on leg	Percent of load	%	Weight on leg	Percent of load	%	Weight on leg	Percent of load	%	Weight on leg	Percent of load	%			
N_30	22.88888240	71%		22.88888888	81%		27.88888817	82%		22.88888814	80%				
N_120	22.71877888	71%		22.24088812	70%		22.87012248	78%		22.08888888	48%				
OS_30	22.88818888	82%		22.2122174	70%		24.08887888	87%		24.78887888	88%				
OS_120	22.20808888	82%		22.78422812	71%		24.78841888	80%		22.87872210	82%				