Nonlinear Biomechanics of Fibroblast Mechanosensing in Fibrous ECM: Modelling, Analysis and Computation

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Cells Use Stress to Find Each Other in Fibrous Darkness

with (IACM Team)

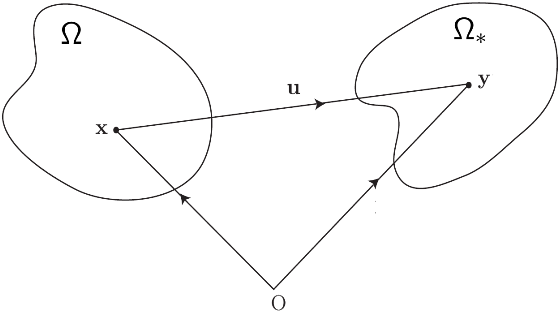
Georgios Grekas
Charalambos Makridakis

and
Guruswami Ravichandran
Jacob Notbohm
Ayelet Lesman
David Tirrell

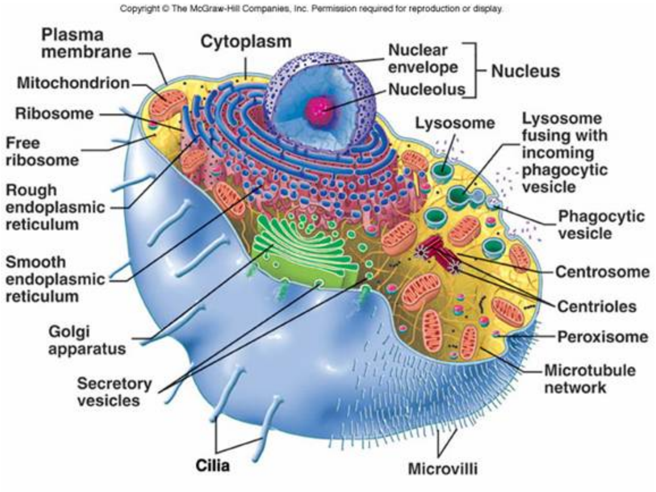
Continuum Mechanics

Continuum Mechanics

mathematical modeling of forces/deformations
in deformable solids



Cell Biology



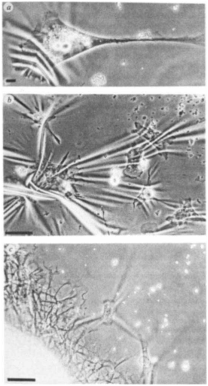
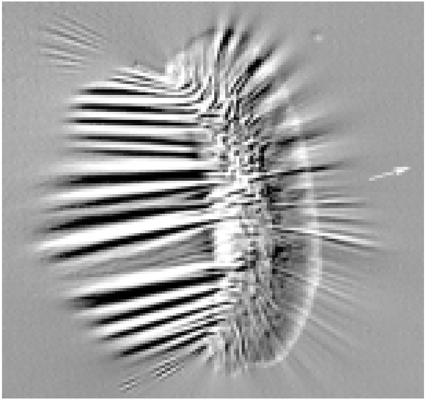


Fig. 1 Tissue culture cell spreading on thin layers of silicone rubber. The traction by which the cells spread and propel themselves is shown by the wrinkles formed in the rubber sheet. Silicone

Fibroblasts cause wrinkling of 2D silicone substrate

Harris, Stopak, Wild 1981



(a)

Moving Fish Epidermal Keratocytes
cause wrinkling of 2D silicone substrate
Burton, Park , Taylor, 1999

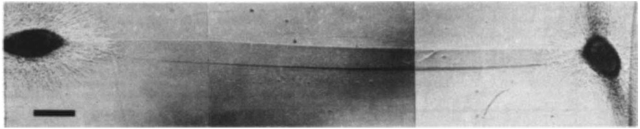


Fig. 4 Two centre effects form in collagen gels even when explants are separated by a distance spanning over 1.5 cm. Collagen fibres become aligned into long axially oriented tracts interconnecting two centres of traction. Heart explants from 8-day chick embryos after 96 h in culture. Scale bar, 1 mm.

Explants induce densification bands in 3D fibrin extracellular matrix

Harris, Stopak, Wild 1981

Fibroblasts exert “huge” forces onto their surroundings

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WHY?

mechanosensing

cells sense forces/stresses/deformations

mechanosensing

cells sense forces/stresses/deformations

tensotaxis

cells protrude/migrate toward regions of higher tension

mechanosensing

cells sense forces/stresses/deformations

tensotaxis

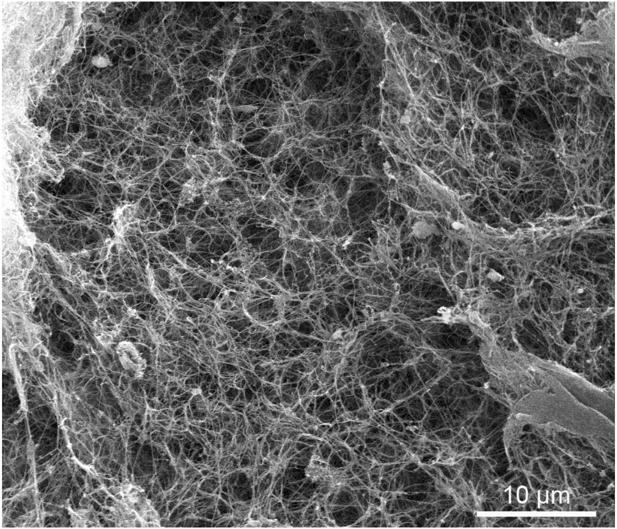
cells protrude/migrate toward regions of higher tension

durotaxis

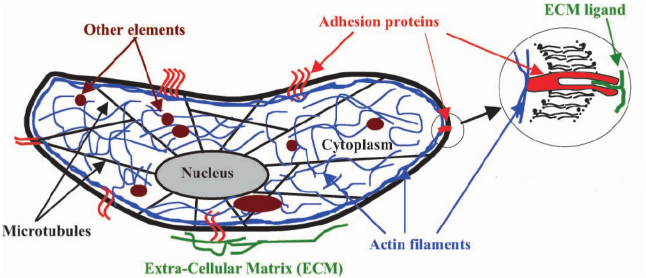
cells protrude/migrate toward regions of higher stiffness

What does the ECM look like?

