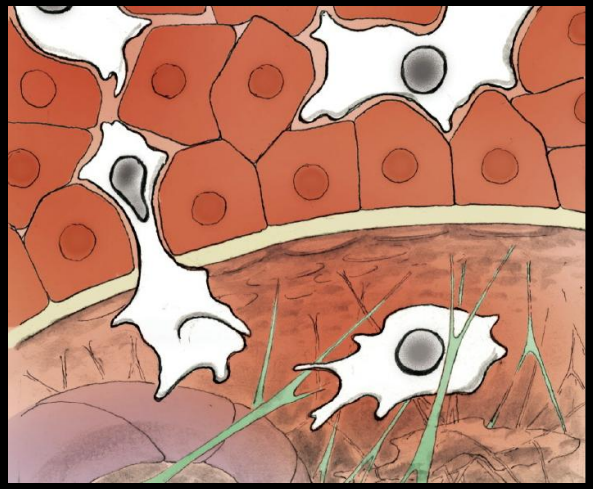
Single Cell Motility in Artificial Tissue



Rhoda J. Hawkins

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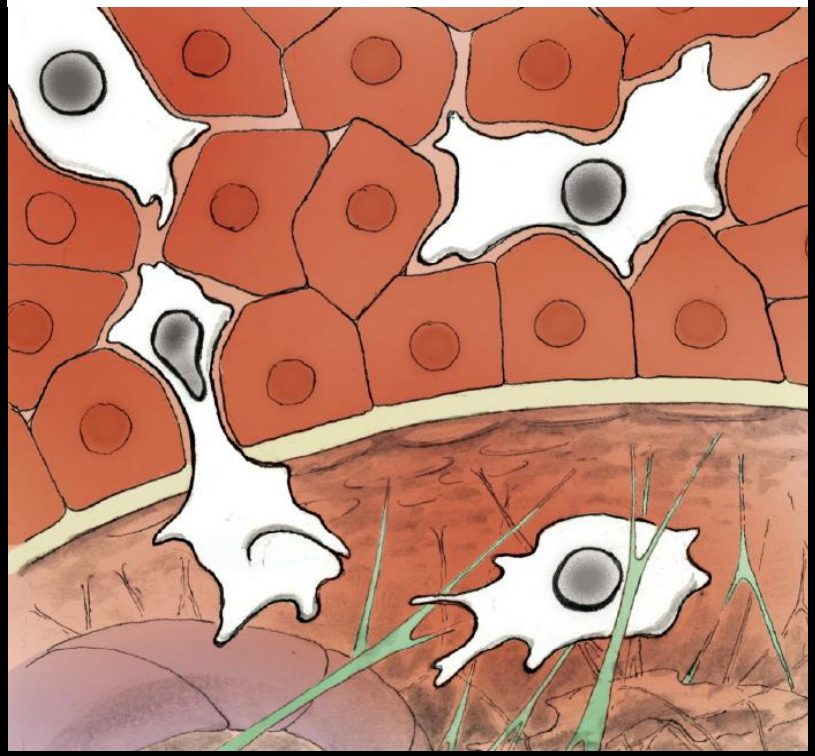
25th June 2012

Metastasis - cell migration in **tissues**

dense cells



extracellular matrix

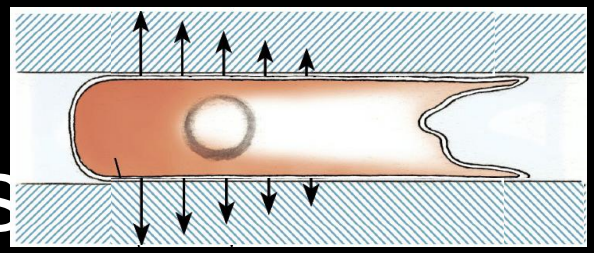
By better understanding the mechanics of cell motility could we learn to stop metastasis?

Plan

Introduction

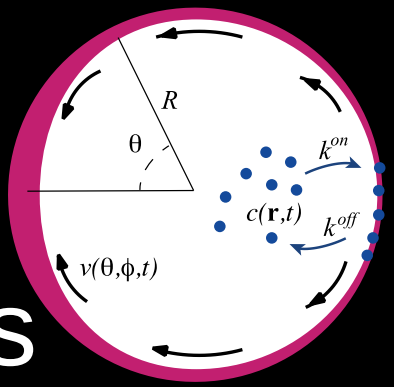
- Cell migration on 2D & in 3D confinement

Cell Motility in microchannels



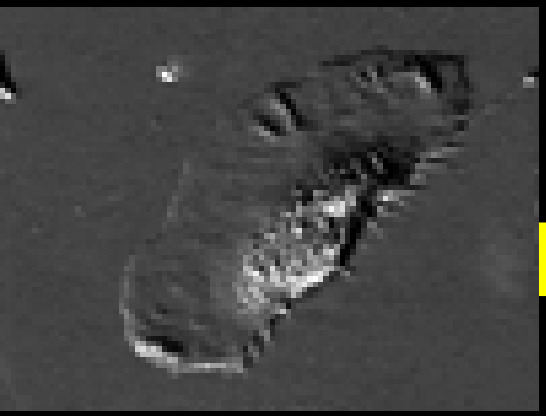
- Experiments – microchannels
- Model & results

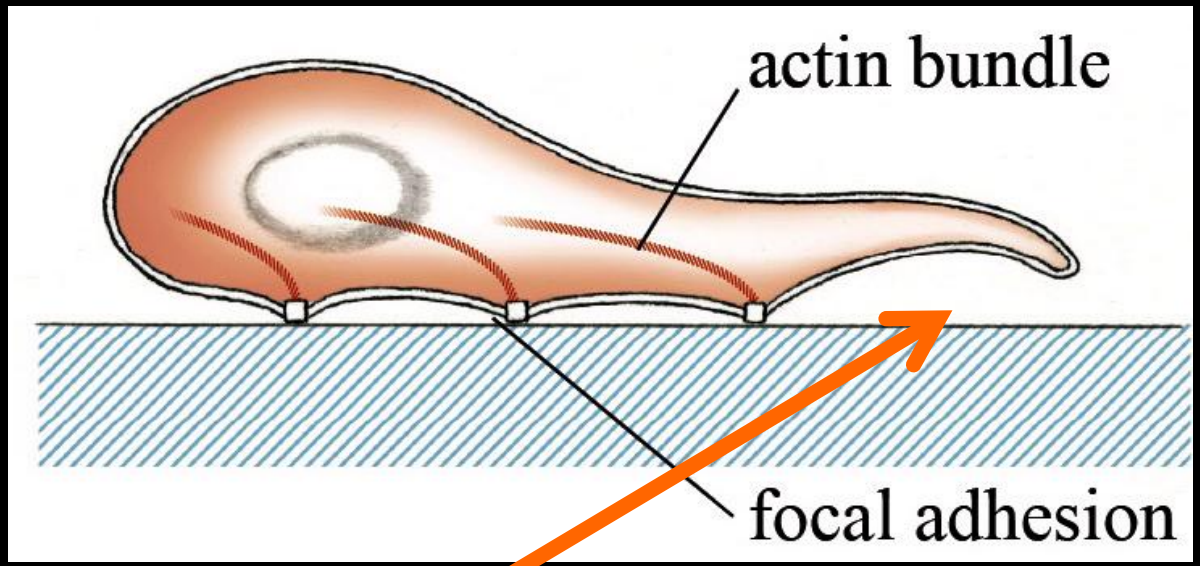
Cell Motility in 3D matrigel



- Experimental system
- Model & linear stability analysis
- Simulations & comparison with experiments

surface





Lamellipodium

Integrins → adhesion → move

Lammermann, Sixt et al. Nature, 2008

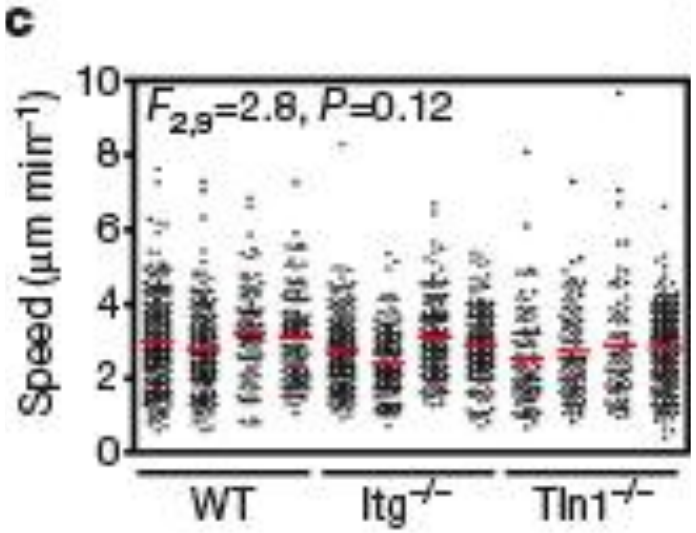
The role of adhesion

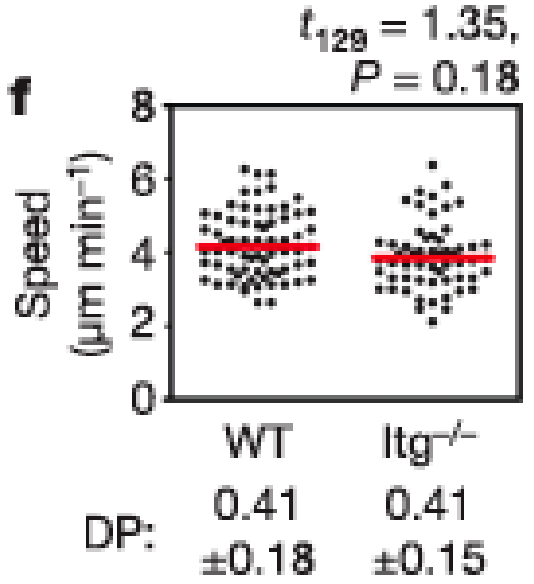
Integrin knockouts

Integrin needed for migration on 2D surface

Wild type & integrin knockouts migrate in vitro (collagen matrices)

& in vivo (mice)



