Modeling microsurgical interventions in morphogenesis

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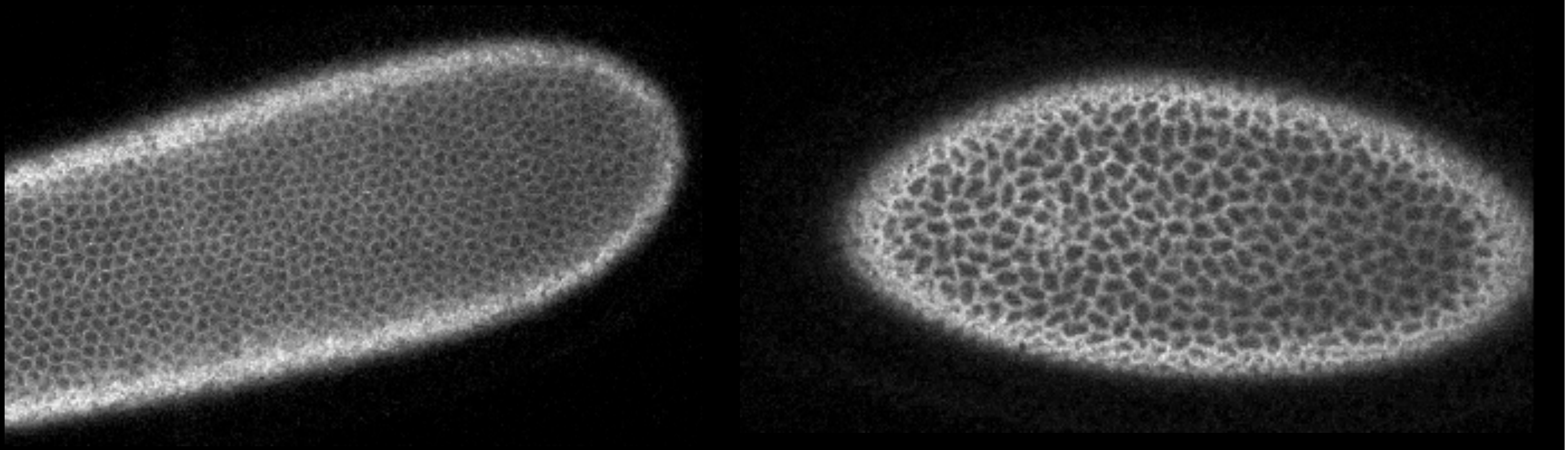
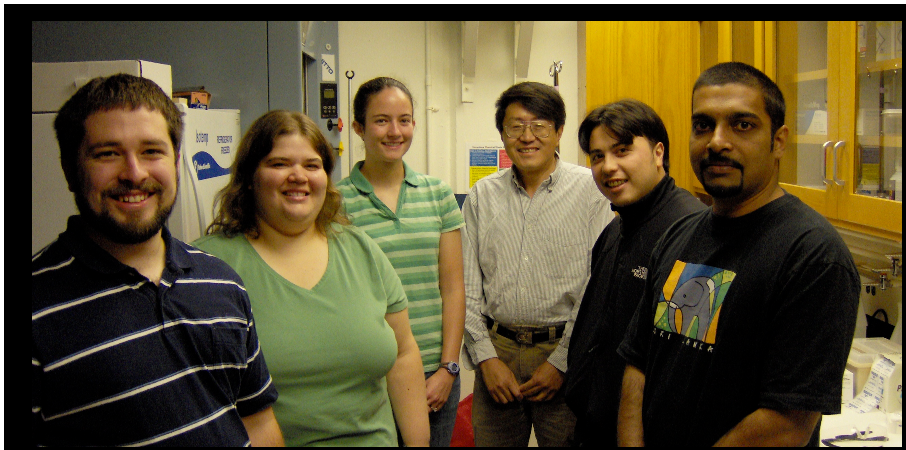


". . . it is critical that we complement the popular molecular and biochemical approaches to the control of morphogenesis with nuts-and-bolts analyses of the physics of how morphogenetic processes occur."

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



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Outline

What does it take to explain a morphogenetic process?
- The biologist’s perspective
- The physicist’s perspective

. . . and never the twain shall meet?

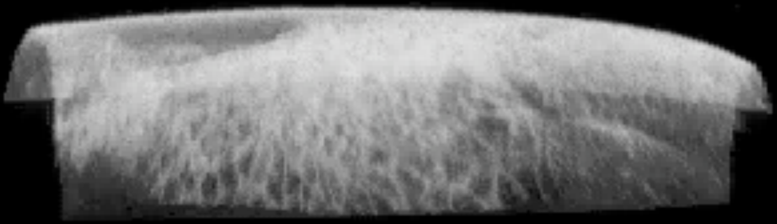
Making <u>quantitative</u> measurements with laser-microsurgery.
- Time- and length-scales of plasma/cavitation dynamics during laser ablation *in vivo*.
- What can we learn from spatial recoil patterns?
- What can we learn from the recoil kinetics (given fast enough time resolution)?

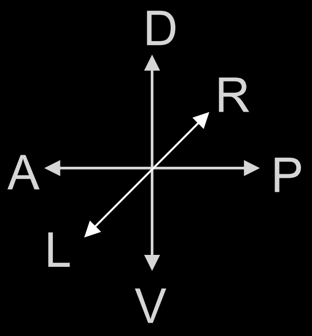
Reproducing the experimental results *in silico*
- Cell-level finite-element modeling
- Finding the appropriate stress distribution ...
- and the appropriate passive viscoelastic response

What does it take to explain a morphogenetic process?

Step #0 for both the biologist and the physicist:

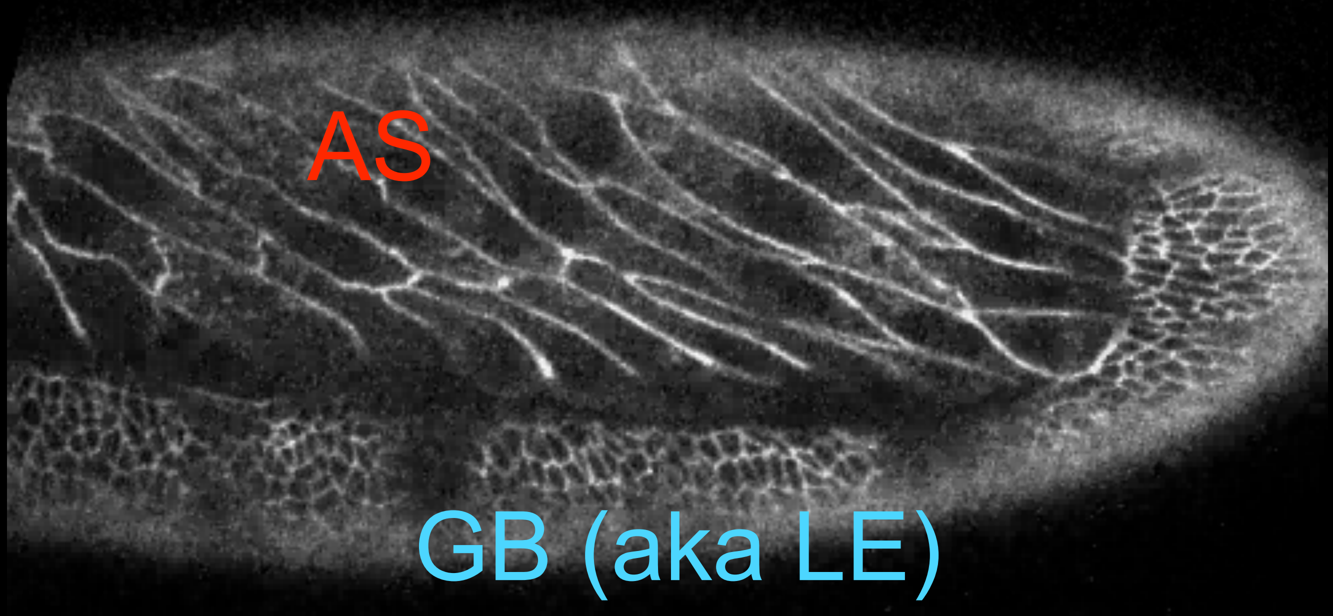
describe the cell and tissue movements - quantitatively and in 3D.



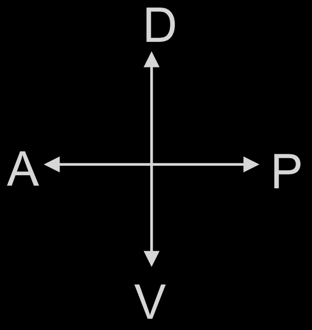


Late Germband Retraction

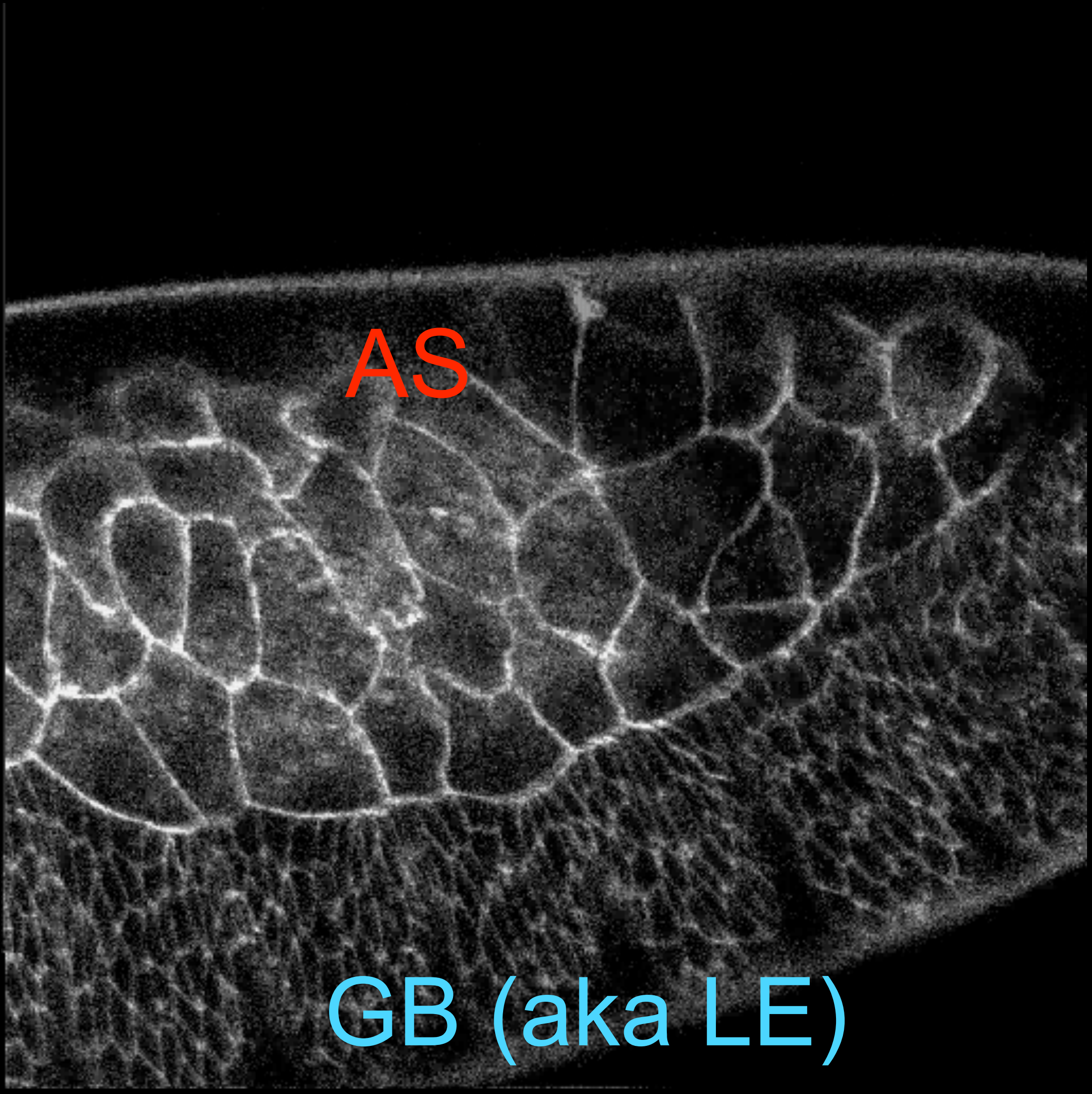
(end of Stage 12)

