Micromechanical Imaging Analysis of Bulk vs. Local Properties Concerning Mesenchymal Stem Cell Heterogeneity

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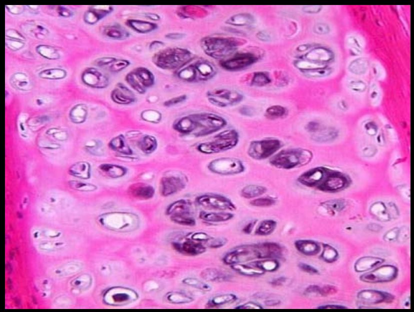
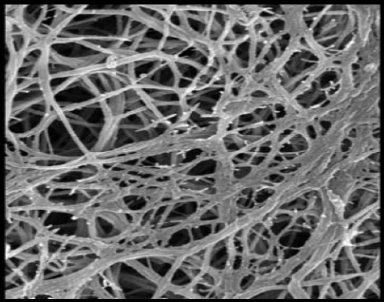
Tissue Engineering

- "An interdisciplinary field that applies the principles of **engineering** and **life sciences** toward the development of **biological substitutes** that restore, maintain, or improve **tissue function** or a whole **organ**" (Langer and Vacanti)



+ Soluble



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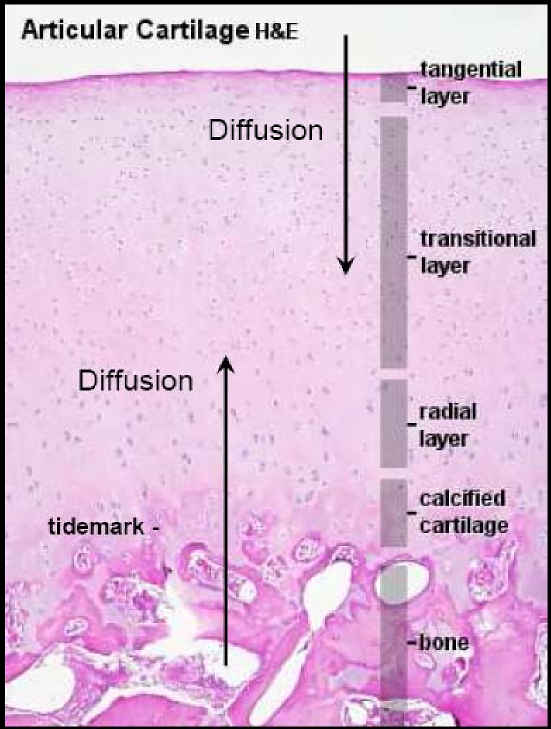
RPI Department of Biomedical Engineering, Clinical Orthopedics and Related Research, September 4, 2007.

Articular Cartilage

- Viscoelastic tissue that can withstand high loads
- Function
  - Transfer loads across joints
  - Low friction joint articulation
- Problems repairing
  - Cells are 1% of tissue by volume
  - Lacks vascular supply



