

# 1) Moving Sausages

## 2) The First Universal Law of Cell Migration

$$\tau = Ae^{\beta v}$$



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# The first universal law of cell migration?

An unexpected outcome of the First World Cell Race:  
Cytoskeletal flows impose a universal coupling  
between cell speed and cell persistence

$$\tau = Ae^{\beta v}$$

Persistence time

Instantaneous speed

Theory

Exp. Vienna

Exp. Paris



Raphael Voituriez

Nir Gov

J.F. Ruprecht

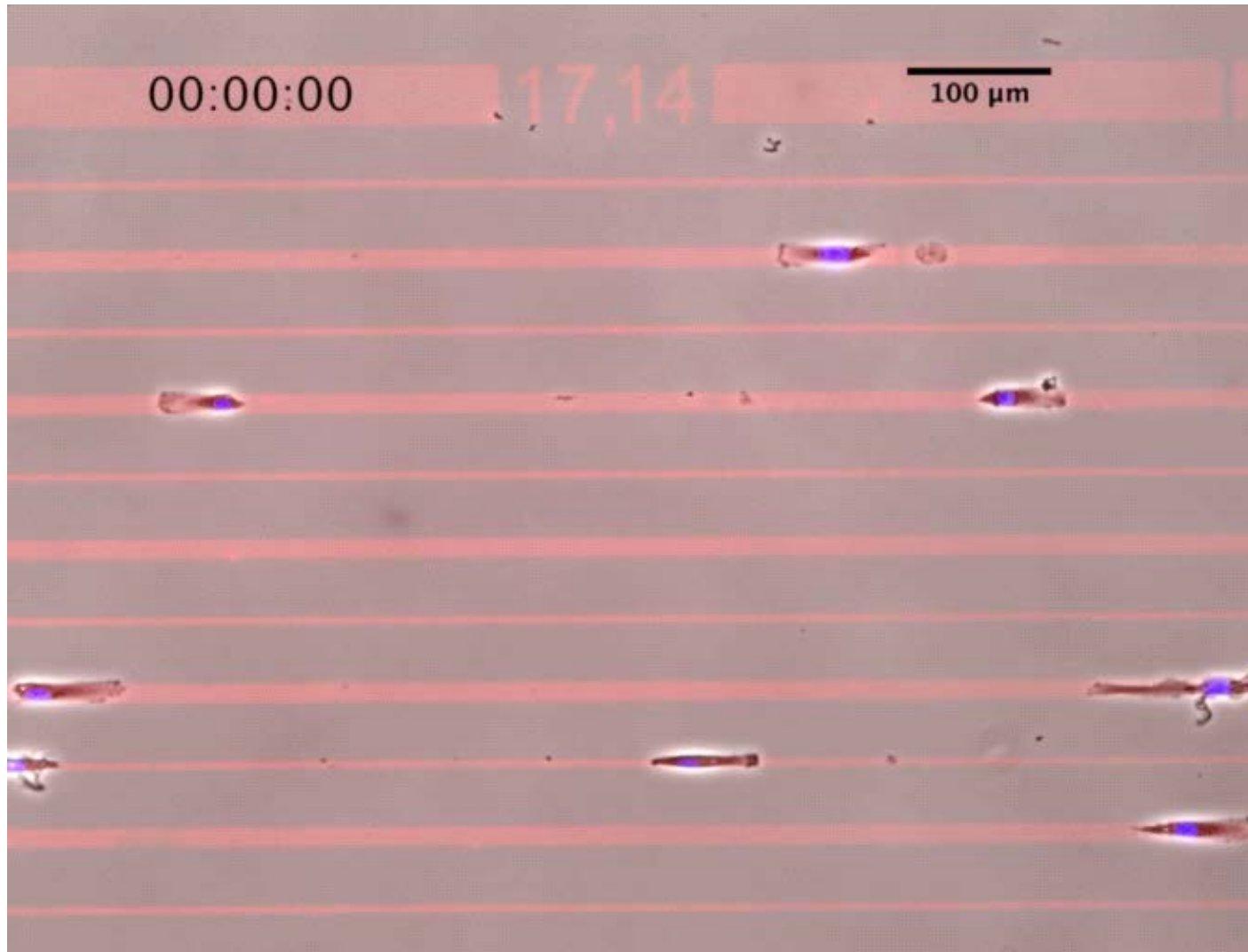
Verena Rupprecht

Stefan Wieser

Paolo Maiuri

F. Lautenschlaeger

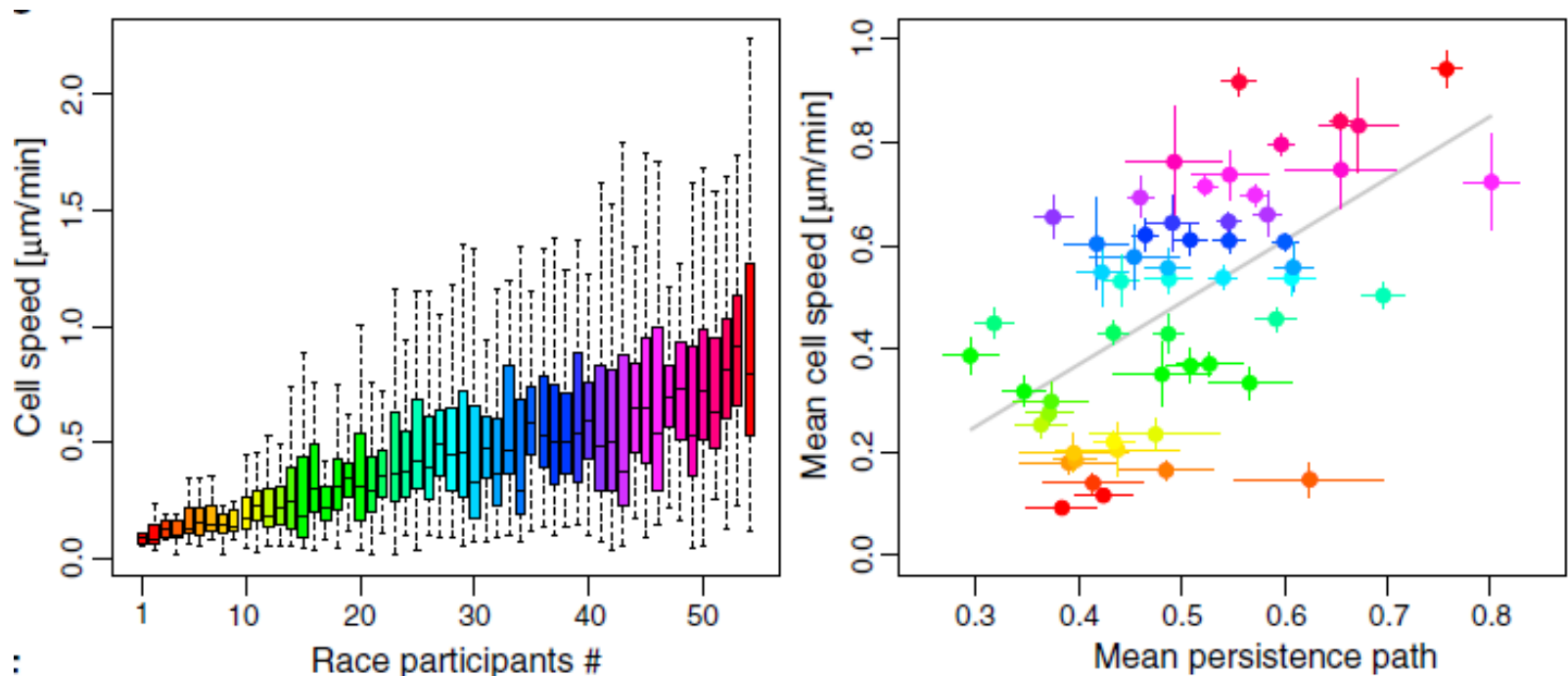
# Micropatterns for migrating cells: the First World Cell Race (2011)



Movie: T. Vignault

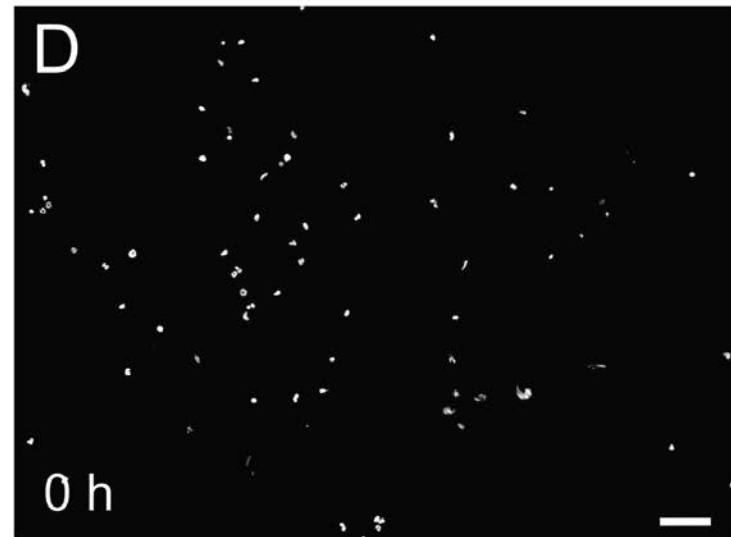
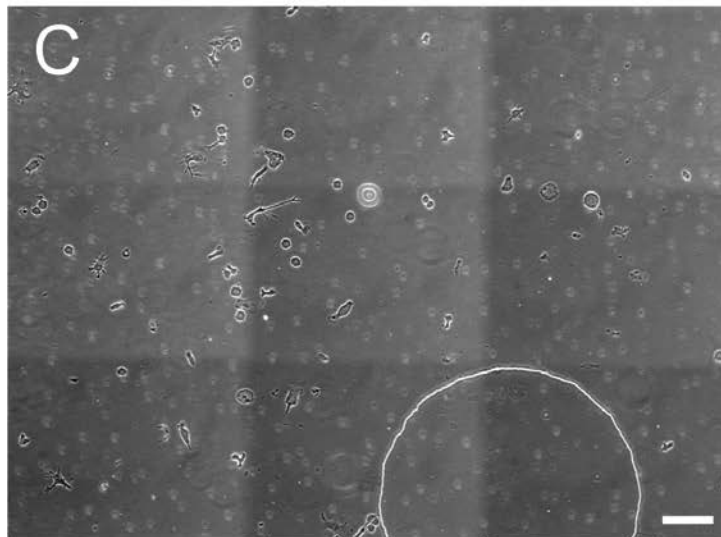
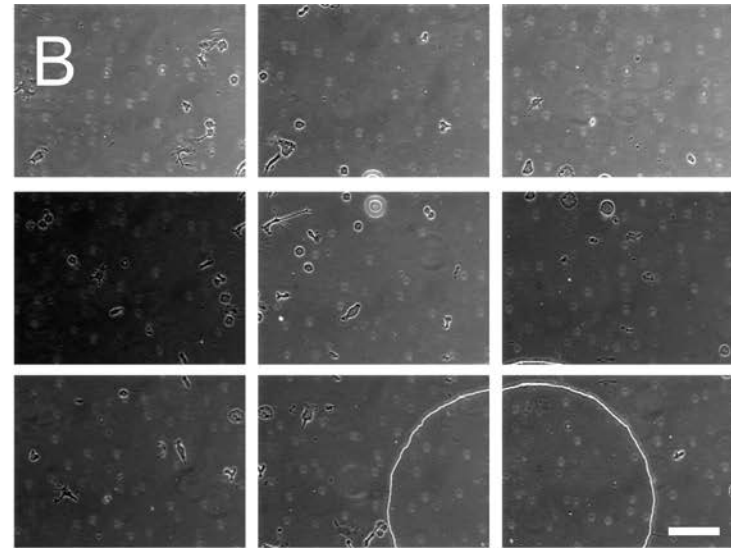
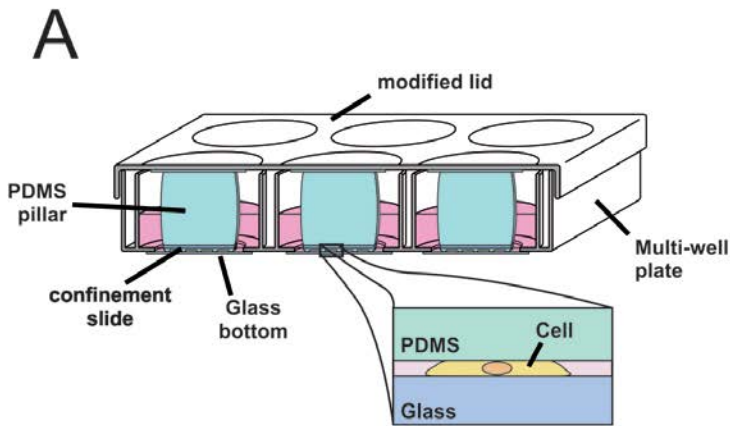
(see also Pouthas et al., JCS 2008 and Doyles et al., JCB 2009)

# Faster cells are also more persistent, revealing a coupling between the motor and the steering wheel

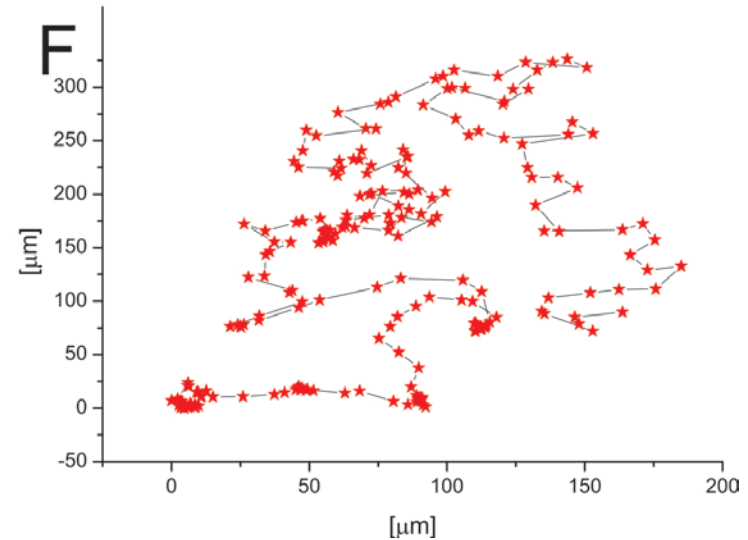
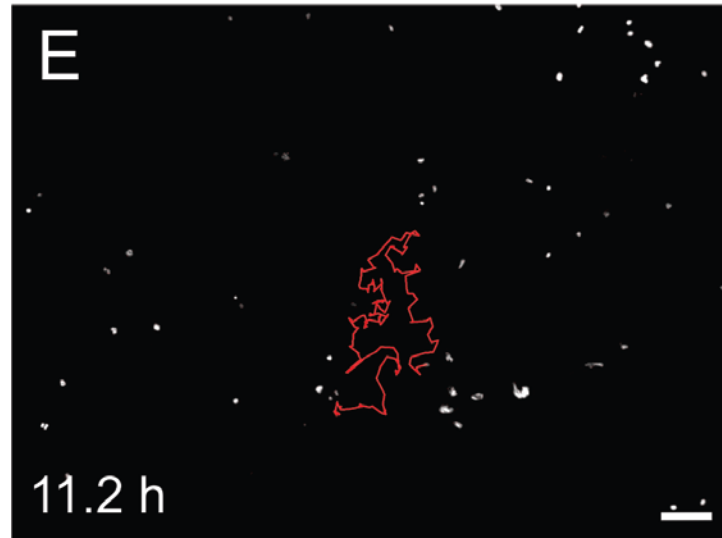


|    |                  |    |                |    |                |    |                |    |                |
|----|------------------|----|----------------|----|----------------|----|----------------|----|----------------|
| 1  | h.Bre.E.Car.1    | 12 | h.Skin.E.Car.2 | 23 | h.Skin.E.Mel.2 | 34 | m.Emb.C.Tra.3  | 45 | m.SGI.E.Sar    |
| 2  | r.Emb.M.Tra      | 13 | h.Skin.E.Car.1 | 24 | h.Bre.E.Fib.5  | 35 | h.Bre.E.Ad.c.7 | 46 | m.Emb.C.Tra.2  |
| 3  | m.Mus.M.Tra      | 14 | h.Skin.E.Mel.1 | 25 | h.Kid.E.Ad.c   | 36 | h.Bre.E.Ad.c.5 | 47 | h.Skin.C.Tra   |
| 4  | m.Emb.C.Tra.8    | 15 | h.Pro.C.Ad.c   | 26 | h.Bre.E.Fib.4  | 37 | h.Skin.E.Pri   | 48 | m.Emb.C.Tra.1  |
| 5  | h.Bre.E.Car.2    | 16 | h.Cer.E.Car    | 27 | m.Bre.E.Ad.c.2 | 38 | h.Bre.E.Ad.c.8 | 49 | m.Bre.E.Ad.c.4 |
| 6  | h.Bre.E.Car.3    | 17 | m.Bre.E.Ad.c.3 | 28 | h.Bla.E.Car    | 39 | m.Bre.E.Ad.c.1 | 50 | h.Bre.E.Fib.3  |
| 7  | h.LyNo.Epi.Mel.2 | 18 | h.Skin.E.Mel   | 29 | m.Hip.N.Tra.1  | 40 | h.Bre.E.Ad.c.3 | 51 | m.Emb.C.Tra.5  |
| 8  | m.Emb.C.Tra.7    | 19 | h.Bre.E.Ad.c.1 | 30 | m.Emb.C.Tra.4  | 41 | h.Skin.E.Tra.2 | 52 | h.Bre.E.Fib.2  |
| 9  | h.Bre.E.Car.4    | 20 | h.Skin.E.Tra.1 | 31 | h.Bre.E.Ad.c.4 | 42 | m.Hip.N.Tra.2  | 53 | h.Bre.E.Ad.c.2 |
| 10 | h.LyNo.E.Mel.1   | 21 | h.Col.E.Ad.c.2 | 32 | h.BM.C.SC      | 43 | h.Bre.E.Fib.6  | 54 | h.Emb.C.Pri    |
| 11 | h.Bre.E.Fib.1    | 22 | h.Col.E.Ad.c.1 | 33 | h.Alv.M.Rha    | 44 | h.Bre.E.Ad.c.6 |    |                |

# Recording long cell tracks: not so simple



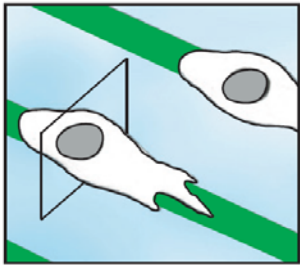
# Recording long cell tracks: not so simple



Important points:

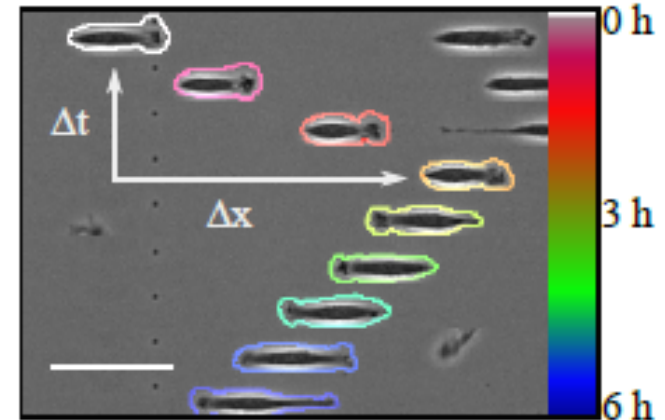
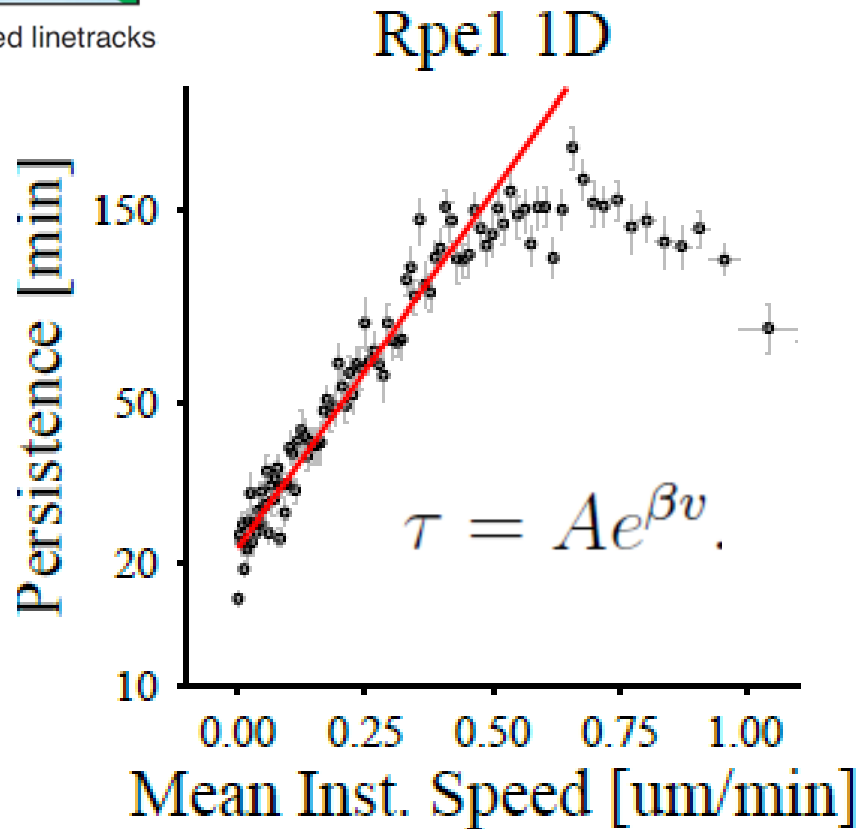
- 1) No convective flows: requires a roof
- 2) Long tracks: stitching
- 3) A focus keeping system

# Persistence is an exponential function of speed



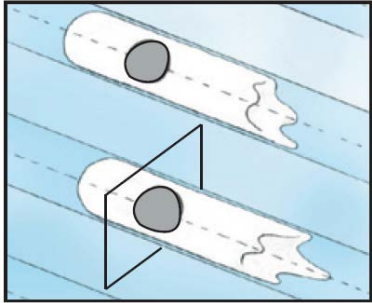
micropatterned linetracks

## Mesenchymal cells on 1D adhesive tracks



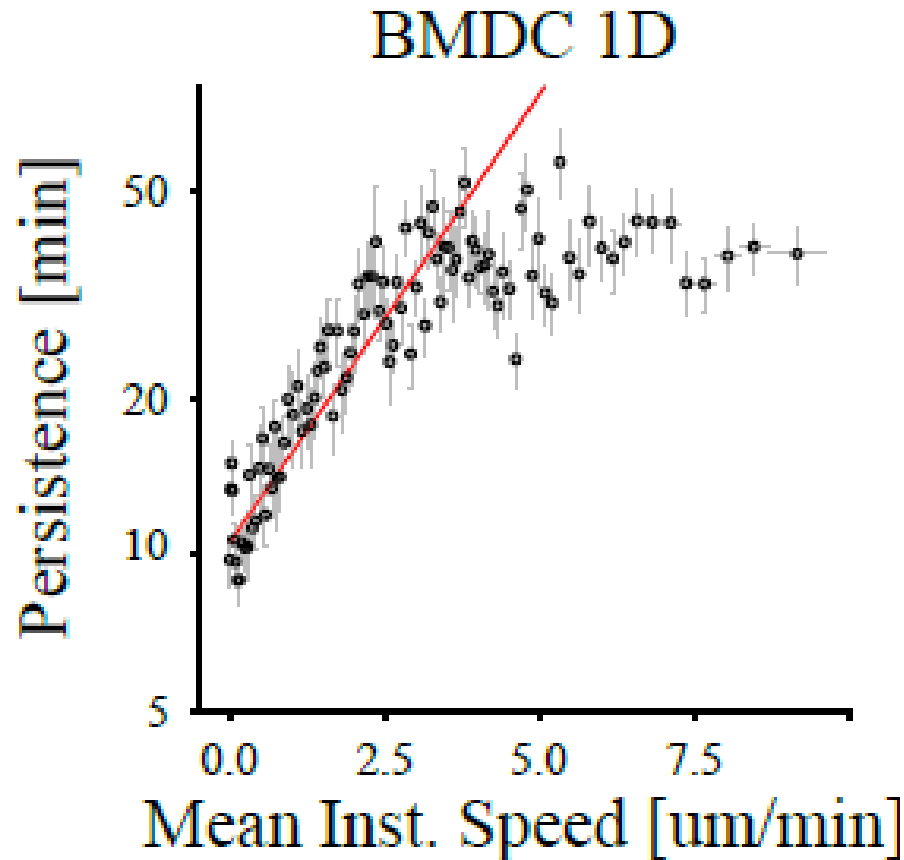
Persistence Time =  $\Delta t$   
Mean Instantaneous Speed =  $\Delta x / \Delta t$

# Persistence is an exponential function of speed



inside microchannels

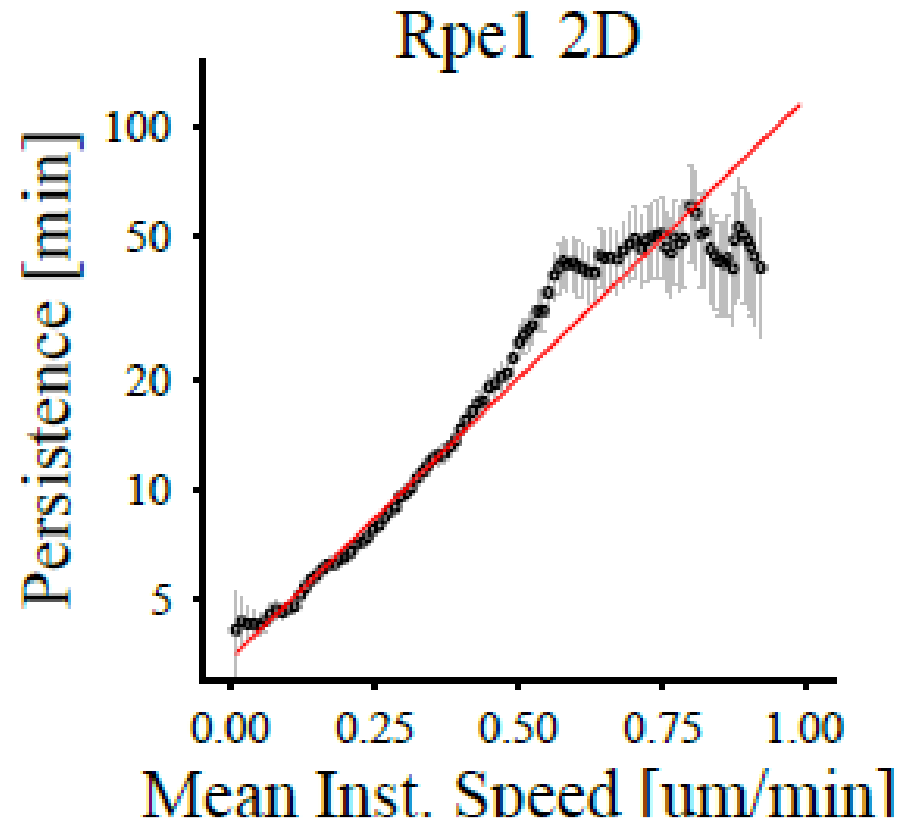
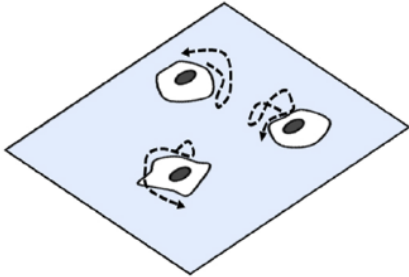
## Amoeboid cells inside 1D channels





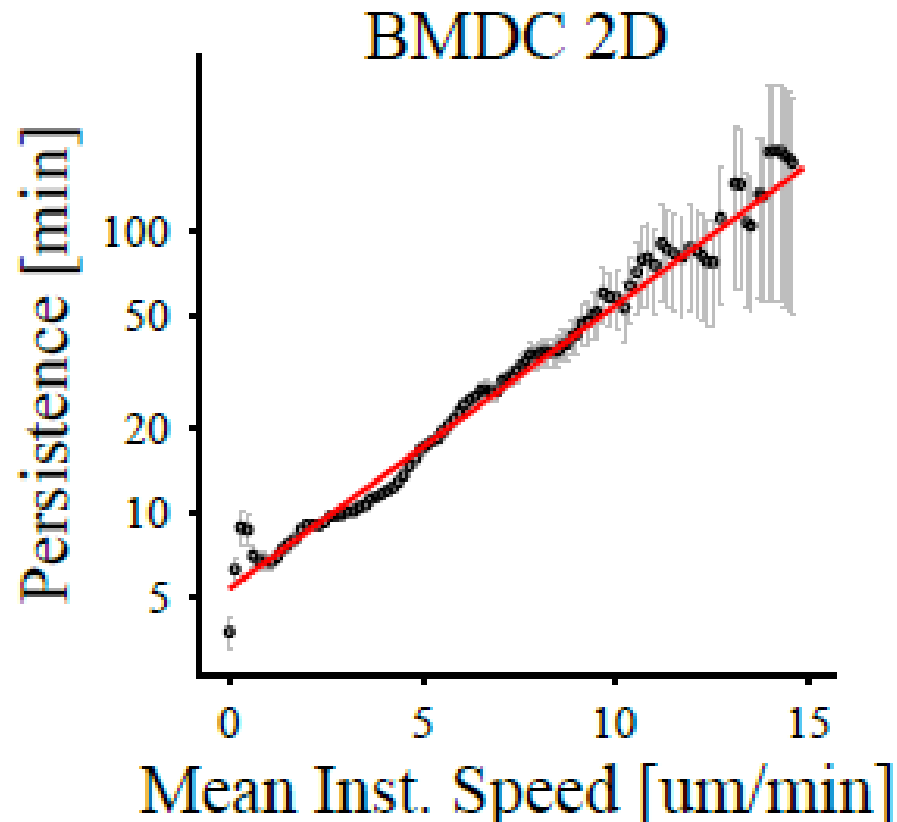
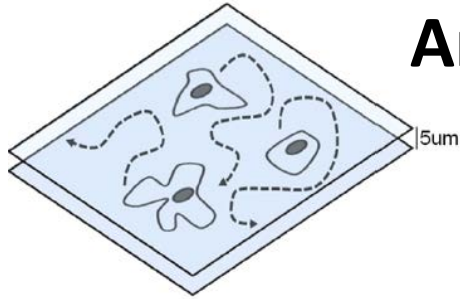
# Persistence is an exponential function of speed

## Mesenchymal cells on 2D adhesive substrate



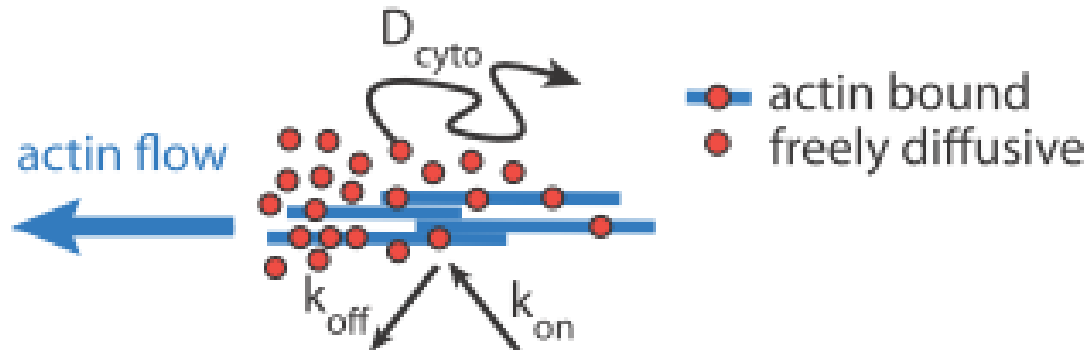
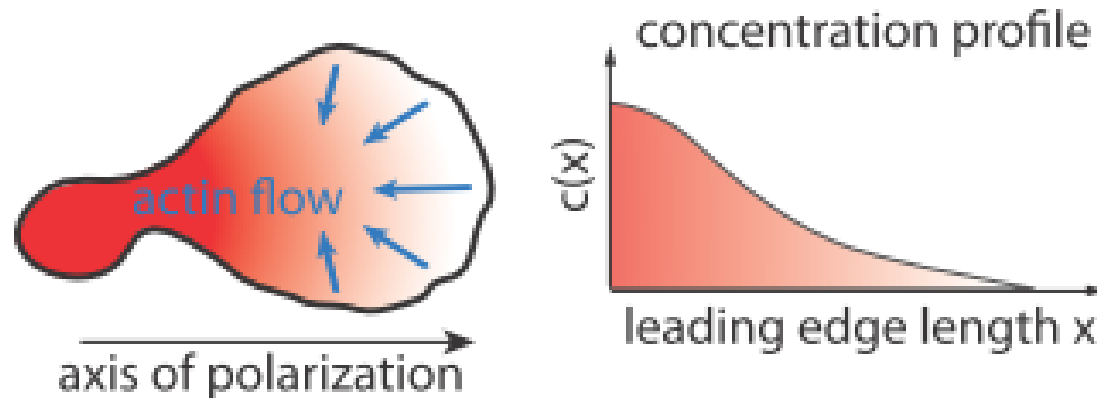
# Persistence is an exponential function of speed

## Amoeboid cells on 2D confined substrate

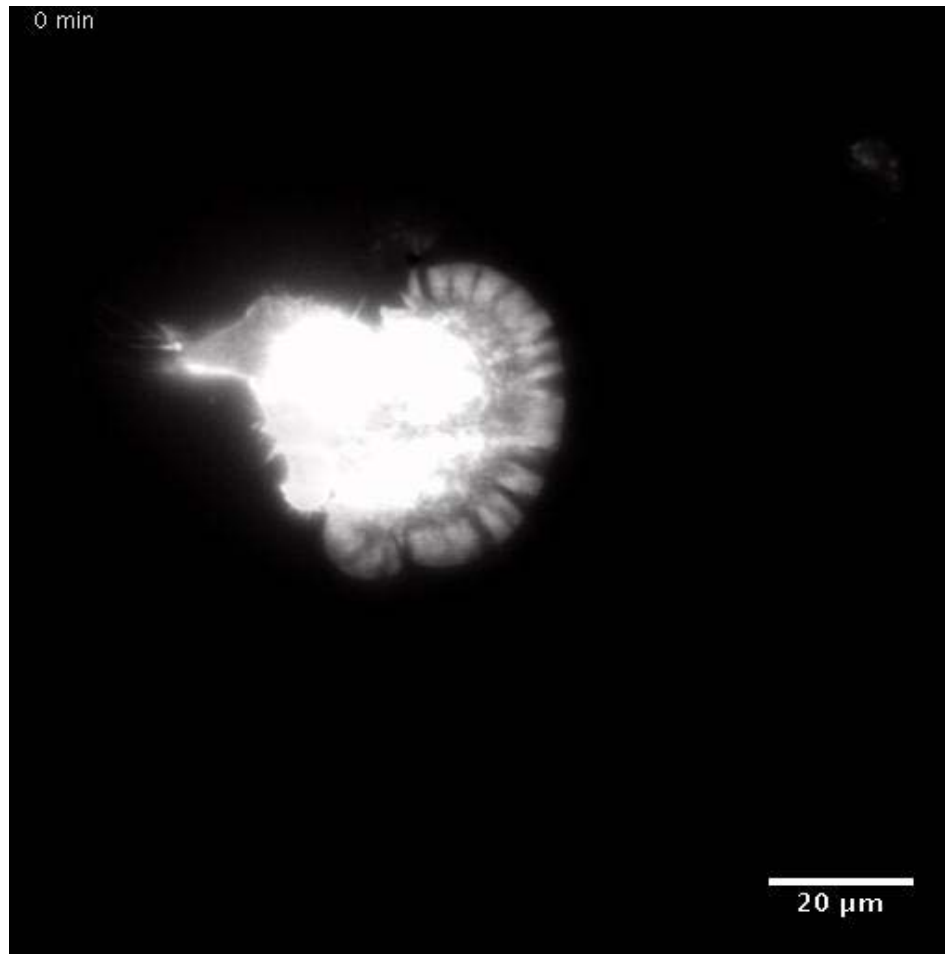


Coupling speed to persistence:  
all cells rely on a net flow of actin  
from front to back in order to move  
and this flow can transport other proteins

### Protein Redistribution

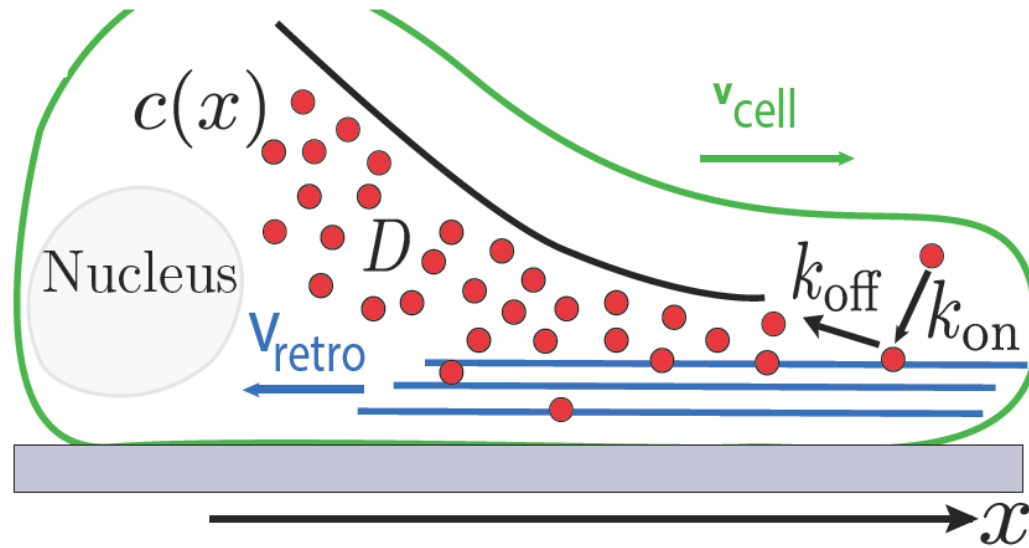


# The actin retrograde flow



(BMDC under agarose, Lifeact-GFP, TIRF, from Stefan Wieser, M. Sixt lab)