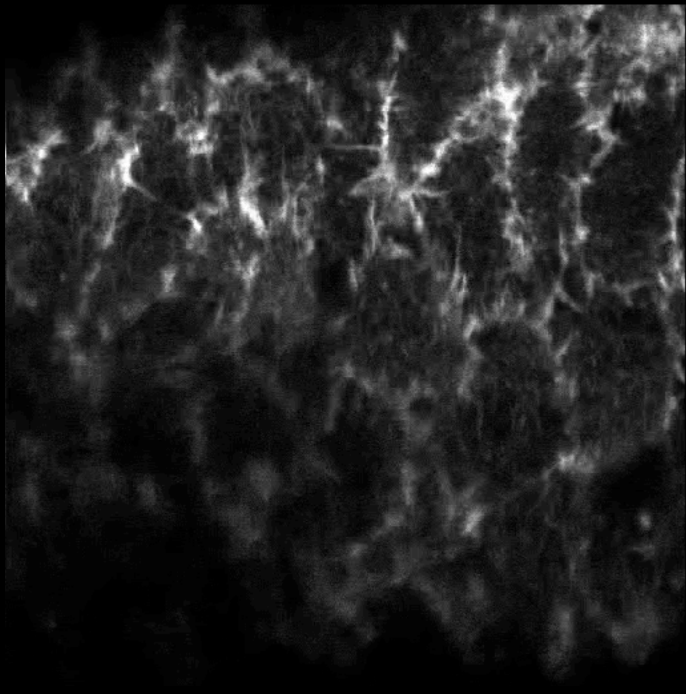


LATERAL
VIEW

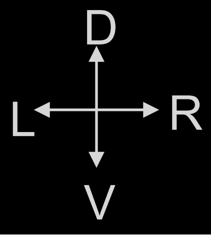


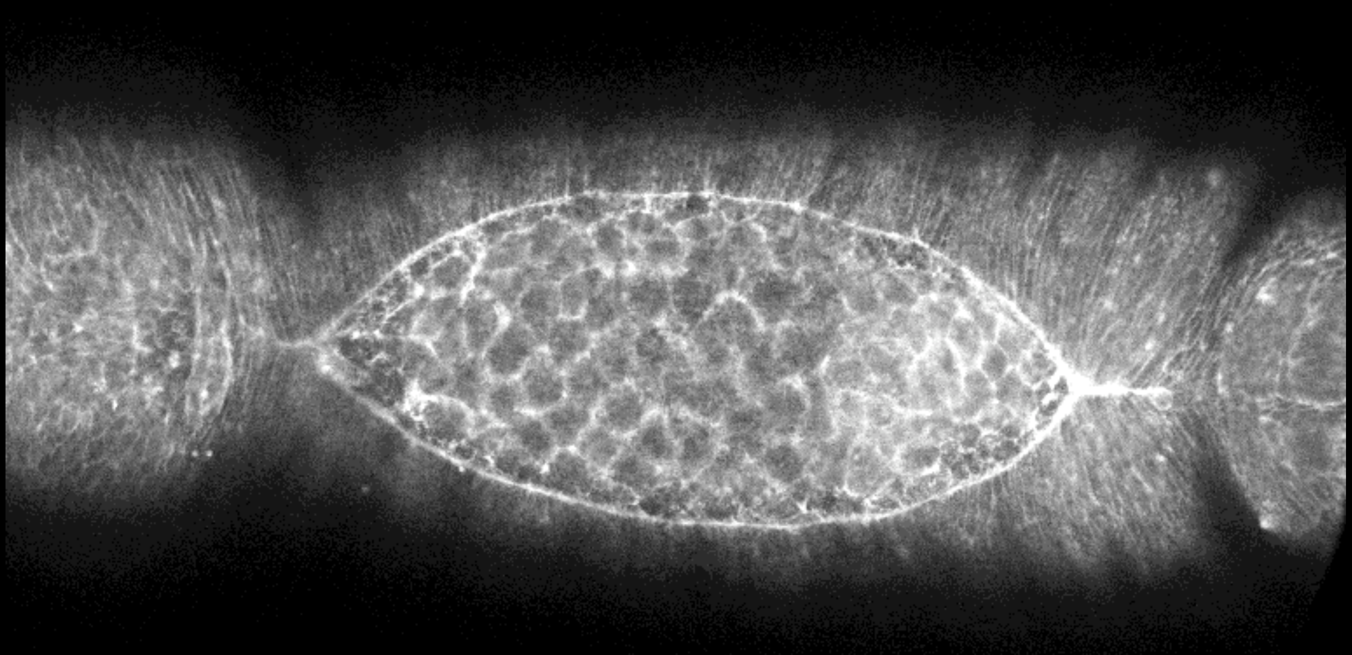
Early in Dorsal Closure

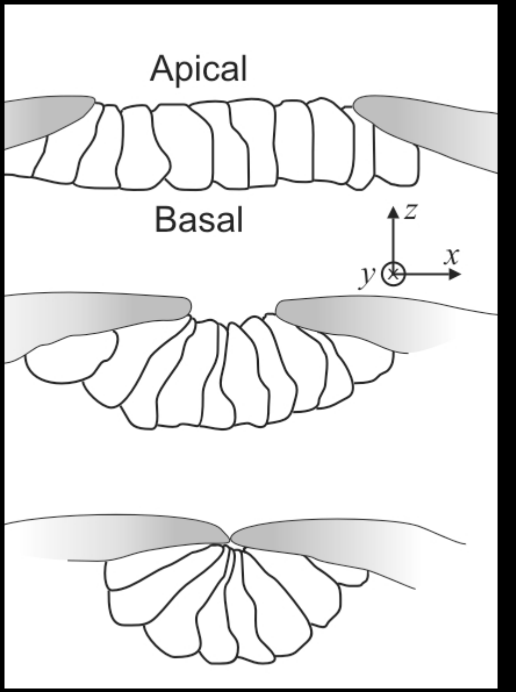




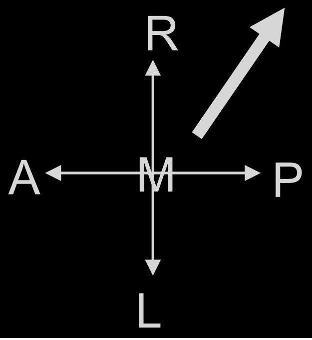
Mid to late Dorsal Closure (Stage 14 to 15)





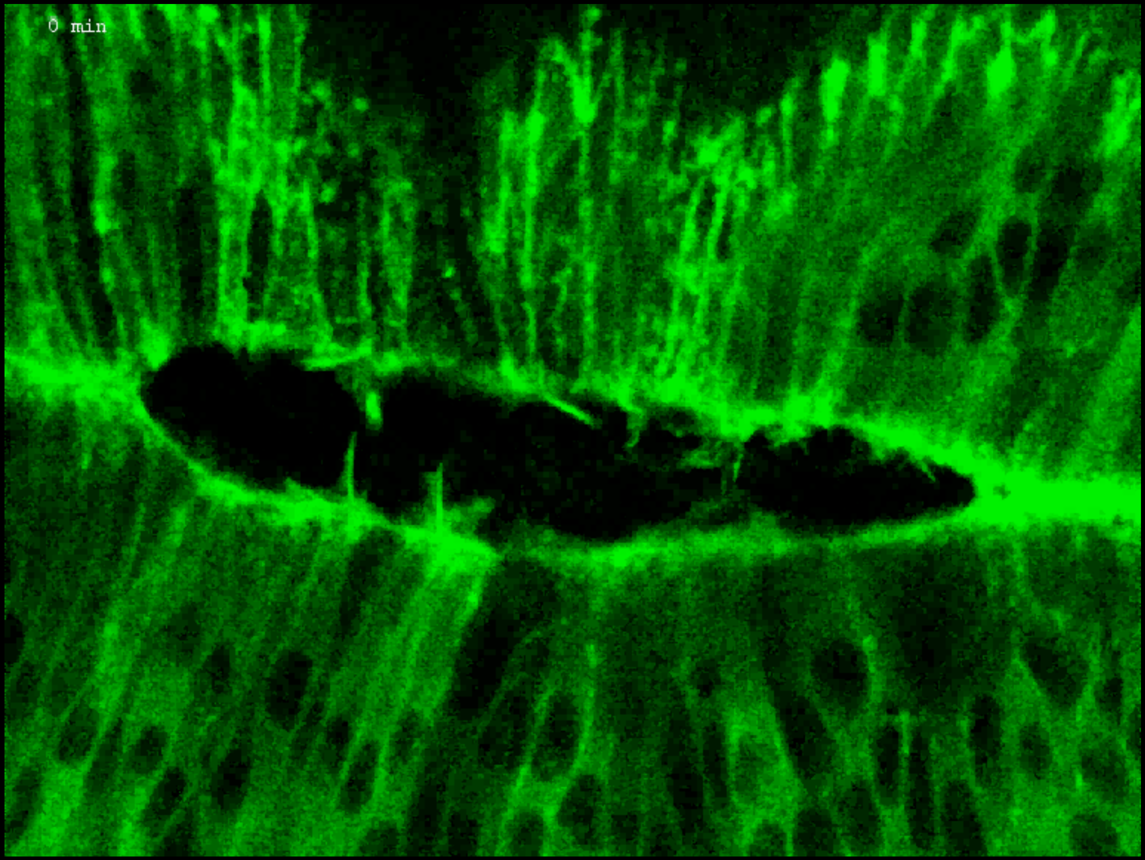


CROSS-SECTIONS
⊥ TO ANTERIO-
POSTERIOR AXIS

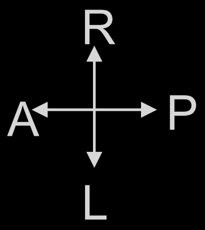


DORSAL VIEW

GFP-actin expressed in LE using Gal4/UAS system

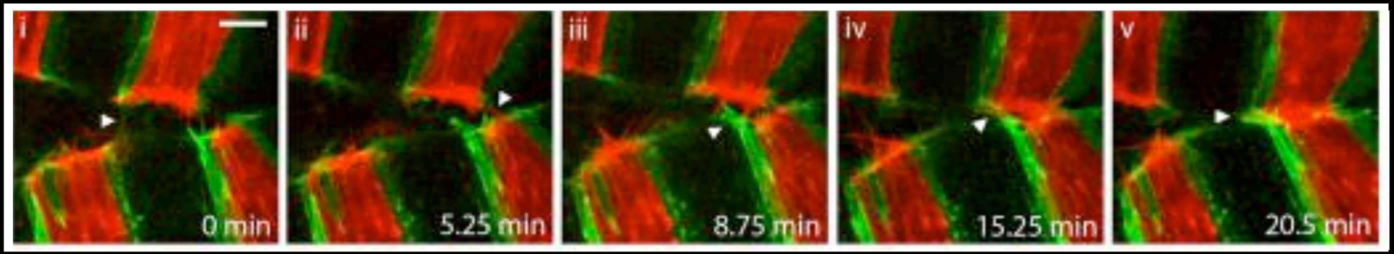


DORSAL VIEW



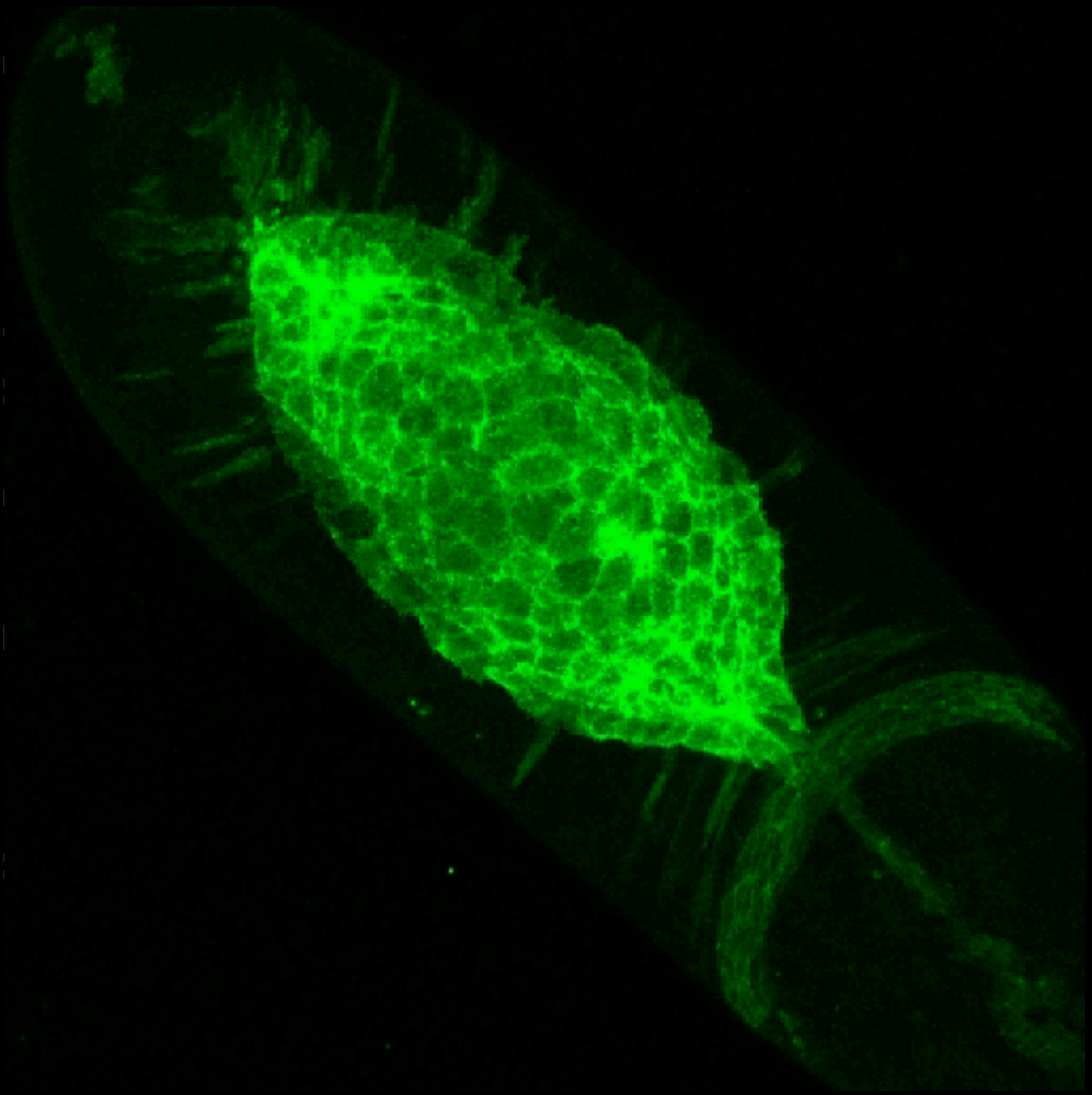
end of Stage 14,
start of Stage 15

courtesy of A. Jacinto and P. Martin



Millard and Martin (2008) Development 135: 621–626.

sGMCA expresssed in AS using Gal4-UAS system

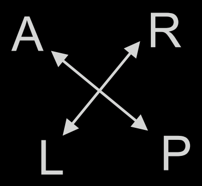


courtesy of J. Weimann and D.P. Keihart

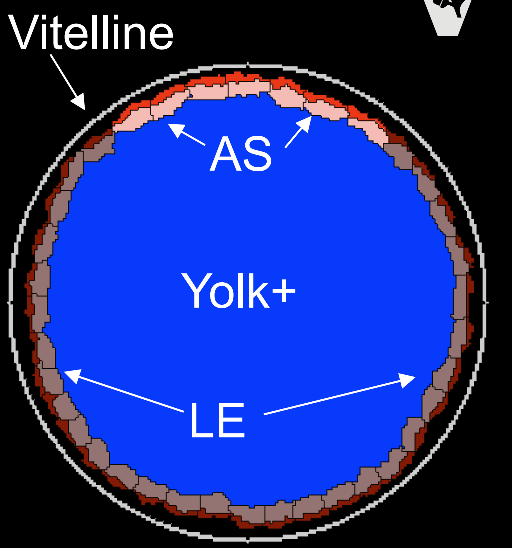
Dorsal Closure

(Stage 14-15)

DORSAL VIEW



Necessary Components and Properties



Cells - LE - Polarization = epithelial, planar cell polarity
Behaviors = adhesion (segment specific)
shape change
purse-string formation (1st row)
filopodia extension (1st row)

AS - Polarization = epithelial
Behaviors = adhesion
shape change - pulsatile, persistent
apoptosis

Yolk+ - Polarization = None
Behaviors = adhesion
volume change?