Network of collagen/fibrin fibers



The Role of Focal Adhesions



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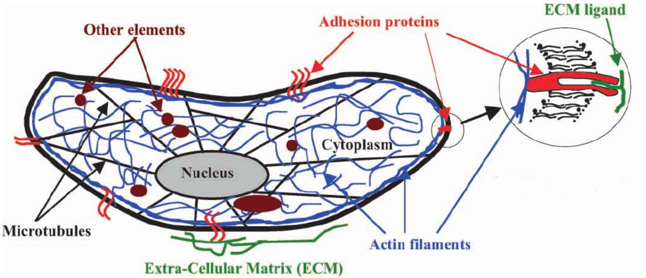
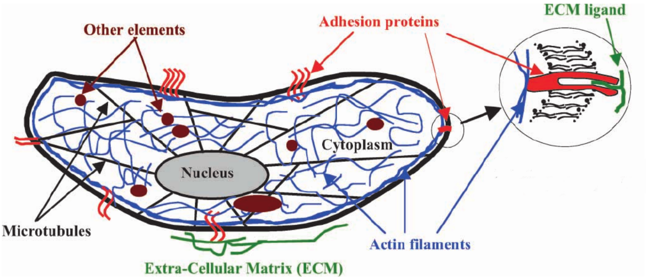


Adhesions allow the cell to exert traction on the ECM

The Role of Focal Adhesions



Adhesions allow the cell to exert traction on the ECM

Adhesions act as force/ stress/ deformation detectors.

Mechanosensing is active!!

Fibroblasts Use Stress, But WHY?

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To see

Fibroblasts Use Stress, But WHY?

To see

and be seen

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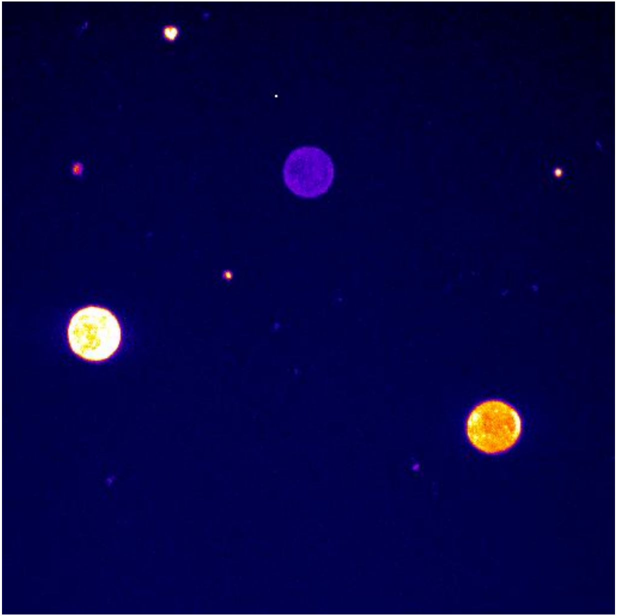
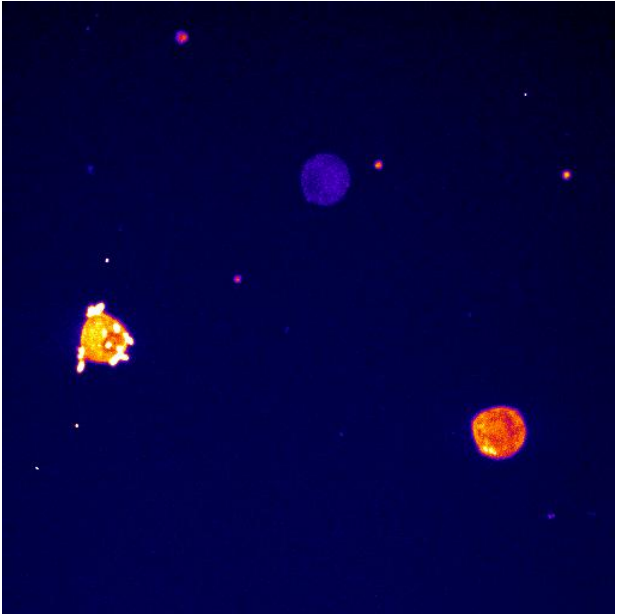
To detect and approach each other by spreading/protruding

To remodel the matrix around them (possibly for tissue morphogenesis Stopak-Harris, 1982)



Notbohm 2015



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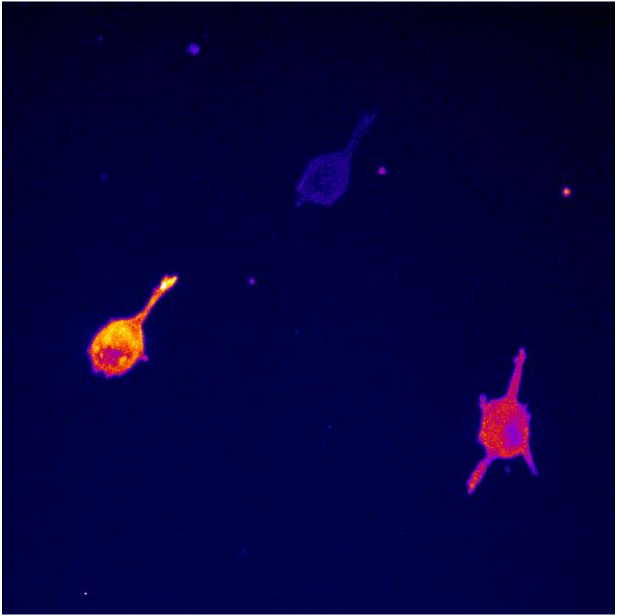

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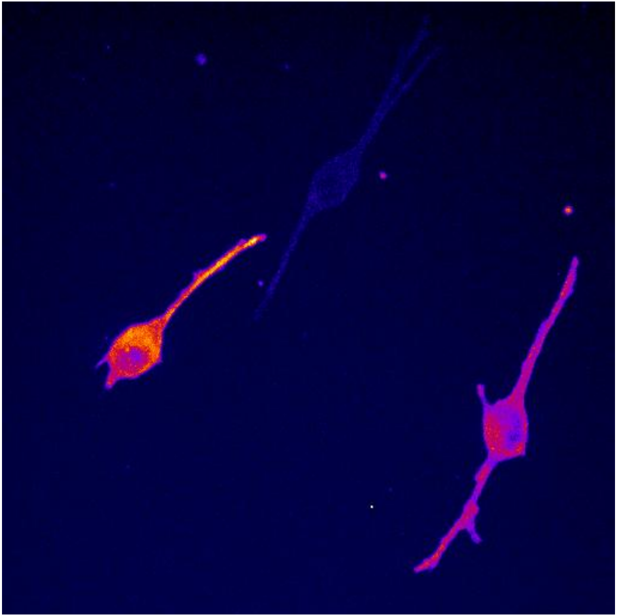

