Mechanisms of Embryonic Wound Healing: Experiment and Modeling

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Embryo Physics Course
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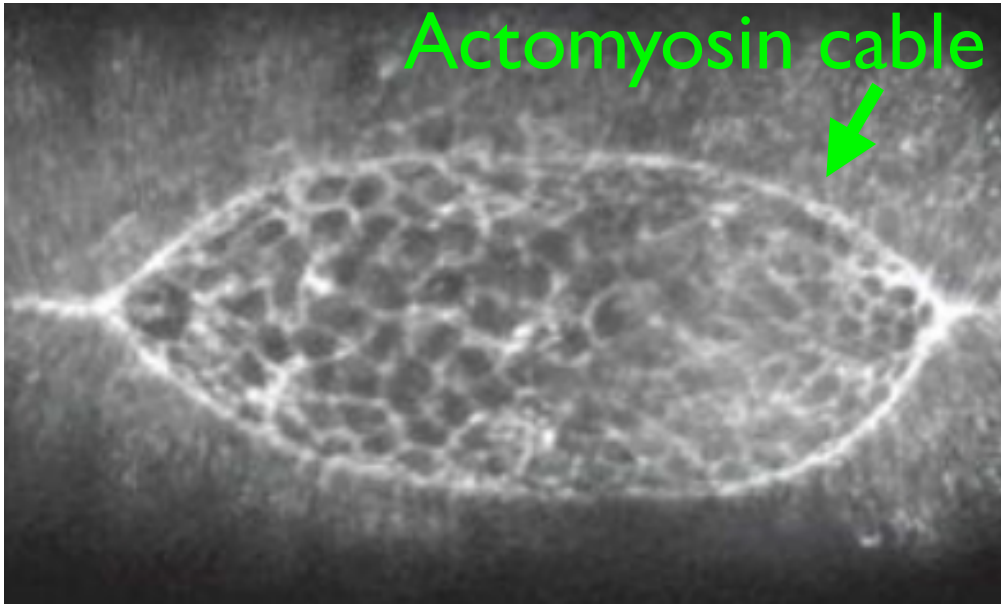
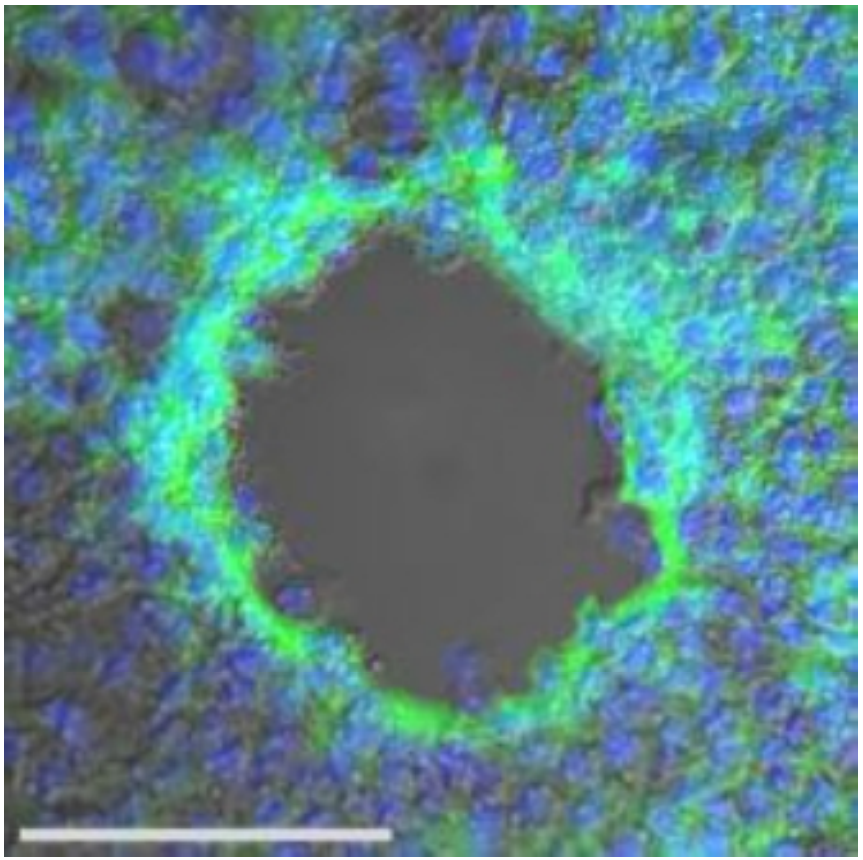
Embryonic Wounds

- Different from adult wounds
  - heal quickly, no scars
- Same cellular machinery as morphogenesis
  - actomyosin cable
  - filopodia



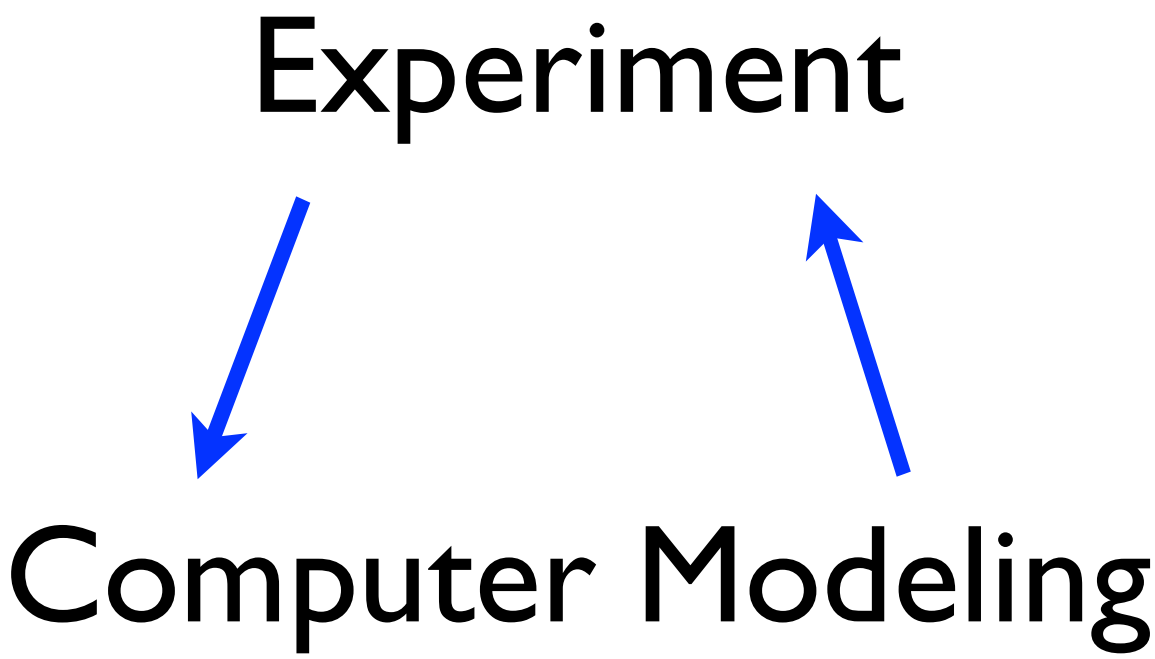
Drosophila dorsal closure
GFP-Moesin label
Rodriguez-Diaz, HFSP J 2 (2008)

Chick Model

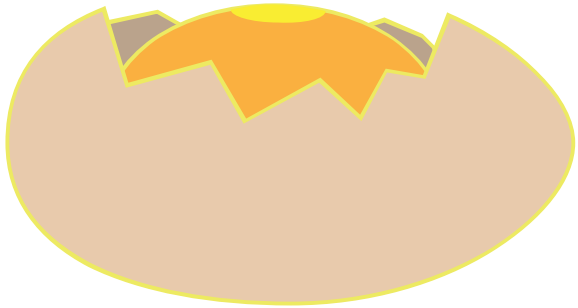


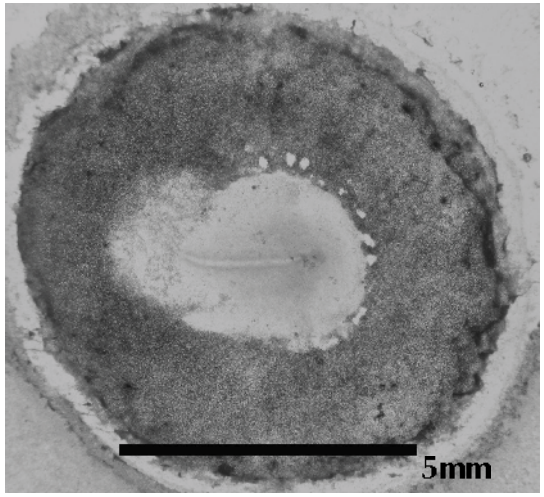
100μm F-Actin Nuclei

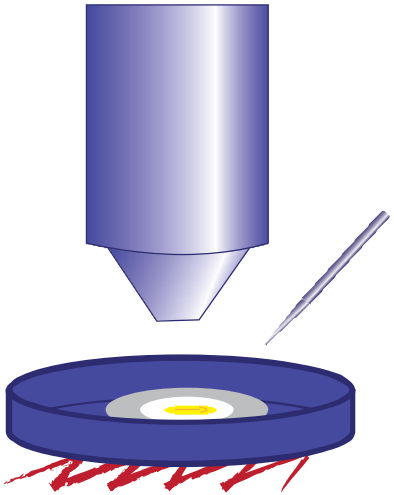
- Chick model system
  - Simple 2D geometry
- Goal: role of mechanical stress in morphogenesis

