Cagliari - 3 MAGGIO 2022
Università degli Studi di Cagliari - Dipartimento di Fisica

Come vetro fuso, come uno stormo. Uno sguardo fisico sull'invasione tumorale

Fabio Giavazzi, Università degli Studi di Milano

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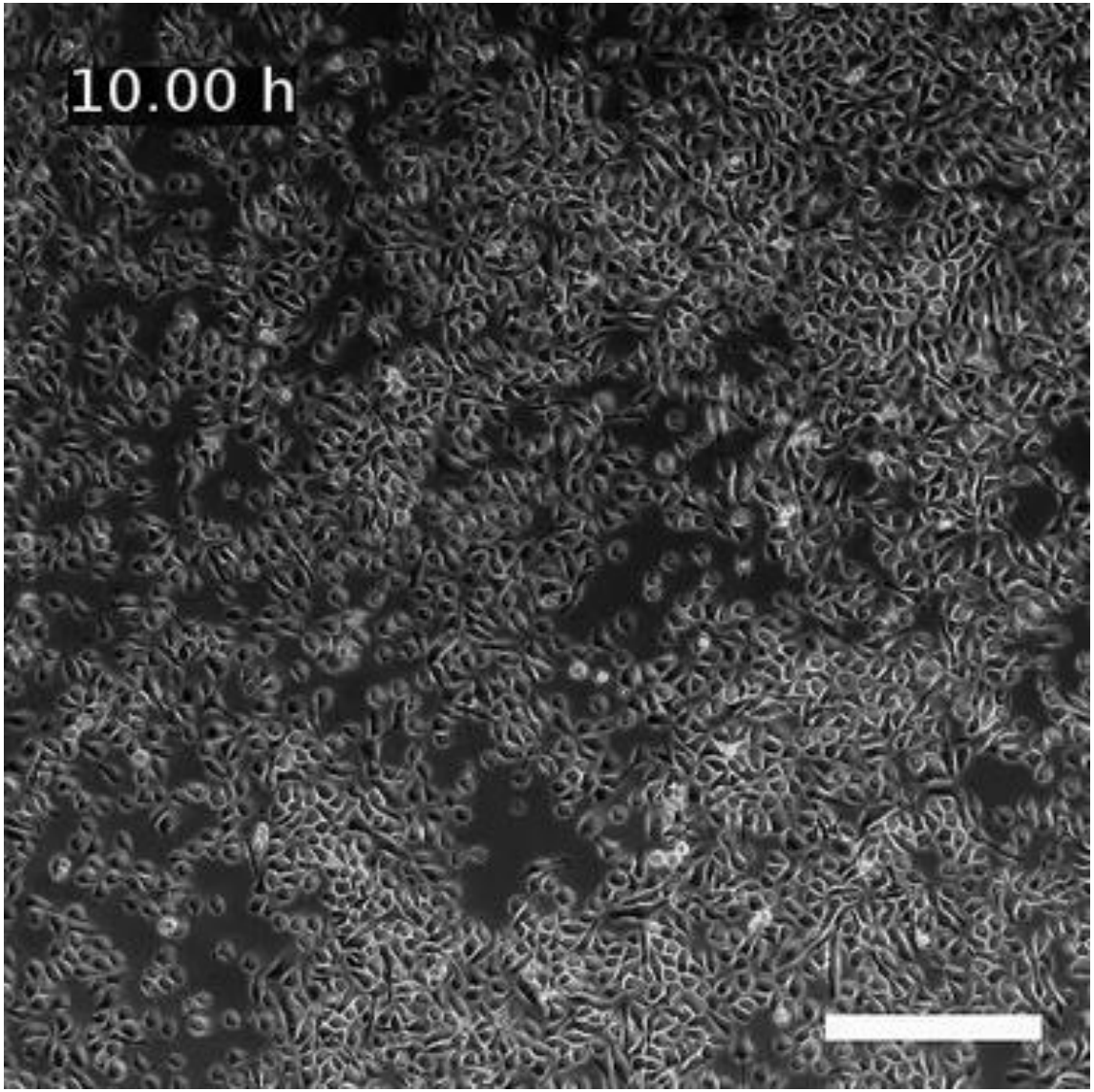
1. **L'epitelio è una barriera solida e impenetrabile**
2. **Le cellule epiteliali isolate sono molto «attive»…**
3. **…fino a che non raggiungono uno stato stazionario e quiescente.**

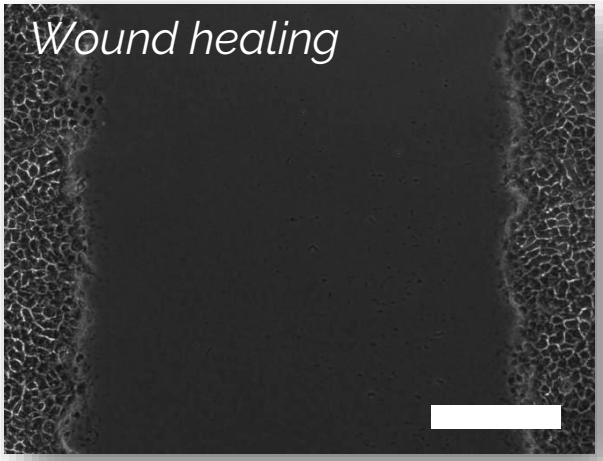
Il était une fois... la Vie (Siamo fatti così)
Albert Barillé, circa 1988

Qualche anno dopo...

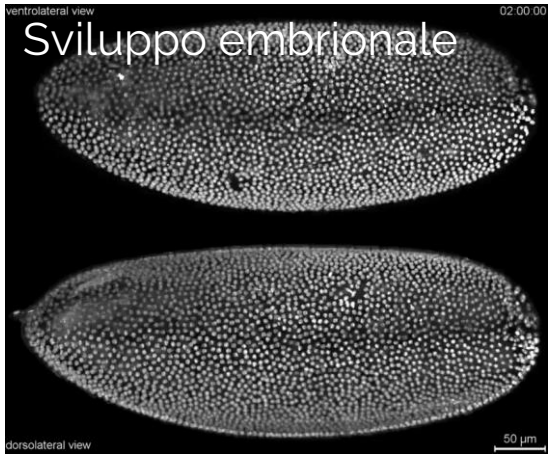


HBEC cells (Scale bar: 300 μm.)
S Garcia *et al.*, PNAS **112**, 15314-15319 (2015)

Jamming e unjamming transitions



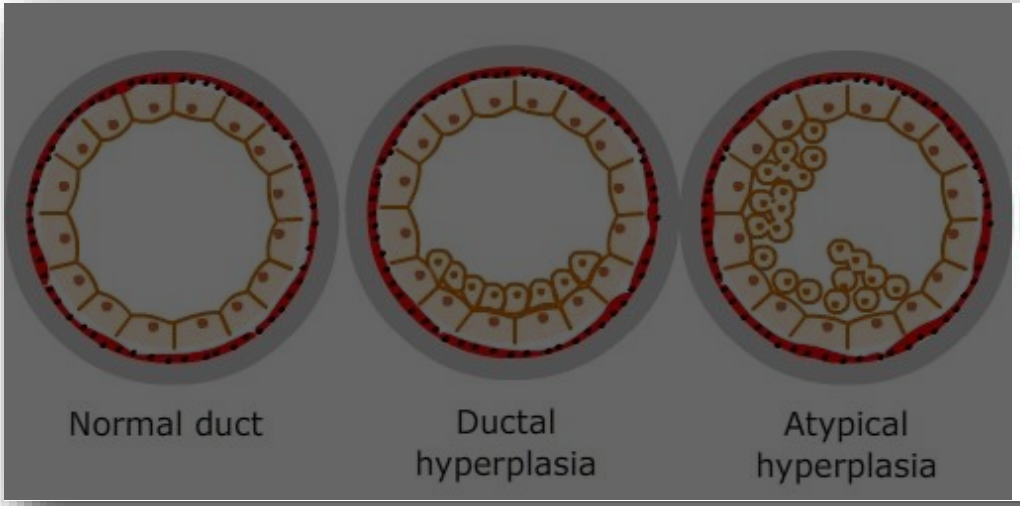
MCF10A epithelial mammary cells
Phase contrast microscopy
Duration: 24 hours -
Scale bar: 300 µm



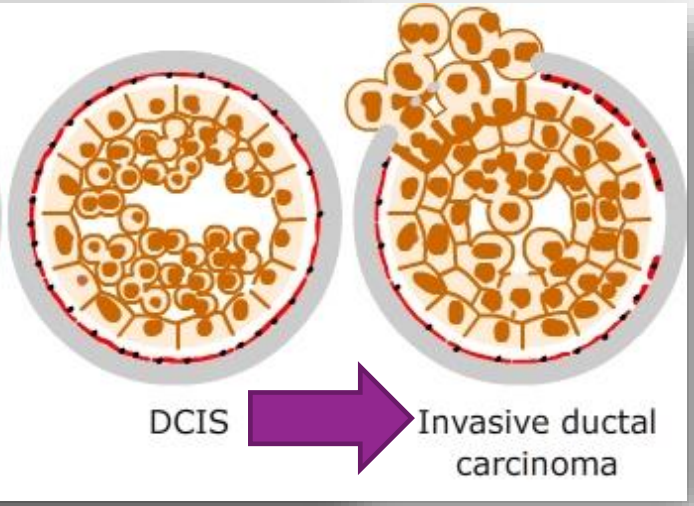
Drosophila melanogaster embryo
Light sheet microscopy

R. Tomer et al. Nat. Methods **9**, 755 (2012)

Carcinoma duttale *in situ*

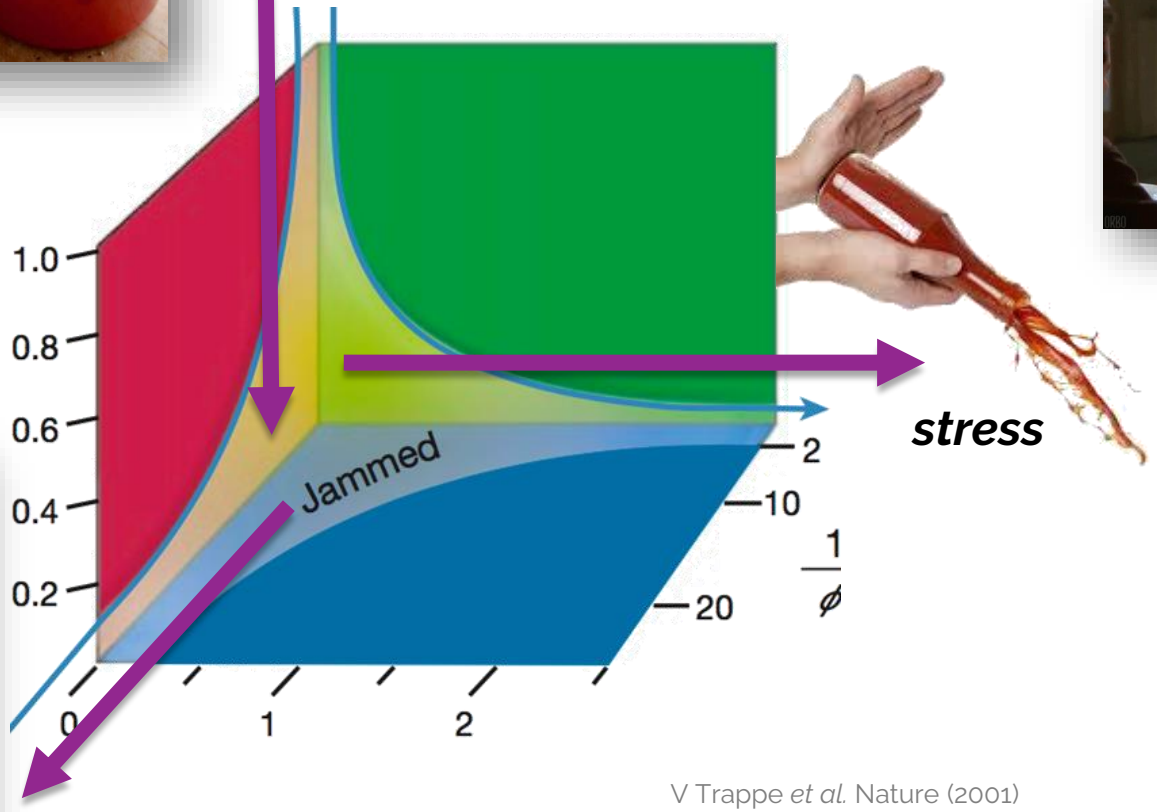


Carcinoma duttale invasivo



Jamming e unjamming transitions

temperatura/
energia di interazione attrattiva

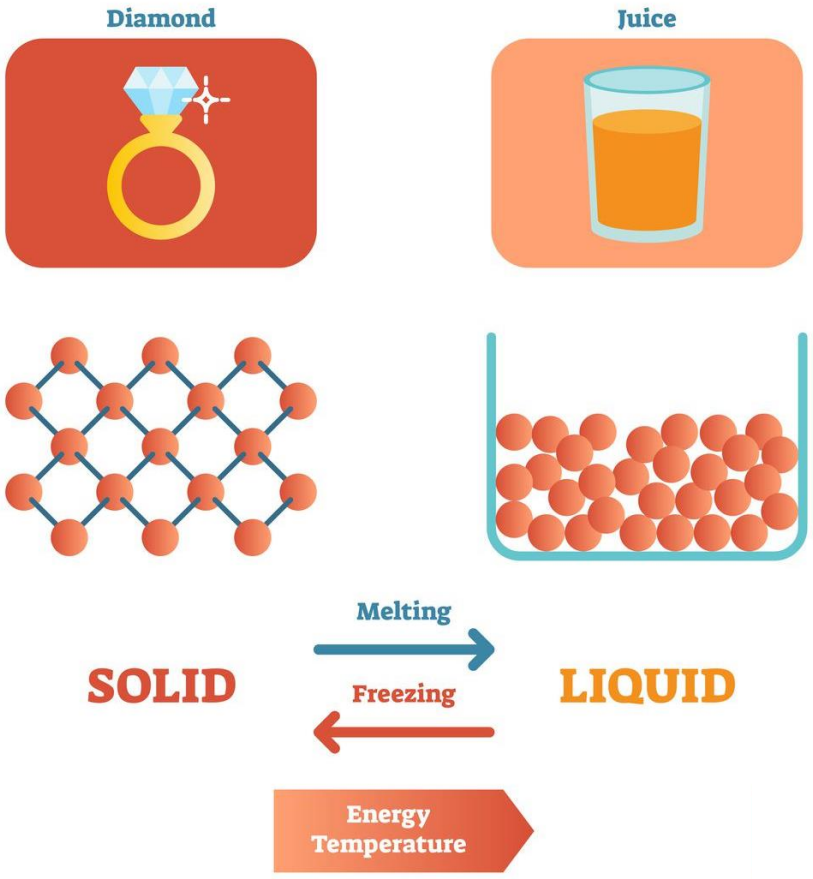


1/densità

V Trappe *et al.* Nature (2001)

