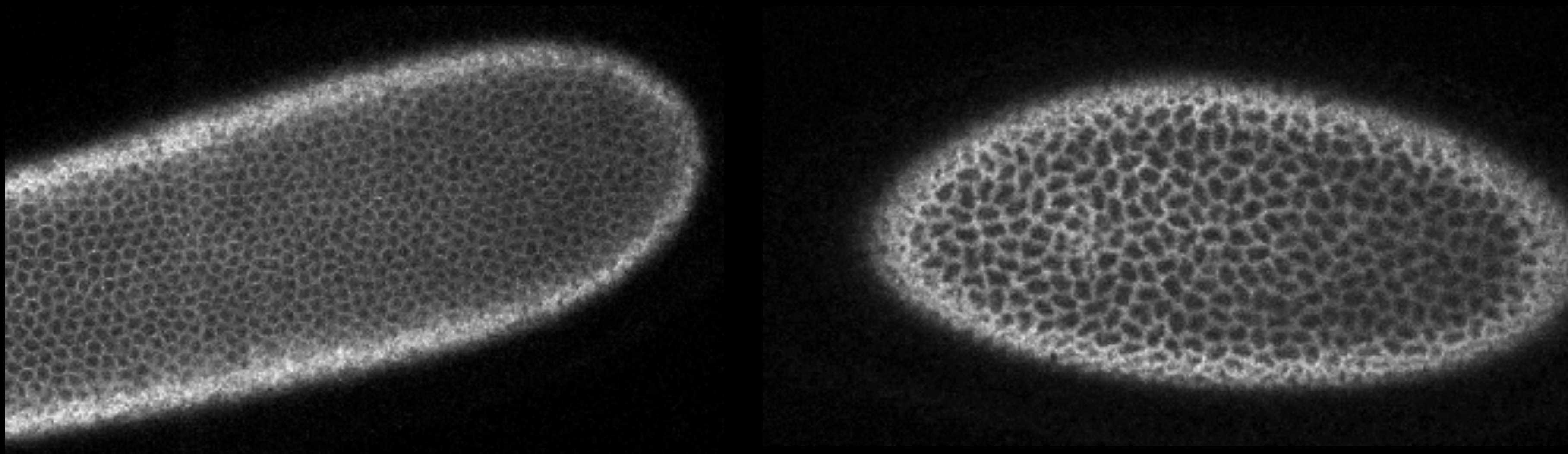
Dissecting cellular biomechanics with a laser

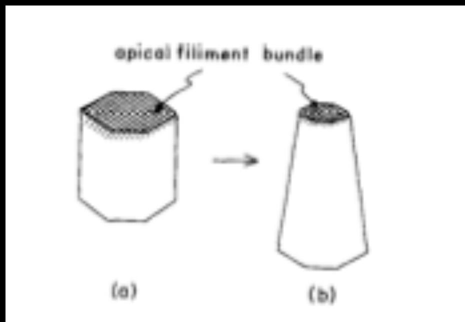
M. Shane Hutson - *Dept of Physics & Astronomy, Dept of Biological Sciences, Vanderbilt Institute for Integrative Biosystem Research & Education (VIIBRE)*



". . . it is critical that we complement the popular molecular and biochemical approaches to the control of morphogenesis with nuts-and-bolts analyses of the physics of how morphogenetic processes occur."

- M.A.R. Koehl, *Sem. Dev. Biol.* **1**: 367 (1990).

Physicists/Engineers: build (code) a model



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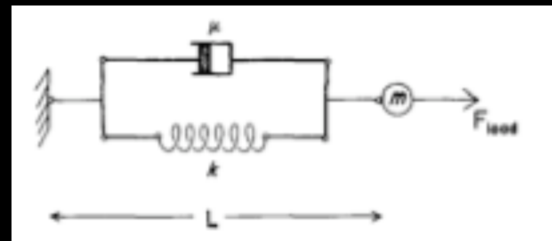

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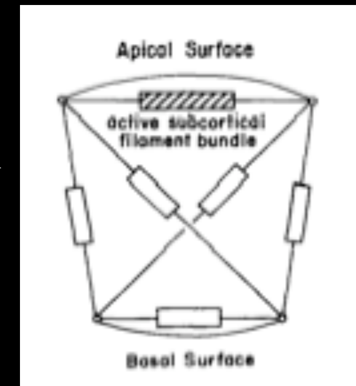
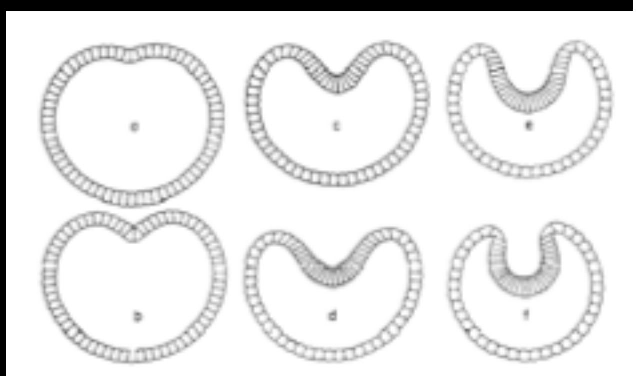
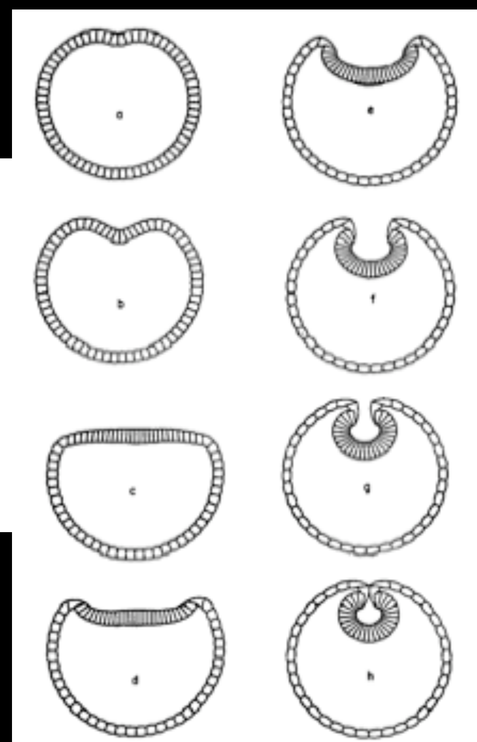
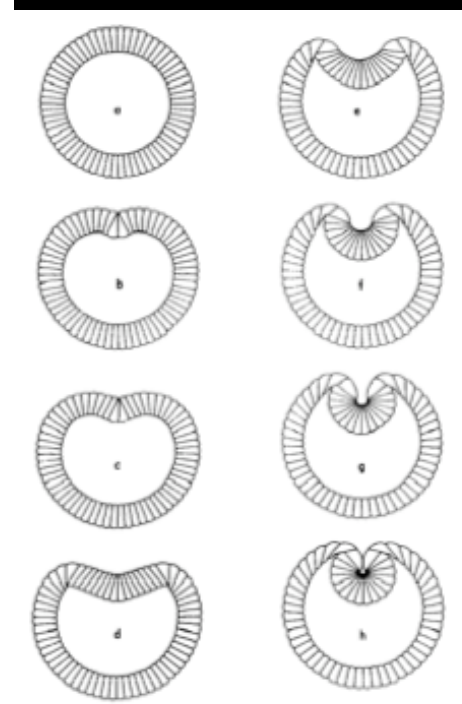




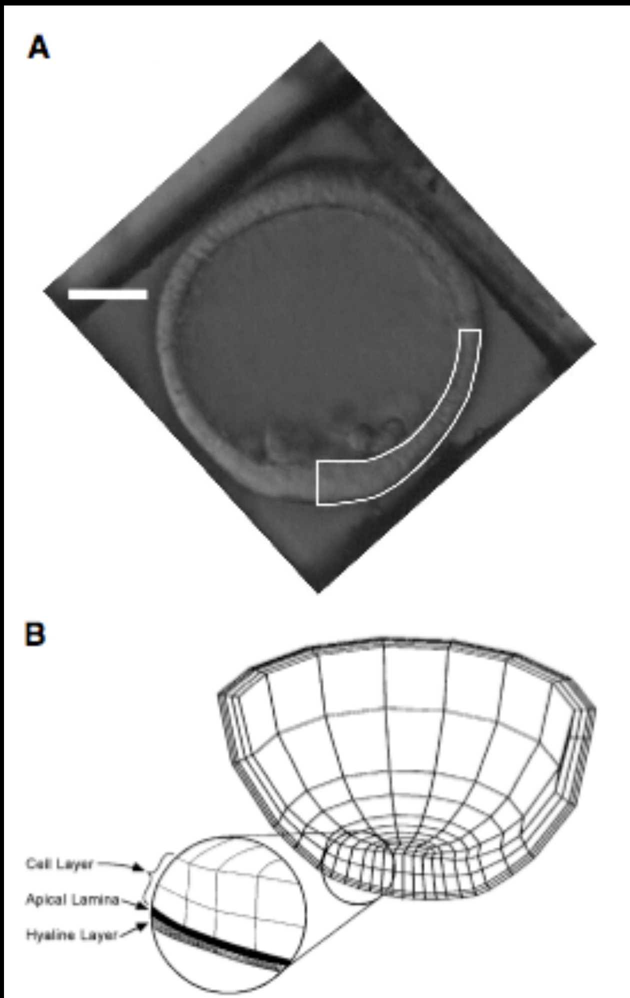


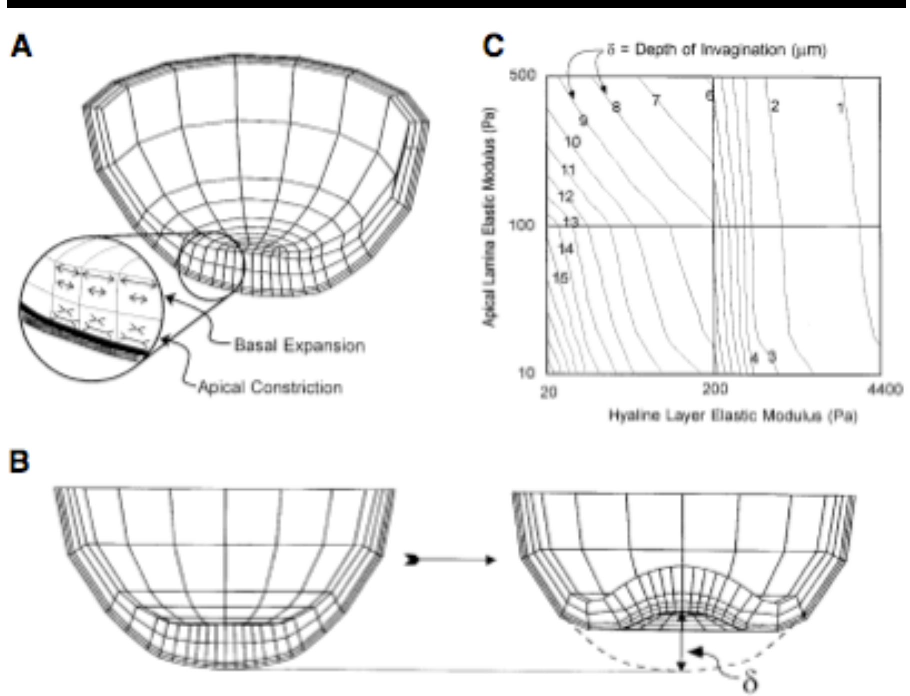


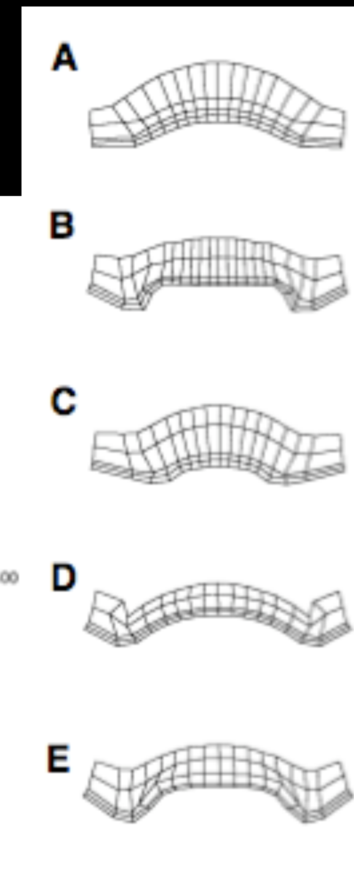
G.M. Odell et al (1981)
Developmental Biology 85: 446-462.