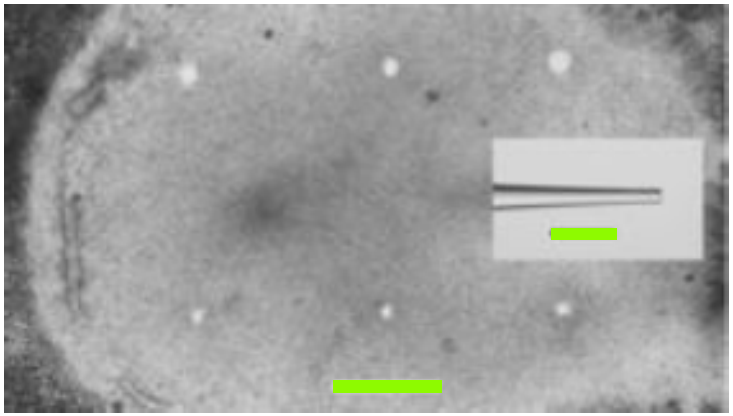

Experiment
Computer Modeling

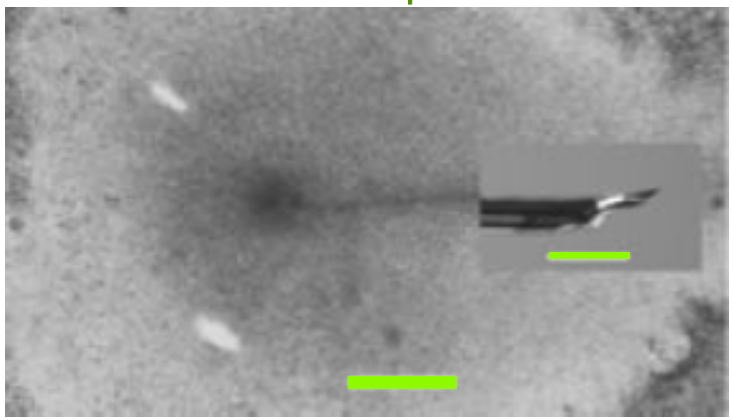
Methods

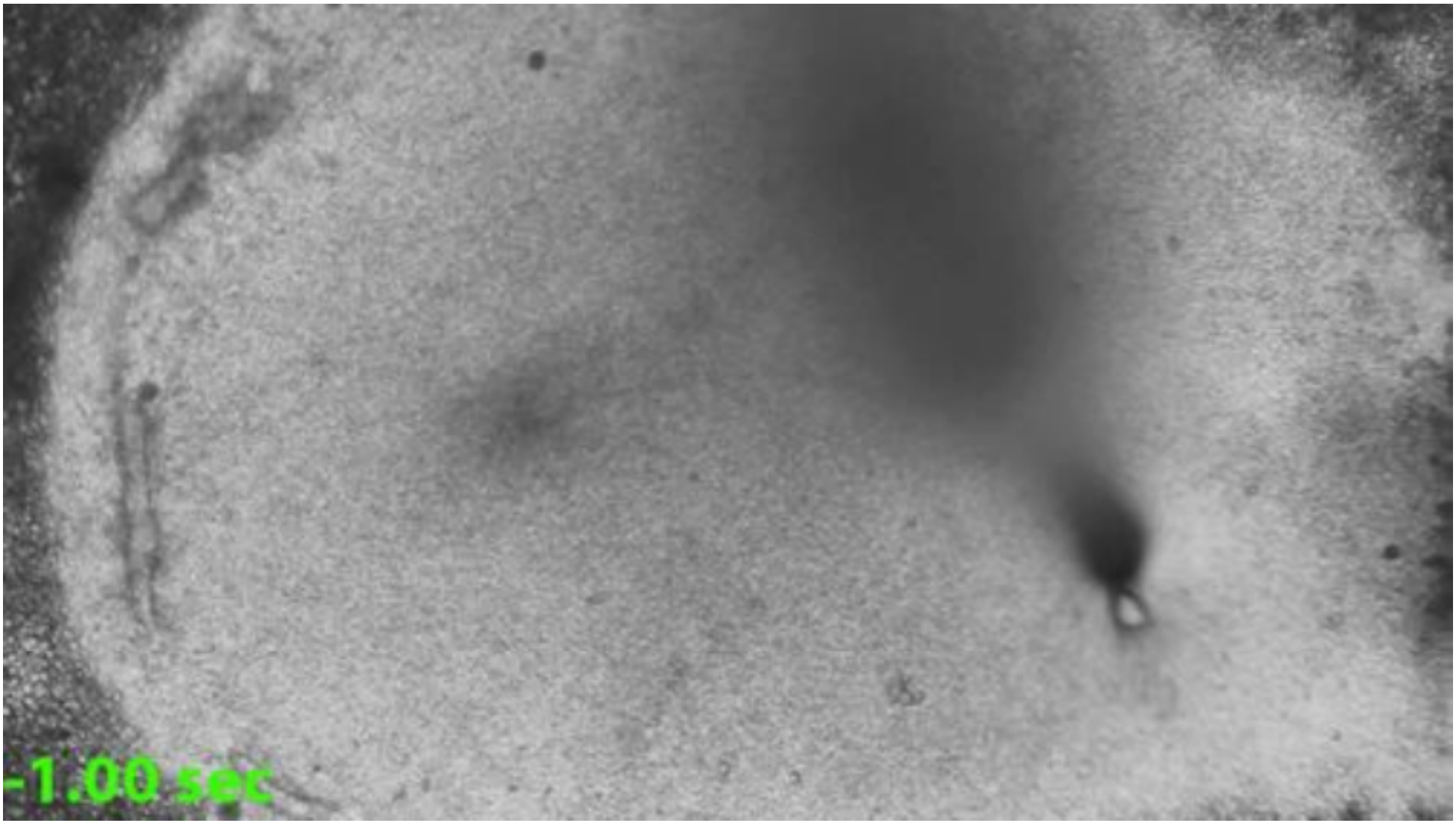








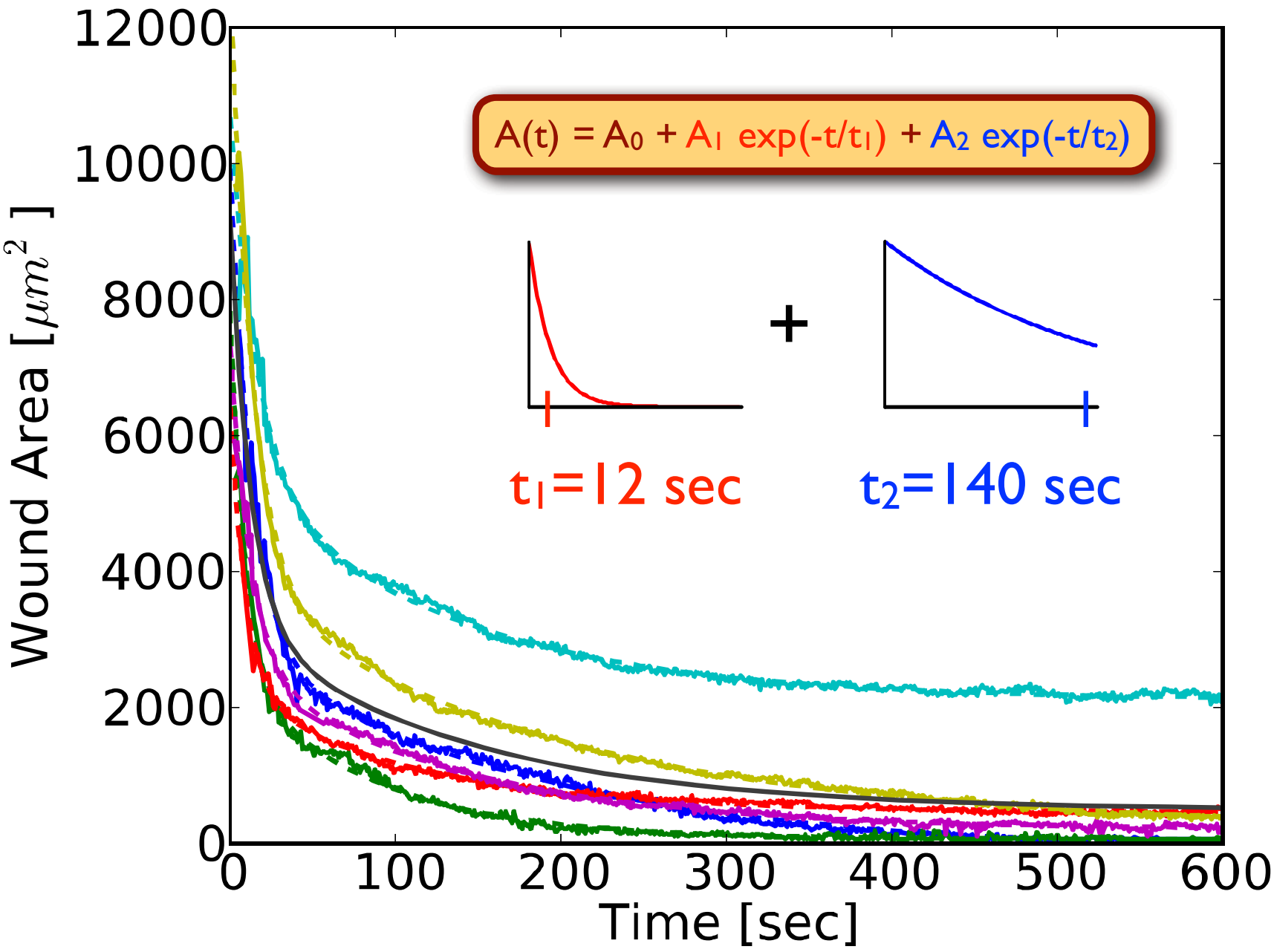




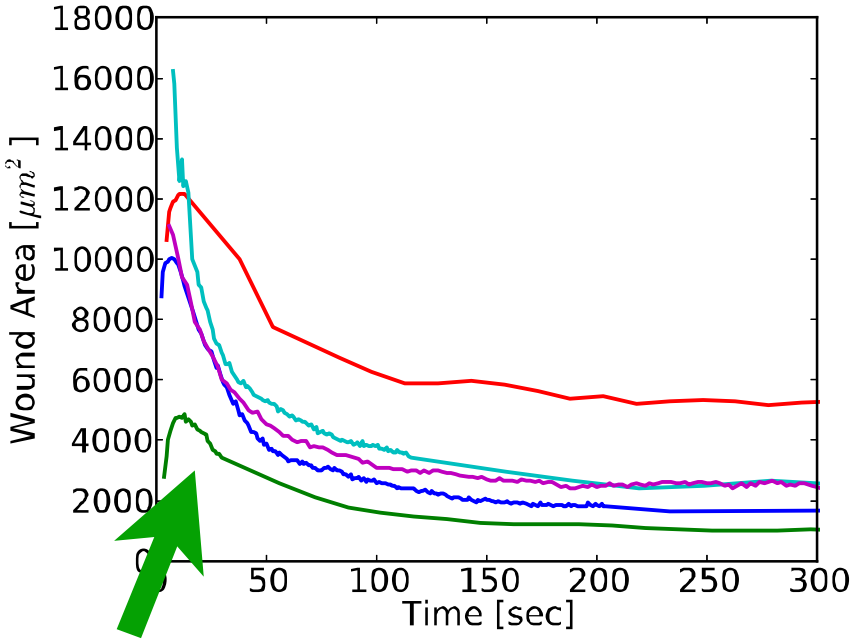
http://youtu.be/U5oPjgUFJdo

Image analysis

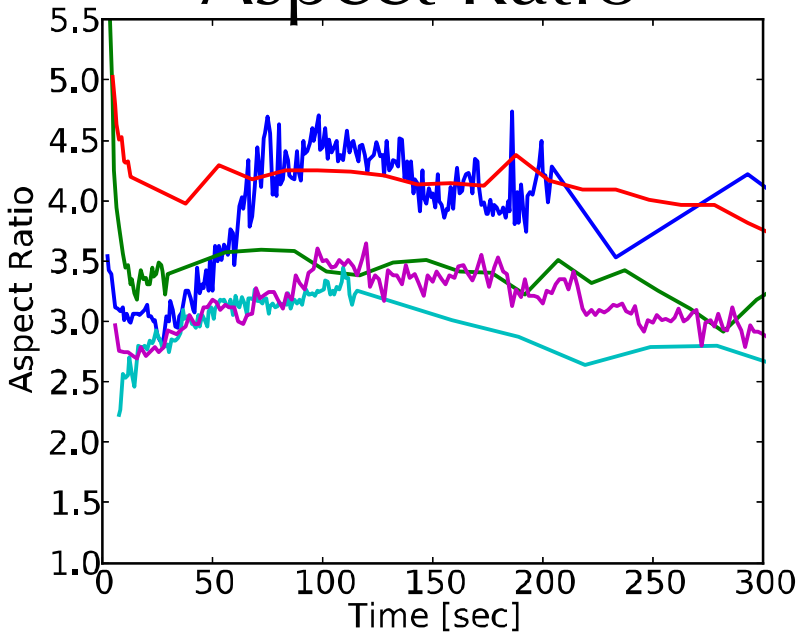
- Threshold to isolate wound
- Measure area, aspect ratio versus time

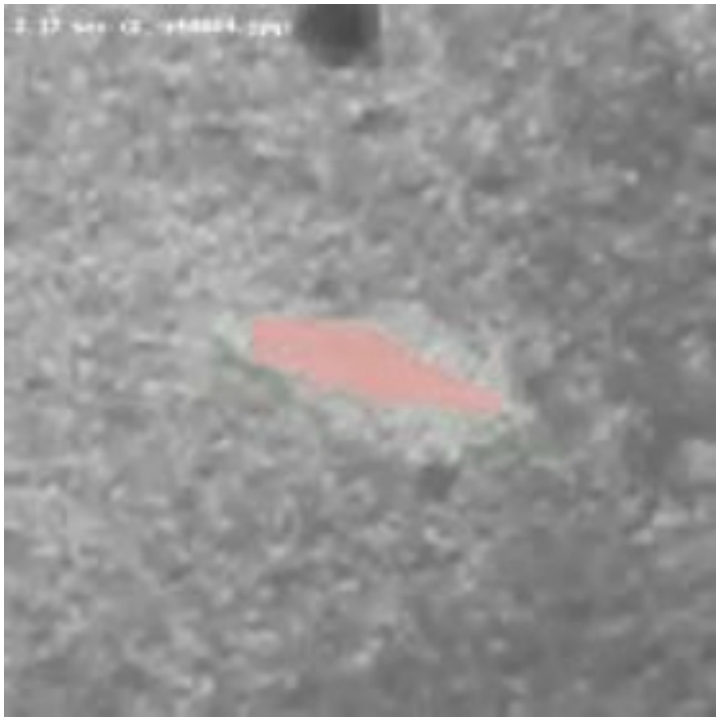

12000
10000
8000
6000
4000
2000
0
Wound Area [μm^2]
0
100
200
300
400
500
600
Time [sec]
A(t) = A0 + A1 exp(-t/t1) + A2 exp(-t/t2)
+
t1=12 sec
t2=140 sec