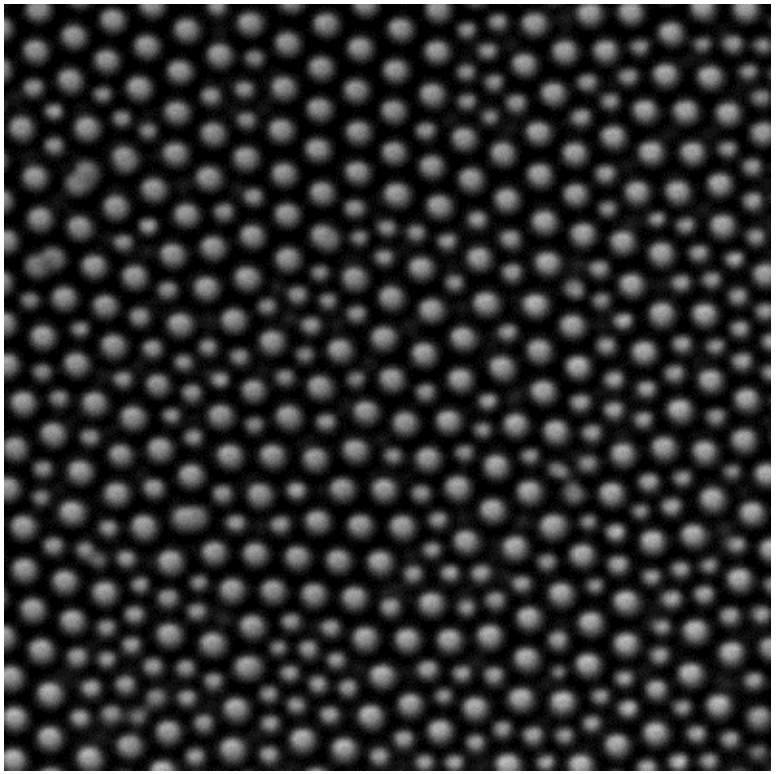
…un altro ricordo di infanzia…

States of Matter



Transizioni solido-liquido in sistemi amorfi

$\phi = 71\%$

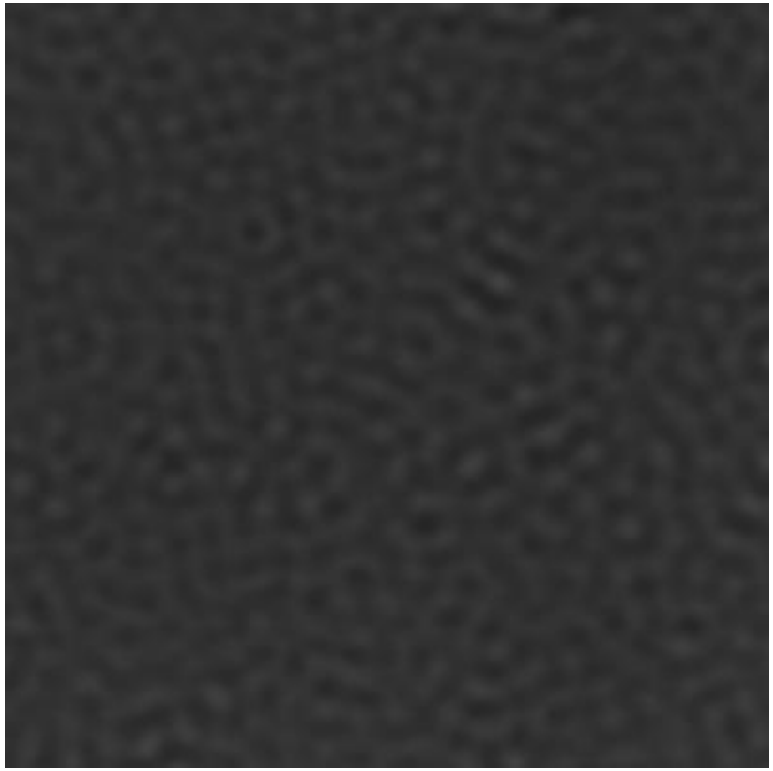


$\phi = 79\%$

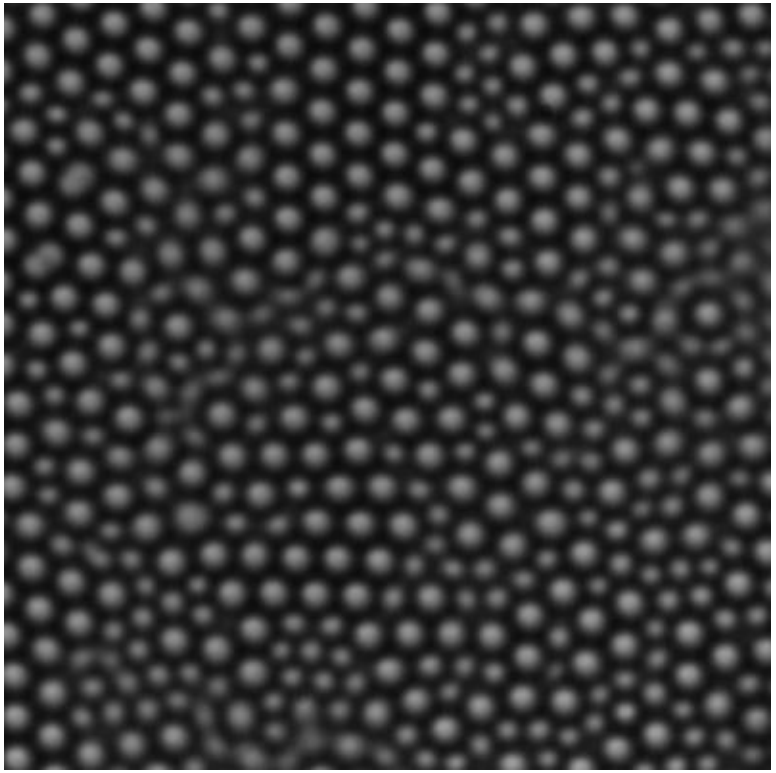
BF microscopy
Duration: 30 min
Image size: 60 μm

R Pastore *et al.*, J. Chem. Phys. **156**, 164906 (2022)

Transizioni solido-liquido in sistemi amorfi



$\phi = 71\%$

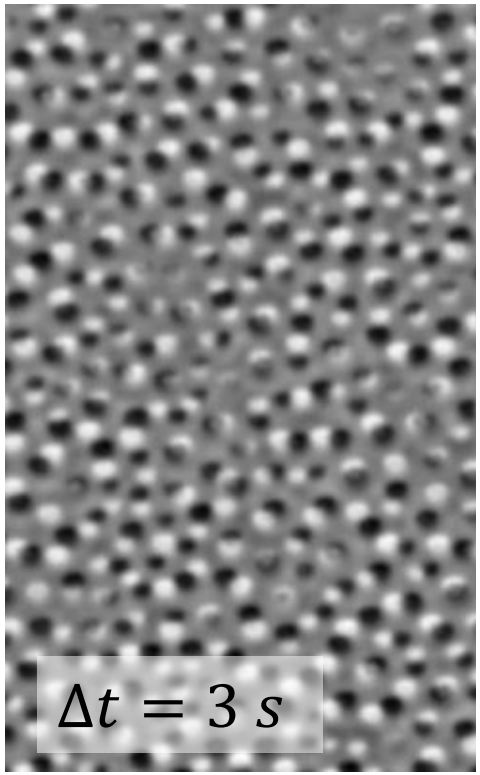


$\phi = 79\%$

Intensità media

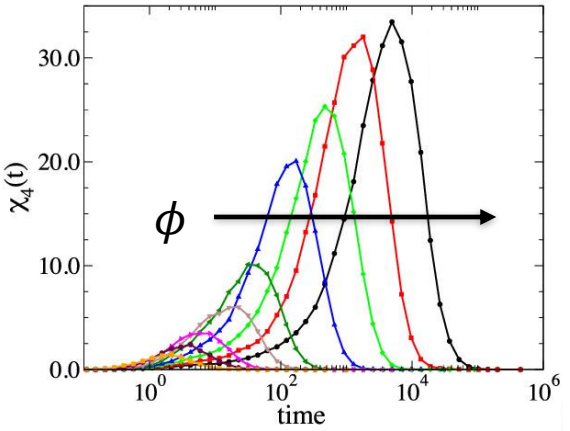
R Pastore *et al.*, J. Chem. Phys. **156**, 164906 (2022)

Transizioni solido-liquido in sistemi amorfi: eterogeneità dinamica

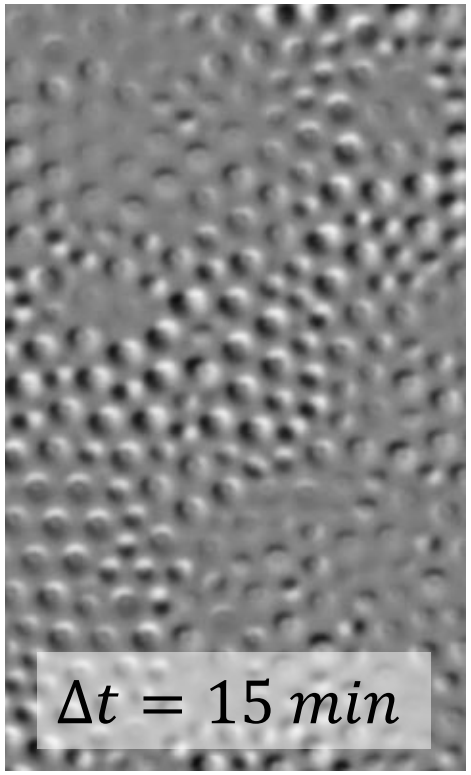


$\phi = 71\%$

Suscettività dinamica



La posizione del picco di χ_4 corrisponde al tempo di rilassamento, la sua 'altezza al numero medio di particelle coinvolte in un riarrangiamento collettivo.



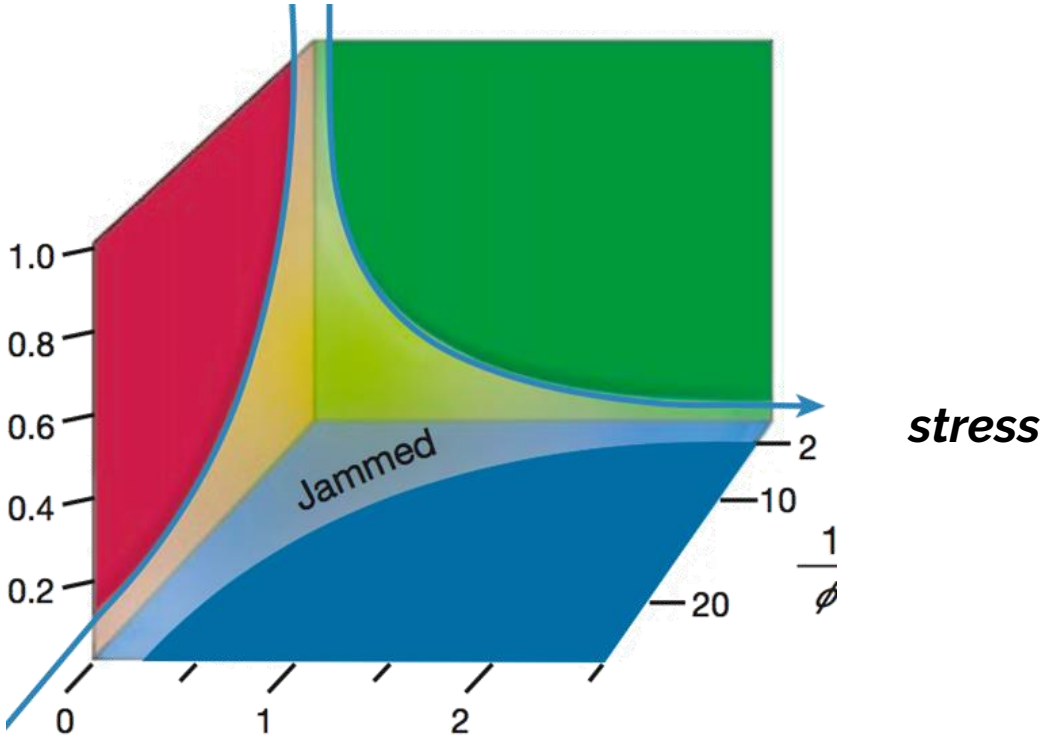
$\phi = 79\%$

Mappe di attività
(differenze di immagini)

