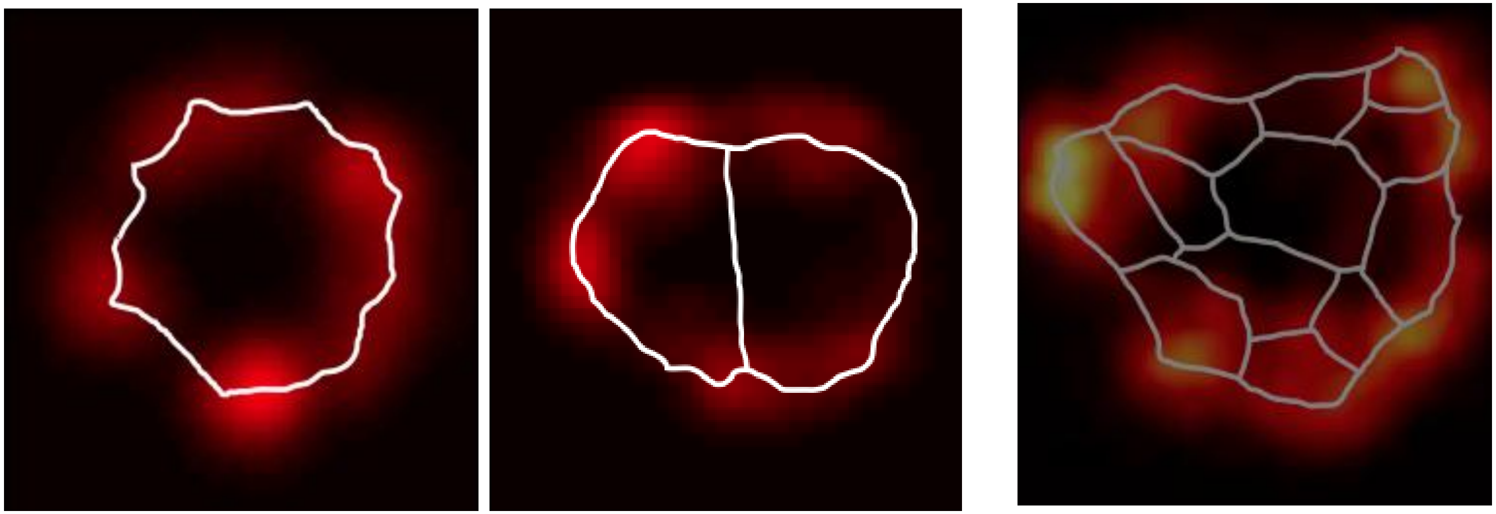
Mechanics and Geometry of Adherent Cells and Cell Layers



Shiladitya Banerjee

James Franck Institute

The University of Chicago

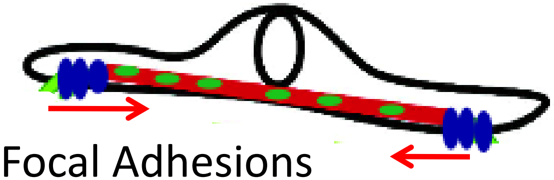
Active Matter: Cytoskeleton, **Cells, Tissues** and Flocks - KITP 2014

Adherent cells exert traction forces on elastic substrates

A.K. Harris *et al*, Science (1980)

Silicon gel wrinkling => Cell is prestressed

What controls traction force generation in isolated cells?

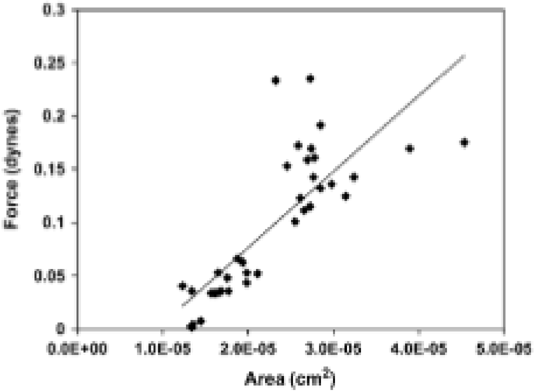


- Mechano-chemical factors: ECM stiffness, Cytoskeletal Tension, Focal Adhesions
- Geometric factors: Cell spread area, adhesion geometry

Interplay between cell-cell and cell-ECM adhesions in muticellular colonies

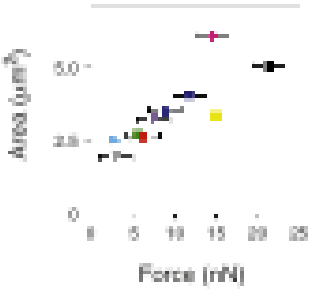
Are there *universal* force-geometry relations across tissue length scales?

What factors control force generation during cell-matrix adhesion?



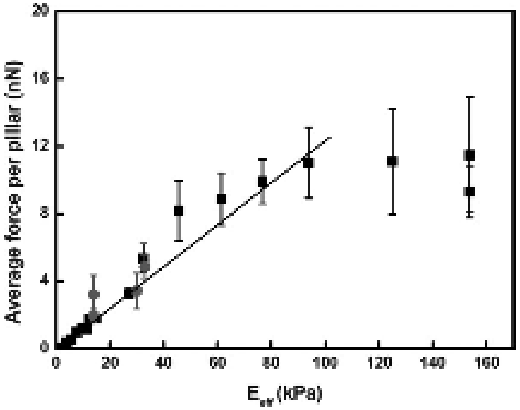
Reinhart-King C.A. *et al*, Biophys J. 2005.

Cell Spread Area



N.Q. Balaban et al, Nat. Cell. Bio. 2001

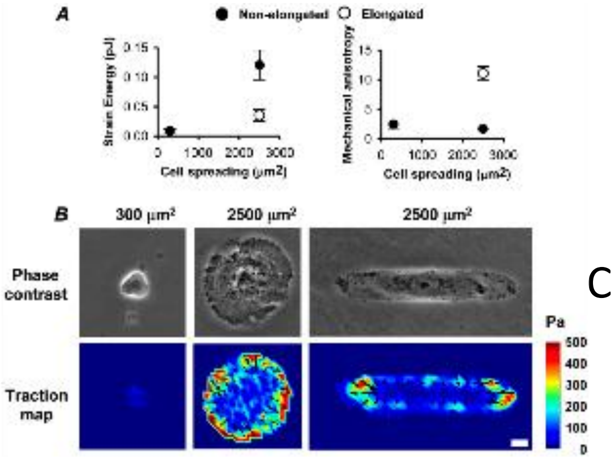
Focal Adhesions



Substrate Stiffness

M. Ghibaudo *et al*, Soft Matter 2008.

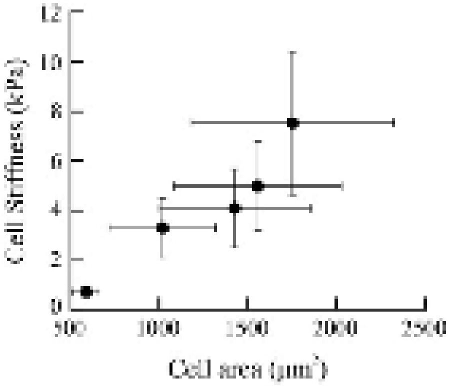
Cellular Forces/Stresses



P. Roca-Cusachs *et al*, Biophys J. 2008.

Cell Shape

Cell Mechanical Properties

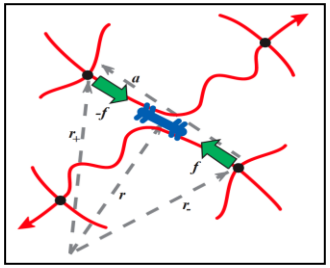


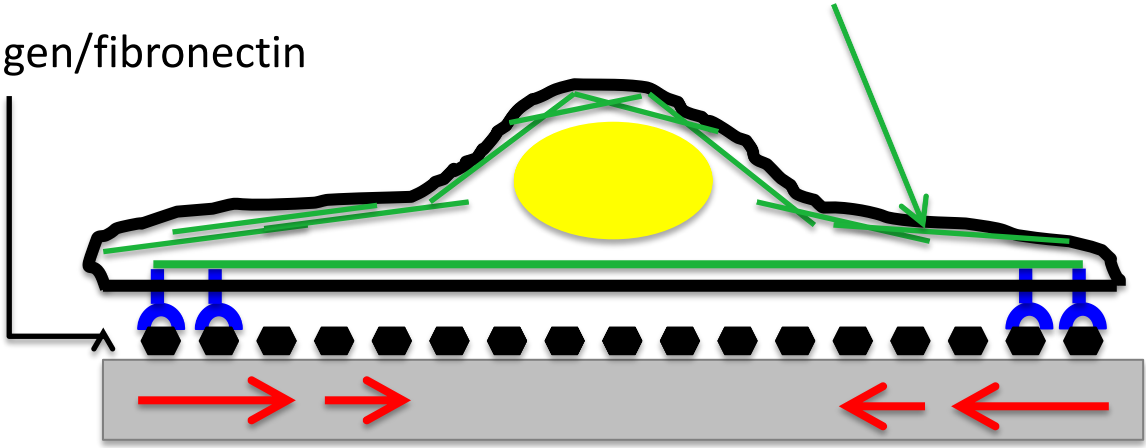
J. Solon *et al*, Biophys J. 2007.

Need to isolate roles of mechanical and geometrical constraints

Minimal Model for force generation

Cells adhere to substrates via focal adhesions and pull on the substrate through contractile forces generated in the actomyosin network → traction stresses regulated by complex signaling.





Actin cytoskeleton → stress fibers & cortex

Collagen/fibronectin

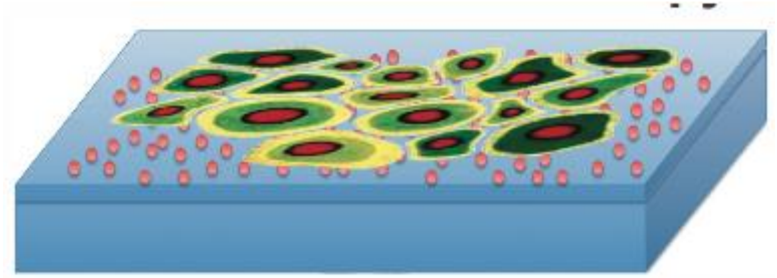
focal adhesions

substrate

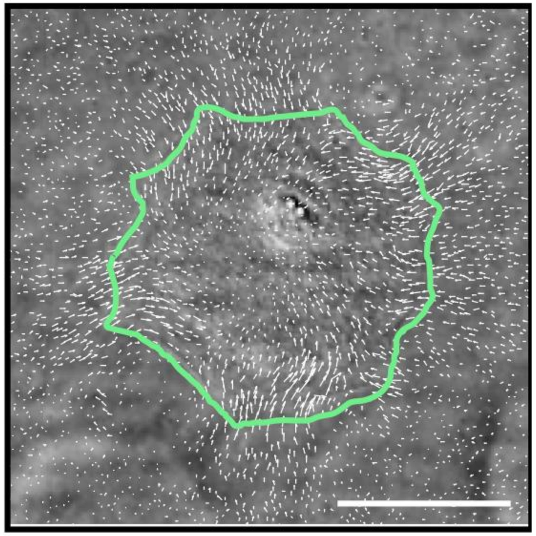
Traction Forces

Traction Force Microscopy (TFM)

Traction stresses inferred using linear elasticity from the displacements of embedded beads.



