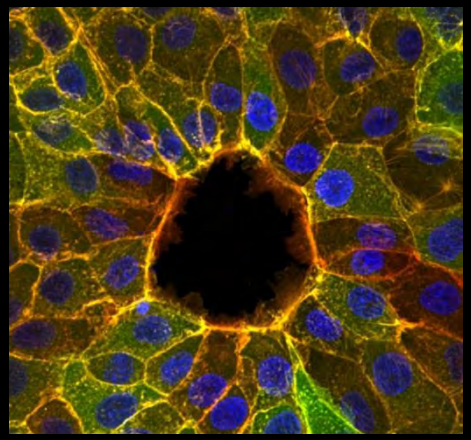
TRACTION and STRESS MICROSCOPY for CELLS: the WOUND HEALING CASE

Embryo Physics Course

APRIL 2, 2014



Vito Conte, A. Brugués, E. Anon, J.H. Veldhuis, J. Colombelli, J.J. Muñoz,

G.W. Brodland, B. Ladoux and Xavier Trepat

ibec

UNIVERSITY OF WATERLOO
CONCORDIA CUM VERITATE

IRB BARCELONA

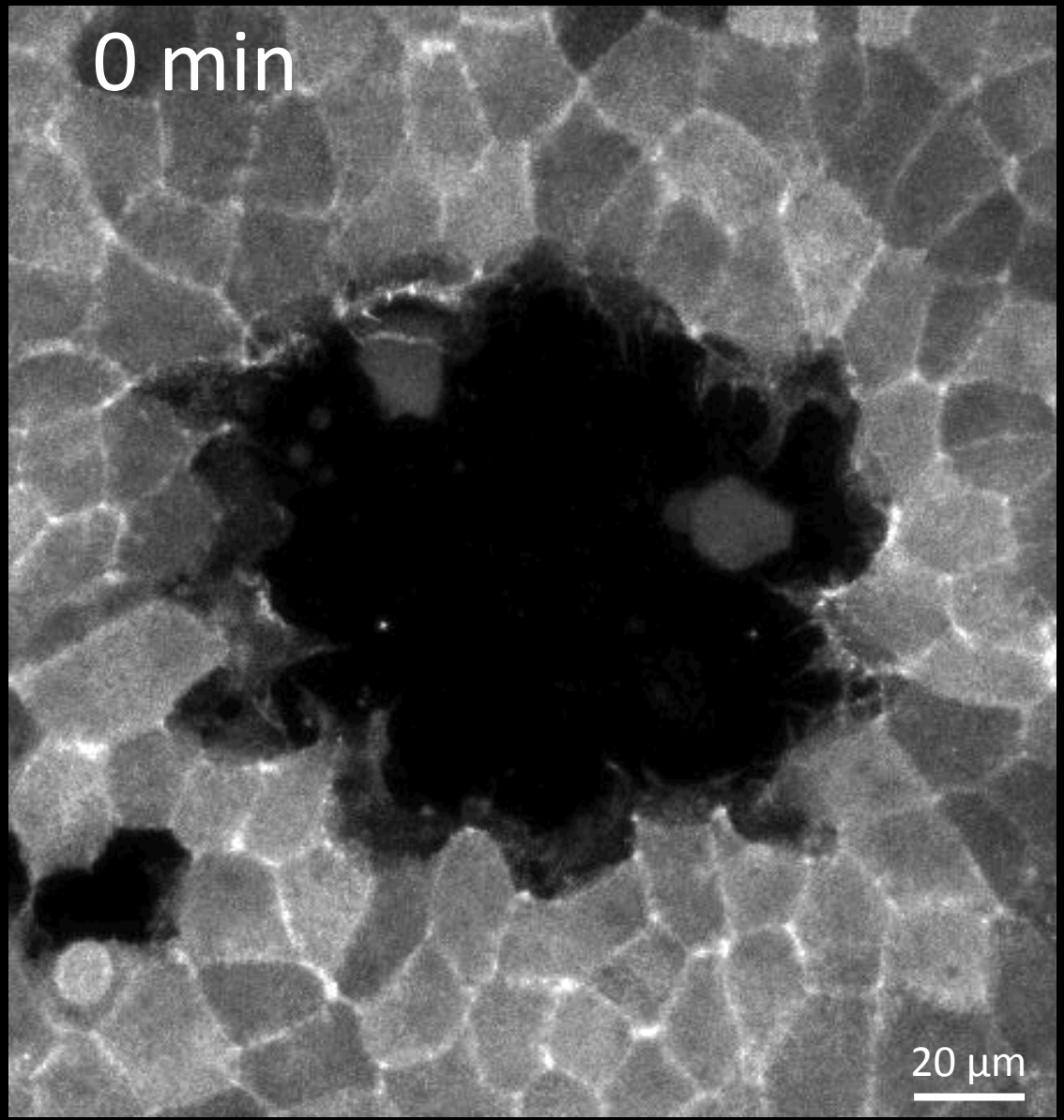
UPC

université PARIS DIDEROT PARIS 7

WHAT IS THE MECHANISM DRIVING EPITHELIAL WOUND HEALING?

AgustíBrugués
Ester Añón

with
Julien Colombelli
Benoit Ladoux

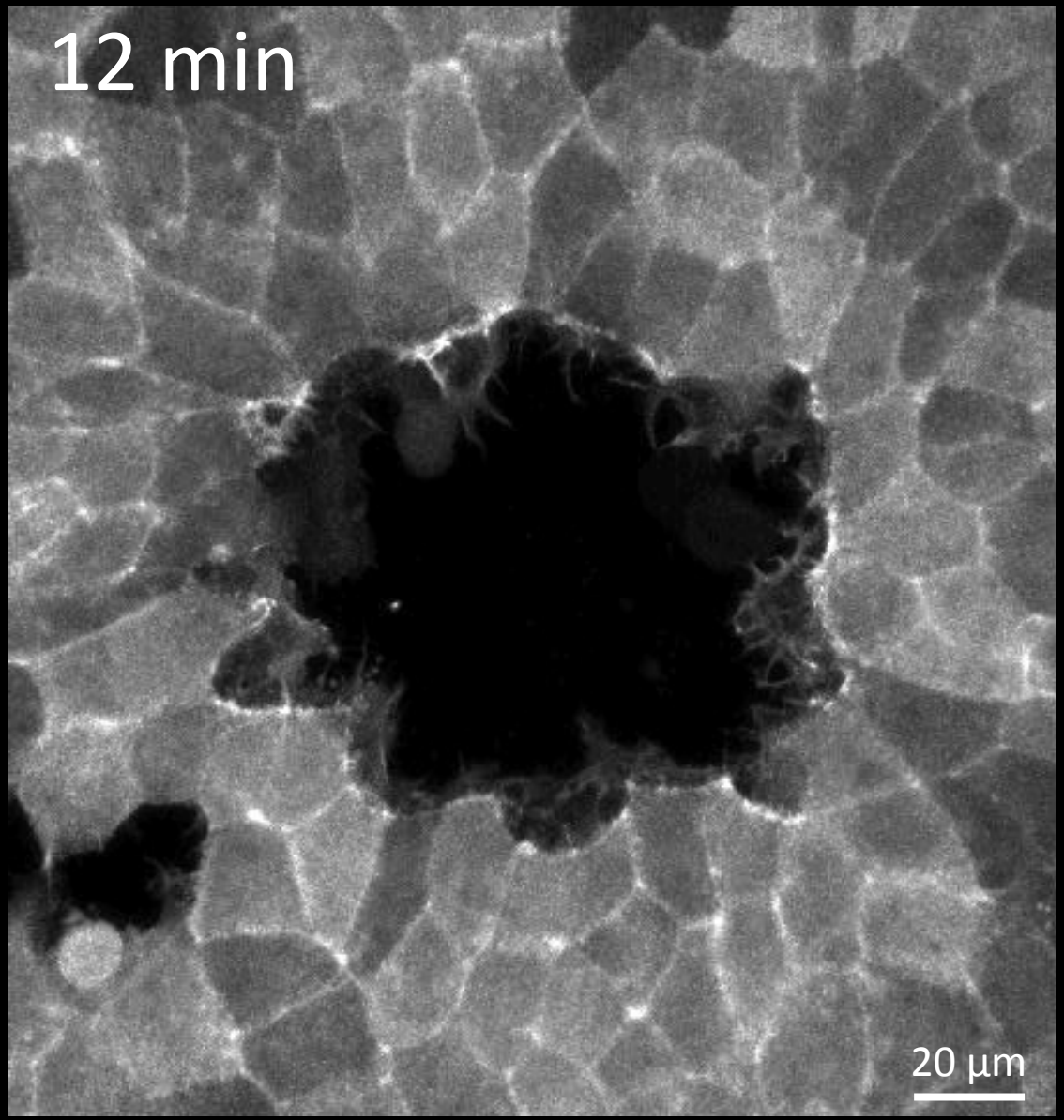


Lifeact

Xavier Trepat

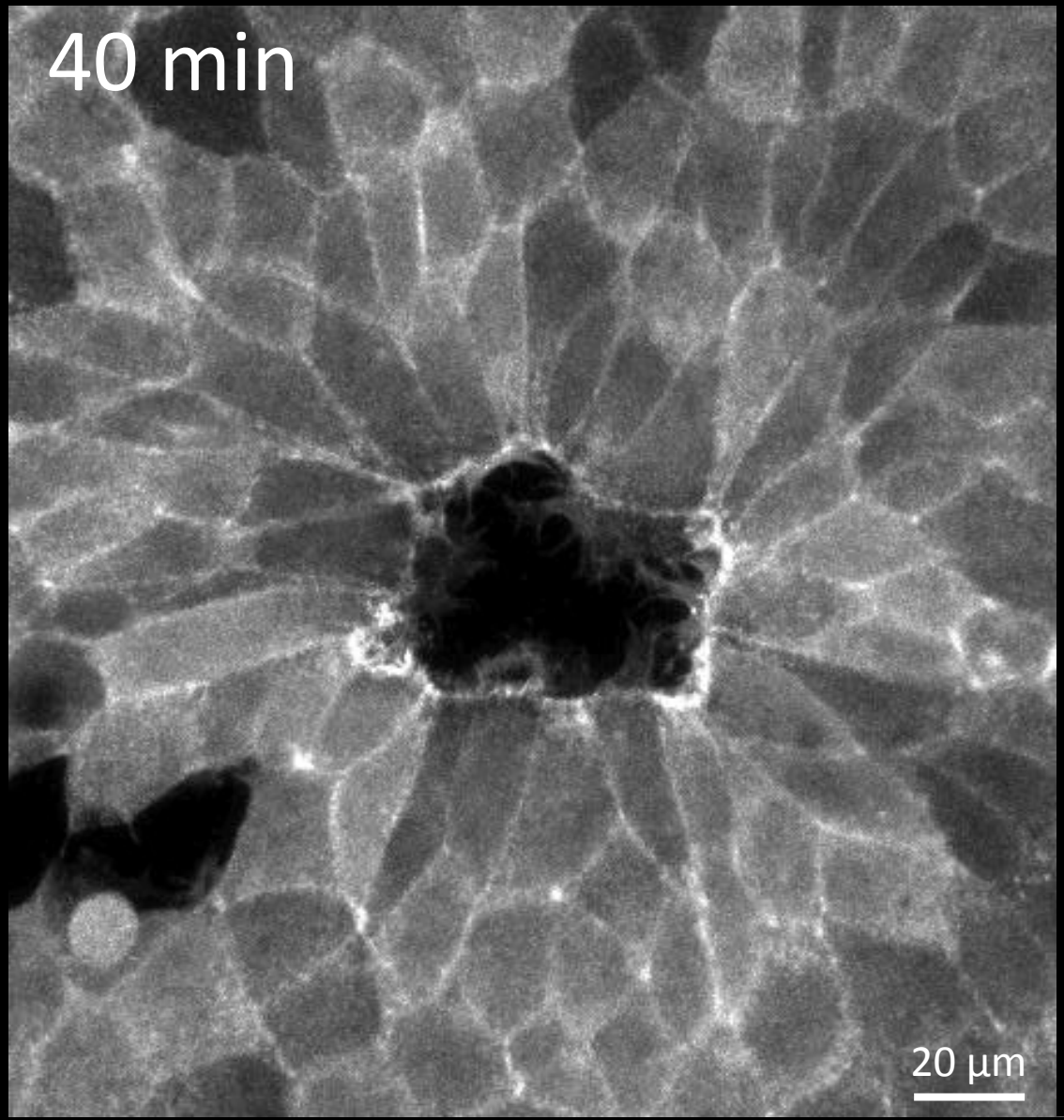
ibec

WHAT IS THE MECHANISM DRIVING EPITHELIAL WOUND HEALING?



Lifeact

WHAT IS THE MECHANISM DRIVING EPITHELIAL WOUND HEALING?



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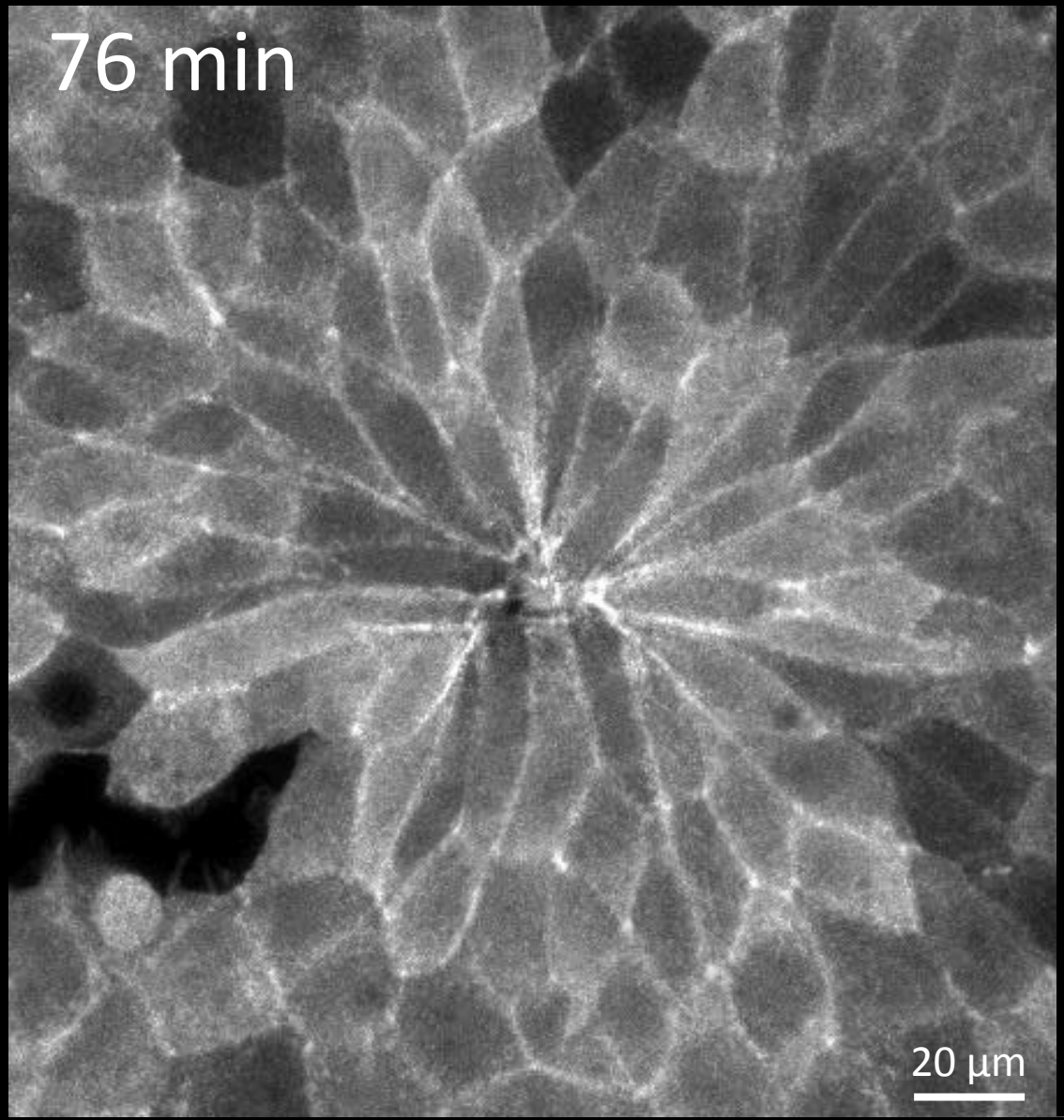
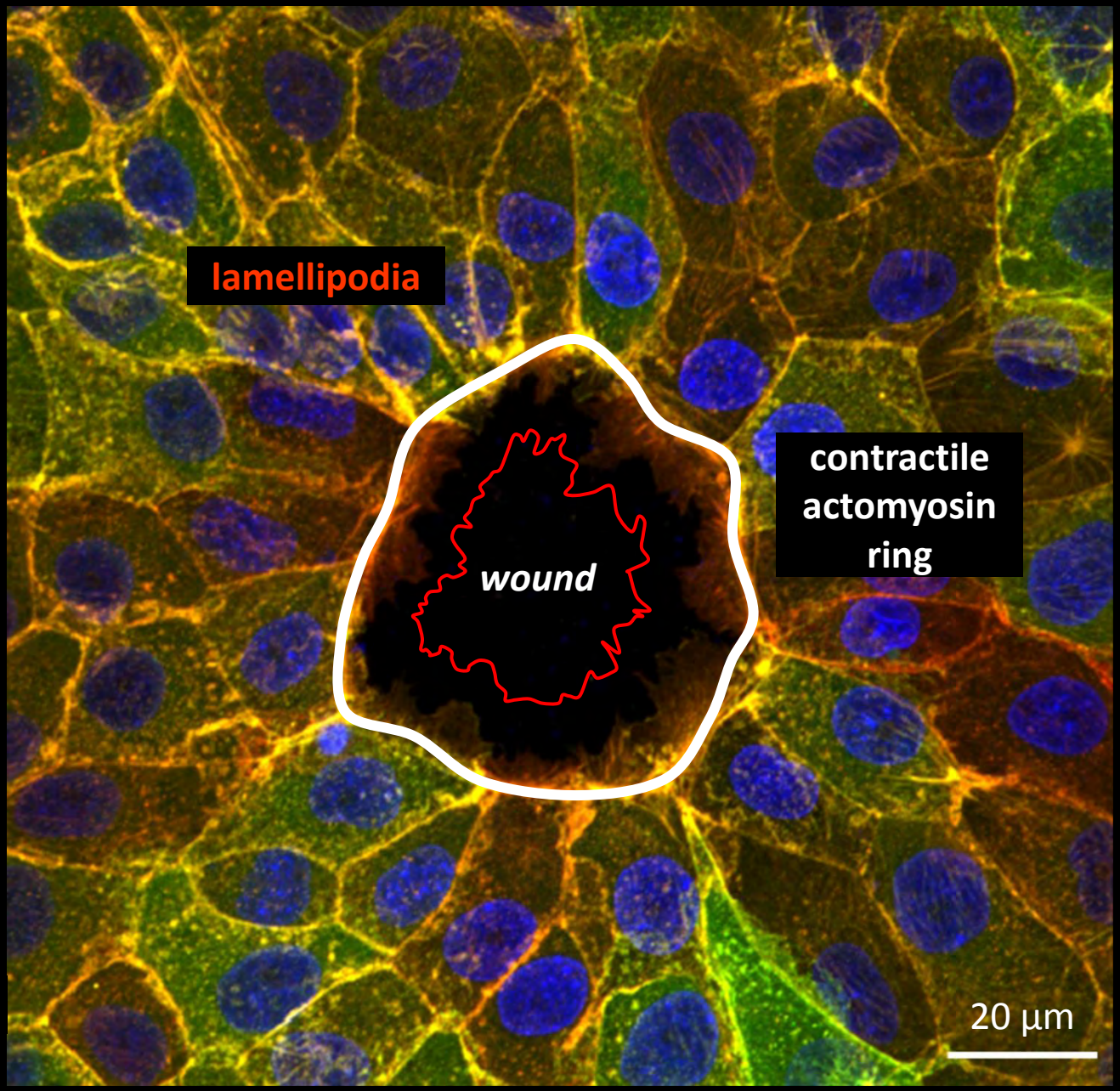