Area



Aspect Ratio



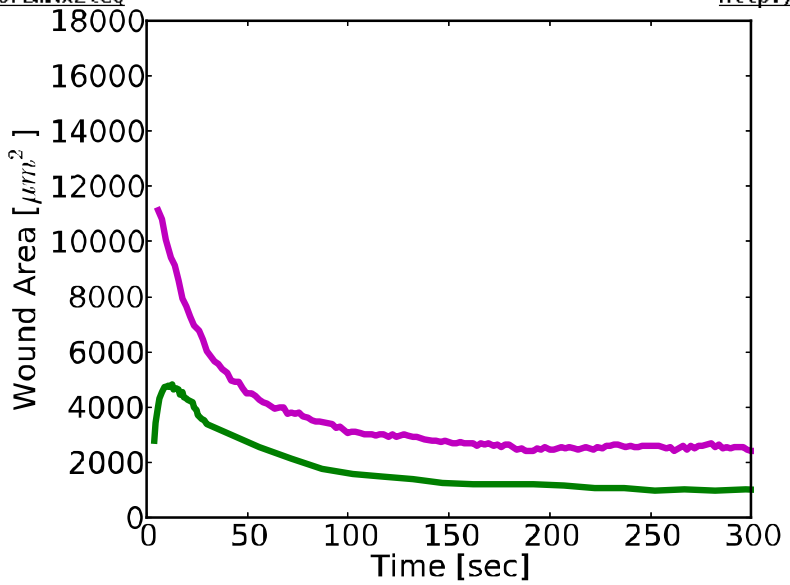


http://youtu.be/8_IlnLw2aSI

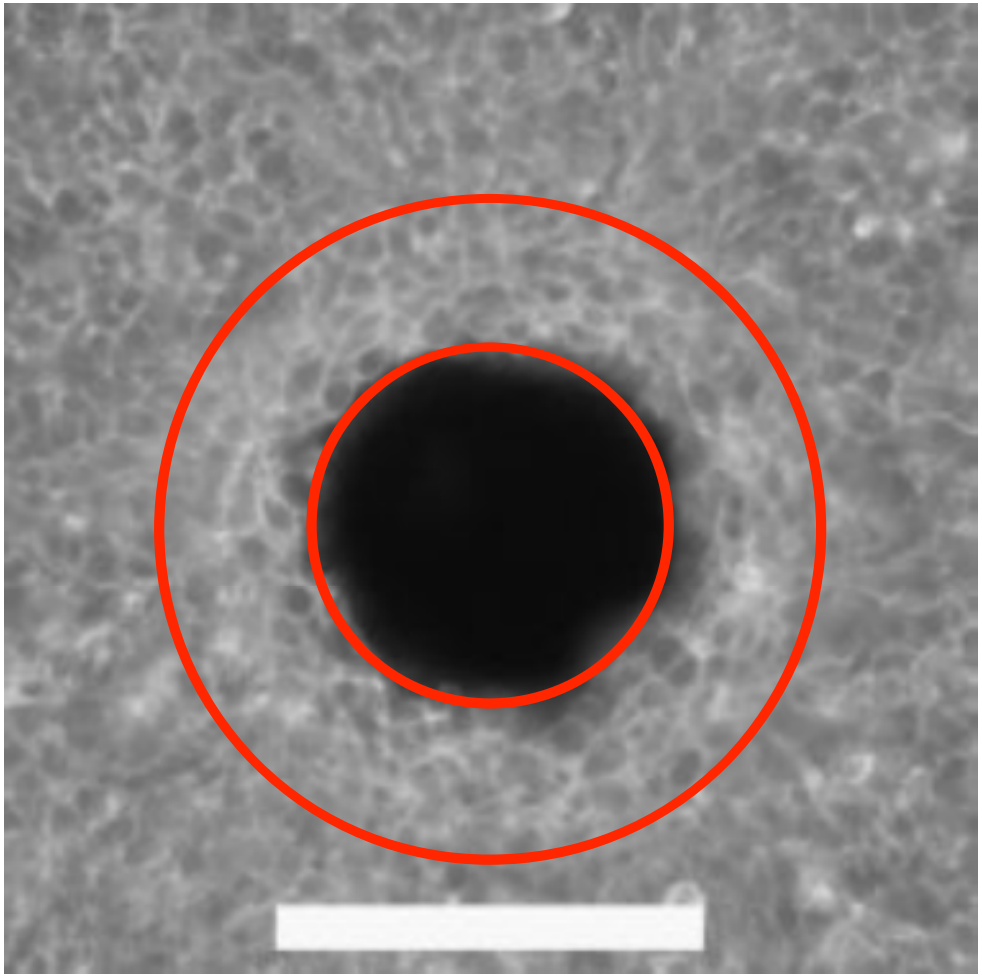
- Area: fast, slow phase
- AR: roughly constant
  - Not round, not slit-like
- Sometimes, area initially *increases*.

http://youtu.be/r8FBmNXZleQ

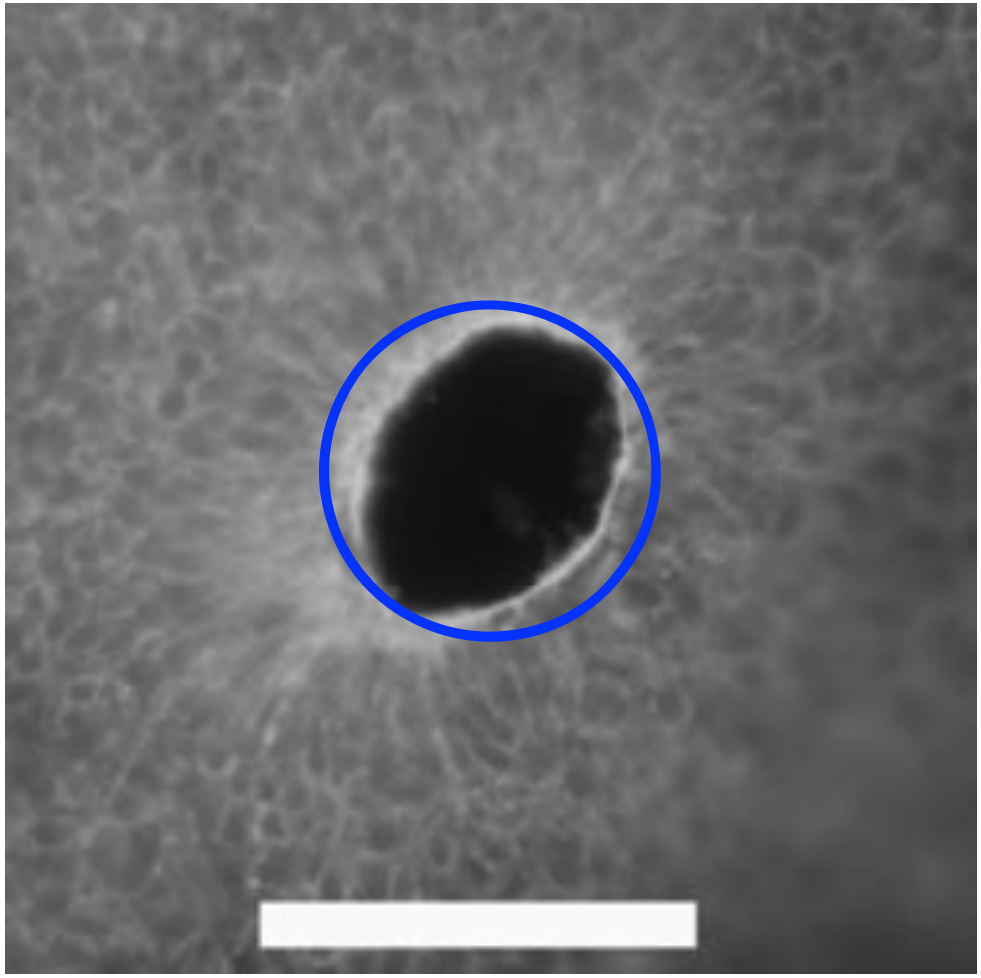
http://youtu.be/S9dbo0GjA1w



Actin Staining



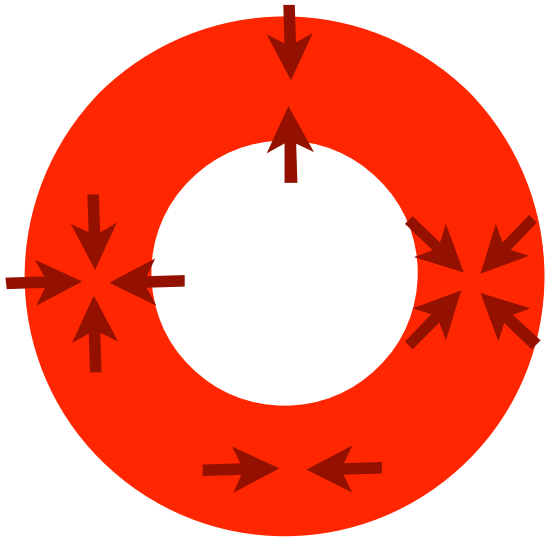
20 sec



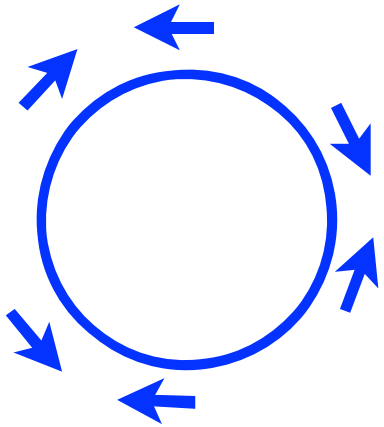
10 min

Scale bar 100µm

Hypothesis



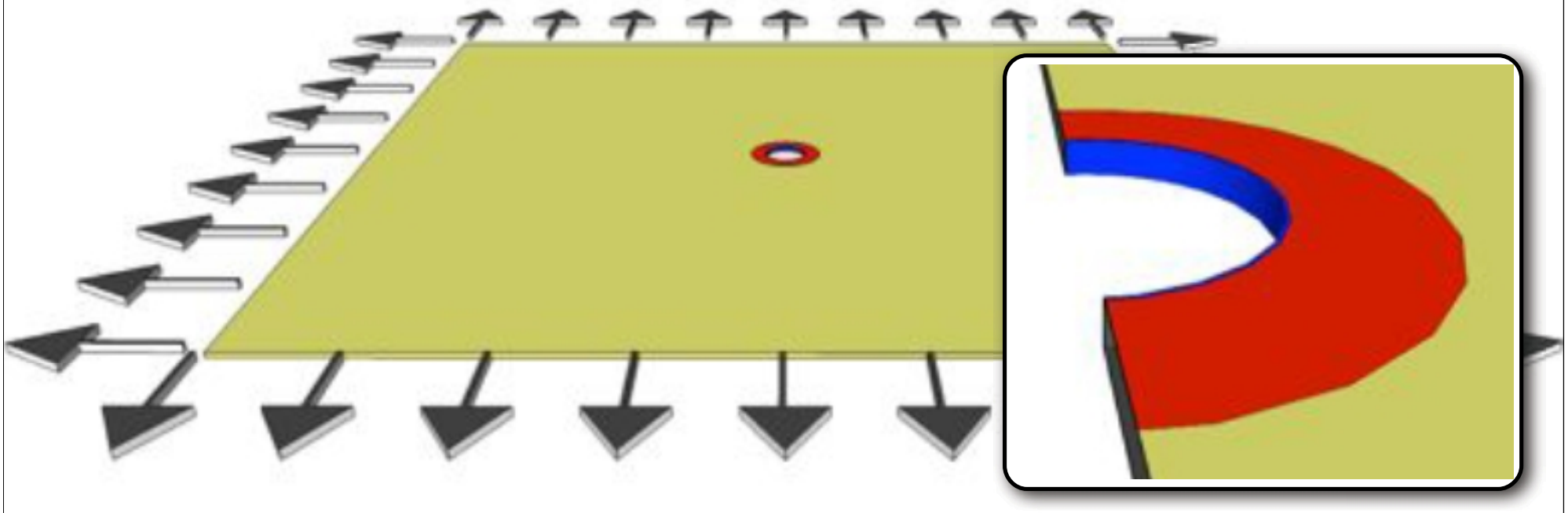
1 Cells in thick ring contract quickly

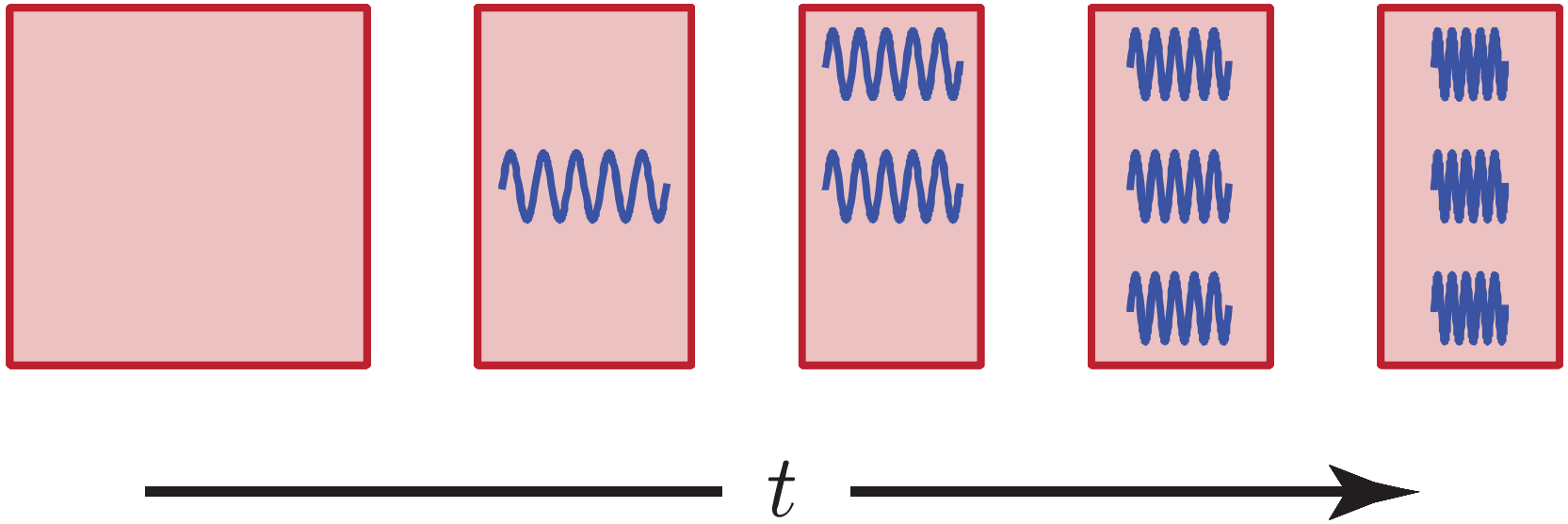


2 Actin fibers form and contract more slowly (actomyosin purse string)

Modeling

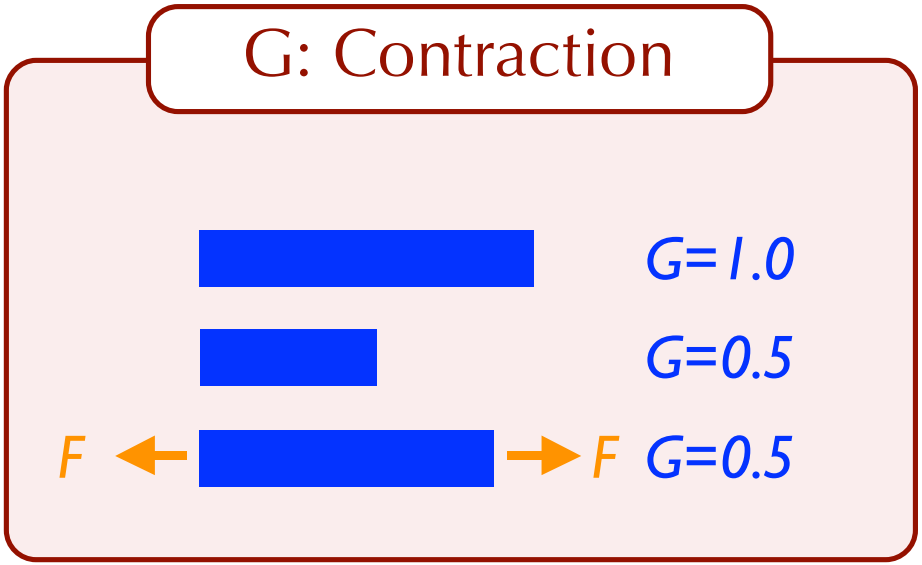
- Embryo a continuum membrane under tension
- "Cells" contract rapidly
- Stiff "Fibers" form more slowly and contract