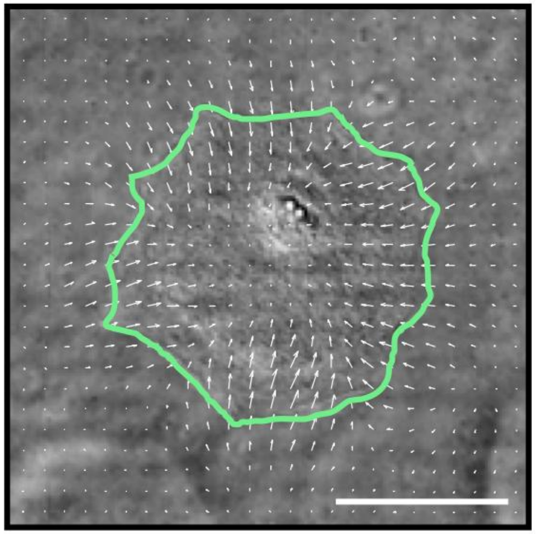
How does the cell deform the substrate?



Substrate displacement field

$$\mathbf{u}_s$$

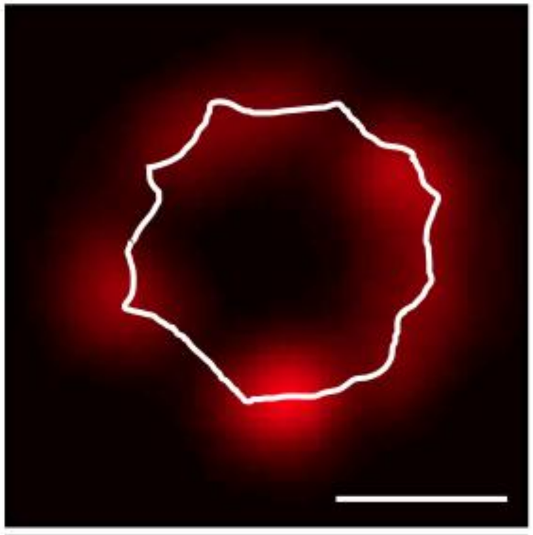
How does the cell pull?



Traction stress

$$\mathbf{T} = \mathbf{G}^{-1}_{elastic} * \mathbf{u}_s$$

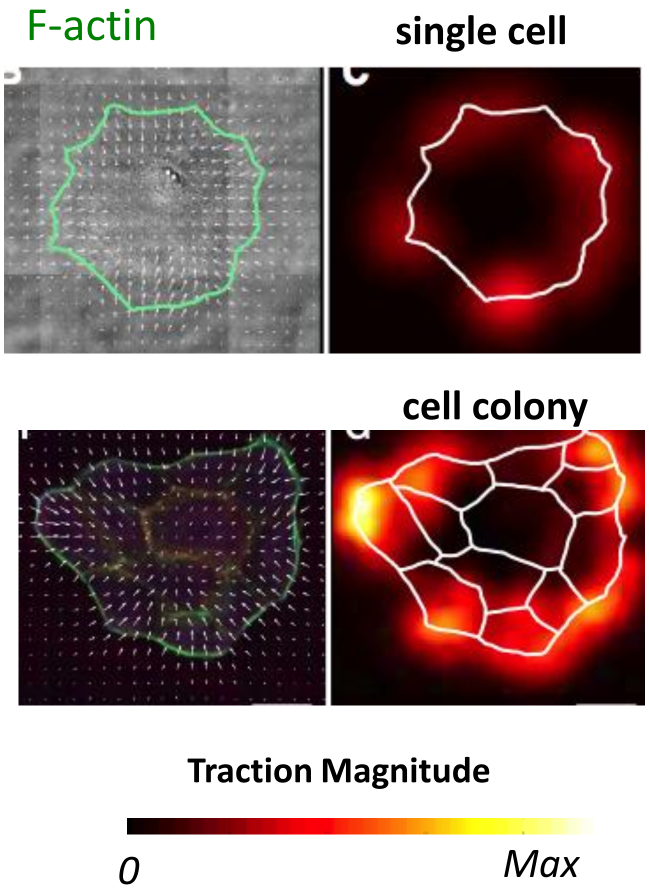
How much energy does it spend to deform the substrate?



Strain energy

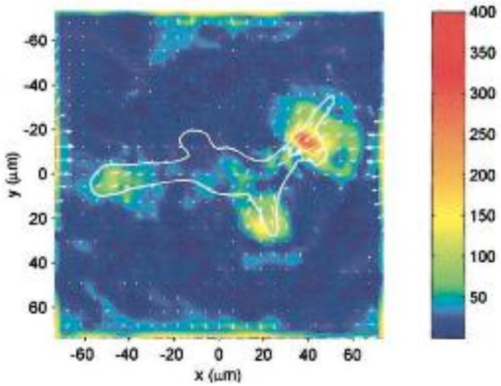
$$W = \frac{1}{2}\int dA\ \mathbf{T}(\mathbf{r}) \cdot \mathbf{u}_s(\mathbf{r})$$

Traction stresses are localized at the edges of cell/cell colony



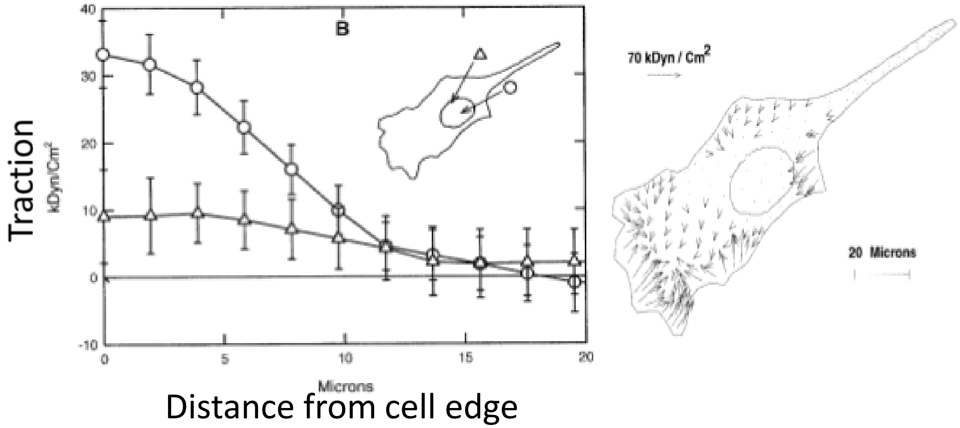
Stationary Keratinocytes

Mertz, Banerjee et al, PRL, 2012



Smooth muscle cells

Butler et al, AJPCP 282, C595 (2001).



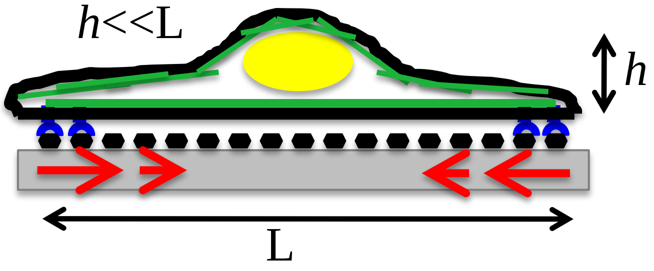
Migrating fibroblasts

Dembo and Wang, Biophys J 76, 2307 (1999).

What factors determine the localization length scale?

Adherent Cell as a Contractile gel

Assuming Mechanical Equilibrium



Cell
Thin Film
Plane Stress Approx.

$$\partial_\beta \sigma_{\alpha\beta} = 0 \ (\alpha, \beta \in x, y, z)$$

$$\partial_z \sigma_{iz} + \partial_j \sigma_{ij} = 0 \ (i, j \in x, y)$$

$$h \partial_j \bar{\sigma}_{ij} = \sigma_{iz}|_{z=0} \equiv T_i$$

(Thickness Averaged)

Traction Stress

$$\vec{T} = Y_a(\vec{u} - \vec{u}^s)$$

Focal adhesions are harmonic springs
Deforming in-plane

$\vec{u}$: Cell's in-plane displacement field

$\vec{u}^s$: Substrate's in-plane displacement field

$$\vec{u}^s(\vec{r}) = \int_{\vec{r}'} G^s_{ij}(\vec{r} - \vec{r}')T_j(\vec{r}')$$

SB and MCM
EPL 2011
PRL 2012
NJP 2013

Contractile gel model…

For a thin substrate $h_s \ll L$: $$G^s_{ij}(r-r') = \frac{1}{\mu_s/h_s}\delta_{ij}\delta(r-r')$$

Effective Force Balance : $$h\partial_j\sigma_{ij} = Y u_i$$

Substrate Rigidity : $$Y = \left(\frac{1}{Y_a} + \frac{h_s}{\mu_s}\right)^{-1}$$

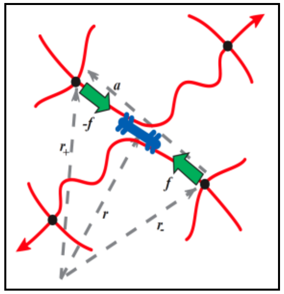
Cellular Constitutive Relation :

$$\sigma_{ij} = B\nabla\cdot\vec{u}\,\delta_{ij} + \mu\left(\partial_i u_j + \partial_j u_i - \nabla\cdot\vec{u}\,\delta_{ij}\right) + \sigma_a\,\delta_{ij}\ (\sigma_a > 0)$$

Compression

Shear

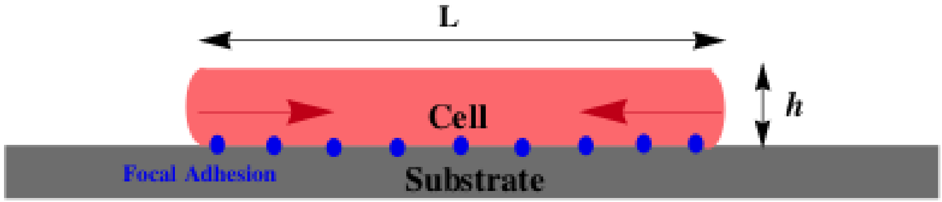
Acto-myosin Contractility



Stress–free Boundary Condition *:* $$\sigma_{ij}n_j = 0$$

$\vec{n}$: unit normal to cell boundary

One-dimension : Elongated cells



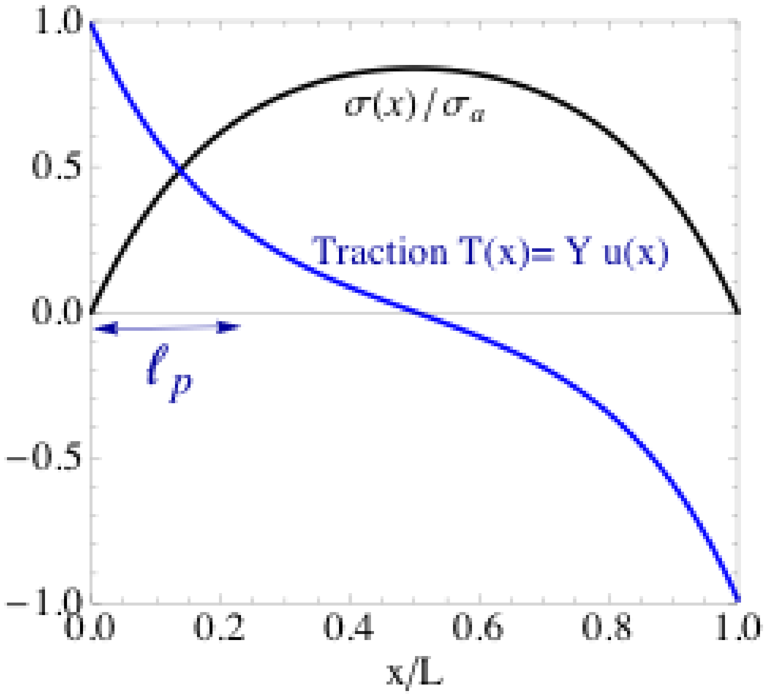
Force Balance → $h\partial_x\sigma = Yu$