Solids - Vitelline - Mechanics = elastic (very stiff)
ECM? - Mechanics = viscoelastic (very flexible)

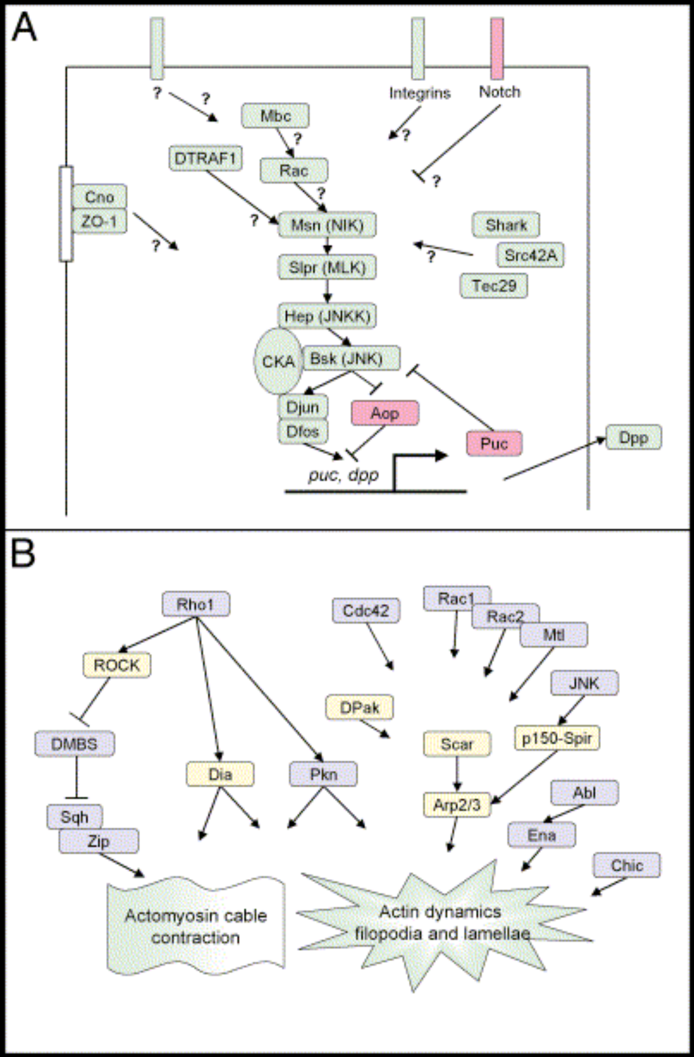
Fluids - Perivitelline - confined (constant volume)

Genes affecting cell motility in dorsal closure (from *The Interactive Fly*)

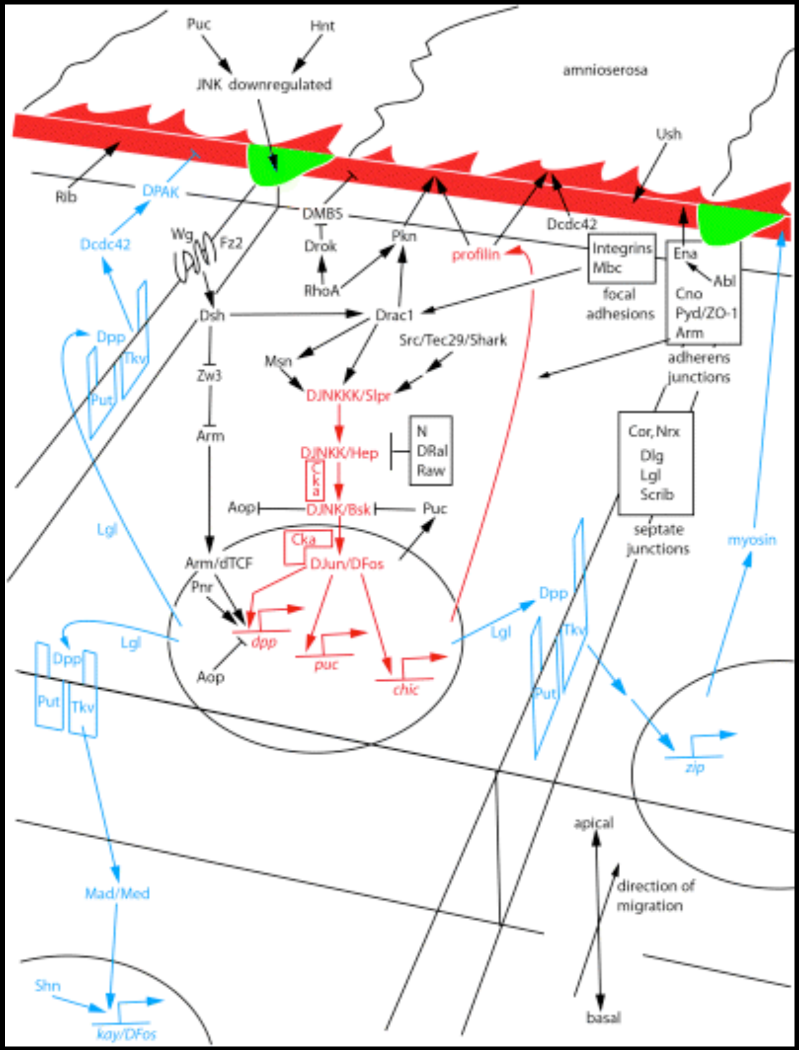
* Anterior open
* basket (also known as JNK)
* Btk family kinase at 29A
* Cdc42
* coracle (a protein 4.1 homolog)
* crossveinless c
* Decapentaplegic
* DJNK (Synonym: Basket)
* dysfusion
* Fps oncogene analog
* Hemipterous
* Jun related antigen
* lethal (2) giant larvae
* misshapen
* myoblast city
* myospheroid (β-integrin)
* Myosin-binding substrate
* neurexin
* PAK-kinase
* peanut
* polychaetoid
* puckered
* Rac1
* ribbon
* scab
* schnurri
* spaghetti squash (regulatory light chain of nonmuscle Myosin II/Zipper)
* Src homology 2, ankyrin repeat, tyrosine kinase
* slipper
* spire
* Src oncogene at 42A
* Tec29
* TGF-ß activated kinase 1
* Transforming growth factor beta at 60A
* trio
* zipper (also known as: Myosin II)

Biologist Step #1: figure out the molecular parts list!

Biologist Step #2: figure out how the parts are connected, i.e. the relevant pathways

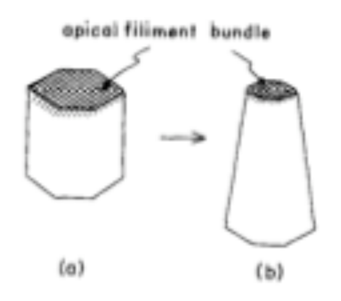


A. Jacinto et al (2002) *Developmental Cell* 3: 9-19.



N. Harden (2002) *Differentiation* 70: 181-203.

Physicist Step #1: build (code) a model



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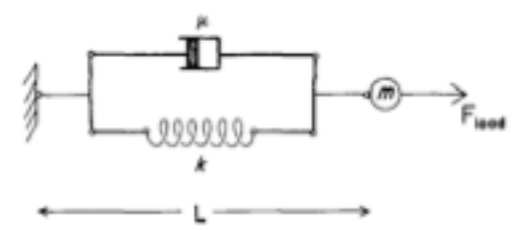
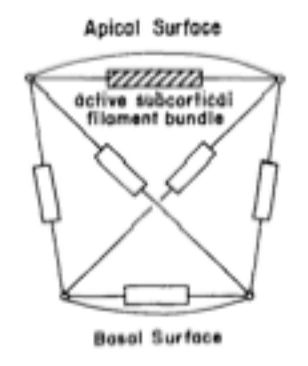
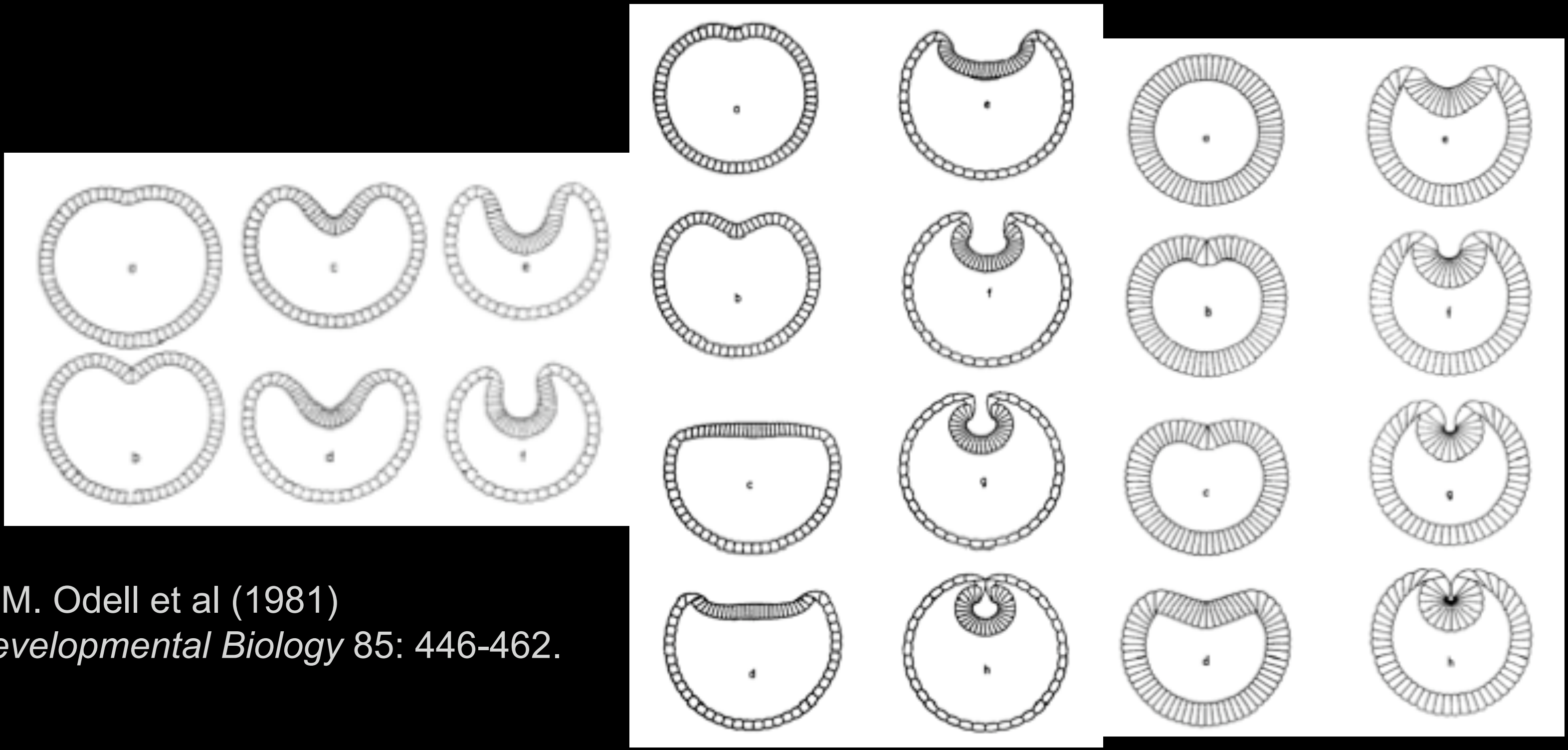


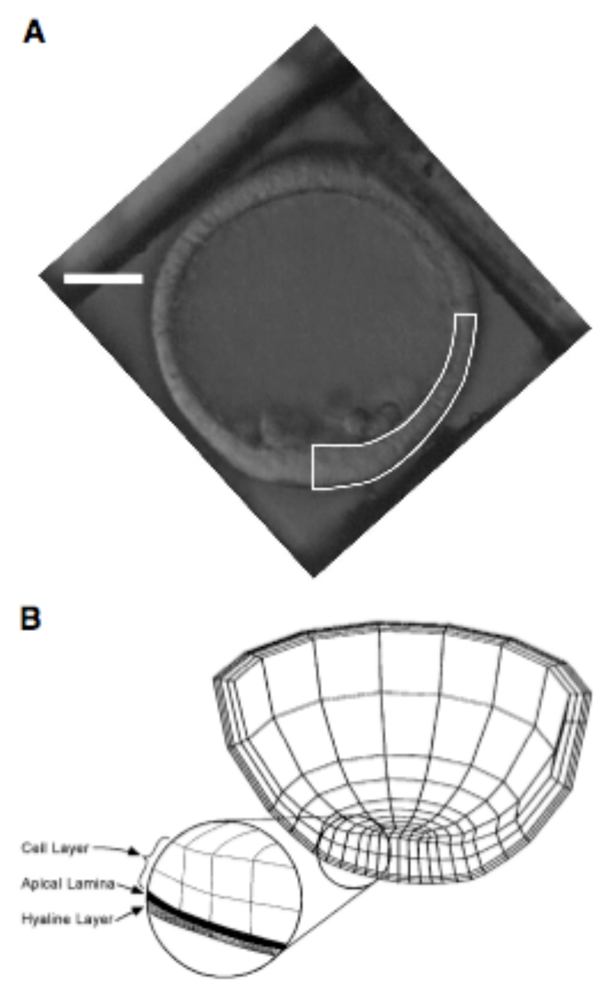


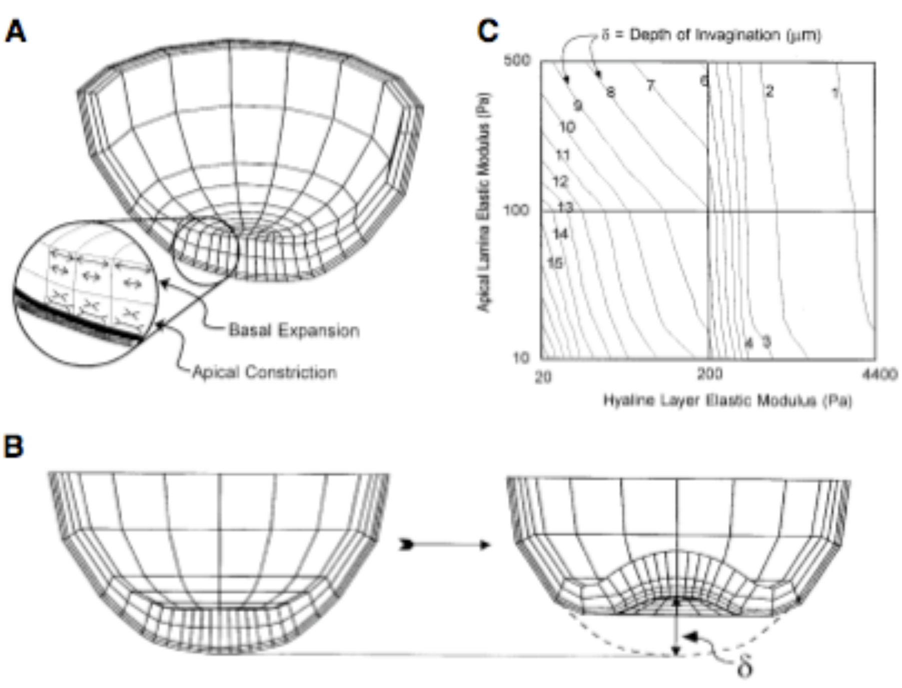


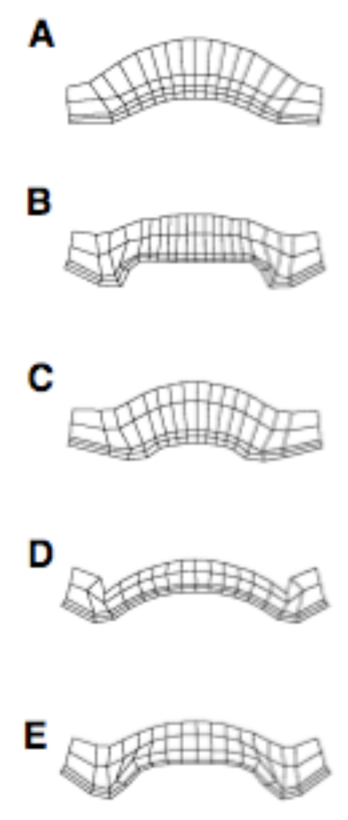
G.M. Odell et al (1981)
Developmental Biology 85: 446-462.