# A simple 1D model for advection of a polarity factor by retrograde flow



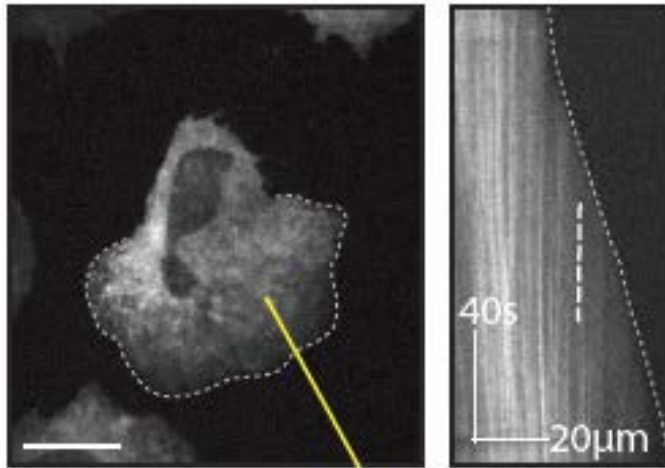
$$\partial_t c(x, t) - \partial_x [V c(x, t)] = D \partial_x^2 c(x, t) + \partial_x \zeta_c$$

$$V = V_0 k_{\text{on}} / (k_{\text{on}} + k_{\text{off}}) \quad D = D_0 k_{\text{off}} / (k_{\text{on}} + k_{\text{off}}) \quad \int_0^L c(x, t) dx = M$$

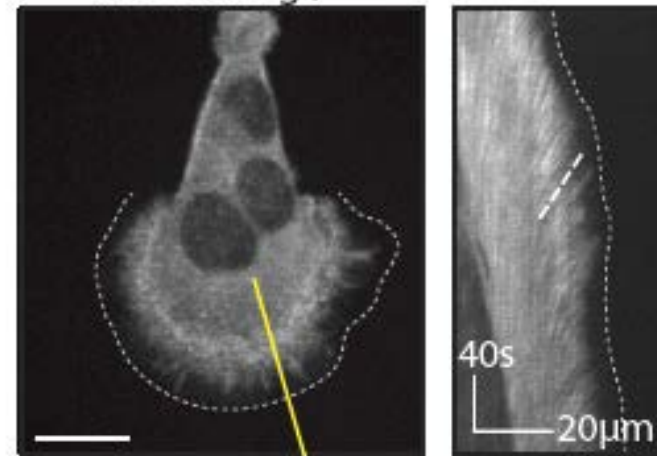
$$\bar{c}(x) = C e^{-Vx/D}$$

# Modulating retrograd flow speed independantly of cell speed

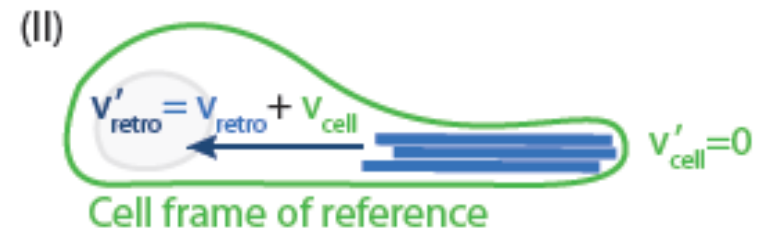
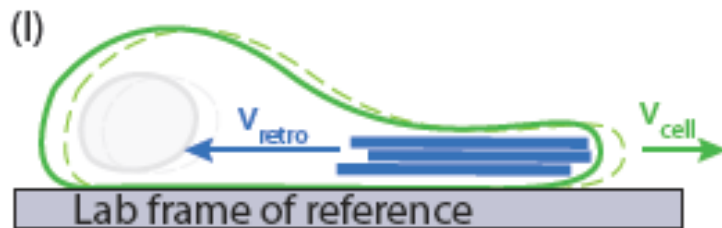
MLC-GFP Wt



MLC-GFP Itg-/-

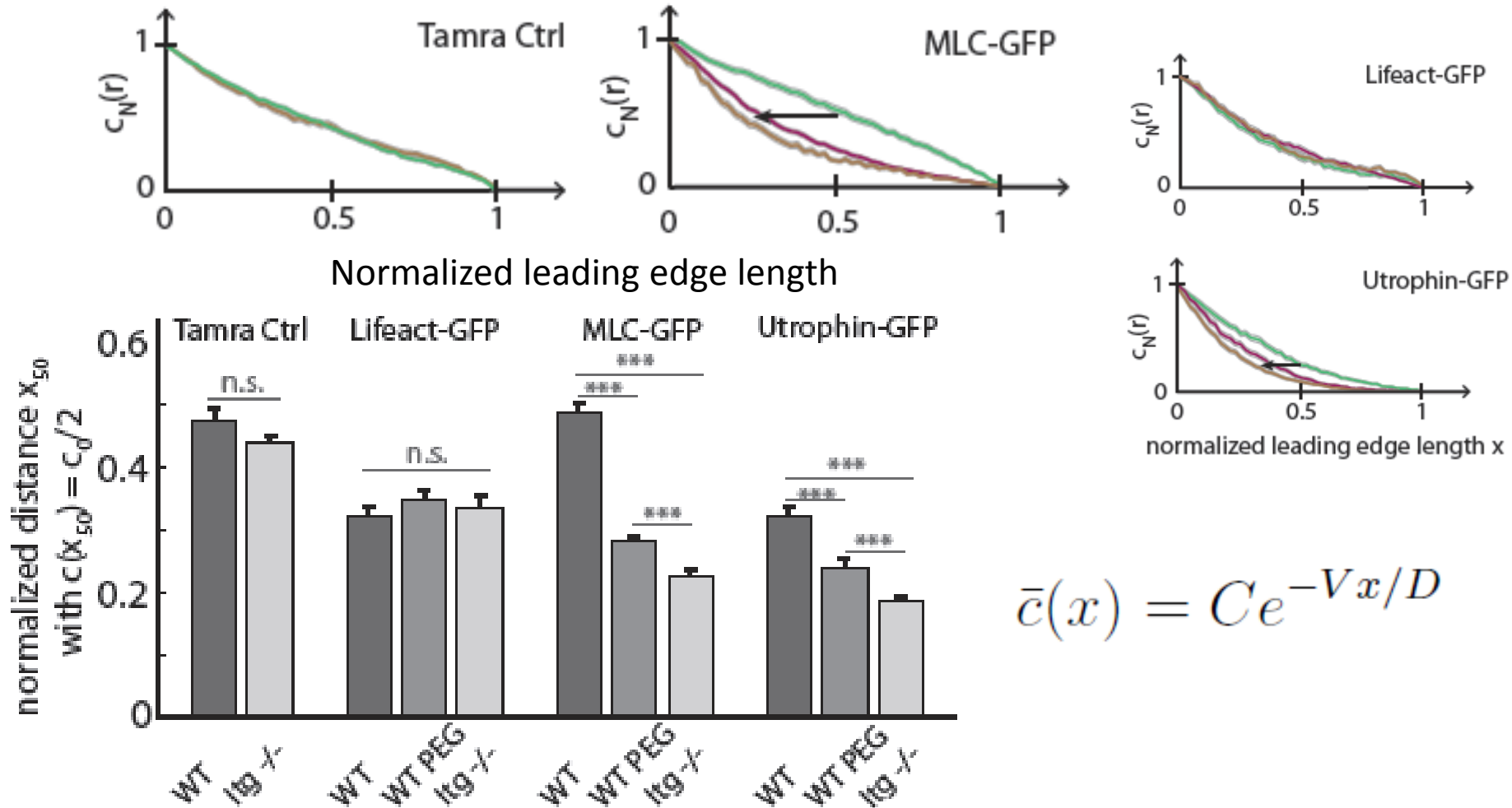


## Reference Systems



(see Renkawitz et al. NCB 2009, Sixt lab)

# Actin flow induces concentration of actin bound proteins



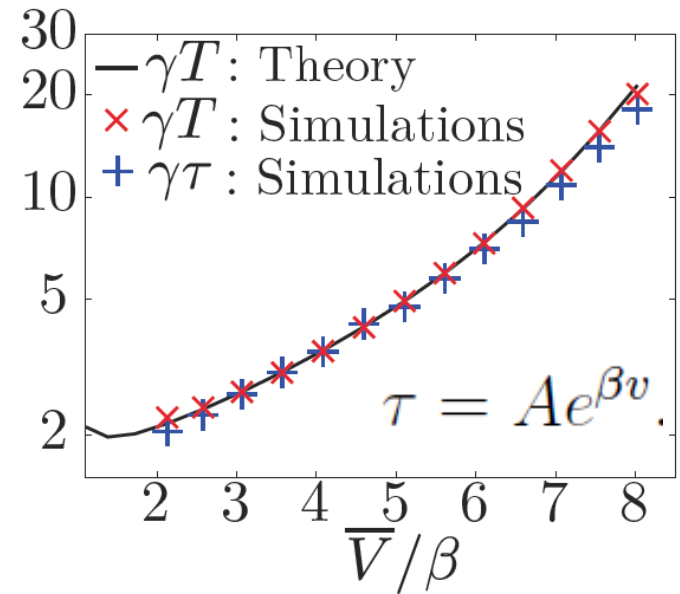
# The model captures the exponential relation between persistence and speed

$$\partial_t c(x, t) - \partial_x [V c(x, t)] = D \partial_x^2 c(x, t) + \partial_x \zeta_c$$

$$V^* = \beta \left( \frac{c_0^n(V)}{C_s^n + c_0^n(V)} - \frac{c_L^n(V)}{C_s^n + c_L^n(V)} \right)$$

$$\partial_t V = -\gamma(V - V^*) + \frac{K}{V} + \sqrt{2K} \zeta_V$$

$$\partial_t \phi = \frac{\sqrt{2K}}{V} \zeta_\phi, \quad L^2/D \ll \gamma^{-1}$$



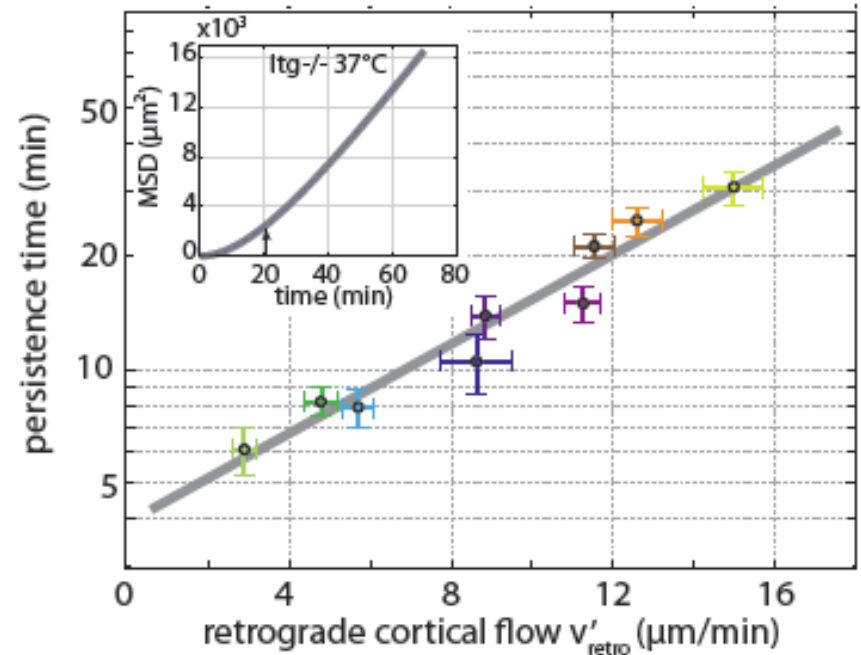
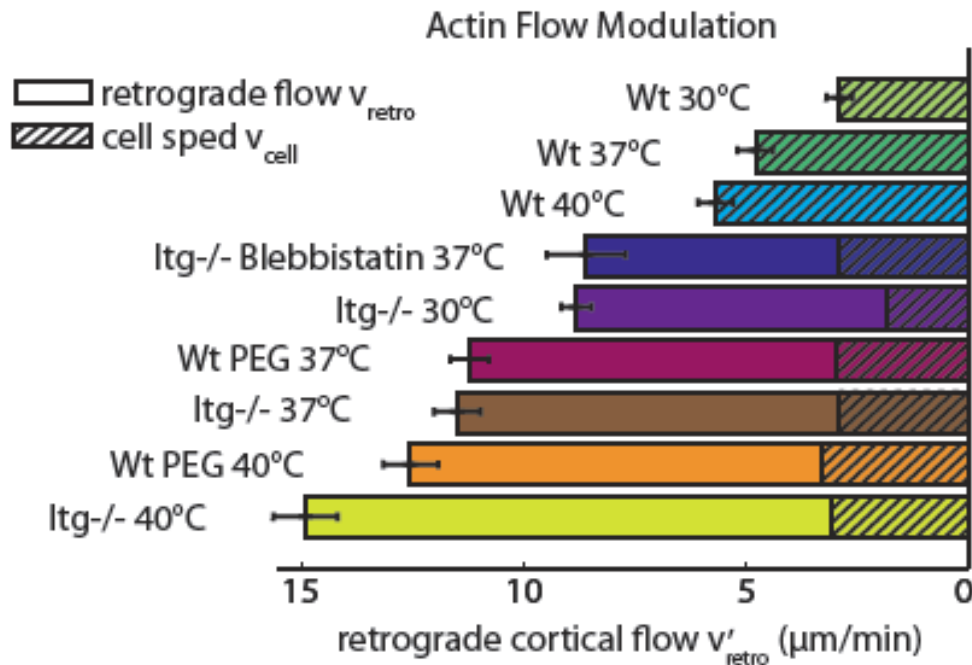
$$\partial_t V = \gamma \mathcal{F}(V) + \sqrt{2\sigma^2(V)} \zeta_V \quad \mathcal{F}(V) = -V + \beta \left( \frac{\bar{c}_0^n(V)}{C_s^n + \bar{c}_0^n(V)} - \frac{\bar{c}_L^n(V)}{C_s^n + \bar{c}_L^n(V)} \right) + \frac{K}{\gamma V}$$

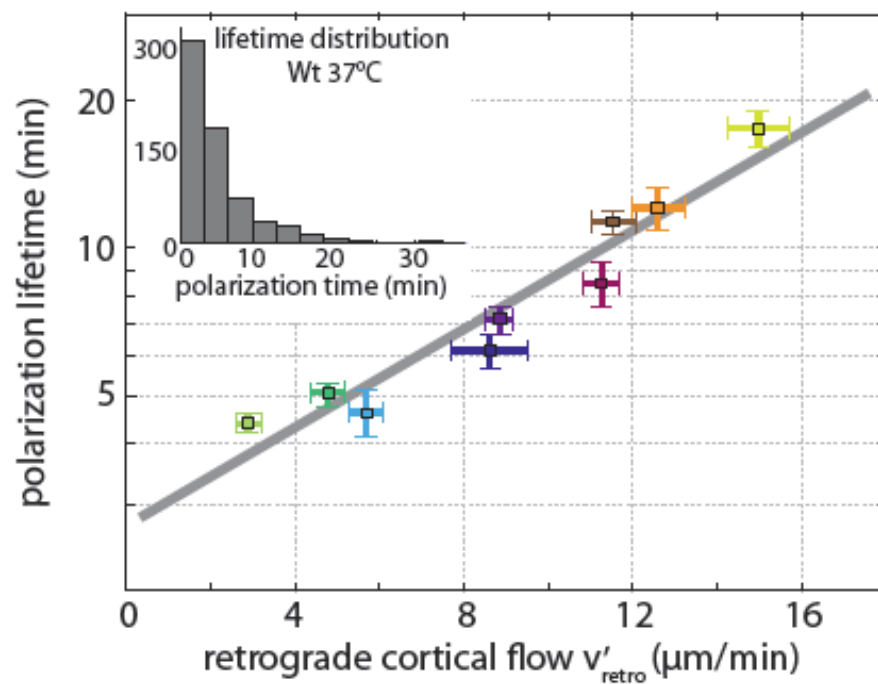
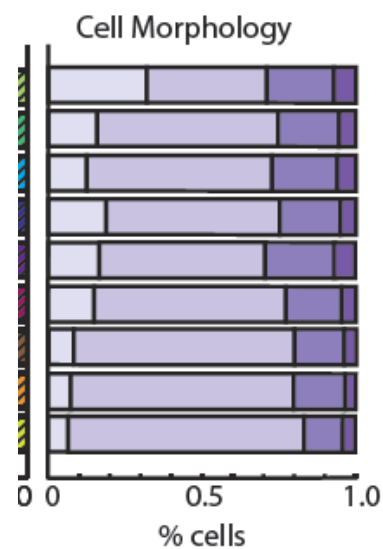
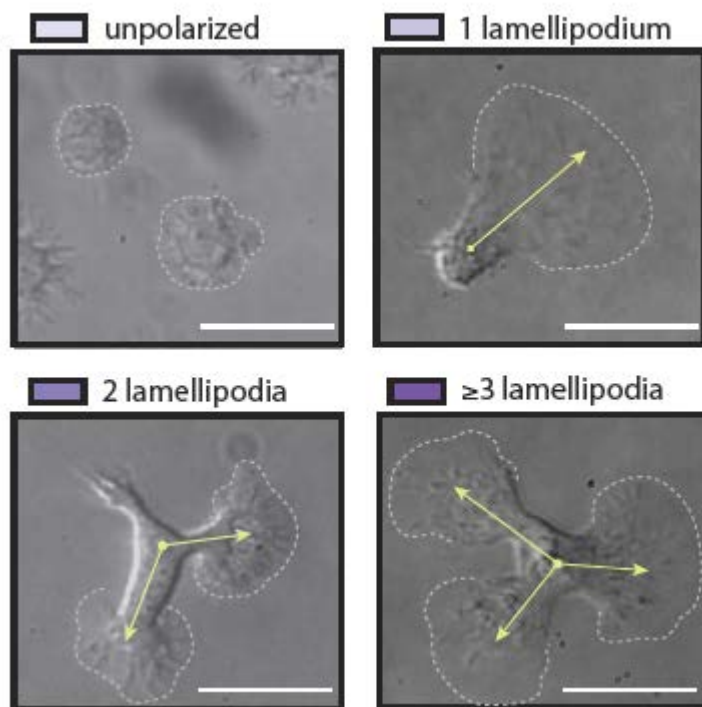
$$P(V) = \frac{C}{\sigma^2(V)} \exp \left( \gamma \int_0^V du \frac{\mathcal{F}(u)}{\sigma^2(u)} \right) \equiv C e^{-\gamma W(V)} \quad \tau_p \simeq A e^{\beta v^\theta}$$

the mean first-passage time at  $v = 0$      $n \simeq 0.9$      $\theta \simeq 1$



# Persistence is correlated to retrograde flow speed, independently of cell speed





The model can generate trajectories  
the cell as an active Brownian particule

$$\partial_t V = \gamma \mathcal{F}(V) + \sqrt{2\sigma^2(V)} \zeta_V$$

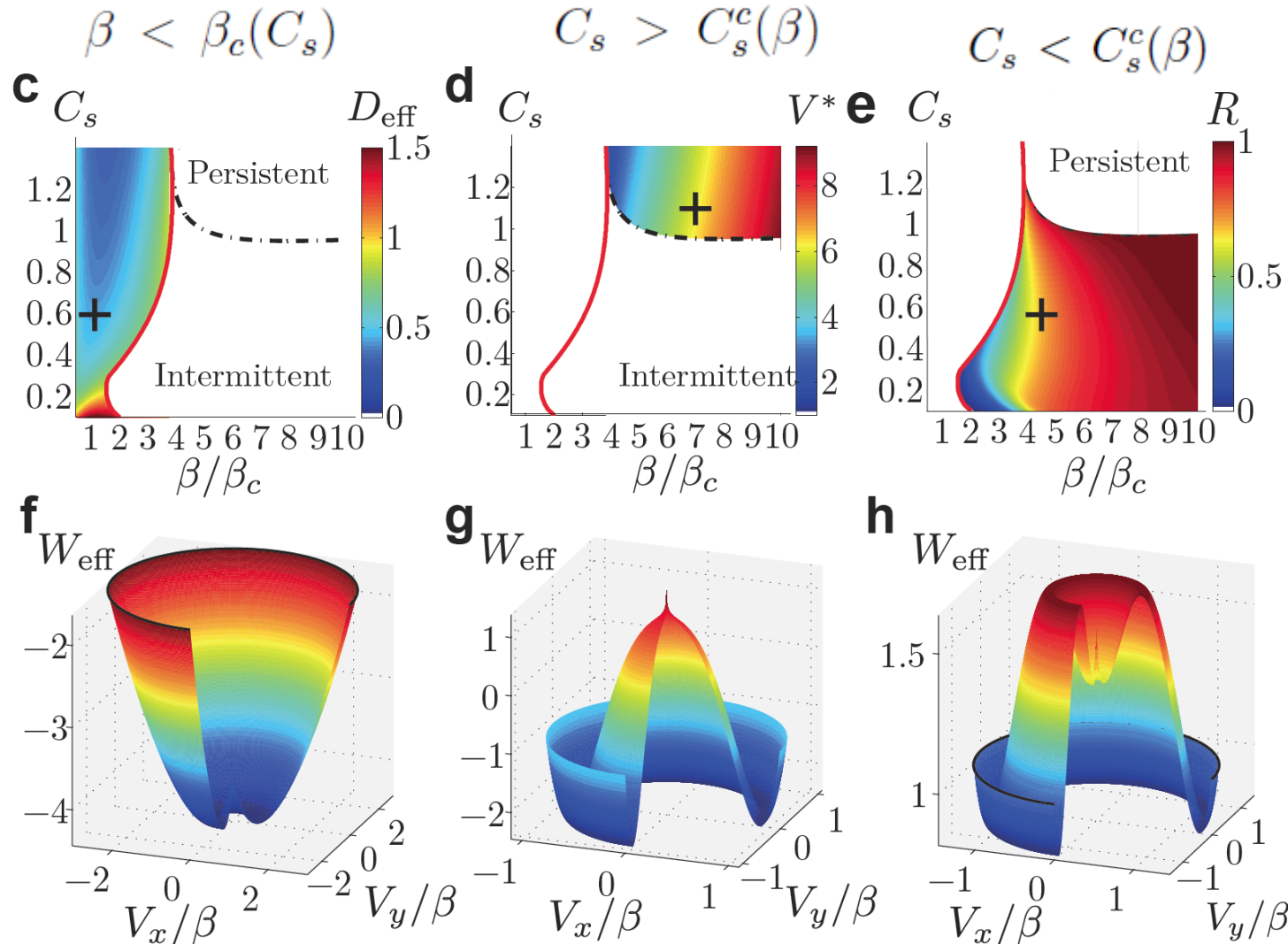
$$\mathcal{F}(V) = -V + \beta \left( \frac{\bar{c}_0^n(V)}{C_s^n + \bar{c}_0^n(V)} - \frac{\bar{c}_L^n(V)}{C_s^n + \bar{c}_L^n(V)} \right) + \frac{K}{\gamma V}$$

$$\sigma^2(V) = K + K_c n^2 \beta^2 C_s^{2n} \left( \frac{\bar{c}_0^{2n-1}(V)}{(C_s^n + \bar{c}_0^n(V))^4} + \frac{\bar{c}_L^{2n-1}(V)}{(C_s^n + \bar{c}_L^n(V))^4} \right)$$

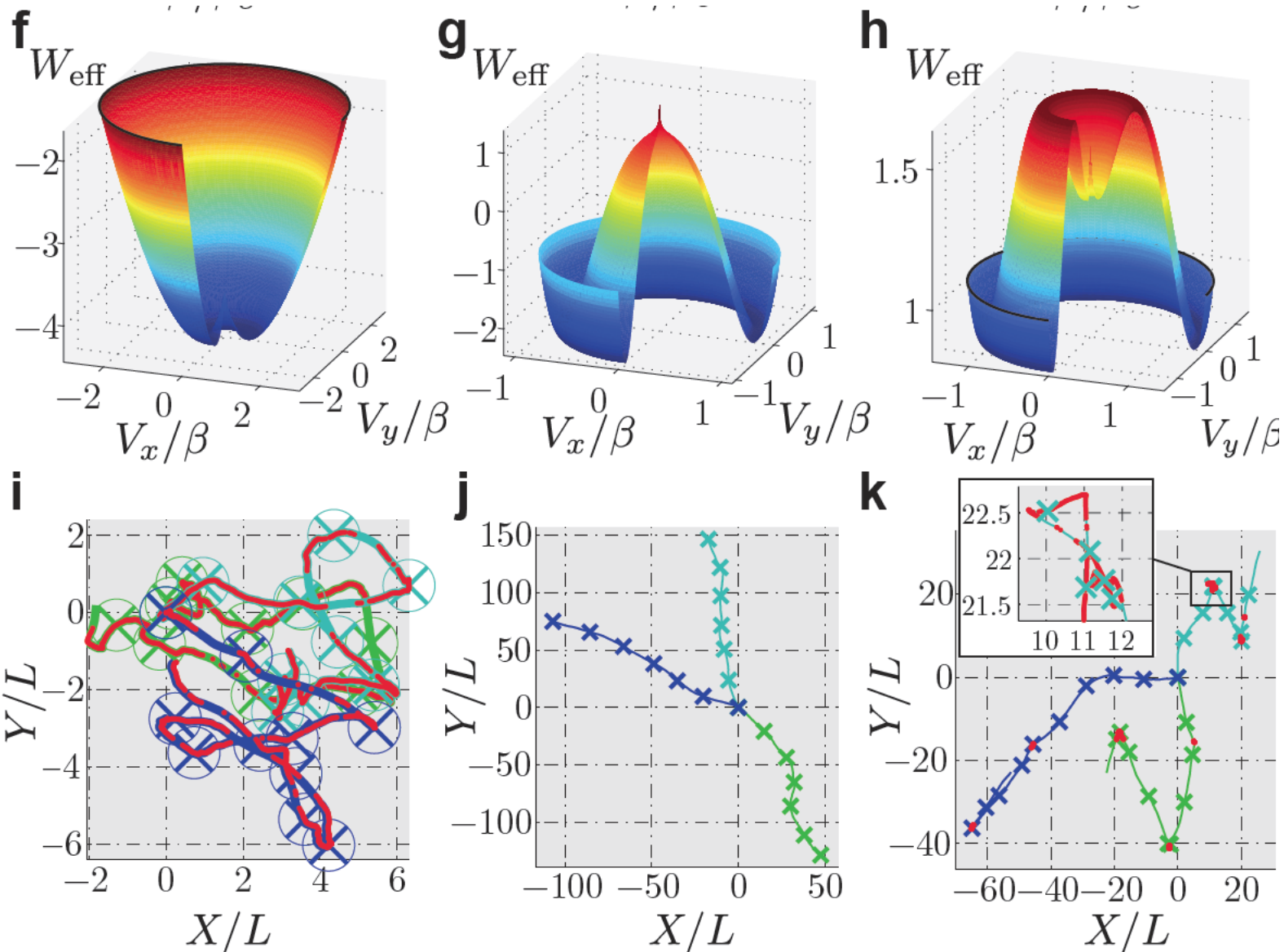
$$P(V) = \frac{C}{\sigma^2(V)} \exp \left( \gamma \int_0^V du \frac{\mathcal{F}(u)}{\sigma^2(u)} \right) \equiv C e^{-\gamma W(V)}$$

# The model predicts a whole phase diagram of cell behaviors

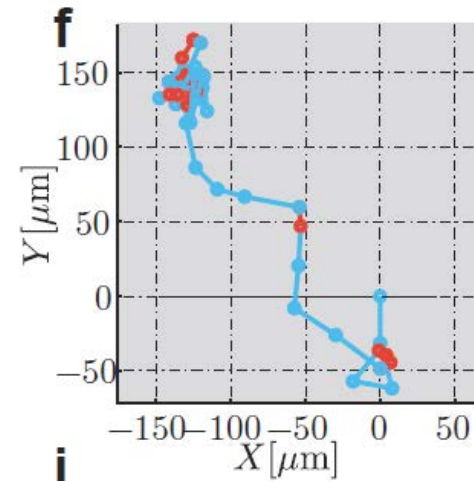
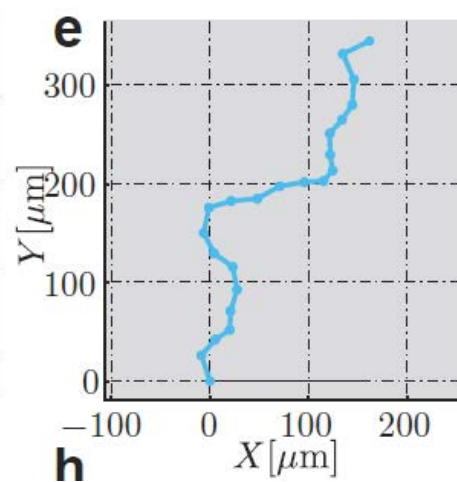
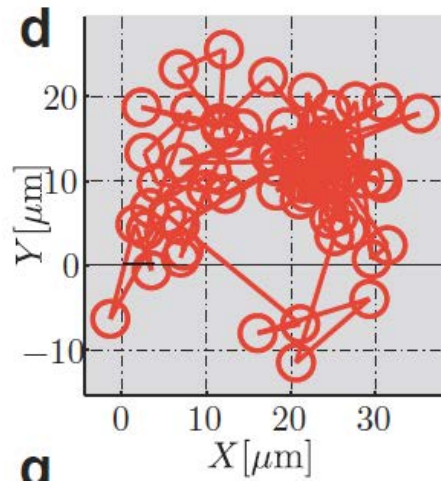
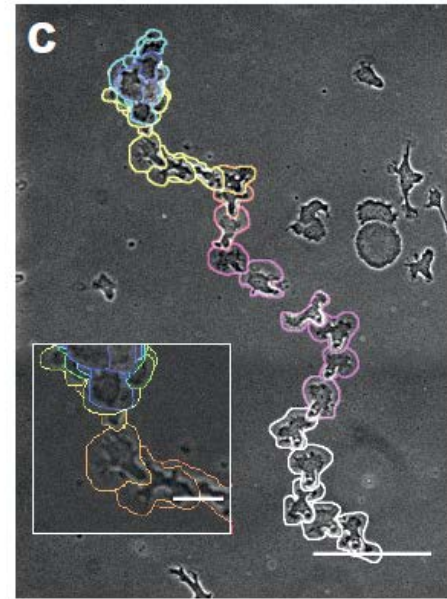
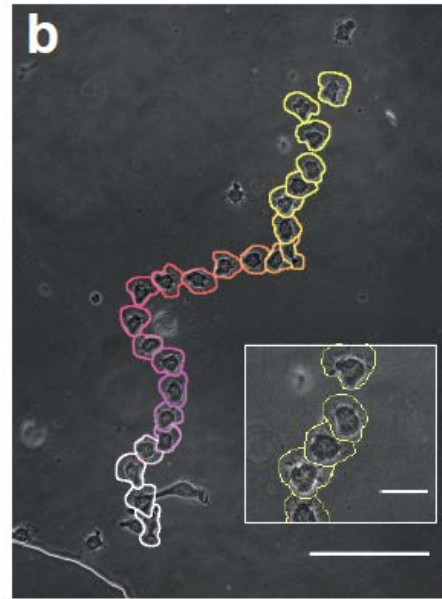
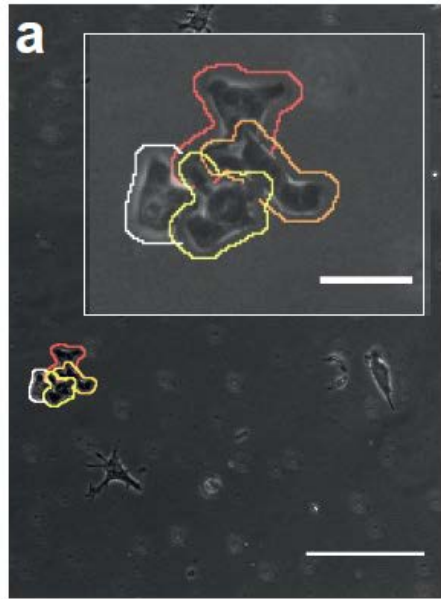
$$\beta > \beta_c(C_s)$$



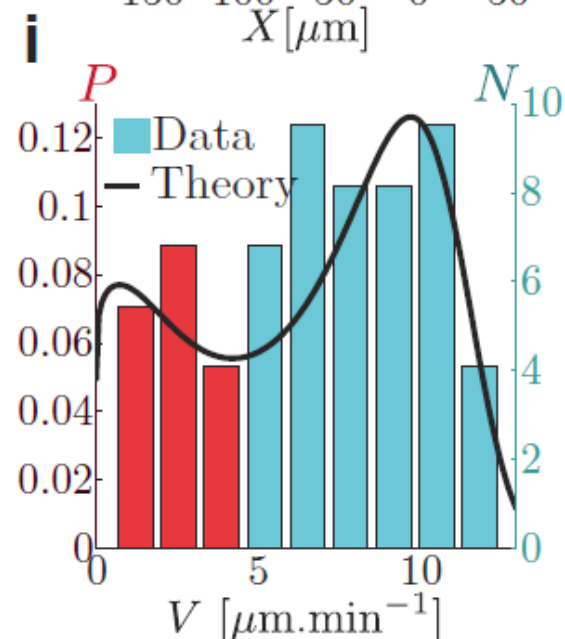
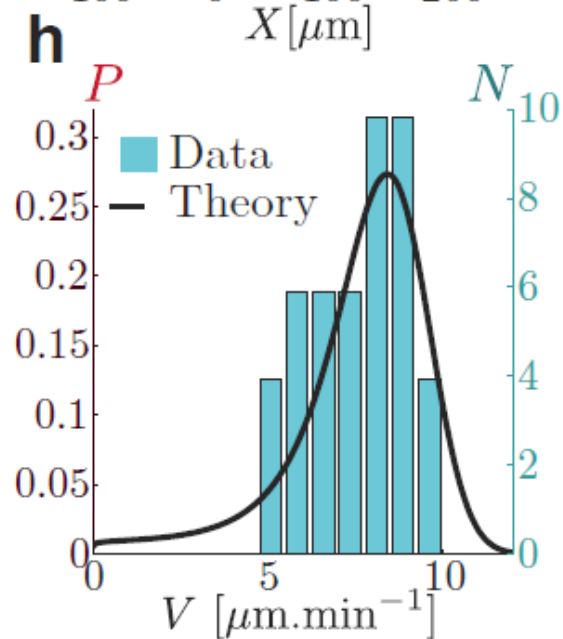
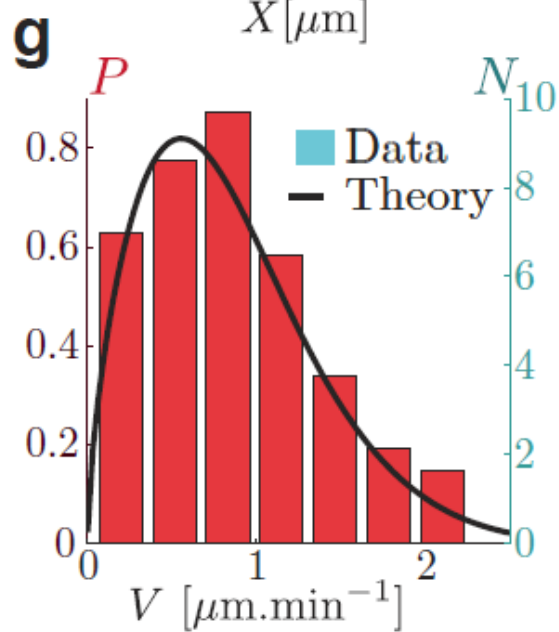
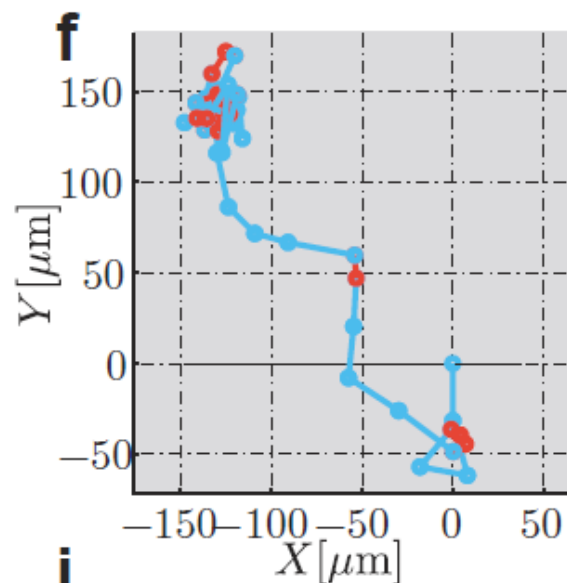
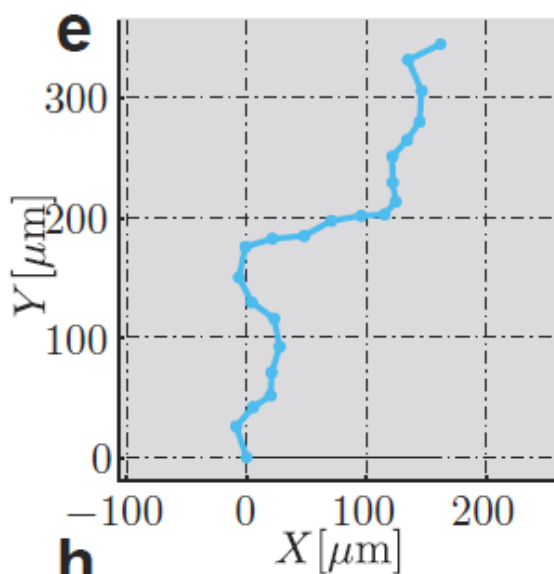
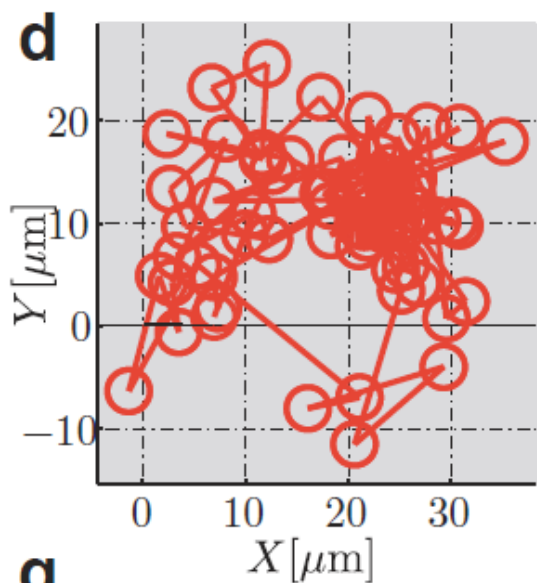
# Simulating trajectories from the model



