

Using light-sheet microscopy and FRET-based talin force sensors to investigate dynamic cell force generation

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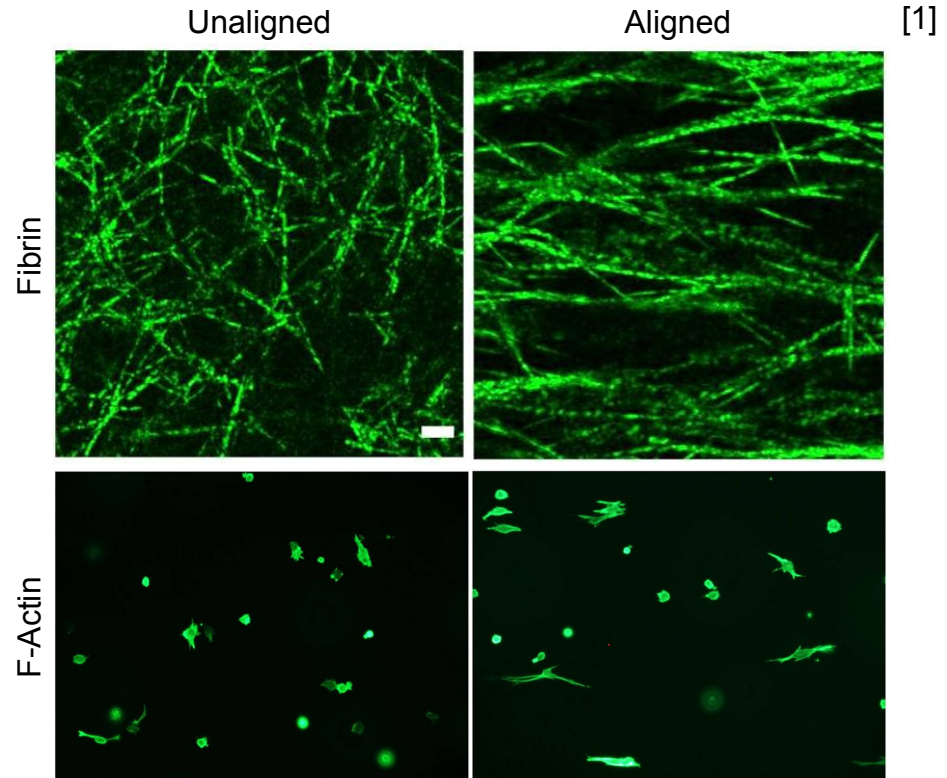


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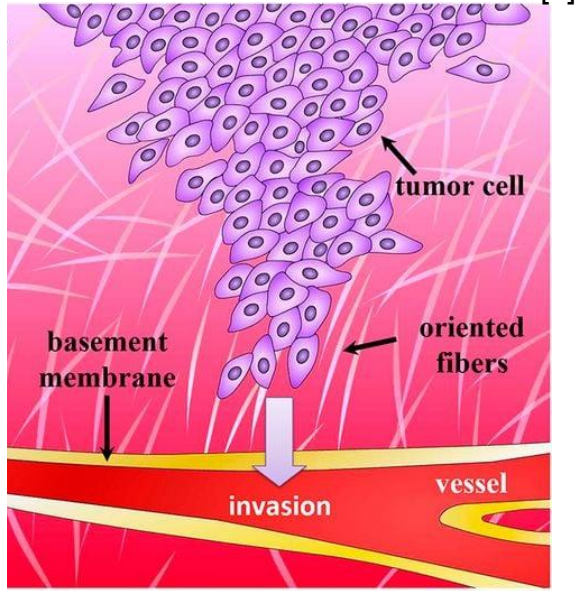
The Extracellular Matrix is a Complex Fibrillar Environment

- Extracellular matrix (ECM) is composed of fibers
- Fiber directionality influences mechanical properties of tissues
- Presence of aligned structures influences cell behavior