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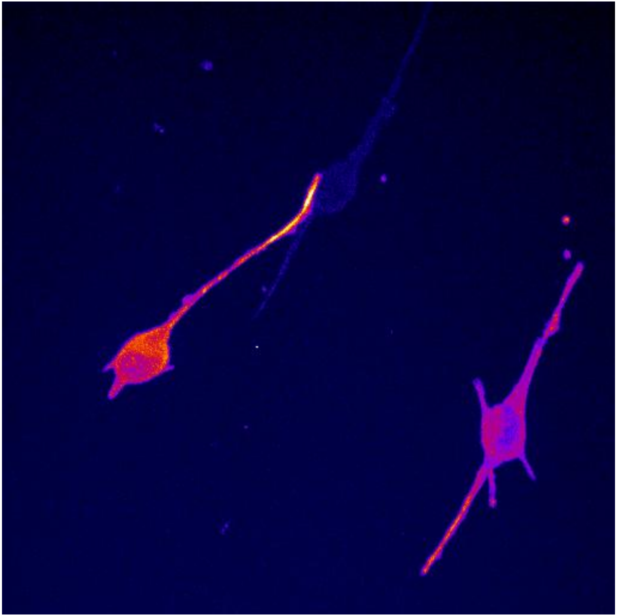
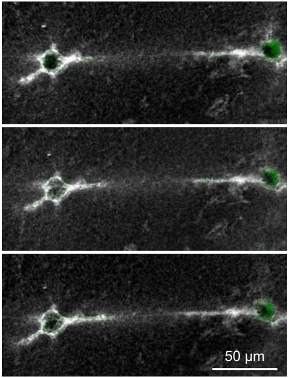


Notbohm-Ravichandran-Lesman-Tirrell 2013



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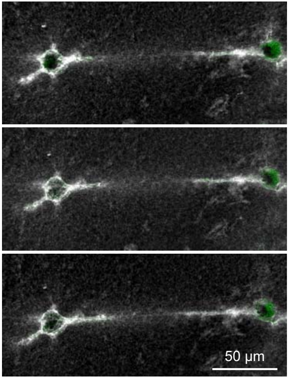
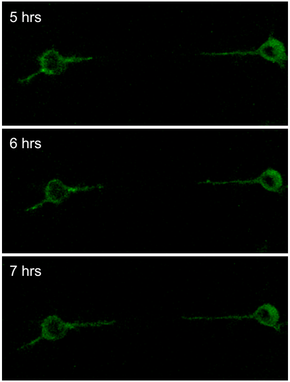
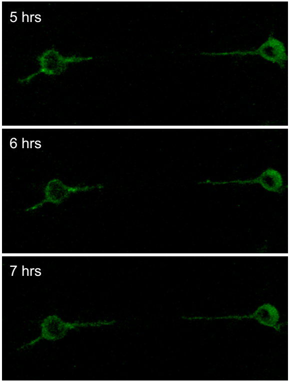


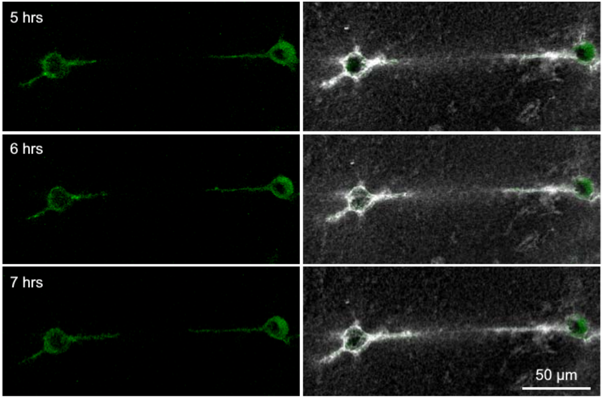
Cells contract and tethers form in the ECM joining them.
Tethers (white) are regions of high ECM density


5 hrs
6 hrs
7 hrs



Later, cells grow appendages along the tethers towards each other.

Green: cell actin.


5 hrs
6 hrs
7 hrs
50 µm

Claim:

This behavior relies on a special nonlinearity of the ECM's mechanical behavior

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This behavior relies on a special (instability) of the ECM's mechanical behavior

this tethering behavior does not occur in homogeneous linear elastic ECM (e.g. hydrogels)

Microbuckling

Individual fibers will buckle under compression

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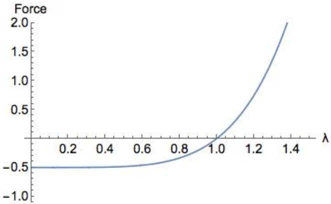
stiffer in tesnion than in compression (rubber band)



A Nonlinear Constitutive Law for Large Deformations

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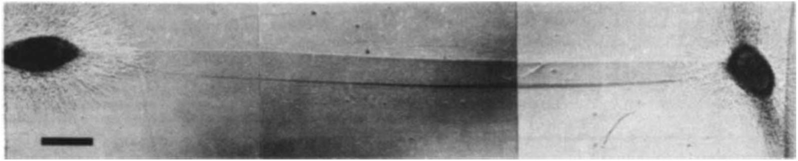

Fig. 4 Two centre effects form in collagen gels even when explants are separated by a distance spanning over 1.5 cm. Collagen fibres become aligned into long axially oriented tracts interconnecting two centres of traction. Heart explants from 8-day chick embryos after 96 h in culture. Scale bar, 1 mm.

Harris-Stopak 1981

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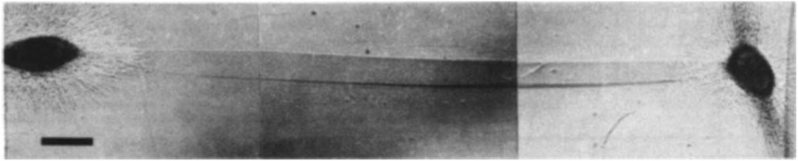

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Macroscopic tether (1.5cm) between contracting (multi-cell) explants (2-3mm) in collagen fibrous ECM

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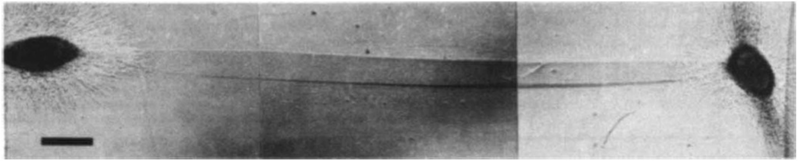

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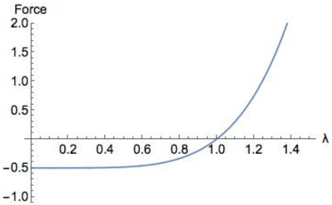
Macroscopic tether (1.5cm) between contracting (multi-cell) explants (2-3mm) in collagen fibrous ECM

Hypothesis: This can be explained by microbuckling.

