Investigation of cell mechanics using Micro/Nanodevices : some examples

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Problematic of Cell mechanics measurements

Issues			Hurdles
Fixed cells	⟷	Living cells	→ Variability → Accessibility (i.e human blood)
Cell lines	⟷	Primary Cells	→ Not fixed → Device biocompatible
Static measurements	⟷	Dynamical measurements	→ Real-time imaging
Qualitative measurements	⟷	Quantitative measurements	→ Data processing
2D Cell culture	⟷	3D Micro environment	→ Creation of a 3D environment mimicking in vivo conditions → Seeding 3D scaffolds

Outline

Problematic
Investigating Migration Mechanism
Primary cells : Macrophages

Micro Devices for cell migration investigation
Review of the State-of-the-art

Micro channel equipped with micro pillar force sensors
Device fabrication
Device characterization
Macrophage migration inside the device
Force amplitude
Force orientation

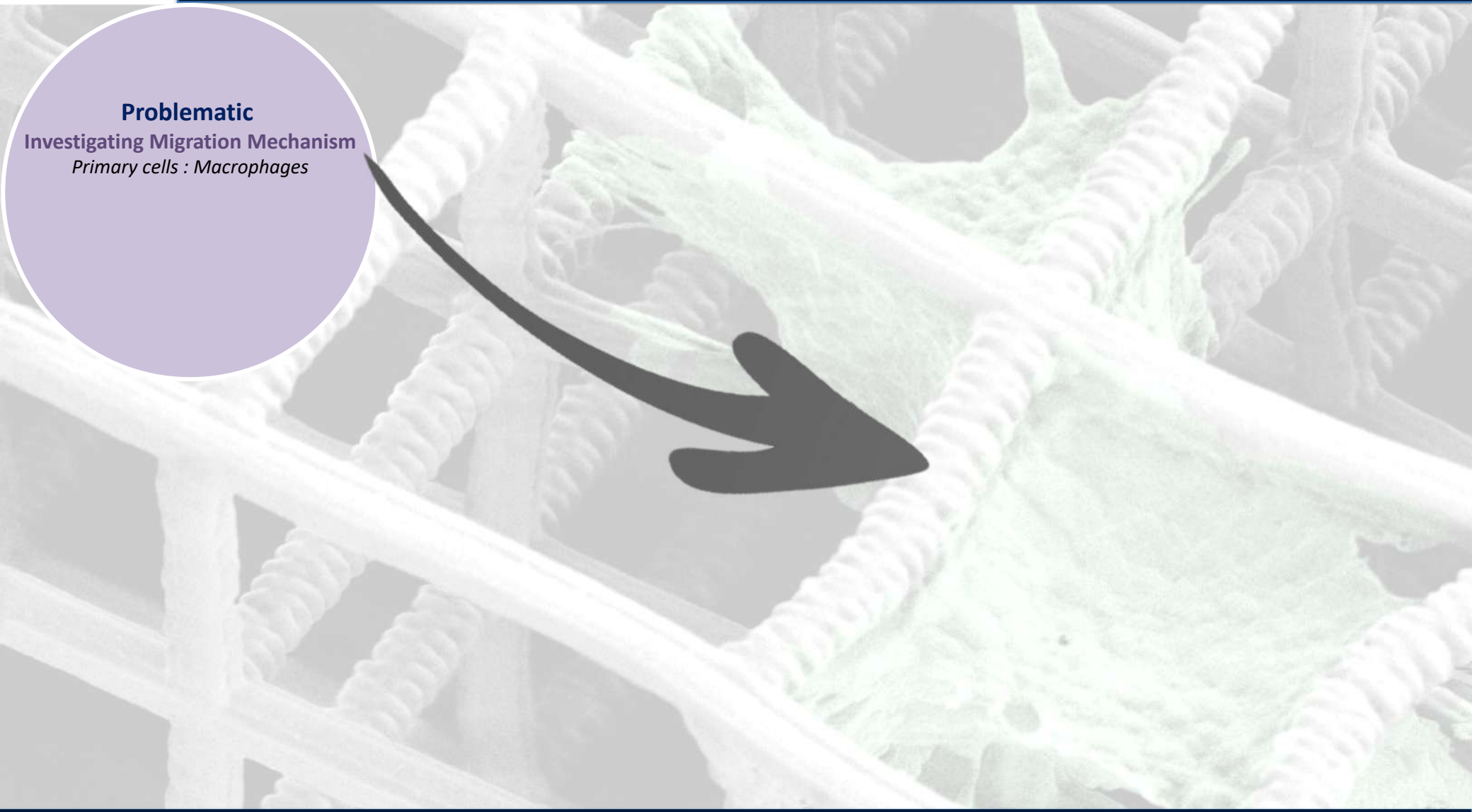
3D scaffold
Scaffold fabrication
Scaffold characterization
Macrophage migration inside the scaffold
Mechanical properties of the scaffold
Stress and Force evaluation