R Pastore *et al.*, J. Chem. Phys. **156**, 164906 (2022)

Jamming e unjamming transitions

temperatura/
energia di interazione attrattiva

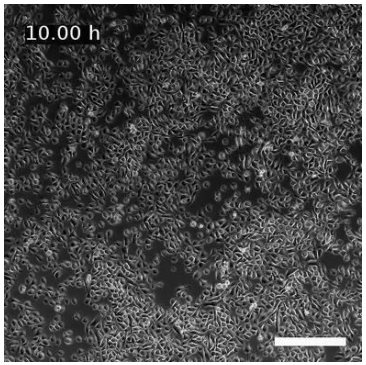


stress

V Trappe *et al.* Nature (2001)

1/densità

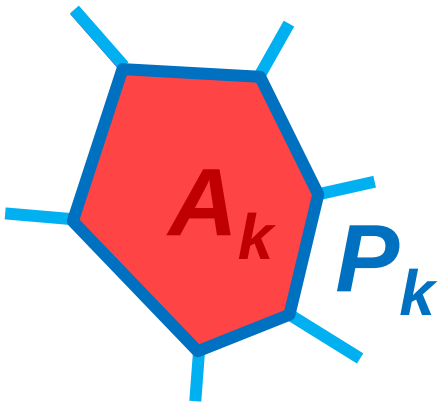
Jamming e *unjamming transitions* nei tessuti biologici

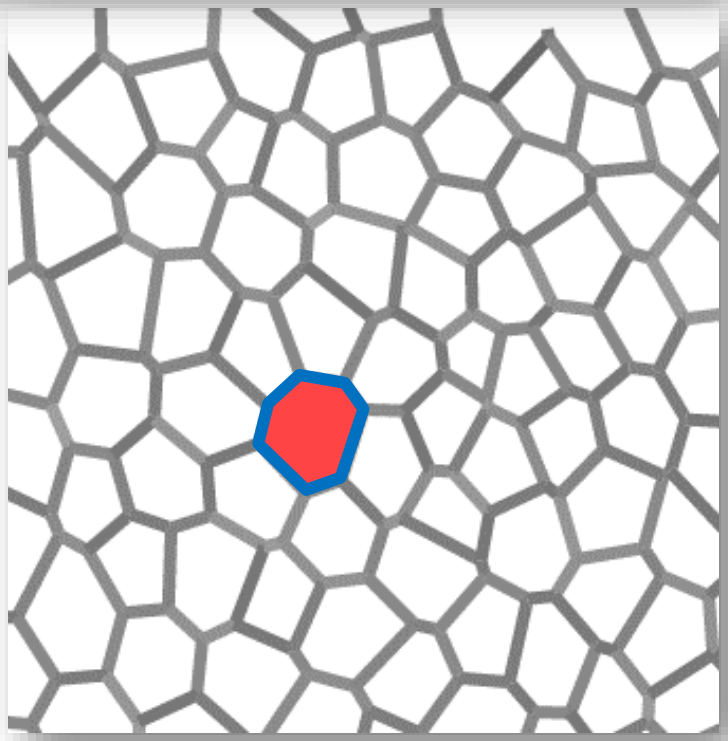


E. ElizabethLawson-Keister *et al.*
Current Opinion in Cell biology (2021)

Jamming transition a densità costante: *vertex model*

$$E = \sum_k \left[K_A (A_k - A_0)^2 + K_P (P_k - P_0)^2 \right]$$



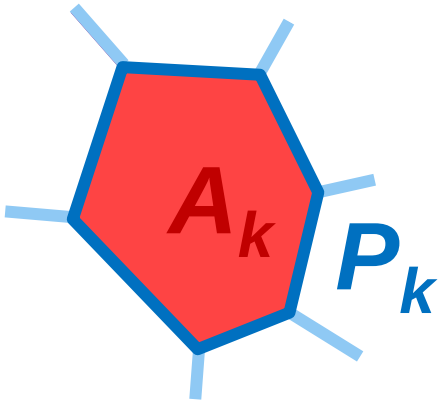


ML Manning et al., *PNAS* **107**, 12517-12522 (2010)
D Bi et al, *Nature Physics* **11**, 1074–1079 (2015)

Jamming transition a densità costante: *vertex model*

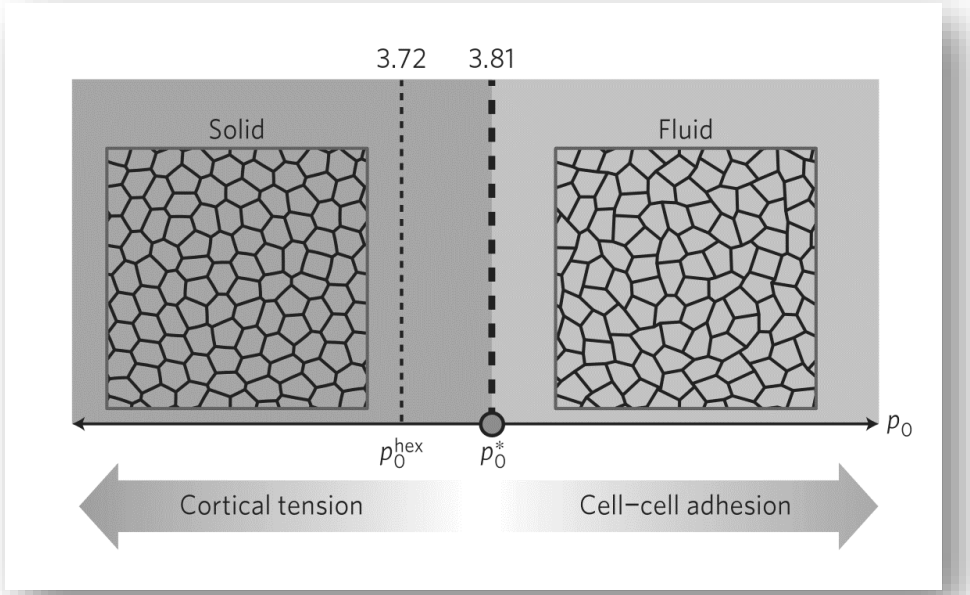
$$E = \sum_k \left[K_A (A_k - A_0)^2 + K_P (P_k - P_0)^2 \right]$$

Termine di *bulk* **Termine interfacciale**
(tensione corticale *vs* adesione cellulare)



$$p_0 = \frac{P_0}{\sqrt{A_0}}$$

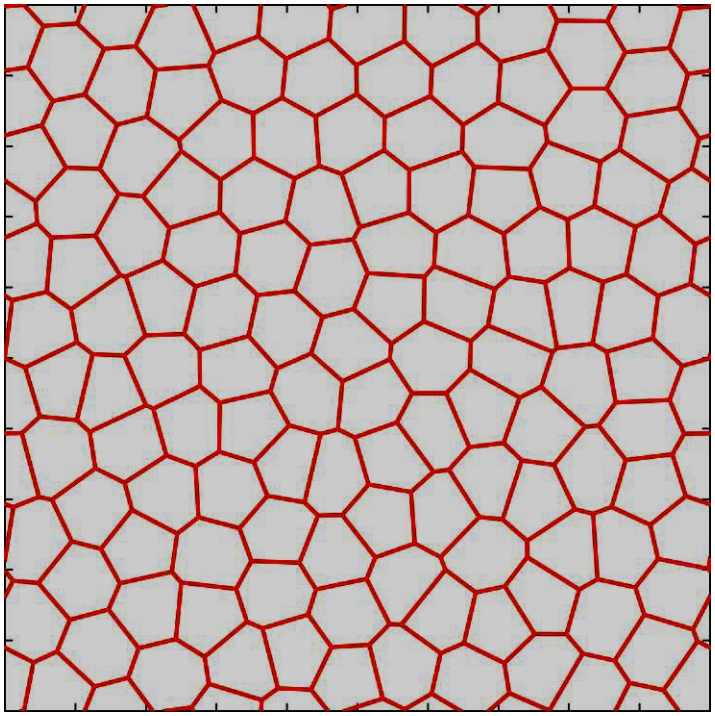
Shape factor



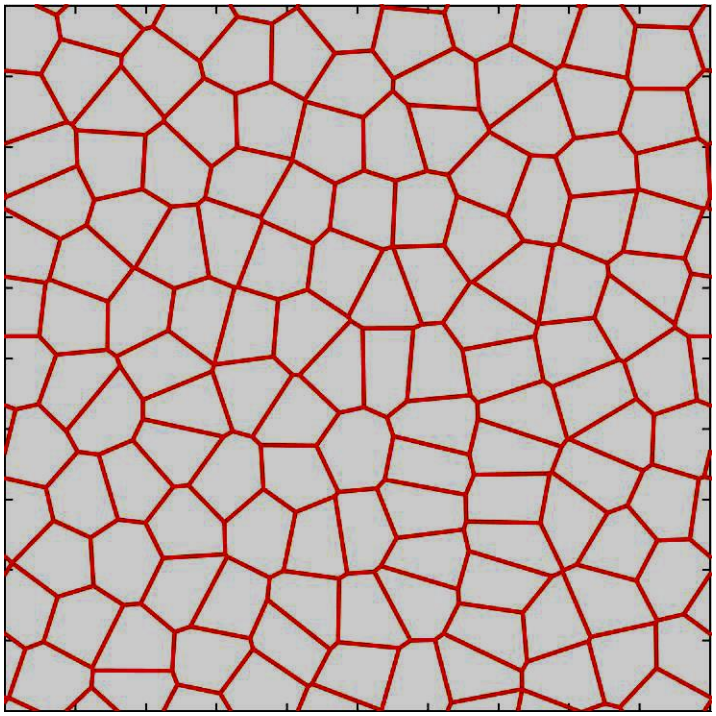
D Bi et al, *Nature Physics* **11**, 1074–1079 (2015)

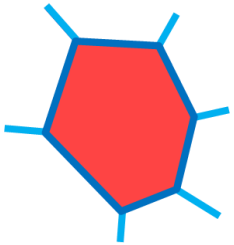
Una transizione «scritta nella forma»

Solid



Fluid





Cortical tension

$$p_0 = \frac{P_0}{\sqrt{A_0}}$$

Cell–cell adhesion

D Bi et al., Phys. Rev. X **6**, 021011 (2016)

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i nostri esperimenti

Unjamming «endocitico»

RAB5A

- È un importante regolatore della attività endocitica della cellula (**internalizzazione di fludi / *recycling* della membrana**)
- E' spesso sfruttato da alcuni **tumori epiteliali** particolarmente aggressivi per promuovere la loro disseminazione

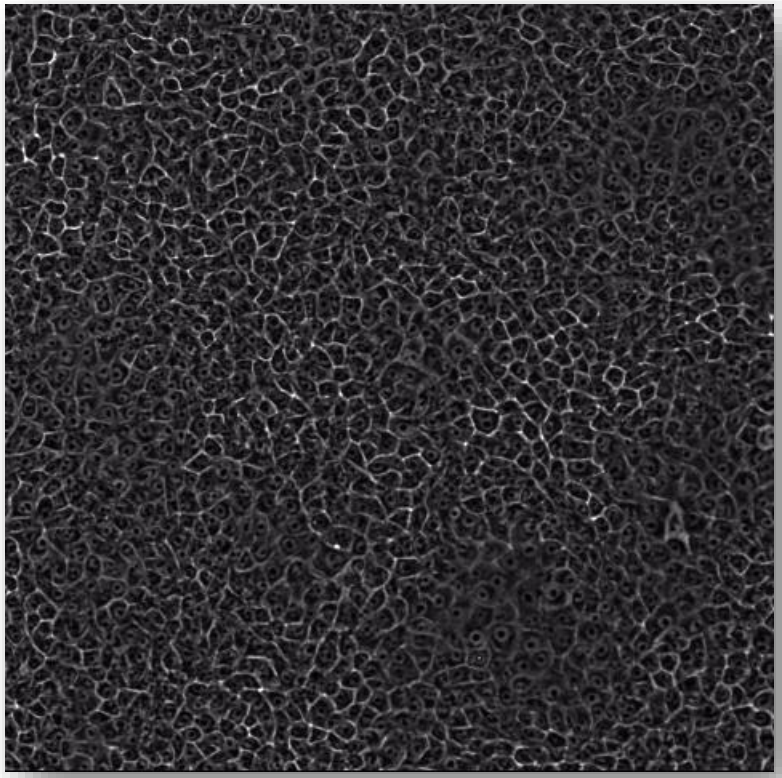
MCF10A cells

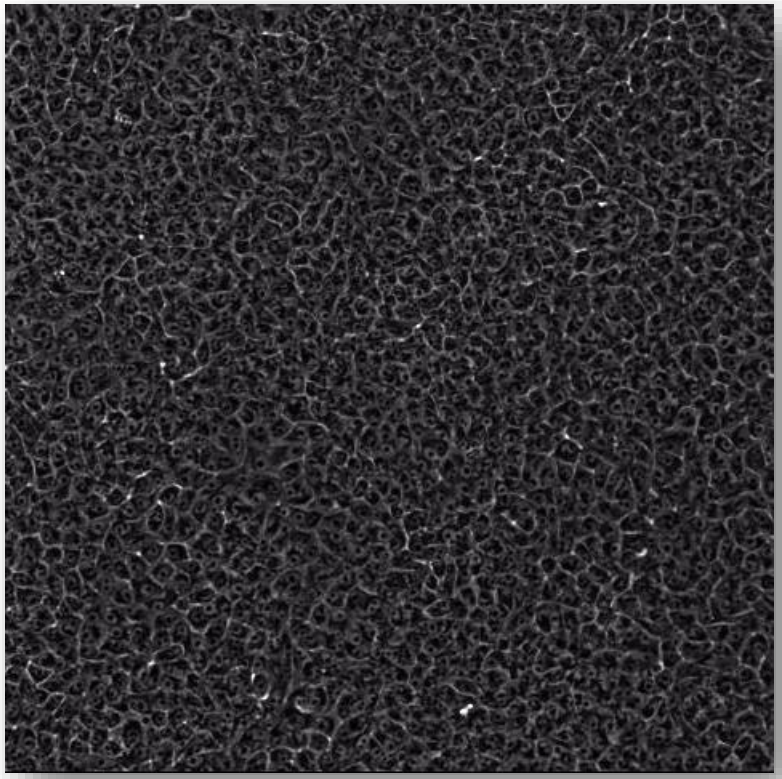
PC microscopy
Duration: 24 h
Image size: 1.3 mm