Elastic Strain Energy Function

Start with a single fiber with force stretch relation $F(\lambda)$ that is weaker in compression than tension and energy

$$\bar{W}(\lambda) = \int_1^\lambda F(\zeta) d\zeta$$



$$F(\lambda) = \mu \left(\lambda^N - 1 \right)$$

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Suppose ECM (2D) has uniform angular distribution of fibers

$$\hat{W}(\lambda_1, \lambda_2) = \frac{1}{2\pi} \int_0^{2\pi} \bar{W}\left(\sqrt{(\lambda_1 cos\theta)^2 + (\lambda_2 sin\theta)^2}\right) d\theta$$

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Explicitly determined

$$W(\boldsymbol{F}), \qquad \boldsymbol{F} = \nabla \boldsymbol{u}$$

Elastic Deformation Energy /unit volume, function of deformation gradient matrix $\boldsymbol{F}$ (strain)

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Nonlinear Stress-Strain Relation

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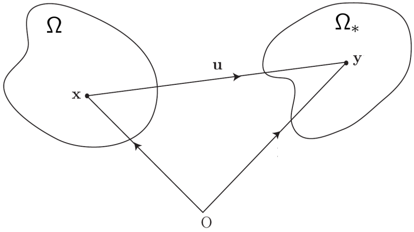

Interesting Properties of W

Uniaxial compression

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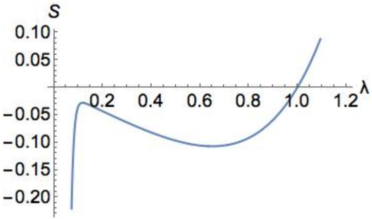

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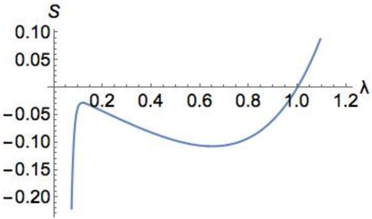

Nonmonotone uniaxial compression:

Interesting Properties of W

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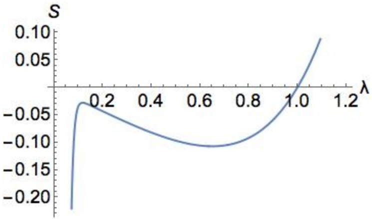

Nonmonotone uniaxial compression:
densification (compressive) phase transition,

Interesting Properties of W

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W is a multi-well isotropic strain energy

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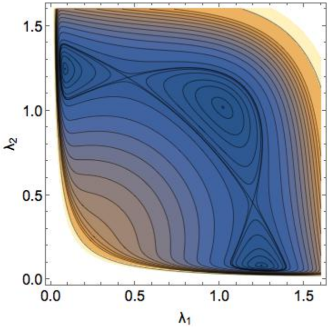
Level curves of $\hat{W}(\lambda_1, \lambda_2)$



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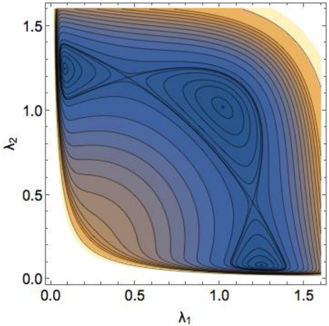

Two wells : two phases (stable states) because of microbuckling

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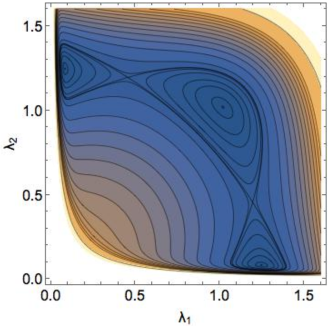

Two wells : two phases (stable states) because of microbuckling
Instabilities, Discontinuities, Interfaces...

Elastic Energy of the ECM

- Model ECM as elastic body with holes (cells/explants)
- Strain Energy Function W.
- Elastic Energy of the ECM

$$\mathcal{E}[\boldsymbol{u}] = \int_{\Omega} W(\nabla \boldsymbol{u}) dV$$

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- Model ECM as elastic body with holes (cells/explants)
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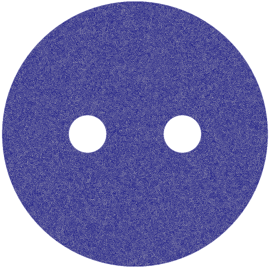
Minimize Energy

Cell Model



Cells are the holes. They contract: they apply centripetal forces proportional to distance from their center.
Explants: same as cells but at a much bigger scale.

Typical Numerical Results (Finite Element Computation)

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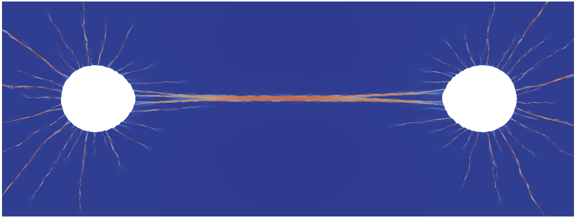

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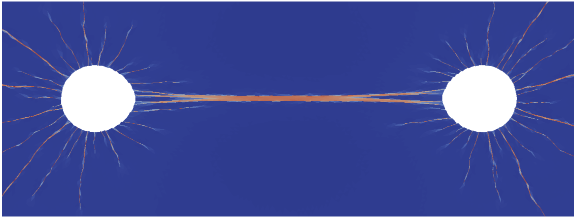
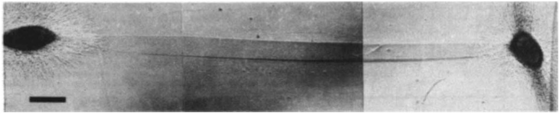


Stopak-Harris 1981



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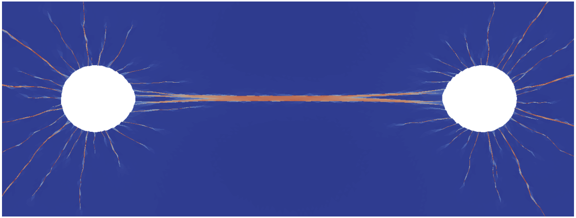