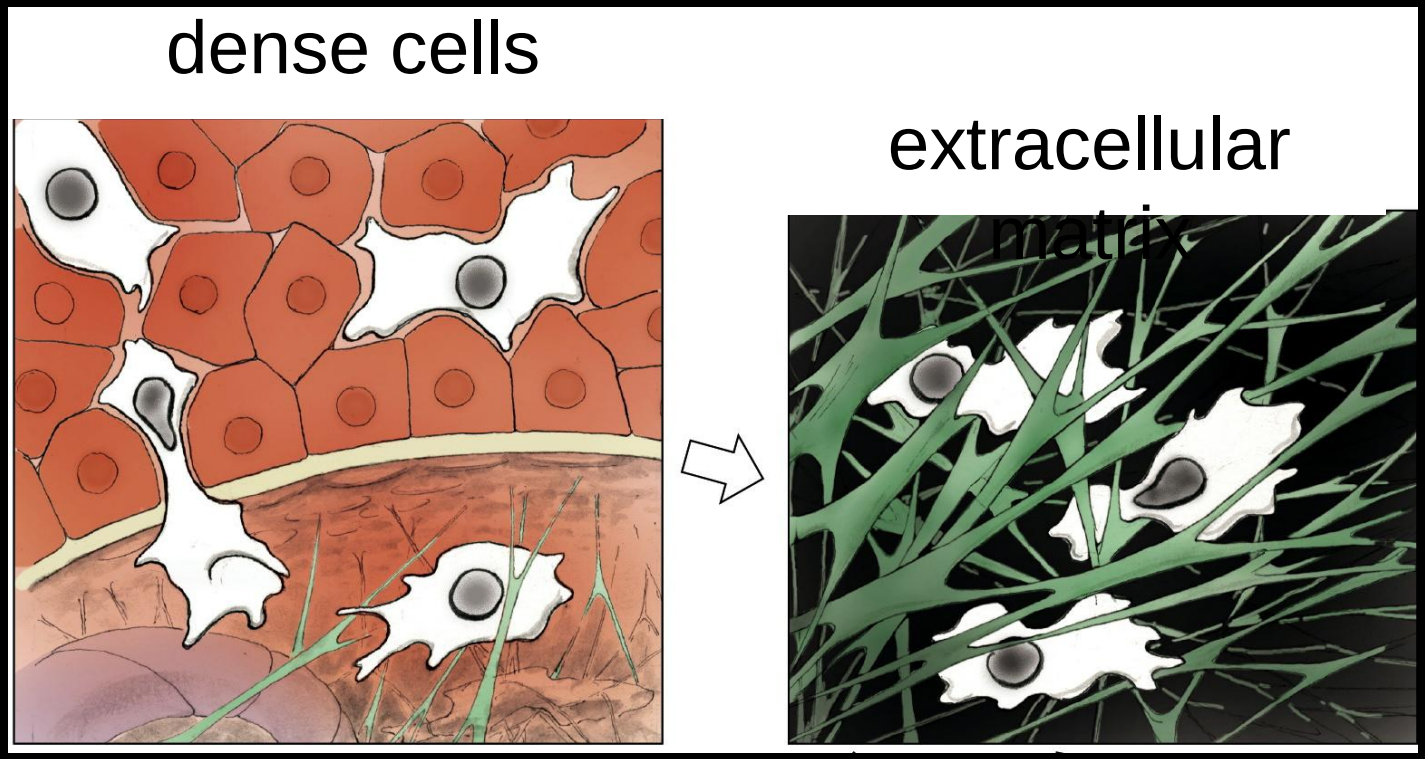


Lammermann, Sixt et al. Nature, 2008

Cell migration in tissues

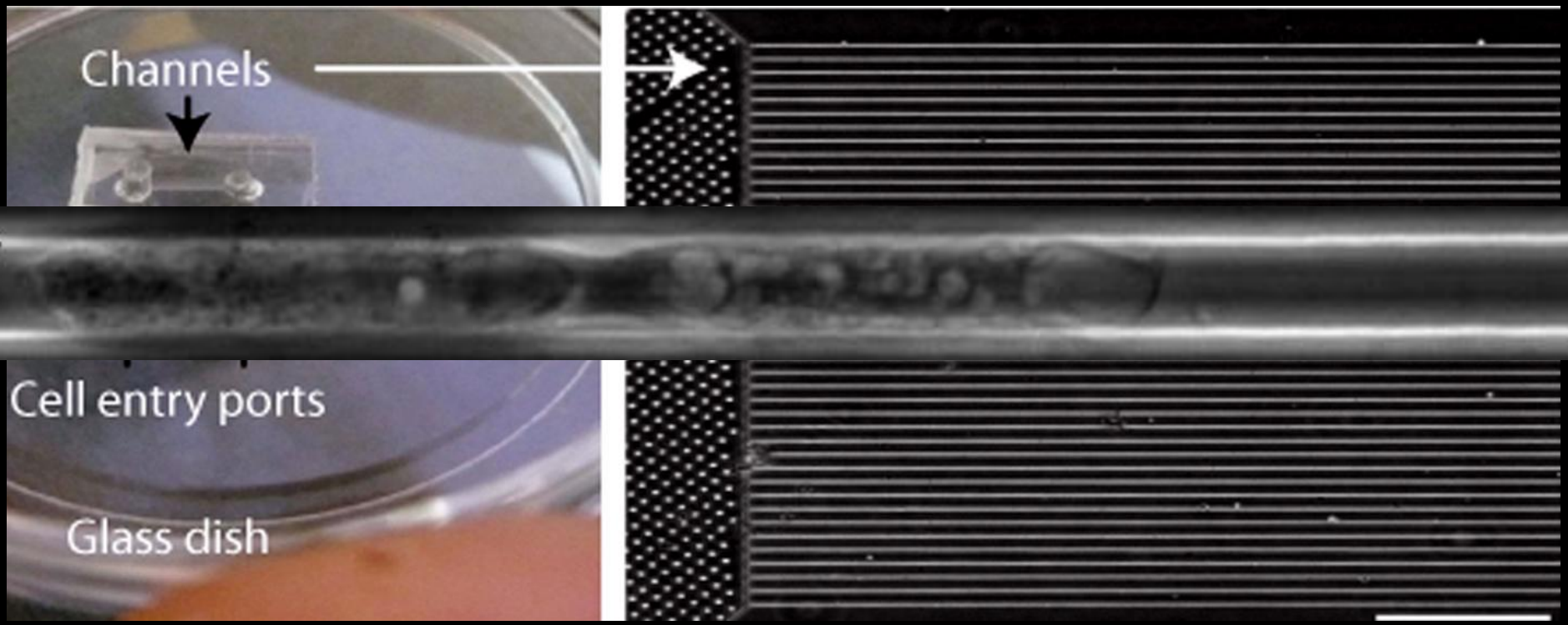
~~Integrins~~ □ ~~adhesion~~ □ move ✓

Lammermann, Sixt et al. Nature, 2008



Is there a different mechanism for motility in confinement?

Migration in microchannels

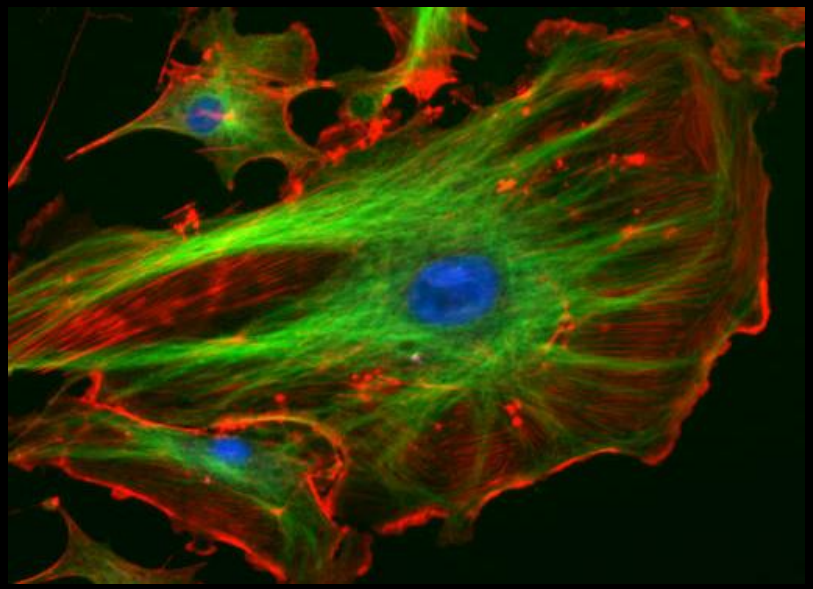


- Simplified quasi 1D model of 3D cell migration
- Isolates effect of confinement
- Controlled & tunable geometry

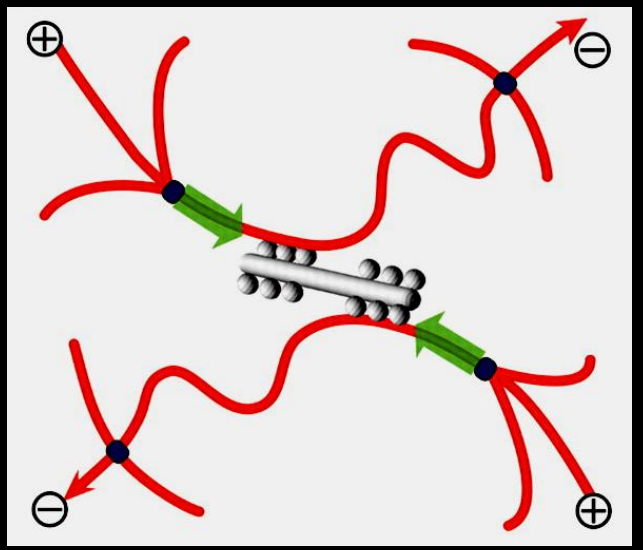
Modelling Cytoskeleton dynamics

Cytoskeleton: out of equilibrium soft matter

 theory of active gels



Cytoskeleton polymers:
microtubules + actin



Molecular motors:
myosin + actin

- contractility
- transport

Active gel equations

$$2\eta u_{ij} = \sigma_{ij} - \frac{\nu}{2}(p_i h_j + p_j h_i) + \frac{1}{2}(p_i h_j - p_j h_i) + \zeta \Delta\mu\, p_i p_j$$

