Active contractility of adherent cells

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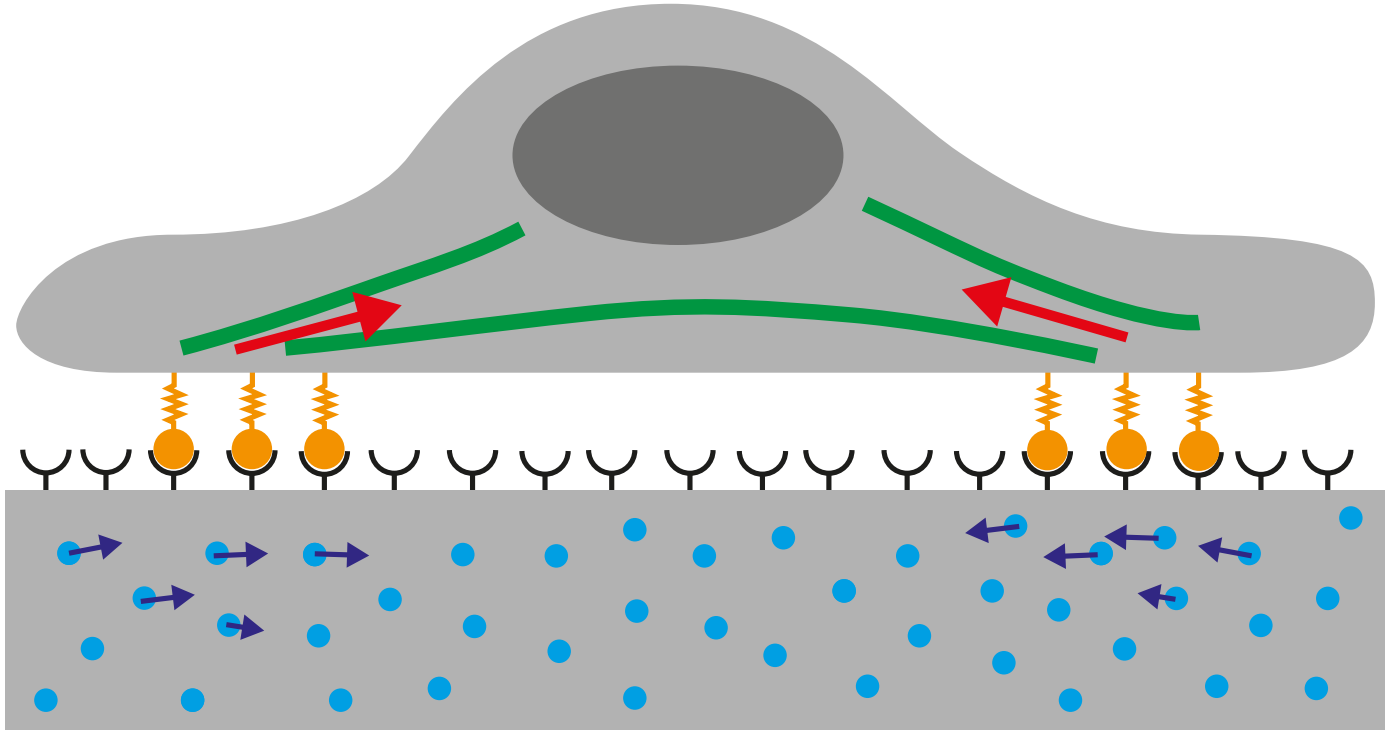
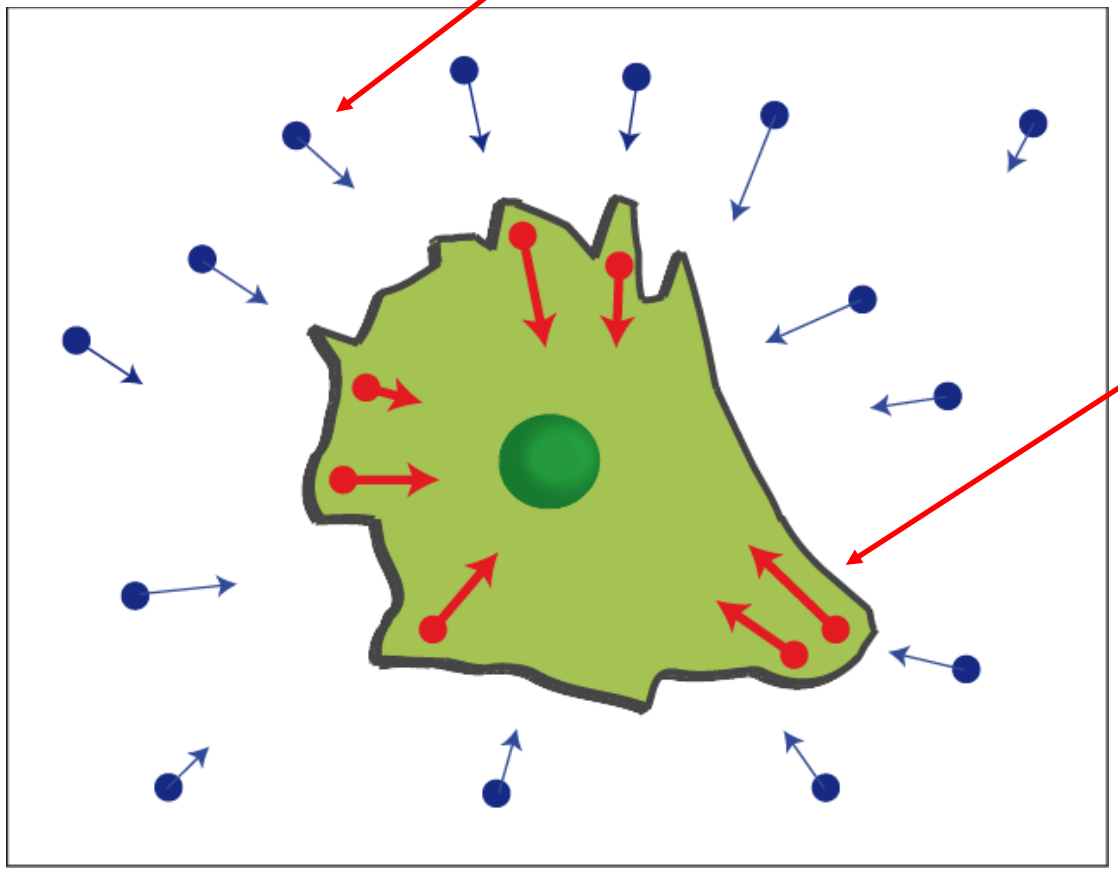
Cells on elastic substrates



Biological cells contract their environment to sense its mechanical properties. The deformation of a soft elastic substrate can be tracked with fluorescent marker beads.

Traction force microscopy (TFM) on soft elastic substrates

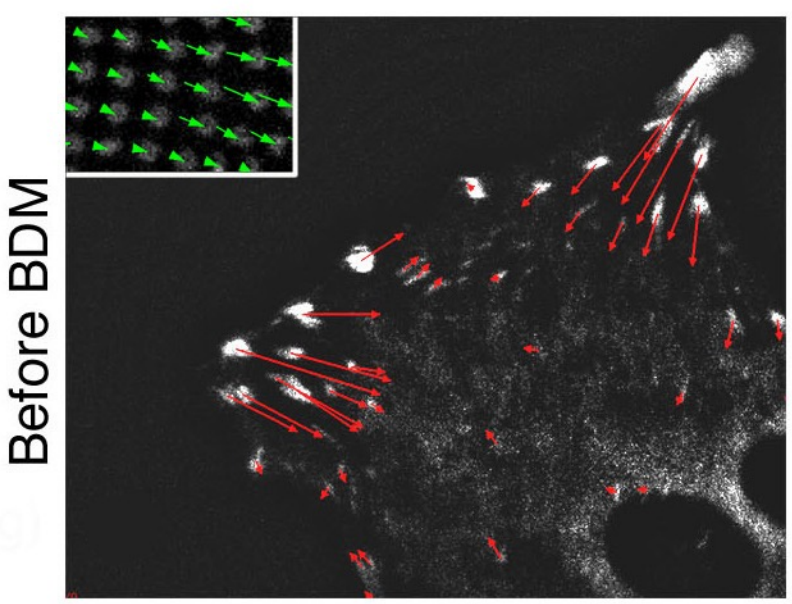
Pick up deformations by extracting movement of fiducial makers with image processing

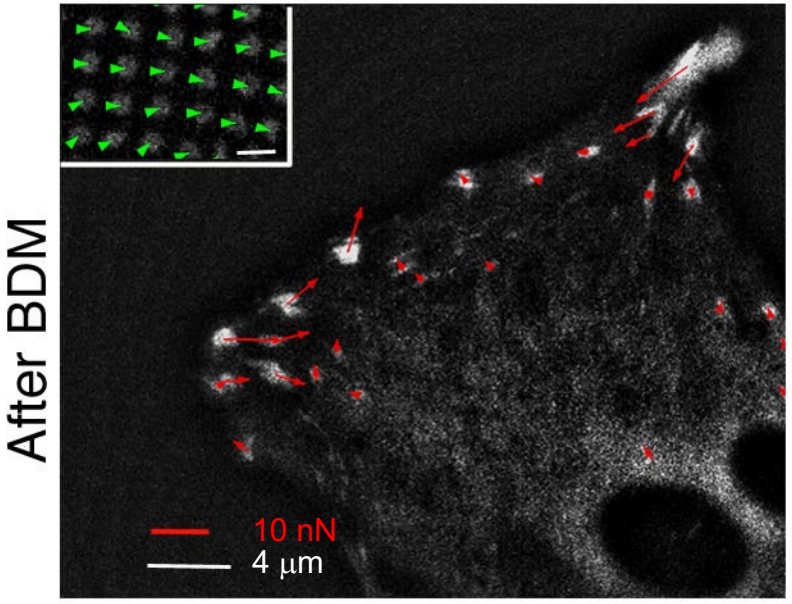


Reconstruct cellular forces, usually by solving the inverse problem of elasticity theory

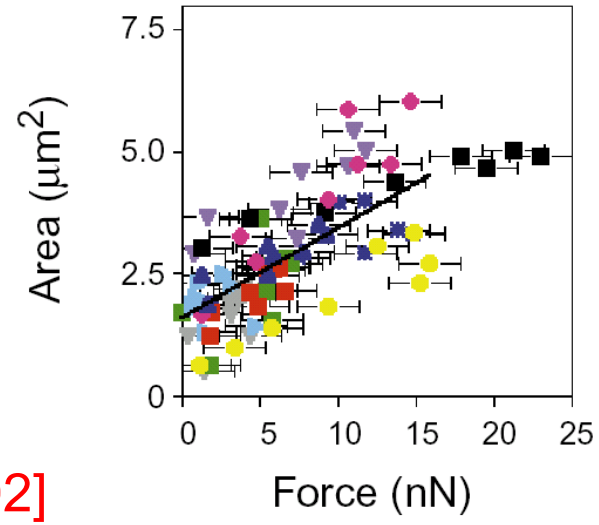
[Balaban+ NCB 2001
Schwarz+ BPJ 2002
Sabass+ BPJ 2008
Plotnikov+ MCB 2014
Schwarz & Soine BBA 2015
Blumberg & Schwarz PLOS ONE 2022]