Lifeact

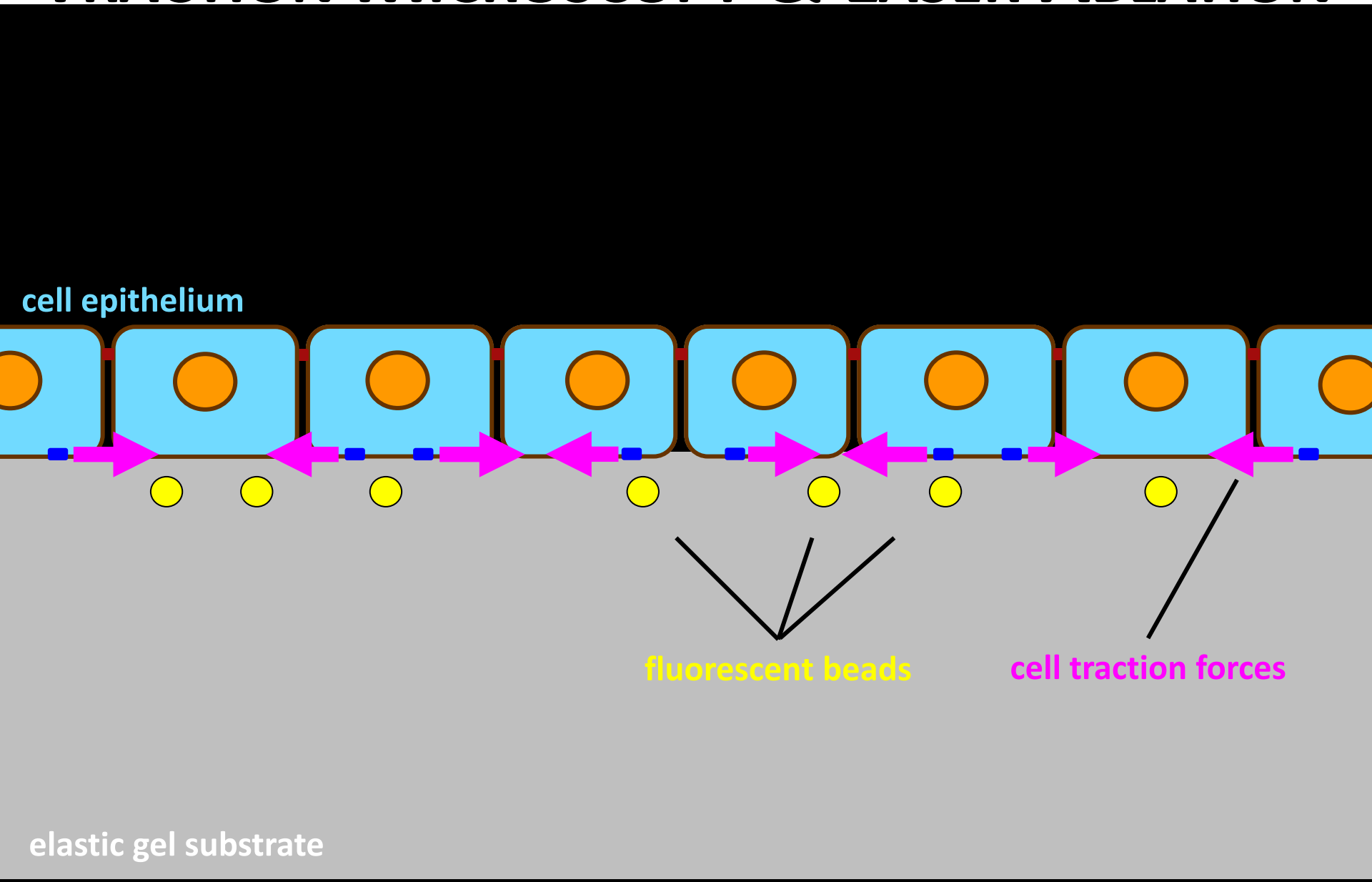
KEY STRUCTURES



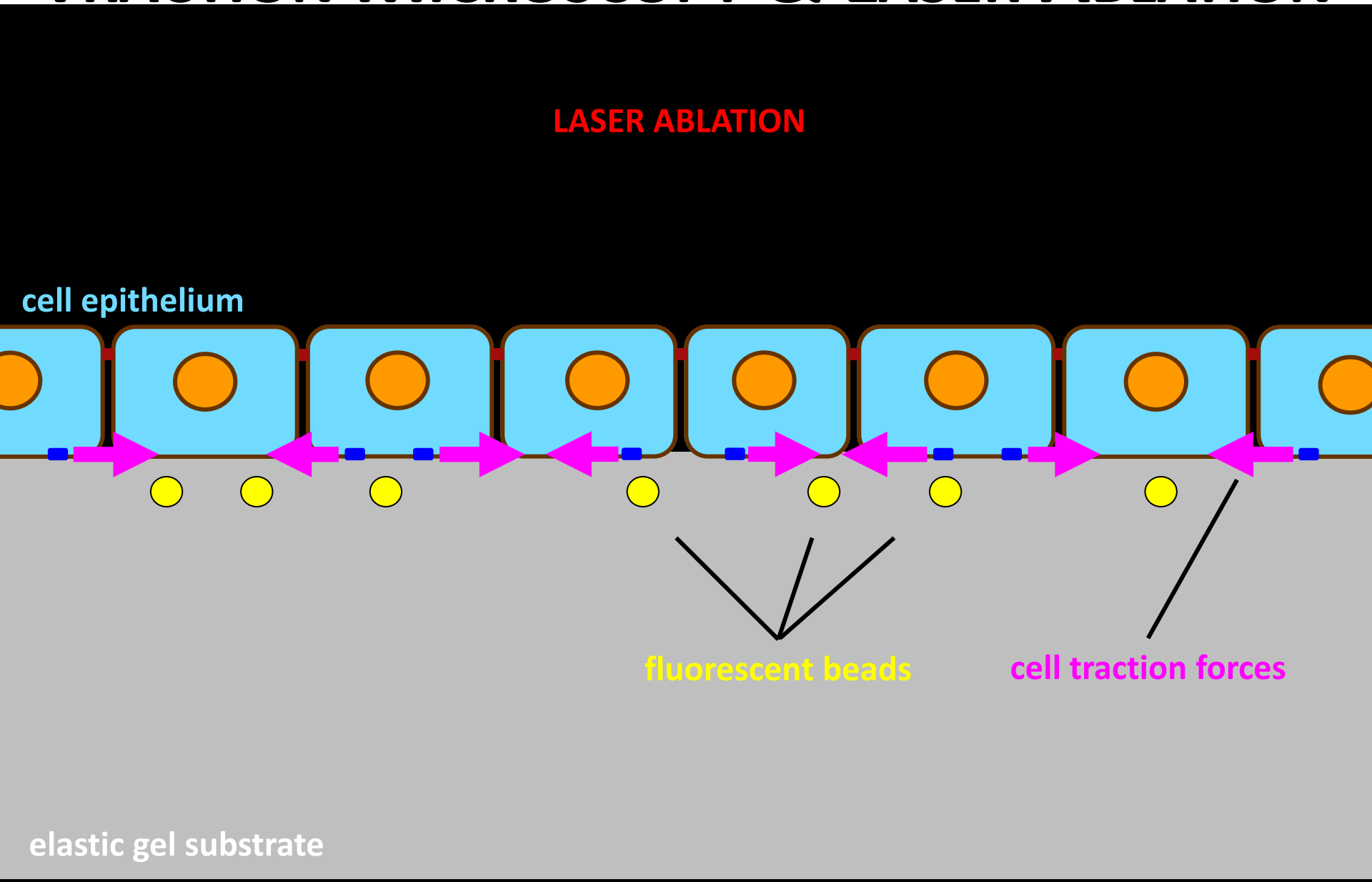
DAPI
Actin

MDCK
epithelium

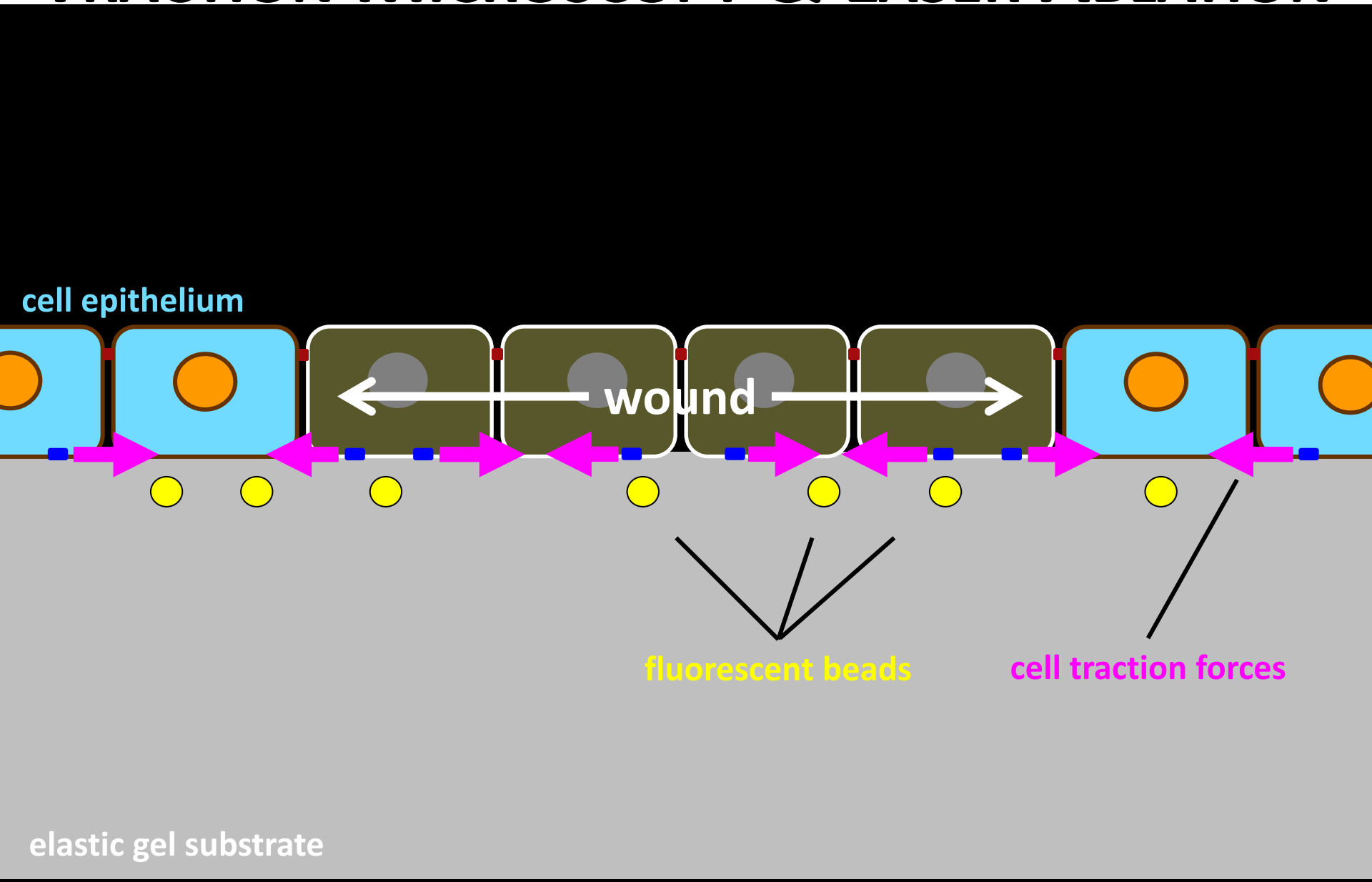
Traction Microscopy & Laser Ablation



Traction Microscopy & Laser Ablation



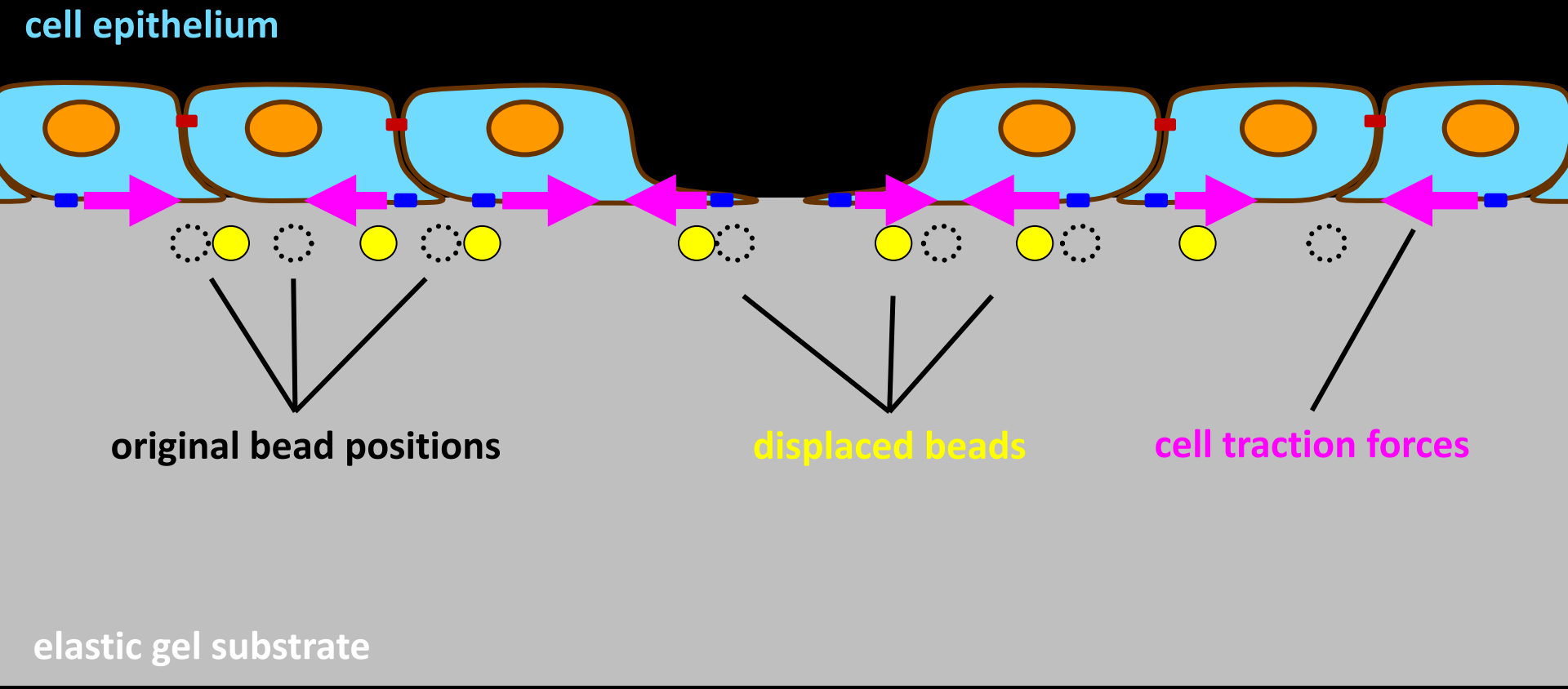
Traction Microscopy & Laser Ablation



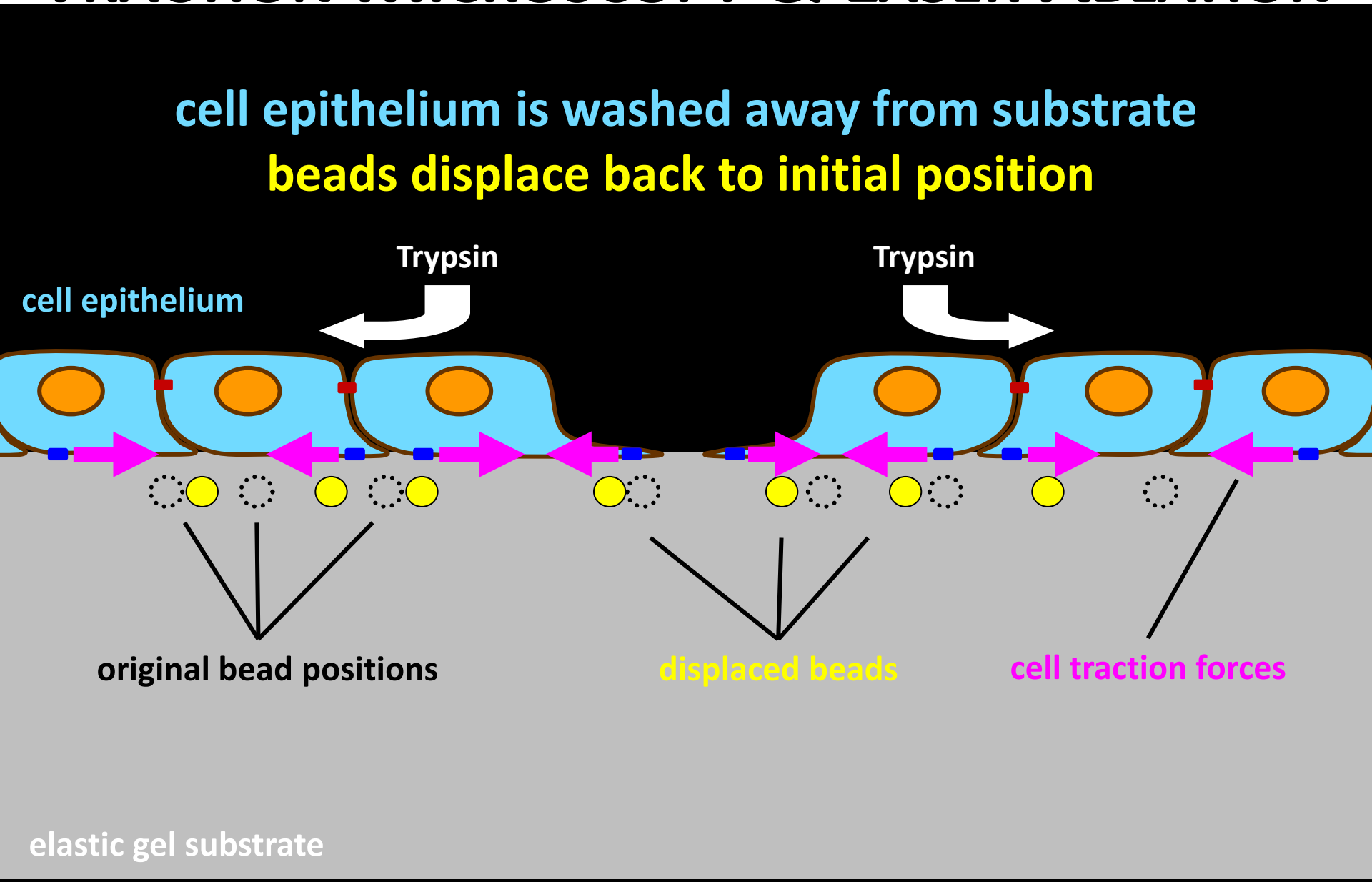
Traction Microscopy & Laser Ablation

cells close the wound region by grabbing on the substrate

substrate deforms and beads displace



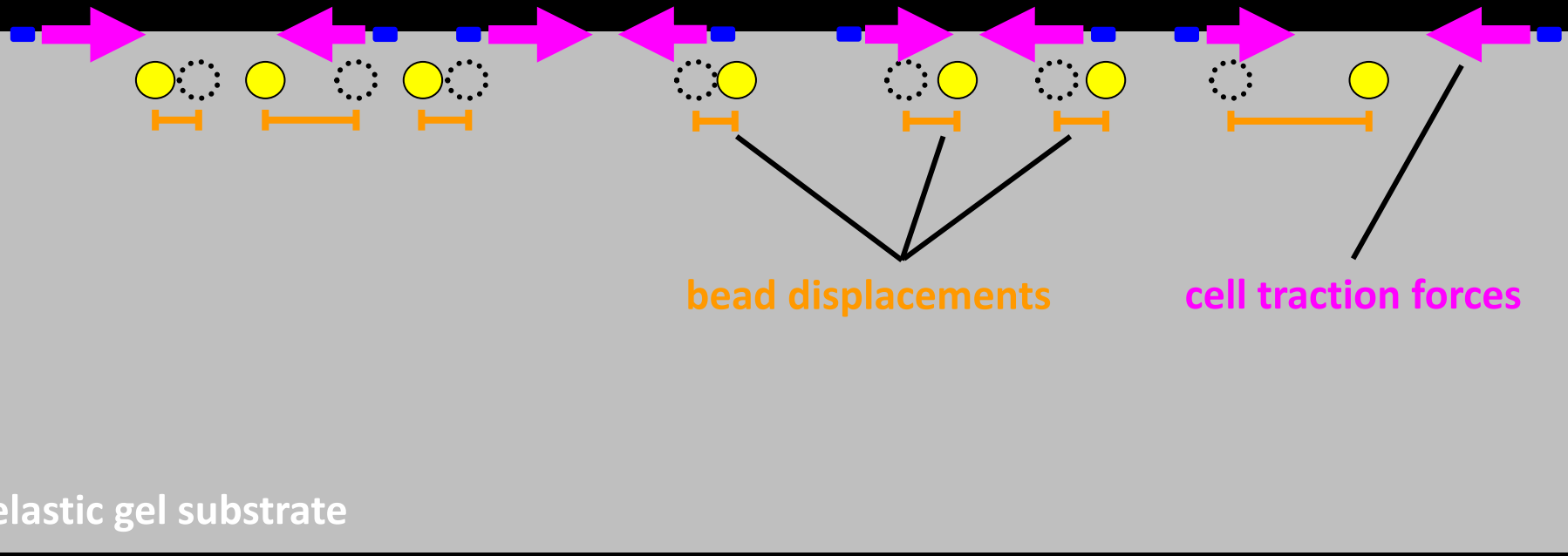
Traction Microscopy & Laser Ablation



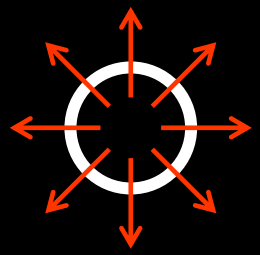
Traction Microscopy & Laser Ablation

bead displacemtents are measured

cell traction forces on the substrate are computed via Inverse Methods



Cell Tractions on the Substrate

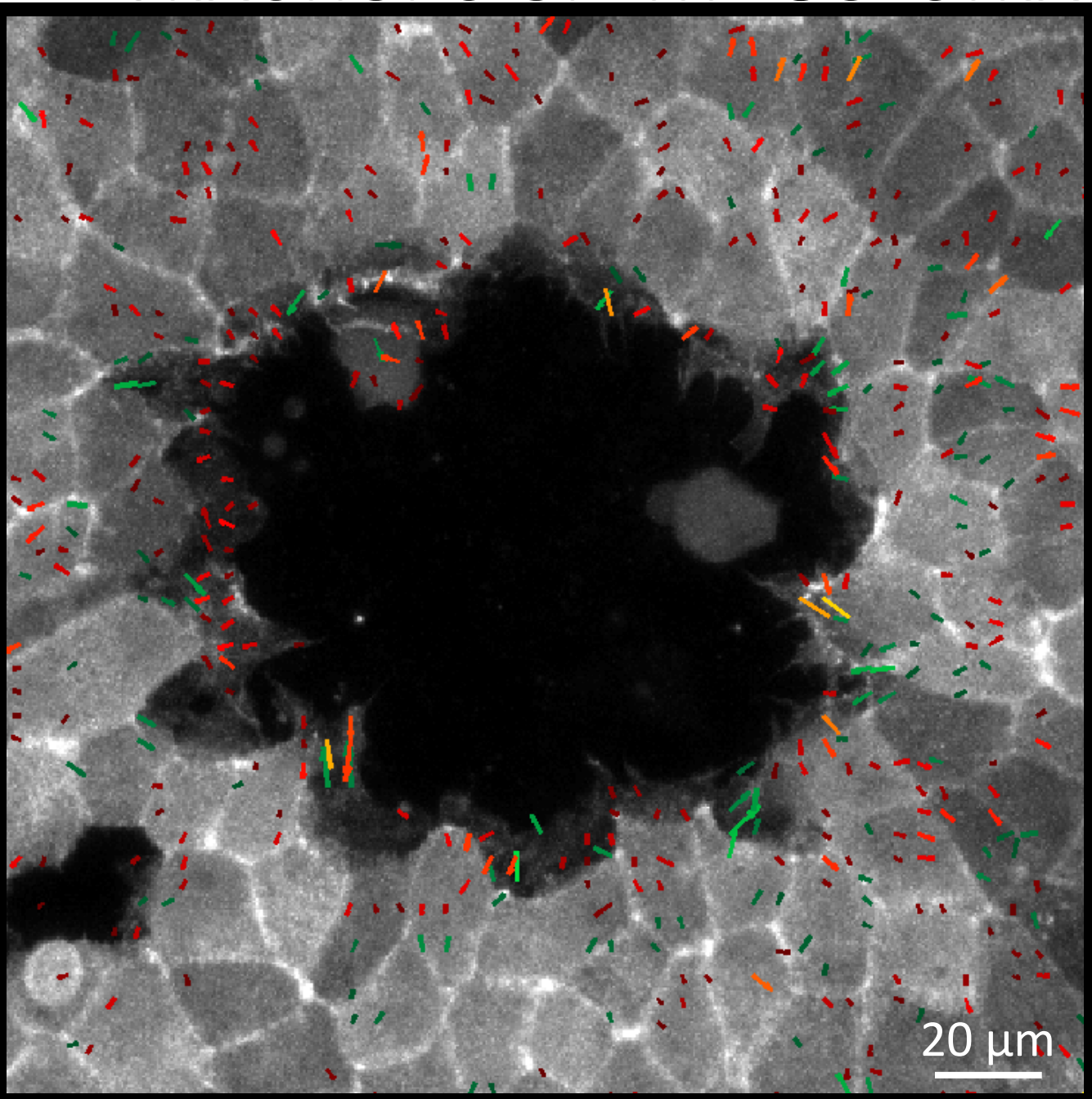


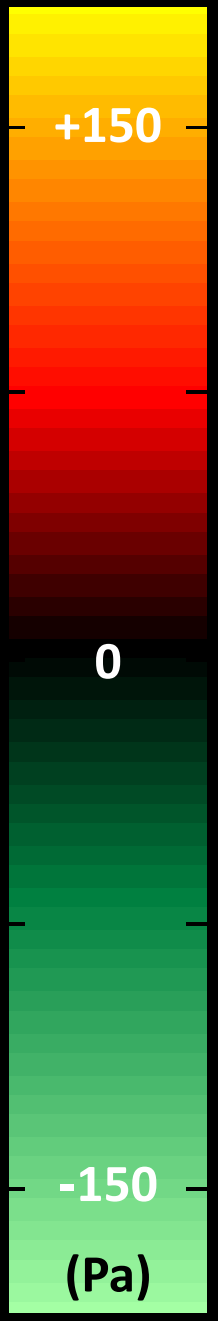
tractions towards wound's exterior

radial direction

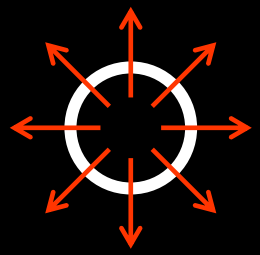
tractions towards wound's interior

Lifeact



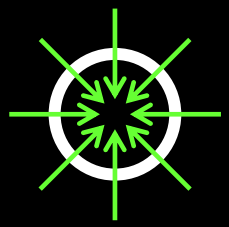


Cell Tractions on the Substrate



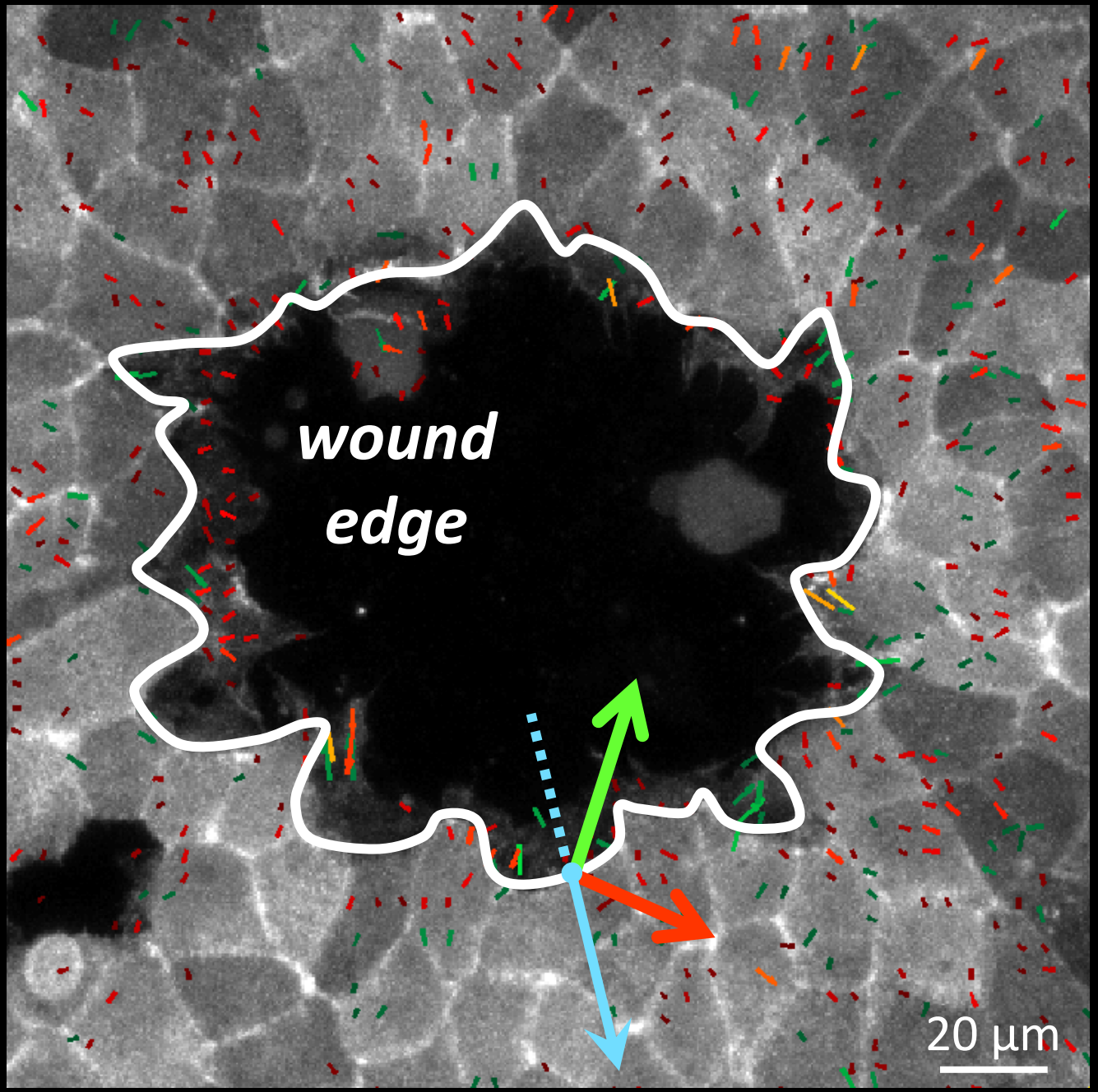
tractions towards wound's exterior

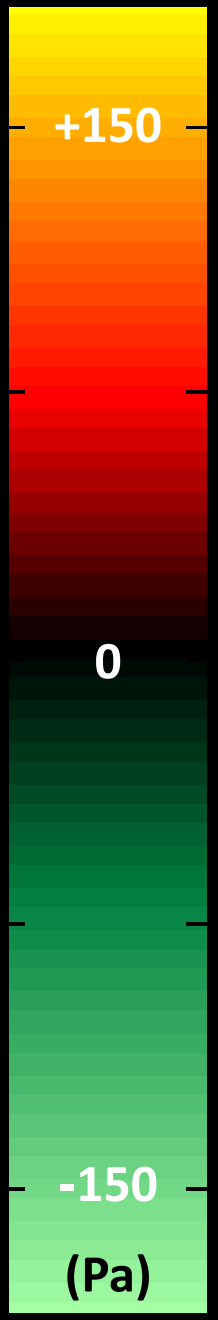
radial direction



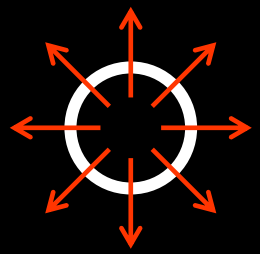
tractions towards wound's interior

Lifeat



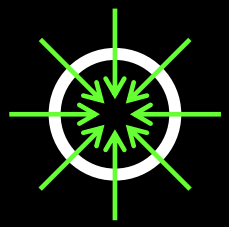


Cell Tractions on the Substrate



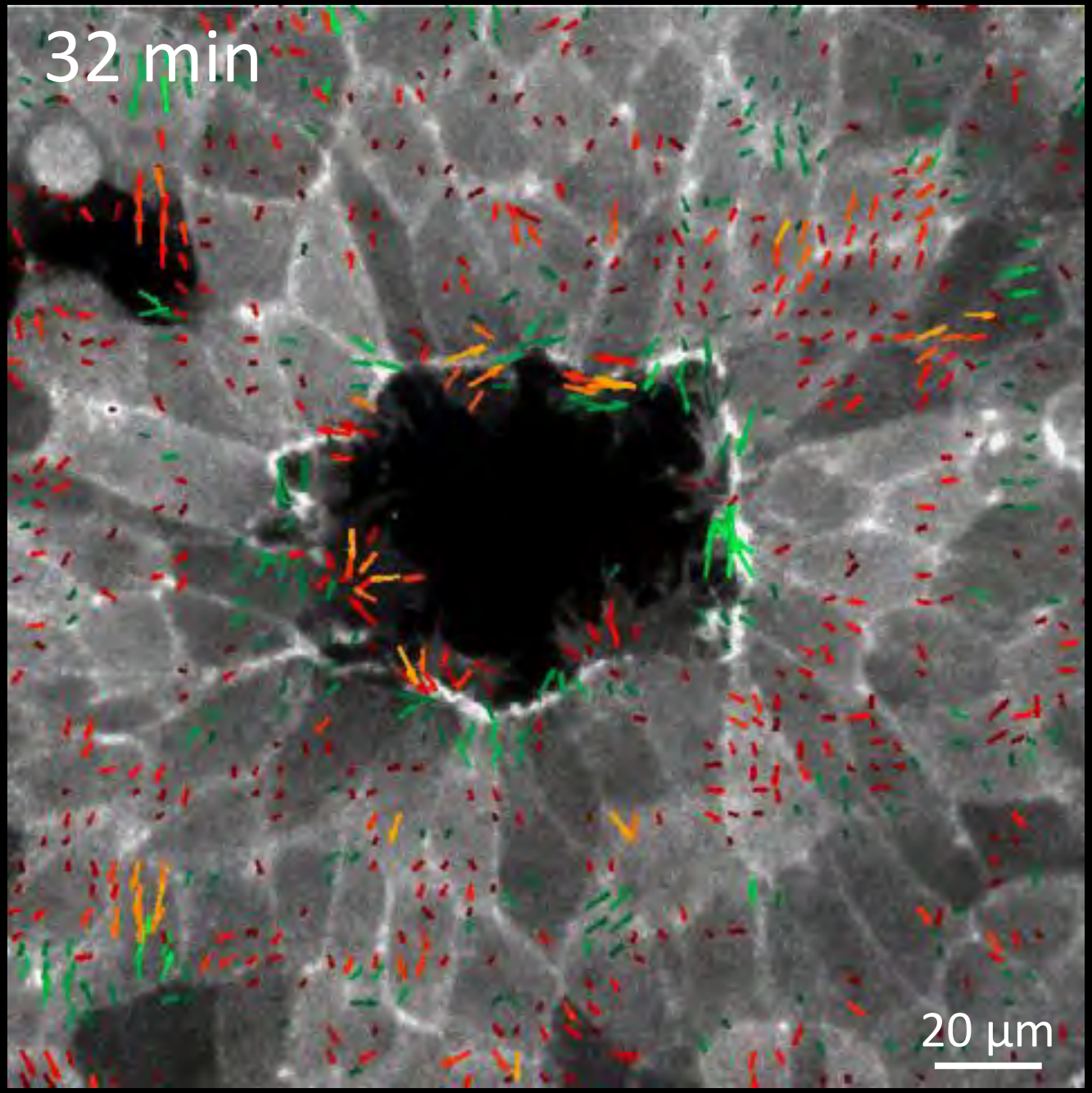
tractions towards wound's exterior

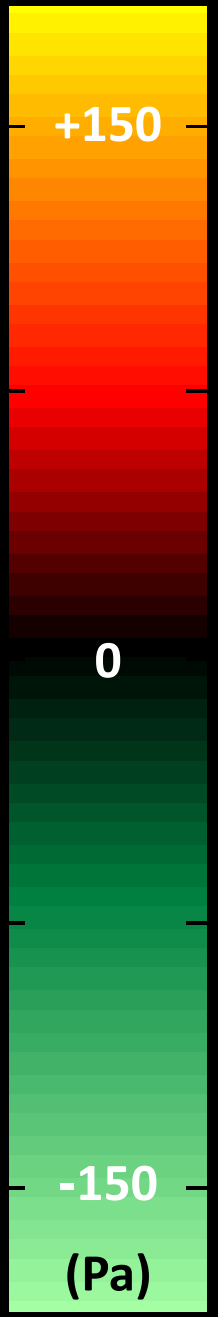
radial direction



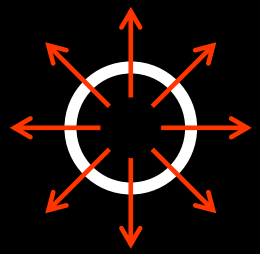
tractions towards wound's interior

Lifeact





Cell Tractions on the Substrate



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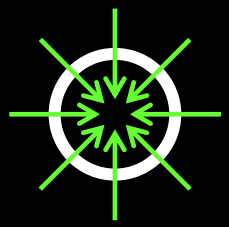

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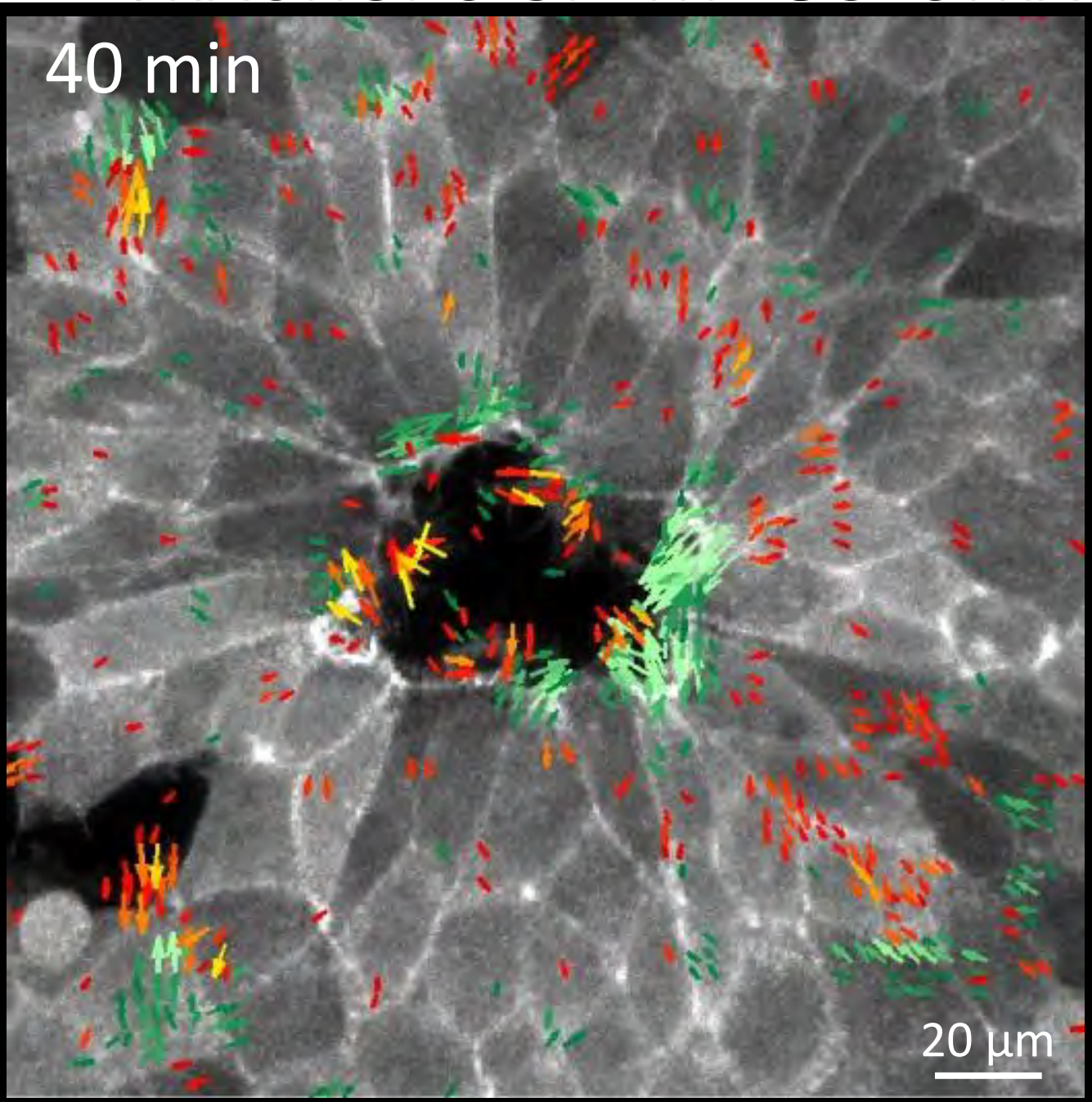
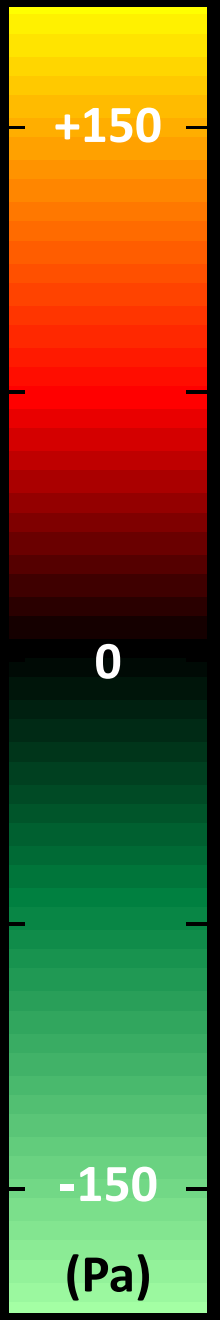
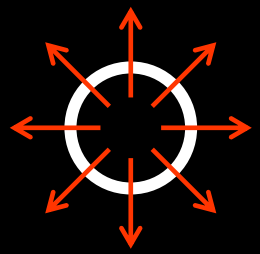




Cell Tractions on the Substrate



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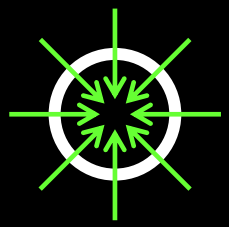
radial direction



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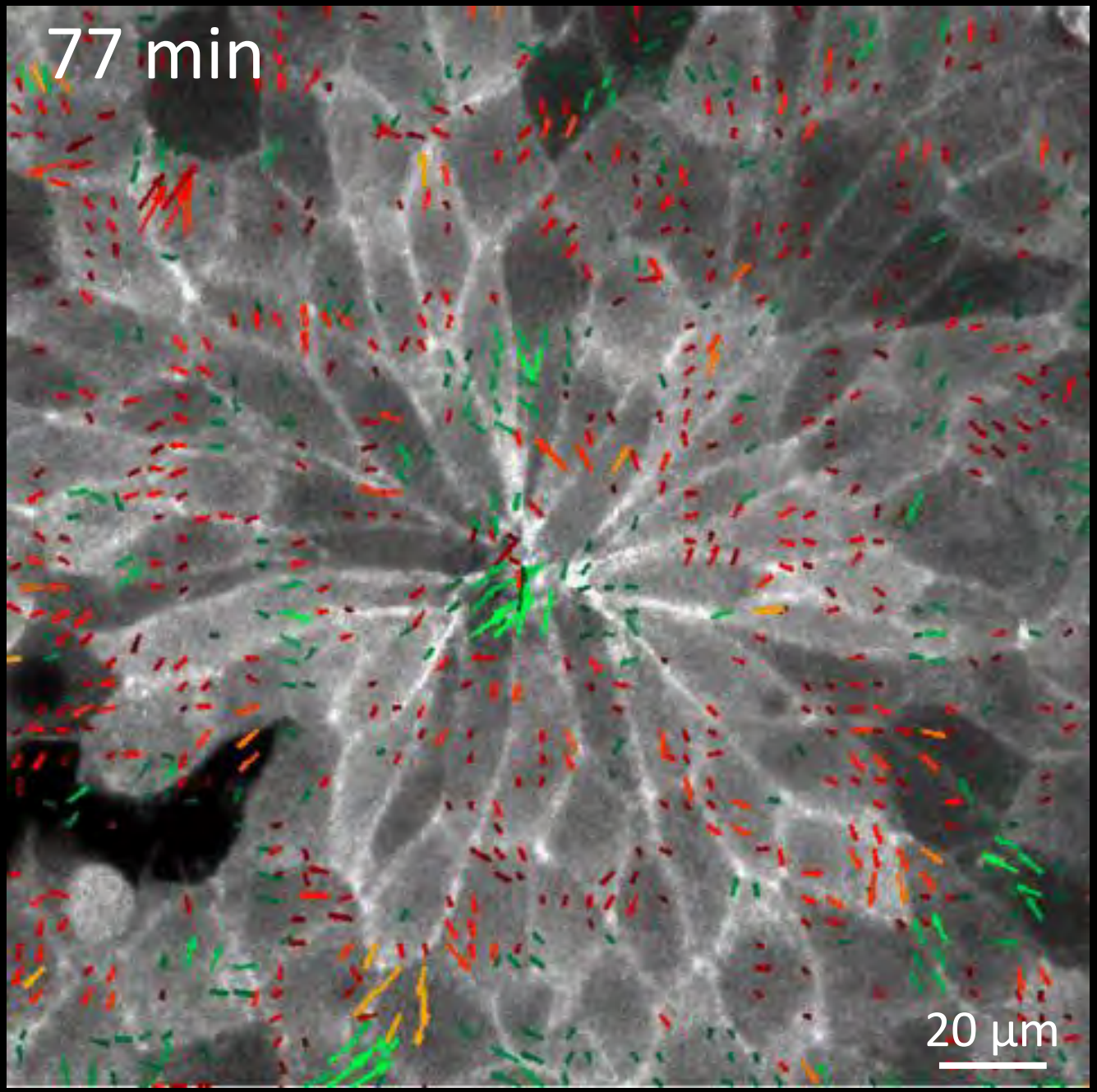
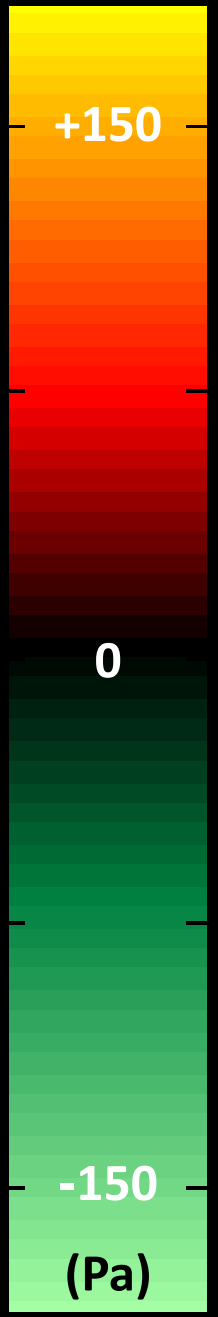
Lifeact