Outline

Problematic

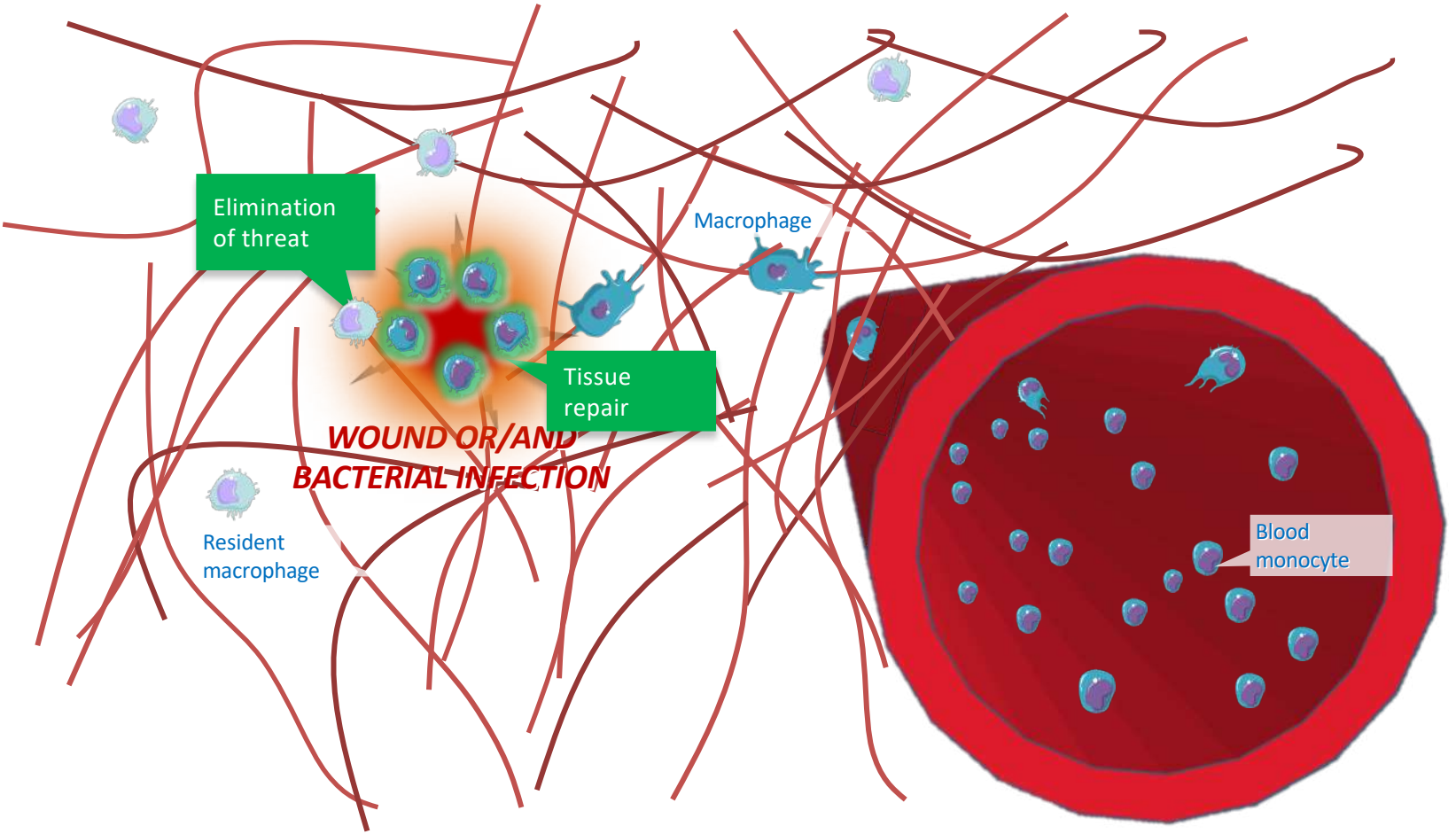
Investigating Migration Mechanism

Primary cells : Macrophages



Human Macrophages

Immune role of macrophages



Resident Macrophages
Wound and infected tissue

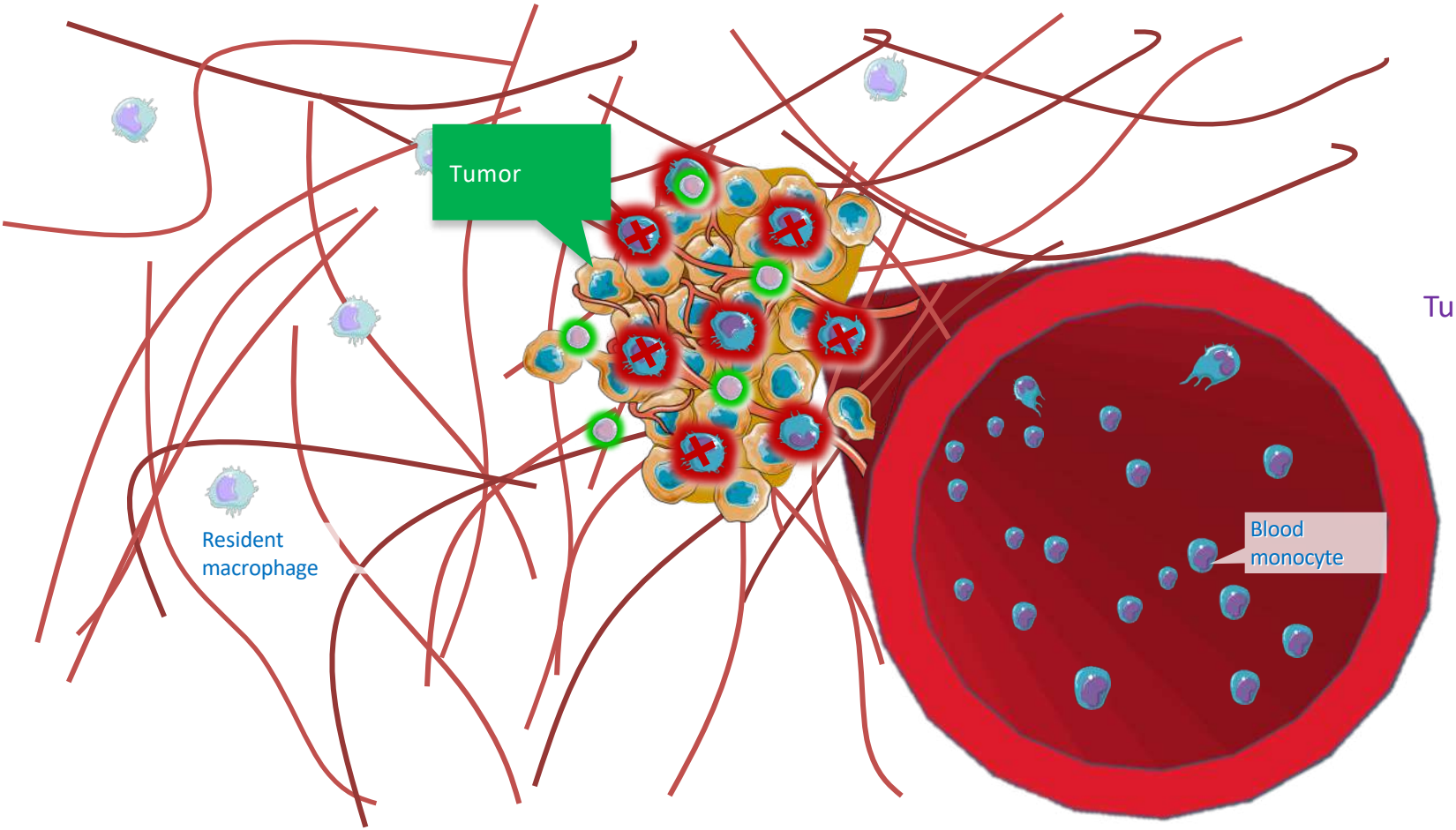
Monocytes
Bloodstream

Macrophage Migration
Tissue

Macrophage 1 & 2
Wound and infected tissue

Human Macrophages

ROLE OF MACROPHAGES IN TUMORAL CONTEXT



Macrophages

↓

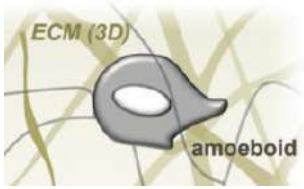
Tumor associated macrophages

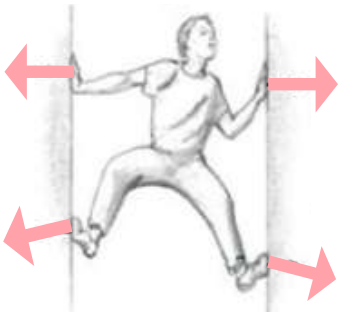
↓

Circulating Tumoral Cells

Specific force patterns

Porous matrix

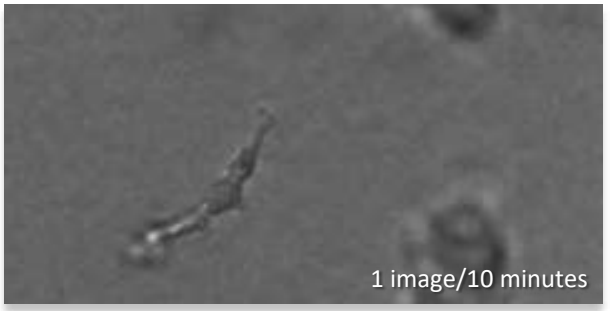


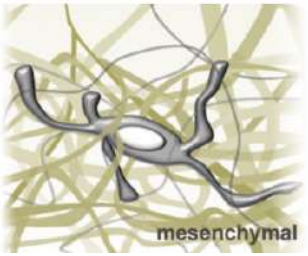


V= 40 µm/h

Van Goethem et al, 2010, Journal of immunology

Non-porous matrix





V= 10-30 µm/h

Heuzé et al, 2013, Immunological reviews

Outline

Problematic

Investigating Migration Mechanism

Primary cells : Macrophages

Migration modes : From amoeboid to Mesenchymal

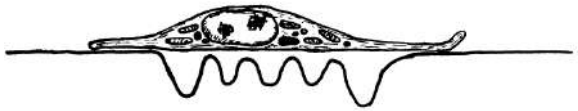
Mechanical forces involved

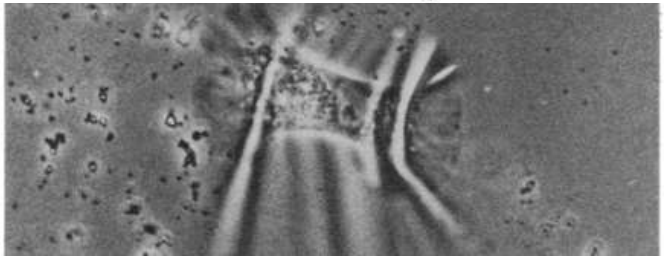
Micro Devices for cell migration investigation

Review of the State-of-the-art

Cell forces measurements

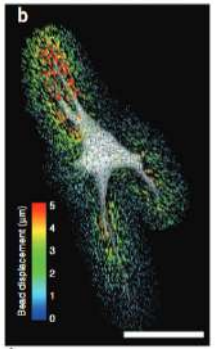
Wrinkling method

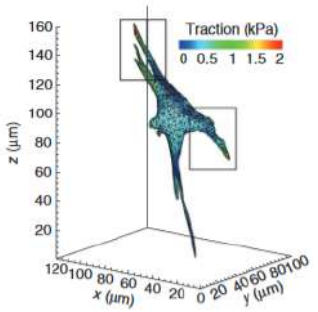




Deformable object : Silicone rubber substrata
Cell : Fibroblast
Force : 10 nN/µm
Harris et al, Science, 1980

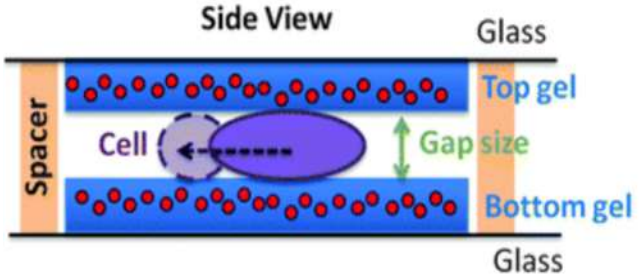
Traction Force Microscopy (2D,3D)





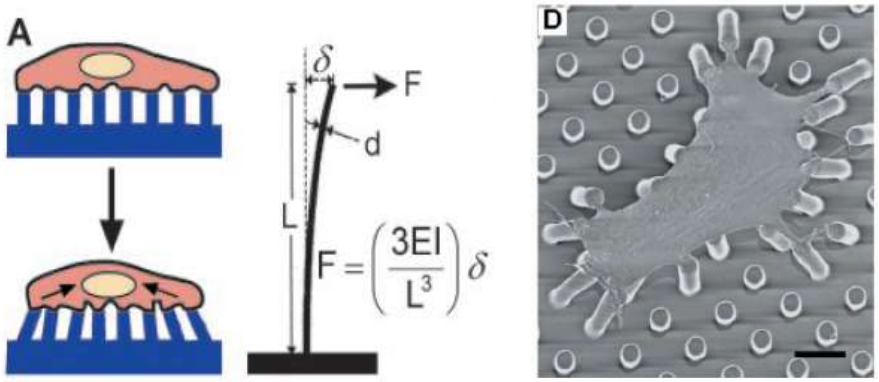
Deformable object : Elastic hydrogel matrices
Cell : NIH 3T3
Stress : 2 kPa
Legant et al, Nature methods, 2010

TFM under induced Spatial confinement



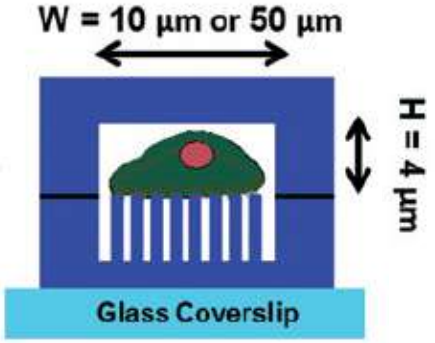
Deformable object : Polyacrylamide gels
Cell : dHL60
Stress : 800 Pa
Yip et al, Integrative biology, 2015

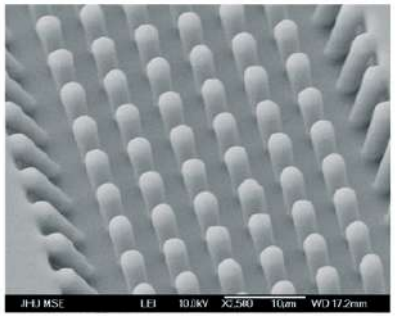
State-of-the-art : Available tool to measure cell-generated forces



Deformable object : PDMS Pillars
Cell : Smooth muscle cell
Force : 10 nN
Tan et al, PNAS, 2003

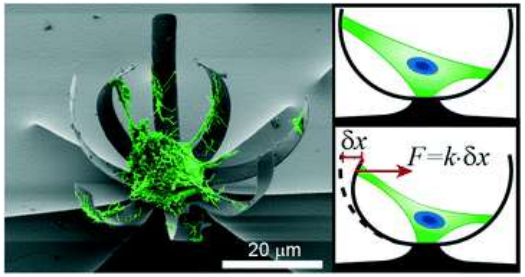
From 2D to confining devices



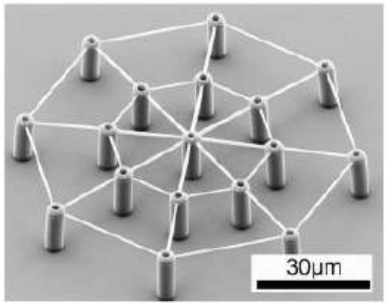


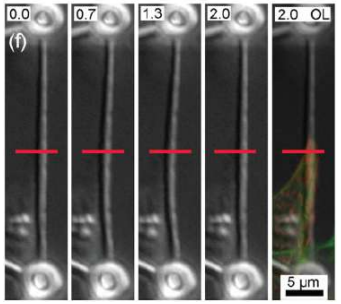
Deformable object : PDMS Pillars
Cell : NIH-3T3 and HOS cells
Force : 4 nN
Raman et al, PNAS, 2013

Inputs of 3D technologies



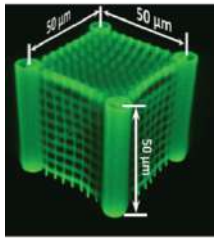
Deformable object : µ-Flower
Cell : MEFs cell
Force : quelques nN
Marelli et al, Lab on a Chip, 2013

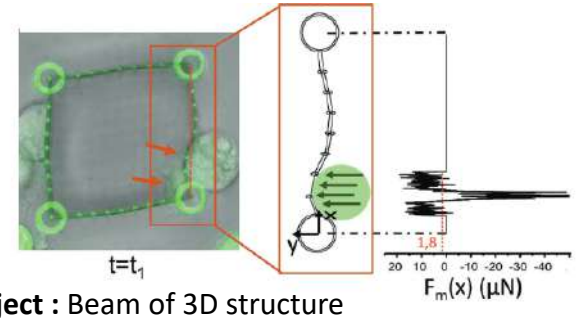




Deformable object : Beam of 3D structure
Cell : chicken cardiomyoc
Force : 20 nN
Klein et al, advanced materials, 2010

