Cell volume cell stiffness and cell motion

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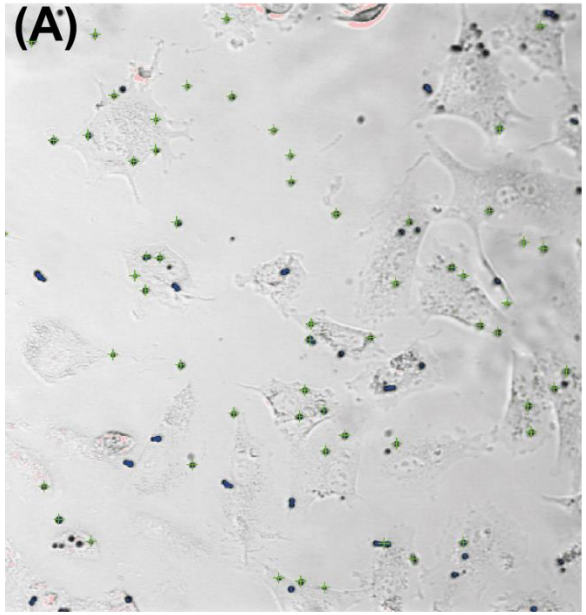
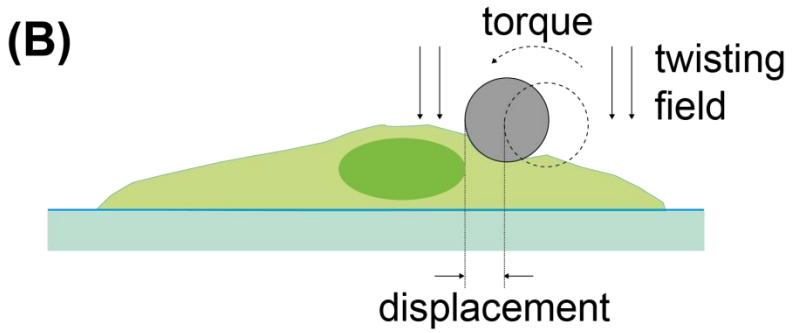
1. Bulk osmotic modulus of cells
2. Substrate stiffness and cell volume
3. Dynamic arrest in motion of cells

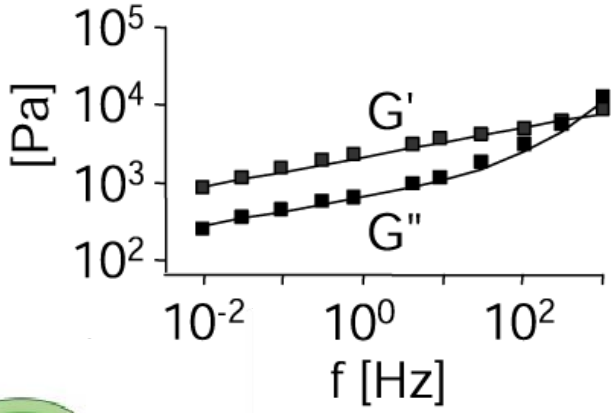
http://weitzlab.seas.harvard.edu/

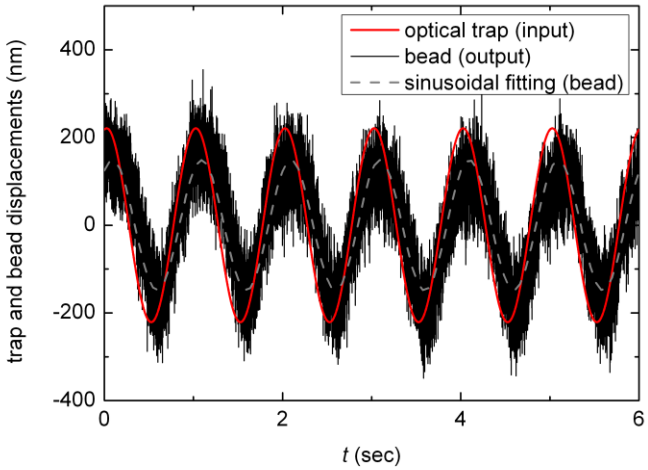
Active Systems
GIST, 6/27/14

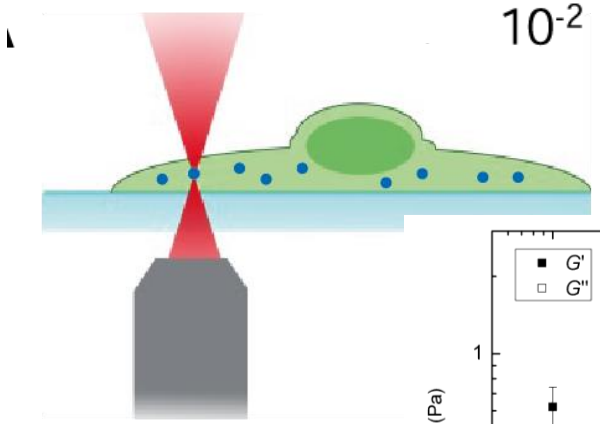
Cell stiffness measurements

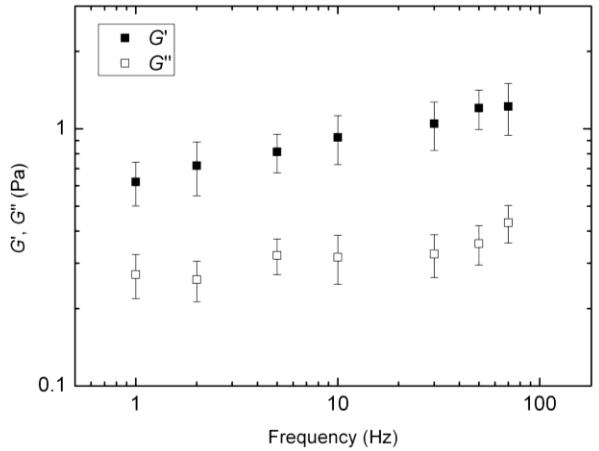












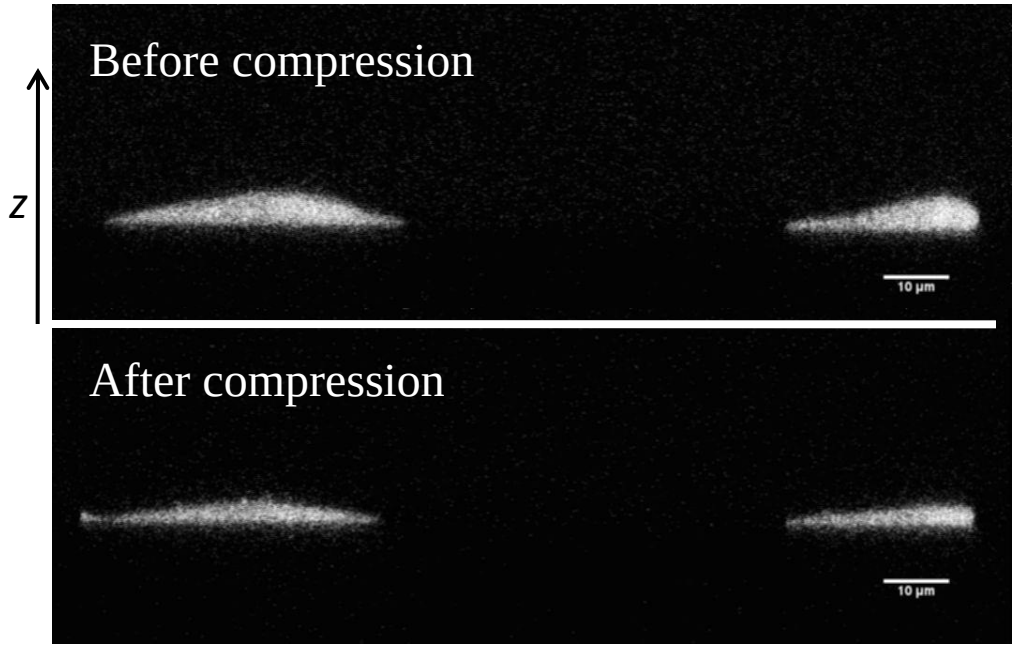
Control of cell volume

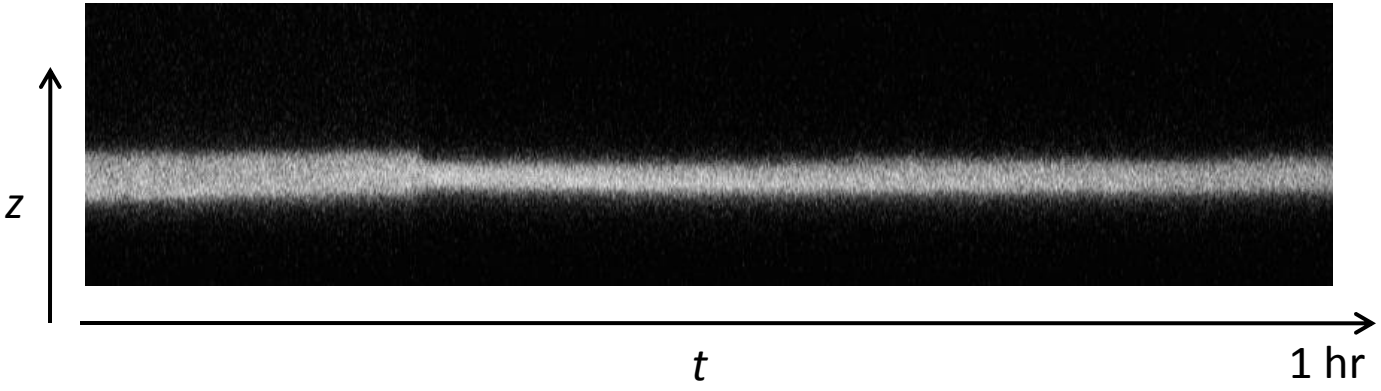
- What controls cell volume
- What are consequences of cell volume

Bulk modulus (osmotic)

- Susceptibility to change in volume
- Not due to compression of water
- Due to change in volume of cell

Compression of cell volume with PEG

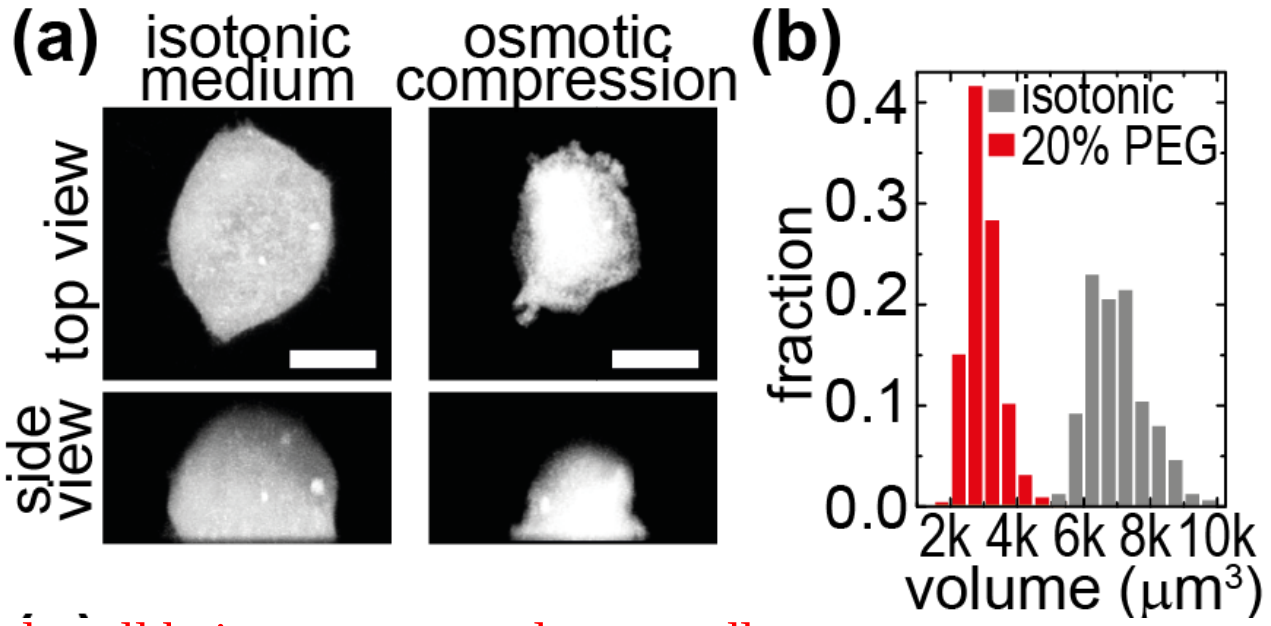




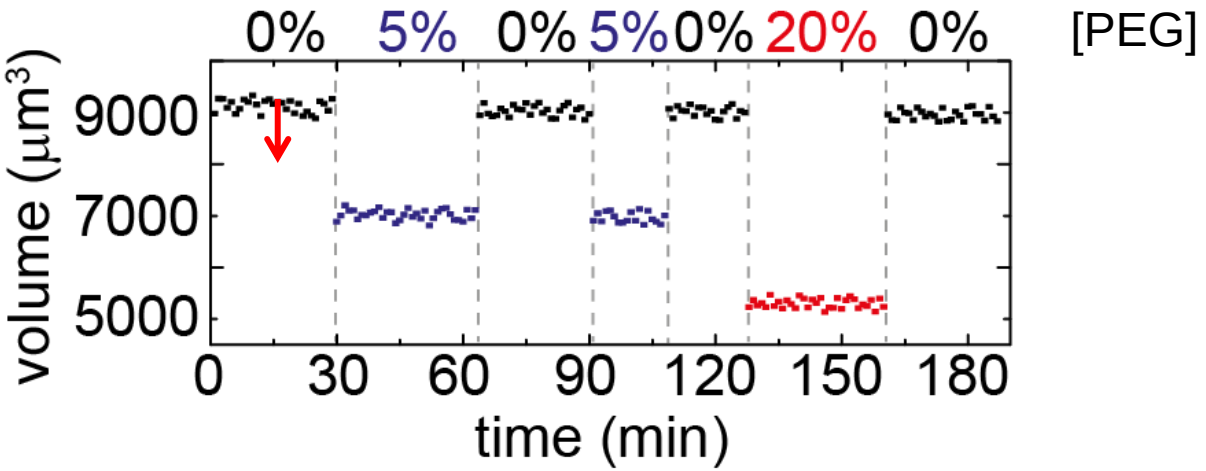
Kymograph

Cell volume responds immediately to change in osmotic pressure

Cell volume controlled by osmotic pressure (PEG)



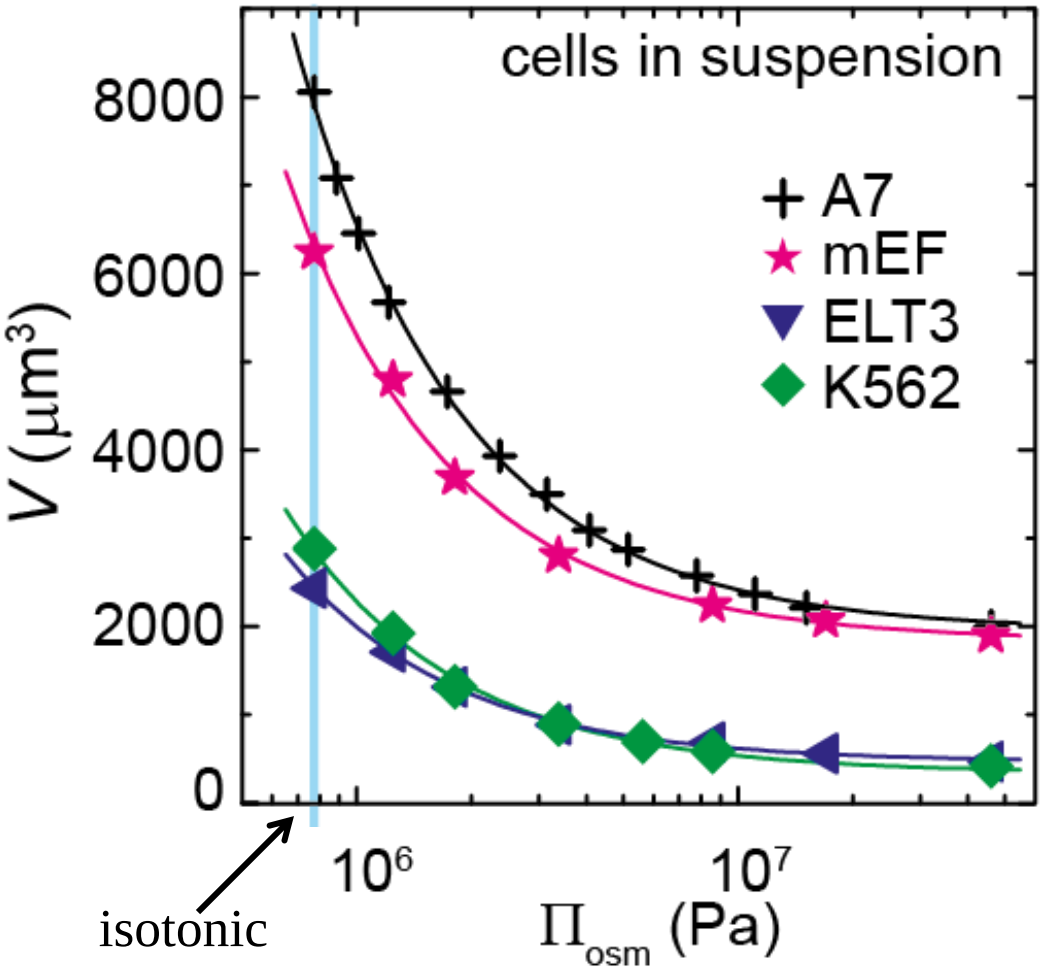
One single cell being compressed repeatedly.



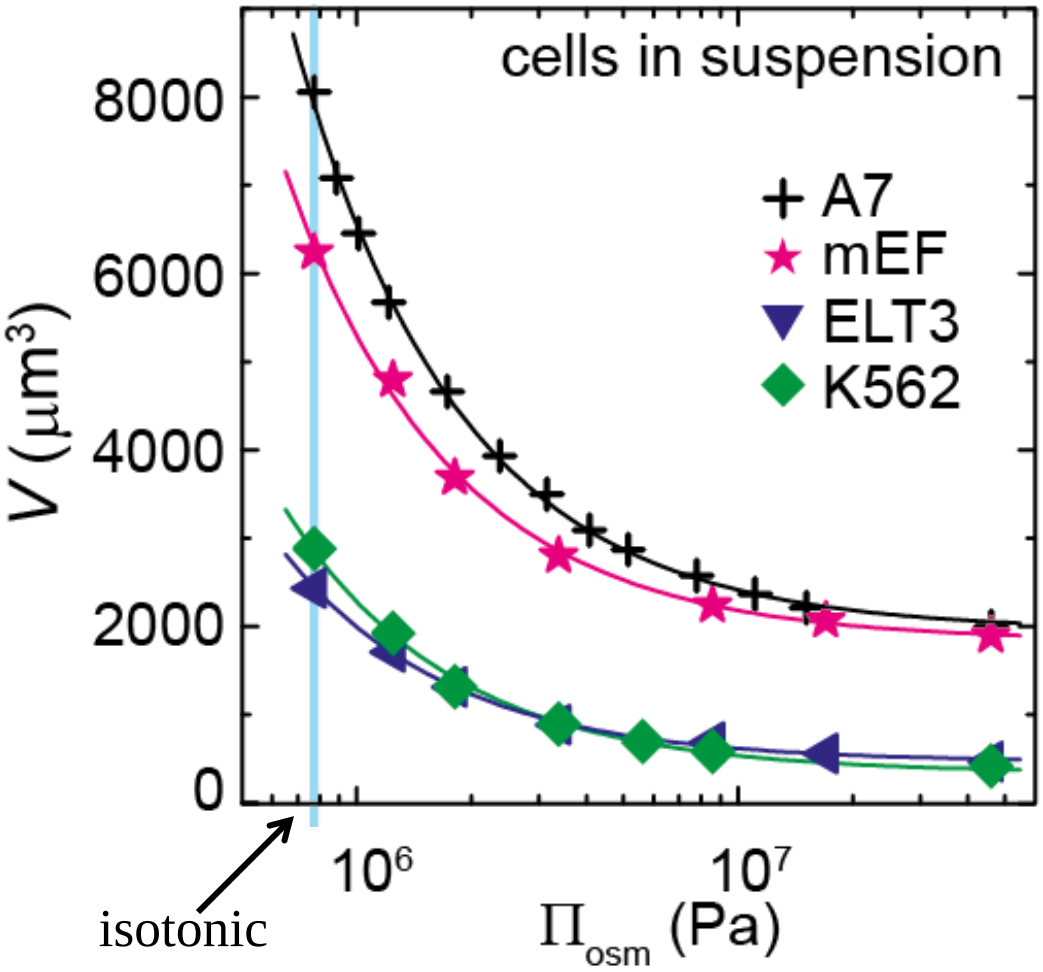
Control of cell volume

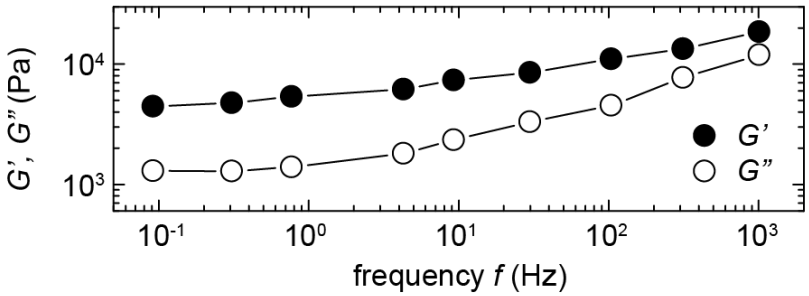
- Water eflux through membrane
- Depends on osmotic pressure

Π-V relationship



Π-V relationship





Shear modulus is orders of magnitude less that Π

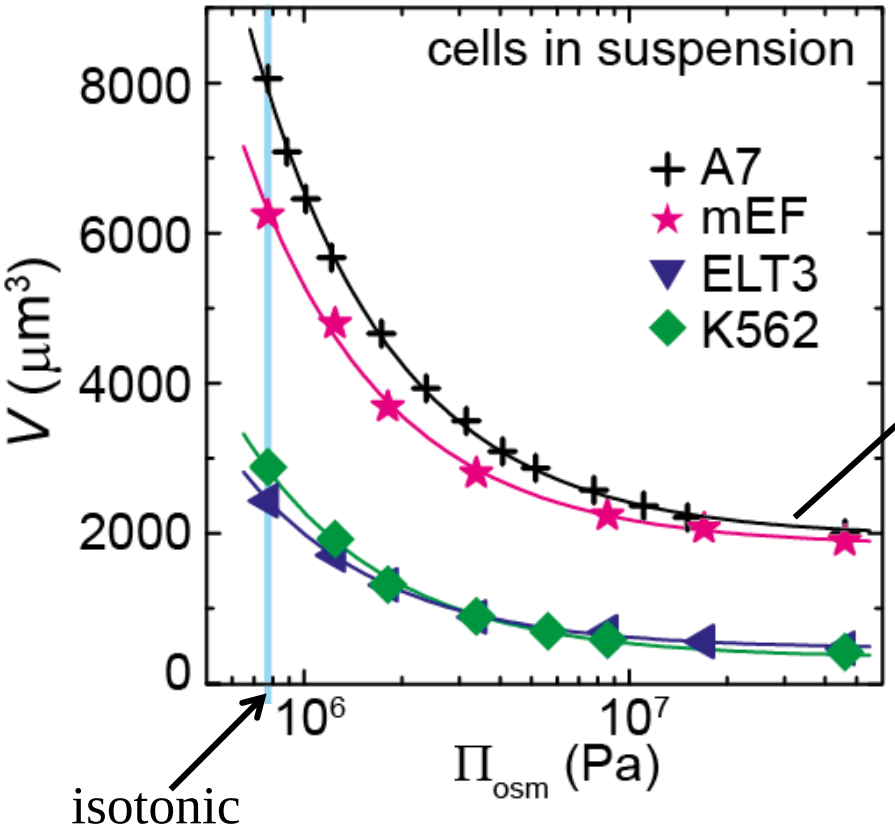
→ No pressure drop across membrane

Control of cell volume

- Water eflux through membrane
- Depends on osmotic pressure
- Stresses on membrane ~ shear modulus
- Too weak to support pressure difference
- No pressure drop across membrane

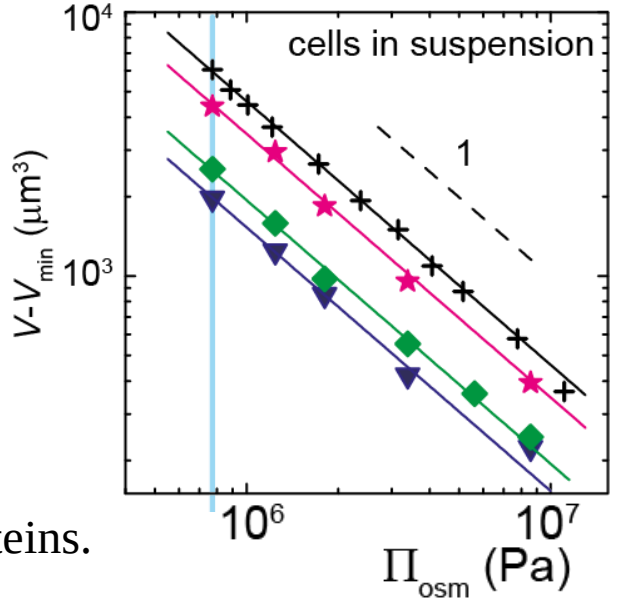
→ Pressure inside = pressure outside

Equation of State: van der Waals for hard spheres



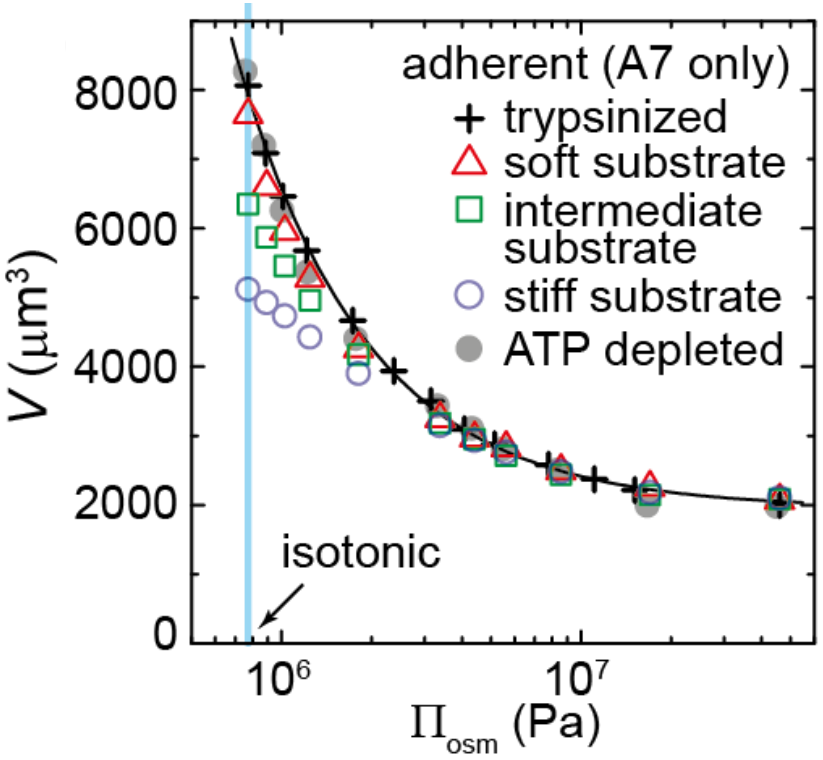
Fitted lines: $\Pi = \frac{Nk_BT}{V - V_{\min}}$.

log-log plot

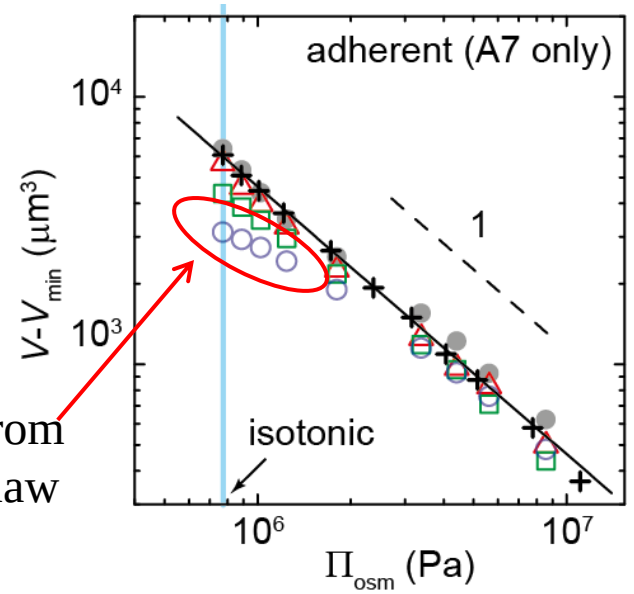


The equation of state of cells is dominated by intracellular ions, while $V_{\min}$ is occupied by cellular materials, mainly proteins.

Substrate stiffness dependence of equation of state

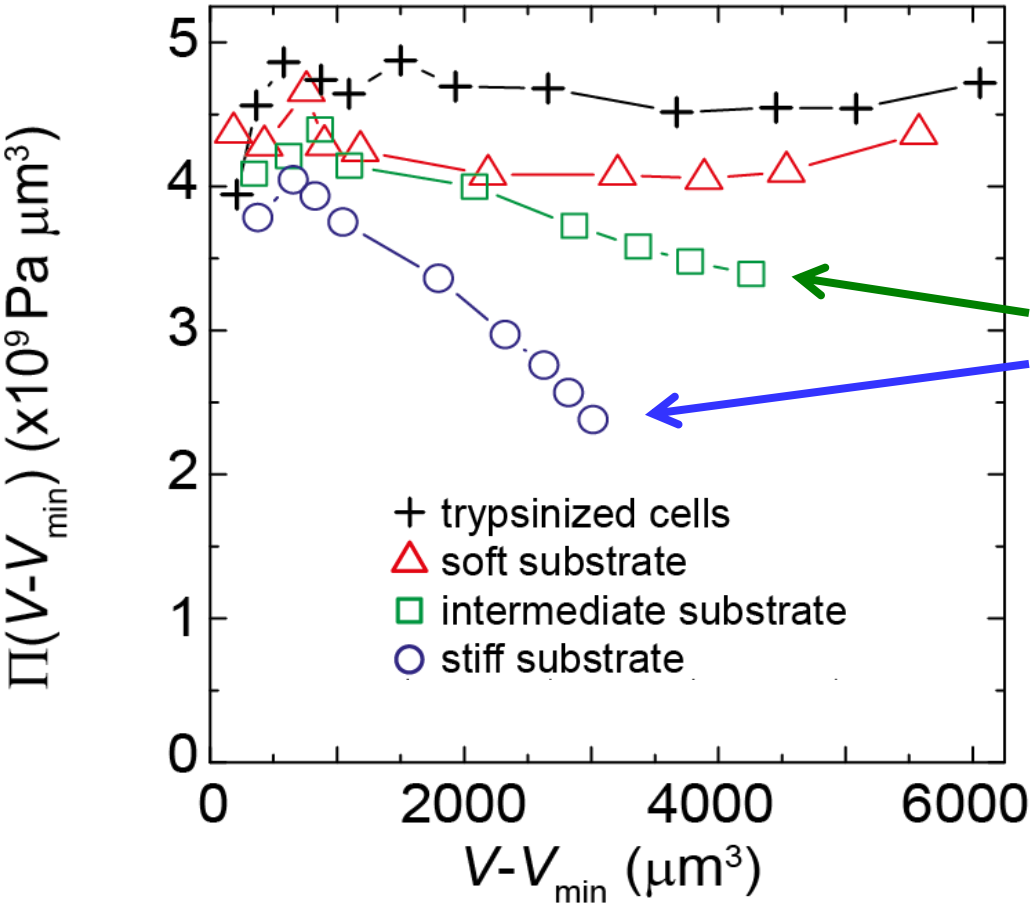


log-log plot



Deviation from VDW gas law

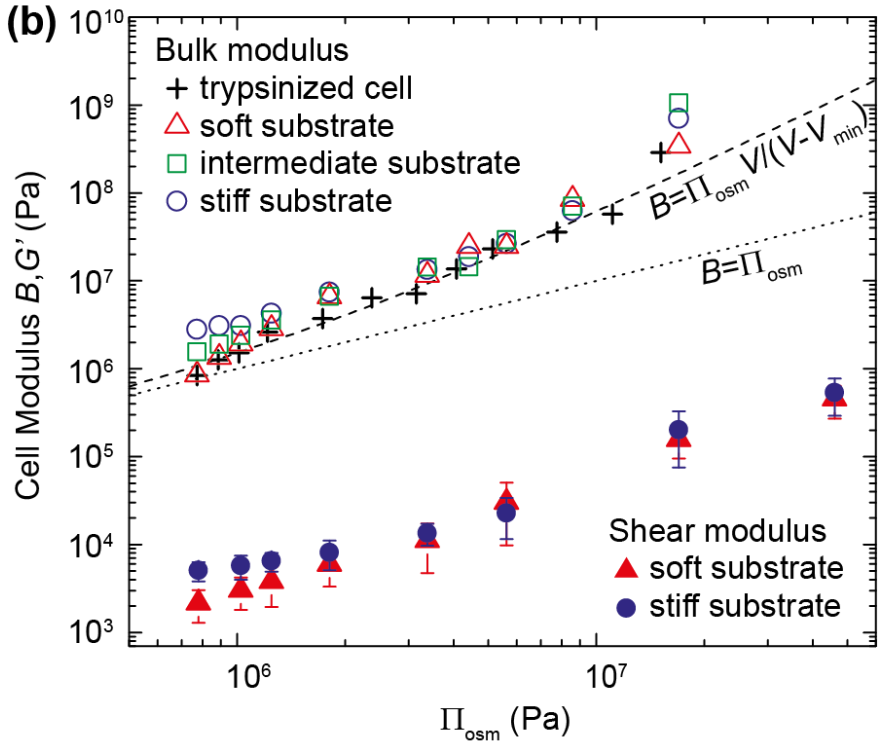
Number of intracellular osmolytes



Volume is regulated on stiffer substrates

The concentration of osmolyte estimated is on the order of 10^2 mM; the number agrees with intracellular ions measured experimentally.