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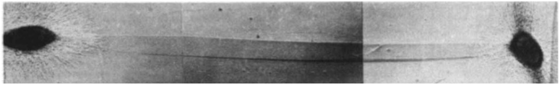

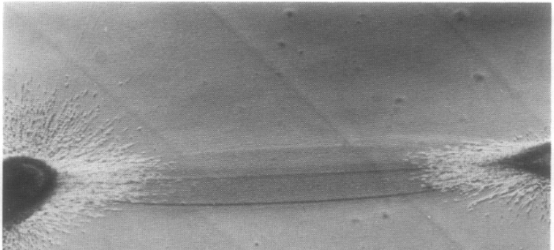

Mesh Dependence

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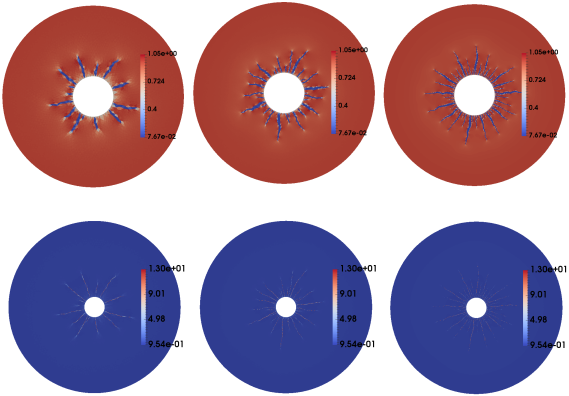

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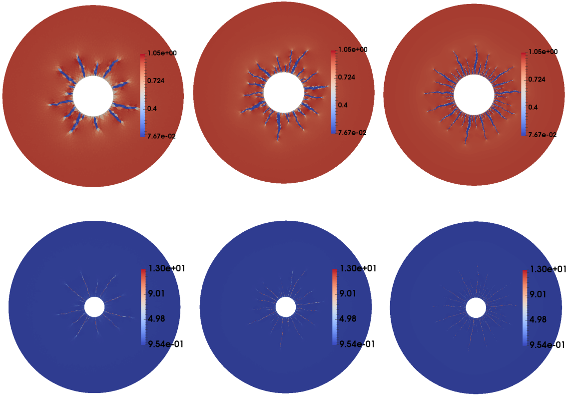
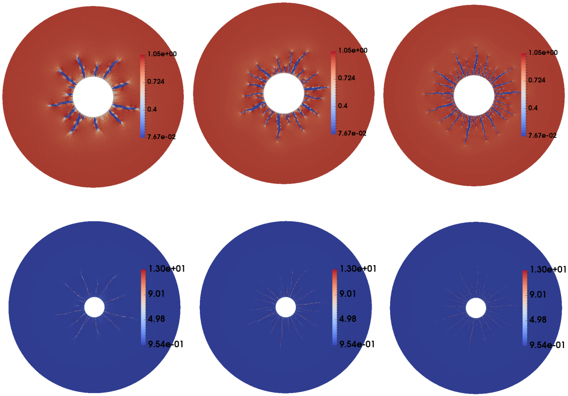


Discontinuities, Oscillations

Mesh Dependence



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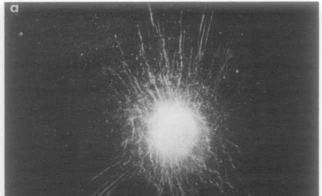
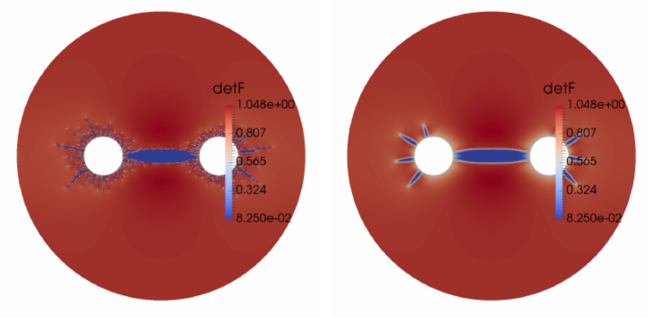


Higher Gradients

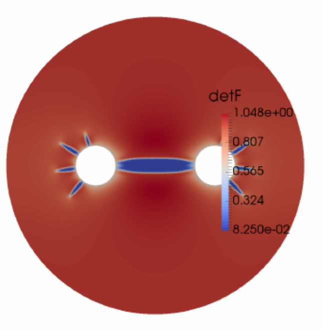
... added to the energy to limit gradient oscillations. Related to discreteness, bending stiffness of the fibers and “rotational springs”’ at network nodes.

$$\Phi[\boldsymbol{u}] = \mathcal{E}[\boldsymbol{u}] + \mathcal{C}[\boldsymbol{u}] + \frac{\varepsilon}{2} \int_\Omega |\nabla\nabla \boldsymbol{u}|^2$$



(a) $\epsilon = 0$.

(b) $\epsilon_1 > 0$.



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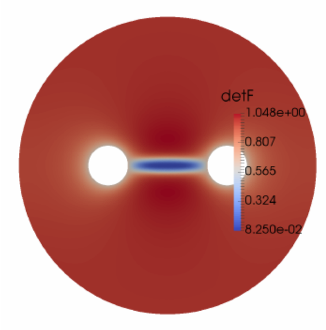
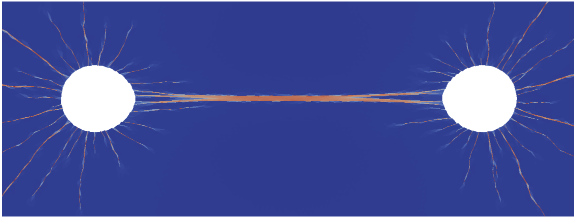
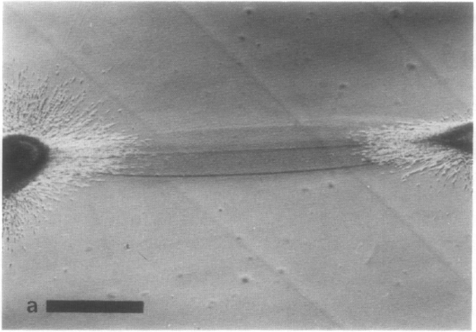


(c) $\epsilon_2 > \epsilon_1$.