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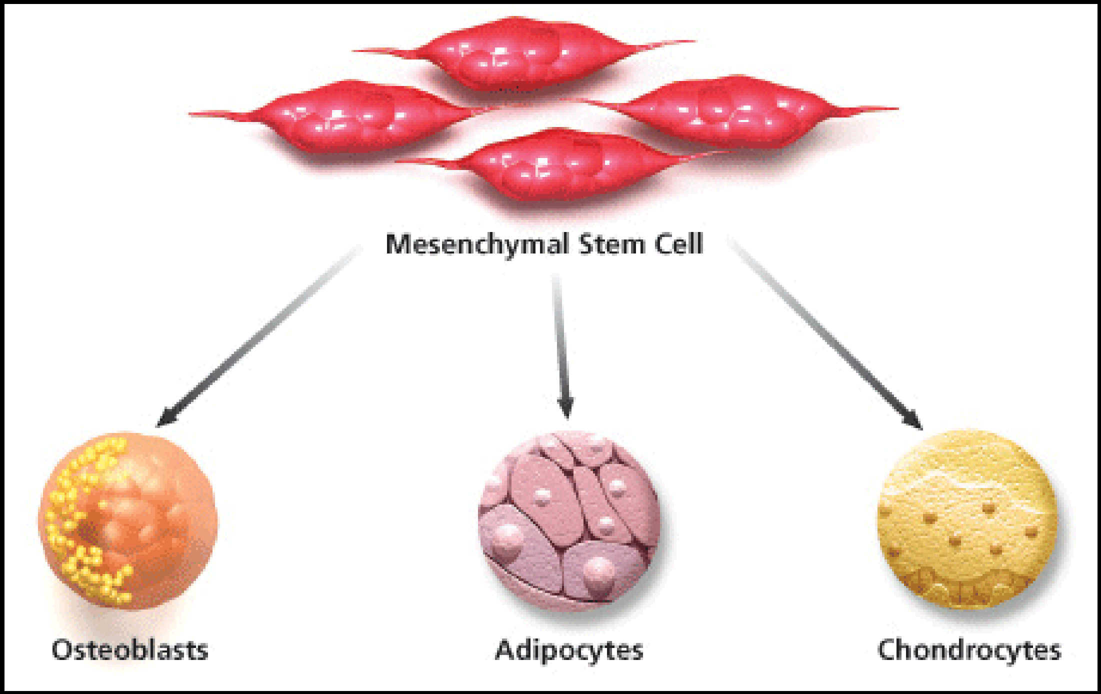
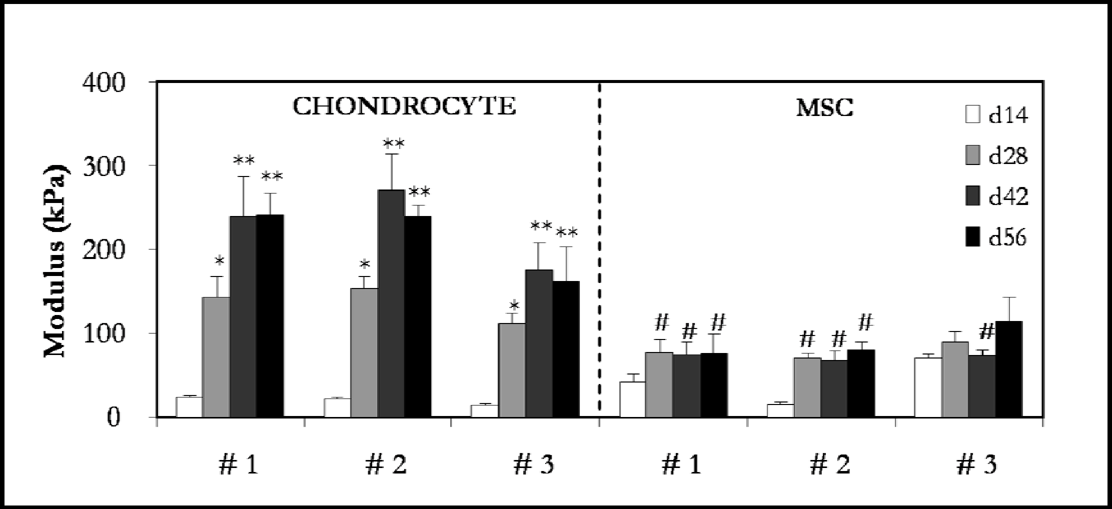
Mesenchymal Stem Cells (MSCs)

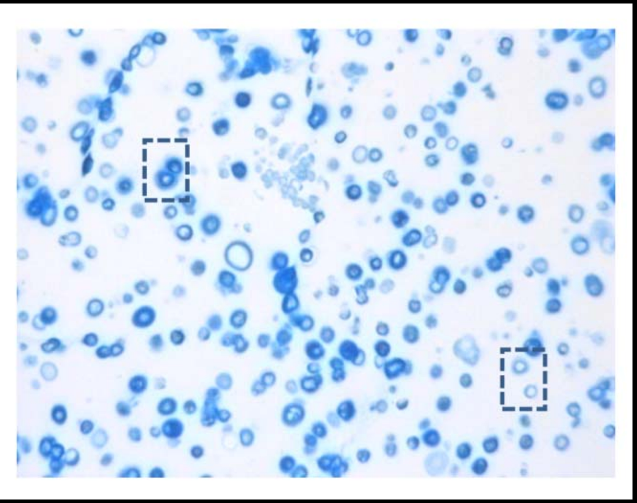
- Harvested from bone marrow
- Tri-differentiation potential



MSCs

- MSCs are a promising cell source for cartilage tissue engineering; however, MSC seeded constructs have yet to be produced that match the mechanical properties of chondrocyte seeded constructs.