. . . and another one . . .

L.A. Davidson et al (1995)
Development 121: 2005-2018.





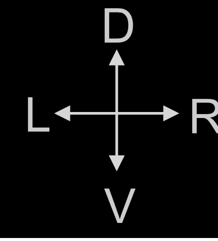


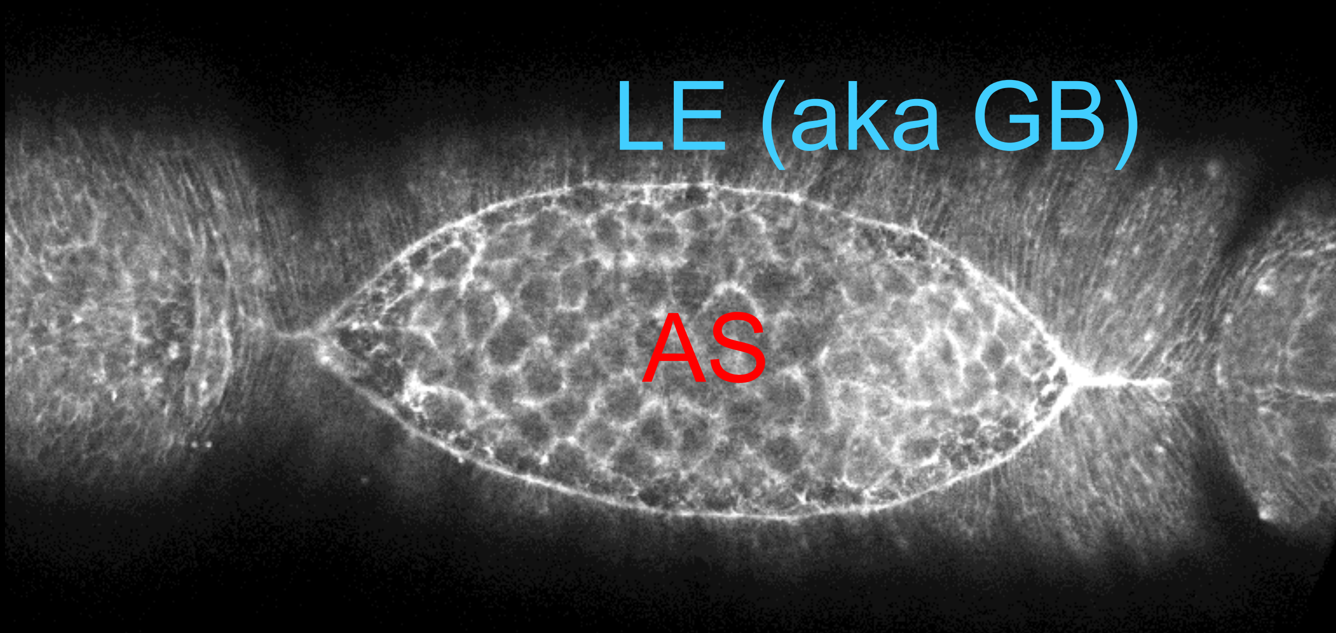
Outline

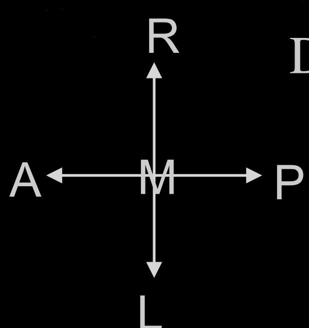
Using laser-microsugery to (in)validate models . . .

1. by evaluating which cells/tissues are critical for a given morphogenetic event.
2. by measuring the subcellular distribution of stress within individual cells – doing so as a function of developmental time and genetic background
3. by isolating individual cells to measure cellular strain.

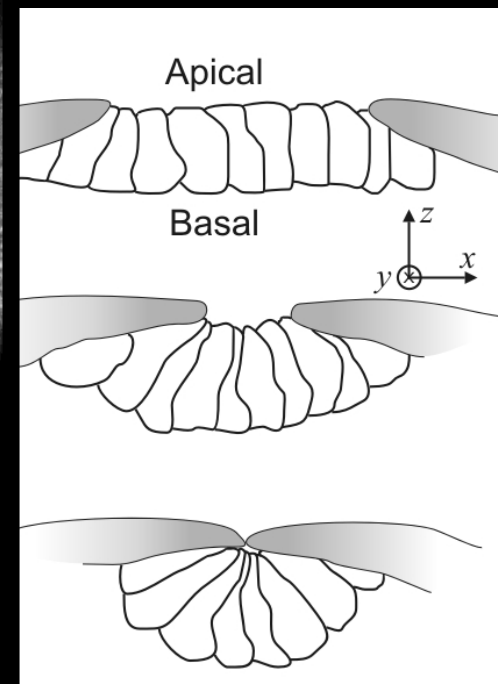
Dorsal Closure (Stage 13/14)





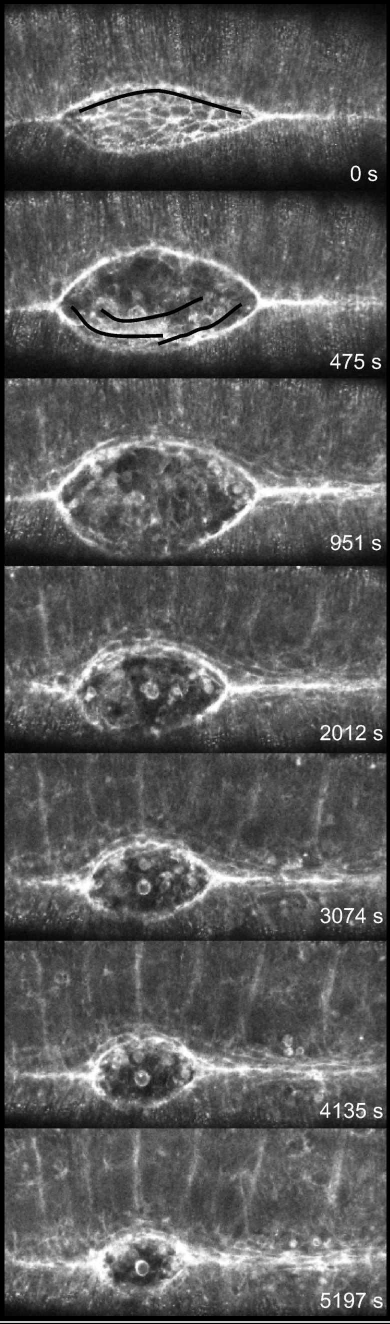


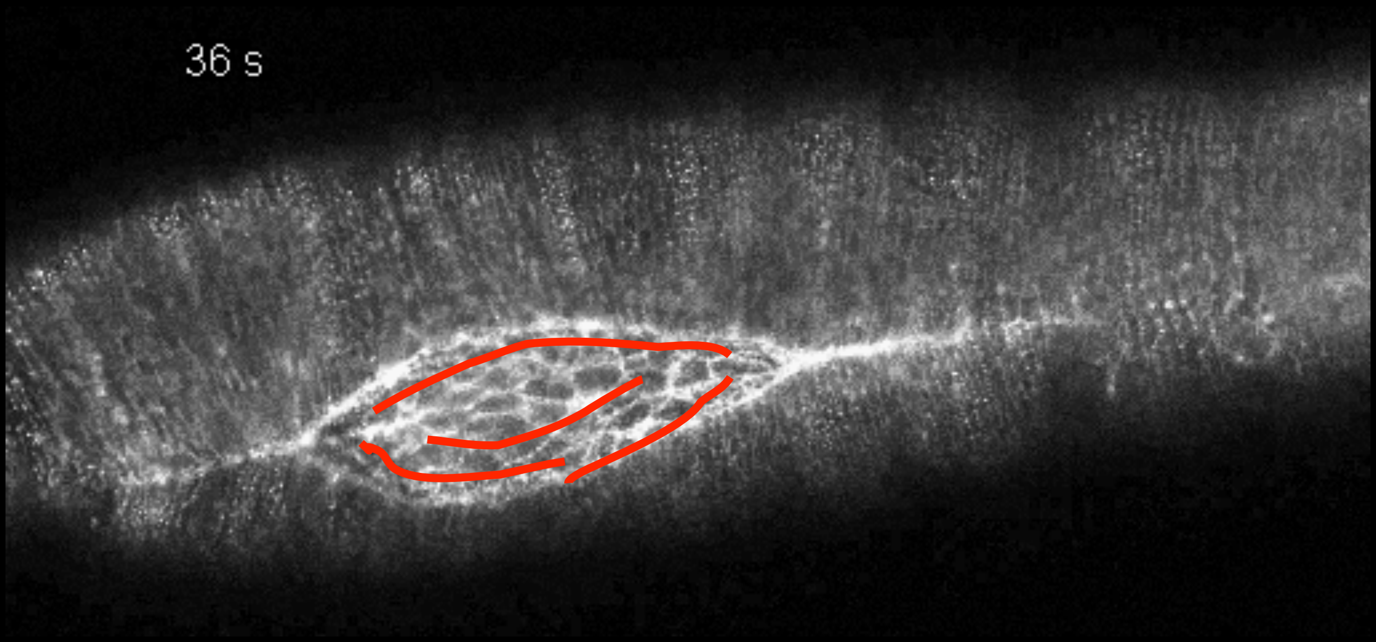
DORSAL VIEW



CROSS-SECTIONS ⊥ TO ANTERIO-POSTERIOR AXIS

The AS is also critically important in dorsal closure.





Hutson, Tokutake, Chang, Bloor, Venakides, Kiehart and Edwards (2003) *Science* 300: 145-149.

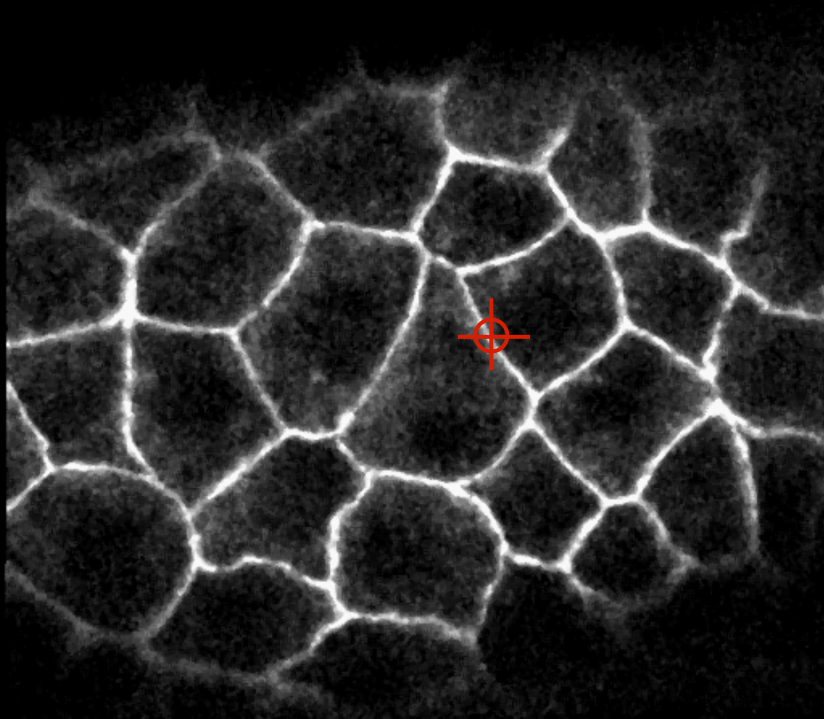
Can laser ablation be a more quantitative tool for studying *in vivo* mechanics?

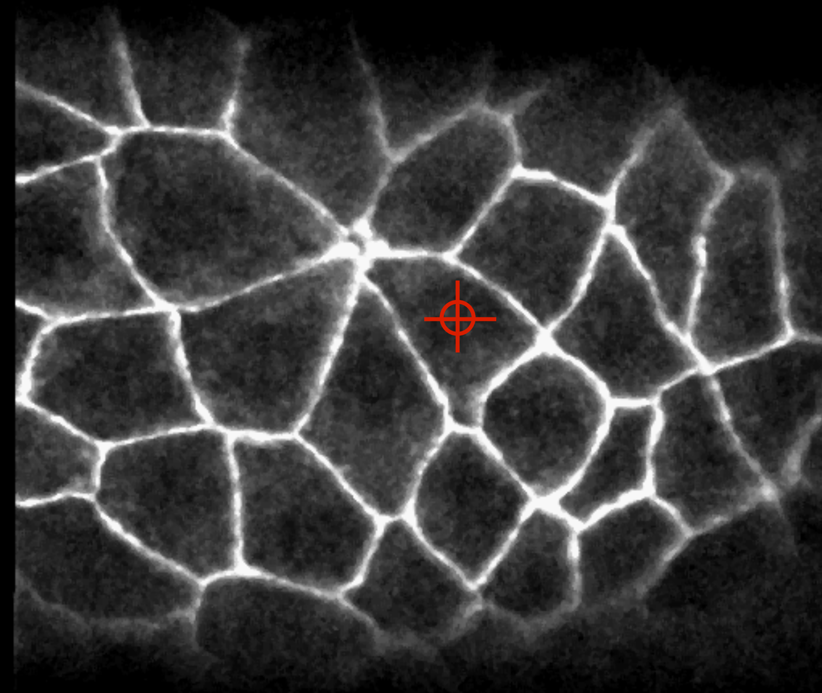
Can we measure the spatiotemporal distribution of mechanical stress . . .