. . . and another one . . .

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

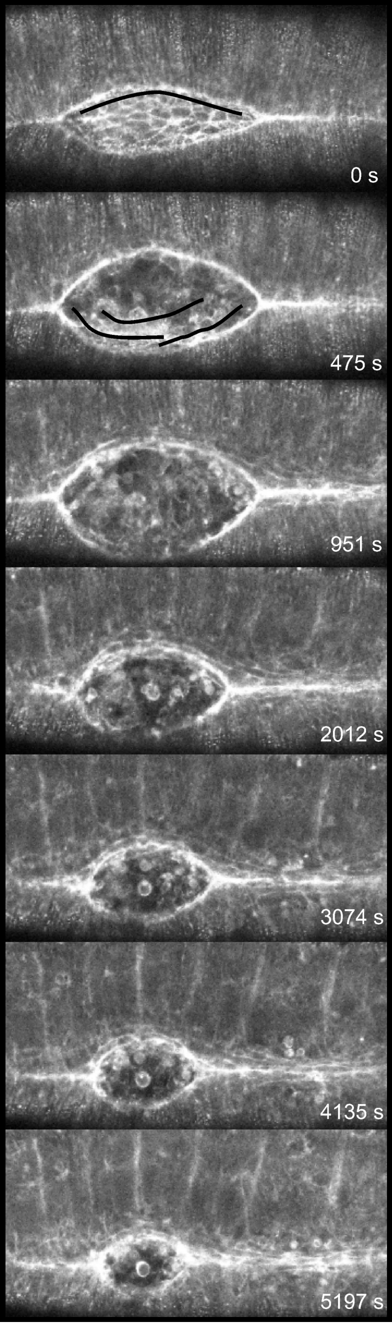




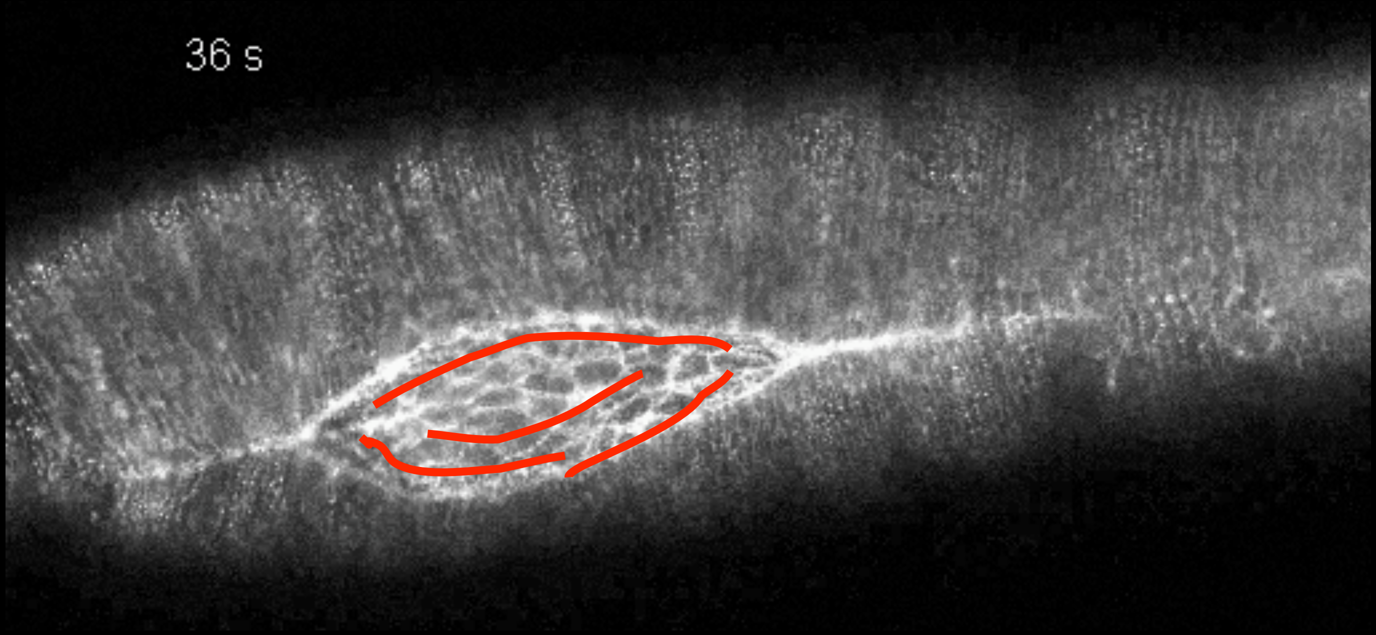


Physicist Step #2: ask biologists to test the model(s)!

. . . and listen for the
deafening roar (or silence).



Challenging hypotheses/models with laser-microsurgery - qualitative

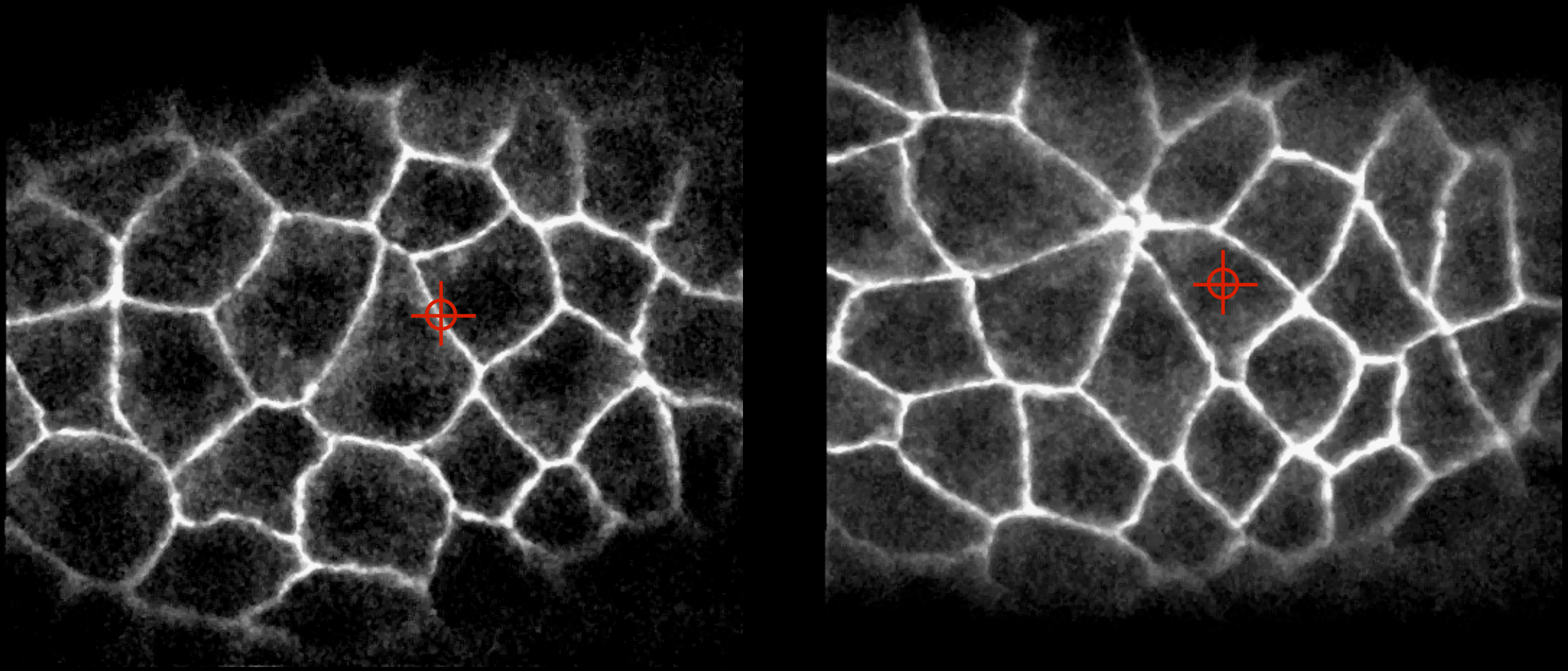


Hutson, Tokutake, Chang, Bloor, Venakides, Kiehart and Edwards (2003) *Science* 300: 145-149.

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

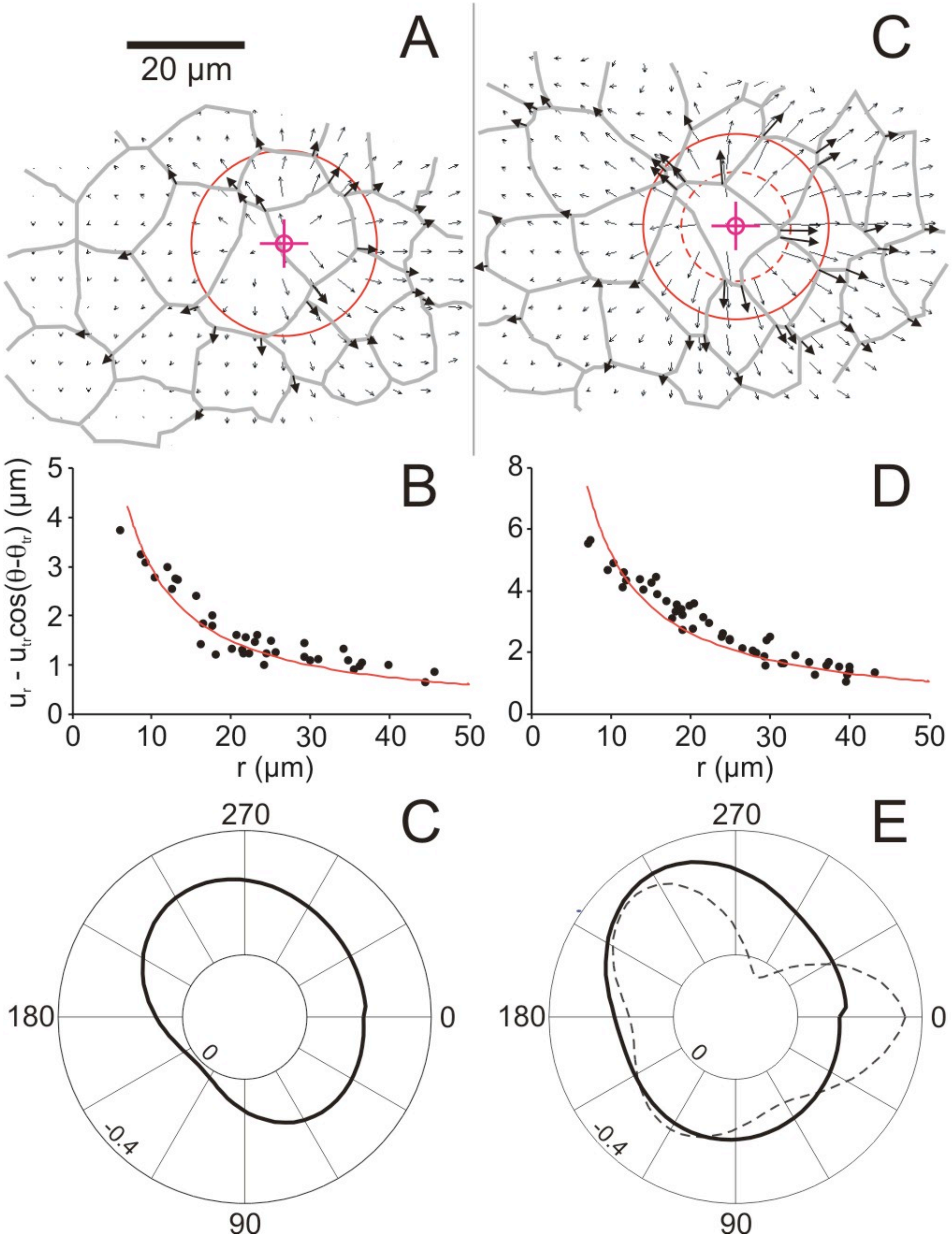
Can we measure the spatiotemporal distribution of mechanical stress in an embryo?

Is an epithelium more like a continuous sheet or a 2D cellular foam ?



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

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



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

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

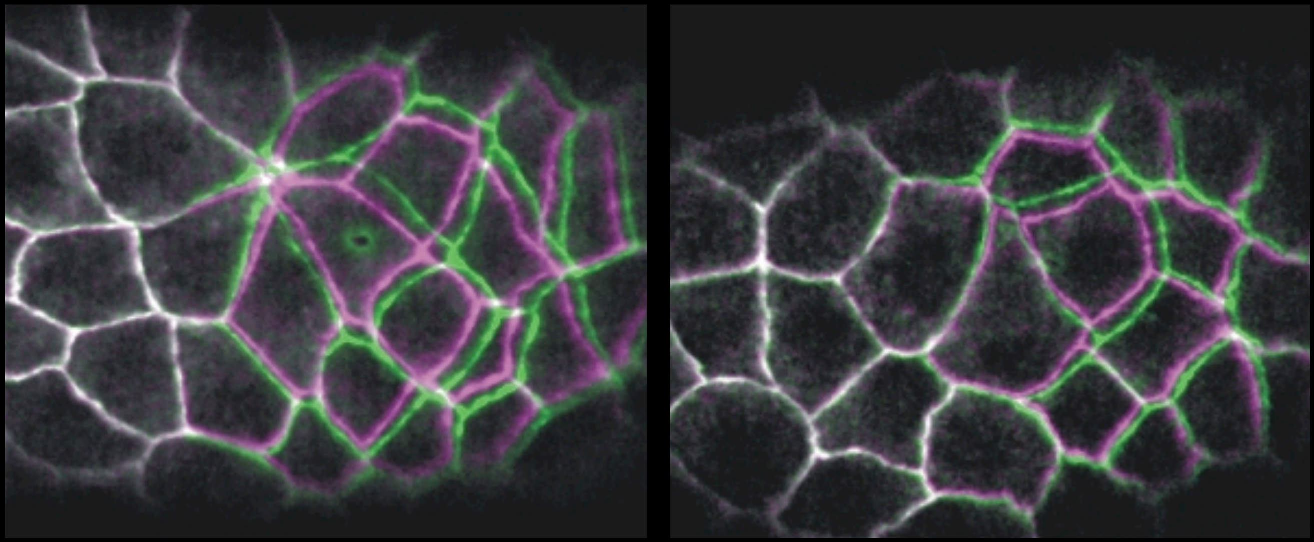
$$u_\theta(r,\theta) = -B_3(r)(\sigma_x - \sigma_y)\sin 2\theta$$
$$+u_{tr}\sin(\theta - \theta_{tr})$$