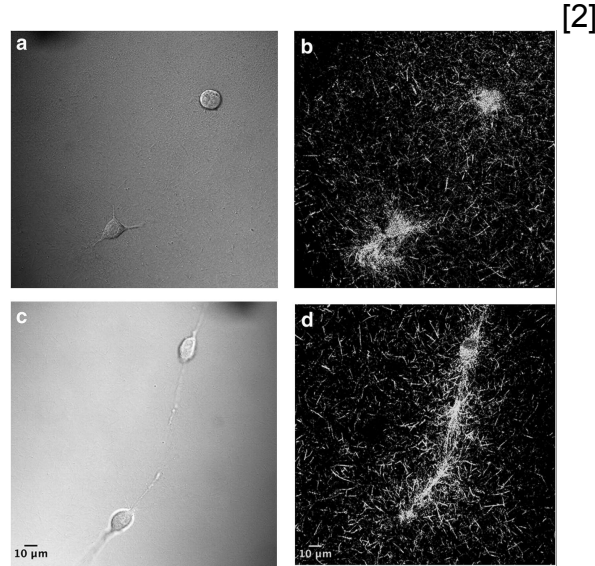


Contact Guidance is a Broadly Observed Phenomenon with Broad Applications

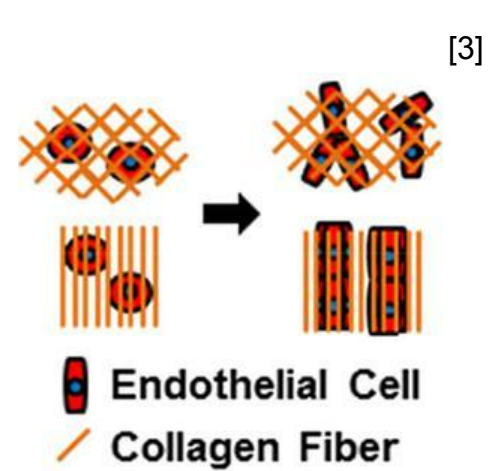
Cancer Biology



Cell Signaling



Tissue Engineering



[1] W. Han, et al., *PNAS* 113(40) 11208-11213, Sept 2016, [2] X. Ma, et al, *Biophysical Journal*, 104(1410-1418): April 2013,

[3] M. McCoy, et al, *ACS Biomater. Sci. Eng.* 2018, 4, 8, 2967–2976

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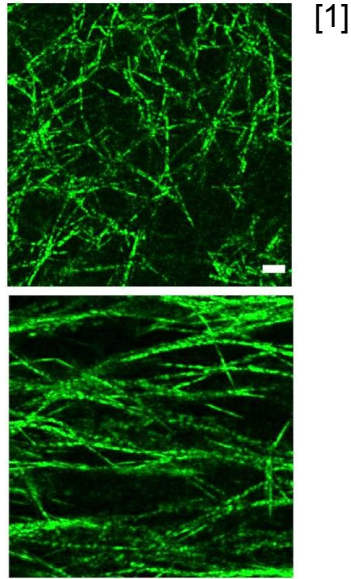


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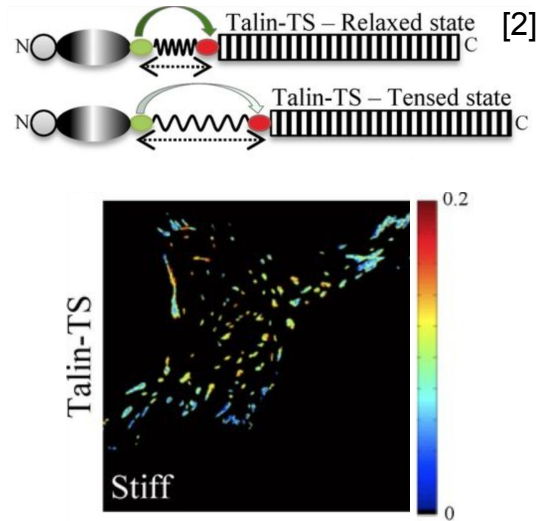
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Project Introduction