. . . at a sub-cellular level

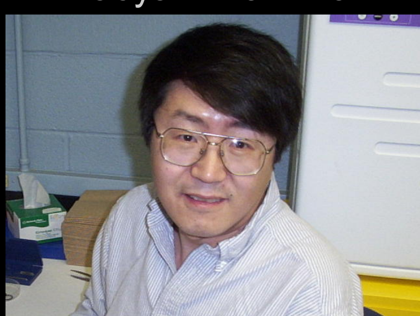
. . . in a living embryo?

Drilling holes in an embryonic epithelium . . .





Xiaoyan ‘Max’ Ma

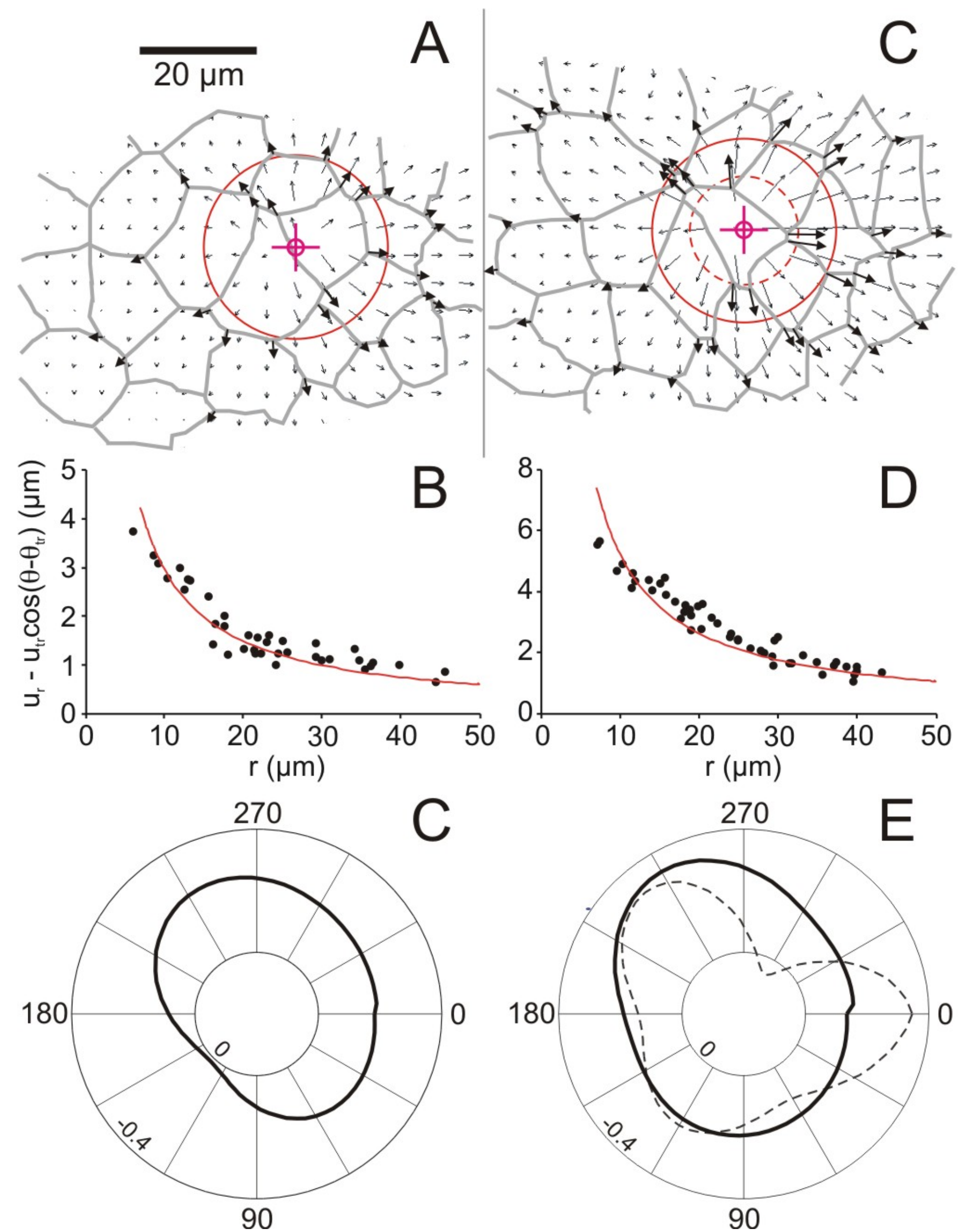


Holley Lynch

Peter Scully

Ma, Lynch, Scully and Hutson (2008) *Physical Biology* 6: 036004

Ma, Lynch, Scully and Hutson (2008) *Physical Biology* 6: 036004



Spatial Information

Relaxation displacements around a circular hole in a thin sheet*:

$$u_r(r,\theta) = B_1(r)(\sigma_x + \sigma_y) + B_2(r)(\sigma_x - \sigma_y)\cos 2\theta + u_{tr}\cos(\theta - \theta_{tr})$$

$$u_\theta(r,\theta) = -B_3(r)(\sigma_x - \sigma_y)\sin 2\theta + u_{tr}\sin(\theta - \theta_{tr})$$

$$B_1(r) = \frac{1+\nu}{2E}\frac{R_0^2}{r}$$

$$B_2(r) = \frac{1+\nu}{2E}\left[\frac{4}{1+\nu}\frac{R_0^2}{r} - \frac{R_0^4}{r^3}\right]$$

$$B_3(r) = \frac{1+\nu}{2E}\left[2\frac{1-\nu}{1+\nu}\frac{R_0^2}{r} + \frac{R_0^4}{r^3}\right]$$

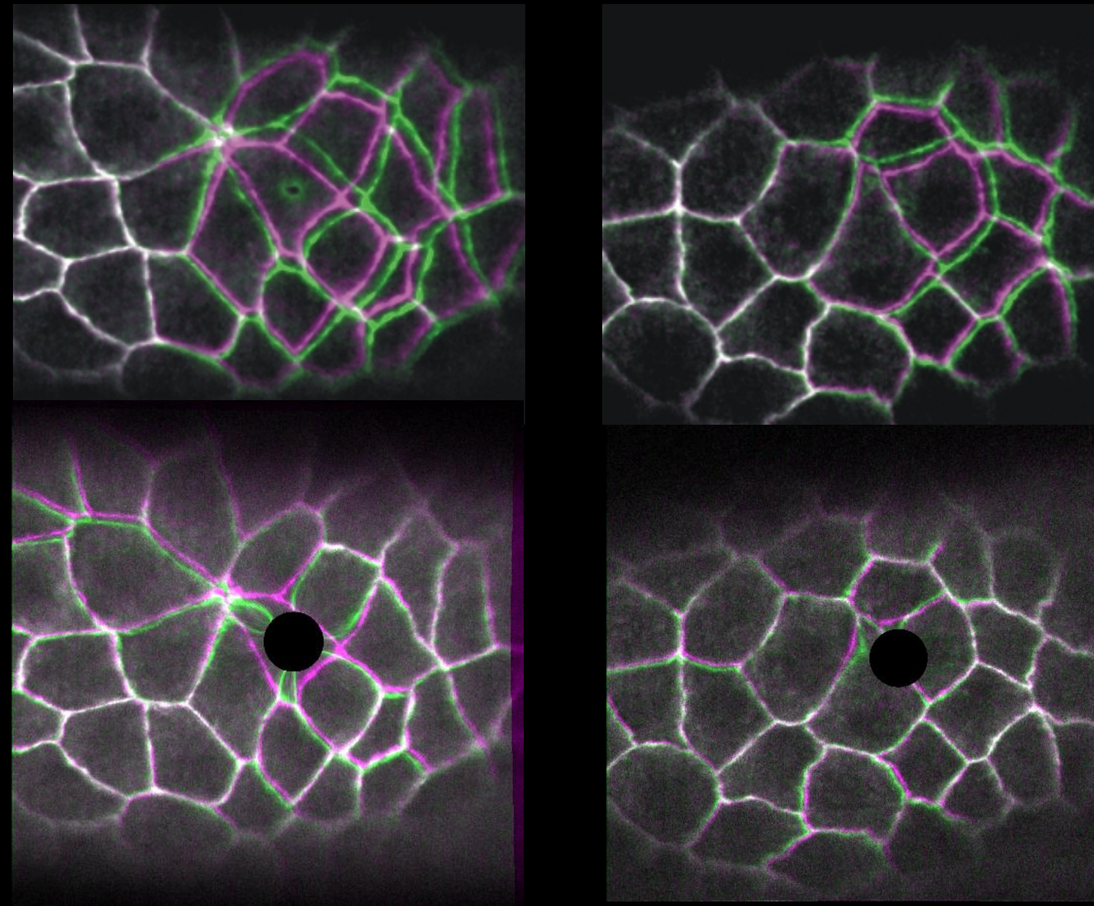
*Assumes a homogeneous, isotropic, linearly elastic material under infinitesimal deformation.

Magenta - pre-ablation stressed state

Green - post-ablation strain-relaxed state

OR

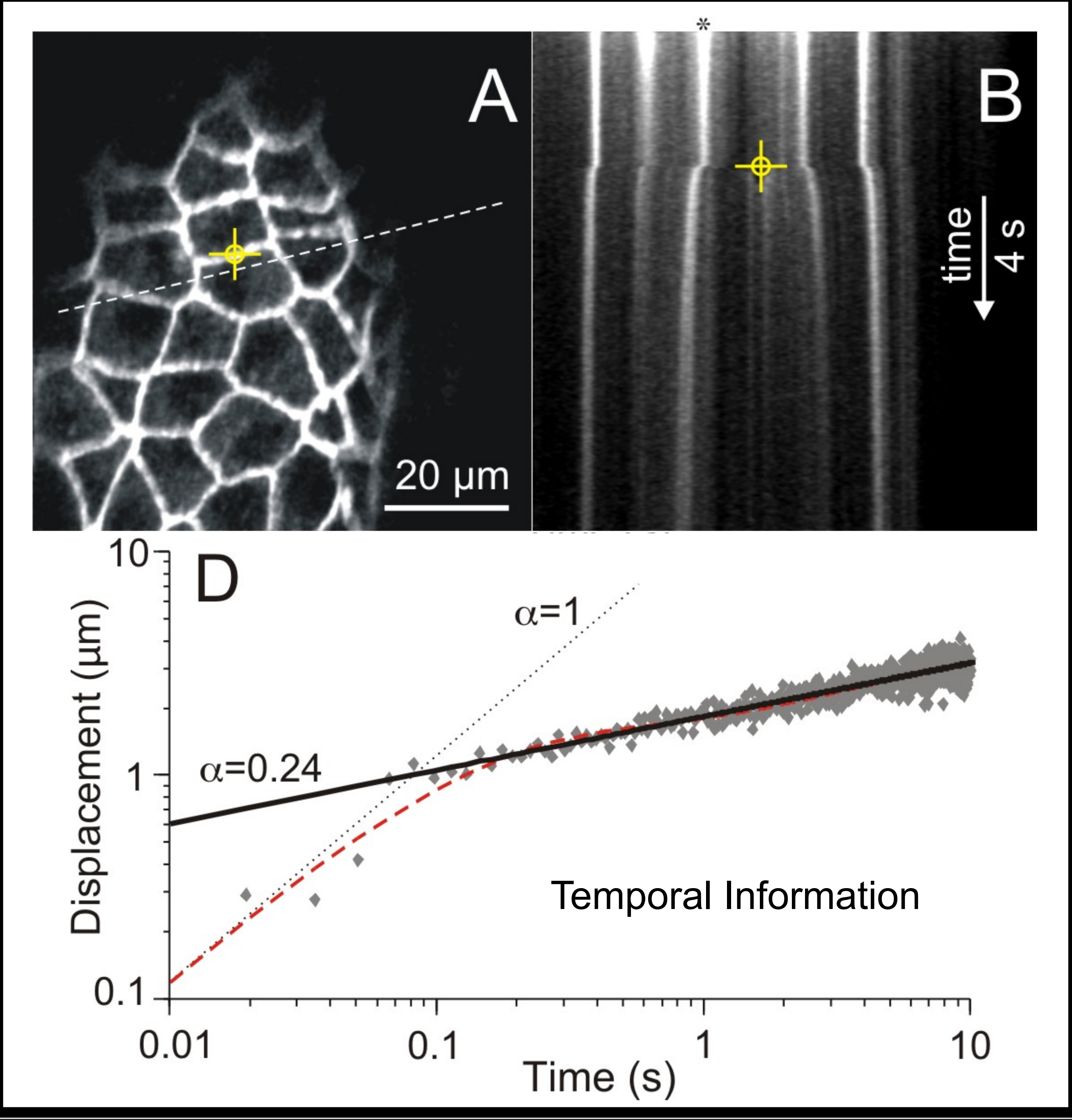
computationally re-strained post-ablation state