# Real cell trajectories



# Model fit on real trajectories

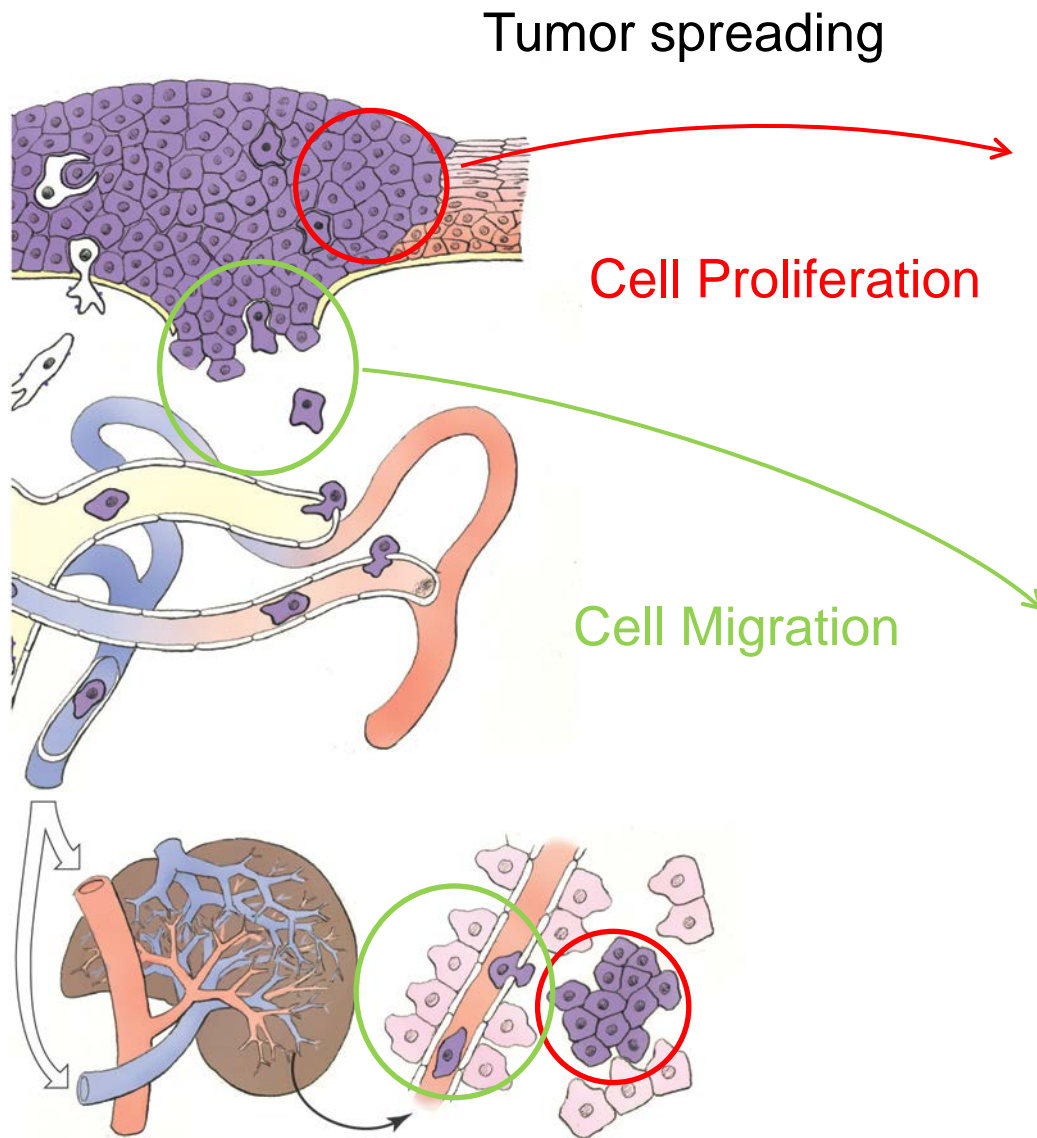


# Conclusions

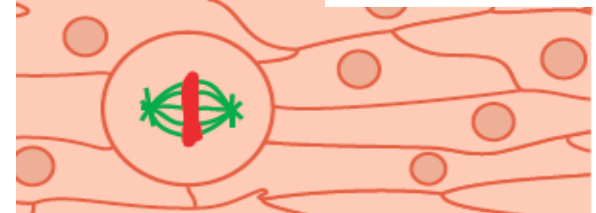
- 1) There is a universal correlation between persistence and speed of migrating cells (the first universal law of cell migrations?)
- 2) Experiments on BMDCs show that persistence is in fact correlated to retrograde actin flow
- 3) A model which assumes advective transport of a polarity factor by the actin flow can predict the exponential relation between persistence and speed
- 4) The same model, when varying parameters, can generate all observed cell trajectories
- 5) Extracting model parameters from real trajectories leads to self-consistent predictions



# Cells under Constrains



## Division under **constrains**

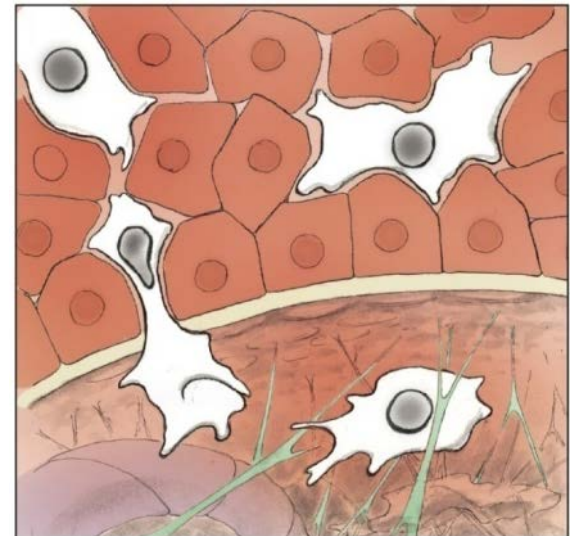


Fink, NCB, 2011

Lafaurie-Janvore, Science, 2013

Lancaster, Dev Cell, 2013

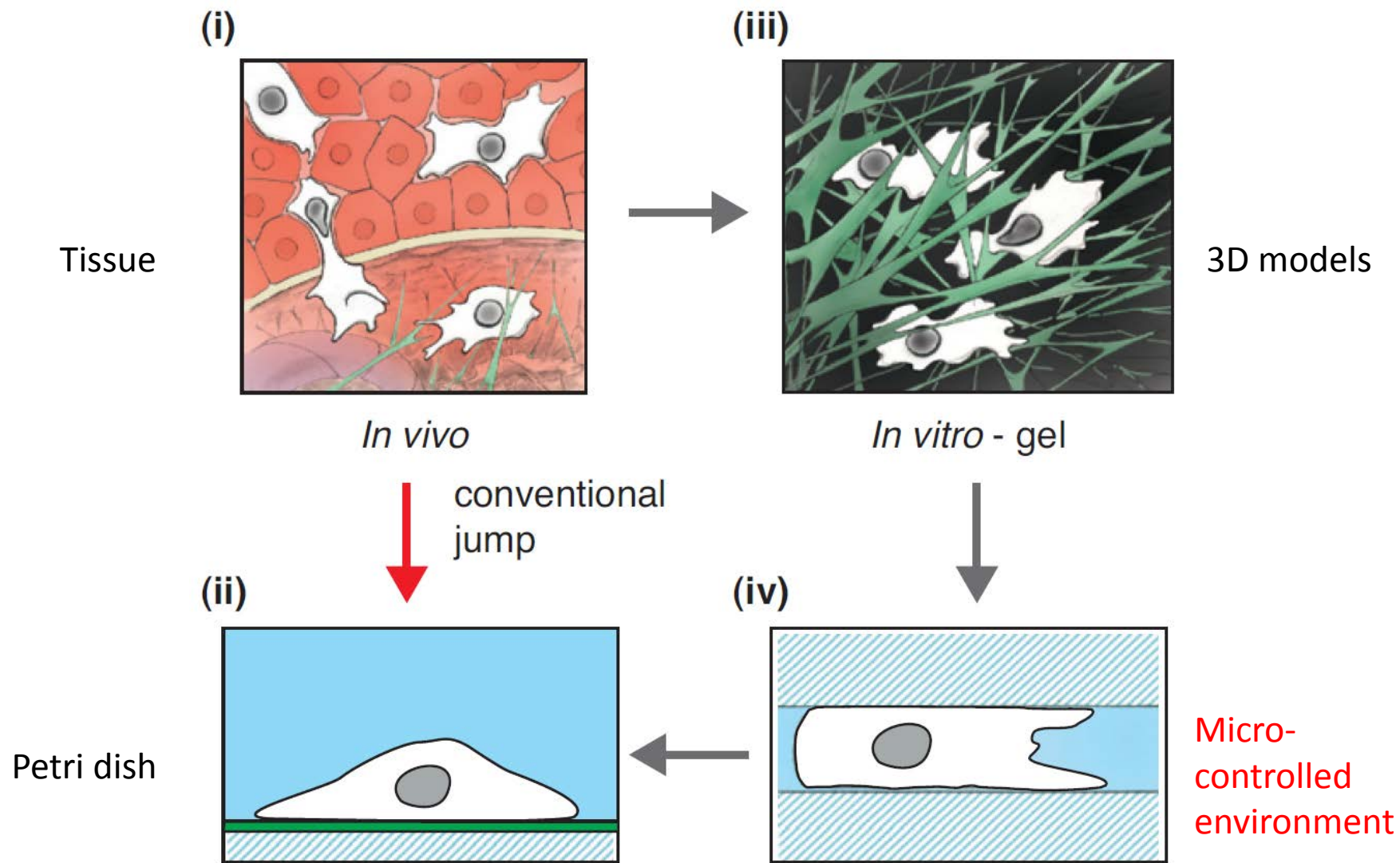
## Migration under **constrains**



Faure-André, Science, 2008

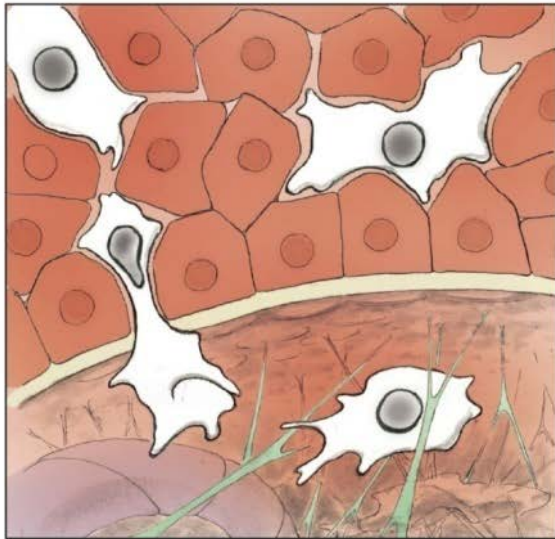
Hawkins, PRL, 2009

# Deconstructing the cell micro-environment

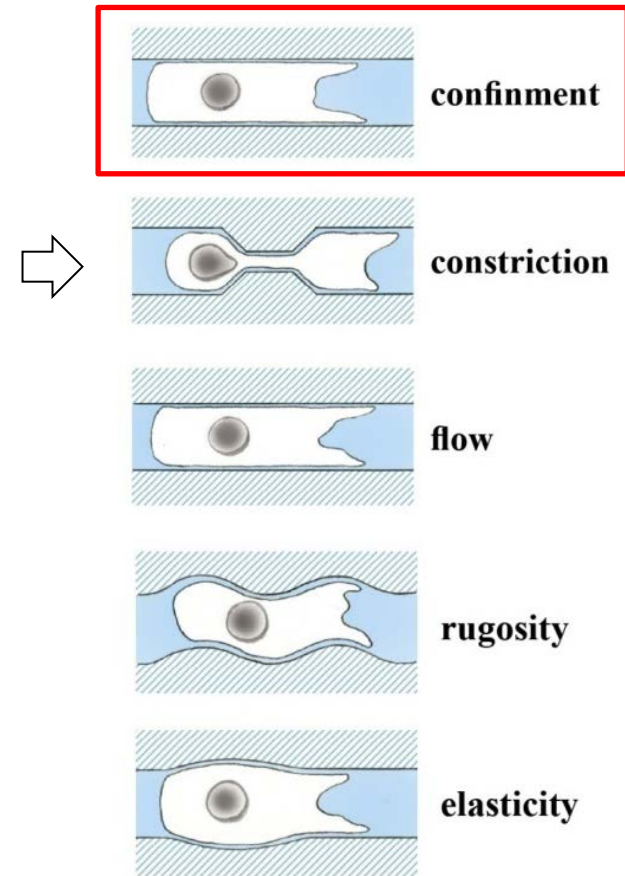


# A reductionist approach to 3D migration

a) *In vivo* interstitial migration



b) *In vitro* migration in 3D



Yanjun Liu



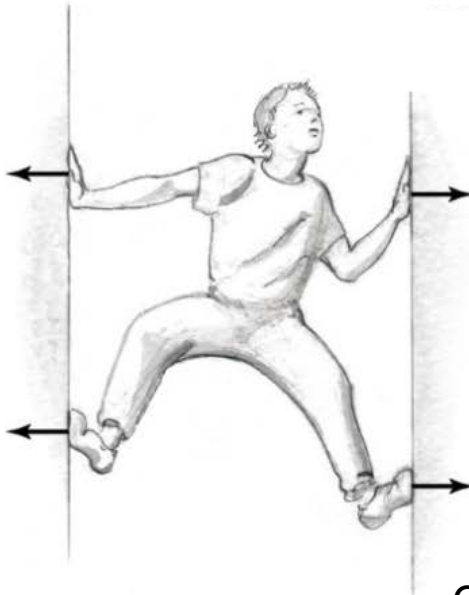
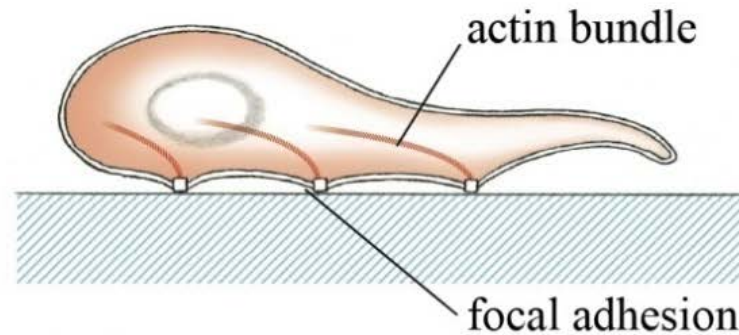
Maël Le Berre



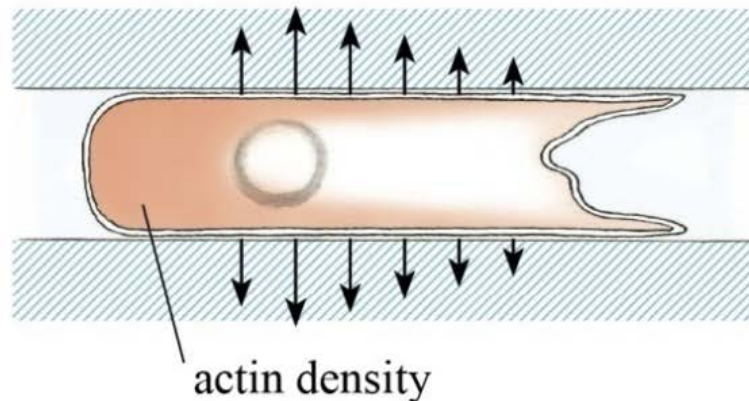
# Exerting force on the substrate: Momentum transfer under confinement



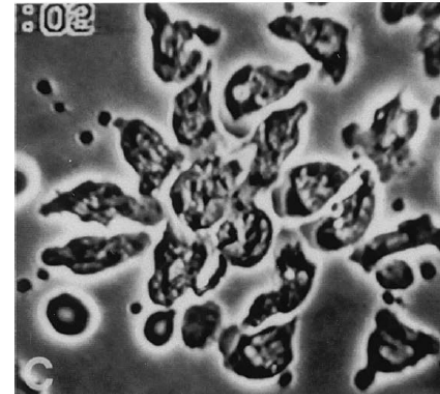
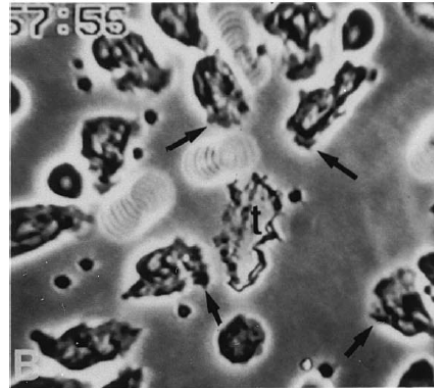
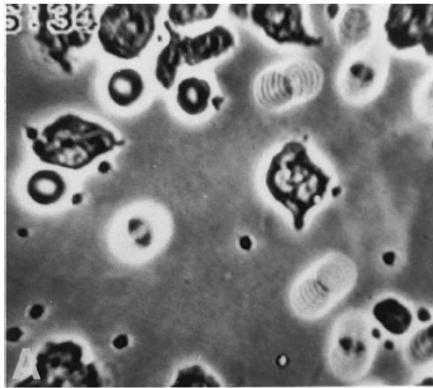
Adhesive: mesenchymal migration, protrusive, slow



Confined: amoeboid migration, contractile, fast

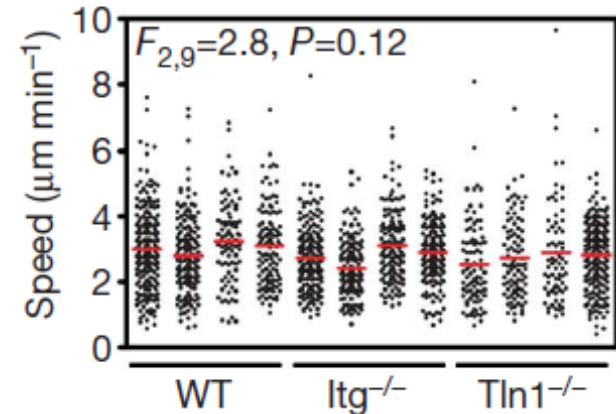
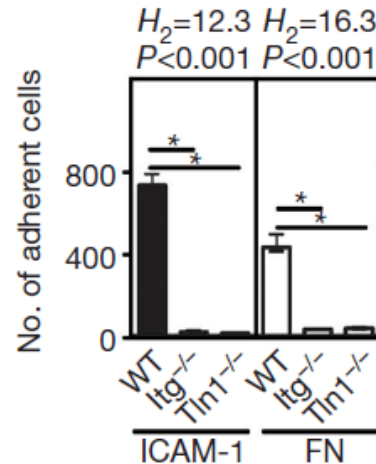
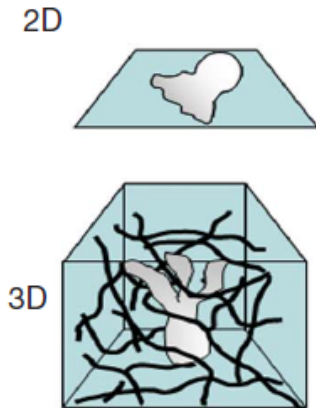


# Chemneying: moving without adhering from Malawista and de Boisfleury to Sixt



Thin preparation  
of PMN  
leukocytes  
treated with  
EDTA

Malawista and de Boisfleury, PNAS 1997; Malawista et al. CMC 2000

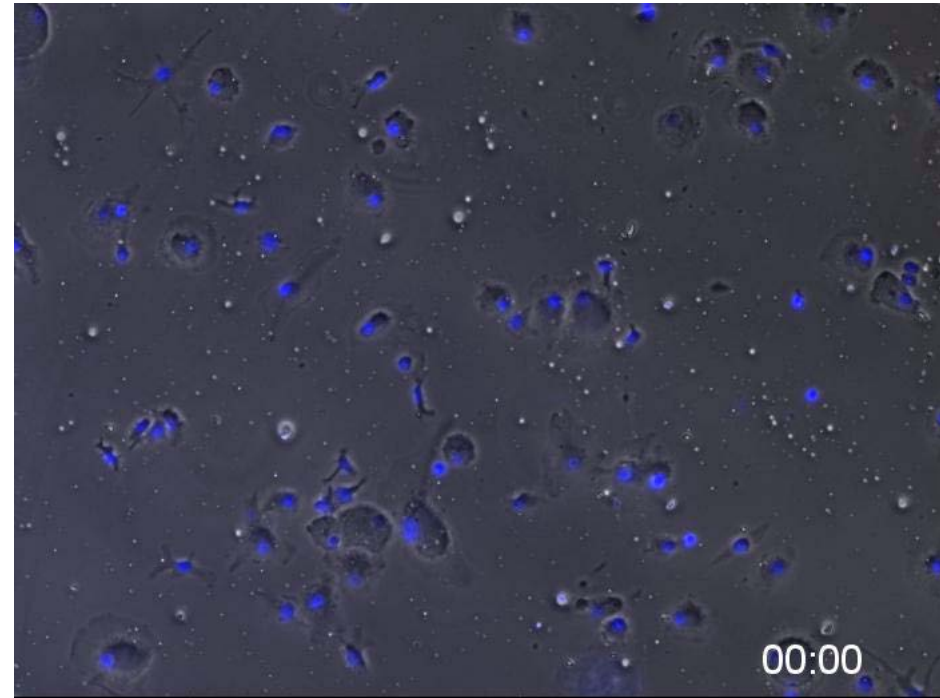
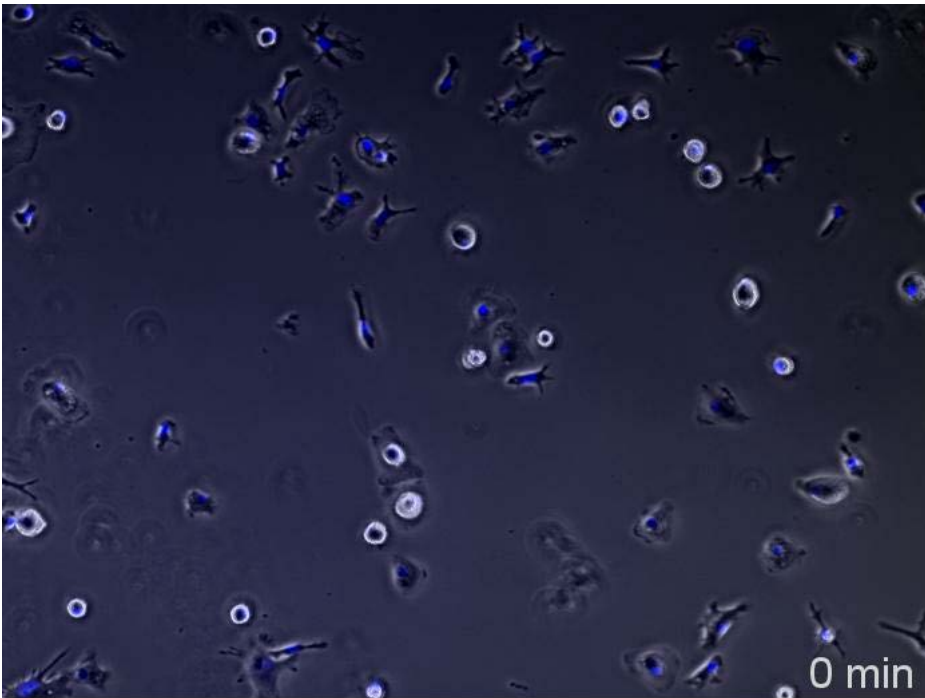


Lämmermann et al., Nature 2008

# Leukocytes need to be confined to reveal their migratory potential

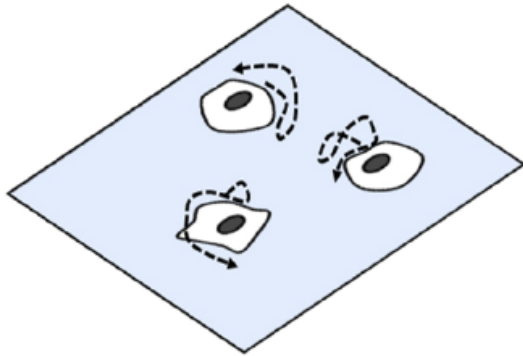
No confinement

5  $\mu\text{m}$  confinement

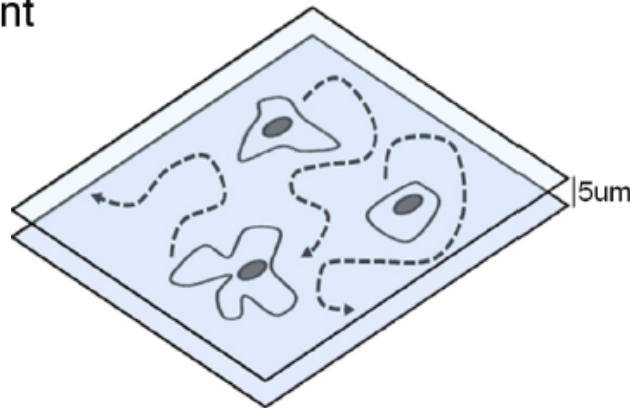


BMDCs migrating on FN coated glass surface

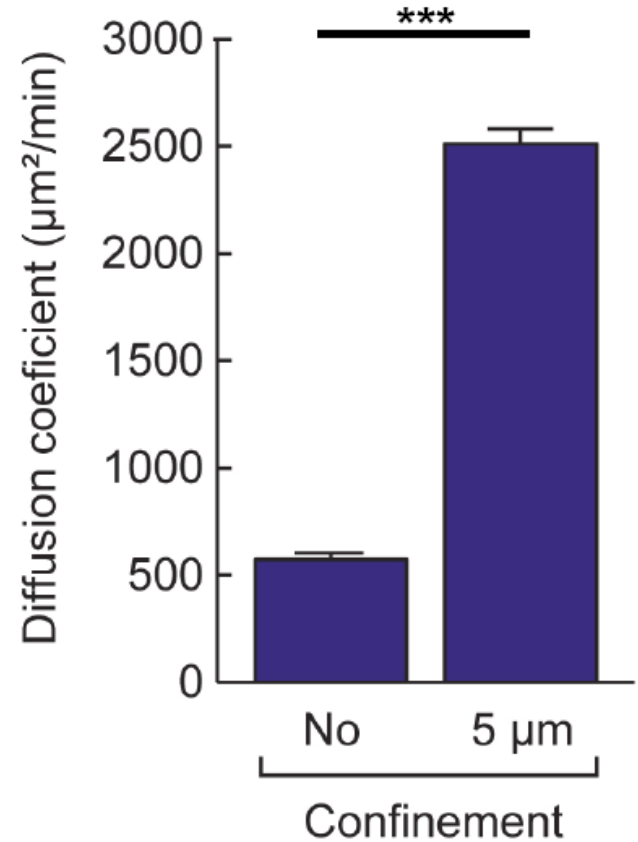
# Leukocytes need to be confined to reveal their migratory potential



No confinement

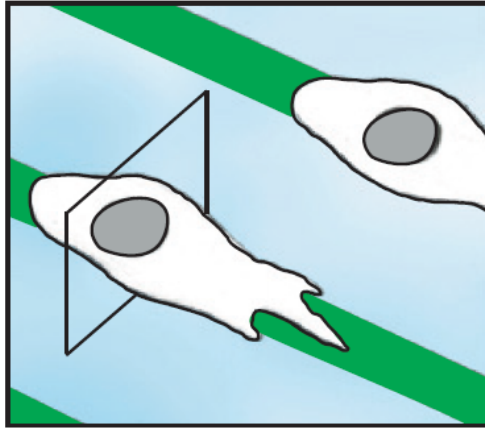


5µm 2D confinement



# Micro-patterns and micro-channels: two simple tools with multiple applications

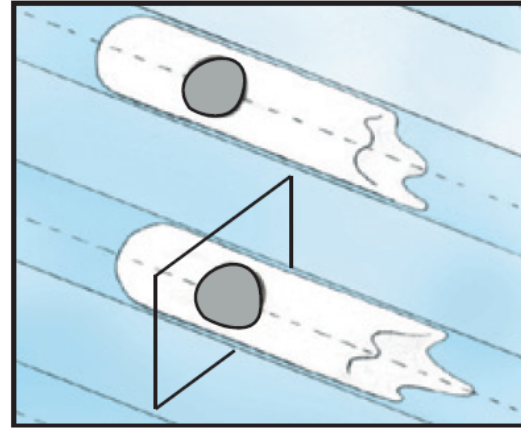
(i)



Control of  
cell adhesion

micropatterned linetracks

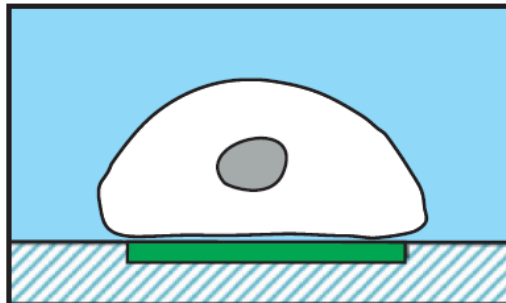
(iii)



Control of  
cell confinement

inside microchannels

(ii)

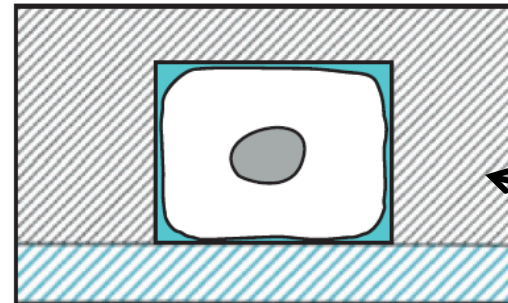


Culture medium

Fibronectine

Coverslip →

(iv)



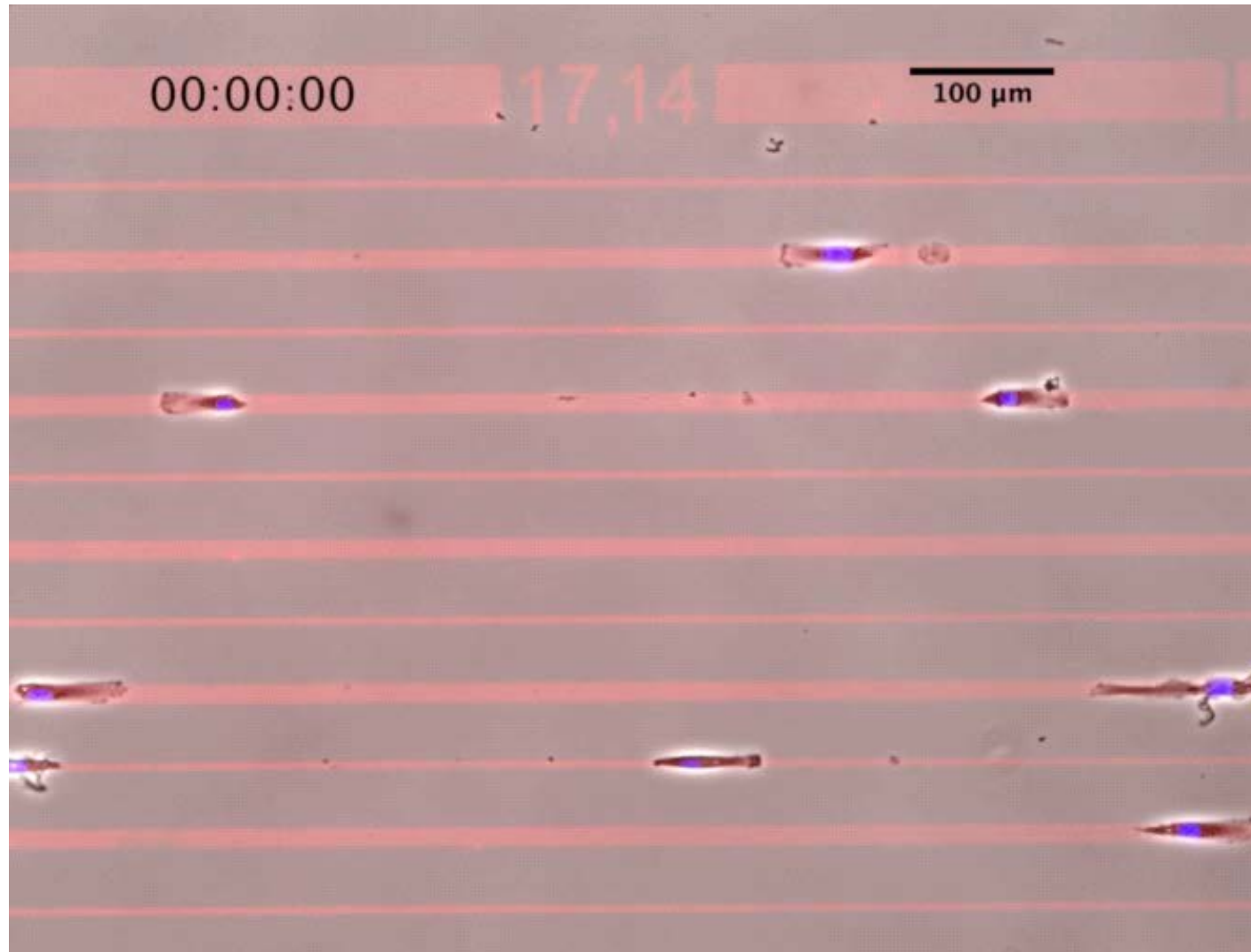
Culture medium

← Confining material  
(PDMS, hydrogel)

← Coverslip



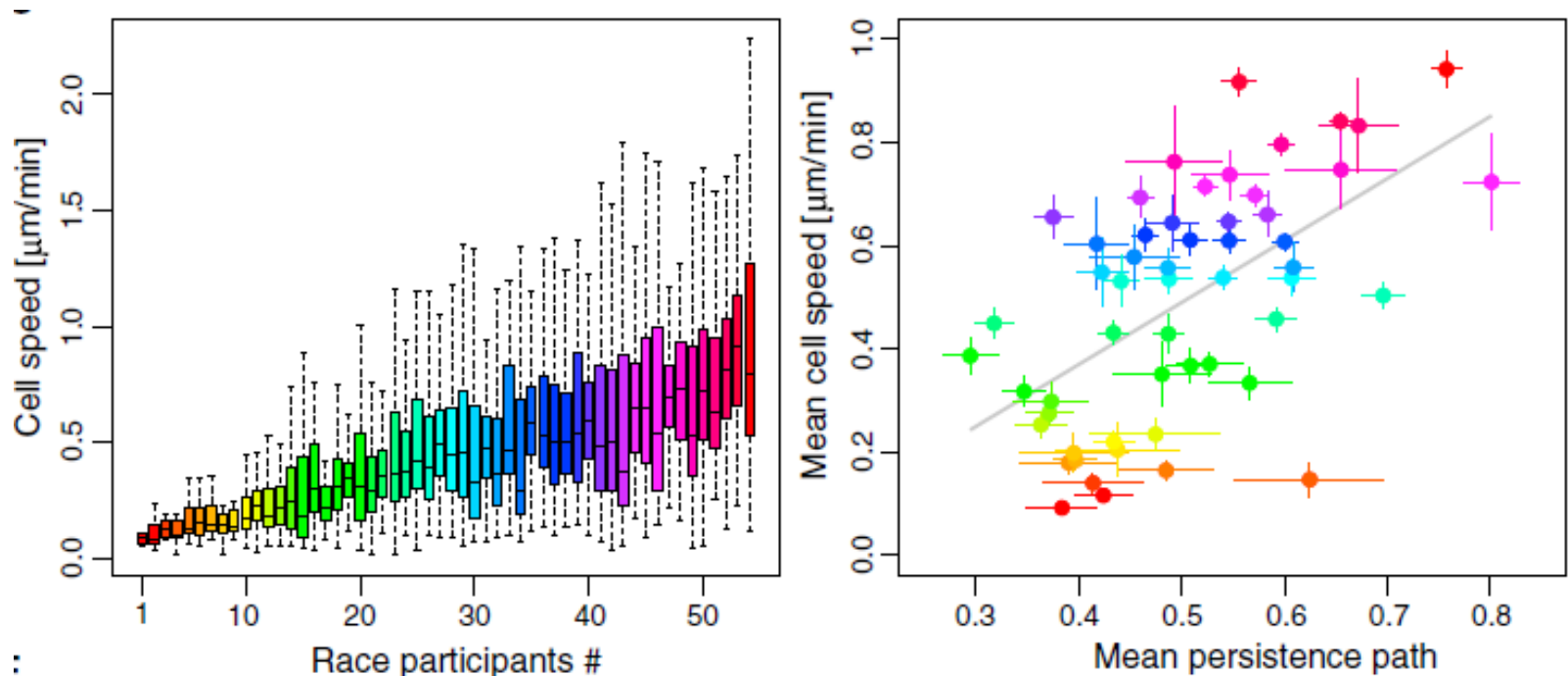
# Micropatterns for migrating cells: the First World Cell Race (2011)