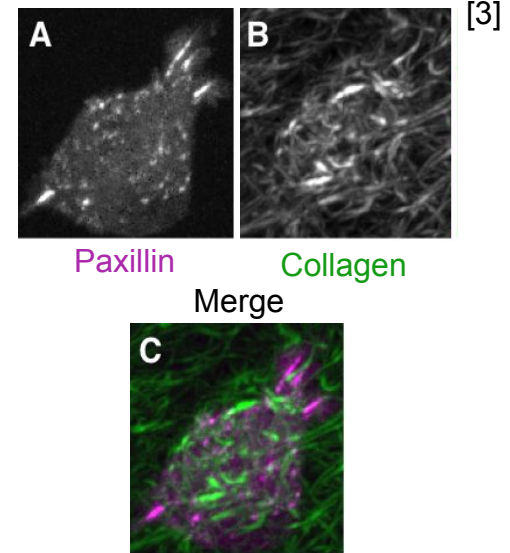
Fibrin Hydrogels



FRET-Talin Sensors

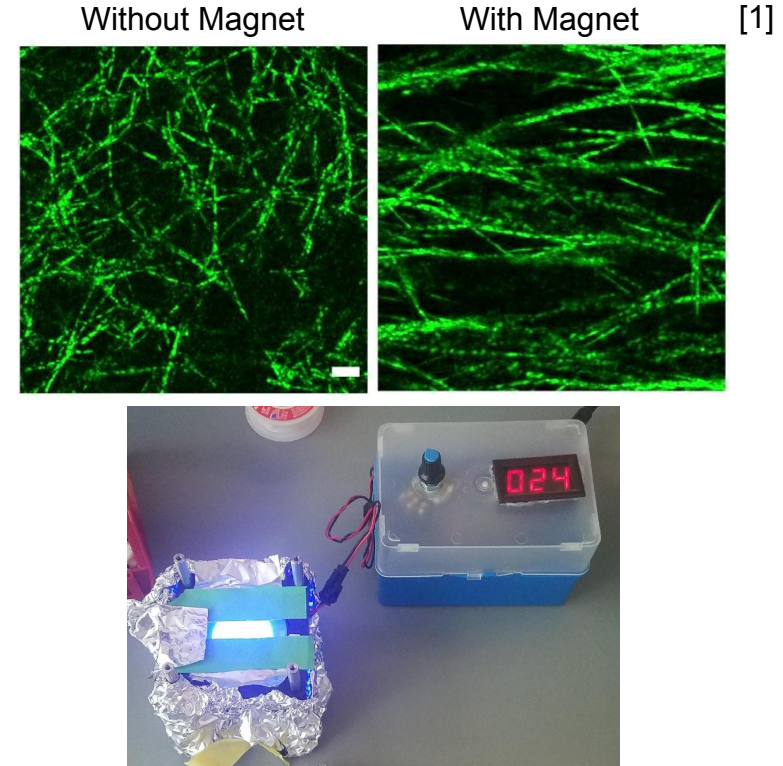


Axially-Swept Laser Sheet Microscopy



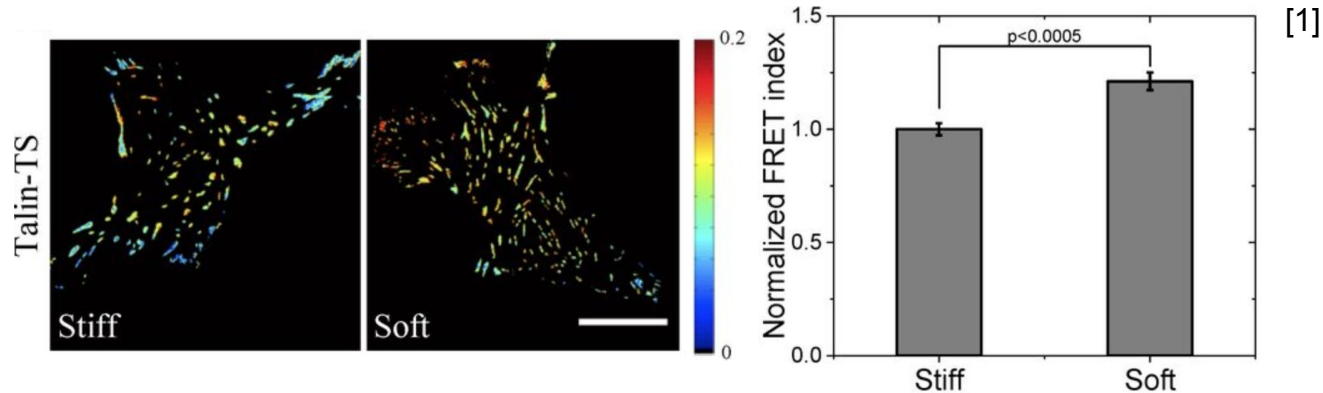
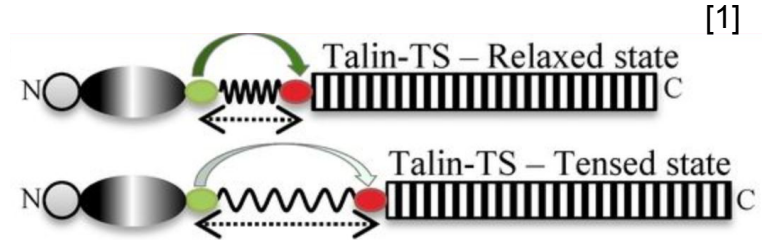
Fibrin Hydrogels Have Independently Tunable Properties