ϕ^f : Fiber Volume Fraction

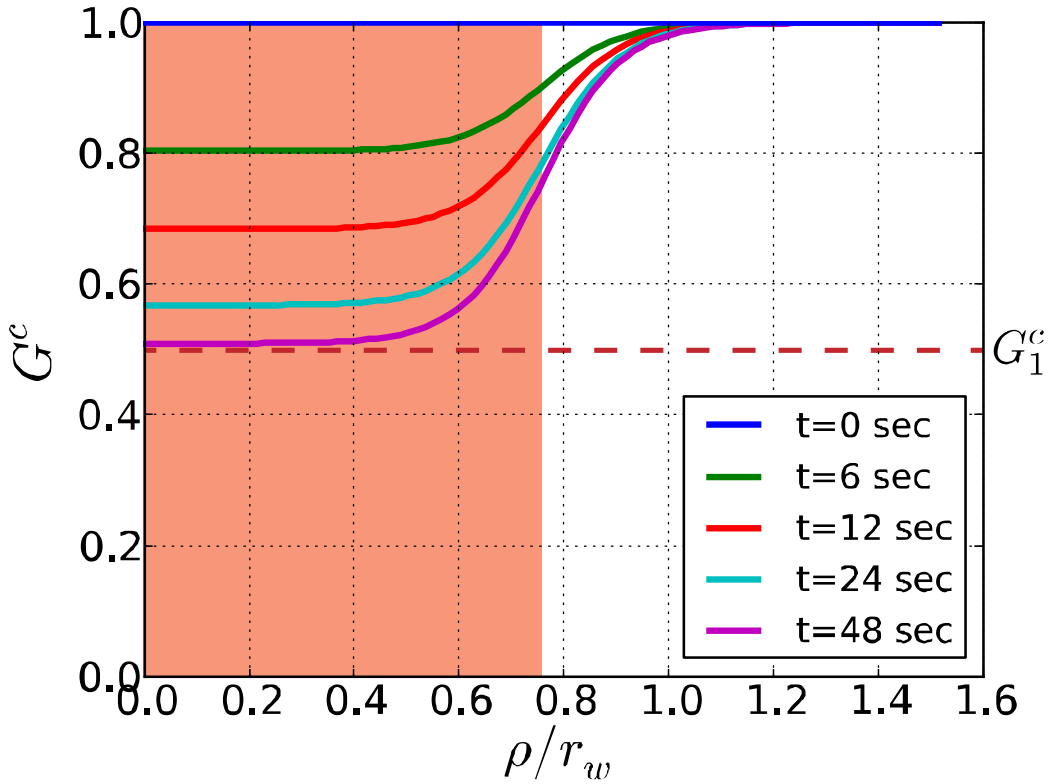
$$\sigma_{total} = \sigma_{cell} + \sigma_{fiber}$$



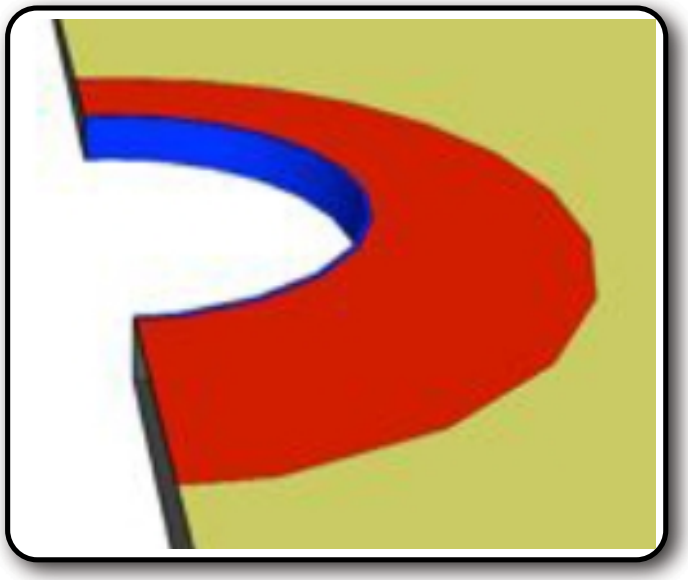
- G<1: Contraction
- Cells (G^c), Fibers (G^f) contract independently

Rodriguez et al., J. Biomech. 27 (1994)