The typical force per adhesion is a few nN



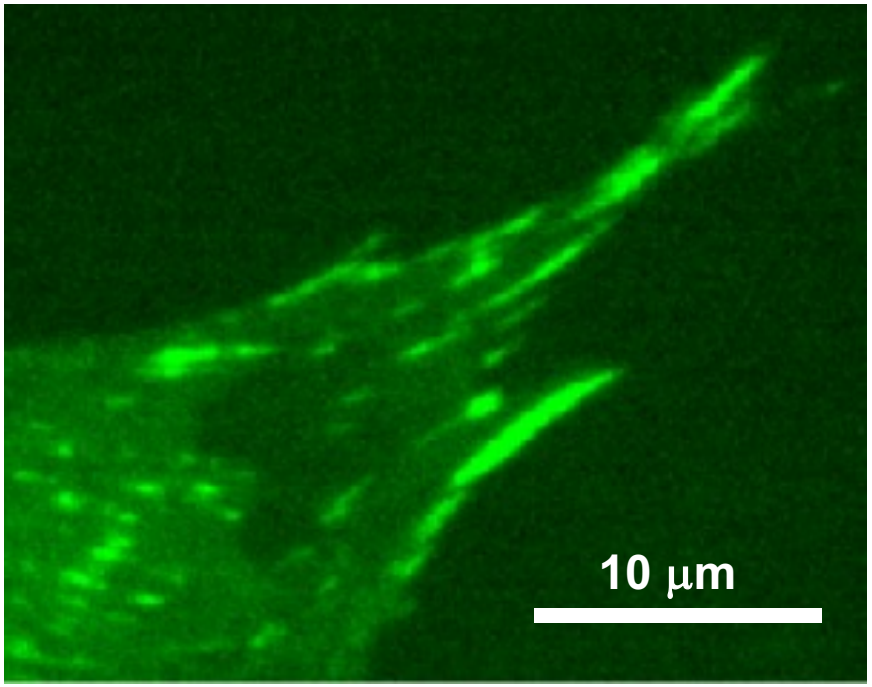


- Force is localized to sites of adhesion
- Adhesion size correlates with force
- Stress constant 5.5 nN/μm^2=5.5 kPa

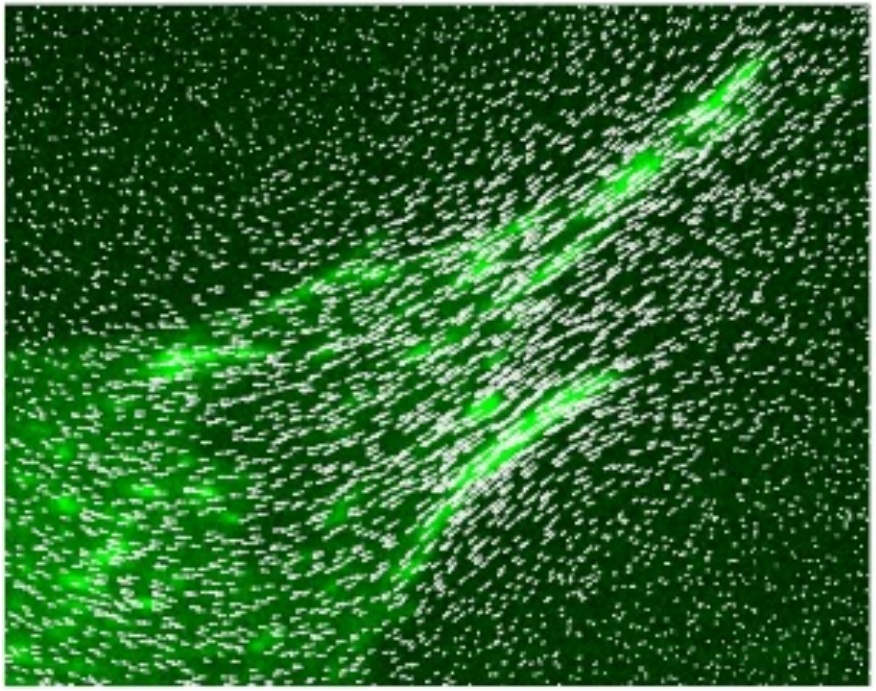


[Balaban et al. NCB 2001, Schwarz et al. BPJ 2002]

High resolution traction force microscopy



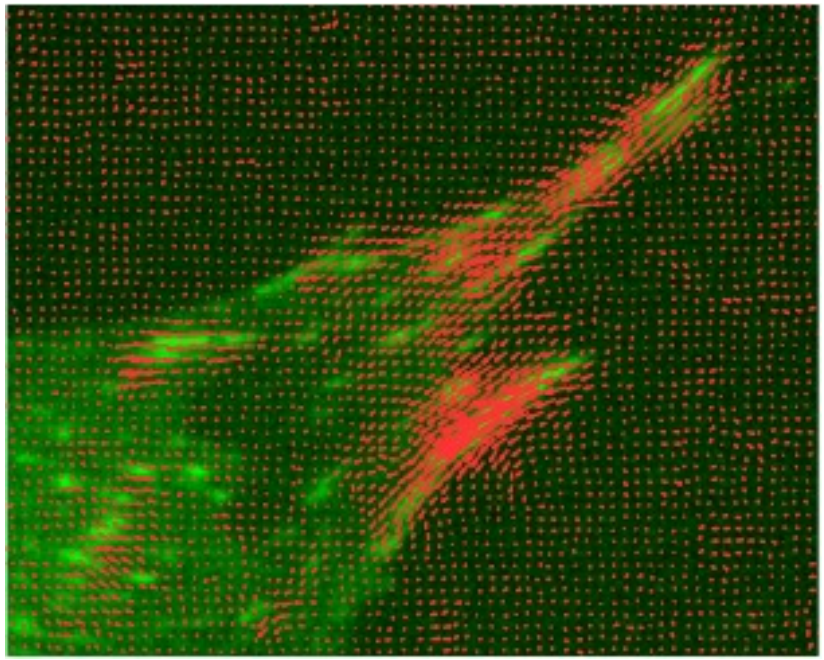
Fibroblast adhesion structures (paxillin) on 15 kPa polyacrylamide-substrate with two differently colored fluorescent nanobeads



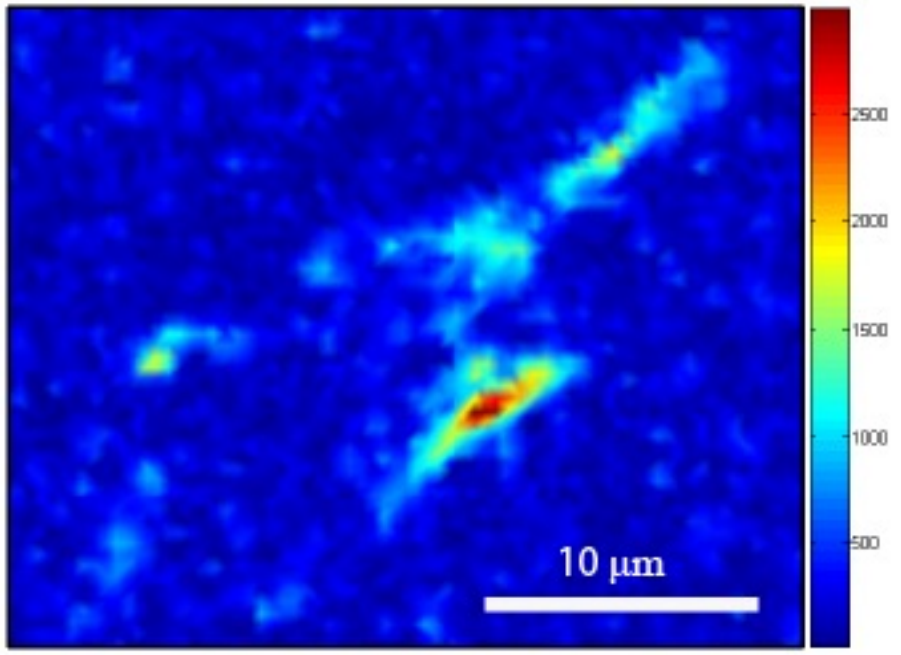
Displacement field extracted with correlation-based particle tracking velocimetry (mesh size 500 nm)

[Sabass+ BPJ 2008, Plotnikov+ MCB 2014]

Fourier Transform Traction Cytometry



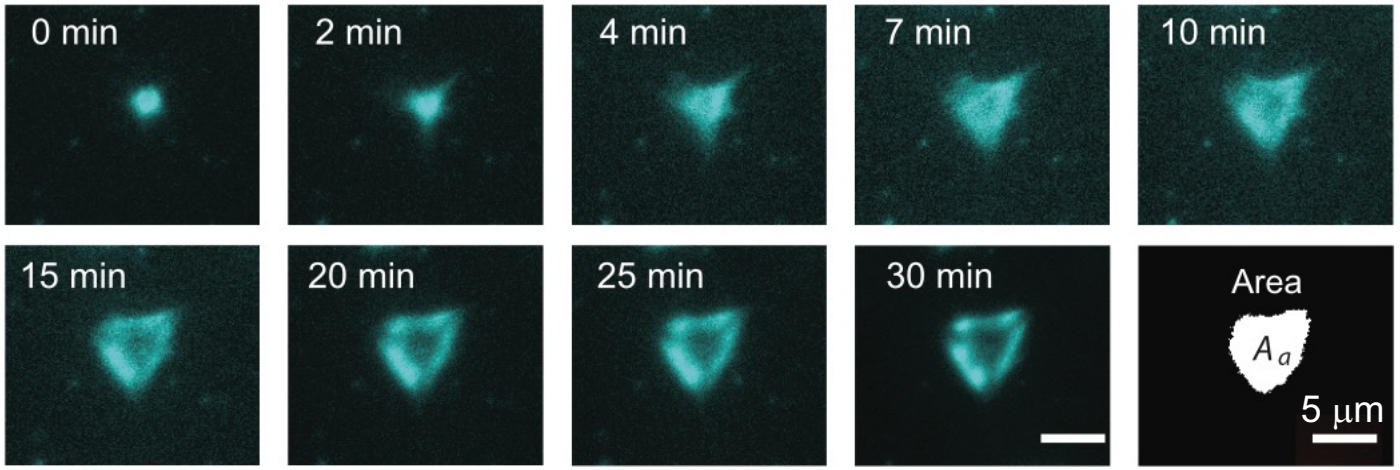
Traction vector field (spatial resolution 1 μm)

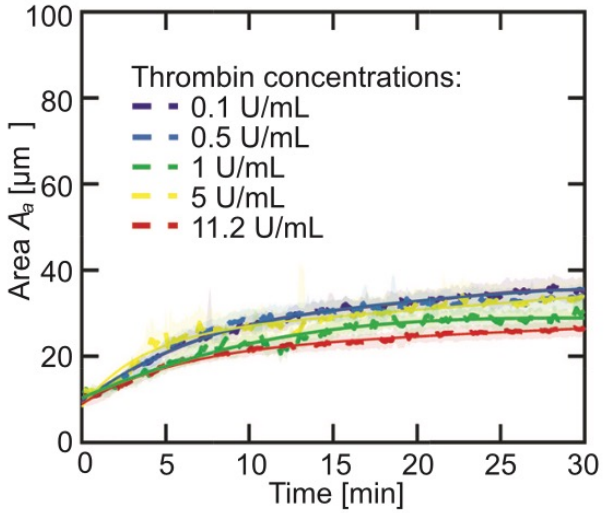


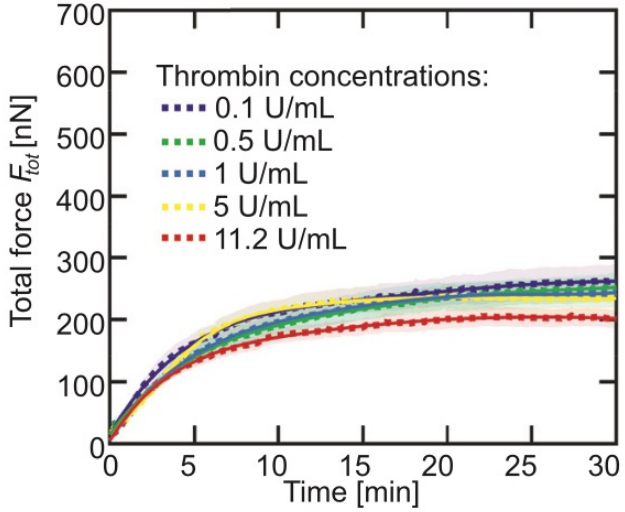
Traction magnitude (in Pa, resolution few 100 Pa)

Traction forces nicely co-localize with the adhesion signal.