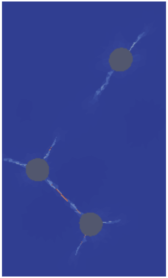
Length Scale $\sqrt{\varepsilon} << $ explant size

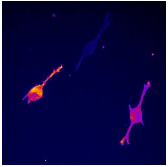


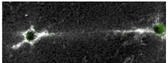


Length Scale $\sqrt{\varepsilon} \approx$ cell size

Compare of simulations with experiments to calibrate ε based on number of protrusions.







Conclusions

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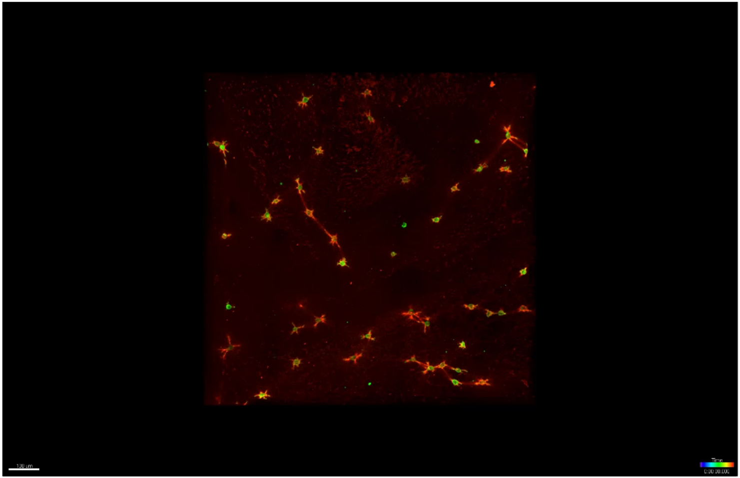
- yes but why?

Cell Networks

Lesman-Notbohm-Ravichnadran-Tirrell unpublished experiments

Cell-cell interactions in 3D



100 µm

Labeled fibrin: 10mg/ml,
3T3 Fibroblast: 3 K cells/10 µl

Cell Networks

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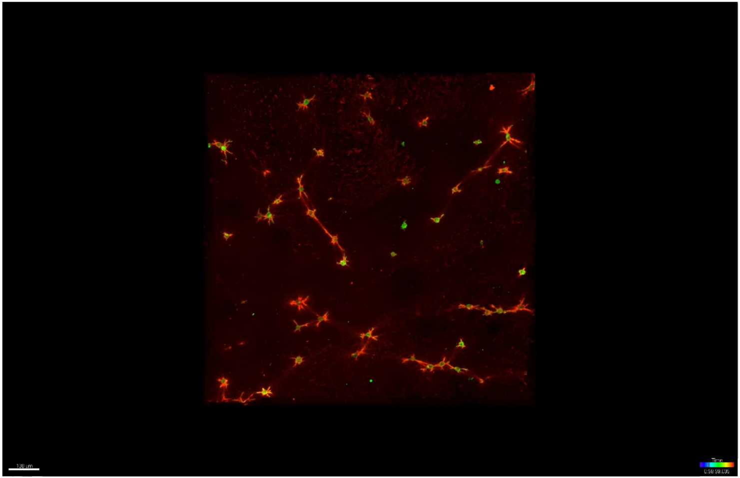

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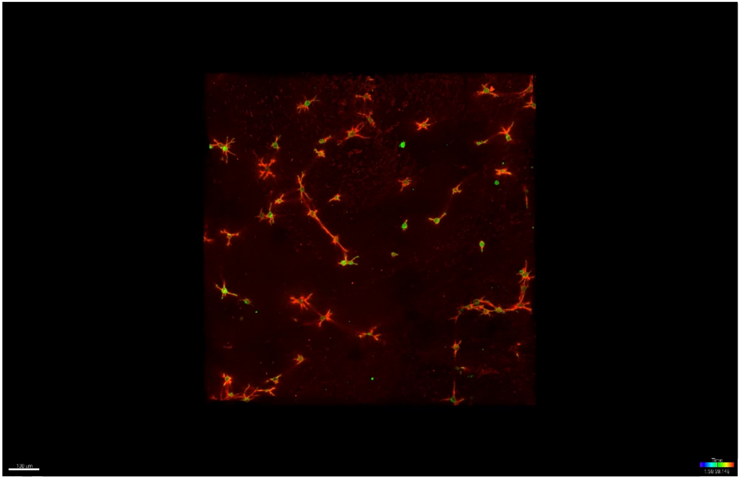

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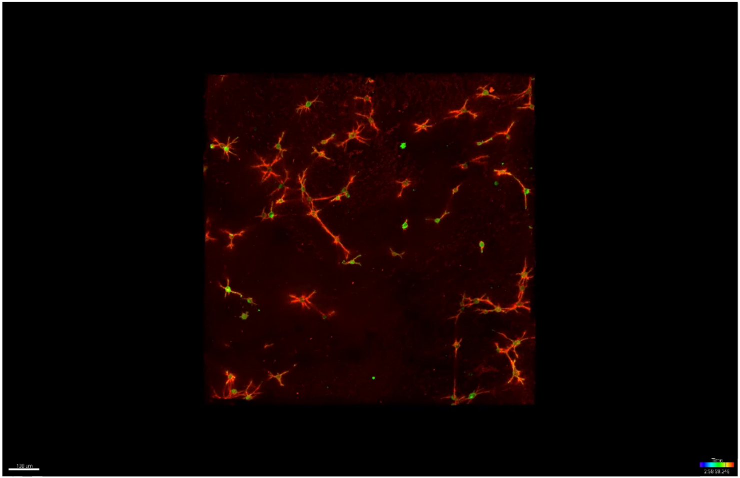

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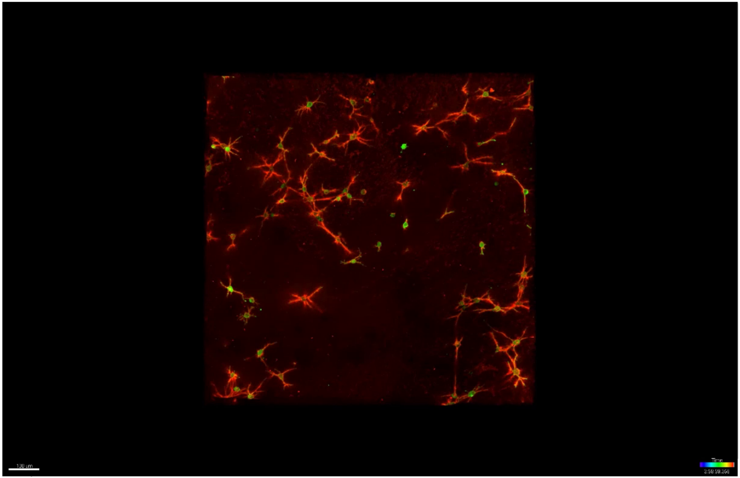