Cells

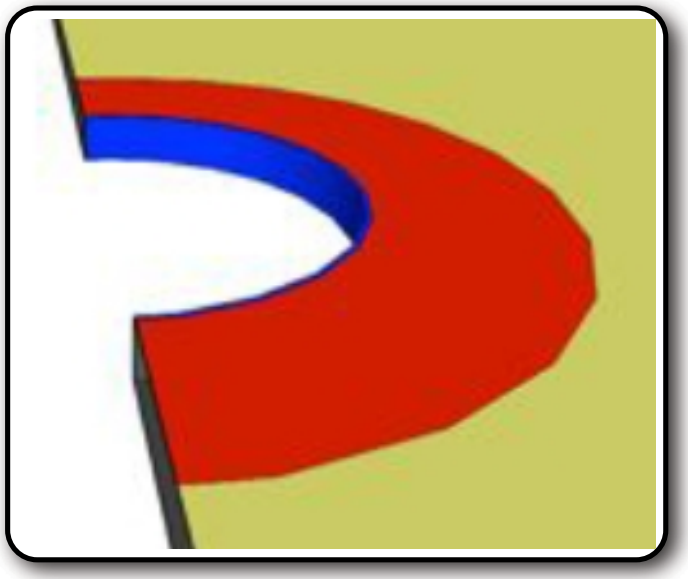


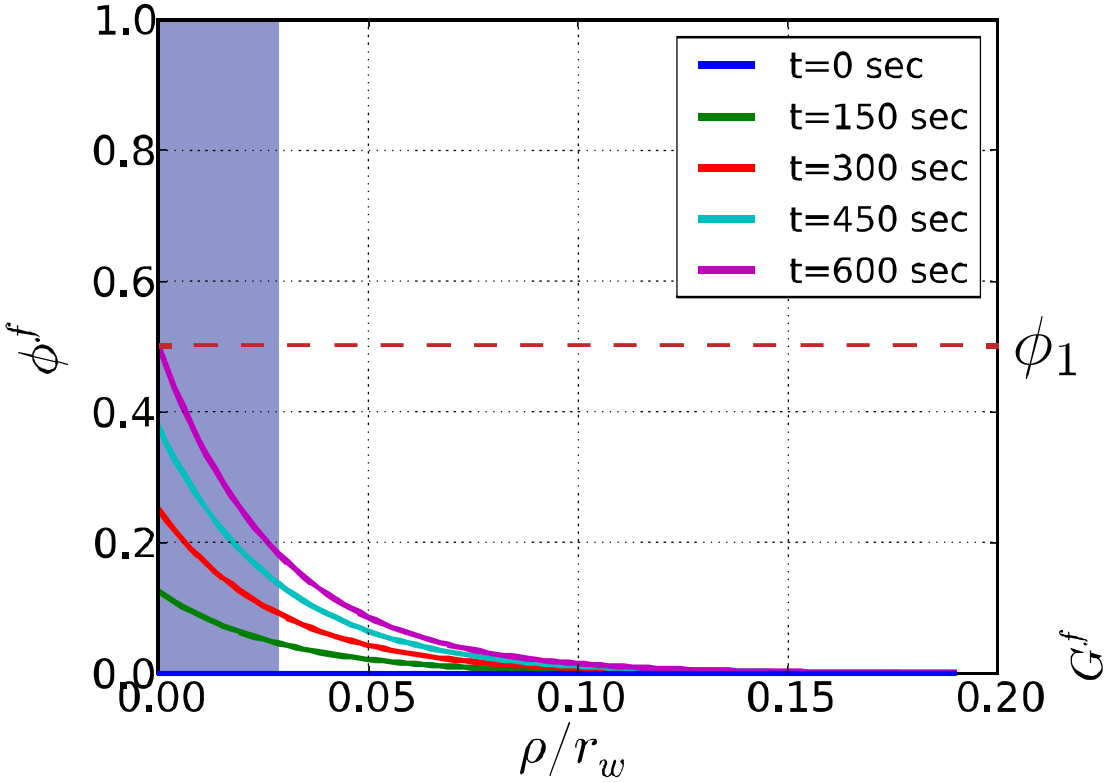
Distance from Wound Edge



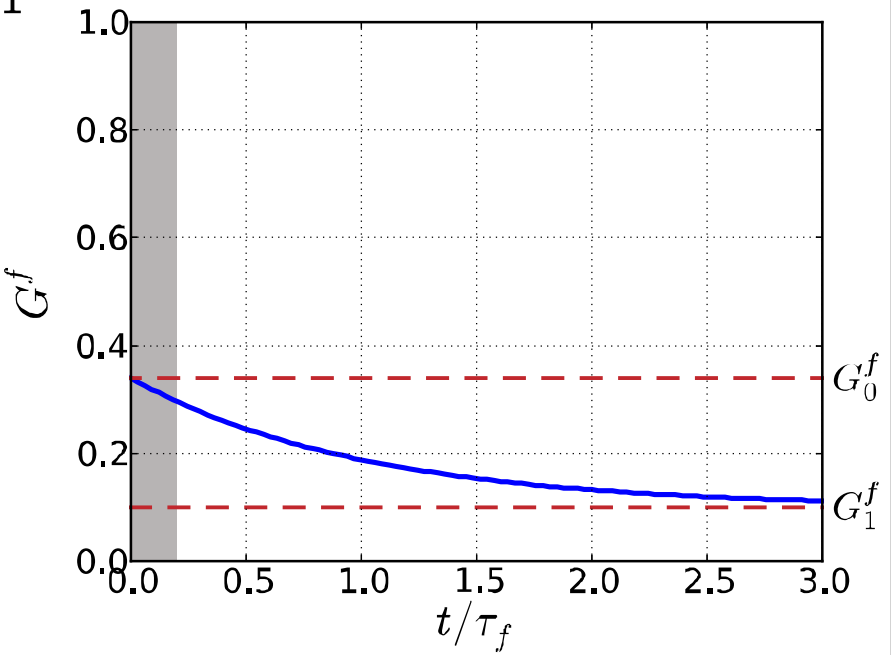
- Cells contract rapidly in broad ring

Fibers





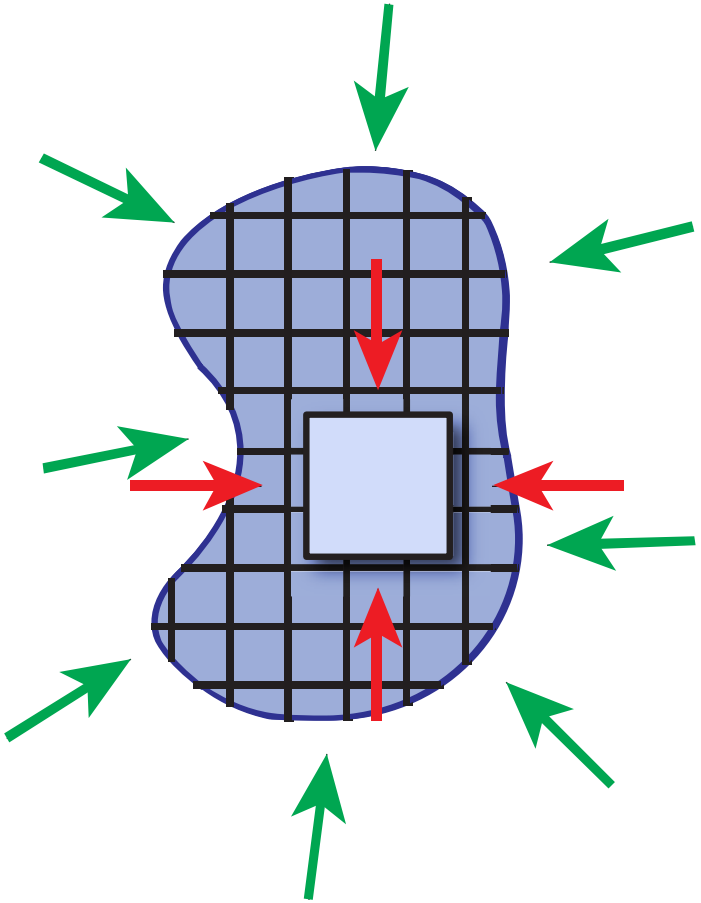
Distance from Wound Edge



Time

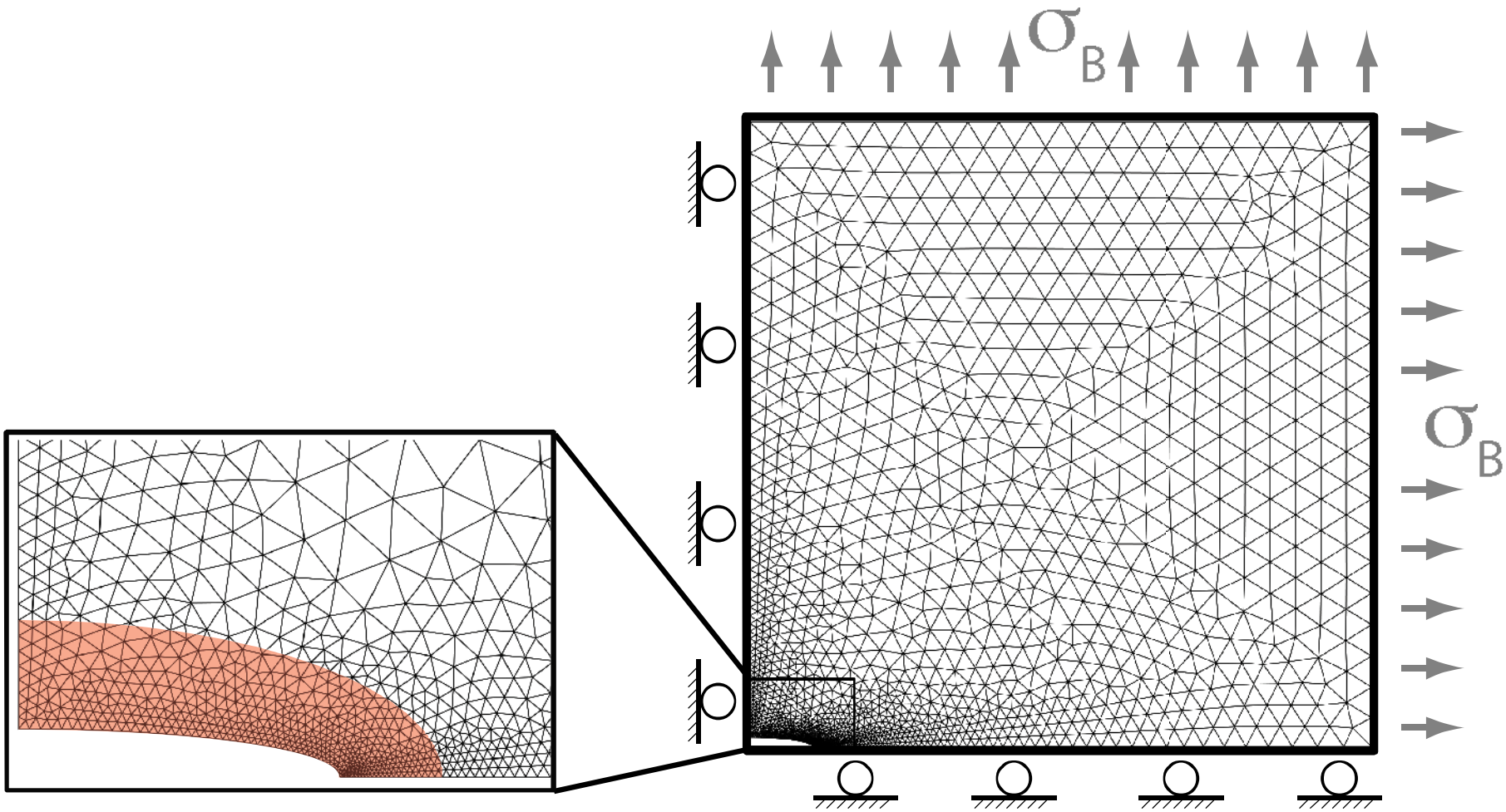
- Fibers form and contract more slowly.

Finite Element Model

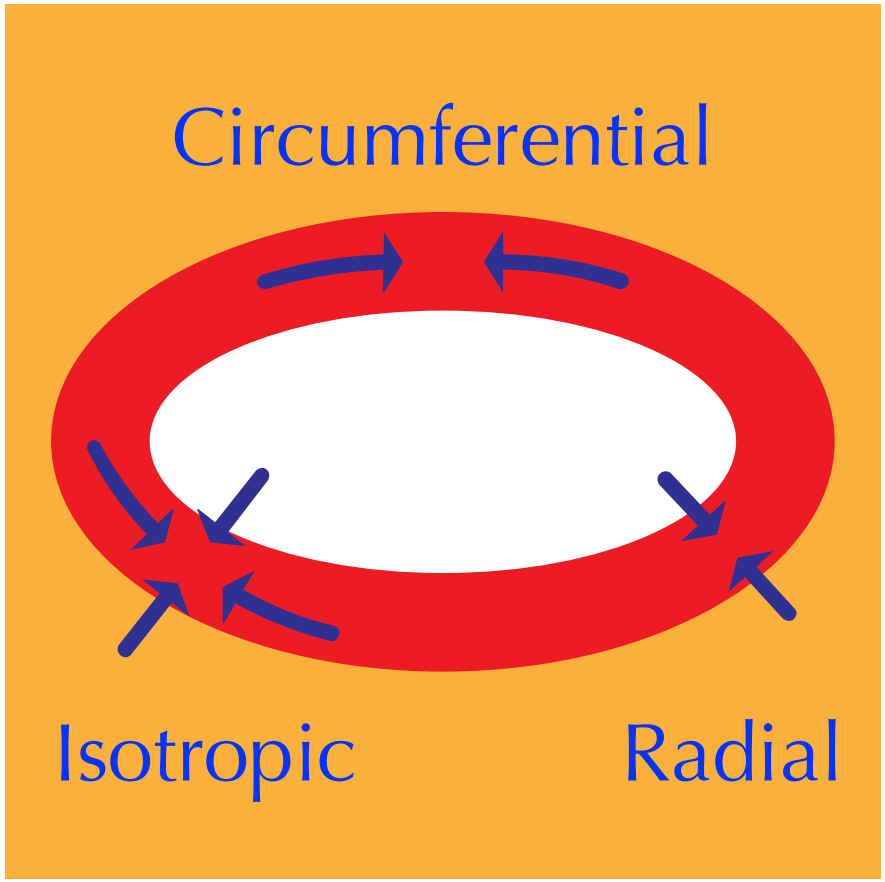


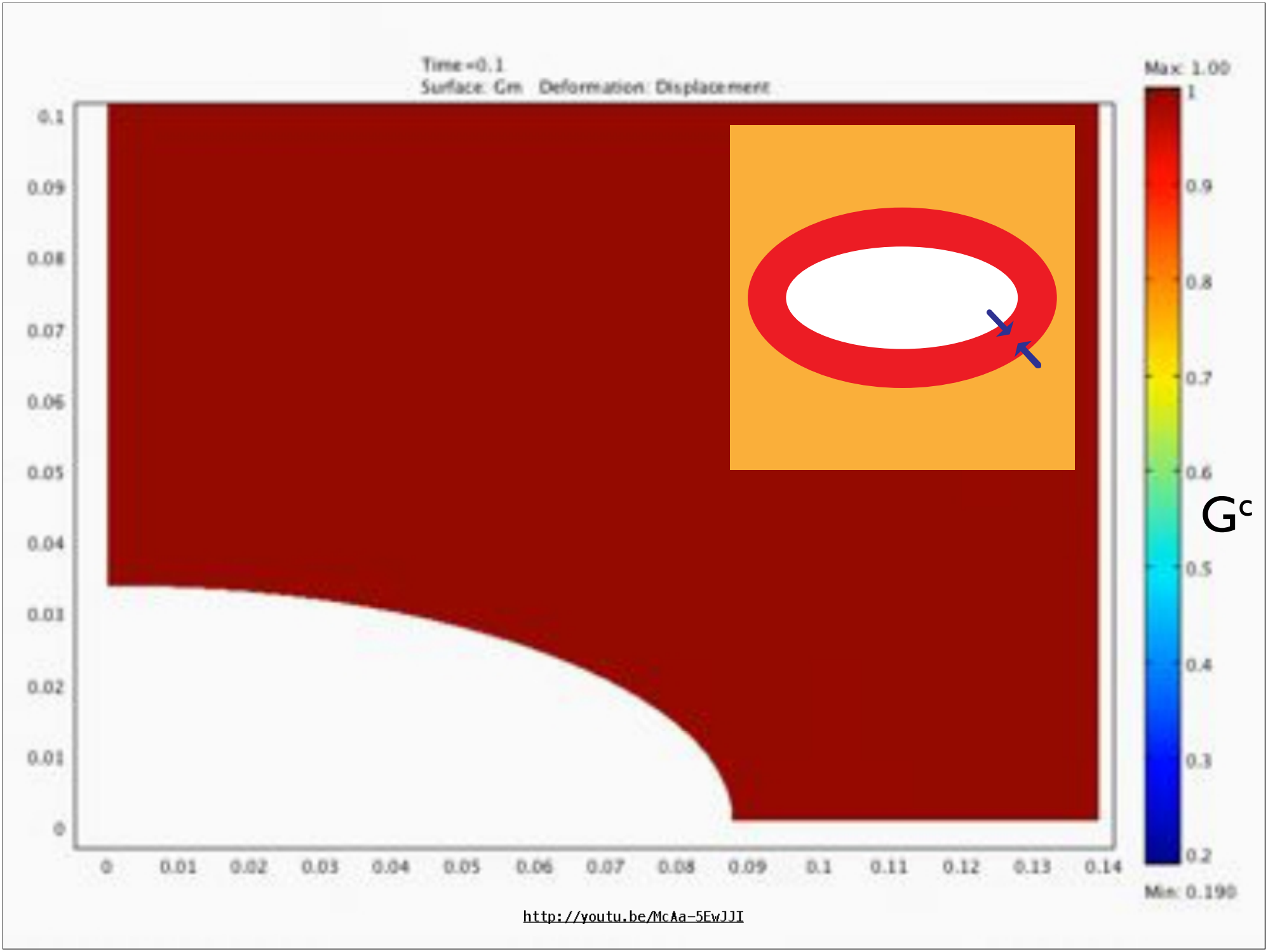
- Break up complex geometry into simple elements
- Laws of mechanics satisfied for each element

Finite Element Model



Cell Contraction





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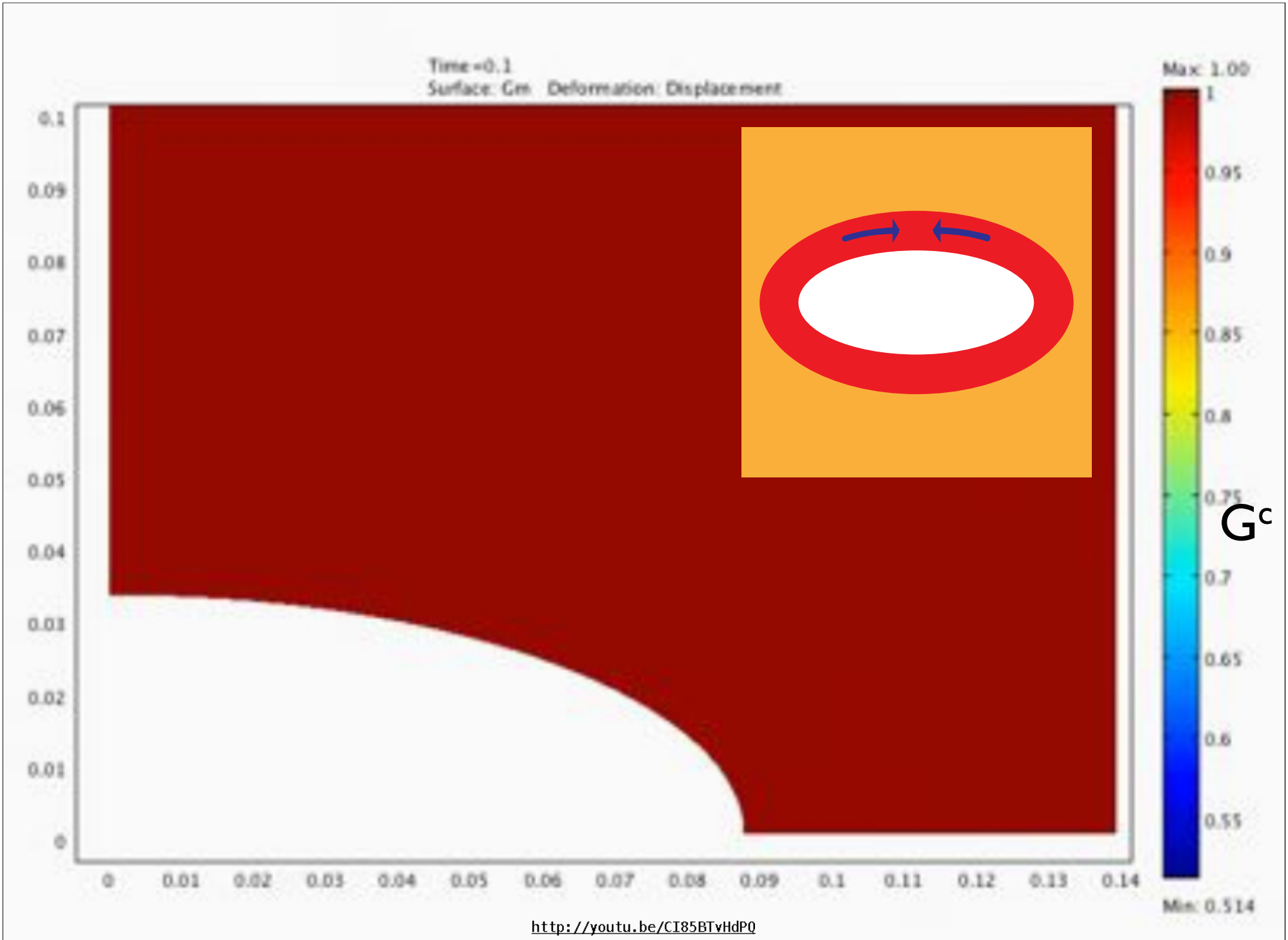