Spreading of blood platelets



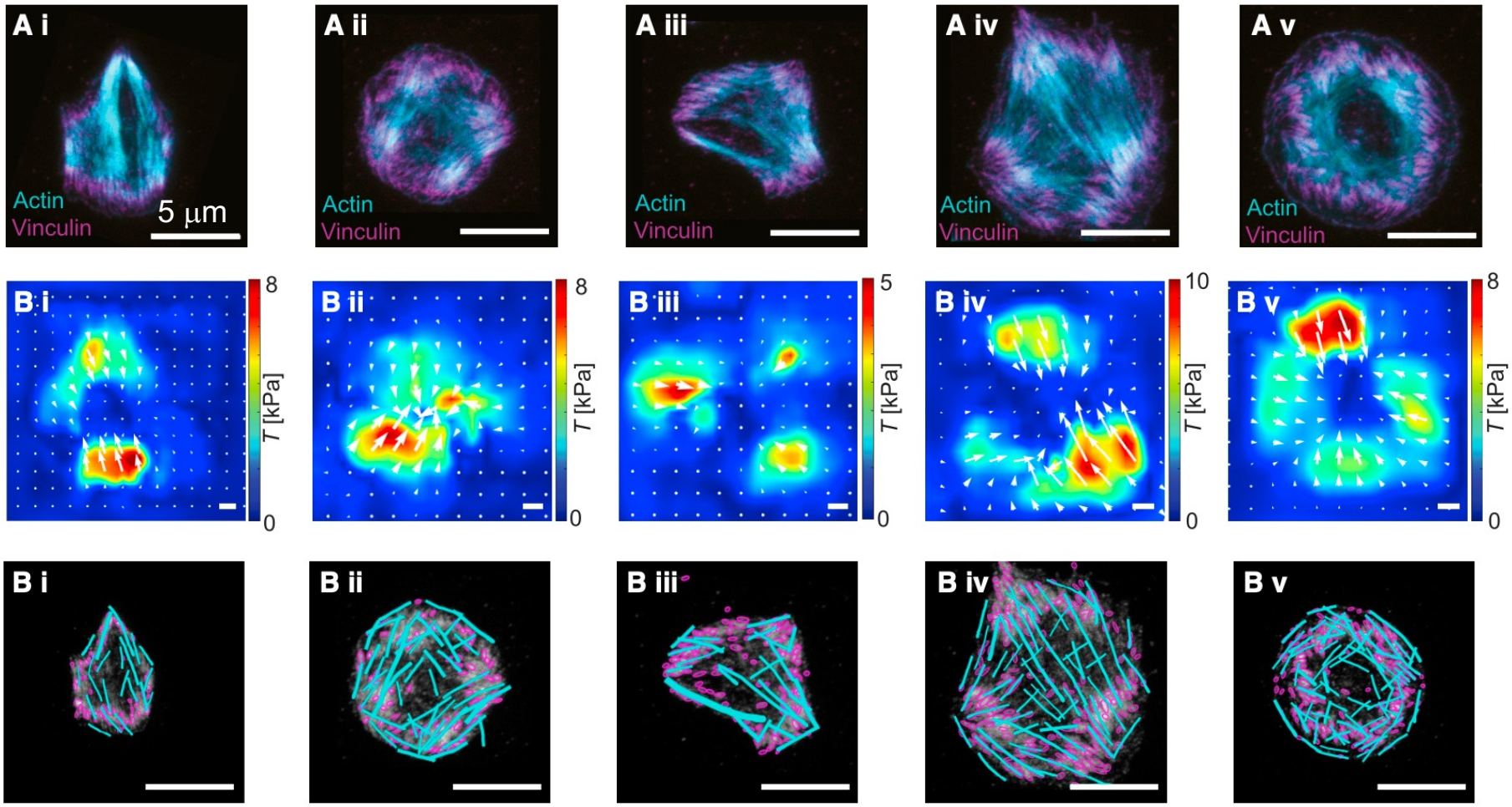




Adhesion area and overall force are generated together.

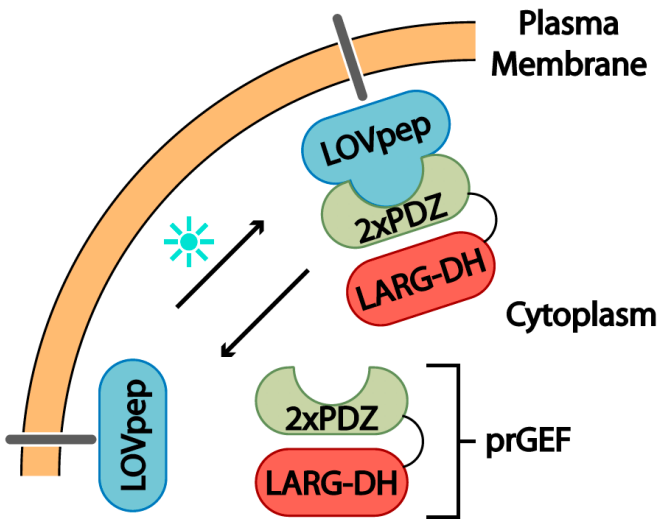
[Zelena, Blumberg et al. Biophys J 2023]

High resolution analysis with STED

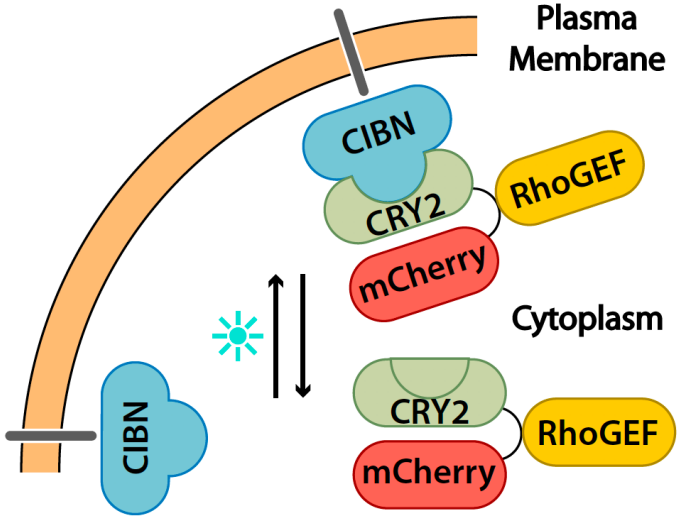


[Zelena, Blumberg et al. Biophys J 2023]

Optogenetic control of Rho-activation



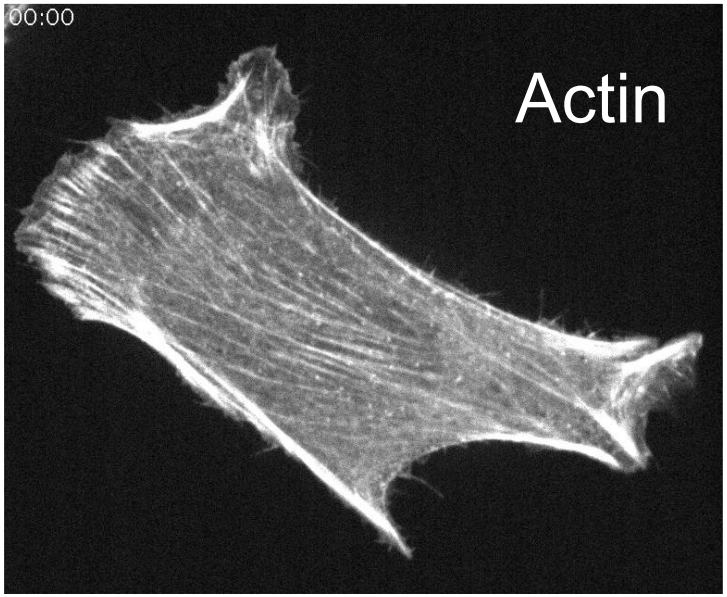
LOV-domain
[Wagner and Glotzer JCB 2016]
[Oakes Nat Comms 2017]