From static to dynamical cell force measurements





Deformable object : Beam of 3D structure
Cell : SH-SY5Y, MCF7 and MDA-MB-231 Cell
Force : 1.5 µN
Lemma et al, advanced materials, 2017

Objectives

Problematic
Investigating Migration Mechanism

→ *Primary Human Macrophages Migration*

→ *Validation of dedicated Microdevices*

→ *Dynamical Cell force measurements*

→ *Spatial confinement*

→ *Optical Imaging*

→ *3D*

Micro channel equipped with micro pillar force sensors

3D scaffold

Outline

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Primary cells : Macrophages

Migration modes : From amoeboid to Mesenchymal

Mechanical forces involved

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Device characterization

Macrophage migration inside the device

Force amplitude

Force orientation

Aspect of the Fluidic device

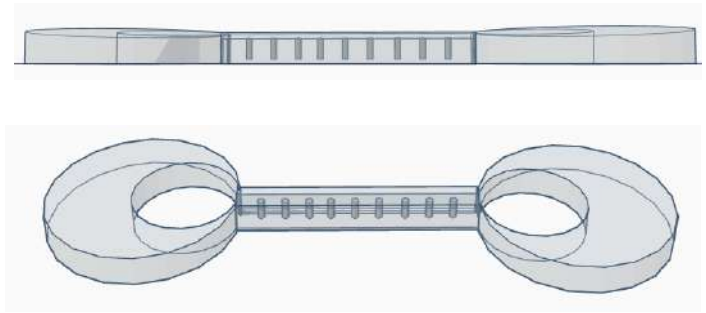
2 Key elements

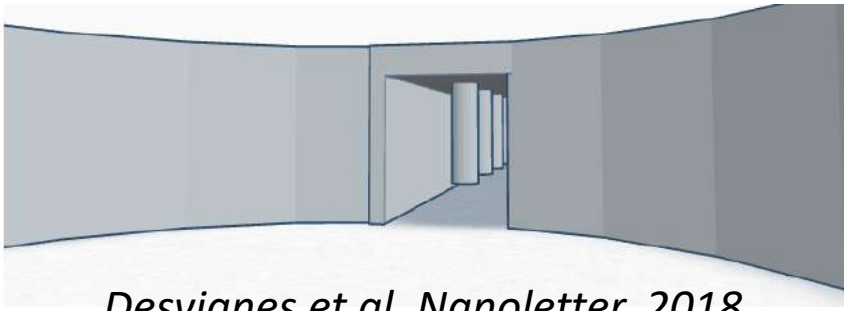
Micro-Channels for Spatial confinement

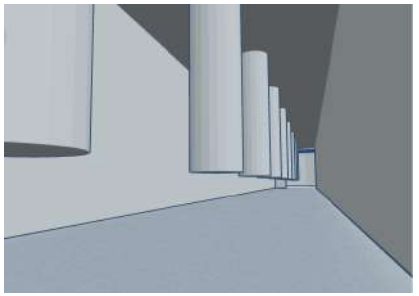
Width	6.5 µm
	12.5 µm
Height	11.25 µm

Micro-Pillars as Force sensors

Pillar diameter	1.86 µm
Pillar Height	8.5 µm

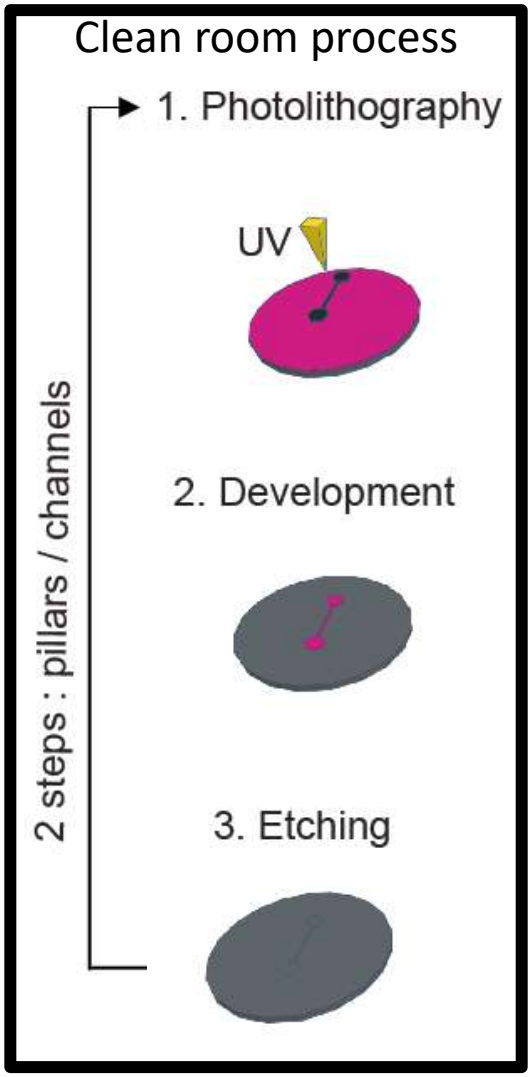


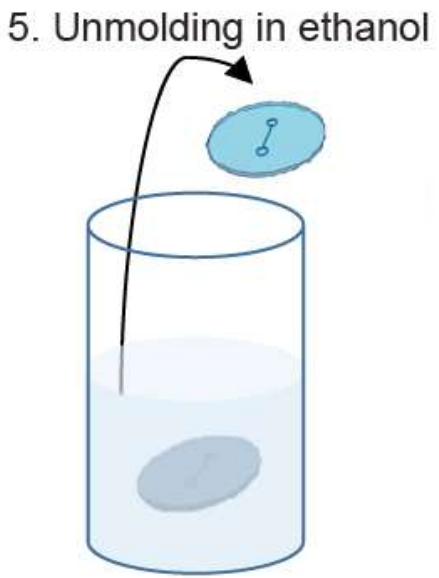


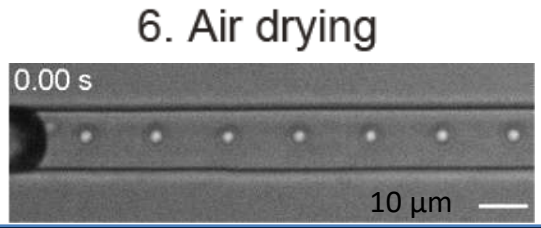