Cellular Stress $\sigma(x) = B\partial_x u + \sigma_a$

$$\boxed{\sigma = \ell_p^2 \frac{d^2\sigma}{dx^2} + \sigma_a}$$

$\ell_p = \sqrt{\frac{Bh}{Y}}$ **Penetration length**

$$\sigma\big|_{x=0,L} = 0$$



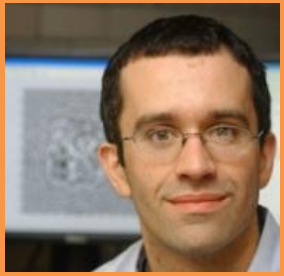
Traction localization and tensile stress buildup

Collective Mechanics of Epithelial Cell Layers

with

Aaron Mertz*

Eric Dufresne*



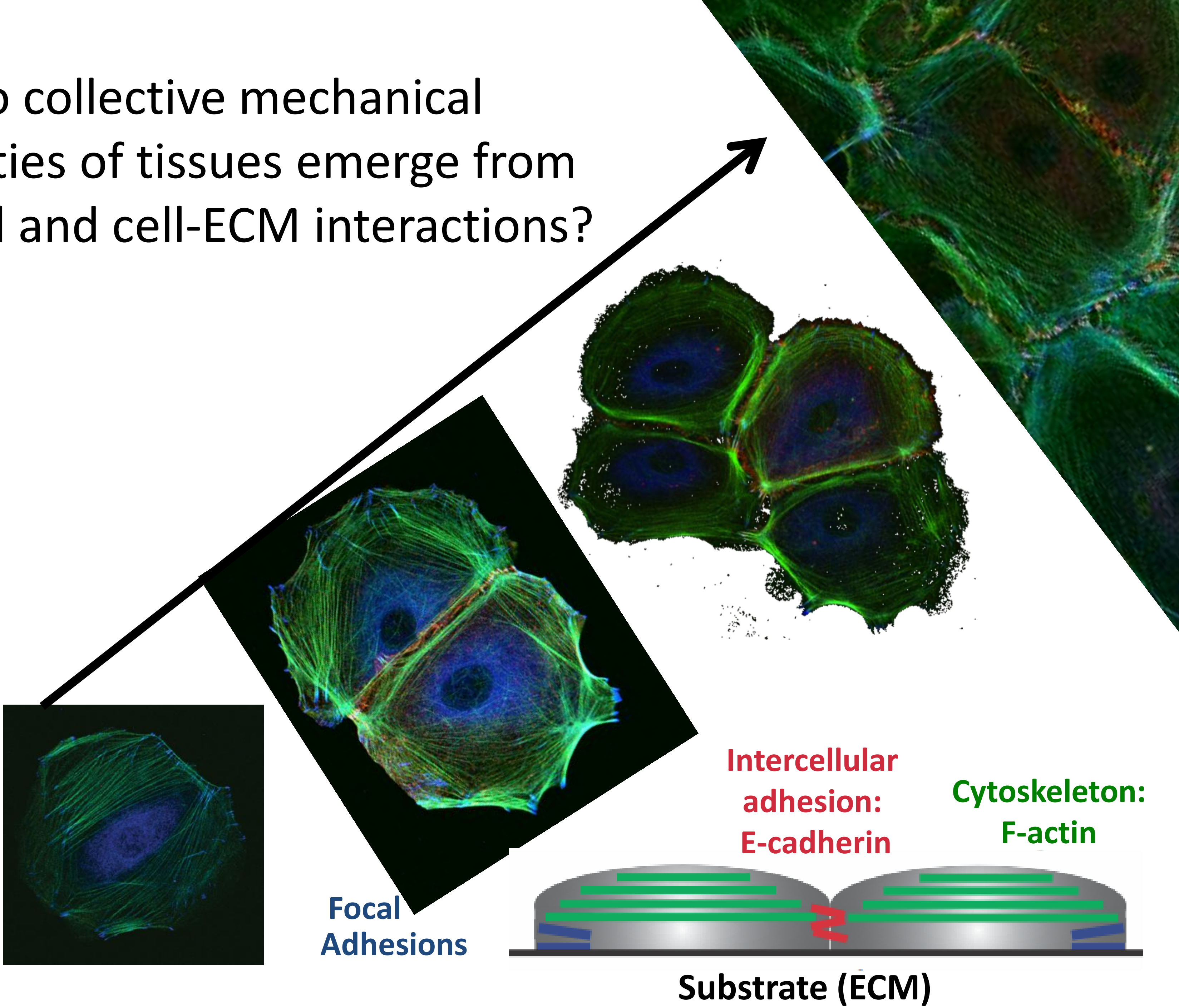
Valerie Horsley*

Cristina Marchetti**

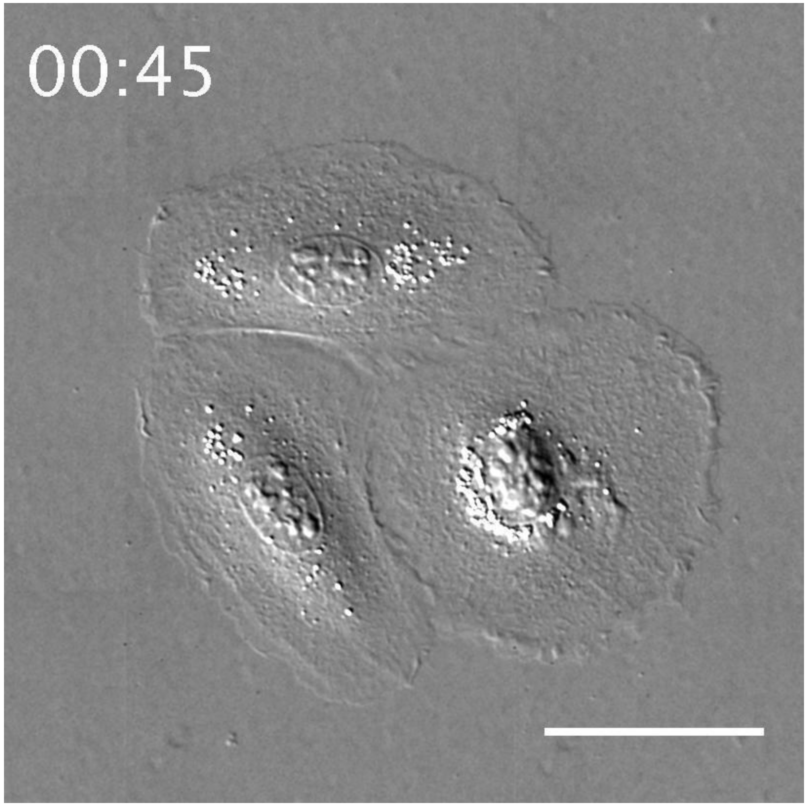
*Yale University

**Syracuse University

How do collective mechanical properties of tissues emerge from cell-cell and cell-ECM interactions?

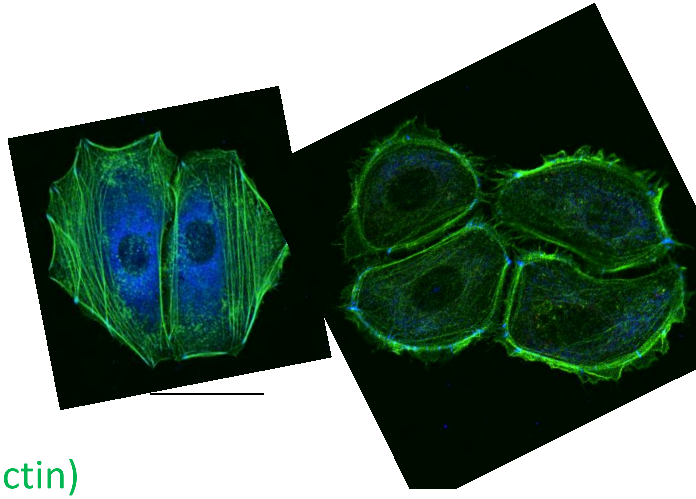


Intercellular adhesions form after calcium elevation



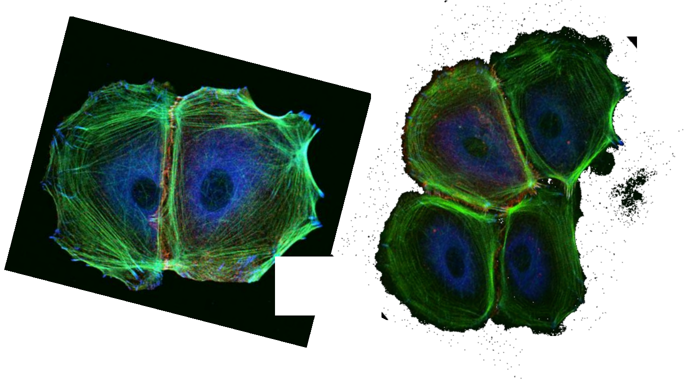
Calcium alters morphology and cohesiveness of colonies

Low calcium



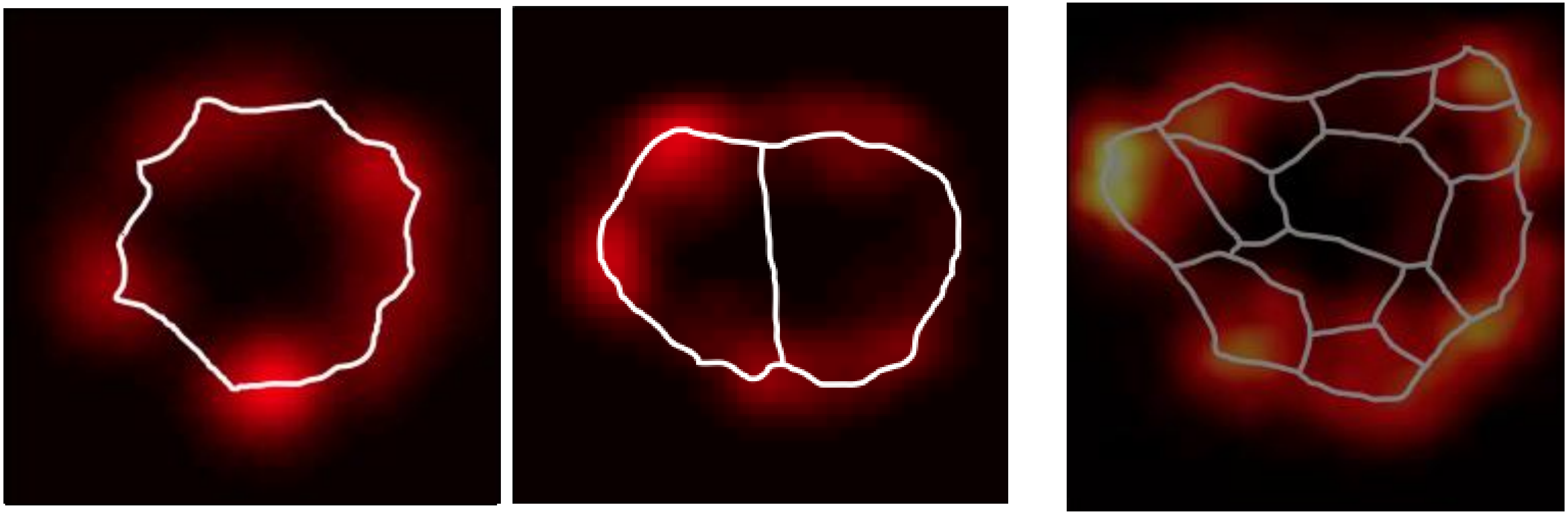
Phalloidin (F-actin)
E-cadherin
Zyxin
Scale bars 50 µm