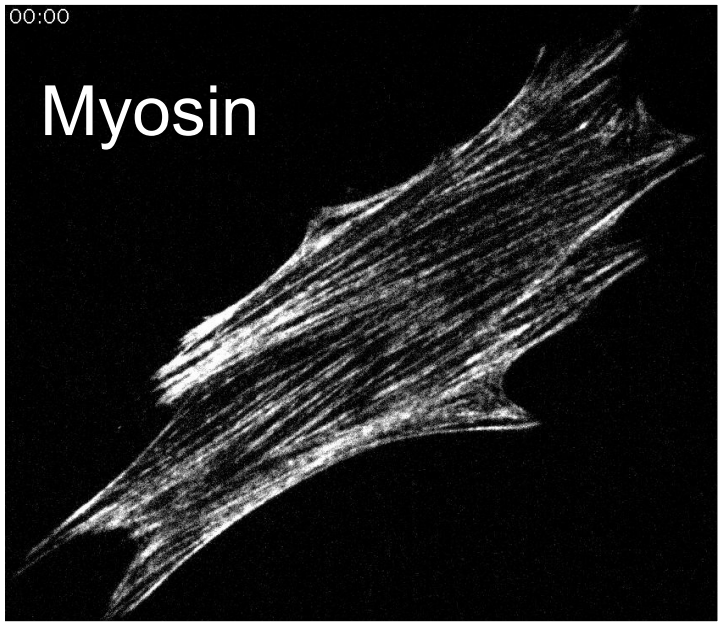


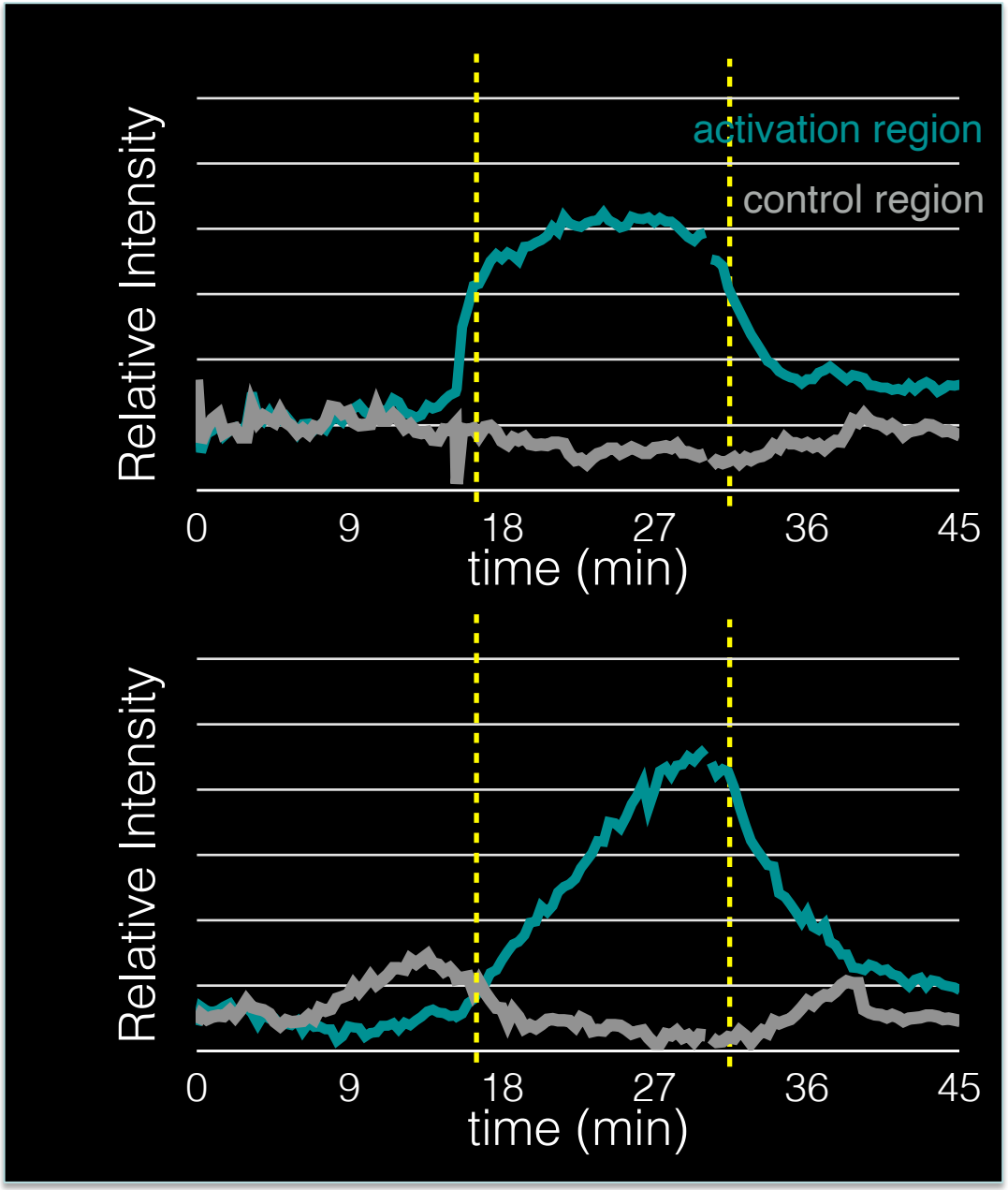
CRY2/CIBN dimerizer system
[Valon BPJ 2015 and Nat Comms 2017]
[Andersen, Wörthmüller BPJ 2023]
[Ruppel, Wörthmüller eLife 2023]

Both systems use light to recruit a Rho-GEF to the membrane, which then triggers actomyosin contractility.

Sub-cellular activation with LOV-system

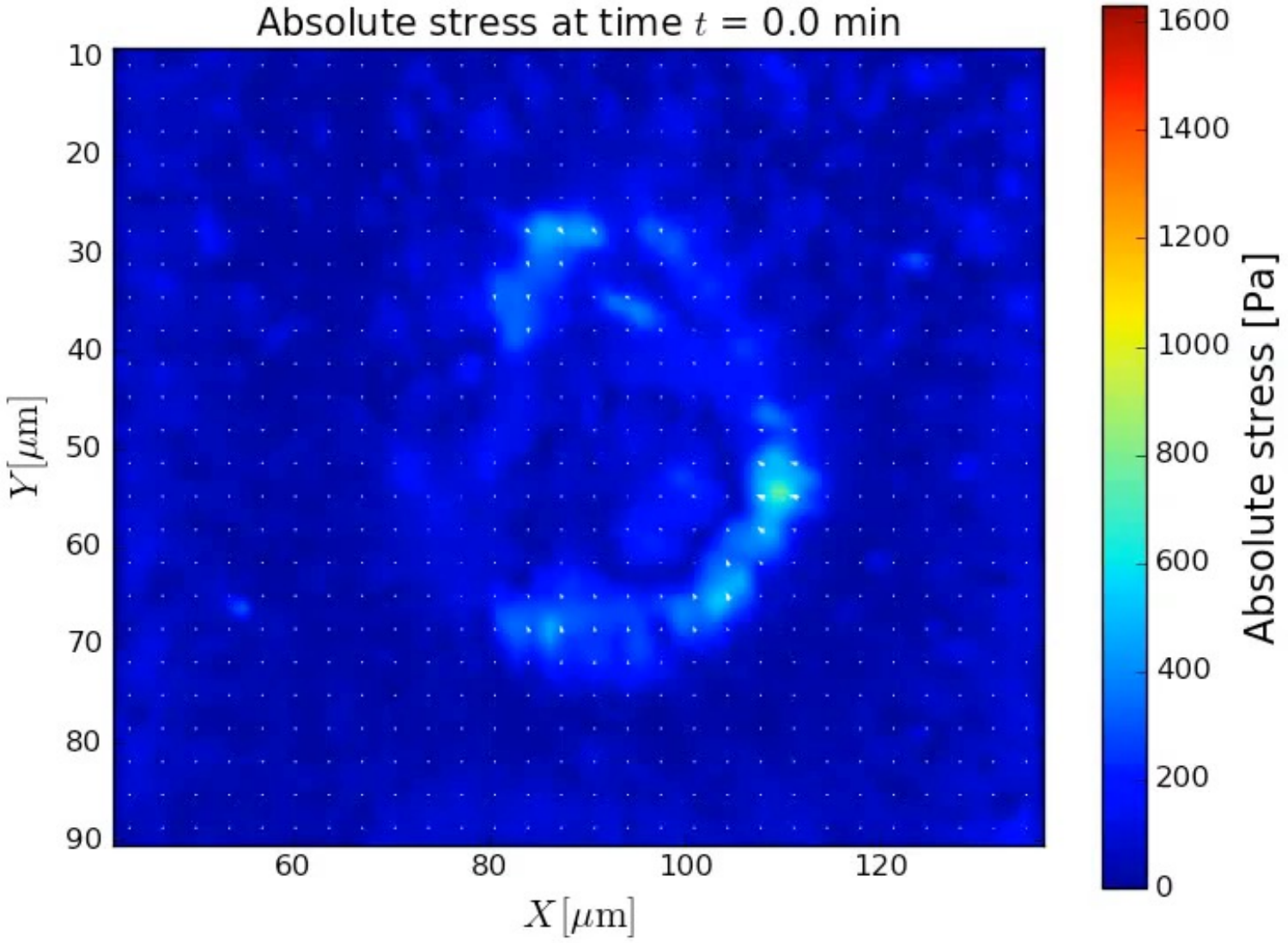




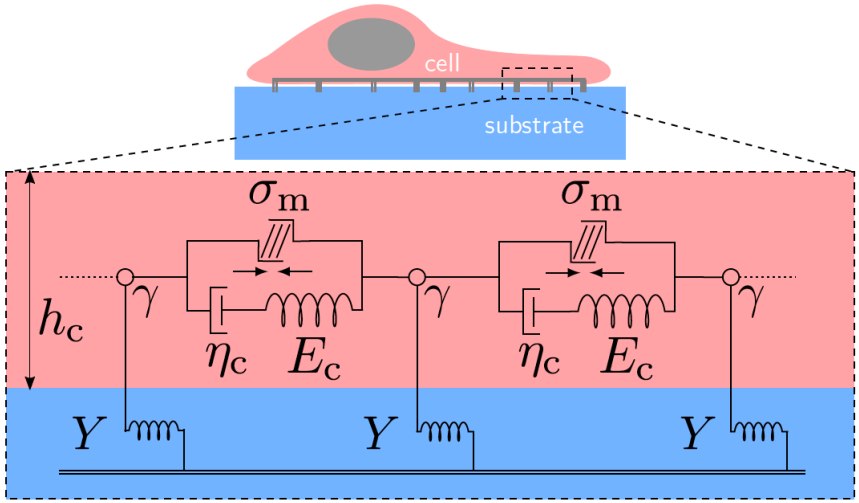


[Oakes Nat Comms 2017]

Optogenetic activation of traction forces

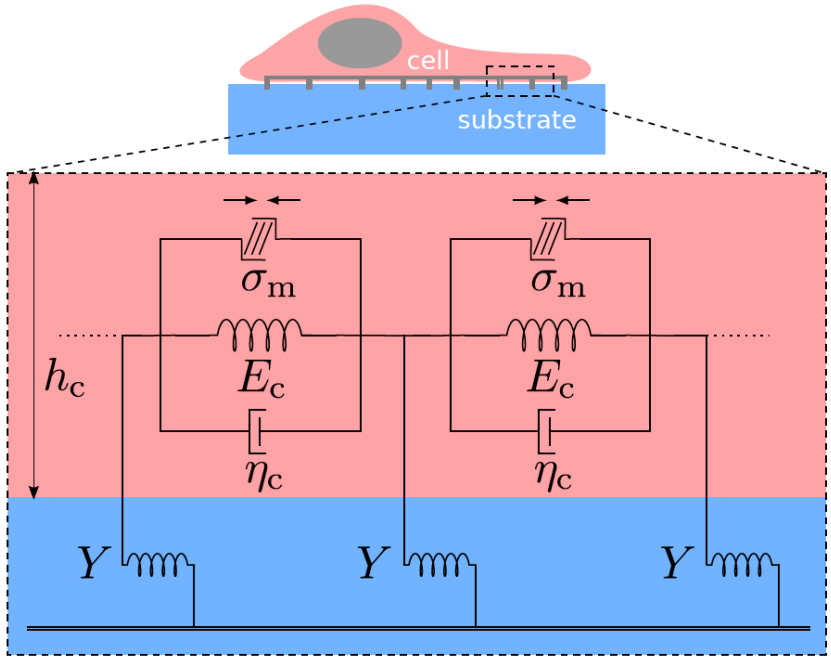


Two viscoelastic models for cells



active Maxwell model (viscous)

$$\frac{\sigma_{ij}-\sigma_{ij}^{\mathrm{m}}}{\tau_\mathrm{c}}+\left(\dot{\sigma}_{ij}-\dot{\sigma}_{ij}^{\mathrm{m}}\right)=\frac{\partial}{\partial t}\left(\lambda\epsilon_{kk}\delta_{ij}+2\mu\epsilon_{ij}\right)$$



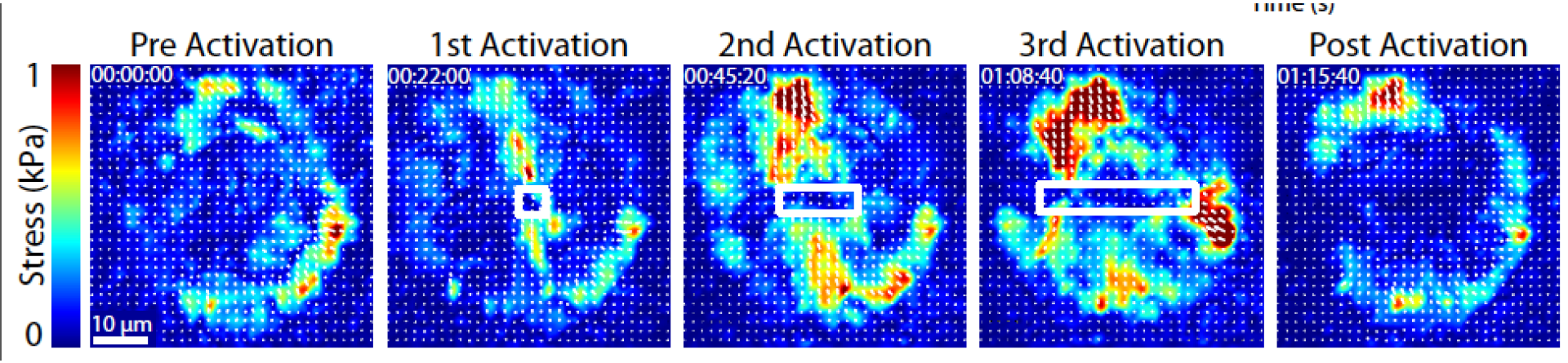
active Kelvin-Voigt model (elastic)