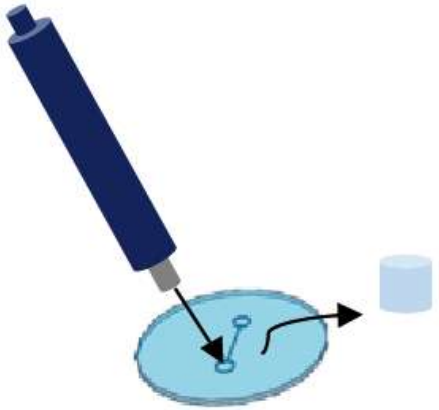
Desvignes et al, Nanoletter, 2018

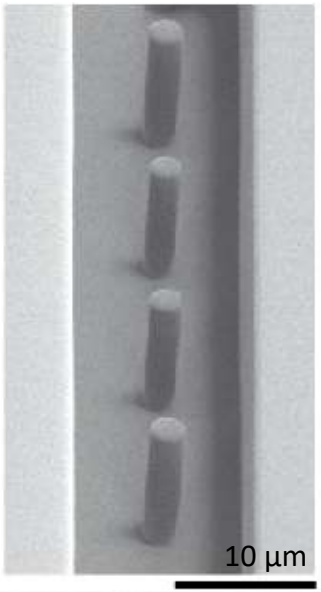
Device fabrication

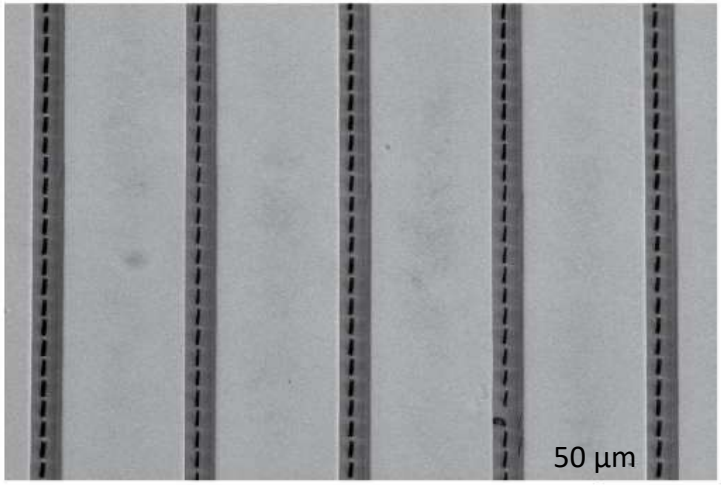




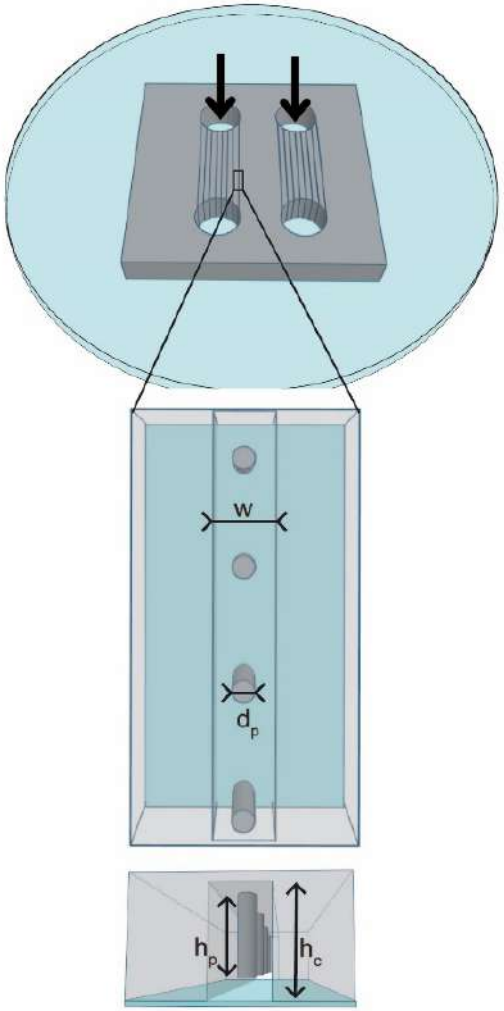


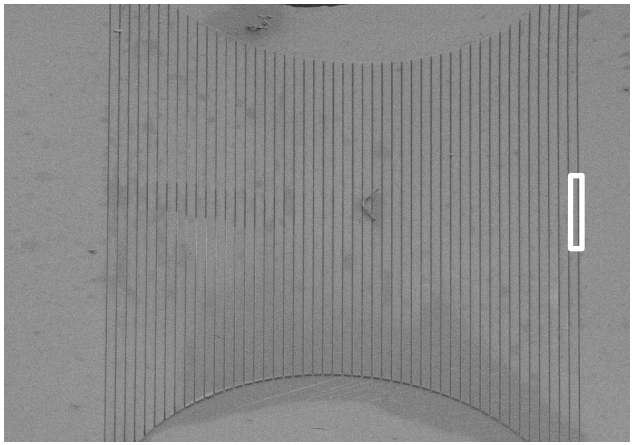


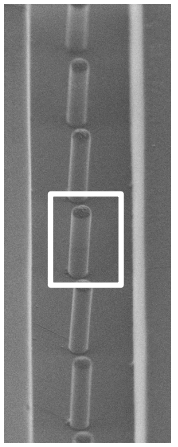


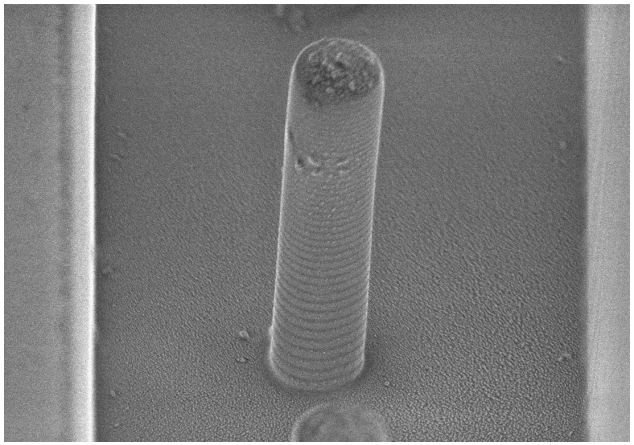


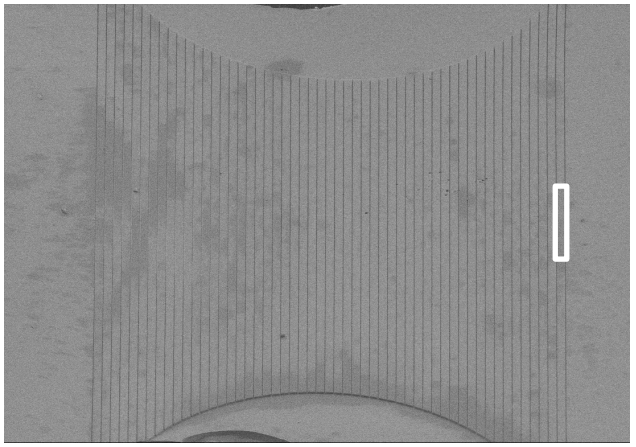
Device characterisation

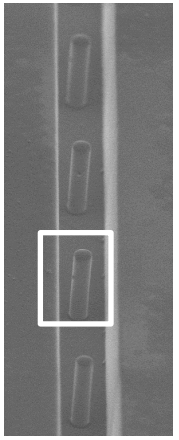


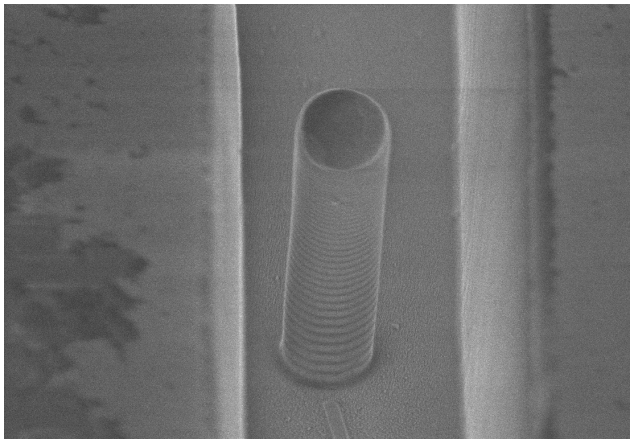






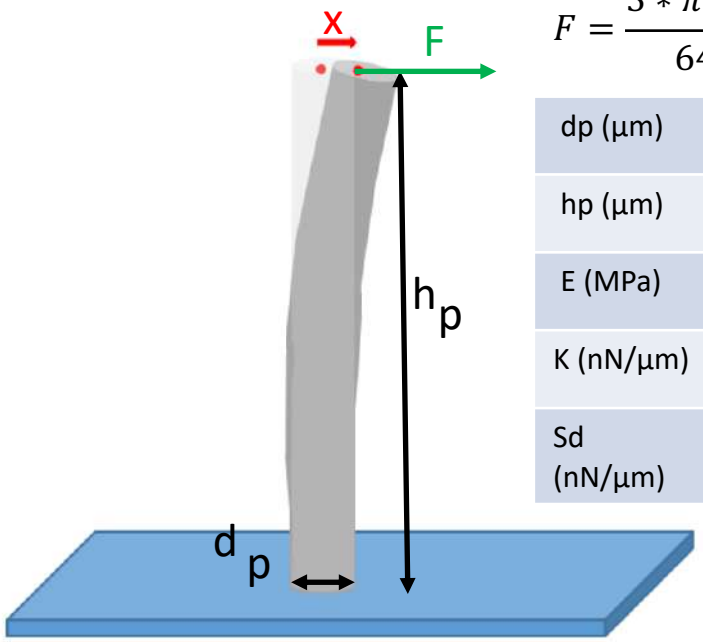






Pillar stiffness

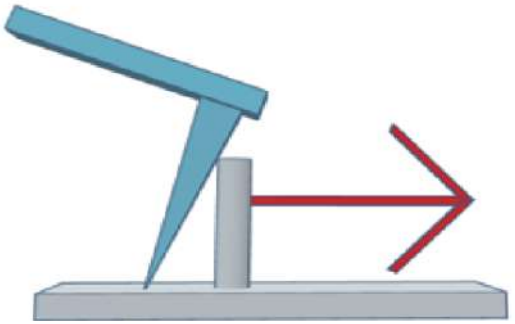
Pillar stiffness estimated with beam theory



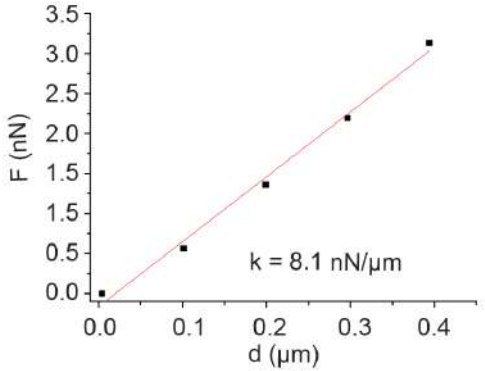
$$F = \underbrace{\frac{3 * \pi * E * {d_p}^4}{64 * {h_p}^3}}_{K} X$$

dp (µm)	1.86
hp (µm)	8.5
E (MPa)	2
K (nN/µm)	6
Sd (nN/µm)	+/- 4

Pillar stiffness measured by AFM

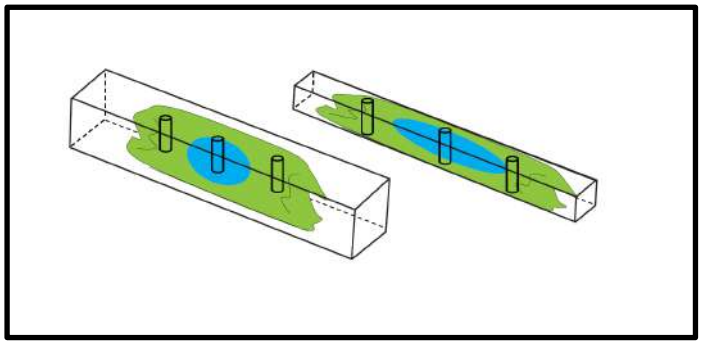


Typical result



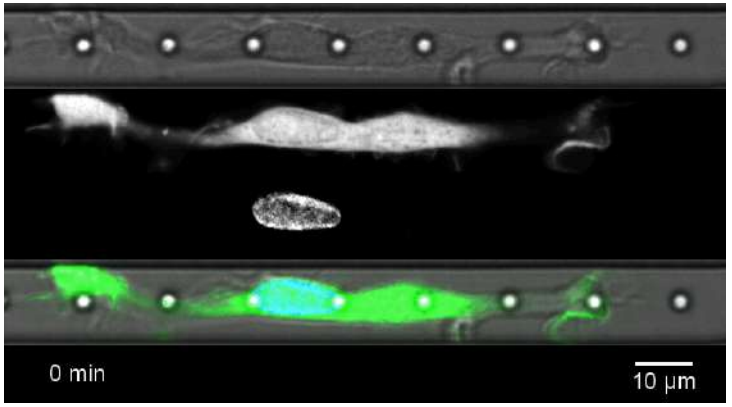
K_{mean} = 7,1 nN/µm (Sd : 1.9 nN/µm)

Macrophage Migration inside pillar-equipped Micro-channels



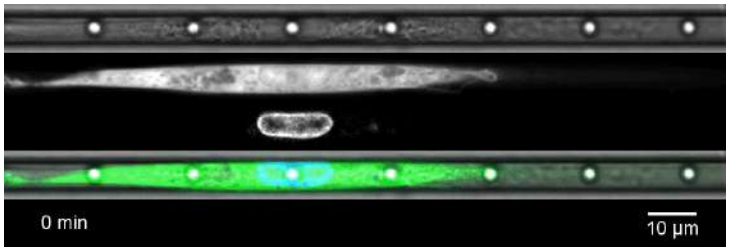
- → Primary monocytes derived in macrophages.
- → After 48h.
- → 5% CO2
- → 37°C
- → Immersion inside culture medium (no flux)