Parameters:

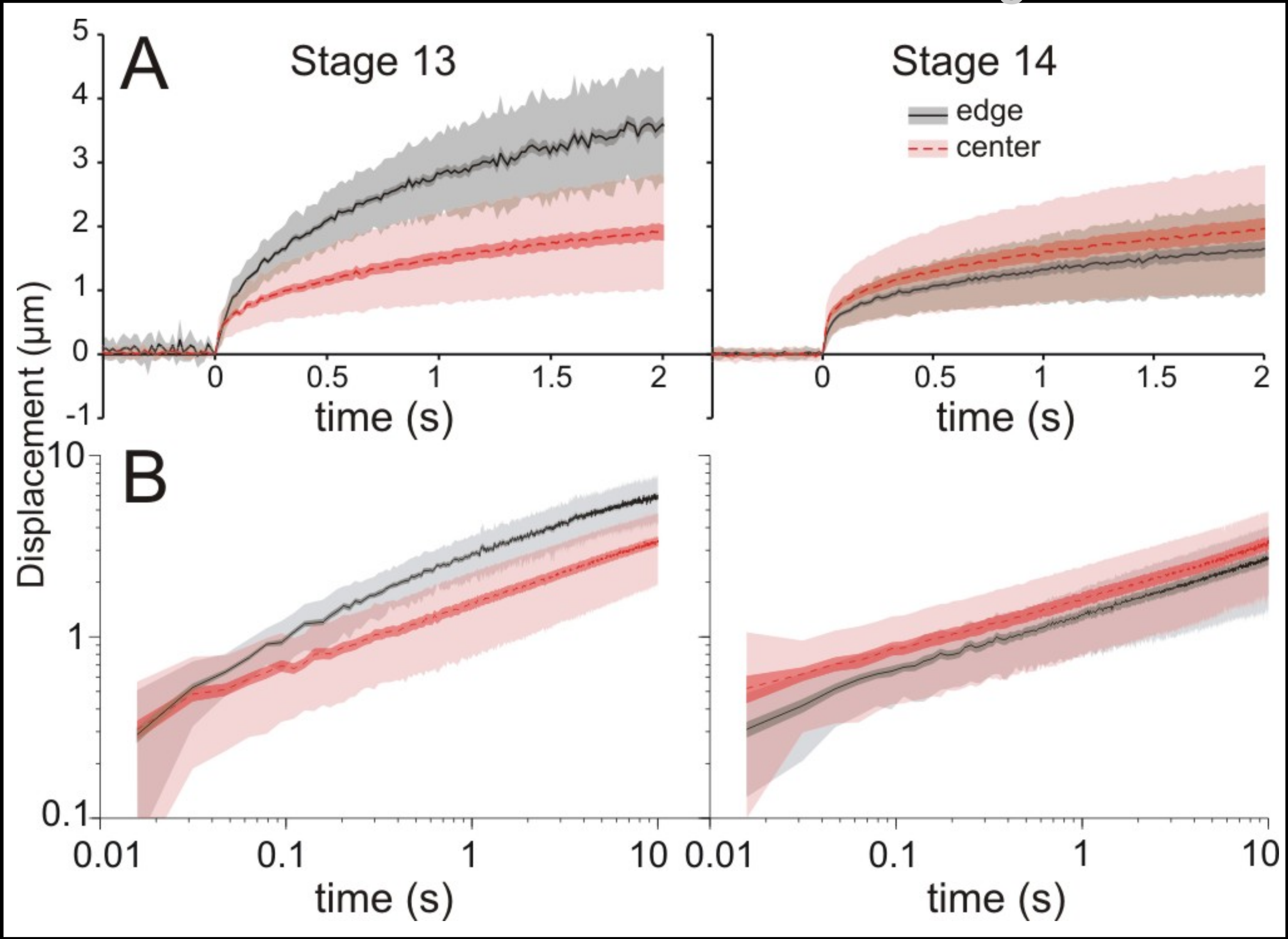
assume r_0=5 μm, ν = 0.33

	Edge Wound	Cell-center Wound
Pre-ablation average strain:	0.8	1.6
Post-ablation c-of-m translation:	5.6 μm @ 342°	7.6 μm @ 332°
Pre-ablation stress anisotropy:	0.01	0.02
Principle stress direction:	75°	55°



Ma, Lynch, Scully and Hutson (2008) *Physical Biology* 6: 036004

Recoils at different sites and stages



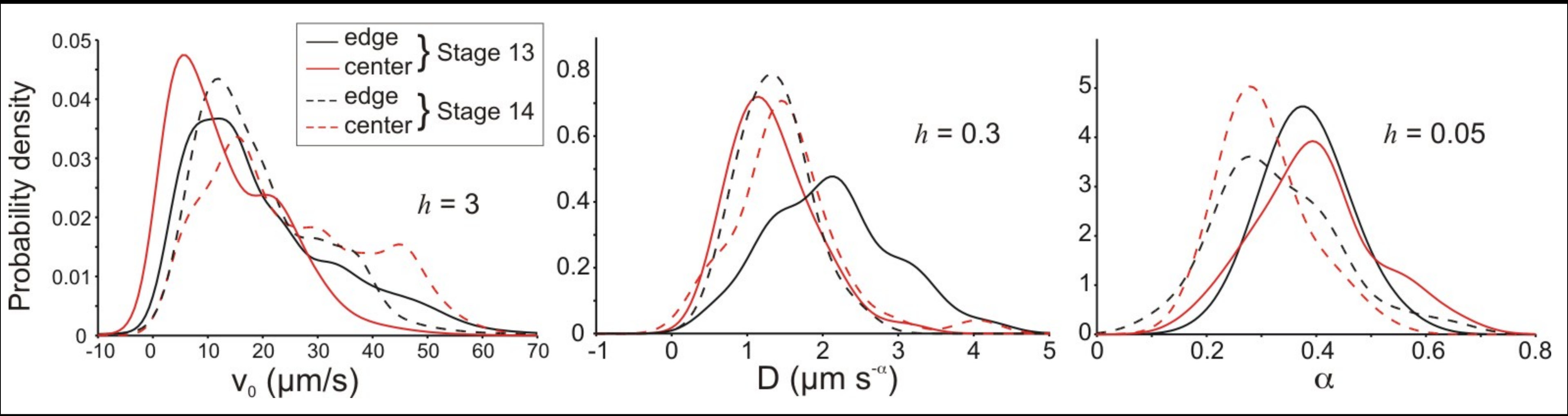
Ma, Lynch, Scully and Hutson (2008) *Physical Biology* 6: 036004

$$\frac{-v_0}{r_0} \approx \gamma_0 = \frac{\sigma}{\eta} \qquad \frac{-u(t)}{r_0} = \frac{-D}{r_0} t^\alpha \approx \varepsilon(t) = \frac{\sigma}{G_0'} t^\alpha$$

stress

limiting Newtonian viscosity

stiffness



Compare to	1 to 3 μm/s	(Hutson et al 2003, Peralta et al 2007, Toyama et al 2008)
	< 0.3 μm/s	(Rauzi et al 2008, Farhadifar et al 2007)
	0.5 to 1 μm/s	(Kumar et al 2006)

Conclusions I . . .

1. Spatial recoil patterns resemble what you'd expect for a hole in a homogeneous thin sheet; the arrangement of cell edges has a limited secondary impact.
2. Biphasic recoil kinetics are consistent with a soft glassy material that transitions to a Newtonian fluid at high-frequency (short times)
3. α decreases from Stage 13 to 14 ⟶ tissue solidifies during closure
4. Stress concentration (1.6-fold) on cell edges in Stage 13; uniform stress in Stage 14
5. Stage-dependences of other parameters imply coupled constraints. Still enough to exclude 5 of 7 published models for apical constriction.

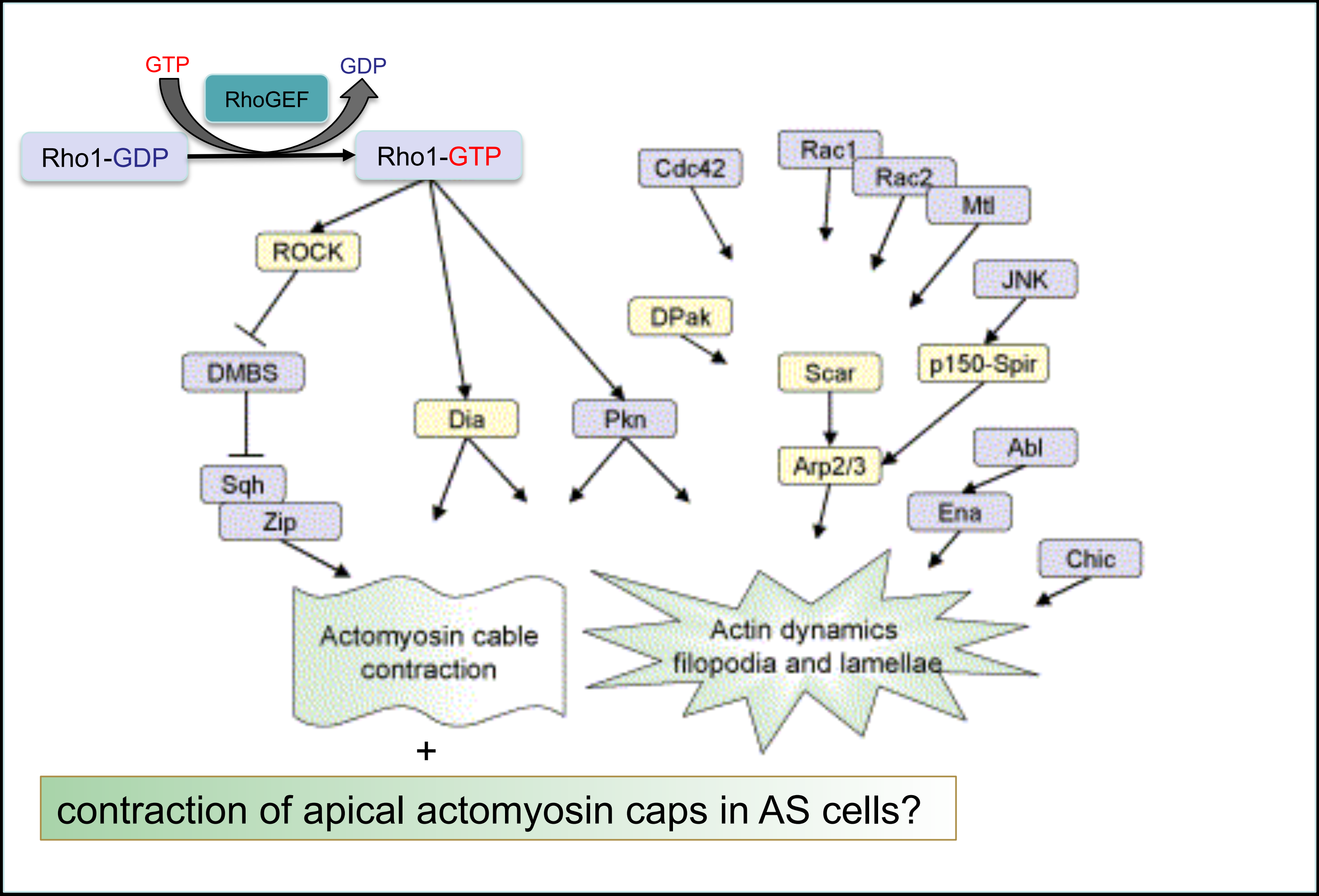
$$\frac{\sigma_{C,14}}{\sigma_{C,13}} = (2.06 \pm 0.28)\frac{\sigma_{E,14}}{\sigma_{E,13}}$$