CTRL

RAB5A

Unjamming «endocitico»





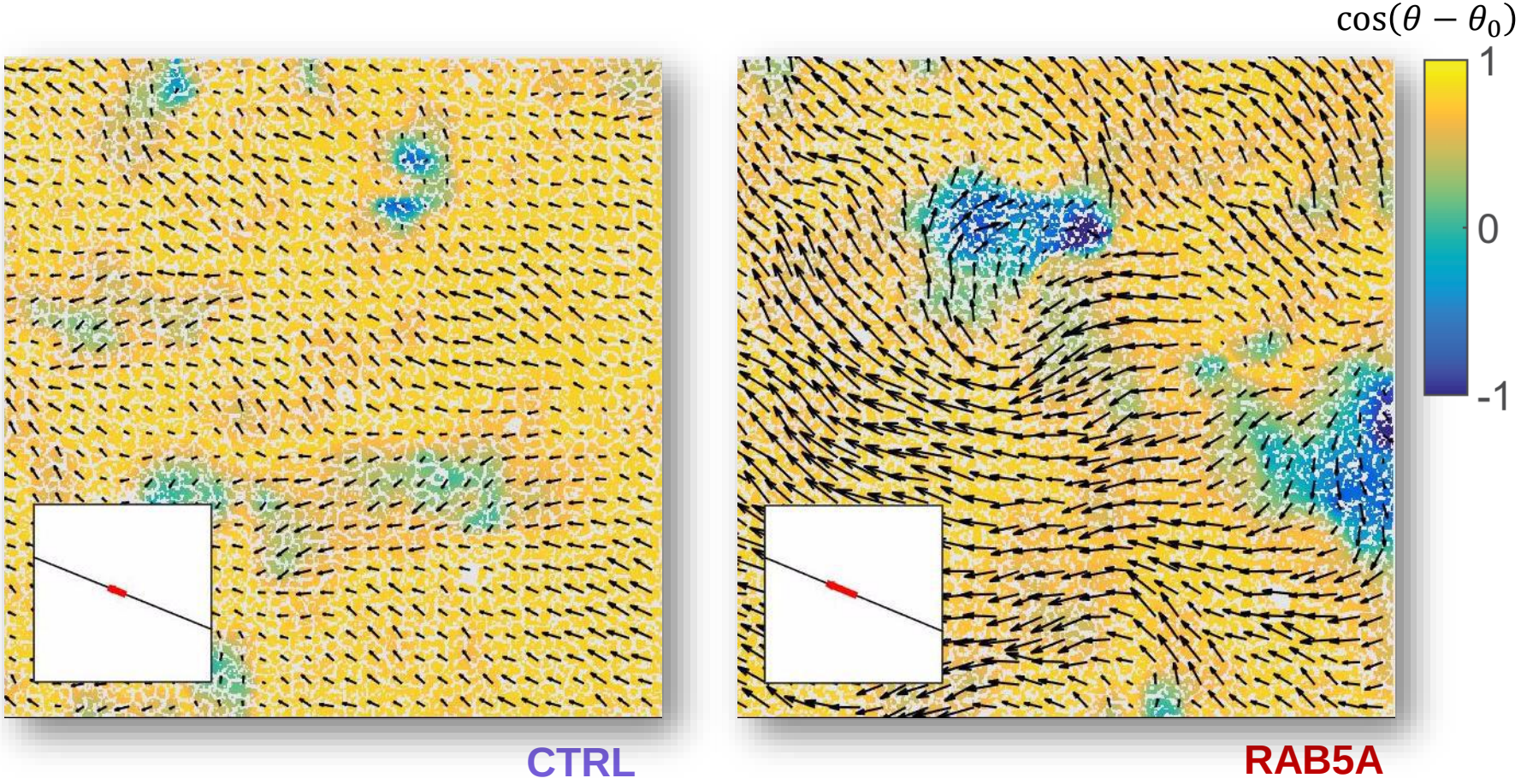
MCF10A cells

CTRL

RAB5A

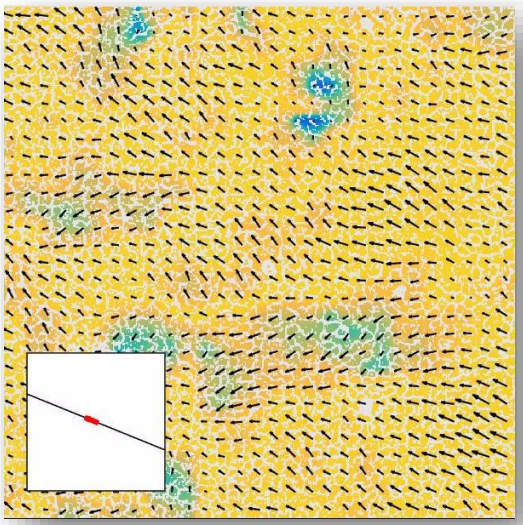
PC microscopy
Duration: 24 h
Image size: 1.3 mm

Unjamming «endocitico»

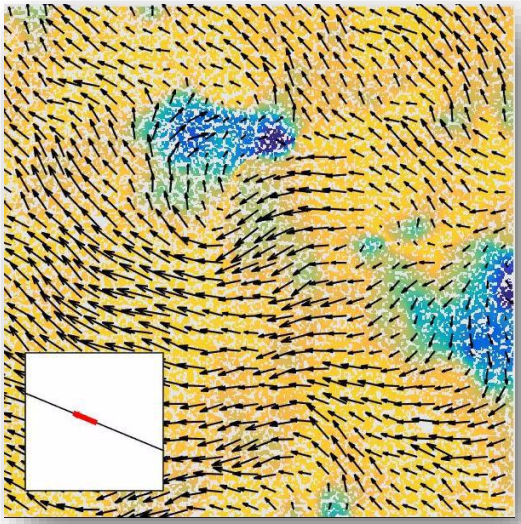


PIV Maps
Duration: 24 h
Image size: 1.3 mm

Unjamming «endocitico»

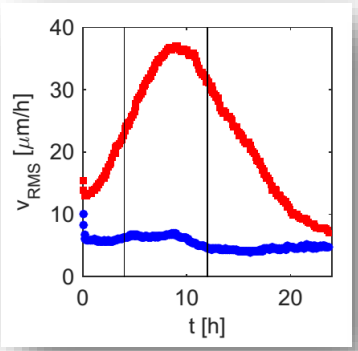


CTRL

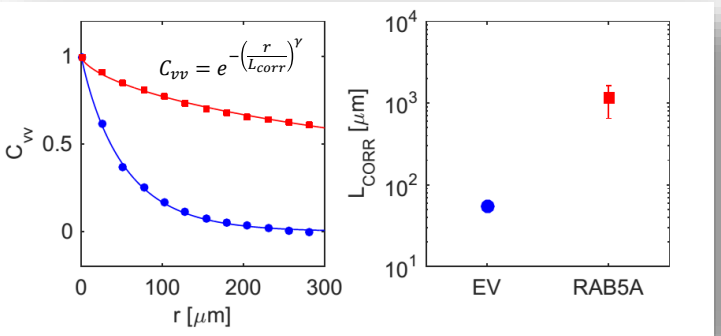


RAB5A

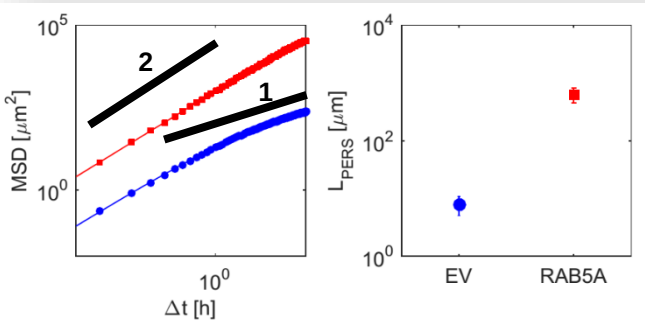
Velocità di migrazione



Correlazione spaziale di velocità



Persistenza del moto di singola cellula



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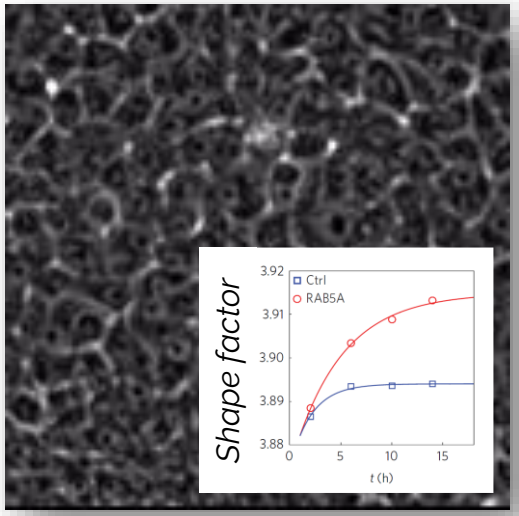
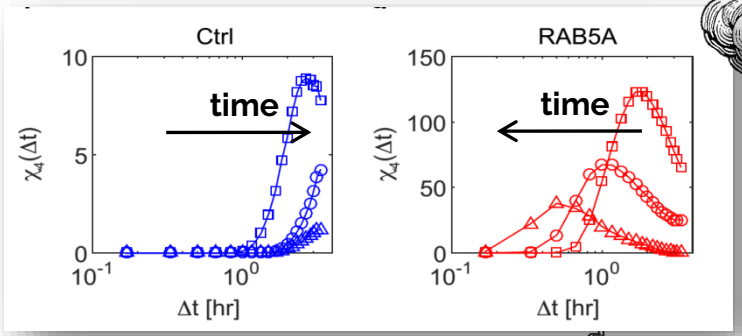
Unjamming «endocitico»

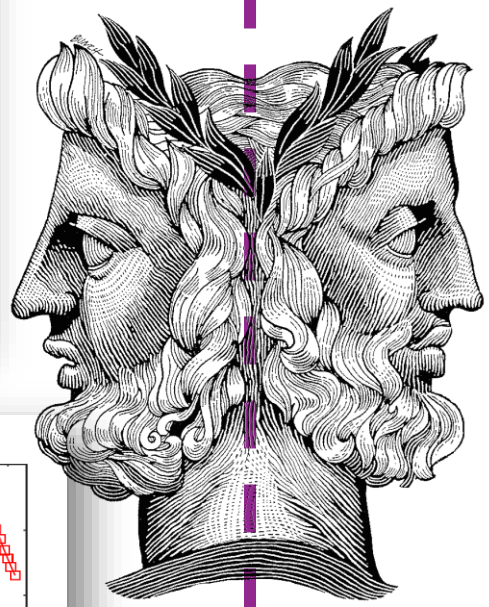
Localmente? È un fluido!

~100 mm



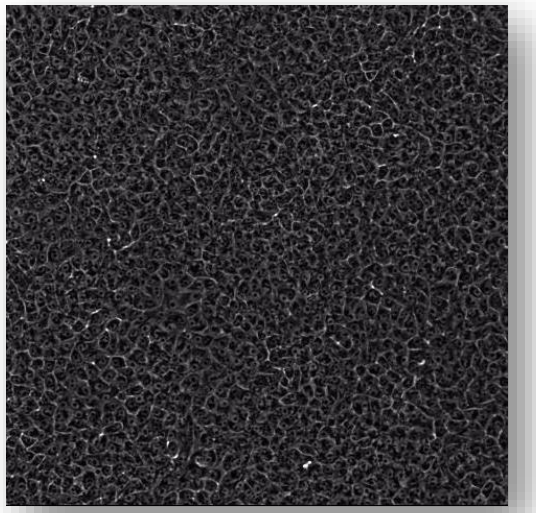


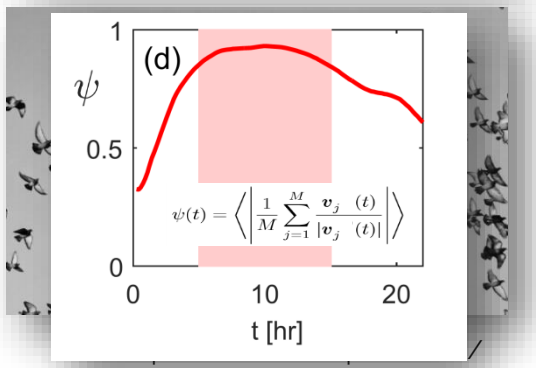
χ_4 : suscettività dinamica



Globalmente? È uno stormo!