- Fibrin is a fibrillar structure found in blood clots
- This material has been used to create 3D hydrogel scaffolds
- Independently tunable parameters that affect microstructure mechanics:
 - Alignment
 - Stiffness



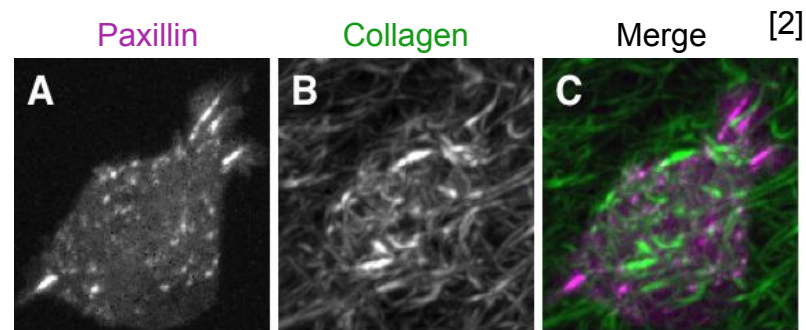
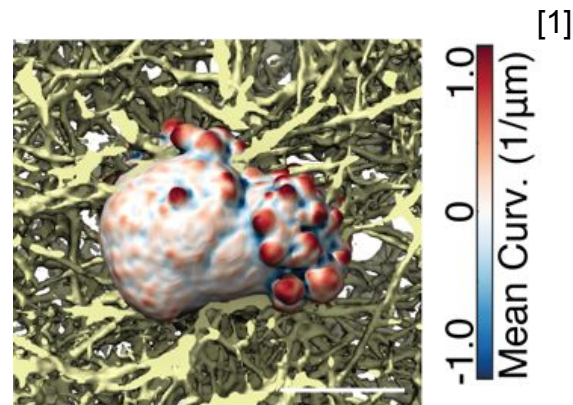
FRET-Talin Tension Cells Provide Insight into Force Generation

- FRET signal decays with increased tension
- Cells operate as both actuators and sensors, generating and responding to tension