passive fluid

liquid crytstal stress-polarisation coupling

polar terms

active stress

$$\frac{\mathrm{D}p_i}{\mathrm{D}t} = \frac{1}{\gamma} h_i - \nu u_{ij} p_j$$

rotational viscosity

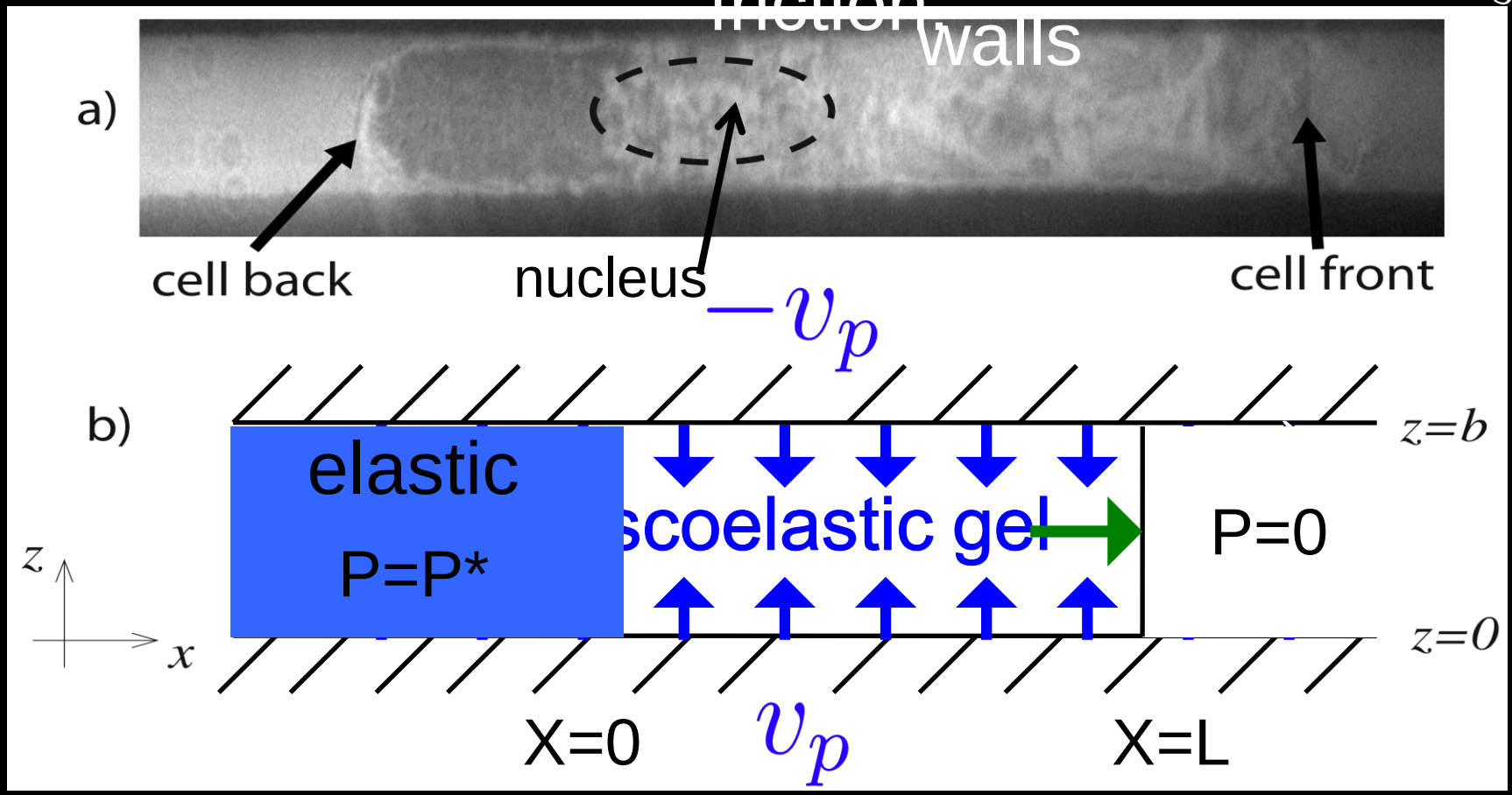
molecular field conjugate to polarisati

$$h_i = -\frac{\delta F}{\delta p_i} + \lambda' p_i$$

Model

Viscous friction: $\sigma_{xz} = -\zeta v_x$

actin polymerisation perpendicular to walls



friction, Darcy flow, pressure

Flow velocity *forwards* opposed to *retrograde* flow on surface

finite *L* even with small bare friction

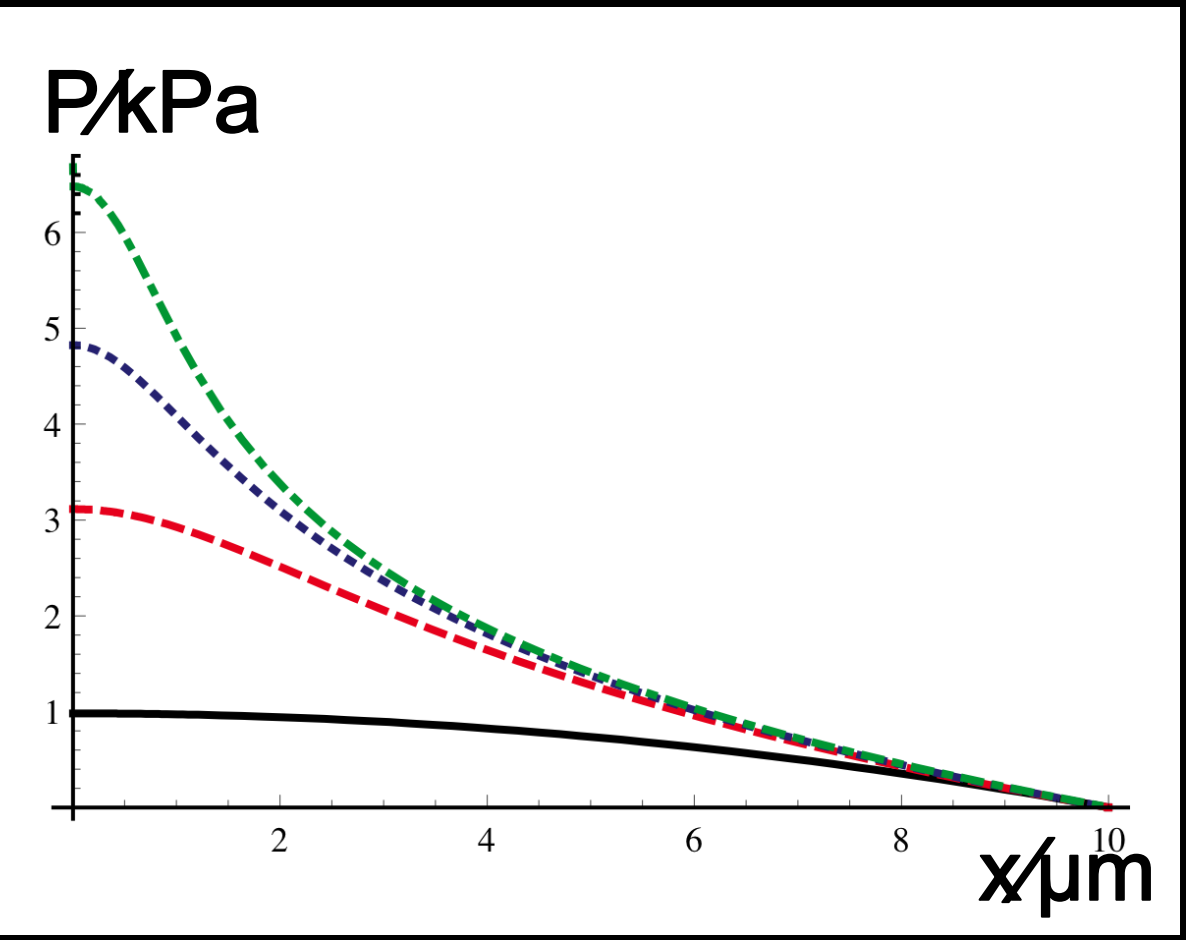
regimes:

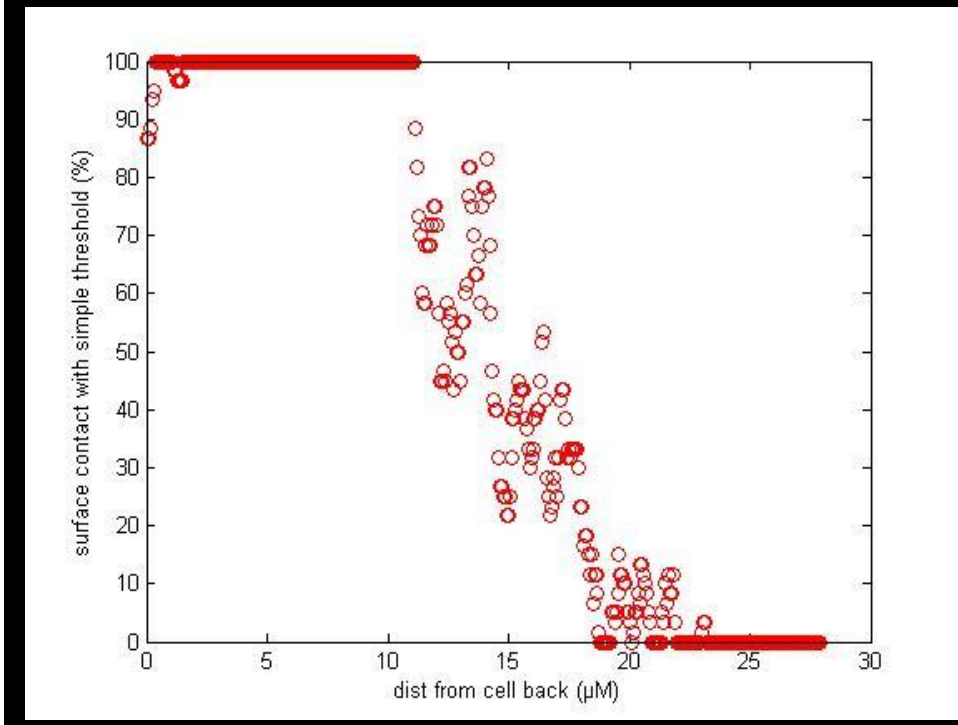
$$v(x) = -\frac{l^2}{12\eta}\left(1 + \frac{P^*}{P}\frac{l}{\xi}\right)\frac{dP}{dx}$$