~1 mm

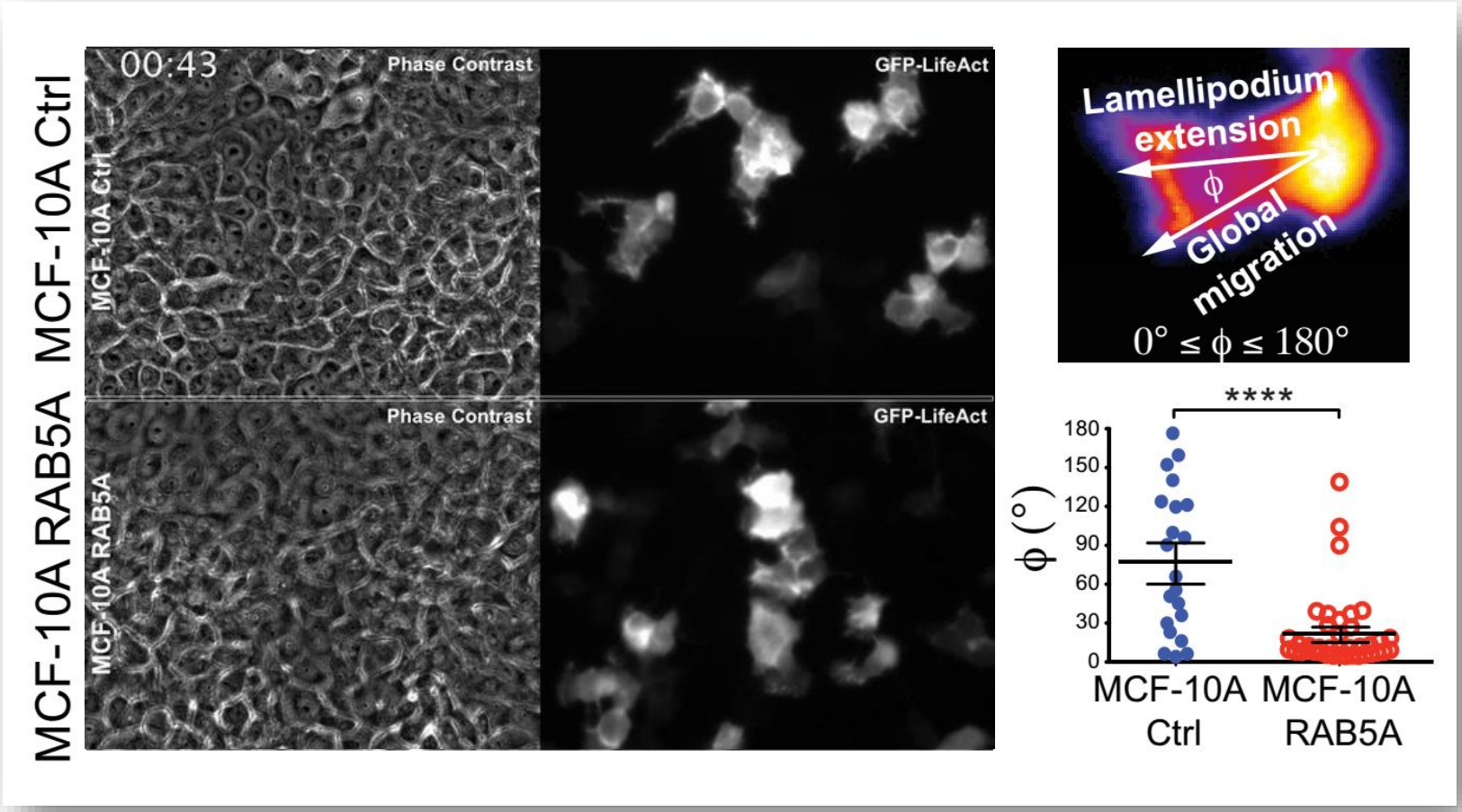




Parametro d'ordine polare

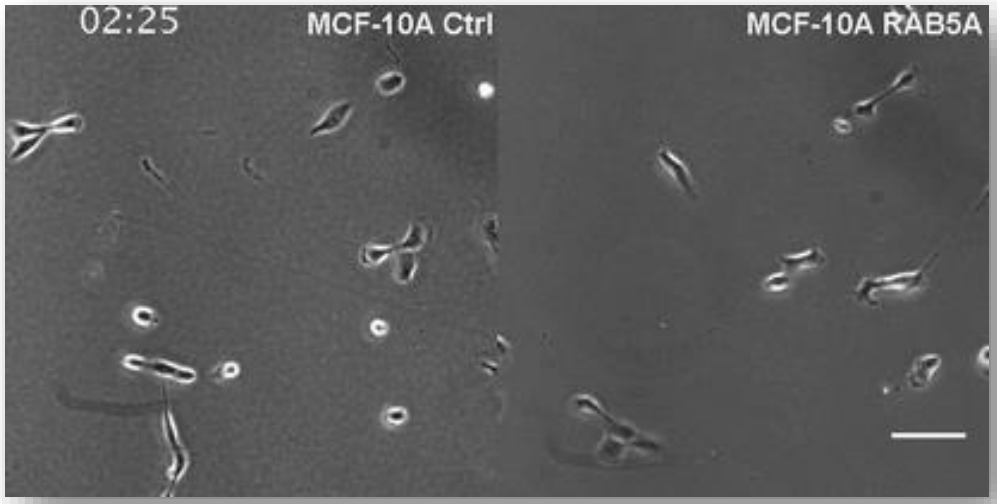
Globalmente? E' uno stormo!

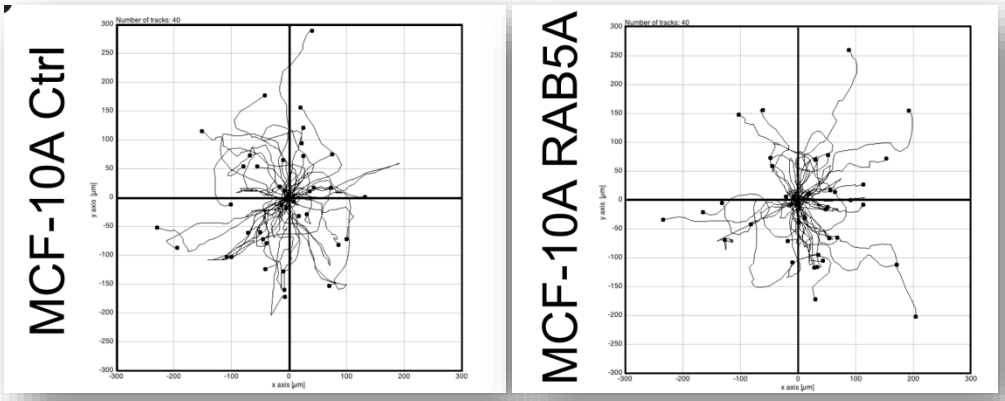
Estensione di persistenti *cryptic lamellipodia* orientati lungo la direzione della migrazione collettiva



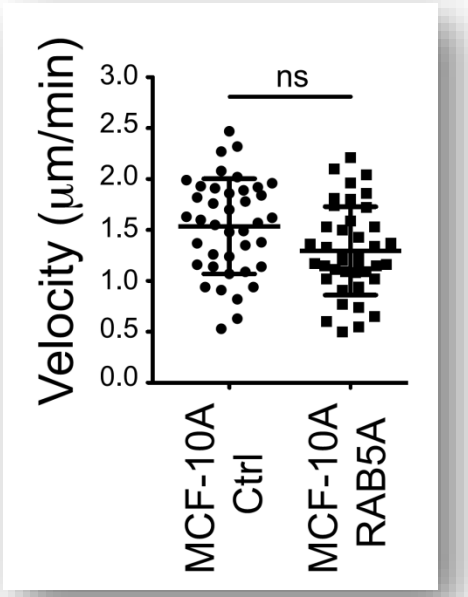
…non una proprietà di singola cellula!

CTRL **RAB5A**





Traiettorie di singola cellula



Velocità media di migrazione

Un modello minimale...

Interazione cellula-cellula

$$E = \sum_k \left[K_A (A_k - A_0)^2 + K_P (P_k - P_0)^2 \right]$$

+

Motilità cellulare e *self-propulsion*

$$\frac{d\boldsymbol{r}_k}{dt} = \mu \boldsymbol{F}_k + v_0 \hat{\boldsymbol{n}}_k \qquad \boldsymbol{F}_k = -\nabla_{\boldsymbol{r}_k} E$$

+

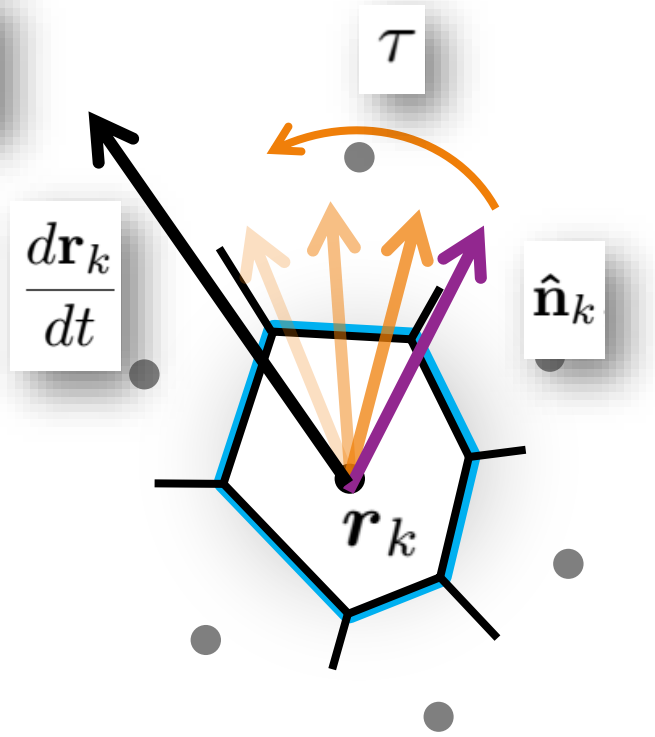
Dinamica di allineamento

$$\partial_t \theta_k = \frac{1}{\tau} \sin\left(\theta_k - \phi_k\right) + \eta_k$$

Rumore

Direzione della velocità di *self-propulsion*

Direzione della risultante delle forze

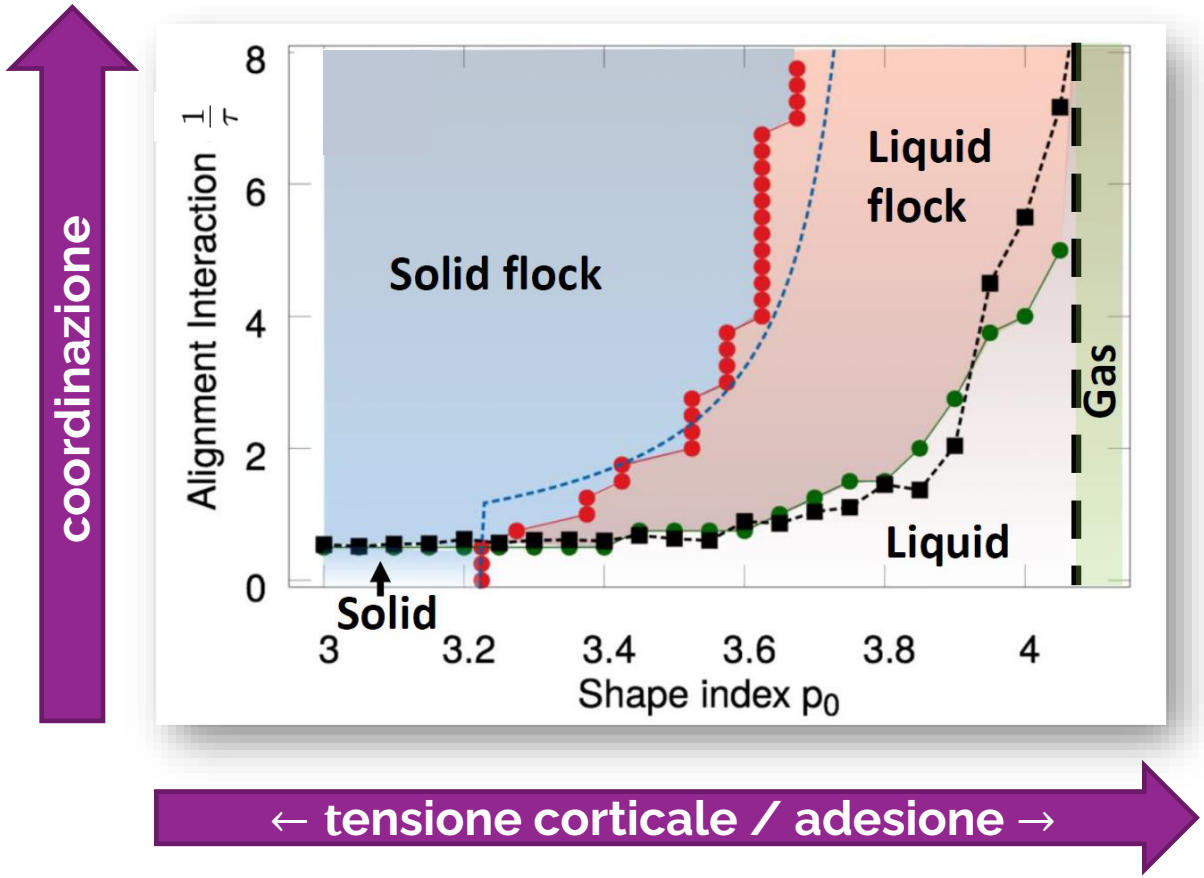


D Bi et al., *Phys. Rev. X* **6**, 021011 (2016)

B Szabo. et al. *Phys Rev E* **74**, 061908 (2006)

F Giavazzi, M Paoluzzi et al. *Soft Matter* **14**, 3471 (2018)

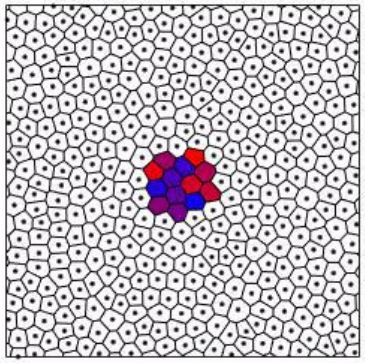
Un modello minimale...