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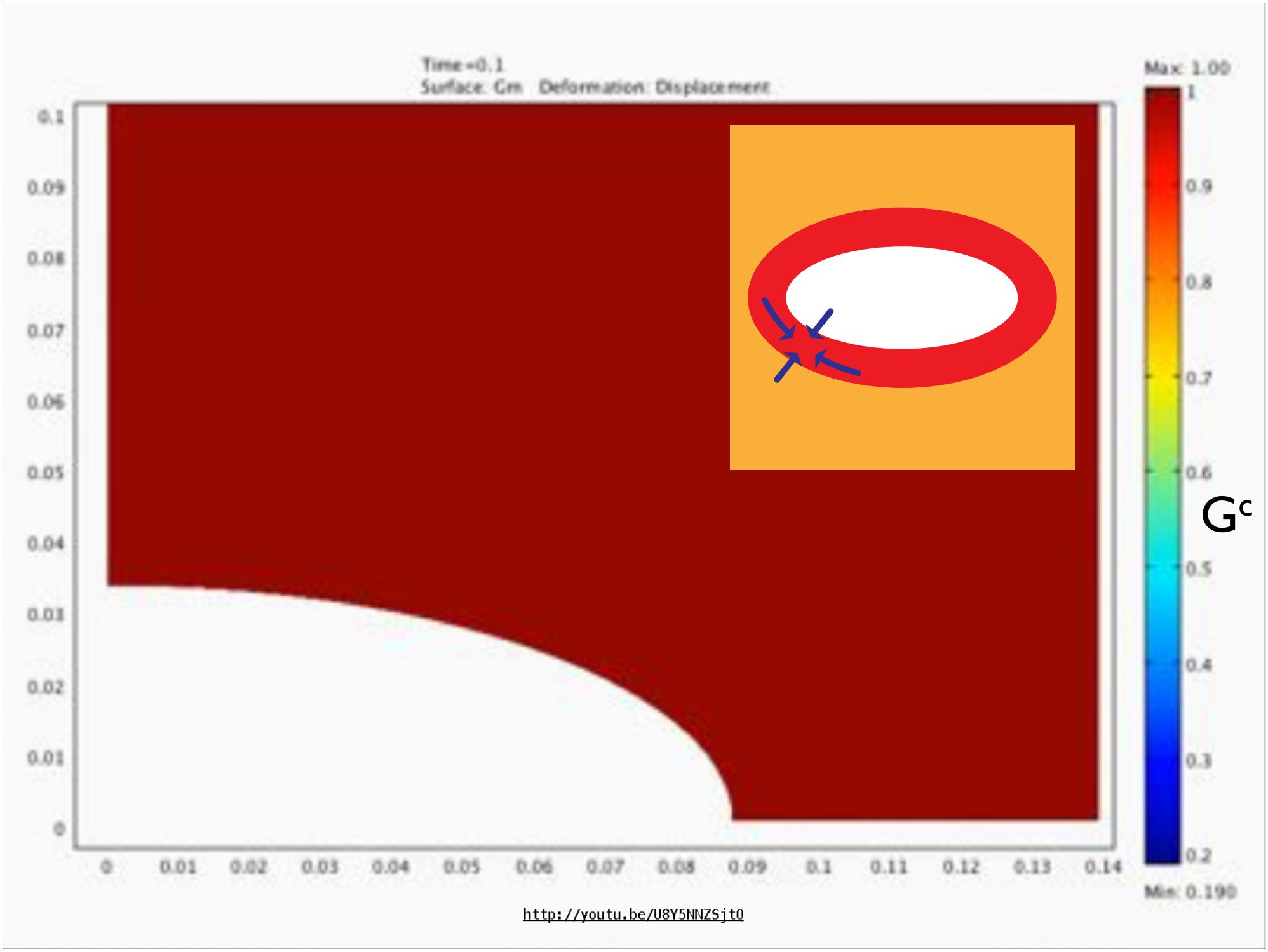
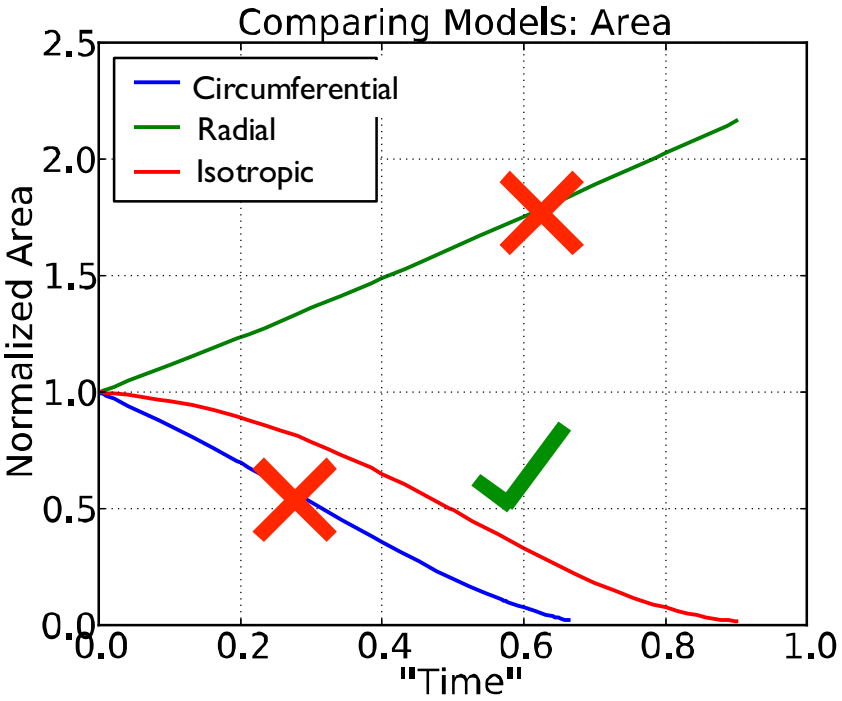
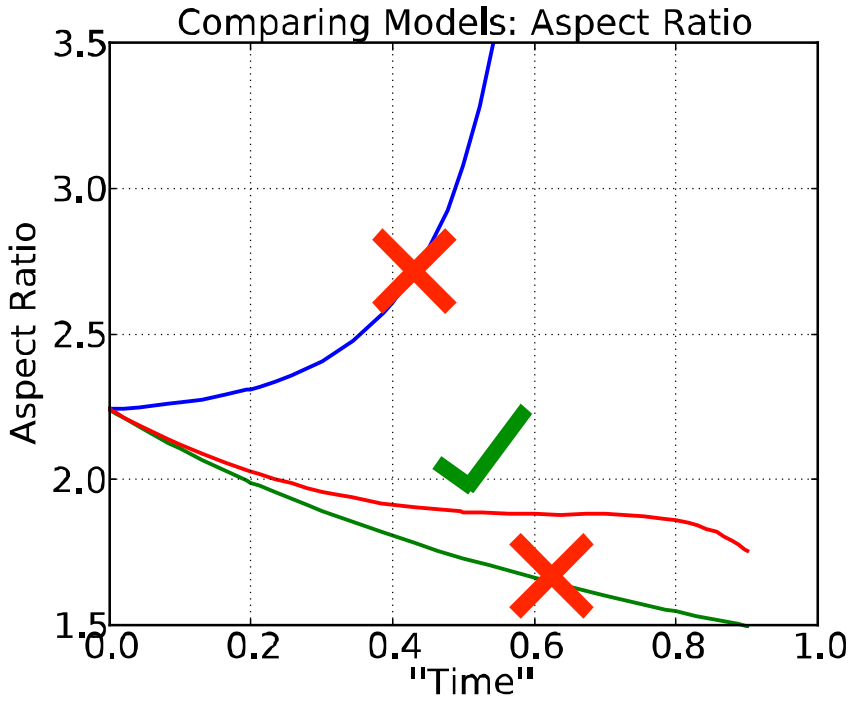
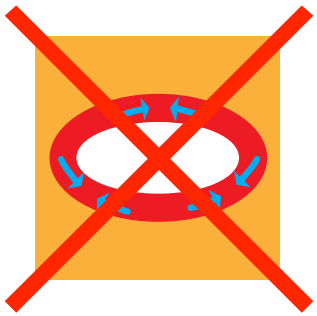


http://youtu.be/U8Y5NNZSjtQ

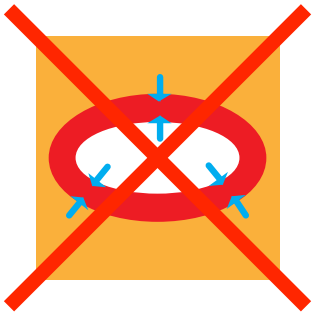
Modeling Results



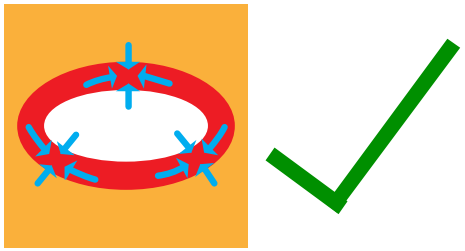




Circumferential



Radial



Isotropic

Putting it all together...

- Fibers and cells in model
- Parameters
  - Geometry
  - Material Properties
  - Rates of Contraction, Formation
- Unknowns: from match of model to experiment