High calcium



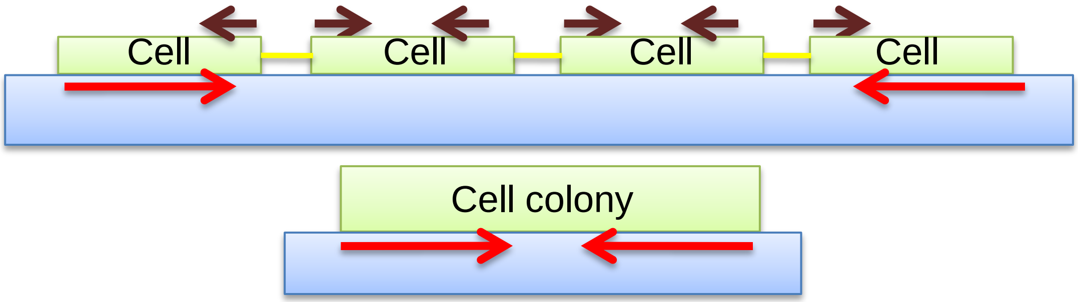
Cohesive Cell Colonies – strong intercellular adhesion

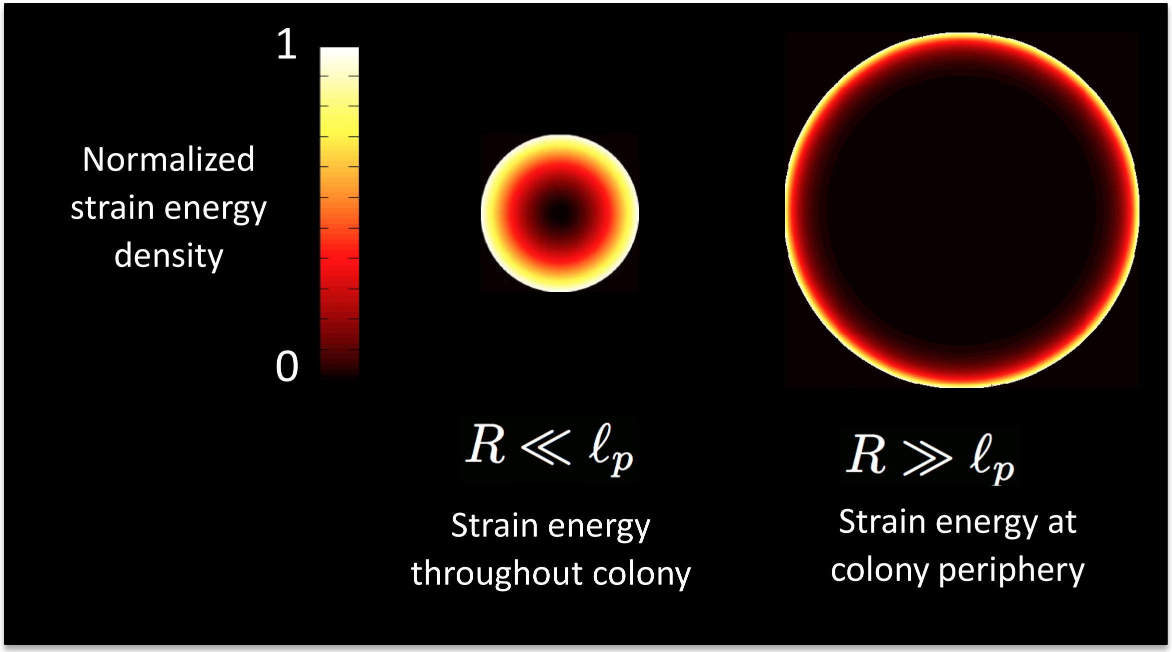
High Calcium Medium



Similar distribution of strain energy regardless of cell number for highly cohesive colonies !

Strongly cohesive colonies – Contractile Gel Model



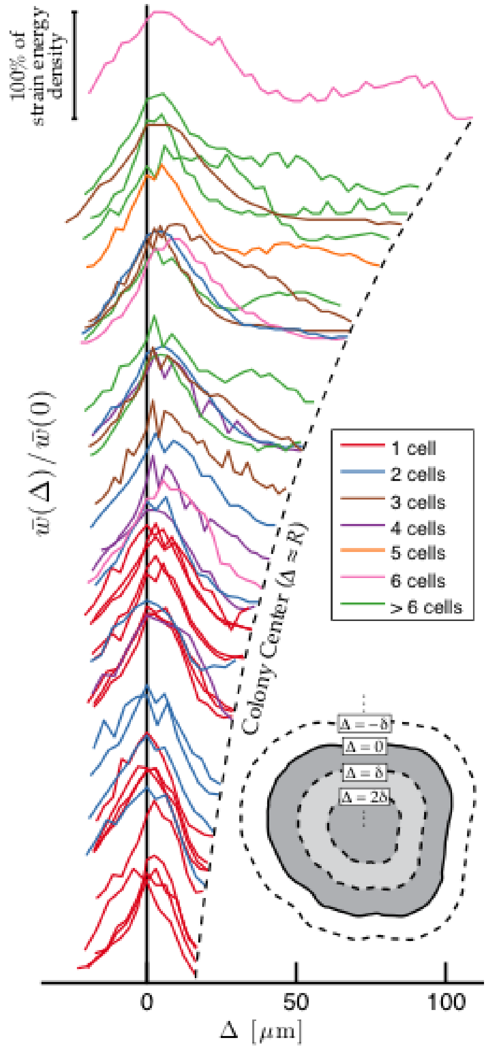


$R \ll \ell_p$

Strain energy throughout colony

$R \gg \ell_p$

Strain energy at colony periphery



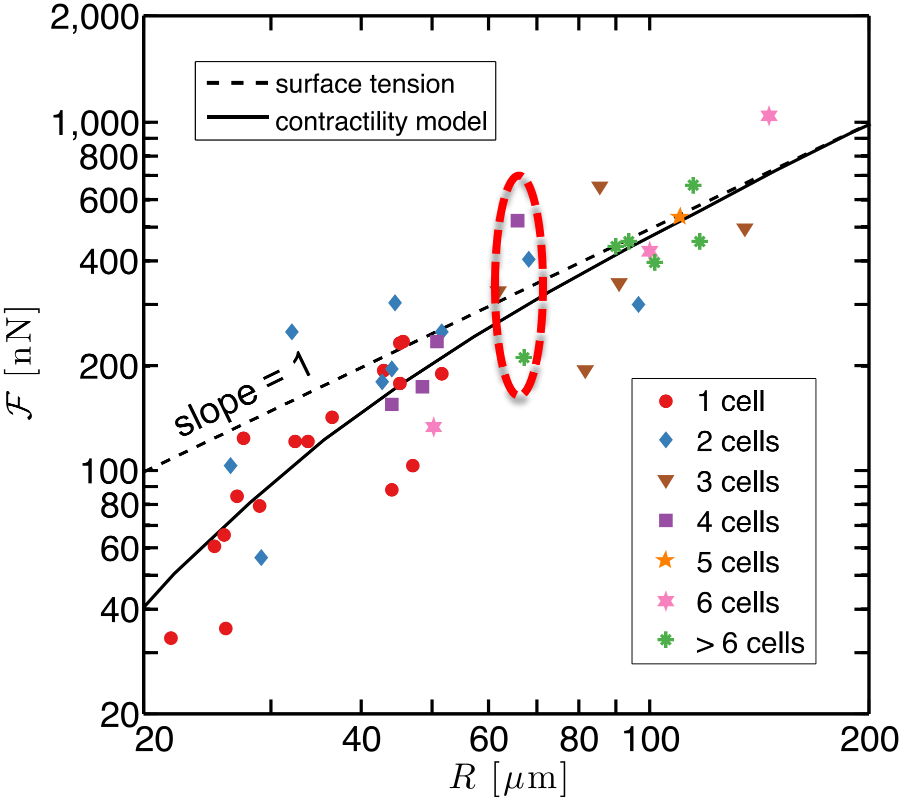
Model Predictions

$$R \ll \ell_p \quad : \quad \mathcal{F}(R) \propto R^3$$

$$R \gg \ell_p \quad : \quad \mathcal{F}(R) \simeq 2\pi h \sigma_a R \propto R$$

Effective surface tension?

Scaling of Traction Forces with colony radius

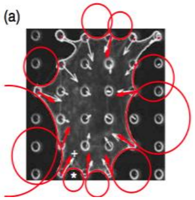


Mertz, Banerjee *et al.*, PRL 2012

- **Total traction** grows monotonically with **colony radius** and **not** the number of cells.
- Linear scaling at large colony radius suggests emergence of an ***effective surface tension*** originating from contractility.

$$\frac{F(R)}{2\pi R} \approx h\sigma_a \approx (8 \pm 2) \times 10^{-4}\, N/m$$

Micropillars

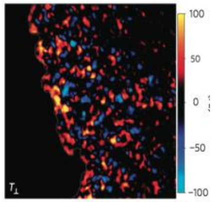


Single HUVEC

$$\gamma \approx 2 \times 10^{-3}\ \mathrm{N/m}$$

Bischofs *et al.*, Physical Review Letters, 2009, expanding on Lemmon *et al.*, Mechanics & Chemistry of Biosystems, 2005