Basis for Study

Imaging techniques have provided valuable information about chondrocyte seeded constructs and native cartilage tissue such as:

- Matrix Production
- Cell Response to Load
- Cell Mechanical Properties

Apply these techniques to MSCs

Part 1

Goal

- Assess cell deformation as a function of matrix production over time

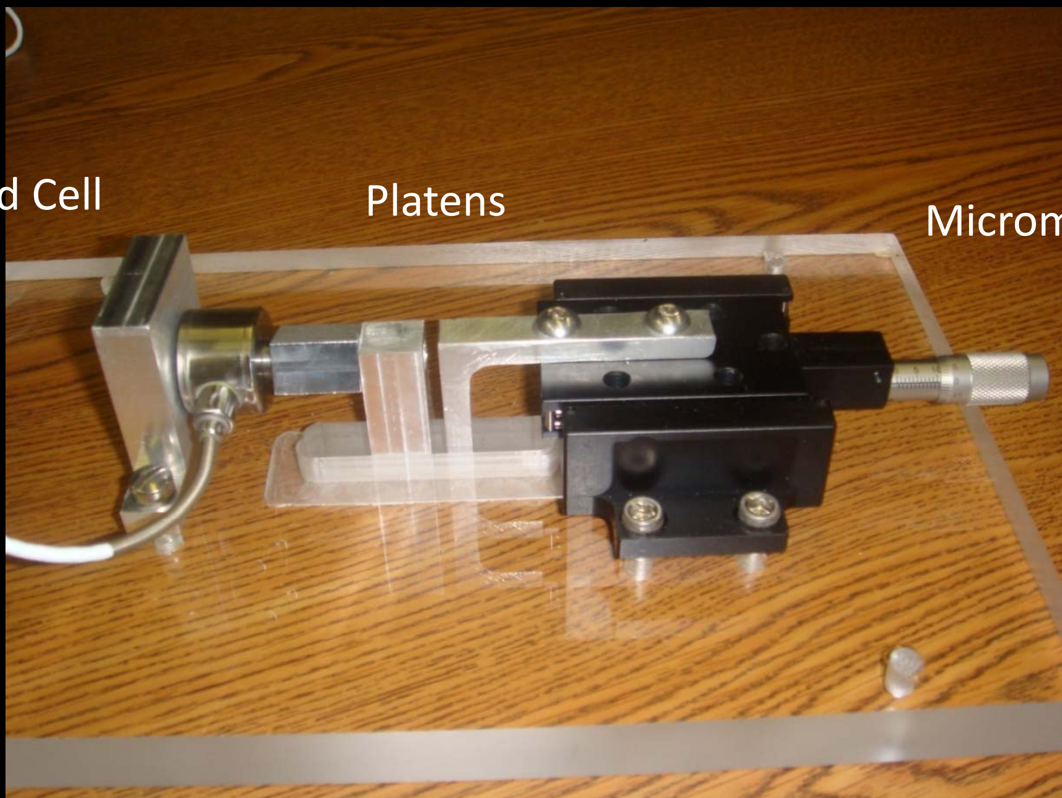
Utilizing

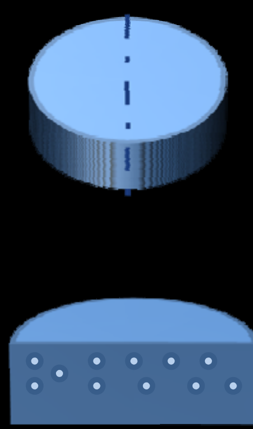
- Confocal Microscopy
- Matlab analysis

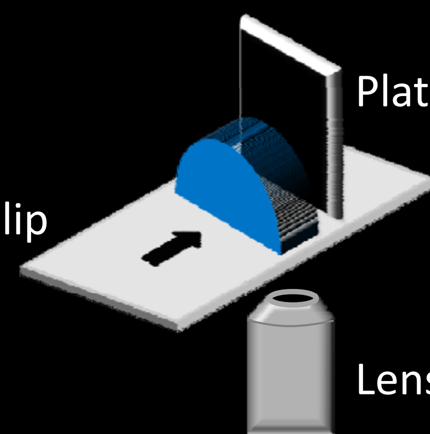
Method

- Harvest bovine MSCs and Chondrocytes from femur and tibia.
- Encapsulate in 2% agarose constructs (4mm Ø, 2.25mm width) at a density of 20M cells/ml.
  - Agarose increases likelihood round phenotype of MSCs
- Culture in chemically defined media with or without TGF-β3 (CM+ or CM-)