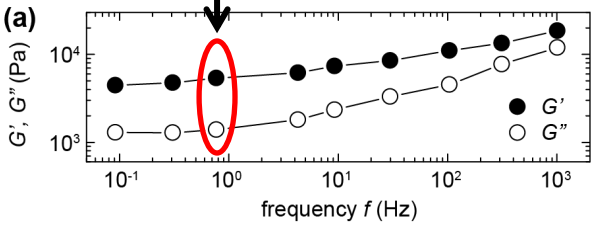
Bulk (osmotic) modulus of cells



Bulk modulus

$B=-V\mathrm{d}P/\mathrm{d}V$

Shear modulus measured with OMTC at 0.75 Hz



- Bulk modulus B >> shear modulus G
- Cytoskeleton plays negligible role

Cell volume is strongly regulated

→ What are the consequences

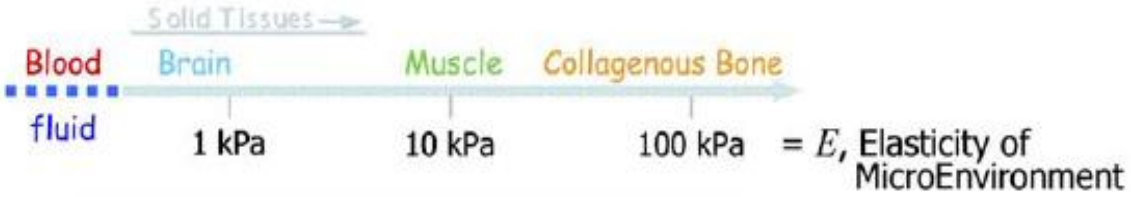
Cells have many different stiffnesses

Neurons Fat Muscle Bone

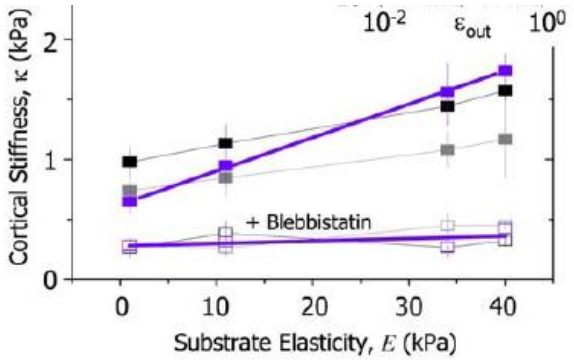
Cell Stiffness → 

Cell stiffness depends on stiffness of environment

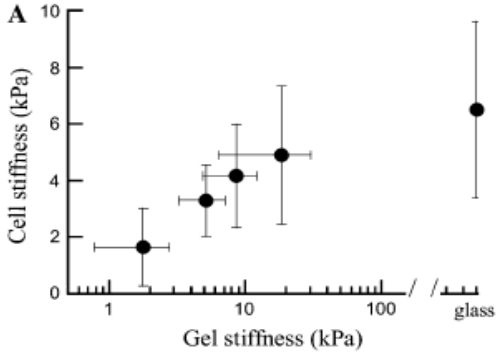
Stiffness of environment:



Cell Stiffness:



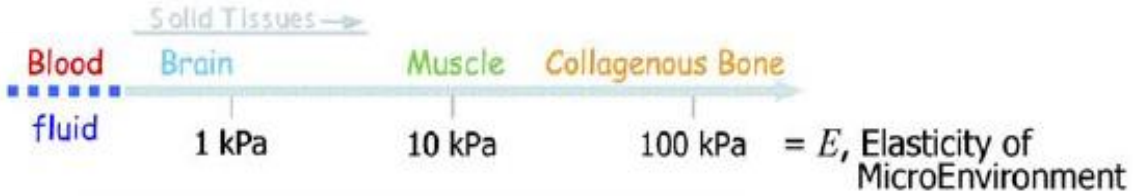
hMSC, Discher group. *Cell* 2006



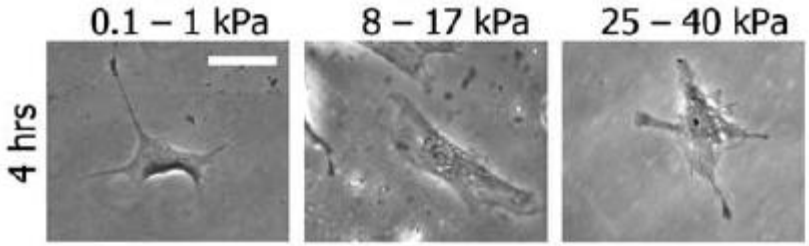
Fibroblast, Janmey group. *Biophysical J* 2007

Stem-cell fate depends on stiffness of environment

Stiffness of environment:



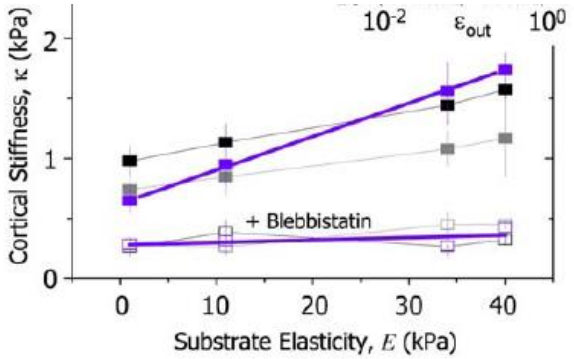
Cell Fate:



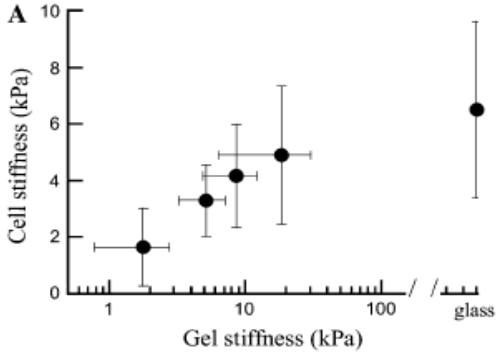
Discher group. *Cell* 2006

Stem cell differentiation

Cell Stiffness:



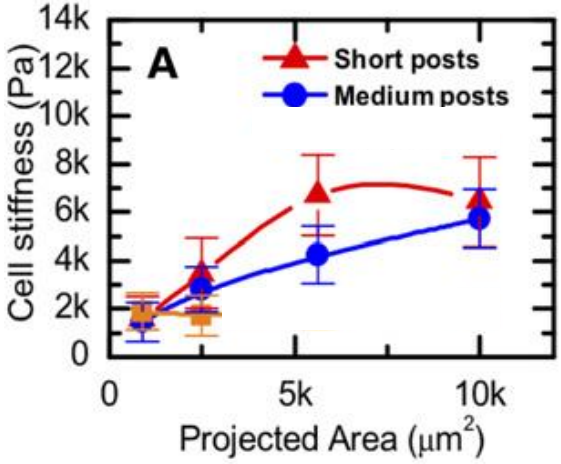
hMSC, Discher group. *Cell* 2006



Fibroblast, Janmey group. *Biophysical J* 2007

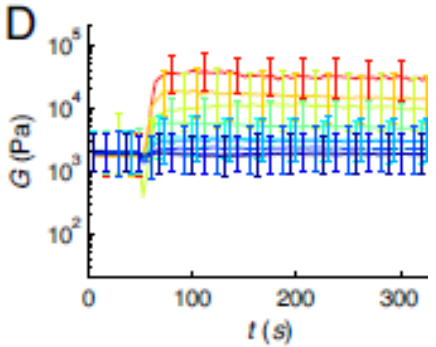
Cells adjust stiffness to their environments

Cell stiffness changes when growing on adhesive patterns with various sizes.



Tee et al. *Biophysical J* (2011)

Cell stiffness changes with osmolarity.

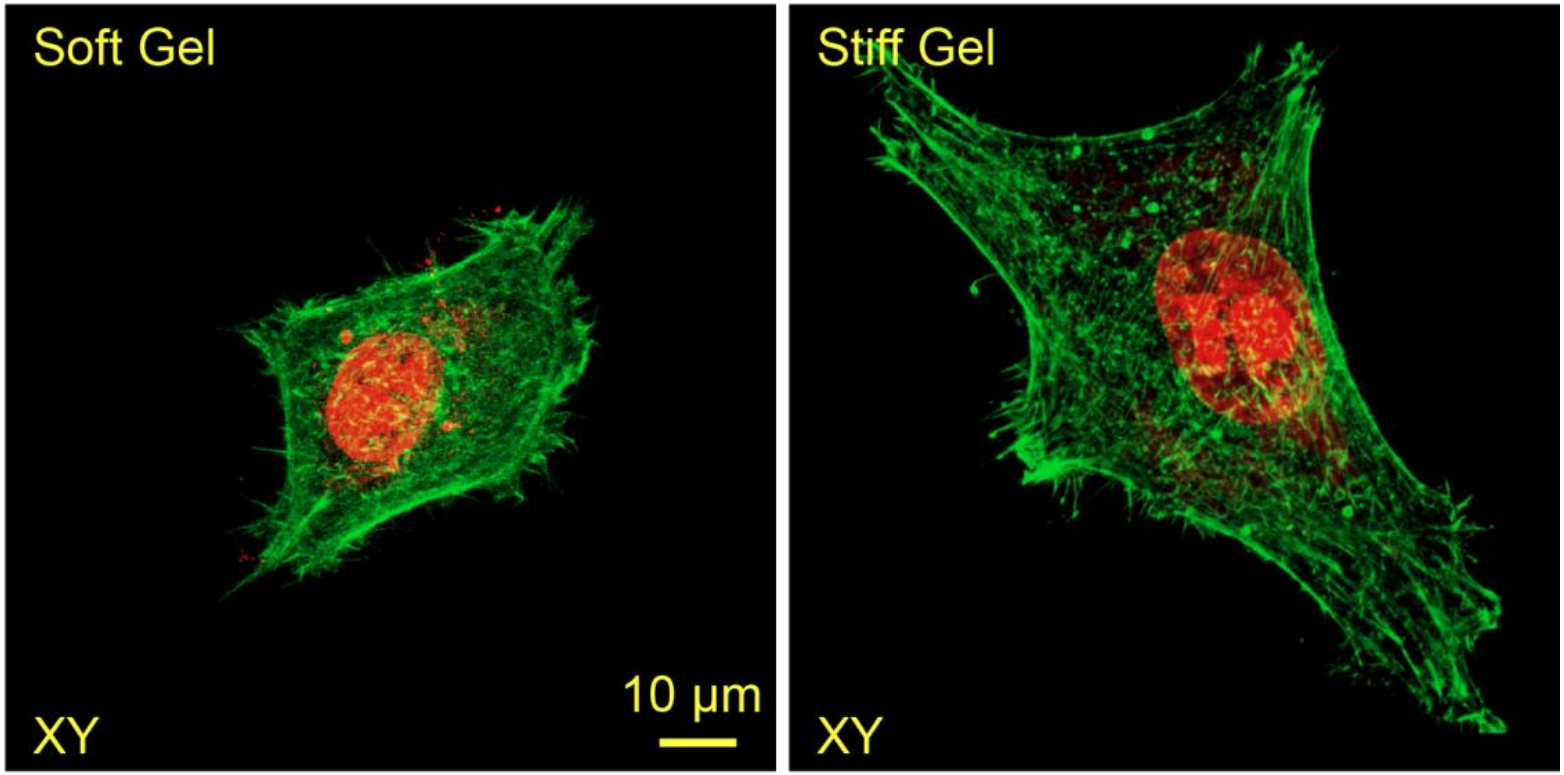


Zhou et al. *PNAS* (2009)

Why does cell stiffness change?
What control parameters are involved?

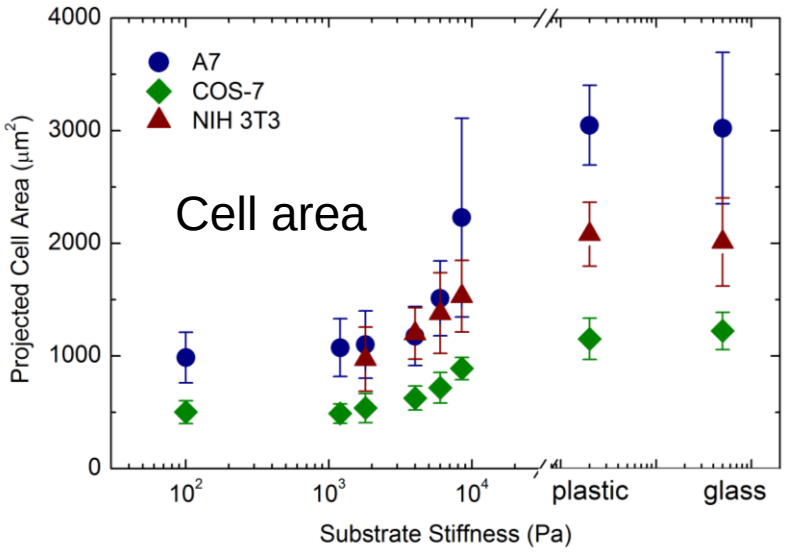
Cell morphology

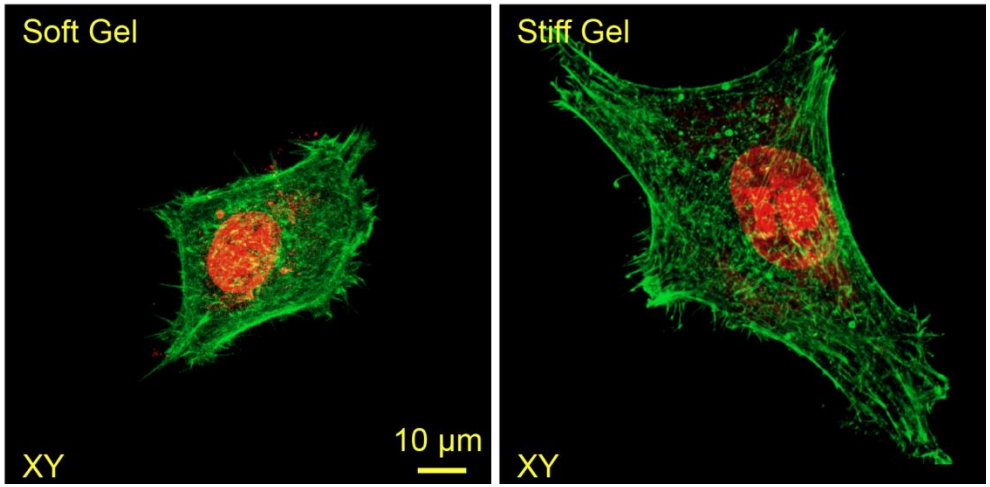
Cell area changes with substrate stiffness



Projected area of cell increases with substrate stiffness.

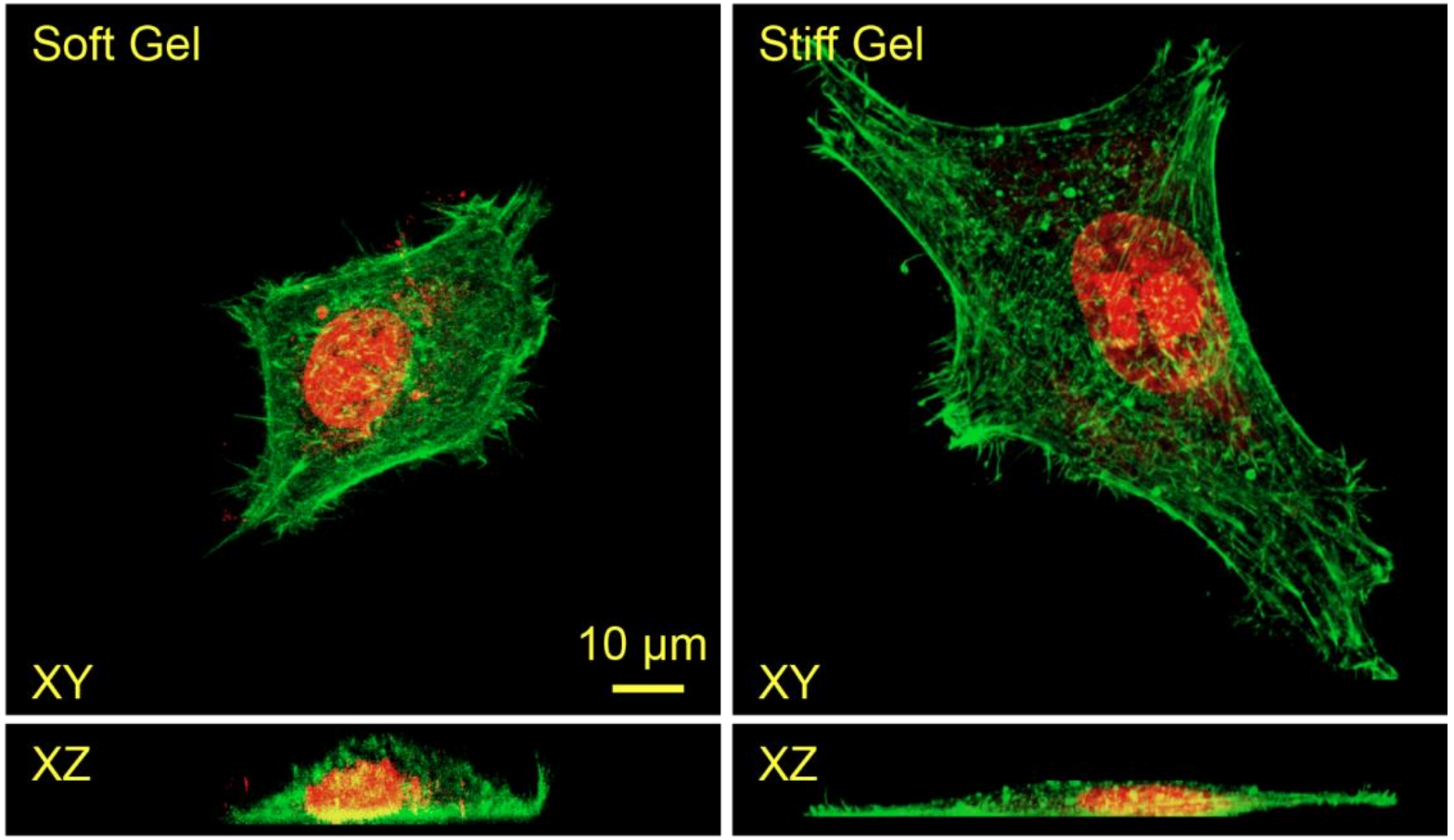
Cell area increases with substrate stiffness





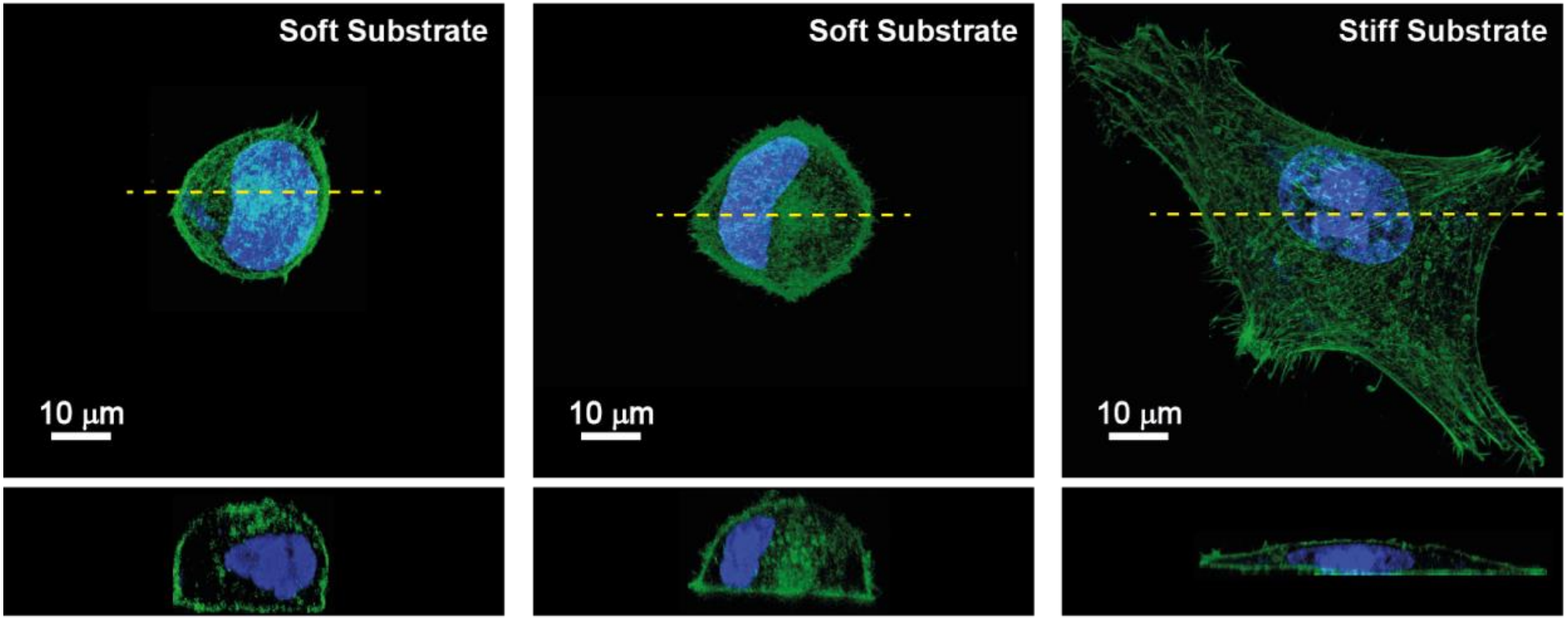
Projected cell area increases with increasing substrate stiffness.

Cell height decreases with substrate stiffness



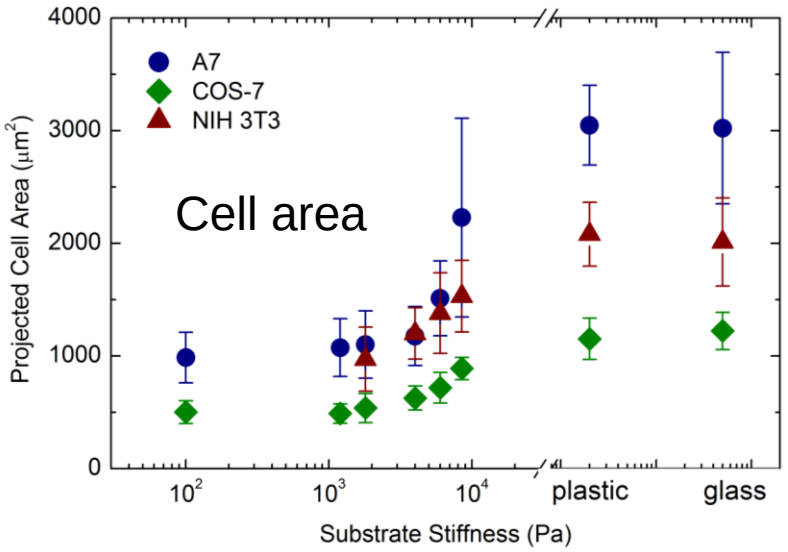
While the projected cell area increases with increasing substrate stiffness, the cell height decreases.

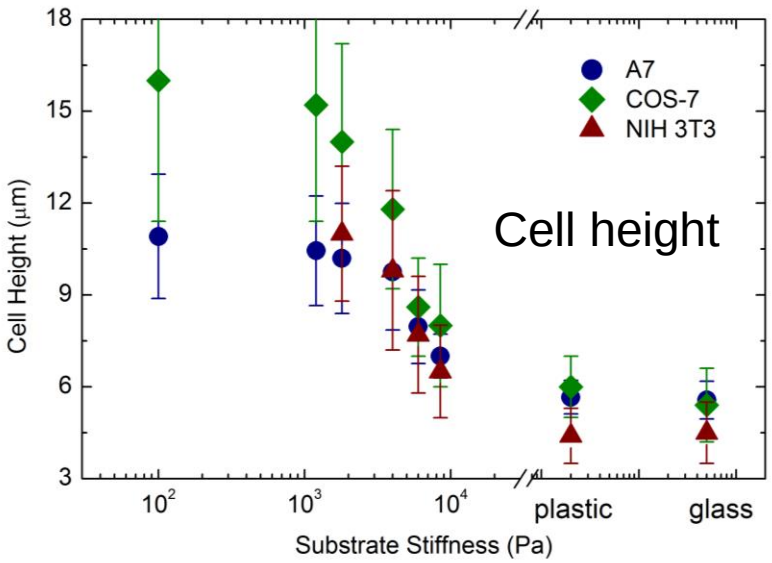
Cell height decreases with substrate stiffness

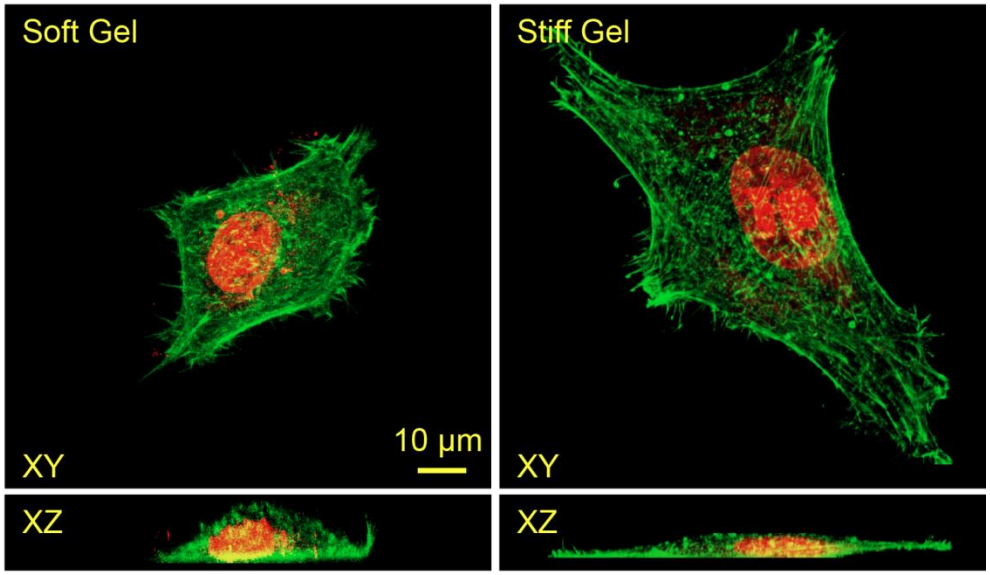


Discrete slice through cell

Cell height decreases with substrate stiffness

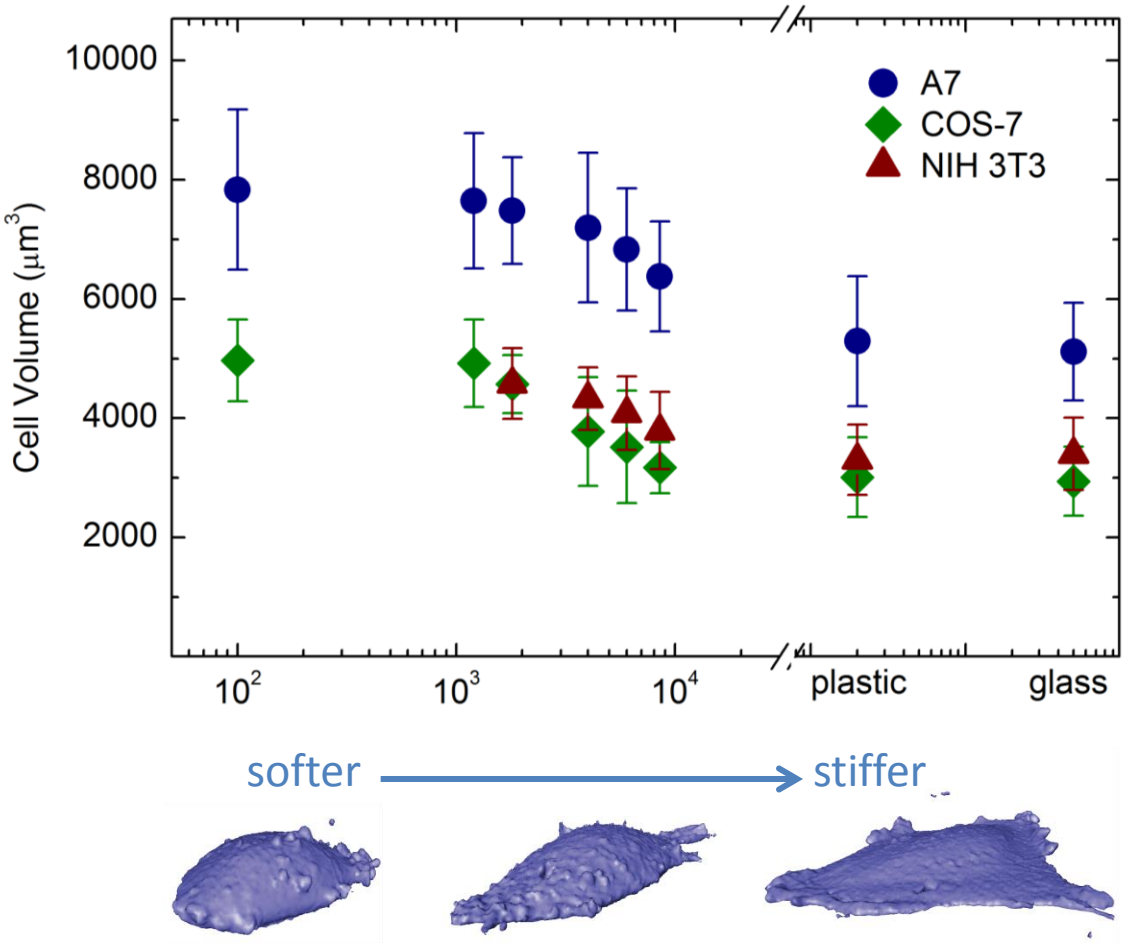




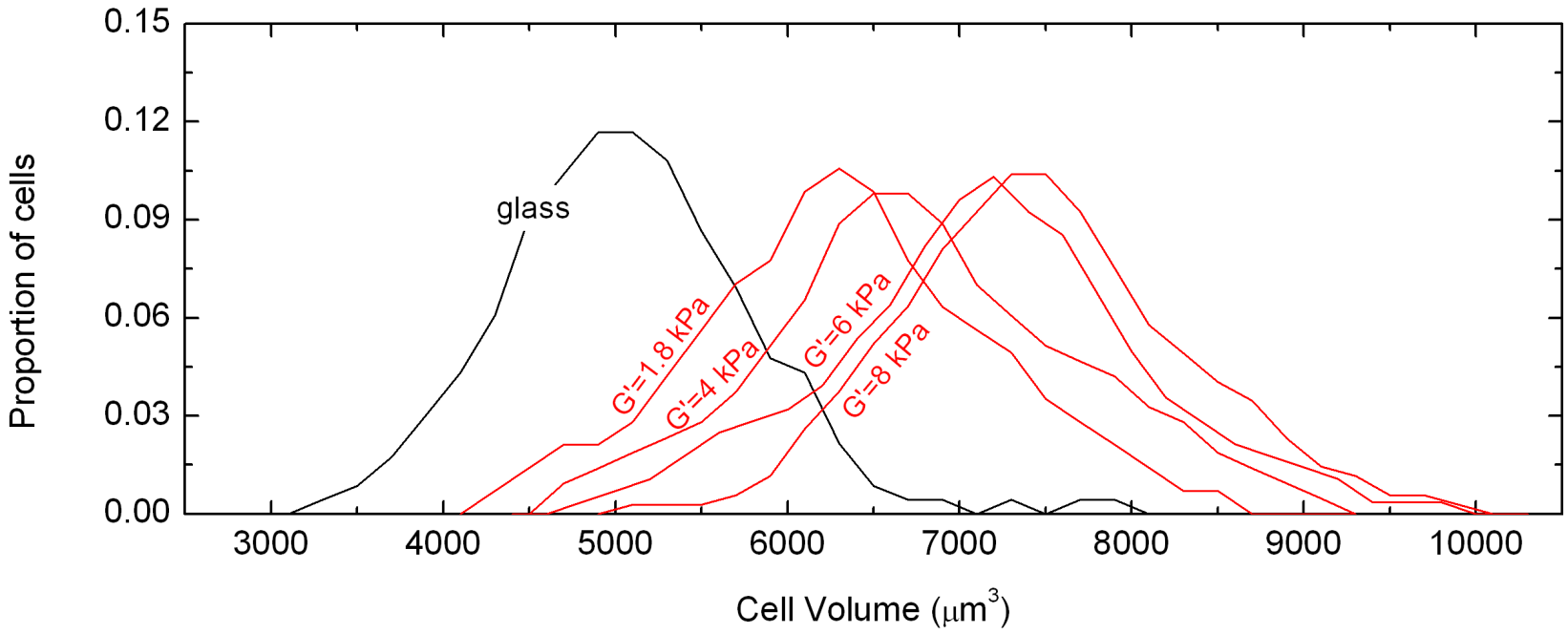


While the projected cell area increases with increasing substrate stiffness, the cell height decreases.