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

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

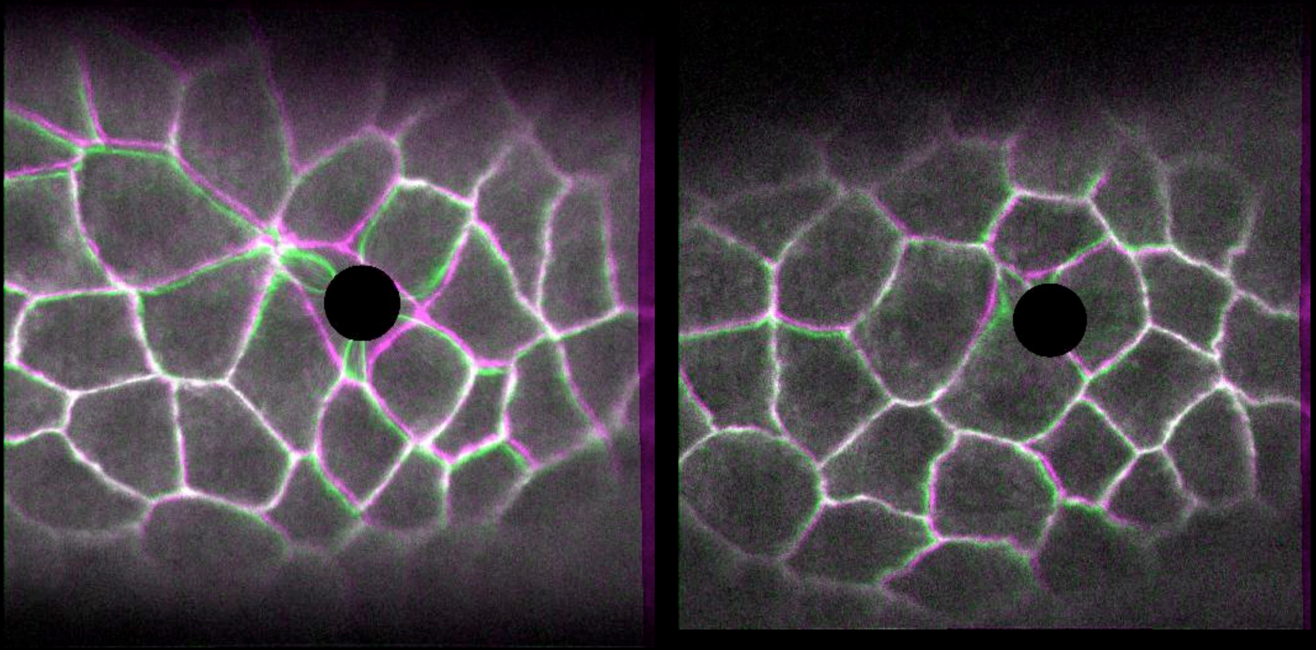
$$B_3(r) = \frac{1+\nu}{2E}\left[2\frac{1-\nu}{1+\nu}\frac{R_0^2}{r} + \frac{R_0^4}{r^3}\right]$$

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



Magenta - pre-ablation stressed state

Green - post-ablation strain-relaxed state



Magenta - pre-ablation stressed state

Green - computationally re-strained post-ablation state

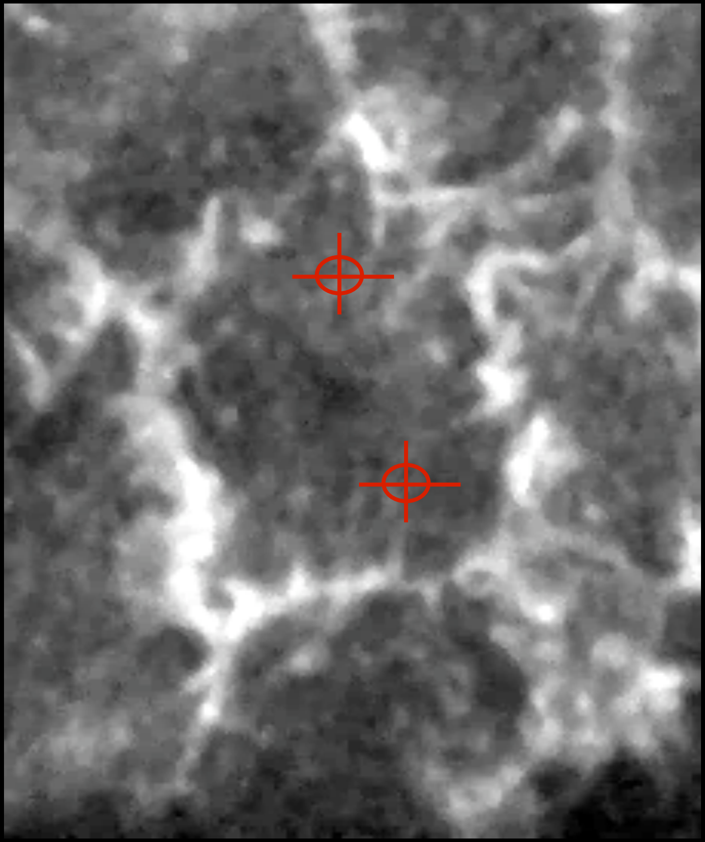
Parameters from Re-straining

Assume r_0=30 pixels, ν = 0.33

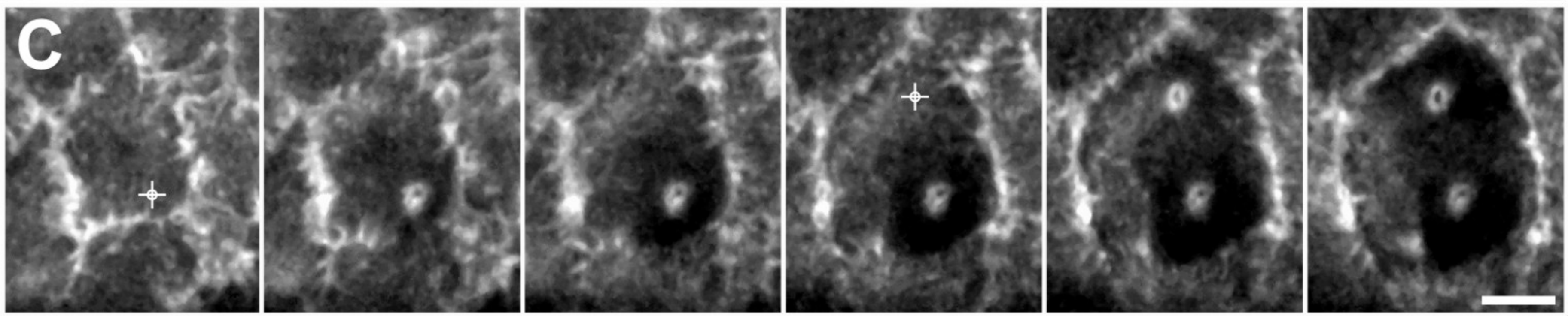
	Edge Wound	Cell-center Wound
Pre-ablation average strain:	0.8	1.6
Post-ablation c-of-mass translation:	5.6 µm @ 342°	7.6 µm @ 332°
Pre-ablation stress anisotropy:	0.01	0.02
Principle stress direction:	75°	55°

So what cellular structures carry the in-plane tension?

Double wounds in a GFP-moesin embryo



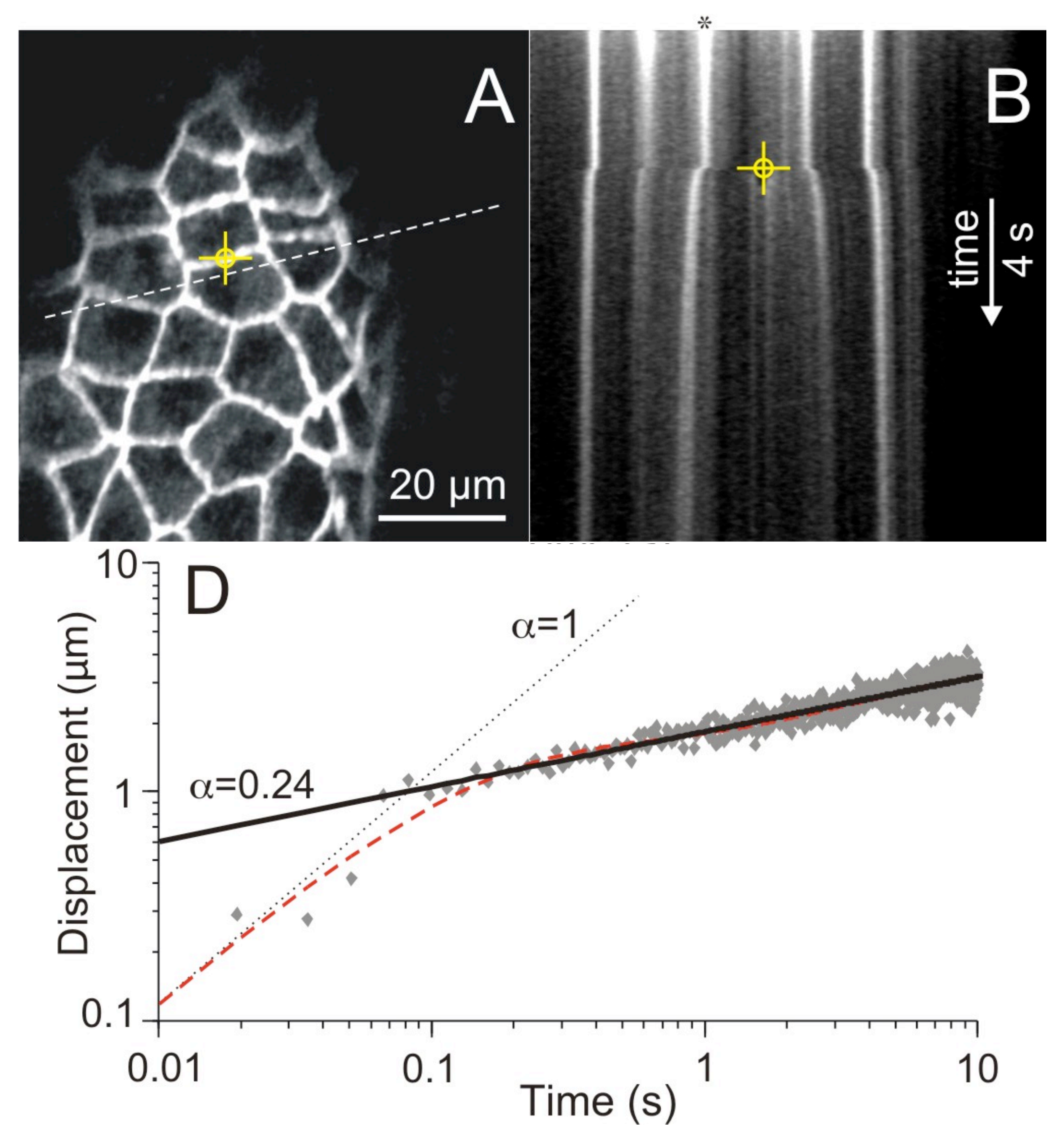
Ma, Lynch, Scully and Hutson (2008) *Physical Biology* 6: 036004



Conclusions I (via spatial recoil patterns)

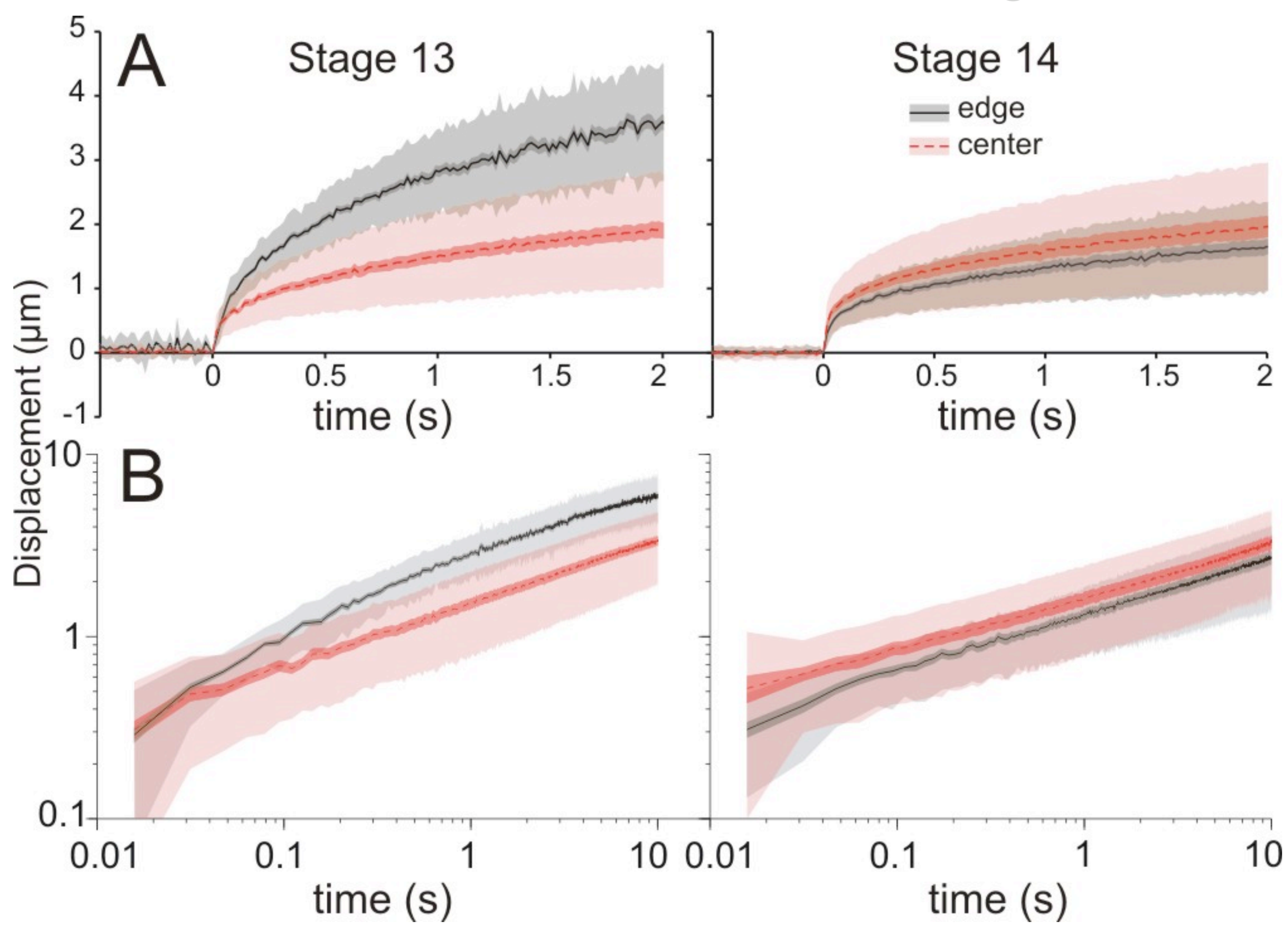
1. The spatial recoil patterns primarily resemble what you'd expect for a hole in a homogeneous thin sheet - much more so than what you'd expect for a 2D foam.
2. The arrangement of cell edges has a limited secondary impact.
3. The in-plane stress in each cell appears to be carried by its apical actin network.

But cells are viscoelastic. We need to look closely at the recoil kinetics.