Flat elastic substrate



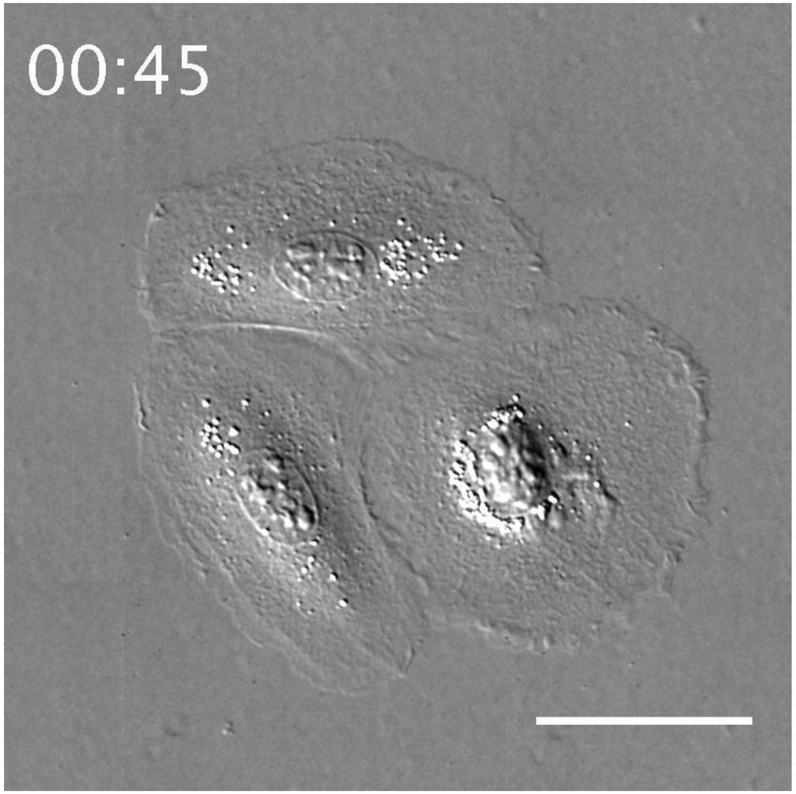
Sheet of MDCK epithelial cells

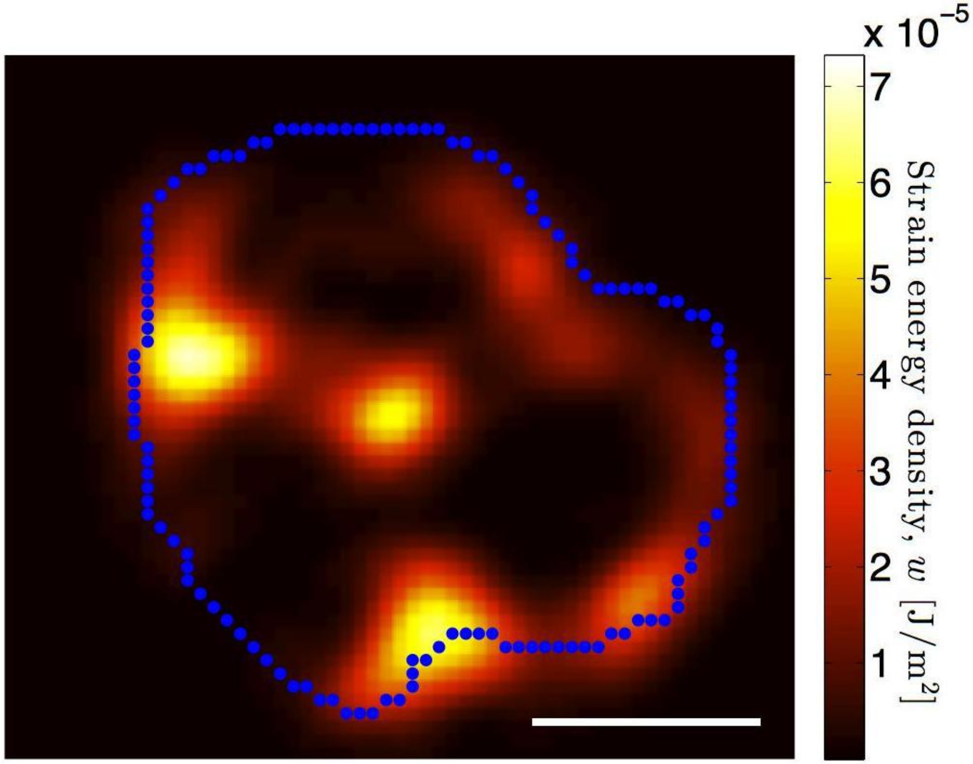
$$\gamma \approx 7 \times 10^{-4}\ \mathrm{N/m}$$

adapted from Trepat *et al.*, Nature Physics, 2009

Large colonies appear to behave like liquid droplets wetting a surface !