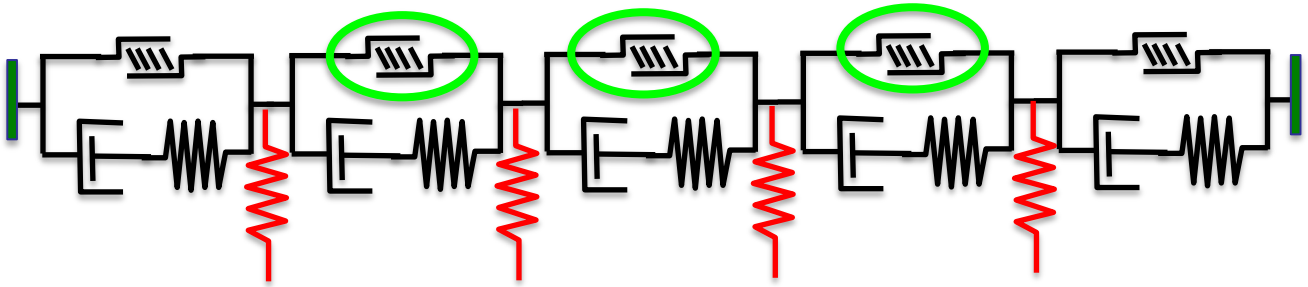
$$\sigma_{ij}-\sigma_{ij}^{\mathrm{m}}=\left(1+\tau_\mathrm{c}\frac{\partial}{\partial t}\right)\cdot\left(\lambda\epsilon_{kk}\delta_{ij}+2\mu\epsilon_{ij}\right)$$

force balance with elastic foundation $\partial_j\sigma_{ij}=Y\left(\mathbf{x}\right)u_i$

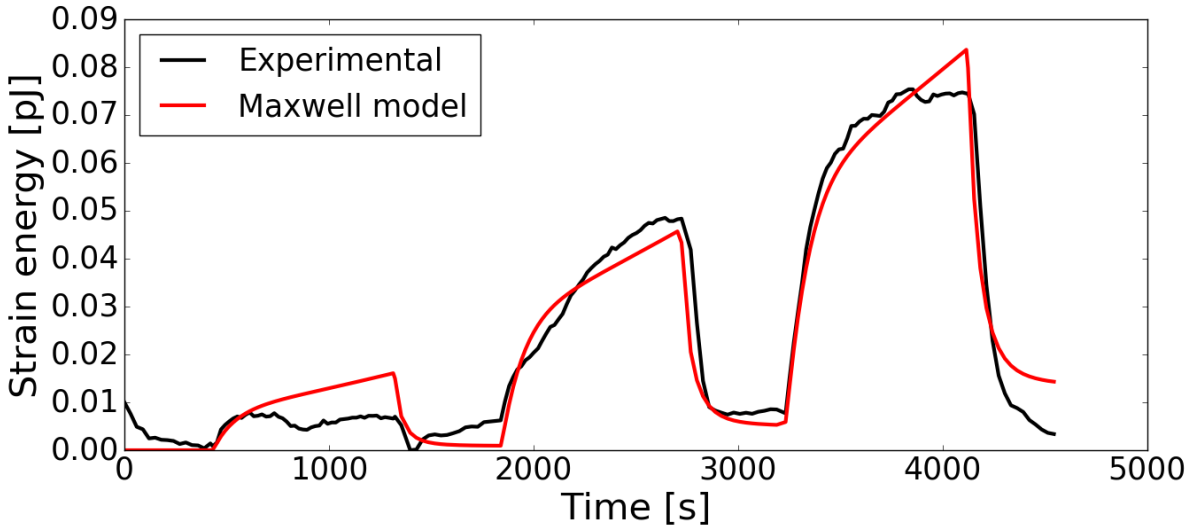
numerical solution with finite element software FEniCS in weak formulation

Optogenetic activation of Rho





active Maxwell chain coupled to elastic foundation



E = 9.8 kPa

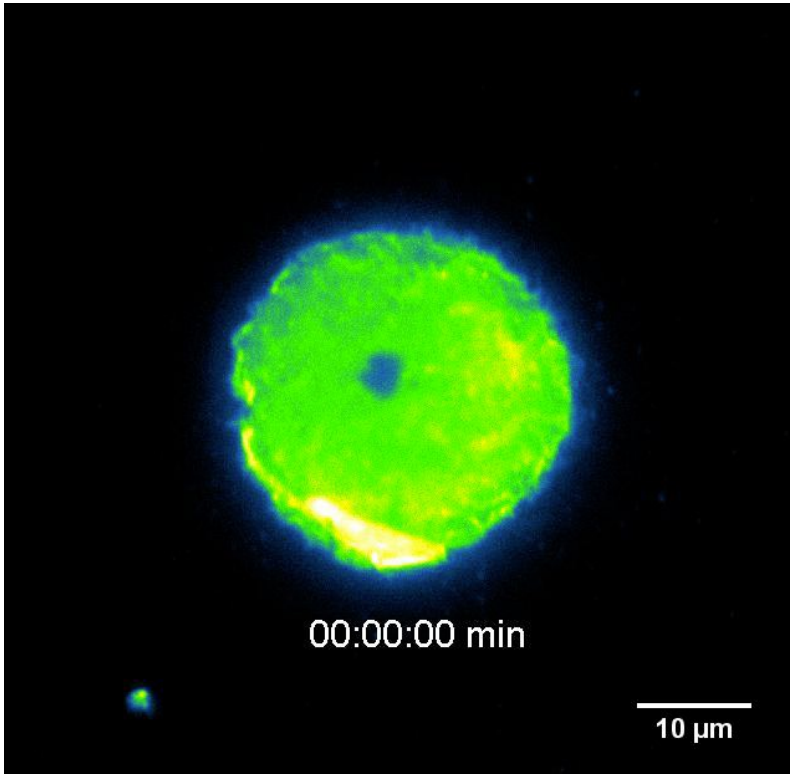
η = 18 MPa*s

γ = 6.7e16 Pa*s/m^2

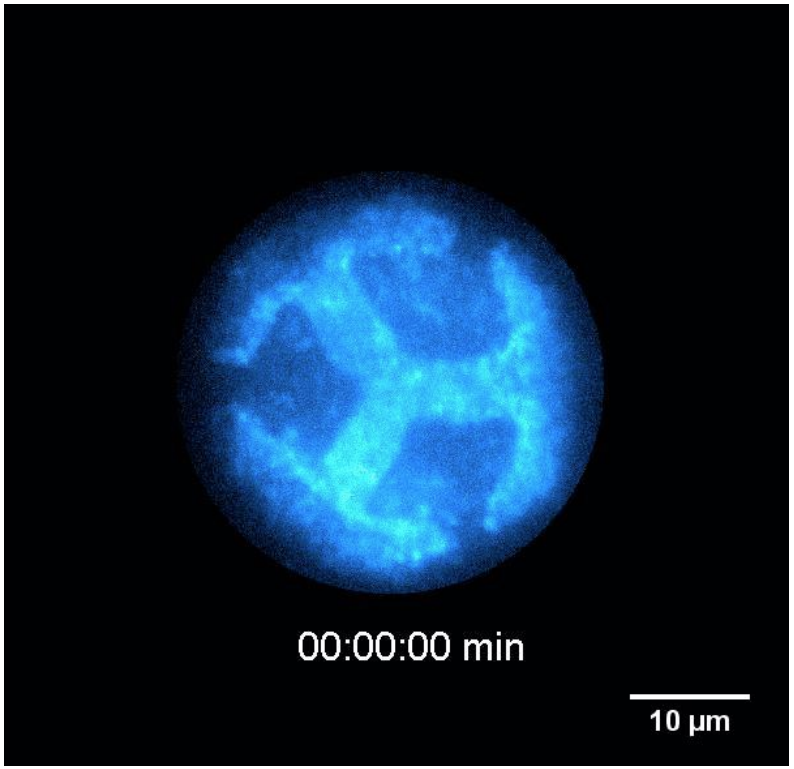
σ = 2.8 kPa

[Oakes Nat Comms 2017]

Optogenetic activation of whole cells



disc

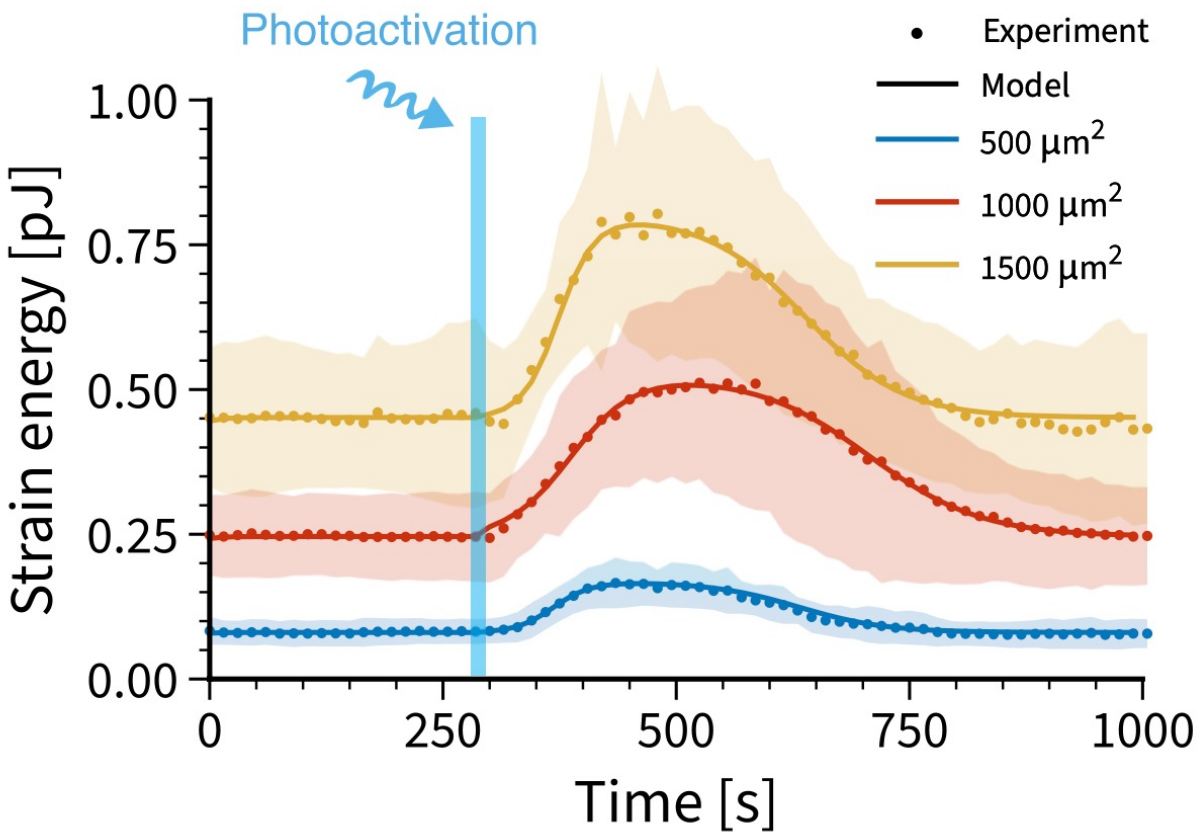


hazard

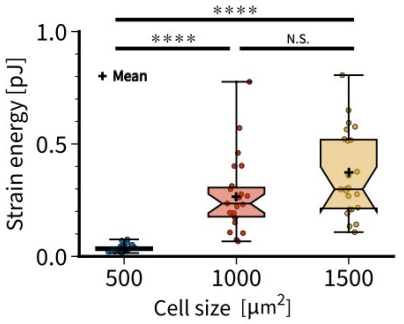
Fibroblasts on micropatterned elastic substrates, optogenetic activation of contractility by CRY2/CIBN-system

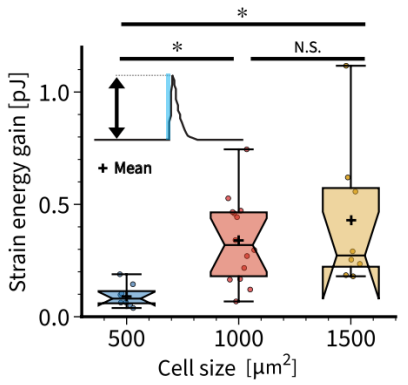
[Andersen, Wörthmüller et al. Biophys J 2023]

Tensional homeostasis



After photoactivation, cells on discs contract for a few minutes and then return to baseline („tensional homeostasis“).