Channel width 12 µm



Channel
Cytoplasm/Cell Tracker
Nucleus/Hoechst
Merge

Channel width 6 µm

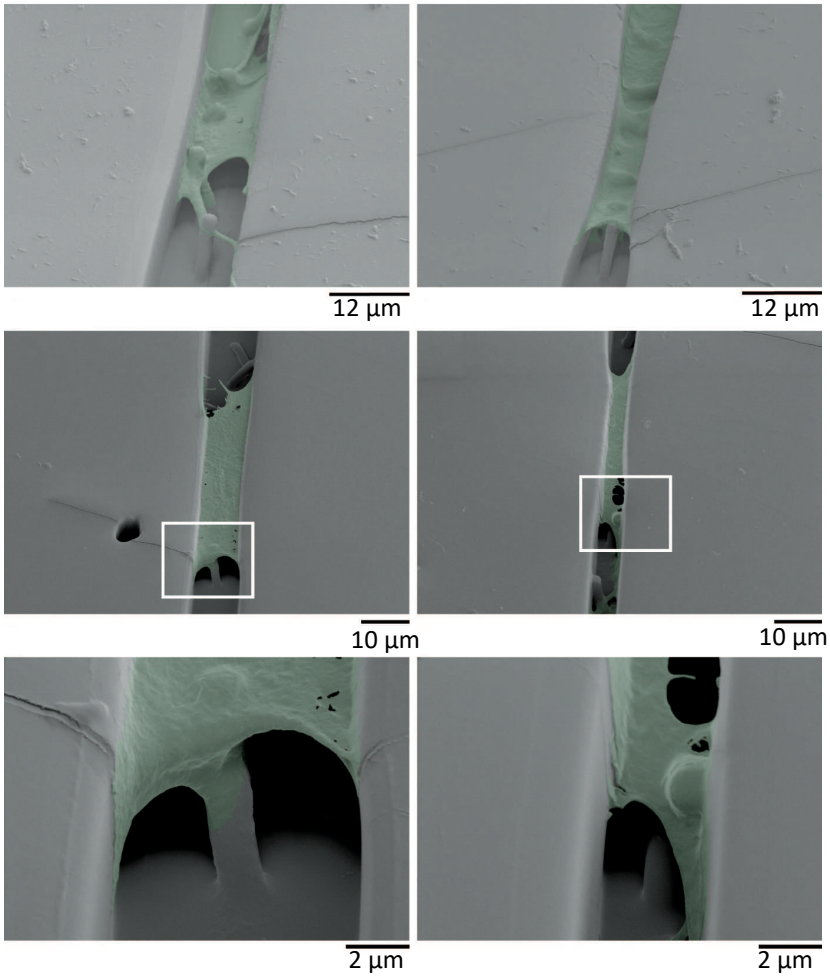


Channel
Cytoplasm/Cell Tracker
Nucleus/Hoechst
Merge

Migration direction

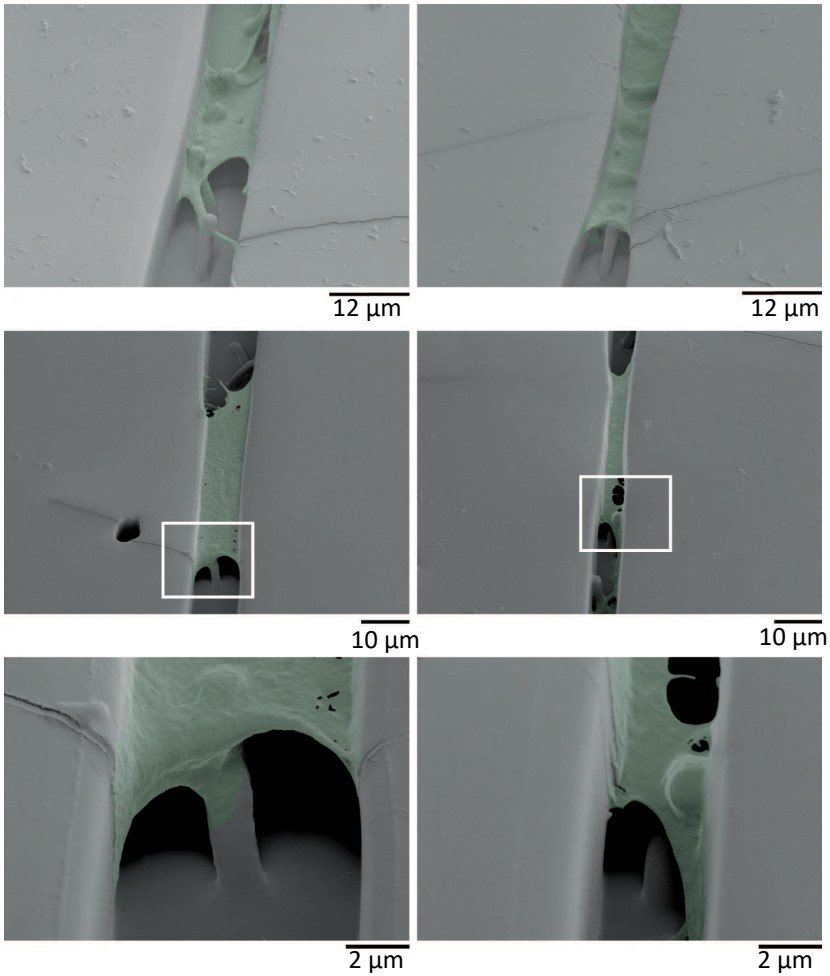
Macrophage/Device contacts

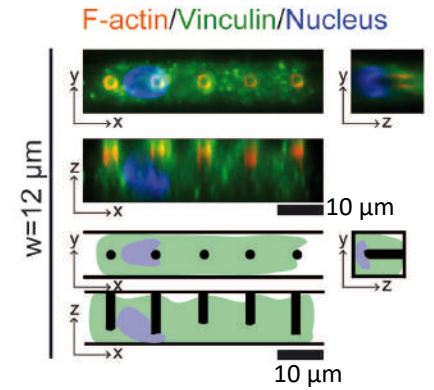
Fixed macrophages observed by Scanning Electron Microscope (SEM)

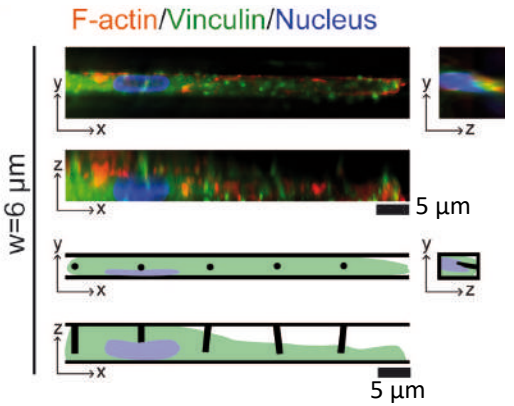


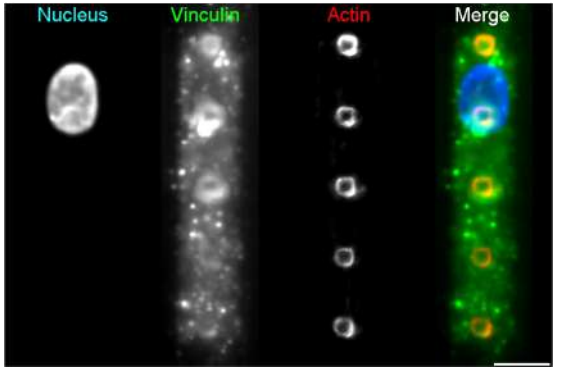
Macrophage/Device contacts

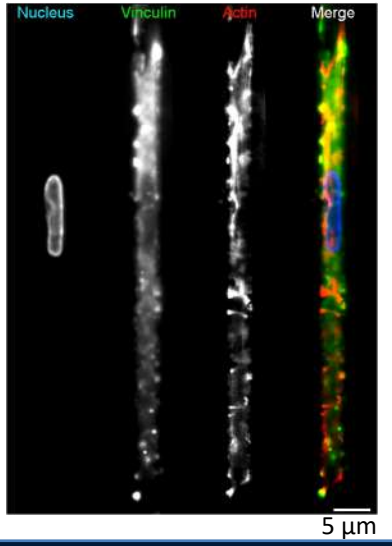
Fixed macrophages observed by Scanning Electron Microscope (SEM) and fluorescent microscopy