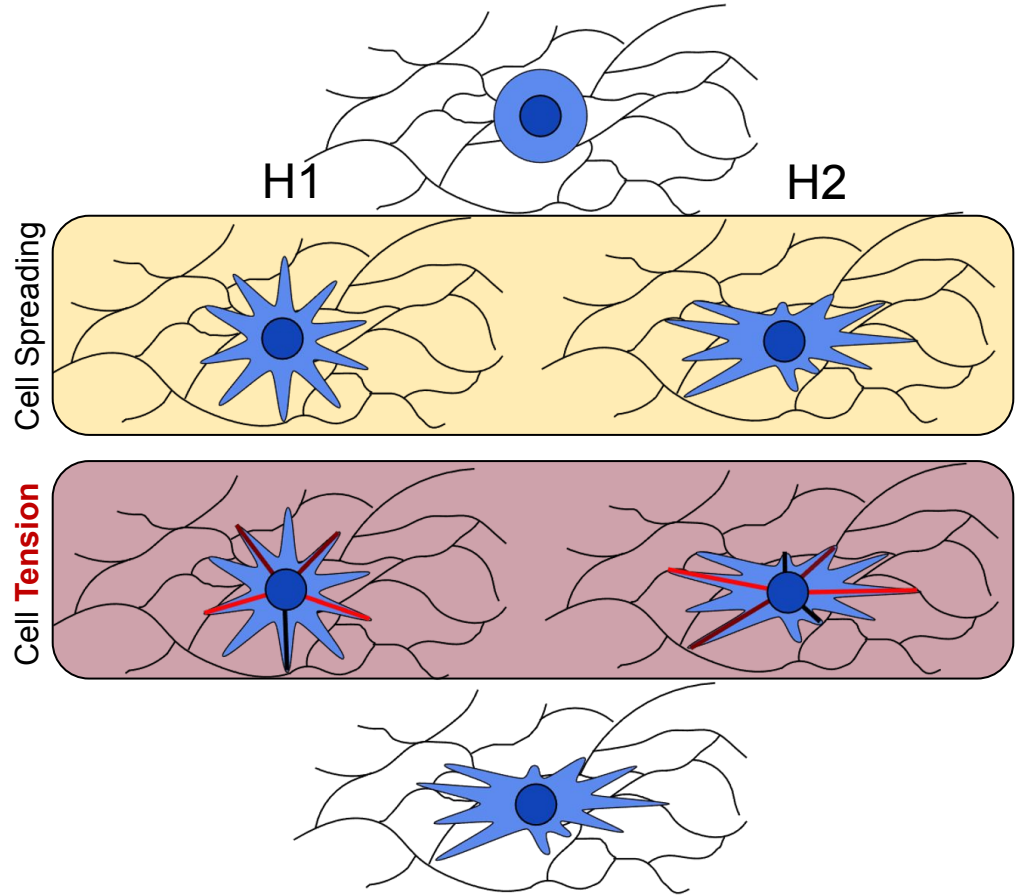
Axially-Swept Light Sheet Microscopy Enables High Resolution 3D Imaging

- 350 nm resolution with 1 second volumetric imaging
- Existing workflows can characterize gross cell morphology
- Imaging modality can localize signal in 3D cell volume
- Capacity to image both ECM and biosensor targets



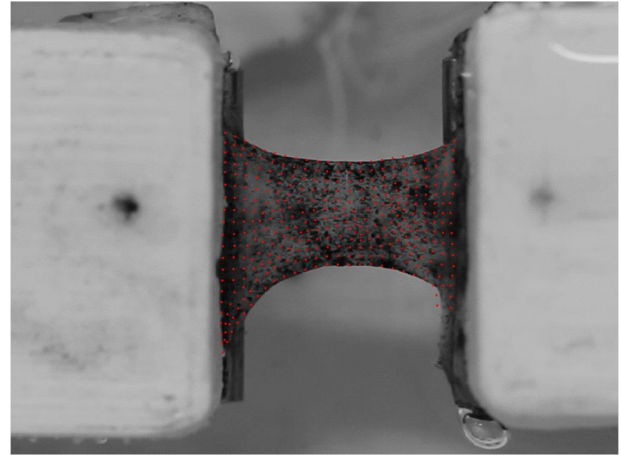
Experimental Design

- Seed cells in complex microenvironments
- Hypothesis 1: Cells spread isotropically and force generation drives polarization
- Hypothesis 2: Cells spread anisotropically and force generation reinforces polarization



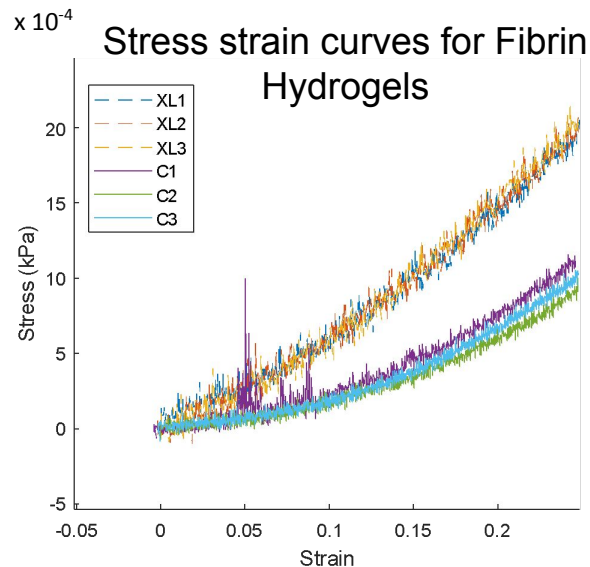
Recent Work: Exploring Fibrin Hydrogel Mechanical Space