Cell volume decreases with substrate stiffness

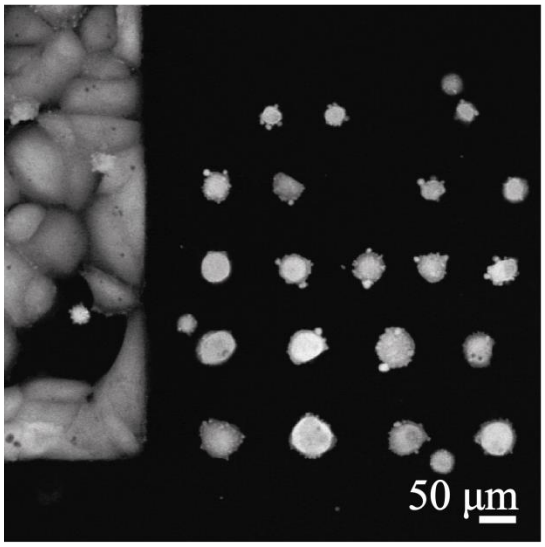


A7 Cell size distribution



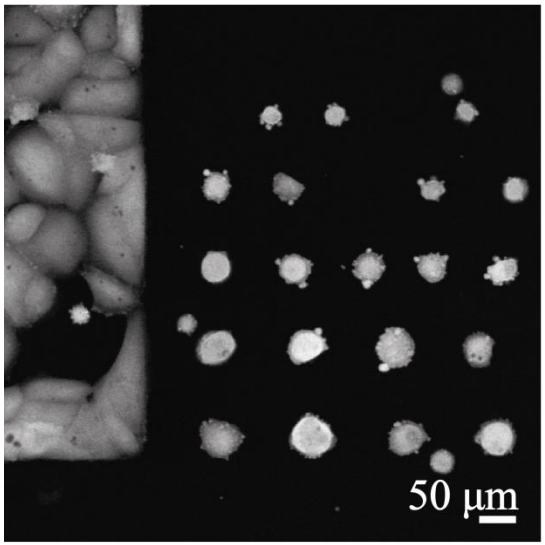
~200 cells per distribution

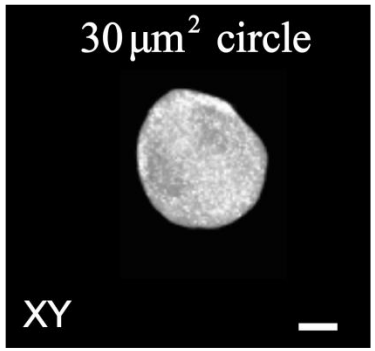
Control cell spreading by adherence area