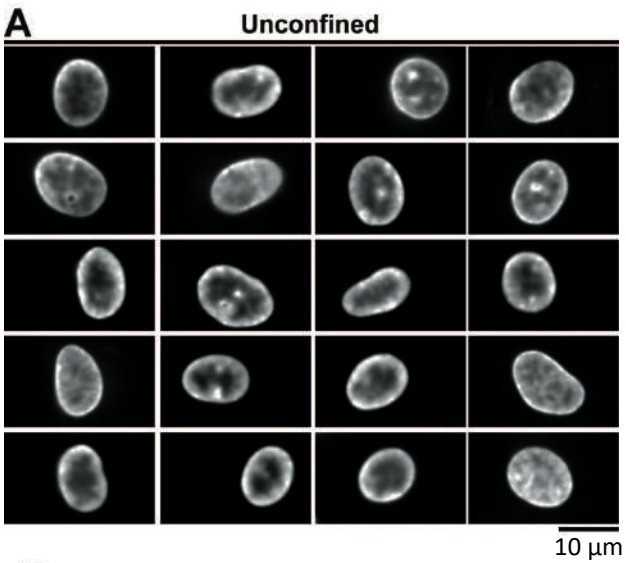


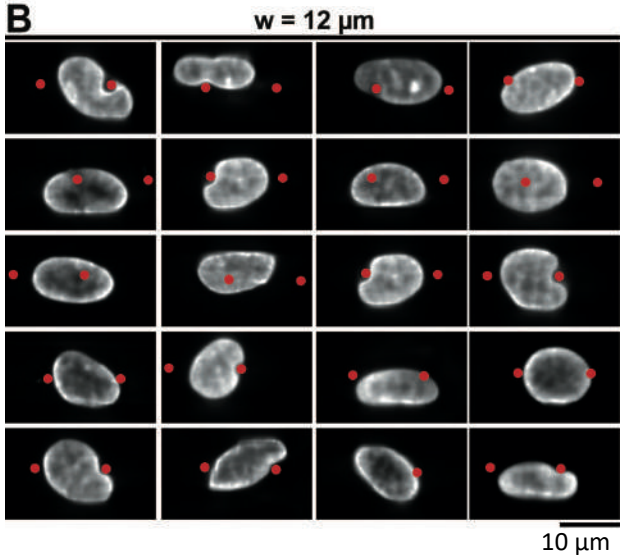


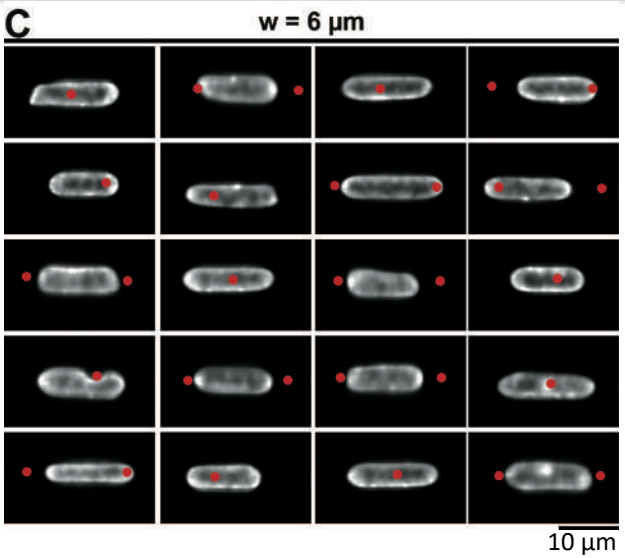


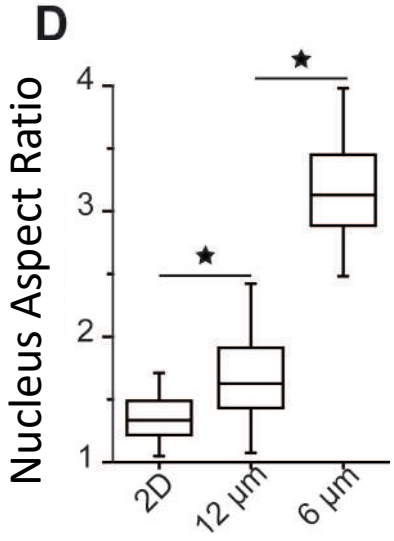


Nucleus shape under confined migration



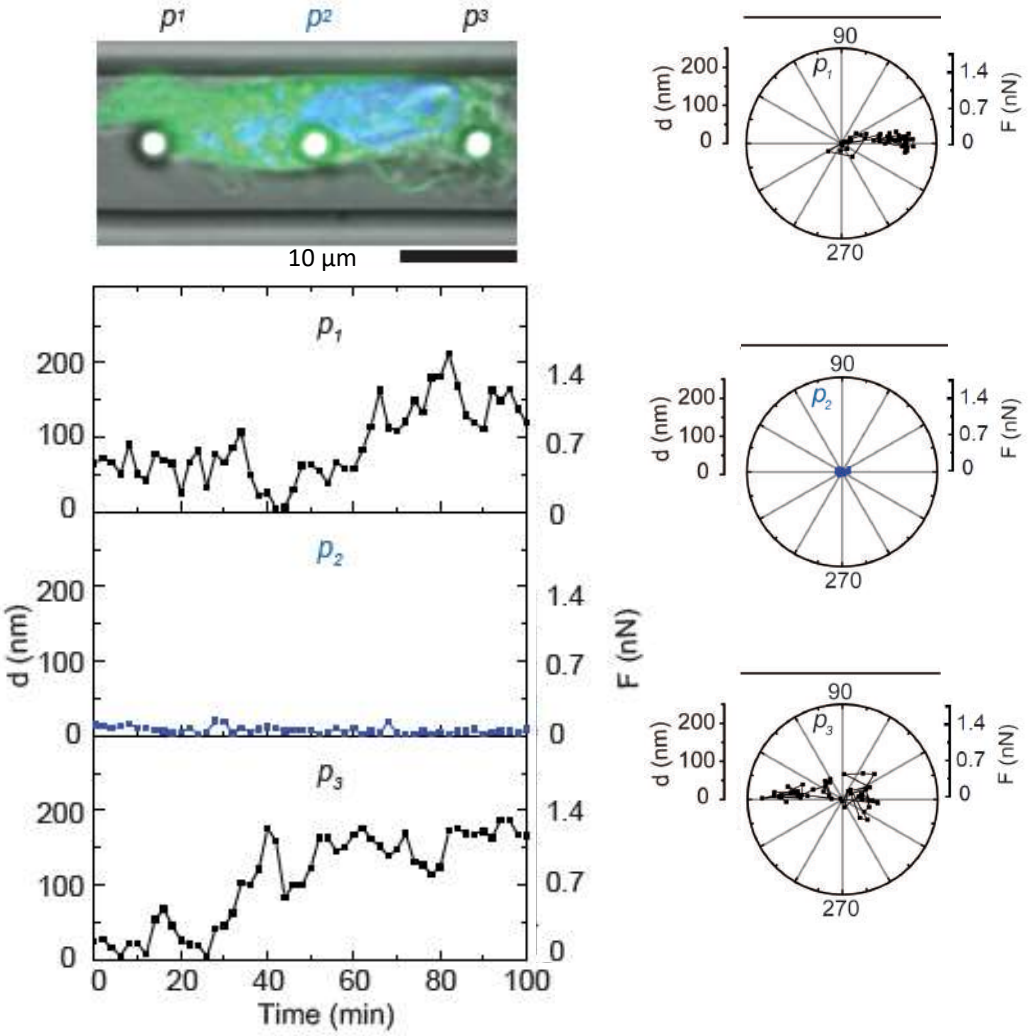


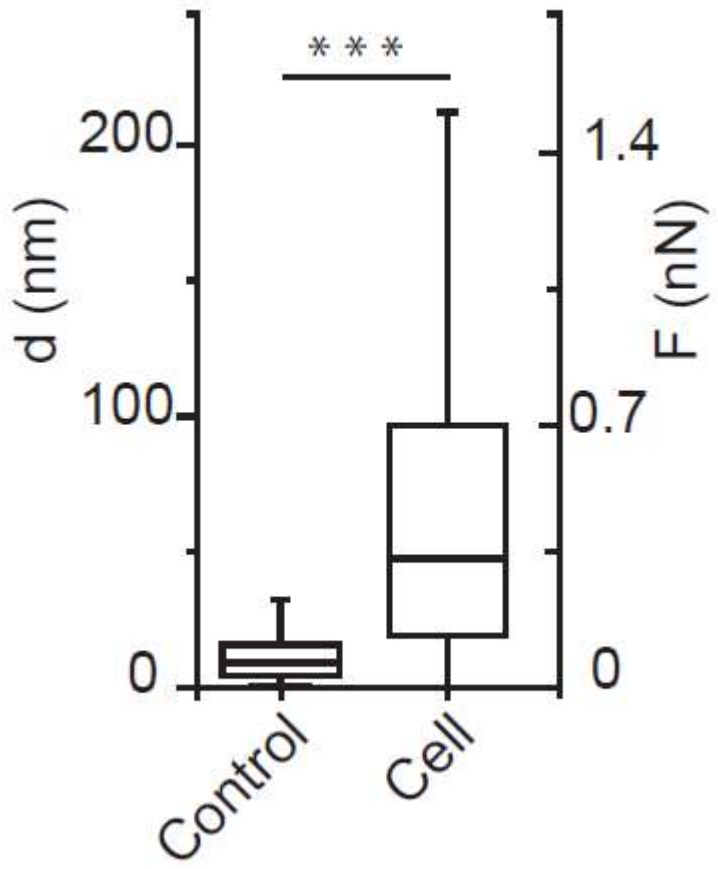




Characterization of top pillar displacement

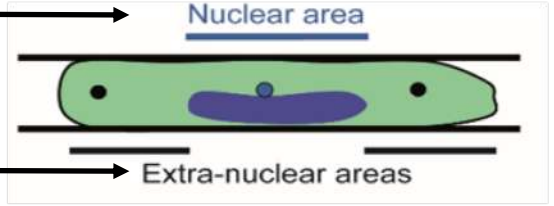
Channel width 12 µm



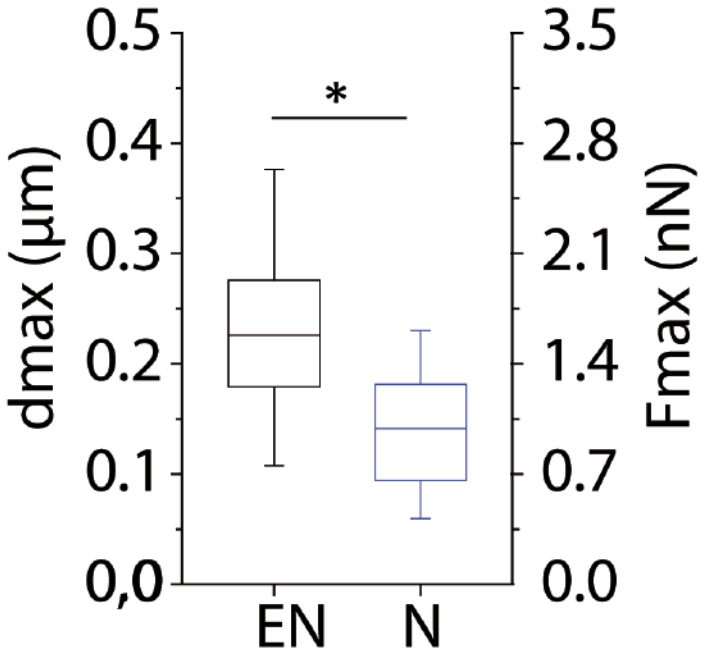


Maximal forces

Maximal Forces
In two cellular regions

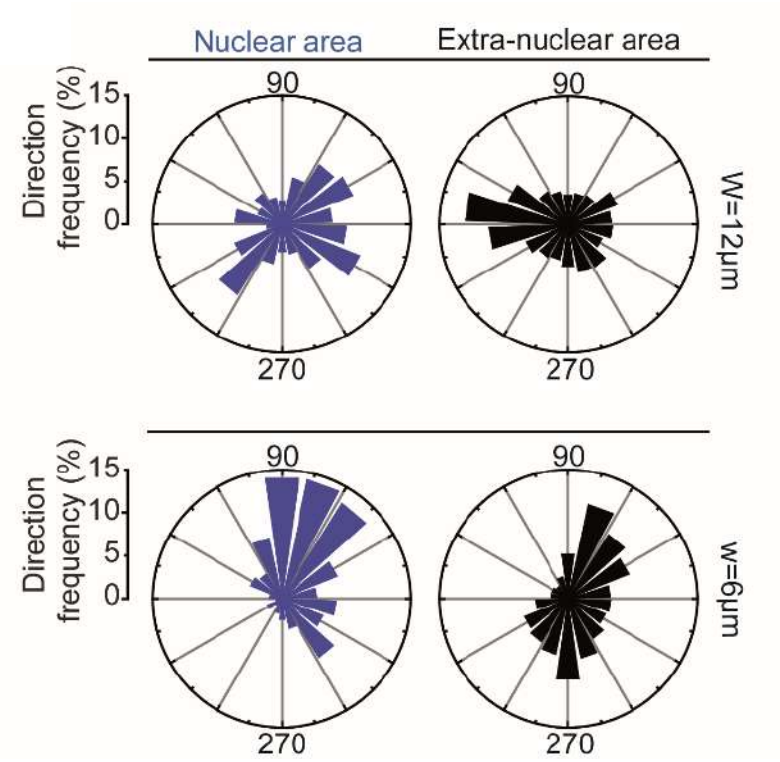


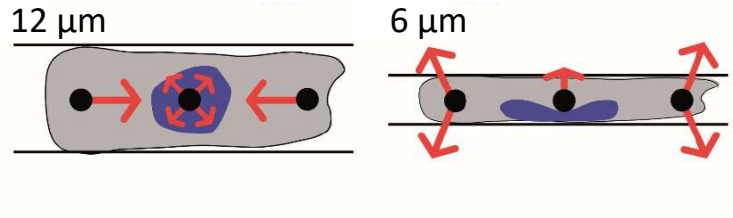
→ Confinement independent (6 or 12 µm channel width)



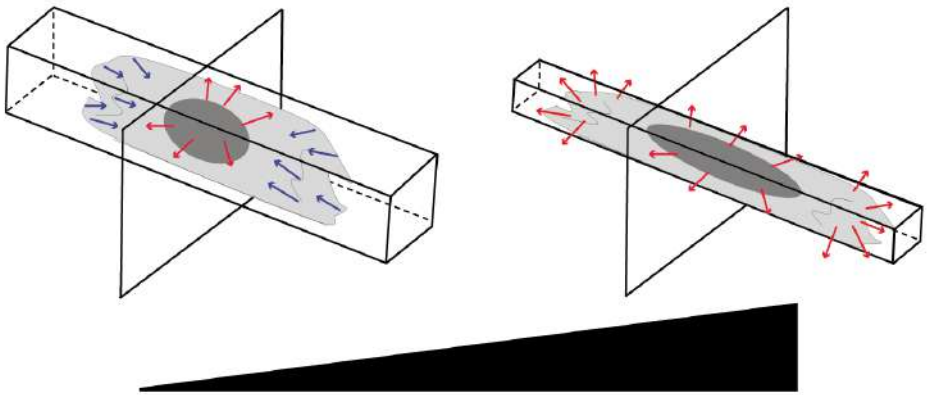
Macrophages applied maximal forces at cell edges (Front and Back).