$P(P > P^*) = \infty$ elastic

$P(P < P^*) = 0$ viscous

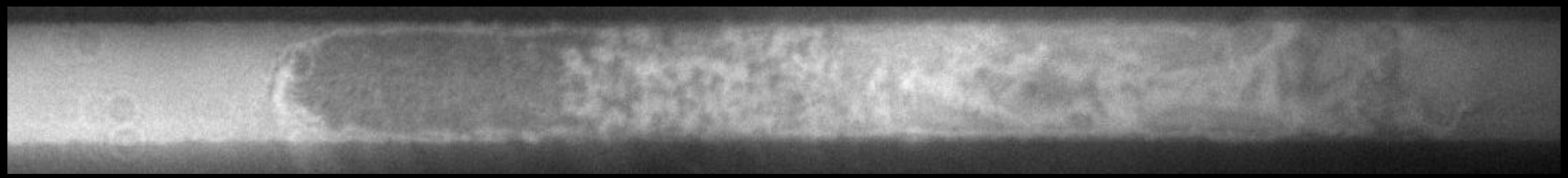
Results – Pressure profile





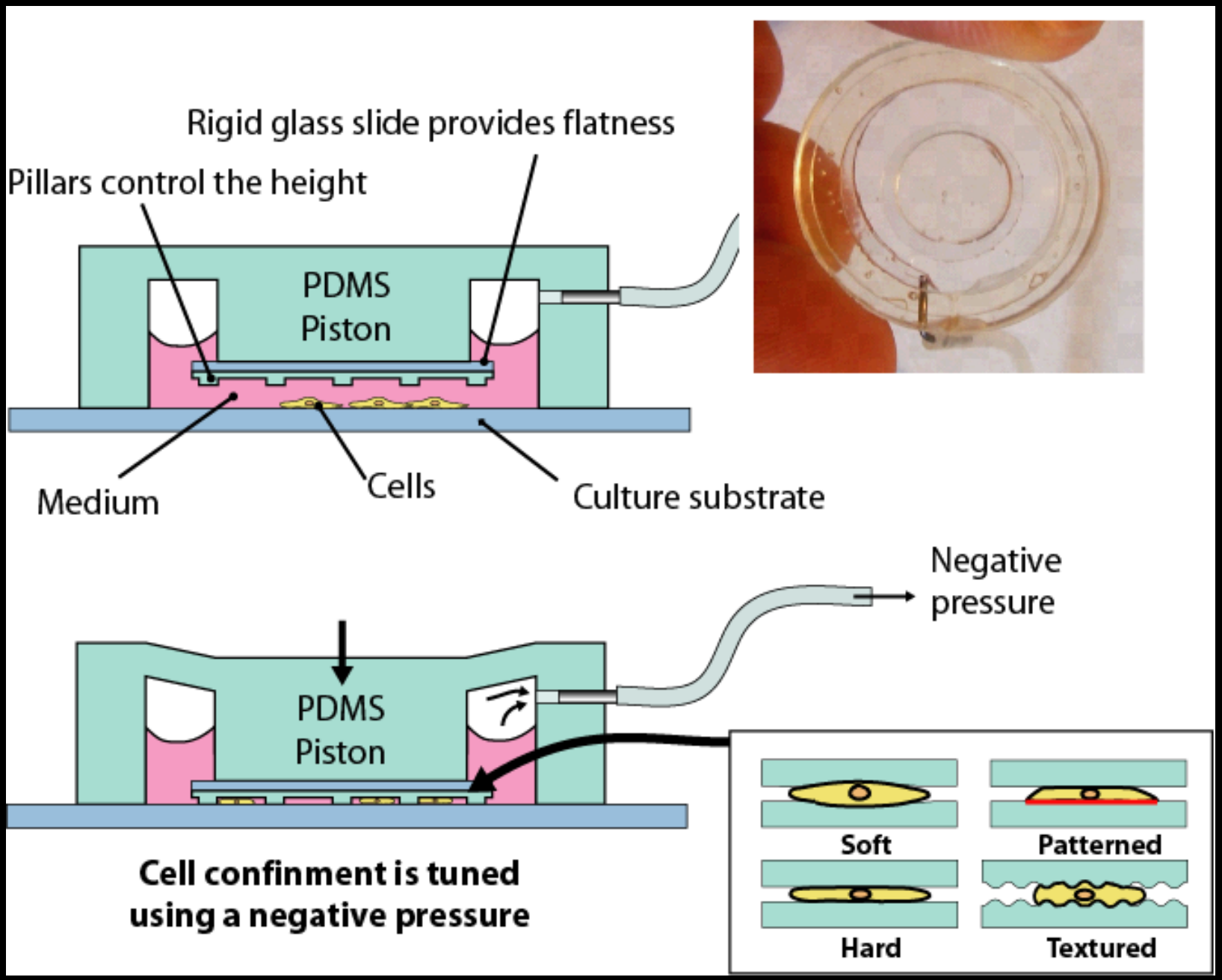
theory

RICM experiment



Cell crusher

Maël Le Berre



Cell crusher

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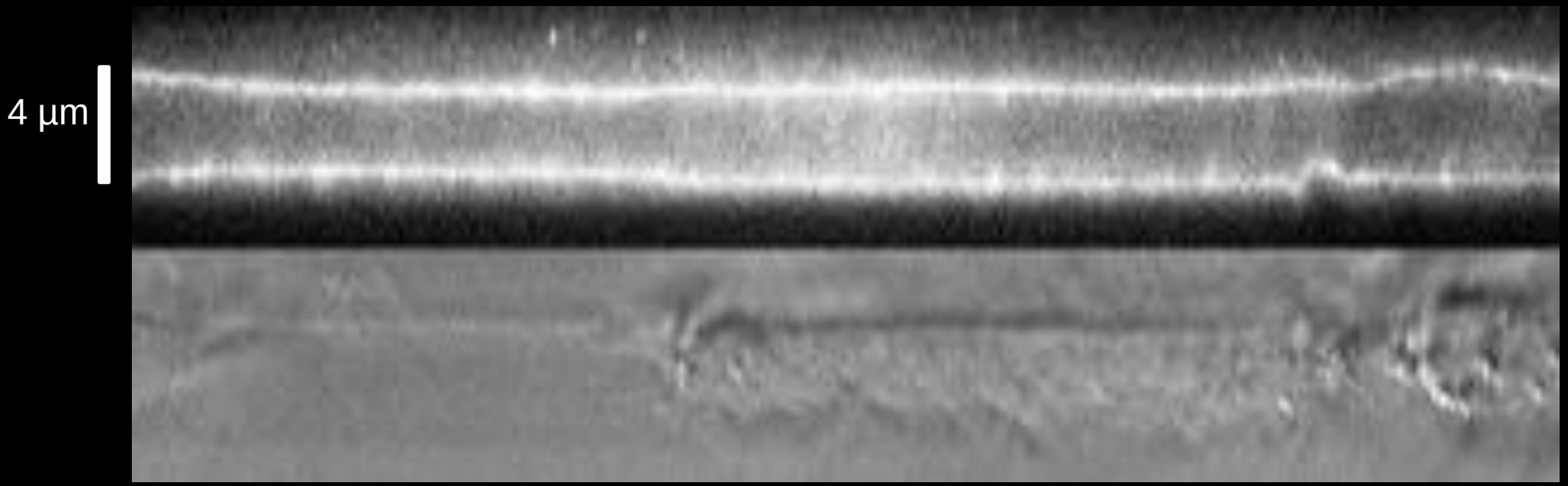
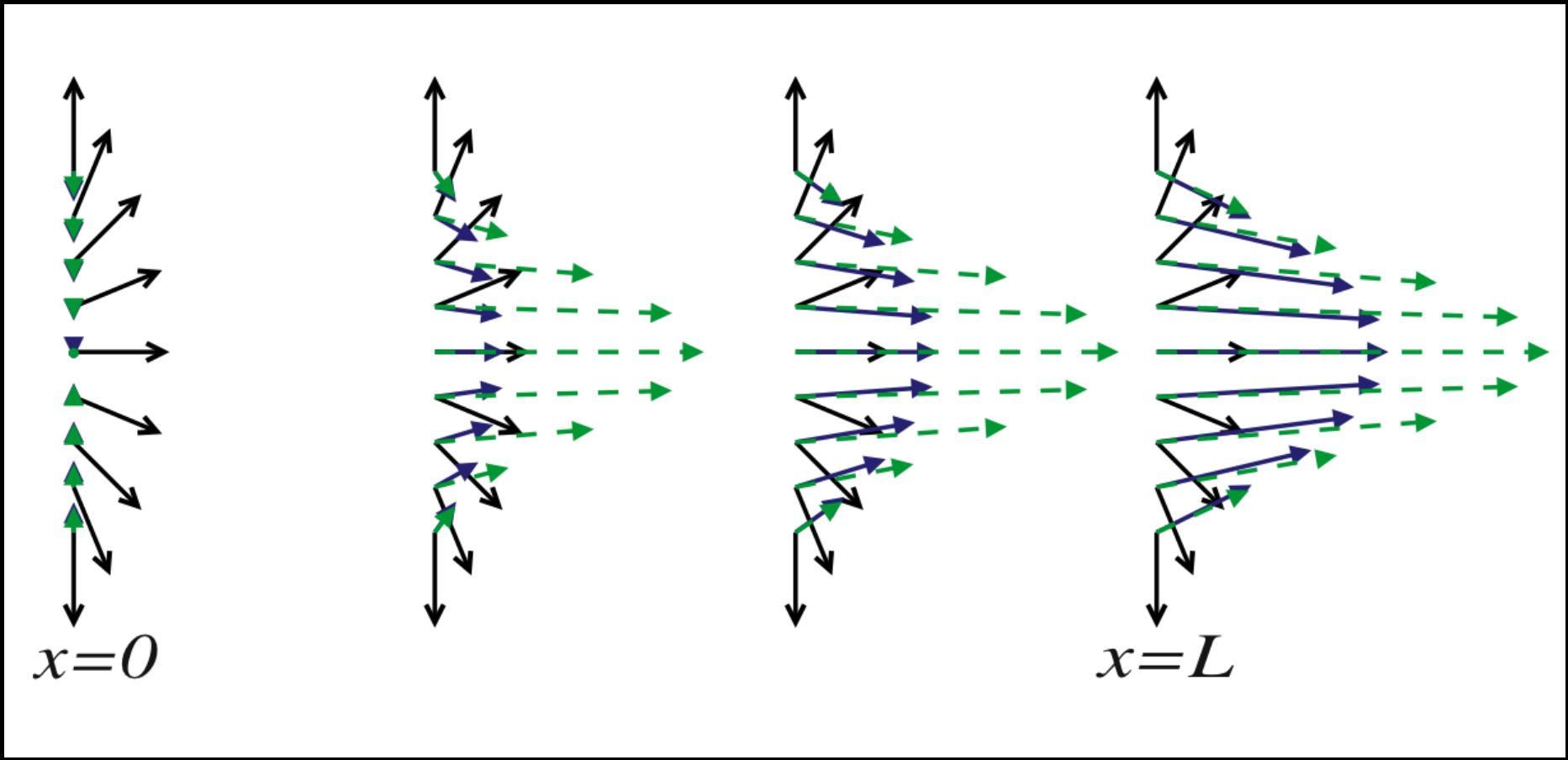


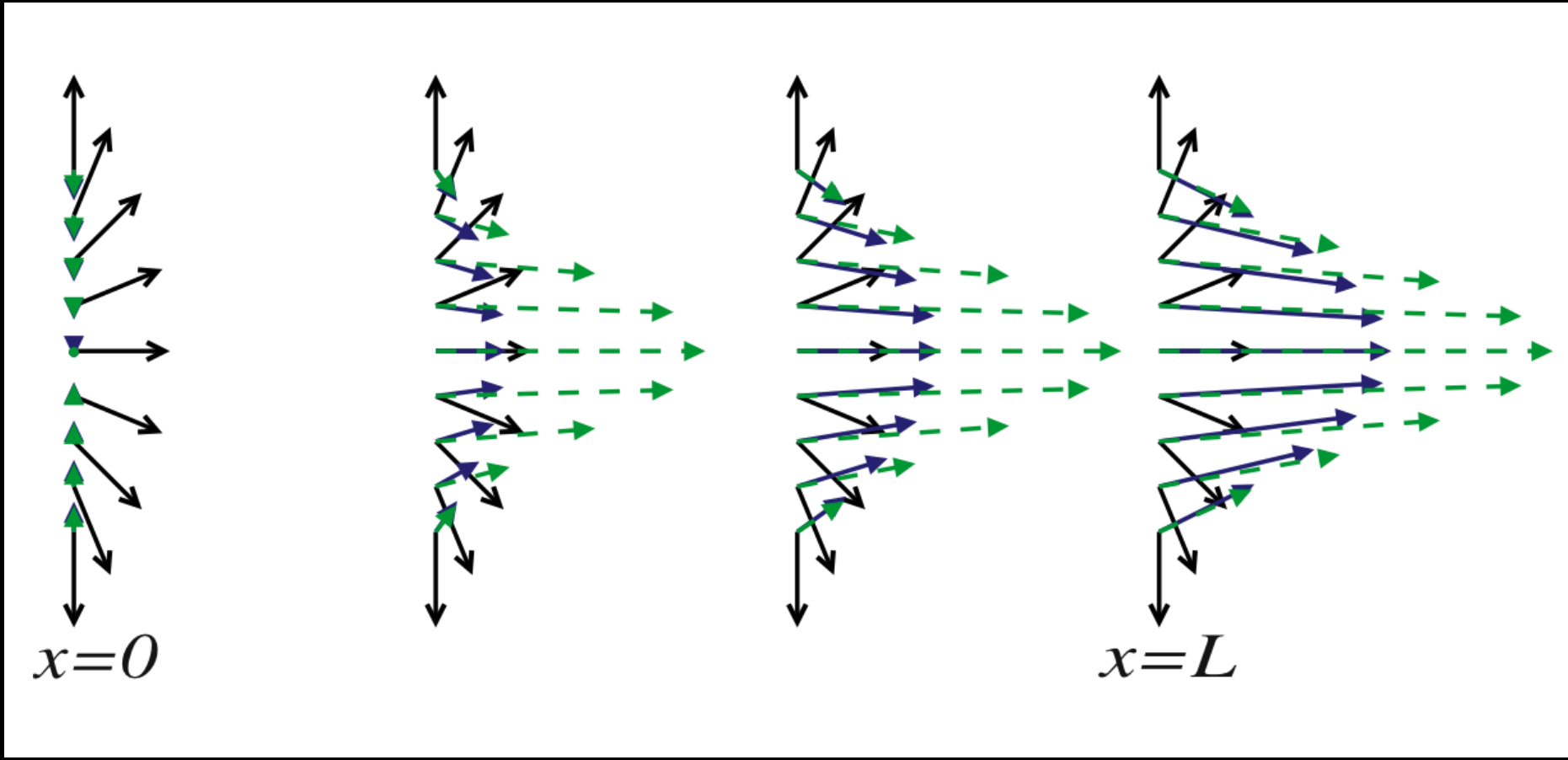
channel 4x4µm polyacrylamide, coating fibonectin-Alexa488, rigidity: 20kPa

velocity



polarisation $\theta(z) = -\frac{\pi}{2}(1 - \frac{2z}{b})$

Adding myosin motor activity

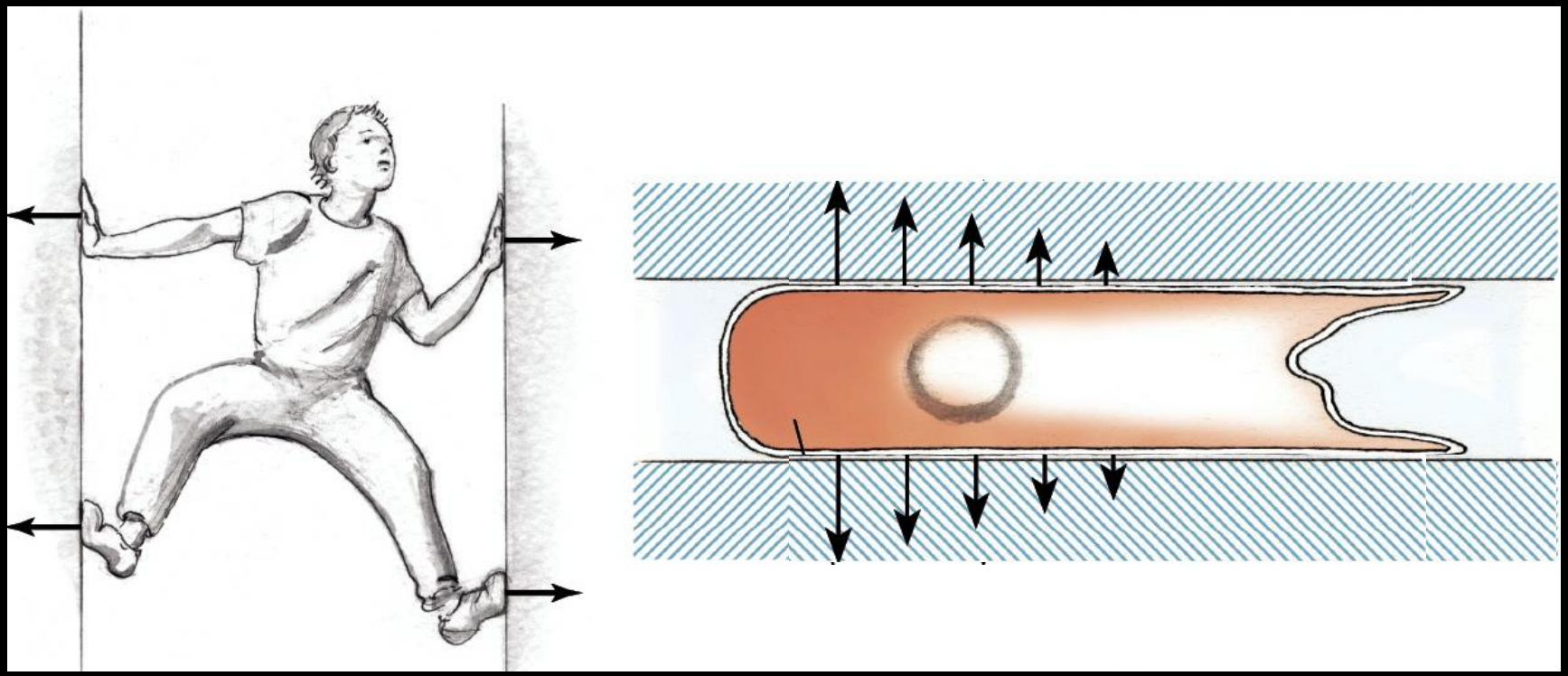


Darcy's law with active myosins

$$v(x) = -\frac{b^2}{12\eta}(1+\tilde{\xi}^{-1})\frac{\mathrm{d}P}{\mathrm{d}x} - \frac{b\tilde{\zeta}(x)\Delta\mu}{4\pi\eta}$$