Movie: T. Vignault

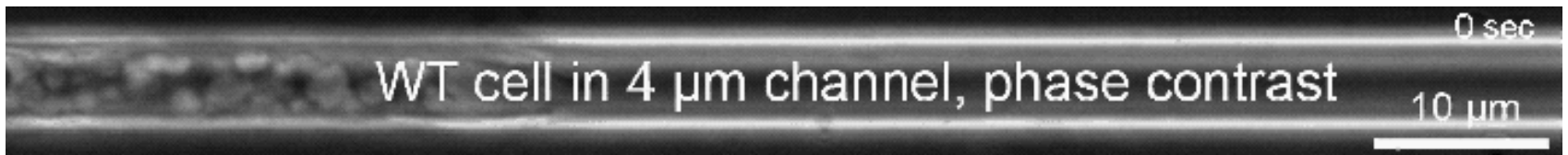
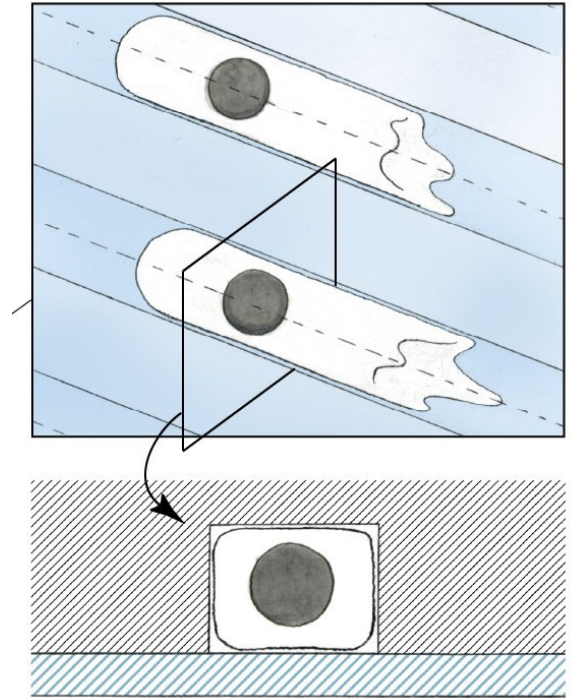
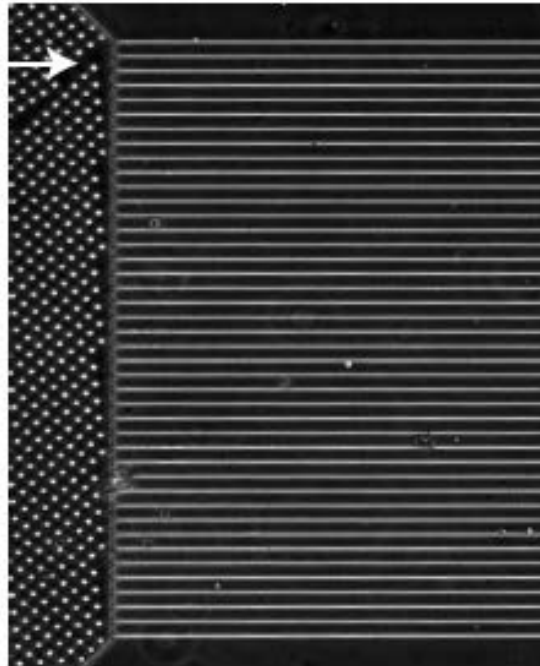
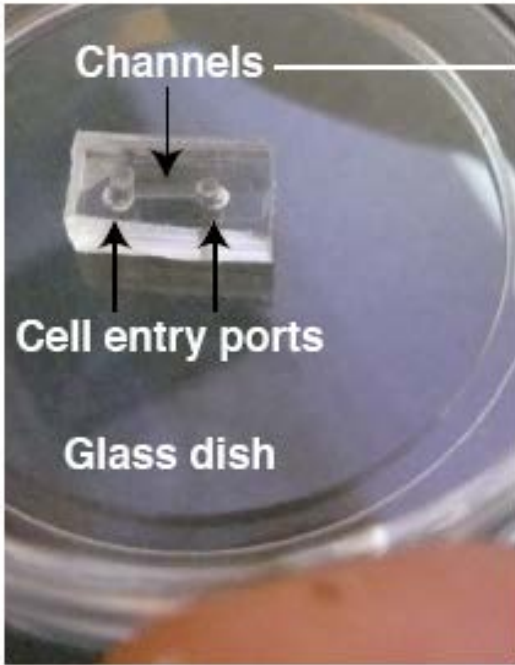
(see also Pouthas et al., JCS 2008 and Doyles et al., JCB 2009)

# Faster cells are also more persistent, revealing a coupling between the motor and the steering wheel



|    |                  |    |                |    |                |    |                |    |                |
|----|------------------|----|----------------|----|----------------|----|----------------|----|----------------|
| 1  | h.Bre.E.Car.1    | 12 | h.Skin.E.Car.2 | 23 | h.Skin.E.Mel.2 | 34 | m.Emb.C.Tra.3  | 45 | m.SGI.E.Sar    |
| 2  | r.Emb.M.Tra      | 13 | h.Skin.E.Car.1 | 24 | h.Bre.E.Fib.5  | 35 | h.Bre.E.Ad.c.7 | 46 | m.Emb.C.Tra.2  |
| 3  | m.Mus.M.Tra      | 14 | h.Skin.E.Mel.1 | 25 | h.Kid.E.Ad.c   | 36 | h.Bre.E.Ad.c.5 | 47 | h.Skin.C.Tra   |
| 4  | m.Emb.C.Tra.8    | 15 | h.Pro.C.Ad.c   | 26 | h.Bre.E.Fib.4  | 37 | h.Skin.E.Pri   | 48 | m.Emb.C.Tra.1  |
| 5  | h.Bre.E.Car.2    | 16 | h.Cer.E.Car    | 27 | m.Bre.E.Ad.c.2 | 38 | h.Bre.E.Ad.c.8 | 49 | m.Bre.E.Ad.c.4 |
| 6  | h.Bre.E.Car.3    | 17 | m.Bre.E.Ad.c.3 | 28 | h.Bla.E.Car    | 39 | m.Bre.E.Ad.c.1 | 50 | h.Bre.E.Fib.3  |
| 7  | h.LyNo.Epi.Mel.2 | 18 | h.Skin.E.Mel   | 29 | m.Hip.N.Tra.1  | 40 | h.Bre.E.Ad.c.3 | 51 | m.Emb.C.Tra.5  |
| 8  | m.Emb.C.Tra.7    | 19 | h.Bre.E.Ad.c.1 | 30 | m.Emb.C.Tra.4  | 41 | h.Skin.E.Tra.2 | 52 | h.Bre.E.Fib.2  |
| 9  | h.Bre.E.Car.4    | 20 | h.Skin.E.Tra.1 | 31 | h.Bre.E.Ad.c.4 | 42 | m.Hip.N.Tra.2  | 53 | h.Bre.E.Ad.c.2 |
| 10 | h.LyNo.E.Mel.1   | 21 | h.Col.E.Ad.c.2 | 32 | h.BM.C.SC      | 43 | h.Bre.E.Fib.6  | 54 | h.Emb.C.Pri    |
| 11 | h.Bre.E.Fib.1    | 22 | h.Col.E.Ad.c.1 | 33 | h.Alv.M.Rha    | 44 | h.Bre.E.Ad.c.6 |    |                |

# A model system for migration in a confined space: microchannels with sub-cellular dimension



Mice bone marrow derived dendritic cell (BMDC), phase contrast

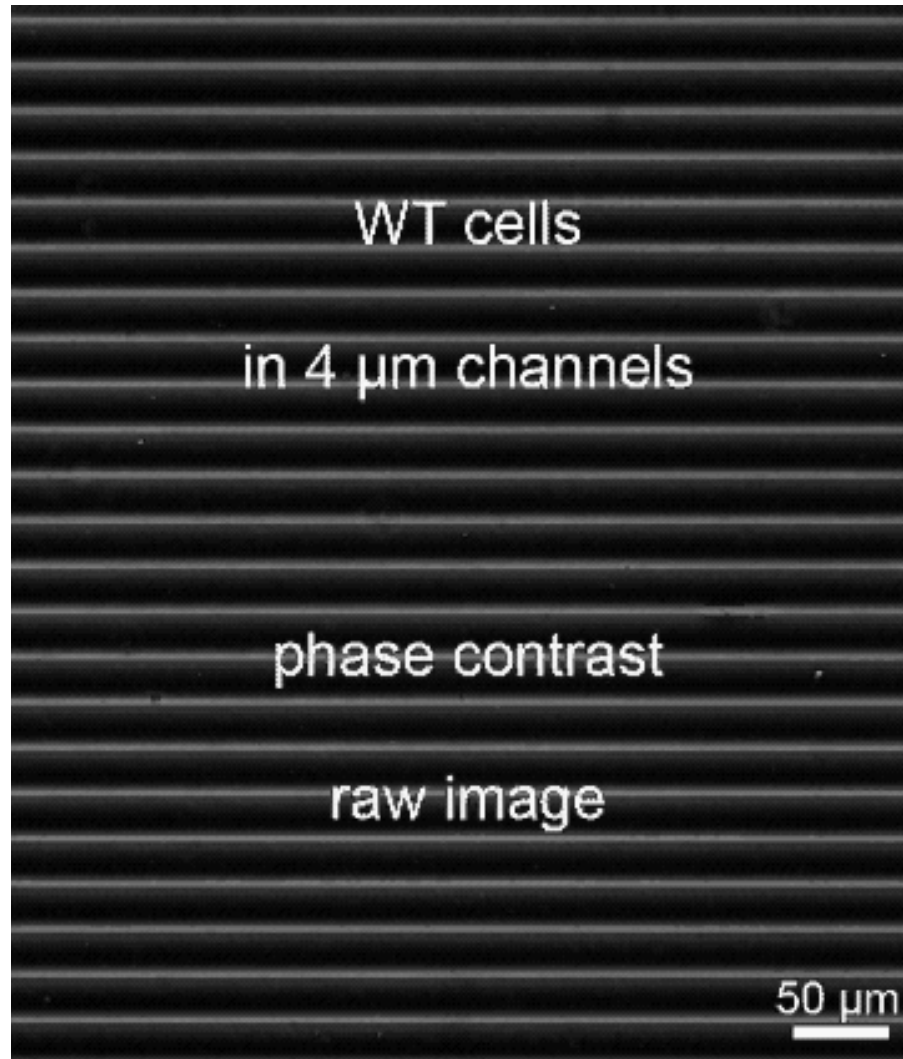
Faure-André, Science, 2008

Hawkins, PRL 2009

Heuze, Meth MB 2011

(also : Jacobelli, Nat Immunol 2010, Fernandez, Blood 2011, Moreau, Immunity 2012)

# Microchannels for migrating amoeboid cells: Another race for immune cells?

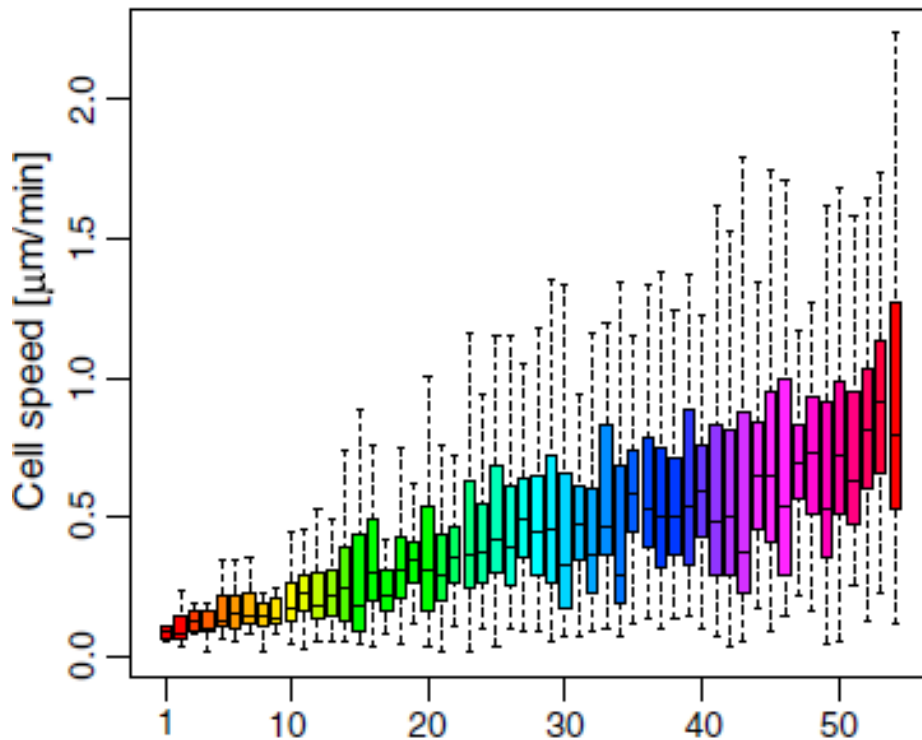


Bone marrow derived dendritic cells from mice (BMDCs)

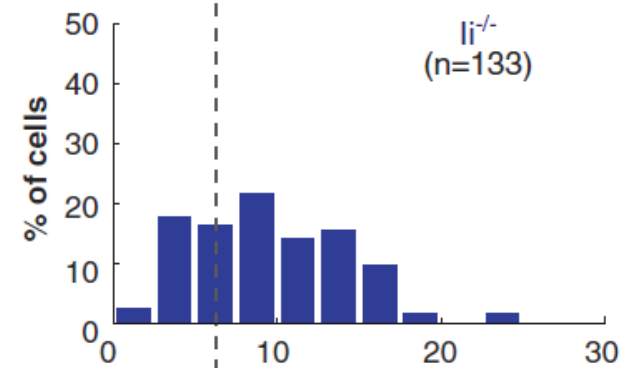
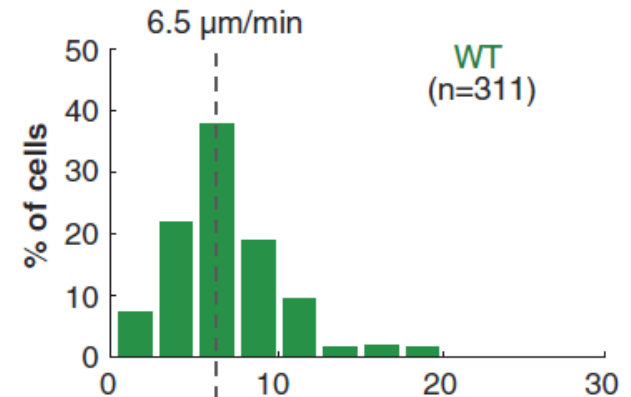
# Amoeboid cells (here BMDCs) in side channels are faster than mesenchymal cells on adhesive lines although they do not move on adhesive lines

WCR cells on lines: **0.1 to 1  $\mu\text{m}/\text{min}$**

BMDCS in channels: **1 to 20  $\mu\text{m}/\text{min}$**



|                    |                   |                   |                   |                  |
|--------------------|-------------------|-------------------|-------------------|------------------|
| 1 h.Bre.E.Car.1    | 12 h.Skin.E.Car.2 | 23 h.Skin.E.Mel.2 | 34 m.Emb.C.Tra.3  | 45 m.SGI.E.Sar   |
| 2 r.Emb.M.Tra      | 13 h.Skin.E.Car.1 | 24 h.Bre.E.Fib.5  | 35 h.Bre.E.Adc.7  | 46 m.Emb.C.Tra.2 |
| 3 m.Mus.M.Tra      | 14 h.Skin.E.Mel.1 | 25 h.Kid.E.Adc    | 36 h.Bre.E.Adc.5  | 47 h.Skin.C.Tra  |
| 4 m.Emb.C.Tra.8    | 15 h.Pro.C.Adc    | 26 h.Bre.E.Fib.4  | 37 h.Skin.E.Pri   | 48 m.Emb.C.Tra.1 |
| 5 h.Bre.E.Car.2    | 16 h.Cer.E.Car    | 27 m.Bre.E.Adc.2  | 38 h.Bre.E.Adc.8  | 49 m.Bre.E.Adc.4 |
| 6 h.Bre.E.Car.3    | 17 m.Bre.E.Adc.3  | 28 h.Bla.E.Car    | 39 m.Bre.E.Adc.1  | 50 h.Bre.E.Fib.3 |
| 7 h.LyNo.Epi.Mel.2 | 18 h.Skin.E.Mel   | 29 m.Hip.N.Tra.1  | 40 h.Bre.E.Adc.3  | 51 m.Emb.C.Tra.5 |
| 8 m.Emb.C.Tra.7    | 19 h.Bre.E.Adc.1  | 30 m.Emb.C.Tra.4  | 41 h.Skin.E.Tra.2 | 52 h.Bre.E.Fib.2 |
| 9 h.Bre.E.Car.4    | 20 h.Skin.E.Tra.1 | 31 h.Bre.E.Adc.4  | 42 m.Hip.N.Tra.2  | 53 h.Bre.E.Adc.2 |
| 10 h.LyNo.E.Mel.1  | 21 h.Col.E.Adc.2  | 32 h.BM.C.SC      | 43 h.Bre.E.Fib.6  | 54 h.Emb.C.Pri   |
| 11 h.Bre.E.Fib.1   | 22 h.Col.E.Adc.1  | 33 h.Alv.M.Rha    | 44 h.Bre.E.Adc.6  |                  |



Faure-André et al., Science 2008

# Amoeboid versus mesenchymal

## Mesenchymal

Proteolysis dependent, high MMP activity

Low Rho-ROCK activity

Elongated morphology with lamellipodial protrusions

Strong attachment to ECM via focal adhesions

## Amoeboid

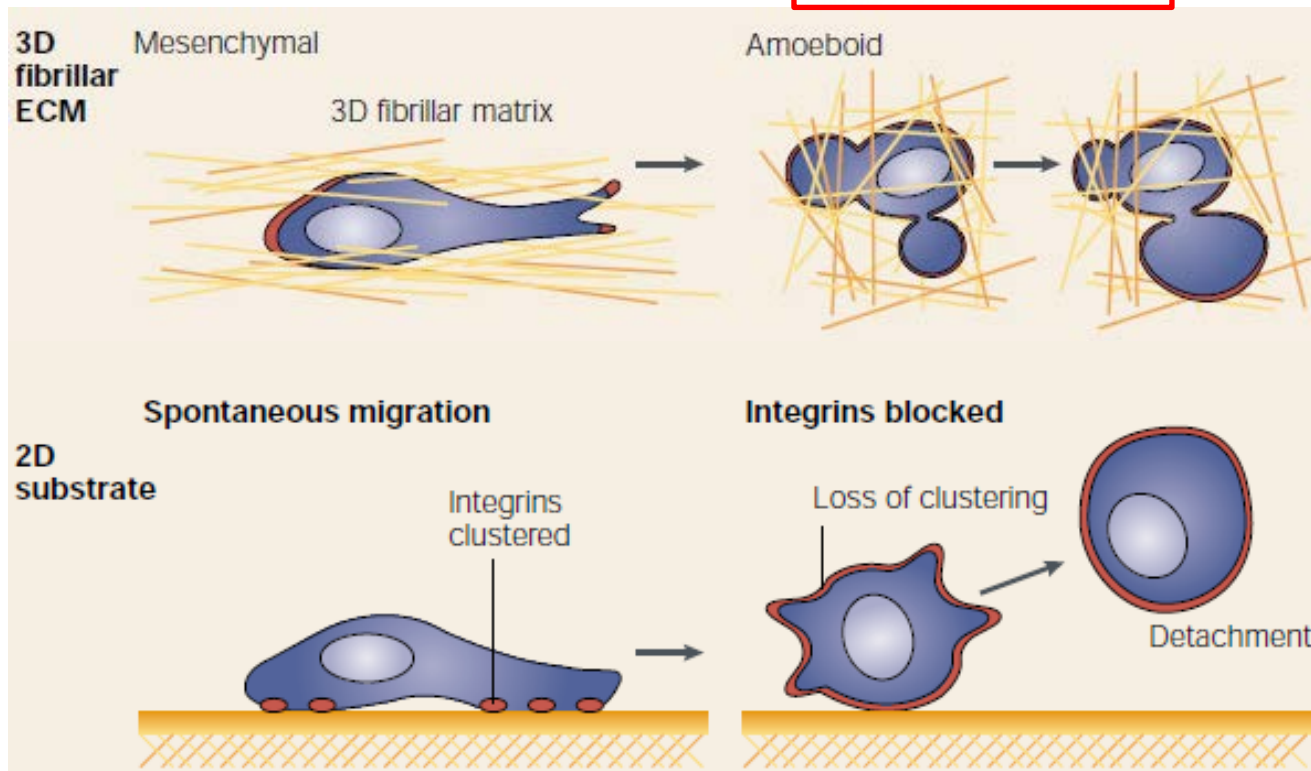
Proteolysis independent, low MMP activity

High Rho-ROCK activity

Rounded morphology with membrane blebbing

High cellular contractility and cortical tension

Weak attachment to ECM



# Elongated to Rounded Transition

Summary on Mesenchymal versus Amoeboid:

(see Sanz-Moreno and Marshall, COCB, 2010)

Elongated morphology/mesenchymal migration is favored by

- strong adhesion
- high protrusive activity (Rac1/Wave2/Arp2/3)

Rounded morphology/amoeboid migration is favored by

- low adhesion
- strong contractility (Rho1/ROCK/Formins/Myosin II)

Important point:

- On **2D** substrates, both elongated and rounded migration are **SLOW**
- Inside **3D** gels or in vivo, elongated cells are still **SLOW** (< 1µm/min) and rounded cells are **FAST** (>1 µm/min)

**WHY? CONFINEMENT!!**

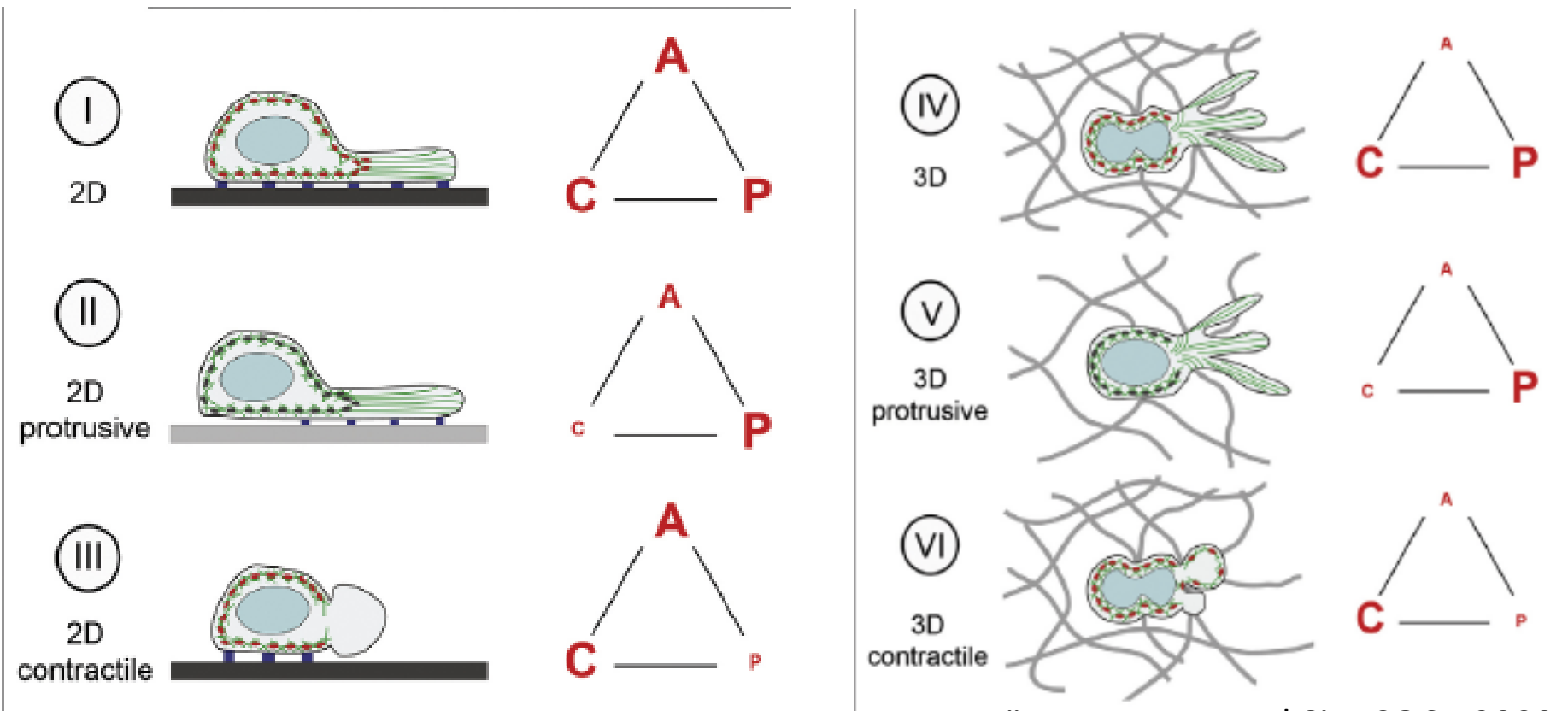
(read Totzluoglu et al. NCB 2013, E. Sahai Lab)

# Migration under confinement: To adhere or not to adhere?

How cells exert force on their surrounding to move?

## Mesenchymal

## Amoeboid

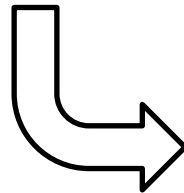
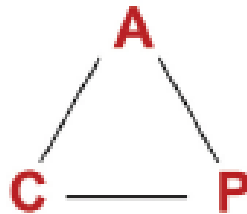
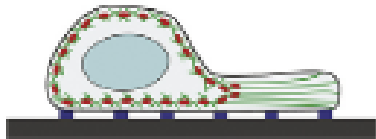




Is it possible, by playing  
on adhesion, confinement, and  
contractility  
to induce mesenchymal cells  
to move like leukocytes?

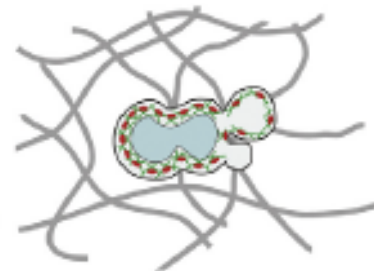
I

2D

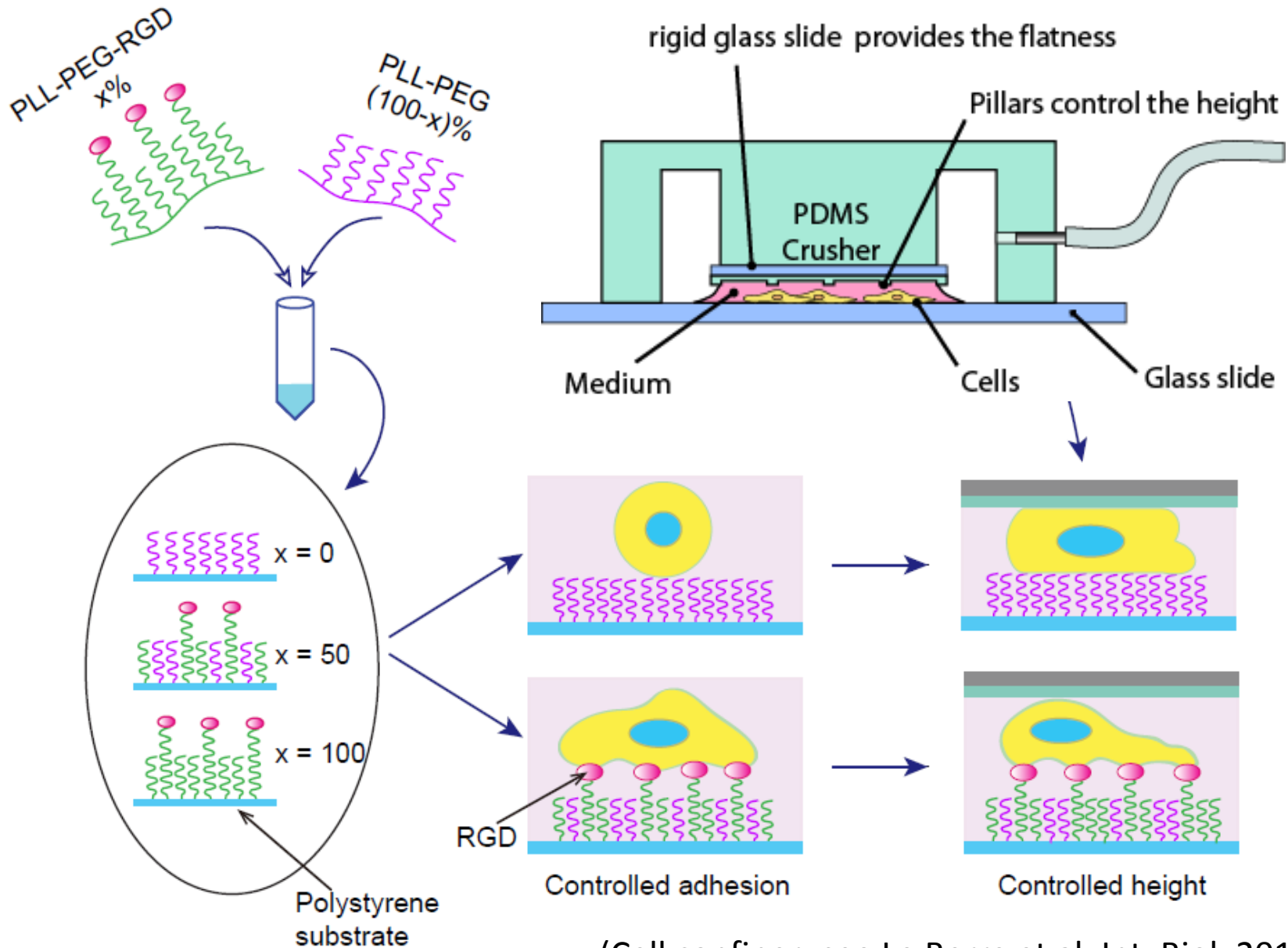


VI

3D  
contractile

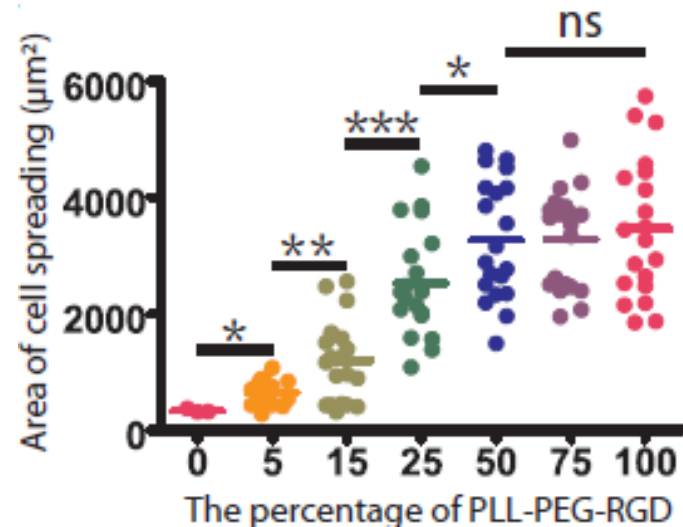
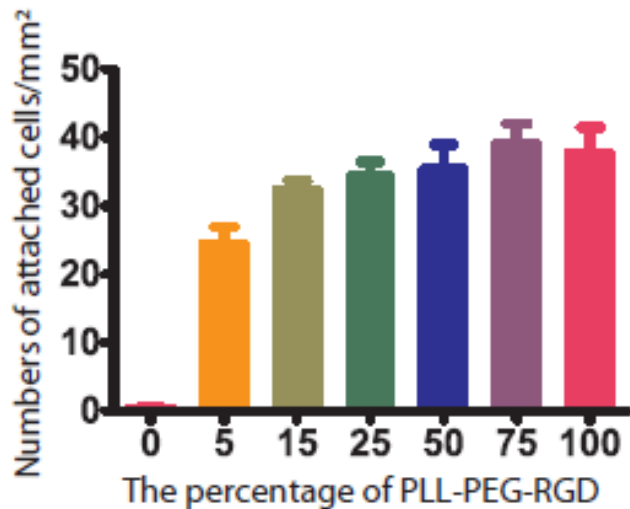
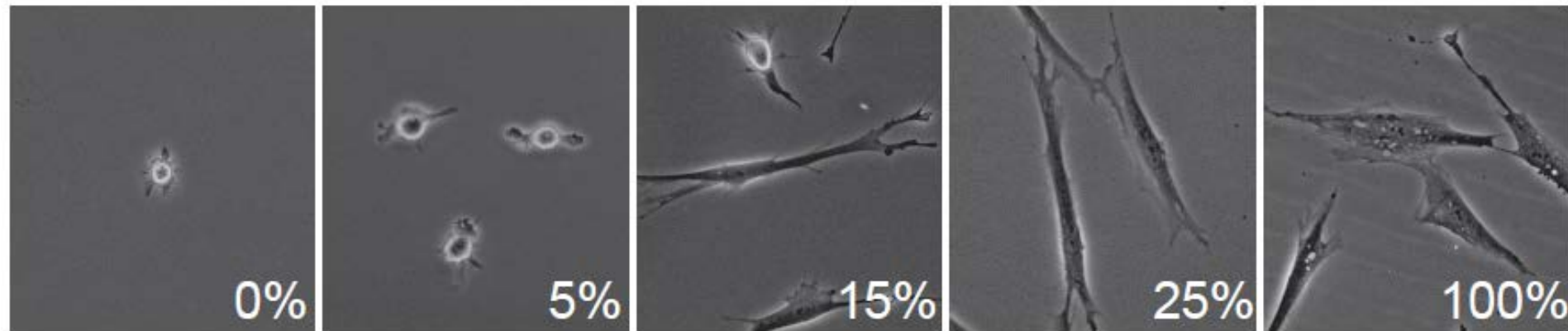


# Quantitative control of confinement and adhesion

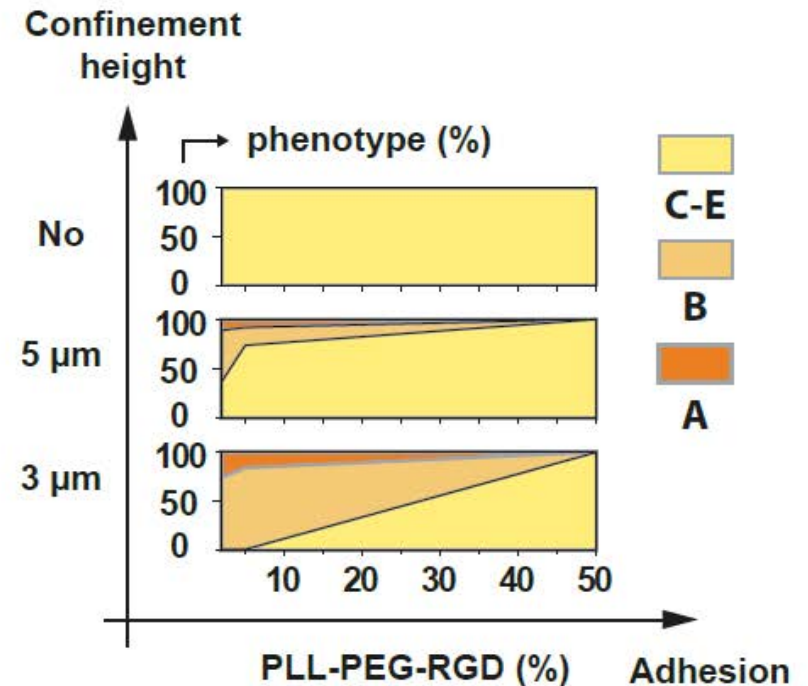
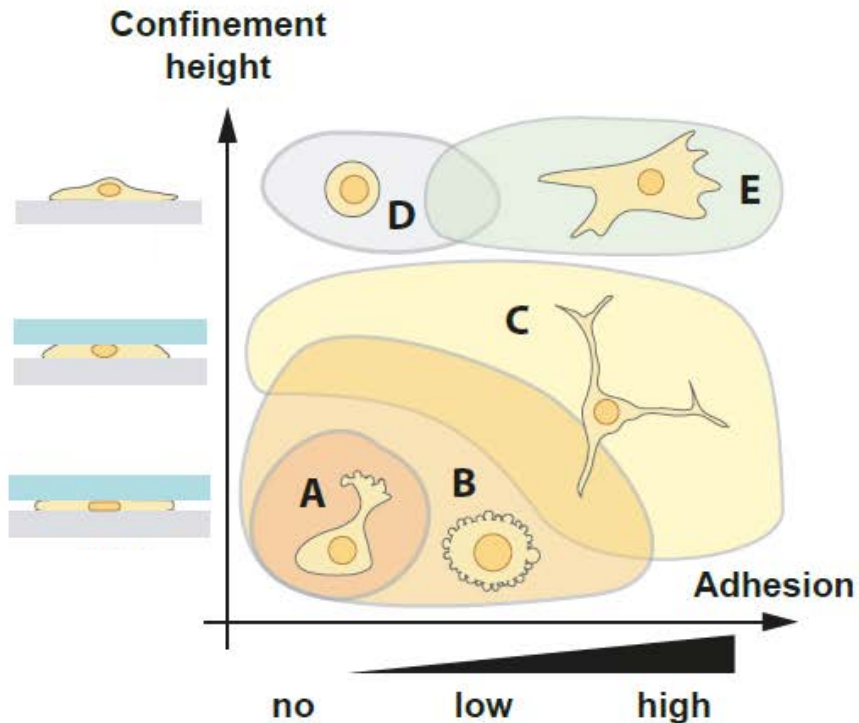
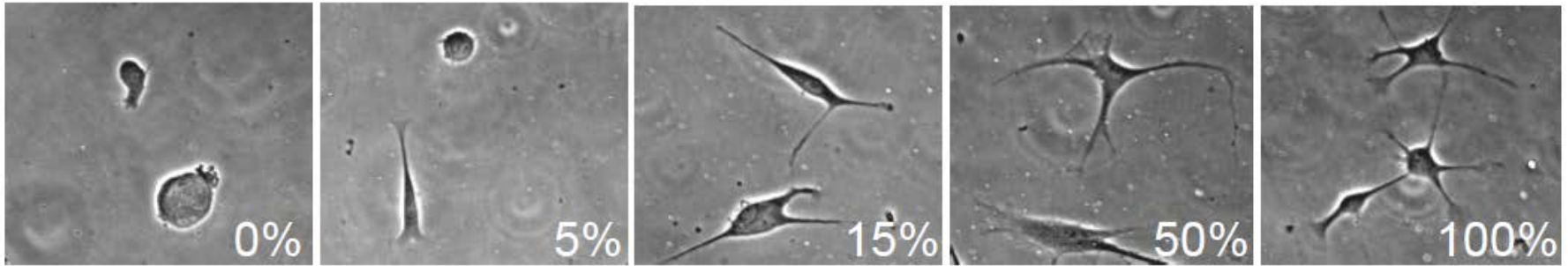
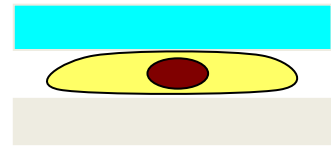


(Cell confiner: see Le Berre et al. Int. Biol. 2012)

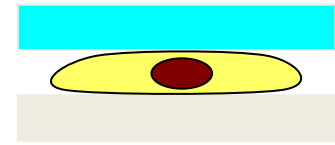
# Primary Fibroblasts (NHDFs) on PLL-g-PEG-RGD/PII-g-PEG surfaces



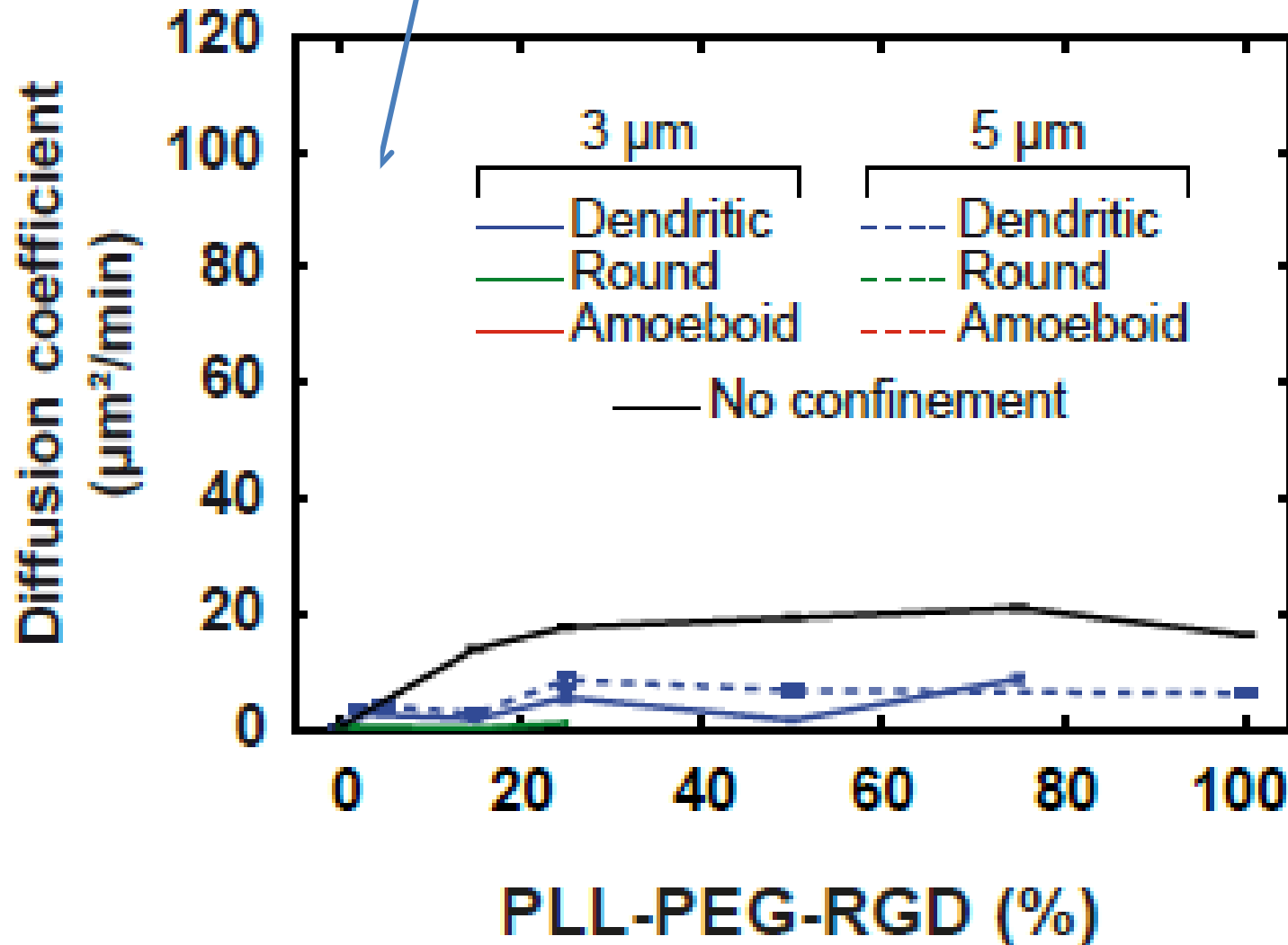
# NHDF morphology under confinement



# NHDF migration under confinement



< 5% RGD: cells cannot bind, but move fast!