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Cell Networks

Hypothesis: Matrix Remodeling

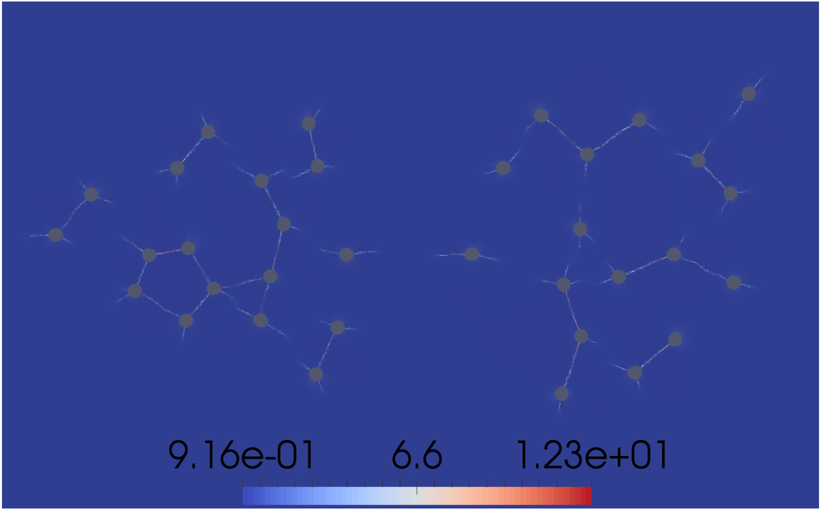
Cell Networks

Hypothesis: Matrix Remodeling

Tether network changes ECM mechanical properties (stiffness)

Cell Networks

2D Simulation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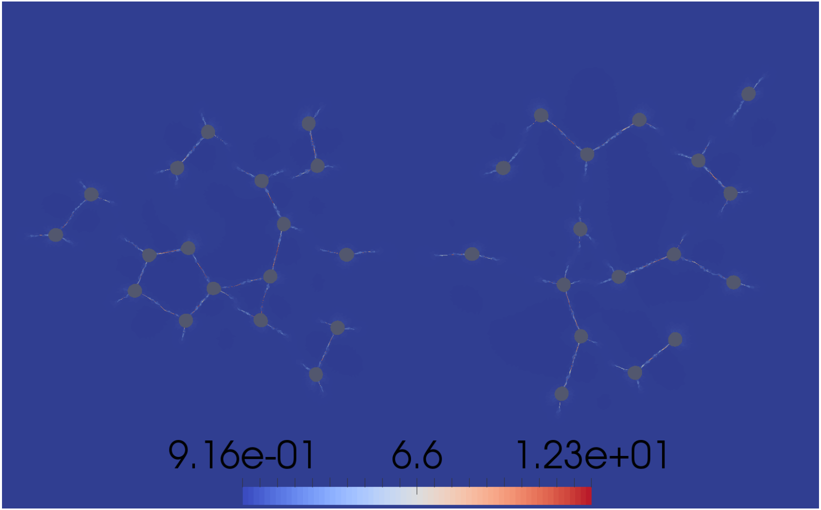
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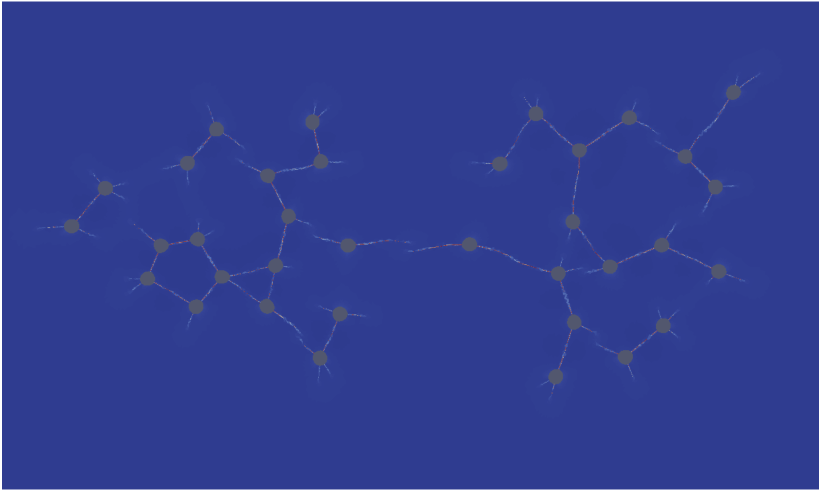