Strain energy localizes to colony periphery as adherens junctions form

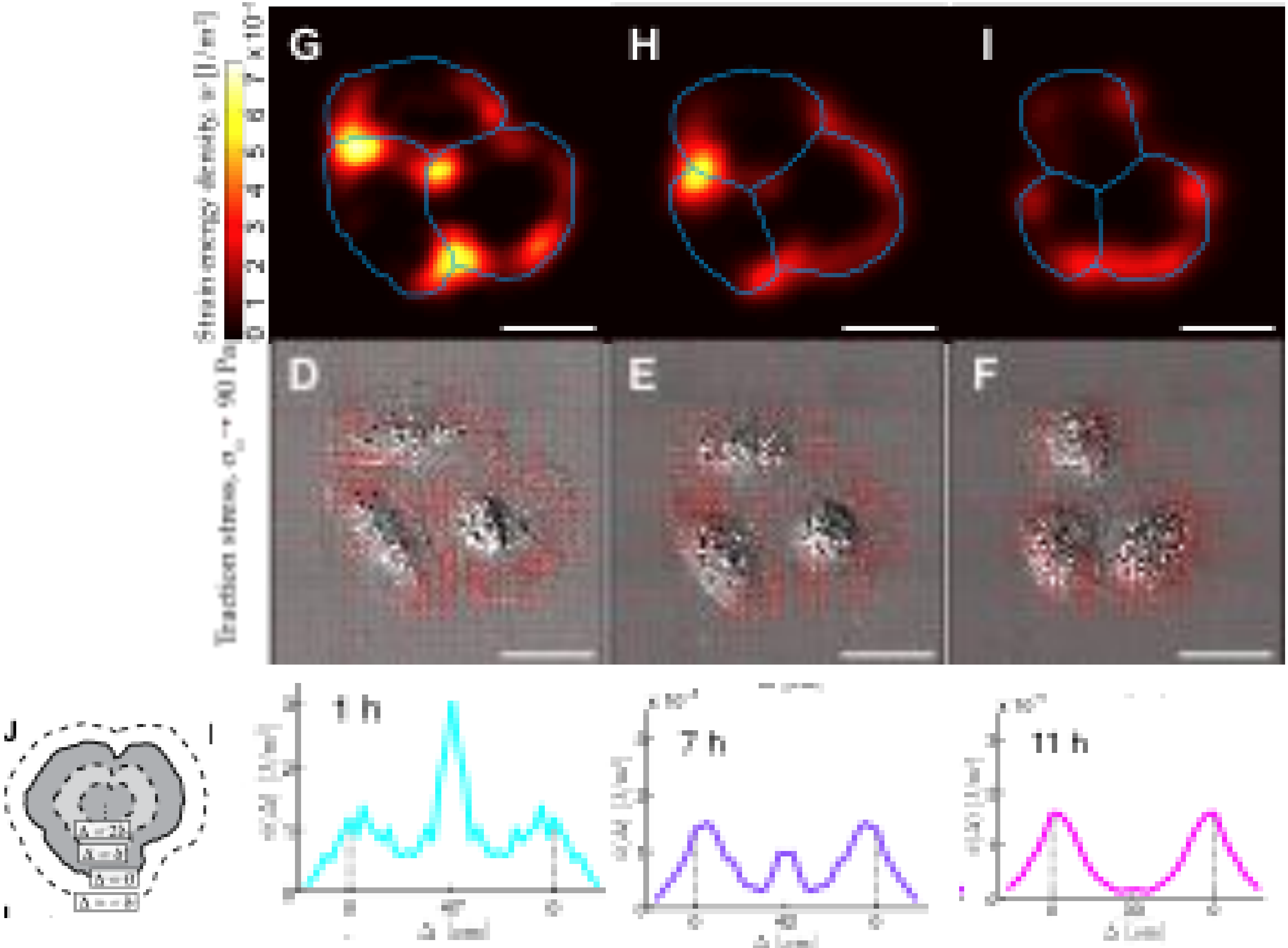




Mertz, Che, Banerjee *et al.*, PNAS, 2013

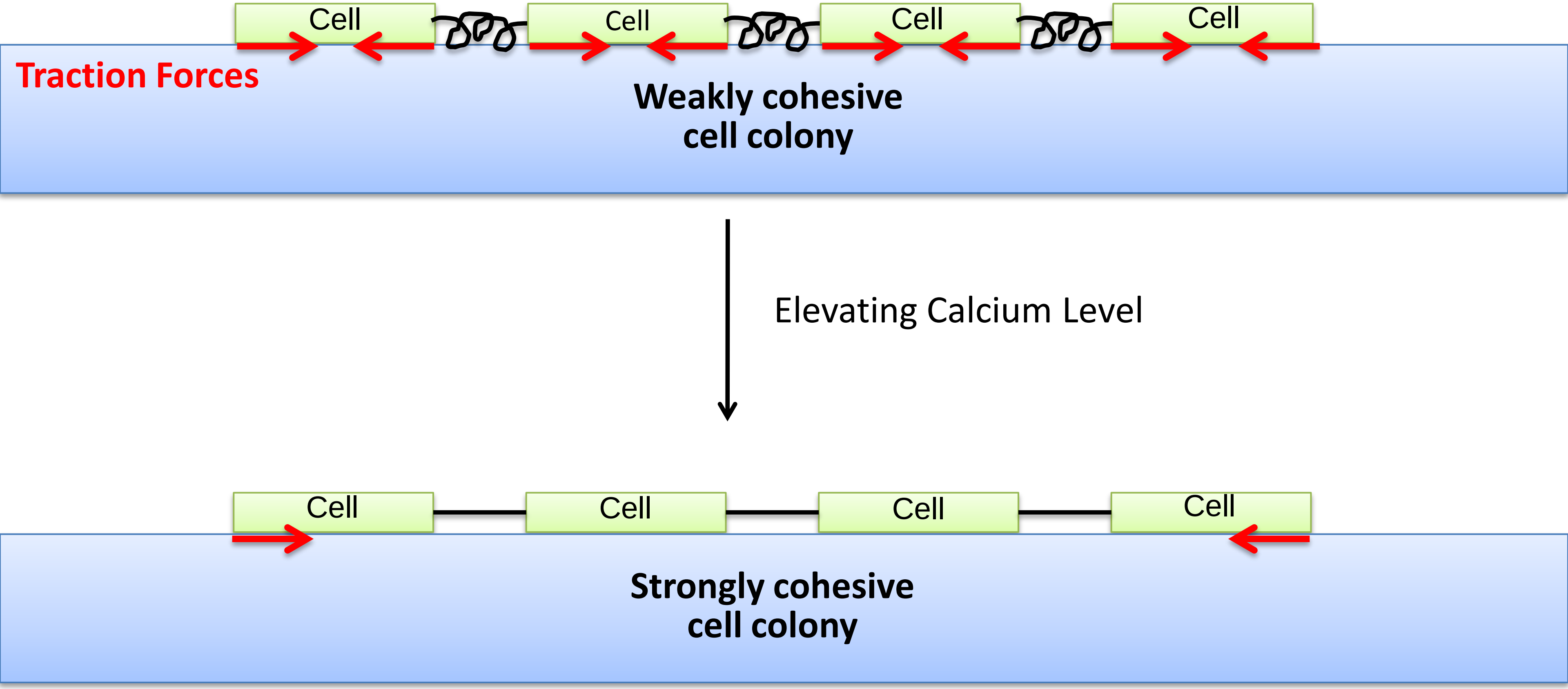
Low Calcium → **High Calcium**

Weak cell-cell coupling → *Strong cell-cell coupling*



Cell-cell adhesion as an elastic bond

Cells adhere to each other via hookean springs with adjustable stiffness



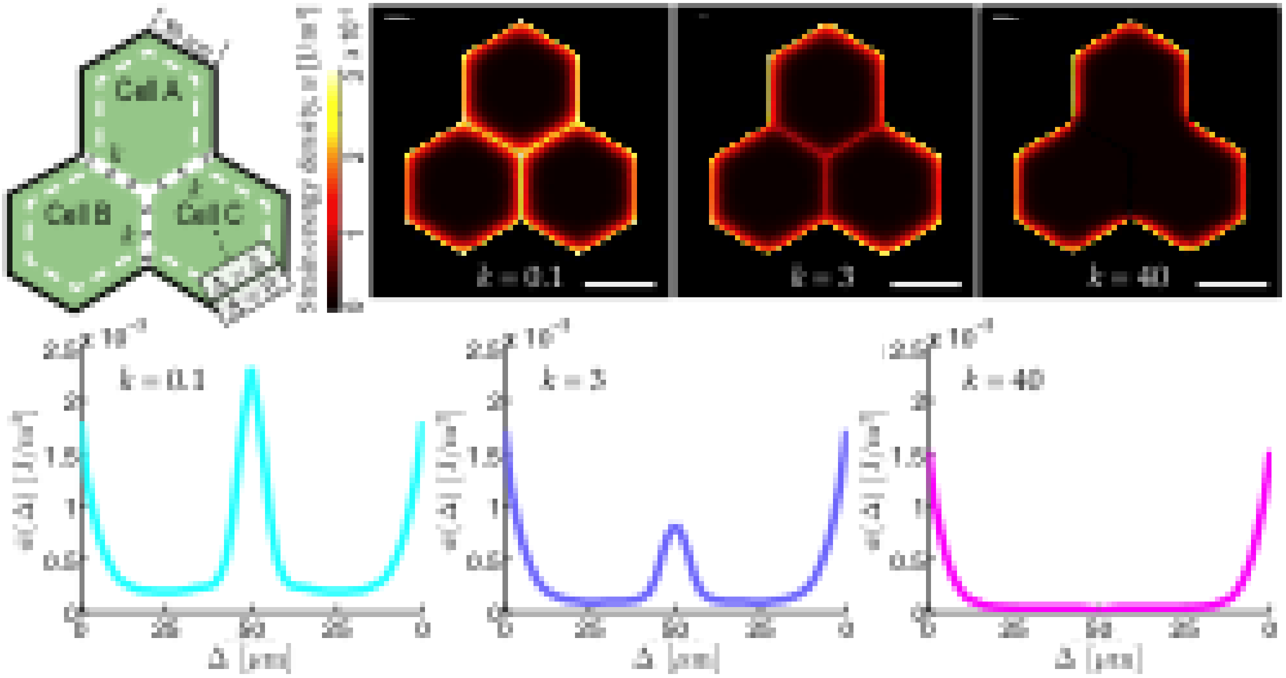
Traction Forces

Weakly cohesive cell colony

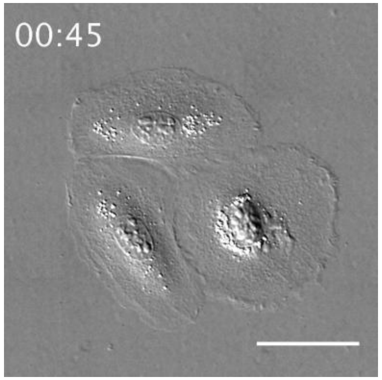
Elevating Calcium Level

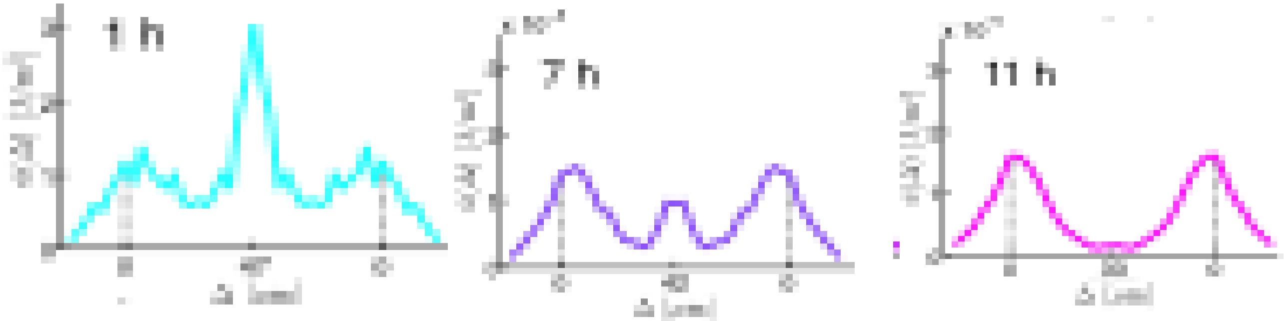
Strongly cohesive cell colony

2D model with shapes and springs captures experimental data



increasing stiffness of cell-cell springs





time after Ca^{2+} addition

Mertz, Che, Banerjee *et al.*, PNAS, 2013

Can Geometry solely regulate traction stresses in adherent cells?

with

Patrick Oakes*

Margaret Gardel*

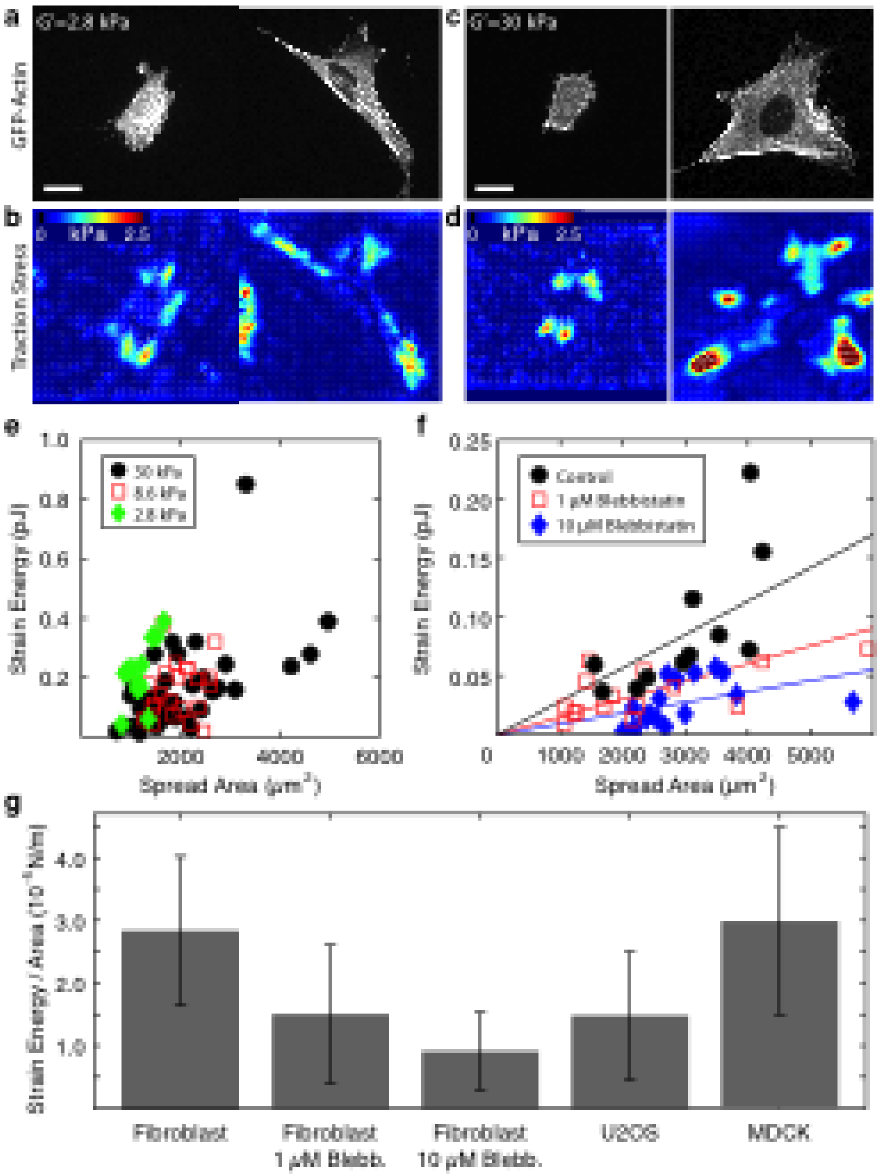
Cristina Marchetti**

*University of Chicago
**Syracuse University

Manuscript Submitted, under review

Adherent Cells maintain a characteristic Surface Tension

Figure 1



Strain Energy

$$W = \frac{1}{2}\int dA\ \mathbf{T}(\mathbf{r})\cdot\mathbf{u}_s(\mathbf{r})$$

$$W \propto A$$

$$\gamma = W/A \simeq 10^{-5}\mathrm{N/m}$$

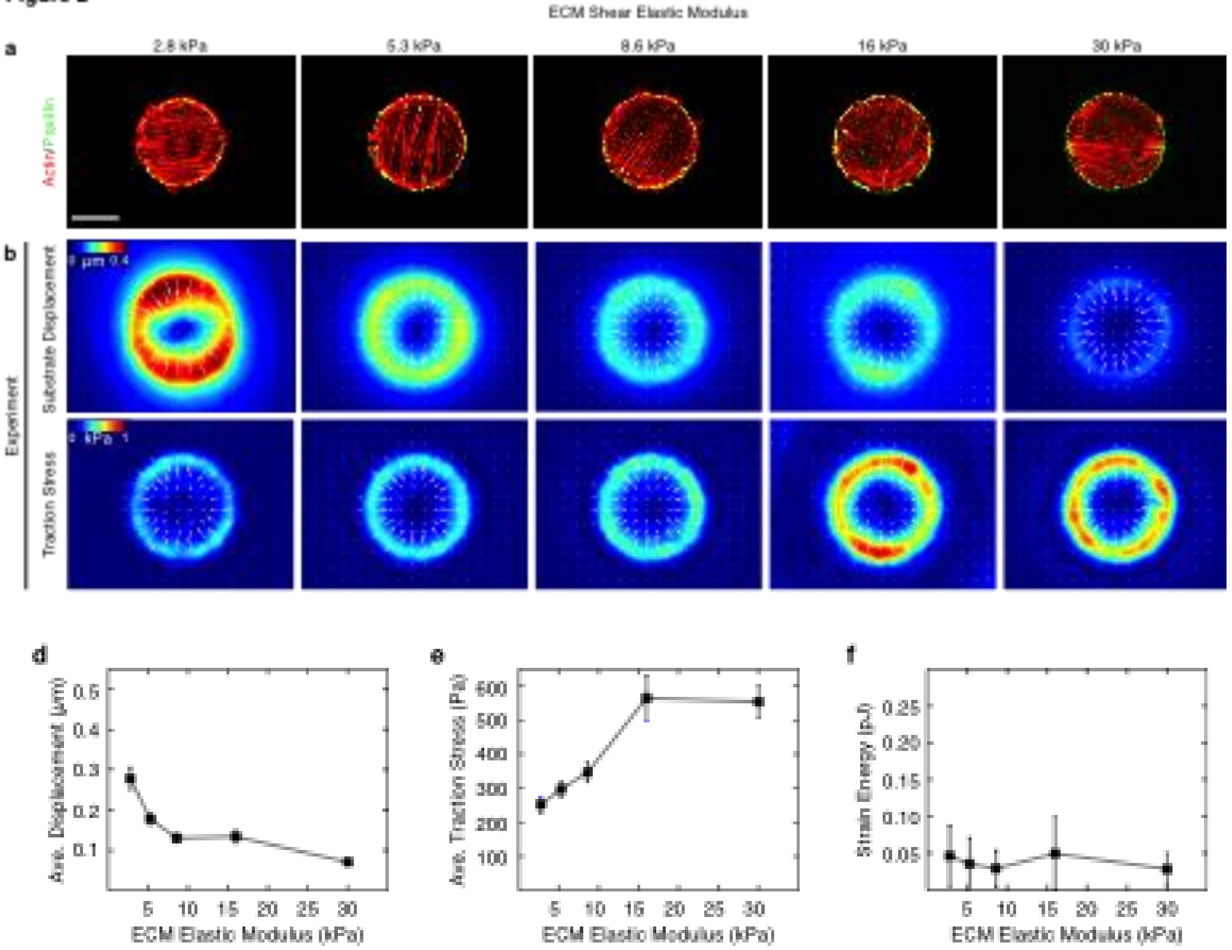
Cellular Surface Tension
dependence

Substrate
Stiffness
(weak)

Myosin-II based
Contractility
(strong)

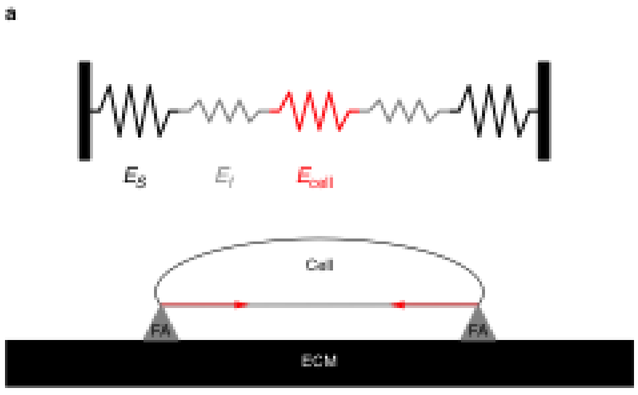
Strain Energy is insensitive to Substrate Stiffness