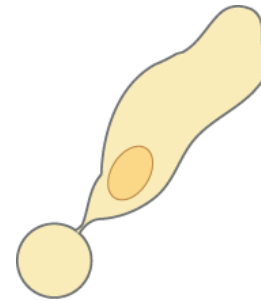


# Is it truly a general phenomenon?

Cell lines assayed: NHDFs, HT29, RPE1, 3T3, MDA-MB231, HEK, HeLa

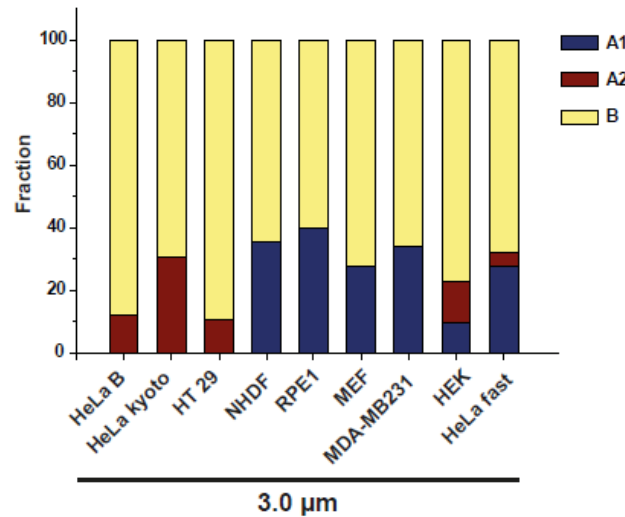
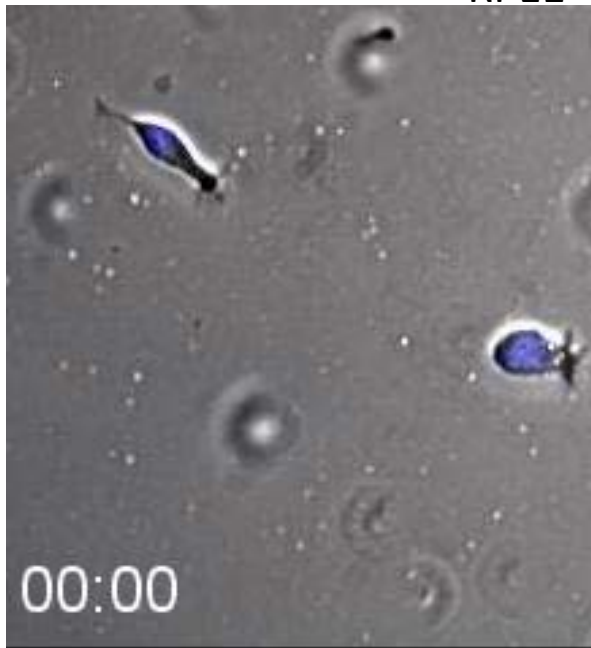


Amoeboid 1 (A1)

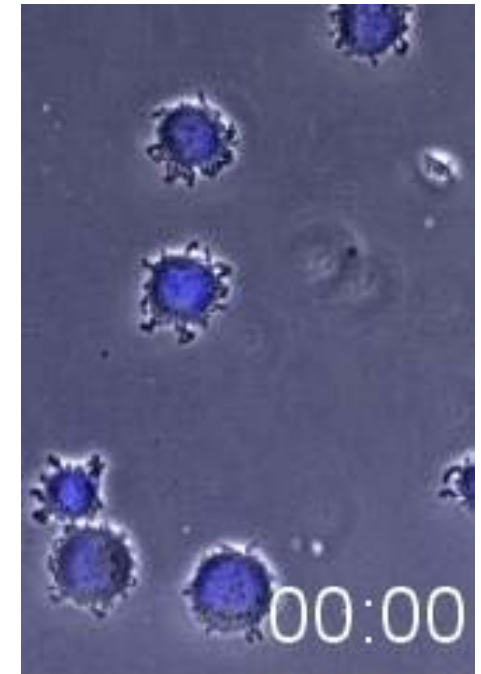


Amoeboid 2 (A2)

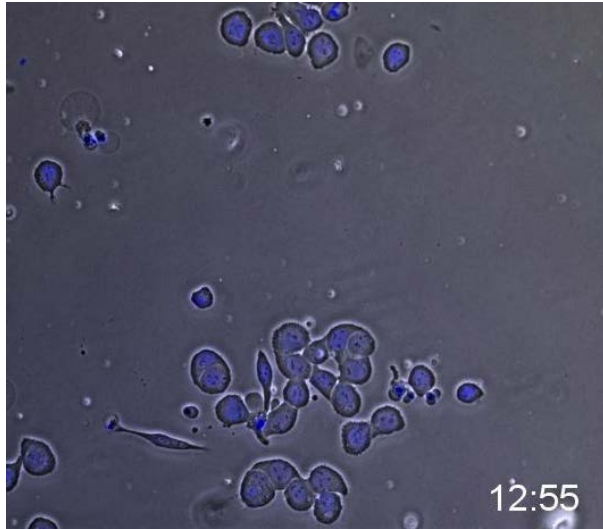
RPE1



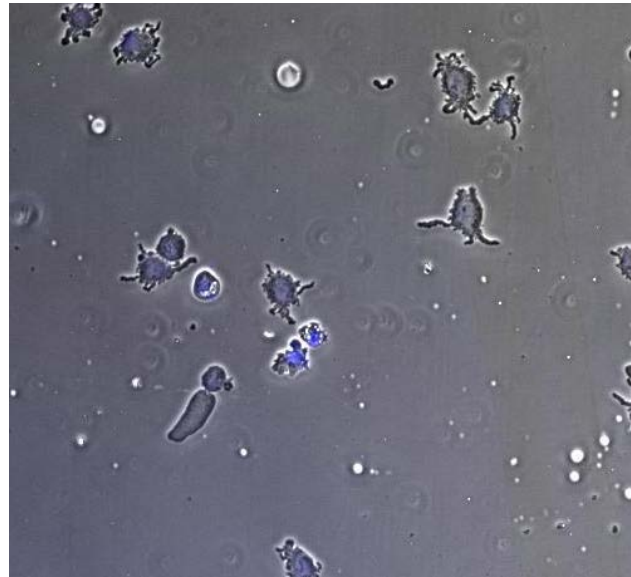
HeLa



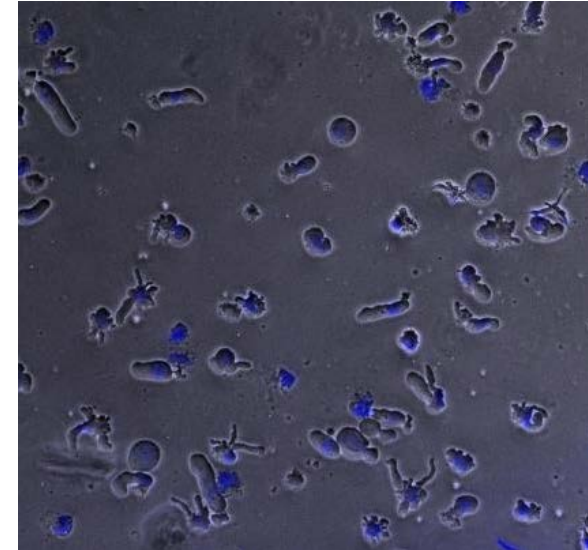
# Fraction of A2 increases with contractility, while inhibiting contractility increases A1



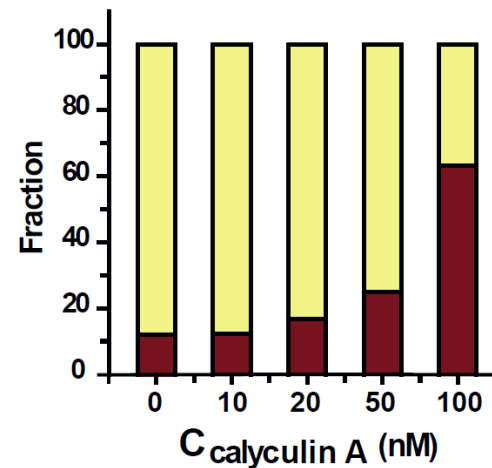
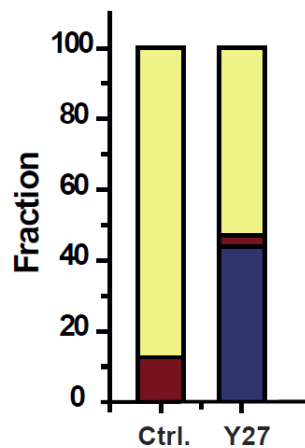
Y27632



Control



Calyculin A



# Turning non motile HeLa cells into fast neutrophil-like cells by purely physical means

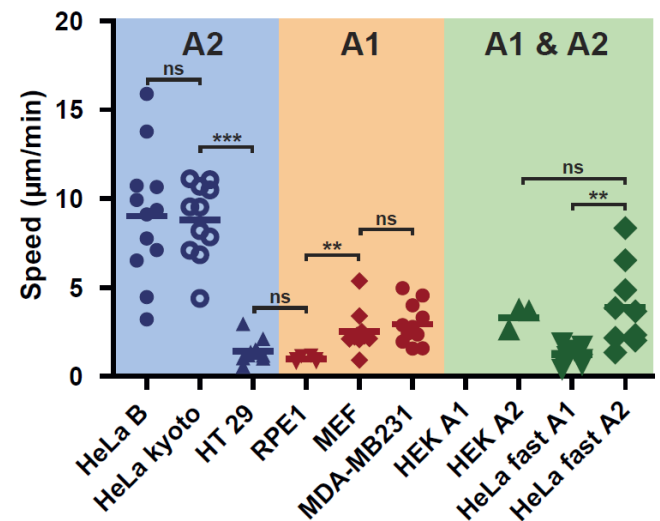
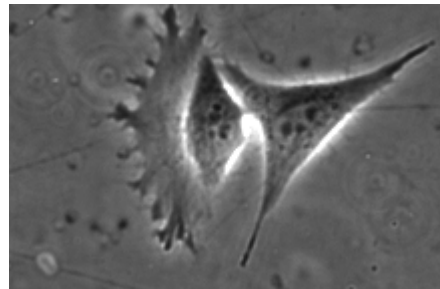
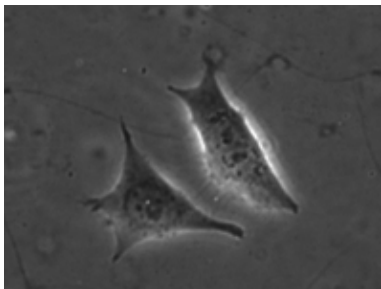


The fastest migrating HeLa cell ever: up to 10  $\mu\text{m}/\text{min}$   
 Movie: Yanjun Liu



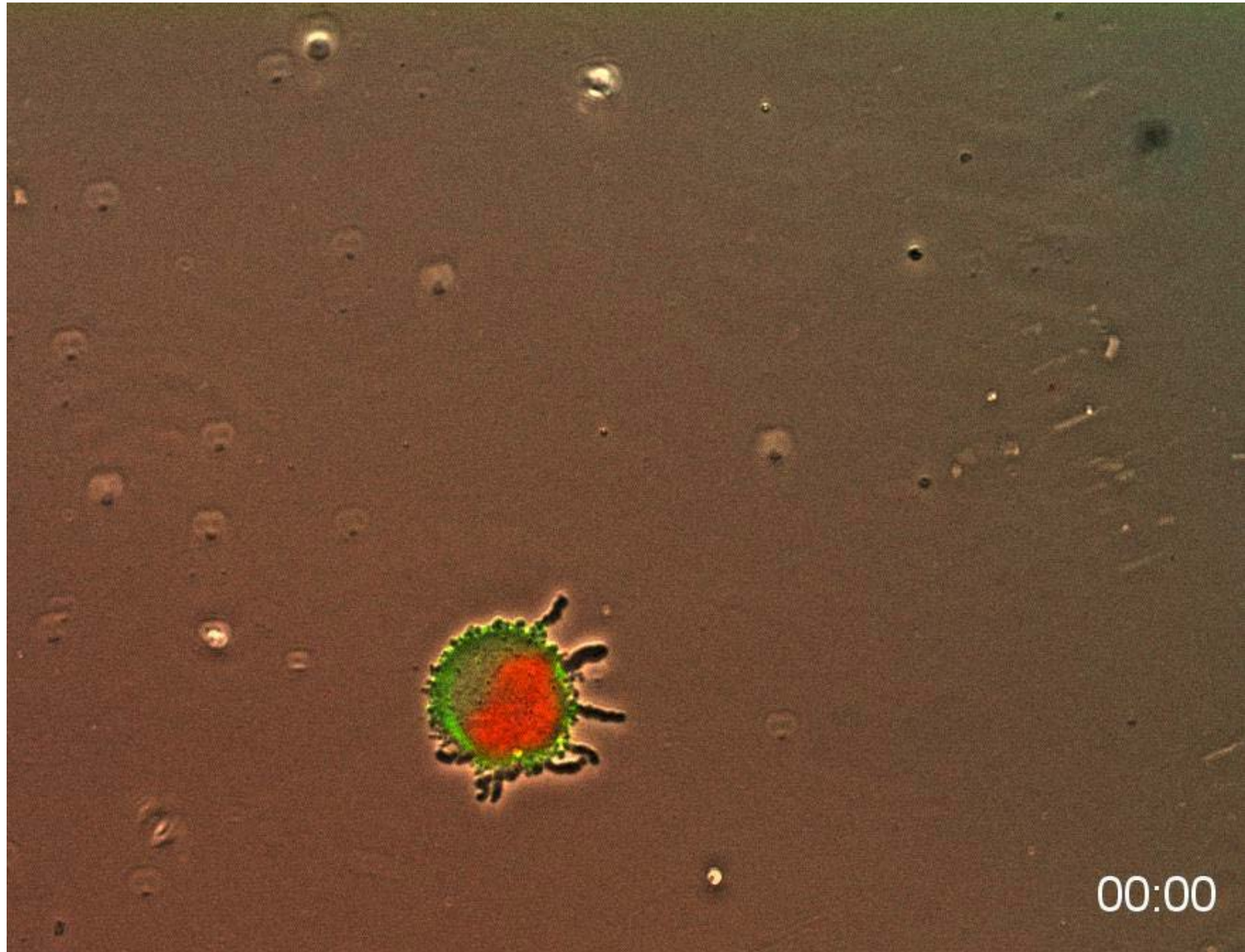
The most famous neutrophil  
 (Movie: Stossel)

## HeLa cells adhesive no confinement





# What is going on with these cells?



Myosin II Nucleus

# What is going on with these cells?

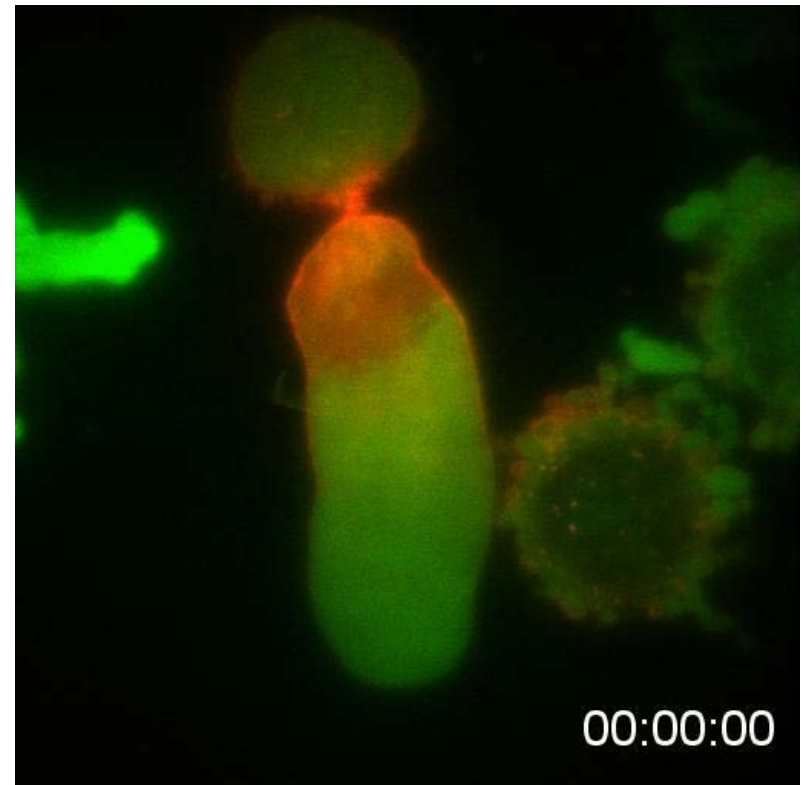
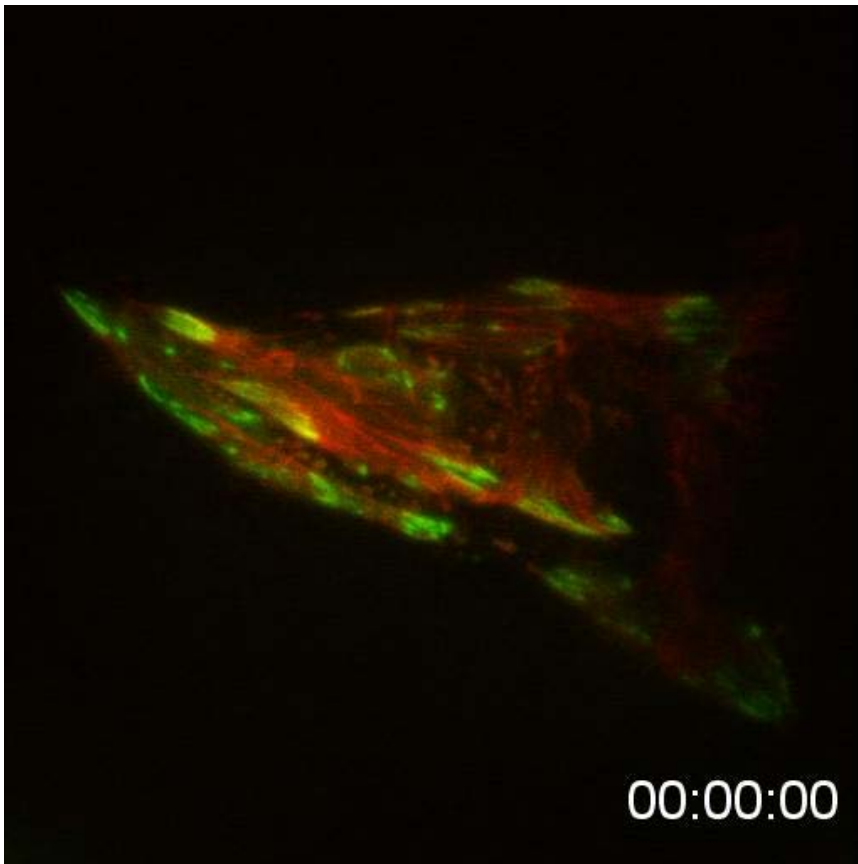
Adhesions (green) plus actin (red)

No confinement  
Fibronectine

Focal adhesions  
Stress fibers

3  $\mu\text{m}$  confinement  
0% RGD

No adhesions  
Flowing cortex

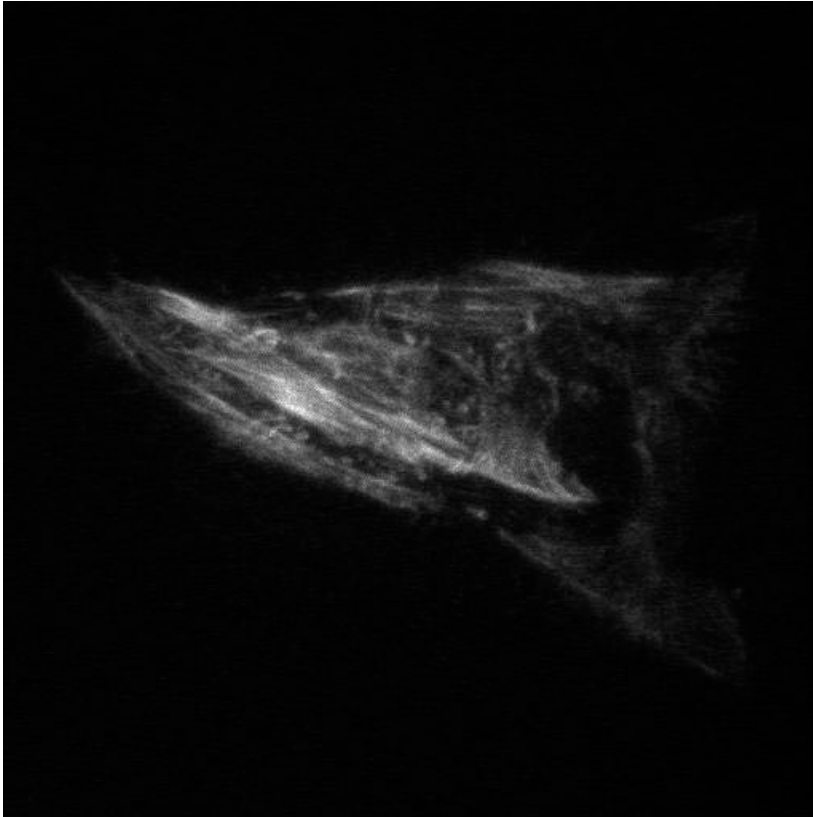


HeLa cells

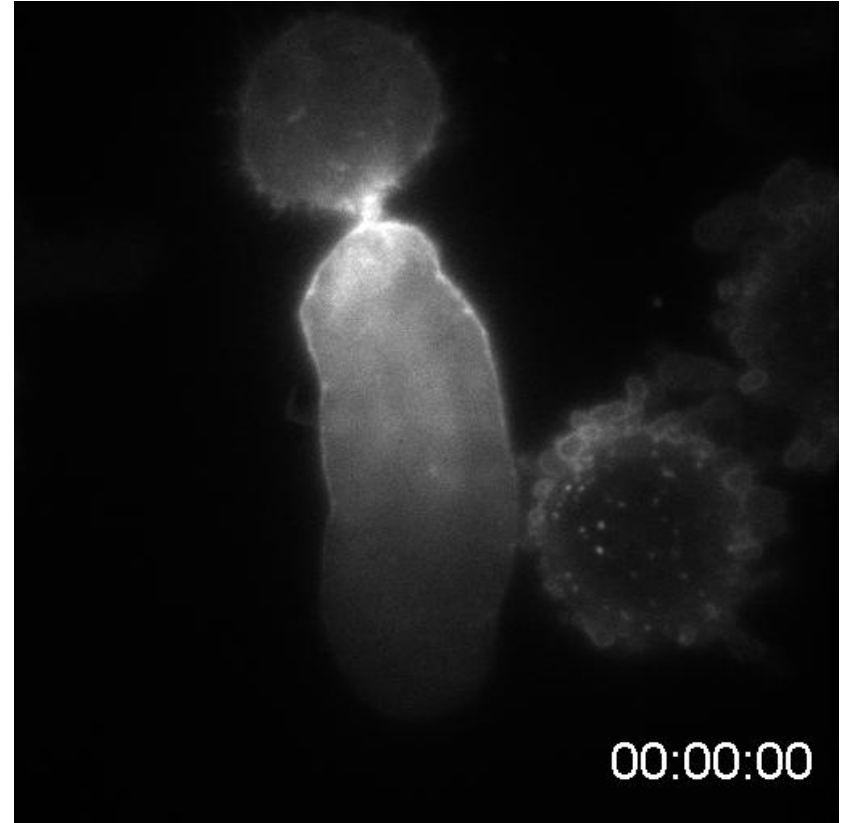
# What is going on with these cells?

## Actin cytoskeleton: Lifeact-mcherry

No confinement  
100% RGD

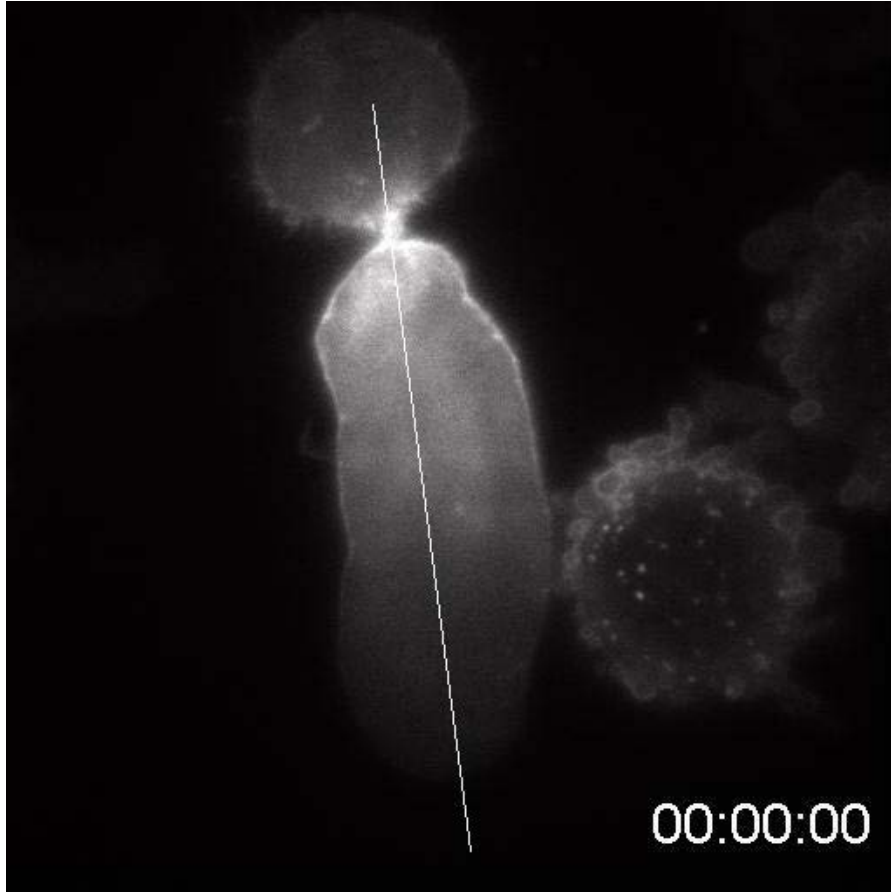


3  $\mu\text{m}$  confinement  
2% RGD

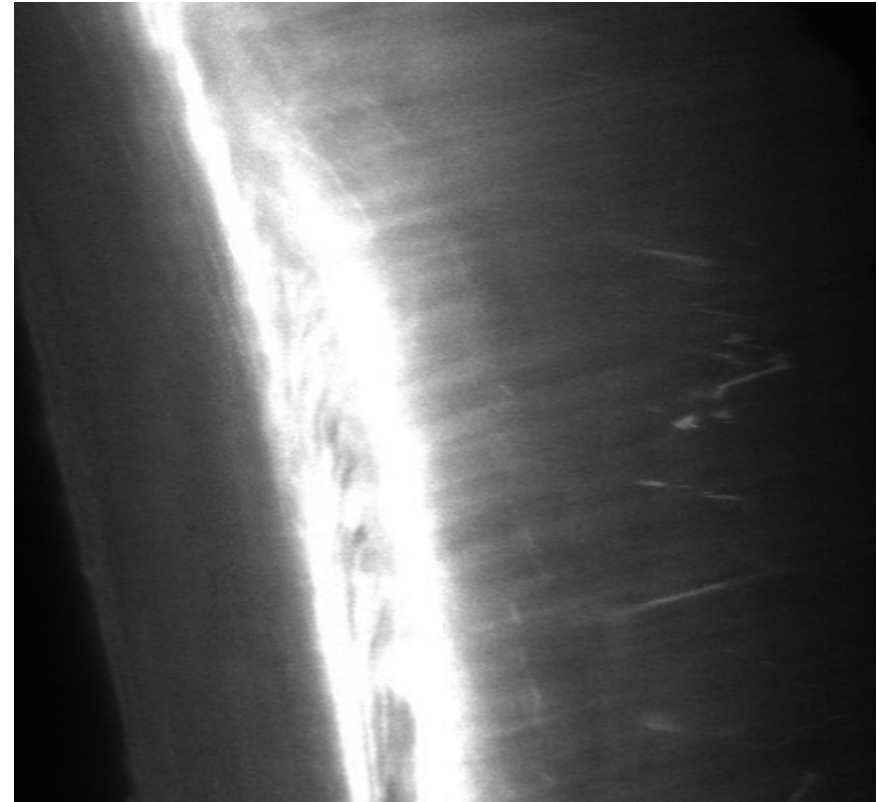


HeLa cells

Actin



Kymograph analysis



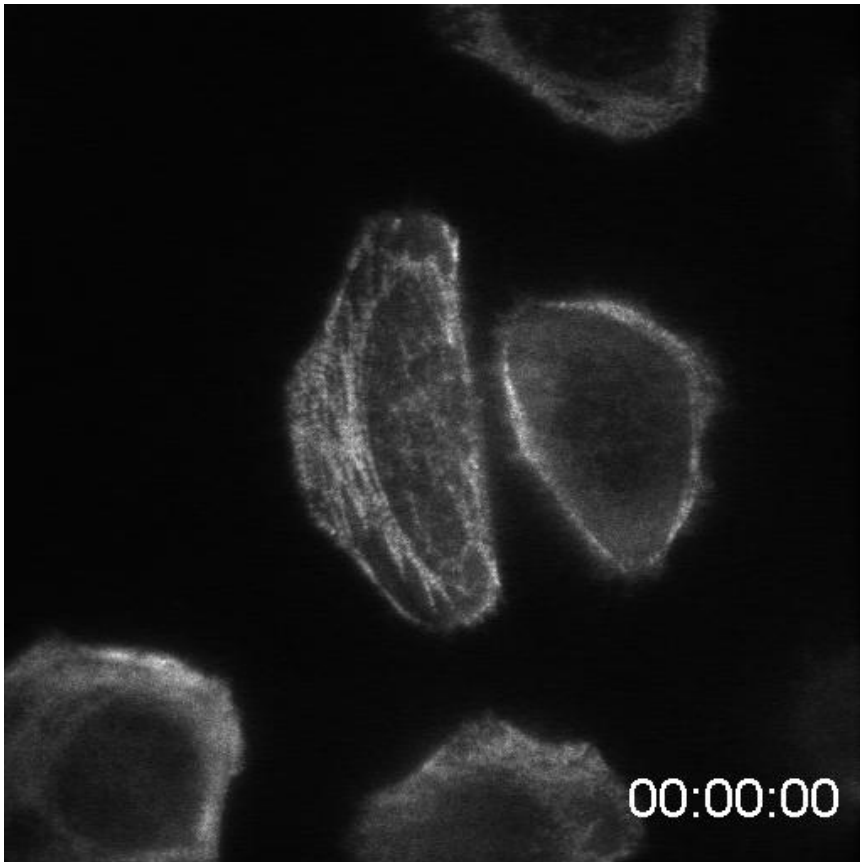
Fast retrograde flow.

Retrograde flow analysis

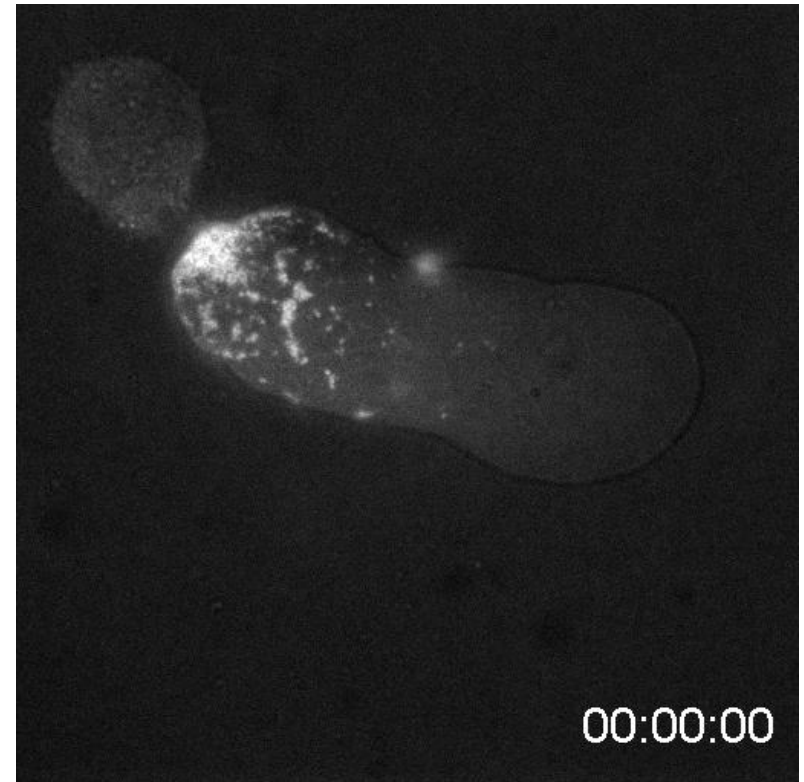
# What is going on with these cells?