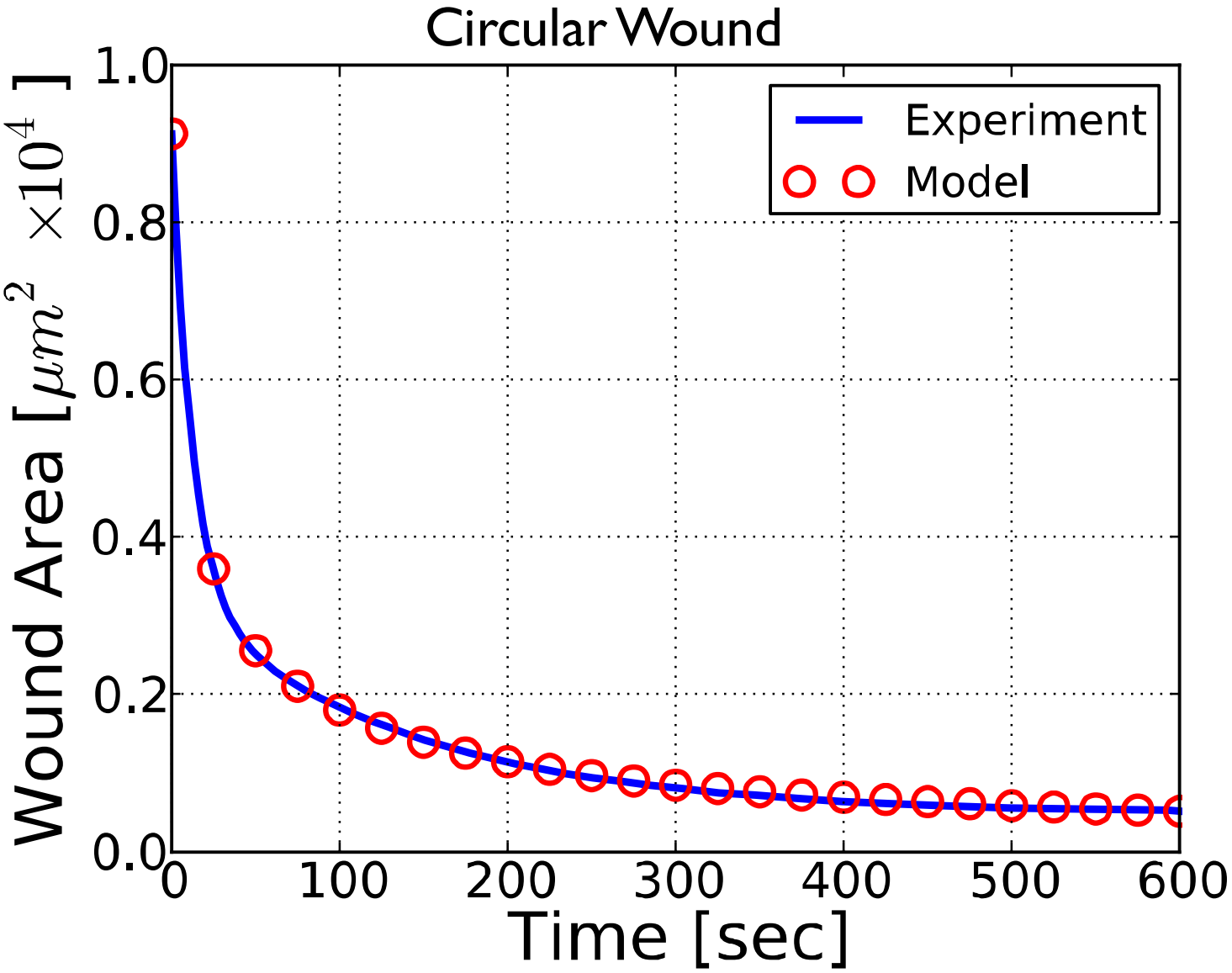
Parameters: Known

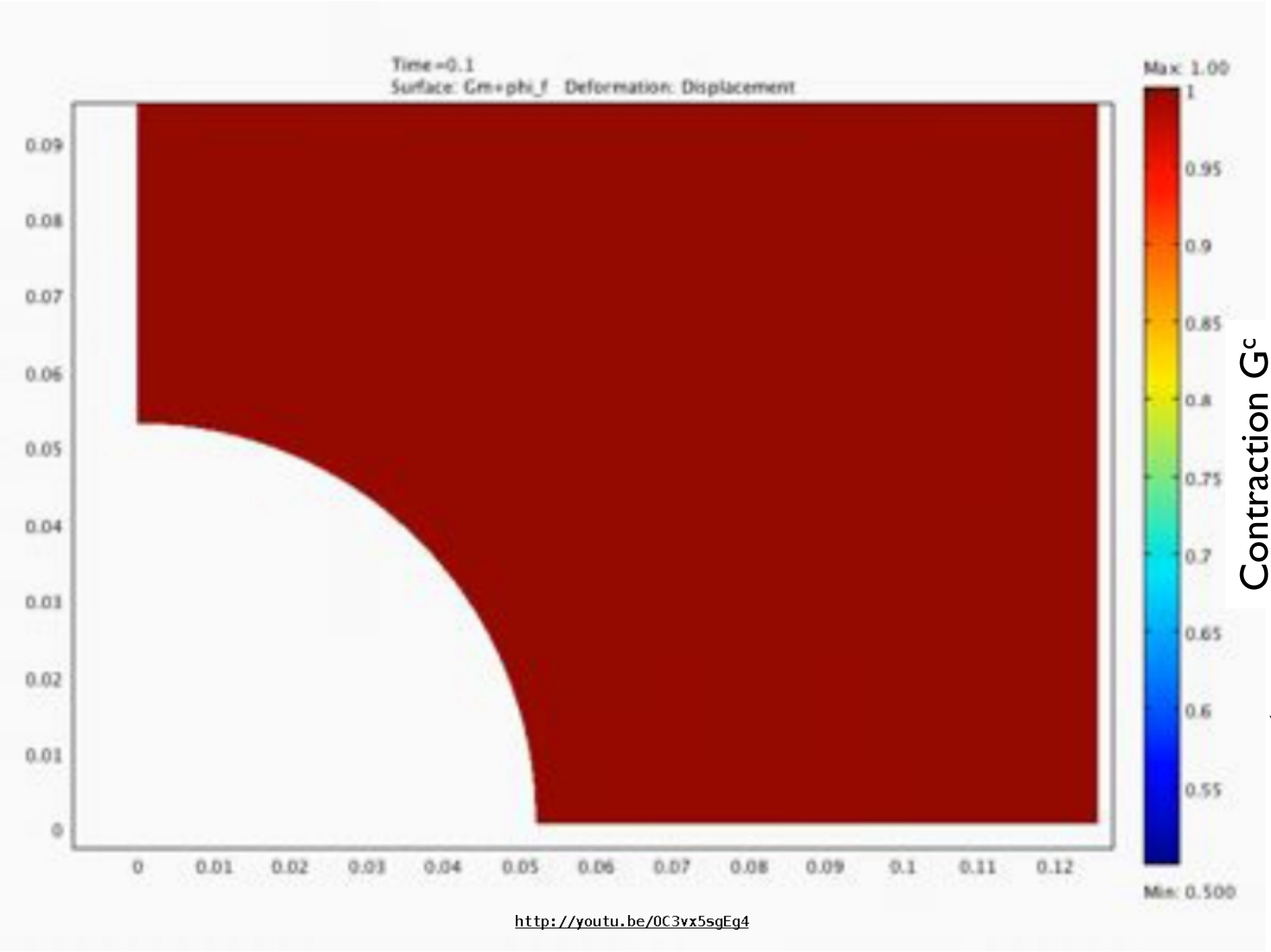
Circular Wound Diameter	110 um
Elliptical Major Axis	180 um
Elliptical Minor Axis	70 um
Cell Ring Width	40 um
Fiber Cable Width	1.5 um
Cell Shear Modulus	40 Pa
Boundary Stress	42 Pa

Parameters: Fit

Fiber Shear Modulus	3200 Pa
Cell Contraction Range	Gc = 1 - 0.55
Cell Contraction Rate	12 sec
Fiber Pre-Stretch	1.65
Final Fiber Contraction	0.15