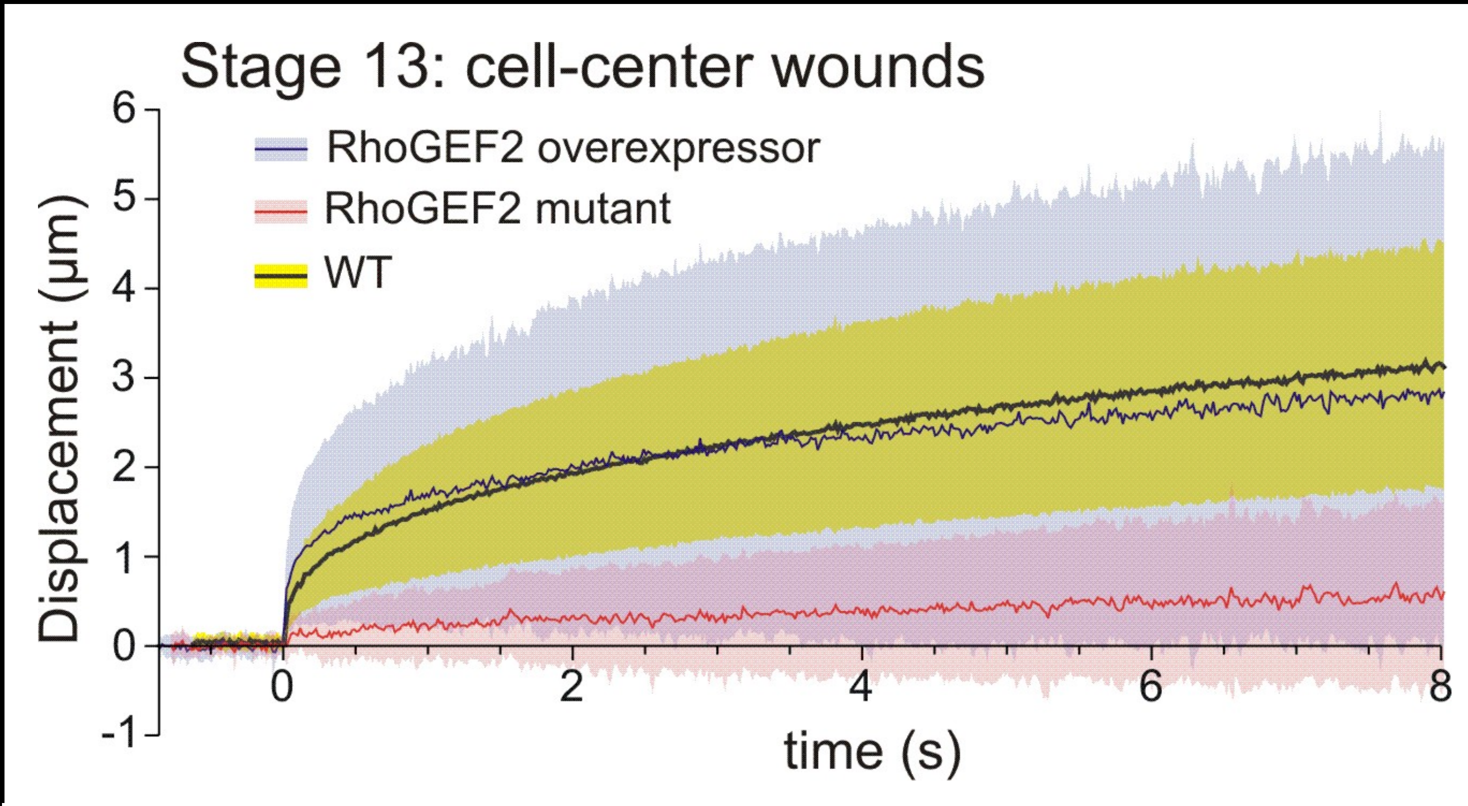
$$\frac{G'_{14}}{G'_{13}} = (1.24 \pm 0.07)\frac{\sigma_{E,14}}{\sigma_{E,13}}$$

$$\frac{\eta_{14}}{\eta_{13}} = (0.77 \pm 0.08)\frac{\sigma_{E,14}}{\sigma_{E,13}}$$

Example scenario - constant η implies
stiffness G' increases 1.6x
stress σ_E increases 1.3x
stress σ_C increases 2.7x

Now, to re-establish contact with the biologists . . .





Marco Antunes
Antonio Jacinto
@ IMM-Lisbon

Category	N	D (μm/s$^{\alpha}$)	α	v_0 (μm/s)
Wild-type	30	1.34 ± 0.07	0.396 ± 0.015	13.4 ± 1.5
RhoGEF2 overexpressor	18	1.68 ± 0.35	0.182 ± 0.035	13.2 ± 2.4
RhoGEF2 mutant	18	0.23 ± 0.09	0.633 ± 0.232	1.8 ± 0.7
ANOVA P-value		9×10^{-6}	0.026	1×10^{-5}
Means difference test (wt vs overexp)		0.16	5×10^{-6}	0.47
Variance ratio test (wt vs overexp)		1×10^{-7}	0.065	0.23

Azevedo, Antunes et al (2011) *Plos ONE* 6: e23964.

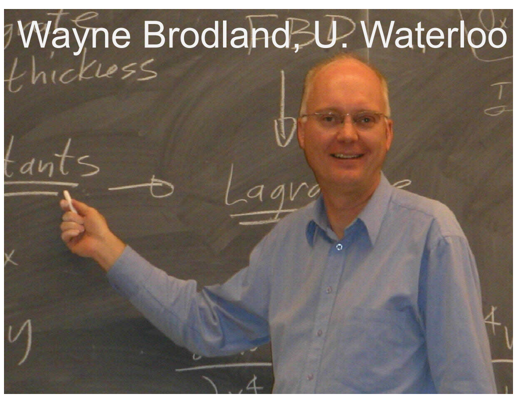
What does a model need to do to reproduce these results?

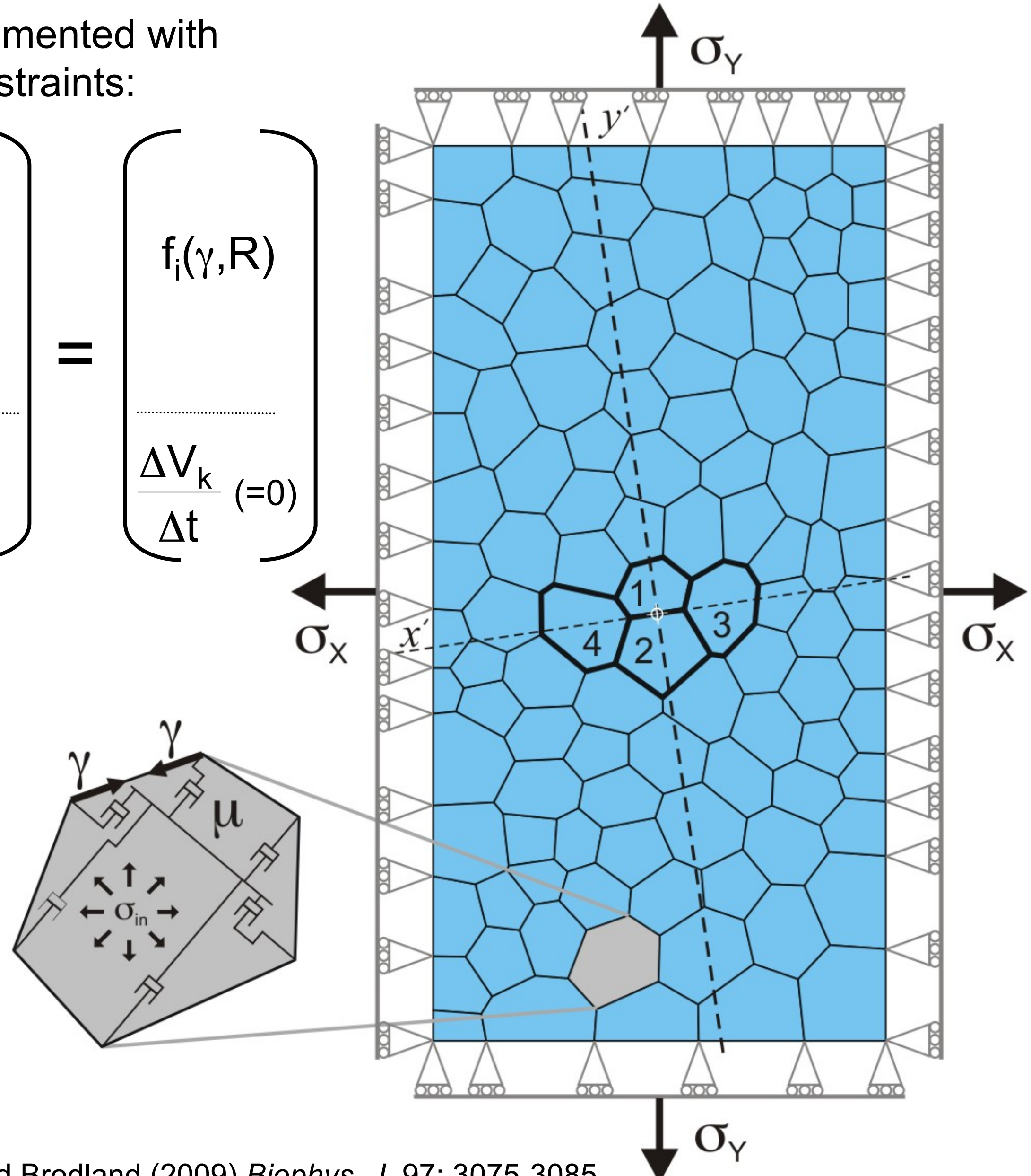
$$[\mathbf{C}]\{\dot{\mathbf{u}}\} = \{\mathbf{f}_\mu\}$$

Augmented with constraints:

$$\begin{pmatrix} C_{ij}(\mu) \\ \text{~1/6 non-zero} & \frac{\partial V_k}{\partial u_i} \\ \frac{-\partial V_k}{\partial u_i} & 0 \end{pmatrix} \begin{pmatrix} \frac{\Delta u_i}{\Delta t} \\ \sigma_{in,k} \end{pmatrix} = \begin{pmatrix} f_i(\gamma, R) \\ \frac{\Delta V_k}{\Delta t} \ (=0) \end{pmatrix}$$