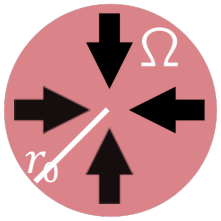




[Andersen, Wörthmüller et al. Biophys J 2023]

Analytical solution for contractile disc

Isotropic contraction



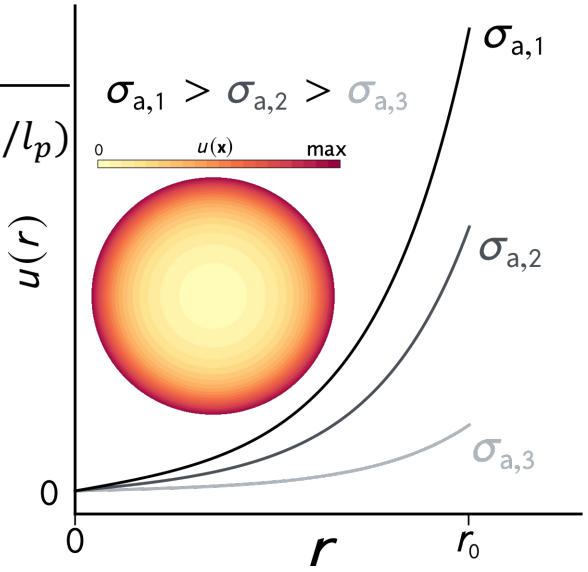
Radial displacement field:

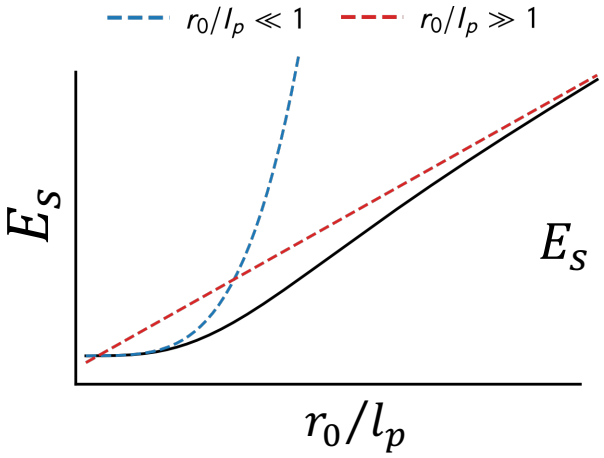
$$u(r) = l_p \frac{\sigma_a}{2\mu + \lambda} \frac{I_1(r/l_p)}{I_0(r_0/l_p) - \frac{2\mu}{2\mu+\lambda}\frac{l_p}{r_0} I_0(r_0/l_p)}$$

Modified Bessel functions of the first kind $I_n(x)$

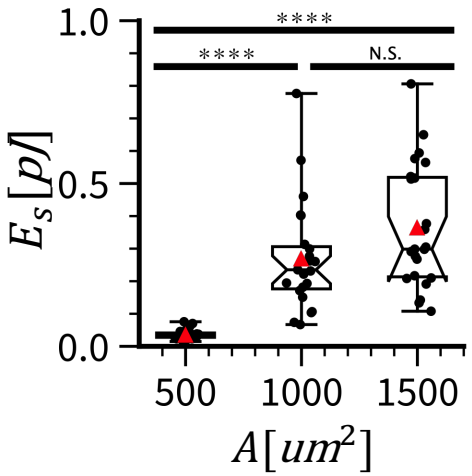
Force localization length $l_p = \sqrt{\frac{h_c E_c}{Y(1-\nu_c^2)}}$

Two-dimensional Lamé parameters λ, μ



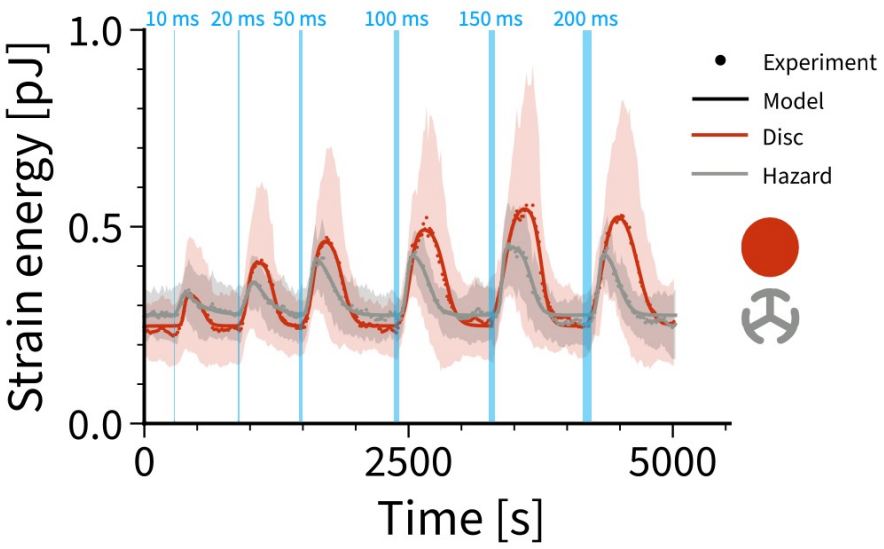


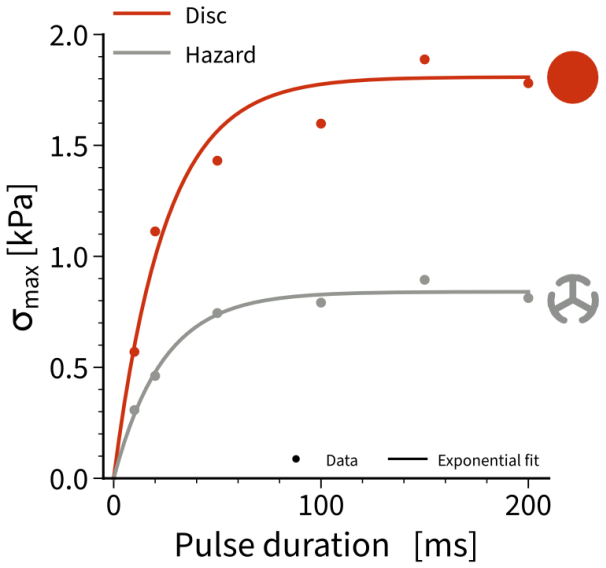
$$E_s \underset{r_0 \gg l_p}{\rightarrow} \frac{\pi \sigma_a^2}{2Y_s} \frac{r_0}{l_p} \sim \sqrt{A}$$



[Edwards and Schwarz PRL 2011, Andersen, Wörthmüller et al. Biophys J 2023]

Saturation with pulse duration





Contractility levels are well below the saturation level, so that the cell can dynamically respond to new signals in both directions.

[Andersen, Wörthmüller et al. Biophys J 2023]

Orders of magnitude estimates

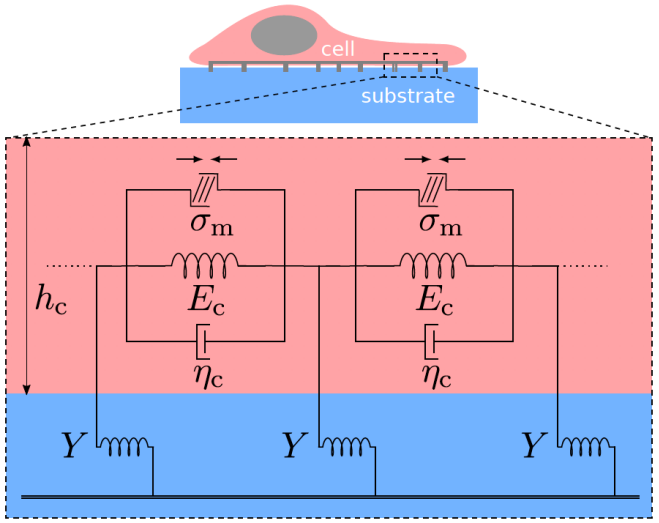
substrate stiffness $$Y = \frac{pN}{nm}\frac{1}{\mu m^2} = \frac{nN}{\mu m^3} = \frac{kPa}{\mu m}$$

localization length $$l_p = \sqrt{\frac{E_c h_c}{Y(1-\nu_c)}} = \sqrt{\frac{10\ kPa\ 1\ \mu m}{kPa/\mu m}} = 3\ \mu m$$

active stress $$\sigma = \frac{F_{tot}}{2\pi r_o h_c} = \frac{\mu N}{2\pi 15 \mu m 1 \mu m} = 10\ kPa$$

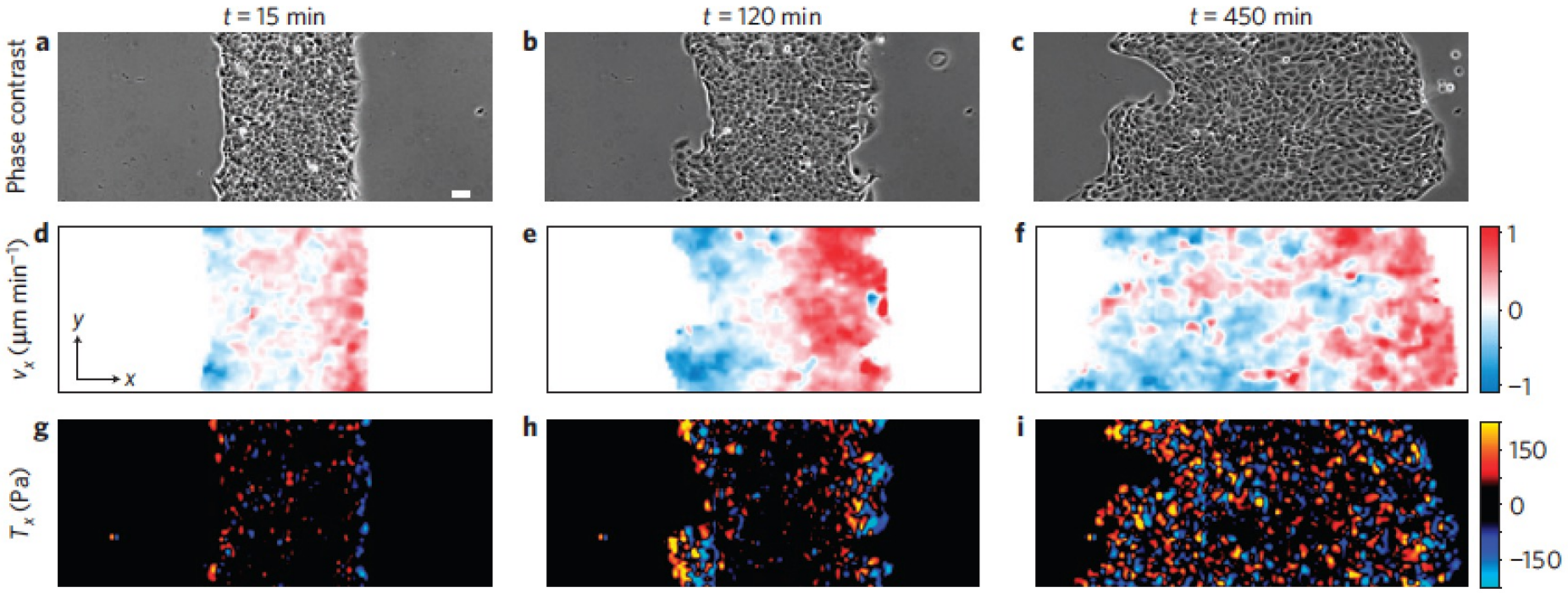
substrate strain energy $$E_s = \frac{\pi(\sigma h_c)^2}{Y}\frac{r_0}{l_p} = pJ$$

relaxation time $$\tau = \frac{\eta}{E} = \frac{100\ kPa\ s}{10\ kPa} = 10\ s$$



[Andersen, Wörthmüller et al. Biophys J 2023]