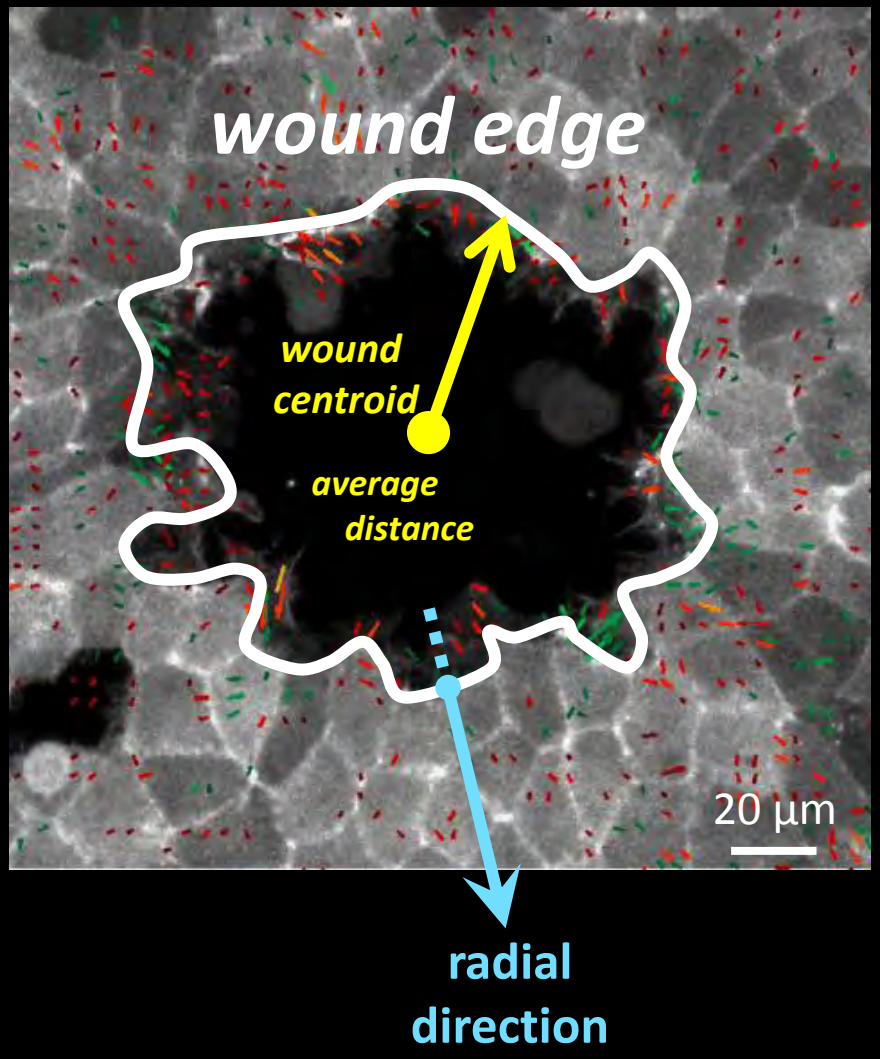




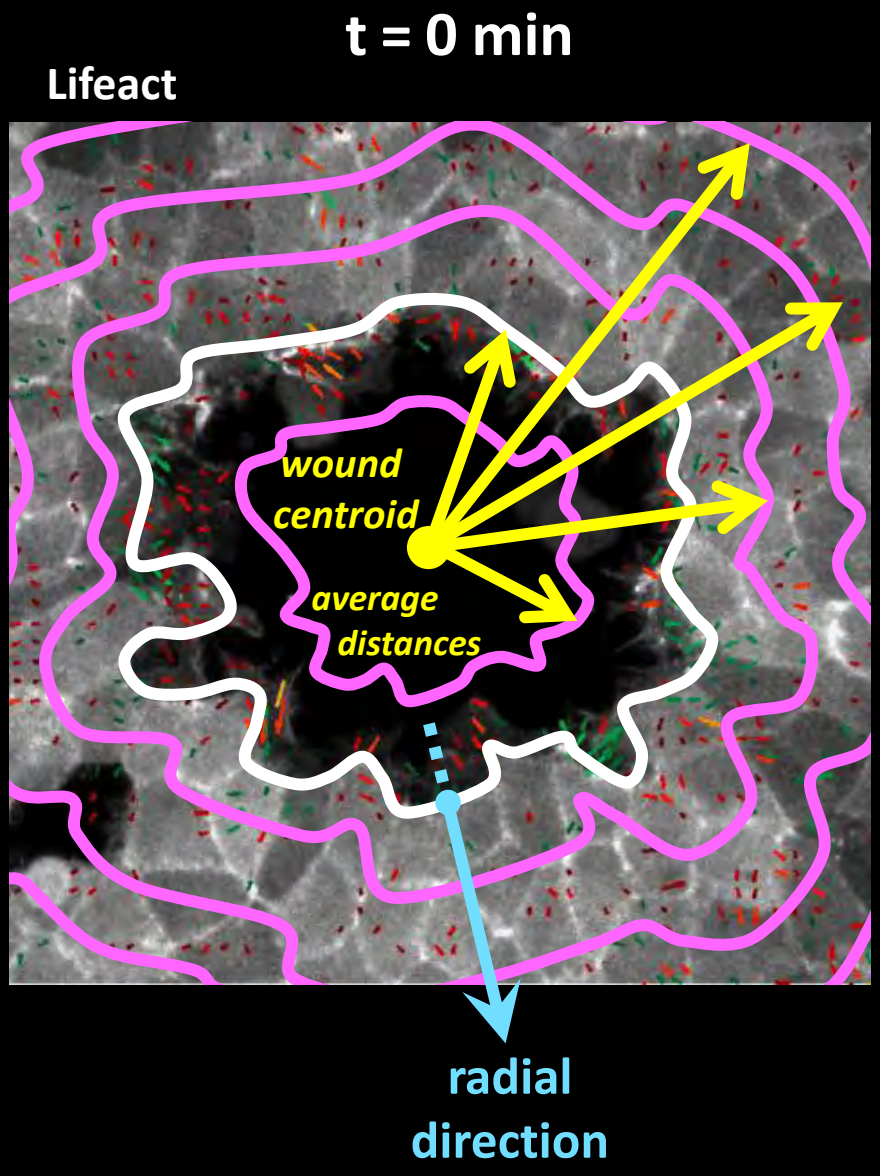
Radial Tractions Average

t = 0 min

Lifeact



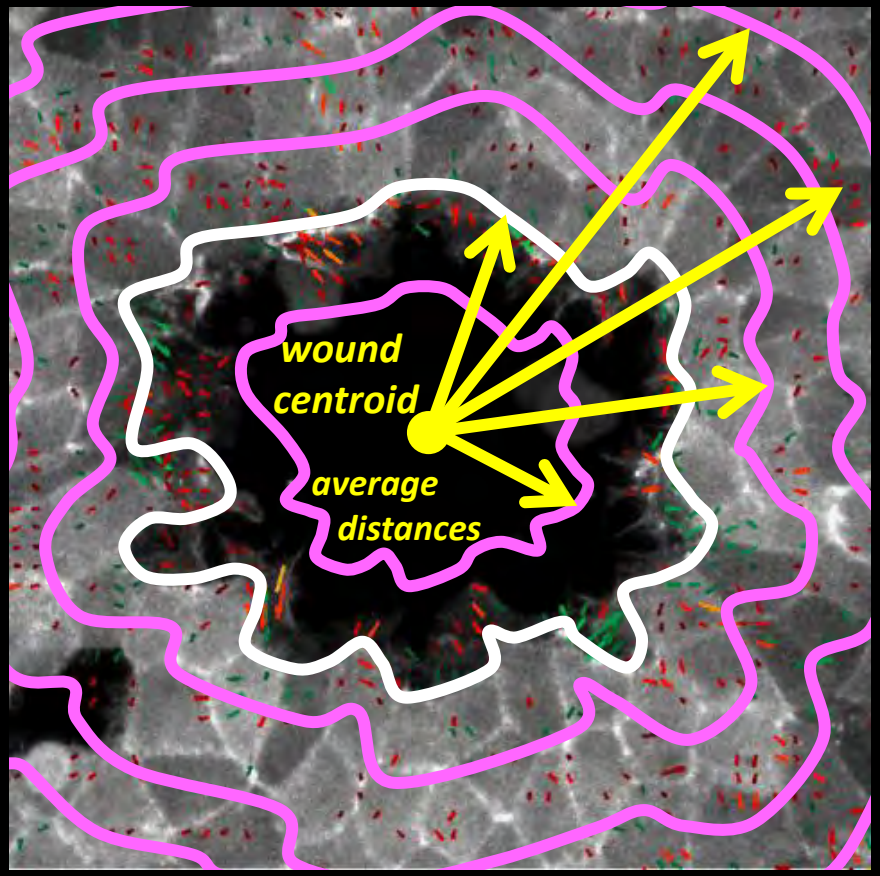
Radial Tractions Average

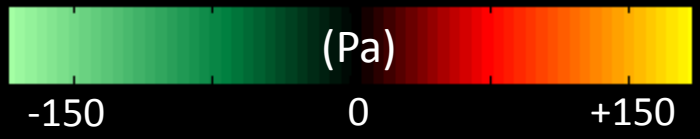


Radial Tractions Average

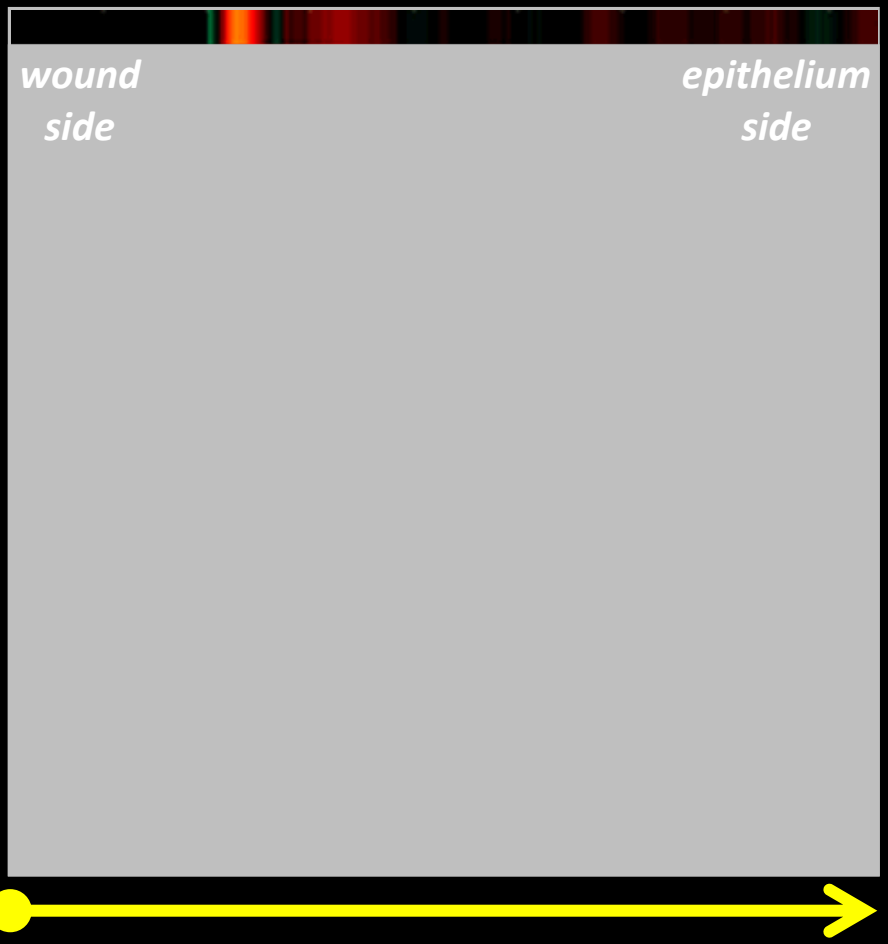
t = 0 min

Lifeact



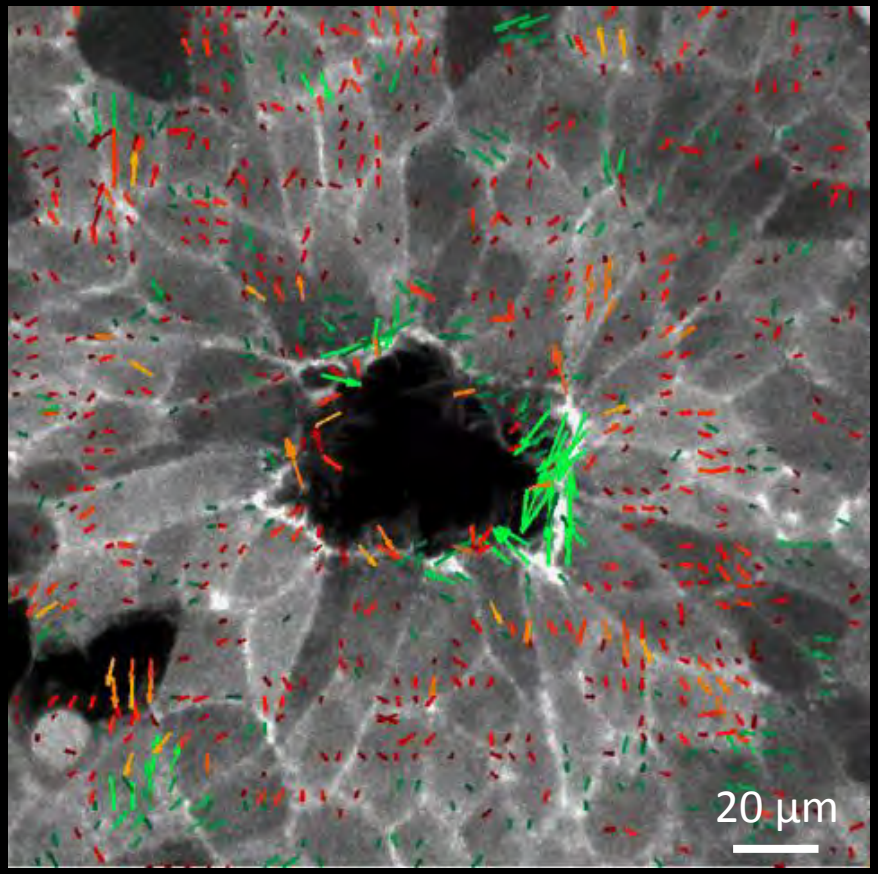


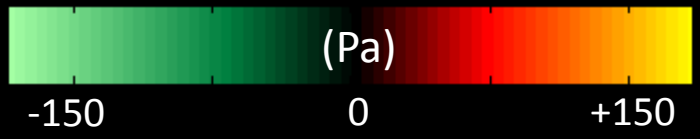
RADIAL TRACTIONS
averages at t = 0 min



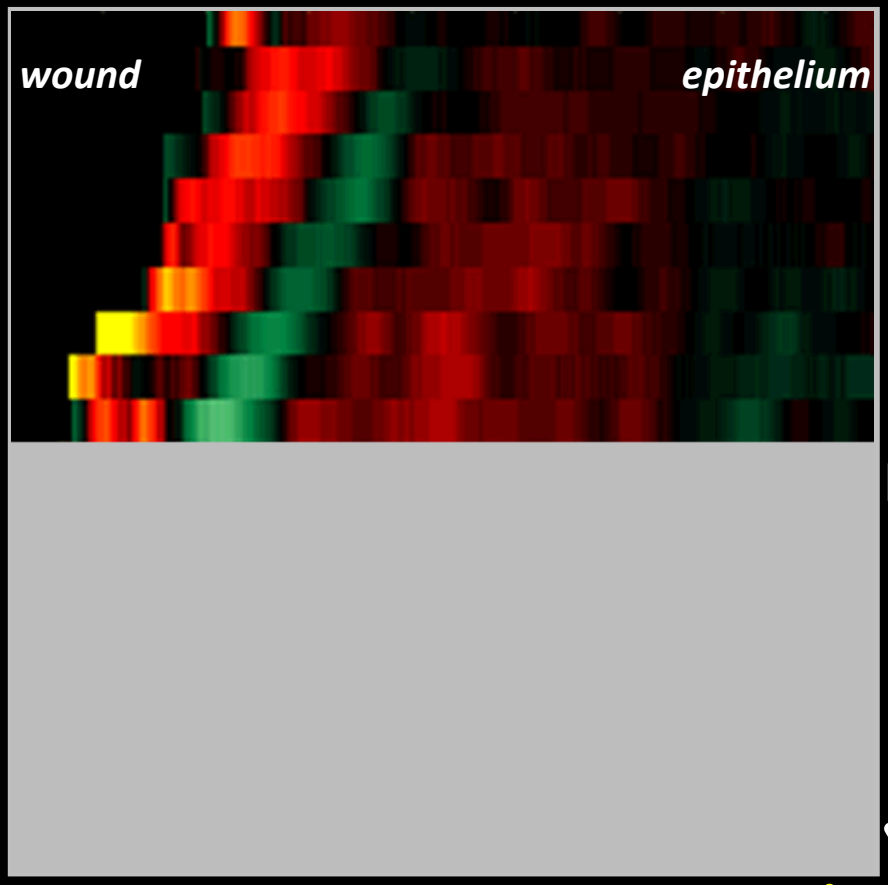
Radial Tractions Average

Lifeact



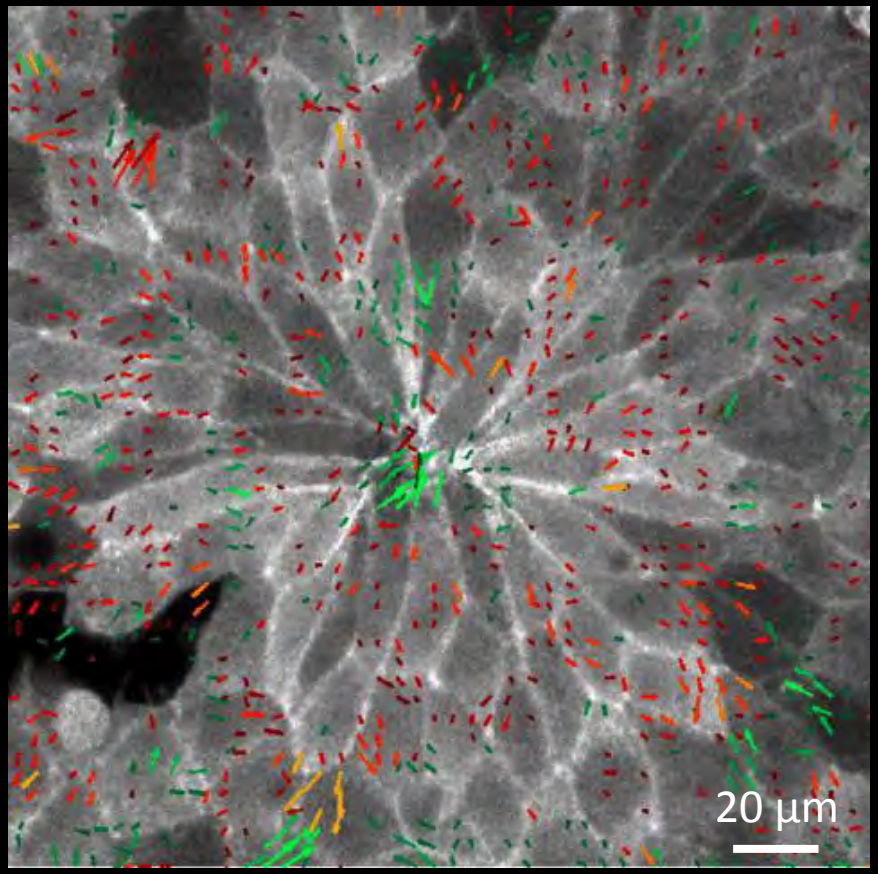


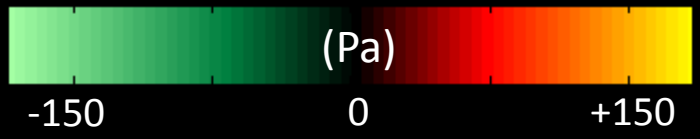
RADIAL TRACTIONS
kymograph



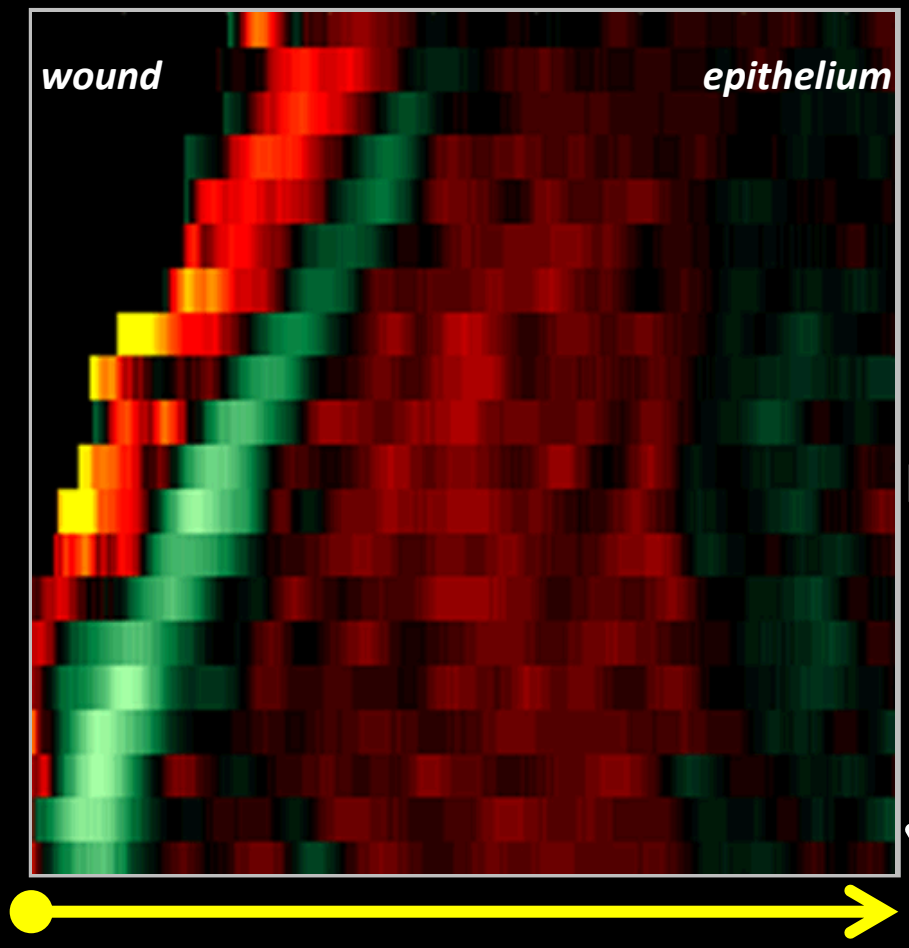
Radial Tractions Average

Lifeact

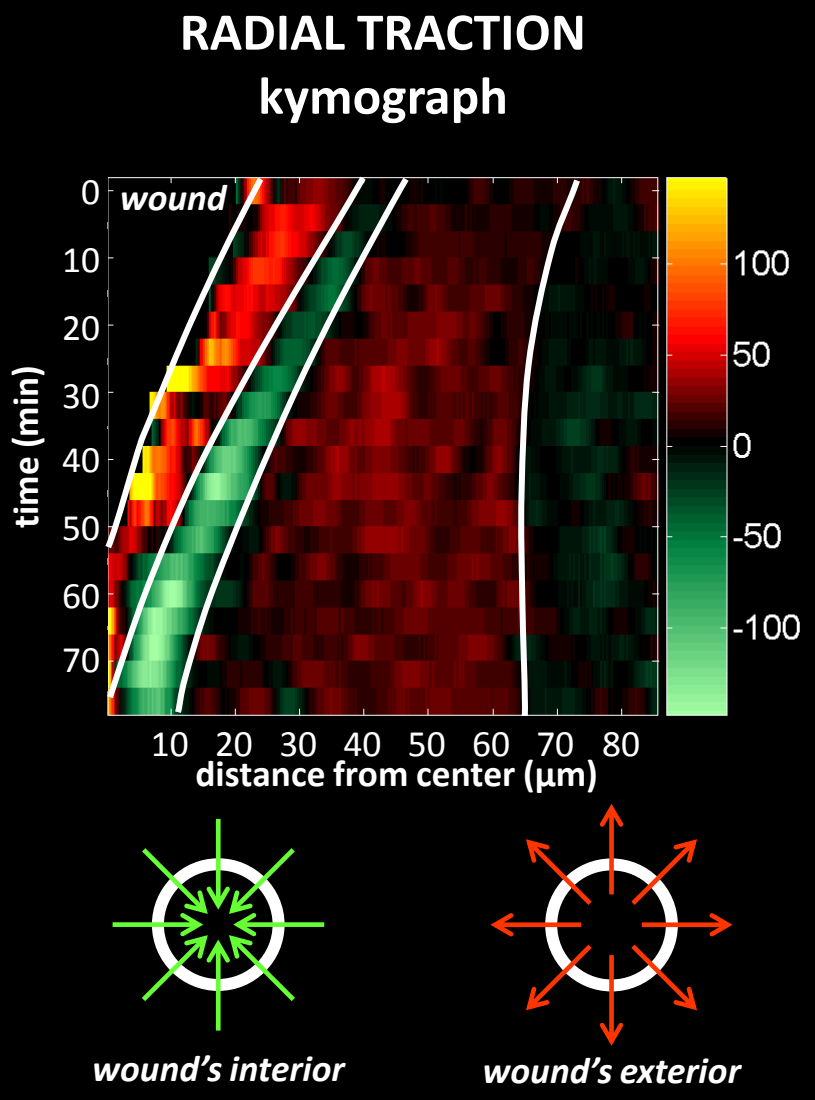




RADIAL TRACTIONS
kymograph

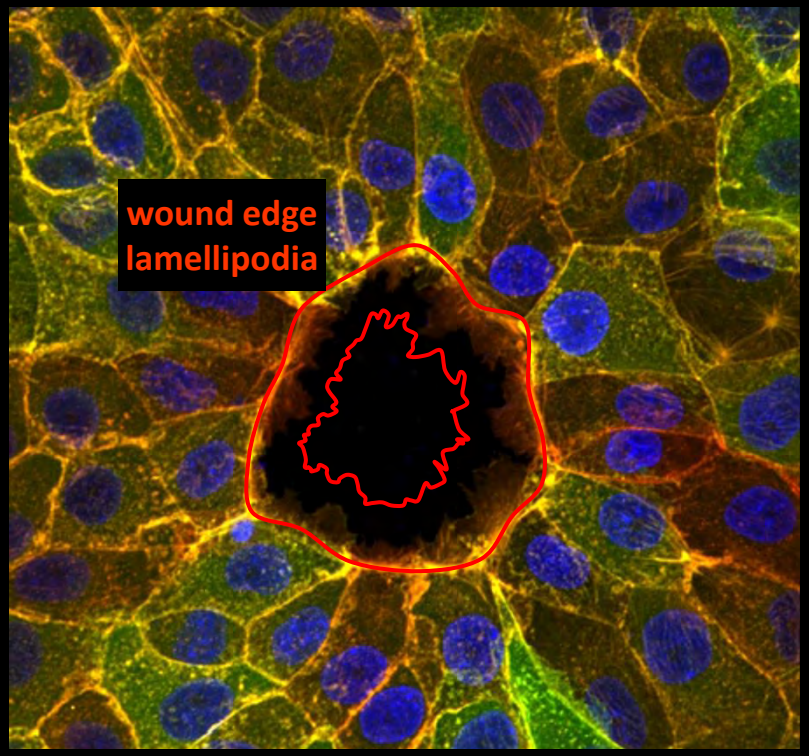


Mechanism Experimental Outline

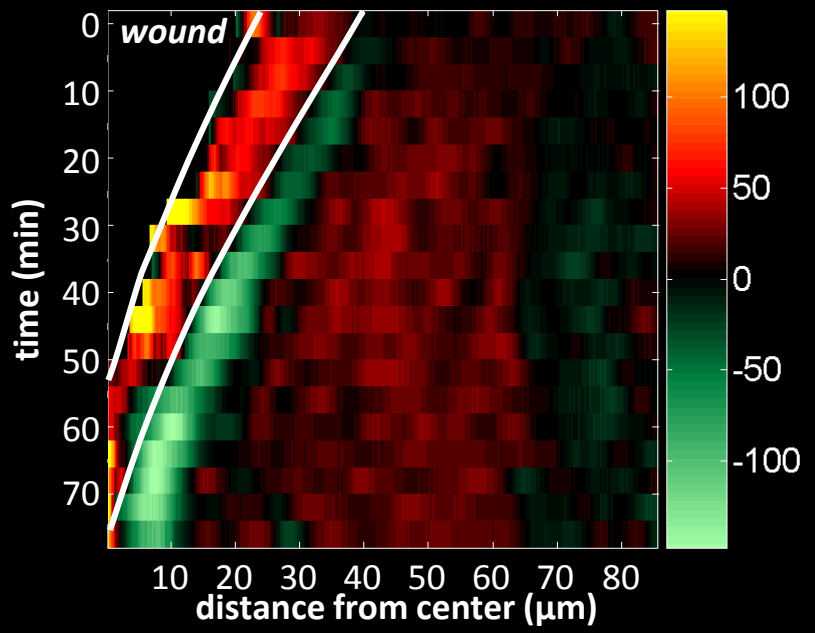


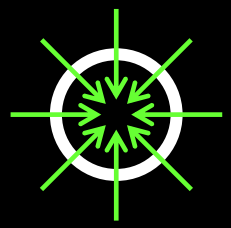
Mechanism Experimental Outline

DAPI
Actin

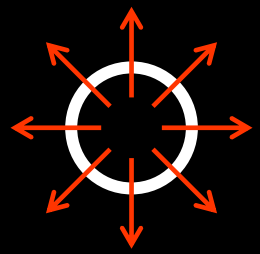


RADIAL TRACTION
kymograph





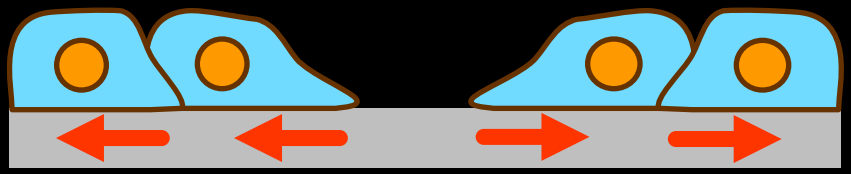
wound's interior

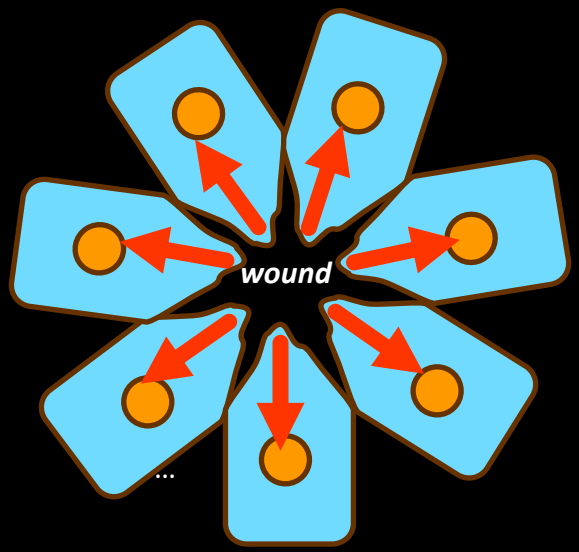


wound's exterior

DECIPHERING THE MECHANISM

CELL CRAWLING
(lamellipodia & filipodia)



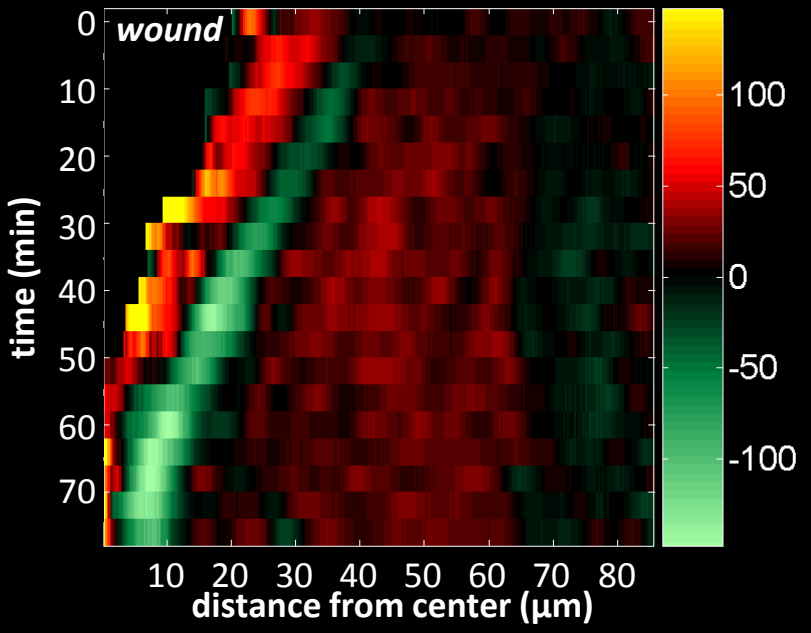


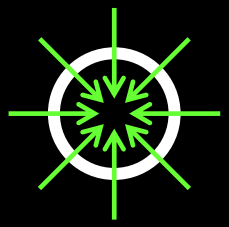
Omelchenko et al. PNAS (2003)

Poujade et al. PNAS (2007)

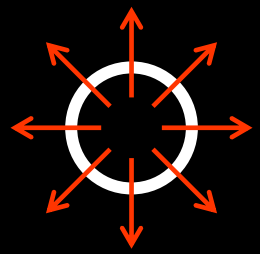
Lee P et al. *PLOS Comp. Biol.* (2011)… etc.

RADIAL TRACTION
kymograph



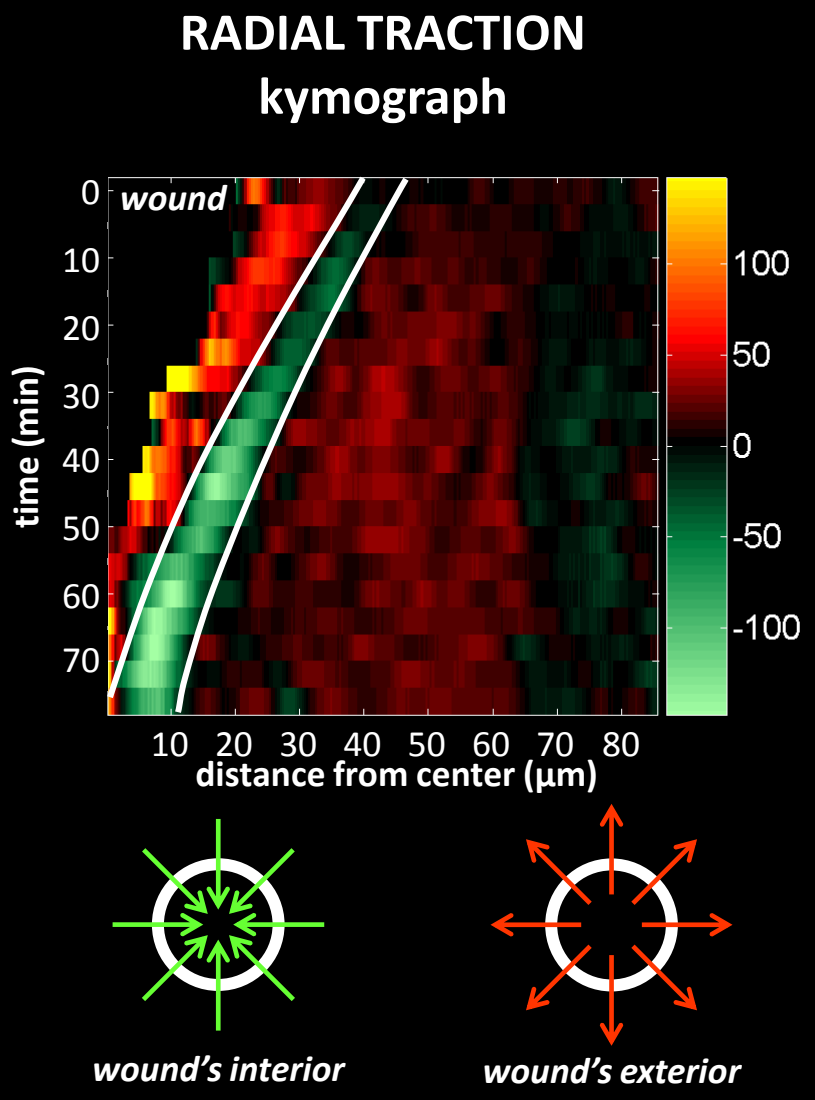


wound's interior



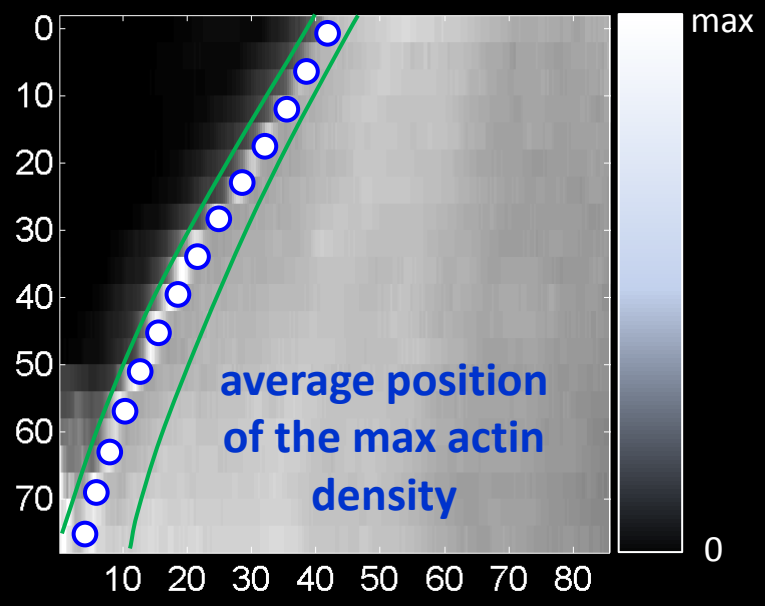
wound's exterior

Mechanism Experimental Outline

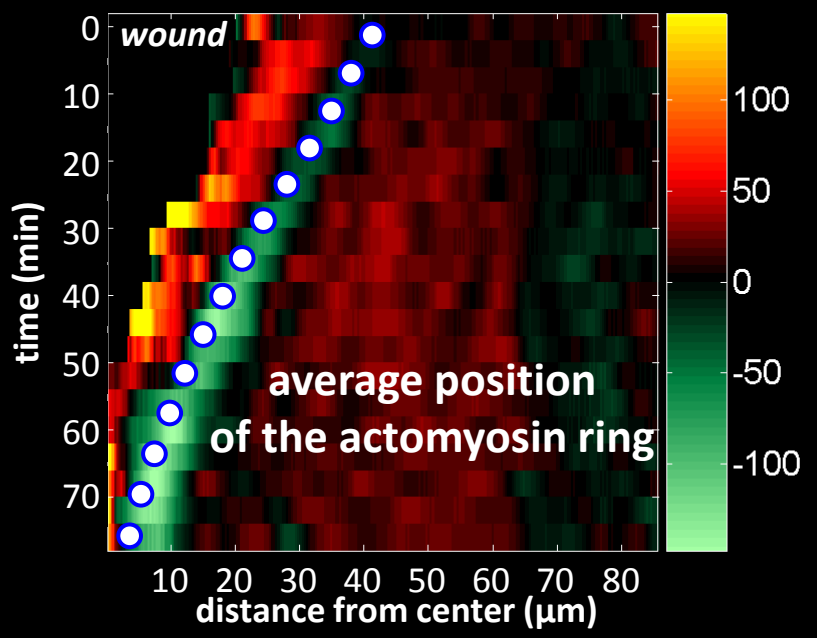


Mechanism Experimental Outline

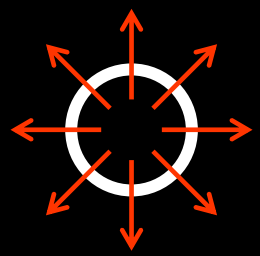
EPITHELIAL ACTIN DENSITY
kymograph



RADIAL TRACTION
kymograph



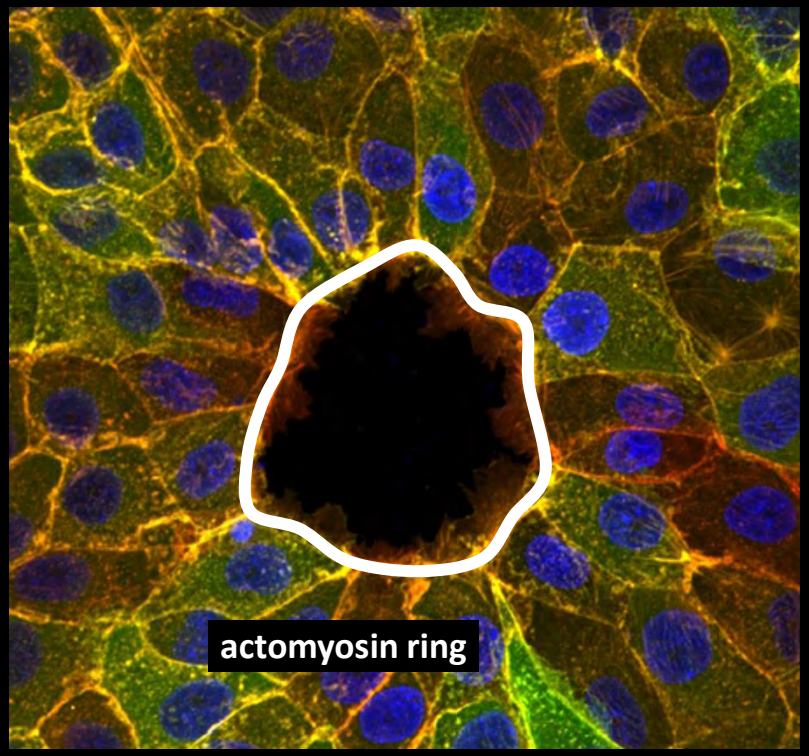
wound's interior



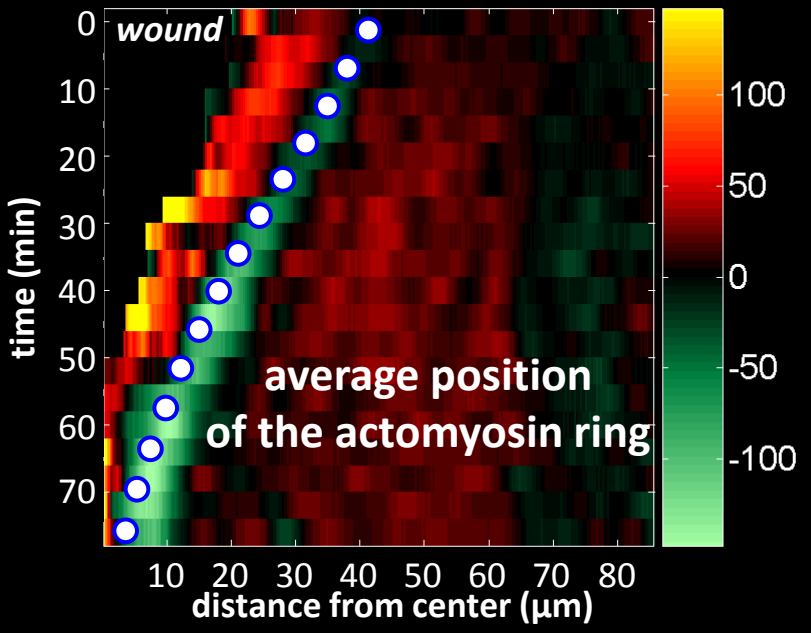
wound's exterior

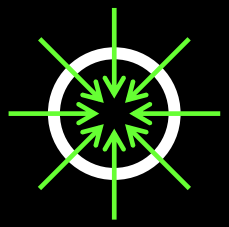
Mechanism Experimental Outline

DAPI
Actin

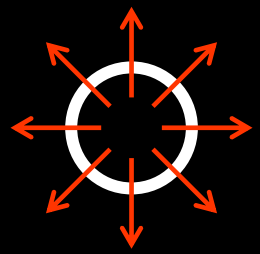


RADIAL TRACTION
kymograph





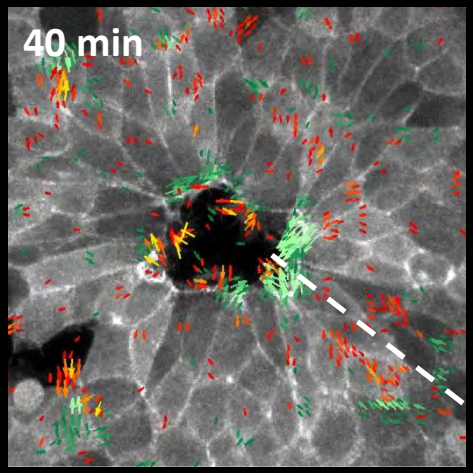
wound's interior



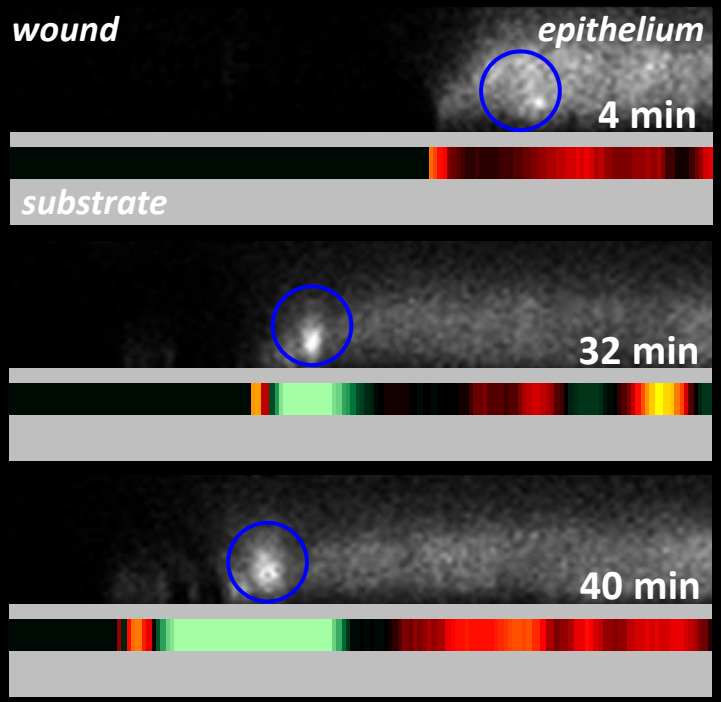
wound's exterior

Deciphering the Mechanism

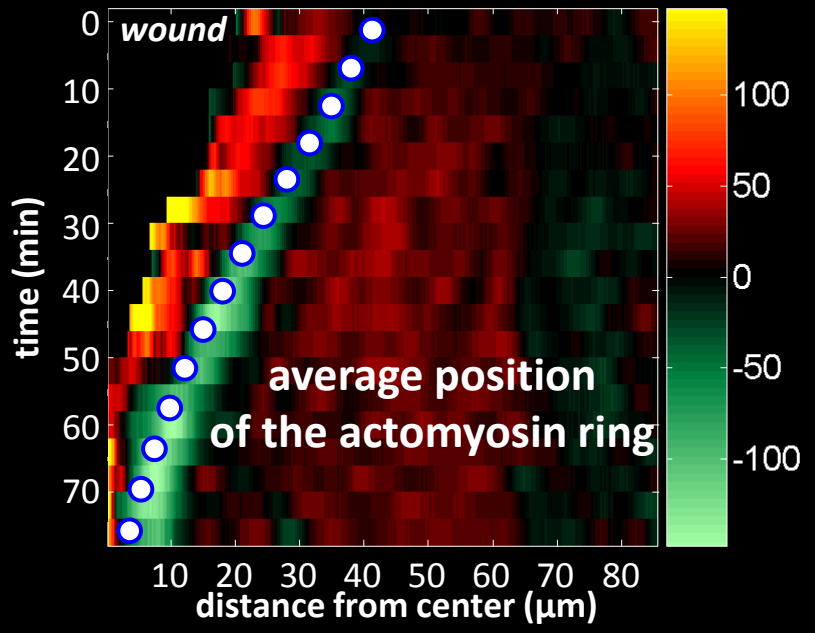
top view

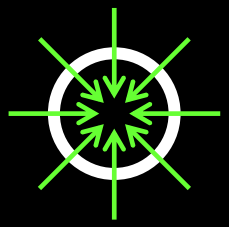


lateral view

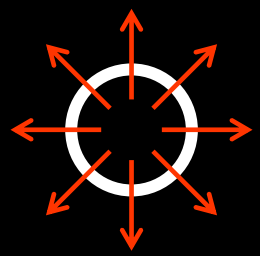


RADIAL TRACTION kymograph





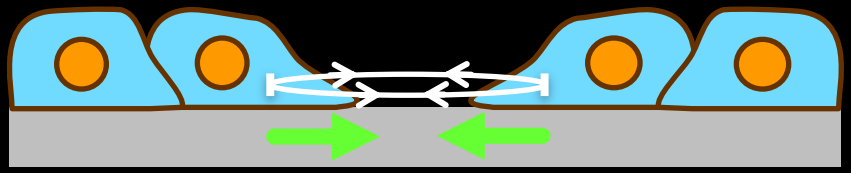
wound's interior

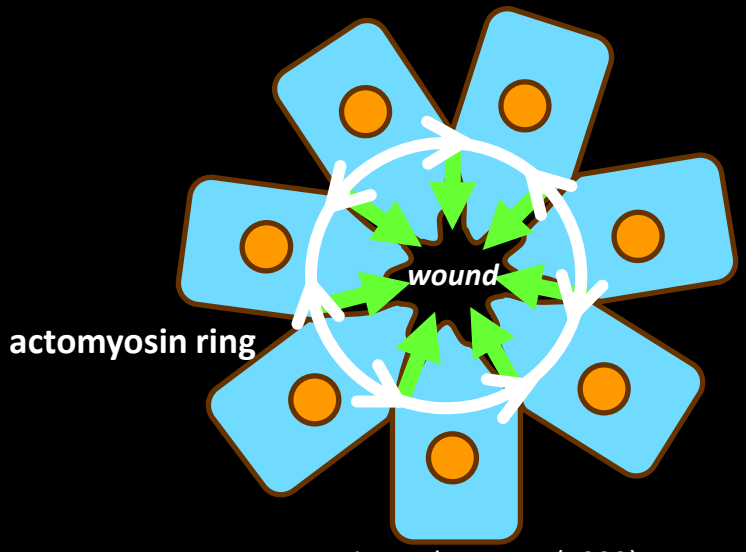


wound's exterior

Deciphering the Mechanism

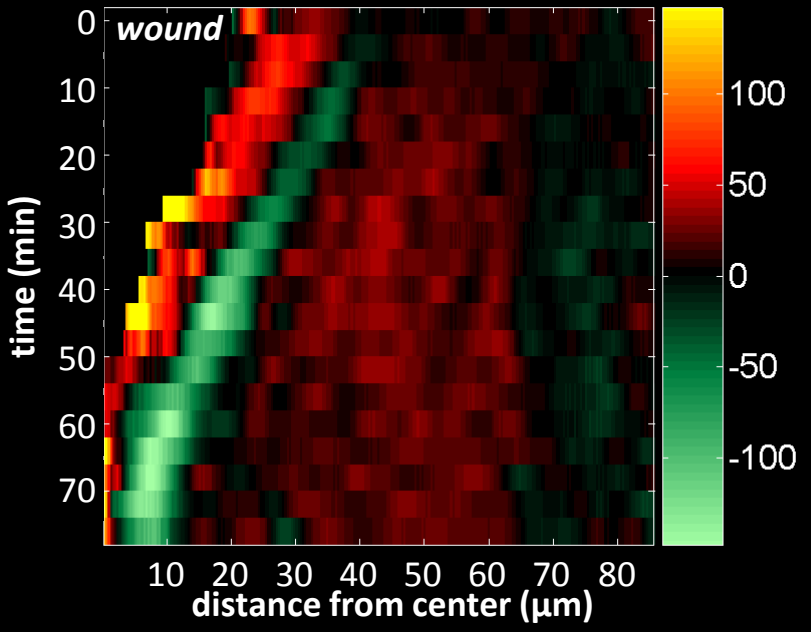
PURSE STRING
(actomyosin cable contraction)

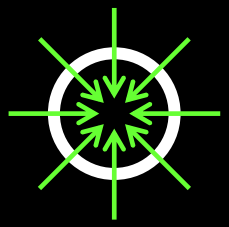




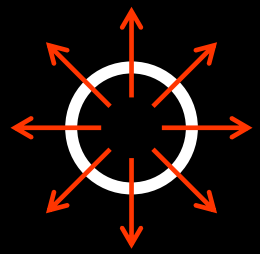
Martin et al. *Nature* (1992)
Davidson et al. Cell Motil Cytoskeleton (2002)
Wood et al. *Nature Cell Biology* (2004)
Tamada et al. *Journal Cell Biology* (2007) …etc.

RADIAL TRACTION
kymograph



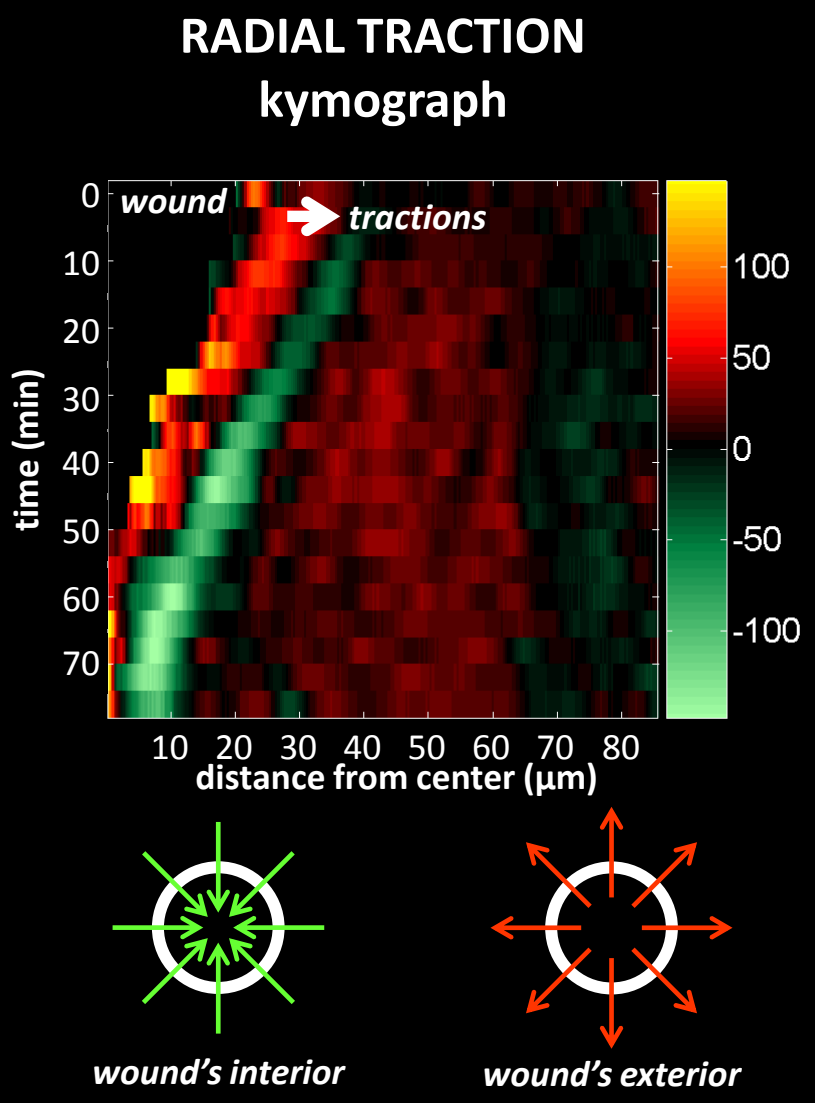


wound's interior



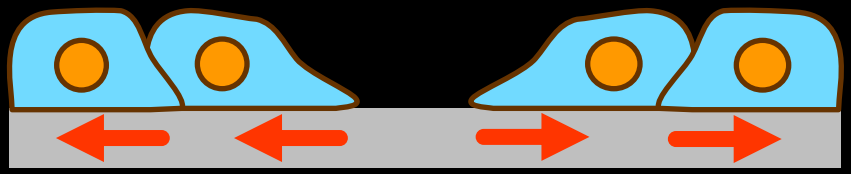
wound's exterior

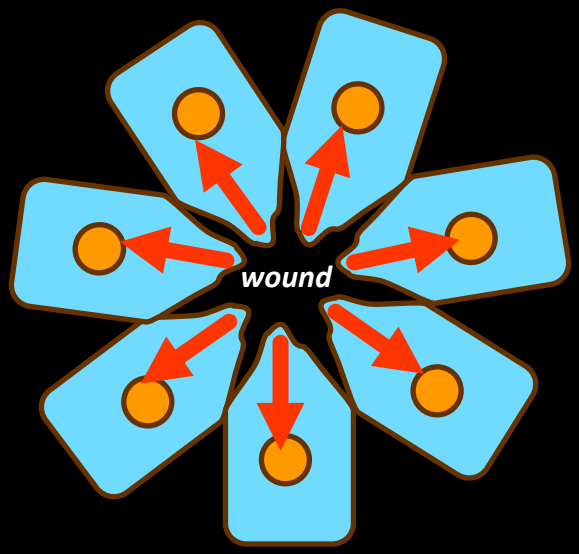
Deciphering the Mechanism



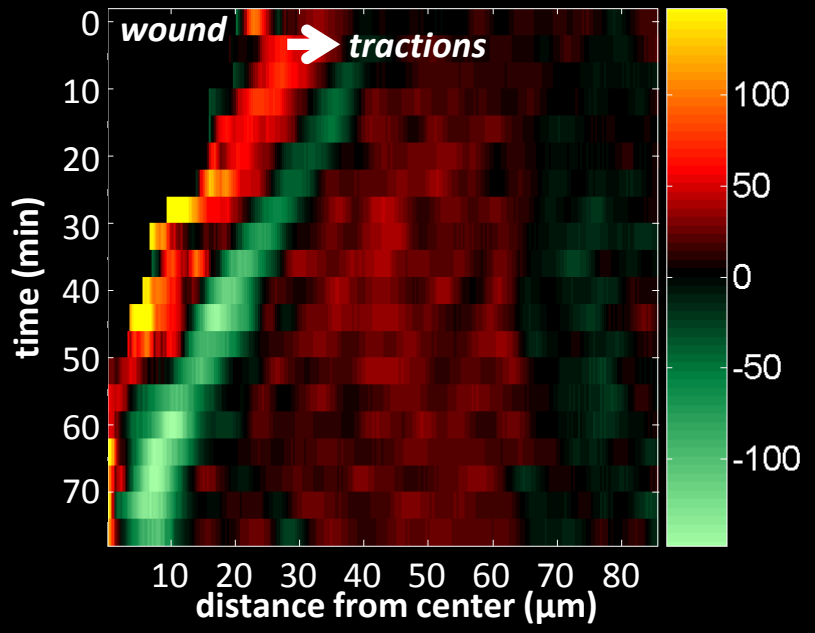
DECIPHERING THE MECHANISM

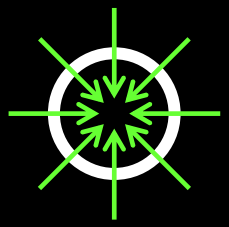
CELL CRAWLING
(lamellipodia & filipodia)



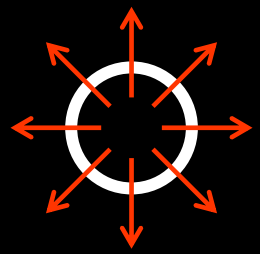


RADIAL TRACTION
kymograph





wound's interior

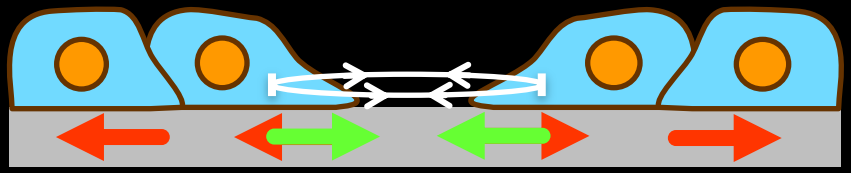


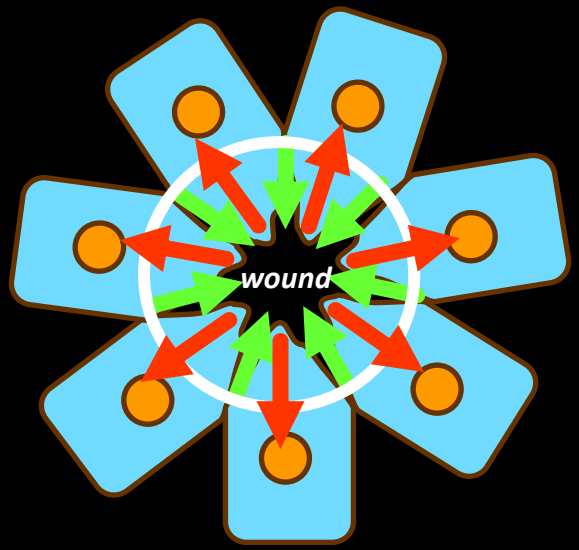
wound's exterior

DECIPHERING THE MECHANISM

CELL CRAWLING & **PURSE STRING**

(lamellipodia & filopodia) **(actomyosin cable contraction)**



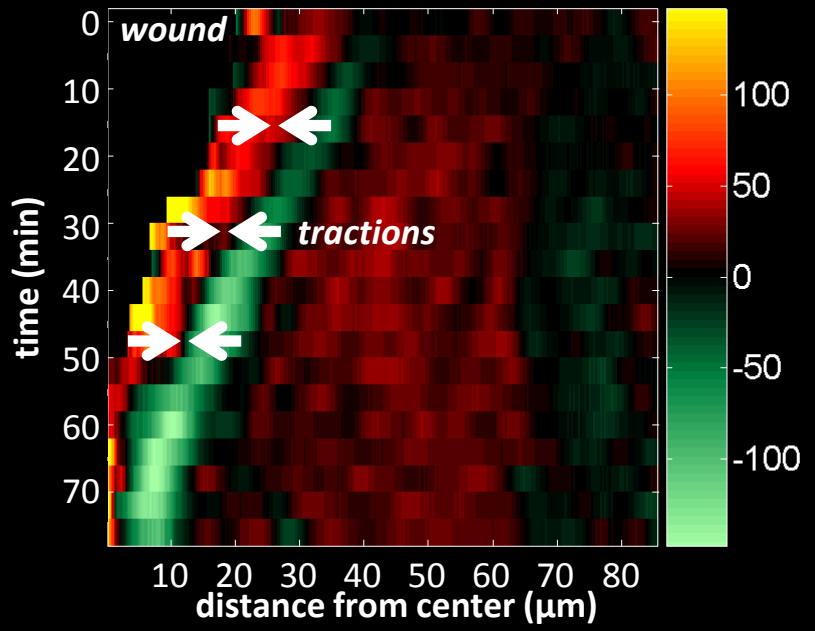


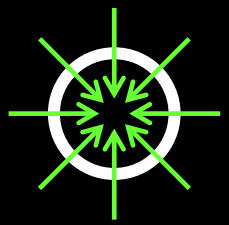
Anon et al. PNAS (2012)

Klarlund PNAS (2012)

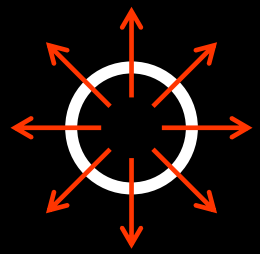
Abreu-Blanco et al. J. Cell Sci. . (2012)… etc.