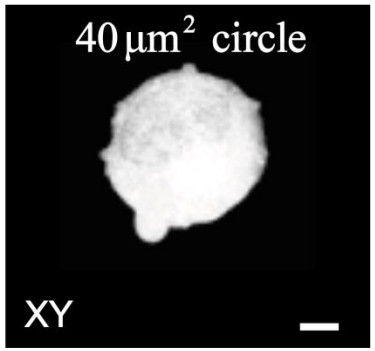


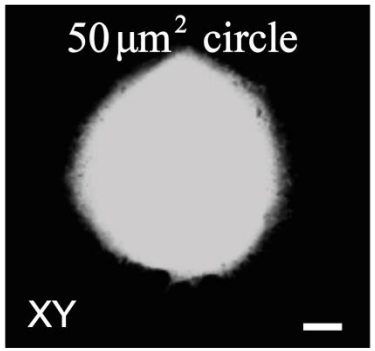
Contact printing of adhesion proteins

Control cell spreading by adherence area



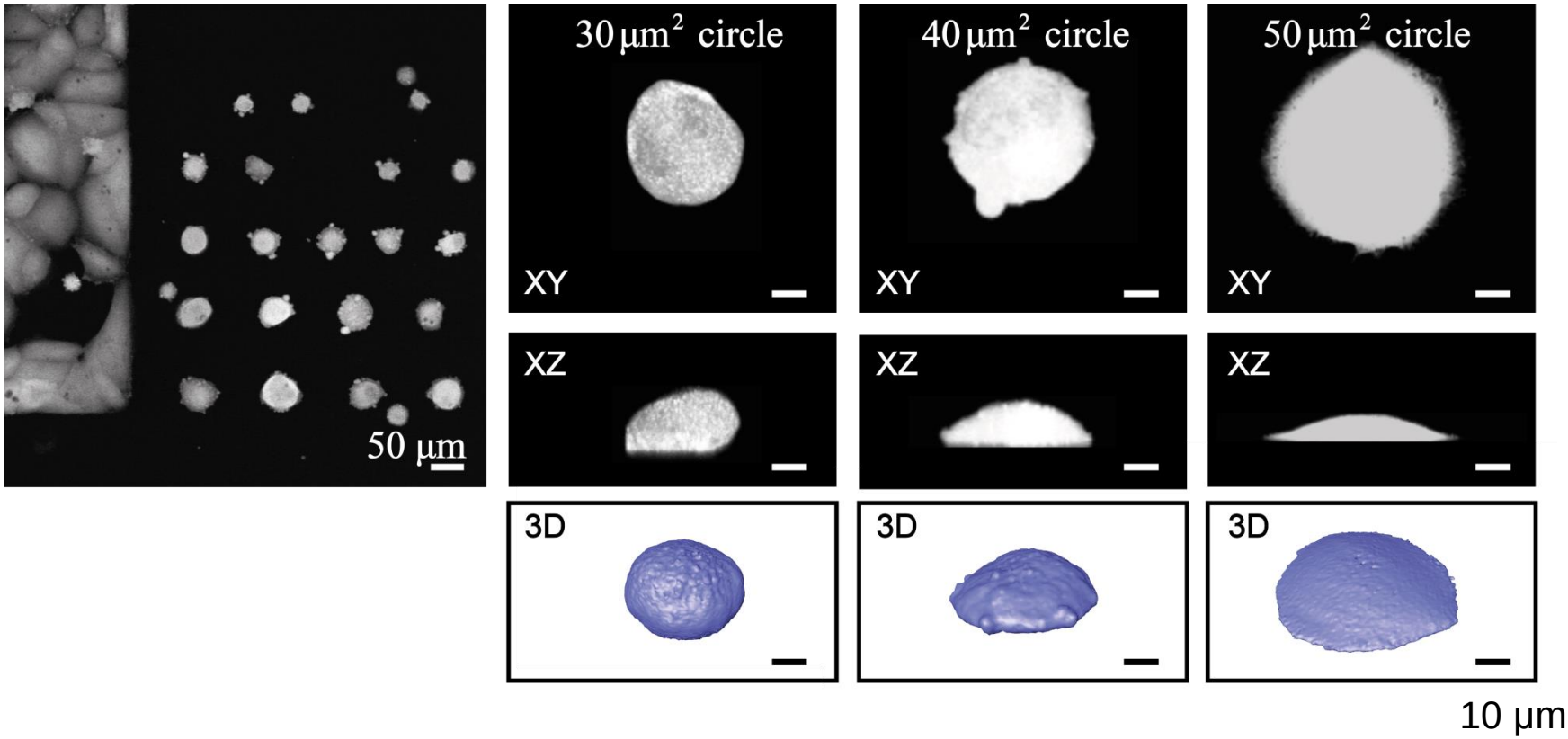




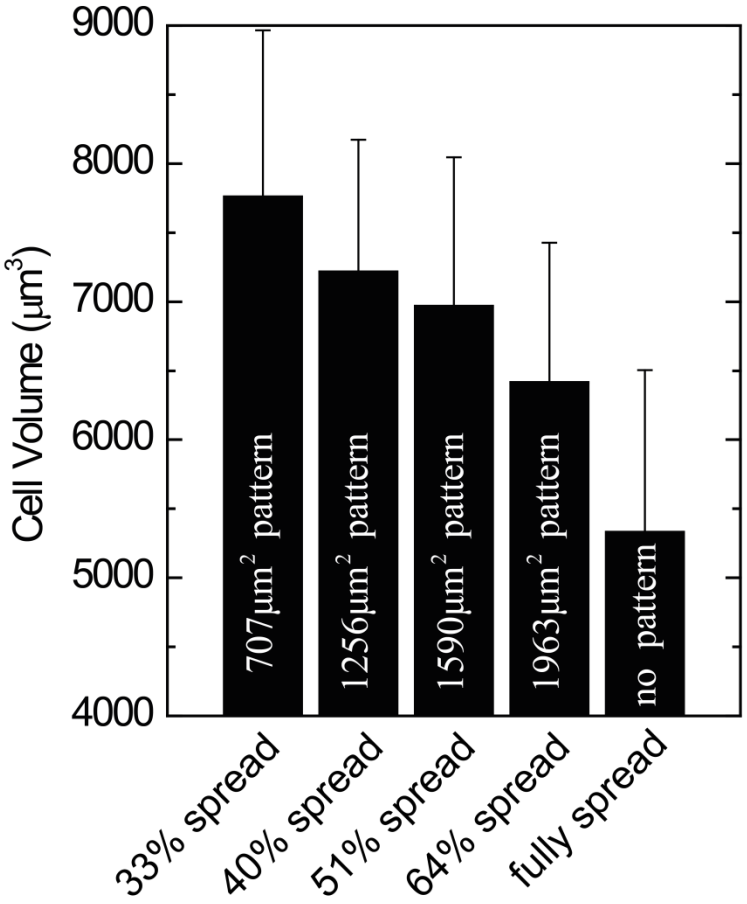


Cells spread to cover only printed area

Control cell spreading by adherence area

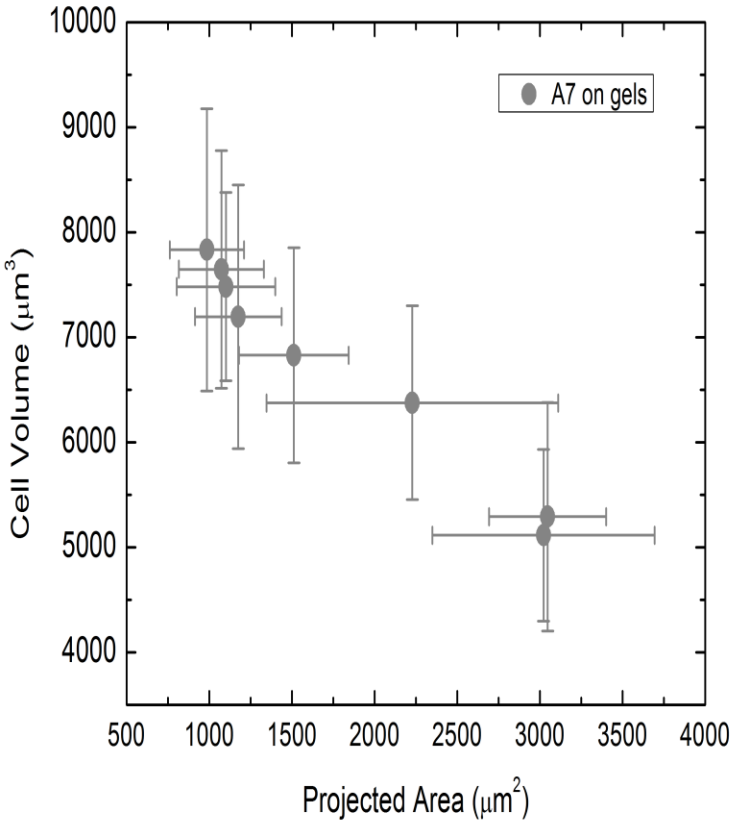


Cell volume decreases with area

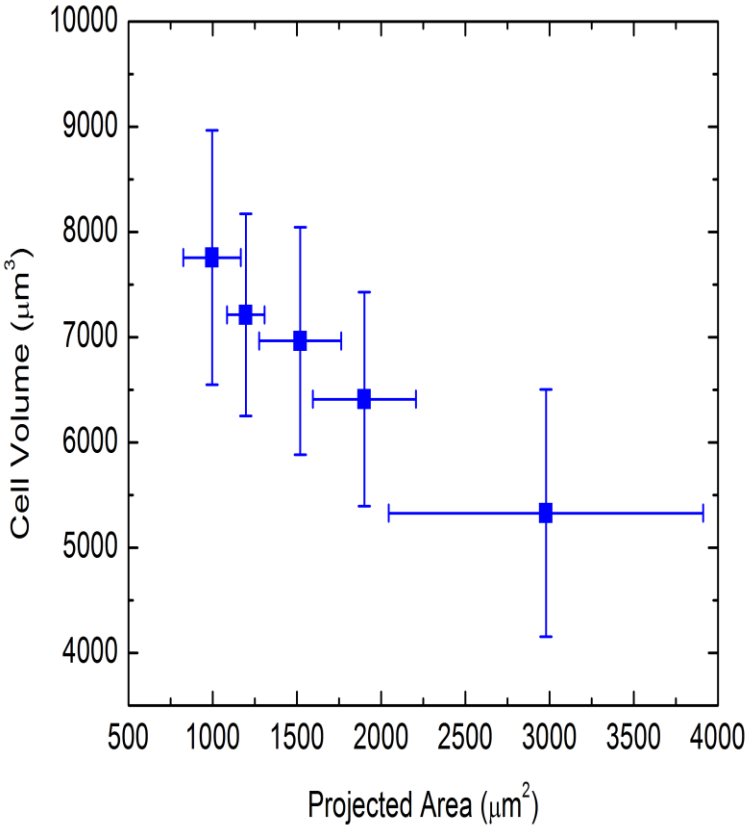


Dependence of cell volume on cell area

Control substrate stiffness

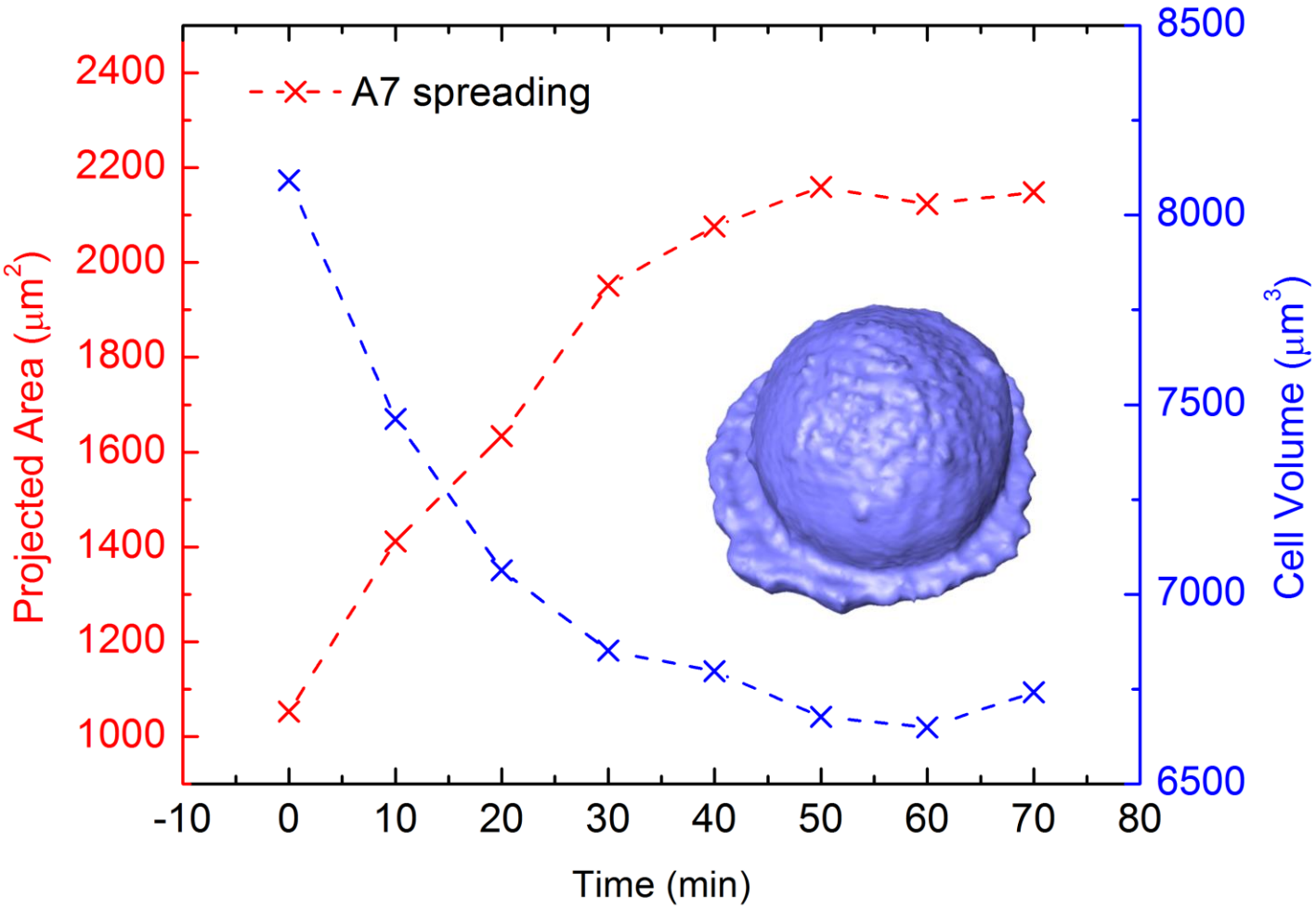


Control adhesive area

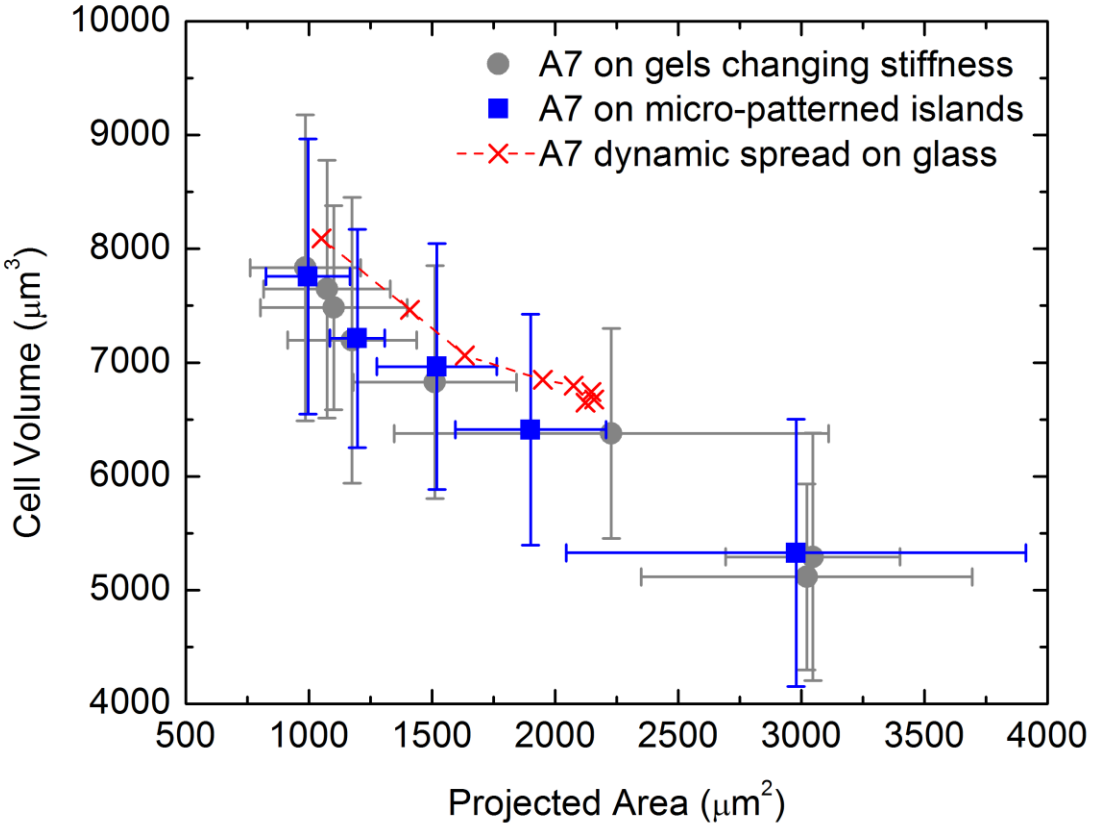


Dynamics of cell volume changes

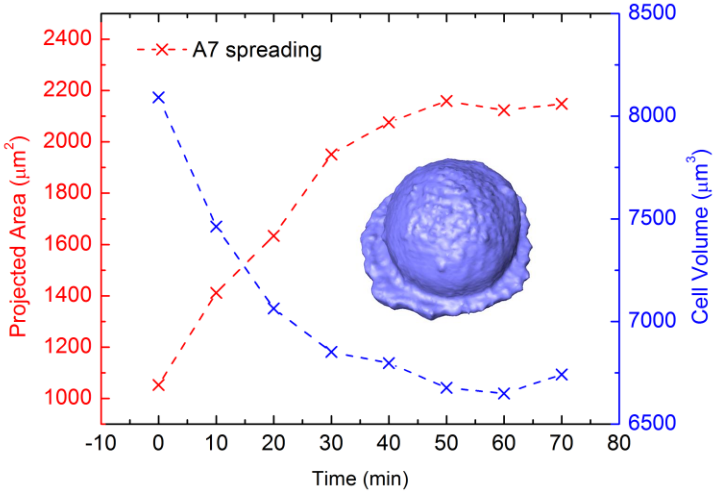
Spreading of a single cell



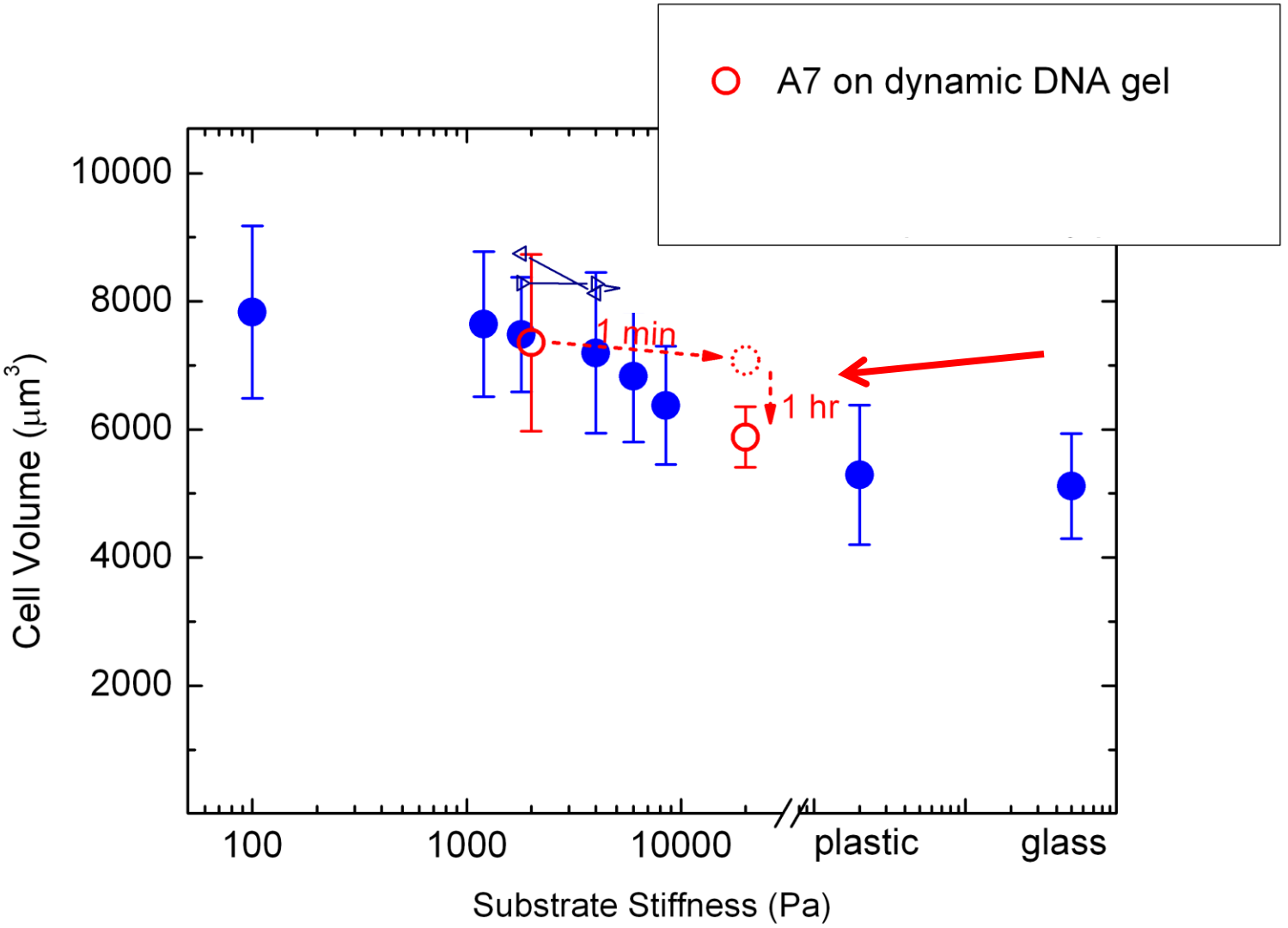
Dynamics of cell volume changes



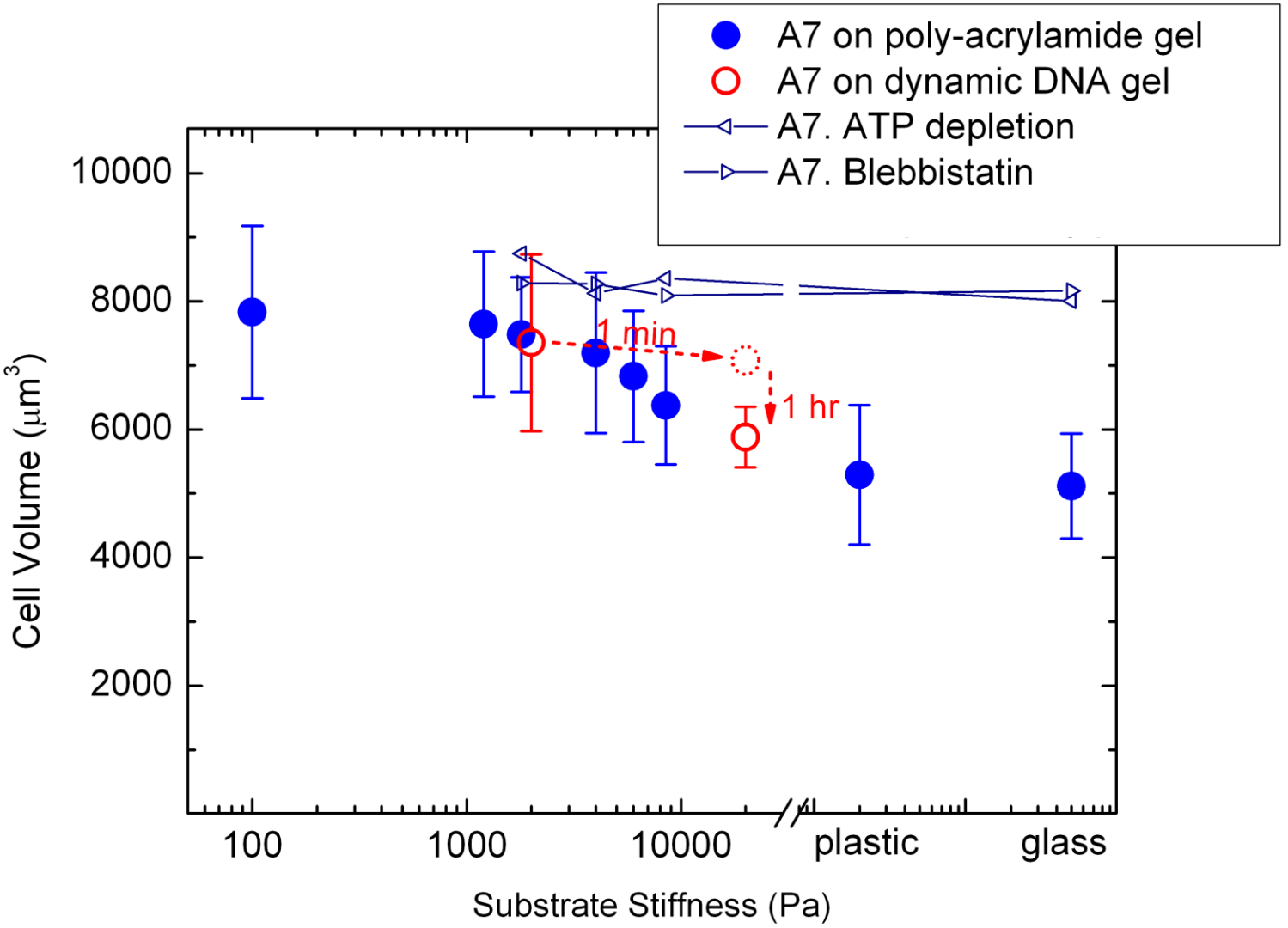
Spreading process of a single cell



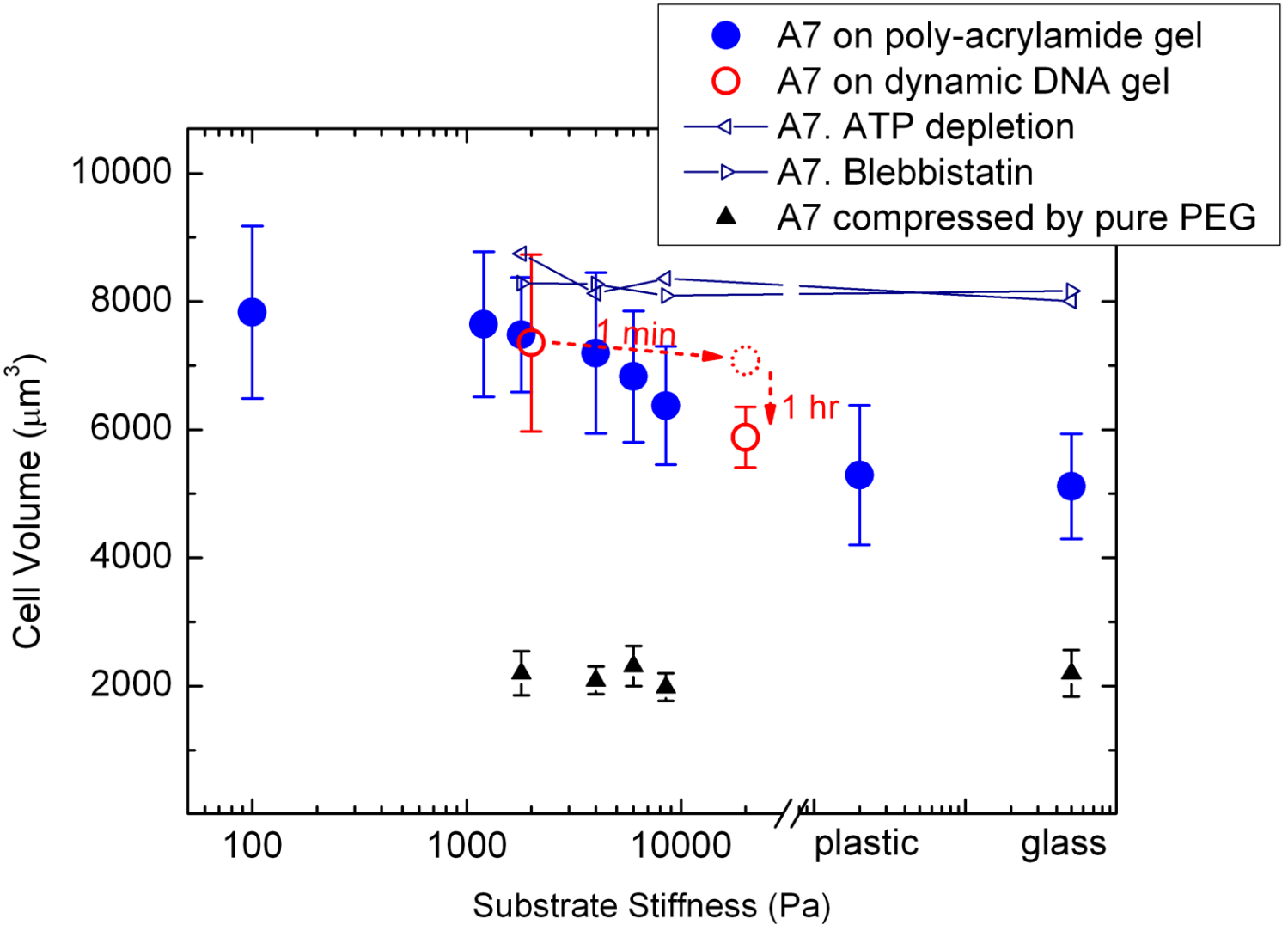
Dynamics of stiffness adjustment



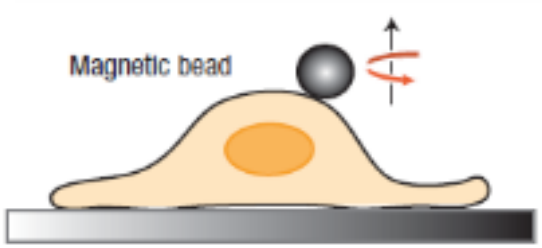
Effect of molecular motors

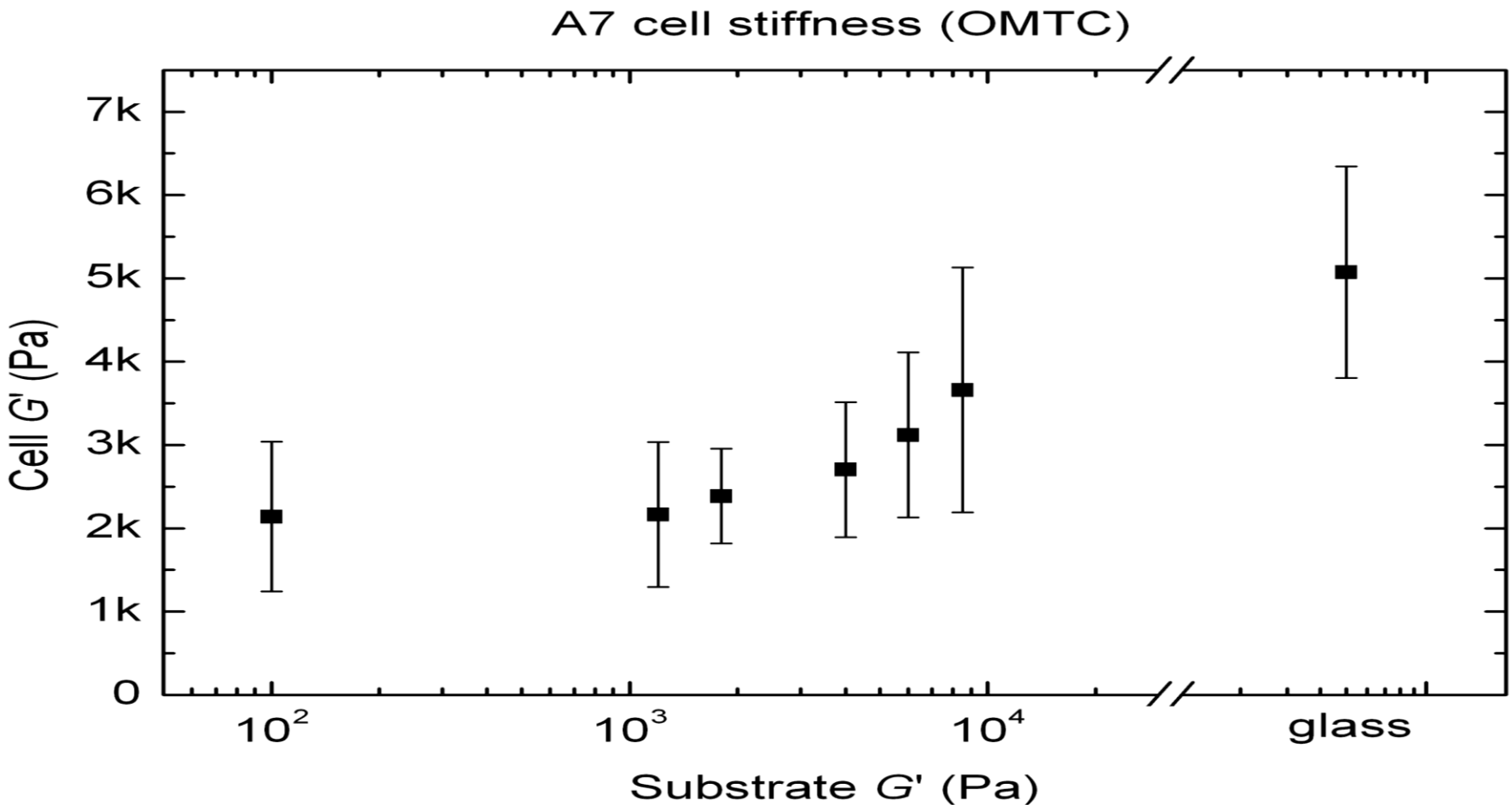


Depends on water content of cell

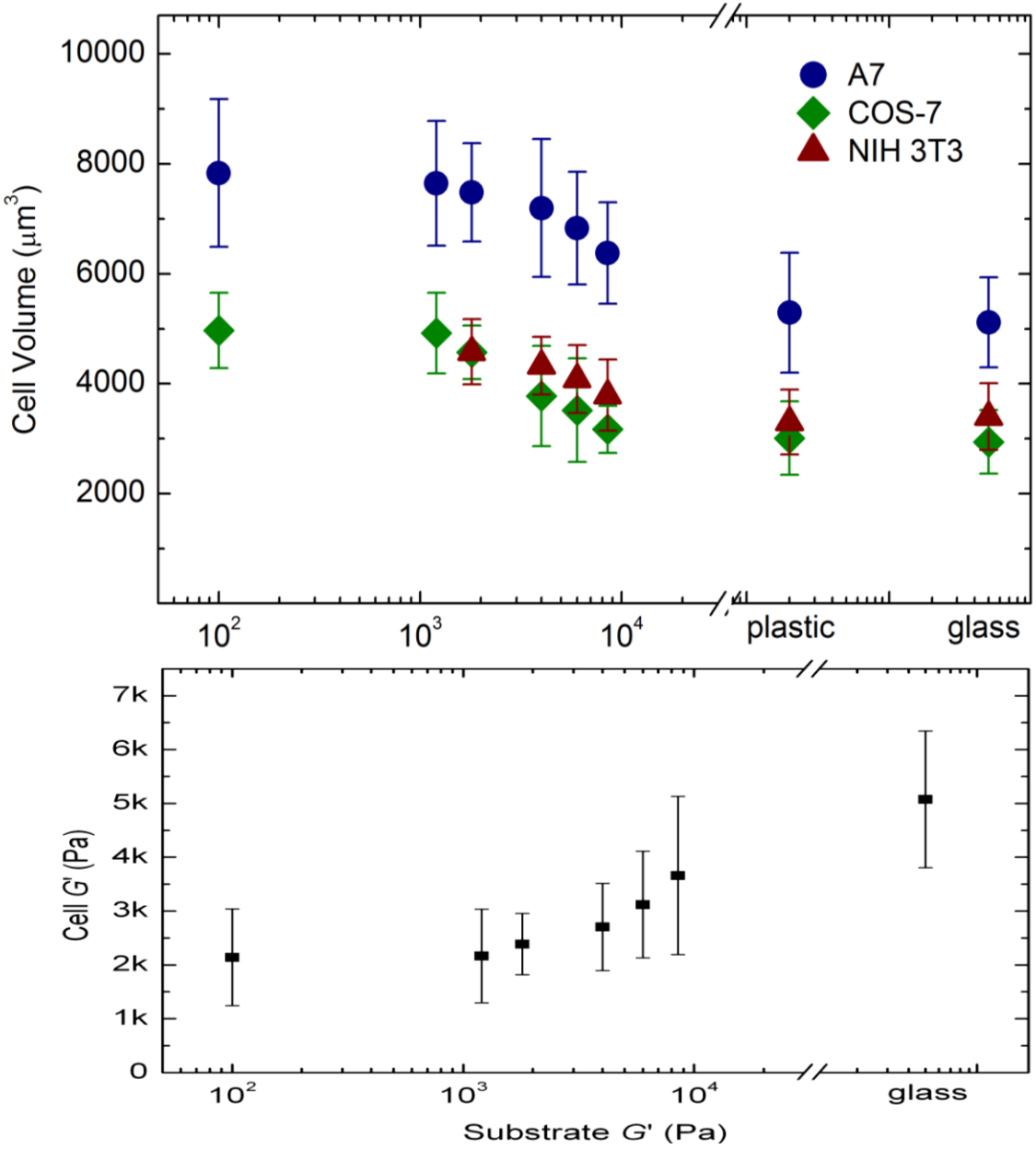


Cell stiffness depends on substrate stiffness

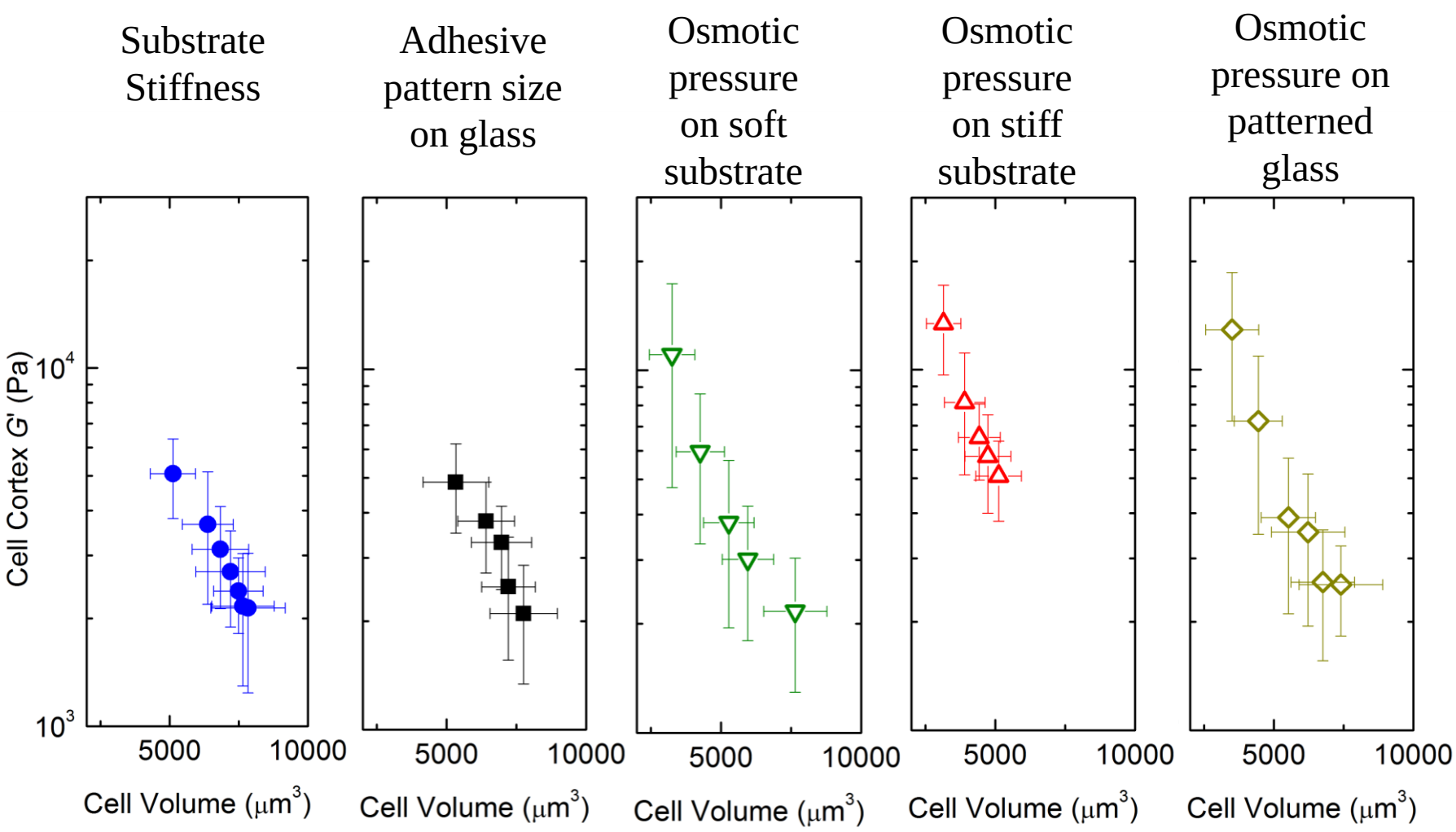




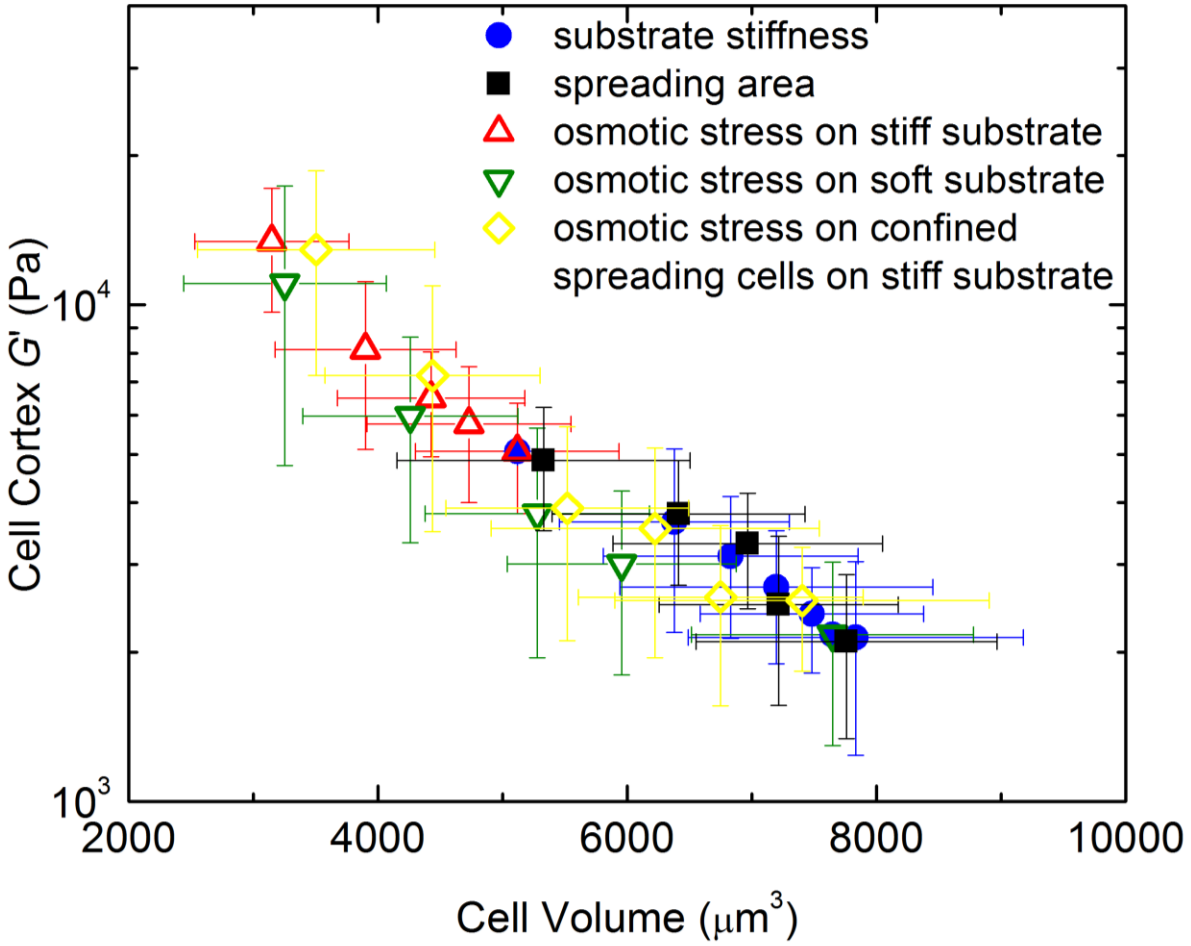
Cell stiffness depends on substrate stiffness