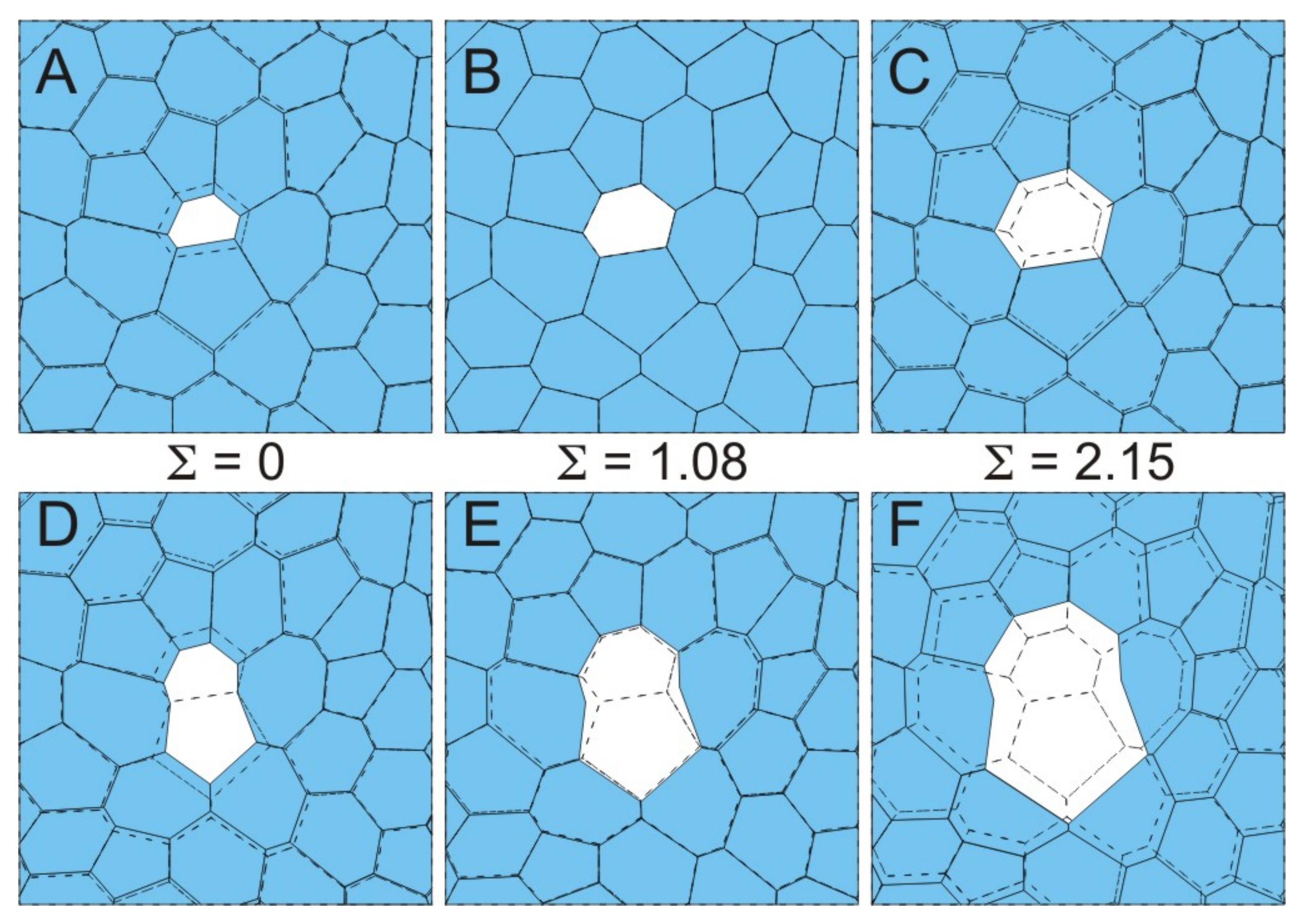
Stiffness or Damping Matrix





Hutson, Veldhuis, Ma, Lynch, Cranston and Brodland (2009) *Biophys. J.* 97: 3075-3085.

Spatial recoil patterns are close to that of a continuous sheet; secondary impact from cell edge arrangement.

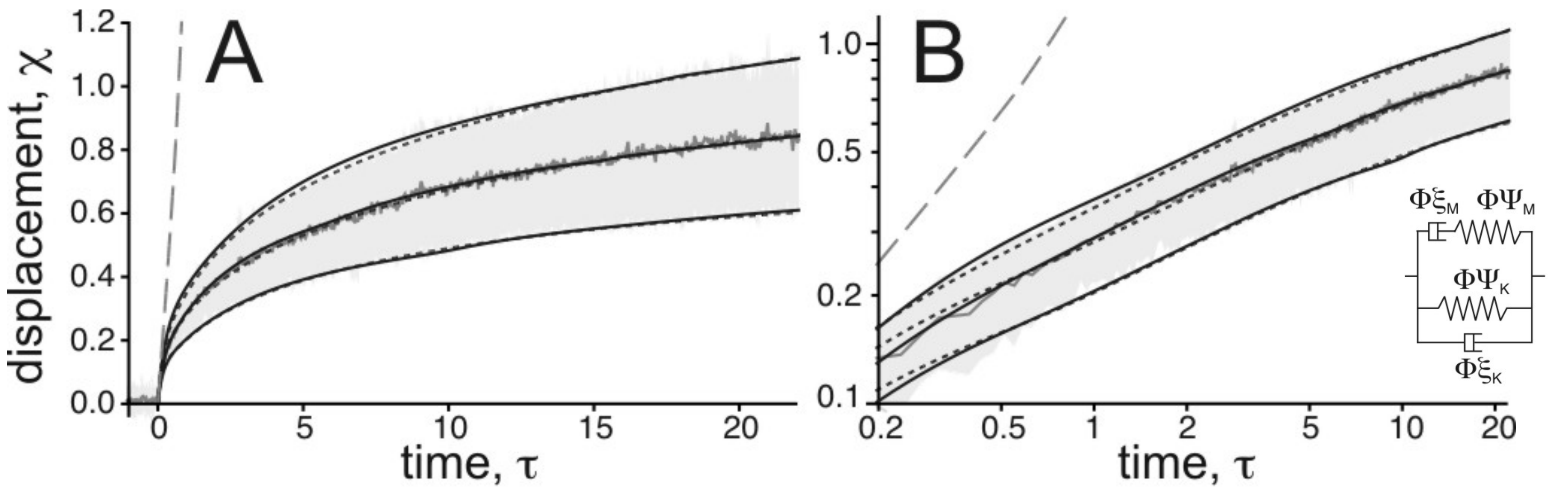


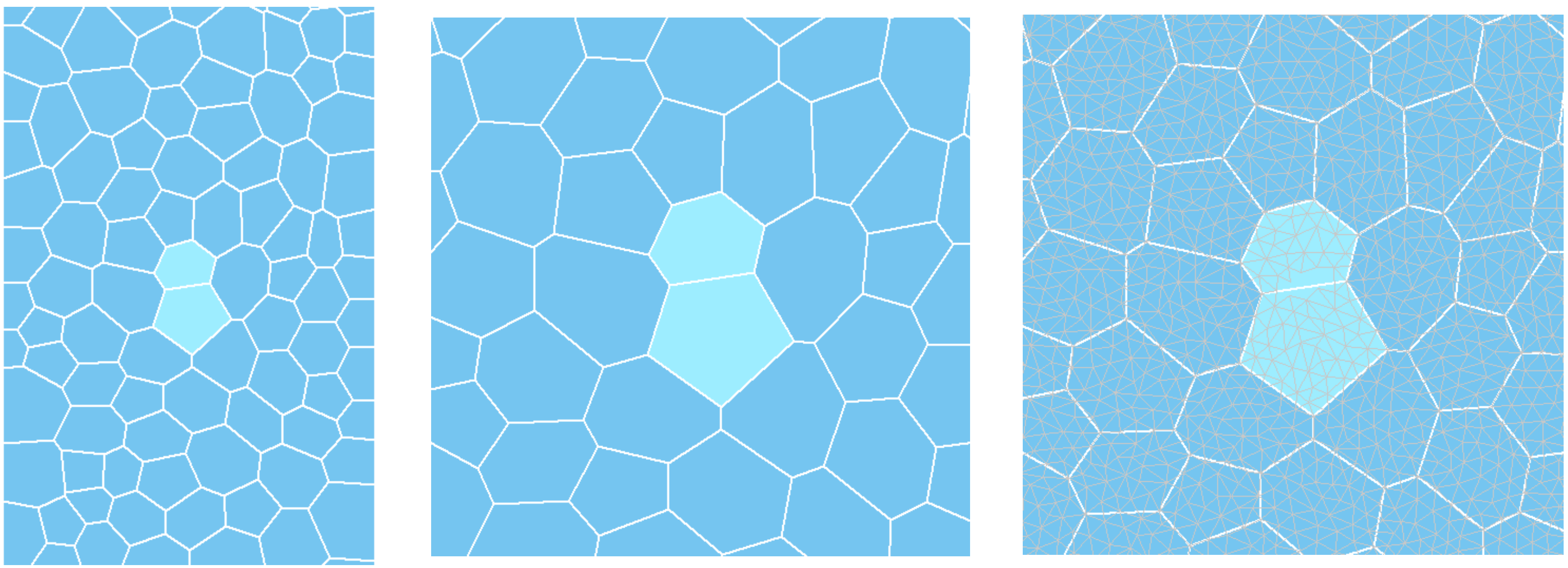
Cell center wound = lose that cell's volume constraint

Cell edge wound = lose that γ, lose volume constraint of two adjacent cells

Hutson, Veldhuis, Ma, Lynch, Cranston and Brodland (2009) *Biophys. J.* 97: 3075-3085.

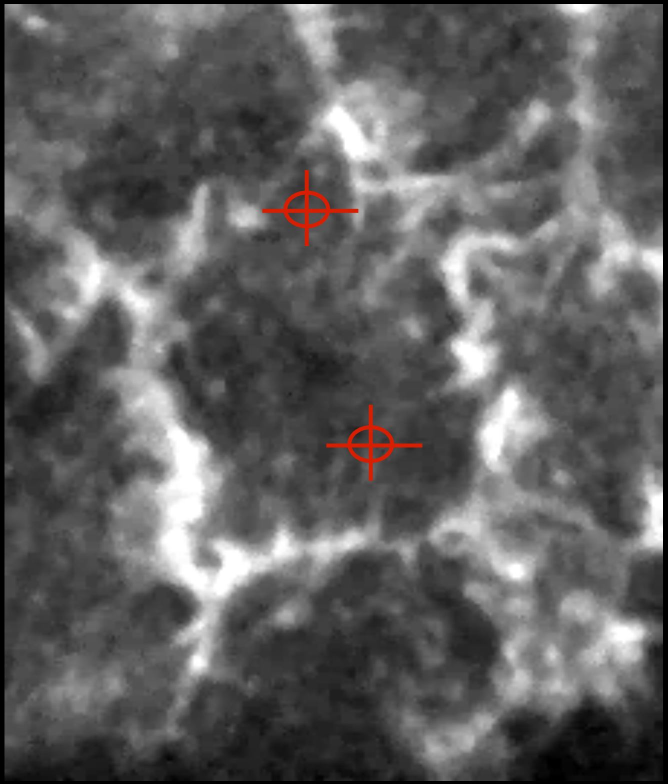
Recoils are biphasic:
roughly linear for t < 0.1 s; weak power-law from 0.1 to 10 s

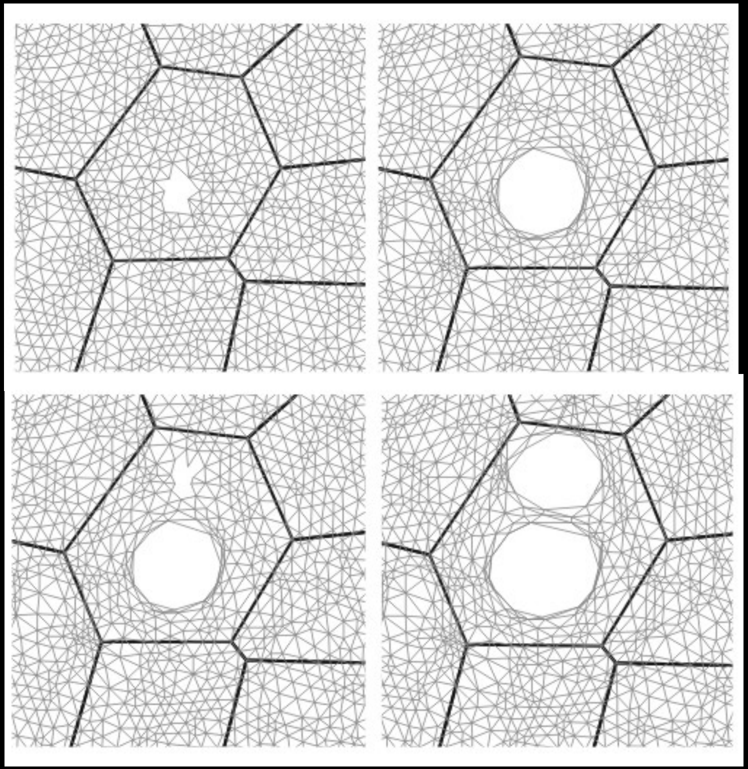




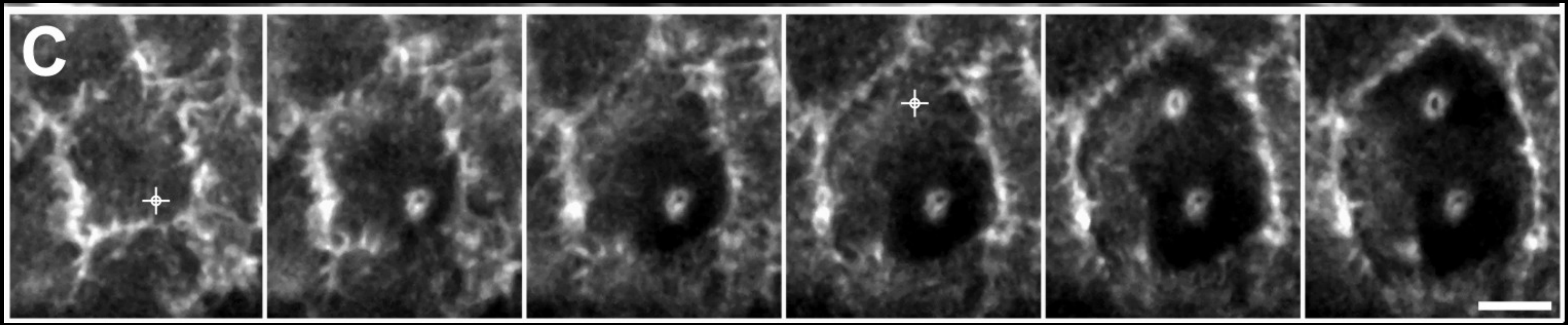
Hutson, Veldhuis, Ma, Lynch, Cranston and Brodland (2009) *Biophys. J.* 97: 3075-3085.