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

Recoils at different sites and stages

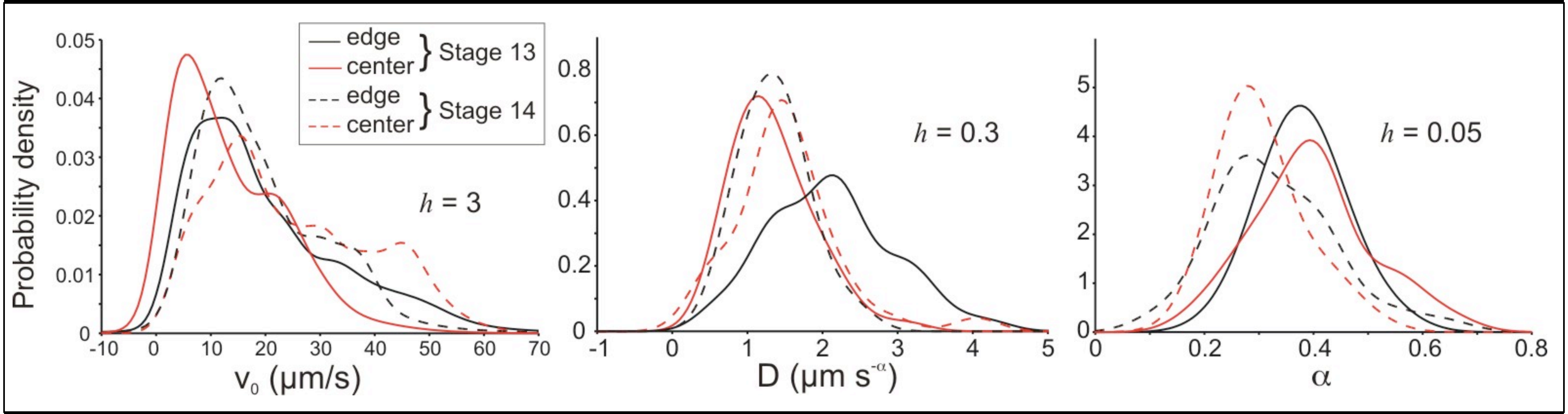


Ma, Lynch, Scully and Hutson (2008) *Physical Biology* 6: 036004 stress

$$\frac{-v_0}{r_0} \approx \gamma_0 = \frac{\sigma}{\eta} \qquad \frac{-u(t)}{r_0} = \frac{-D}{r_0} t^{\alpha} \approx \varepsilon(t) = \frac{\sigma}{G'_0} t^{\alpha}$$

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 II (via recoil kinetics)

1. Biphasic recoil kinetics are consistent with a soft glassy material that transitions to a Newtonian fluid at high-frequency (short times)

stress

$$\frac{-v_0}{r_0} \approx \gamma_0 = \frac{\sigma}{\eta} \qquad \frac{-u(t)}{r_0} = \frac{-B}{r_0} t^{\alpha} \approx \varepsilon(t) = \frac{\sigma}{G'_0} t^{\alpha}$$

limiting Newtonian viscosity

stiffness

2. Stress concentration (1.6-fold) on cell edges in Stage 13; none in Stage 14
3. α decreases from Stage 13 to 14 ⟶ tissue becomes more solid-like
4. Stage-dependences of other parameters imply coupled constraints. These exclude 5 of 7 published models for apical constriction.

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

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

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

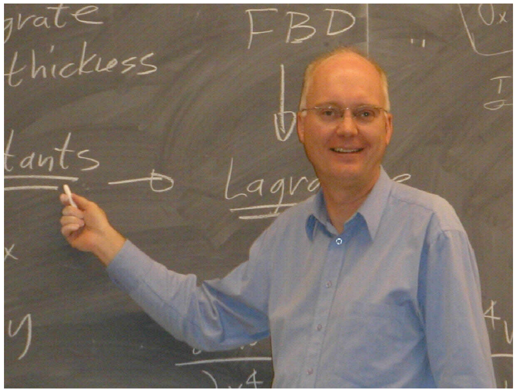
Example scenario - constant η implies
stiffness G' increases 1.6x
stress σ_E increases 1.3x
stress σ_C increases 2.7x

Can we reproduce our experimental observations *in silico*?

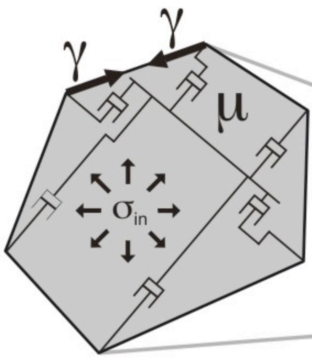
$\mathbf{C}\,\dot{\mathbf{u}} = \mathbf{f}$ Augmented with constraints:

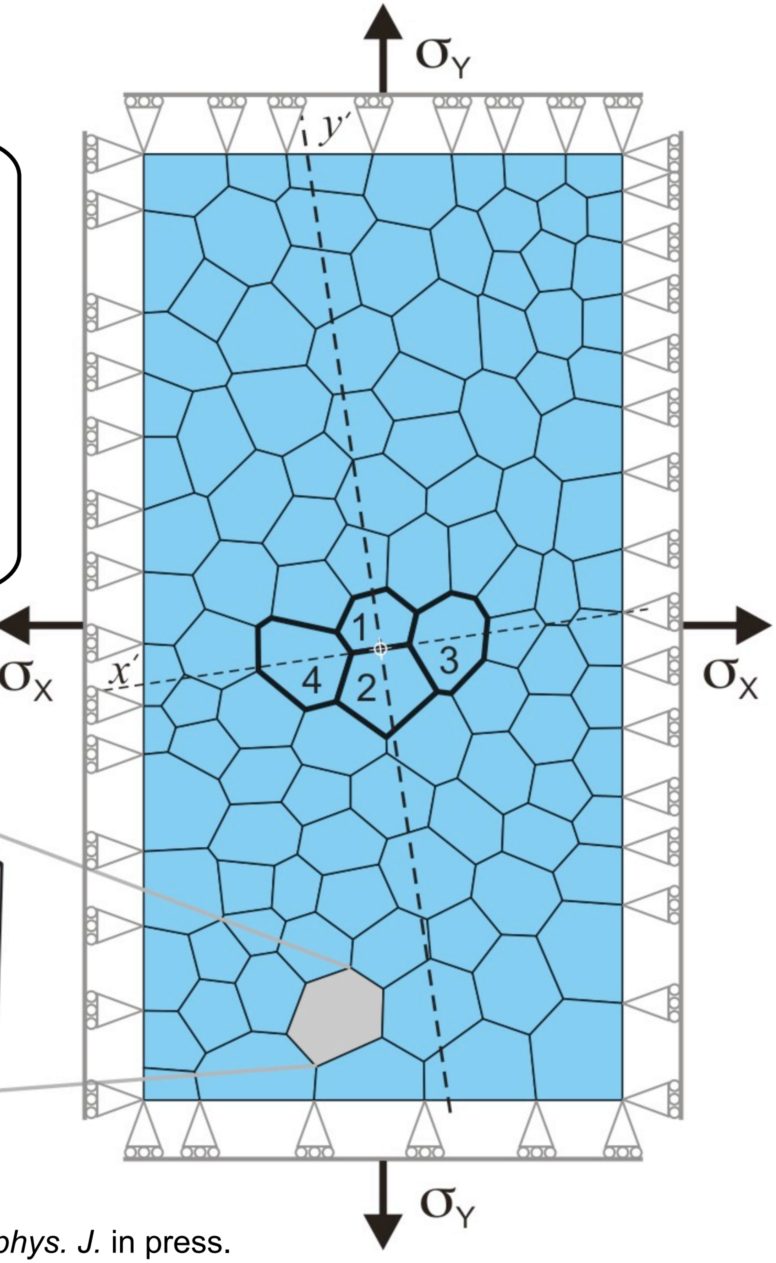
$$\left(\begin{array}{c:c} \begin{matrix} C_{ij}(\mu) \\ \text{~1/6 non-zero} \end{matrix} & \dfrac{\partial V_k}{\partial u_i} \\ \hdashline -\dfrac{\partial V_k}{\partial u_i} & 0 \end{array}\right) \left(\begin{array}{c} \dfrac{\Delta u_i}{\Delta t} \\ \hdashline \sigma_{in,k} \end{array}\right) = \left(\begin{array}{c} f_i(\gamma, R) \\ \hdashline \dfrac{\Delta V_k}{\Delta t}\ (=0) \end{array}\right)$$

Solved iteratively for discrete time steps



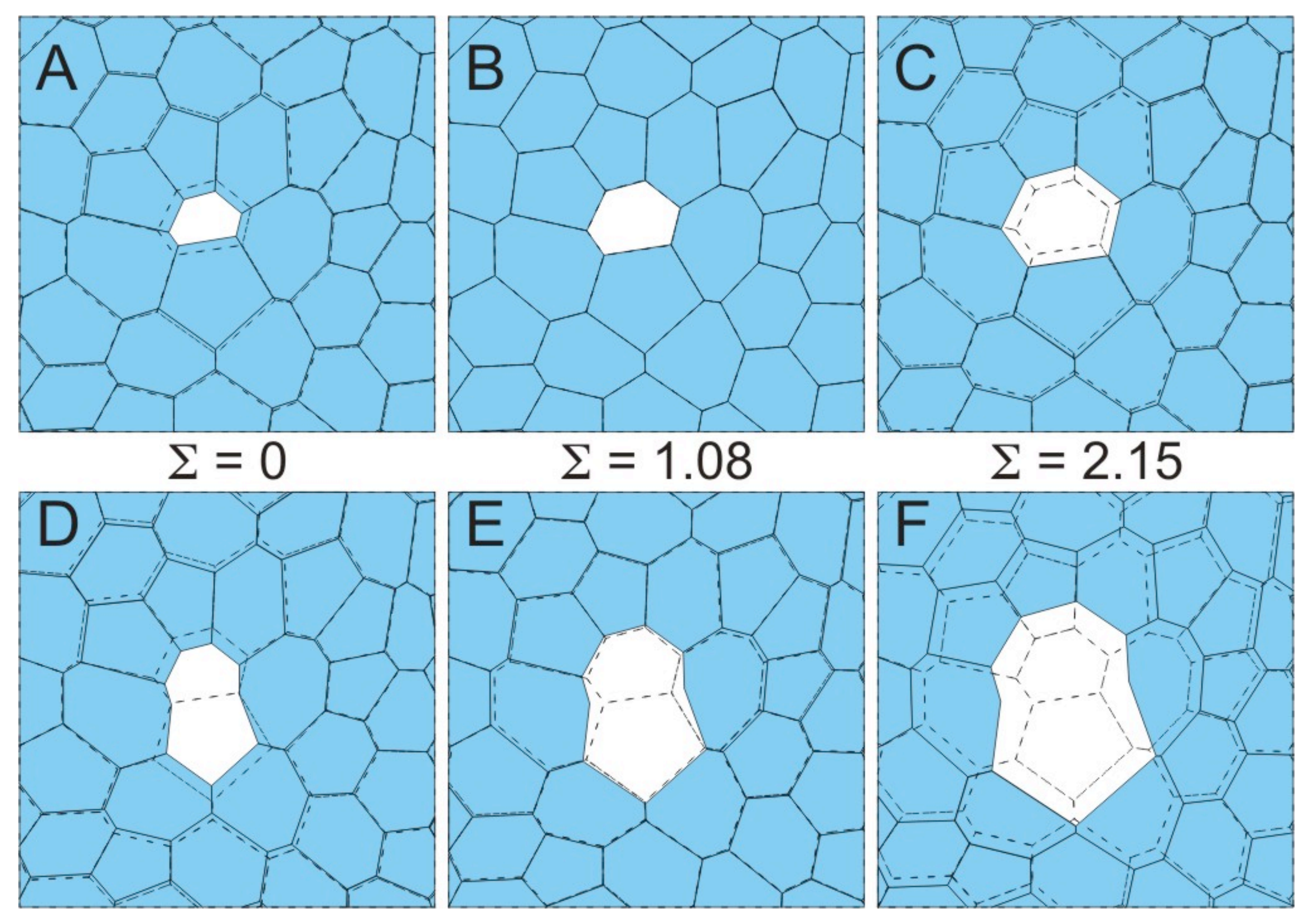
Wayne Brodland, U. Waterloo





Hutson, Veldhuis, Ma, Lynch, Cranston and Brodland (2009) *Biophys. J.* in press.

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

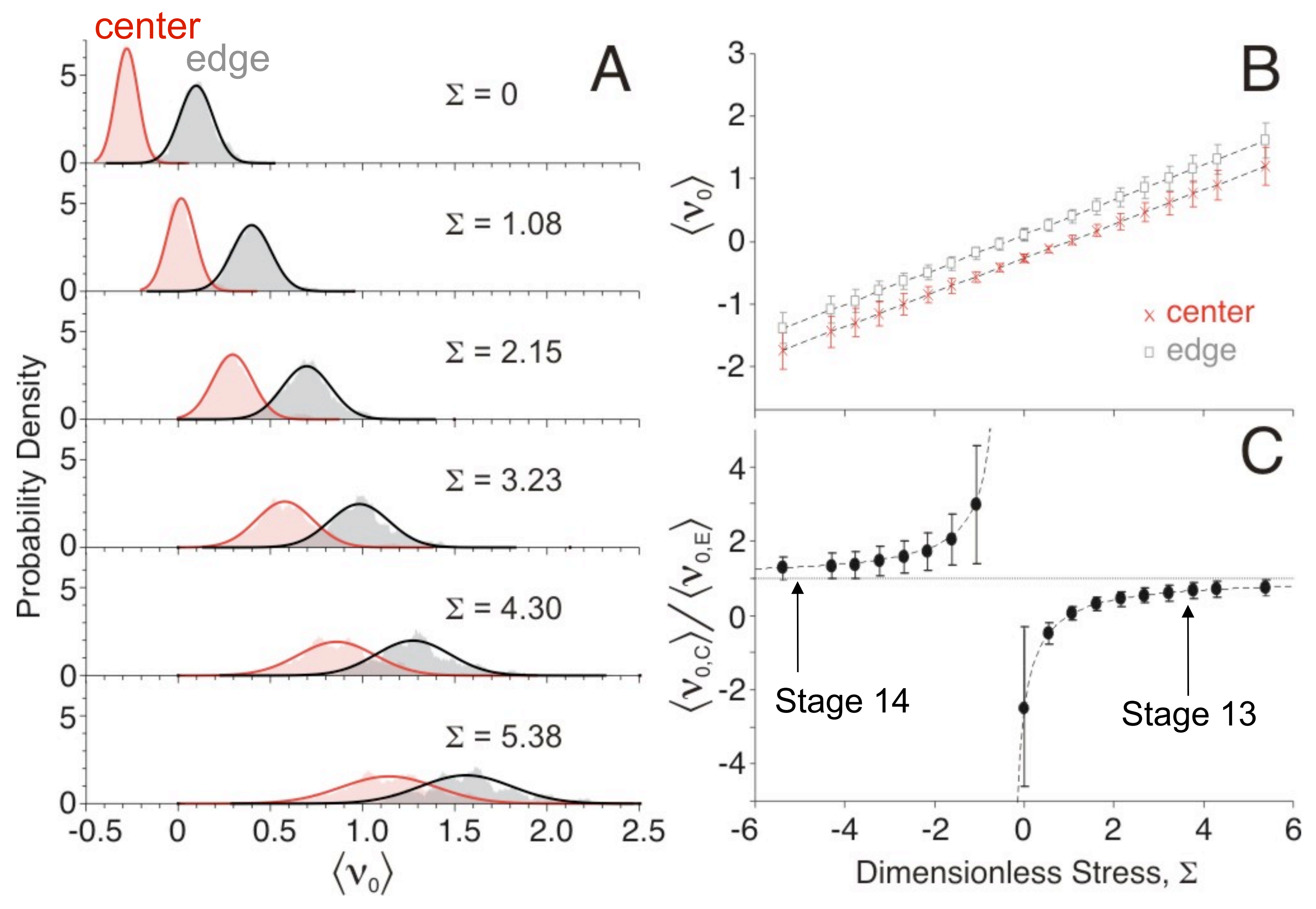


Cell center wound =
lose that cell's
volume
constraint

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

Hutson, Veldhuis, Ma, Lynch, Cranston and Brodland (2009) *Biophys. J.* in press.