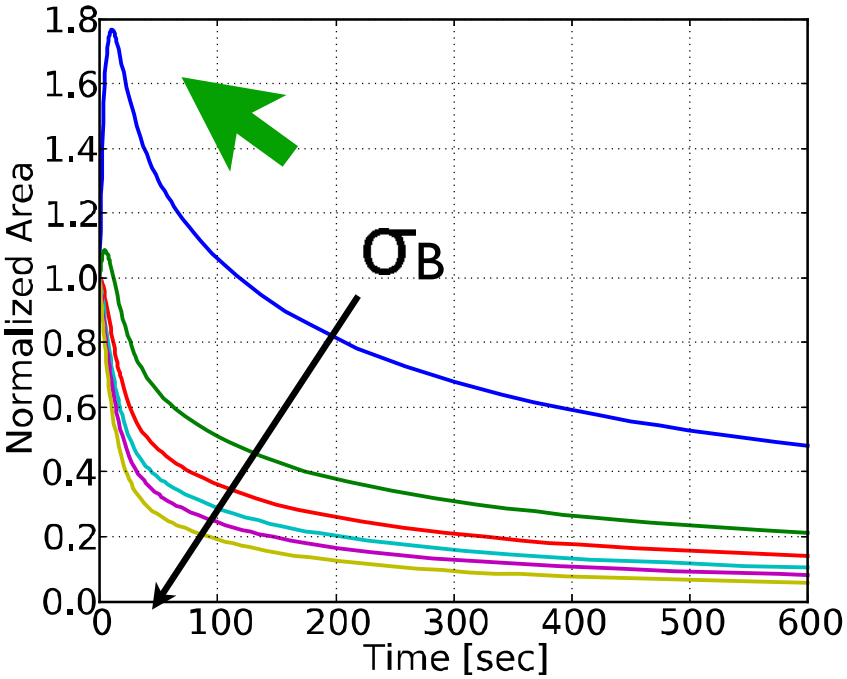
Modeling Results

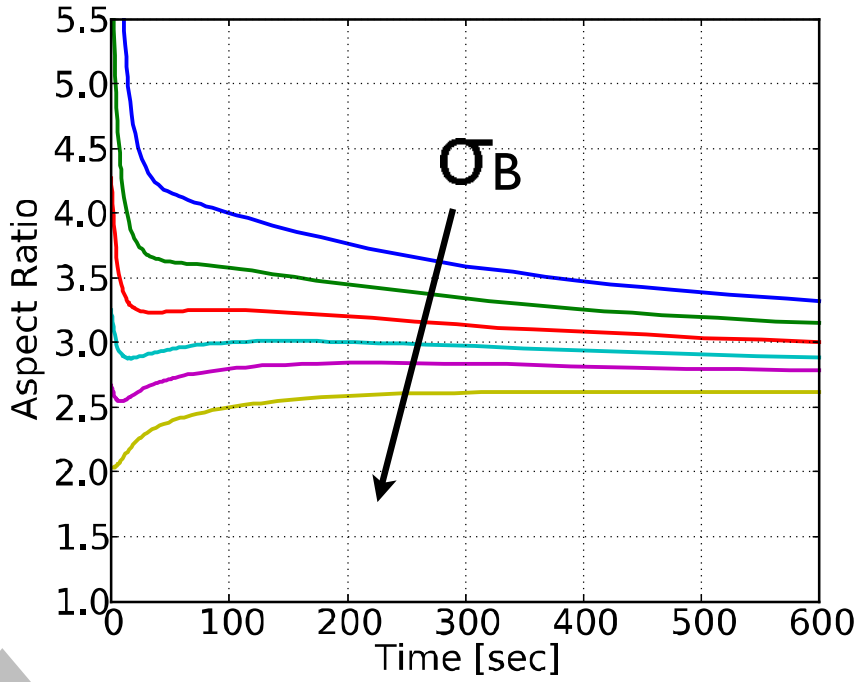


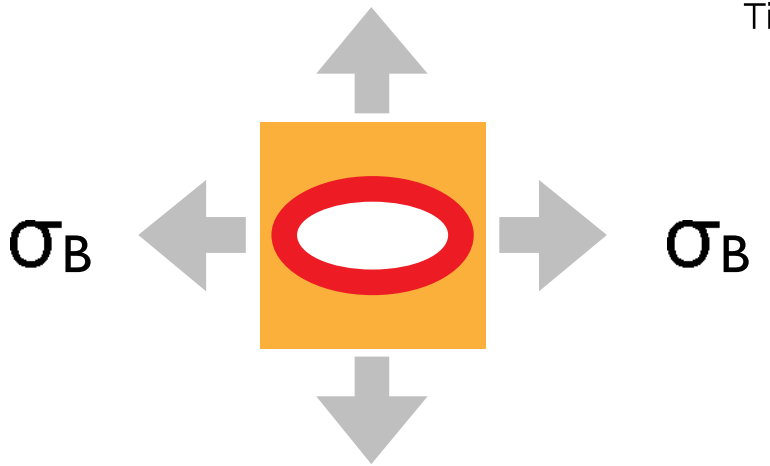


http://youtu.be/0C3vx5sgEg4

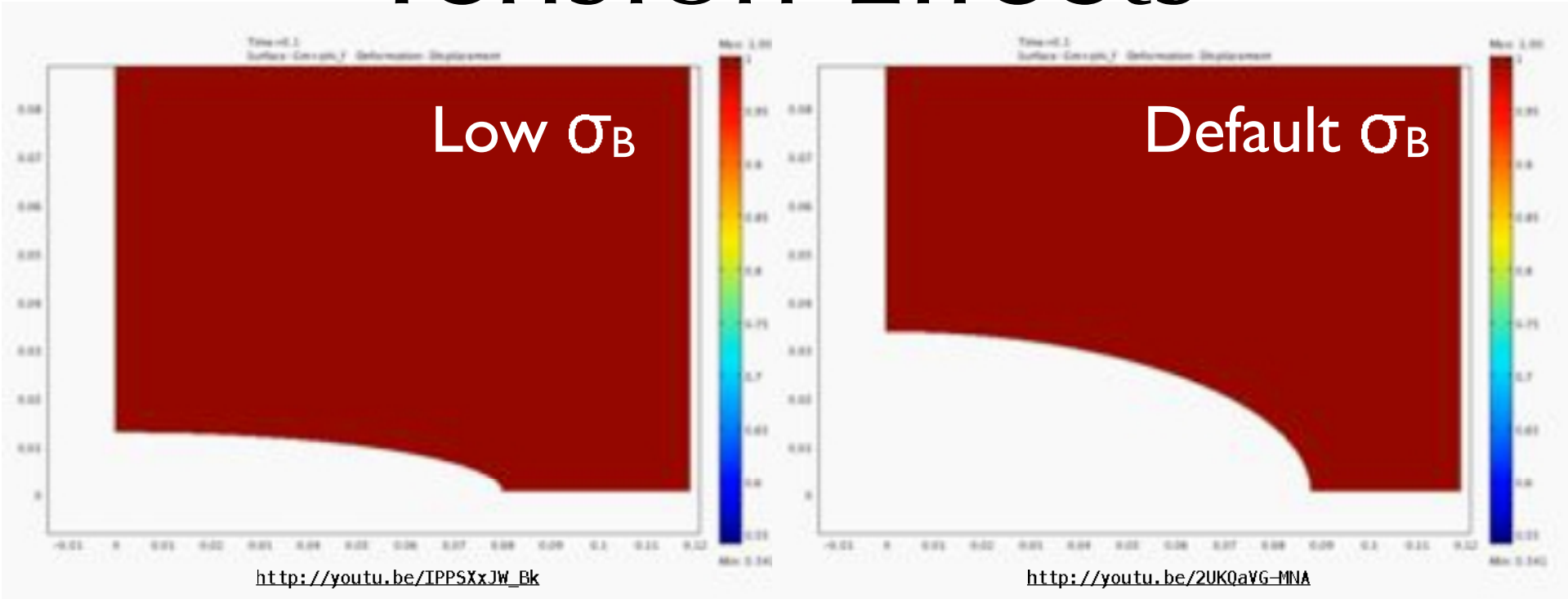
Tension Effects



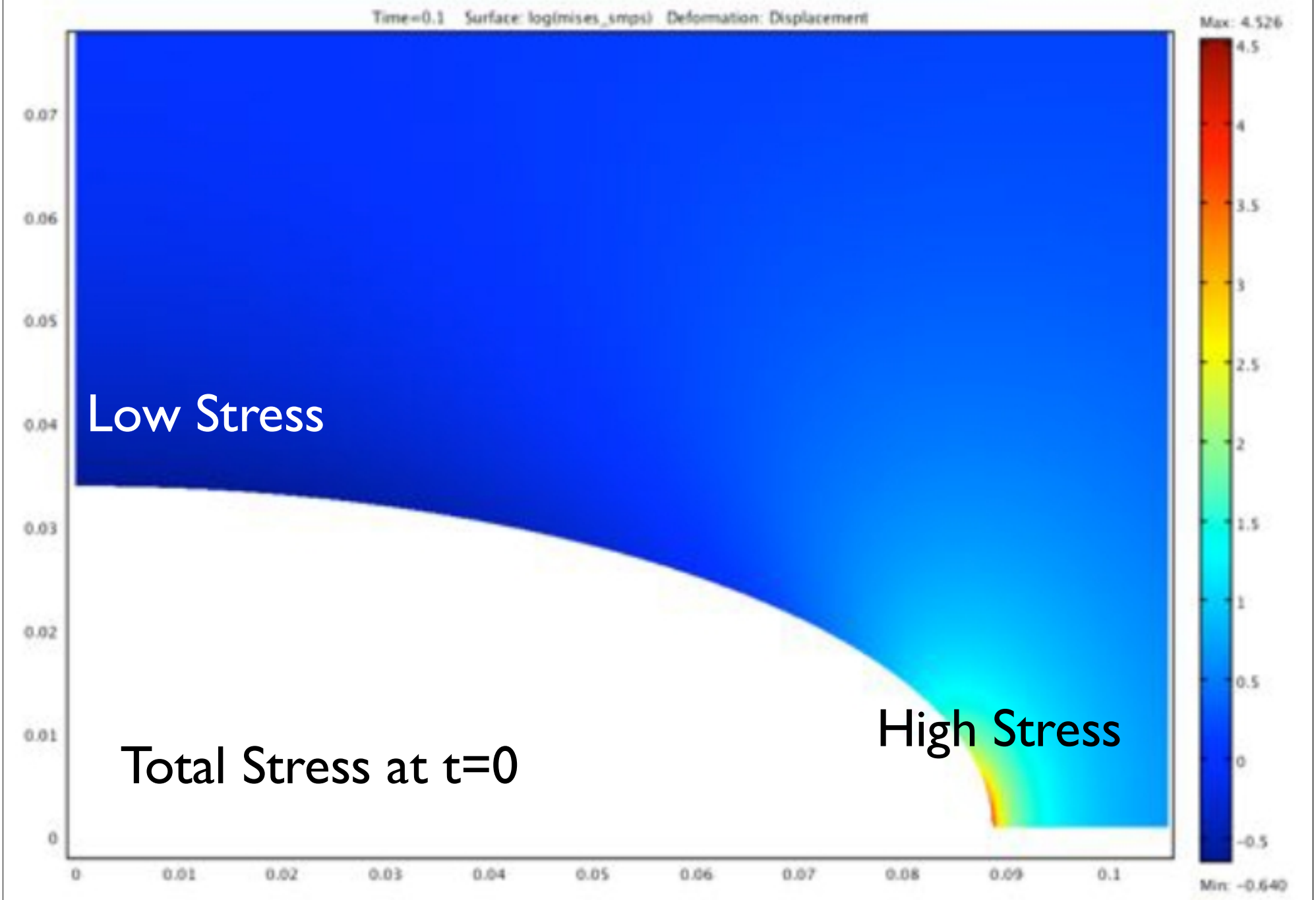


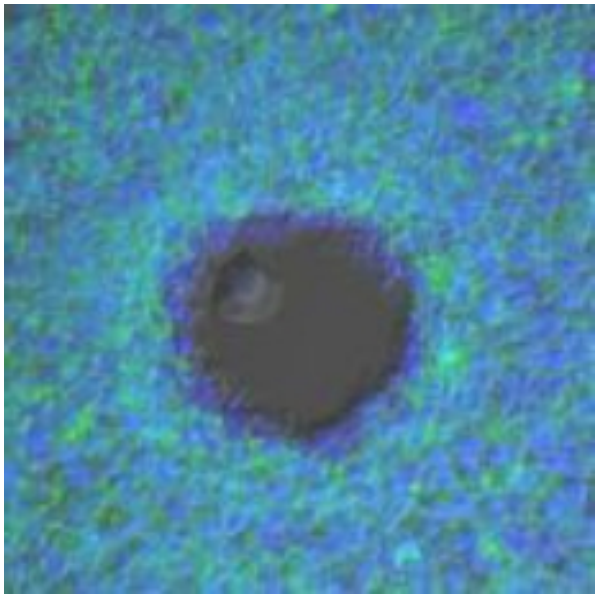


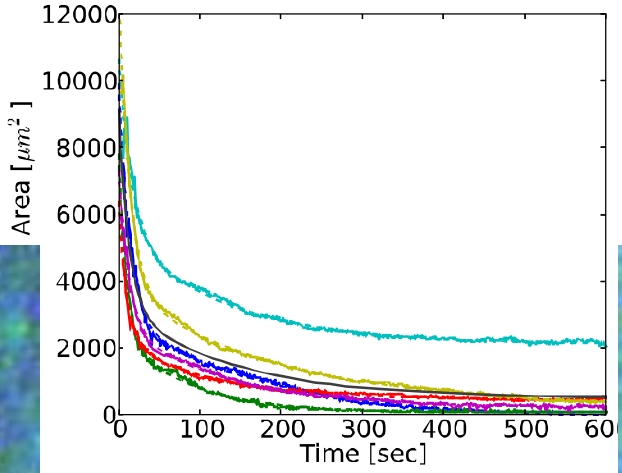
Tension Effects

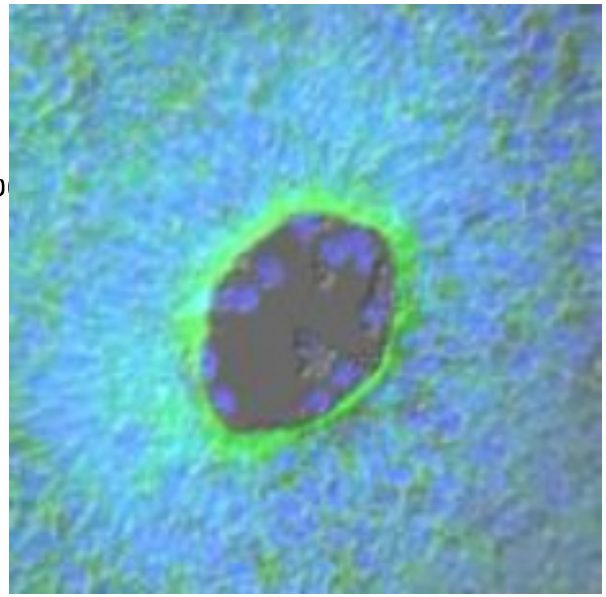


- At low tension, minor axis initially "retracts"
  - Area increases briefly
- Effect not seen at higher tension
- Consistent with experiment


Time=0.1 Surface: log(mises_smps) Deformation: Displacement
Max: 4.526
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
Min: -0.640
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
Low Stress
High Stress
Total Stress at t=0





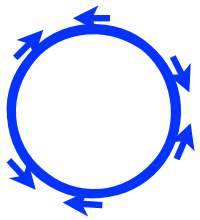


Seconds

Isotropic Contraction

Minutes

Purse String



Thanks to...

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Yunfei Shi

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Elliot Elson

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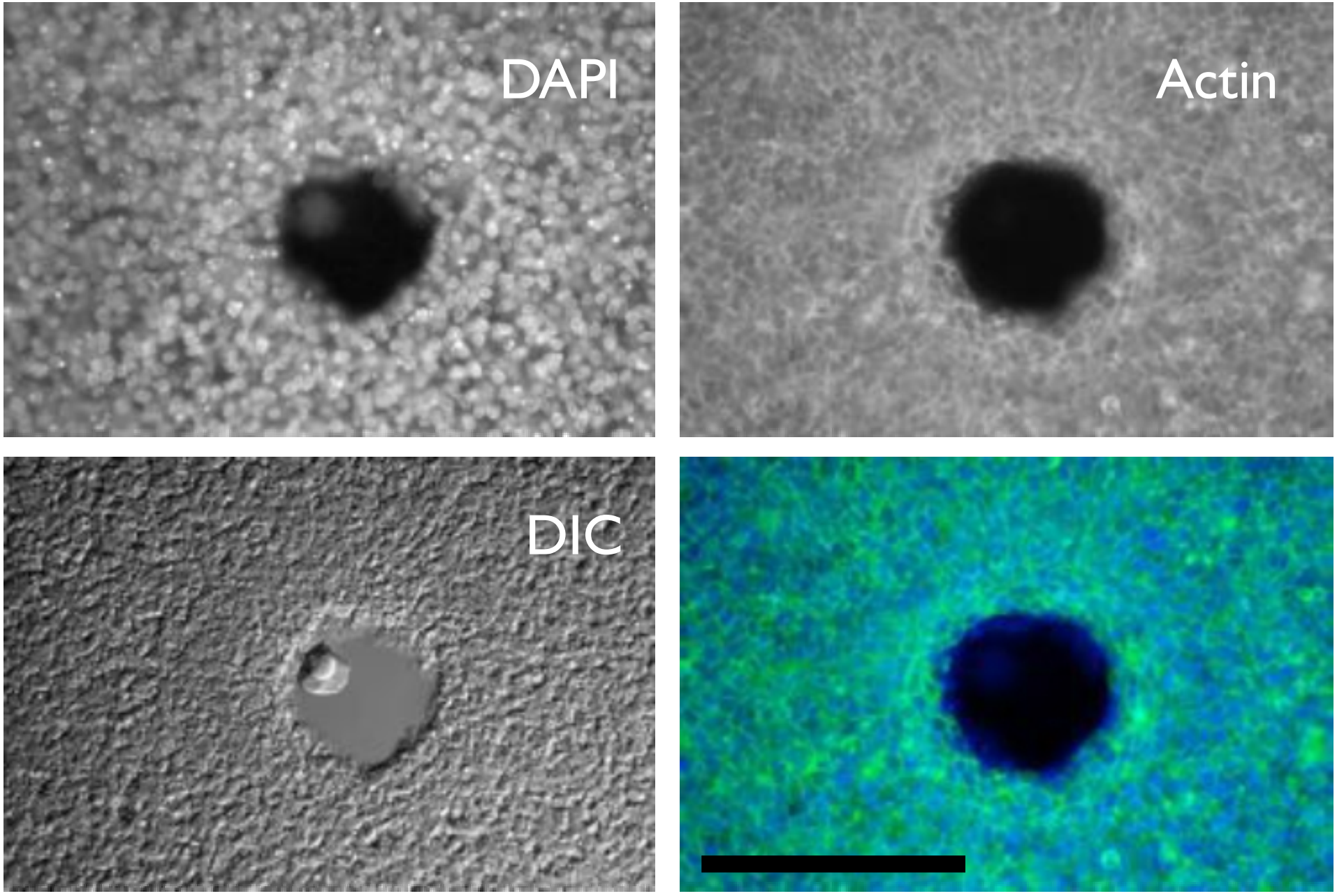
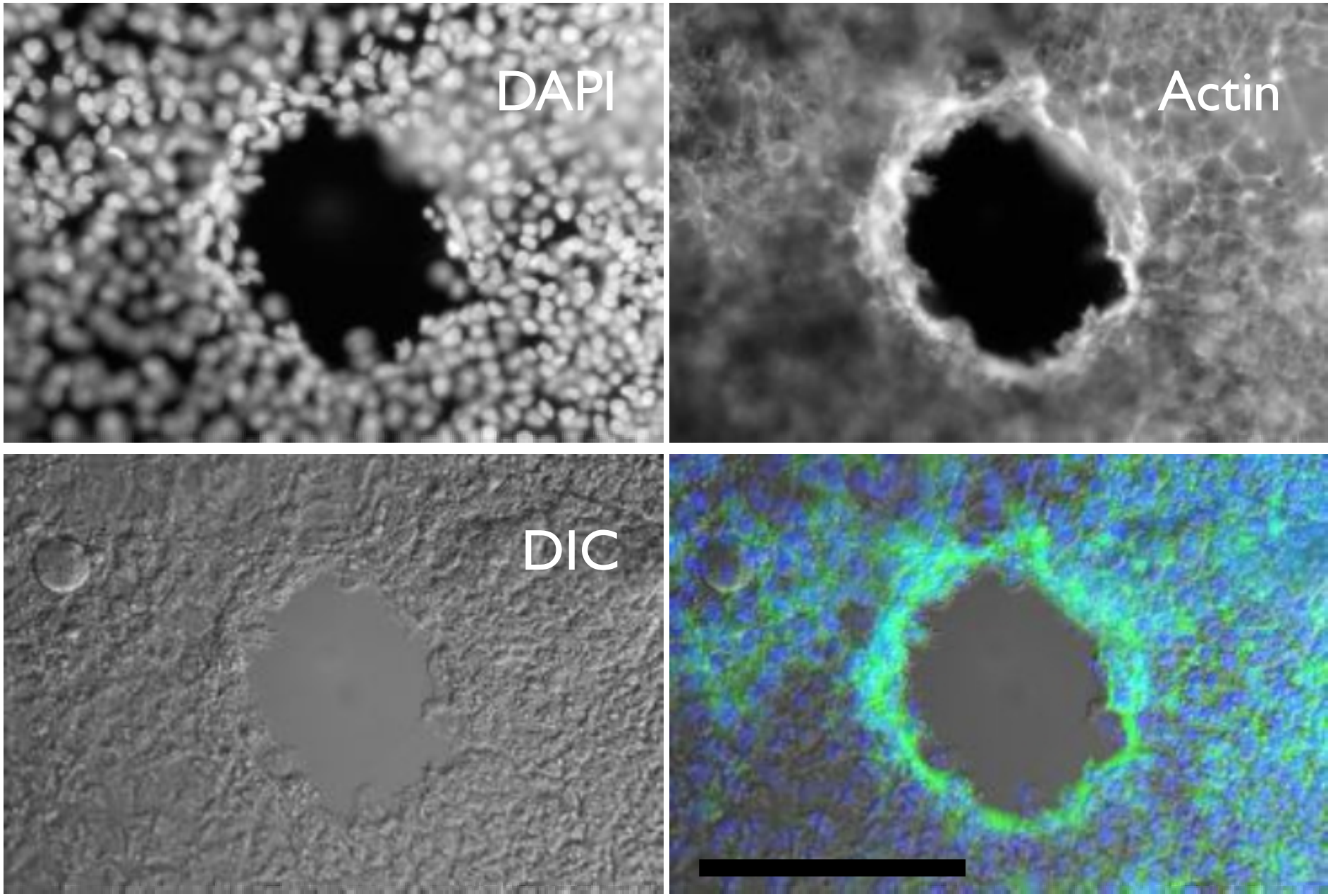
Ectoderm 20 sec



Endoderm 1 min



Ectoderm 11 min