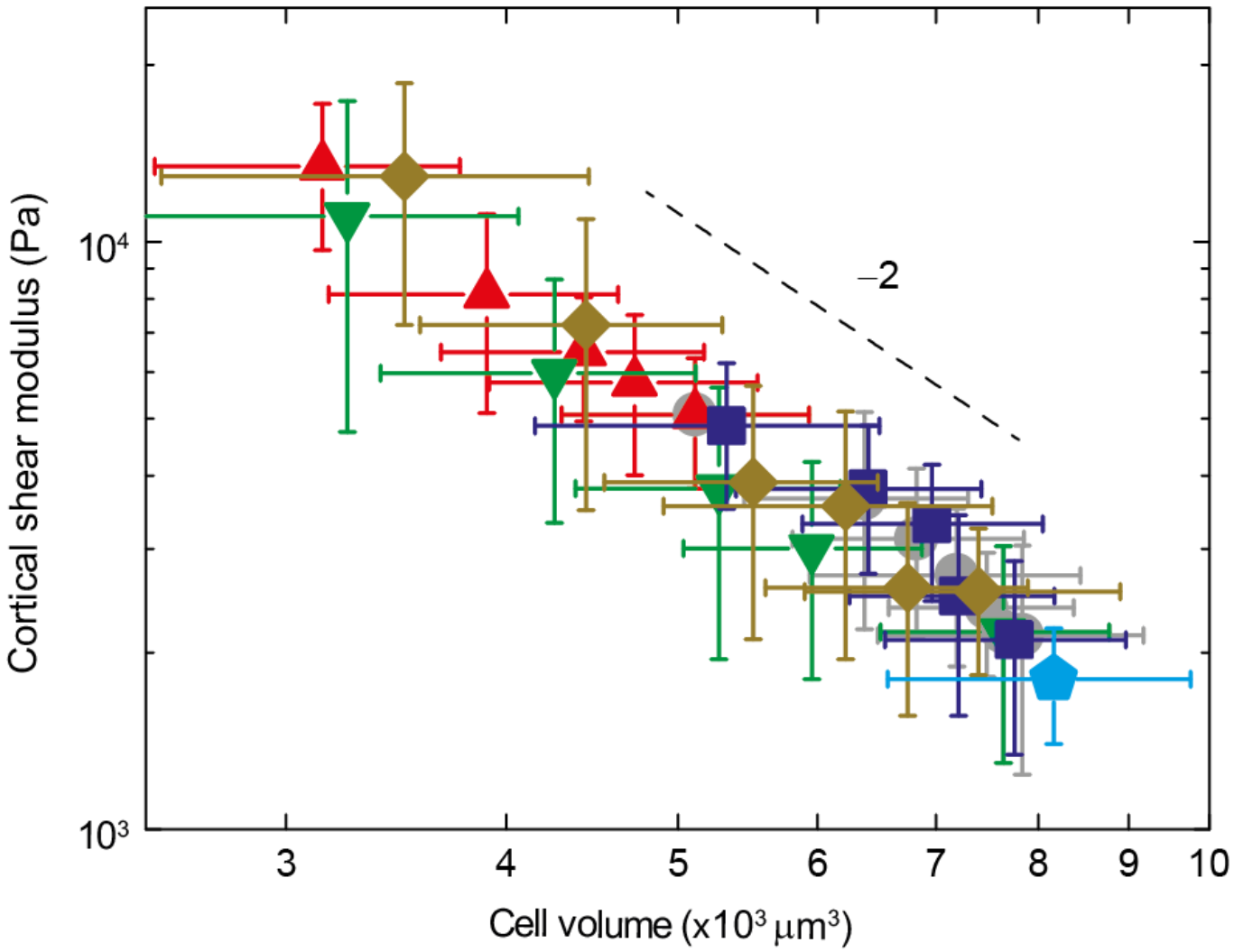
Cell stiffness depends on cell volume



Cell stiffness depends on cell volume

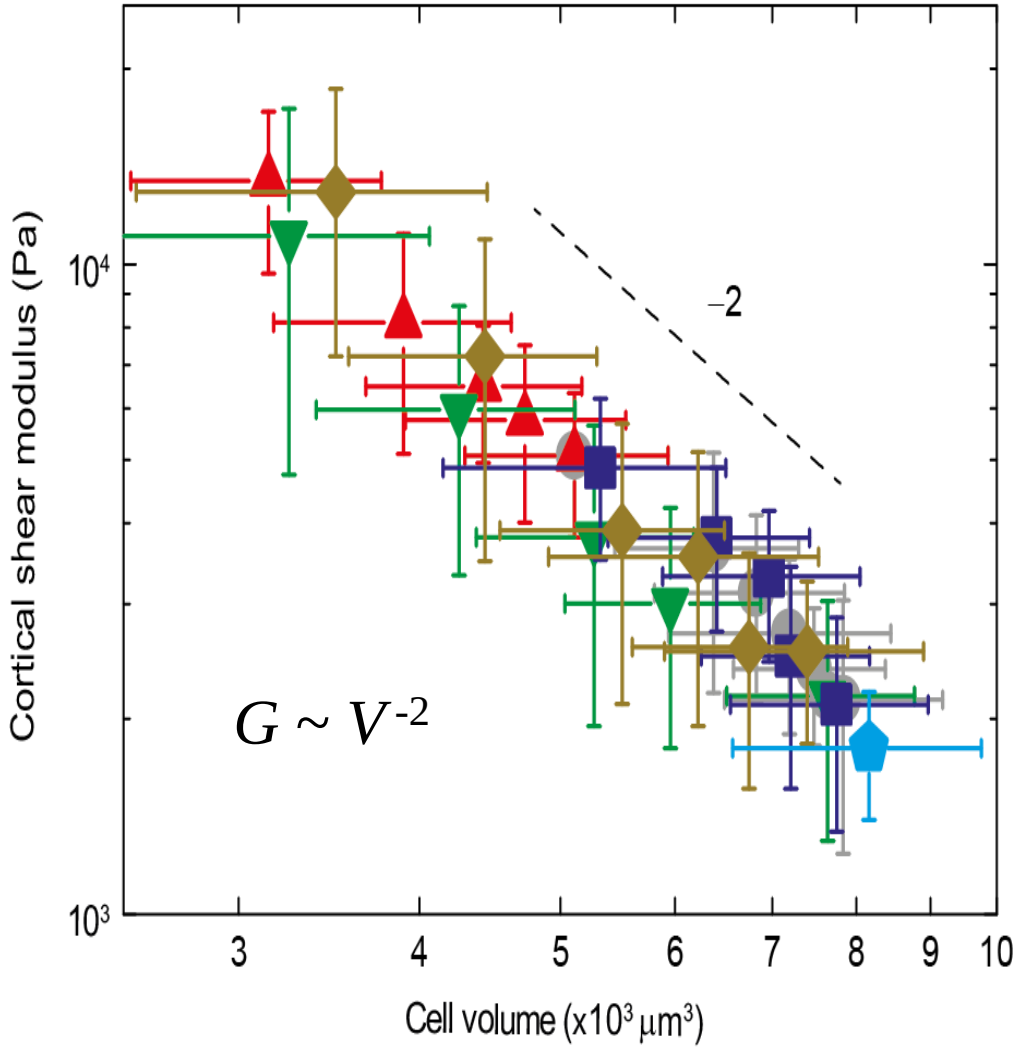


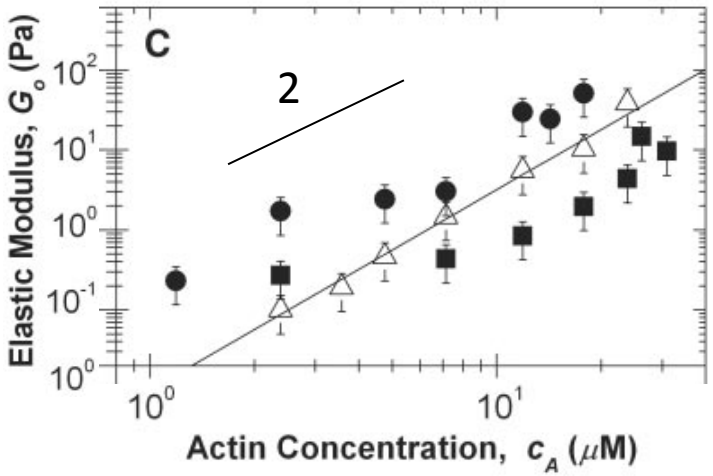
Cell stiffness depends on cell volume



Behaves like reconstituted cross-linked actin networks: $C^{2.2}$

Cell stiffness scaling is same as that of reconstituted actin network

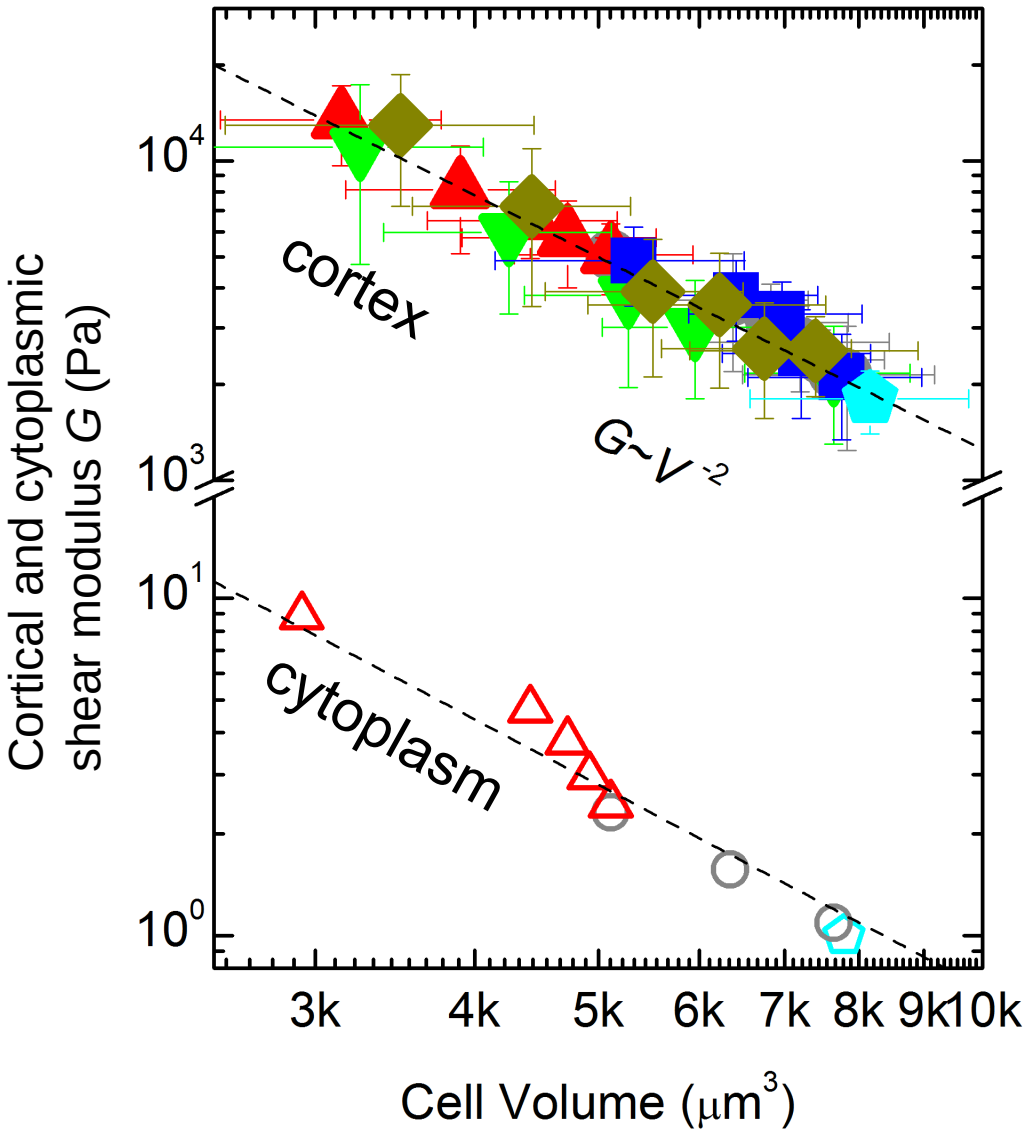




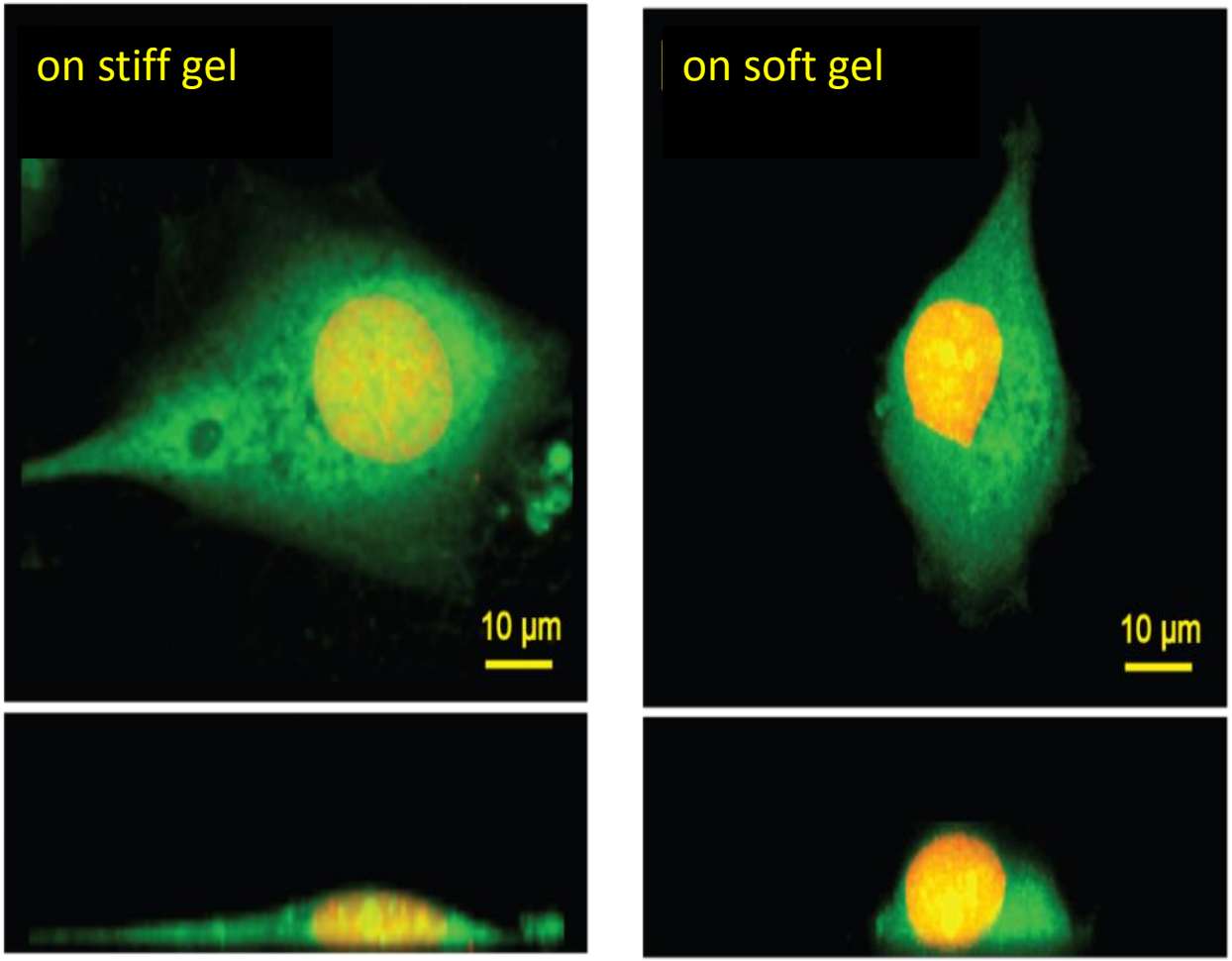
$G \sim C_A^2 \sim V^{-2}$

Gardel et al. *Science* 2004

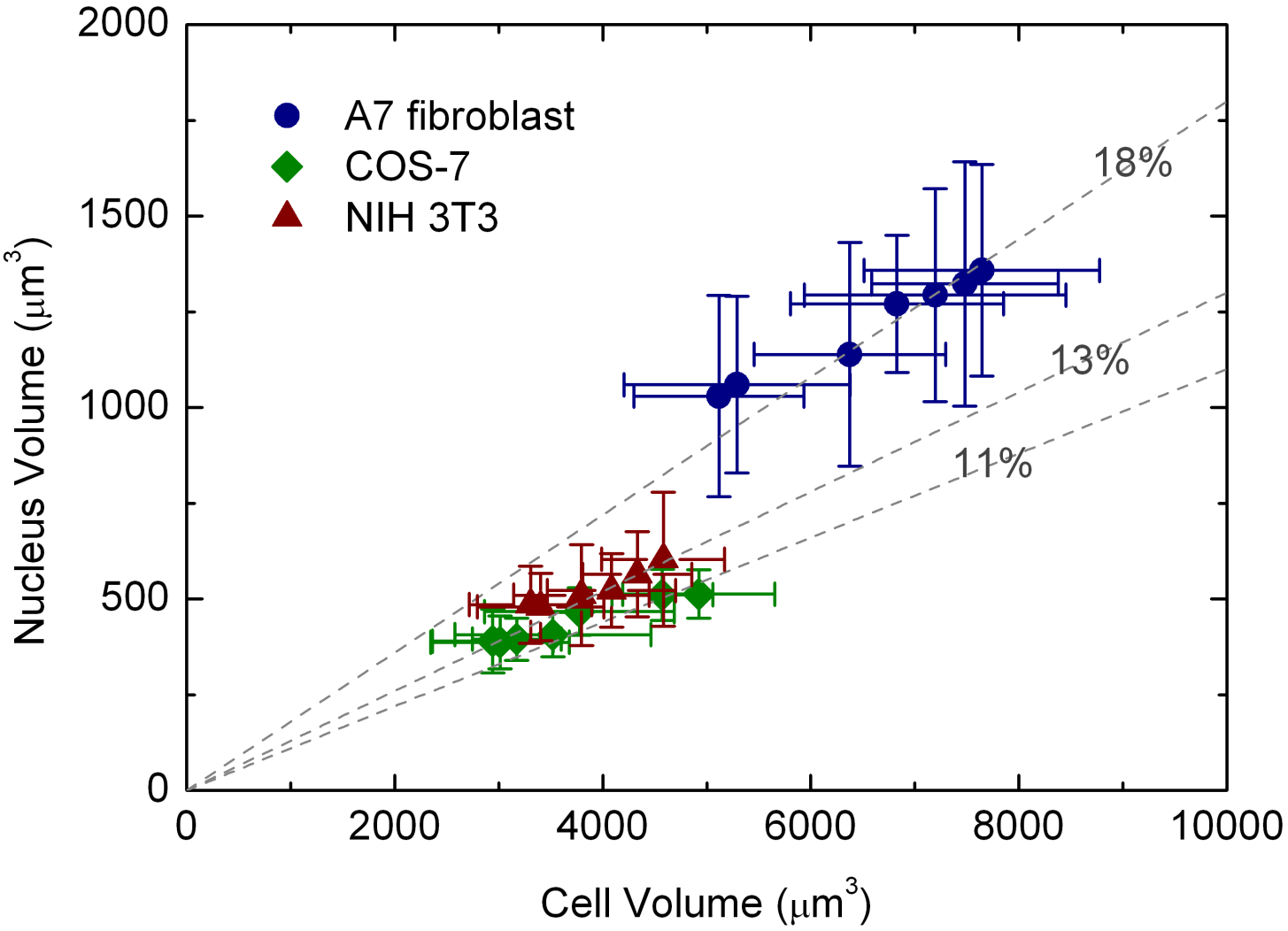
Cortical and cytoplasmic stiffness scales with cell volume



Nucleus volume changes too

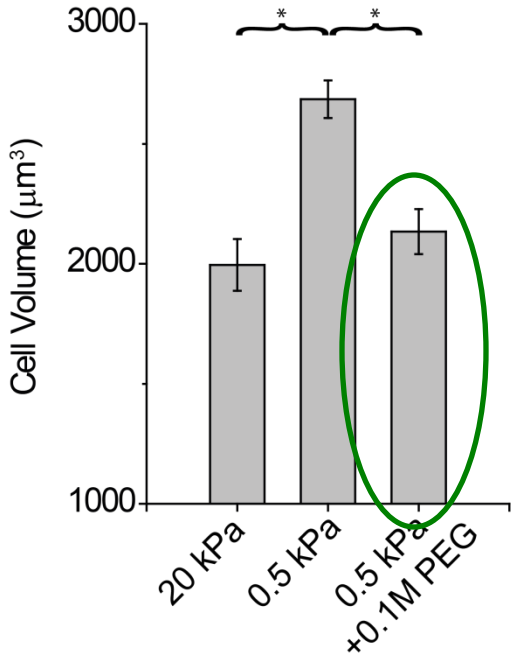


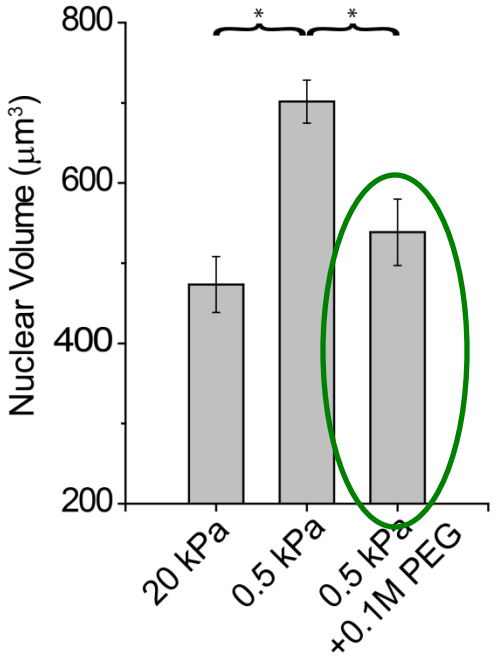
Nucleus volume changes with cell volume



What about stem-cell differentiation?

D1 mouse mesenchymal stem cells on stiff and soft PAA gel substrates (collagen coated)
Compress cell volume osmotically with adding PEG 300.

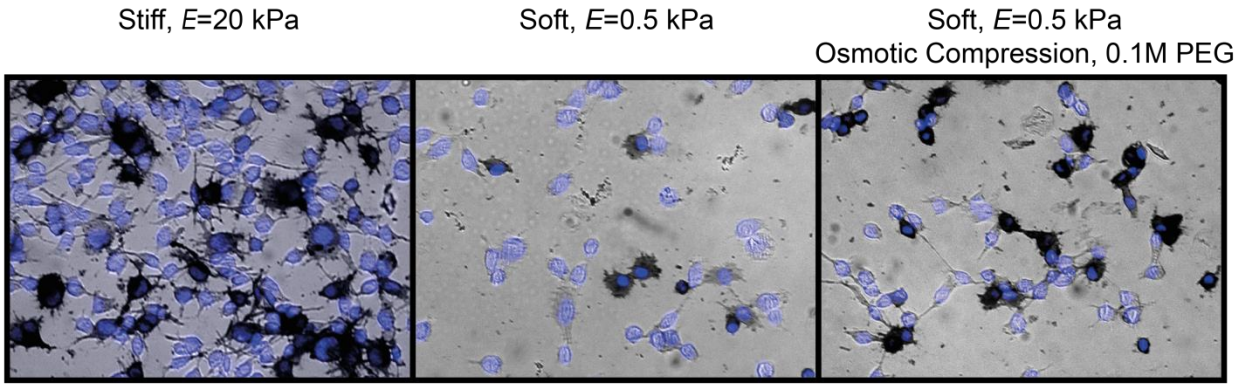




Soft substrate → volume decreased by osmotic pressure

Bar: standard error
* $p < 0.05$

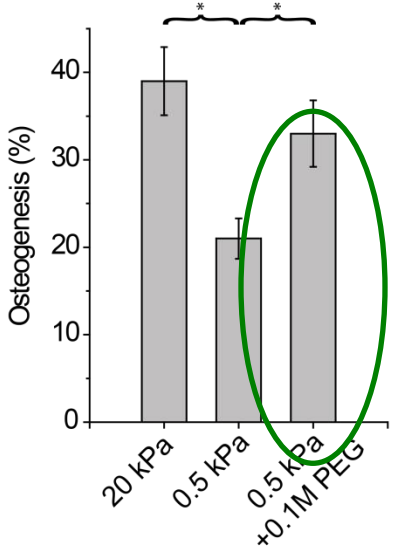
Cell volume affects stem cell differentiation

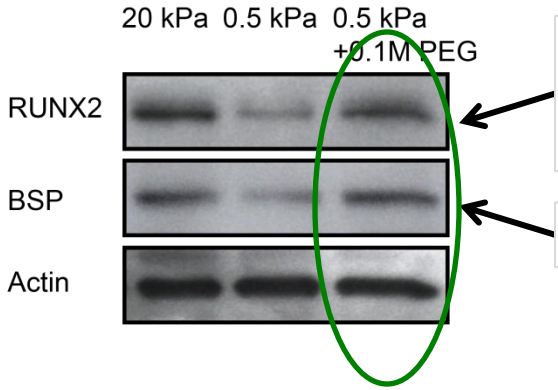


Fixed and stained after 7 days culture.

Alkaline Phosphatase (Bone)

DAPI





Runt-related transcription factor 2, a key transcription factor associated with osteoblast differentiation.

Bone sialoprotein

Western blot after 3 days culture.

Bar: standard error

* $p < 0.05$

Soft substrate → volume decreased by osmotic pressure

Full gene expression analysis

- Gene-chip analysis
- Analysis of mRNA

- Determine origin of dependence

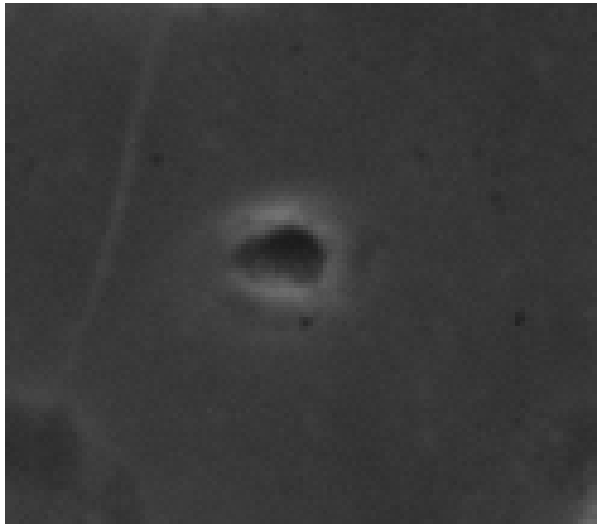
Cell stiffness depends on cell volume

- Cell stiffness correlates with cell volume
- Cell volume controllable by:
  - substrate stiffness
  - adhesion area
  - osmotic pressure
- Nucleus volume tracks cell volume
  - gene expression
  - stem-cell differentiation

Now for something fun

Cell motion on substrates

Single cell versus multi-cell motion

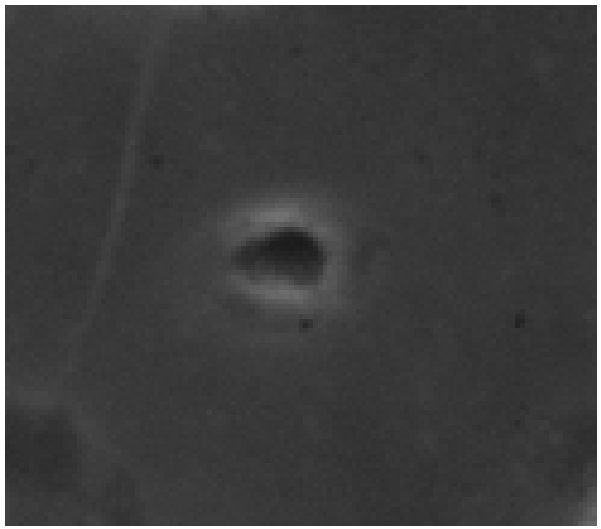


30 μm

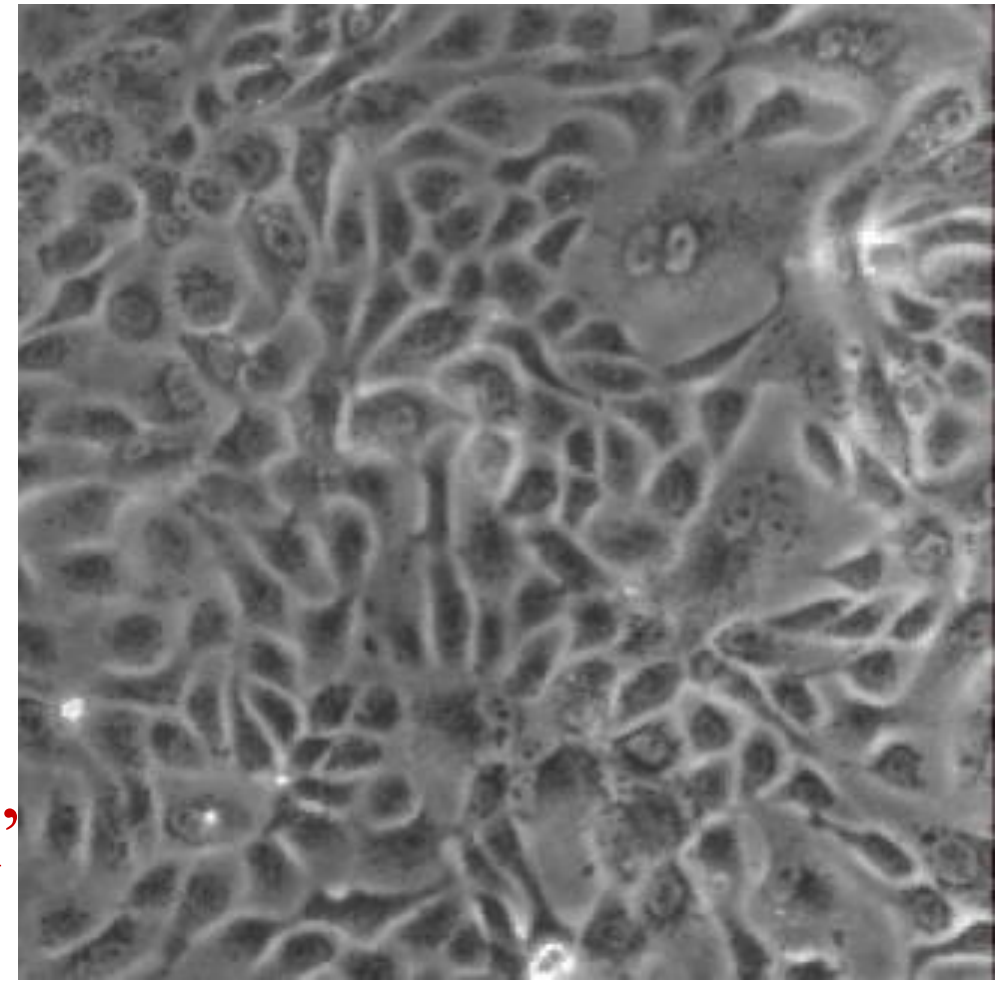
Tissue cells:

- contractile “inchworm”
- spread, stick, release

Single cell versus multi-cell motion



30 μm



30 μm

Tissue cells:

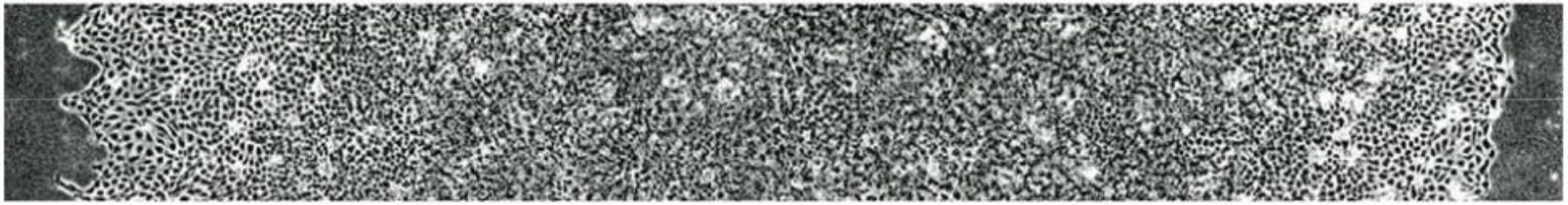
- contractile “inchworm”
- spread, stick, release

Do canonical forms drive collective motion?

Q: how does a fibroblast talk to his friends a millimeter away?

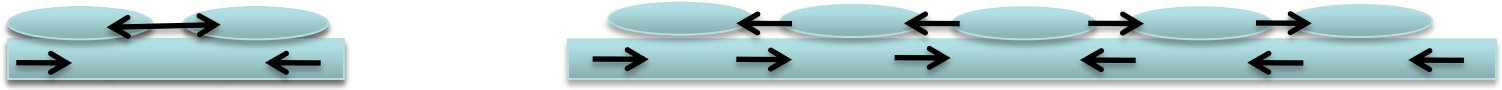
A: cell phone

How do 10^5 – 10^6 cells coordinate?



Trepat, et. al., Nat.Phys. 2009

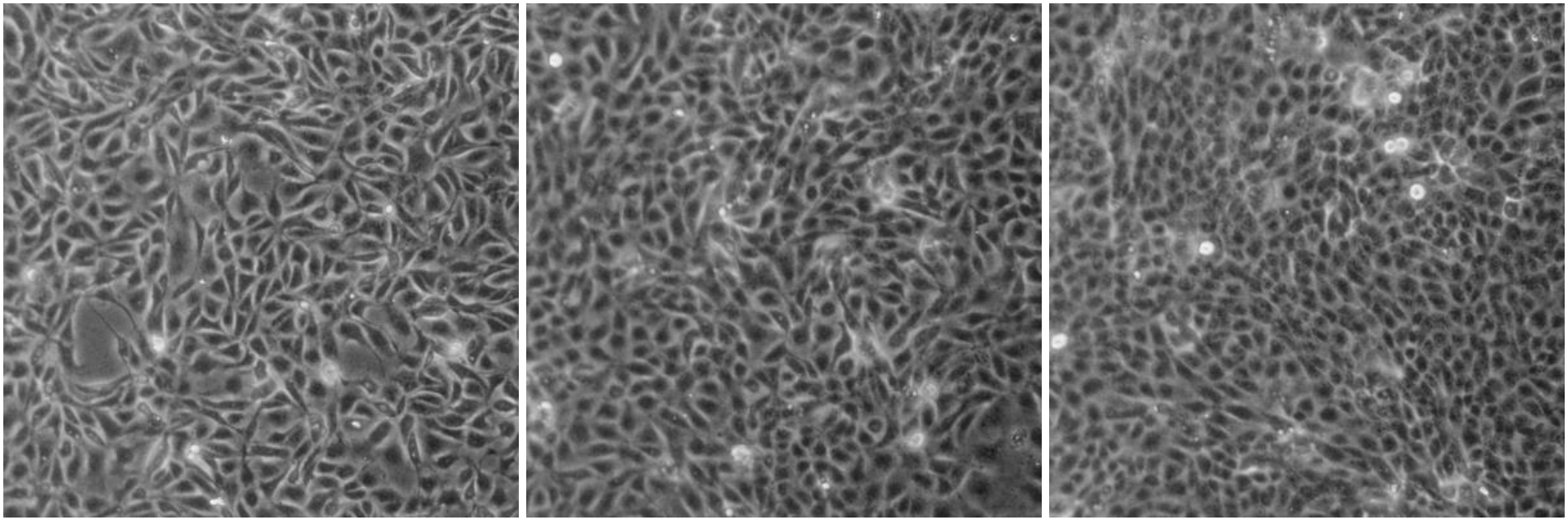
Do cells communicate across millimeters?



tissue-cell collective migration: development, wound healing, tumor invasion

MDCK epithelial cells on polyacrylamide surfaces.

Time evolution of cell behavior

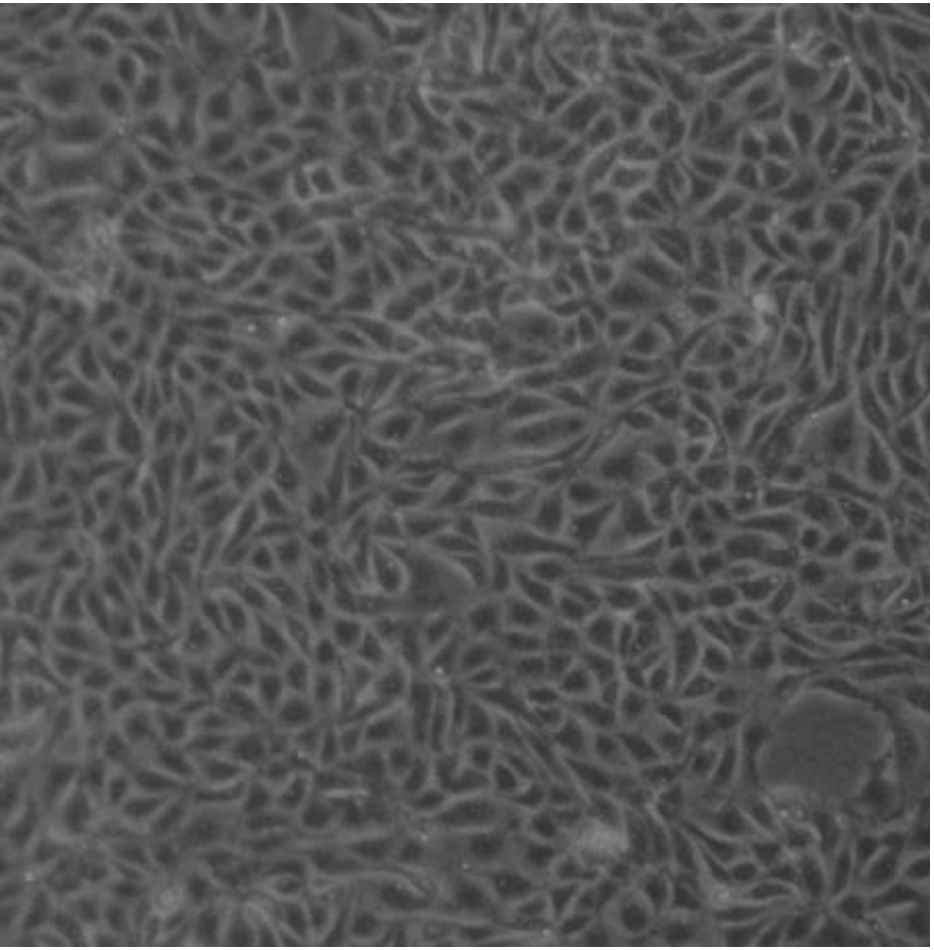


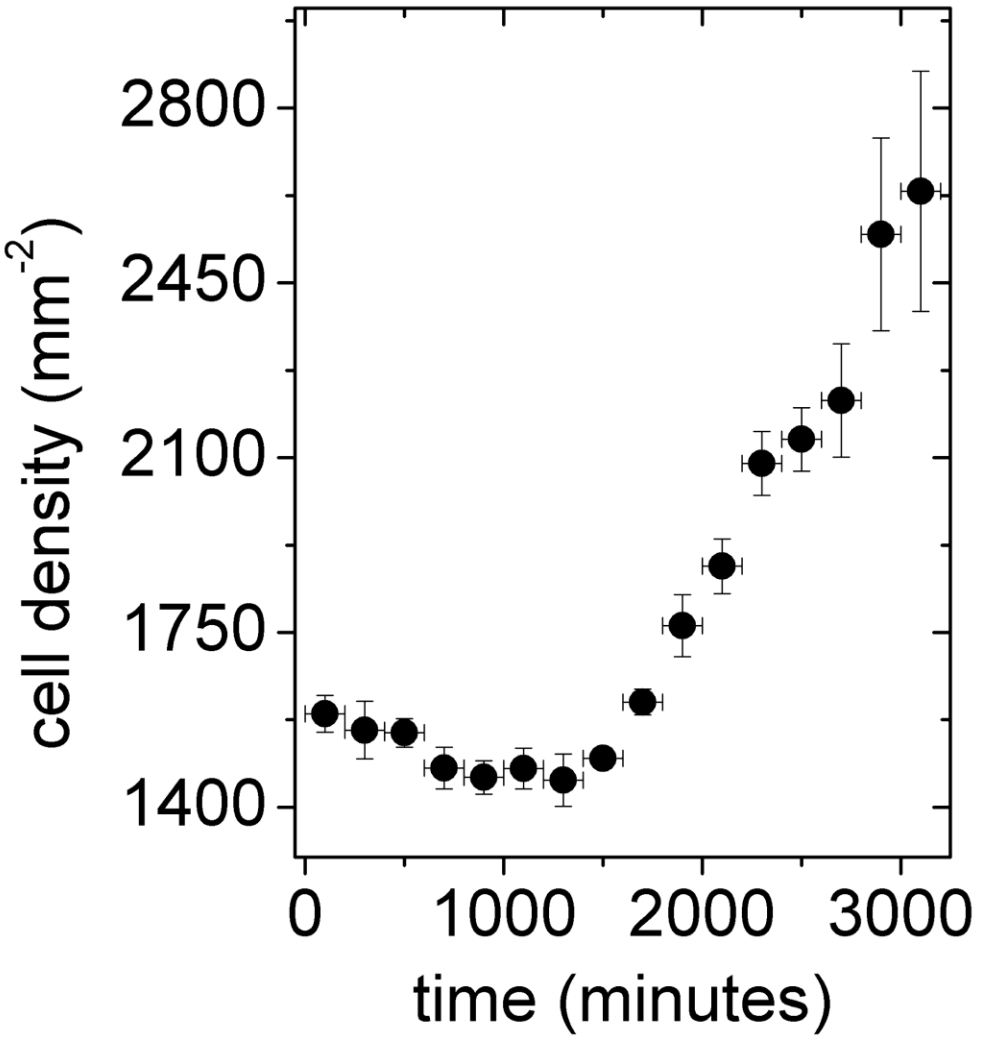
MDCK epithelial cells on polyacrylamide surfaces.

- Speed up time-lapse: cells move, substrate fluctuates.
- 200 mins, ~800 μm field of view. 16 datasets taken in succession.

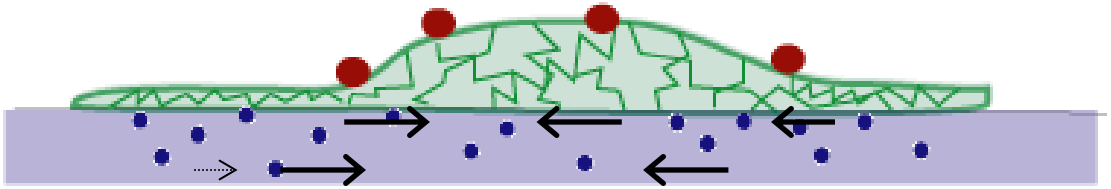
Cell density increases with time

200 mins, ~800 μm field of view. 16 datasets taken in succession.