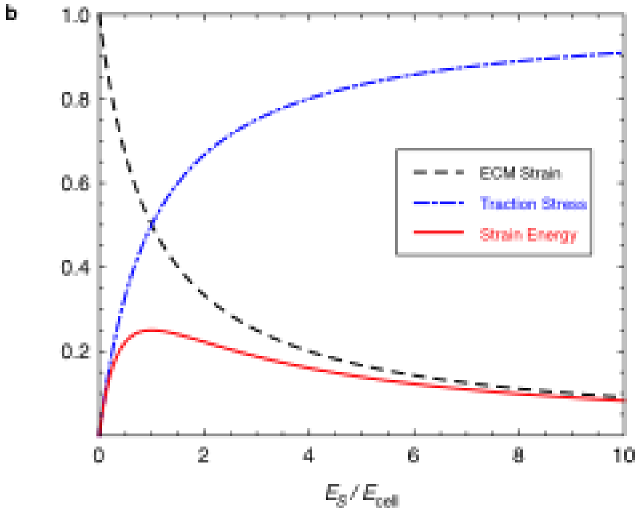
Figure 2



Pattern Area=800 μm^2

Uniform Three-element Model





$$\sigma_{cell} = E_{cell}\epsilon + \sigma_a$$

$$\sigma_f = E_f \epsilon_f$$

$$\sigma_s = E_s \epsilon_s$$

$$\epsilon_s = \sigma_a \frac{E_{\text{eff}}}{E_s(E_{\text{eff}} + E_{\text{cell}})} \qquad E_{\text{eff}} = (E_s^{-1} + E_f^{-1})^{-1}$$

$$T = E_{\text{eff}}\sigma_a/(E_{\text{cell}} + E_{\text{eff}})$$

$$W = \left[\frac{h\sigma_a^2 E_{\text{eff}}^2}{2E_s(E_{\text{cell}}+E_{\text{eff}})^2}\right] A$$

Using, $h \sim 1\ \mu\text{m}$, $E_{\text{cell}} \sim 10$ kPa, $E_{\text{eff}} \sim E_s \sim 10$ kPa, $\sigma_a \sim 1$ kPa

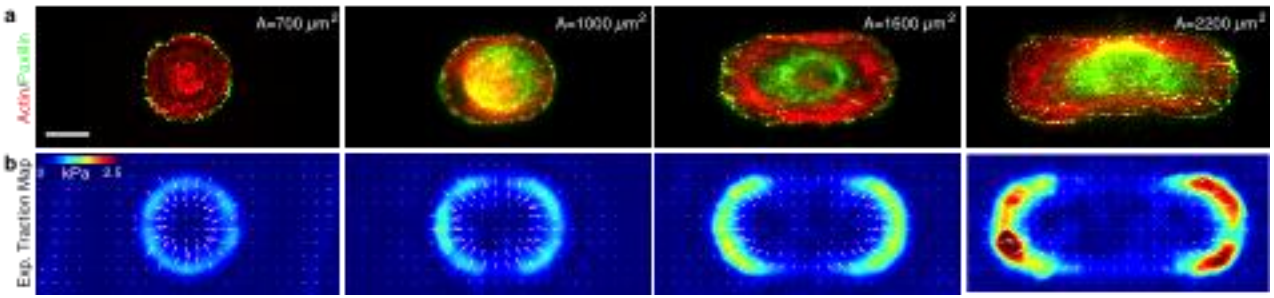
$$\gamma \sim 1.25 \times 10^{-5}\ \text{N/m}$$

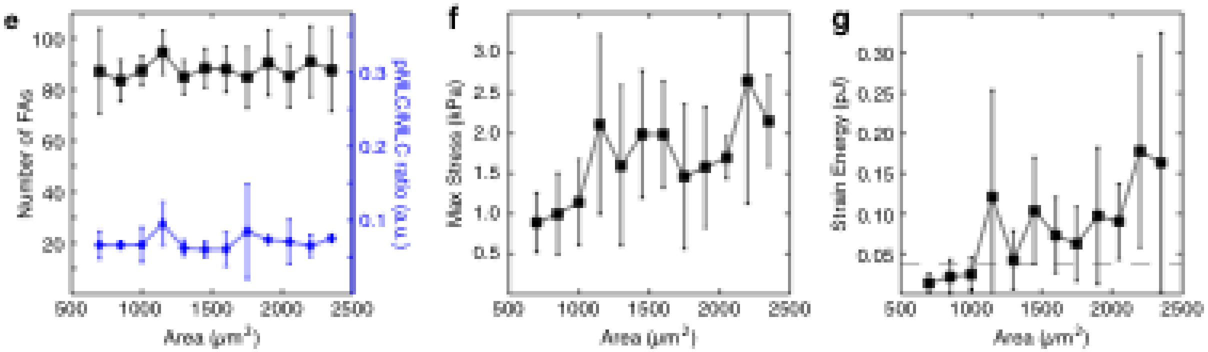
Micropatterns with Constant Curvature

Substrate shear modulus = 16 kPa

Radius of Curvature=15 µm

Figure 3

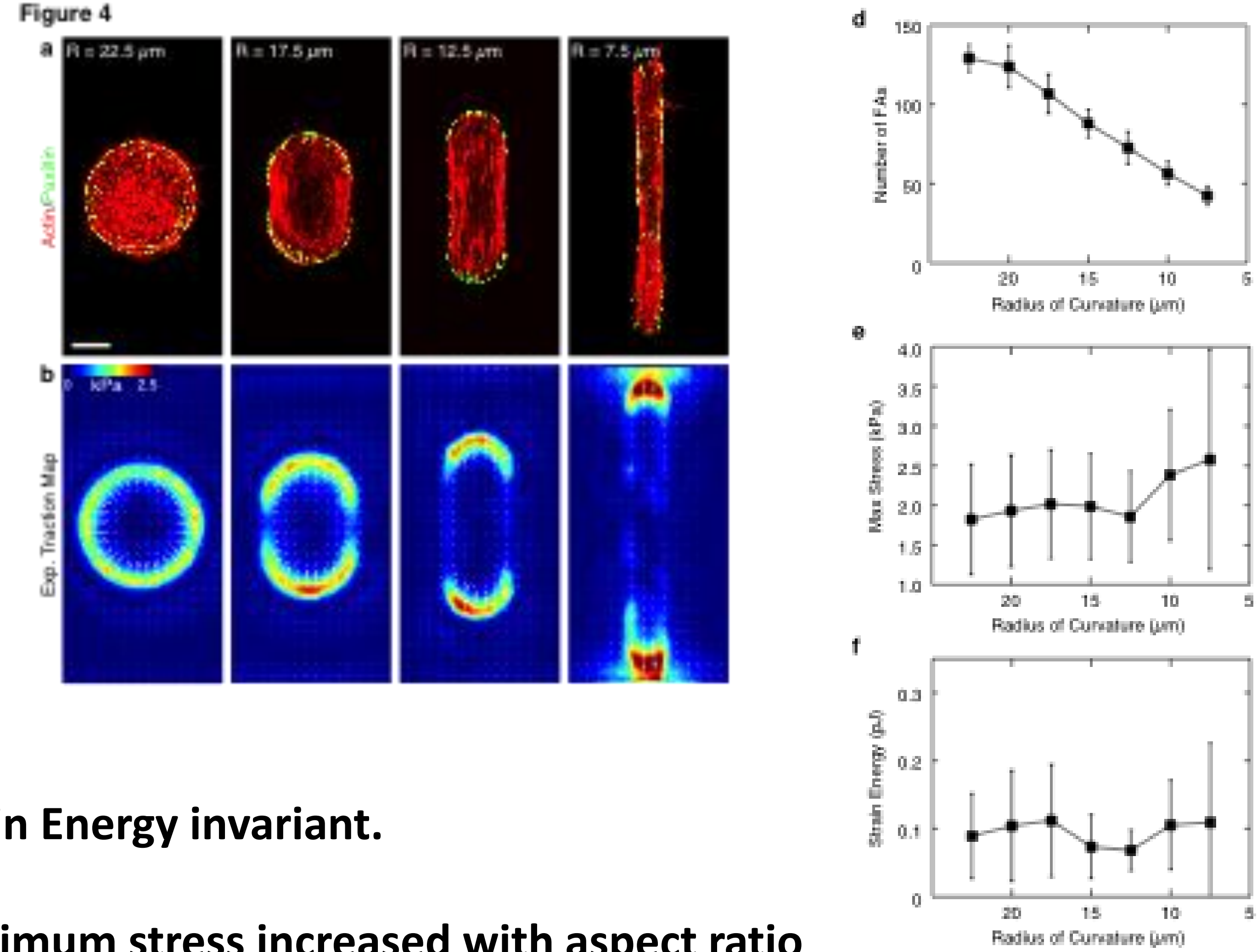




- **Strain Energy scales with Cell Spread Area**
- **Traction Stresses strongly localize to curved regions**

Micropatterns with Constant Area

Pattern Area=1600 µm^2 **Substrate shear modulus = 16 kPa**



- **Strain Energy invariant.**
- **Maximum stress increased with aspect ratio**

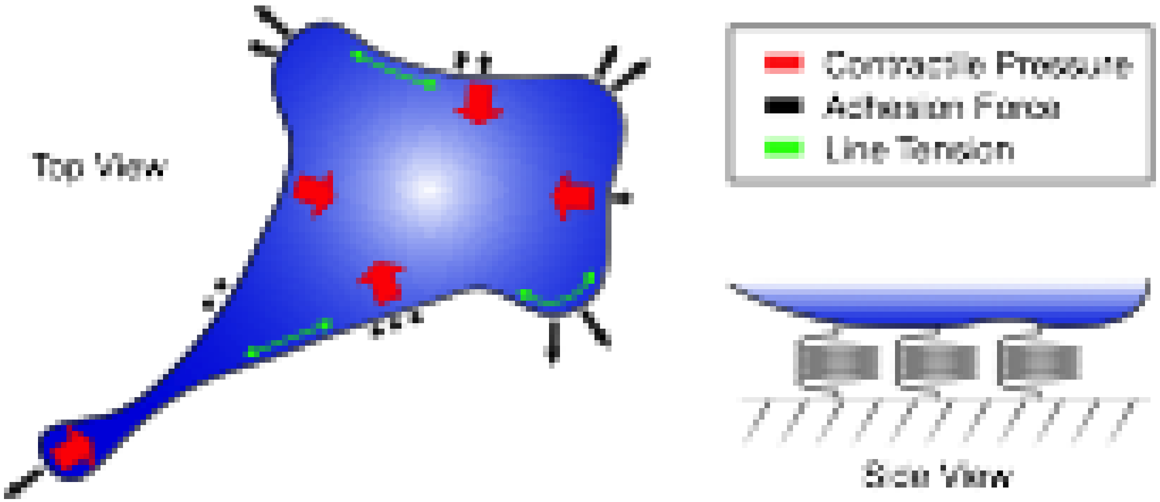
Line Tension

$$U = \frac{h}{2}\int dA\ \sigma_{ij}u_{ij} + \frac{Y}{2}\int dA\ \mathbf{u}^2 + \lambda \oint ds$$