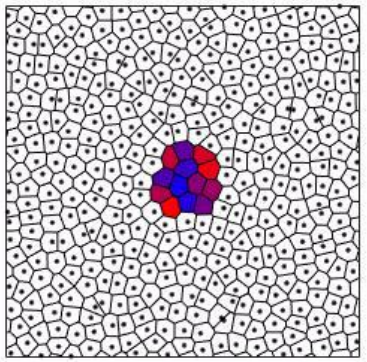
F Giavazzi, M Paoluzzi *et al*. *Soft Matter* **14**, 3471 (2018)

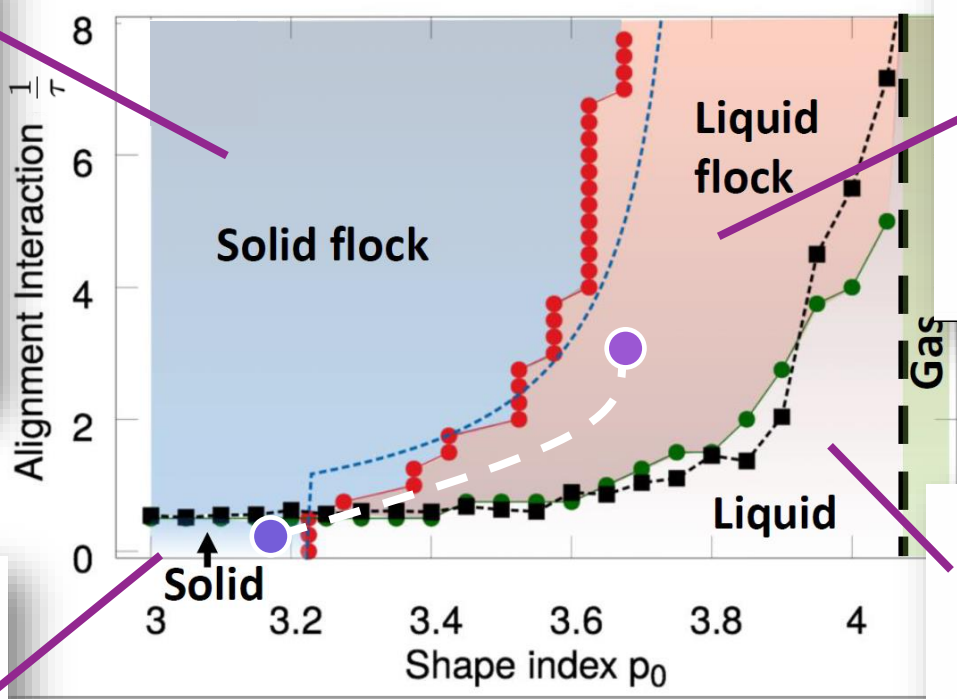
Un modello minimale...

SOLID FLOCK

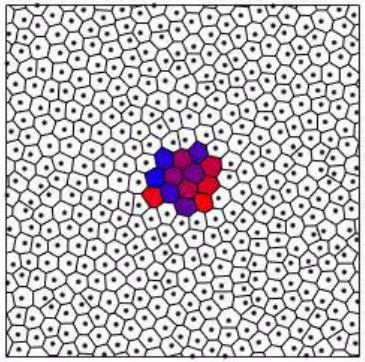


LIQUID FLOCK

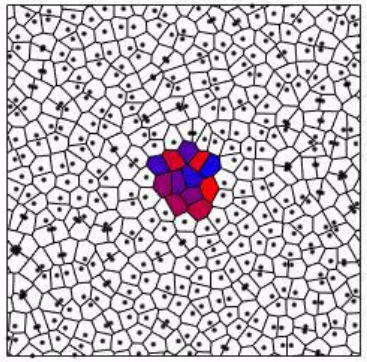




SOLID



LIQUID



← tensione corticale / adesione →

F Giavazzi, M Paoluzzi *et al.* *Soft Matter* **14**, 3471 (2018)

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nature materials

ARTICLES

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Endocytic reawakening of motility in jammed epithelia

Chiara Malinverno[1†], Salvatore Corallino[1†], Fabio Giavazzi[2*], Martin Bergert[3], Qingsen Li[1], Marco Leoni[4], Andrea Disanza[1], Emanuela Frittoli[1], Amanda Oldani[1], Emanuele Martini[1], Tobias Lendenmann[3], Gianluca Deflorian[1], Galina V. Beznoussenko[1], Dimos Poulikakos[3], Kok Haur Ong[5], Marina Uroz[6,7,8,9], Xavier Trepat[6,7,8,9], Dario Parazzoli[1], Paolo Maiuri[1], Weimiao Yu[5], Aldo Ferrari[3*], Roberto Cerbino[2*] and Giorgio Scita[1,10*]

Soft Matter

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Flocking transitions in confluent tissues†

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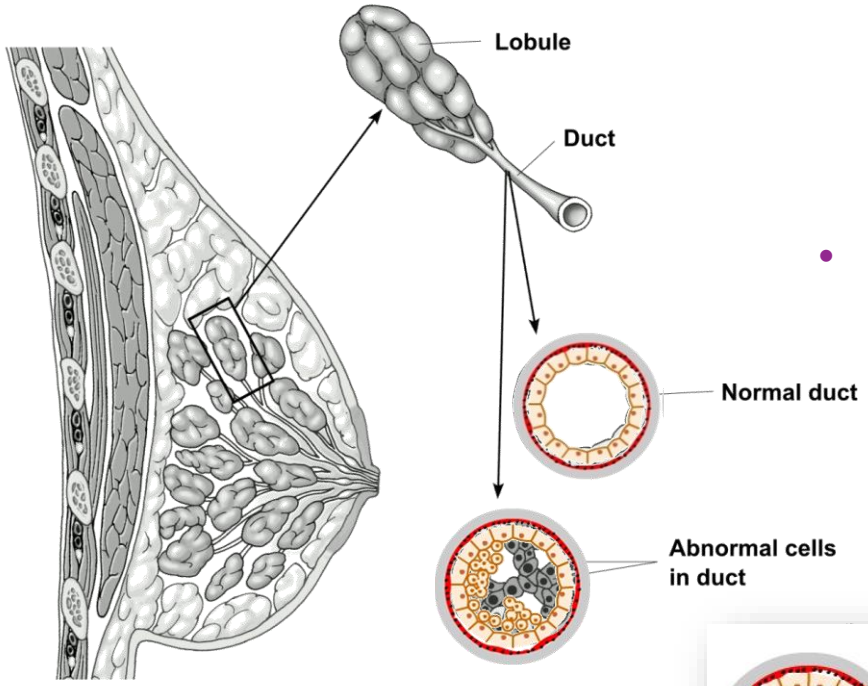
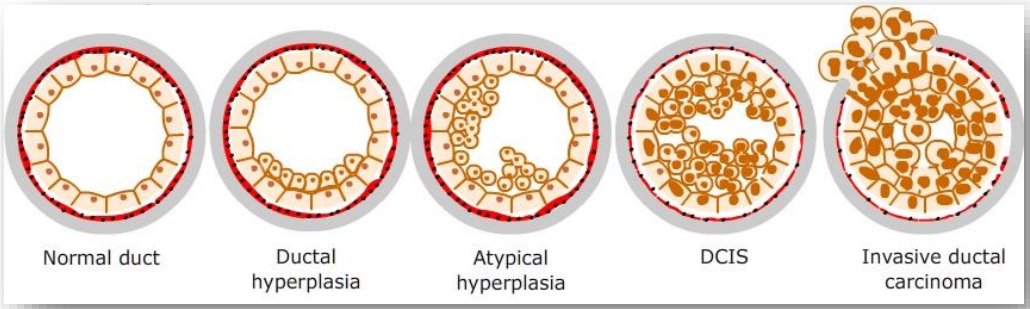


- Isogeniche alle MCF10A, **riproducono** *in vitro* **la progressione da** Carcinoma duttale in situ (**DCIS**) **a** carcinoma duttale invasivo (**IDC**)
- Durante la fase DCIS, **l'estremo confinamento e l'elevata densità** cellulare portano ad un notevole stress compressivo, **sopprimono la motilità cellulare** e tendono a ritardare o bloccare la progressione del tumore.

Ductal **C**arcinoma
In **S**itu



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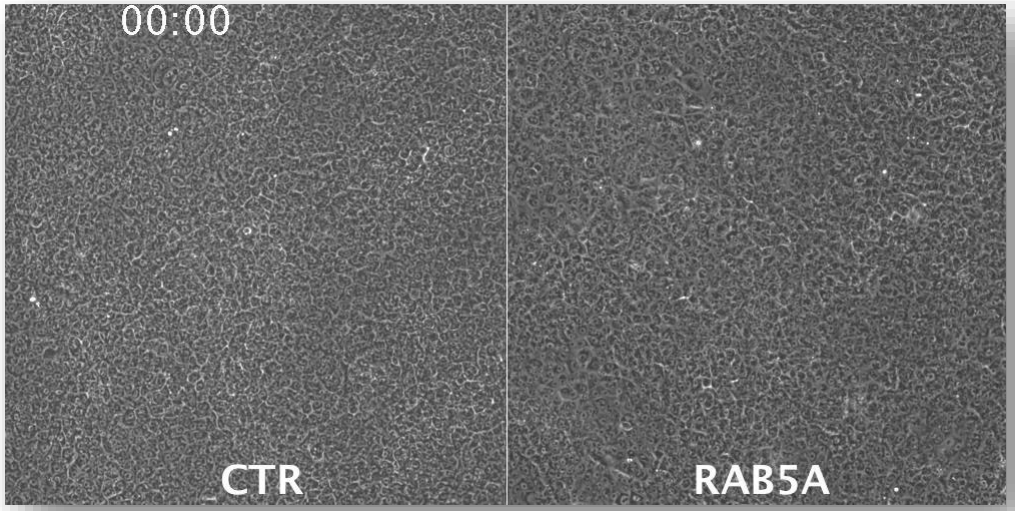
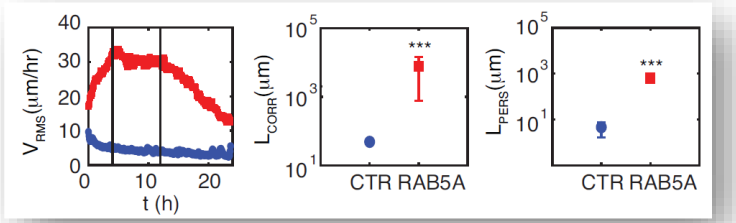
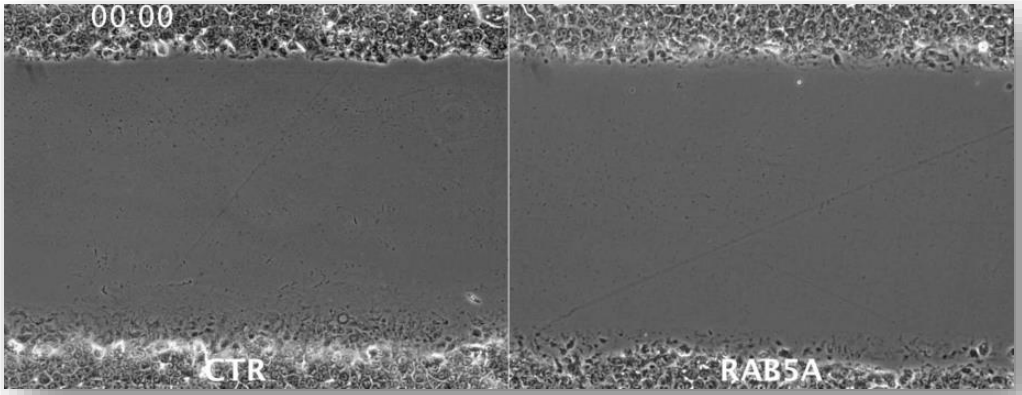