- Uniaxial tensile testing



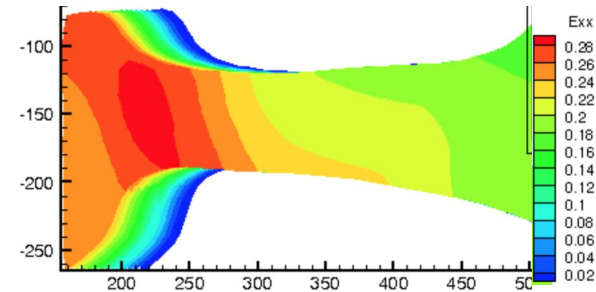
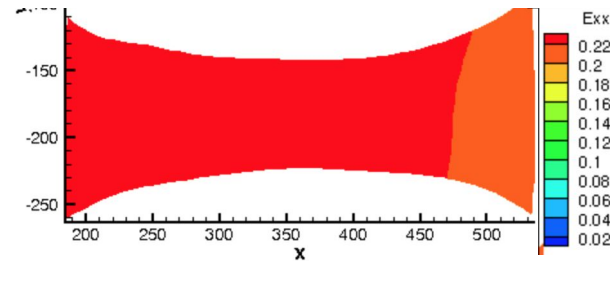
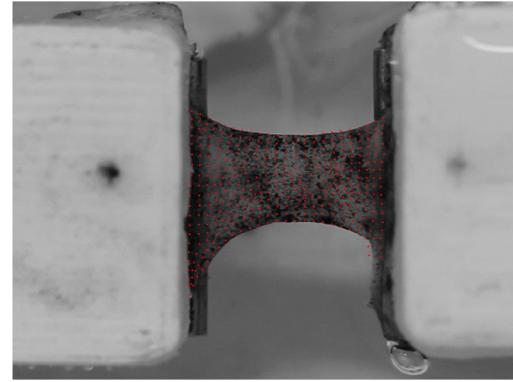
Recent Work: Exploring Fibrin Hydrogel Mechanical Space

- Uniaxial tensile testing
- X-linking increases mechanical stiffness of gels in tension



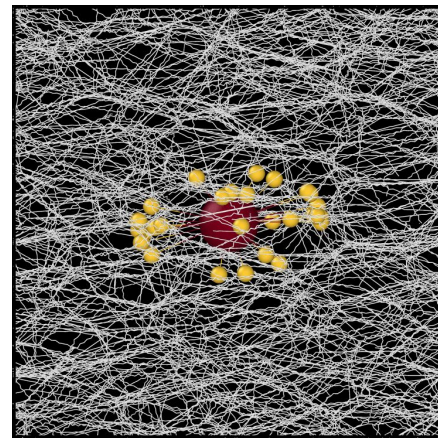
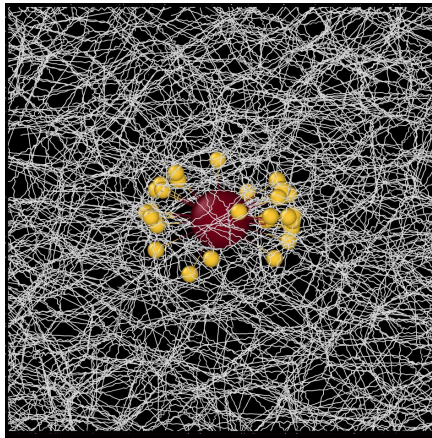
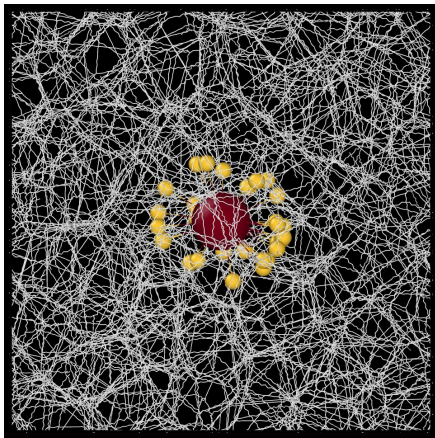
Recent Work: Exploring Fibrin Hydrogel Mechanical Space