RADIAL TRACTION kymograph





wound's interior

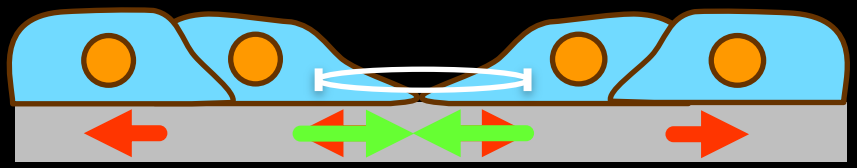


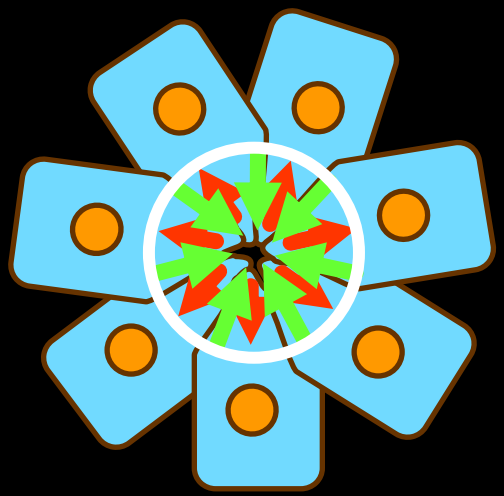
wound's exterior

DECIPHERING THE MECHANISM

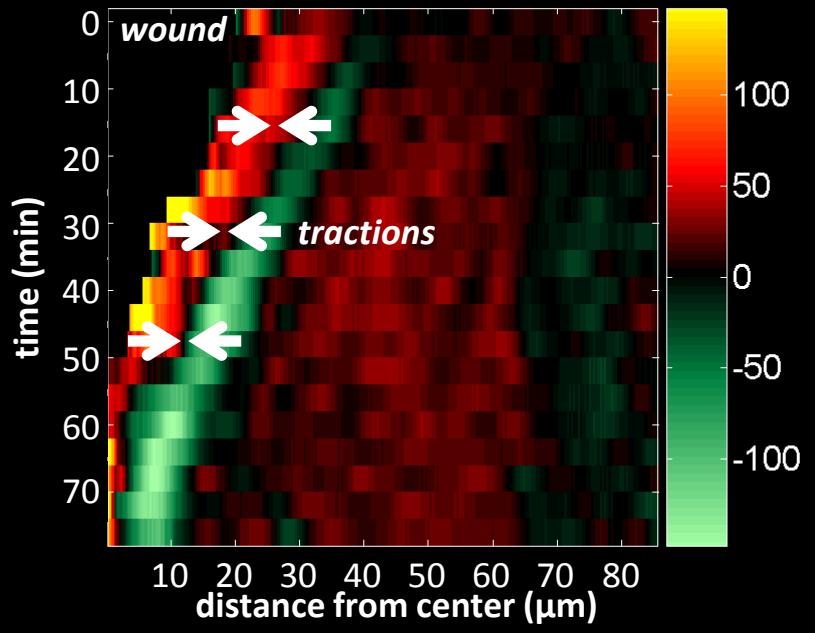
CELL CRAWLING & **PURSE STRING**

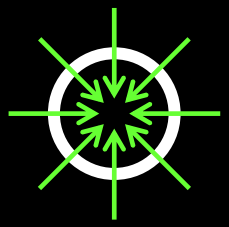
(lamellipodia & filopodia) (actomyosin cable contraction)



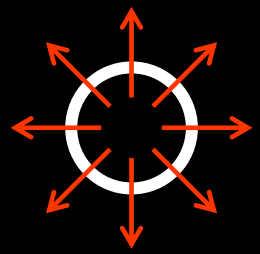


RADIAL TRACTION kymograph





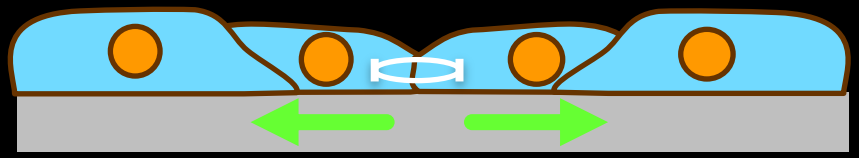
wound's interior

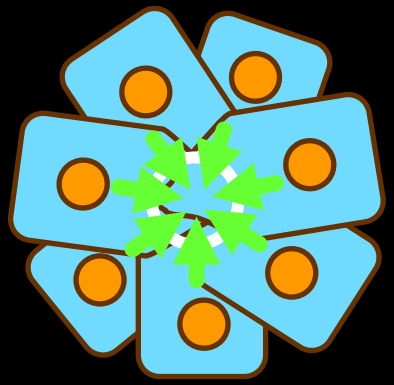


wound's exterior

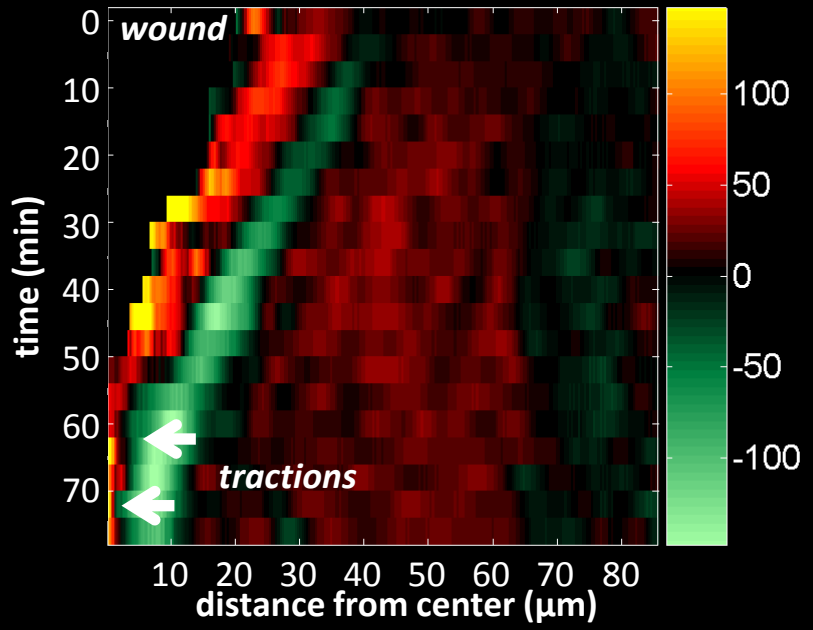
DECIPHERING THE MECHANISM

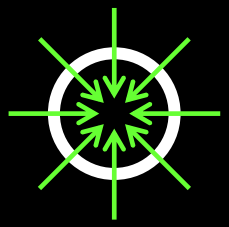
PURSE STRING
(actomyosin cable contraction)



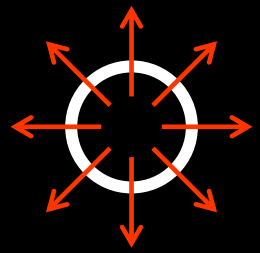


RADIAL TRACTION
kymograph





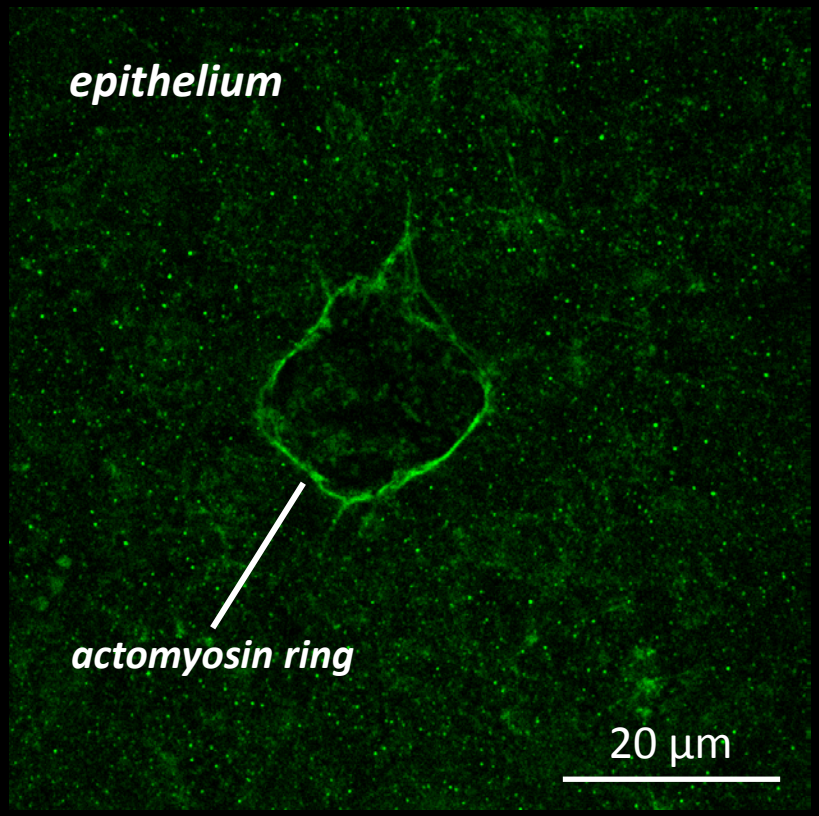
wound's interior



wound's exterior

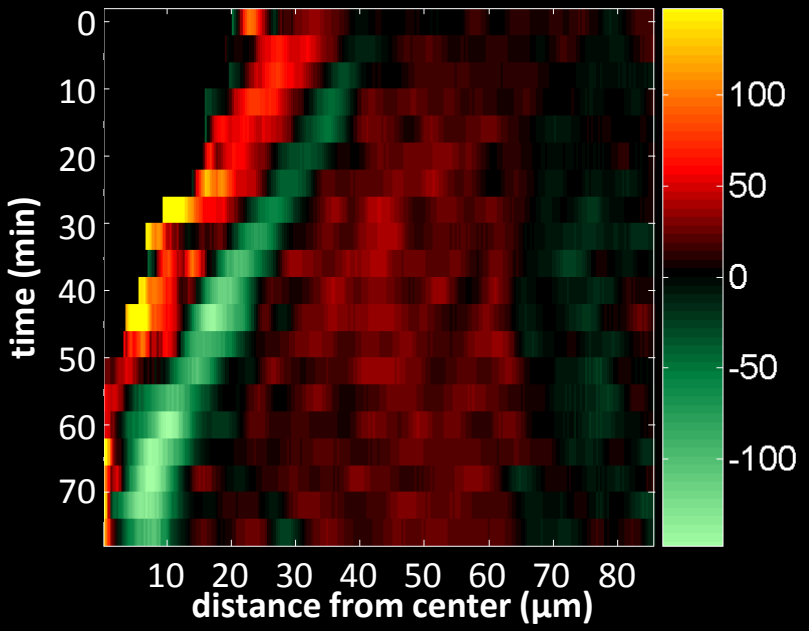
EXPERIMENTAL VALIDATION of the MECHANISM

EGTA CALCIUM CHELATION
(weakened cell-cell junctions)



phospho-myosin

RADIAL TRACTION
kymograph



EXPERIMENTAL VALIDATION of the MECHANISM

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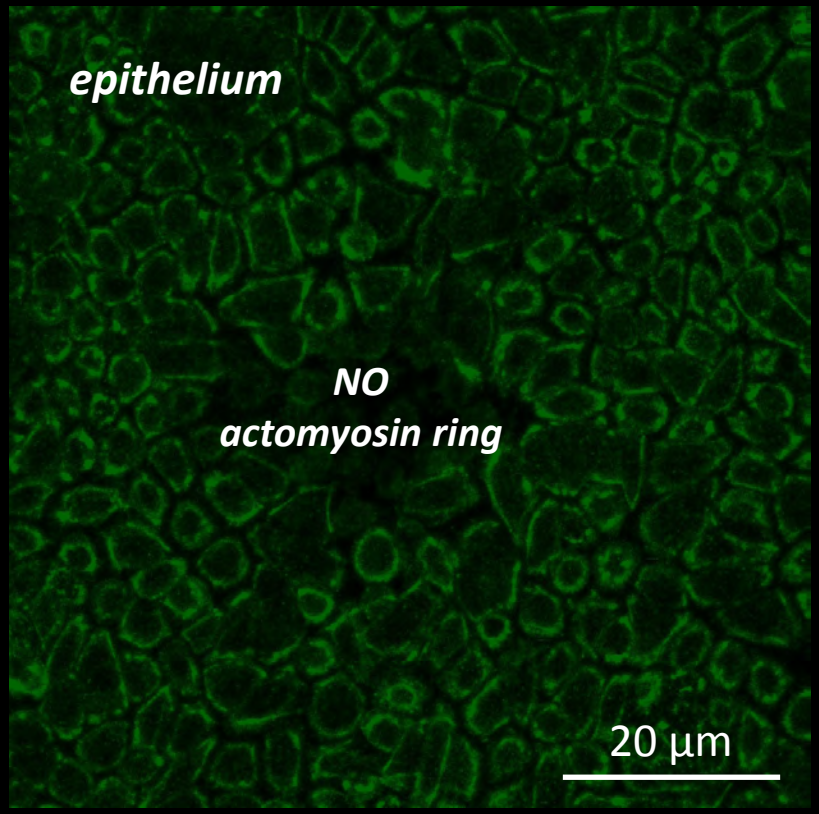
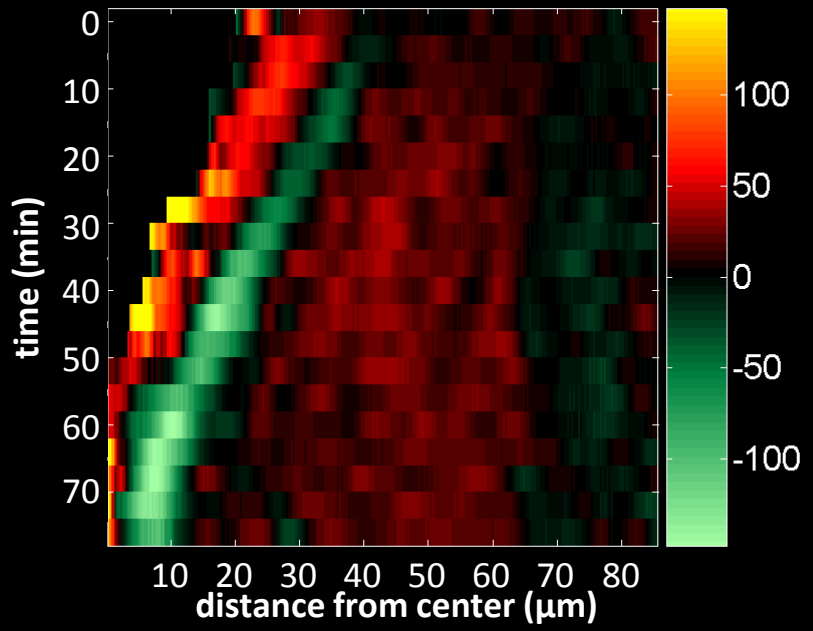
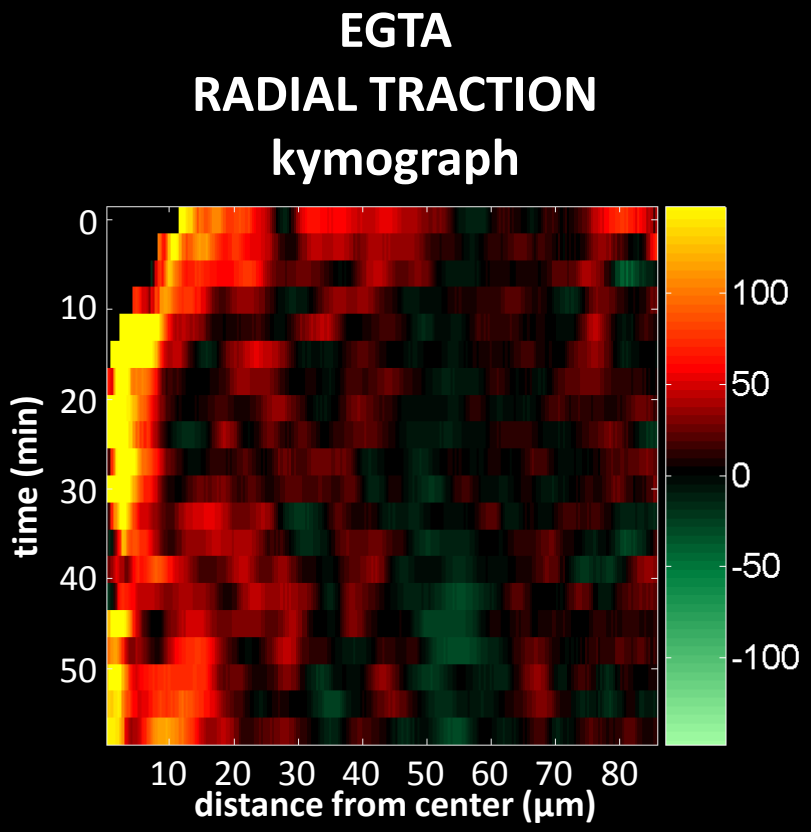


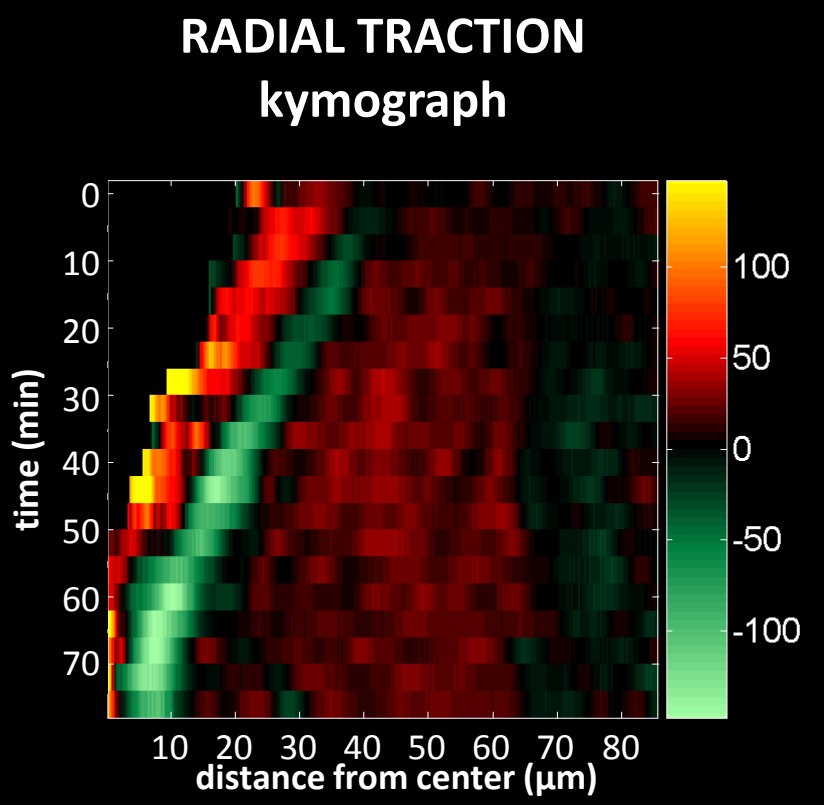
phospho-myosin

RADIAL TRACTION
kymograph

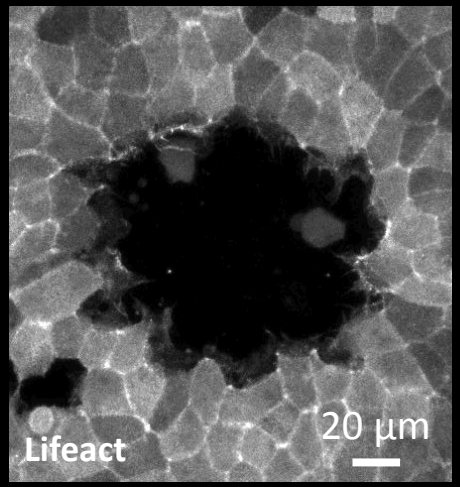


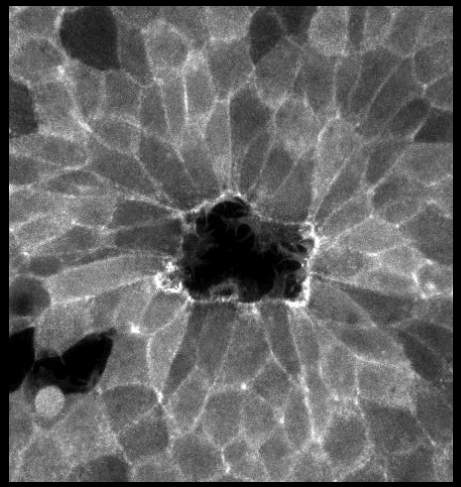
EXPERIMENTAL VALIDATION of the MECHANISM