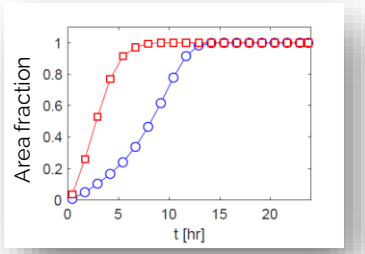
CTRL **RAB5A**

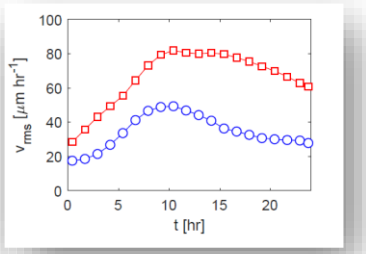
L'over-espressione di RAB5A causa *unjamming e flocking*





"a wound that never heals"





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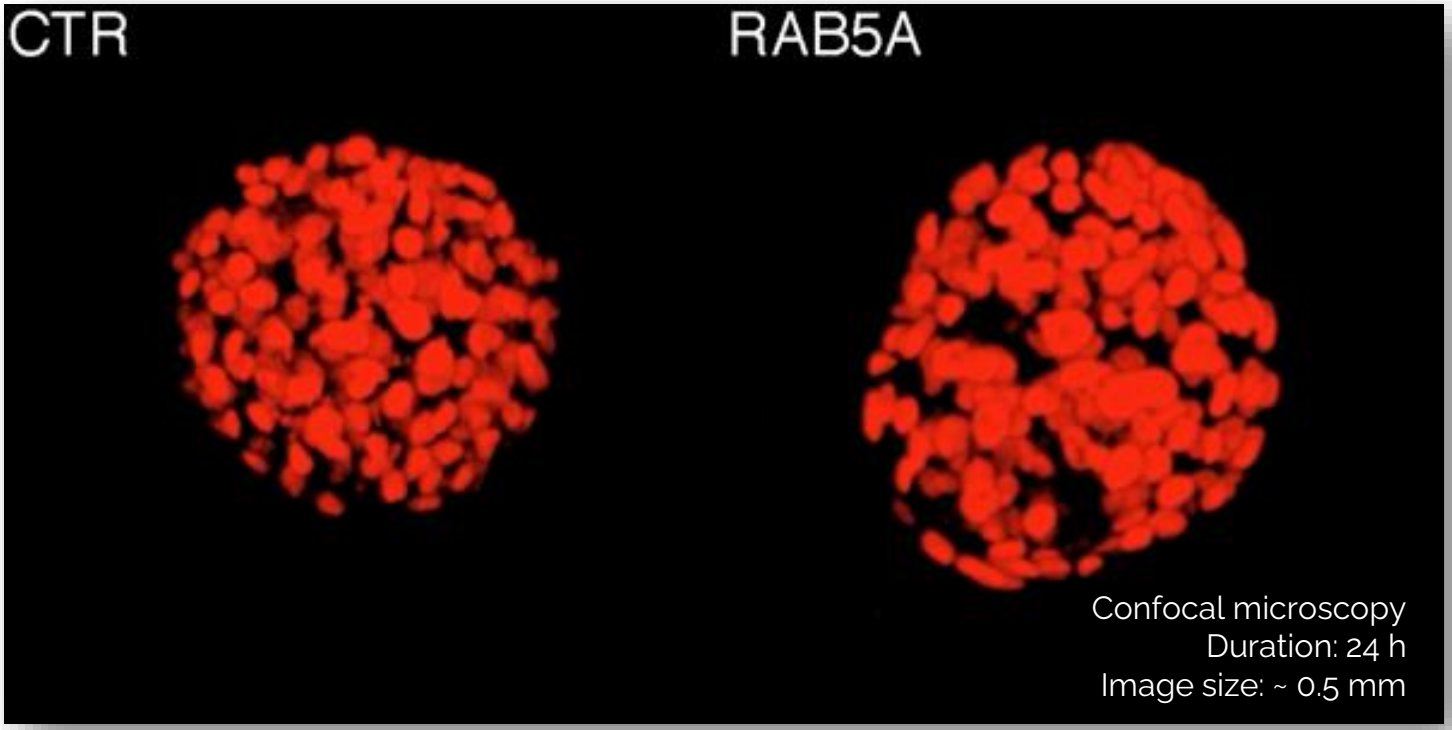
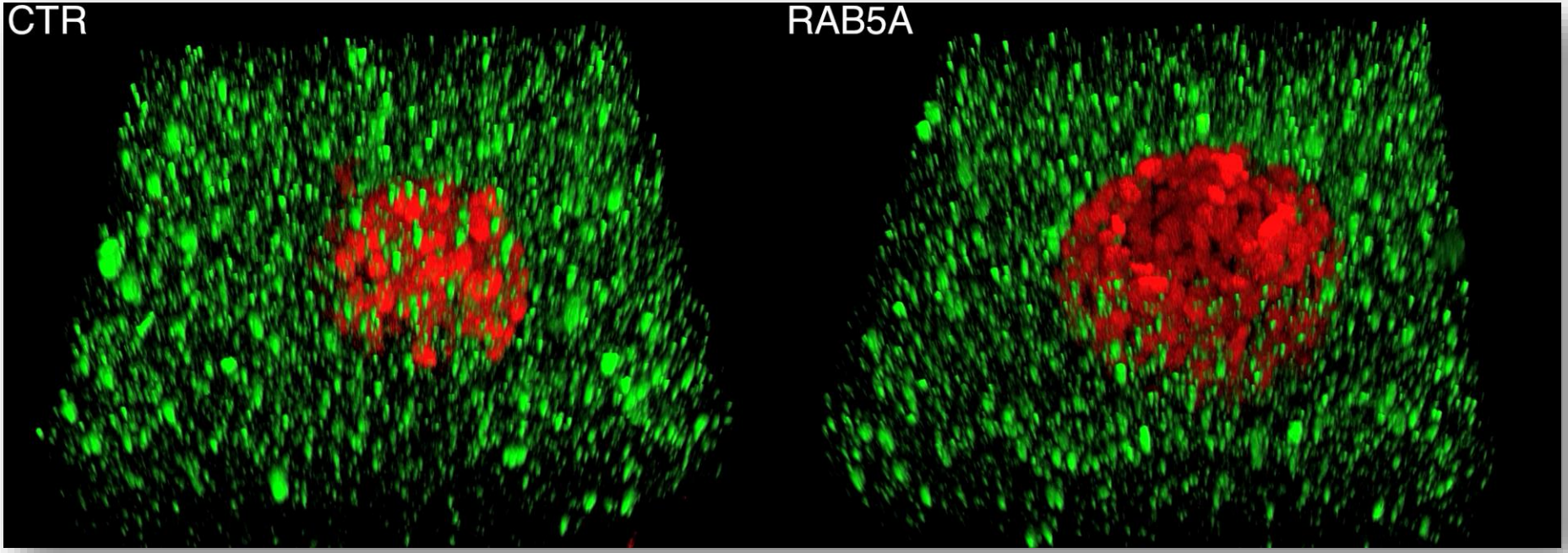


Abbiamo cresciuto aggregati tridimensionali (sferoidi) di cellule CTR and RAB5A, che sono stati poi impiantati in una matrice di collagene piuttosto rigida ($c = 6\frac{mg}{ml}$, $E \approx 140\ Pa$) per simulare una condizione di DCIS.

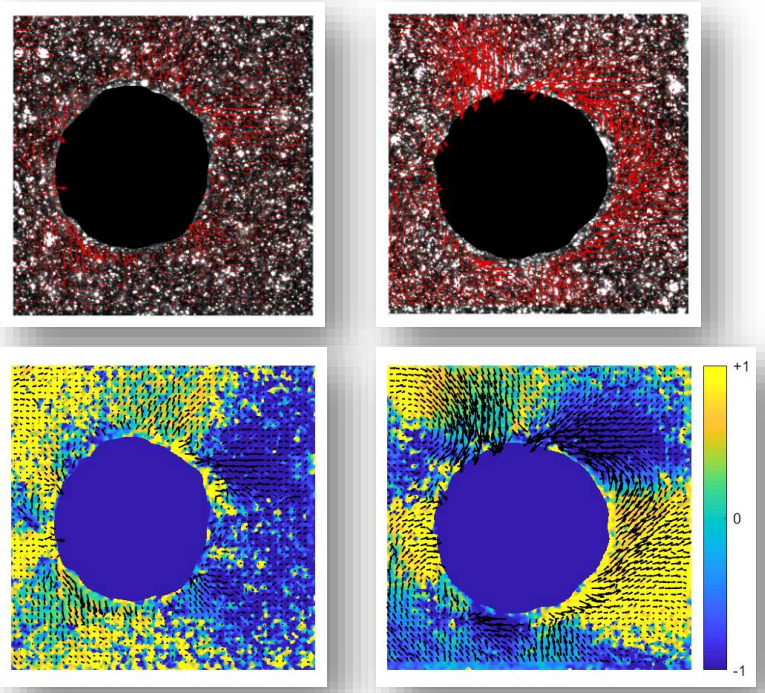
Fluctuation stress microscopy



Per misurare le forze (*stress*) esercitate dagli sferoidi sulla matrice extracellulare abbiamo disperso dei traccianti flurescenti all'interno della matrice stessa, in modo da poterne visualizzare la deformazione.

Fluctuation stress microscopy

CTR **RAB5A**



Cosine of the angle with the horizontal direction

Optical flow velocimetry $\rightarrow \boldsymbol{v}(\boldsymbol{x}, t)$

Funzione di correlazione della derivata spaziale della velocità

$$C_{ij}(\boldsymbol{x}, t) = \langle \partial_i v_j(\mathbf{x}, t + t_0) \partial_i v_j(\boldsymbol{x}, t_0) \rangle$$

Deformazione quadratica media

$$\langle \Delta \epsilon_{ii}{}^2 (\boldsymbol{x}, \tau) \rangle = \lim_{\tau \to \infty} 2 \int_0^{\tau} (\tau - |t|) C_{ii}(\boldsymbol{x}, t) dt$$

Kubo, R. The fluctuation-dissipation theorem. RPP **29**, 255 (1966

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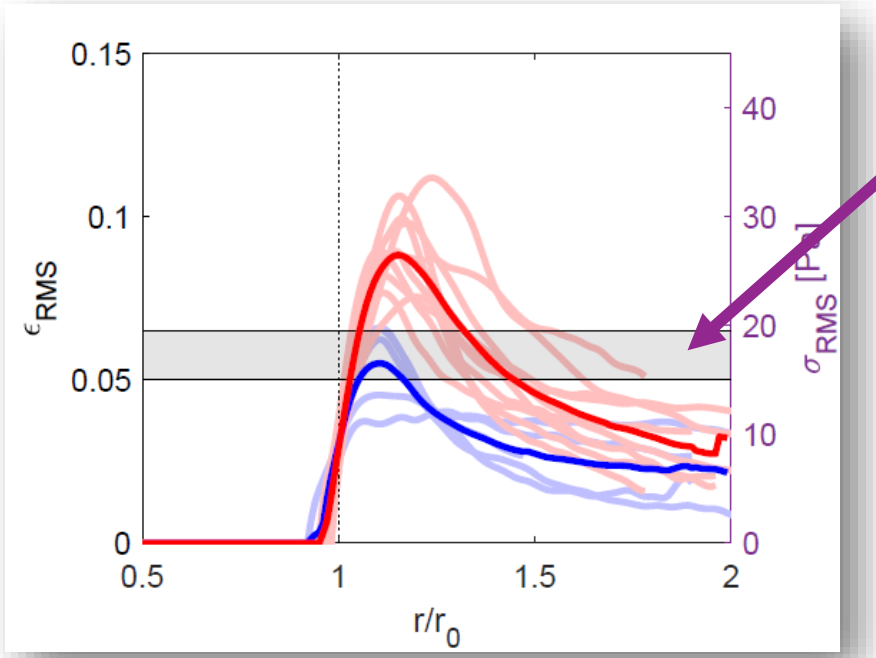
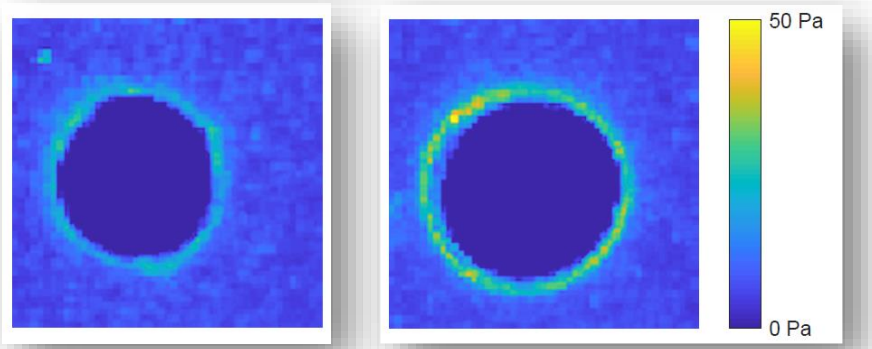
Fluctuation stress microscopy

CTR **RAB5A**

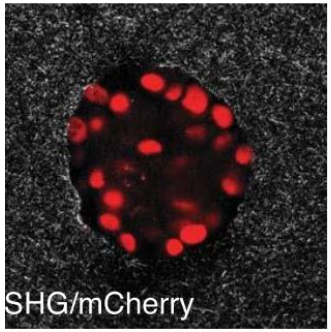


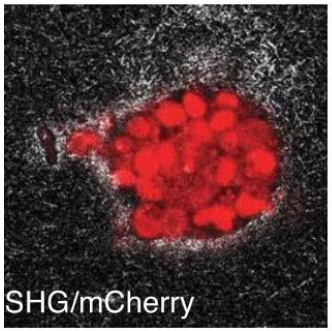


Soglia per la deformazione irreversibile del collagene
(da Vader, Kabla, Weitz, Mahadevan. PLoS One **4**, e5902, 2009)

Gli *stress* esercitati dagli sferoidi RAB5A sulla matrice extracellulare **sono sistematicamente al di sopra della soglia**, mentre questo non succede mai per CTR.