- X-linking increases mechanical stiffness of gels in tension
- Strain tracking techniques can observe local differences in stiffness
- Strain tracking can observe differences in local bulk stiffness



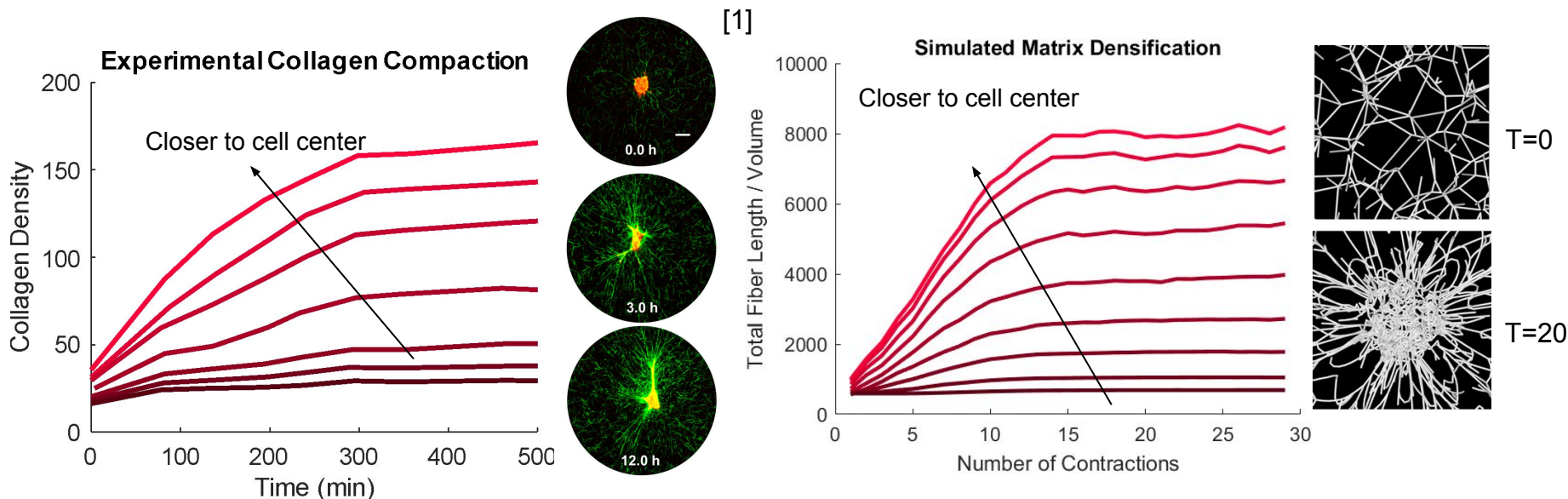
Recent Work: 3D Discrete Fiber Model of Cell Compaction

- 3D discrete fiber model of cell-mediated compaction in initially unaligned and aligned fiber networks
- Generating simulated datasets that can be compared against experimental results



Recent Work: 3D Discrete Fiber Model of Cell Compaction

- Model recapitulates experimental observations of plateauing matrix densification and radial alignment of collagen

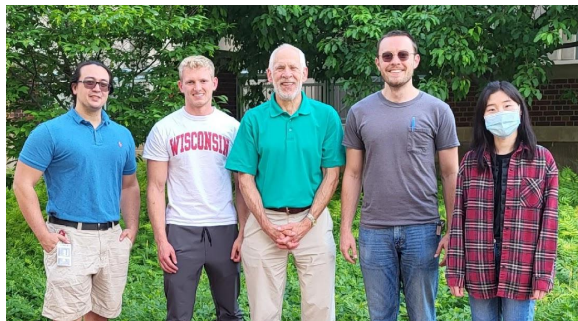


Upcoming Work

- Newly transected cells coming from collaborator
- Microscope construction is complete and will be transported by the summer

Acknowledgements

Tranquillo Lab



Driscoll Lab



Collaborators

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Martin Schwartz, Yale University

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