Myosin motor: MYH9-GFP (Myosin II A)

No confinement  
100% RGD



3  $\mu\text{m}$  confinement  
2% RGD



HeLa cells

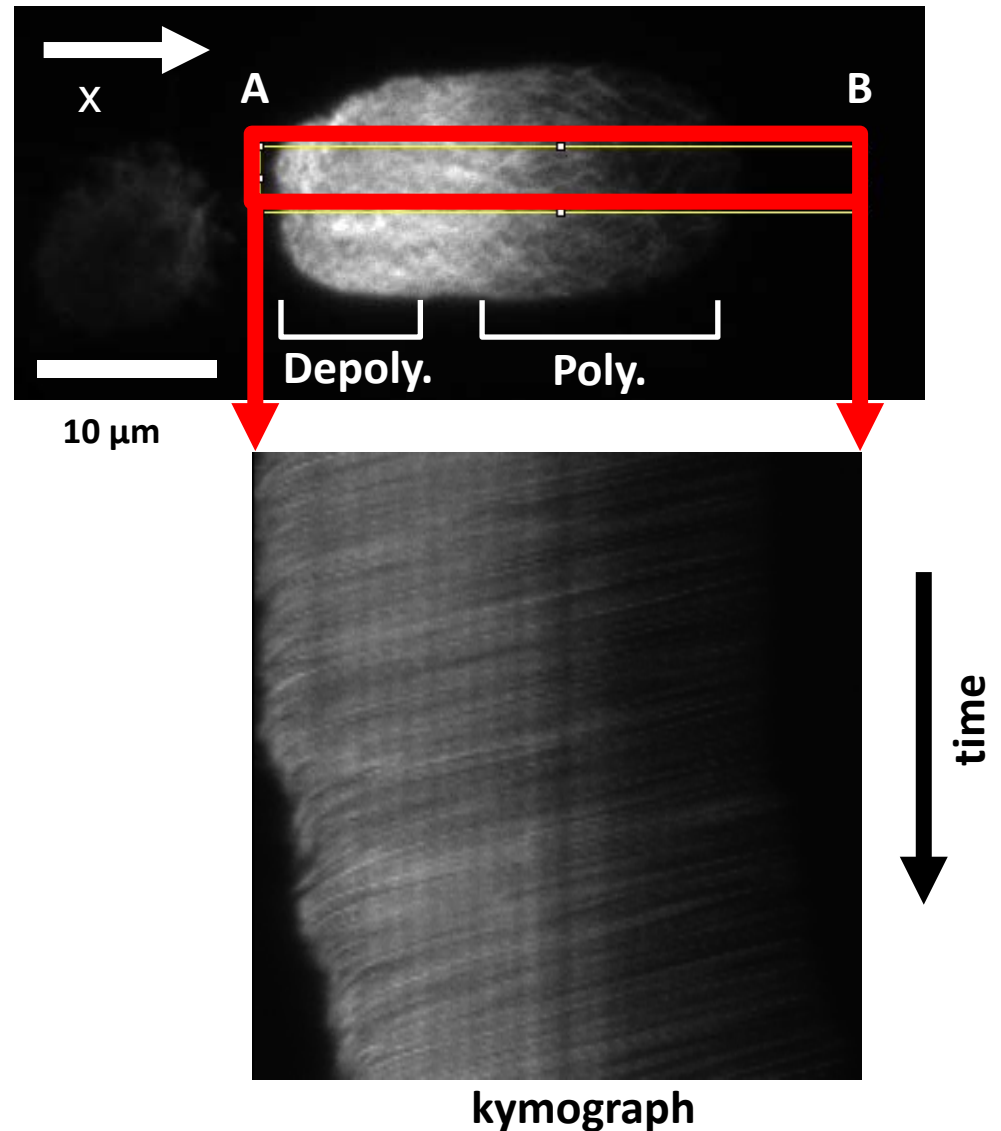
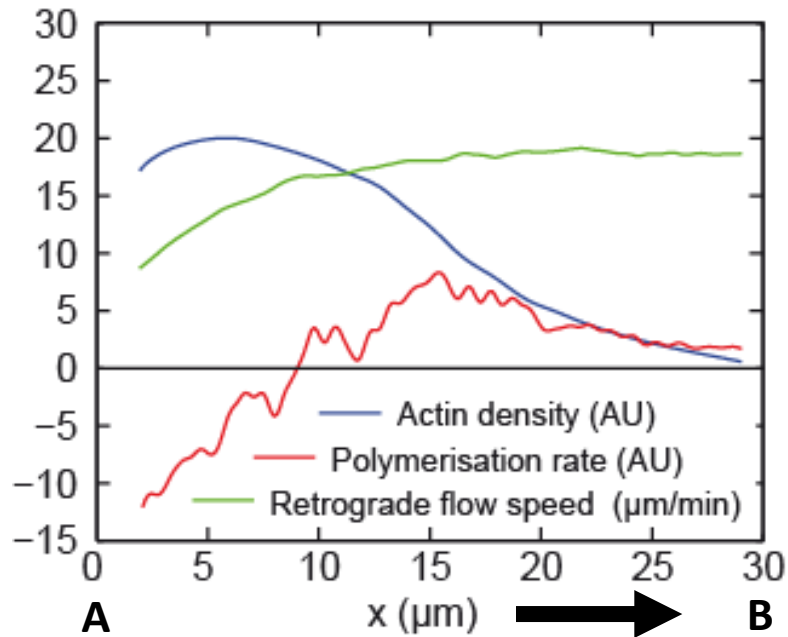


# Actin polymerisation

Actin polymerisation rate:

=

$\text{Div}(\text{Actin density} \times \text{retrograde flow})$



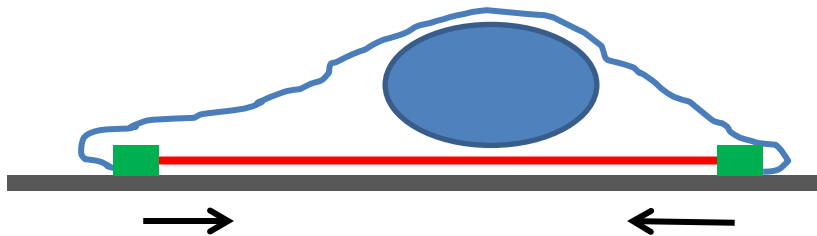
# What is going on with these cells?

Focal adhesions

Stress fibers

Pulling

Slow motility ( $\sim 0.1 \mu\text{m}/\text{min}$ )



No confinement  
100% RGD

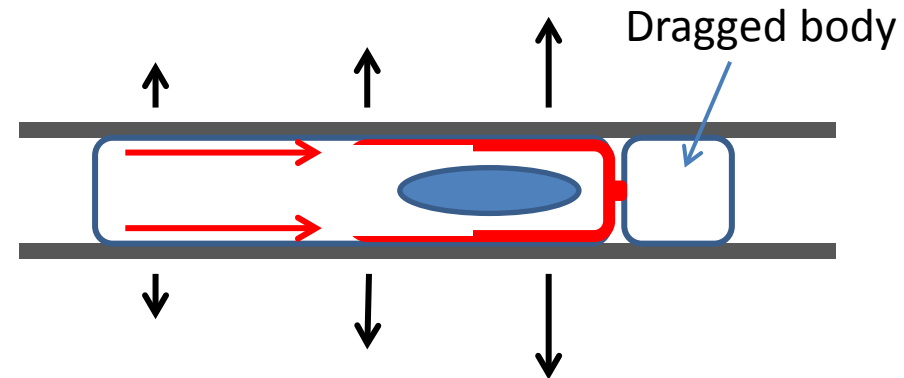
No adhesions

**Flowing cortex**

Pushing

Fast motility ( $\sim 5-10 \mu\text{m}/\text{min}$ )

Speed and polarity strongly  
depend on contractility



3  $\mu\text{m}$  confinement  
0% RGD



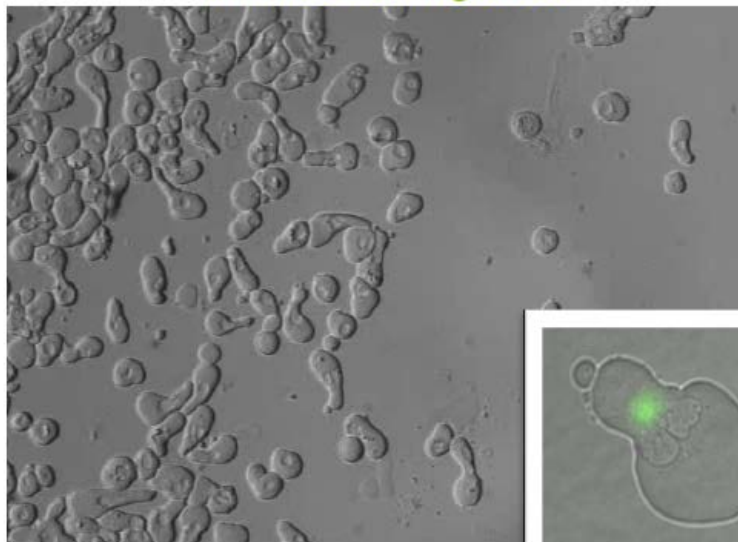
# These cells move like neutrophils and even like... fish embryonic cells (under confinement)



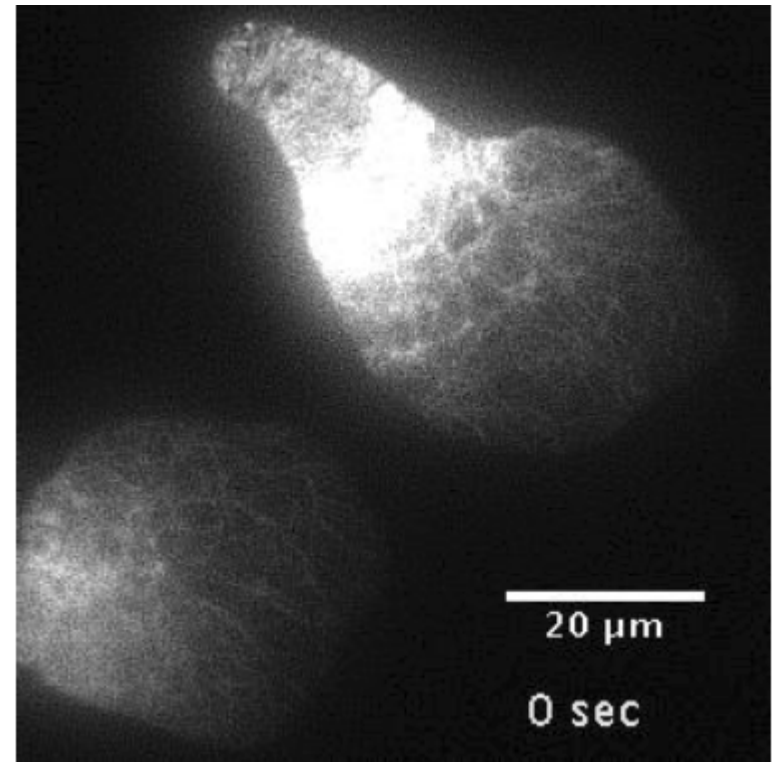
Unpolarized cells



*in-vitro cell migration*

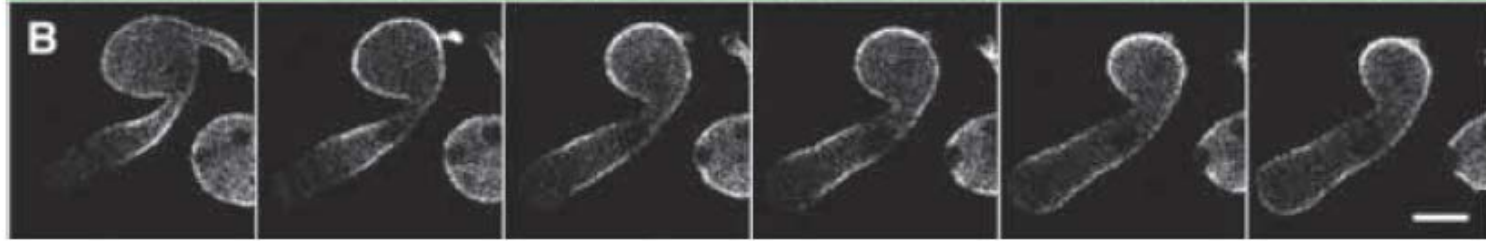
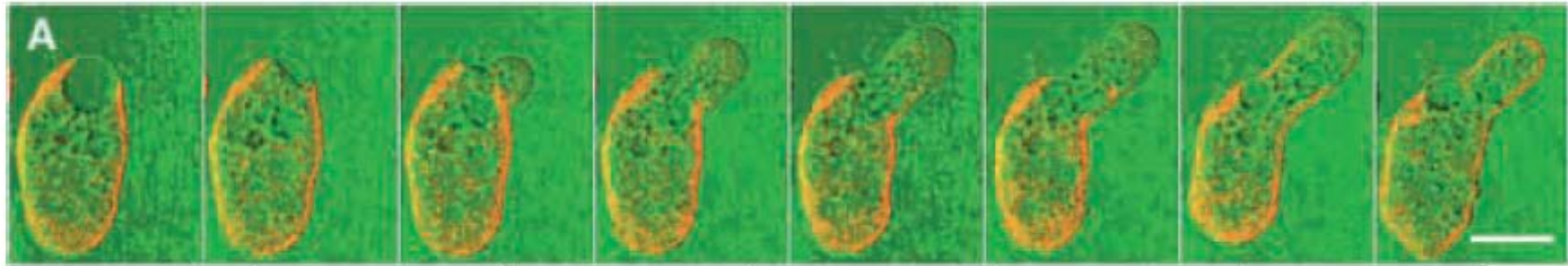


Highly polarized phenotype

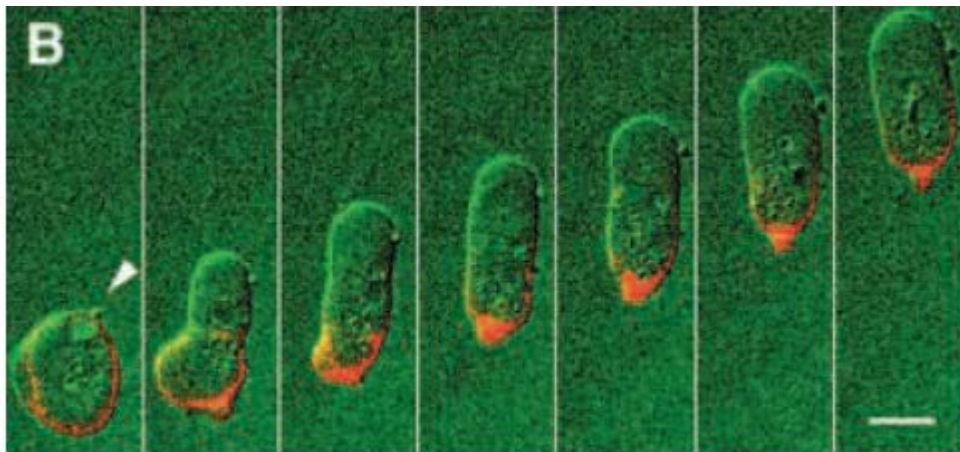


(from Verena Ruprecht,  
CP Heisenberg lab, IST, Vienna)

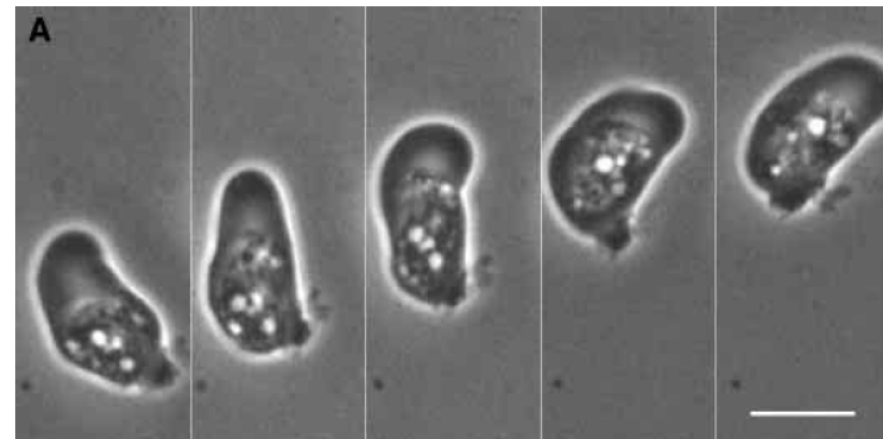
# It is really amoeboid migration: Dicty can do it too! (when treated with quinine)



Myo II

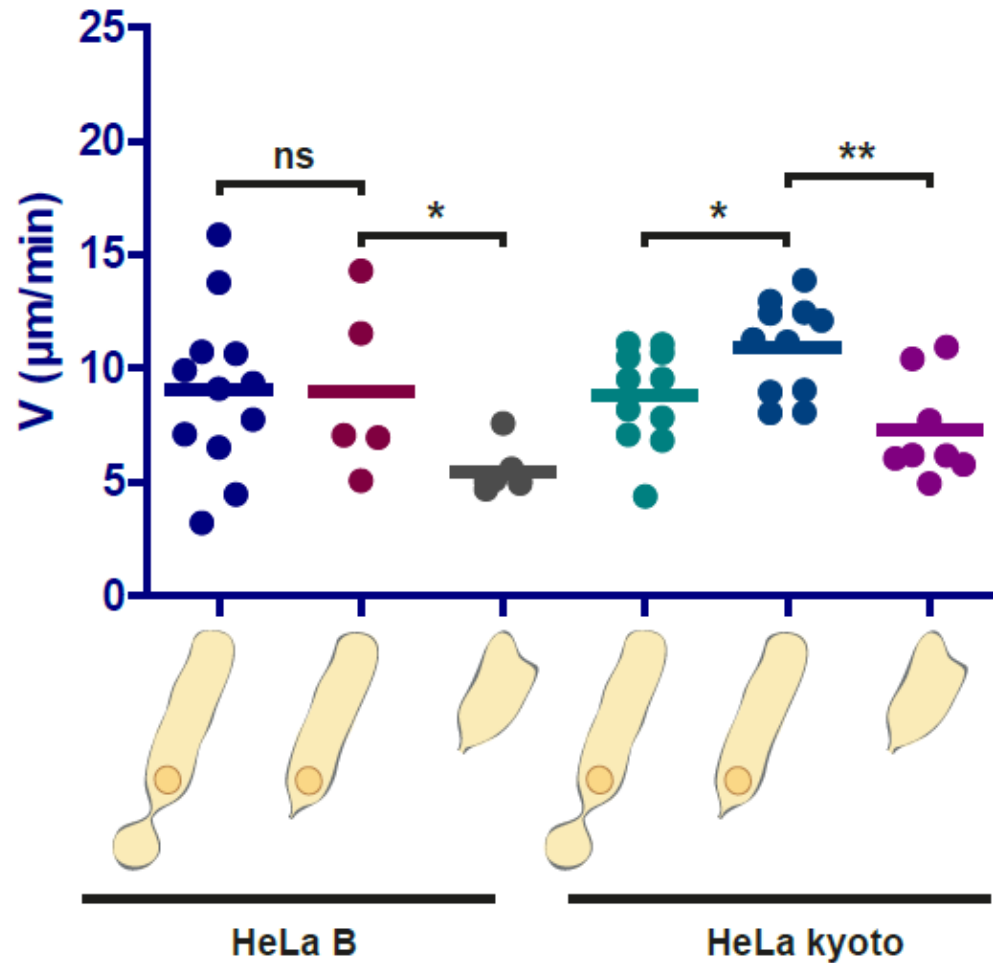


Actin

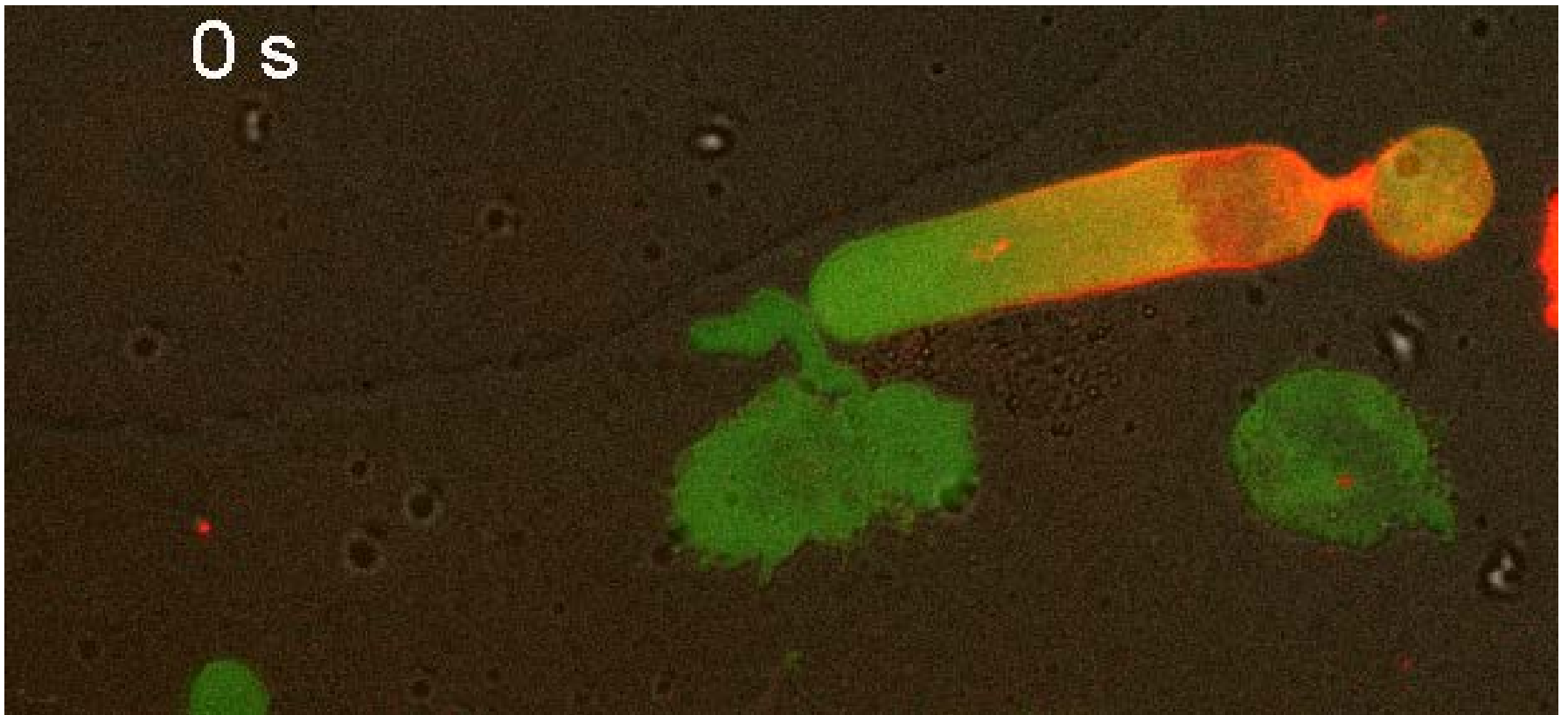


And Inouye, JCS, 2001

# A primitive and universal flow/friction/pressure driven migration?



One more migrating sausage...  
HeLa cells as a model system  
to study fast amoeboid migration!!

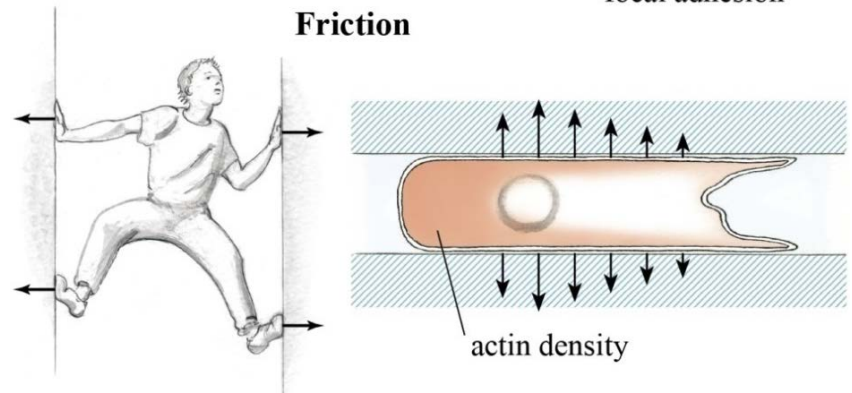
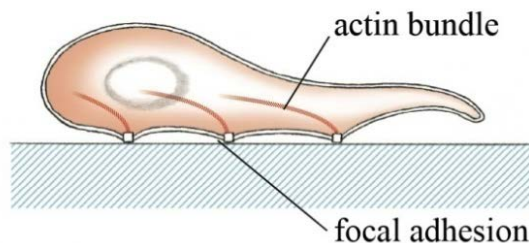


# Conclusions

- 1) At high adhesion, cells migrate slower under confinement (lamelipodial/adhesion driven motility): they use their force to pull on the substrate, not to move
- 2) At low adhesion, cells migrate faster under confinement (amoeboid/friction driven motility), they use their force to contract, generating a pressure gradient
- 3) Mesenchymal cells moving under low adhesion/strong confinement display amoeboid morphology and behavior: HeLa as a model system for fast amoeboid migration!!



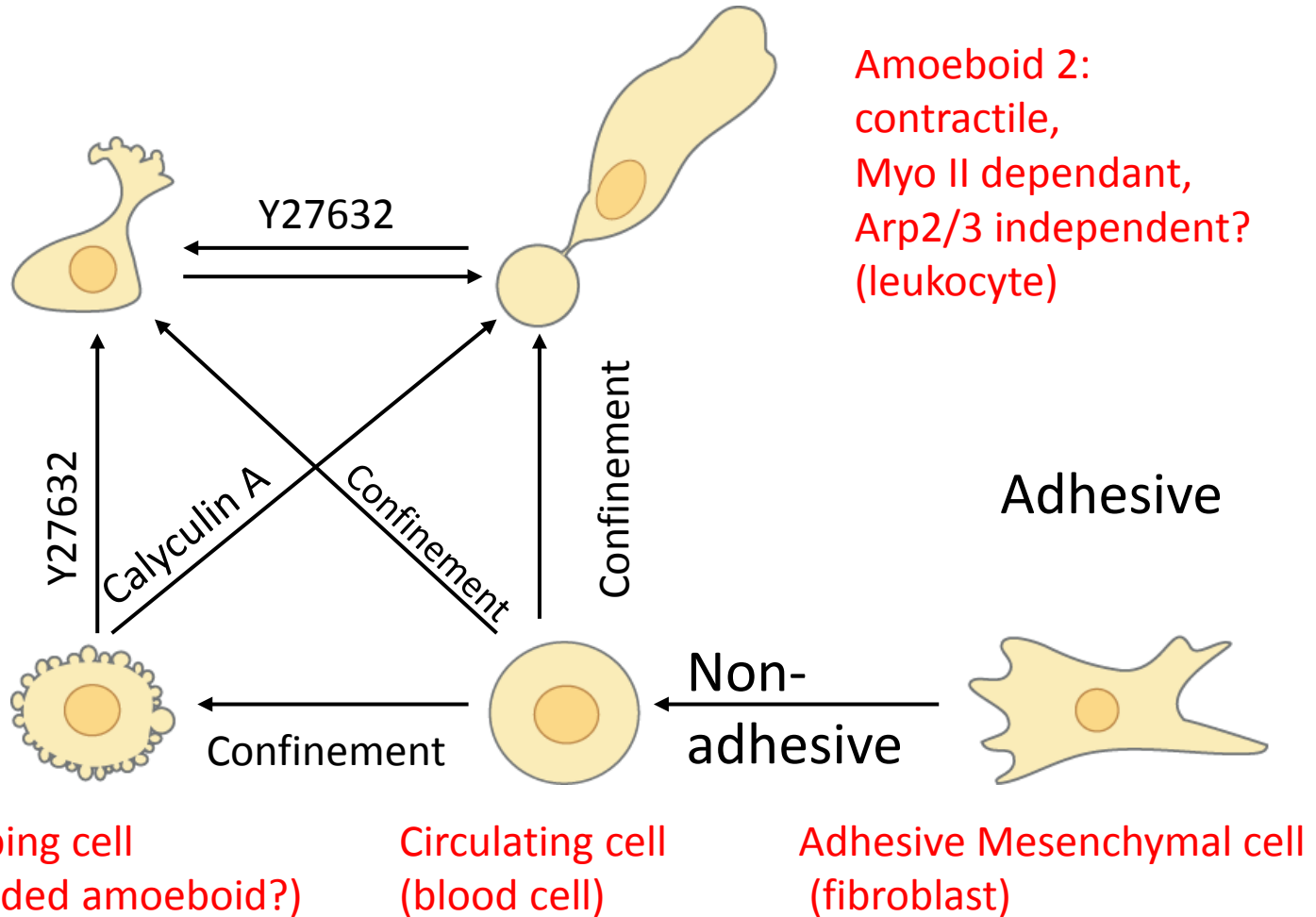
**Adhesion**



# A phase diagram for non-adhesive cell migration?

Amoeboid 1:  
protrusive  
non contractile  
Myo II independent,  
Arp2/3 dependant?  
(cancer cell?)

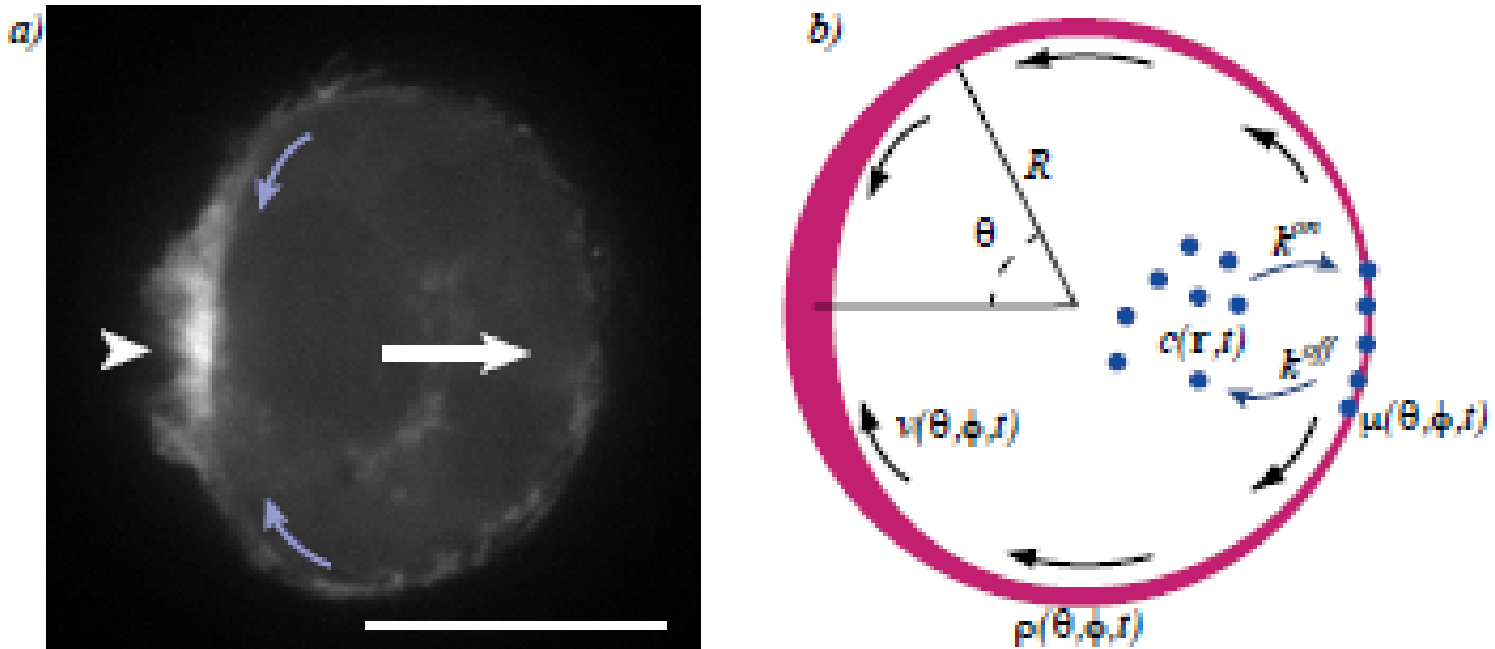
Amoeboid 2:  
contractile,  
Myo II dependant,  
Arp2/3 independent?  
(leukocyte)



But there is a limit to migration under confinement: the cell nucleus  
(check Wolf et al., JCB 2013)

# The flowing cortex: A spontaneously polarizing motile state

Actin in MDA-MB231 cell migrating inside matrigel



Poincloux, PNAS 2011, Hawkins Biophys J 2011

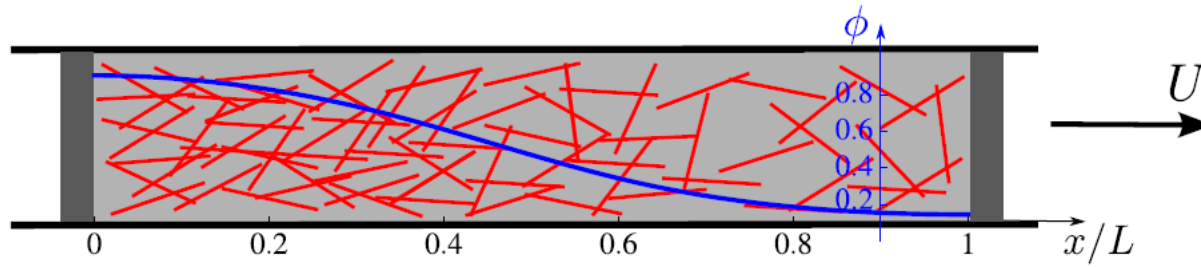


Rhoda Hawkins (Univ. Sheffield, UK)

Raphaël Voituriez (UPMC, Paris)



# The flowing cortex: A phase diagram of the motile states



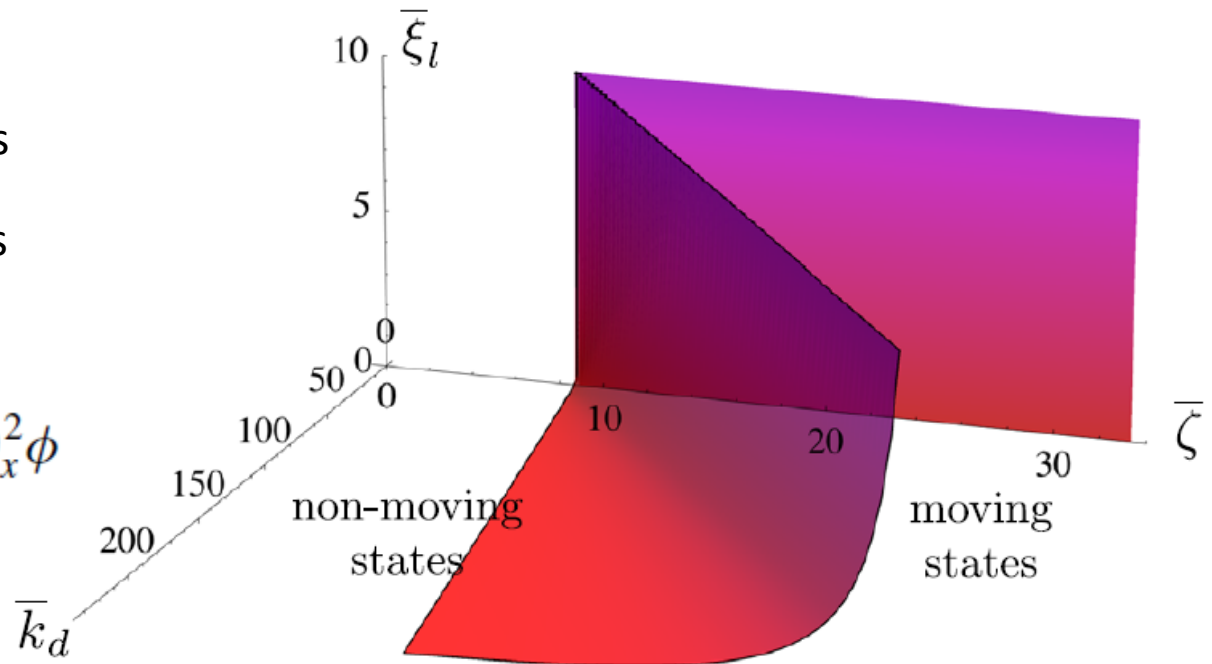
$$\partial_x (\sigma^n - \Pi) = \xi J_p$$

$$\sigma^n = \zeta \phi \quad \text{Active stress}$$

$$\eta_p \partial_x J_p \quad \text{Passive stress}$$

$$\xi J_p = \xi \phi v_p$$

$$\Pi = \alpha(\phi - \phi_0)^3 - \gamma \partial_x^2 \phi$$



Zeta: activity (contractility)

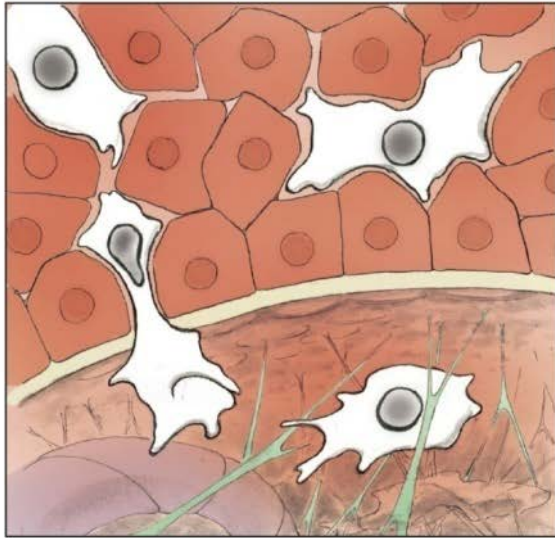
Xi: friction

Kd: depoly rate

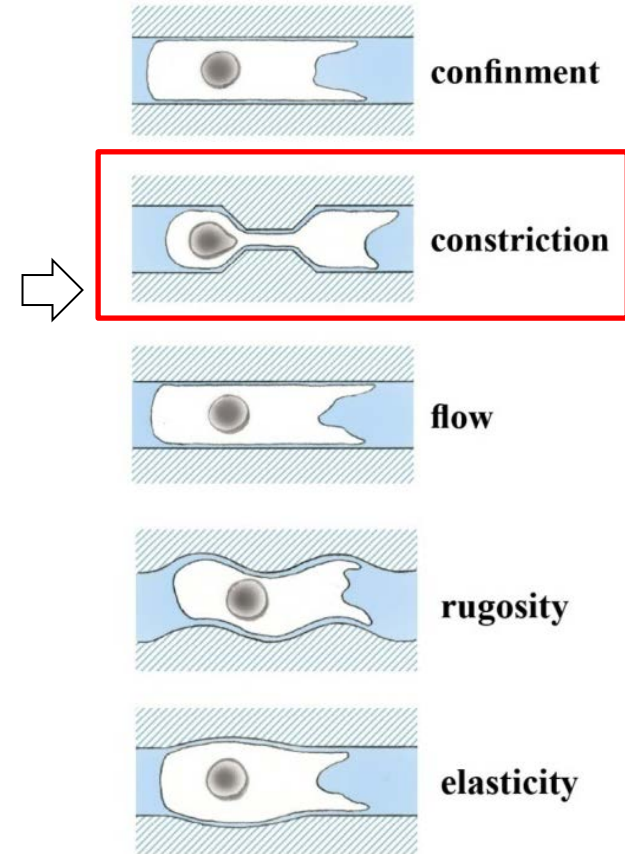


# A reductionist approach to 3D migration

a) *In vivo* interstitial migration

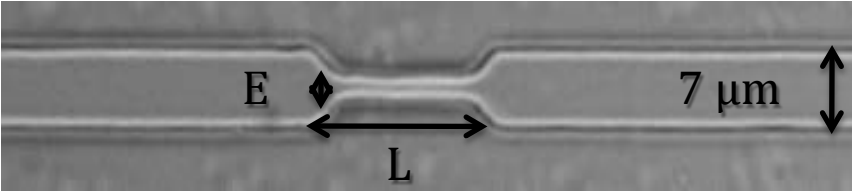
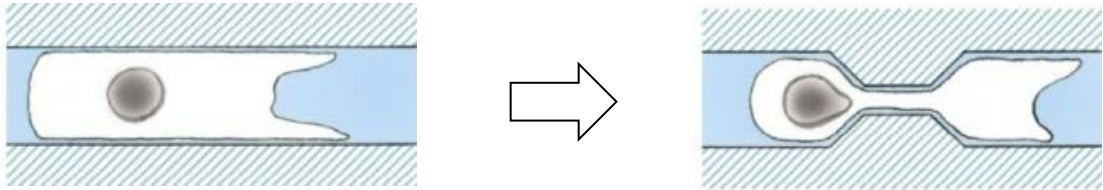


b) *In vitro* migration in 3D



Hawa-Racine Thiam

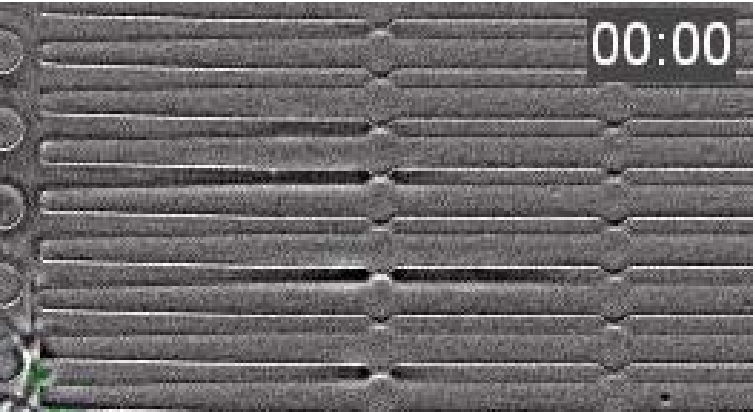
# Constrictions in microchannels: a new tool to specifically assay the capacity of cells to deform their nucleus