CTR **RAB5A**





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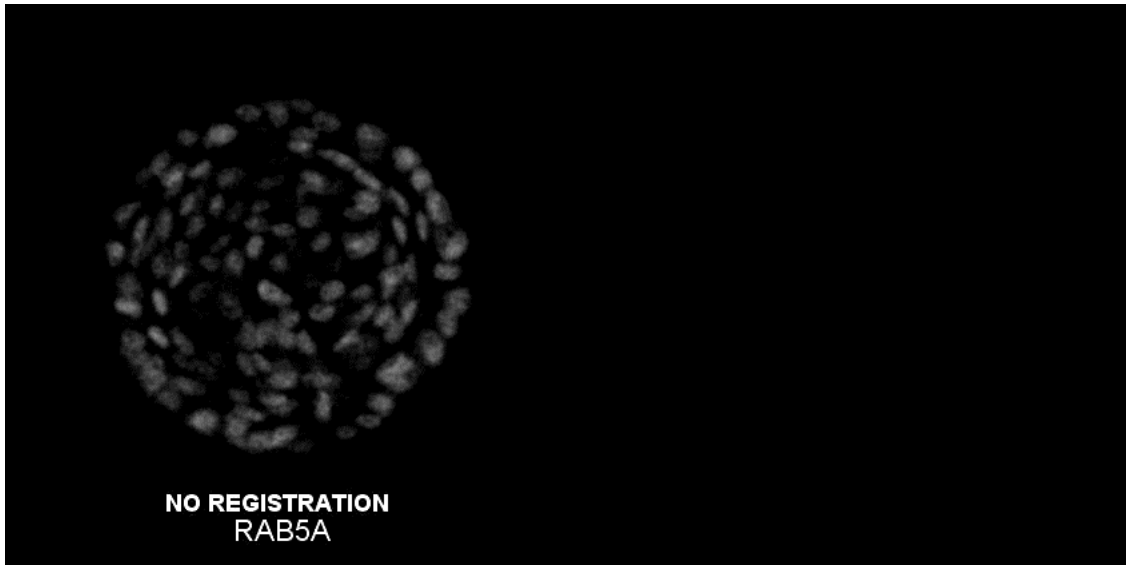
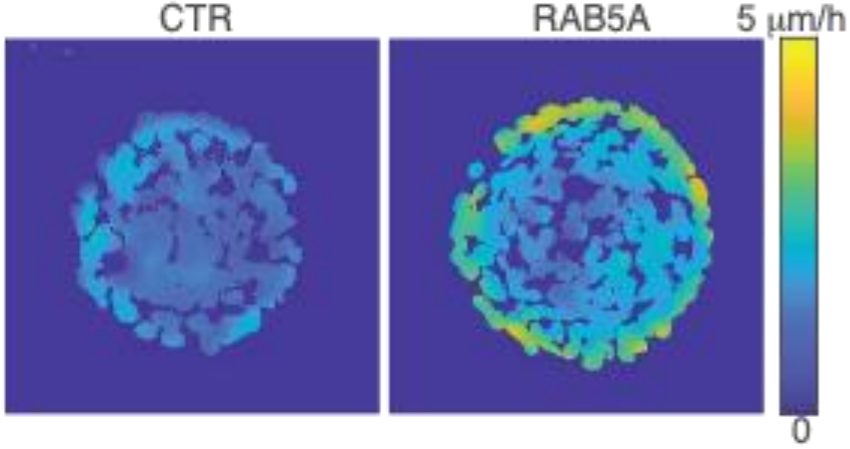
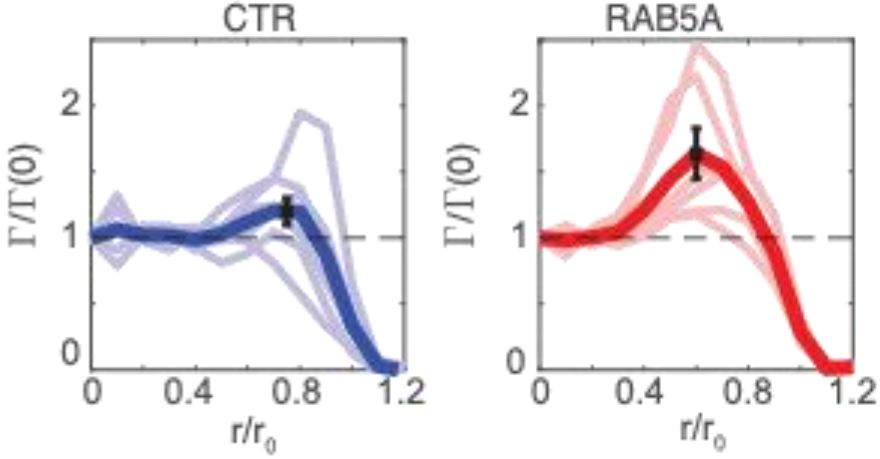


Gli sferoidi RAB5A presentano un marcato **gradiente radiale** di attività, **con una "crosta"** estremamente **fluida**.

Confocal section
Duration: 24 h
Image size: ~ 0.5 mm



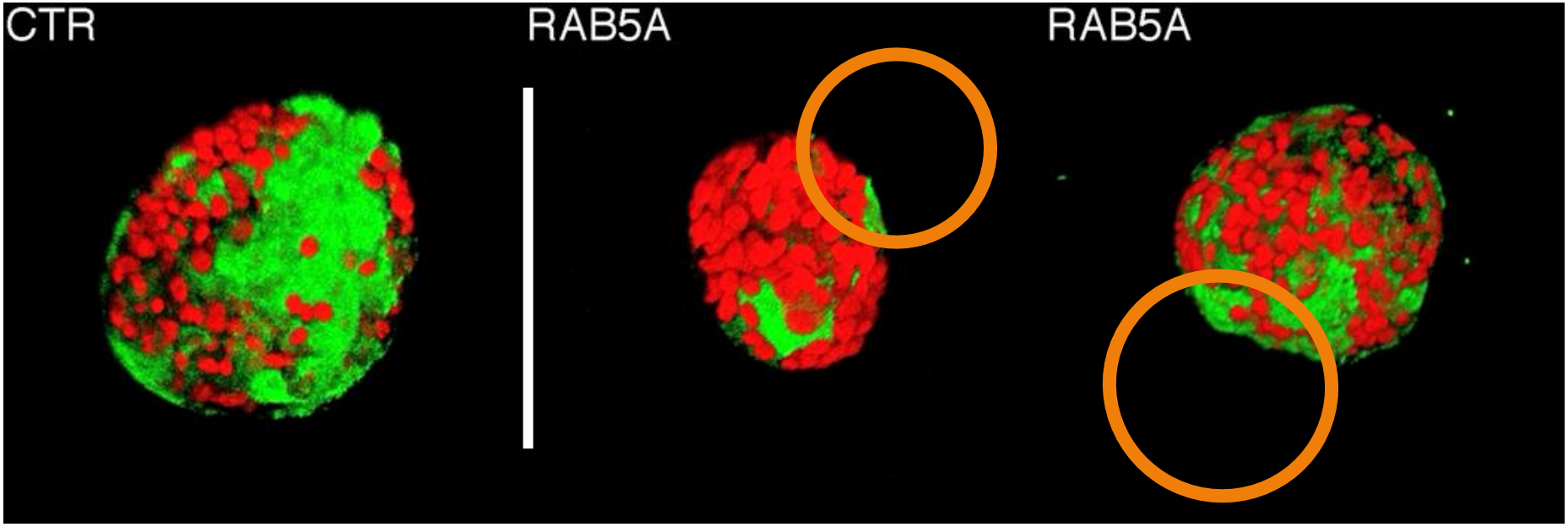
RMS velocity map in the co-rotating reference frame



Activity profile (rate of cell-cell rearrangement)

Unjamming e invasione collettiva

confinamento *invasive bud* *Invasive strand*



Sulla superficie degli sferoidi, la combinazione di **ampi *stress* meccanici** generati dal moto collettivo e **fluidificazione locale** innesca il processo di invasione all'interno della matrice extracellulare.

A Palamidessi *et al.*, Nature Materials **18**, 1252 (2019)

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ARTICLES
https://doi.org/10.1038/s41563-019-0425-1
nature materials

Unjamming overcomes kinetic and proliferation arrest in terminally differentiated cells and promotes collective motility of carcinoma

ROYAL SOCIETY OF CHEMISTRY

Soft Matter

PAPER

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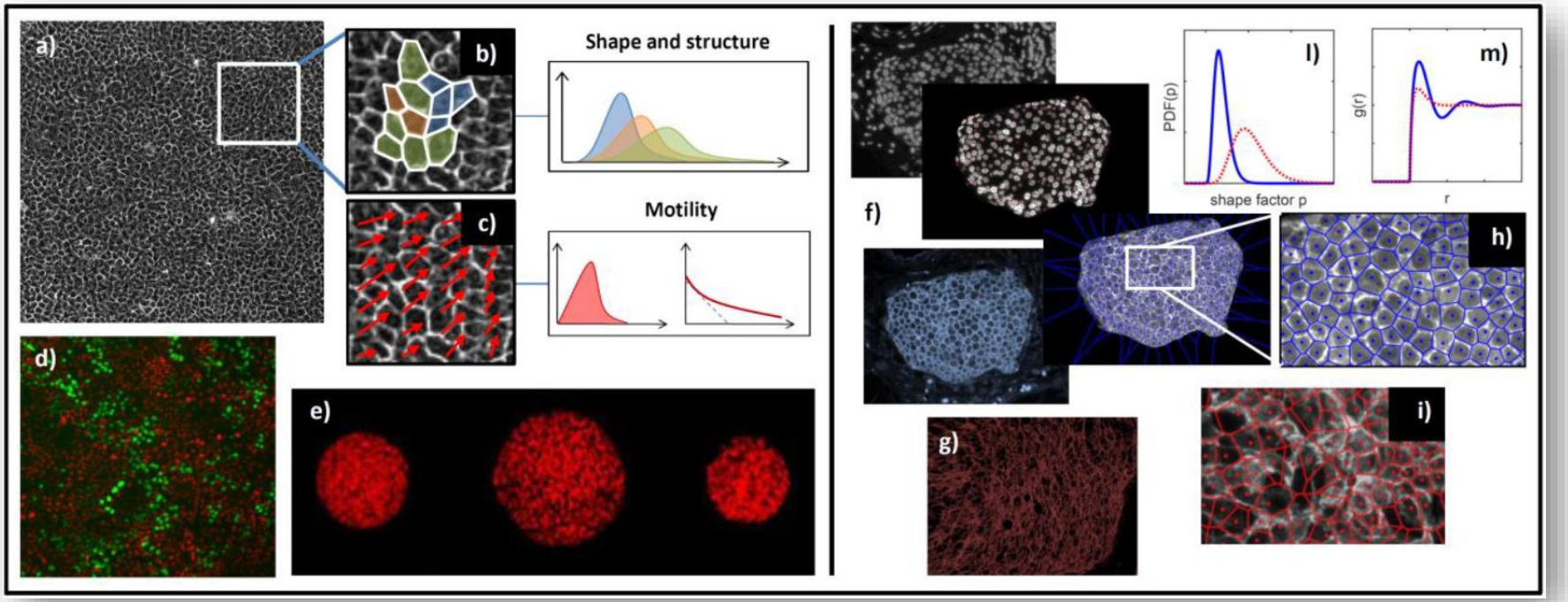
Disentangling collective motion and local rearrangements in 2D and 3D cell assemblies†

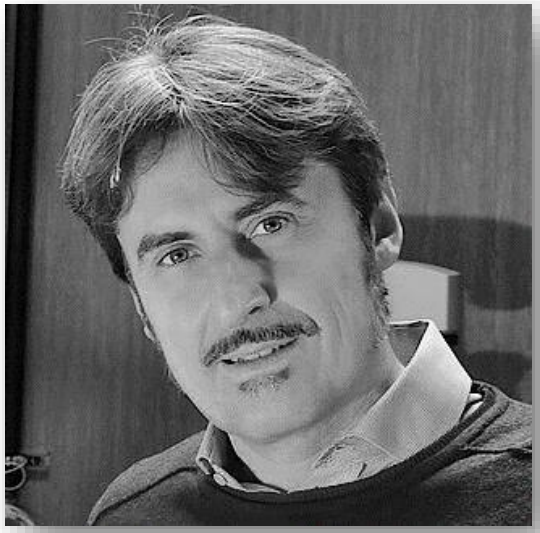
Tissue fluidification promotes a cGAS/STING-mediated cytosolic DNA response in invasive breast cancer

Predire l'invasione?



Una delle maggiori sfide aperte consiste nella individuazione di maker **statici** di *unjamming* che permettano di prevedere l'evoluzione di un tessuto tumorale sulla base della sua **struttura** e della **morfologia** delle cellule.

Giorgio Scita
Biologo cellulare



Roberto Cerbino
Fisico

Claudio Tripodo
Anatomo patologo

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All-optical Fingerprinting of Metastatic Potential

Biophysics-based structural markers of unjamming in epithelial tissues