Custom Unconfined Compression Device



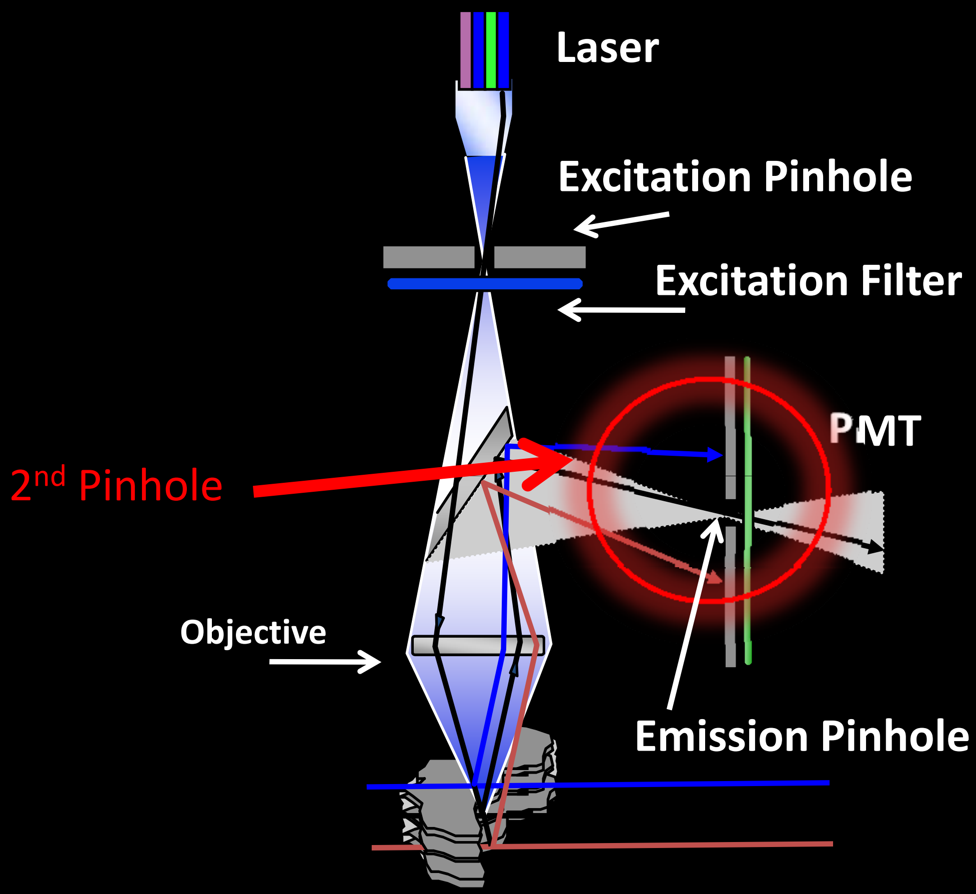




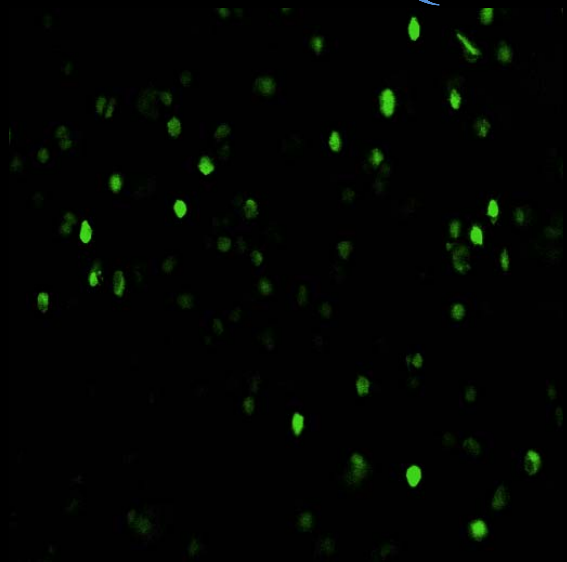
Confocal Microscopy

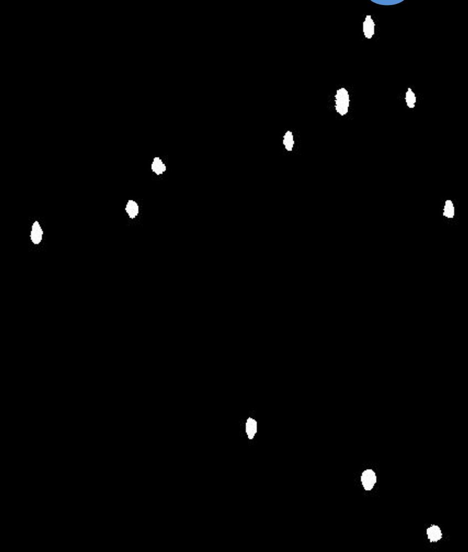
Benefits

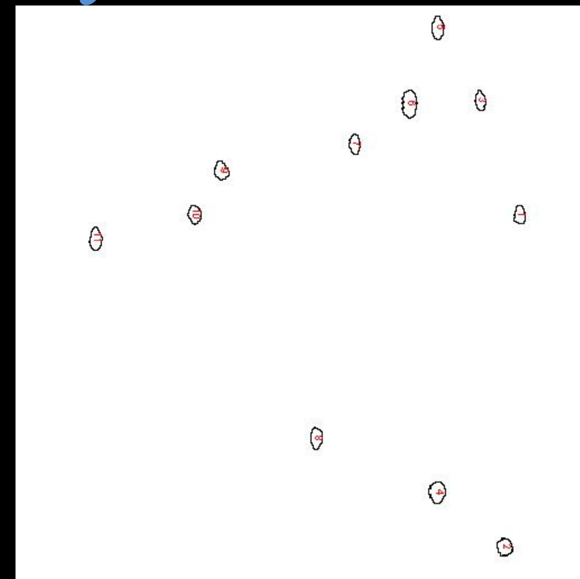