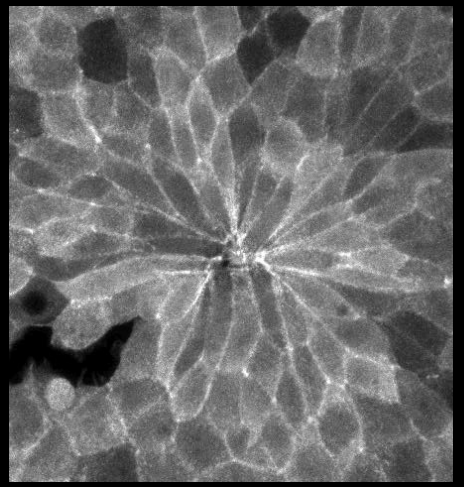




WE EXPERIMENTALLY QUANTIFIED AND DETAILED A CANDIDATE MECHANISM DRIVING EPITHELIAL WOUND HEALING





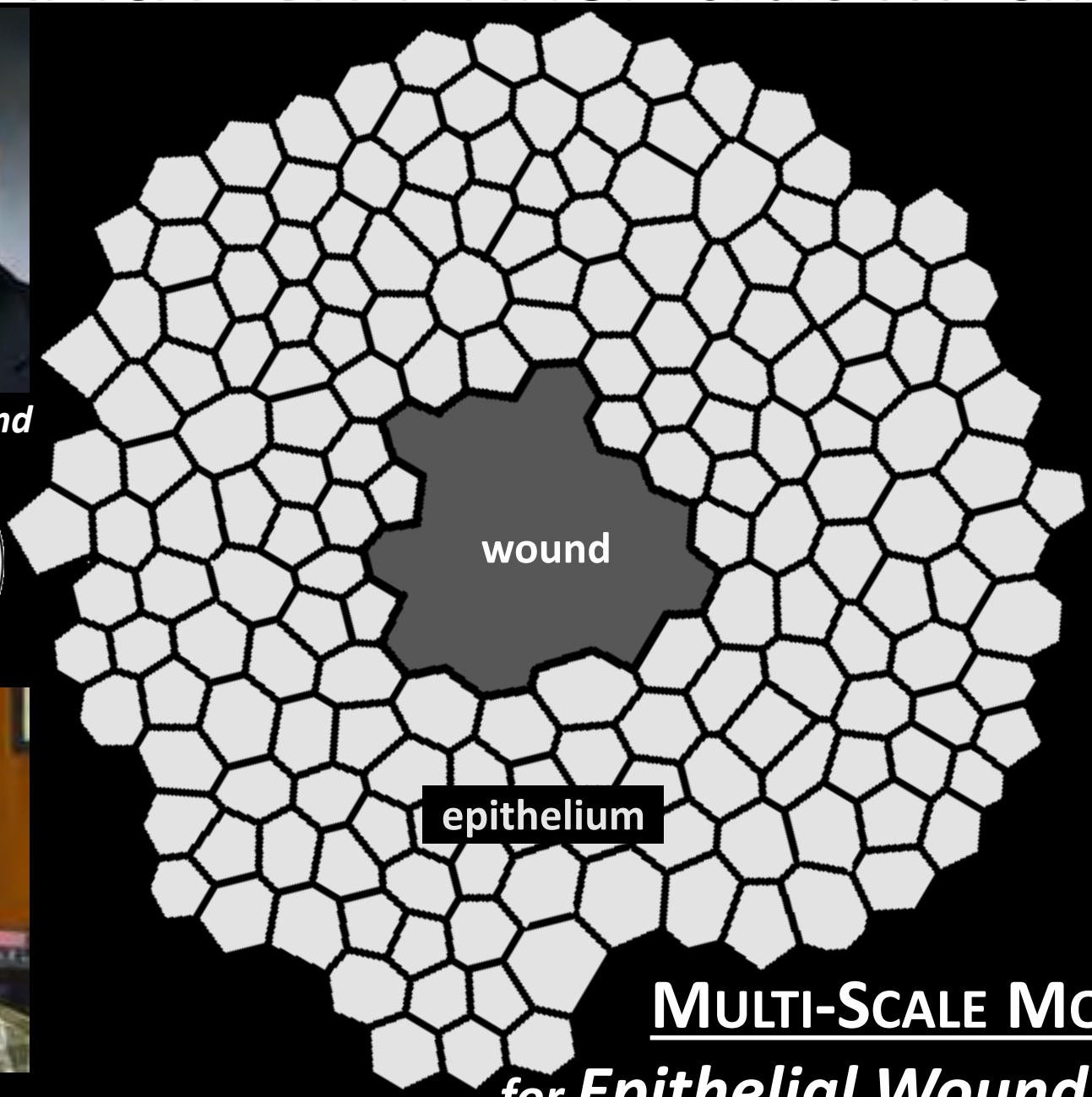


CELL CRAWLING + PURSE STRING

Mechanical Validation of the Mechanism

Wayne Brodland

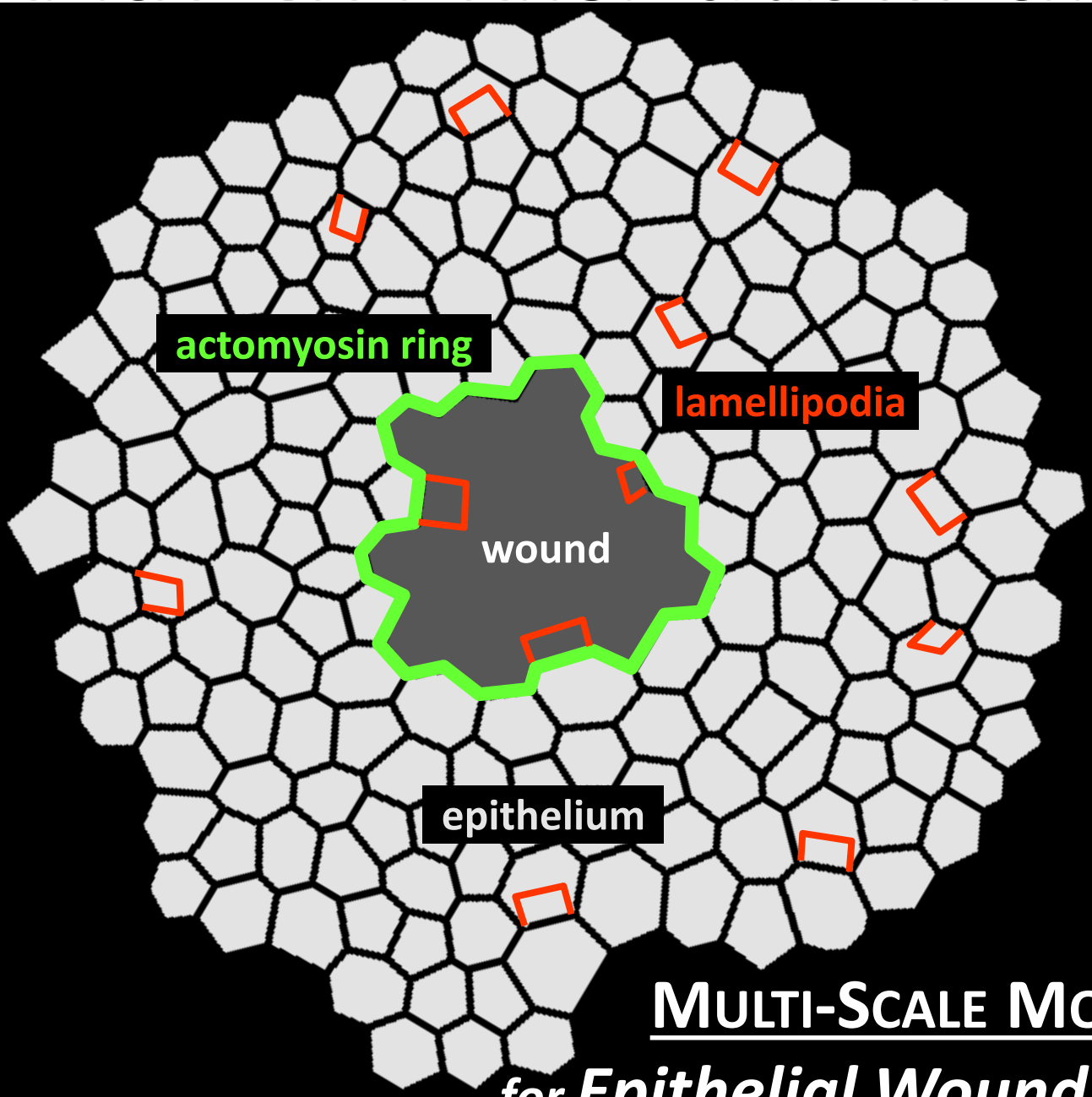
Jim Veldhuis



Multi-Scale Model

for Epithelial Wound Healing

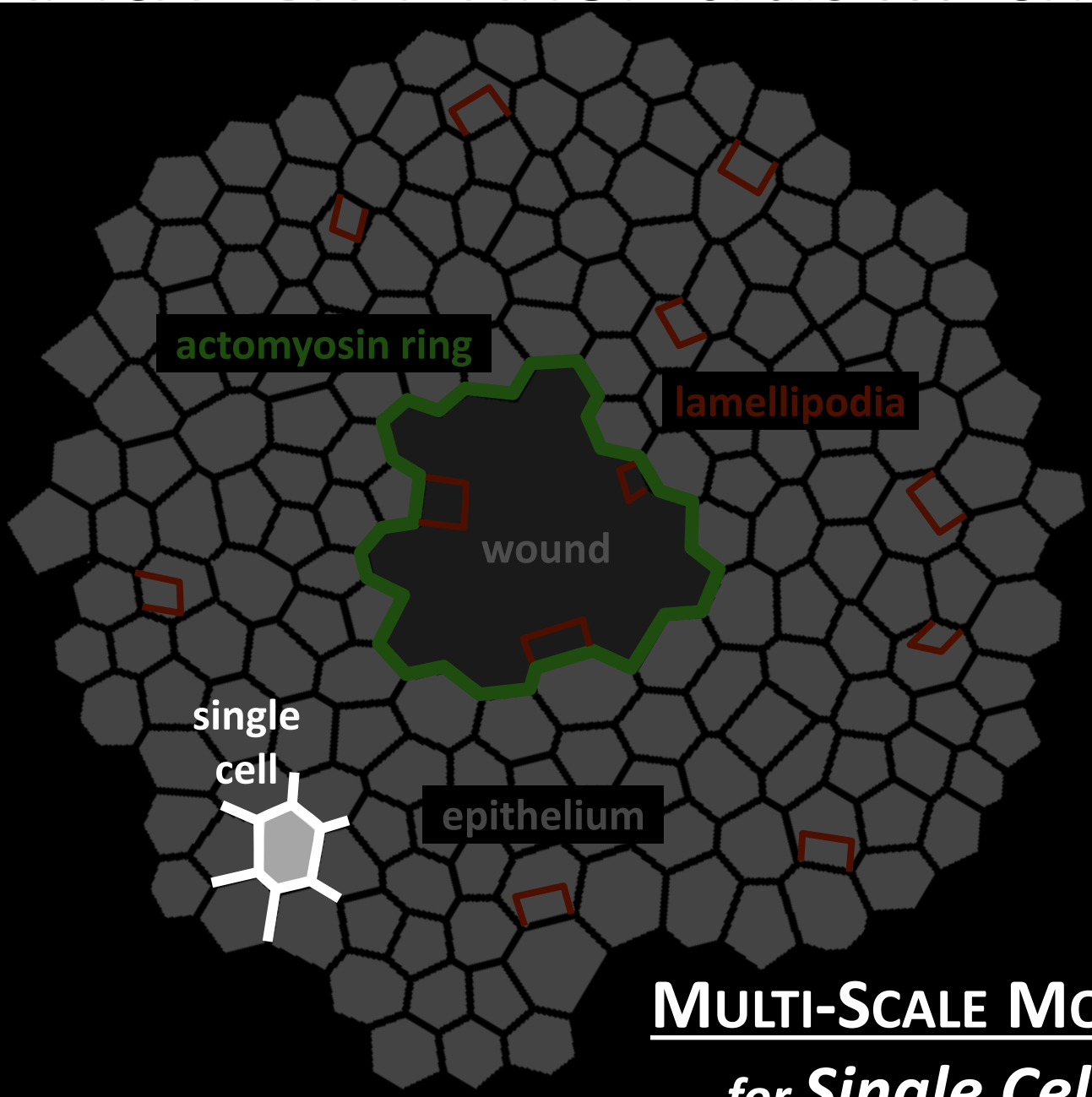
Mechanical Validation of the Mechanism



Multi-Scale Model

for Epithelial Wound Healing

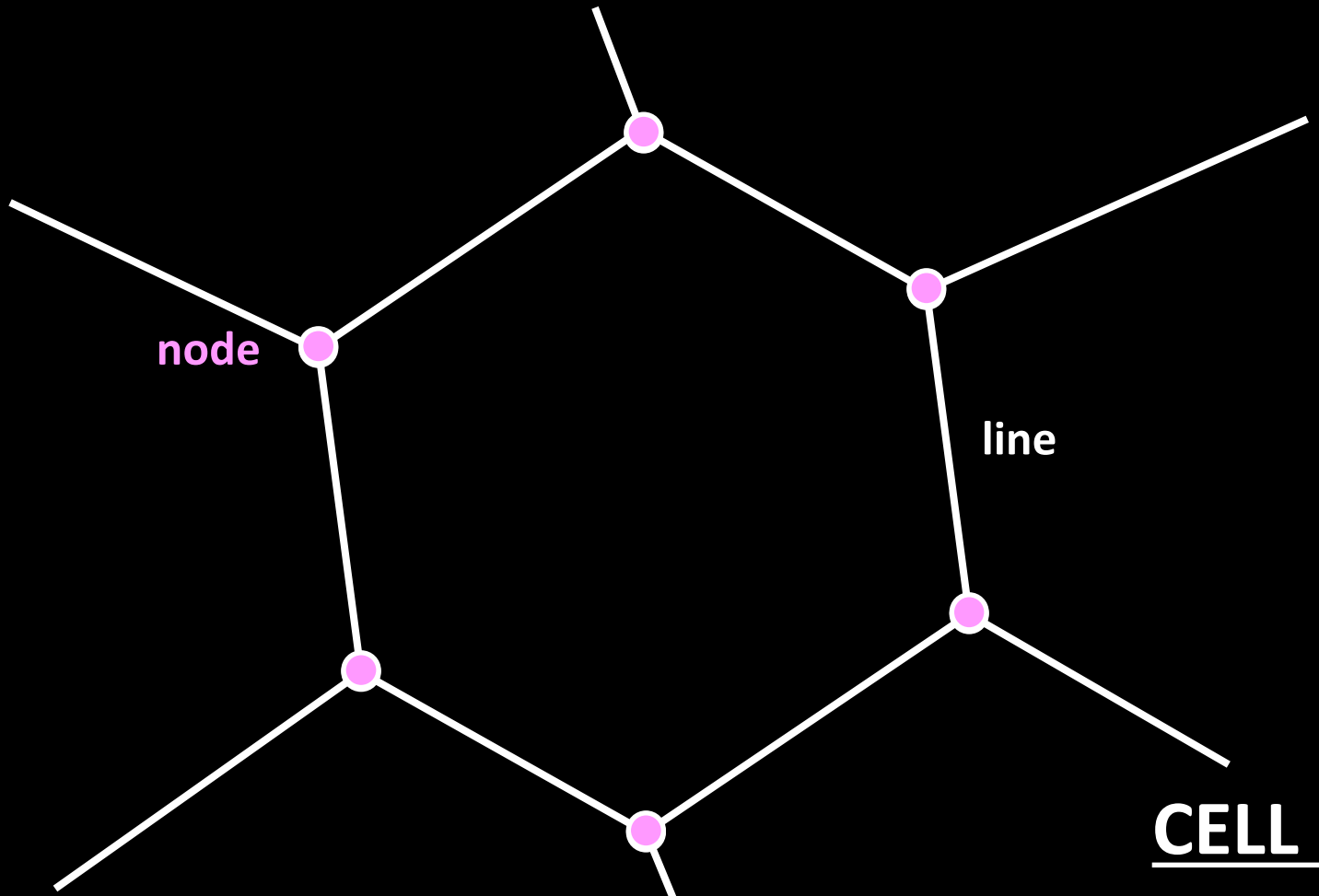
Mechanical Validation of the Mechanism



Multi-Scale Model

for Single Cell

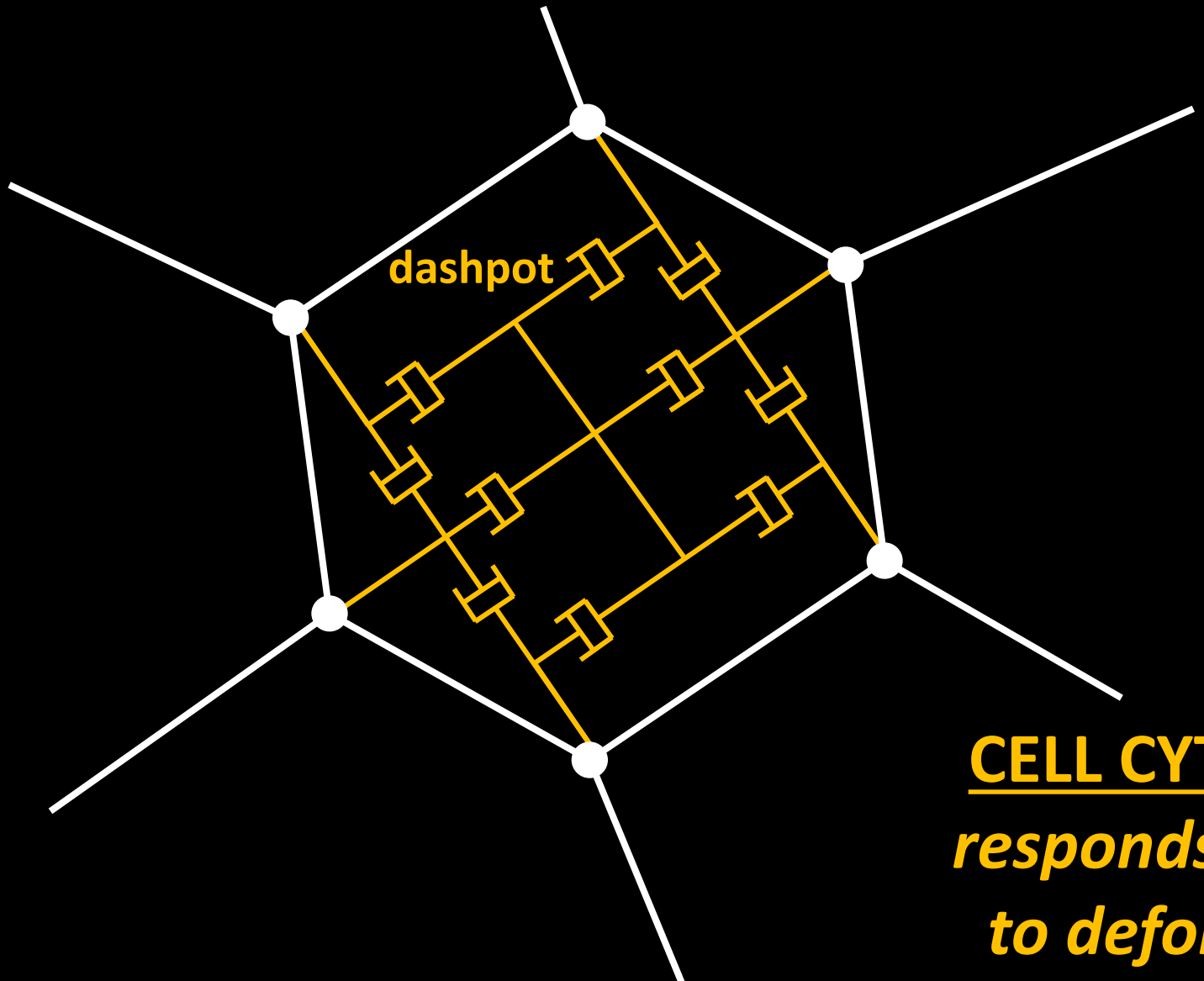
MULTI-SCALE MODEL for SINGLE CELL



CELL UNIT

geometry of nodes
joined through lines

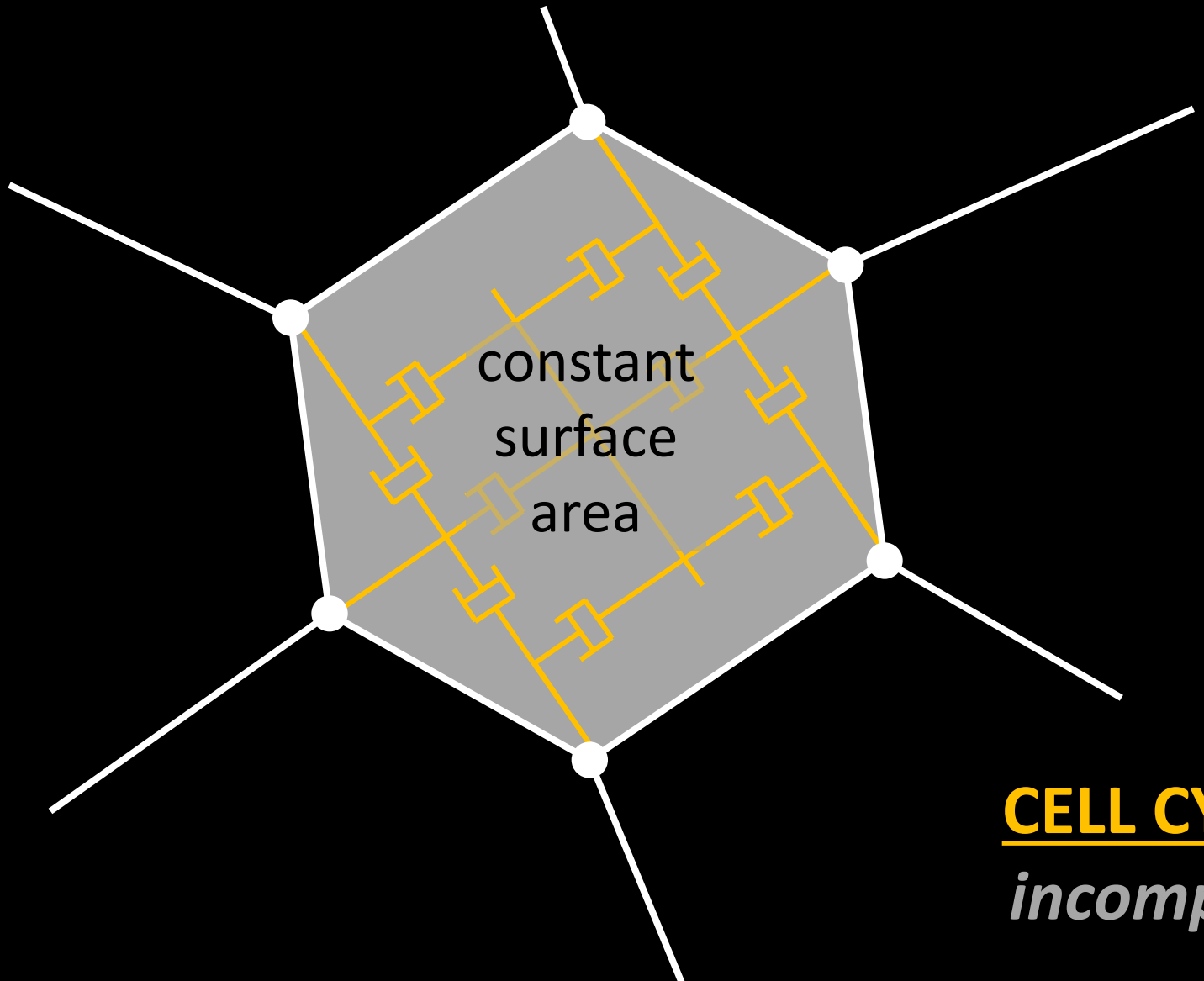
MULTI-SCALE MODEL for SINGLE CELL



CELL CYTOPLASM

responds viscously to deformations

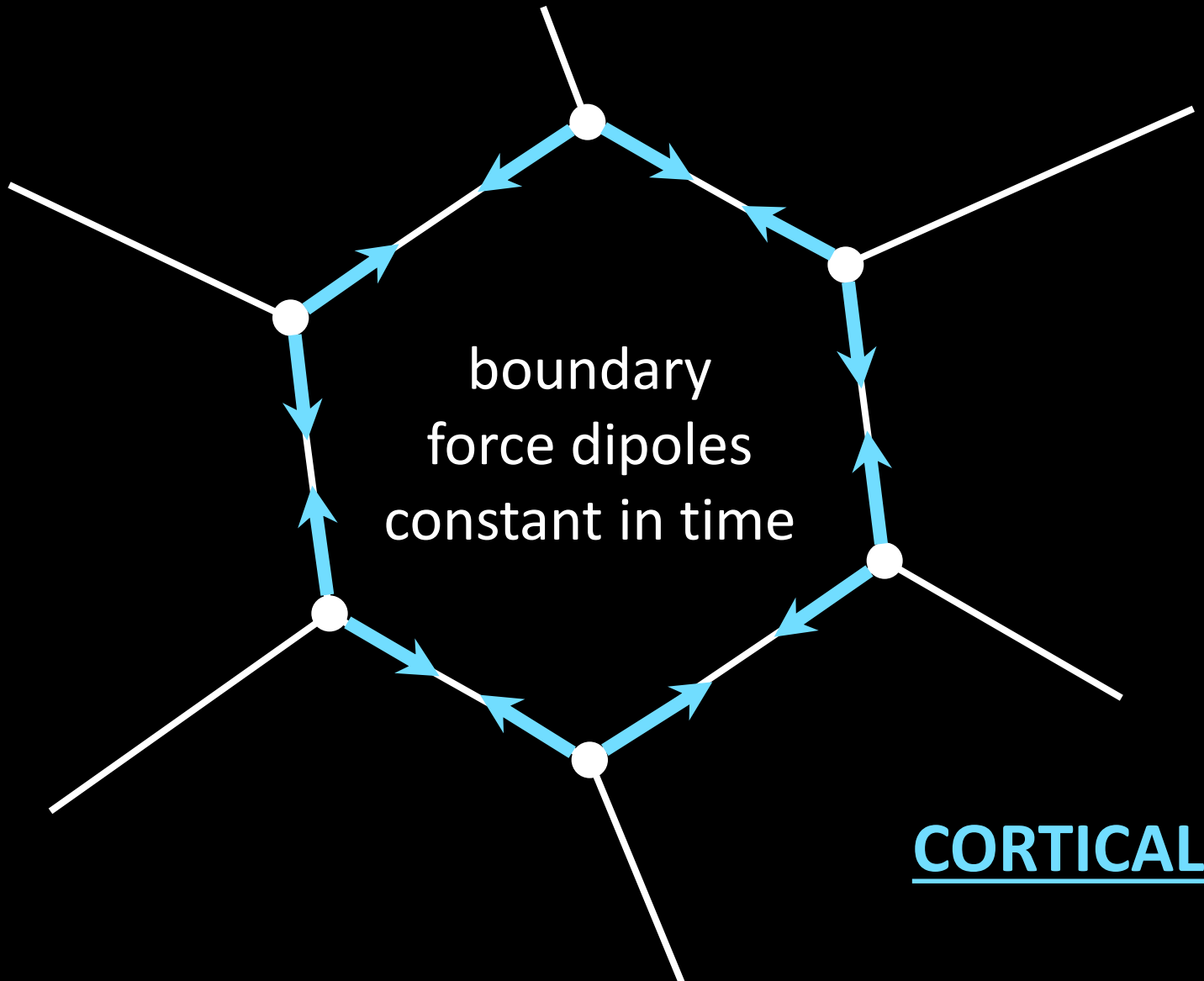
MULTI-SCALE MODEL for SINGLE CELL



CELL CYTOPLASM

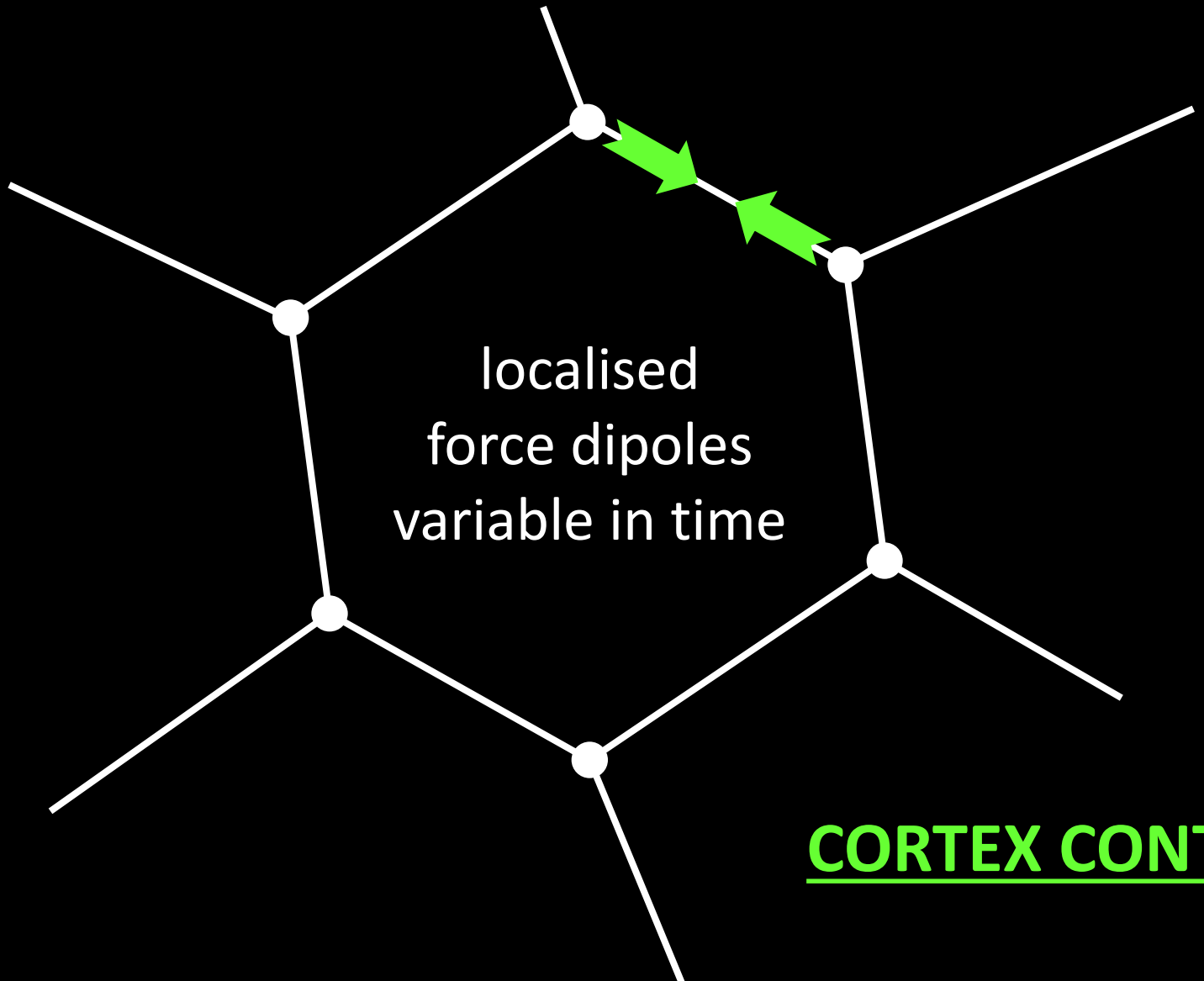
incompressibility

MULTI-SCALE MODEL for SINGLE CELL



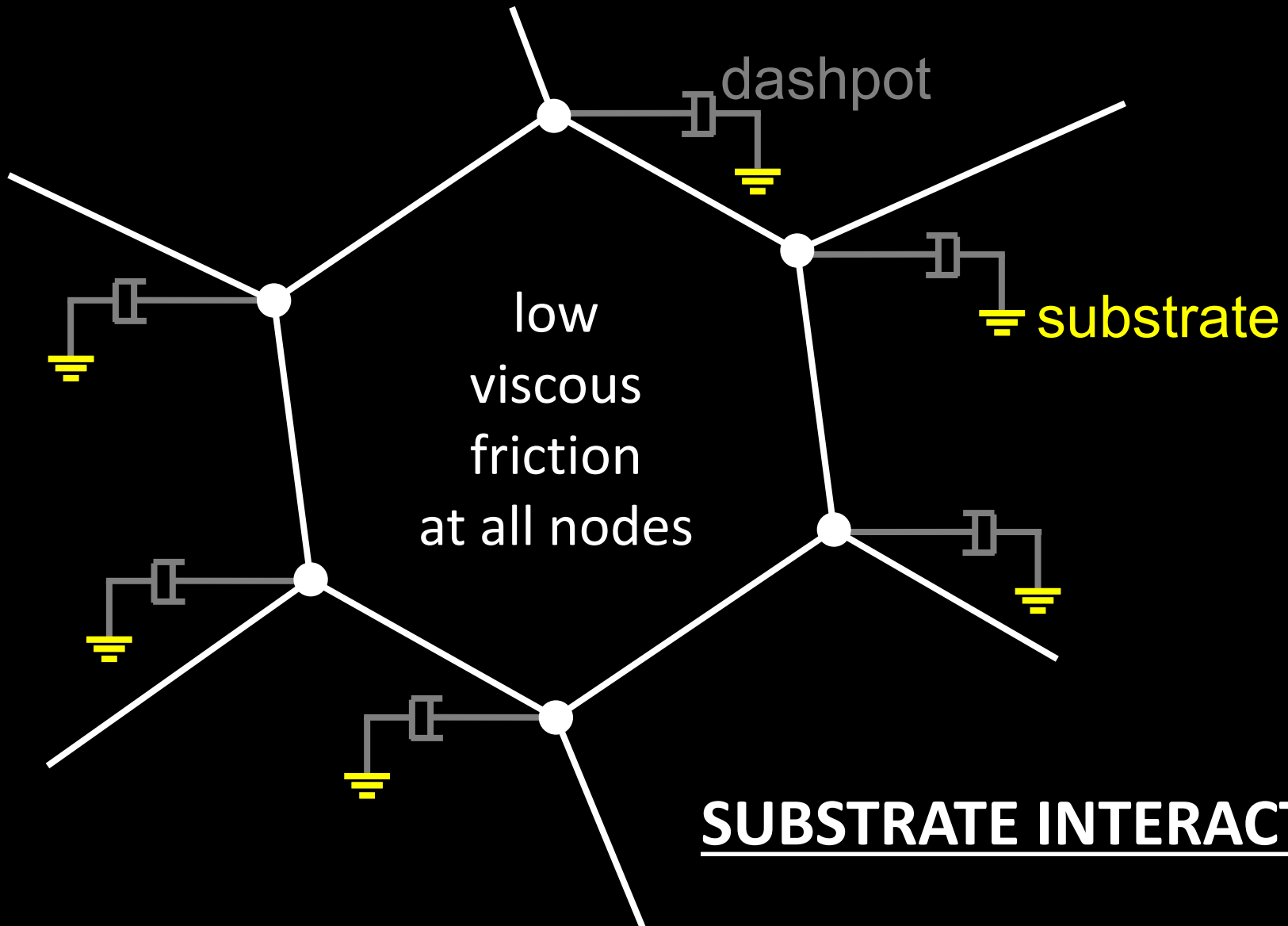
CORTICAL TENSION

MULTI-SCALE MODEL for SINGLE CELL



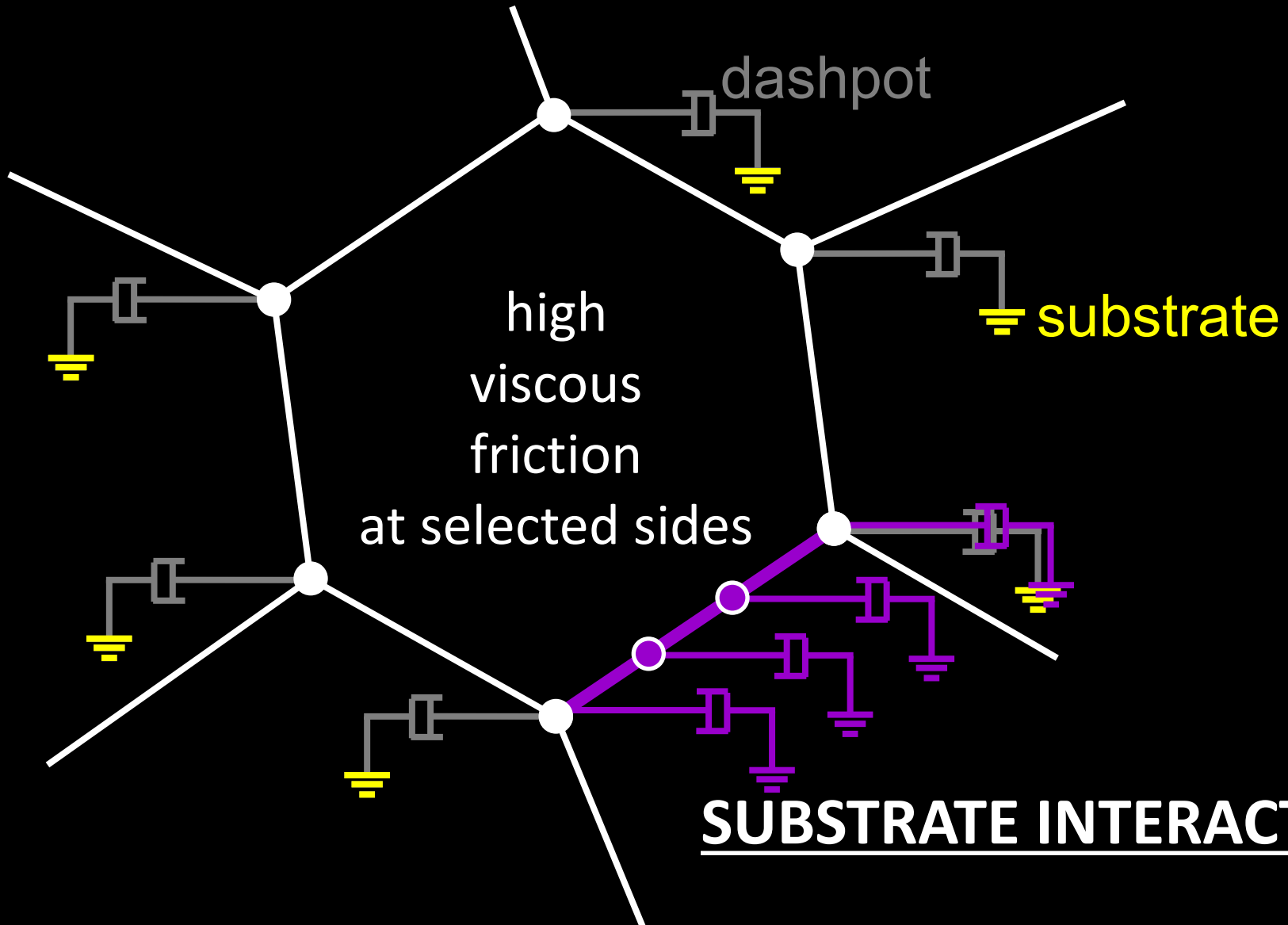
CORTEX CONTRACTILITY

MULTI-SCALE MODEL for SINGLE CELL

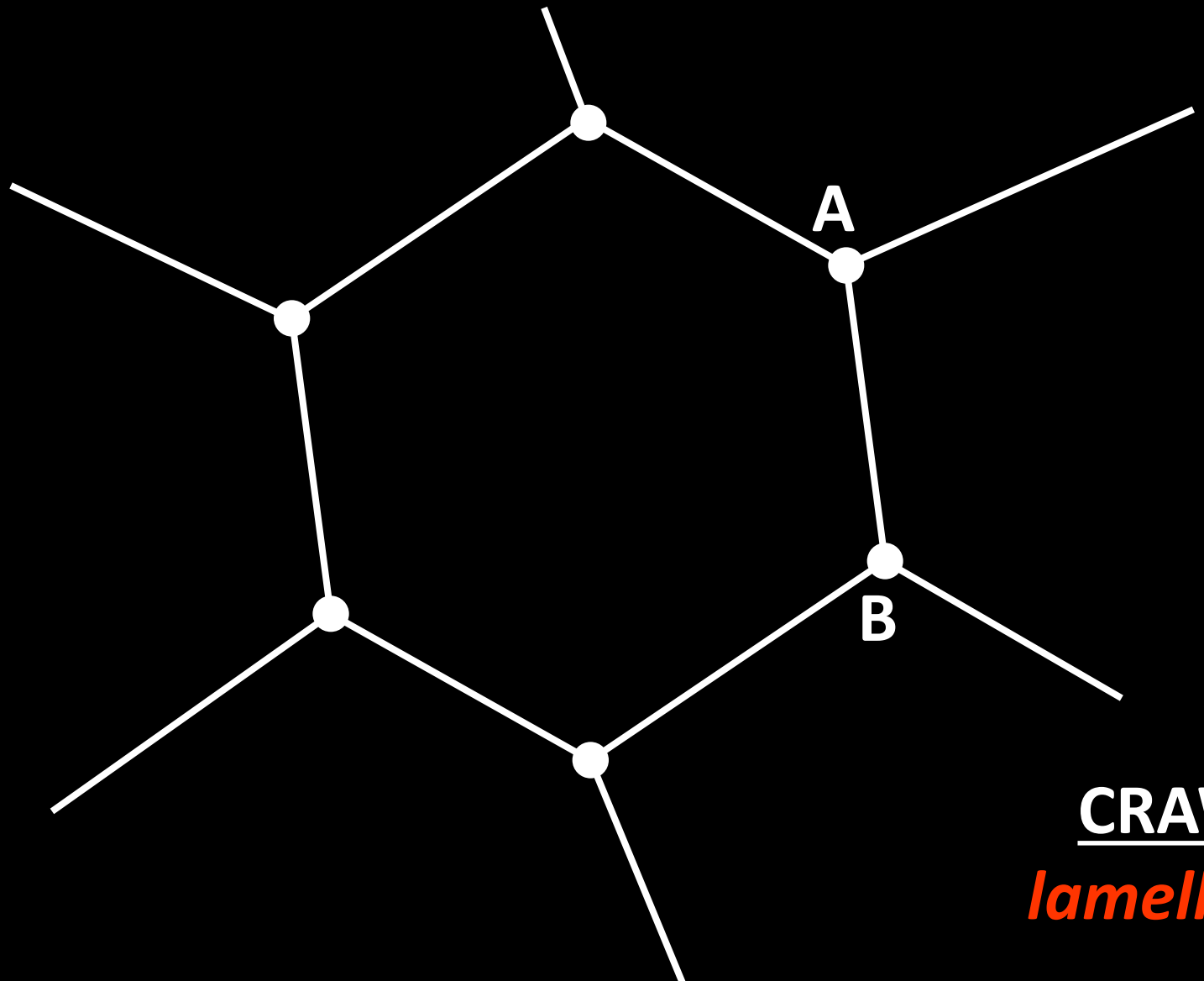


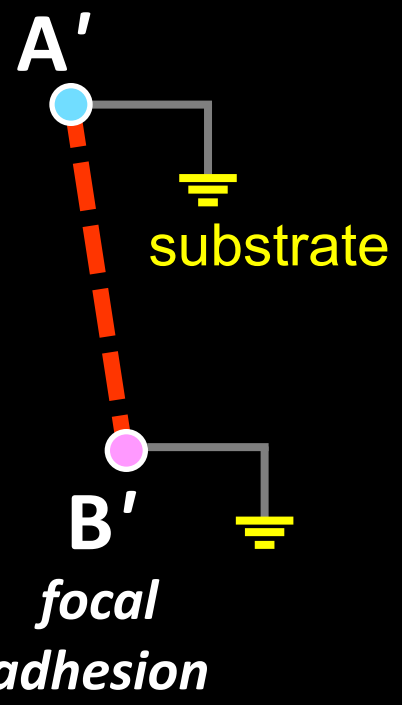
SUBSTRATE INTERACTION

MULTI-SCALE MODEL for SINGLE CELL



MULTI-SCALE MODEL for SINGLE CELL

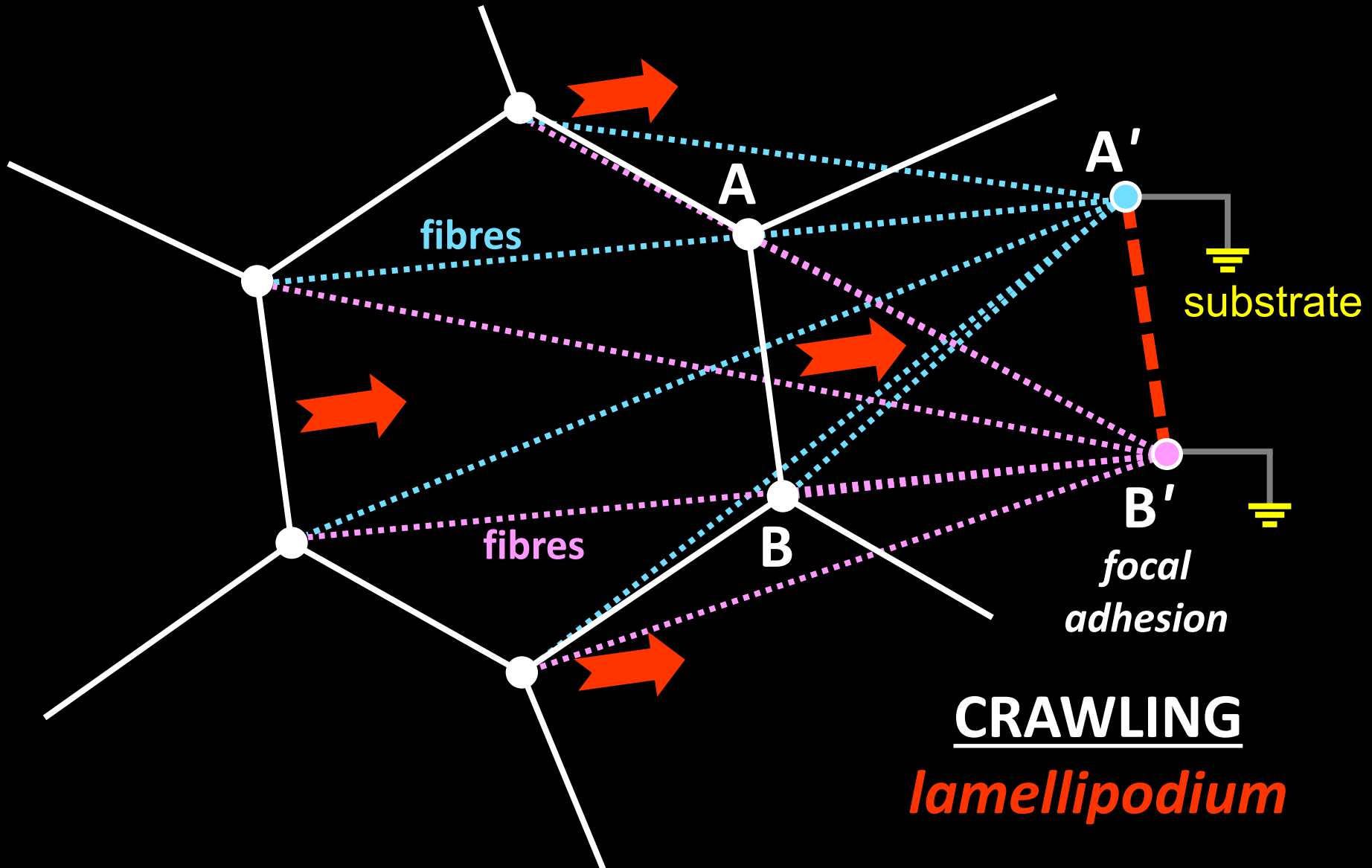




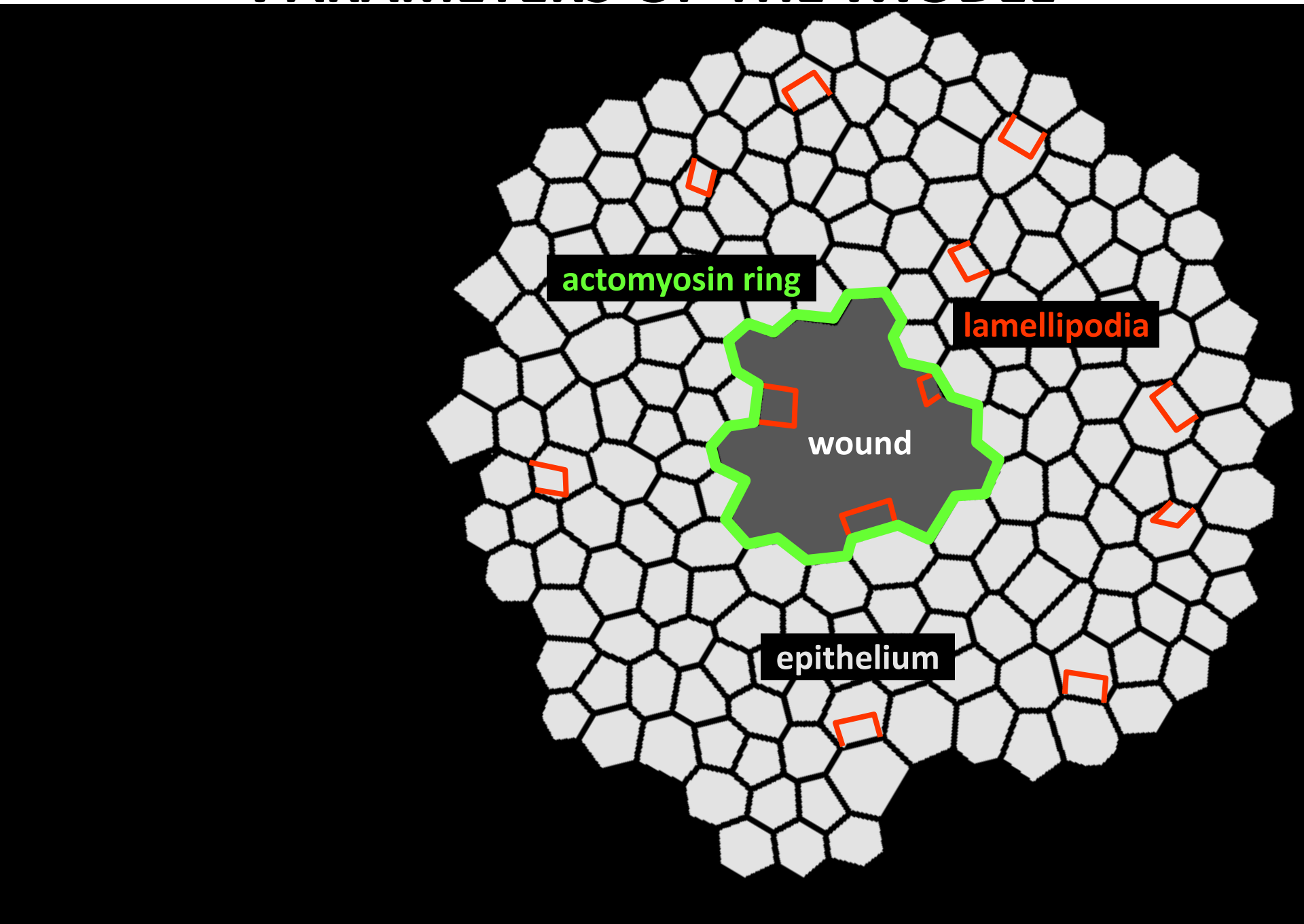
CRAWLING

lamellipodium

MULTI-SCALE MODEL for SINGLE CELL



Parameters of the Model

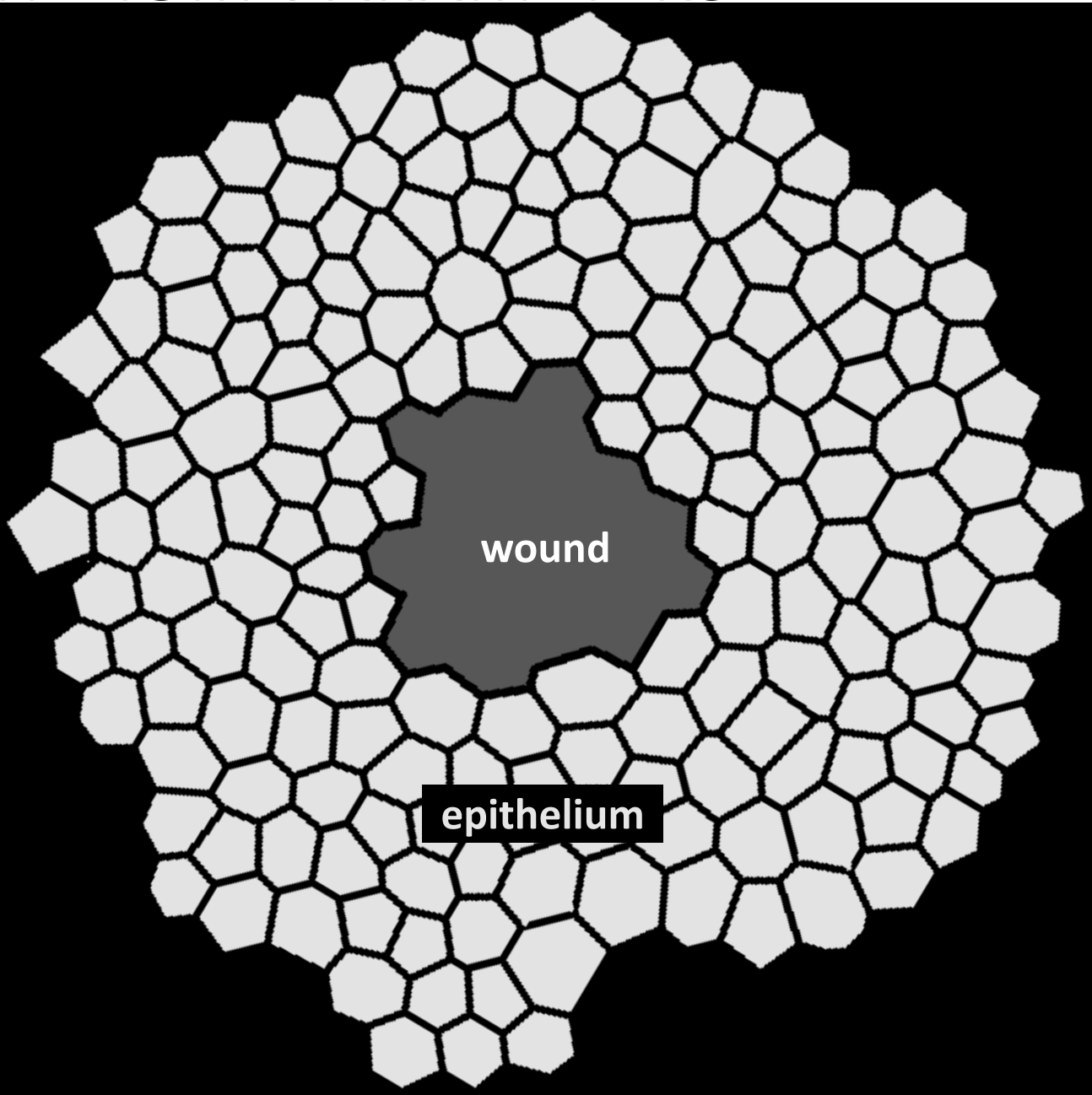


Epithelium Parameters

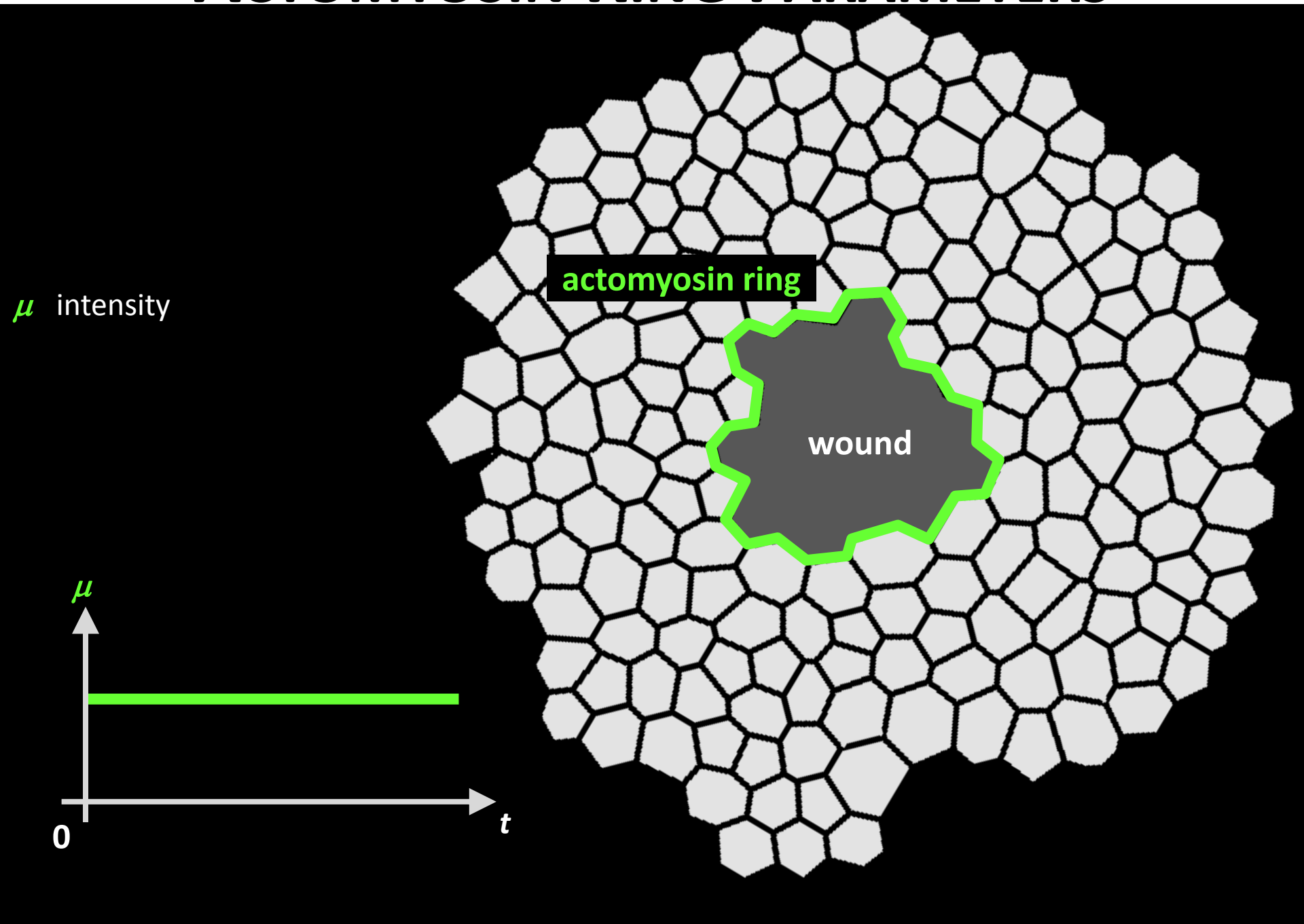
η citoplasm viscosity

χ cortical tension

no prescriptions at boundary



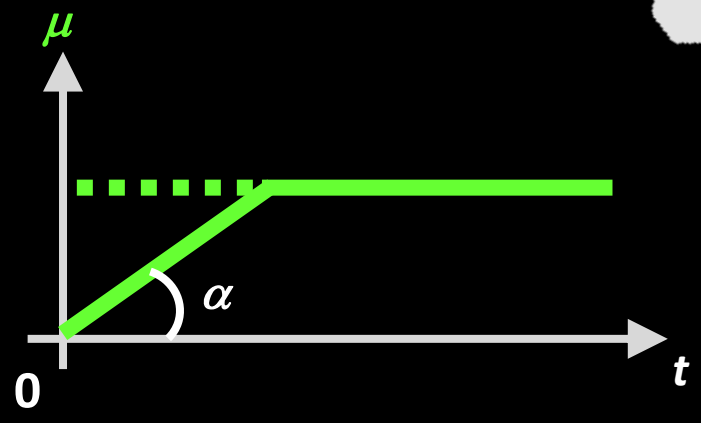
Actomyosin Ring Parameters

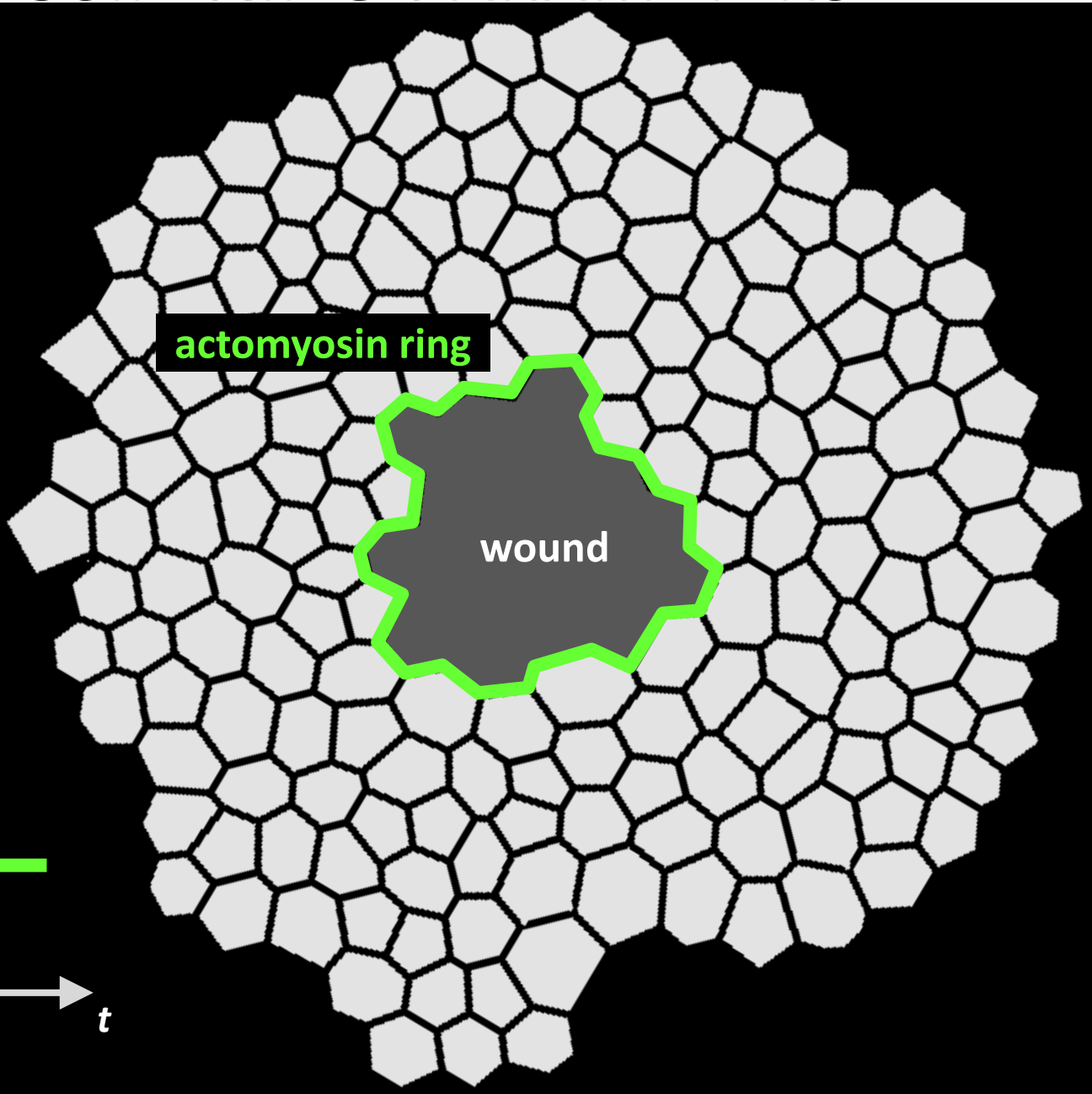


Actomyosin Ring Parameters

μ intensity

α increase rate



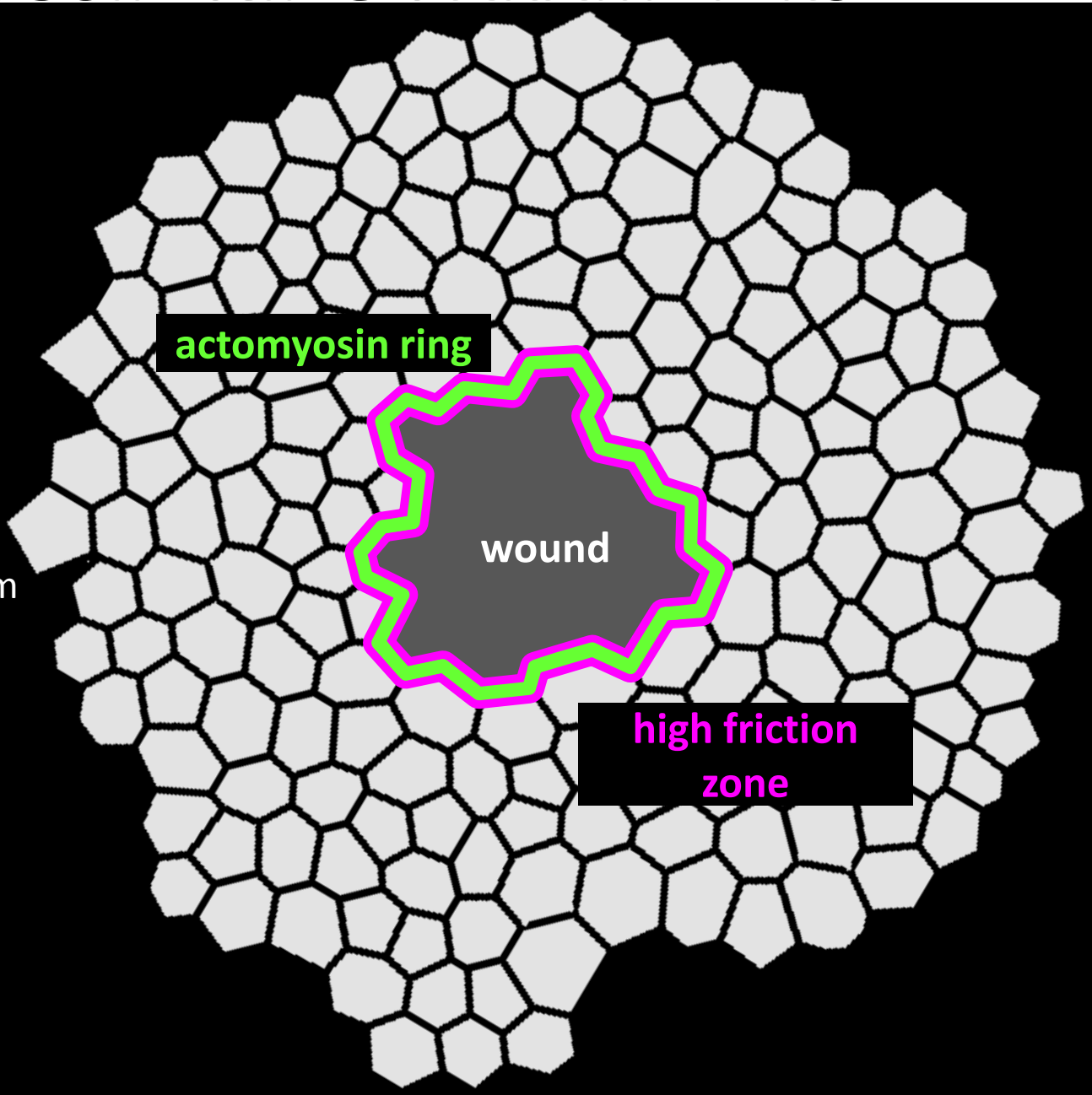


Actomyosin Ring Parameters

μ intensity

α increase rate

ω ring friction w.r.t. epithelium (i.e. high/low friction ratio)