Double wounds in a GFP-moesin embryo





Ma, Lynch, Scully and Hutson (2008) *Physical Biology* 6: 036004

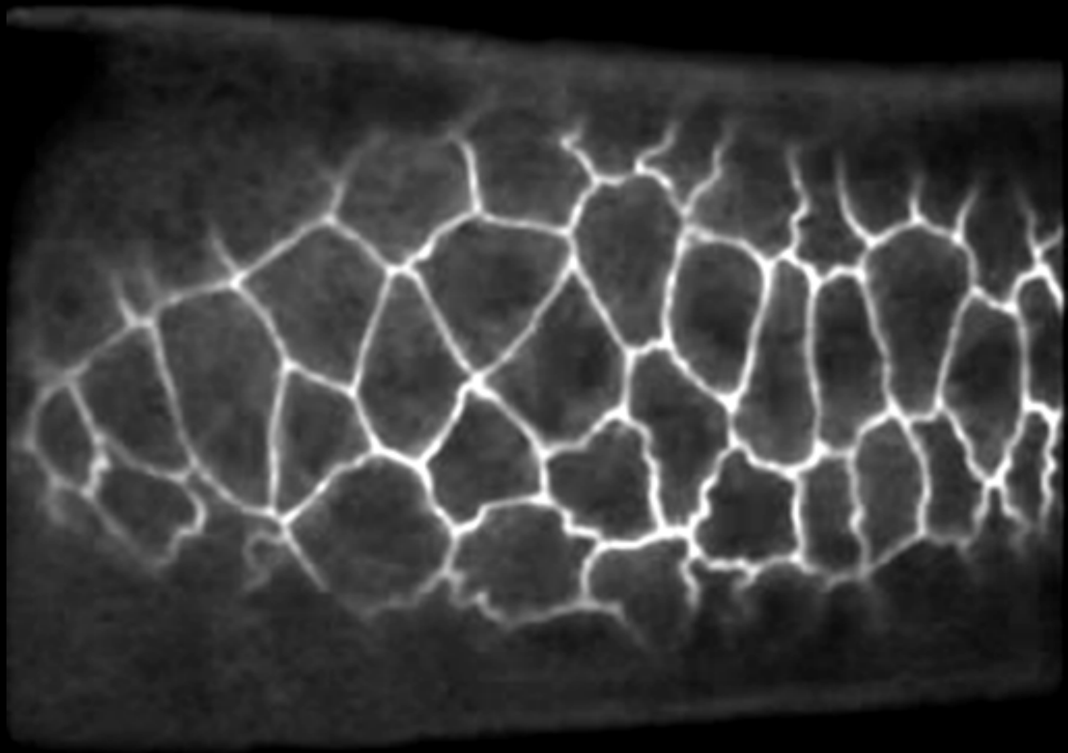


A conflict that needs to be resolved . . .

Cell-level FE models: cellular strain ~0

Continuous sheet fit: cellular strain ~1

Can we mechanically 'isolate' a single AS cell?

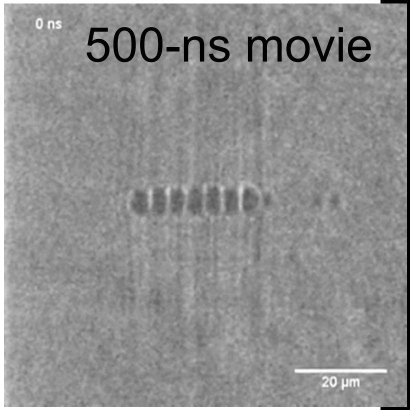


sequential ablations that 'chase' the moving cells.

Not if we use a standard microsurgery system.

An alternative: holographic microsurgery.

Aroshan Jaysinghe



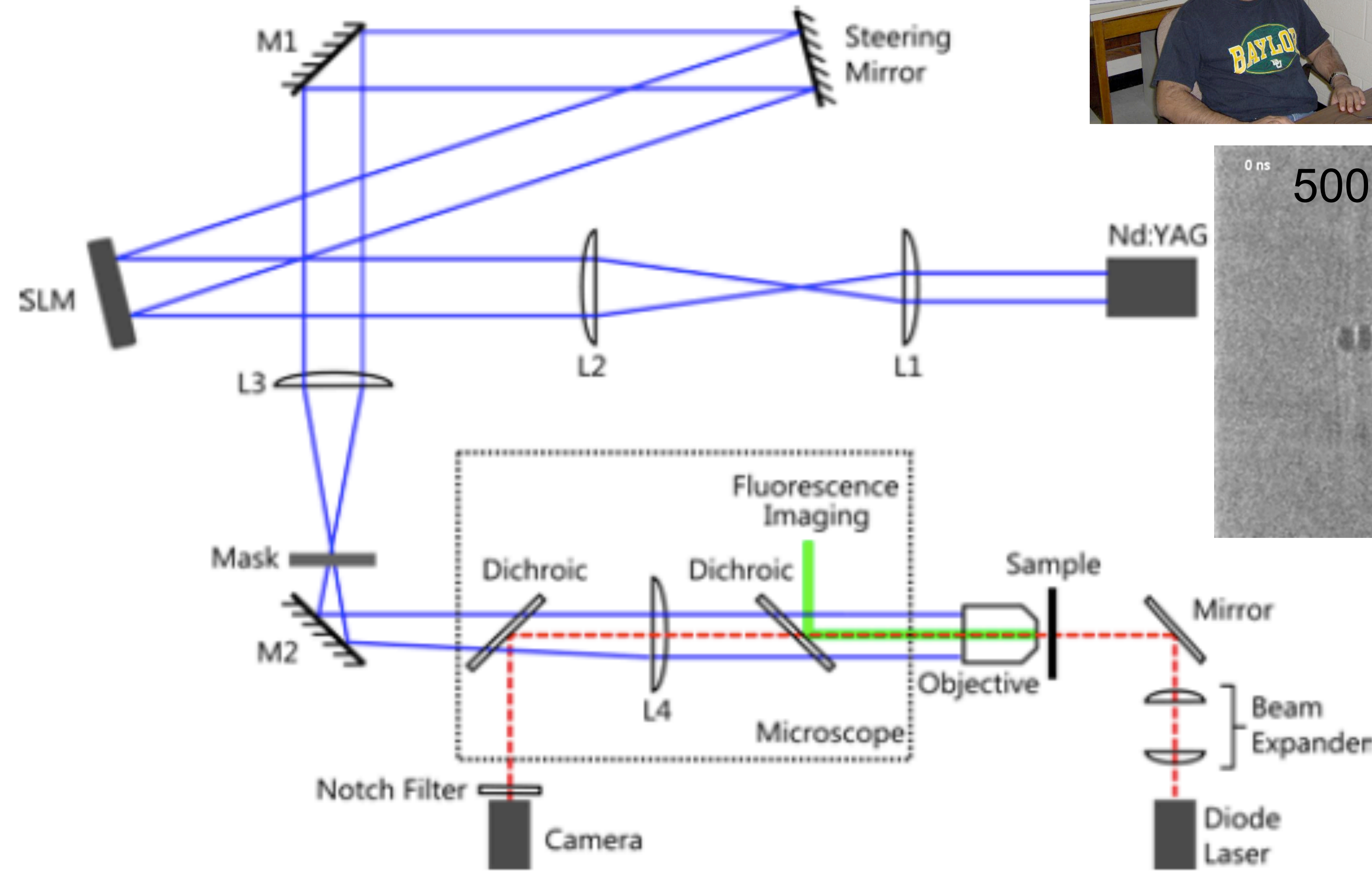
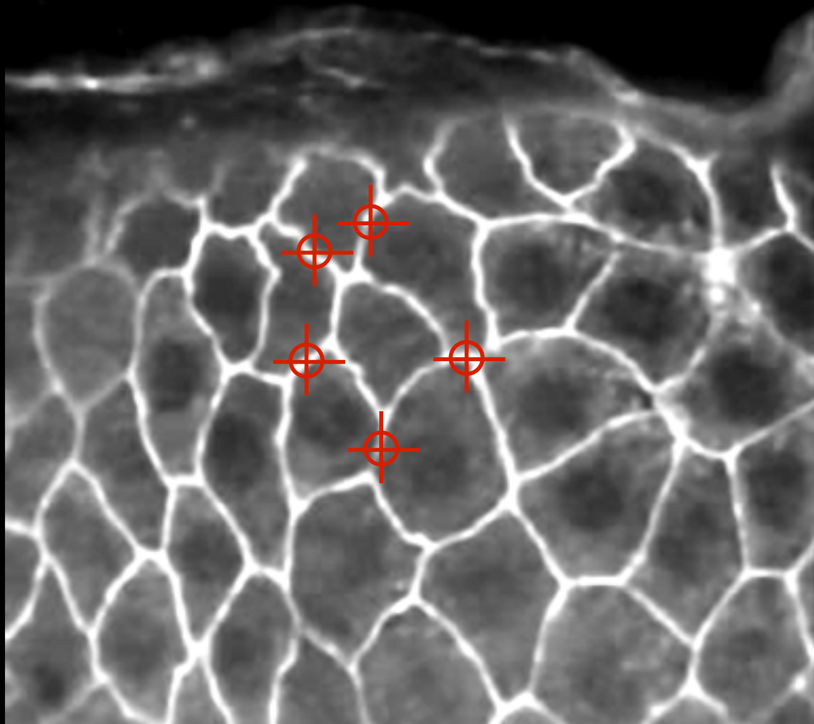
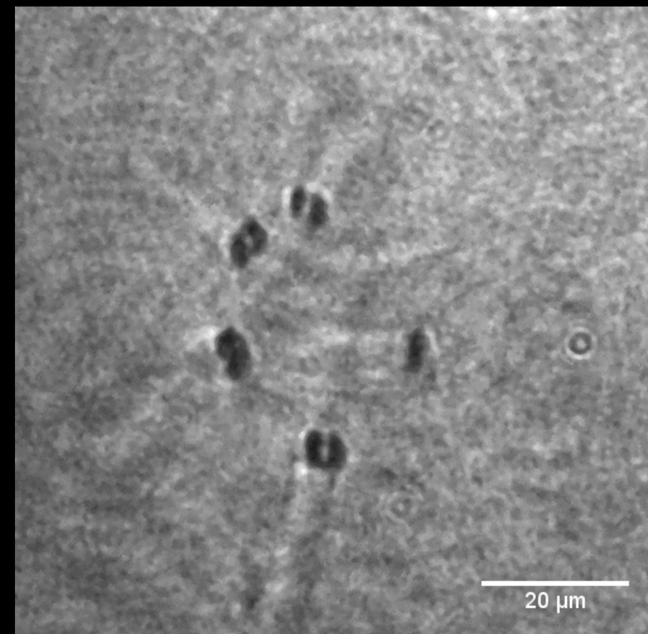


Fig. 1. Optical layout with paths for ablation, high-speed bright-field imaging and confocal fluorescence imaging shown in solid blue, dashed red and thick green lines, respectively.