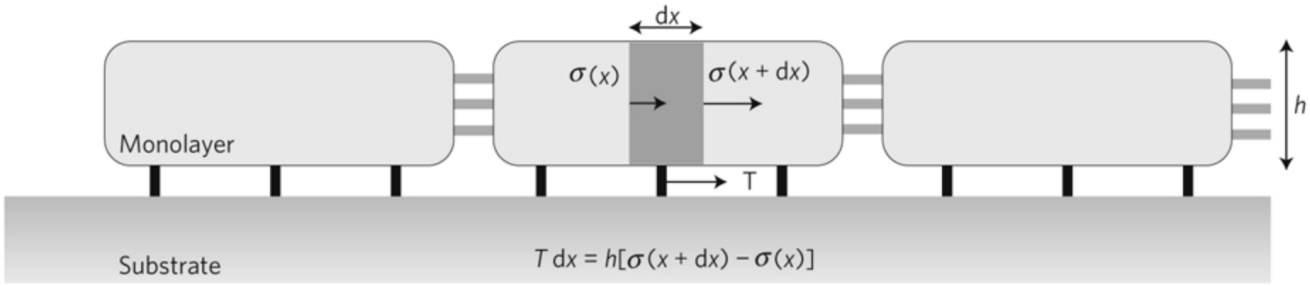
Monolayer stress microscopy



After removing the barriers at the left and the right,
a stripe of cells is expanding into empty space. On soft elastic substrates, traction T can be measured with TFM.

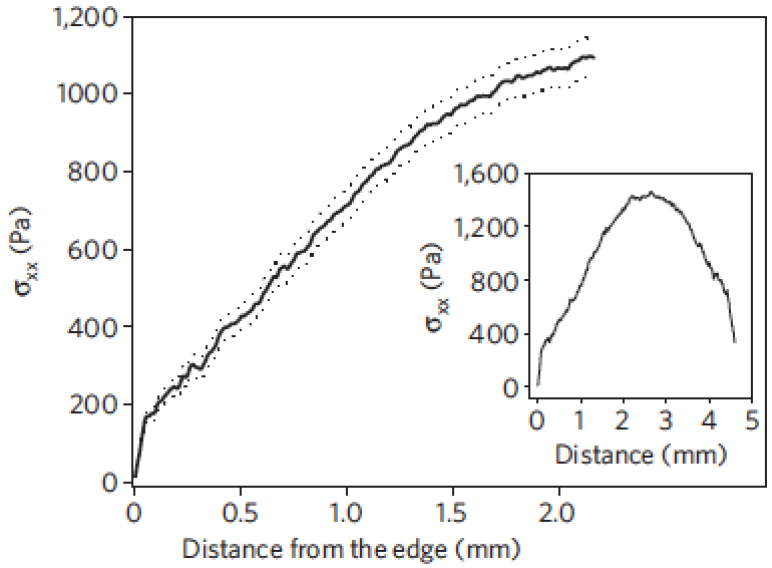
[Trepat+ Nature Physics 2009, Tambe+ Nature Physics 2012]

Internal stress profile



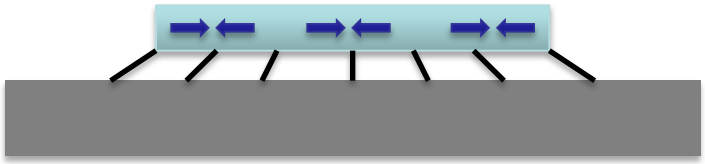
$$\langle \sigma_{xx}(x) \rangle = \frac{1}{h_z h_y} \int_0^x \int_0^{h_y} T_x(x', y')\, dx'\, dy'$$

Internal monolayer stress can be obtained from traction stress by numerical integration



The internal stress profile is parabolic with maximal value around 1.5 kPa

Stress profile from actively contracting stripe



force balance: σ internal stress, u deformation

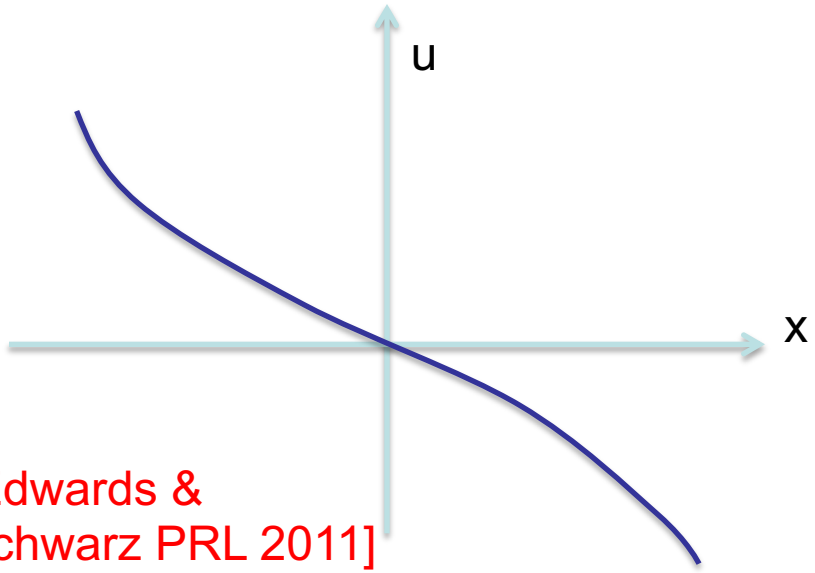
$$\sigma(x) - \sigma(x+dx) - k\,dx\,u\left(x+\frac{dx}{2}\right) = 0 \Rightarrow \frac{d\sigma}{dx} = ku$$

Elastic constitutive law (E 1d modulus, E*P active stress): $\sigma = E(\frac{du}{dx} + P)$

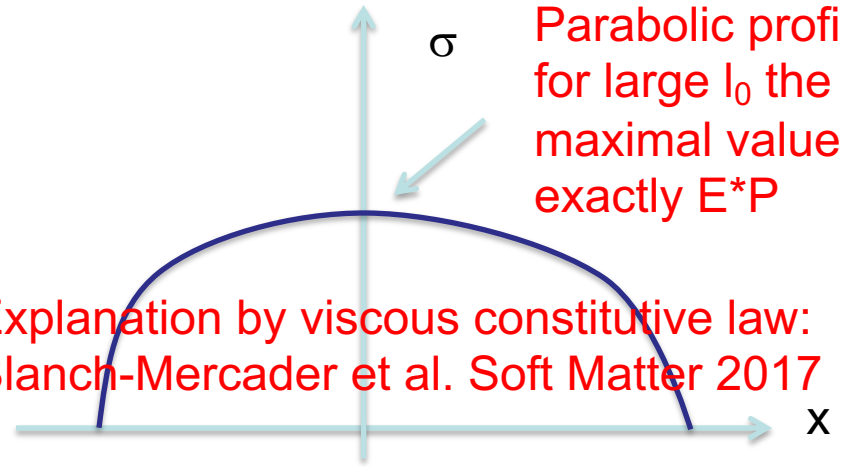
P=const $\Rightarrow \frac{d^2u}{dx^2} - \frac{1}{l^2}u = 0$ $\quad l = \sqrt{E/k}$ localization length

zero stress boundary: $\sigma(\pm l_0) = 0 \Rightarrow \frac{u}{l_0} = -P\frac{\sinh(\gamma x/l_0)}{\gamma\cosh(\gamma)}$

$\gamma = l_0/l$ localization parameter



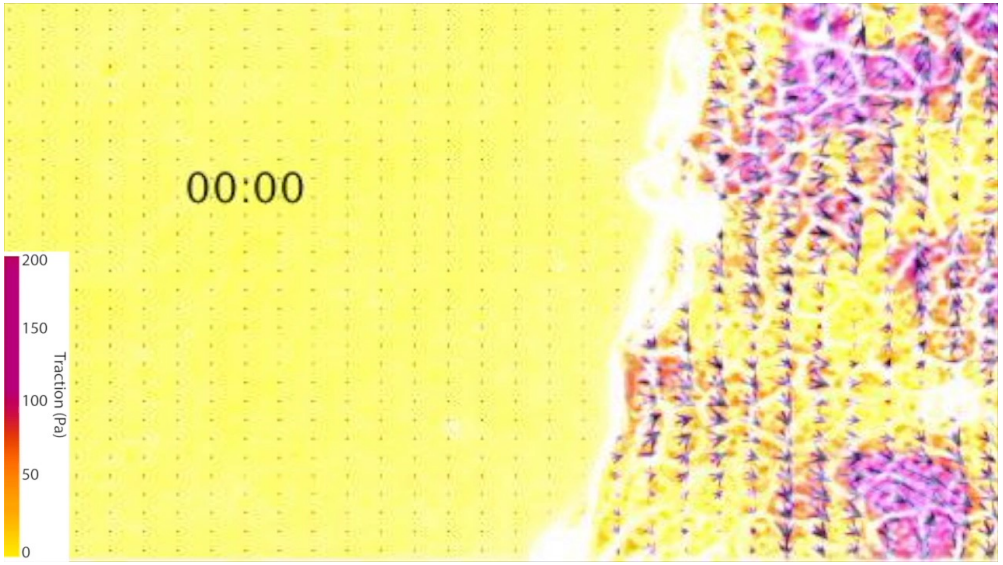
[Edwards & Schwarz PRL 2011]



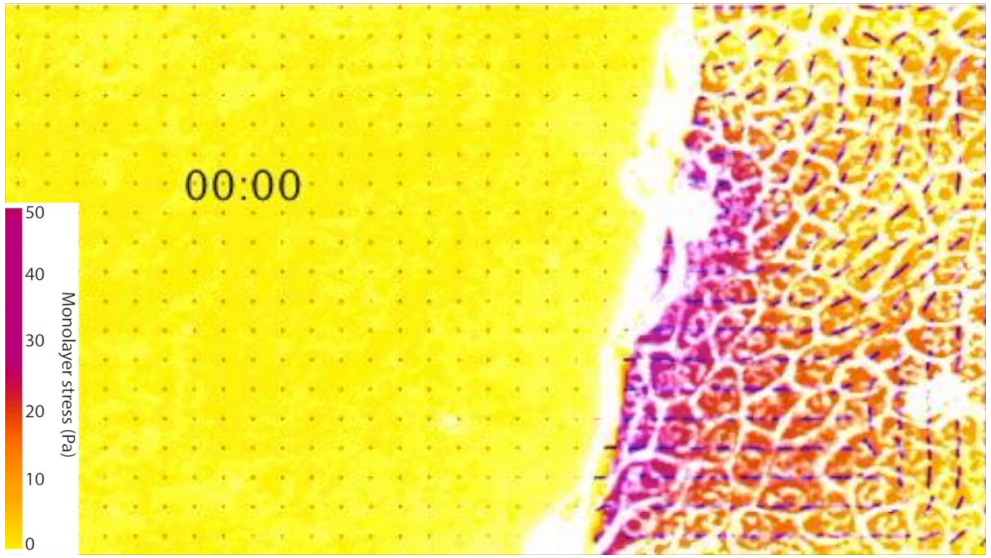
Parabolic profile; for large l_0 the maximal value is exactly E*P