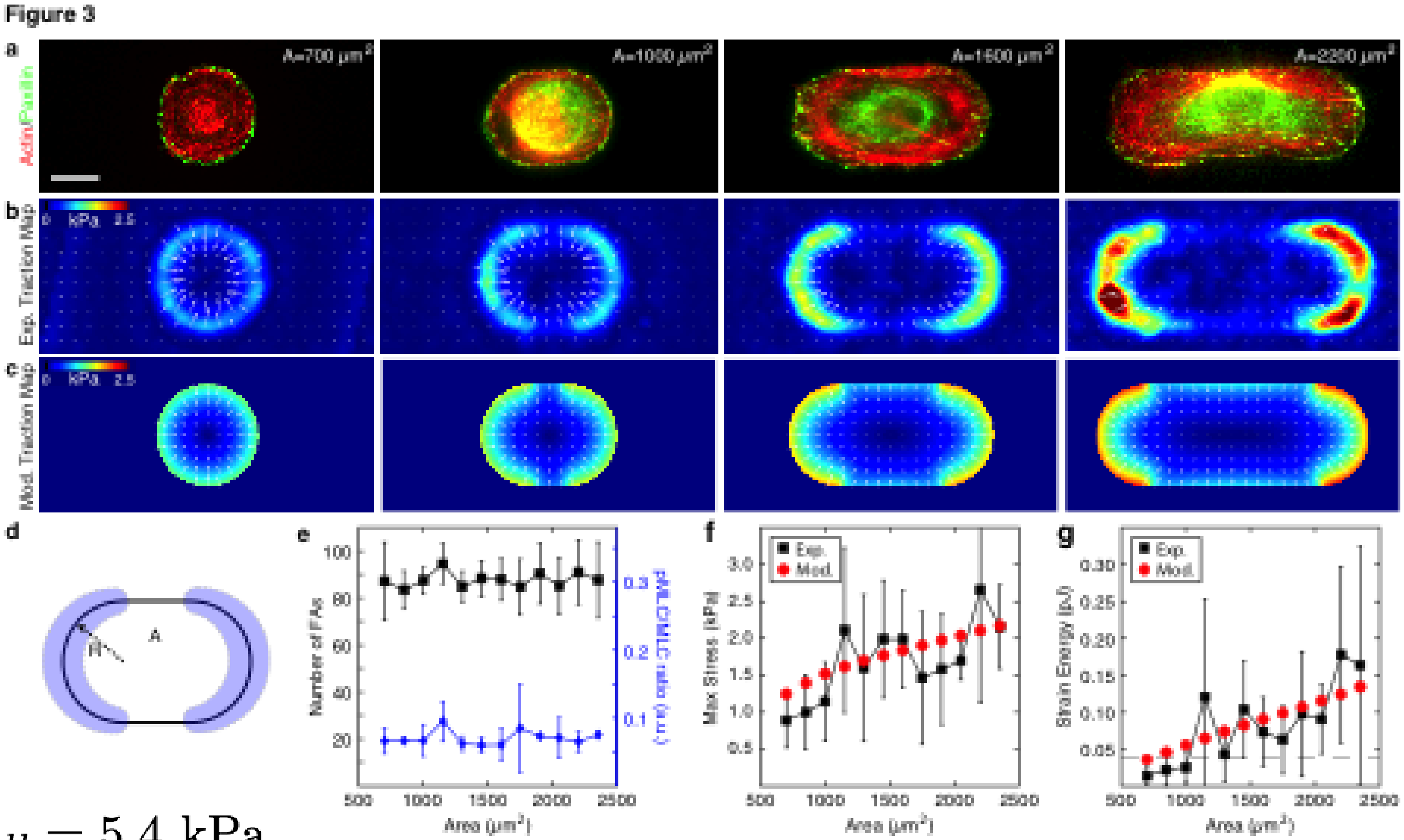
$\lambda > 0$ **Edge Contractility**

Line Tension Scales with Cell Perimeter $\lambda = f_m P$

Force/length exerted by molecular motors acting in parallel across the cell boundary



Micropatterns with Constant Curvature



$$E_{cell} = 5.4 \text{ kPa}$$

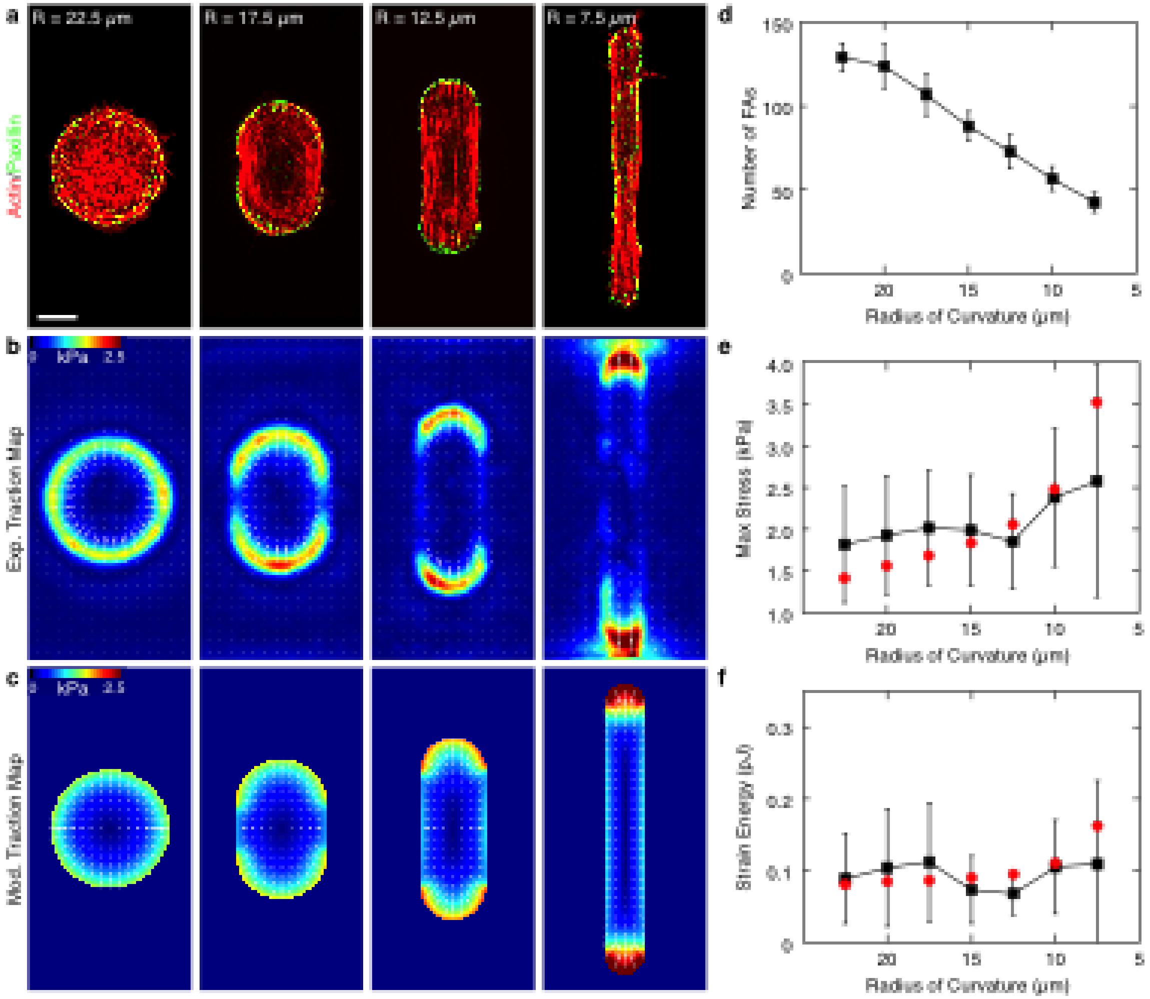
$$\sigma_a = 2.4 \text{ kPa}$$

$$f_m = 0.7 \text{ nN}/\mu\text{m}$$

$$\text{Surface Tension } \gamma = 6.06 \times 10^{-5}\text{N/m}$$

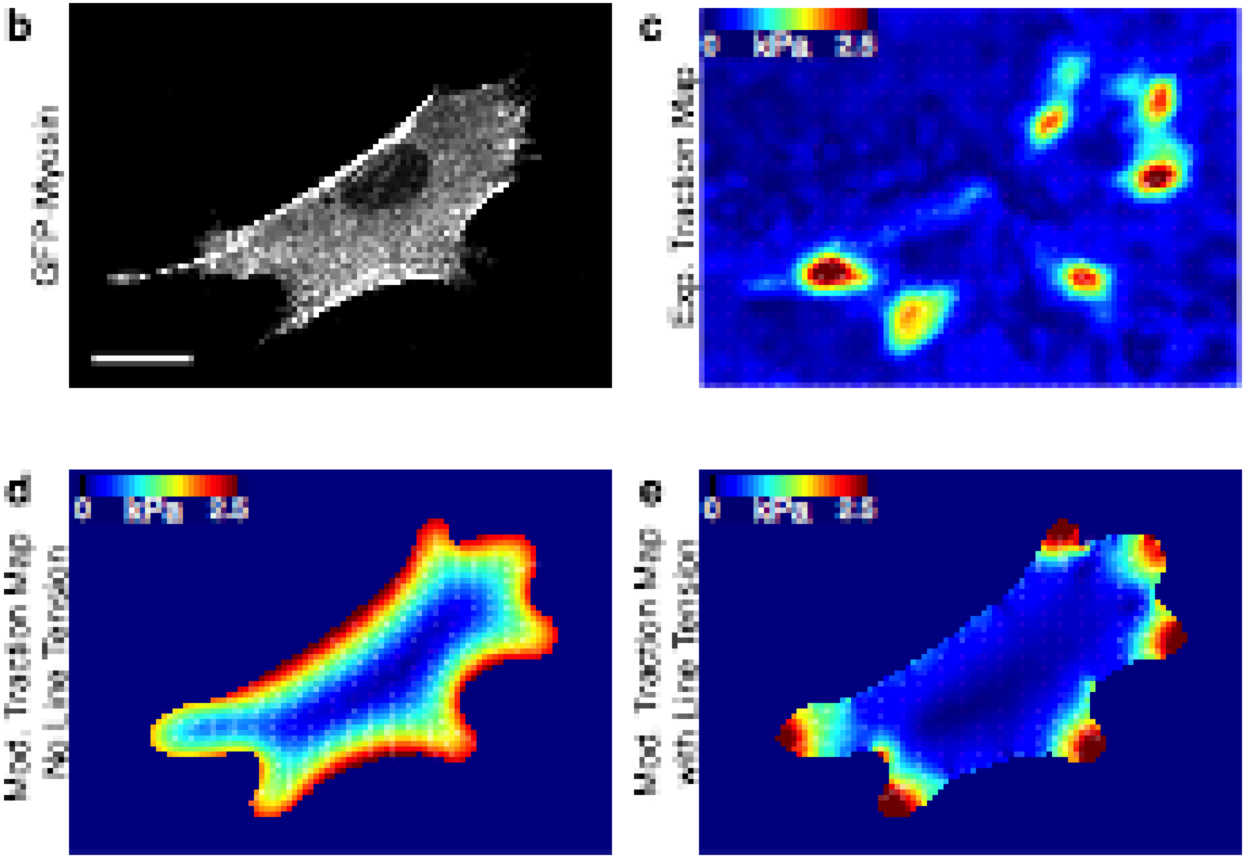
Micropatterns with Constant Area

Figure 4



Unconstrained Fibroblasts

Force Distribution predictions



Concluding Remarks

Single Cells

- *Global Mechanics*: Substrate stiffness, number of focal adhesions and cell shape have little effect on total cellular strain energy.
  Strain Energy of an adherent cell is regulated by its spread area alone, for a particular cell type.

- *Local Mechanics*: Traction stresses are highly sensitive to substrate stiffness, cell shape or adhesion geometry.

Cell Colonies

- Cohesive cell colonies wet the substrate underneath with an effective surface tension.

- Colony surface tension emerges from having strong intercellular adhesions and acto-myosin contractility.

- Cadherin based adhesions organize cell-matrix forces to the periphery of the colony.