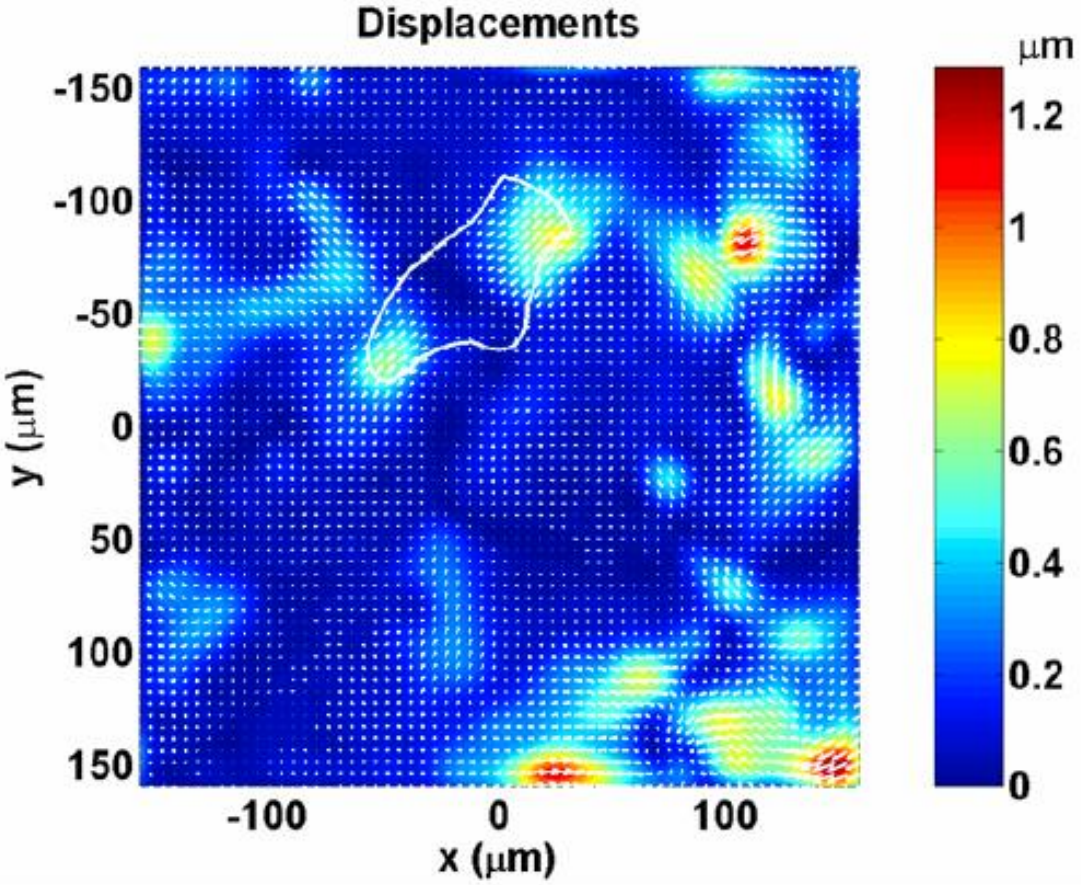




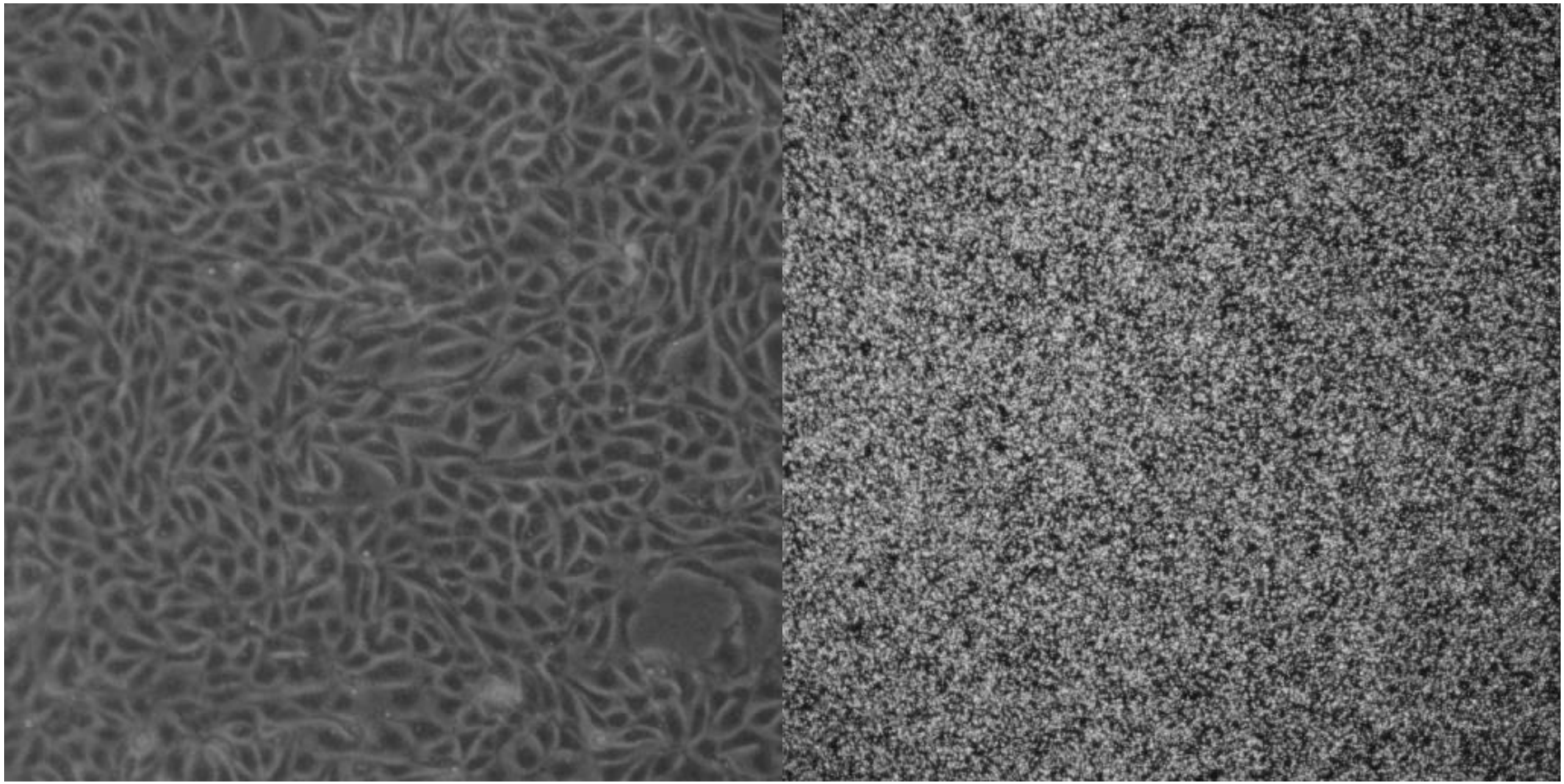
Traction force microscopy



A7 Cells on 1kPa substrate, 24 hrs



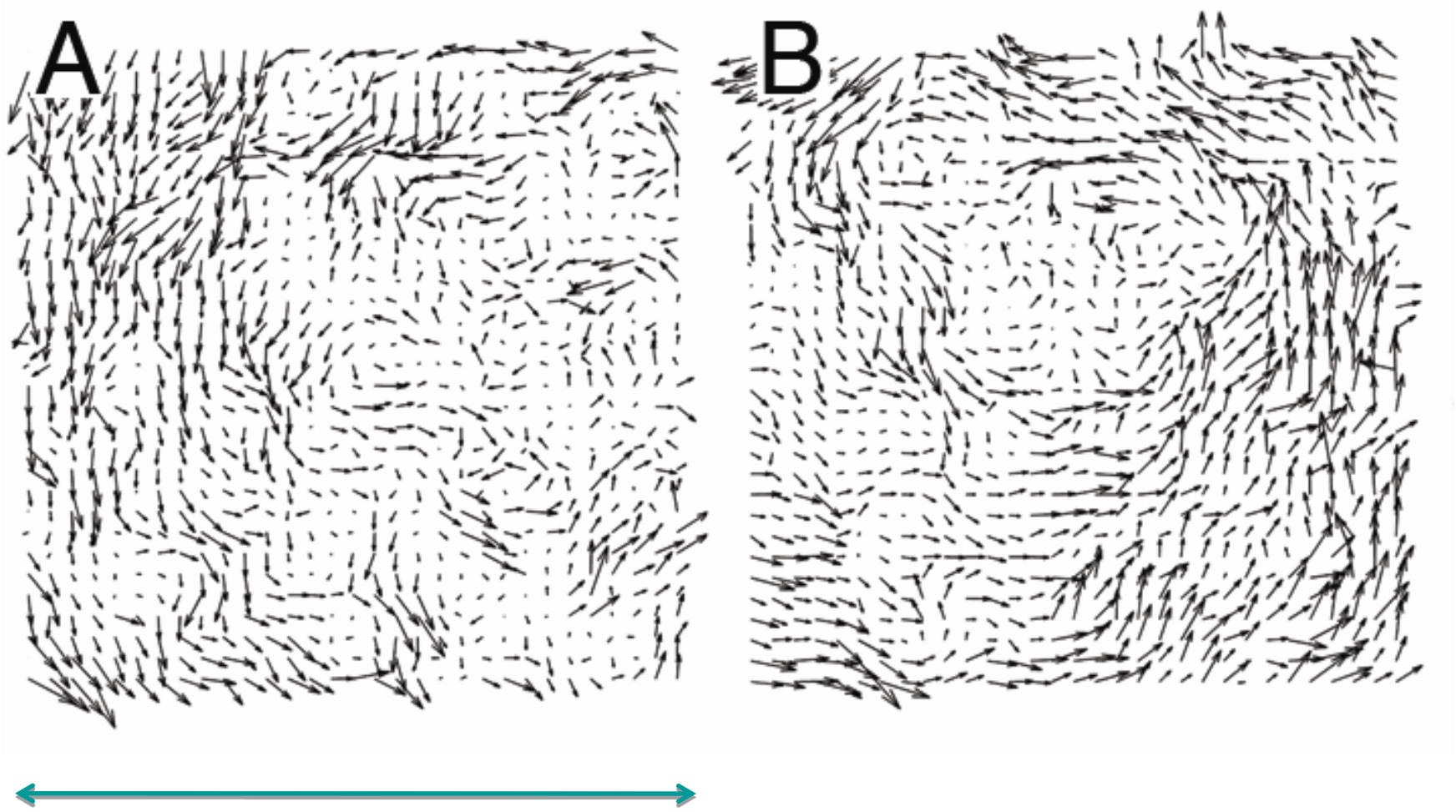
MDCK epithelial cells on polyacrylamide surfaces.



Cells

Substrate

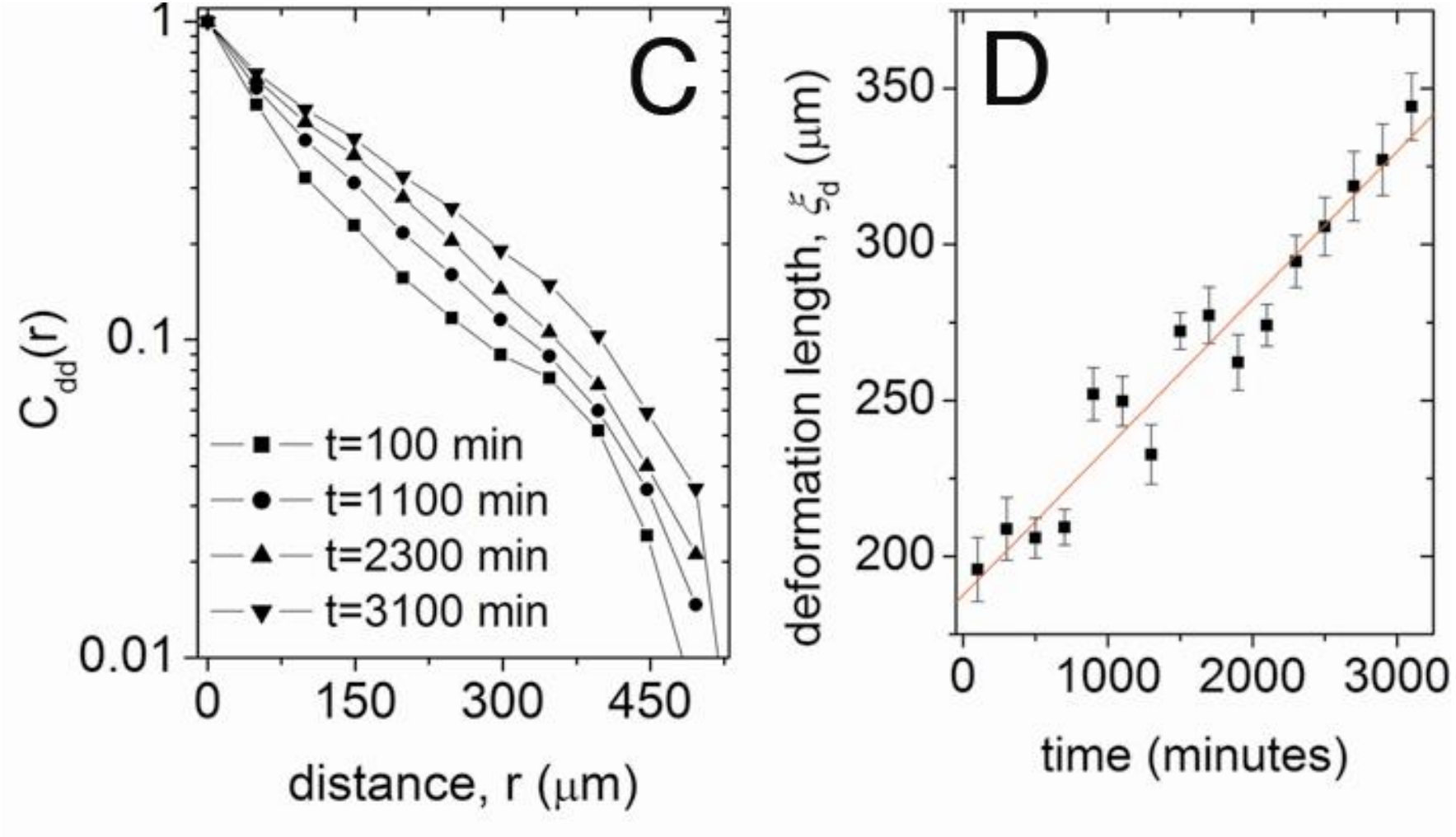
Deformation patterns span hundreds of microns



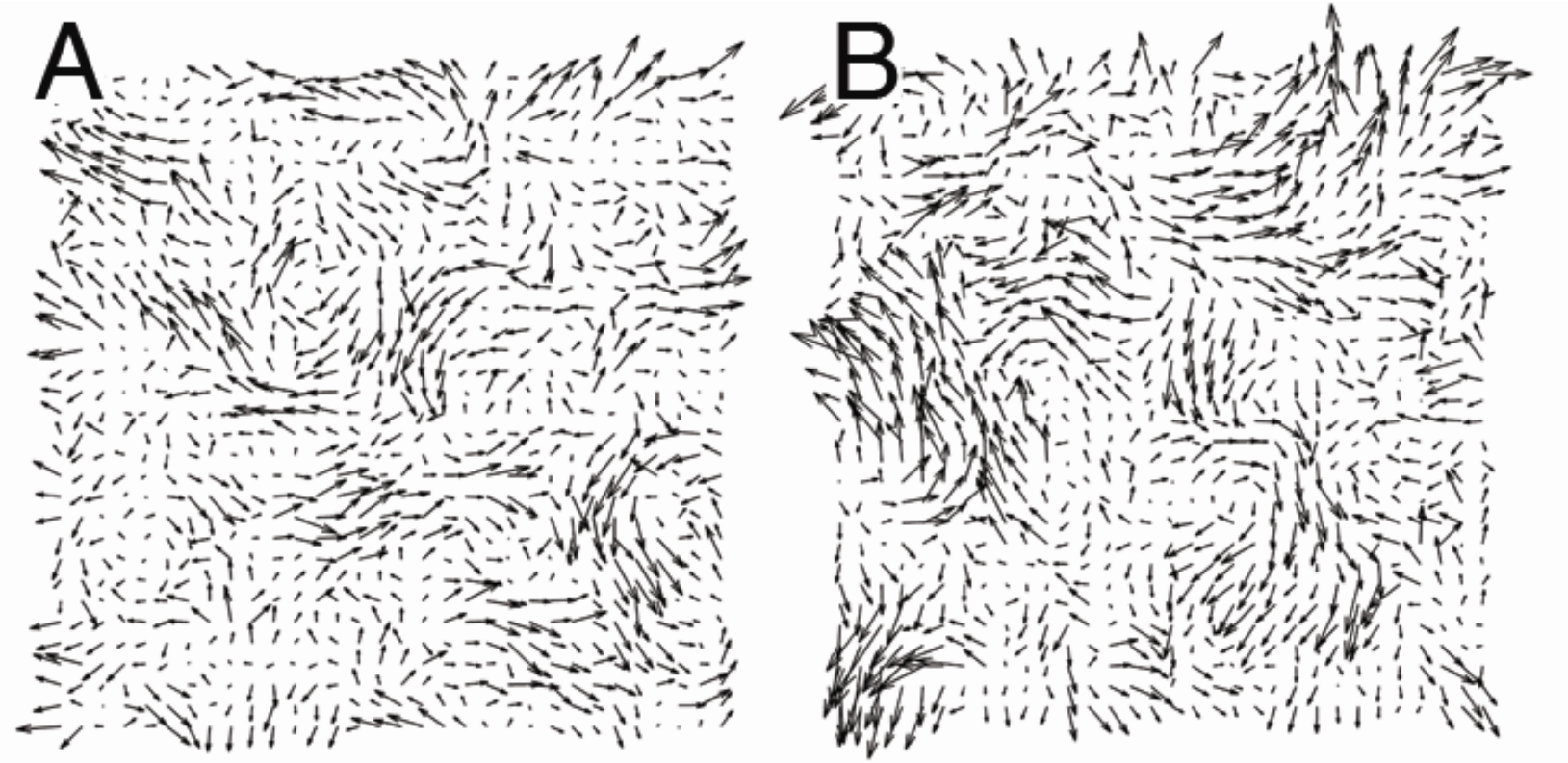
Quantify length-scale: spatial correlation function

$$C_{\mathbf{dd}}(R) = \left\langle \frac{\sum_j \delta\mathbf{d}(\mathbf{r}_j) \cdot \delta\mathbf{d}(\mathbf{r}_j + \mathbf{R})}{\sum_j \delta\mathbf{d}(\mathbf{r}_j) \cdot \delta\mathbf{d}(\mathbf{r}_j)} \right\rangle_{t,\varphi}$$

Correlation length grows in time

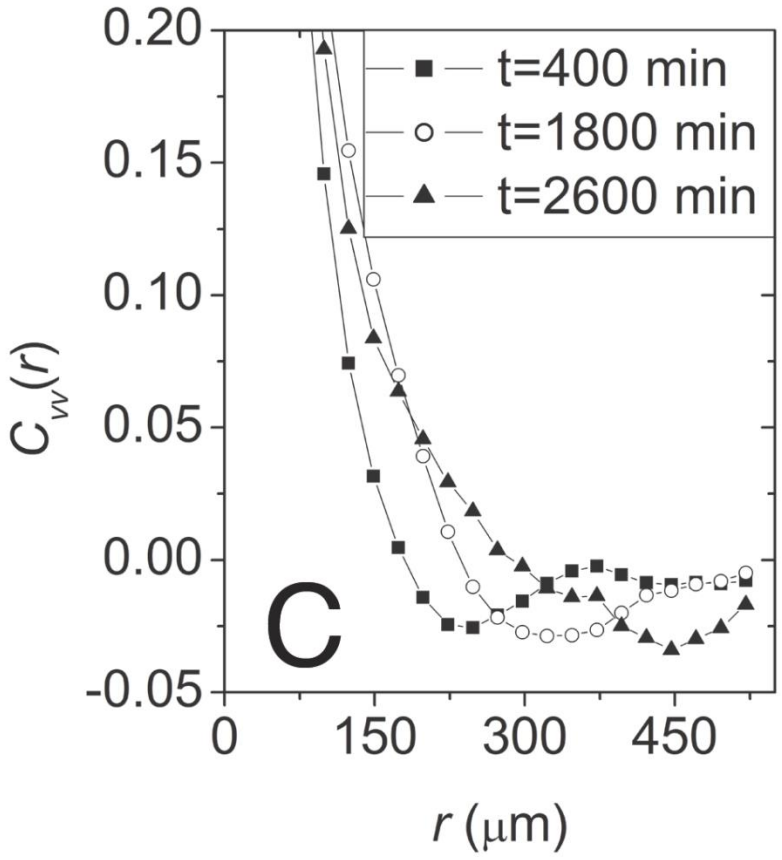


Collective migration: large scale patterns

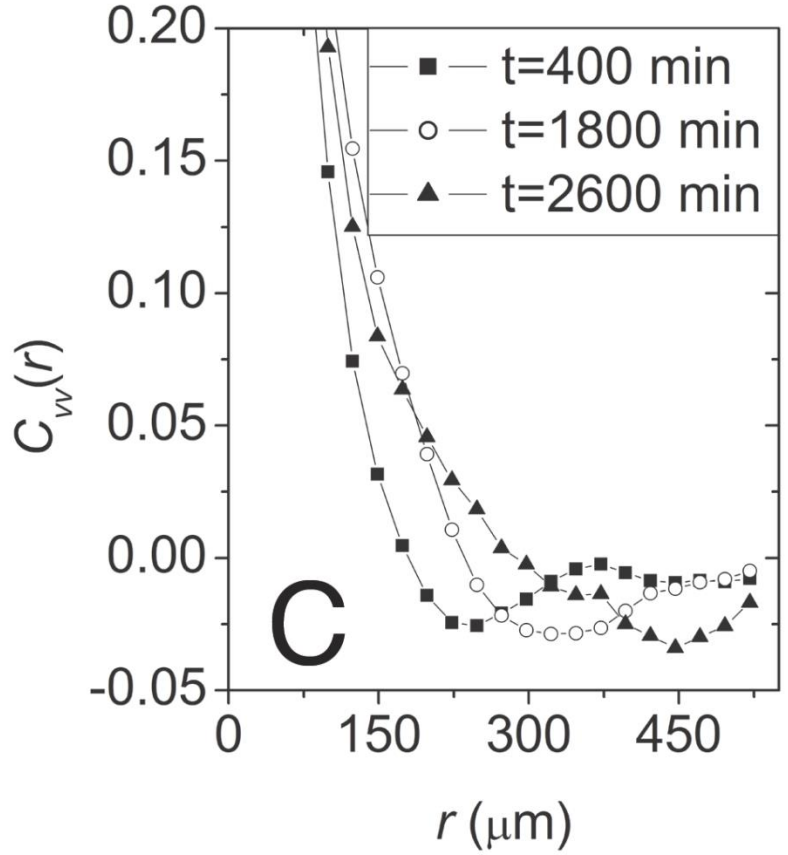


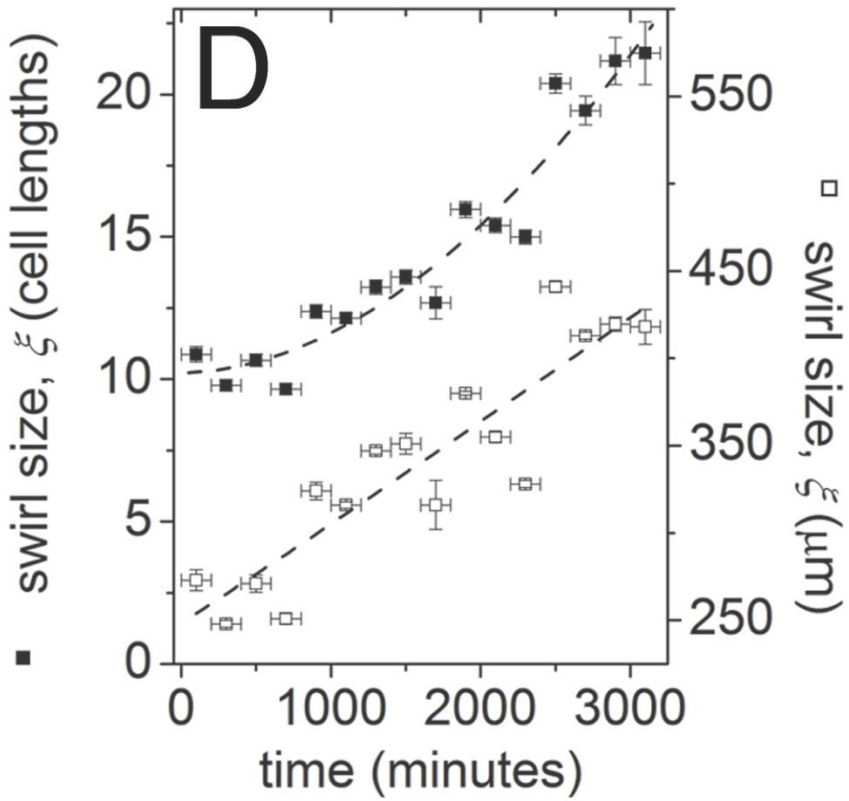
$$C_{\mathbf{vv}}(R) = \left\langle \frac{\sum_j \delta\mathbf{v}(\mathbf{r}_j)\cdot\delta\mathbf{v}(\mathbf{r}_j+\mathbf{R})}{\sum_j \delta\mathbf{v}(\mathbf{r}_j)\cdot\delta\mathbf{v}(\mathbf{r}_j)} \right\rangle_{t,\varphi}$$

Measure correlation length

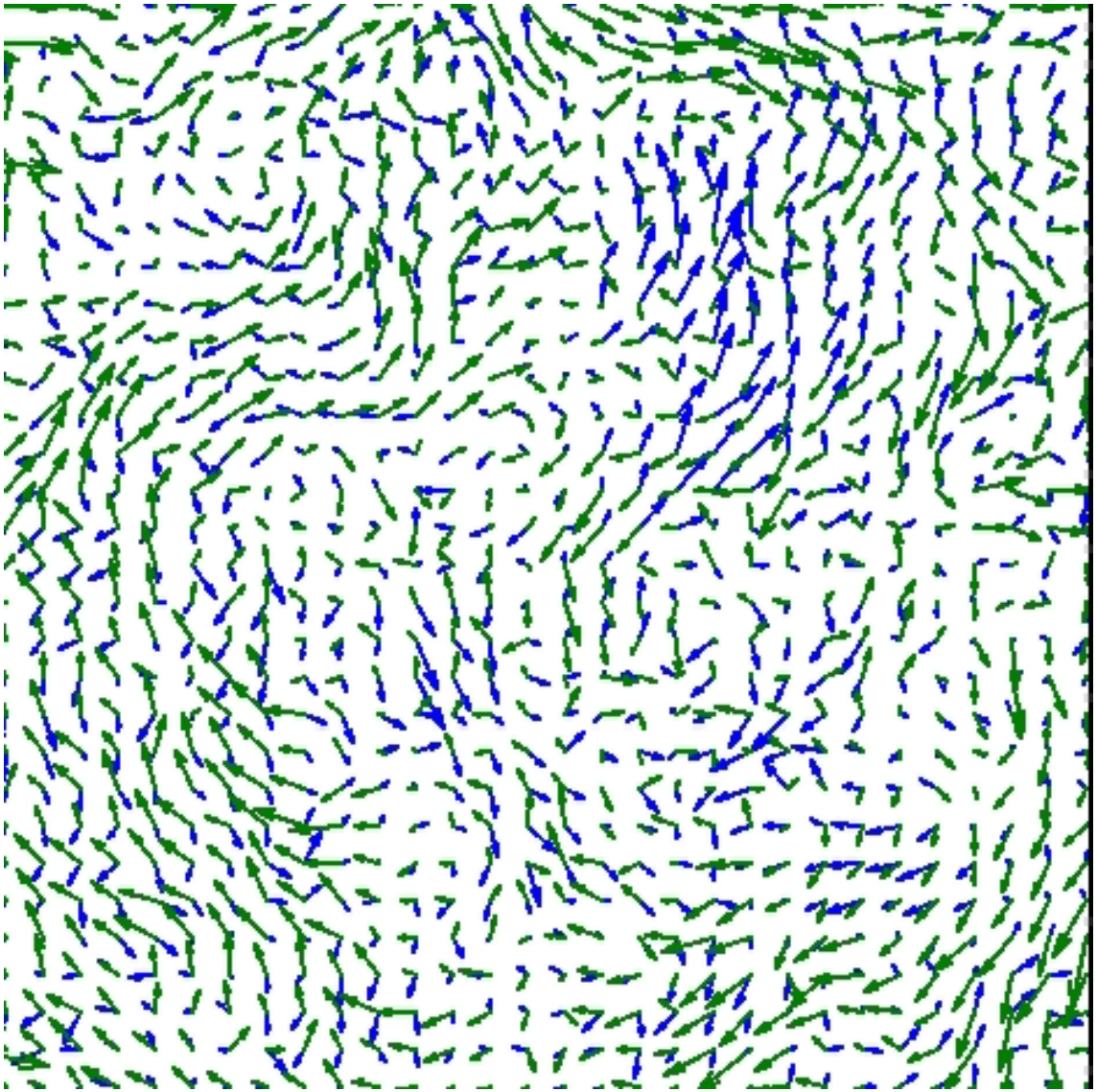


Correlation correlation length grows in time

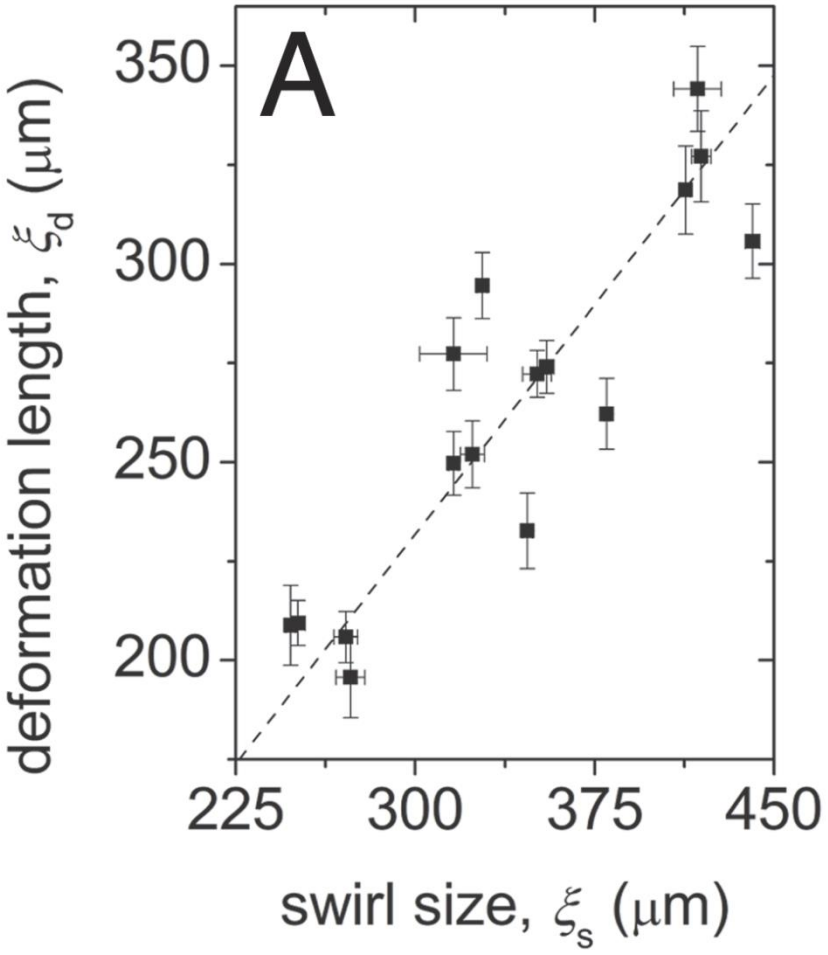




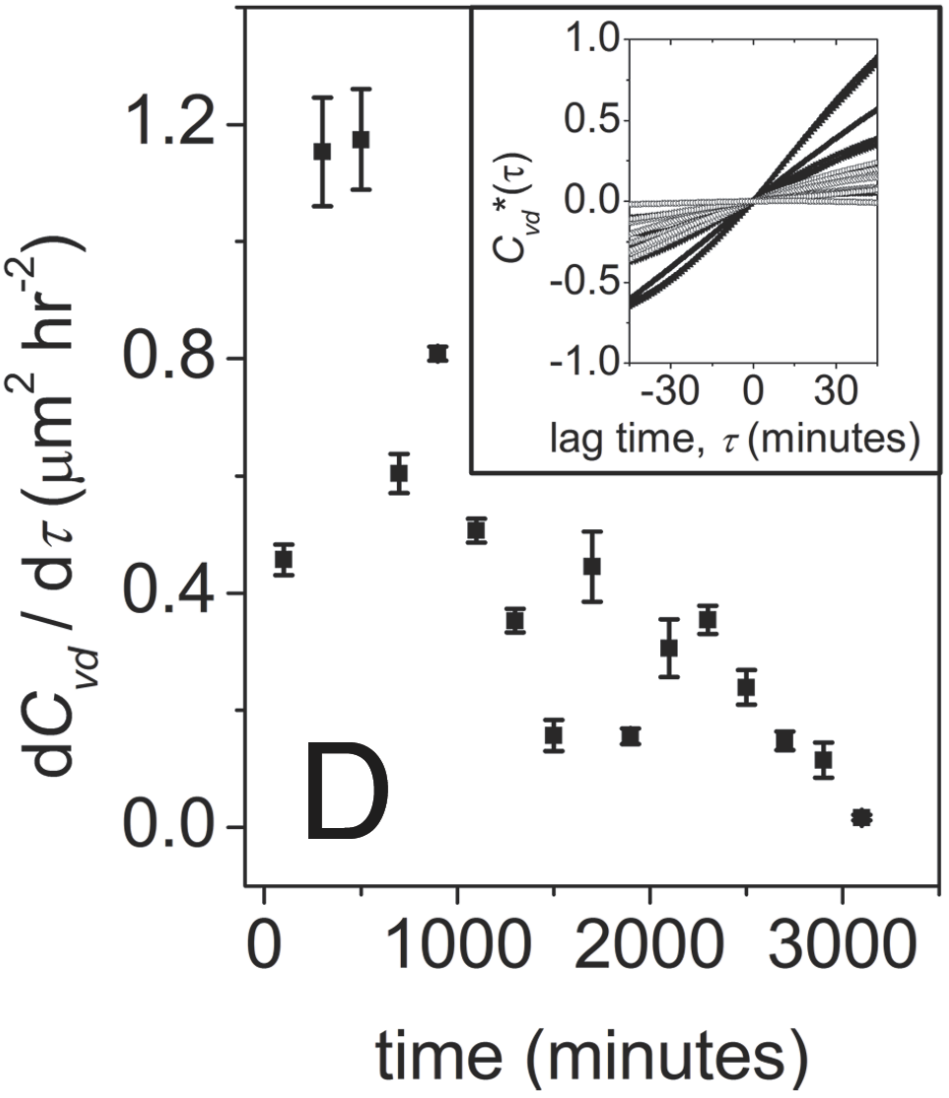
How are the two patterns related?