Explanation by viscous constitutive law: Blanch-Mercader et al. Soft Matter 2017

Expanding epithelial cell monolayer



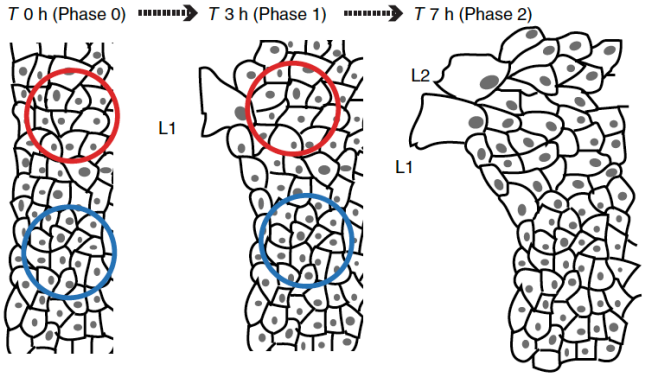
Traction force



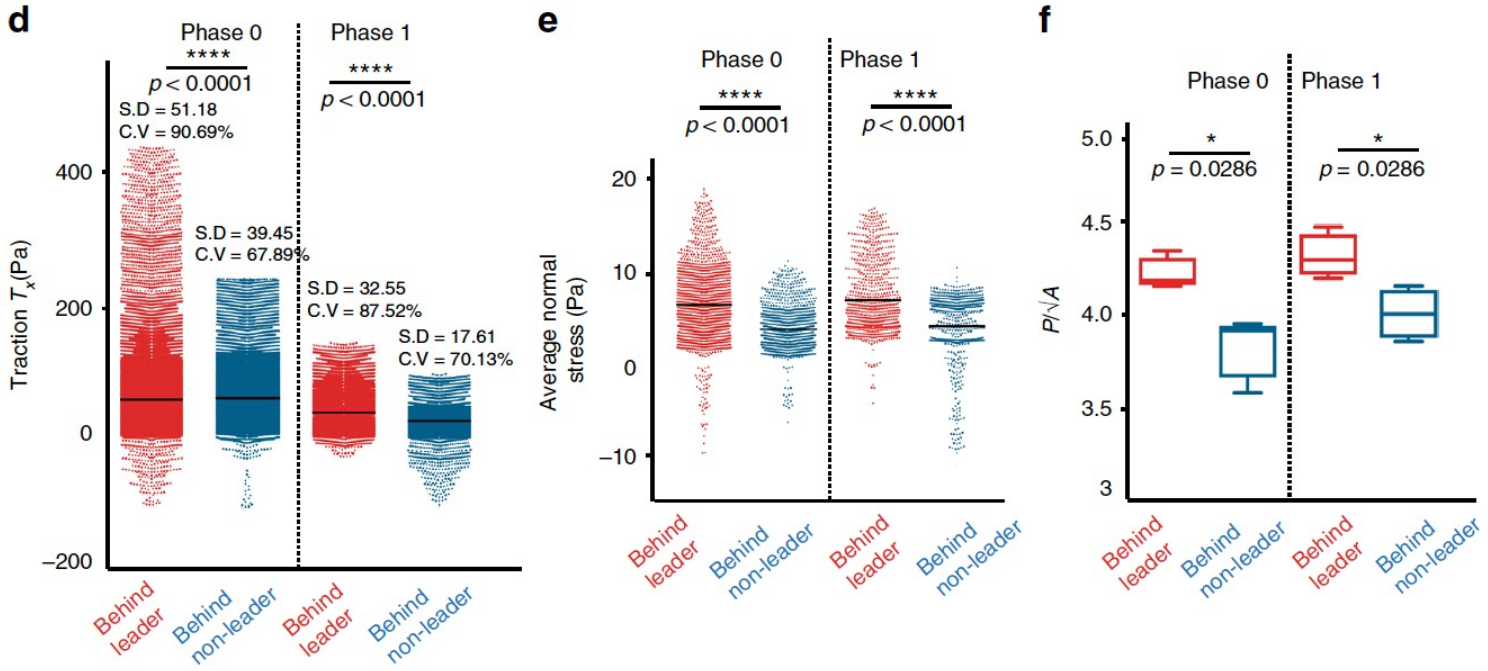
Monolayer stress

[Vishwakarma et al. Nat Comms 2018]

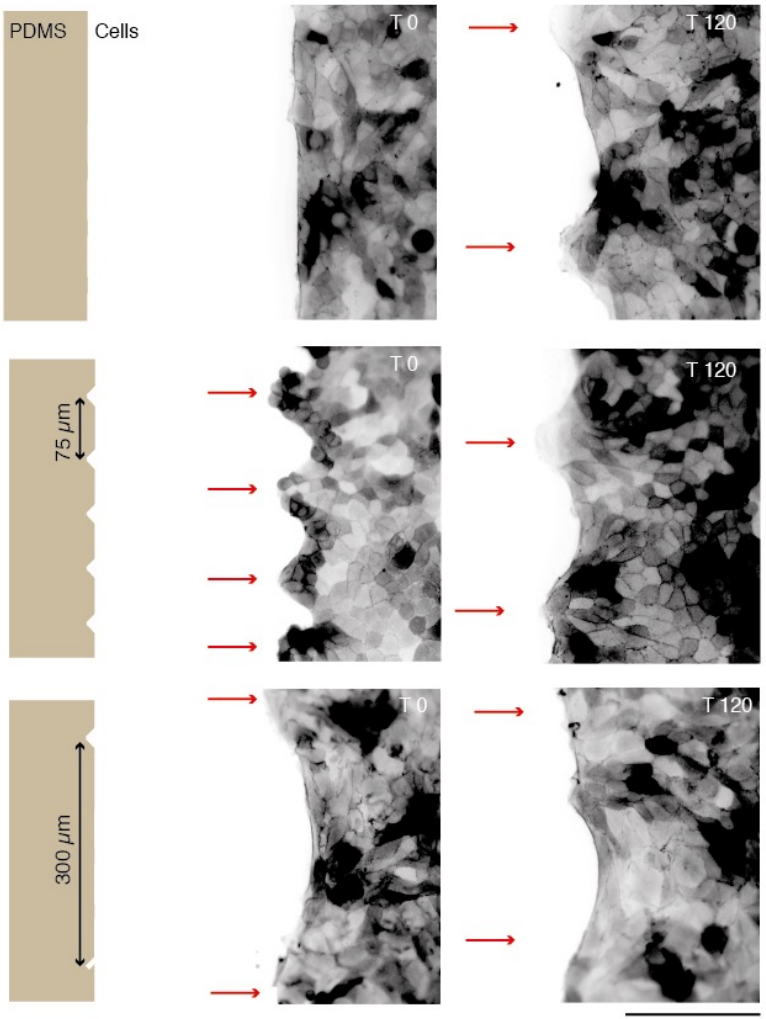
Mechanically active cells in the monolayer select a leader



- **phase 0:** isolated mechanical activity of small groups of cells in the bulk
- **phase 1:** leader cells emerge
- **phase 2:** second generation of leader cells

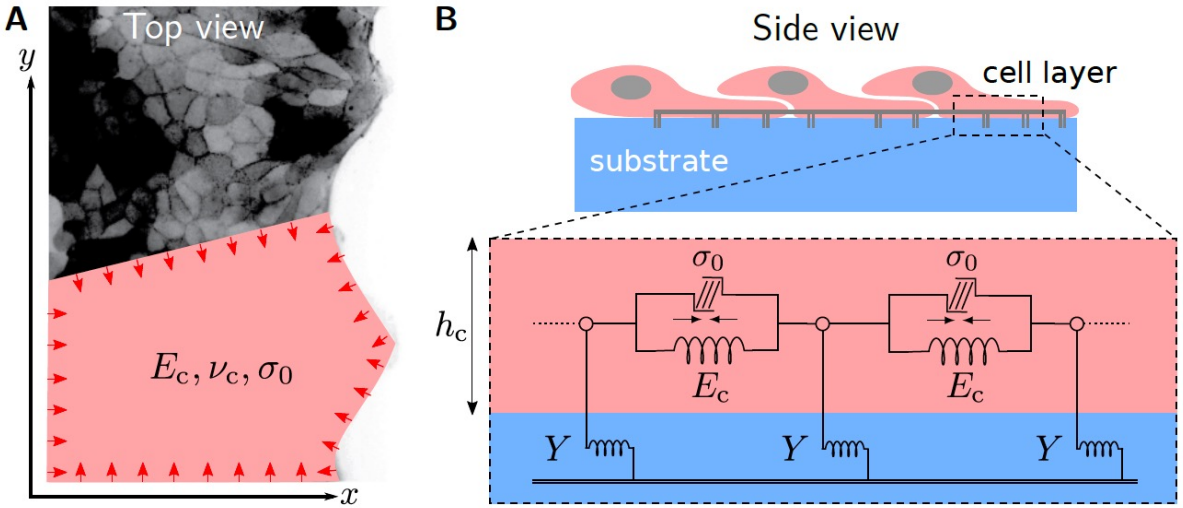


Leader cells keep a prefered distance



Even when initially prepared with different distances using stencils, the leader cell distance goes back to 160 μm with time.

Force penetration length generates a mechanical territory



Actively contracting monolayer with solid-like viscoelasticity and elastic foundation

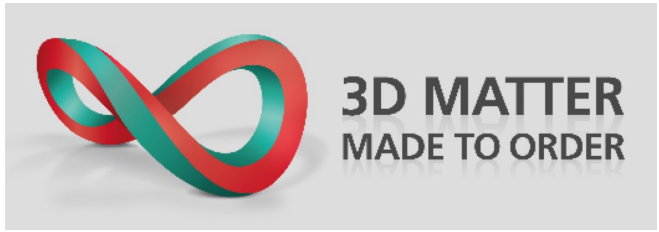
$$\frac{\lambda+\mu}{\lambda+2\mu}u_{j,ij}+\frac{\mu}{\lambda+2\mu}u_{i,jj}+\frac{1}{\lambda+2\mu}\sigma_{0,i}=\frac{u_i}{l_p^2}$$

Navier equation solved with FEniCS

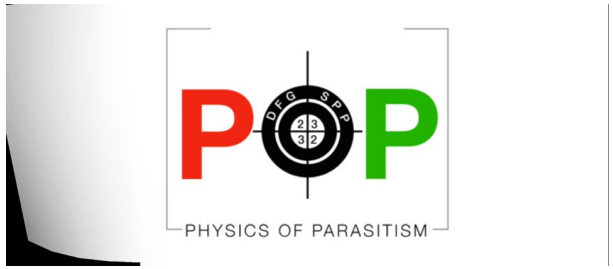
force penetration length $l_p=\sqrt{\dfrac{E_c h_c}{Y\left(1-\nu_c^2\right)}}\approx 160\,\mu m$

Acknowledgements

STRUCTURES
CLUSTER OF
EXCELLENCE



DFG



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