- Specific focal plane
- 3D and 4D imaging



Quantitative Image Analysis







Eccentricity = (Distance between foci)/ Length

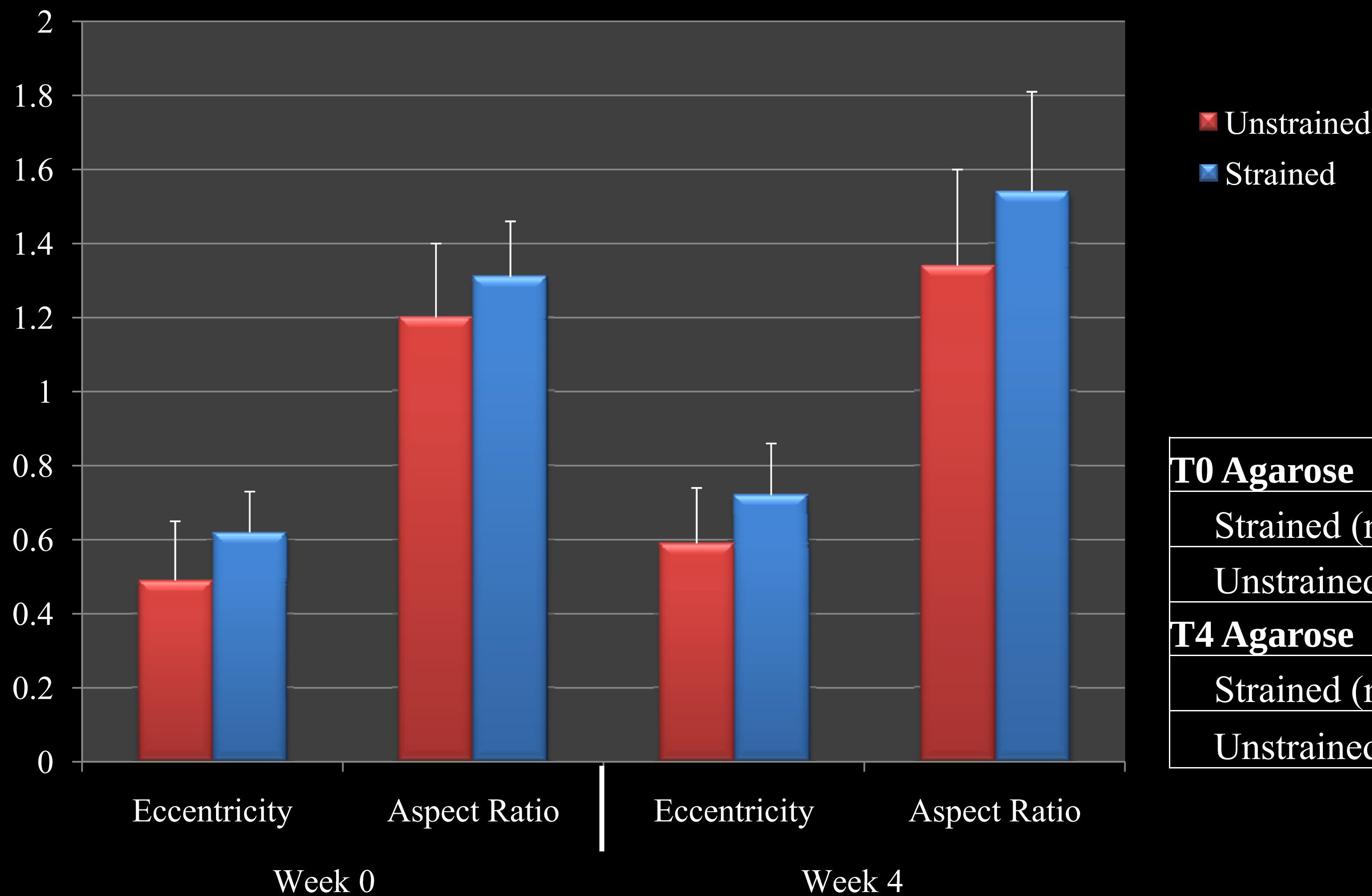
- Eccentricity of 0 = circle
- Eccentricity of 1 = line