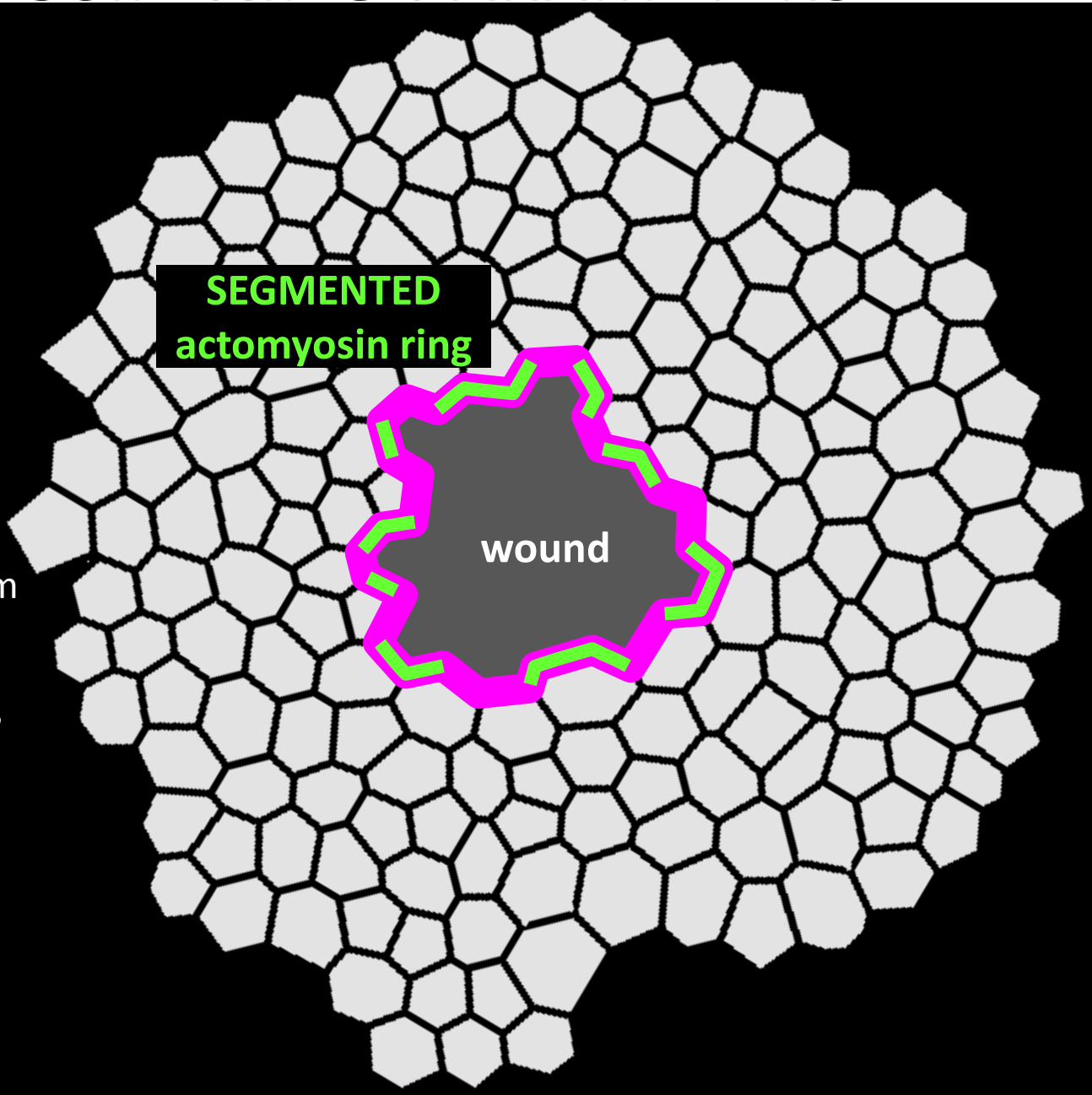
Actomyosin Ring Parameters

μ intensity

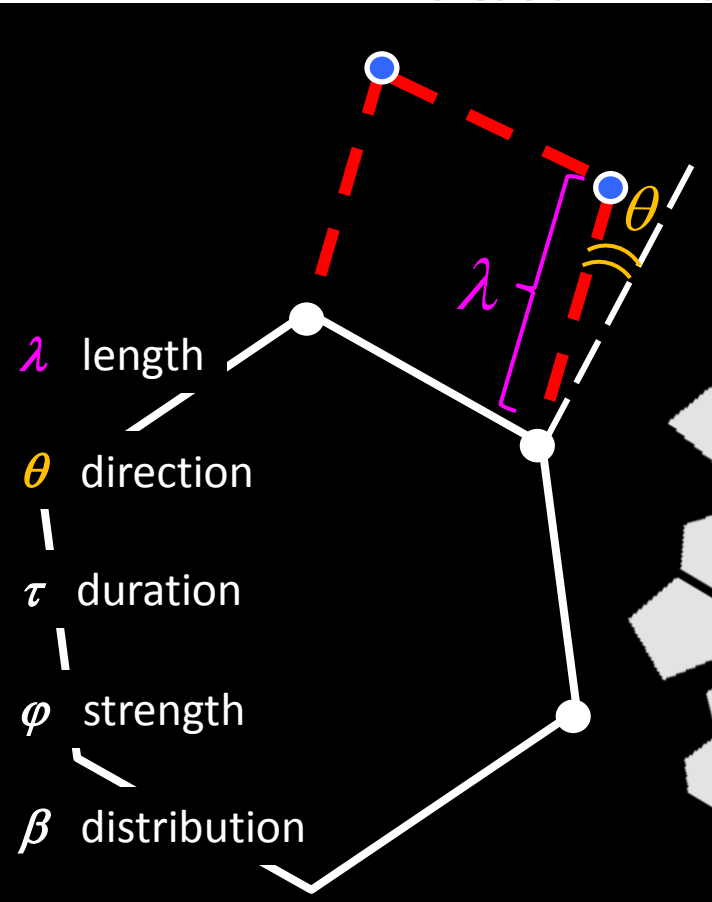
α increase rate

ω ring friction w.r.t. epithelium (i.e. high/low friction ratio)

ρ density of myosin segments



Lamellipodia Parameters



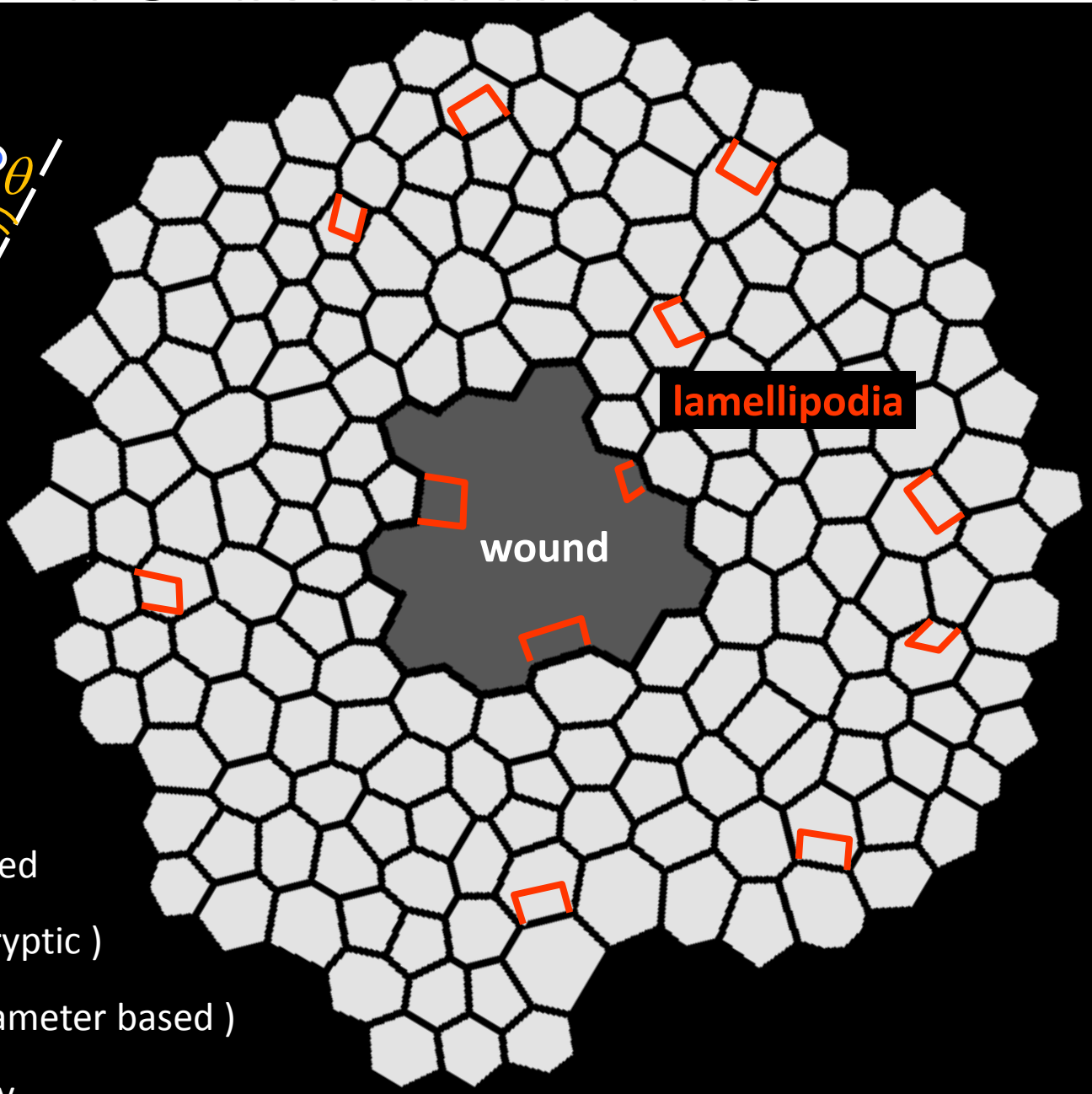
λ length

θ direction

τ duration

φ strength

β distribution



π probability of being generated

w location weight (wound / cryptic)

r reduction factor (wound-diameter based)

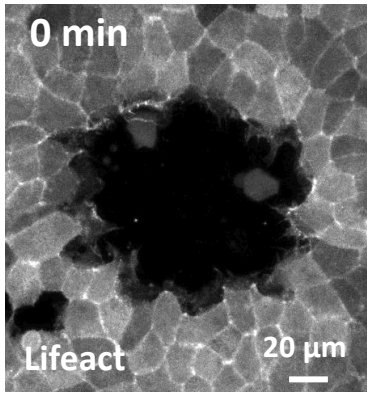
e cell side selection probability

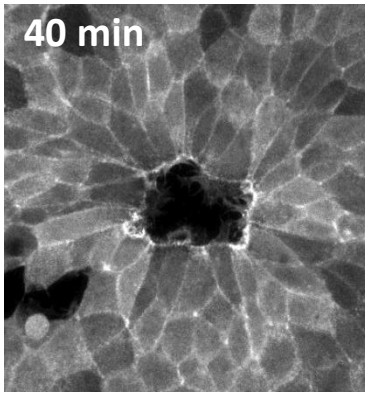
LAMELLIPODIA PARAMETERS VALUES

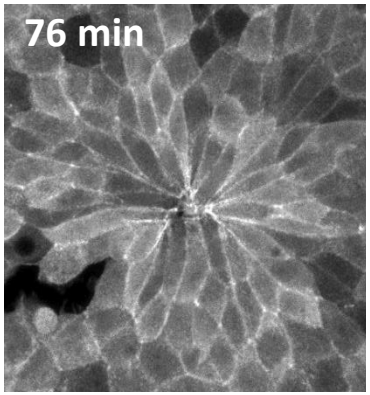
λ	length	λ ***wound lamellipodia 2.5*** λ ***cryptic lamellipodia***
θ	direction	polarization towards wound's centroid: (d,θ) = (0,0) ; (30,$\pm 45°$); (60,$\pm 180°$);
τ	duration	3±1 *time units* (*wound and cryptic*)
φ	strength	wound lamellipodia 25% stronger than cryptic
β	distribution	backward distribution

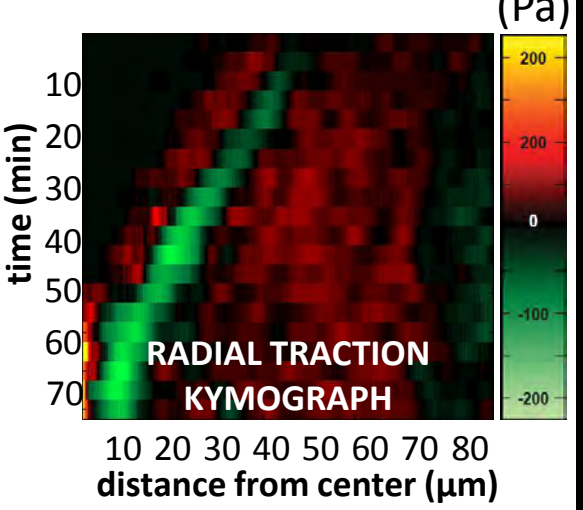
π	probability of being generated	*6 out of 10 cell-sides* (*randomly*)
w	location weight (wound / cryptic)	*1 out of 3 generated lamellipodia is cryptic*
r	reduction factor (wound-diameter based)	*10% current length*
e	cell side selection probability	*uniform*

MECHANICAL VALIDATION of the MECHANISM



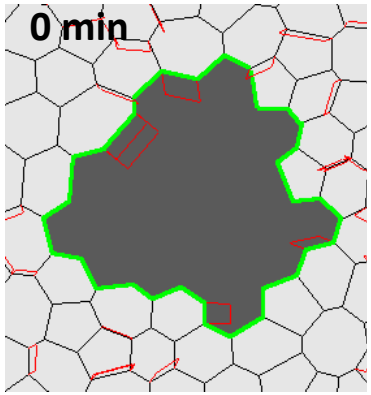


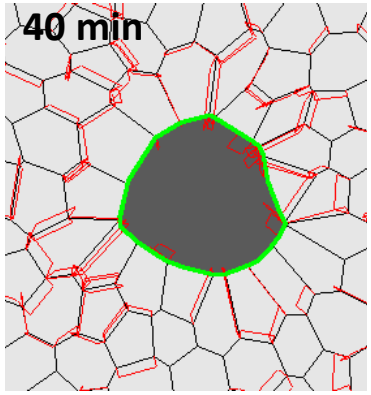


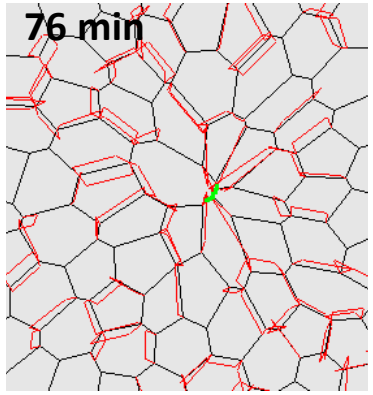


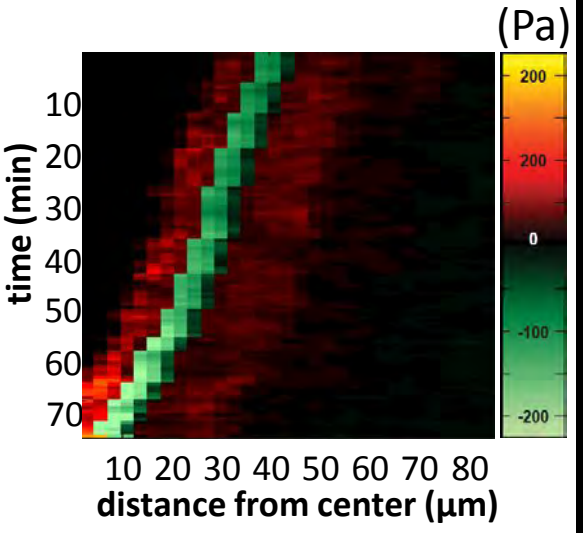
in vitro

in silico





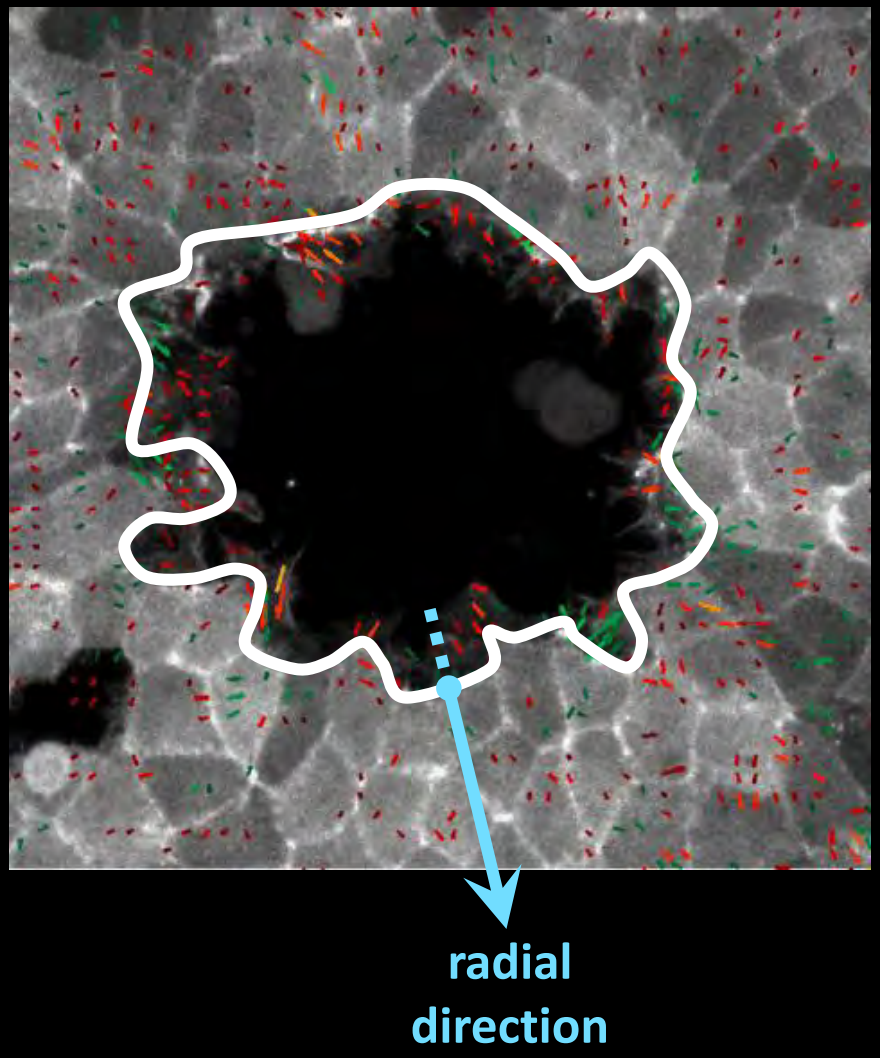




lamellipodia + actomyosin ring

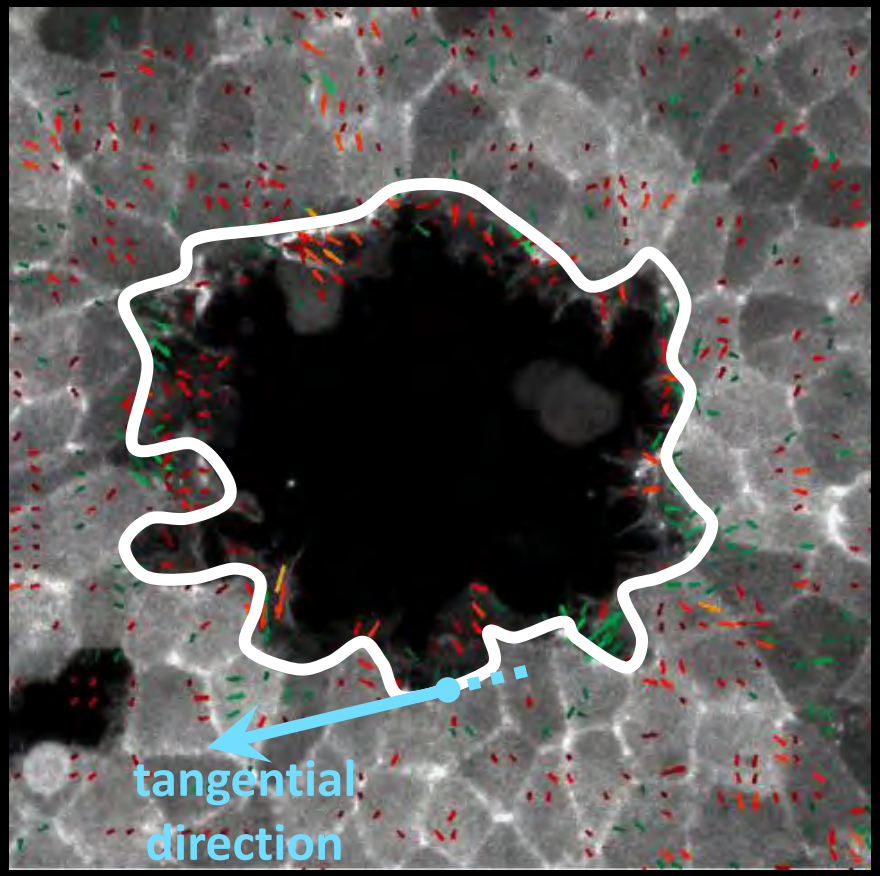
Tangential Tractions Average

Lifeact

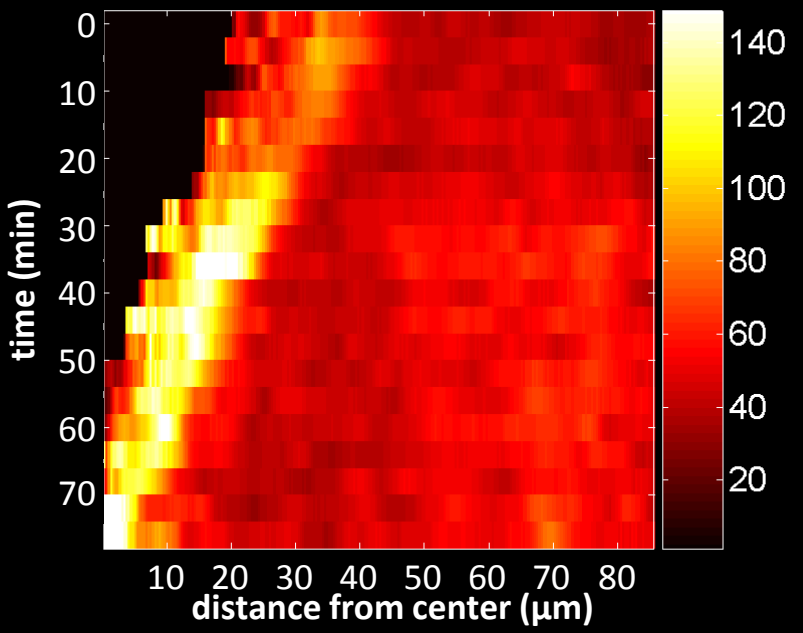


TANGENTIAL TRACTIONS AVERAGE

Lifeact

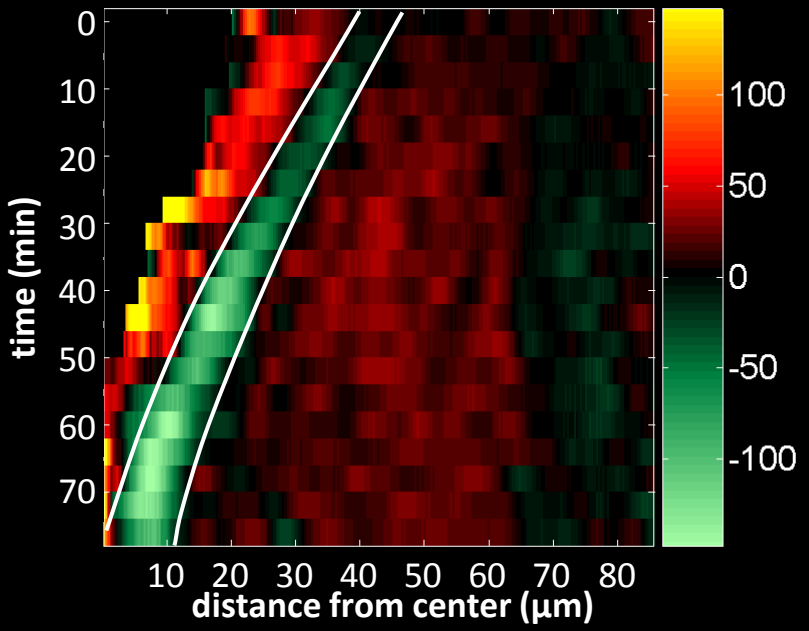


TANGENTIAL TRACTION
kymograph

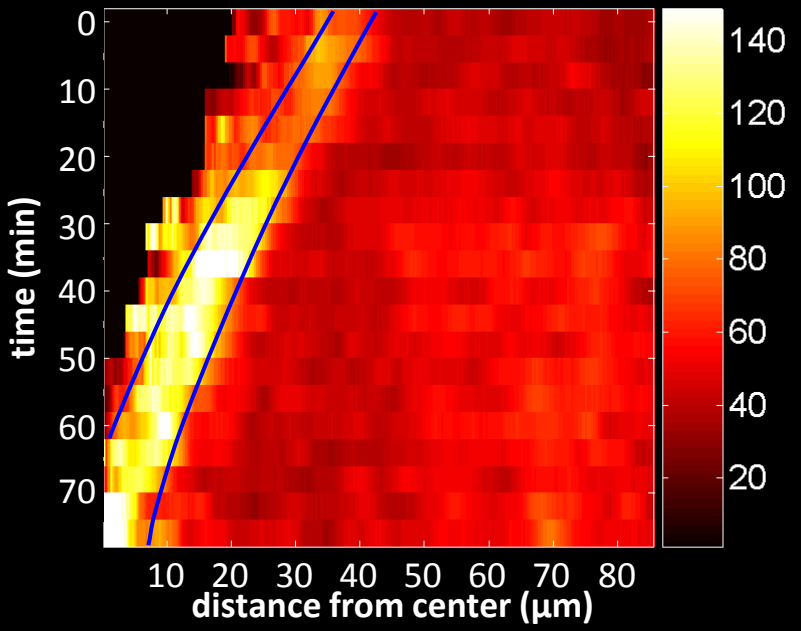


TRACTIONS KYMOGRAPHS

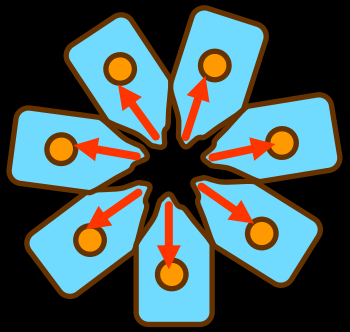
RADIAL TRACTION
kymograph



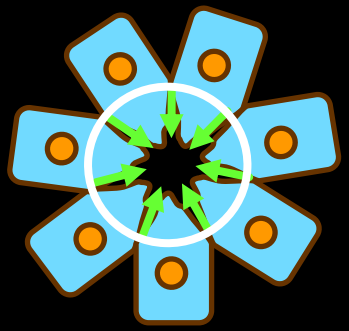
TANGENTIAL TRACTION
kymograph



CELL CRAWLING



PURSE STRING



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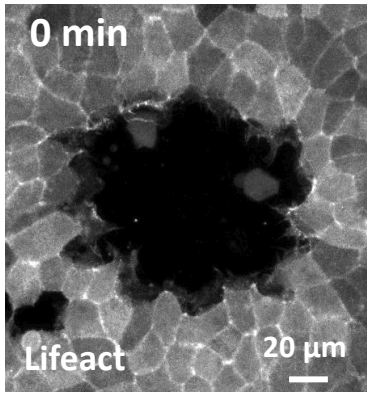
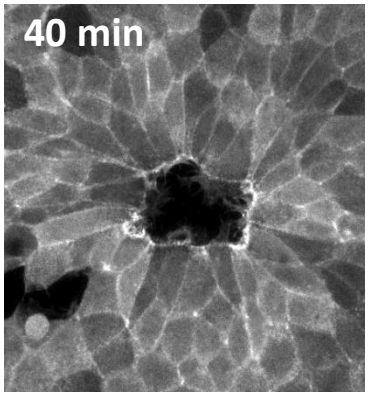
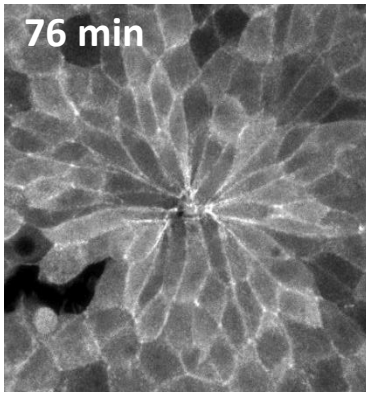
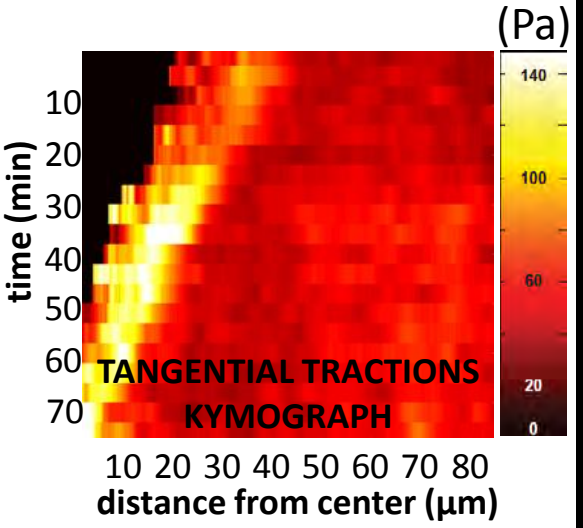
MECHANICAL VALIDATION of the MECHANISM





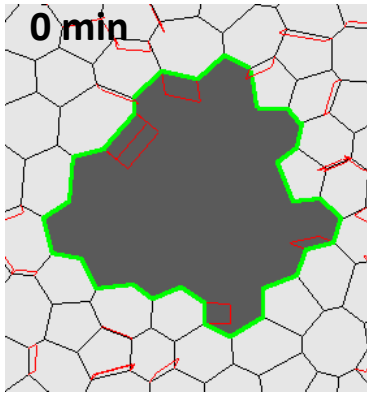


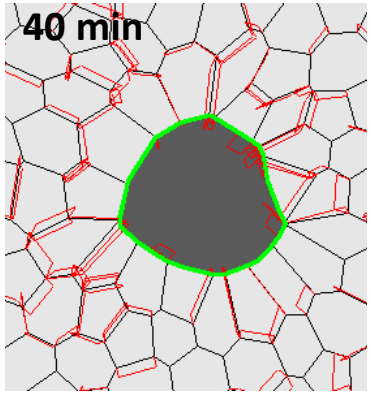


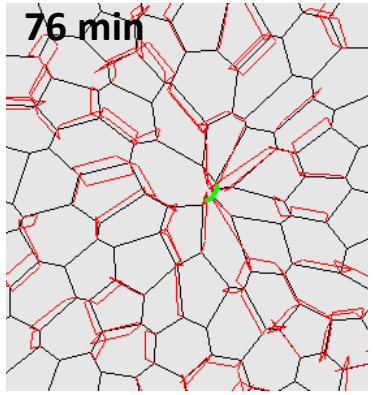
in vitro

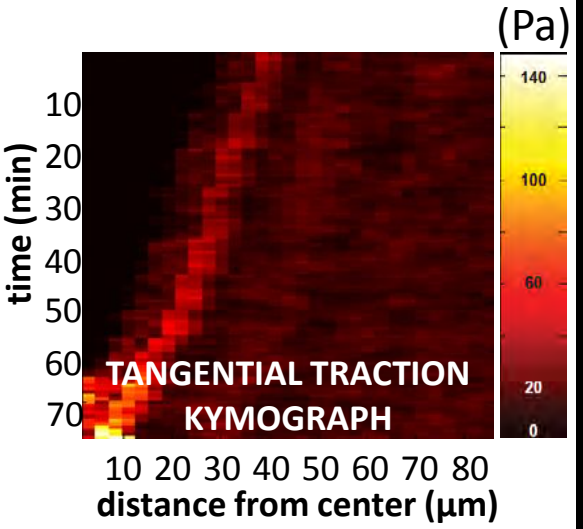
WHAT IS MISSING?

in silico



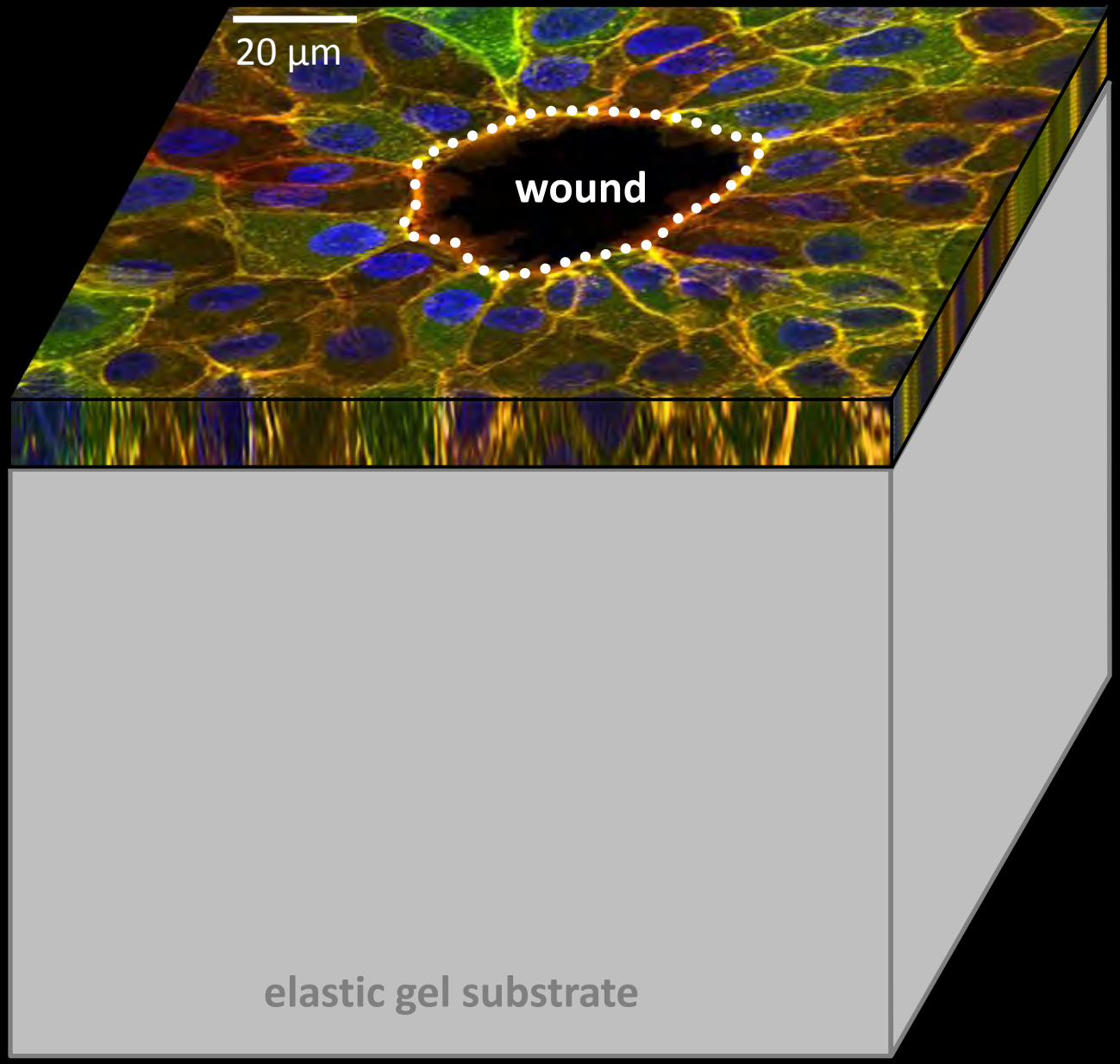




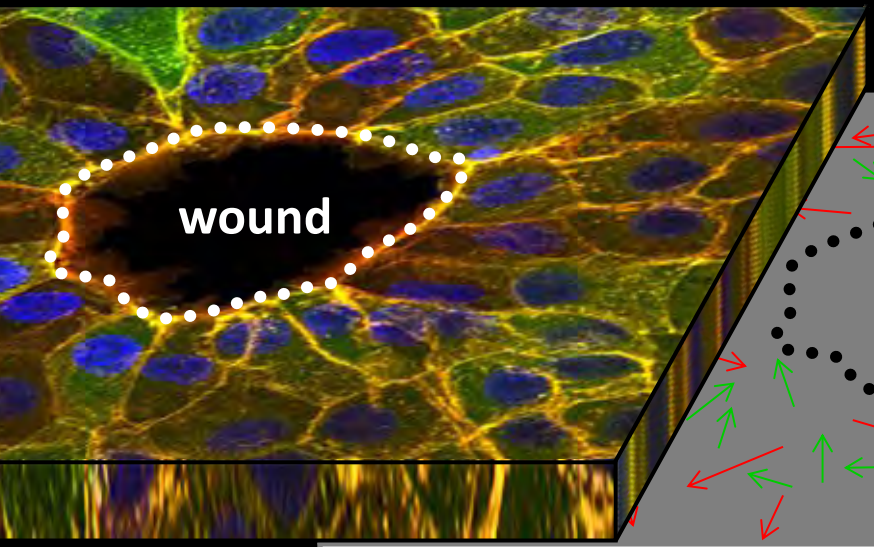


lamellipodia + actomyosin ring

Stress Microscopy in the Substrate



Stress Microscopy in the Substrate



epithelium

wound trace

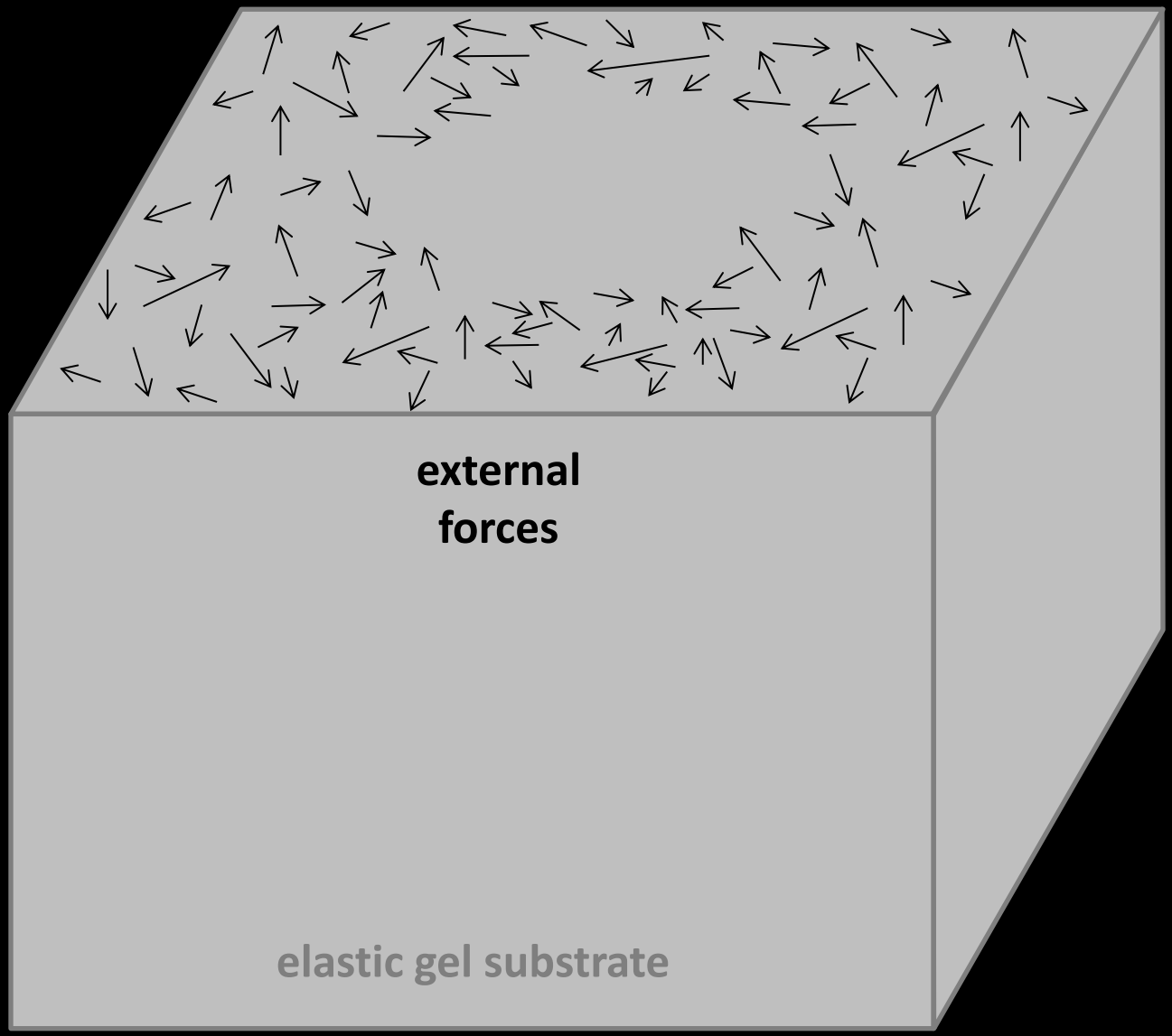
gel interface

elastic gel substrate

tractions towards wound's interior

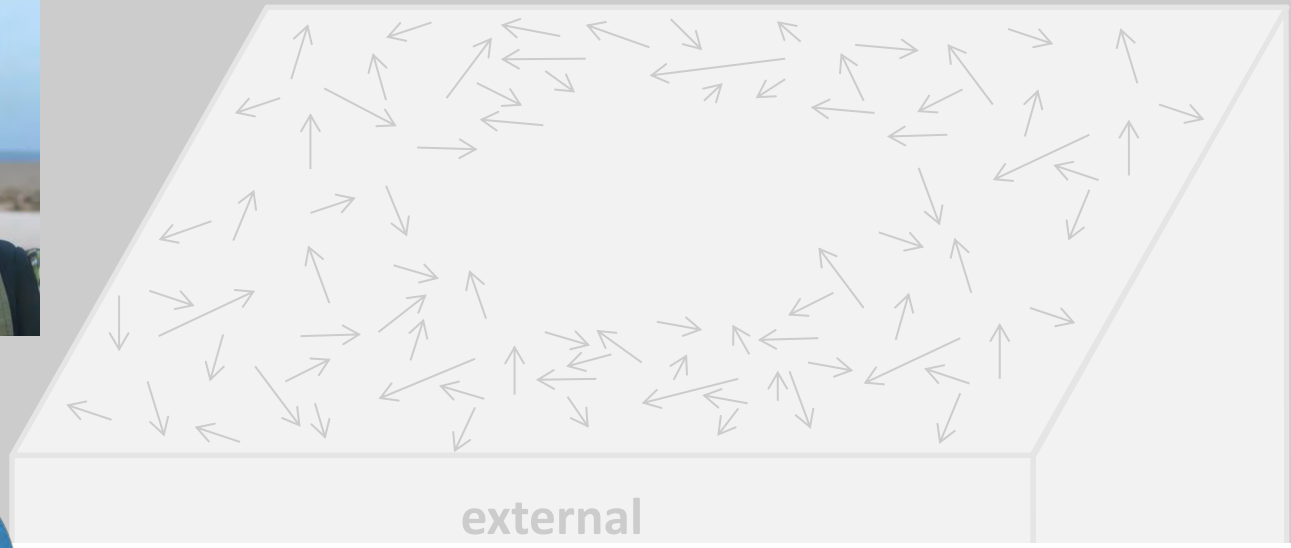
tractions towards wound's exterior

Stress Microscopy in the Substrate



Stress Microscopy in the Substrate

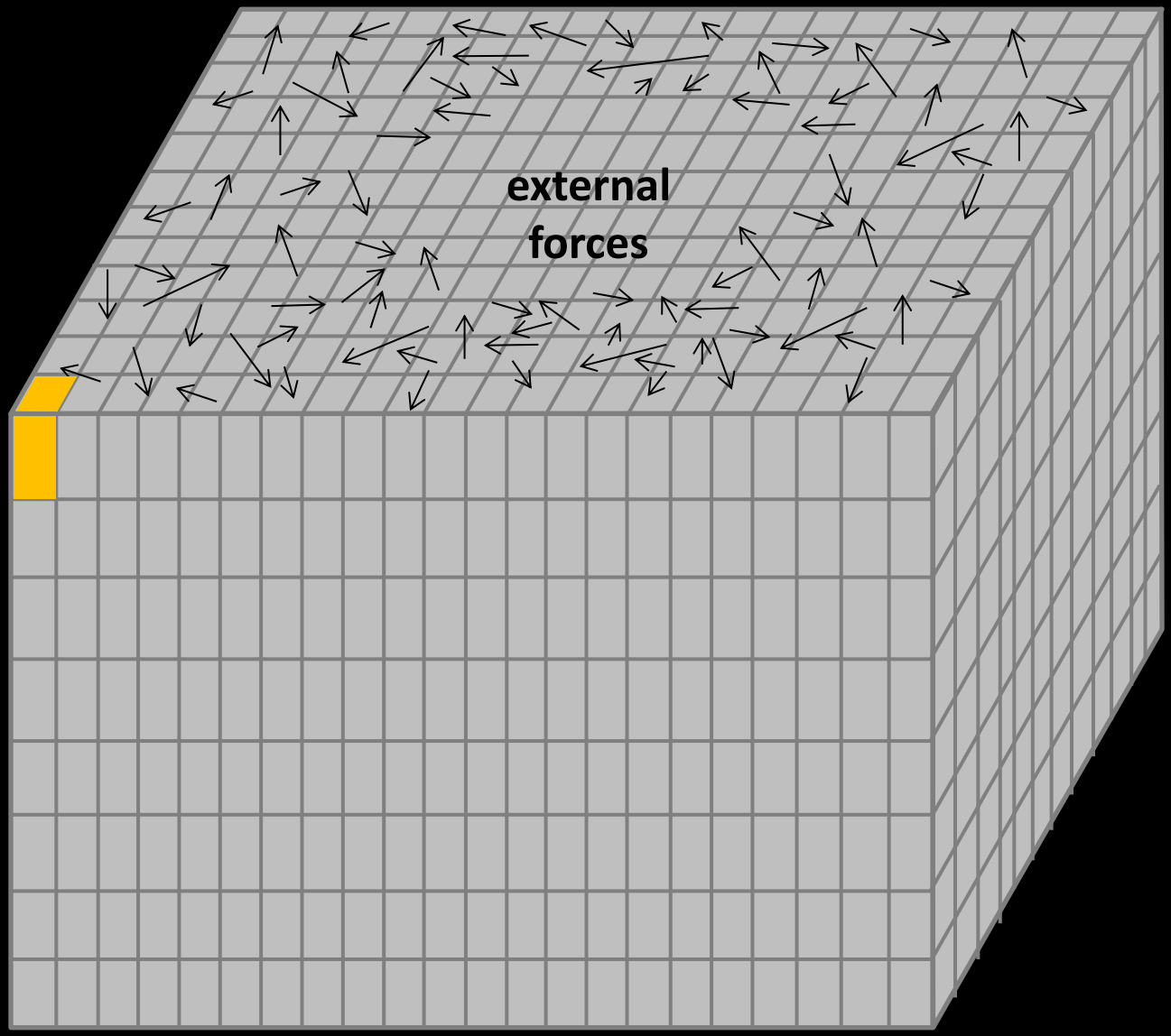
José Muñoz



compressive and **tensile** states
of a solid material
are fully described by a physical quantity called
Stress Tensor