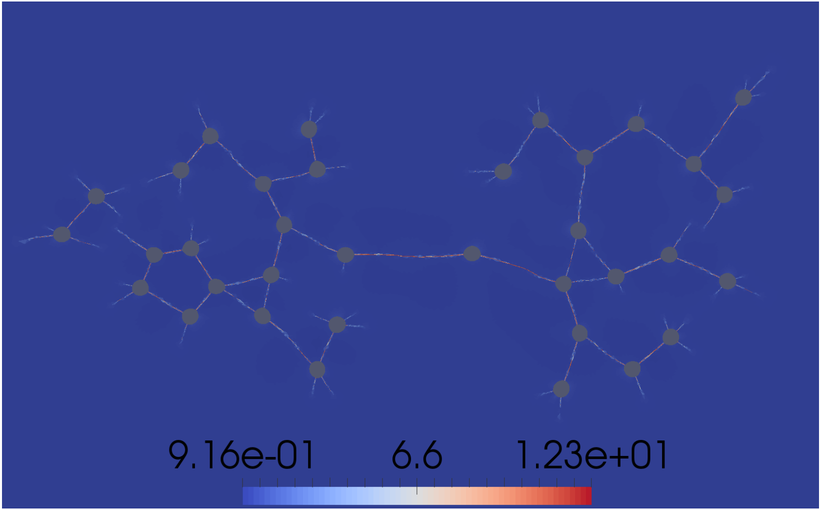
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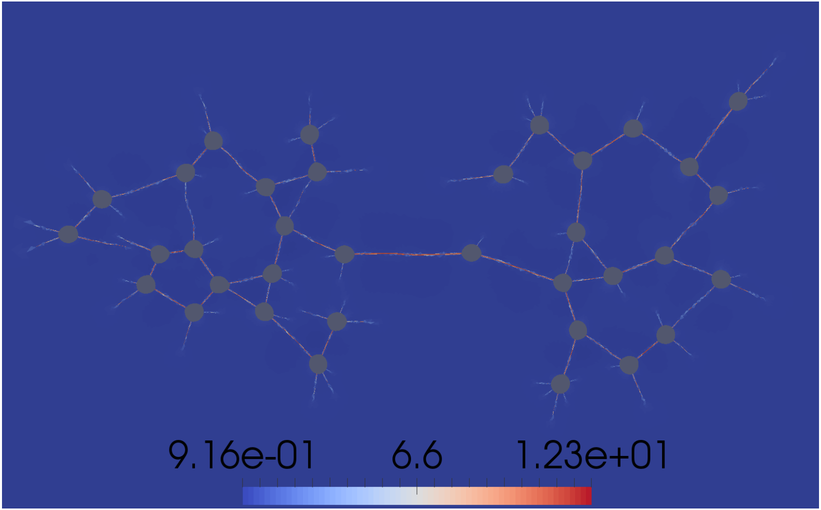
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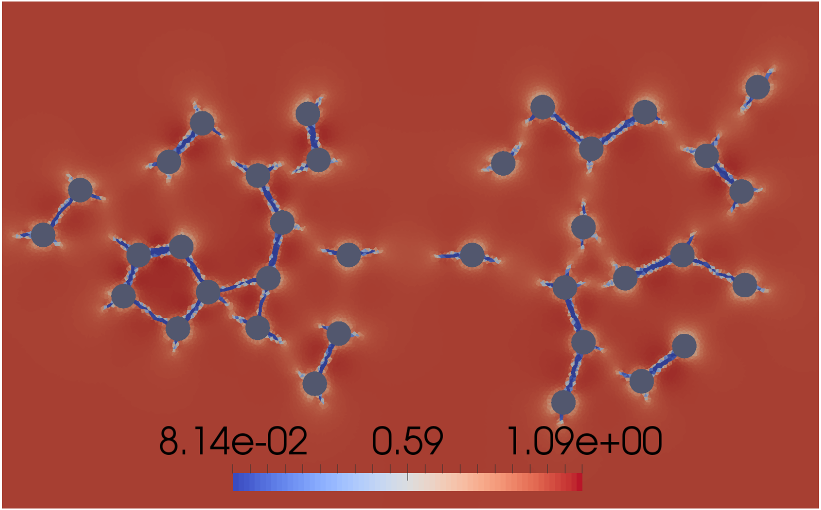
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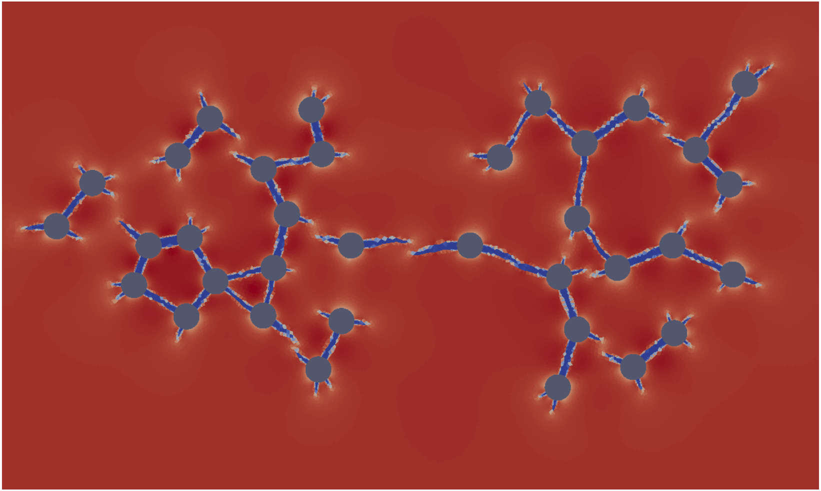

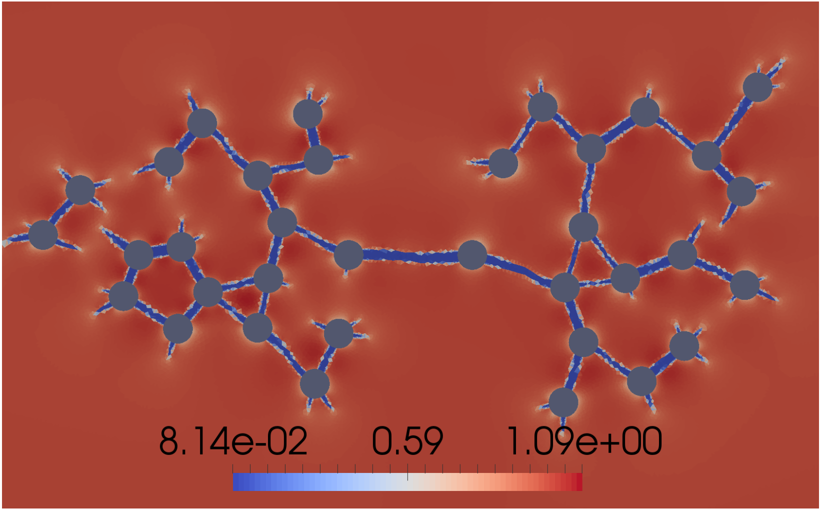
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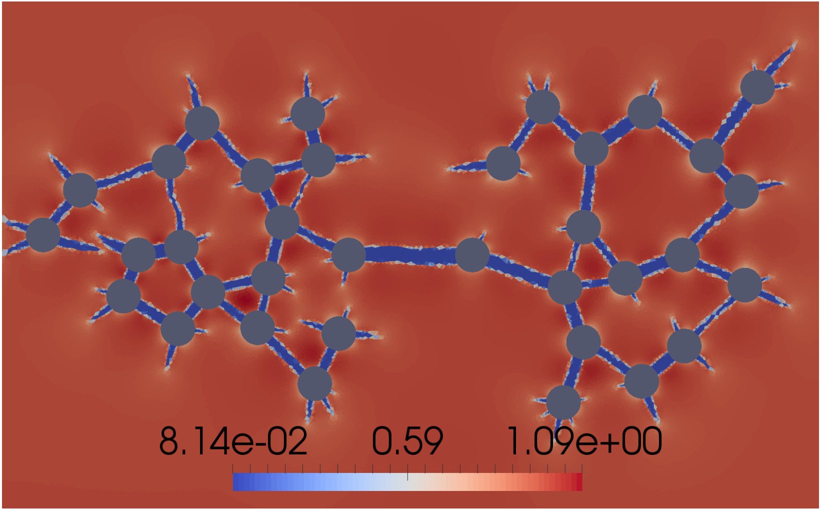
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