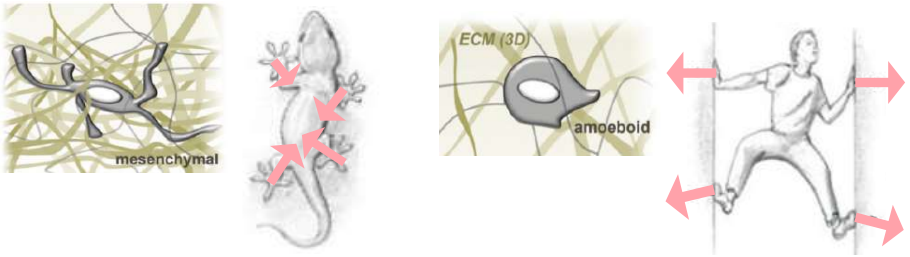
Force orientations





Desvignes et al, Nanoletter, 2018





Van Goethem et al, 2010, Journal of immunology
Heuzé et al, 2013, Immunological reviews

Microfluidic Approach : Main results and conclusions

Problematic
Investigating Migration Mechanism

→ *Primary Human Macrophages Migration*

→ *Validation of dedicated Microdevices*

→ *Dynamical Cell force measurements*

→ *Spatial confinement*

→ *Optical Imaging*

→ *3D*

Micro channel equipped with micro pillar force sensors

→ **Validation of a dedicated Micro-device for the investigation of human macrophage migration under Spatial confinement enabling dynamical cell force measurements with a limit of detection of 64 pN**

→ **Local forces are higher at cell edges than around the nucleus**

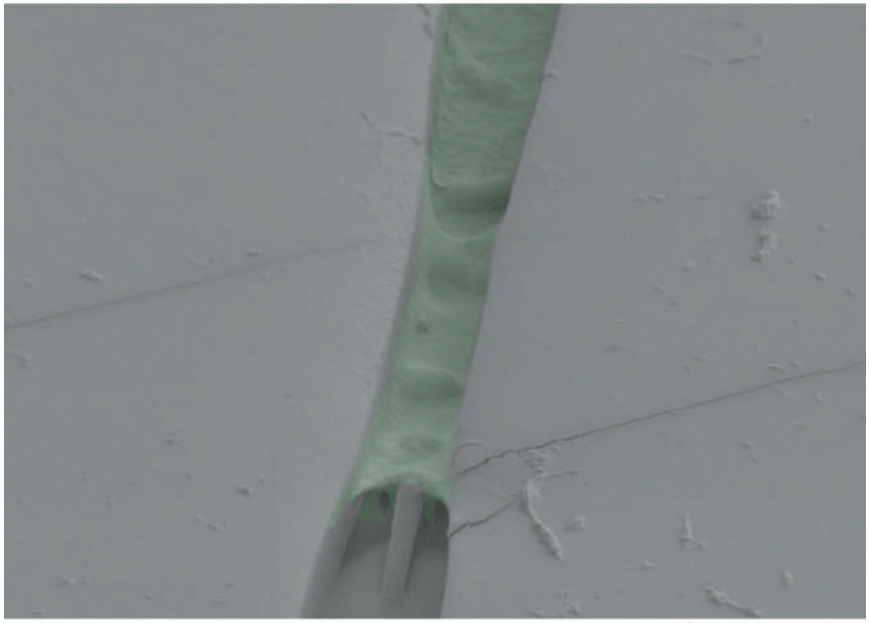
→ **Local migration forces are in the order of 1 nN**

→ **Under strong confinement the nucleus is deformed**

→ **Under strong confinement cell redirects forces from inwards to outwards**

Open Issue

→ **Cell forces exerted against micro-channel edges ?**

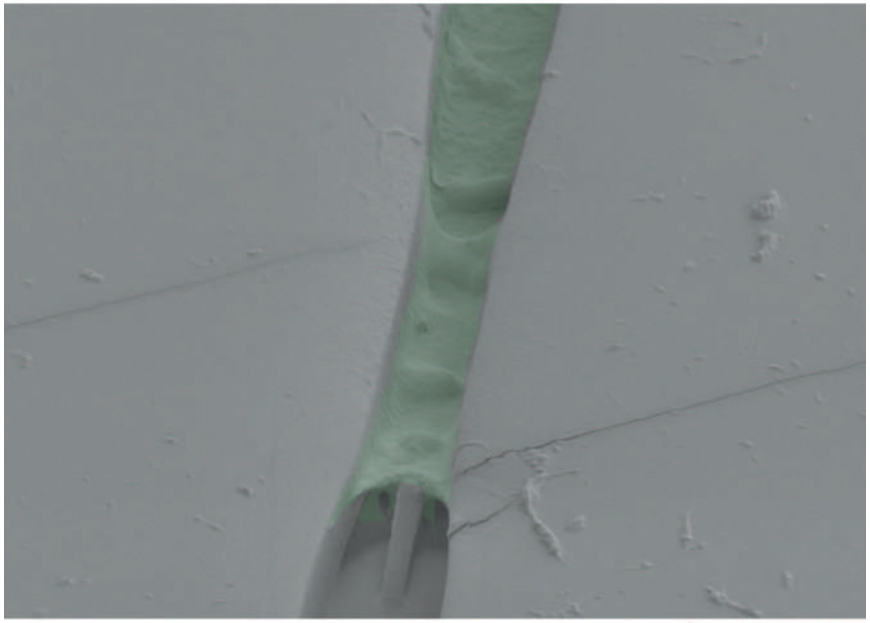


A new approach enabling global mechanica
stress measurement
under spatial confinement and

3D Landscape

Open Issue

→ **Cell forces exerted against micro-channel edges ?**



A new approach enabling global mechanical stress measurement
under spatial confinement and

3D Landscape

3D scaffold

Scaffold fabrication
Scaffold characterization
Macrophage migration inside the scaffold
Mechanical properties of the scaffold
Stress and Force evaluation

Outline

Problematic

Investigating Migration Mechanism

Primary cells : Macrophages

Migration modes : From amoeboid to Mesenchymal

Mechanical forces involved

Micro Devices for cell migration investigation

Review of the State-of-the-art

Micro channel equipped with micro pillar force sensors

Device fabrication

Device characterization

Macrophage migration inside the device

Force amplitude

Force orientation

3D scaffold

Scaffold fabrication

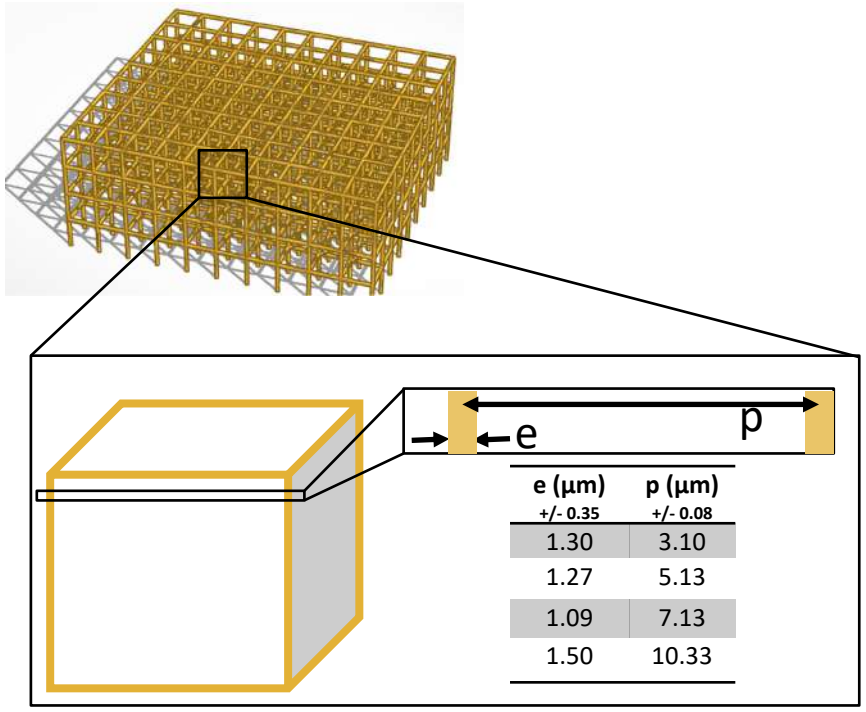
Scaffold characterization

Macrophage migration inside the scaffold

Mechanical properties of the scaffold

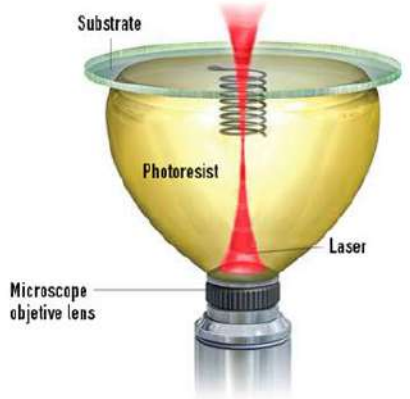
Stress and Force evaluation

Scaffold Fabrication



e (µm) +/- 0.35	p (µm) +/- 0.08
1.30	3.10
1.27	5.13
1.09	7.13
1.50	10.33

1. 2-Photons optical lithography in photosensitive resist (IP-Dip)



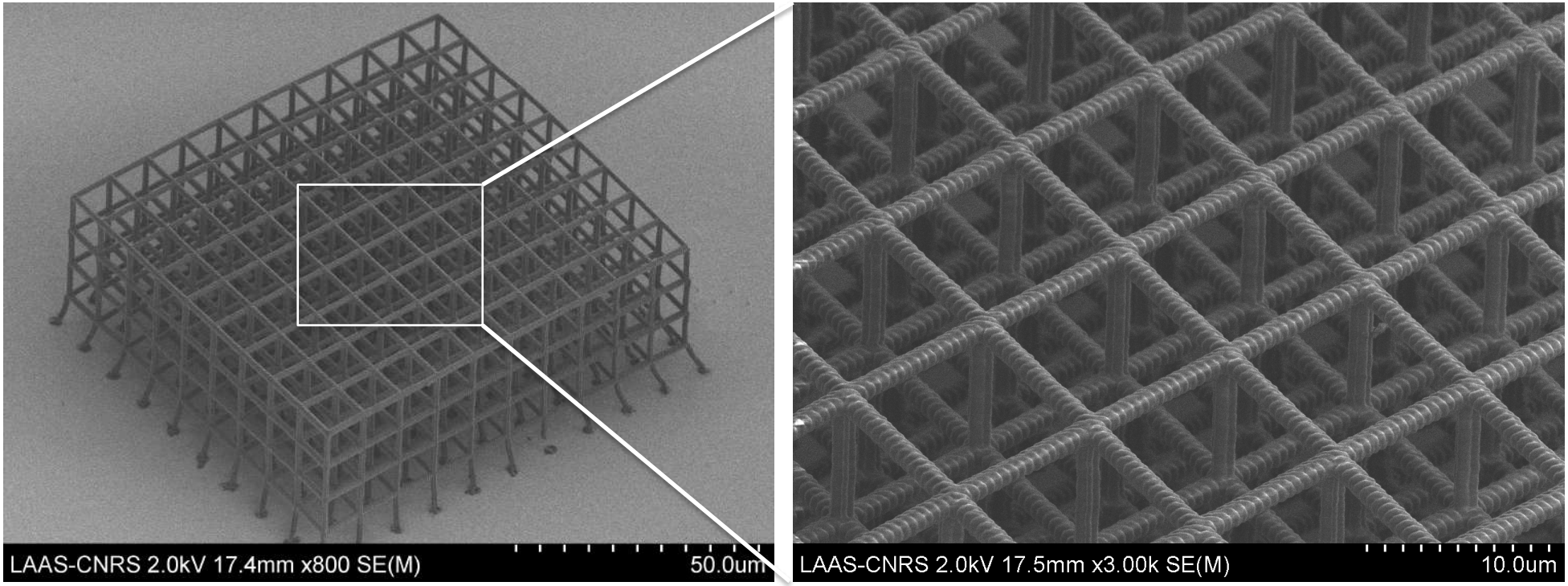
Nanoscribe

- Two-photon absorption DWL
- IP-Dip resist
- Scan speed 10 mm/s
- Laser power 25 mW