σ (x,y,z)

Stress Microscopy in the Substrate



finite
flement

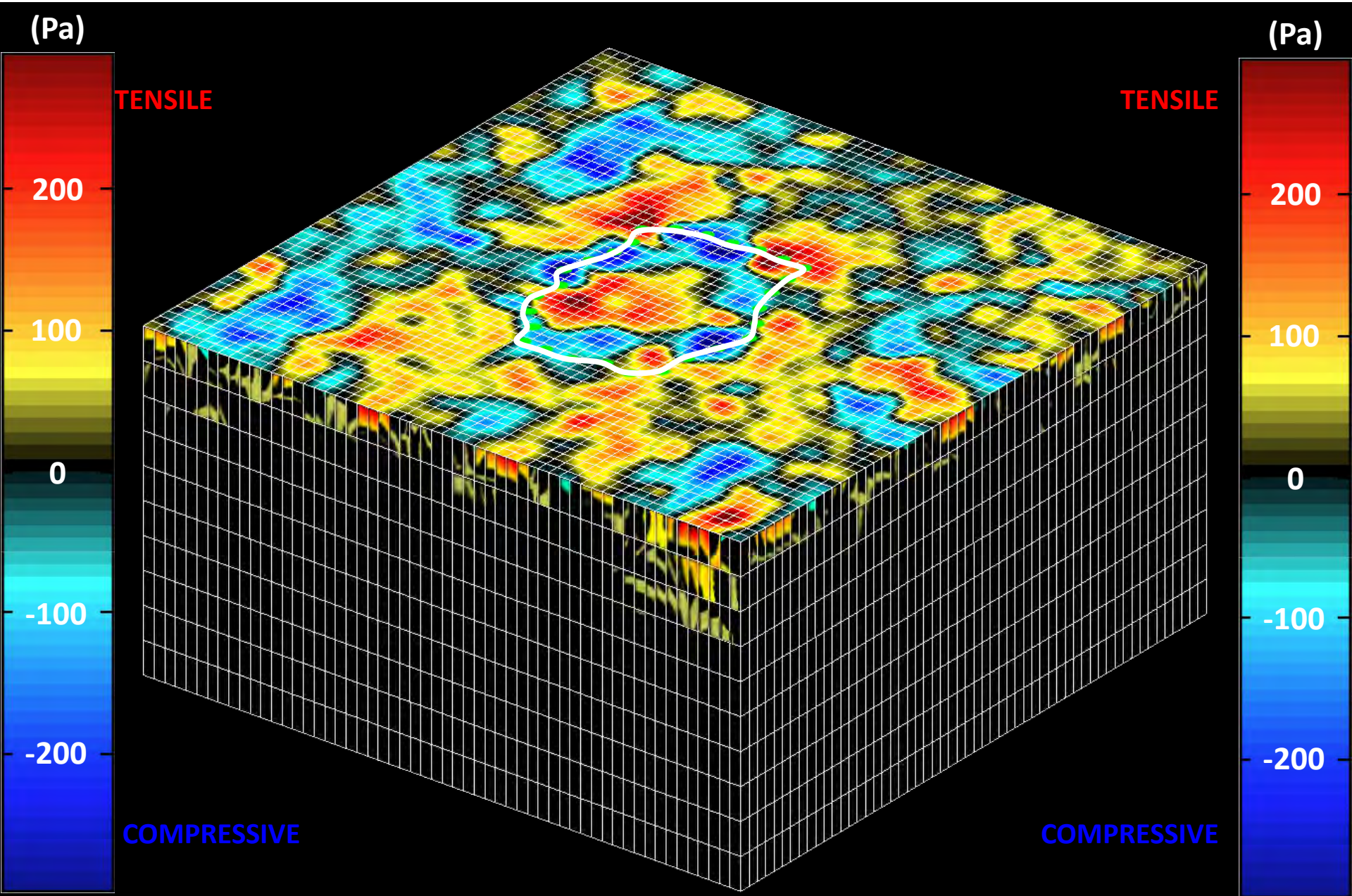
FINITE
ELEMENT
METHOD

Stress
Tensor

σ (x,y,z)

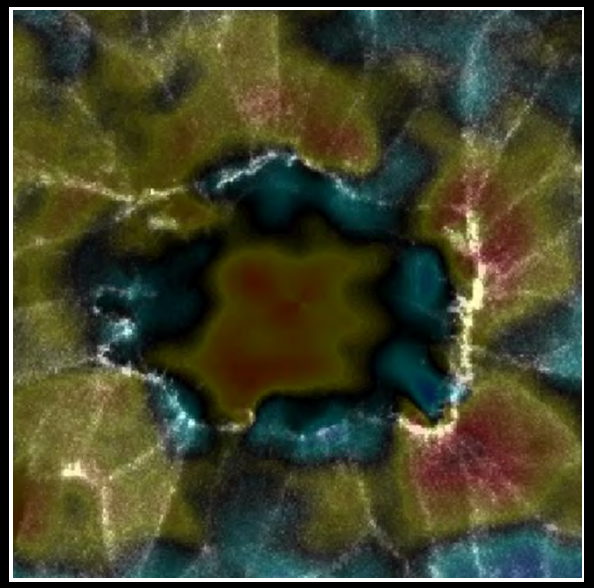
AVERAGE NORMAL STRESS IN THE SUBSTRATE

– AVERAGE COMPRESSION / TENSION –

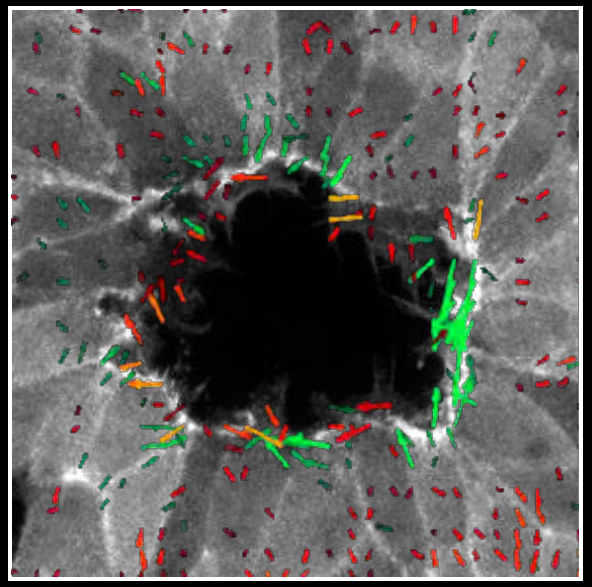


Stress Microscopy in the Substrate

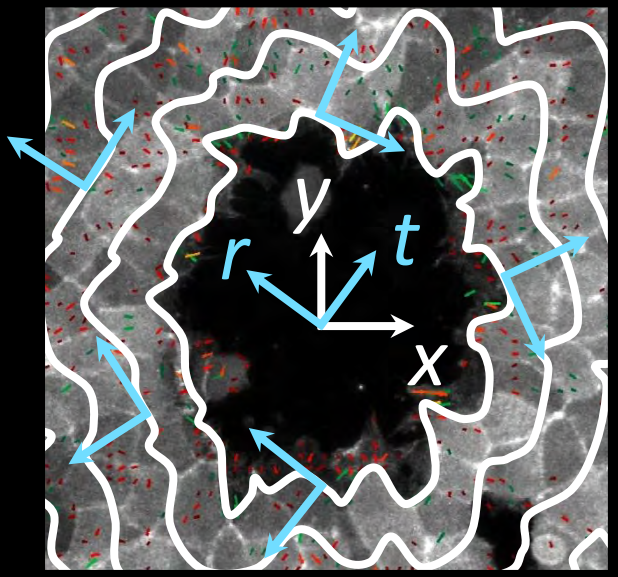
gel substrate radial stress
(Pa)



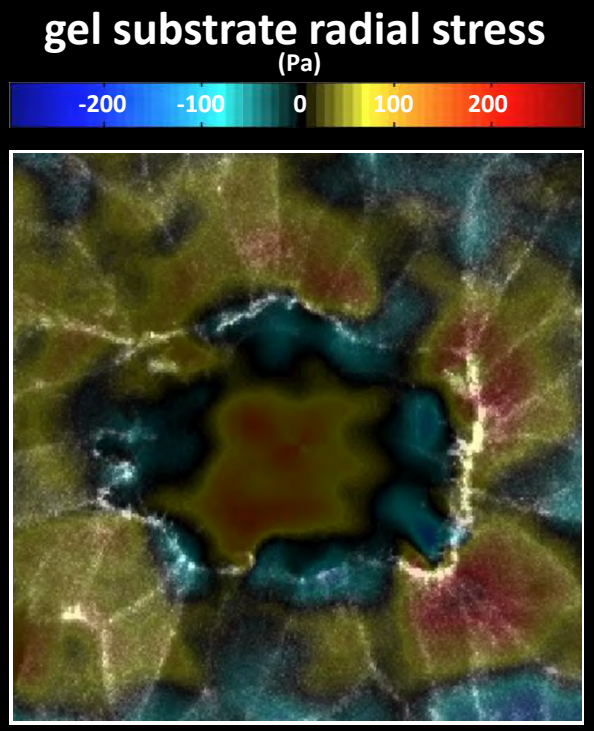
in vitro



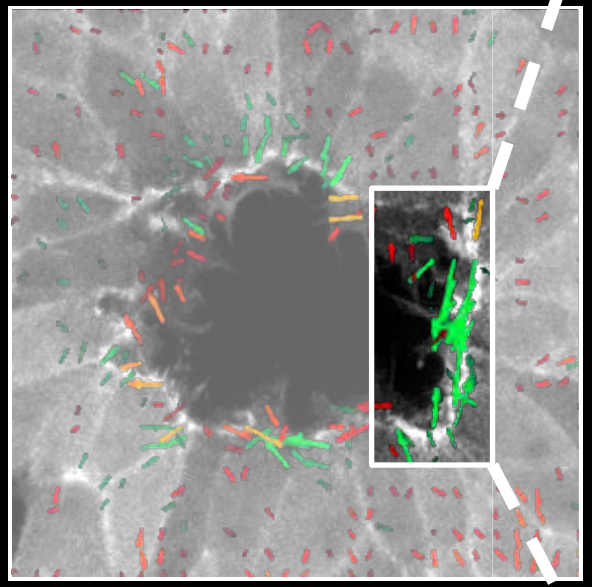
Lifeact

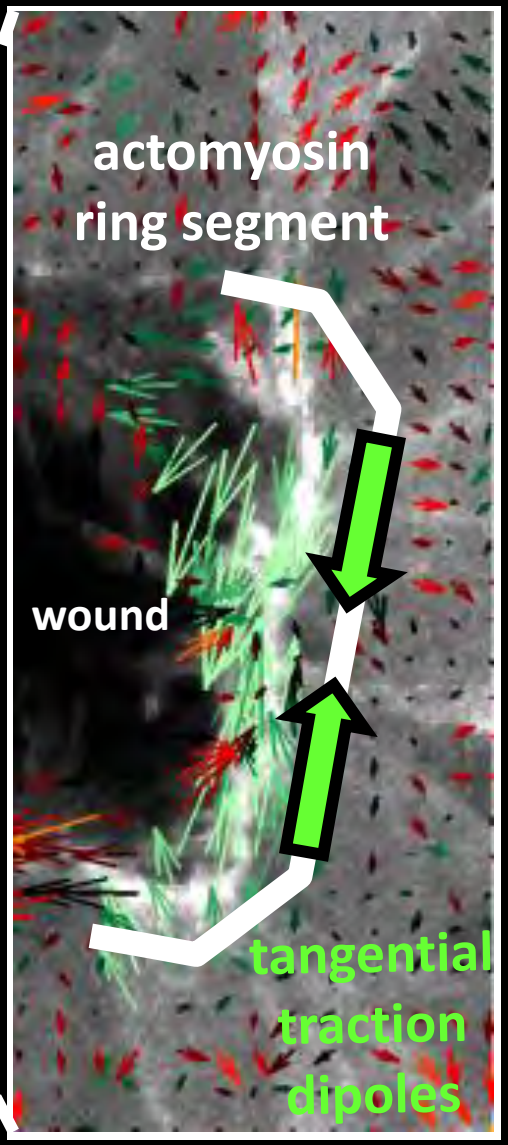


Stress Microscopy in the Substrate



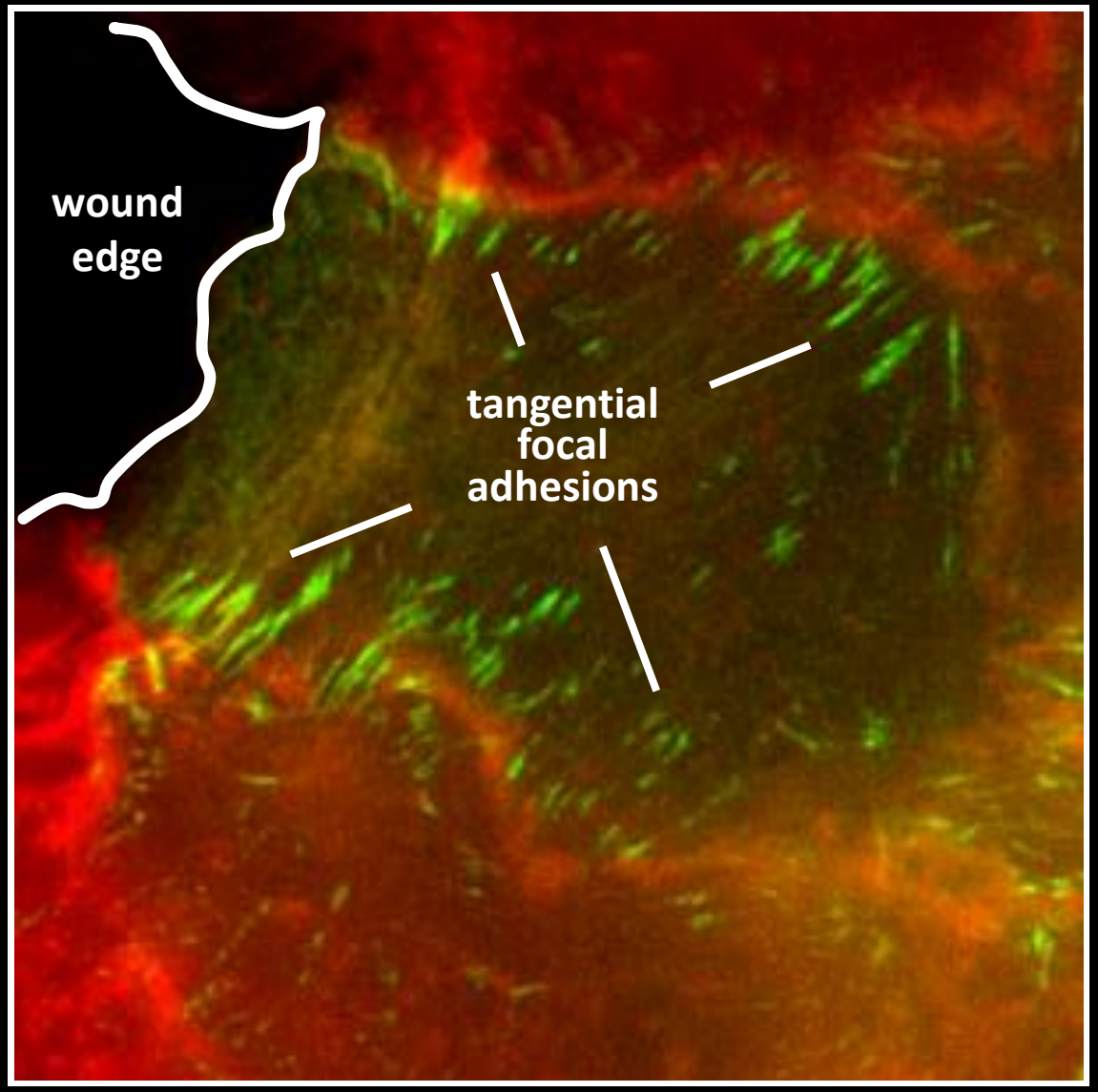
in vitro



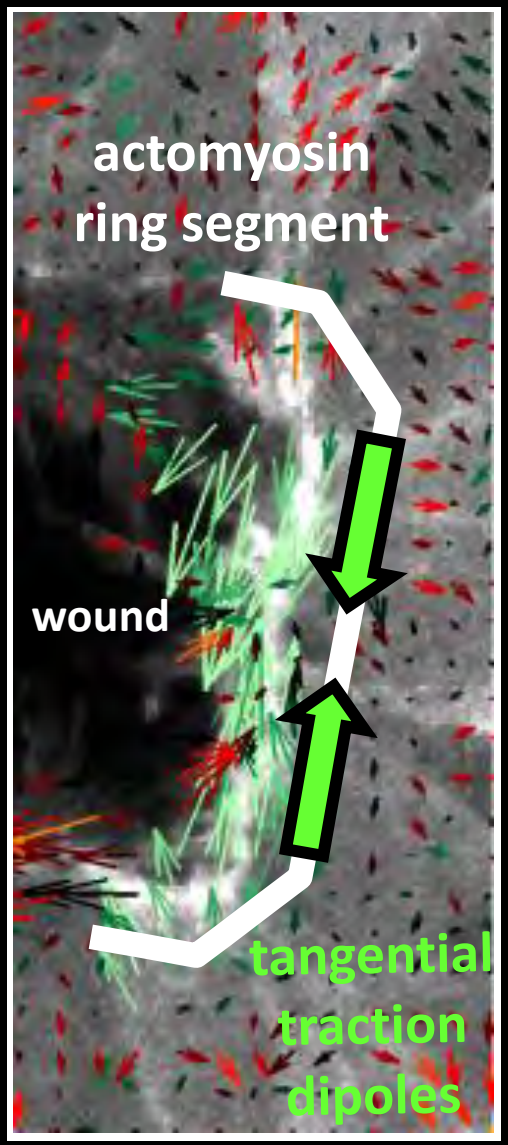


Lifeact

Grounded & Segmented Actomyosin Ring

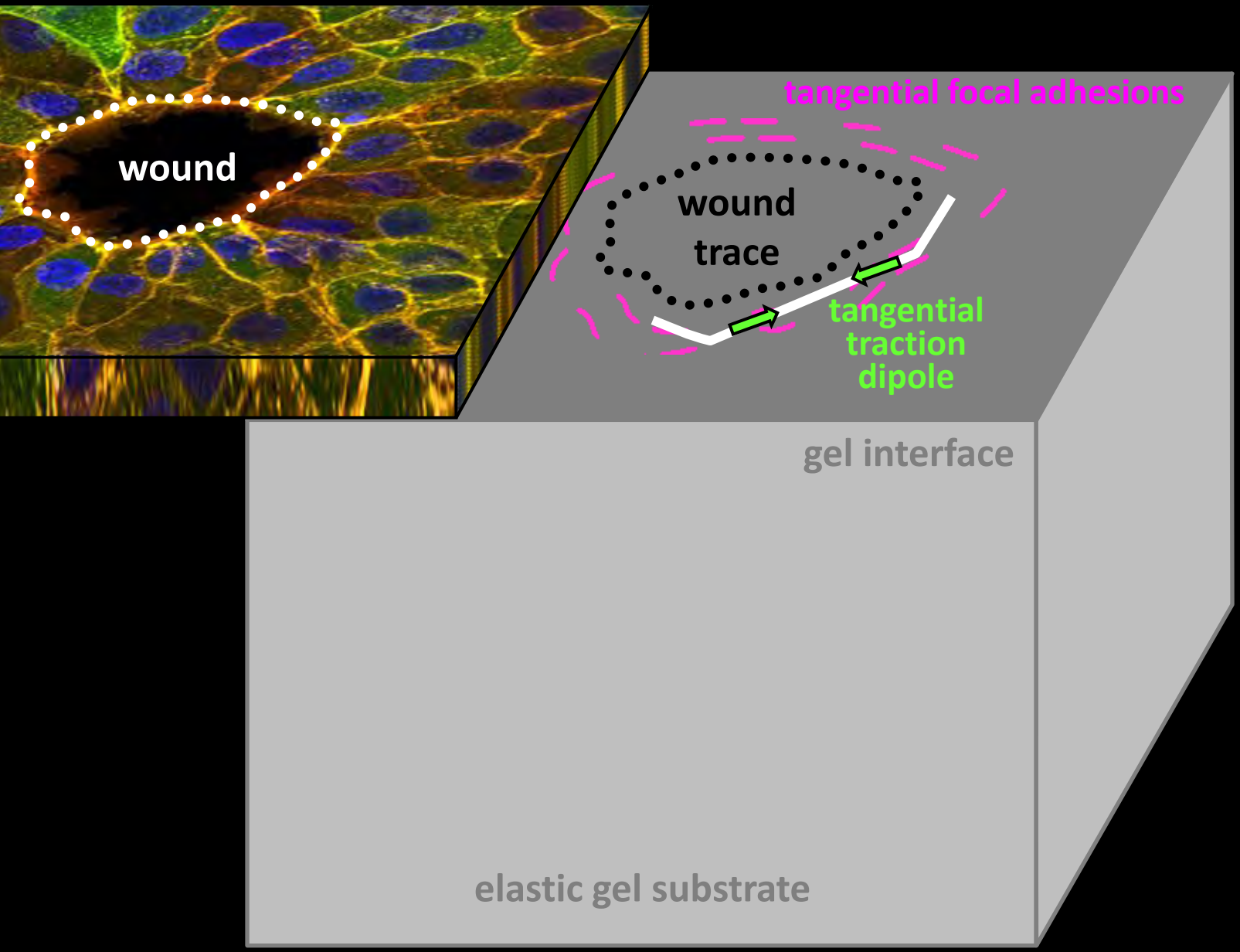


Talin Lifeact

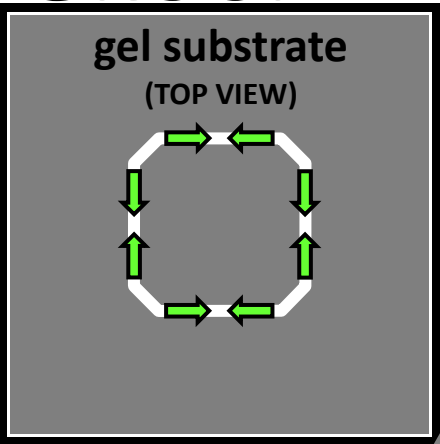


Lifeact

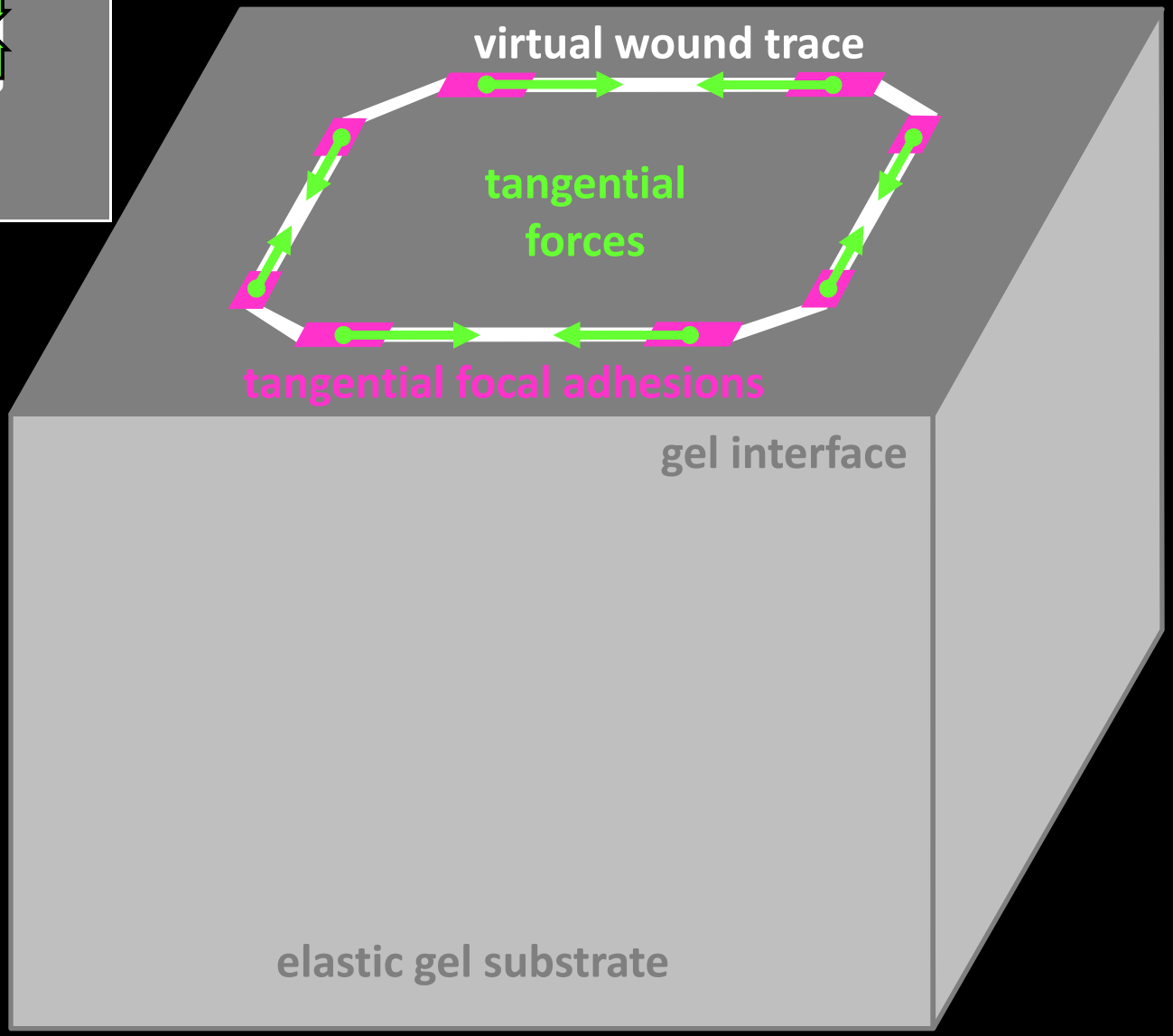
GROUNDED & SEGMENTED ACTOMYOSIN RING



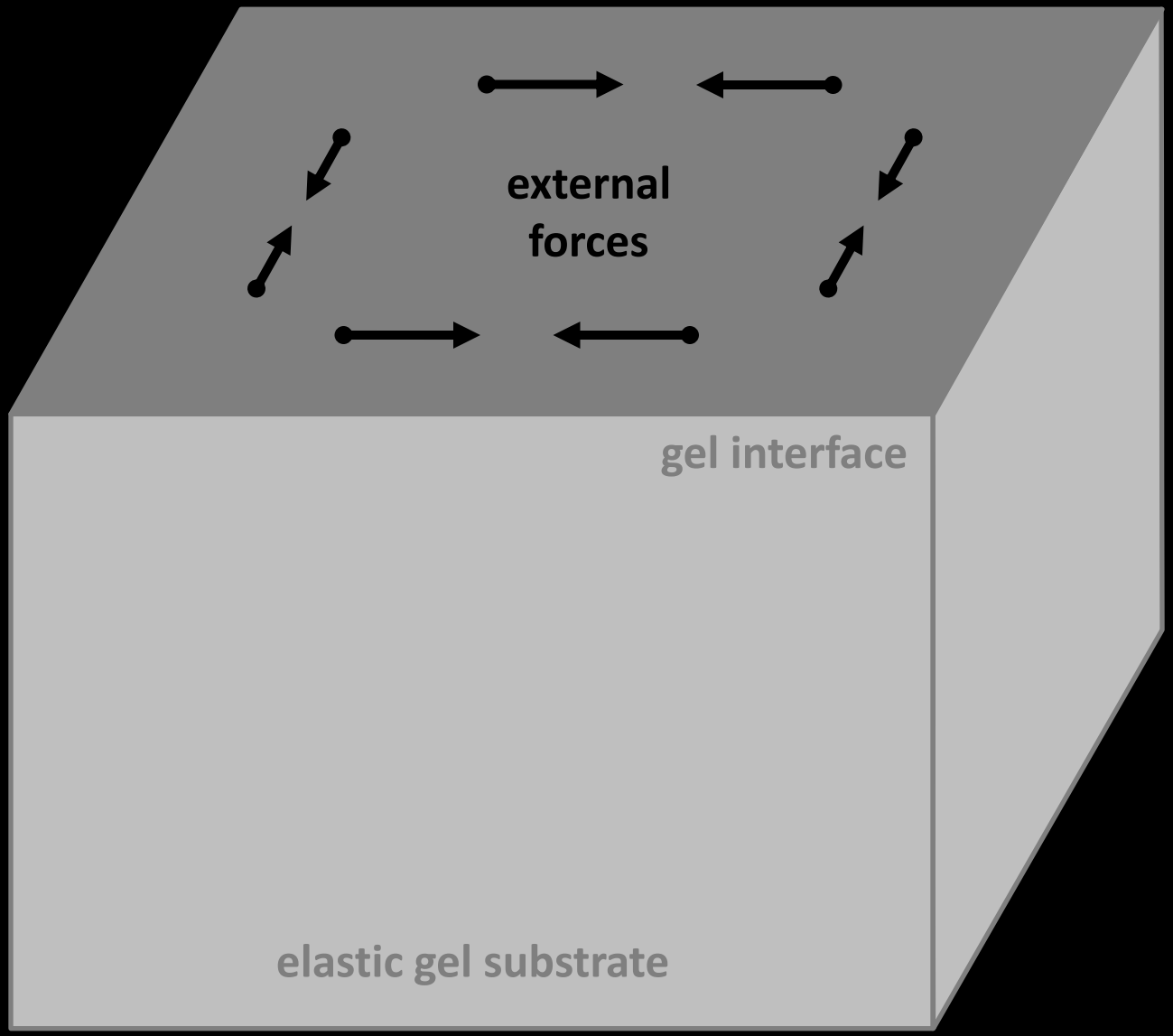
GROUNDED & SEGMENTED ACTOMYOSIN RING



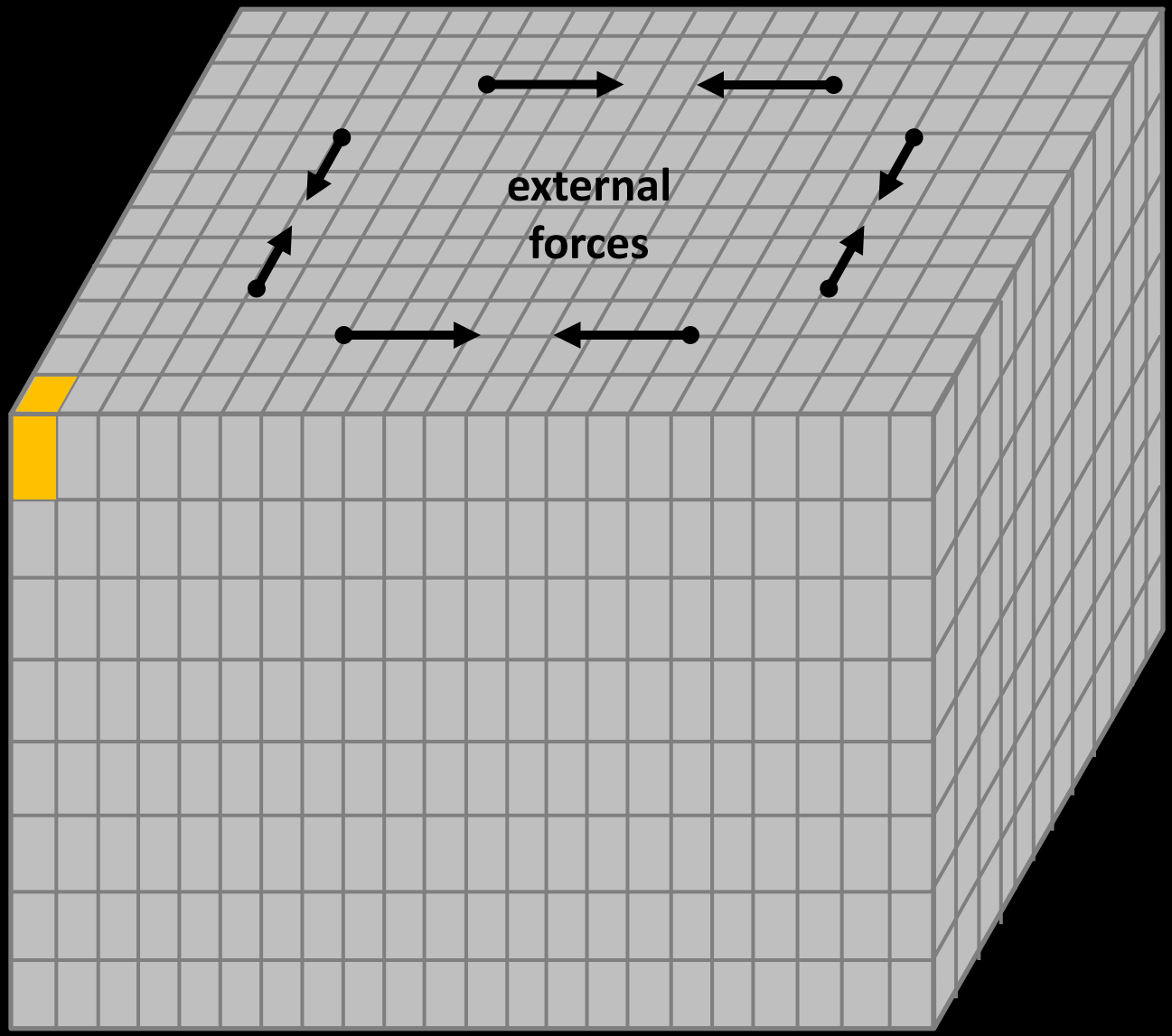
in silico



Grounded & Segmented Actomyosin Ring



GROUNDED & SEGMENTED ACTOMYOSIN RING



finite
flement

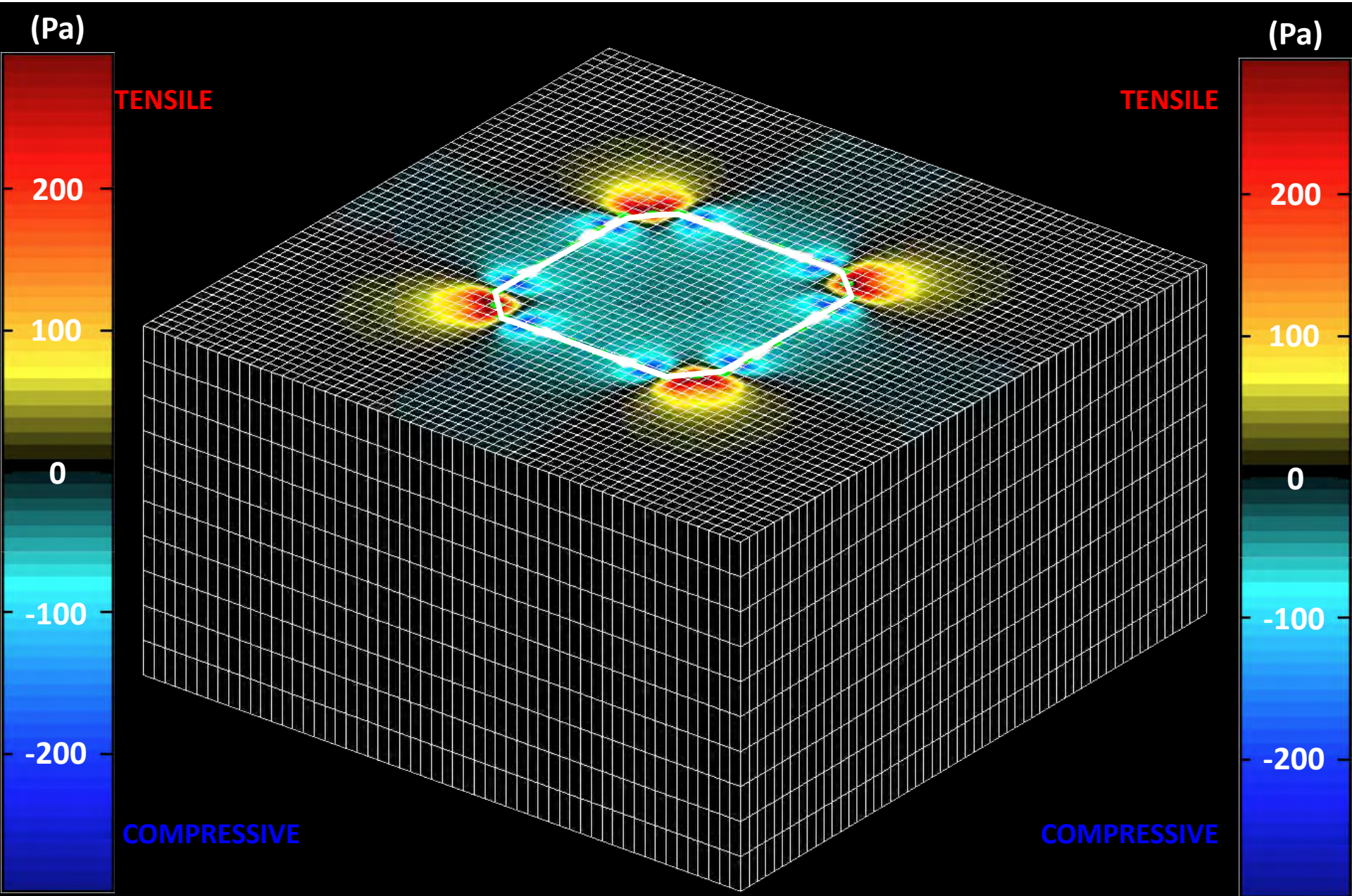
FINITE
ELEMENT
METHOD

Stress
Tensor

σ (x,y,z)

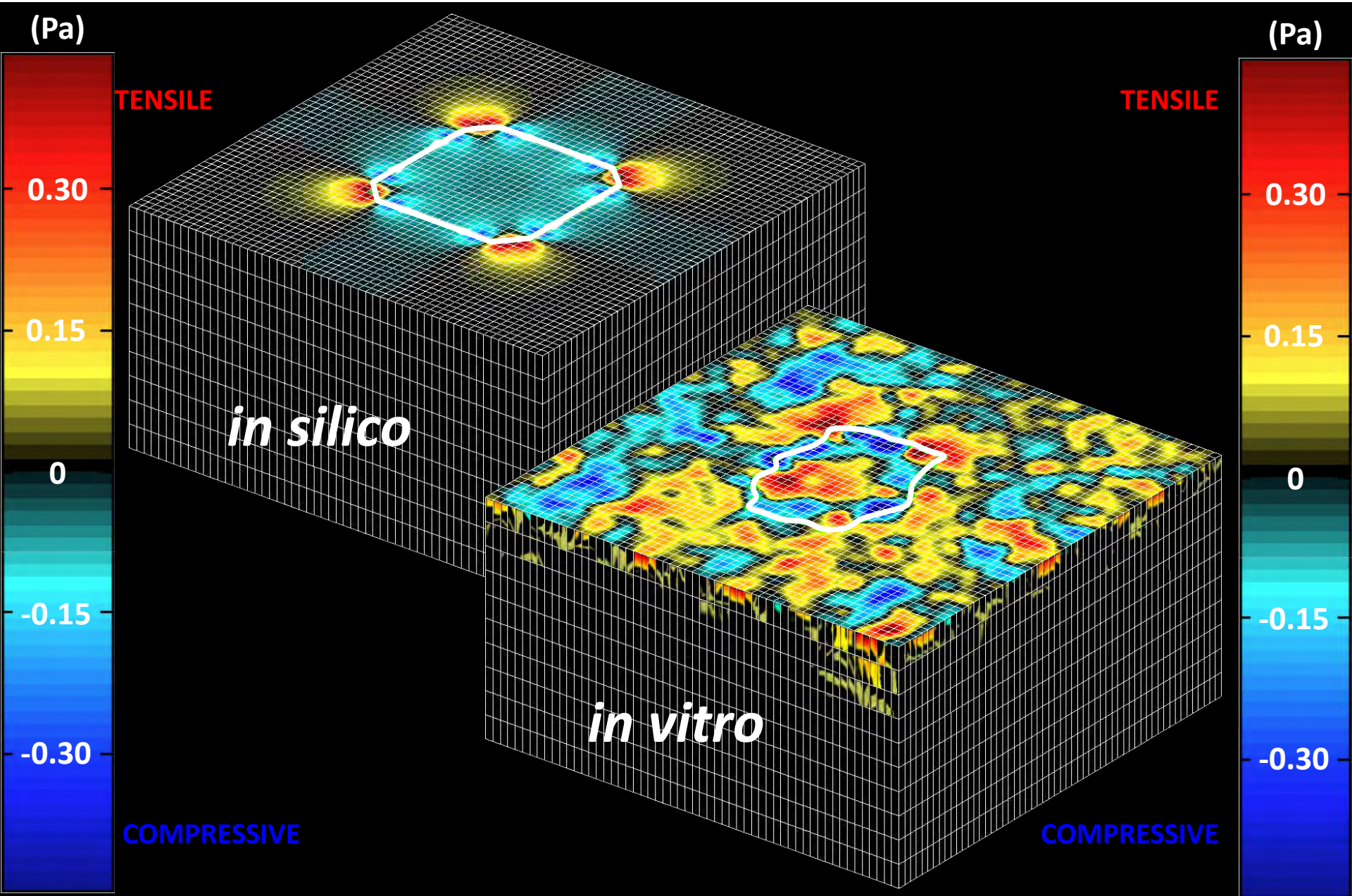
GROUNDED & SEGMENTED ACTOMYOSIN RING

– AVERAGE COMPRESSION / TENSION –

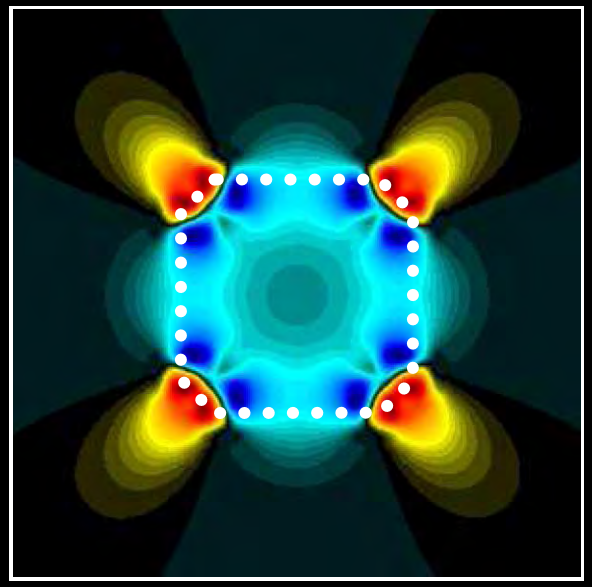


GROUNDED & SEGMENTED ACTOMYOSIN RING

– AVERAGE COMPRESSION / TENSION –



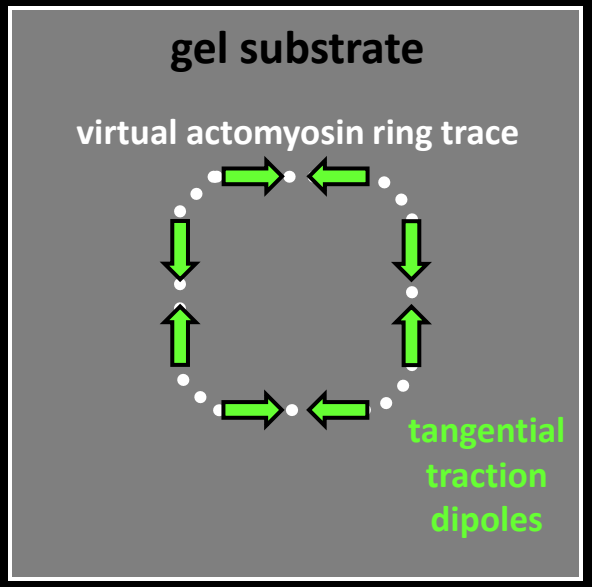
GROUNDED & SEGMENTED ACTOMYOSIN RING



gel substrate tangential stresses

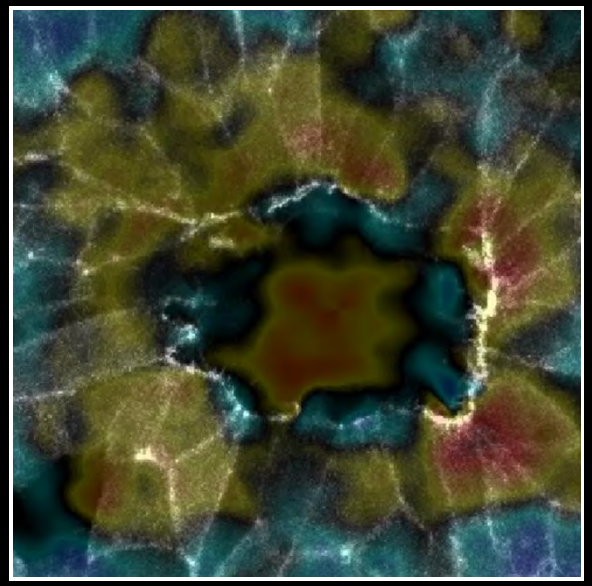
(Pa)