**Screening for constrictions sizes :**  
Length:  $L = 5; 10; 15; 20\mu\text{m}$   
Width:  $E = 1; 1,5; 2; 2,5; 3; 3,5; 4; 5\mu\text{m}$

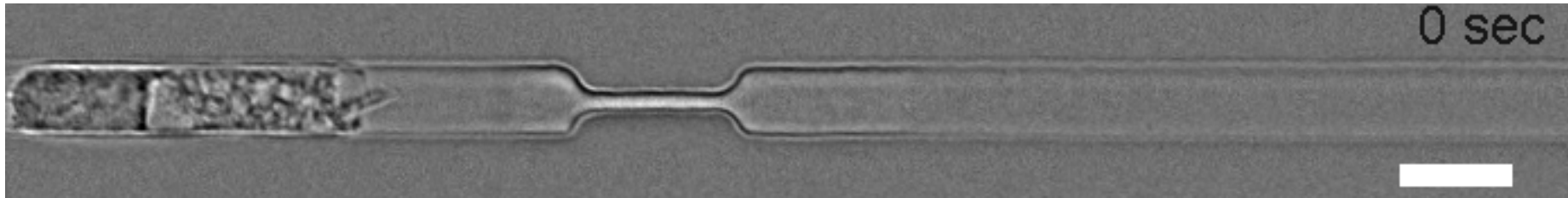
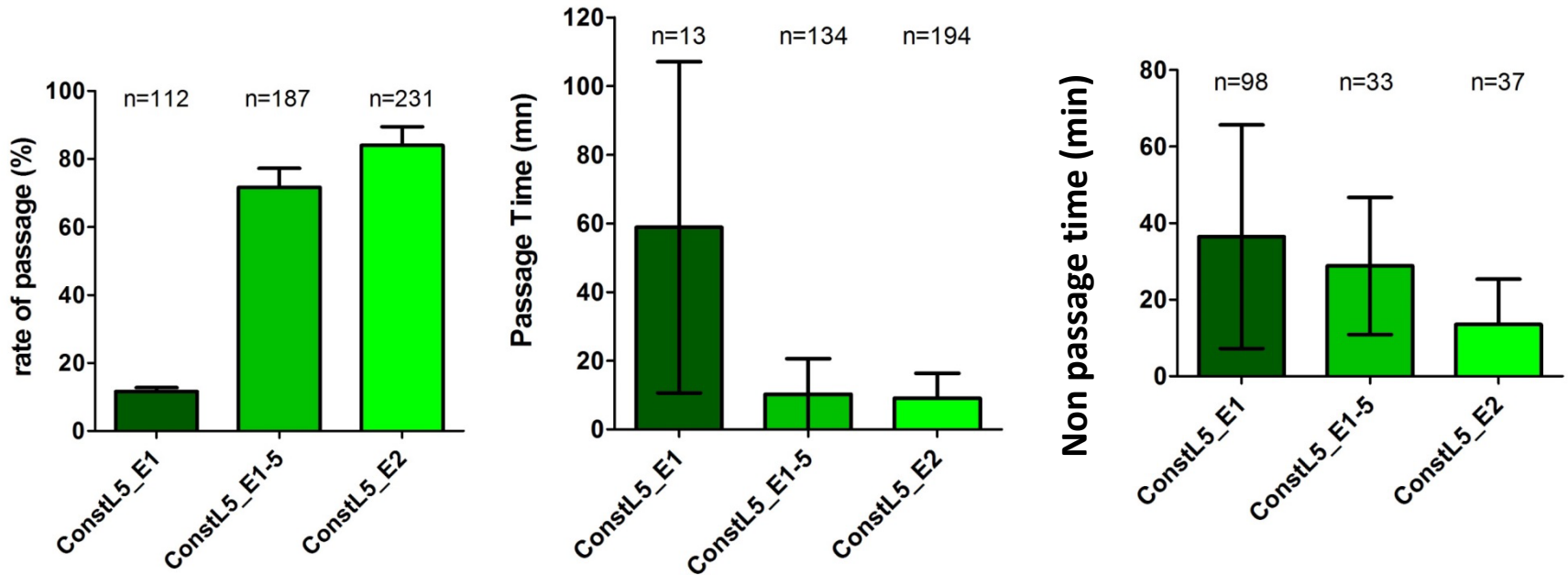
$L = 5 \mu\text{m}; E = 1\mu\text{m}$

$L = 5 \mu\text{m}; E = 2\mu\text{m}$



Bone marrow derived dendritic cells (BMDCs); **Hoechst**

# The cell nucleus is limiting transmigration



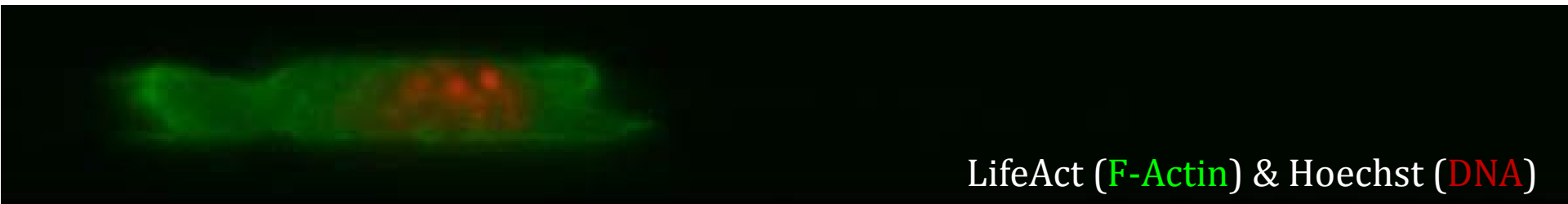
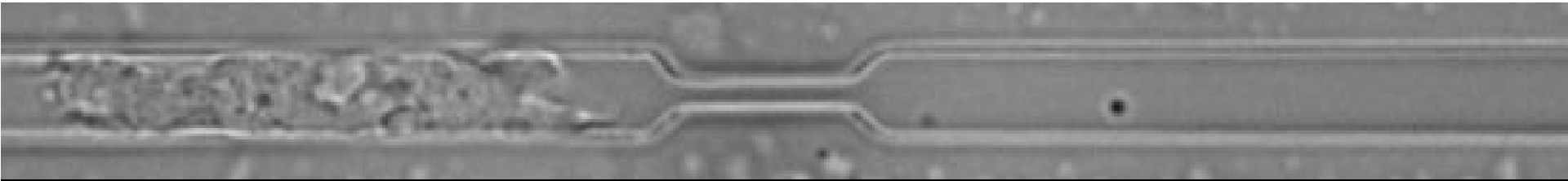
2  $\mu$ m gap

Conclusions from the size screen:

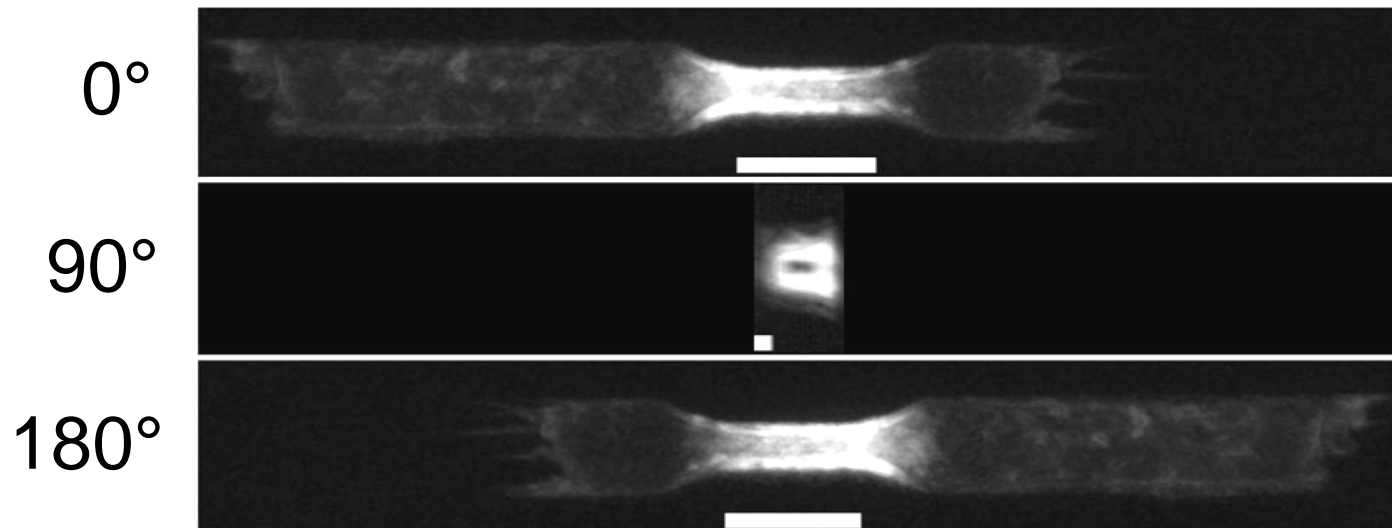
- DCs can pass down constrictions larger than 1  $\mu$ m in width
- There is a sharp transition: most cells pass above 1.5  $\mu$ m
- Cells are slowed down below 4  $\mu$ m

# Actin assembly around squeezed nucleus

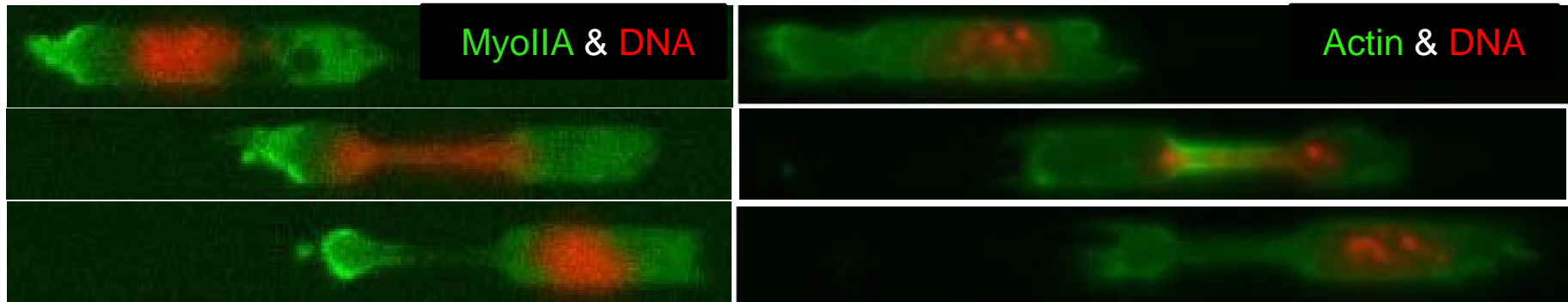
*BMDCs (Ii KO) in a 7 $\mu$ m channels with 15 $\mu$ m long and 2 $\mu$ m thickness constriction*



LifeAct (F-Actin) & Hoechst (DNA)



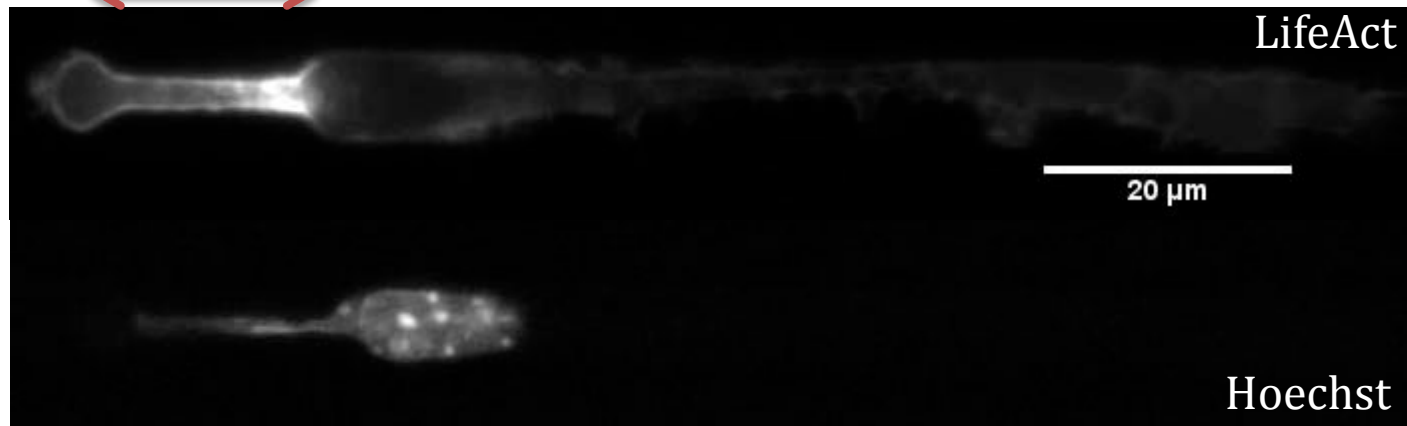
# This actin structure does not contain Myo II



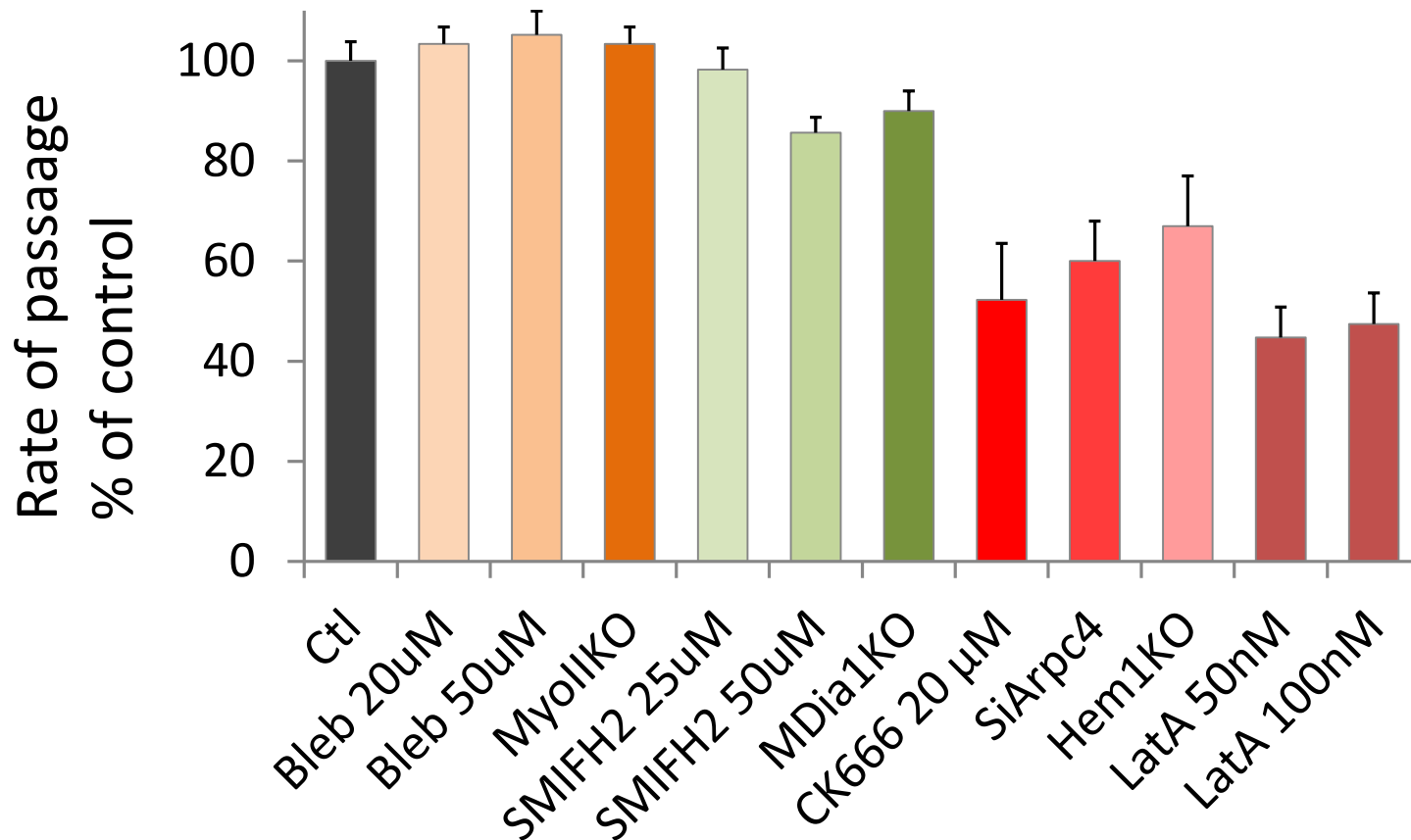
**Constriction**



50  $\mu$ M Blebbistatin

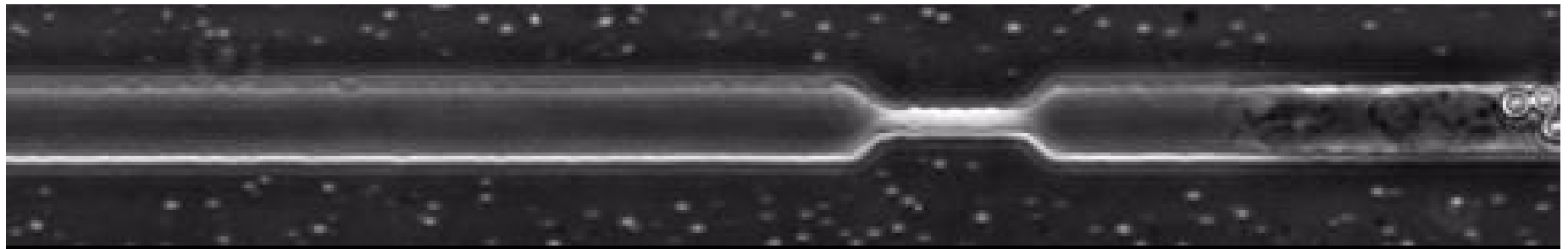
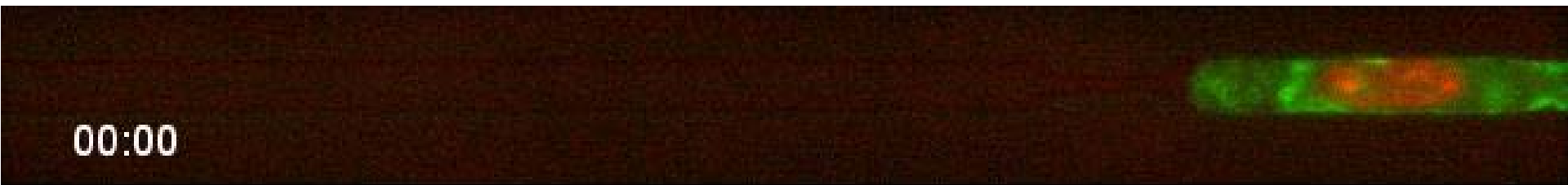
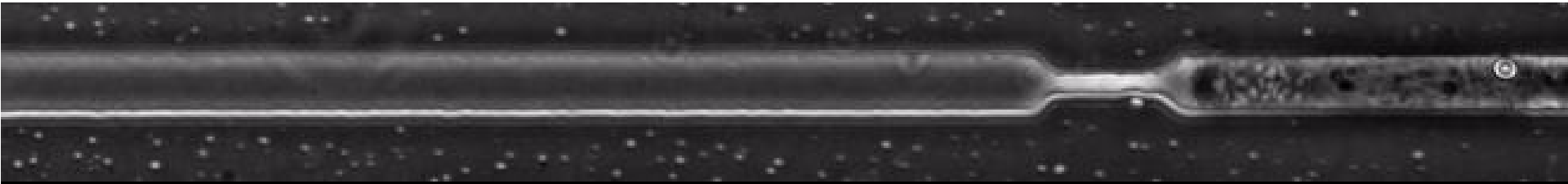


# Transmigration of iDCs depends on Hem1 and Arp2/3 but not on Myo II or formins

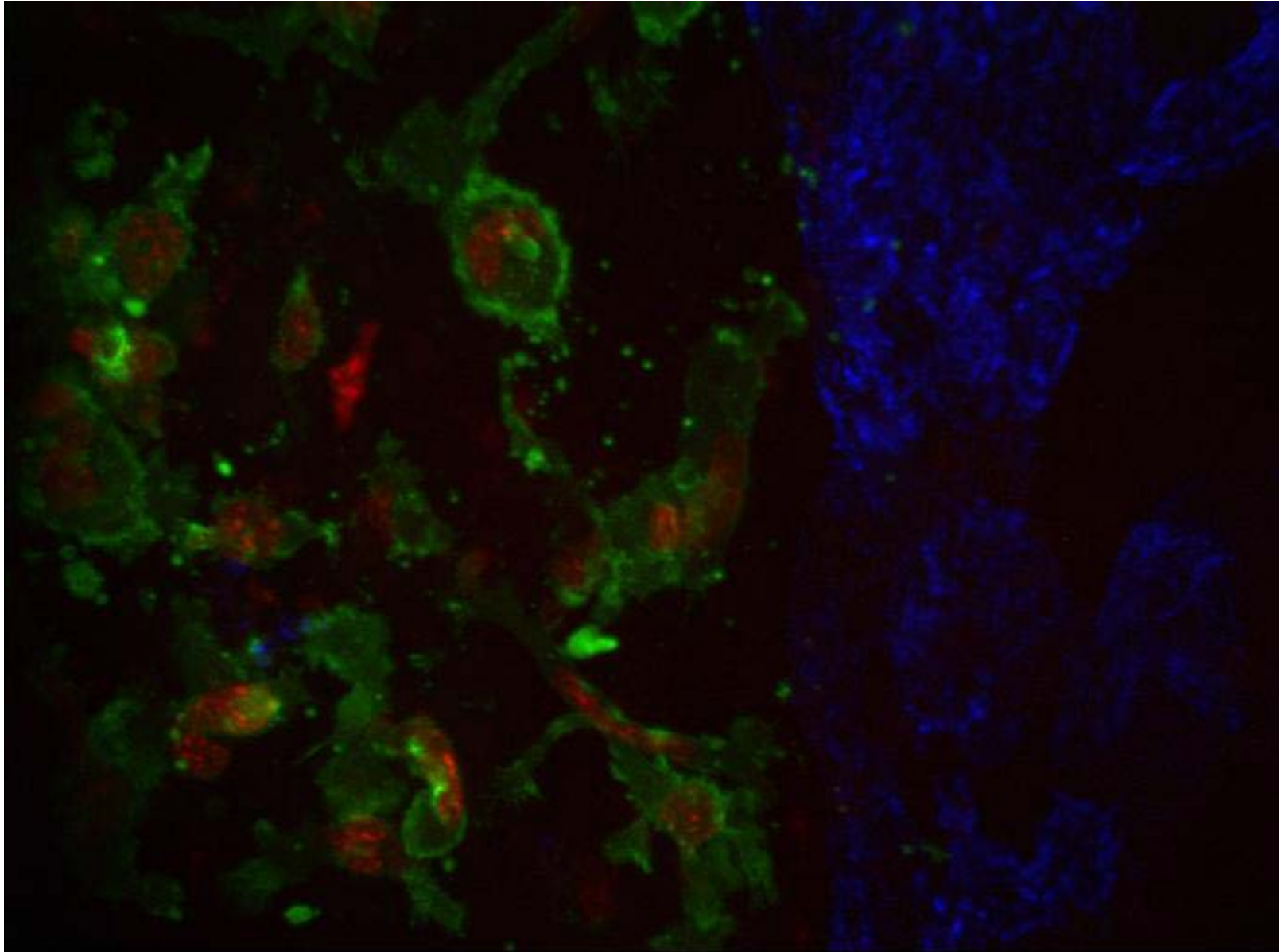


Does not depend either on the LINC complex or the lamina

# Actin nucleation is specifically induced by the nucleus

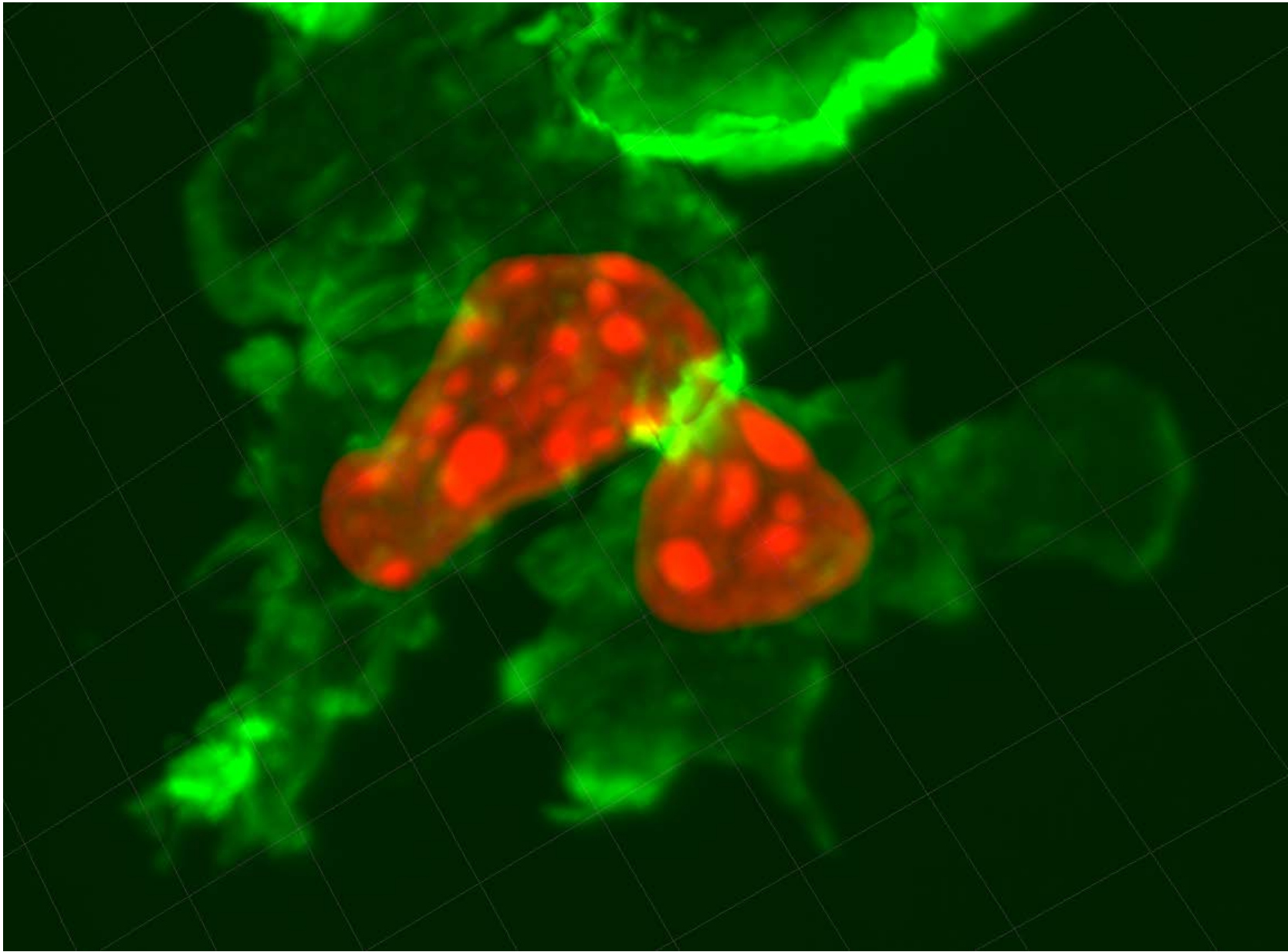


# This ring exists *in vivo*



L1, Hoechst, LYVE (mouse ear explant, movie: Matthew Raab)



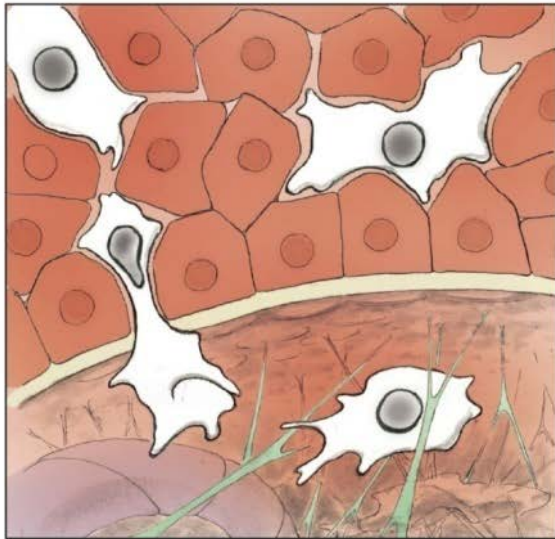


# Conclusions

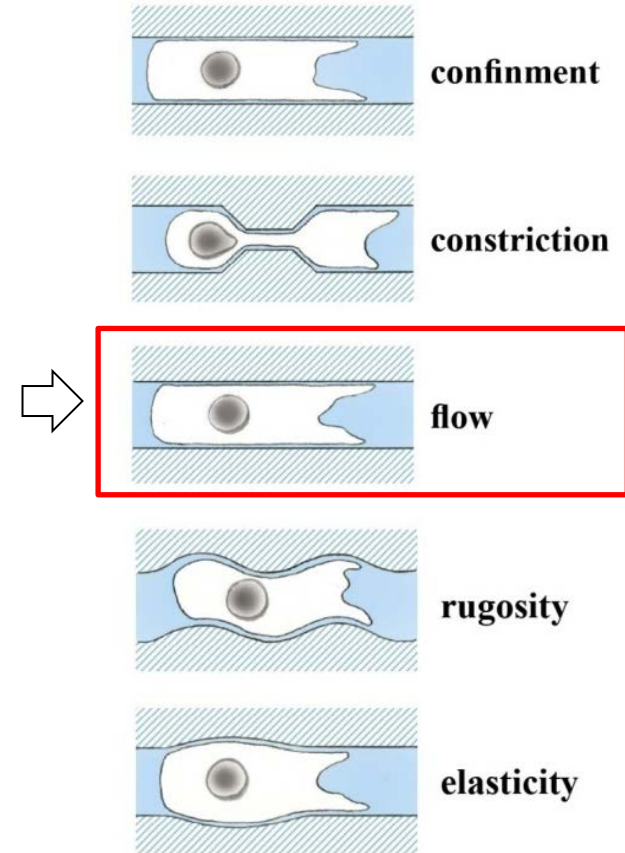
- 1) Below a minimal size, the nucleus becomes limiting and BMDCs use an Arp2/3 dependant MyoII independent mechanism to squeeze it
- 2) BMDCs can concentrate antigens via a Myosin II dependant filtering system and they do not push fluid in front of them ('viscous catching')
- 3) Geometric anisotropy is enough to bias cell migration, independently of adhesion, due to deformation of the visco-elastic cell body

# A reductionist approach to 3D migration

a) *In vivo* interstitial migration



b) *In vitro* migration in 3D



Mélanie Chabaud



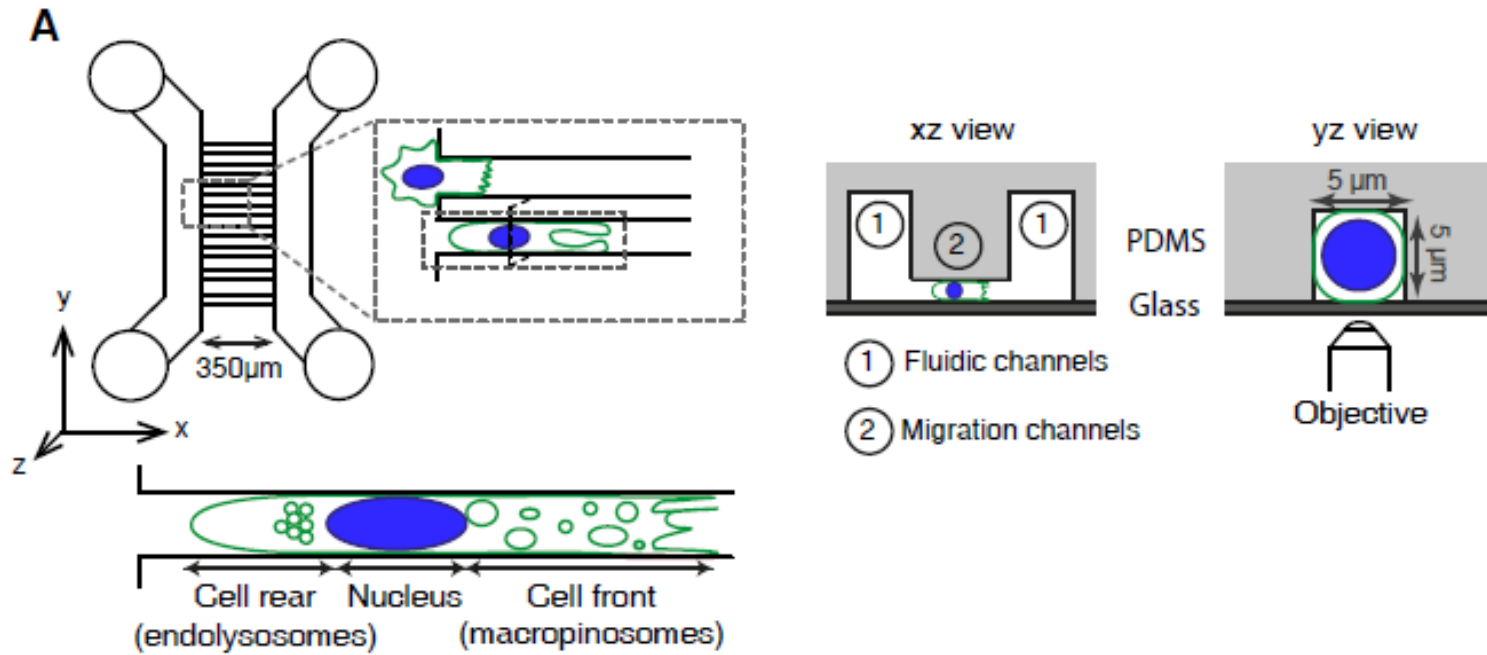
Mélina Heuze



Mathieu Pinot



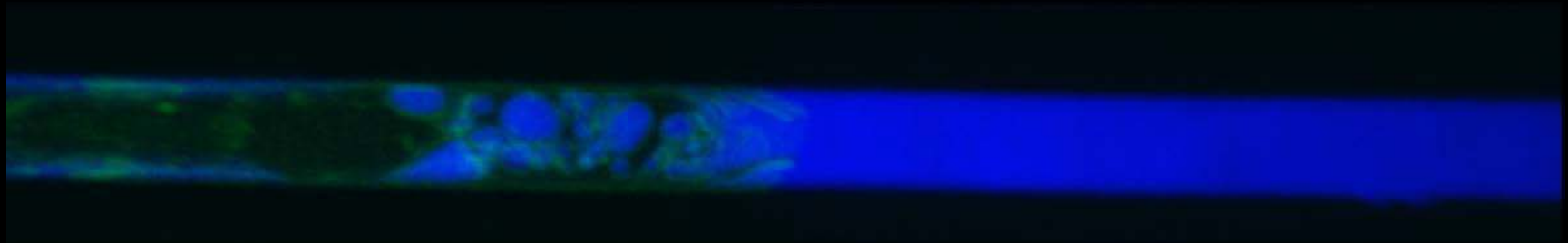
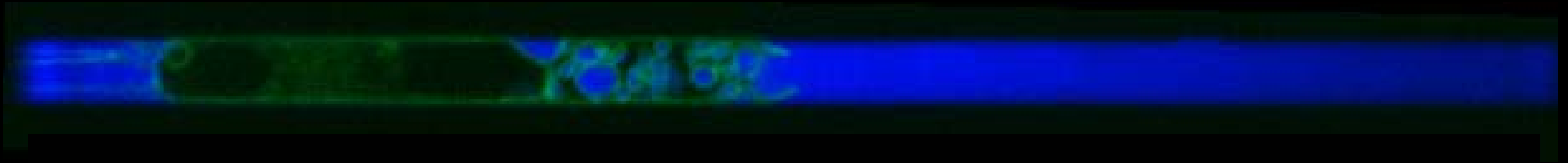
# System