Aspect Ratio = Length/Width

Cell #	Length	Width	Eccentricity	Orientation	Aspect Ratio
1	20.67	10.57	0.86	89.86	1.96
2	16.25	11.31	0.72	82.22	1.44
3	15.61	12	0.64	69.42	1.3
4	19.29	10.29	0.85	89.42	1.87
5	17.95	9.29	0.86	87.39	1.93
6	25.02	12.87	0.86	89.99	1.94
7	19.2	13.67	0.7	87.25	1.4
8	20.84	10.29	0.87	89.19	2.03
9	17.58	8.93	0.86	83.25	1.97
10	15.38	13.62	0.46	77.78	1.13
11	16.8	9.89	0.81	88.34	1.7
Avg.	18.60	11.16	0.77	84.92	1.70
Stdev.	2.84	1.67	0.13	6.43	0.32

Agarose gel 1 strained image 3

Quantitative Analysis



T0 Agarose MSCs	
	Strained (n=90)
	Unstrained (n=116)
T4 Agarose MSCs	
	Strained (n=33)
	Unstrained (n=37)

Goal: Assess cell deformation as a function of matrix production over time

Part 2

Goal

- Observe how the local micromechanical properties of chondrocytes and MSCs translate to the bulk mechanical properties.

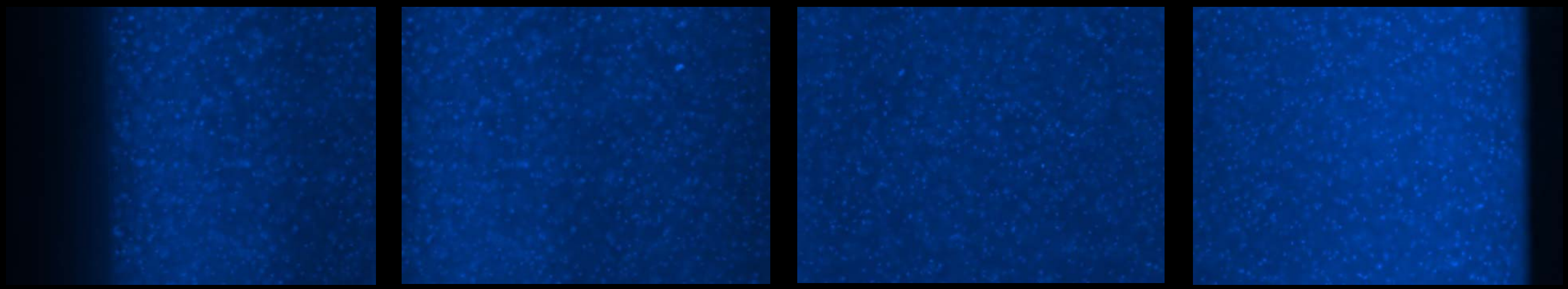
Utilizing

- Fluorescent Microscopy
- Autostitch
- Vic-2D

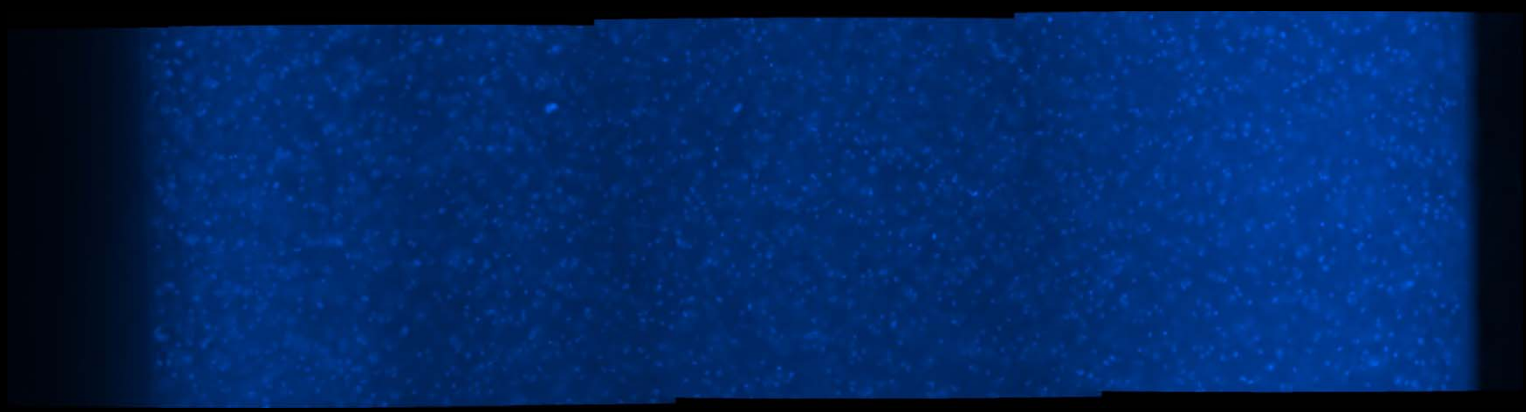
Method

- Chondrocytes & MSCs
  - 2% agarose constructs
- Time points of Weeks 0,1,3,6,9
- Mechanically Test on Microscope
  - Stained nucleus
  - Image the construct strains from 0% to 20% by increments of 4%.