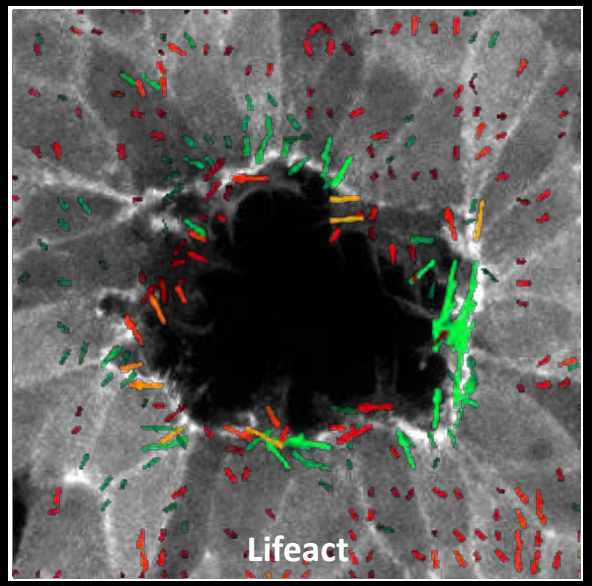
-200 -100 0 100 200



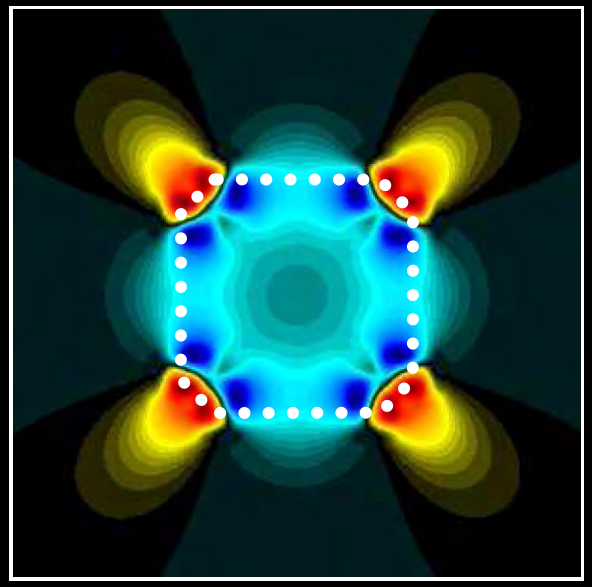
in silico

in vitro





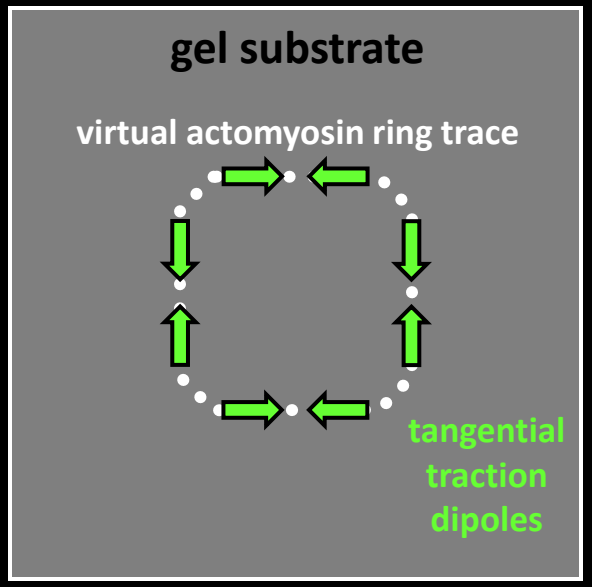
GROUNDED & SEGMENTED ACTOMYOSIN RING



gel substrate tangential stresses

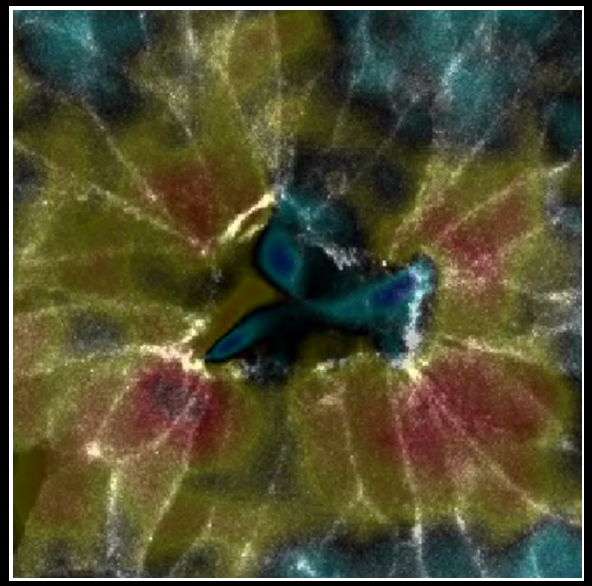
(Pa)

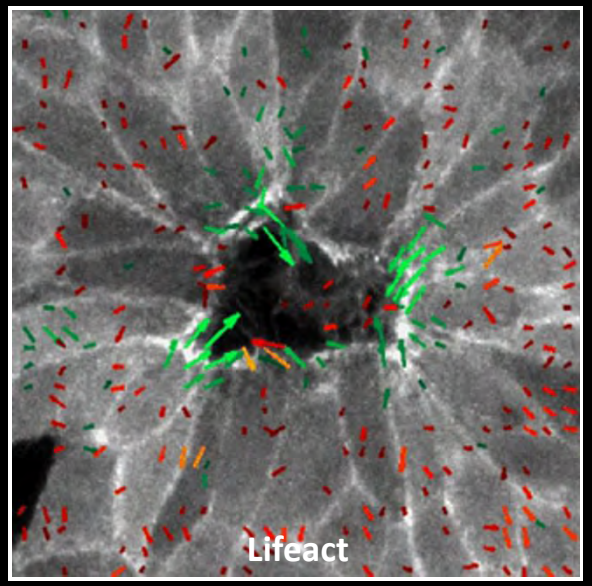
-200 -100 0 100 200



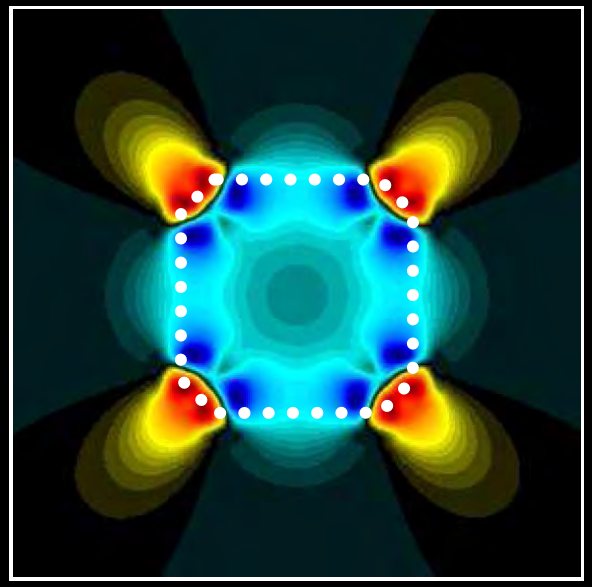
in silico

in vitro





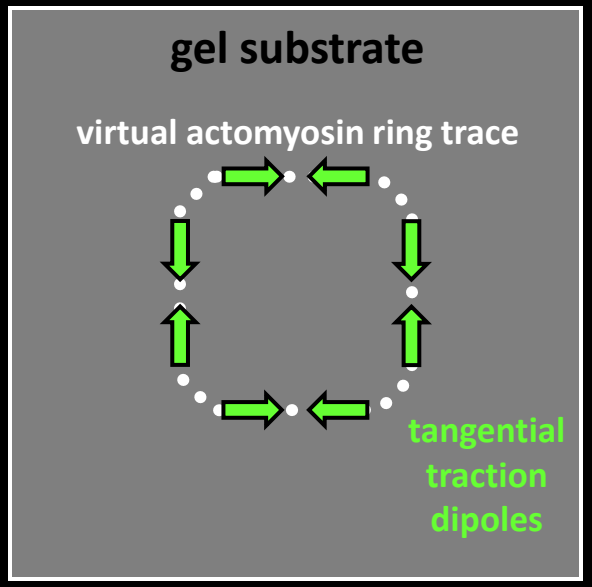
GROUNDED & SEGMENTED ACTOMYOSIN RING



gel substrate tangential stresses

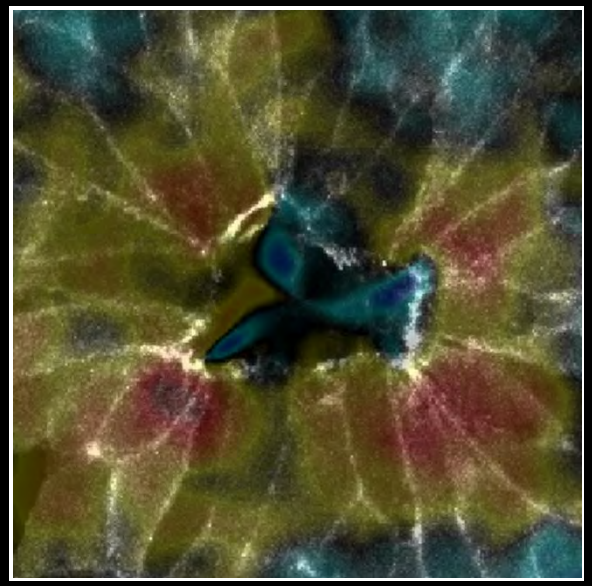
(Pa)

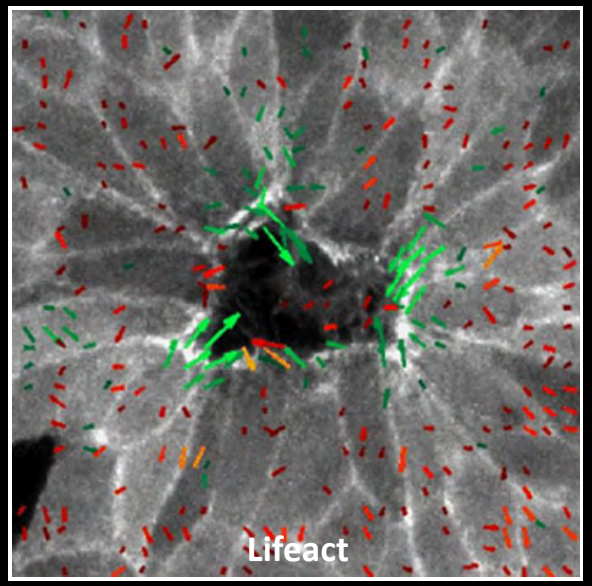
-200 -100 0 100 200



in silico

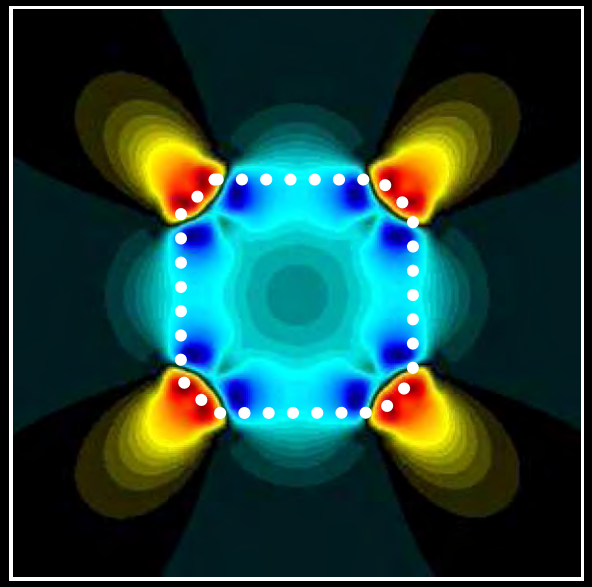
in vitro



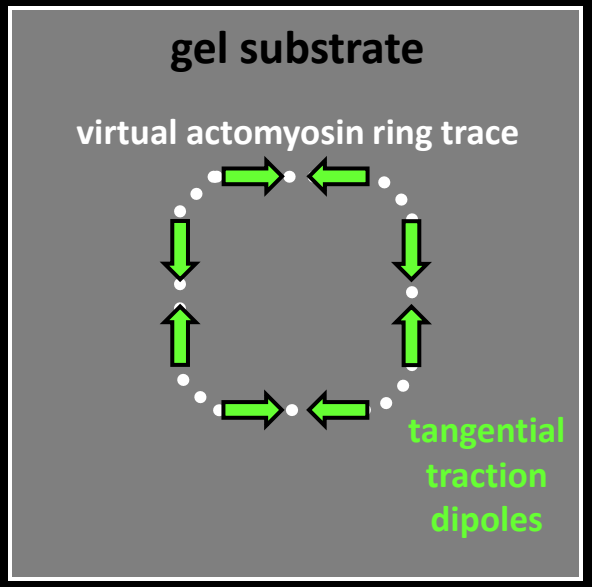


THE ACTOMYOSIN RING IS BASAL, GROUNDED AND SEGMENTED

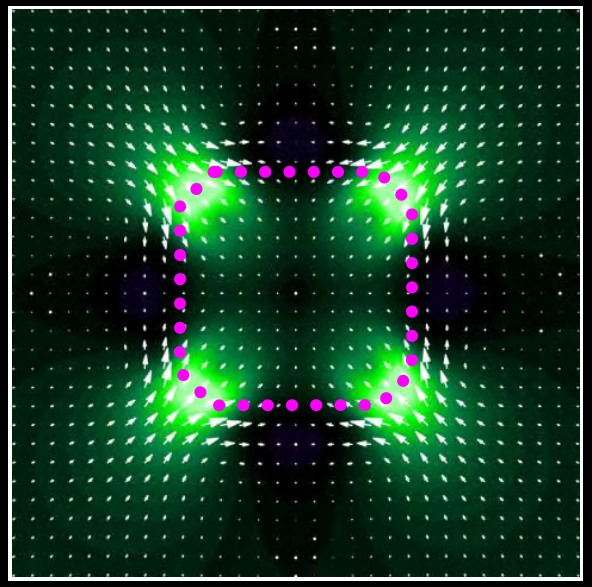
GROUNDED & SEGMENTED ACTOMYOSIN RING



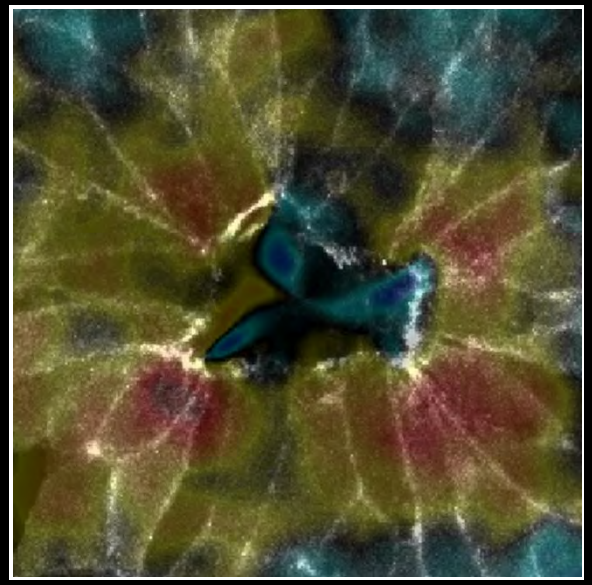
gel substrate tangential stresses
(Pa)
-200 -100 0 100 200

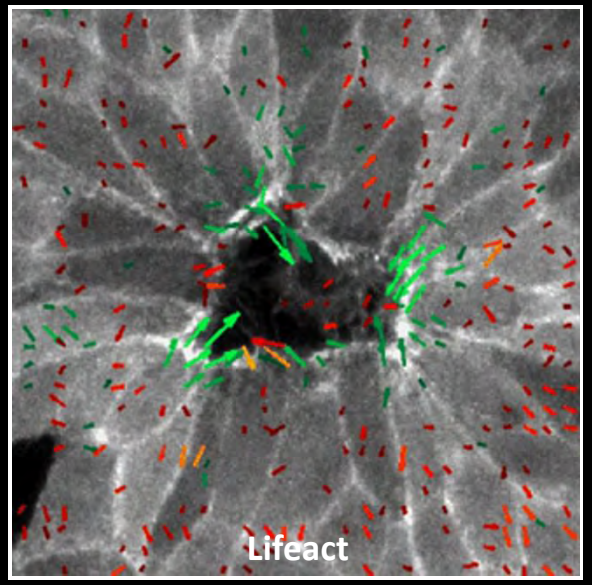


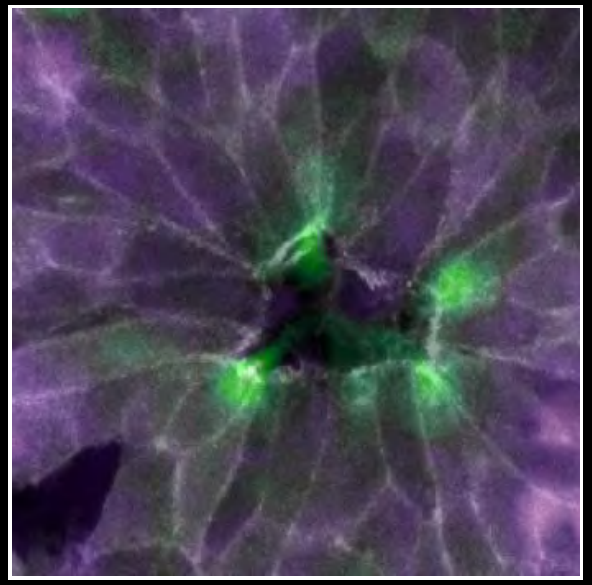
in silico
in vitro



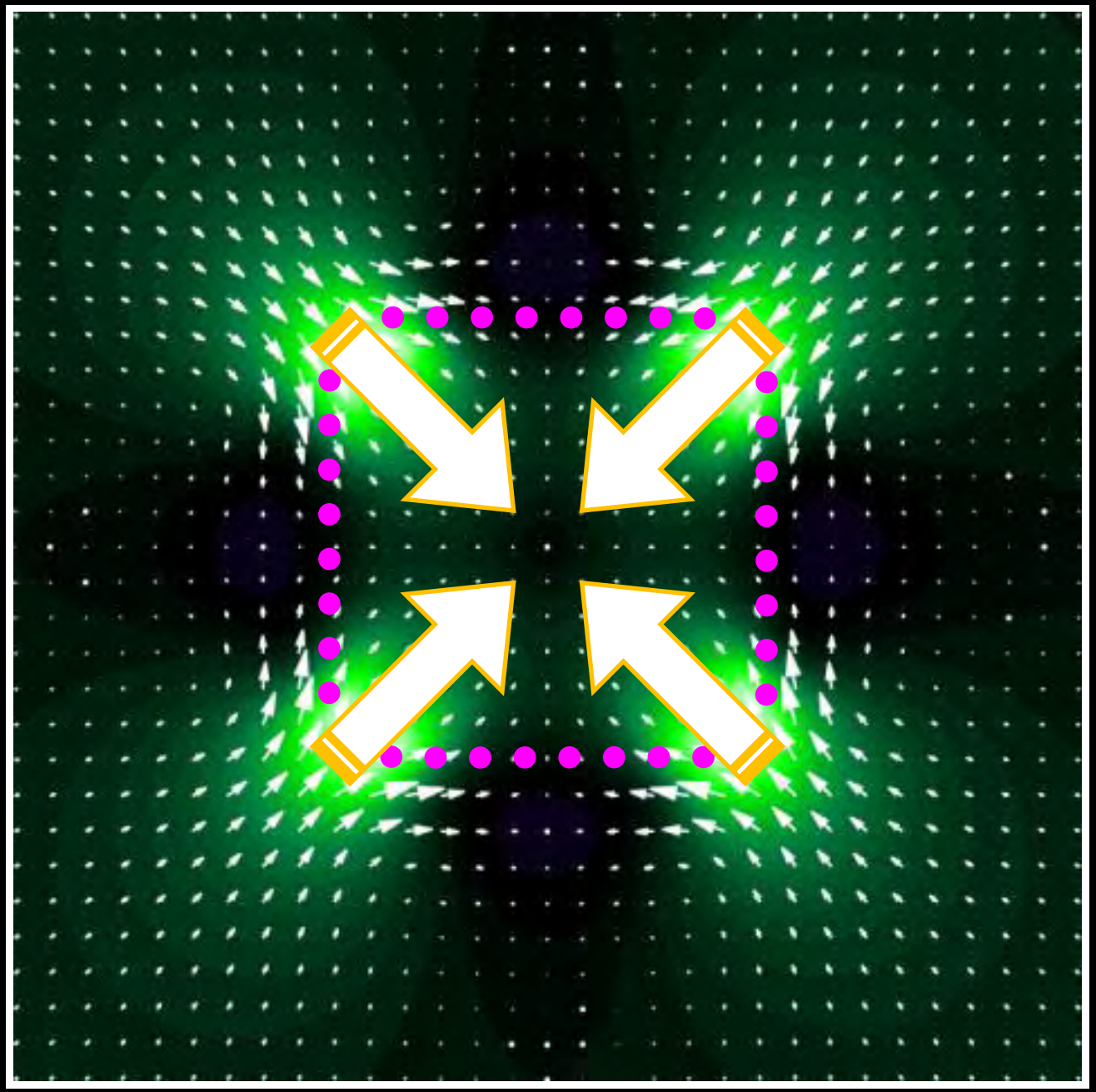
gel substrate displacements
(μm)
-0.3 -0.2 -0.1 0 0.1 0.2 0.3





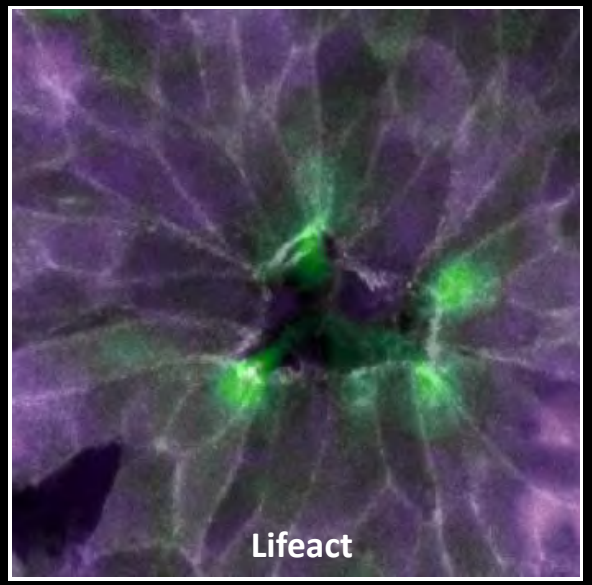


Epithelial Self-Steering Speculation

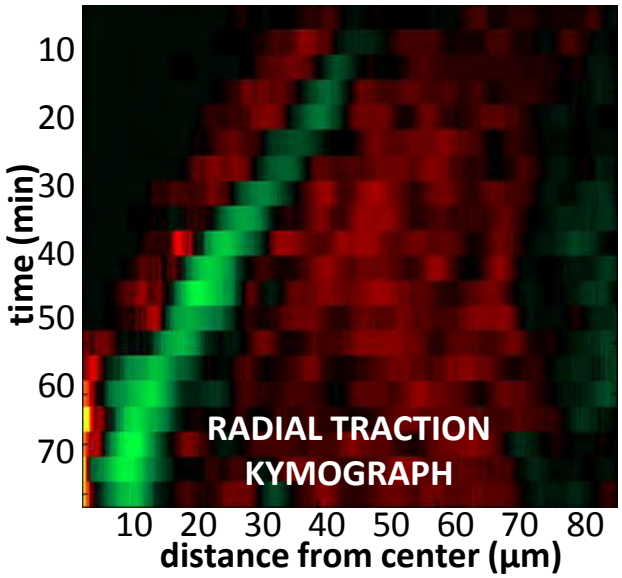


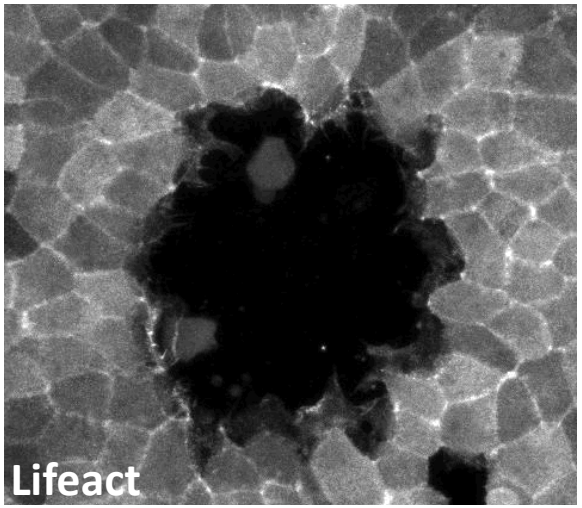
gel substrate displacements
(μm)

-0.3 -0.2 -0.1 0 0.1 0.2 0.3

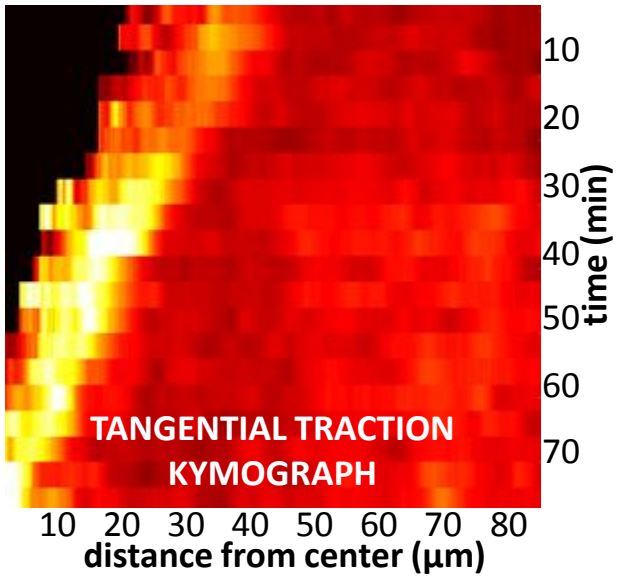


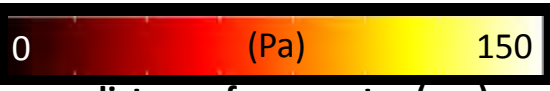
WHAT IS MISSING?



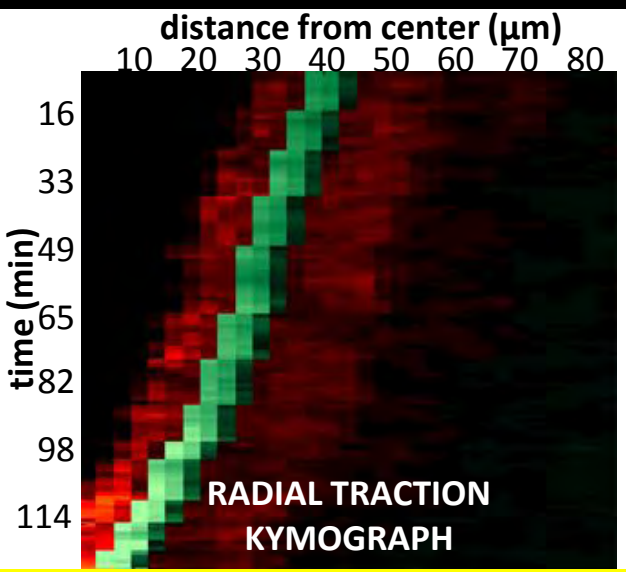


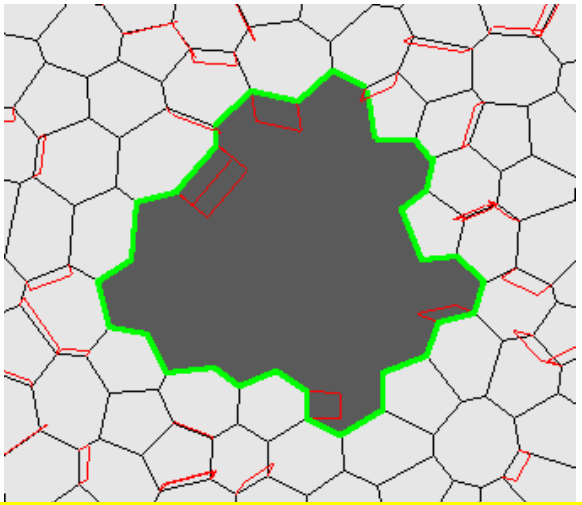
in vitro

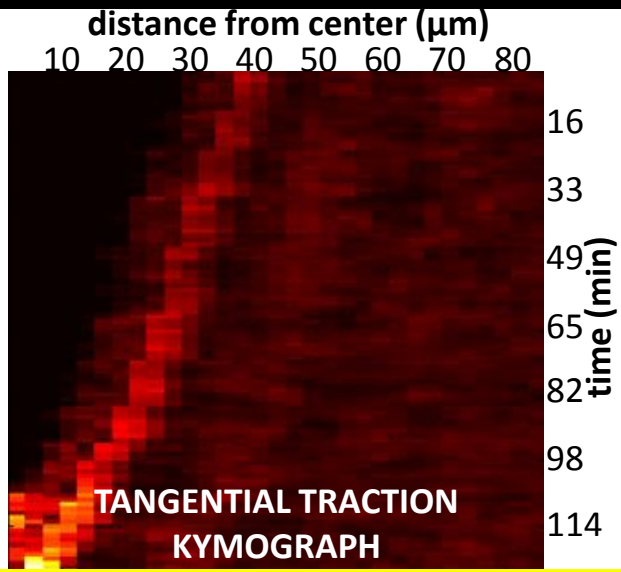




in silico

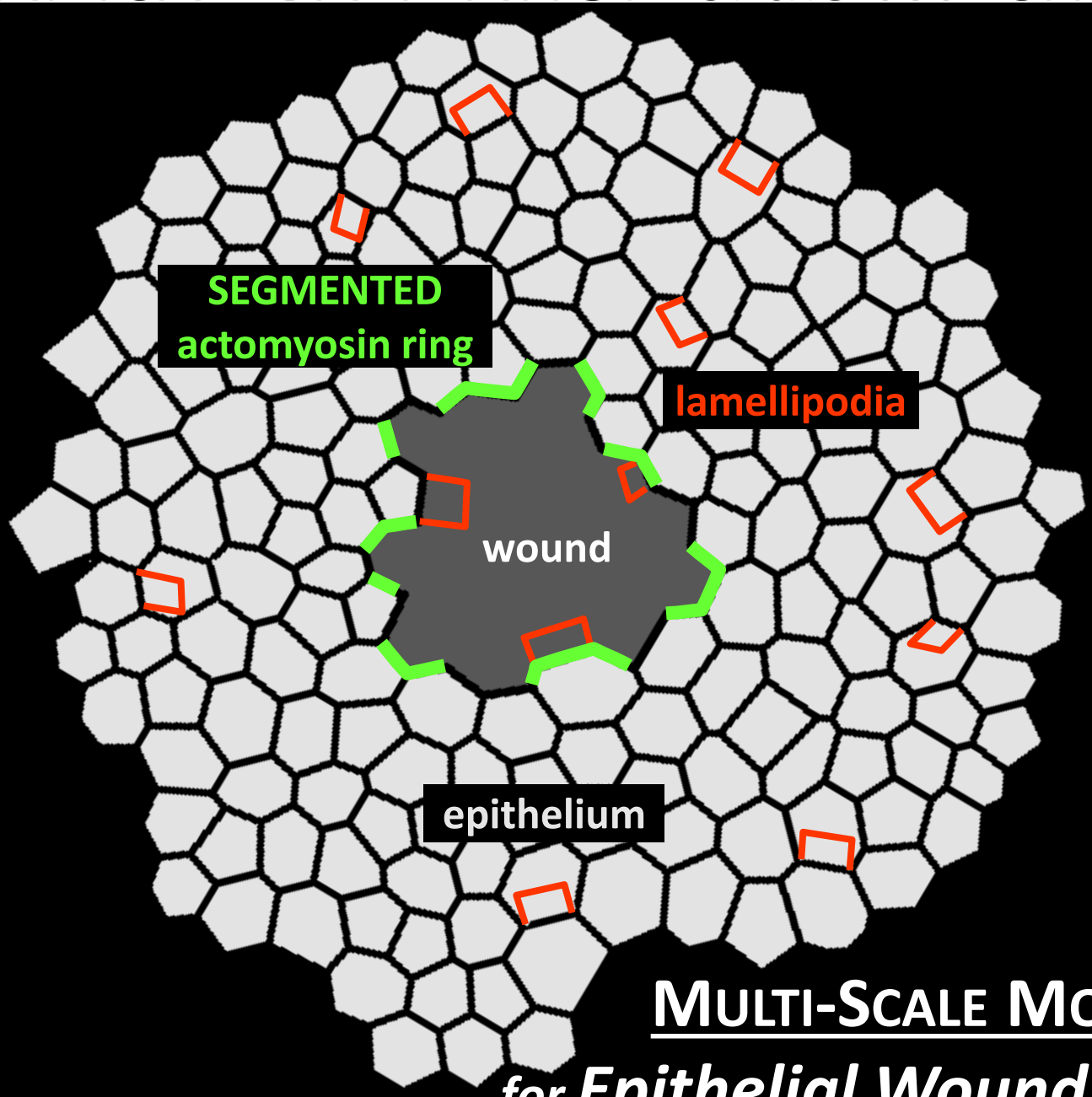






lamellipodia + CONTINUOUS actomyosin ring

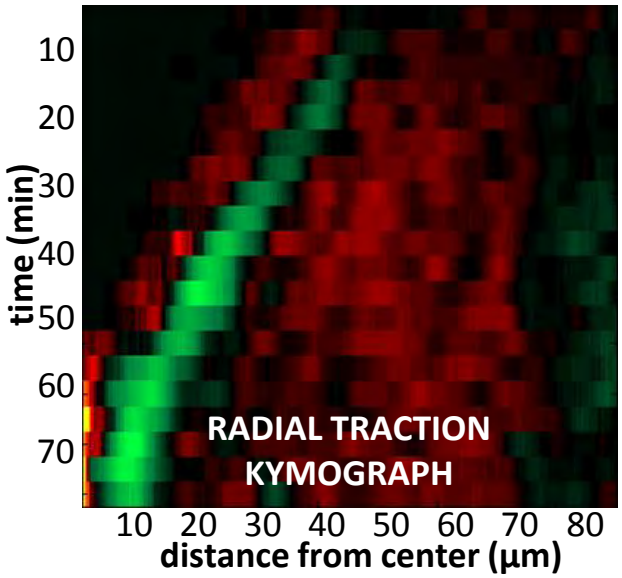
MECHANICAL VALIDATION of the MECHANISM

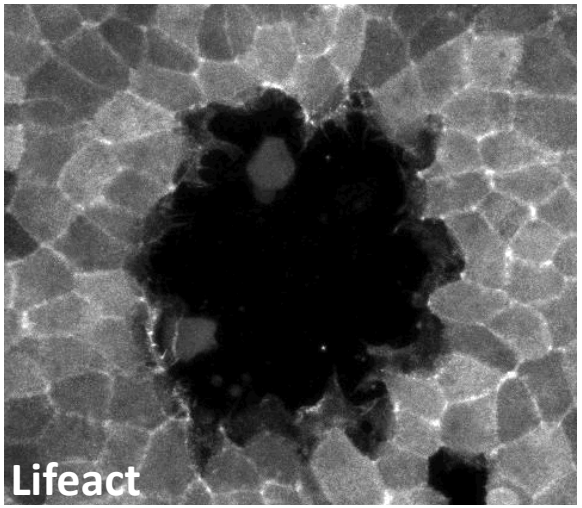


MULTI-SCALE MODEL

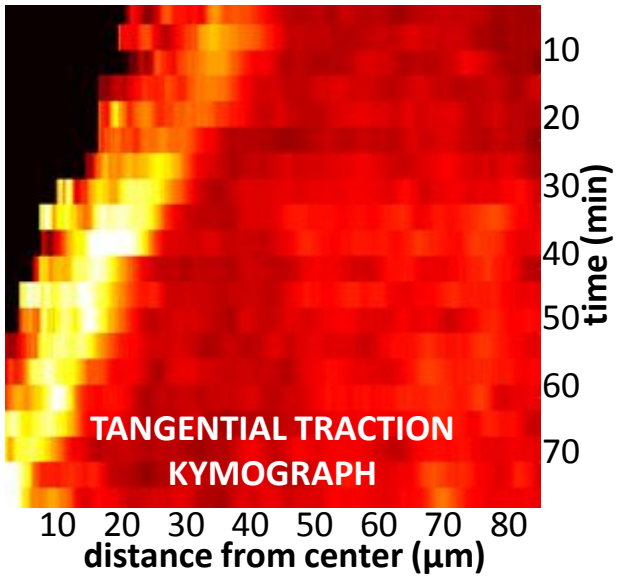
for Epithelial Wound Healing

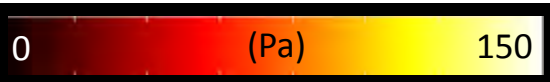
MECHANICAL VALIDATION of the MECHANISM



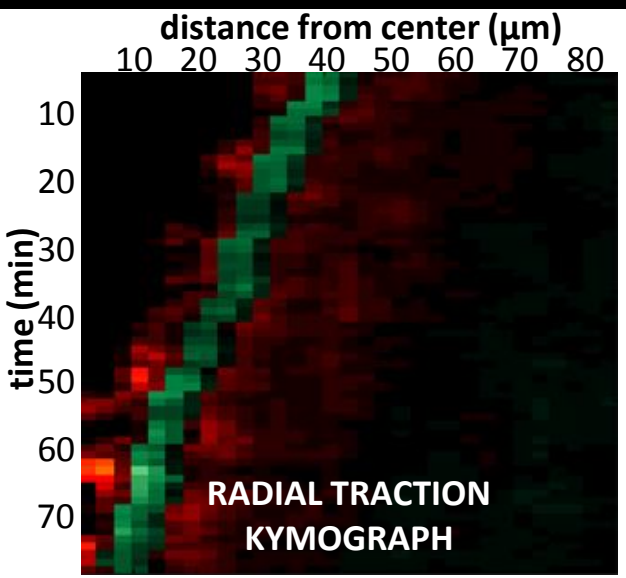


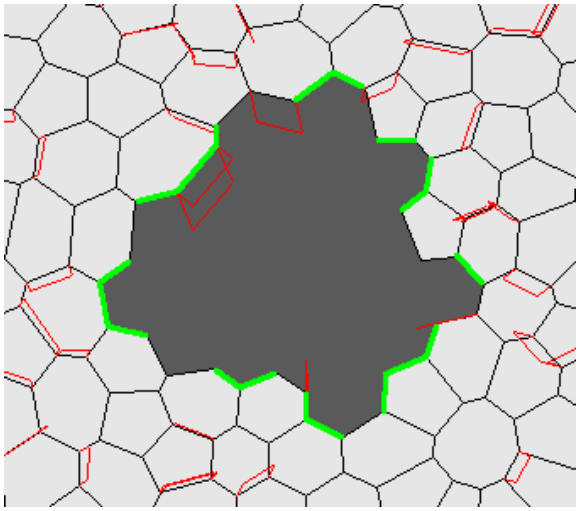
in vitro

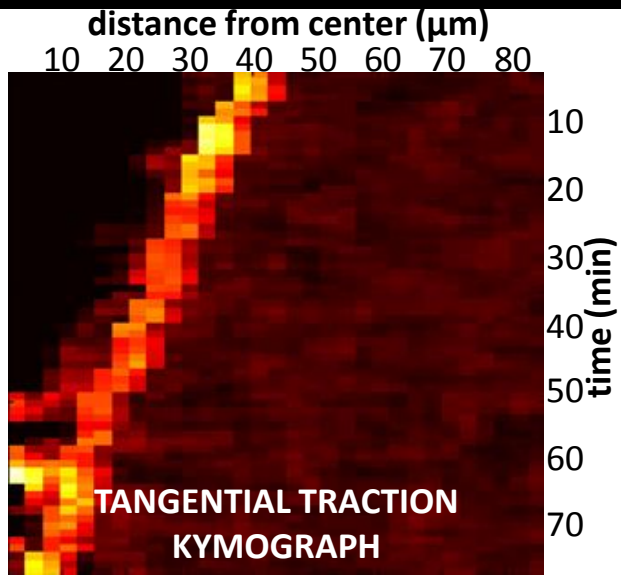




in silico



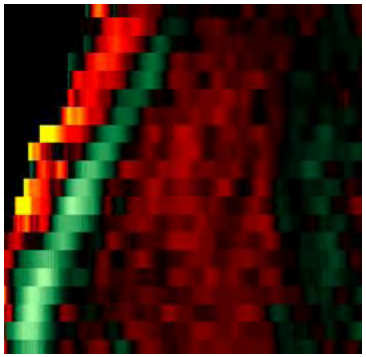




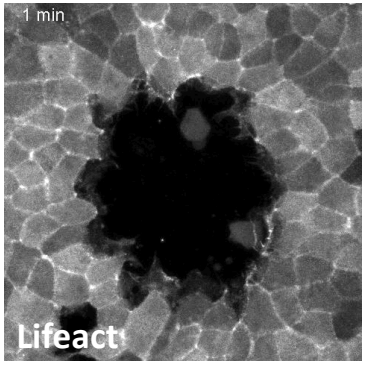
lamellipodia + SEGMENTED actomyosin ring

CONCLUSIONS

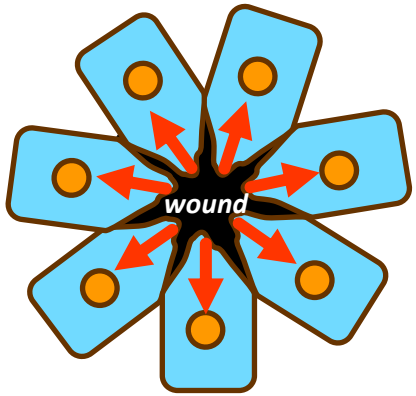
RADIAL



in vitro & in silico



TISSUE DYNAMICS



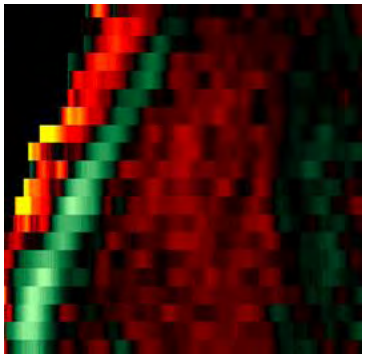
cell crawling

purse string

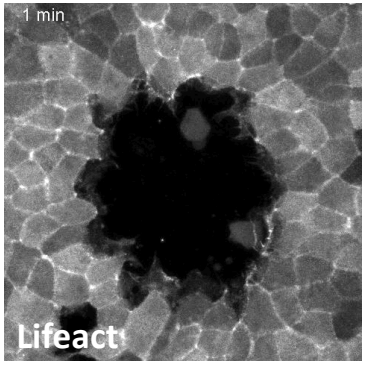
a MECHANISM
for
WOUND HEALING

CONCLUSIONS

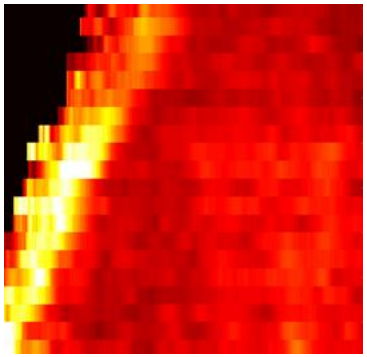
RADIAL



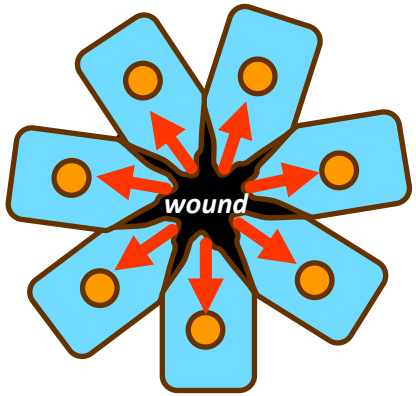
in vitro & in silico



TISSUE DYNAMICS



TANGENTIAL



cell crawling

purse string

a MECHANISM for WOUND HEALING

grounded purse string

ACKNOWLEDGMENTS