Swirl size grows with deformation pattern



Migration follows deformation in time



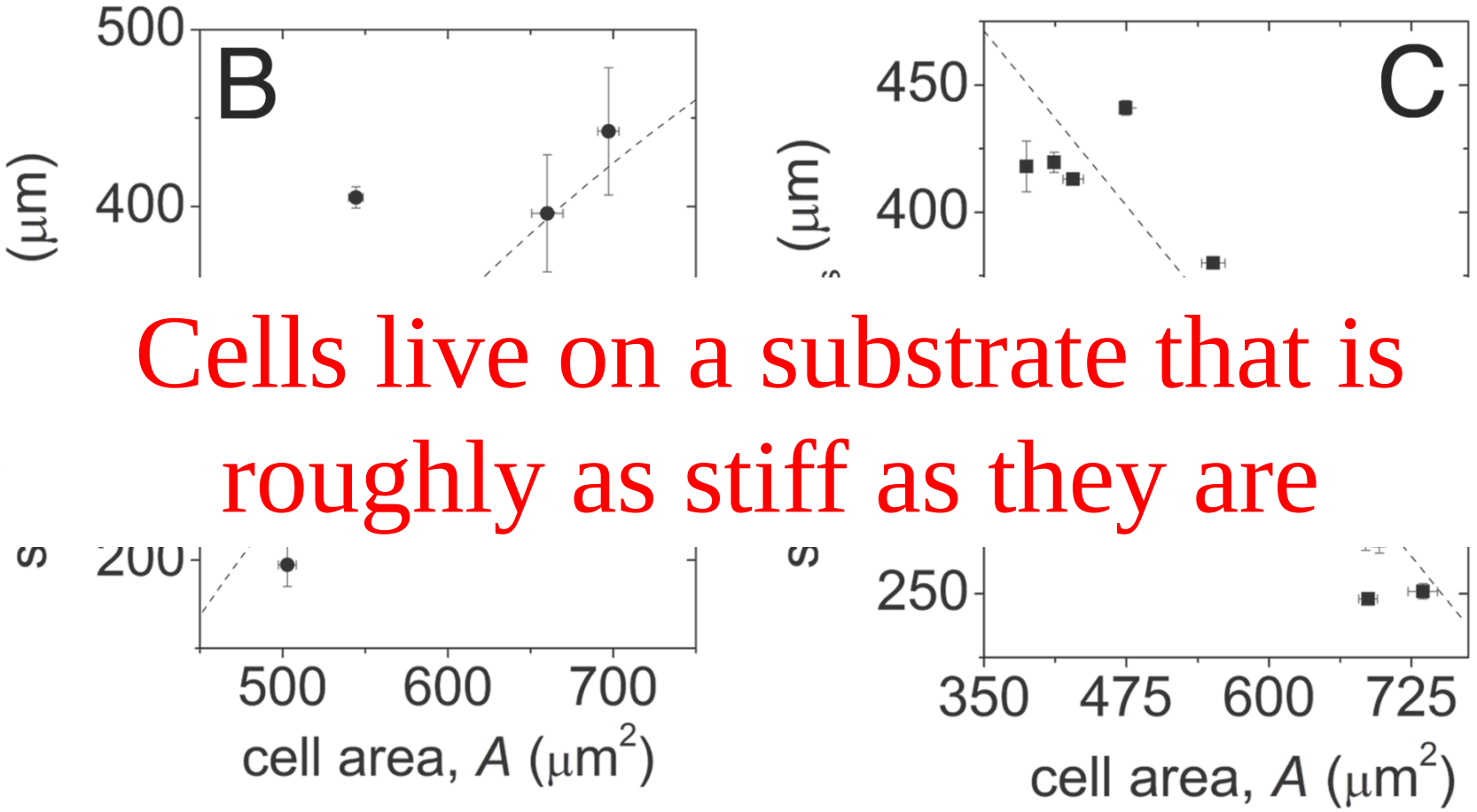
$$C_{\mathbf{dv}}(\tau) = \left\langle \sum_j \delta\mathbf{d}(t_j) \cdot \delta\mathbf{v}(t_j + \tau) \right\rangle_{\mathbf{R}}$$

- Time correlation: positive slope.
- Slope decreases with time.

Substrate deformations: essential

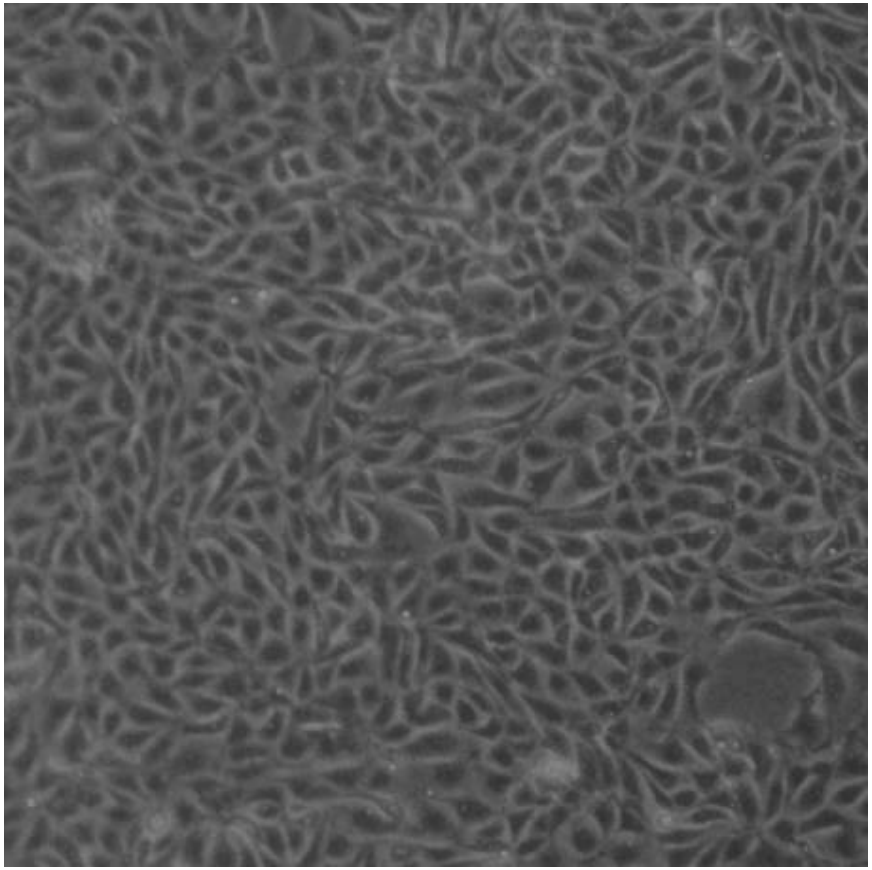
glass

gel



Cells live on a substrate that is roughly as stiff as they are

Cells call long distance: collective substrate deformations guide collective cell migration

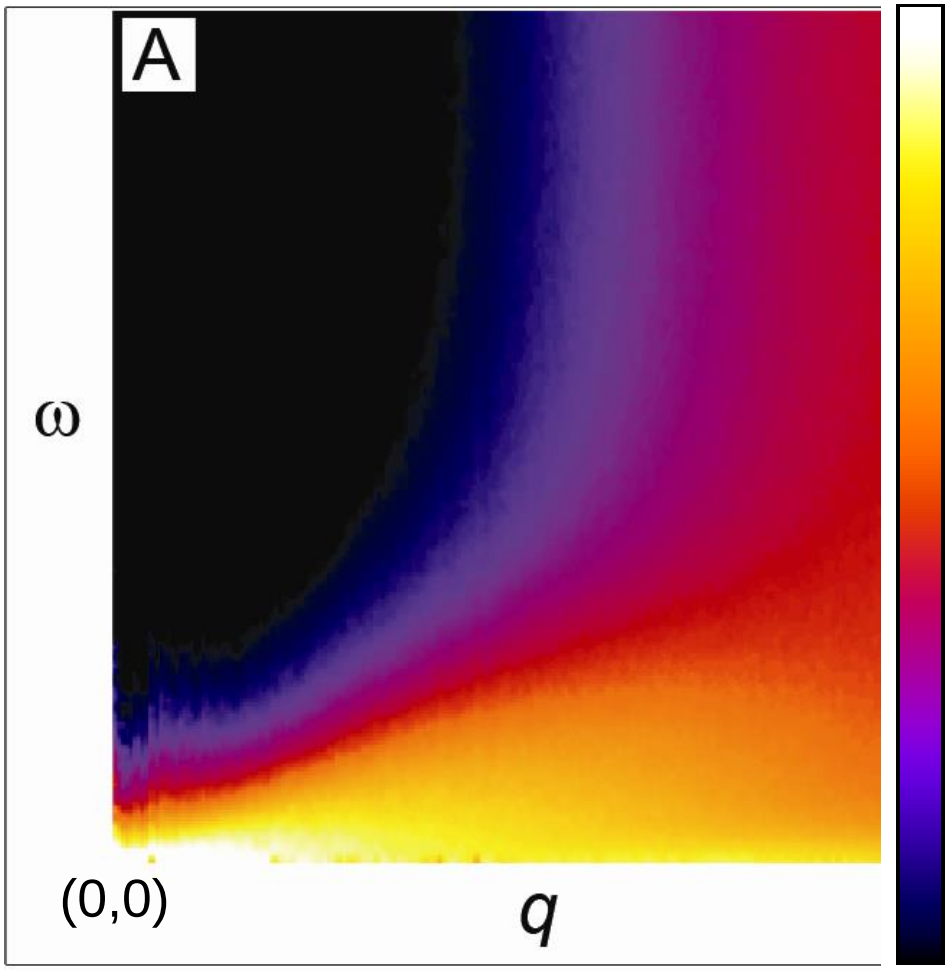


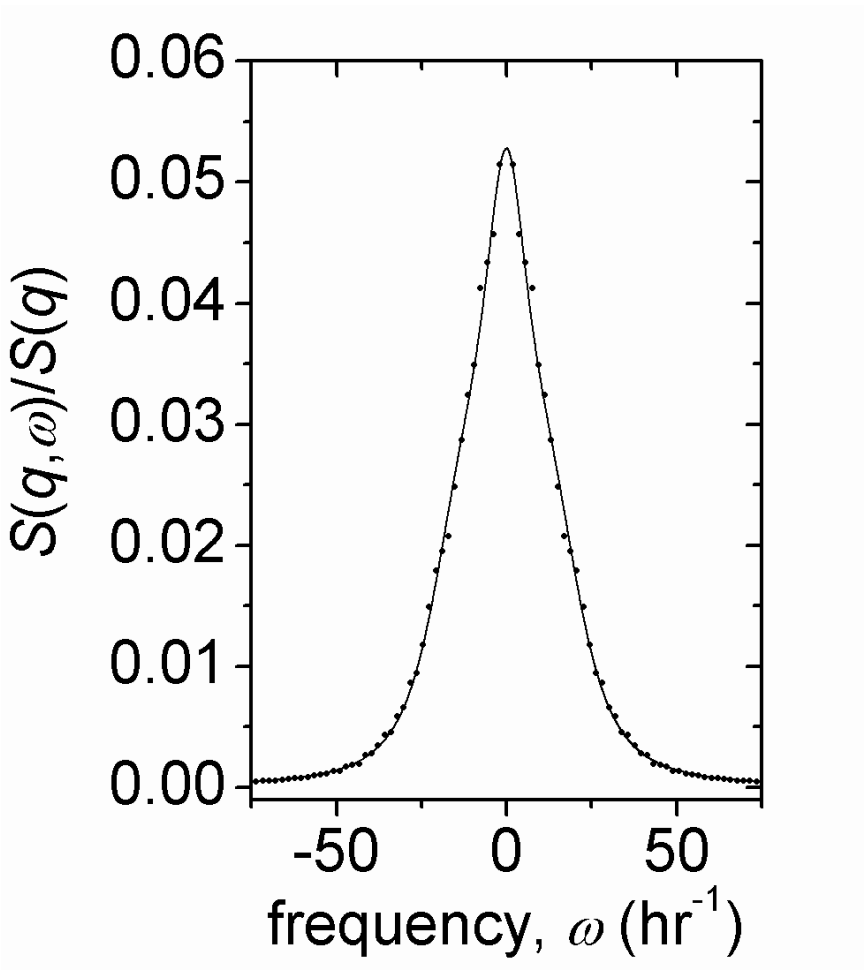
Dynamic structure factor

$$S(q,\omega) = \left| \mathscr{F}[\rho(r,t)] \right|^2$$

- Sound speed, c
- Damping, Γ
- Diffusivity, D_0
- Compressibility, χ
- DOS

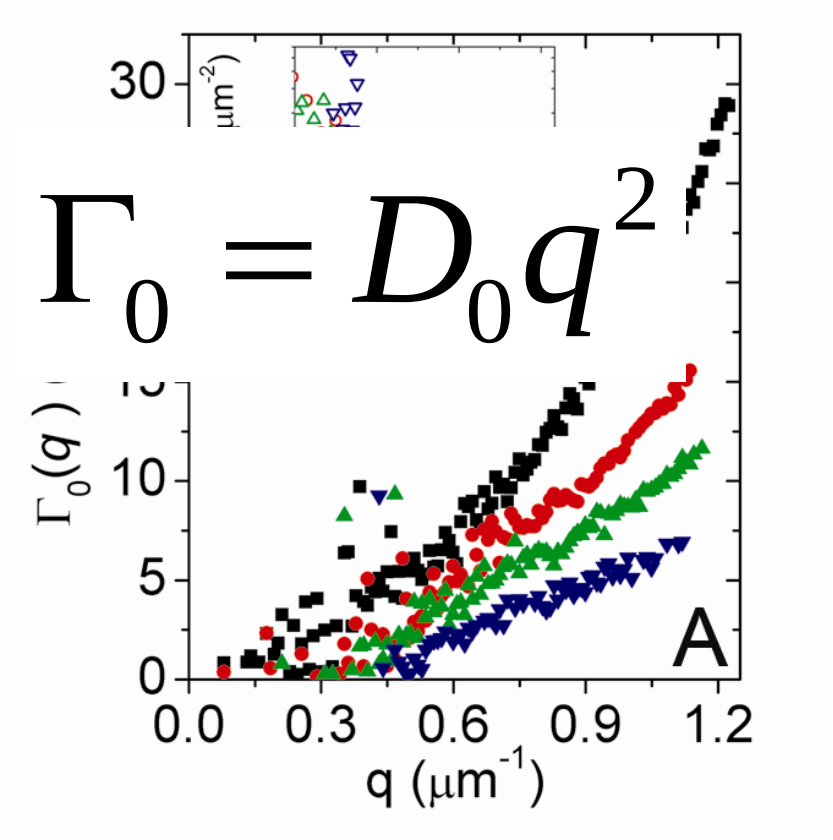
$S(q,\omega)$ of cell motion
pick a q, analyze ω lineshape





Rayleigh-Brillouin triplet

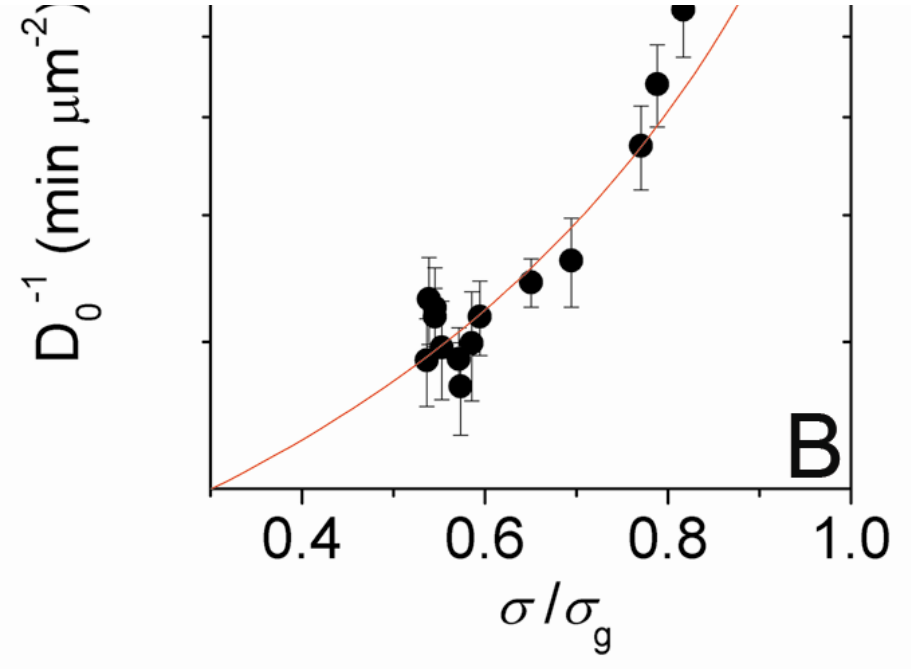
$$\frac{S(q,\omega)}{S(q)} = \frac{I_R(q)\frac{1}{2}\Gamma_0(q)}{\omega^2 + \left(\frac{1}{2}\Gamma_0(q)\right)^2} + \frac{I_B(q)\Omega(q)\Gamma^2(q)}{\left(\omega^2 - \Omega^2(q)\right)^2 + \omega^2\Gamma^2(q)}$$



- Commonly used function in IXS, INS
- **Rayleigh** peak: diffusive fluctuations.
- **Brillouin** peak pressure fluctuations.

Vogel - Fulcher Tammann Hesse (VFTH) equation

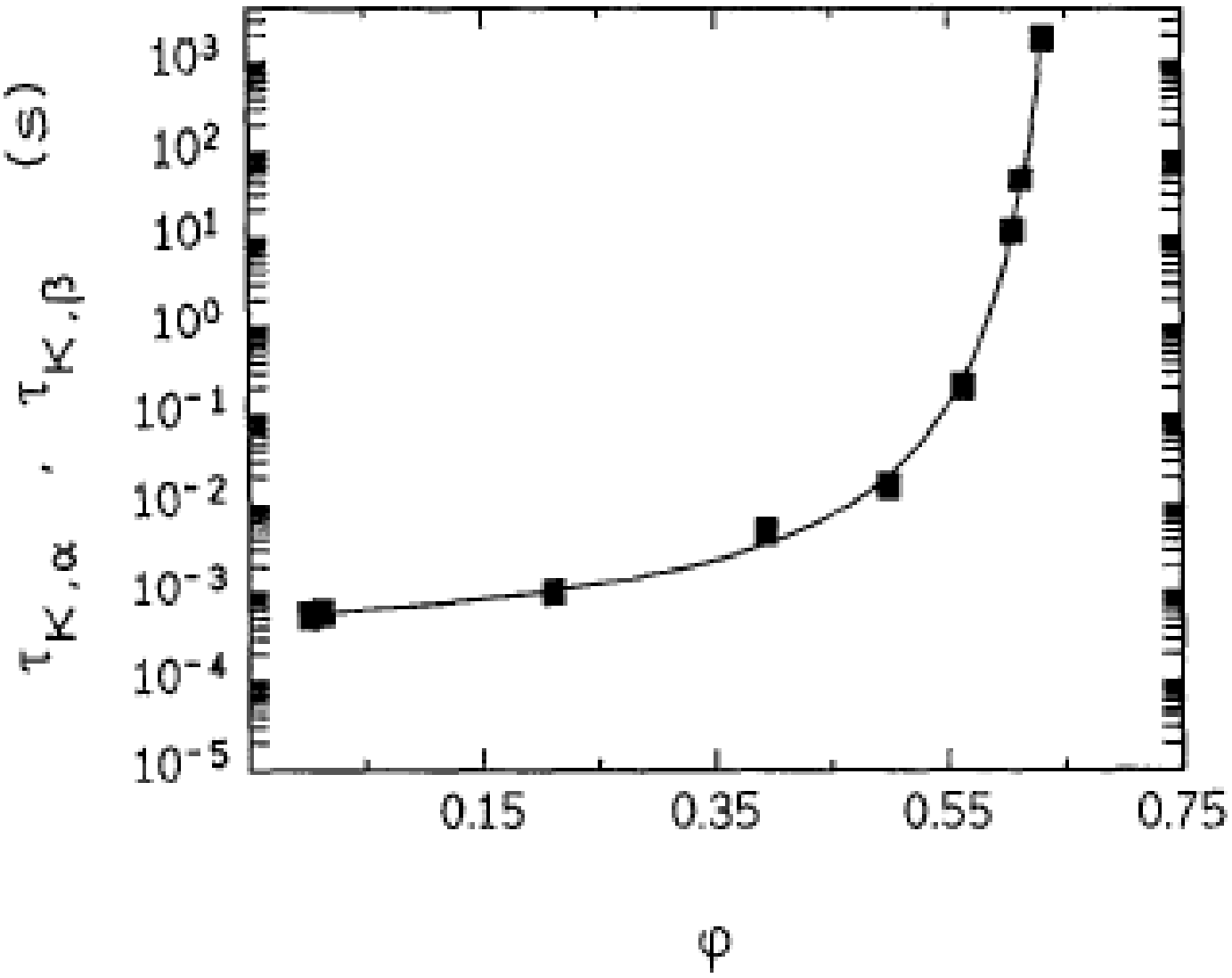
$$D_0^{-1} = D_{\min}^{-1} \exp\left(\frac{A\sigma}{\sigma_0 - \sigma}\right)$$



Assume $D_0 = (\text{cell size})^2/\tau_{div}$, VFTH fit gives $\sigma_g = 2728$ mm^{-2}

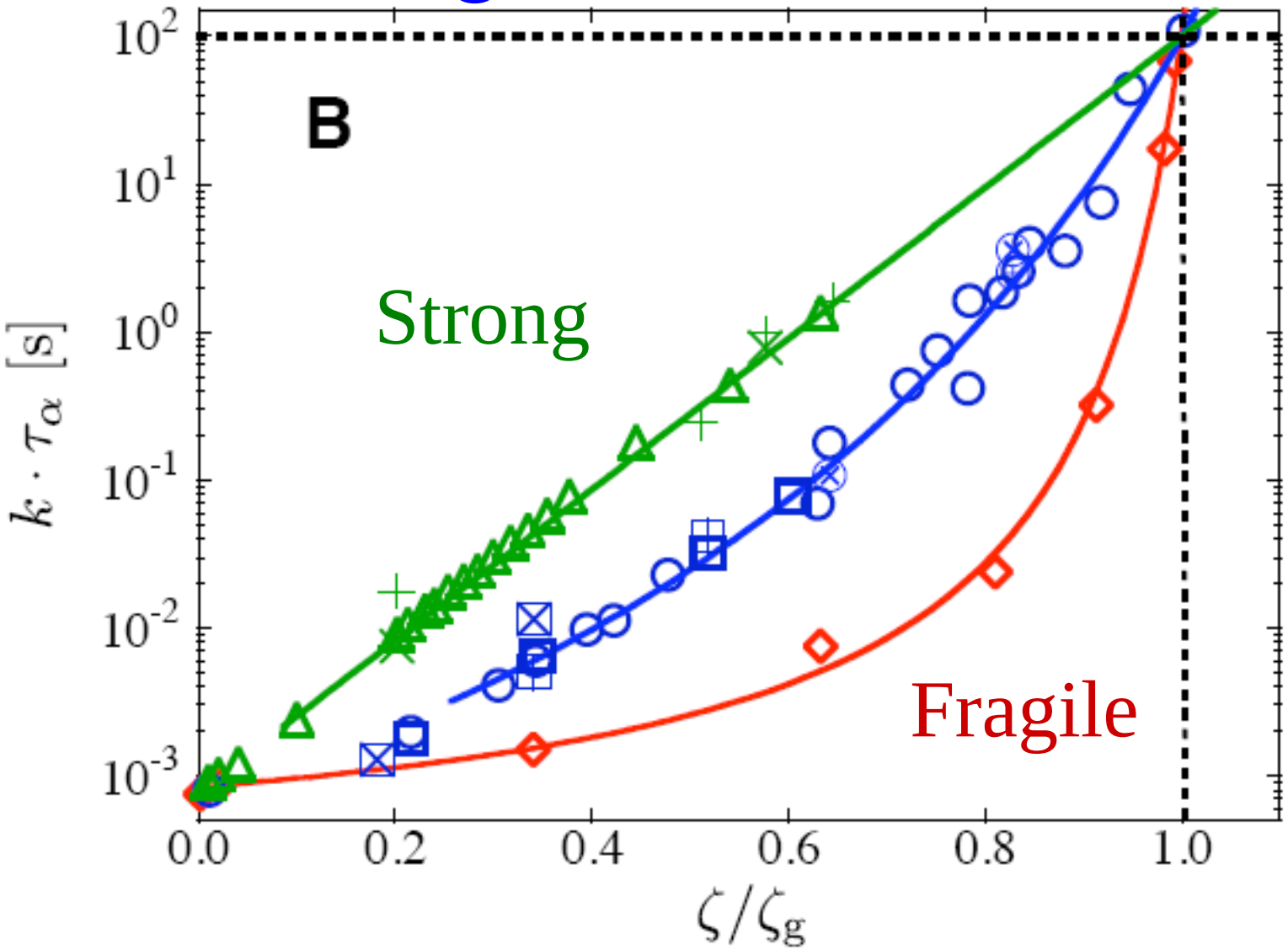
Cell density analogous to inverse temperature, colloidal glass transition

Divergence of time scale for colloidal particles



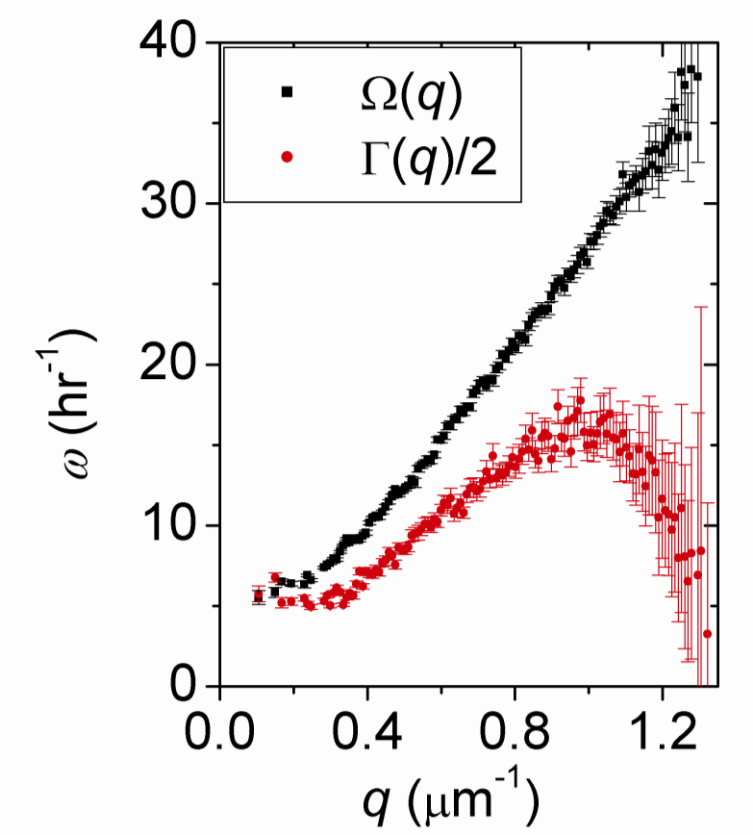
Bartch, Antonietti, Schupp, Sillescu, *J. Chem. Phys.* **97**, 3950 (1992)

Comparison to molecular glasses



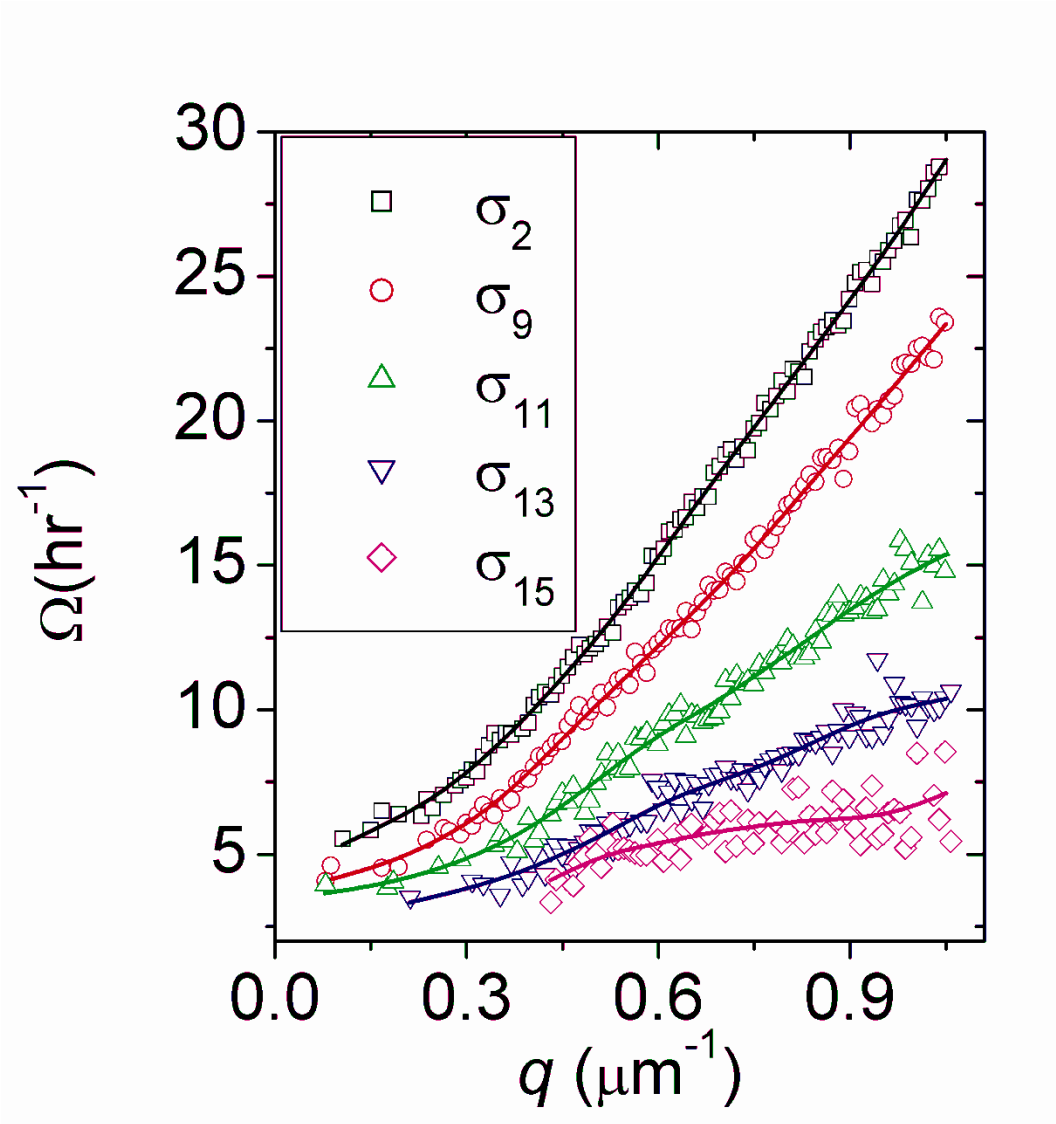
Brillouin peak

$$\frac{S(q,\omega)}{S(q)} = \frac{I_R(q)\frac{1}{2}\Gamma_0(q)}{\omega^2 + \left(\frac{1}{2}\Gamma_0(q)\right)^2} + \frac{I_B(q)\Omega(q)\Gamma^2(q)}{\left(\omega^2 - \Omega^2(q)\right)^2 + \omega^2\Gamma^2(q)}$$



- Commonly used function in IXS, INS
- **Rayleigh** peak: diffusive fluctuations.
- **Brillouin** peak pressure fluctuations.