Conclusion (part 1): cell rock climbers

- In confinement cells build up friction needed to migrate by active actin polymerisation pushing against the walls



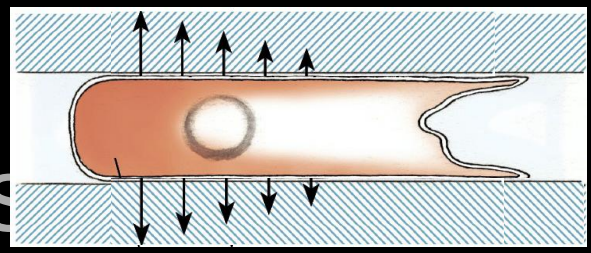
Hawkins et al. PRL **102** (2009) 058103

Plan

Introduction

✓Cell migration on 2D & in 3D confinement

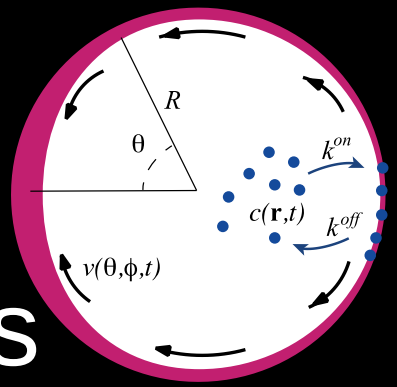
Cell Motility in microchannels



✓Experiments – microchannels

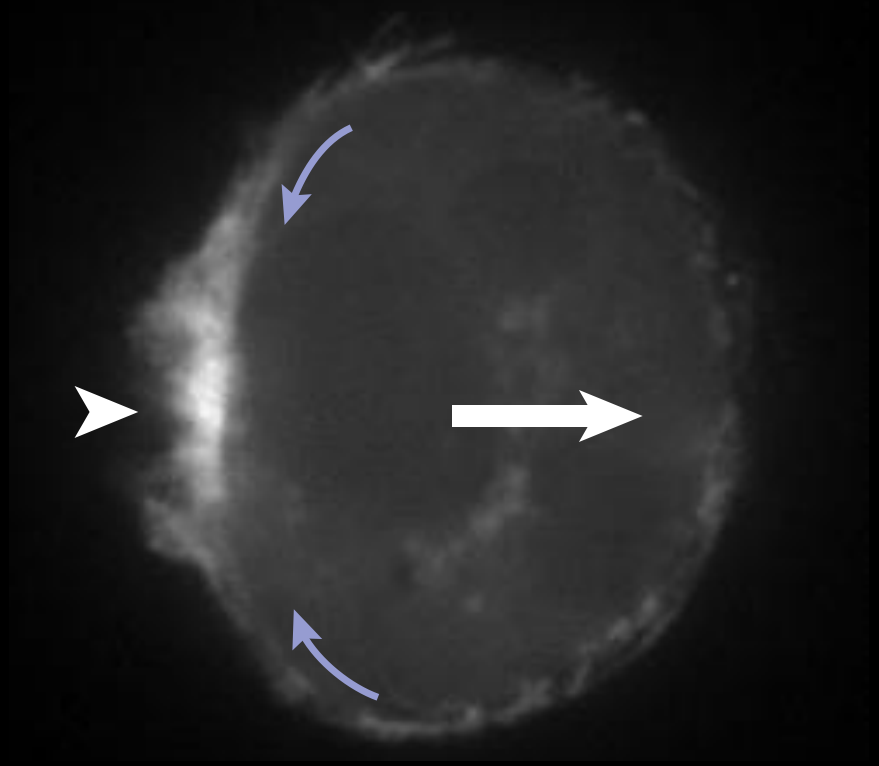
✓Model & results

Cell Motility in 3D matrigel



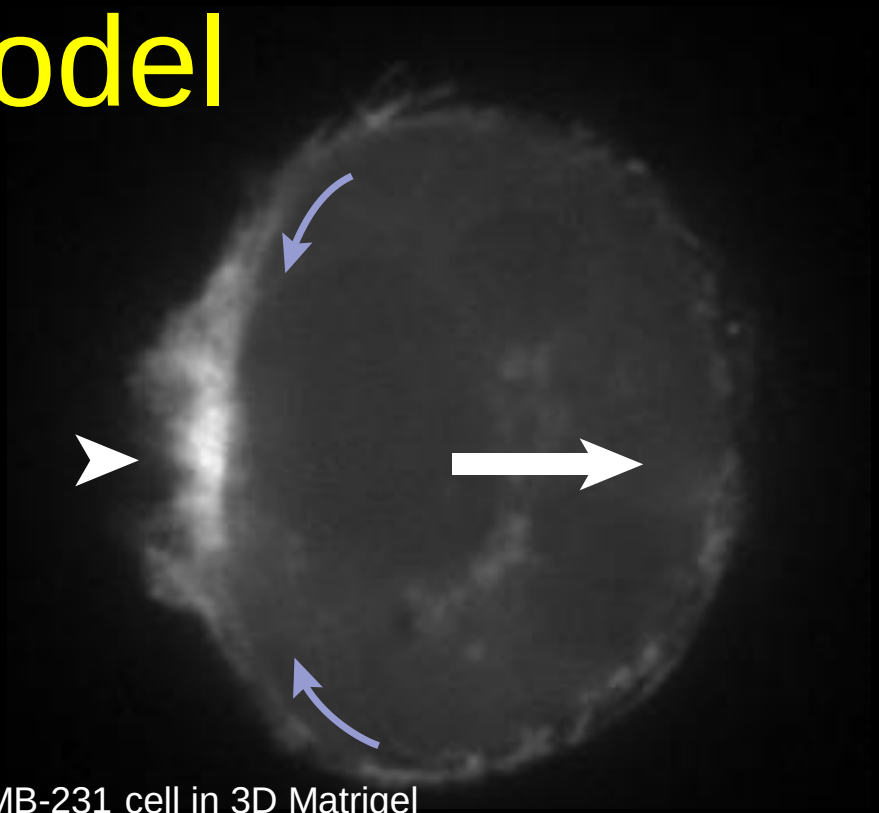
- Experimental system
- Model & linear stability analysis
- Simulations & comparison with experiments

Cells in 3D confinement in matrigel

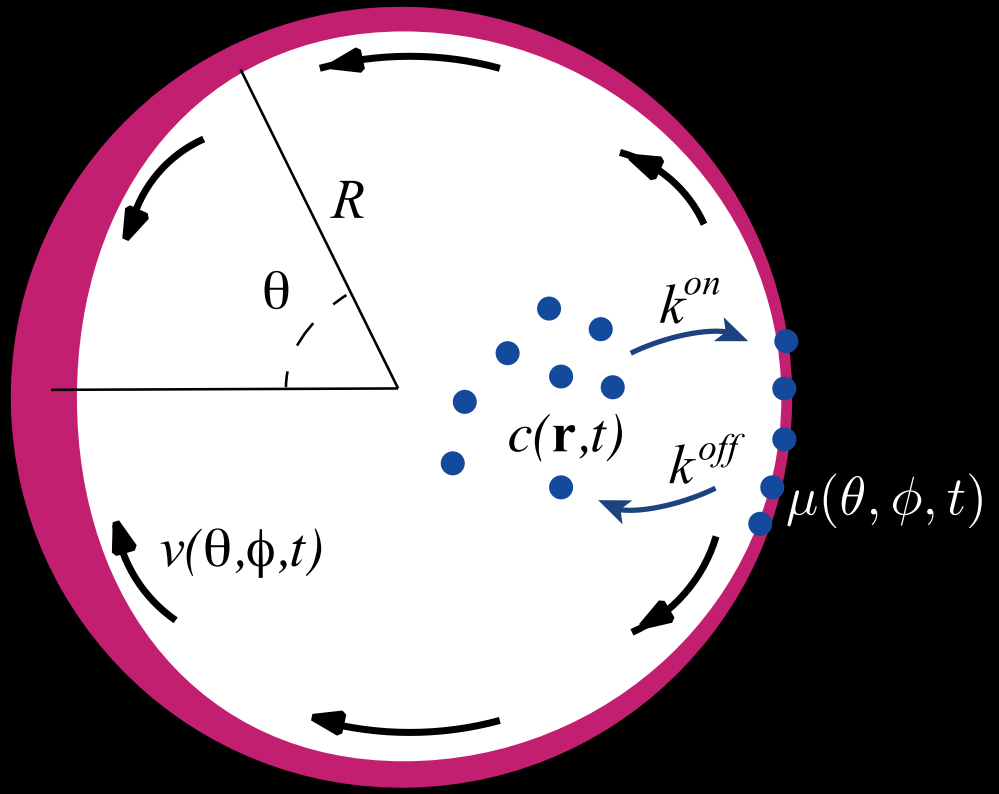


- MDA-MB-231 cell (breast cancer)
- In 3D Matrigel
- Cell expressing mCherry-Lifeact - labels F-actin
- Renaud Poincloux, Philippe Chavrier

Model



MDA-MB-231 cell in 3D Matrigel
Renaud Poincloux, Philippe Chavrier



- Spherical cell with thin shell of (de)polymerising gel
- 2D compressible gel (≅ variable thickness)
- Myosin □ active stress ✕ local myosin concentration
- Myosin (de)attaches to cortex, diffuses in cell bulk & cortex & is carried by the induced actin flow in cortex

Dynamical equations

- mass conservation for 2D compressible gel:

$$\partial_t \delta\rho + \nabla \cdot ((1+\delta\rho)\underbrace{\nabla\psi}_{=\mathbf{v}}) = -k_d \delta\rho$$

- force balance (low Reynolds number):

$$\nabla\left(\underbrace{-\zeta\mu}_{\text{active stress}} - \alpha\delta\rho + \beta\nabla^2\delta\rho\right) = \underbrace{\xi}_{\text{friction}}\nabla\psi$$

- diffusion of myosin in cytoplasm:

$$\partial_t c = D_c \Delta c$$

- conservation of myosin at cortex/cytoplasm interface:

$$-D_c \partial_r c\,|_{r=R} = k^{\mathrm{on}} c(R) - k^{\mathrm{off}}\mu$$

- conservation of myosin in the cortex:

$$\partial_t \mu + \nabla\cdot(\mu\nabla\psi) = k^{\mathrm{on}} c(R) - k^{\mathrm{off}}\mu + \underbrace{D_\mu}_{\text{diffusion in cortex}}\nabla^2\mu$$

analysis

Homogeneous stationary solution:

$$\psi = \delta\rho = 0 \qquad \mu = \mu_0 \qquad c = c_0 = k^{\mathrm{off}}\mu_0/k^{\mathrm{on}}$$