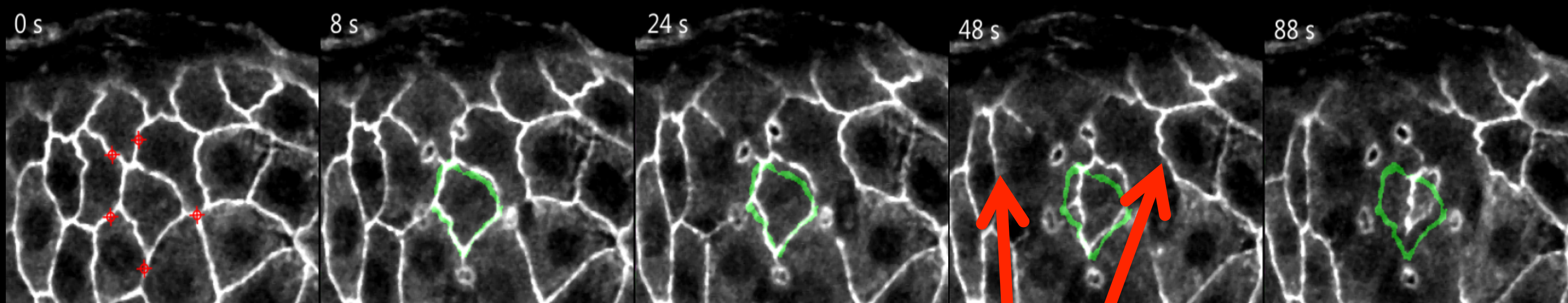
Holographic microsurgery enables “cell isolation” expts



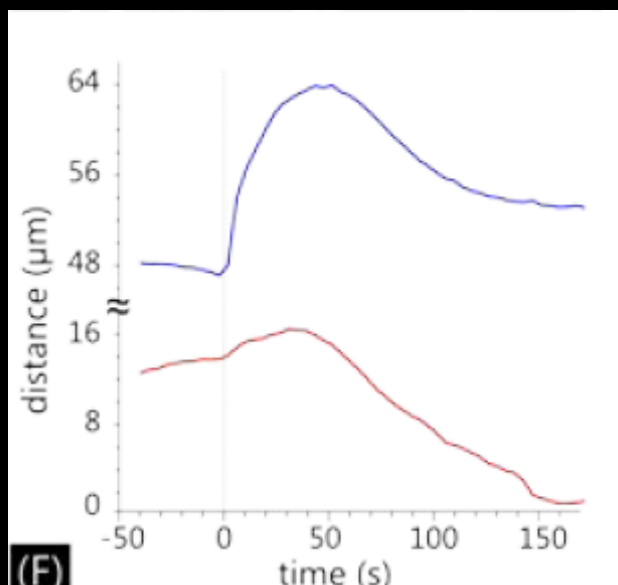


Jayasinghe, Rohner and Hutson (2011) *Biomed. Opt. Express* 2: 2590-2598.

Holographic microsurgery enables “cell isolation” expts



Looks like cellular strain ~0; interesting active(?) contraction at longer times.

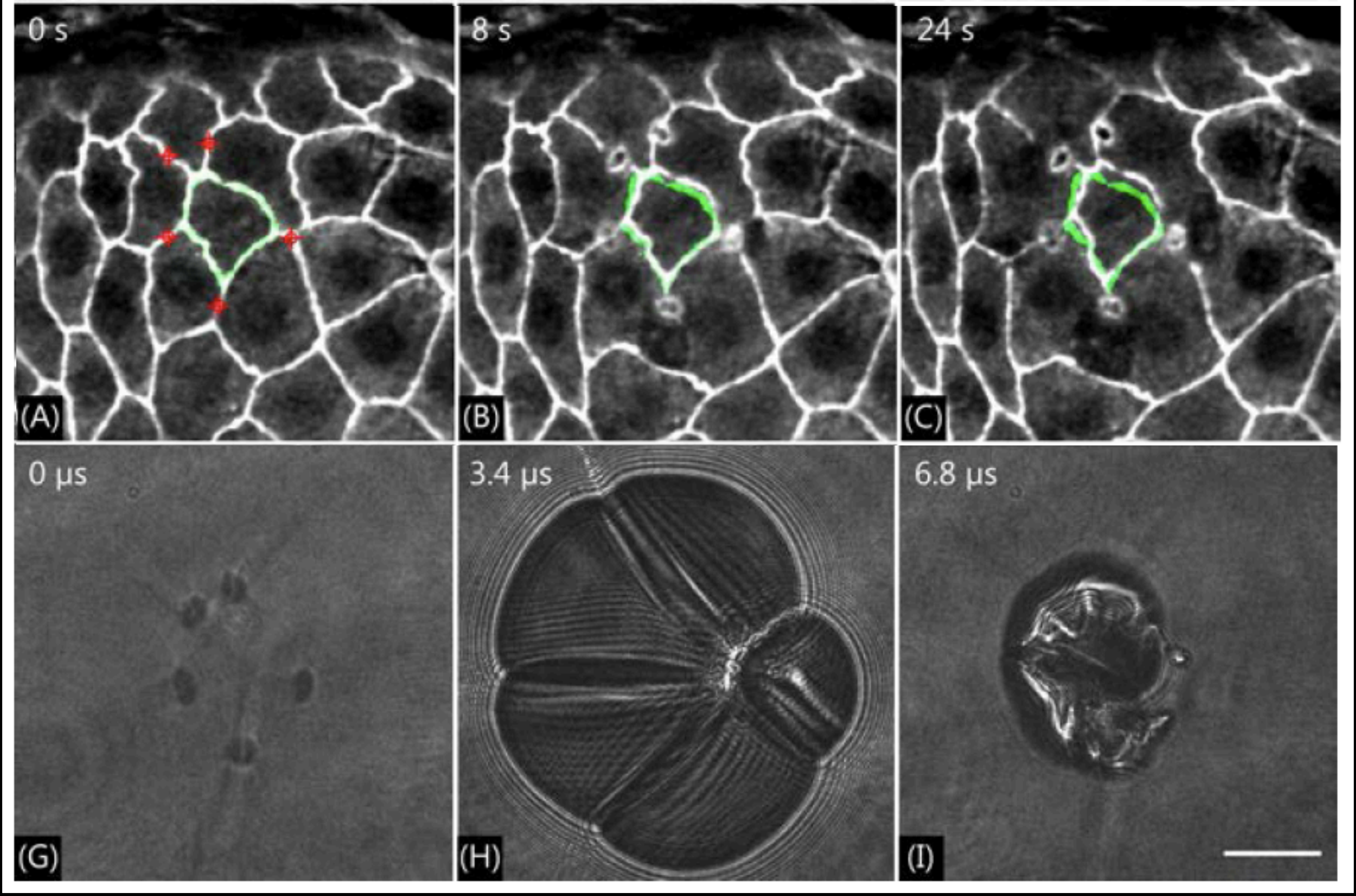


Distance across the outside of the wound

Distance across the isolated cell

Jayasinghe, Rohner and Hutson (2011) *Biomed. Opt. Express* 2: 2590-2598.

A “not yet understood” aspect of laser microsurgery . . .



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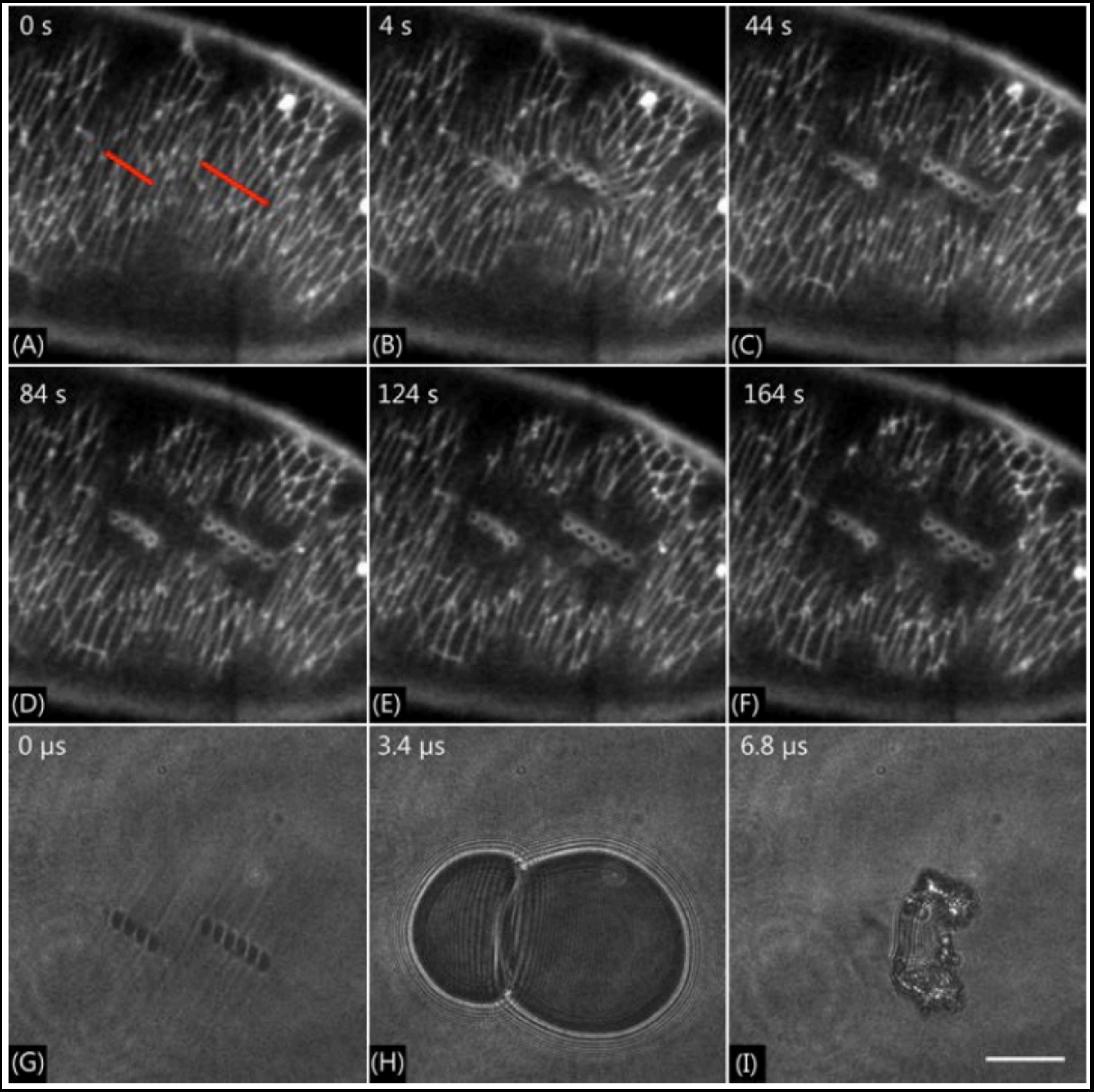
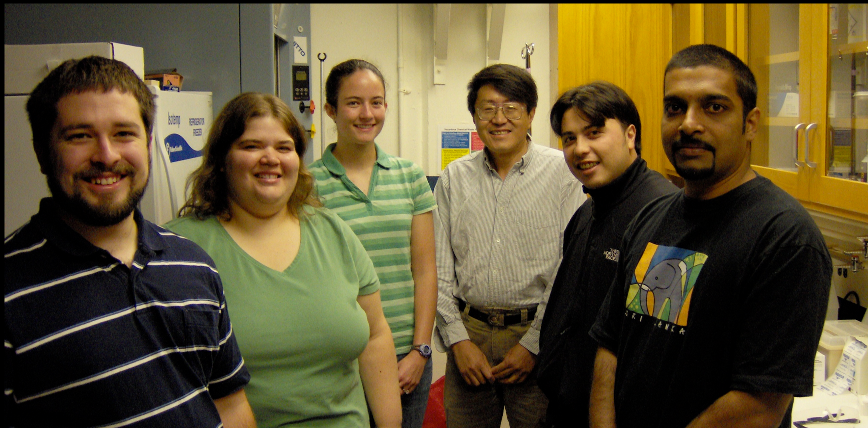


Jayasinghe, Rohner and Hutson (2011) *Biomed. Opt. Express* 2: 2590-2598.

Conclusions III (via cell isolation expts)

1. Cells may carry large mechanical tension WITHOUT being under large strains.
2. Microsurgery in vivo leads to smaller damage regions than the cavitation bubbles would suggest. Why?



David Mashburn
Holley Lynch
Sarah Crews
Ty McCleery
Tomas Yan
Aroshan Jayasinghe
Monica Lacy

DoD Medical FEL Program

Alumni

Jason Rohner
Gilma Adunas
Xiaoyan Ma (PD)
Borislav Ivanov (PD)
Yaowu Xiao (PD)

Undergraduates

Karl Echiverri
Rob Gish
Siri Kadire
Elliott Kim
Peter Scully
Alanna Patsiokas
Paula Angarita - FIU
Trevor Meek - SNU
Mershard Frierson - Fisk
John Kirkham - Rhodes
McRae Linton - Duke
Kevin Parker - Duke
Brett Rosenthal - Duke

Collaborators

G. Wayne Brodland - Waterloo
Antonio Jacinto - IMM-Lisbon
Marco Antunes - IMM-Lisbon
Glenn Edwards - Duke
Dan Kiehart - Duke
Stephanos Venakides - Duke