2. $\langle v_{0,C} \rangle$ is the same or slightly less (~30%) than $\langle v_{0,E} \rangle$



Hutson, Veldhuis, Ma, Lynch, Cranston and Brodland (2009) *Biophys. J.* in press.

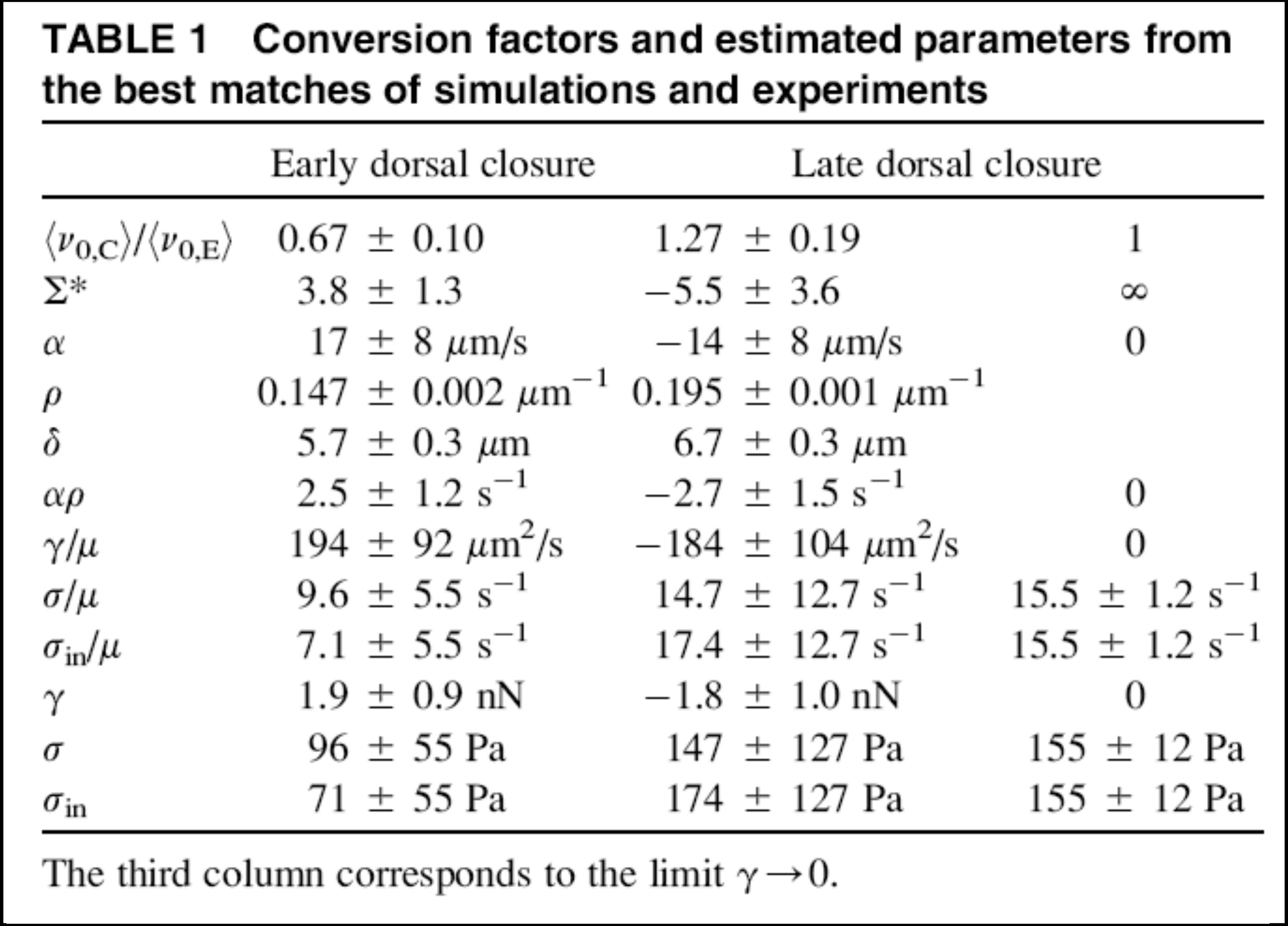
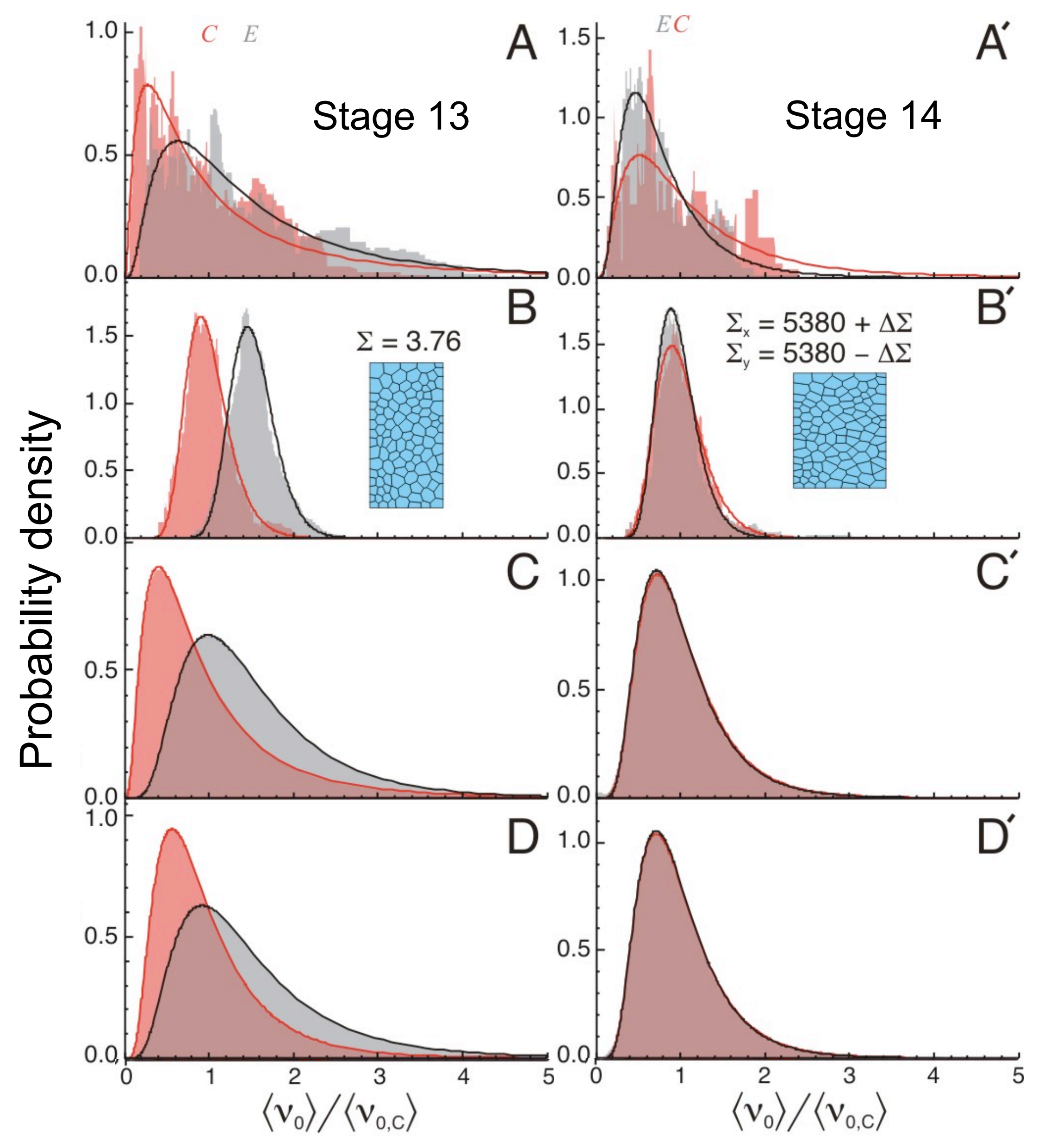


TABLE 1 Conversion factors and estimated parameters from the best matches of simulations and experiments

	Early dorsal closure	Late dorsal closure	
$\langle v_{0,\mathrm{C}} \rangle / \langle v_{0,\mathrm{E}} \rangle$	0.67 ± 0.10	1.27 ± 0.19	1
Σ^*	3.8 ± 1.3	-5.5 ± 3.6	∞
α	$17 \pm 8\ \mu\mathrm{m/s}$	$-14 \pm 8\ \mu\mathrm{m/s}$	0
ρ	$0.147 \pm 0.002\ \mu\mathrm{m}^{-1}$	$0.195 \pm 0.001\ \mu\mathrm{m}^{-1}$	
δ	$5.7 \pm 0.3\ \mu\mathrm{m}$	$6.7 \pm 0.3\ \mu\mathrm{m}$	
$\alpha\rho$	$2.5 \pm 1.2\ \mathrm{s}^{-1}$	$-2.7 \pm 1.5\ \mathrm{s}^{-1}$	0
γ/μ	$194 \pm 92\ \mu\mathrm{m}^2/\mathrm{s}$	$-184 \pm 104\ \mu\mathrm{m}^2/\mathrm{s}$	0
σ/μ	$9.6 \pm 5.5\ \mathrm{s}^{-1}$	$14.7 \pm 12.7\ \mathrm{s}^{-1}$	$15.5 \pm 1.2\ \mathrm{s}^{-1}$
σ_{in}/μ	$7.1 \pm 5.5\ \mathrm{s}^{-1}$	$17.4 \pm 12.7\ \mathrm{s}^{-1}$	$15.5 \pm 1.2\ \mathrm{s}^{-1}$
γ	$1.9 \pm 0.9\ \mathrm{nN}$	$-1.8 \pm 1.0\ \mathrm{nN}$	0
σ	$96 \pm 55\ \mathrm{Pa}$	$147 \pm 127\ \mathrm{Pa}$	$155 \pm 12\ \mathrm{Pa}$
σ_{in}	$71 \pm 55\ \mathrm{Pa}$	$174 \pm 127\ \mathrm{Pa}$	$155 \pm 12\ \mathrm{Pa}$

The third column corresponds to the limit $\gamma \rightarrow 0$.

3. The v_0-distributions are wide and lognormal.



expt

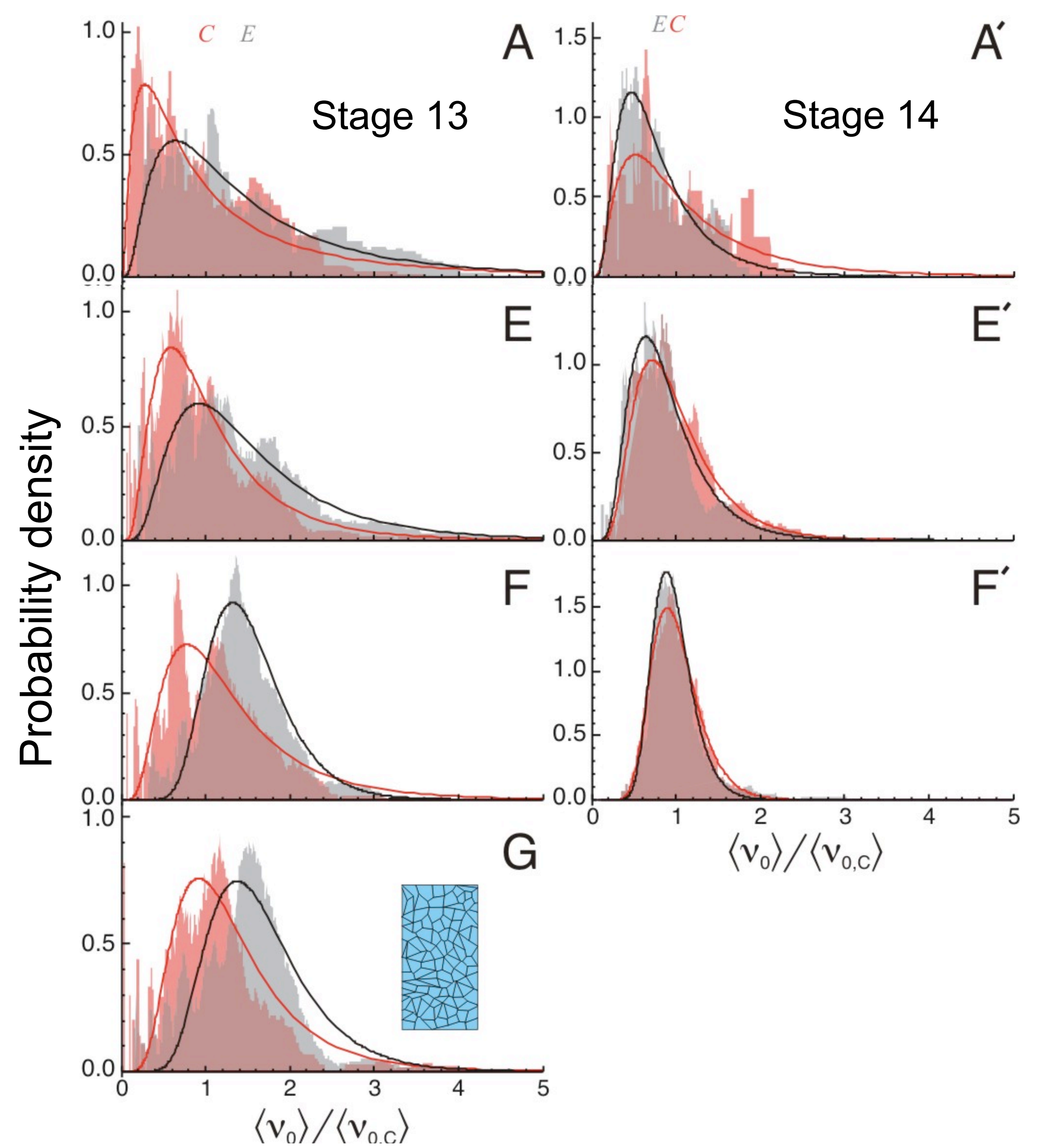
everything uniform

variable Σ (inter-embryo)

variable η (inter-embryo)

Hutson, Veldhuis, Ma, Lynch, Cranston and Brodland (2009) *Biophys. J.* in press.