Spherical harmonic perturbation:

$$c(r,\phi) = c_0 + c_{l,m}(r)Y_{l,m}(\theta,\phi)e^{st}$$

$$c_{l,m}(r) = \tilde{c}_{l,m}I_{l+1/2}(\sqrt{\frac{s}{D_c}}r)/\sqrt{r}$$

1D approximation (neglect curvature)

dispersion relation cubic in s:

$$\Big((s + D_c k^2)(s + k^{\mathrm{off}} + D_\mu k^2) + k^{\mathrm{on}}(s + D_\mu k^2)\Big)\Big(\xi(s + k_d) + k^2(\alpha + \beta k^2)\Big) + \zeta\mu_0 k^2 (s + k_d)(s + k^{\mathrm{on}} + D_c k^2) = 0$$

where $k^2 = l(l+1)/R^2$

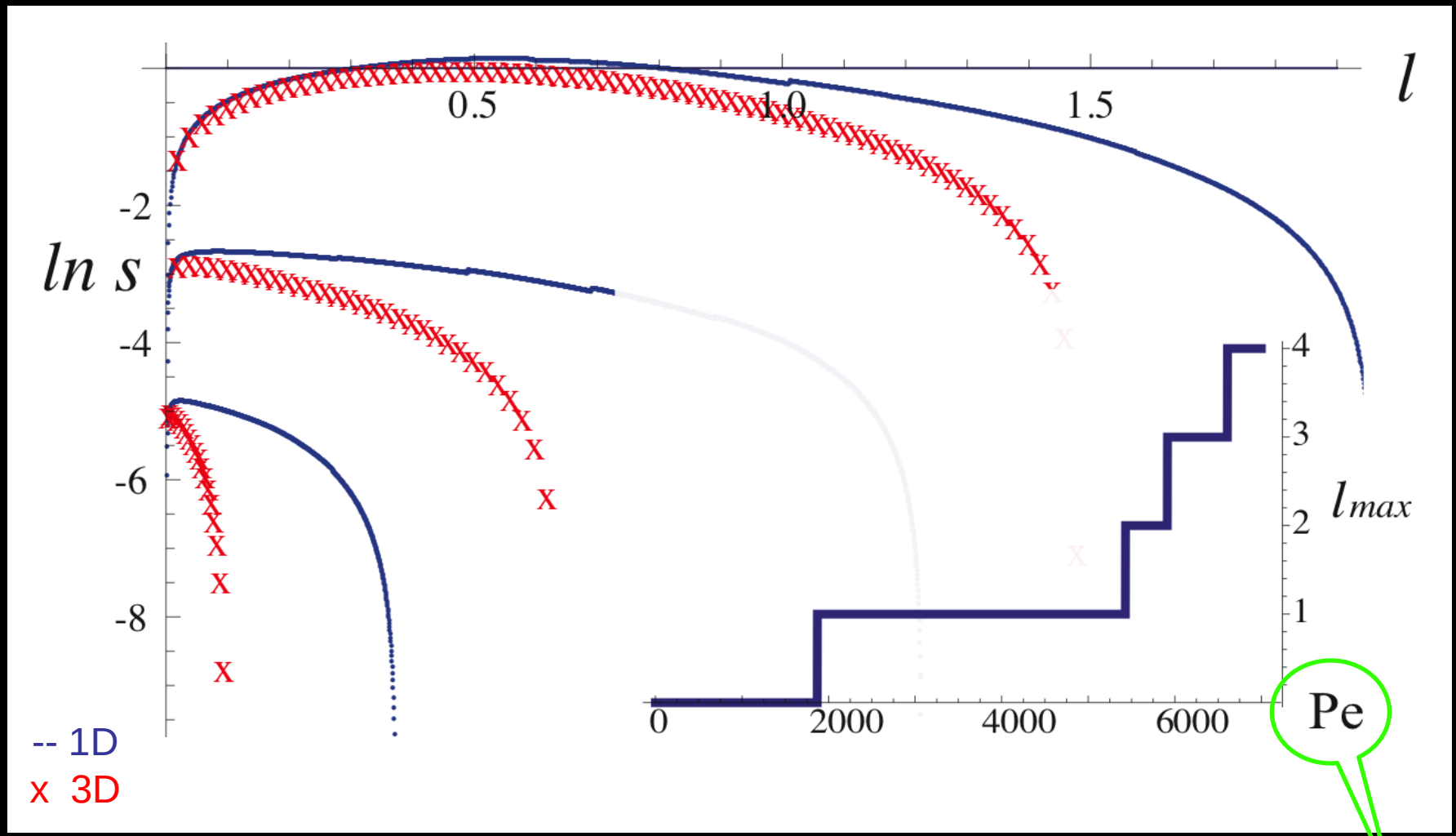
Activity threshold above which instability appears $s > 0$

$$Pe = \frac{-\zeta\mu_0}{\tilde{D}\xi} > Pe_c$$

Critical Péclet number

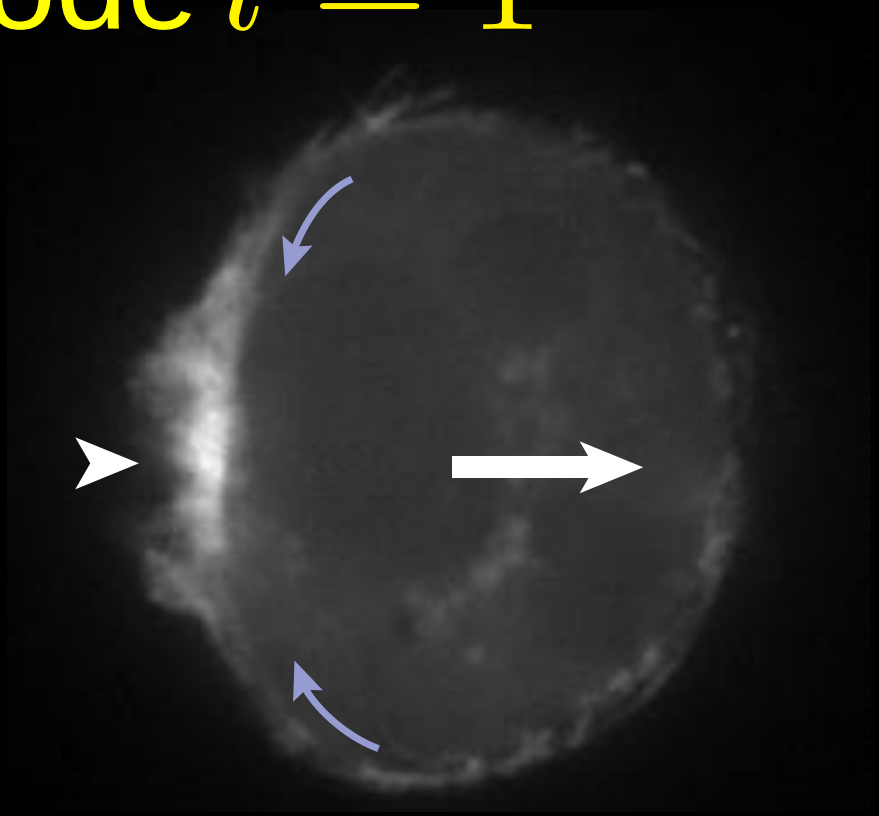
where $\tilde{\mathrm{D}} = \dfrac{\mathrm{D_c k^{off}} + \mathrm{D}_\mu \mathrm{k^{on}}}{\mathrm{k^{on}}}$

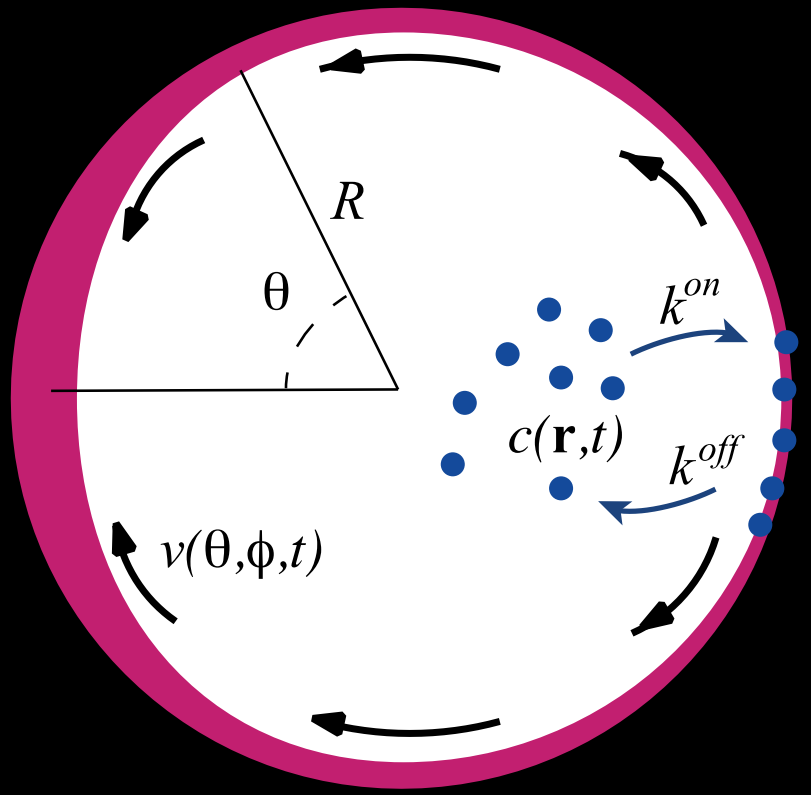
Results – dispersion relation $s(l)$



activity

Mode $l = 1$





MDA-MB-231 cell in 3D Matrigel
Renaud Poincloux, Philippe Chavrier

- cell motion since stress σ_{nt} integrates to non zero force:

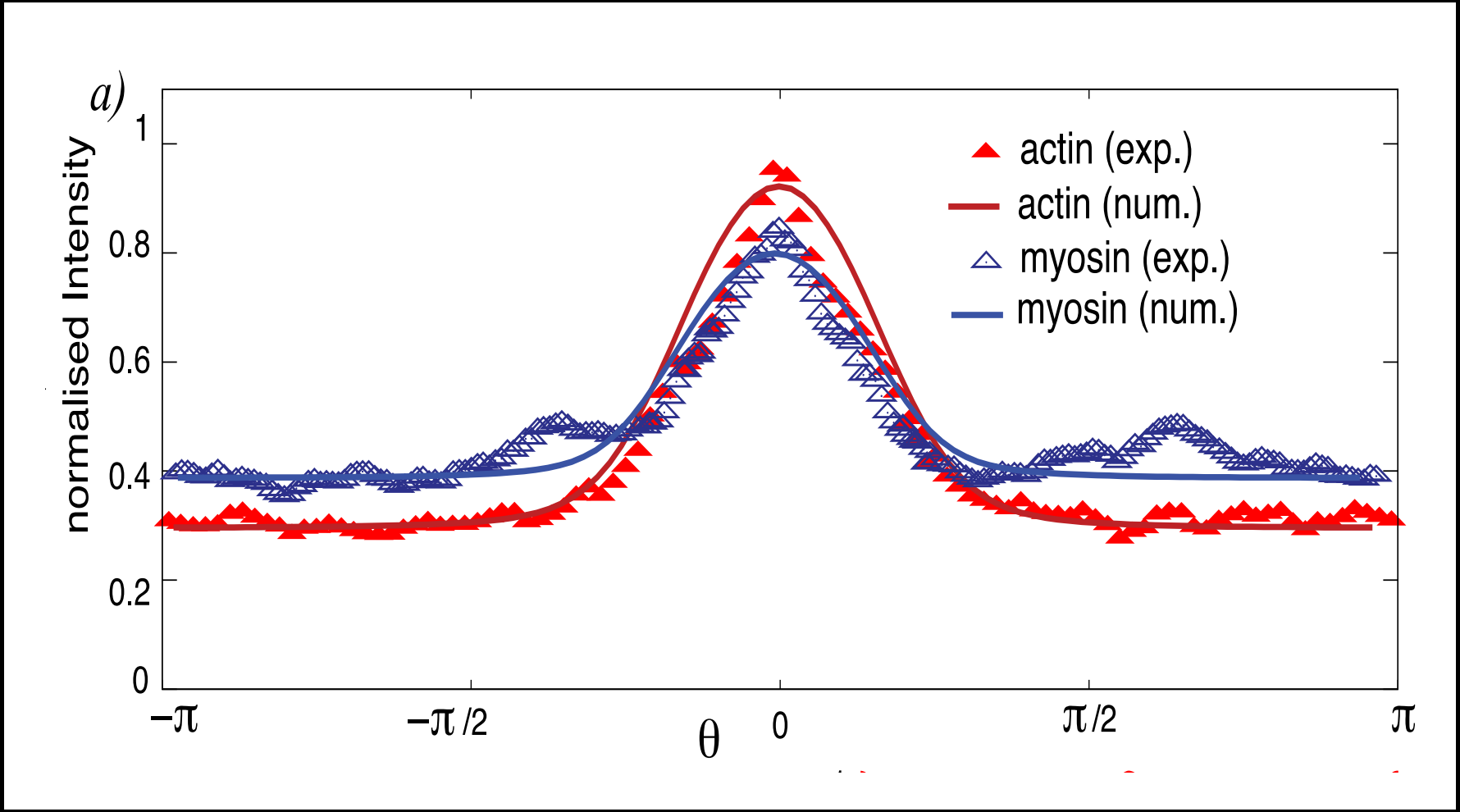
$$F = \frac{8\pi}{3} h_0 R^2 \xi v_0$$

- blebbing due to weakend at front $l > 1 \rightarrow$ several blebs

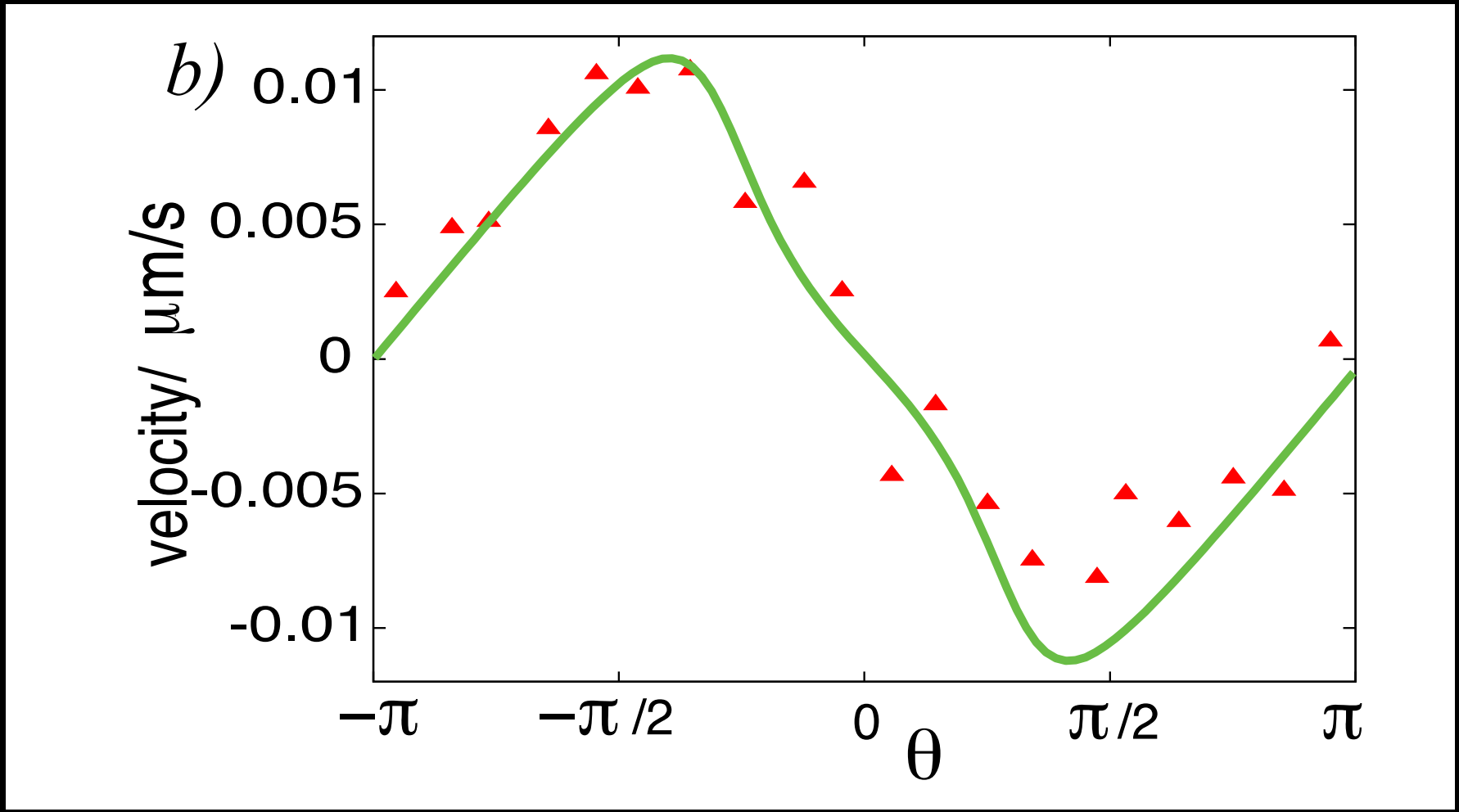
Long time behaviour

- Full nonlinear treatment required
- Did with numerical simulations for 1D
- Compare with experimental measurements:
  - MB-231 tumor cells seeded in 3D matrigel
  - Intensity mCherry-Lifeact labeled actin
  - Intensity of labeled myosin
  - Kymographs give velocity of actin
- Maximum and background intensities fitted

Results – actin & myosin density

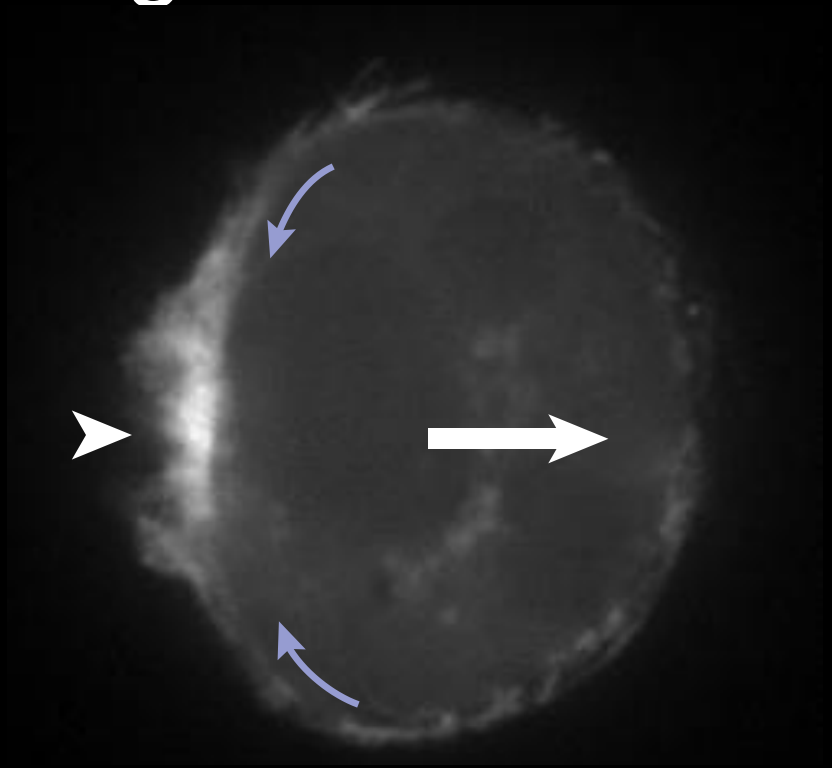


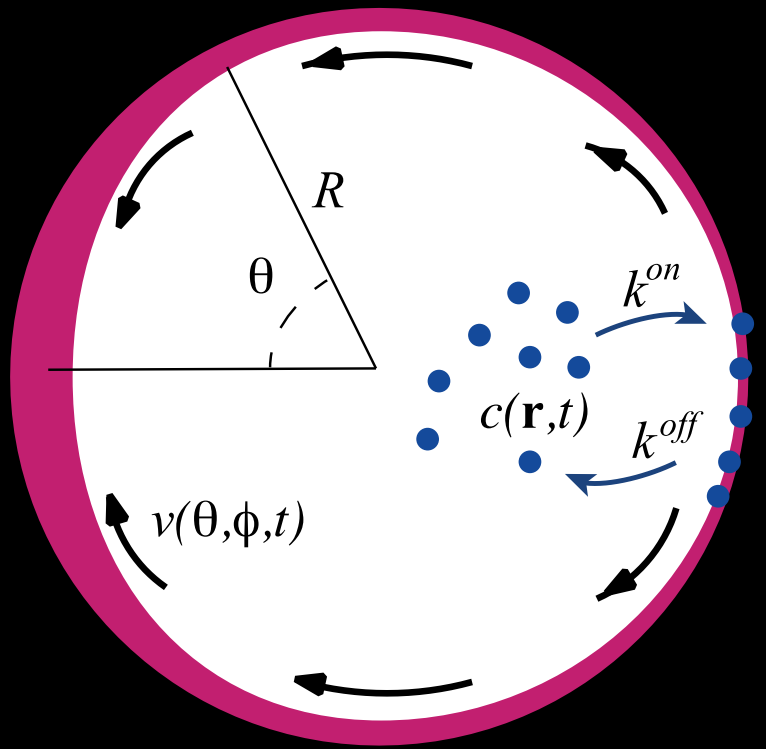
Results – actin velocity



Conclusion (part 2): spherical swimmers

In 3D confinement spontaneous cortical flow generated by acto-myosin contraction can lead to cell migration



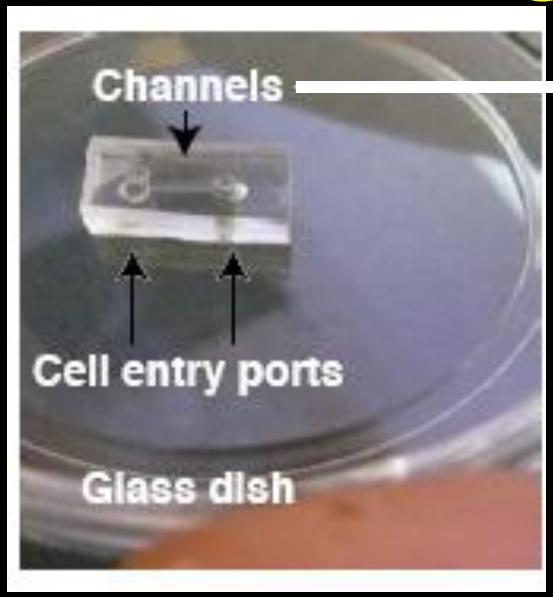


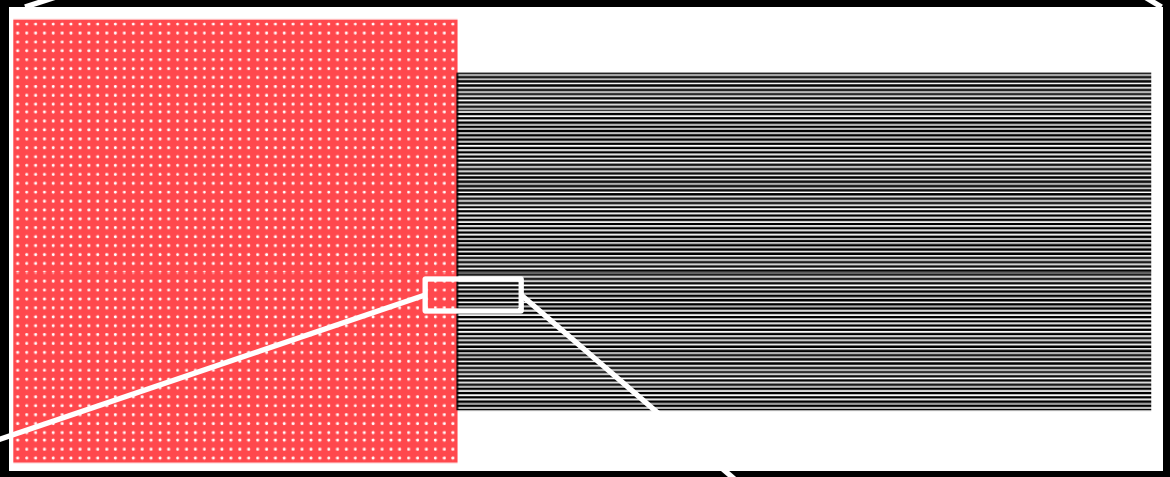
Hawkins et al Biophysical Journal (2011)