Autostitch

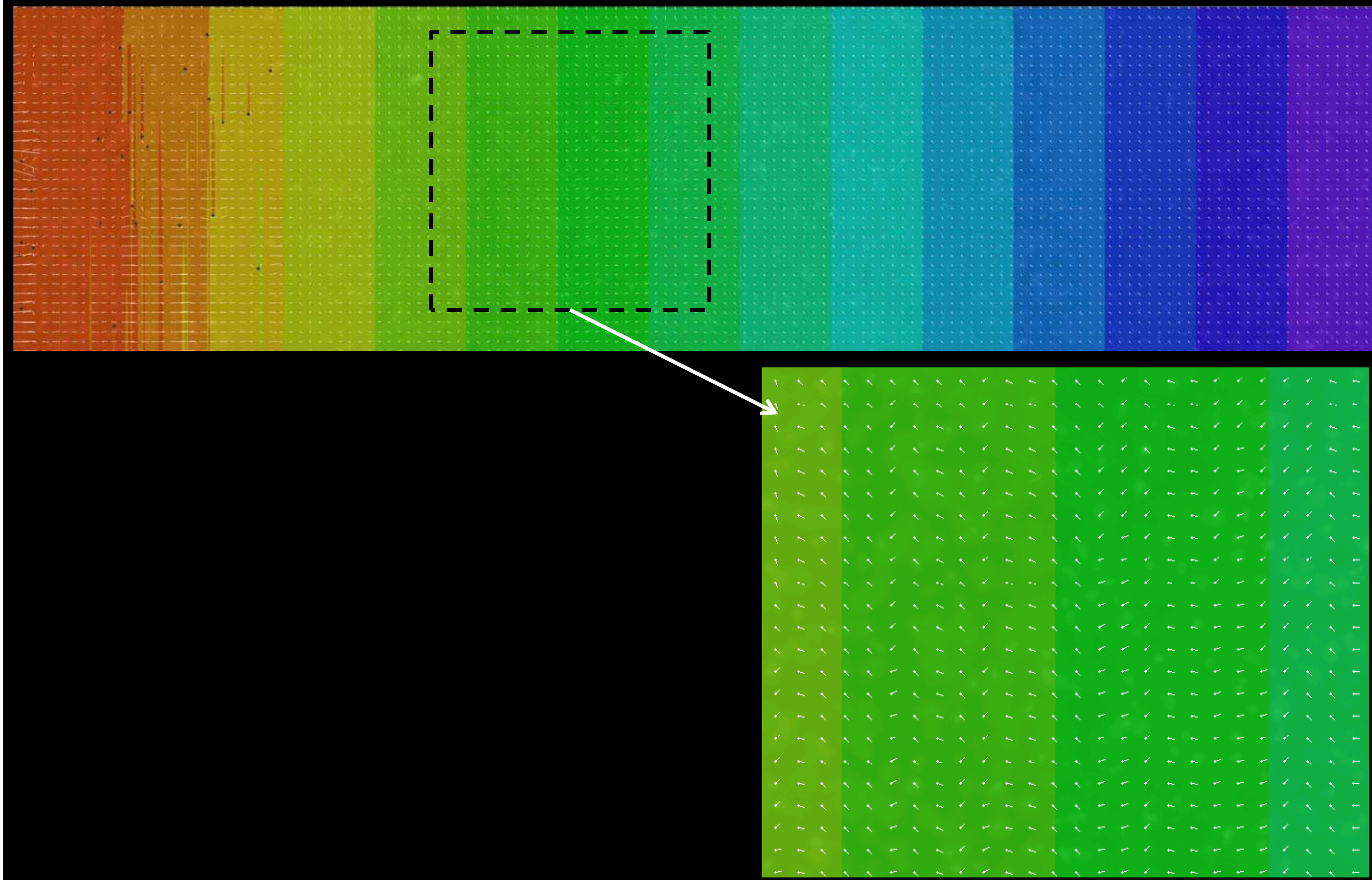


Study1_CM+_Sample2_Measurement1_10x



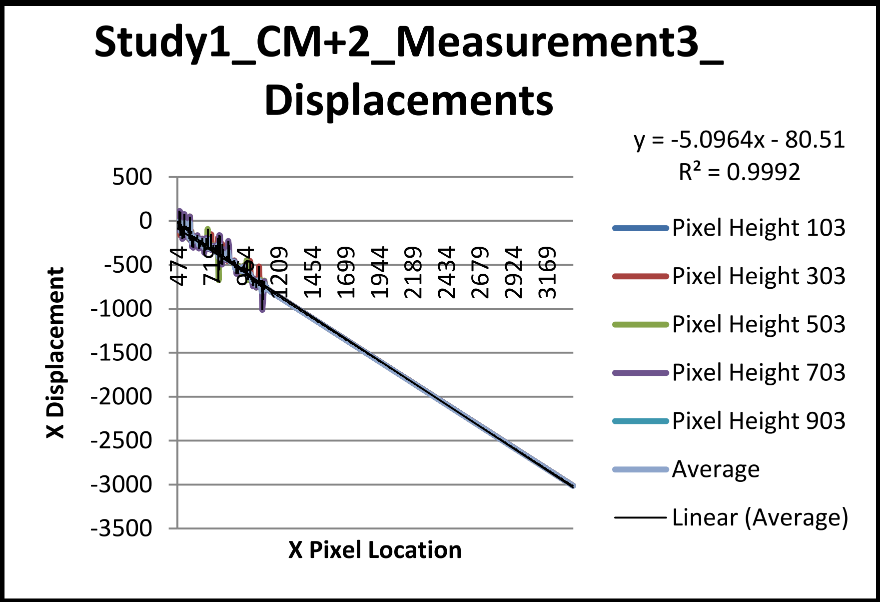
Stitch images together because at 10X the whole construct can’t be imaged

Vic-2D



Vic-2D

- Linear displacement across the construct
- Non-uniform displacement at local points
- Goal: Compare local to bulk mechanical properties



Future Work

- Develop a more sophisticated microscopic compression tester
- Use markers in the agarose to determine the strain in pericellular as well as intracellular matrix
- Compare matrix elaborated by chondrocytes and MSCs at a microscopic and macroscopic level

Conclusions

- With the use of confocal imaging, we may be able to determine cell deformation and matrix elaboration of viable cells through noninvasive methods.
- The analysis of cell, local, and bulk tissue mechanics may provide insight into the subpar bulk mechanics found in MSC constructs.
- Using 2D fluorescent microscopy we can determine the heterogeneity of local matrix properties and relate these to bulk material properties.

Thank You

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WHERE DISCOVERIES BEGIN

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