3. The v_0-distributions are wide and lognormal.



expt

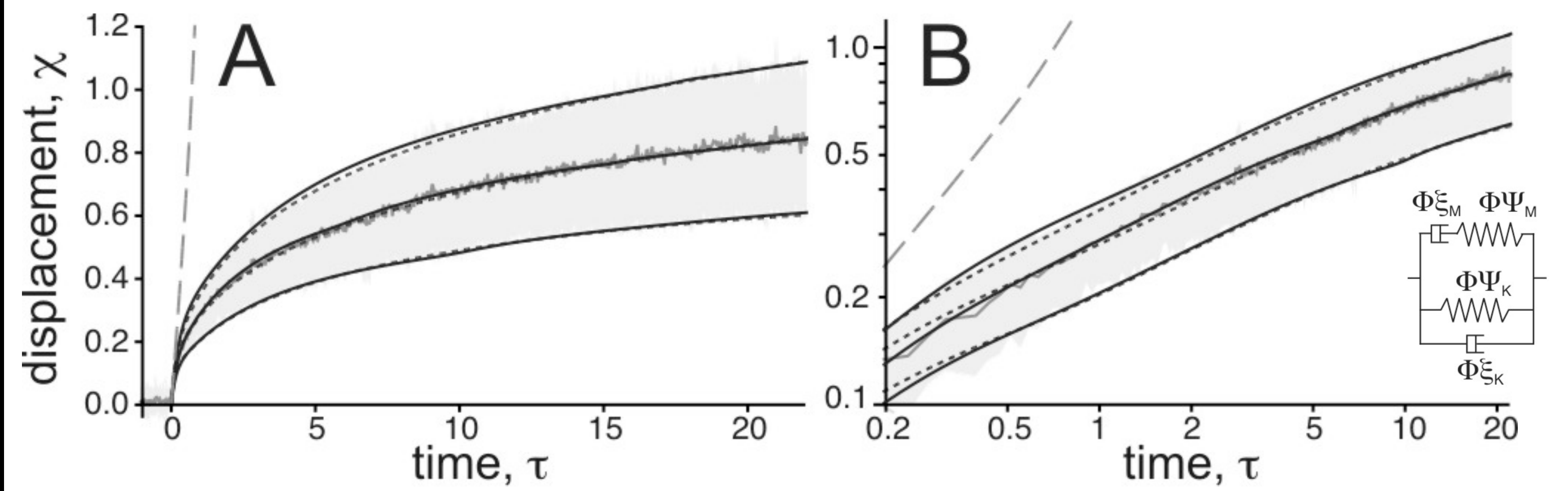
variable η (intra-embryo)

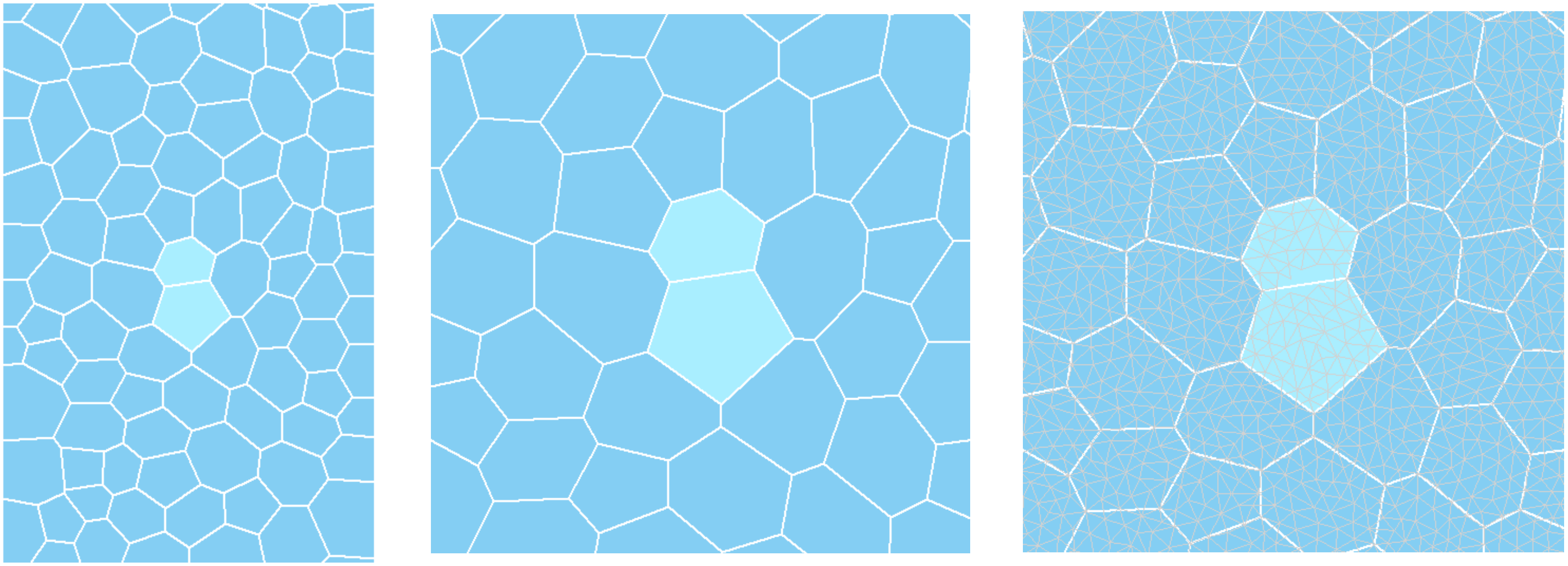
variable γ (intra-embryo)

variable γ re-equilibrated (intra-embryo)

Hutson, Veldhuis, Ma, Lynch, Cranston and Brodland (2009) *Biophys. J.* in press.

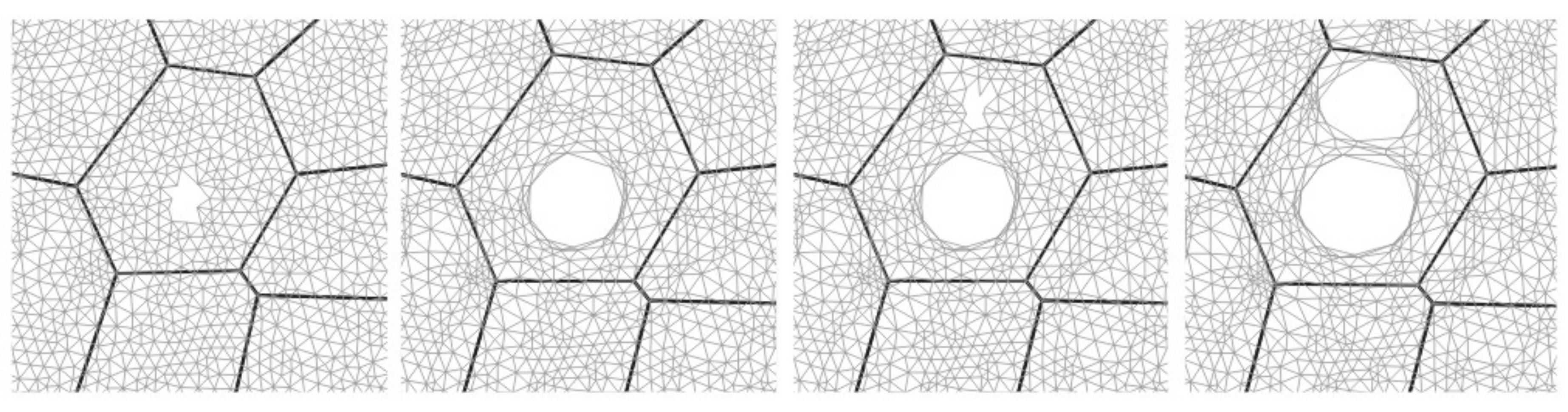
4. Recoils are biphasic:
roughly linear for t < 0.1 s; weak power-law from 0.1 to 10 s





Hutson, Veldhuis, Ma, Lynch, Cranston and Brodland (2009) *Biophys. J.* in press.

5. Successive wounds to adjacent cells produce two recoils. Same happens for successive wounds to different parts of a single cell.

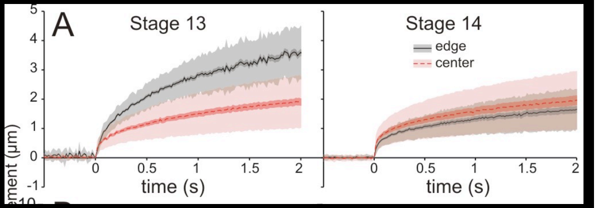


Hutson, Veldhuis, Ma, Lynch, Cranston and Brodland (2009) *Biophys. J.* in press.

Conclusions III (via modeling)

1. We can reproduce all 5 experimental observations using:
   a. cells that carry interfacial (γ) and in-plane (σ_{in}) tensions - where σ_{in} is several times larger than in-plane γ-equivalent
   b. a uniform cytoplasmic viscosity in each cell
   c. a fine intracellular network of linearly viscoelastic elements
   d. wide variability in either the viscosity or dim'less stress (could be inter- or intra-embryo variability)
2. We can get 4 of 5 with the viscoelastic network coarse-grained (i.e. only along cell edges).

CBO Redux: What needs to be specified for the mechanics of each cell?

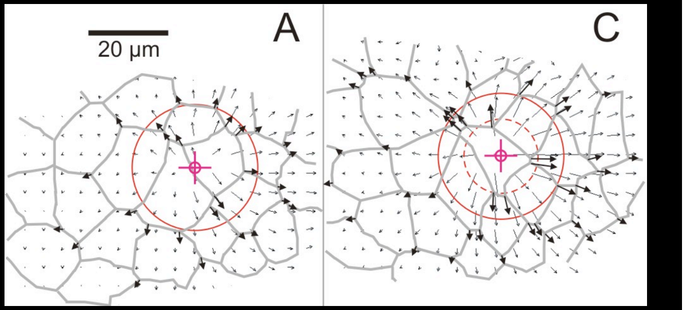


1. viscoelasticity (measured creep function or alternative)

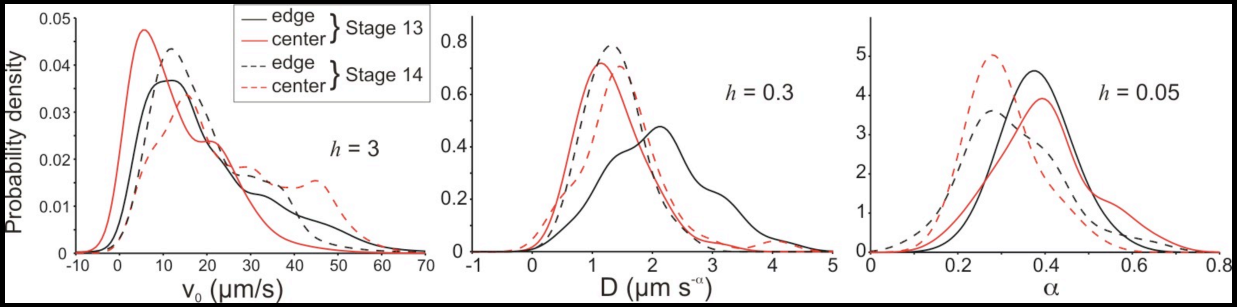
2. current unstressed cell shape
(hole drilling accesses true strain -
from which this can be estimated)

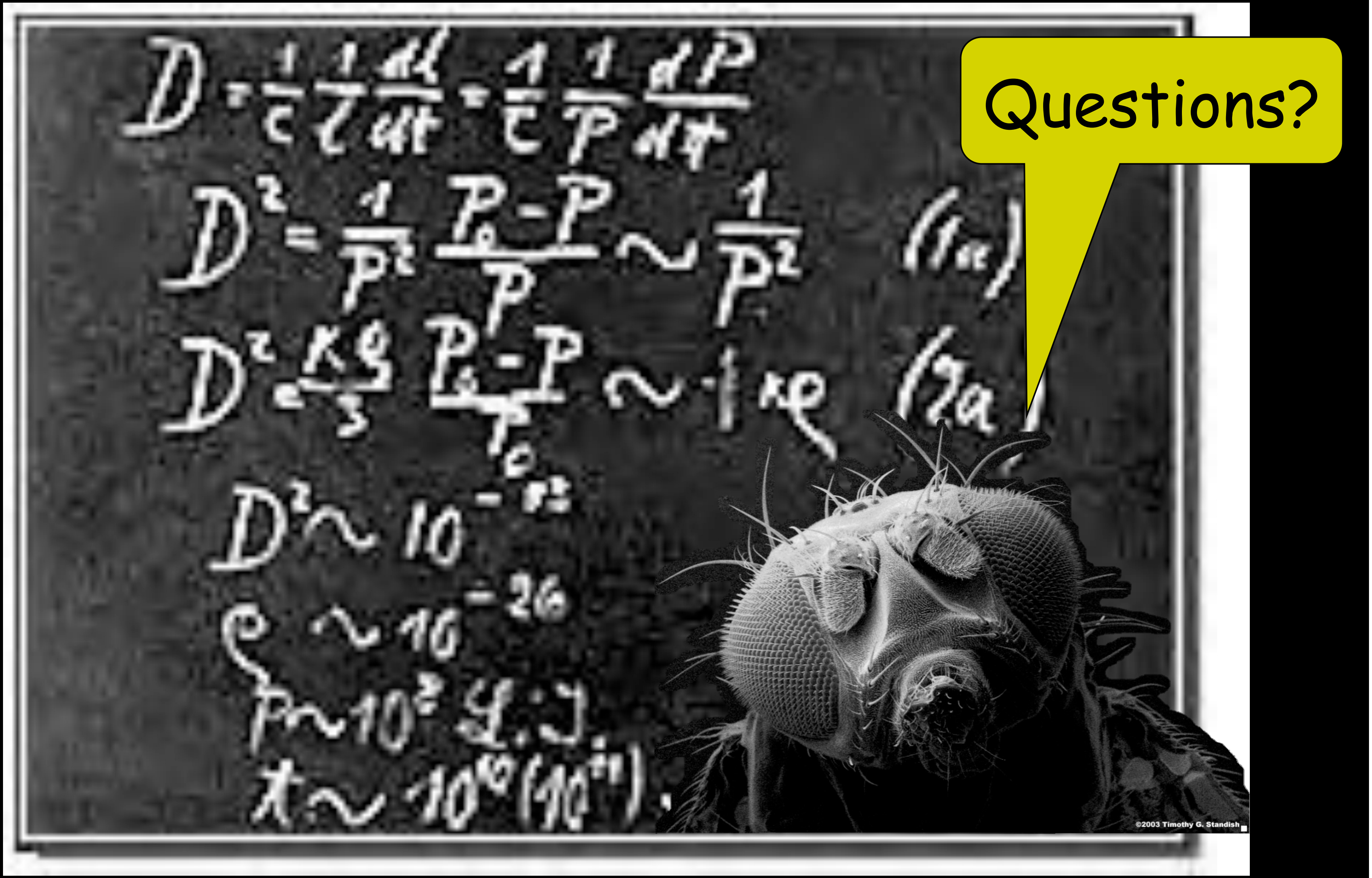
3. volume constraint

4. surface area constraint(s) - multiple if polarized



**** Not just mean values, but distributions!! *****





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