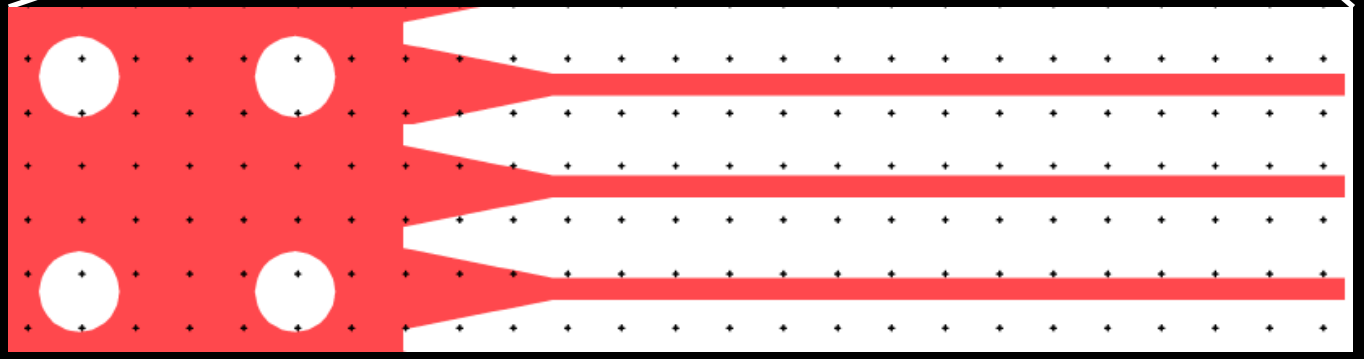
Discussion questions

- How could we stop metastatic cancer cells moving without stopping immune cells moving?
- What (if anything) are useful things (theoretical) physicists could do to guide therapeutics?

Fabricating microchannels

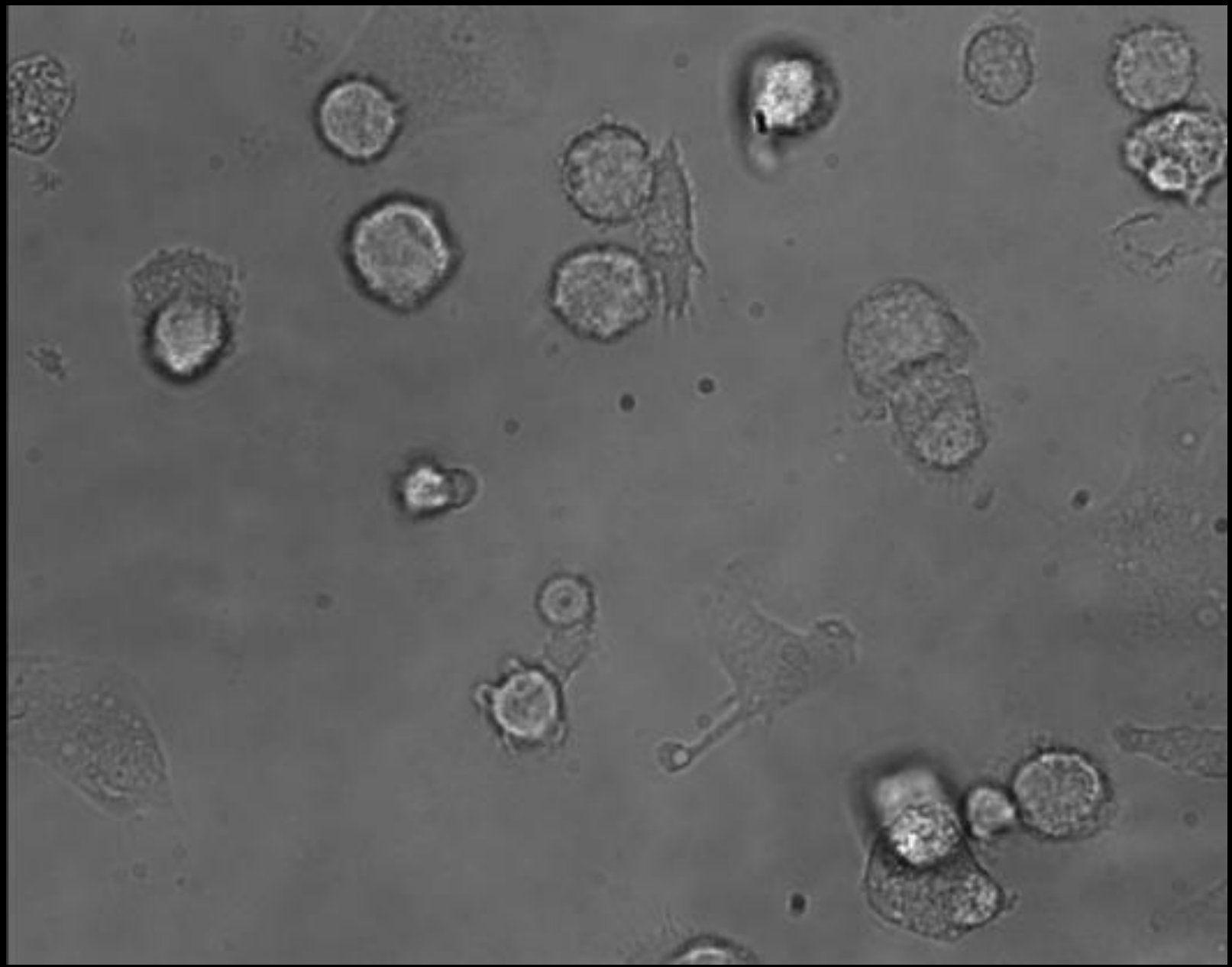




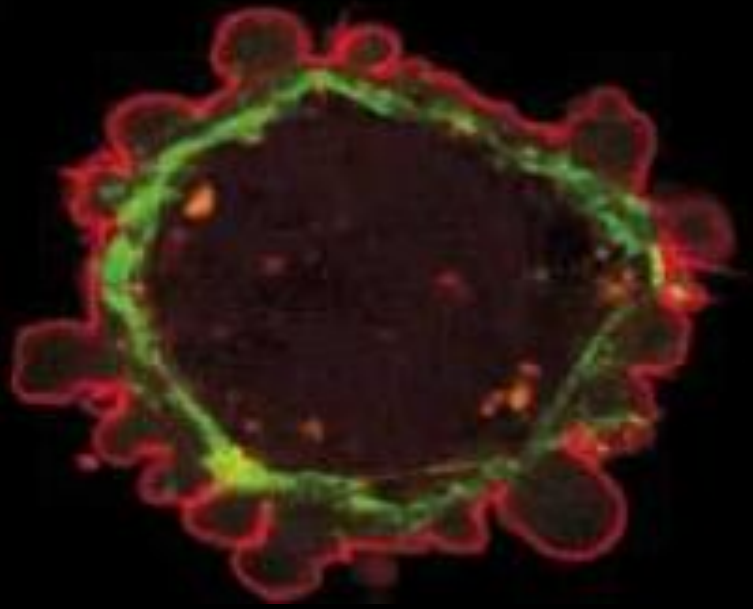


↕ ~µm

Intro: Dendritic cells on a flat surface



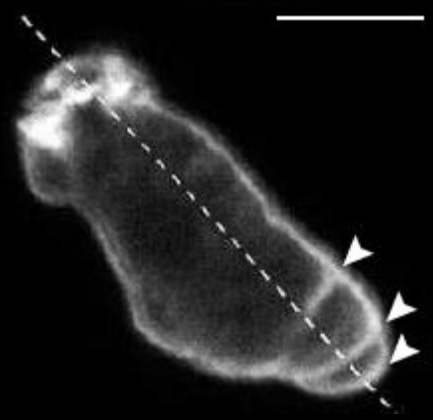
Blebbing



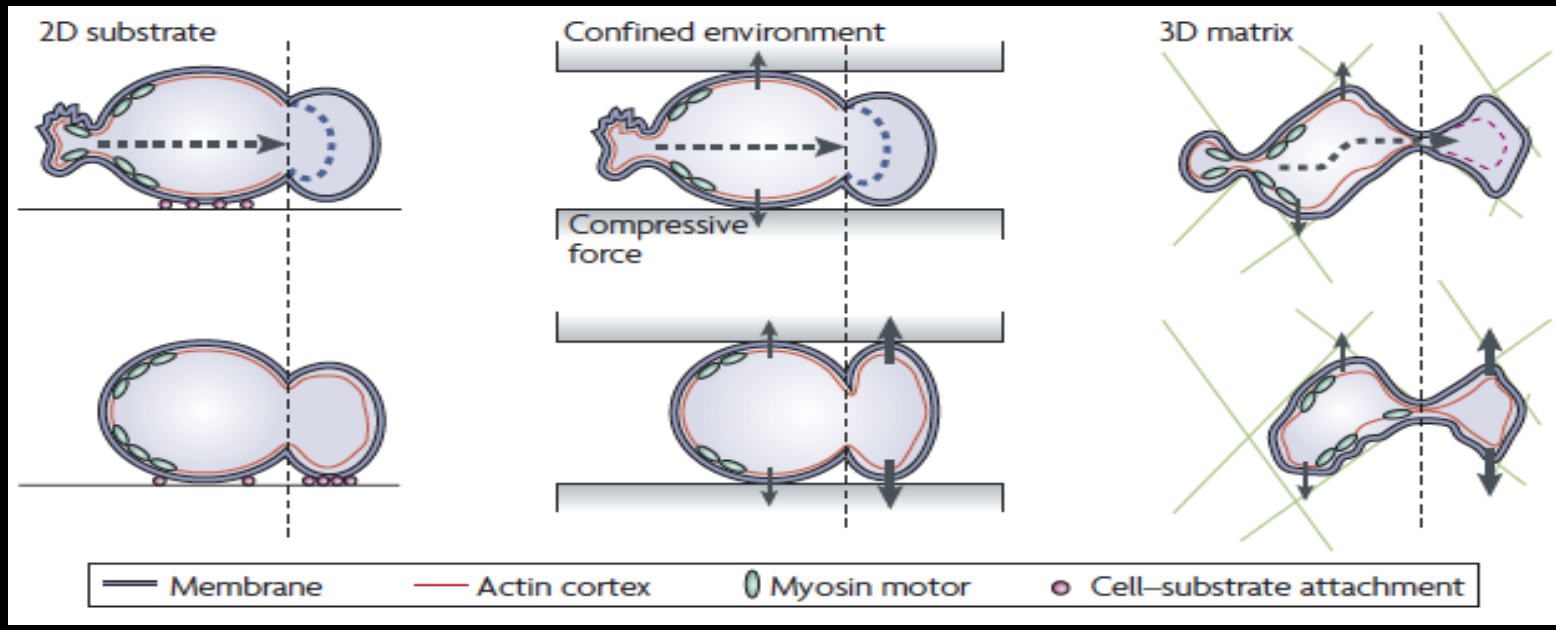
Myosin light chain localization in a filamin-deficient melanoma cell.
Charras J Microsc. 2008 Sep;231(3):466-78

- Blebs are spherical protrusions of the membrane
- Produced by contraction of the actomyosin cortex
- Involved in apoptosis but also division & spreading

Blebbing motility?



Actin cortex of a blebbing *Dictyostelium discoideum.* *Yoshida & Soldati, J. Cell Sci. 119, 3833–3844 (2006)*



Blebbing assisted migration, *Charras & Paluch, Nat Rev Mol Cell Biol. 2008 Sep;9(9):730-6*

- Blebs may play a role in migration in 3D environments
- Alternative migration mechanism to lamellipodium?
- We develop a model for migration due to actomyosin contraction – the same mechanism causing blebs