**Microfluidic device : Normalization of direction and cell shape**

# Antigen uptake in vitro: DCs are filters

And they do not push the fluid in front



4  $\mu$ m

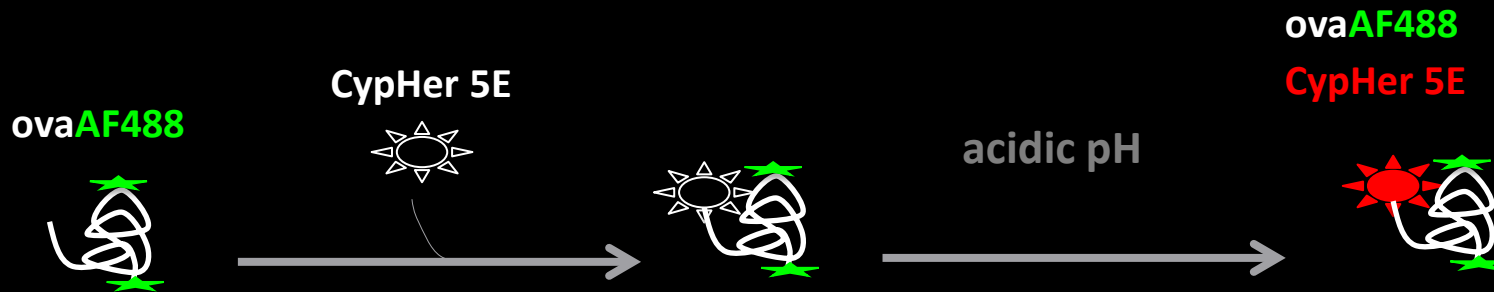


Dextran MHC II

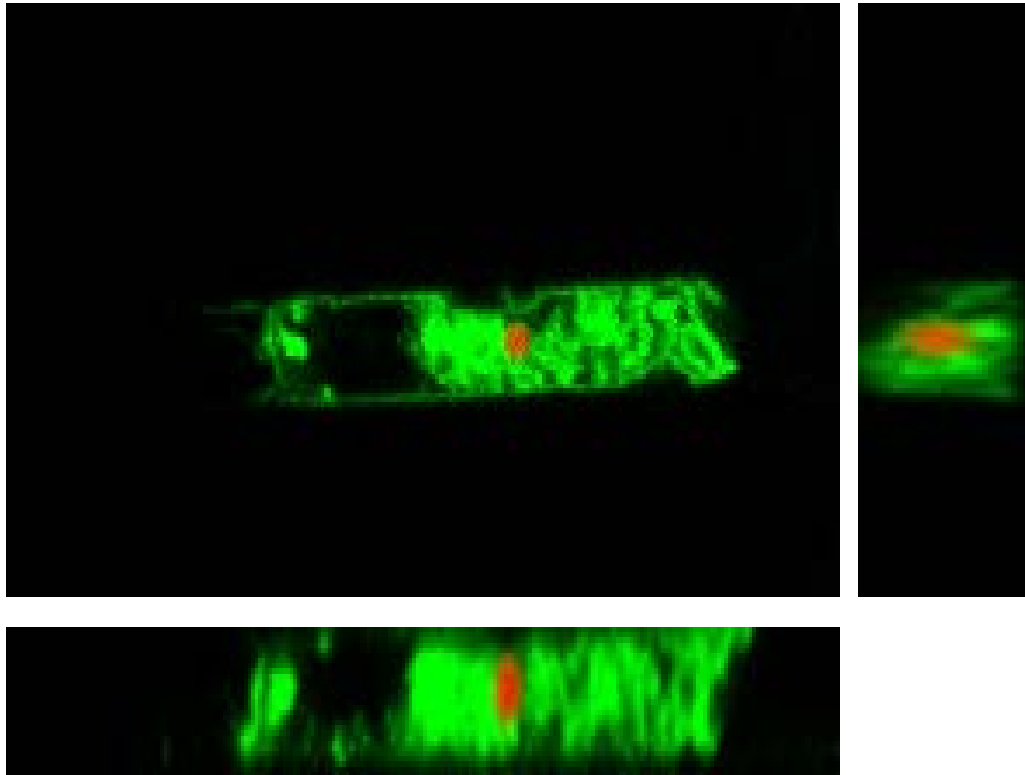
Mélina Heuze and Mélanie Chabaud

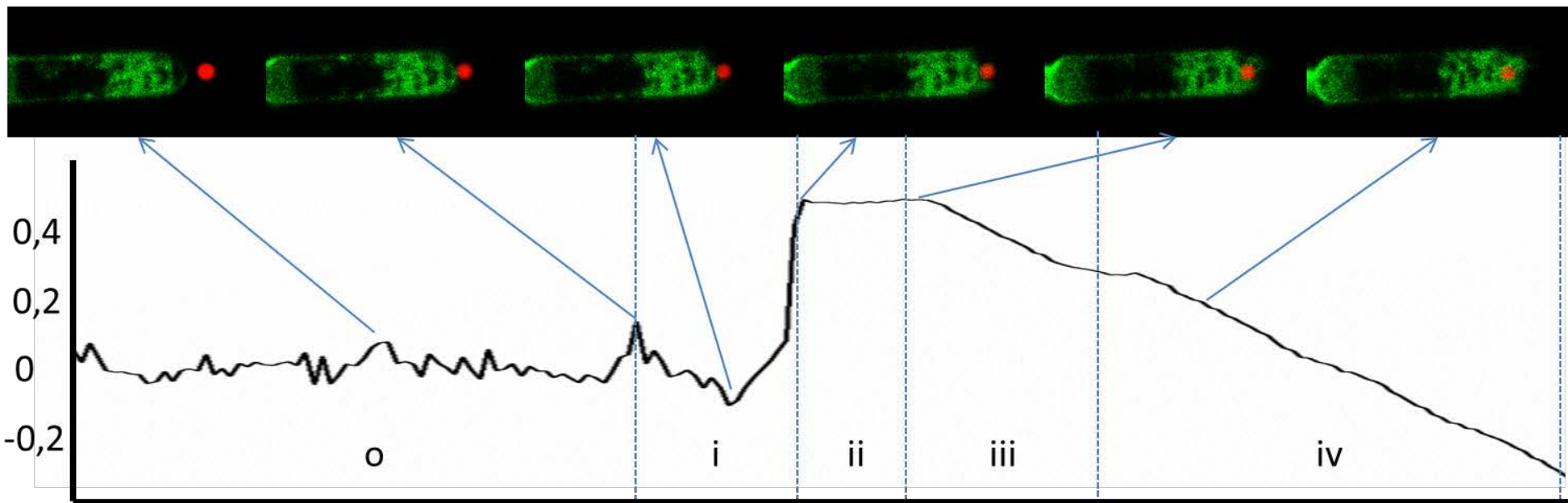
# Fate of internalized material

After 20-40min of exposure to the fluid-phase marker...  
DCs concentrate it in the lysosomal compartments

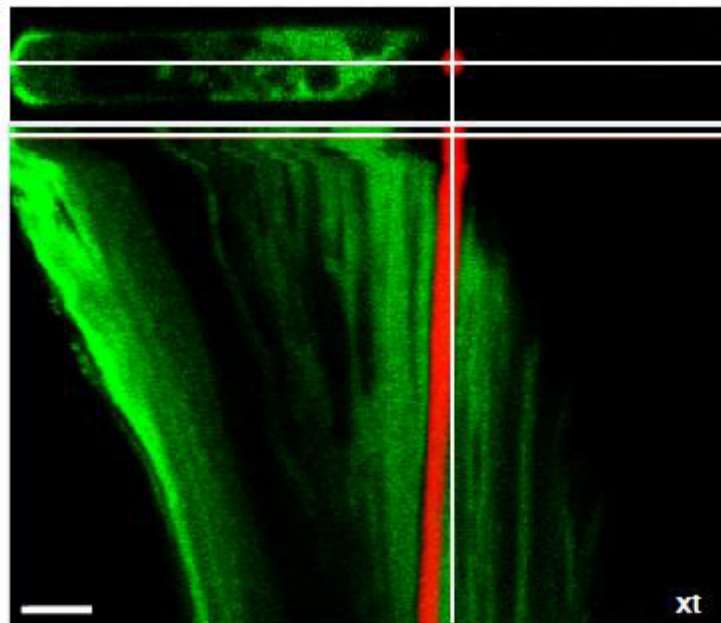


# Eating a bead

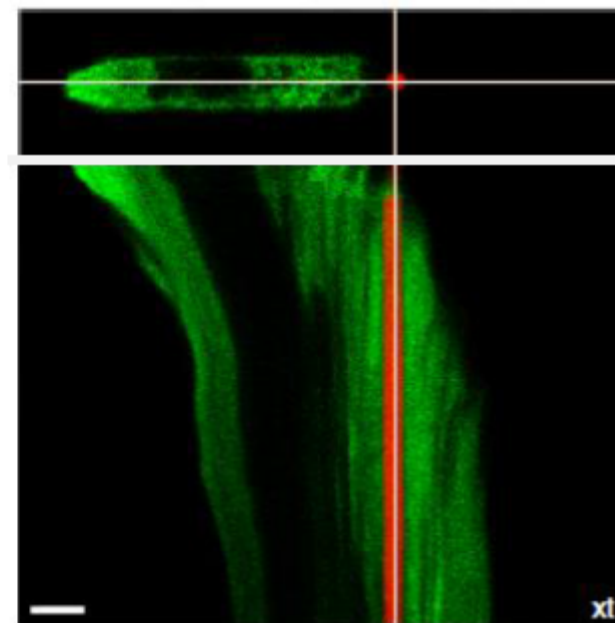




LOW  $P_{TRAP} = 0.25W$



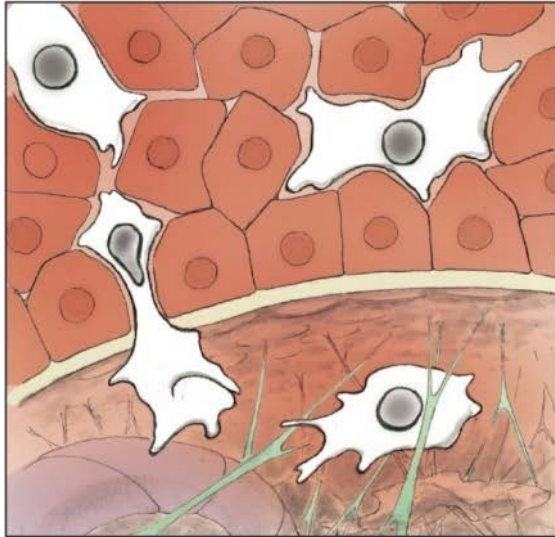
■ HIGH  $P_{TRAP} = 0.95 W$



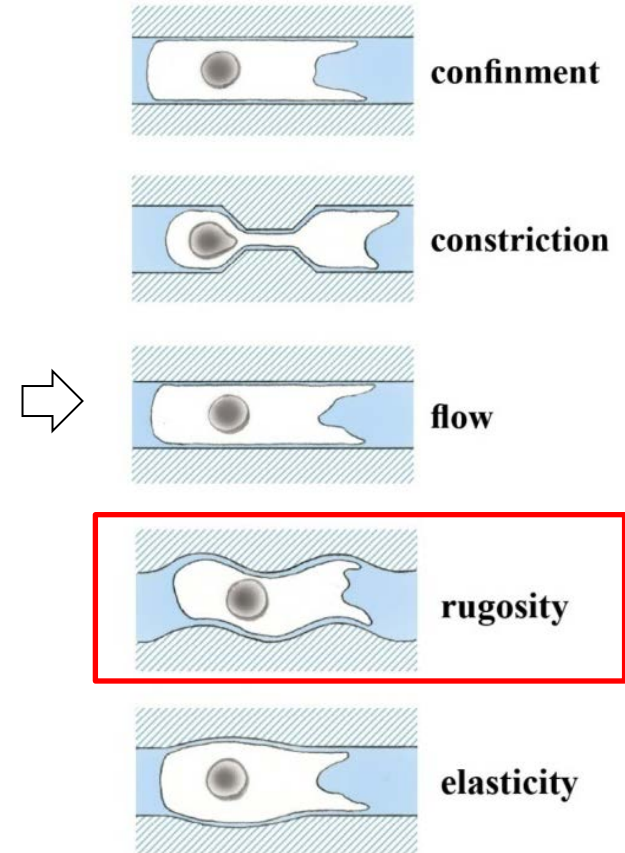


# A reductionist approach to 3D migration

a) *In vivo* interstitial migration



b) *In vitro* migration in 3D

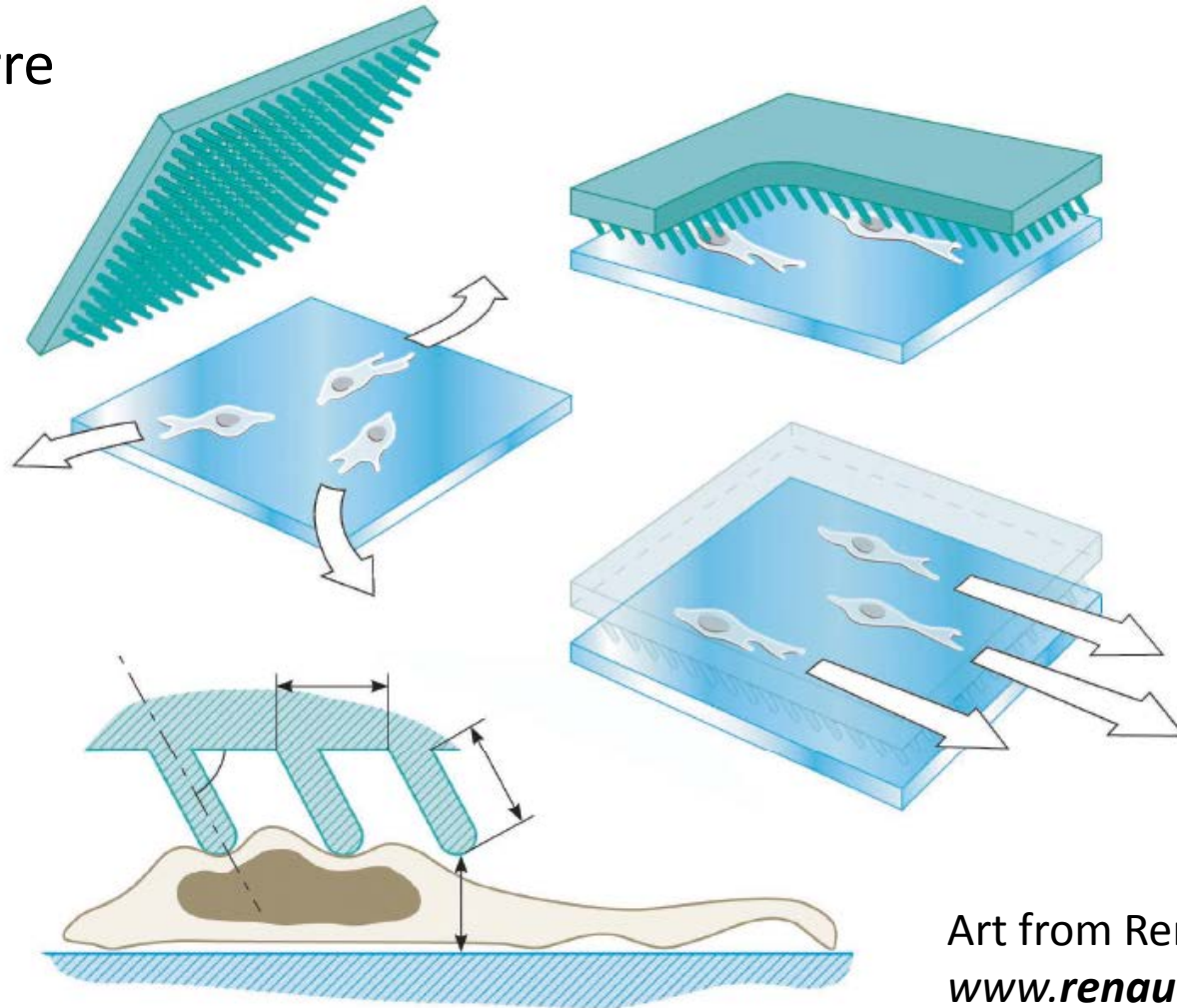


Yanjun Liu Maël Le Berre



# Anisotropic friction: guiding cells with non-adhesive anisotropic micro-structures

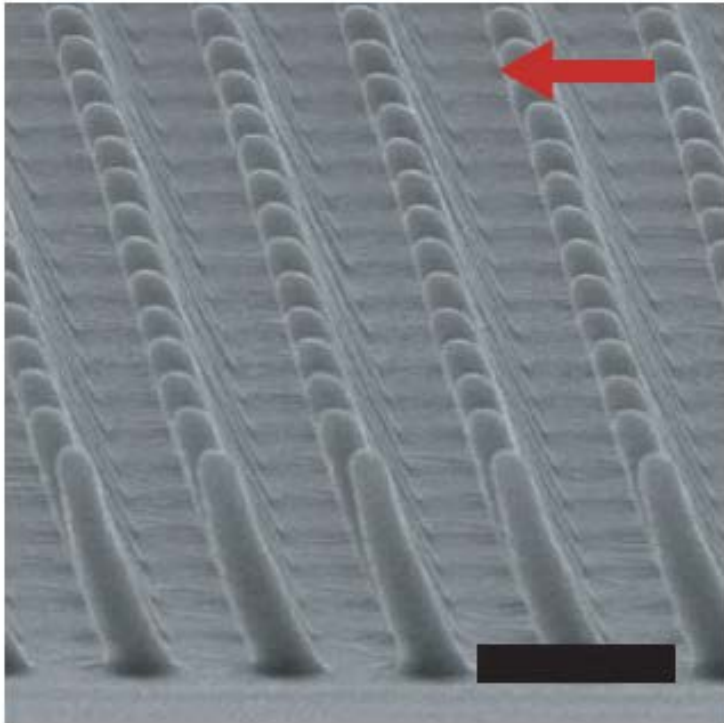
Maël Le Berre  
YanJun Liu



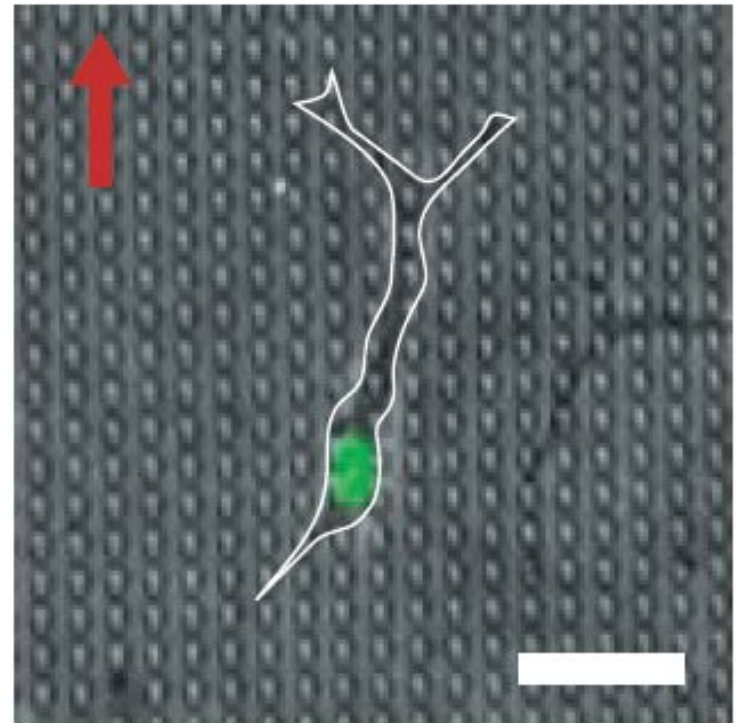
Art from Renaud Chabrier  
[www.renaudchabrier.com](http://www.renaudchabrier.com)

**Nanofabrication:** in collaboration with Y. Chen Lab, ENS Paris

# Cells under tilted micro-pillars



20  $\mu\text{m}$



30  $\mu\text{m}$



Tilted pillar



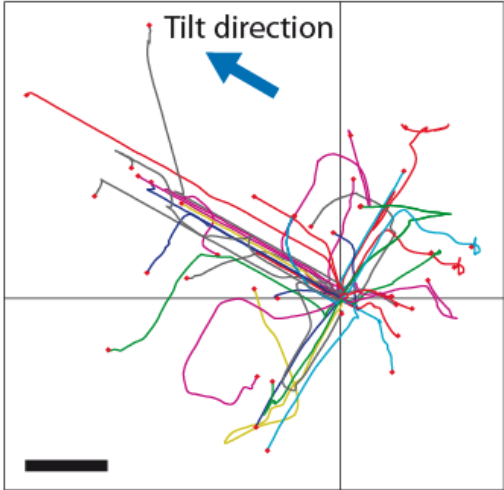
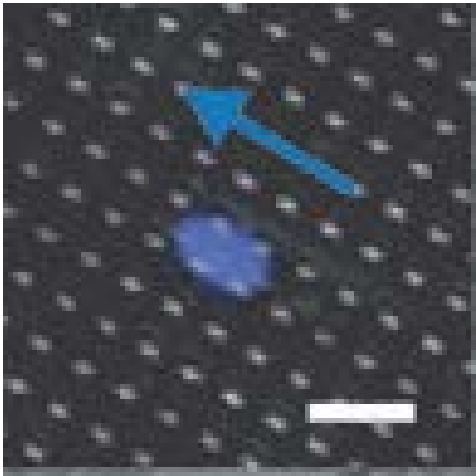
PDMS spacer



Glass coverslip

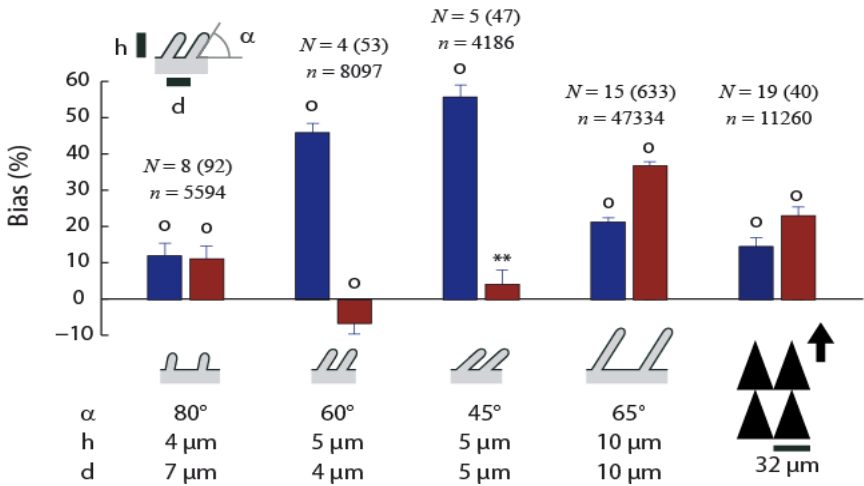
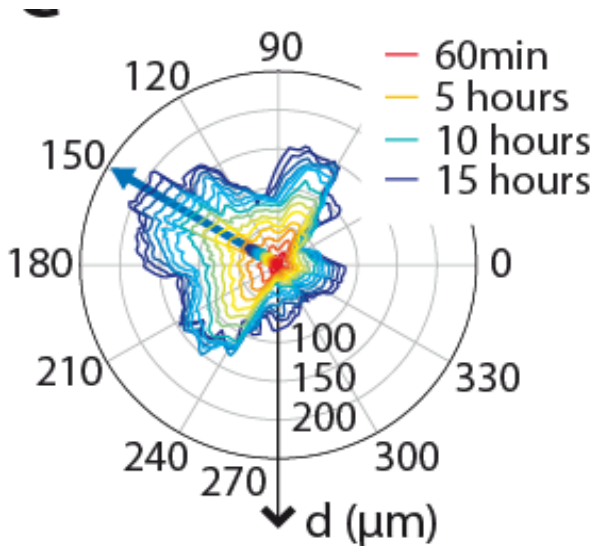


# Trajectory analysis reveals a robust biased migration

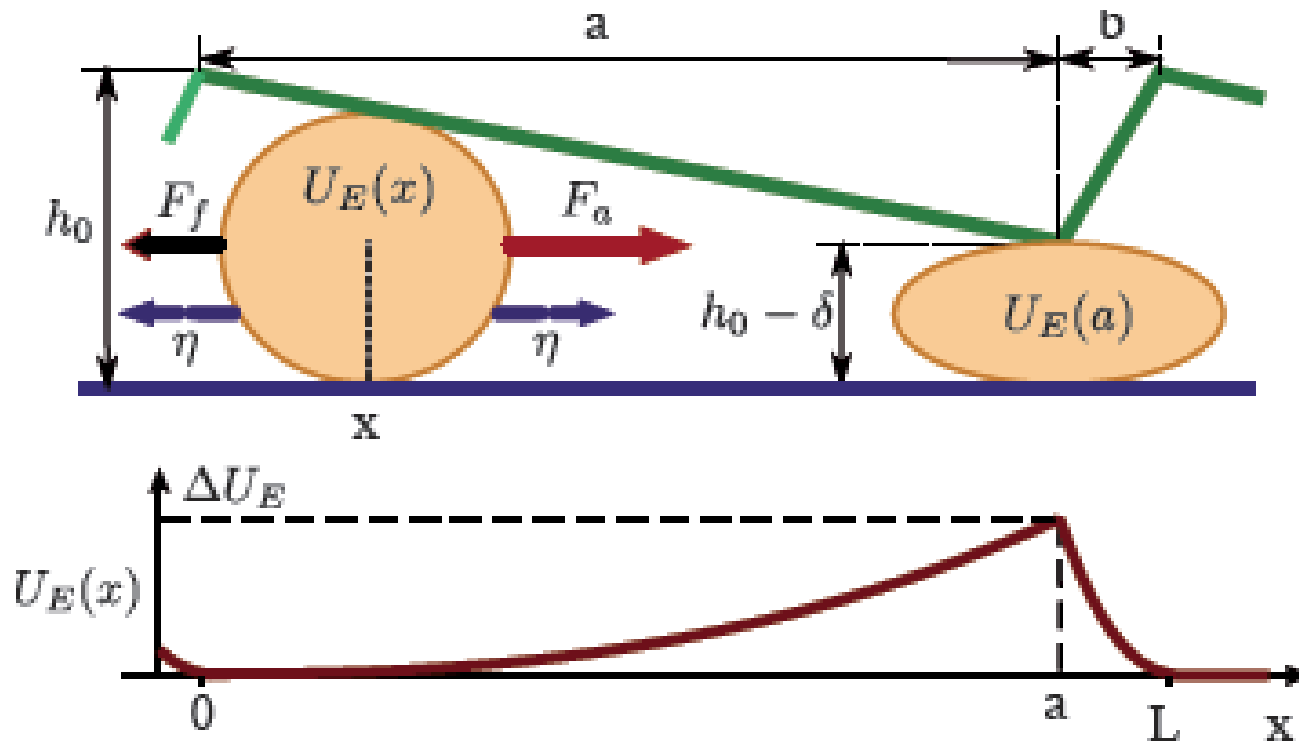


100  $\mu\text{m}$

Whole population bias (50% boundary)



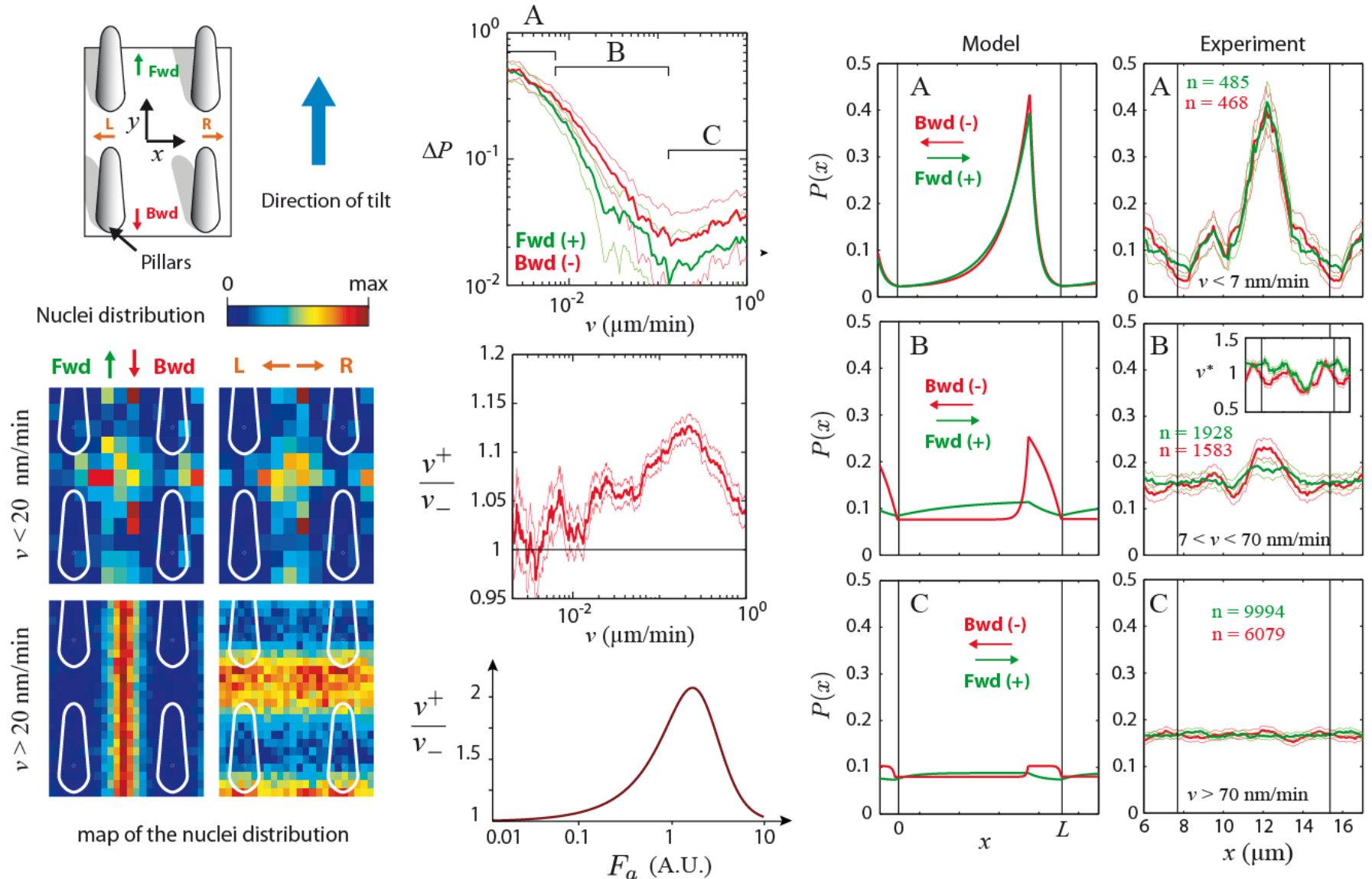
# Simple model of an active Brownian particle in a ratchet potential



$$F_a = \epsilon \bar{F} + \eta(t)$$

$$\lambda d_t x = \epsilon F - \partial_x U_E + \eta$$

# A simple model qualitatively captures the measured statistics on nuclear position



# Conclusions

- 1) Below a minimal size, the nucleus becomes limiting and BMDCs use an Arp2/3 dependant MyoII independent mechanism to squeeze it
- 2) BMDCs can concentrate antigens via a Myosin II dependant filtering system and they do not push fluid in front of them ('viscous catching')
- 3) Geometric anisotropy is enough to bias cell migration, independently of adhesion, due to deformation of the visco-elastic cell body

# Systems Biology of Cell Division and Cell Polarity

## Most wanted Cell Torturers

Franzi



Matthieu

Emmanuel

Hiro

Nicolas

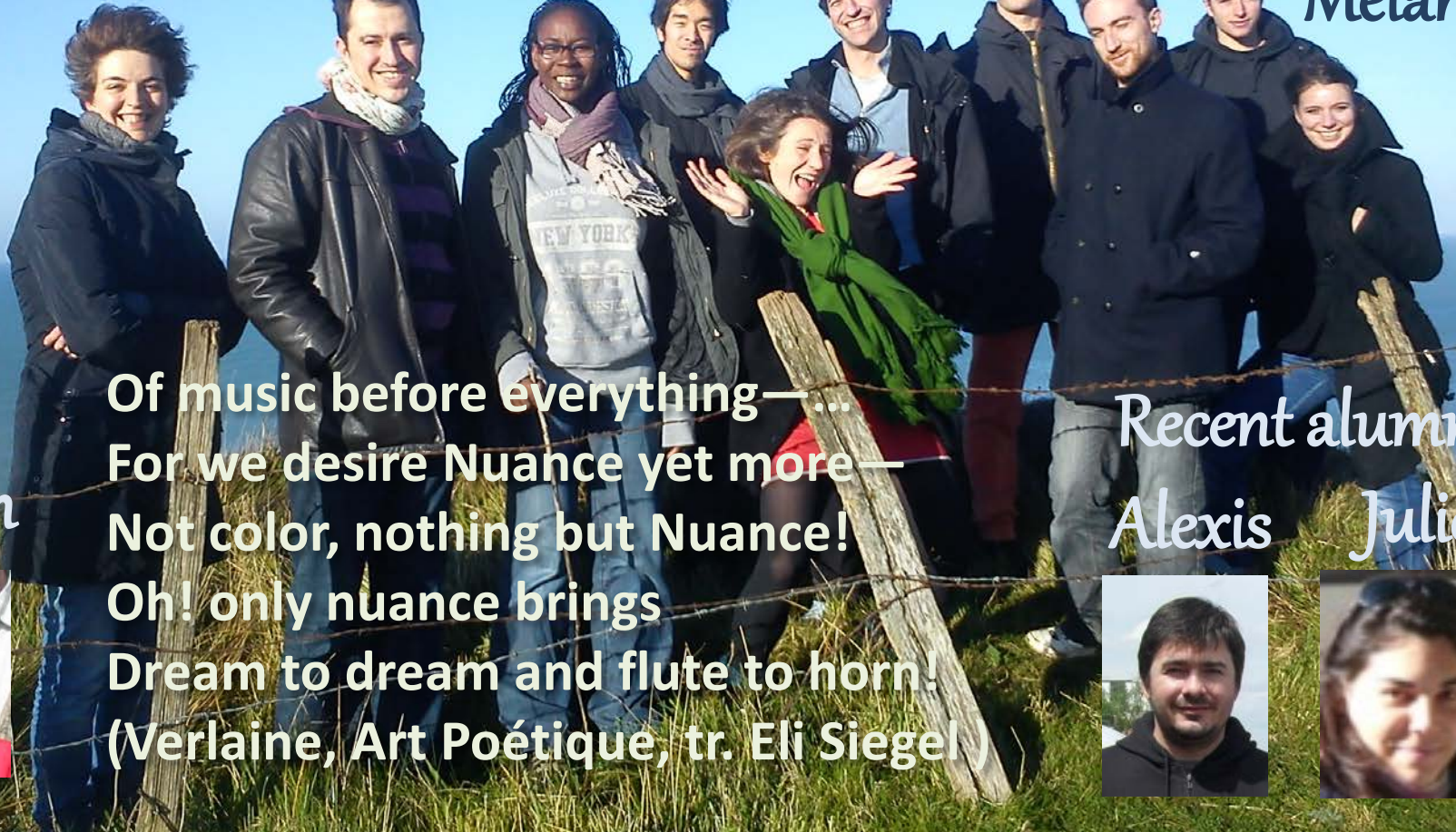
Maël Nicolas

Daria

Hawa Ewa

Mélanie

Paolo



Of music before everything—...  
For we desire Nuance yet more—  
Not color, nothing but Nuance!  
Oh! only nuance brings  
Dream to dream and flute to horn!  
(Verlaine, Art Poétique, tr. Eli Siegel)

Recent alumni

Alexis Julie

Yanjun







# Acknowledgments



## MICEMICO: migration under confinement

- Team of Ana-Maria Lennon (IC, Paris)
- Team of Raphael Voituriez (UPMC, Paris)
- Team of Vassili Soumelis (IC, Paris)
- Team of Danijela Vignjevic (IC, Paris)

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- Yong Chen (ENS Paris)
- Manuel Théry (CEA Grenoble)

## Universal law of cell migration:

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- Verena Ruprecht, CP Heisenberg (IST)
- Nir Gov (Weizmann Institut, Israel)

## Imaging:

- Imaging Platform PICT IBISA
- Nikon Imaging Center

## Plasmids and cells

- Ina Poser (MPI-CBG, Dresden)
- Roland Wedlich-Söldner (MPI, Martinsried)
- more...

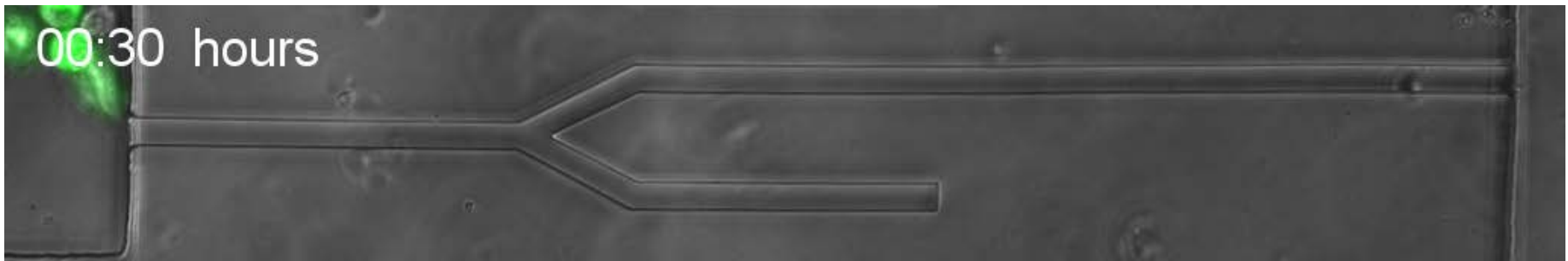
## Current funding



## Former funding



This year cells will race inside micro-channels,  
to give a chance to amoeboid cells to win



Be FAST, PERSISTENT and SMART!

*Beware of the dead-ends and may the best cell win!*

To register or get more information: <http://goo.gl/sk3PQ>

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