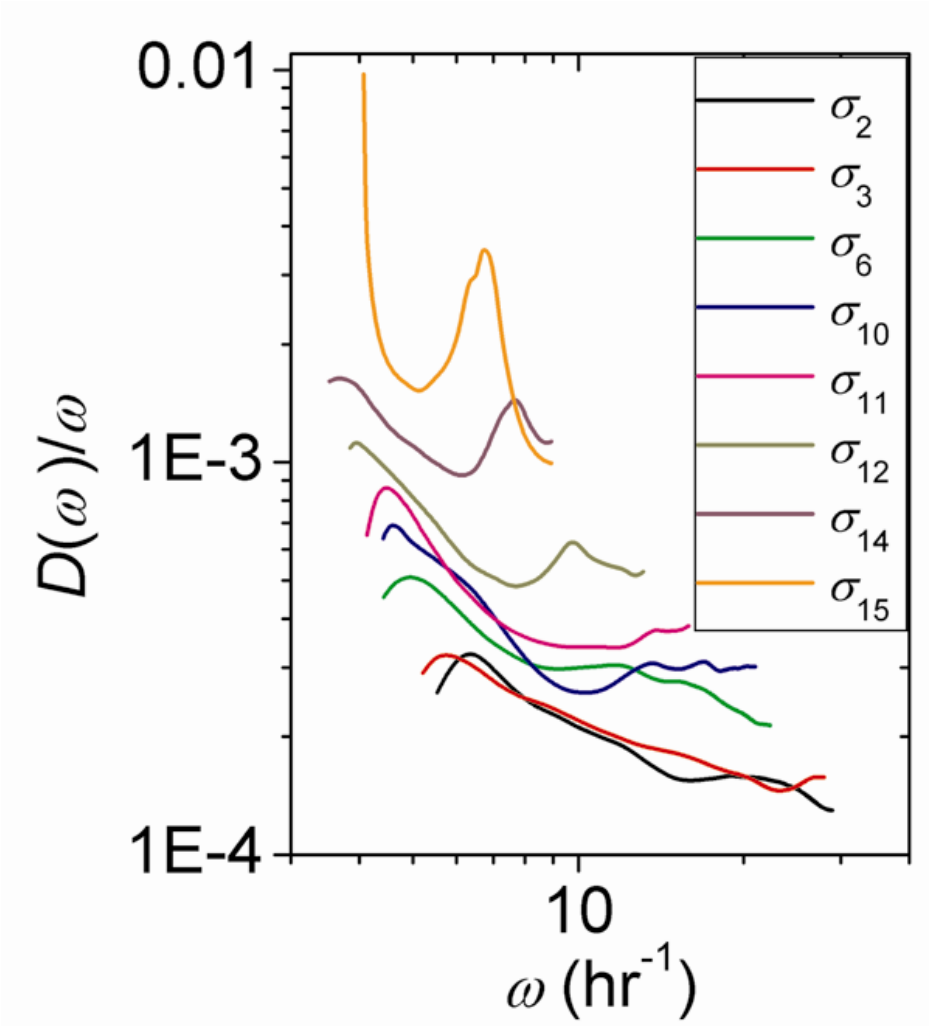
Brillouin peak



Density of States (DOS)

$$D(\omega) \sim q \frac{dq}{d\omega}$$

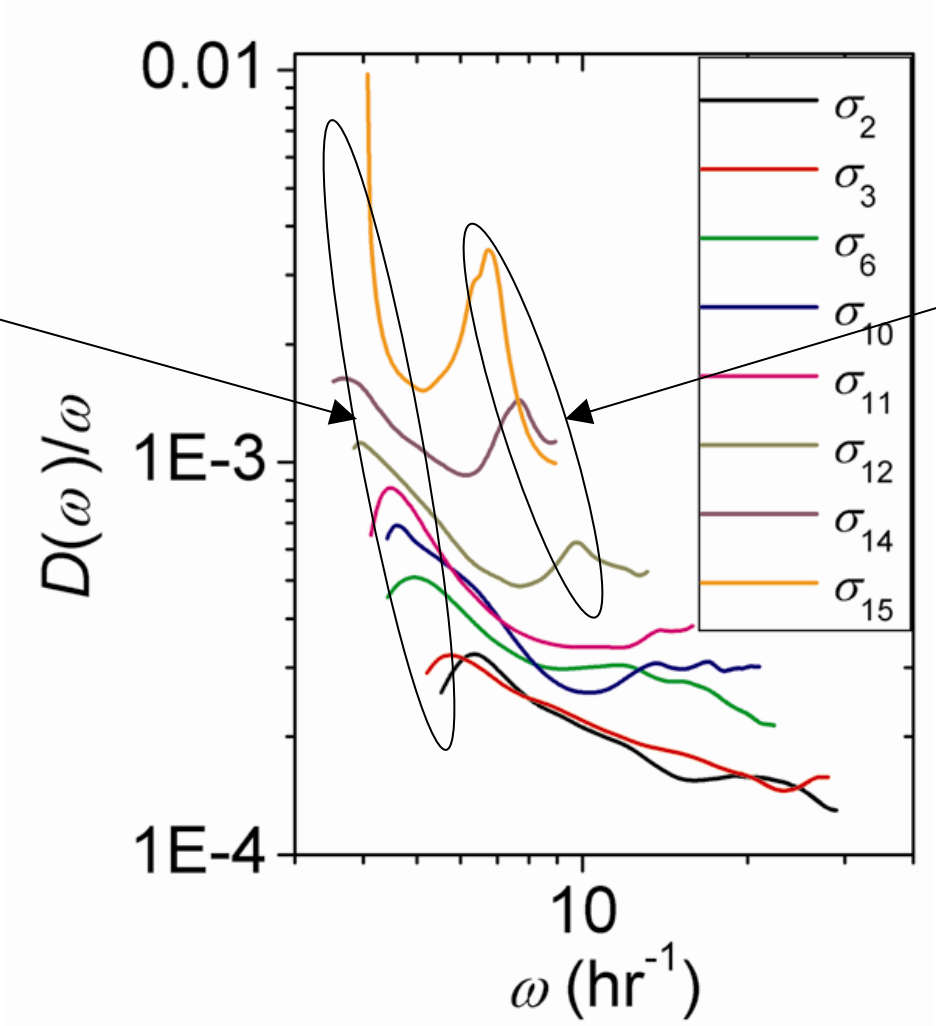
Brillouin peak: Density of States (DOS)



Brillouin peak: Density of States (DOS)

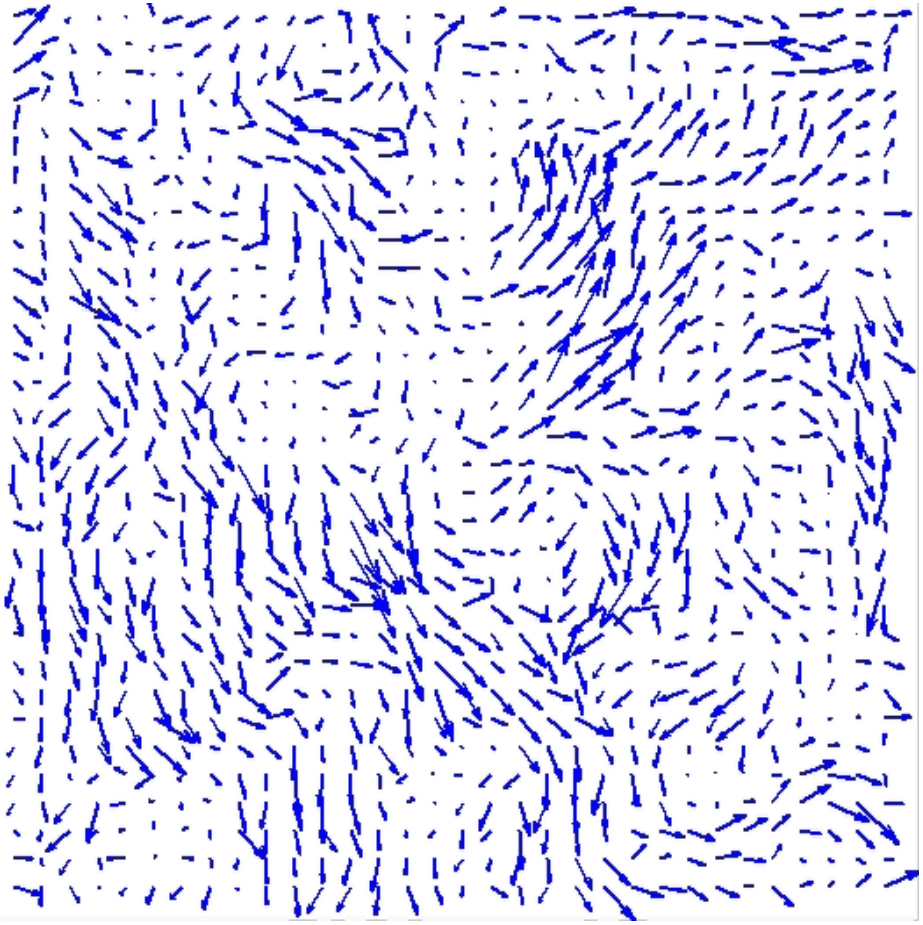
Cell body deformations

Cell Divisions



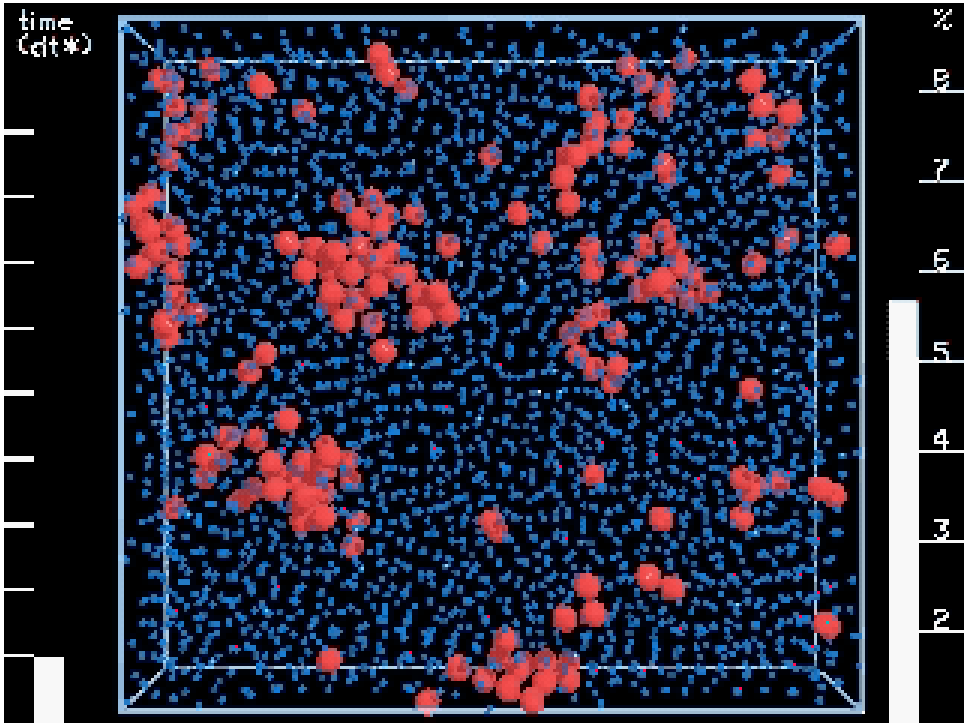
DOS peak: soft modes in glasses

Velocity field: length scale for flow



Structural Relaxations in a Supercooled Fluid

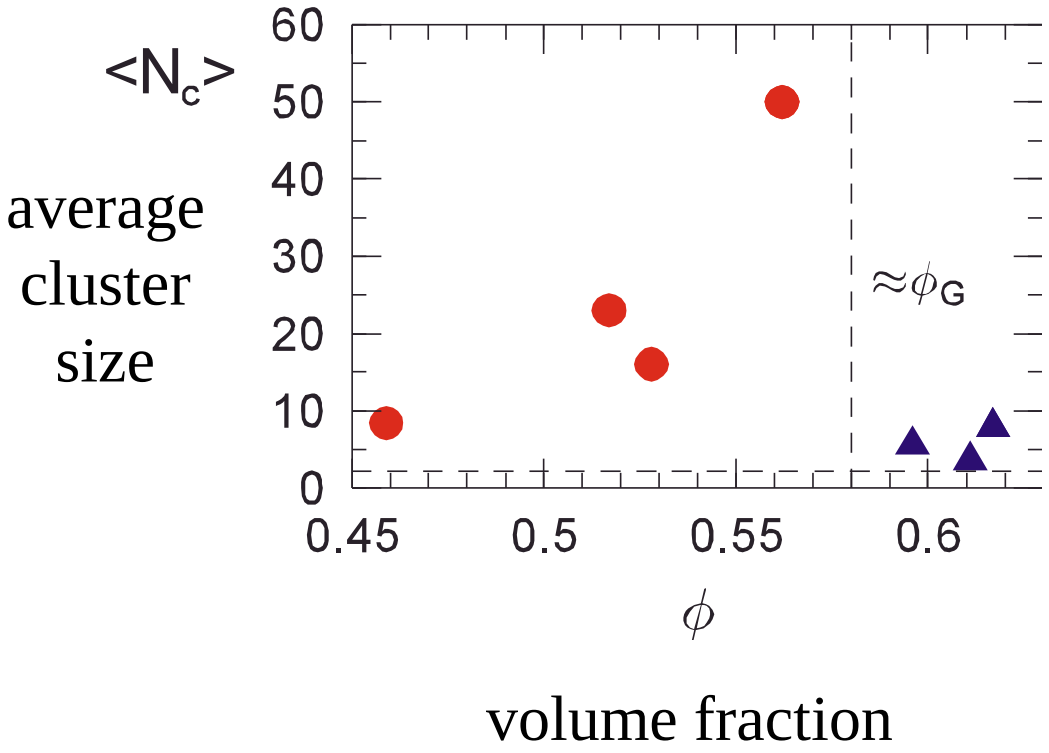
time



Number of relaxing particles

Relaxing particles are highly correlated spatially

Relaxation events are spatially correlated

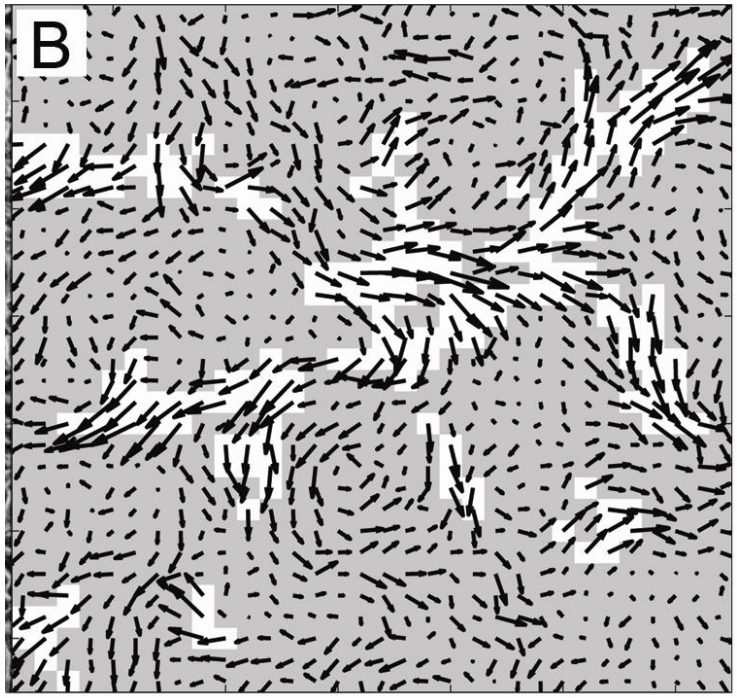


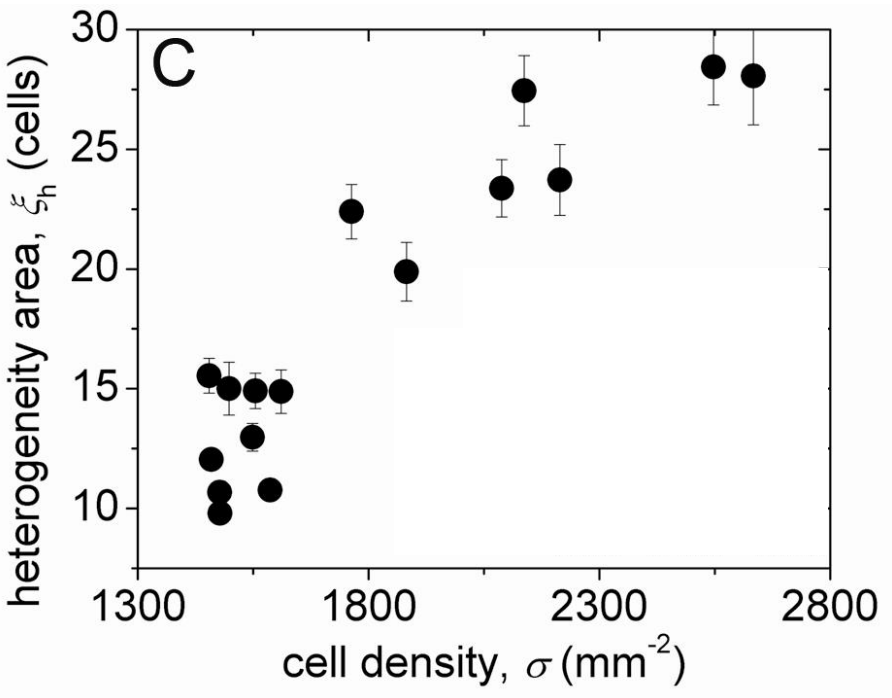
Cluster size grows as glass transition is approached

Dynamic heterogeneities grow

choose all **v**'s within fastest 15%

calculate size of connected regions: ξ_h

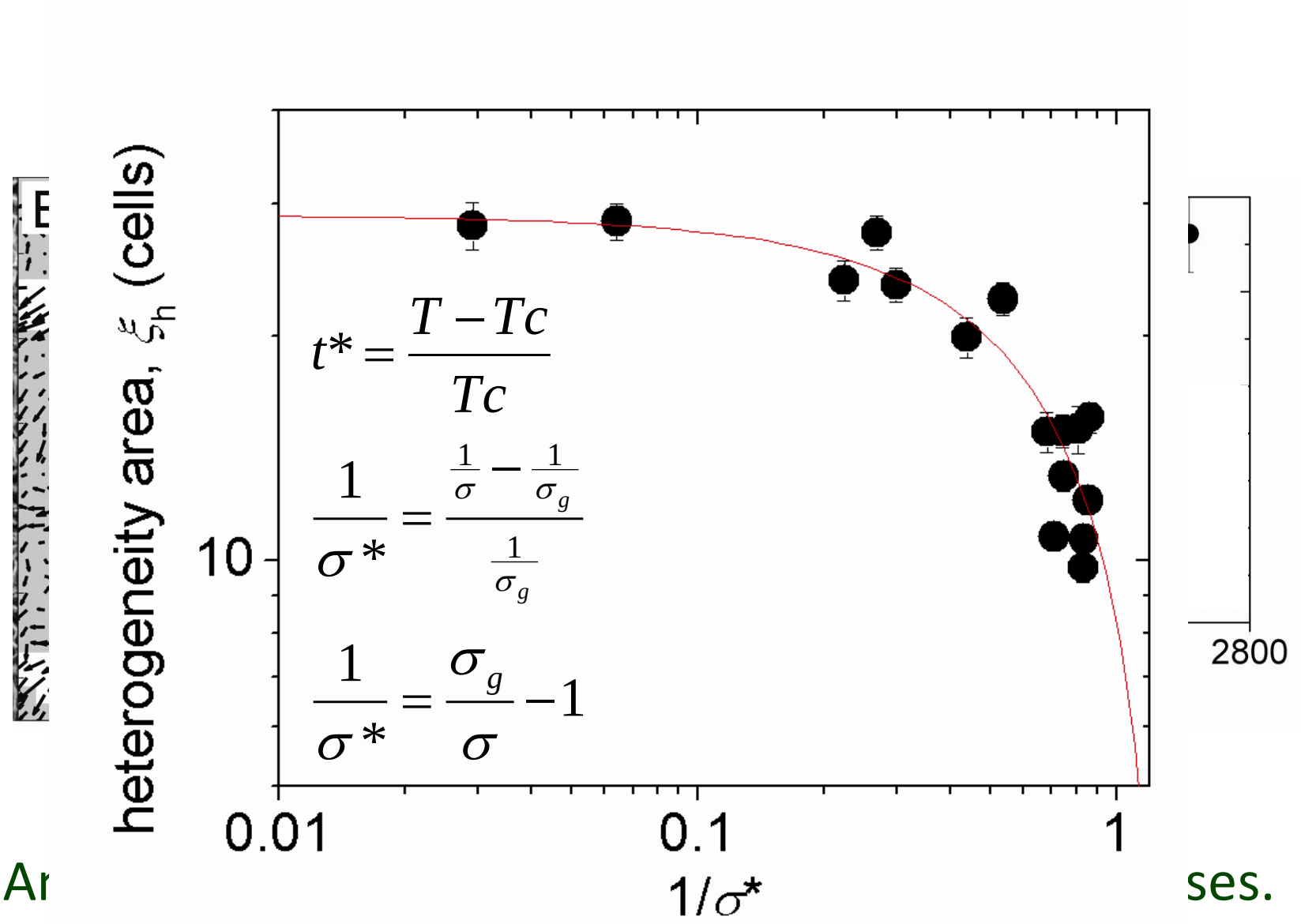




Migration slows down, cluster size grows.

Analog to dynamic heterogenities in colloidal glasses.

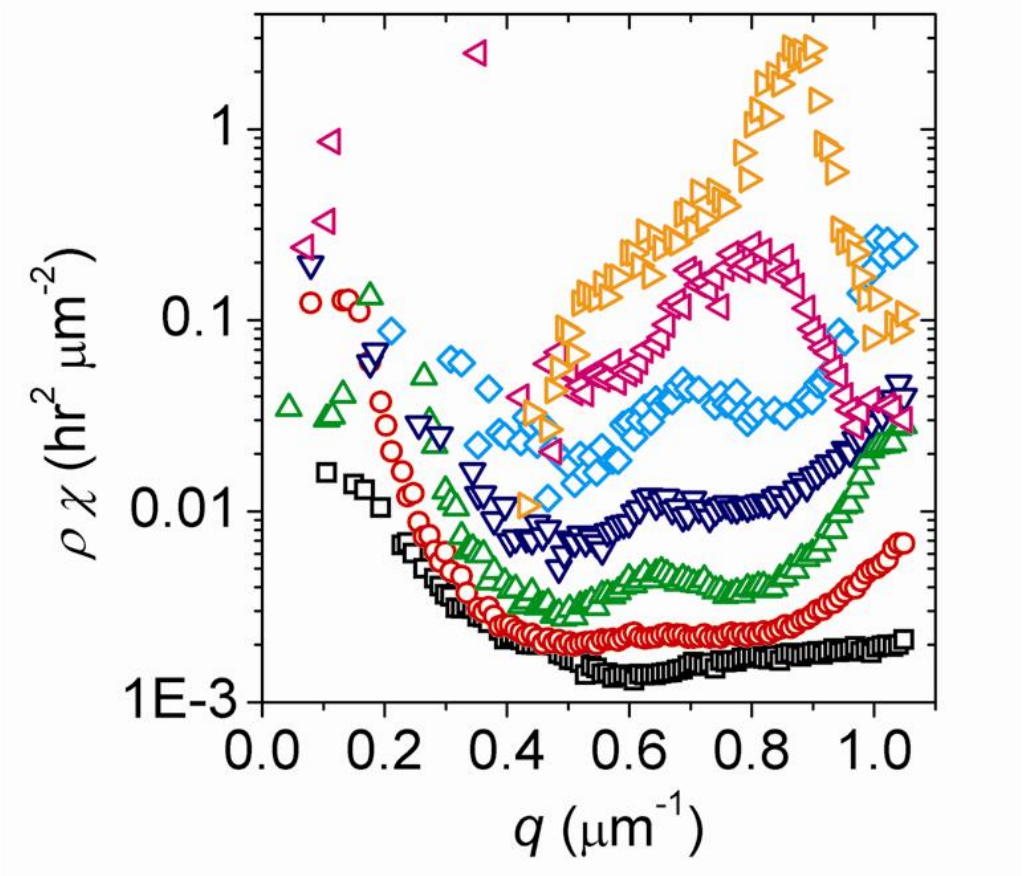
Dynamic heterogeneities grow



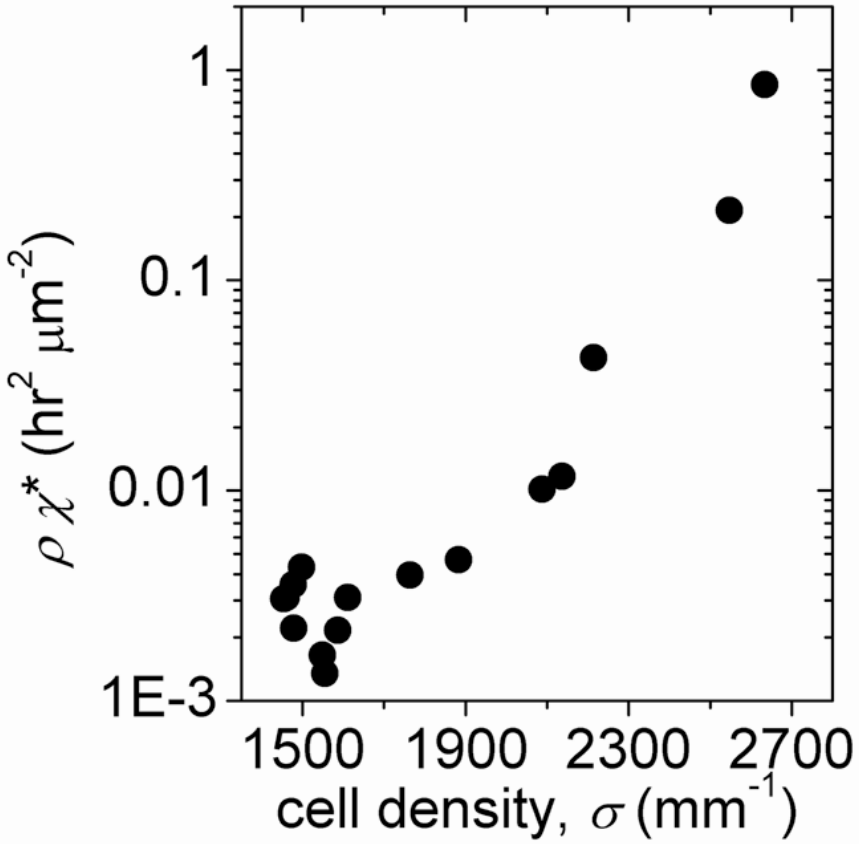
Ar ses.

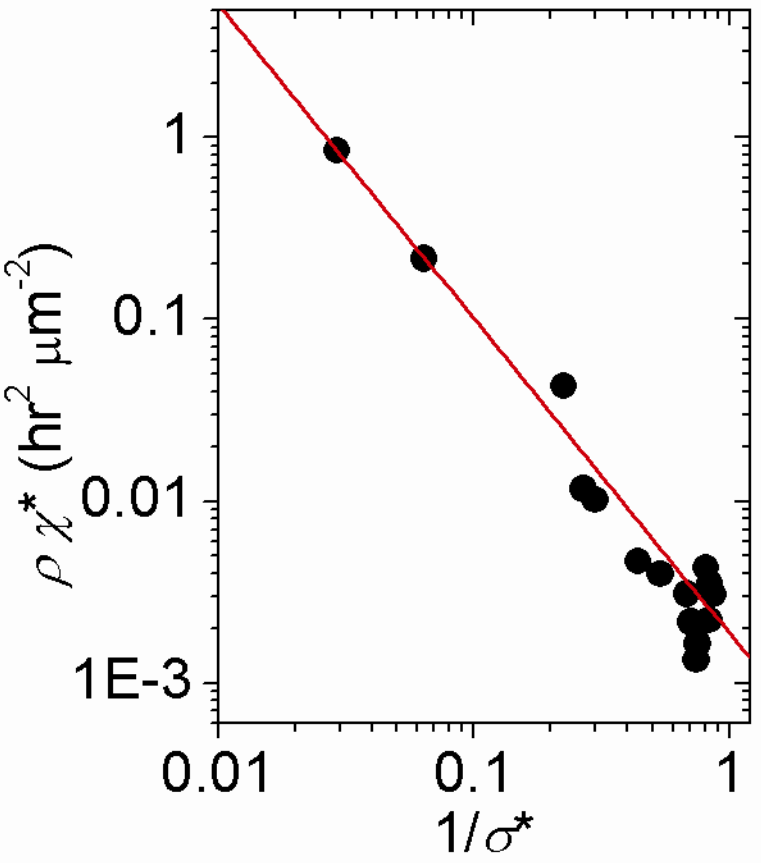
Compressibility

$$\chi = \frac{1}{\rho c^2} \frac{I_R}{2I_B + 1}$$



Compressibility





Wavelength at peak = **0.40+-0.04 cell lengths**.

Exactly the same as **high-ω DOS peak**!

Phase transition from **migration** state to **division** state?

Tissue analogy to glass forming fluid

- Growing dynamic heterogeneities
- Growing relaxation times: fragility
- Cell body deformation: Boson peak in DOS

Implications:

- collective motion is activated.
- fluidize tissue to initiate fast motion.
- wound healing, metastasis, embryonic development.

Conclusions

- Inert particles undergo ‘diffusive’ motion
- Fluctuations driven by motors in cell
- Cell volume depends on cell spreading
- Cell stiffness correlates with cell volume
- Stem-cell differentiation depends on volume

Thank you for your attention