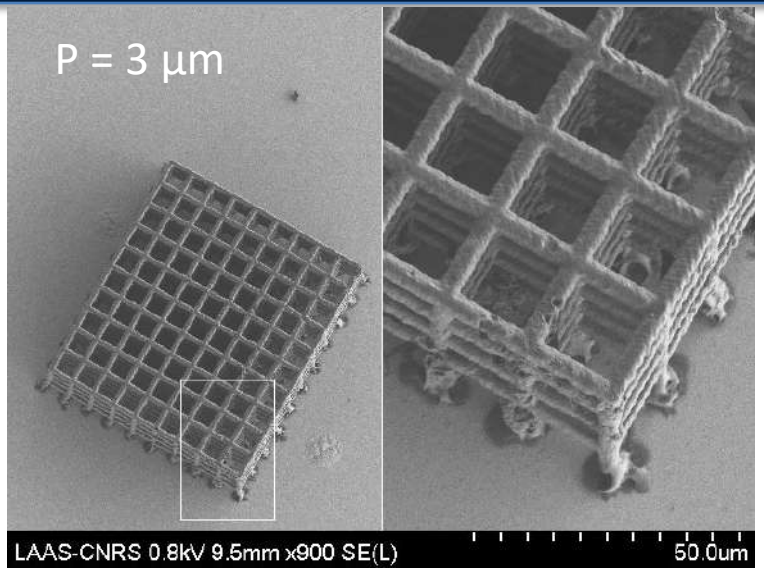
2. Development

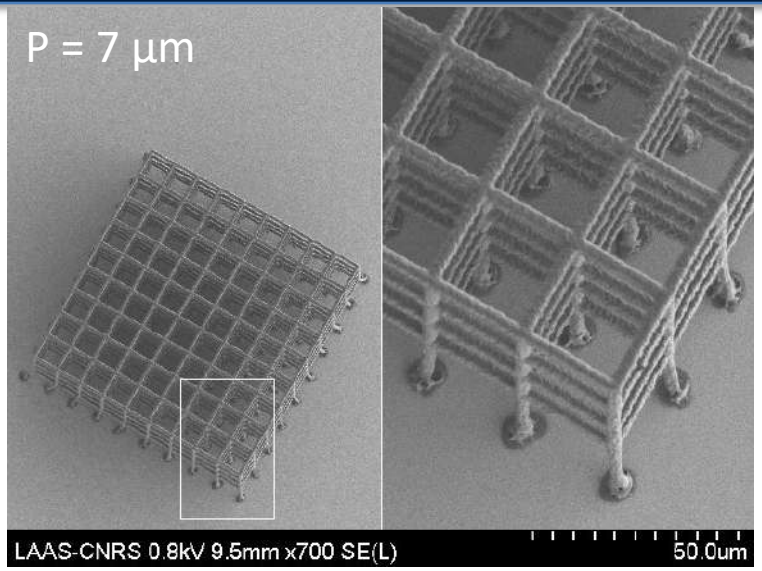
3. Critical point drying for limiting collapse

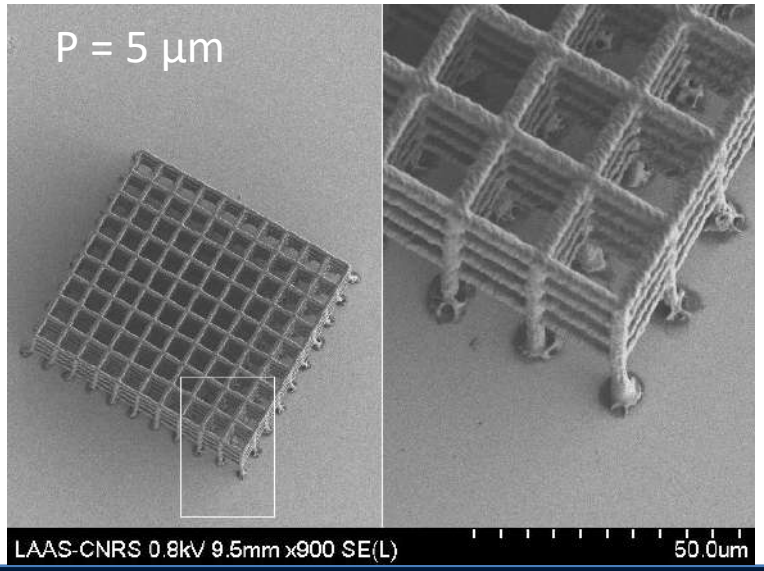
Scaffold Characterization

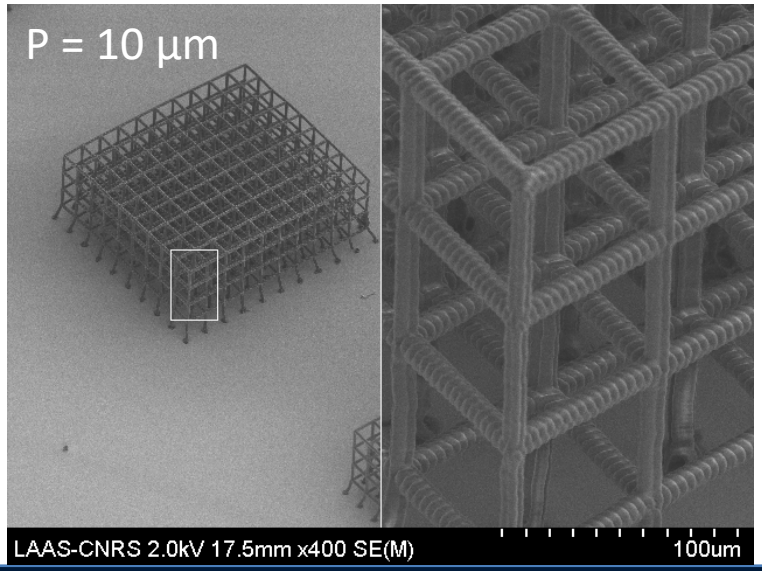


Scaffold Characterization

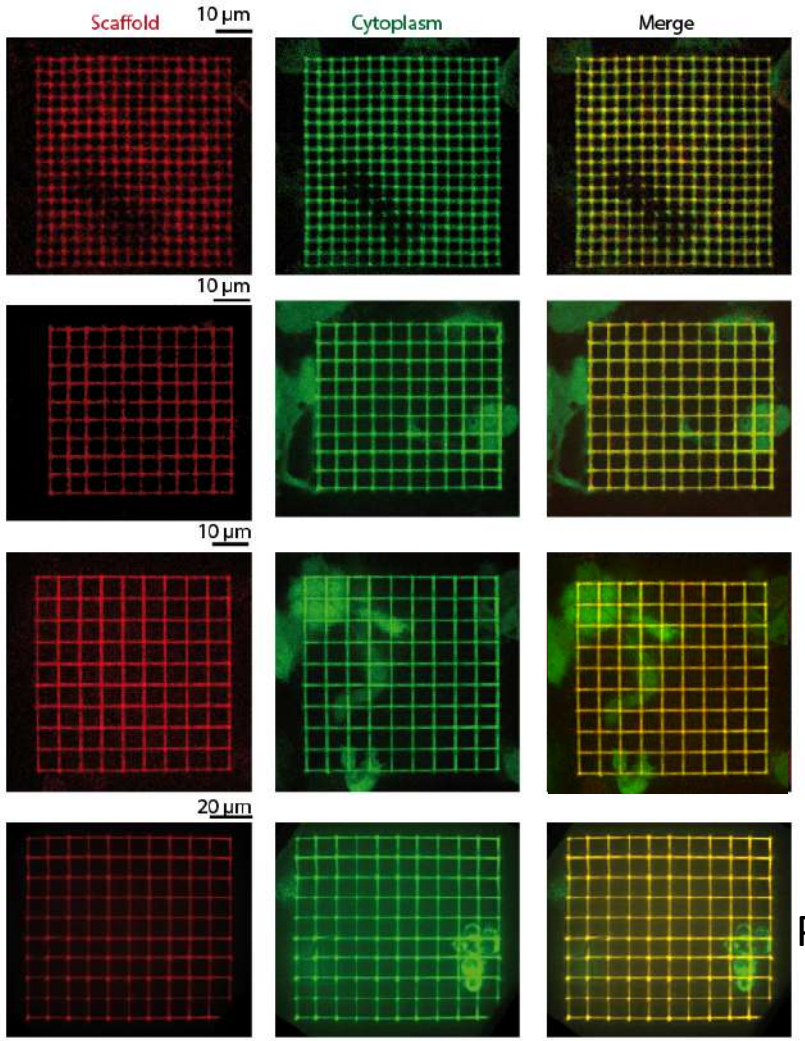




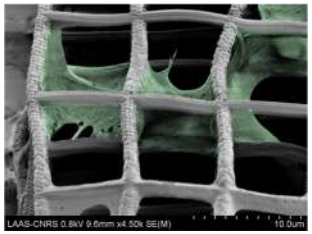


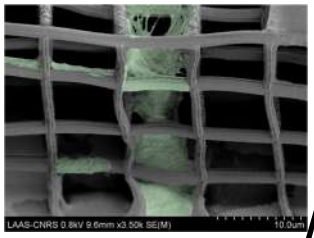


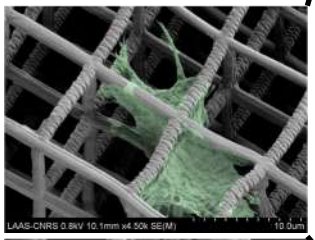
Macrophage Invasion

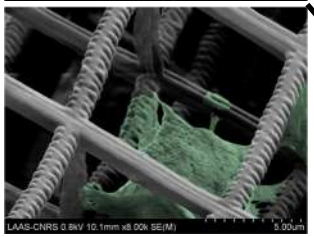


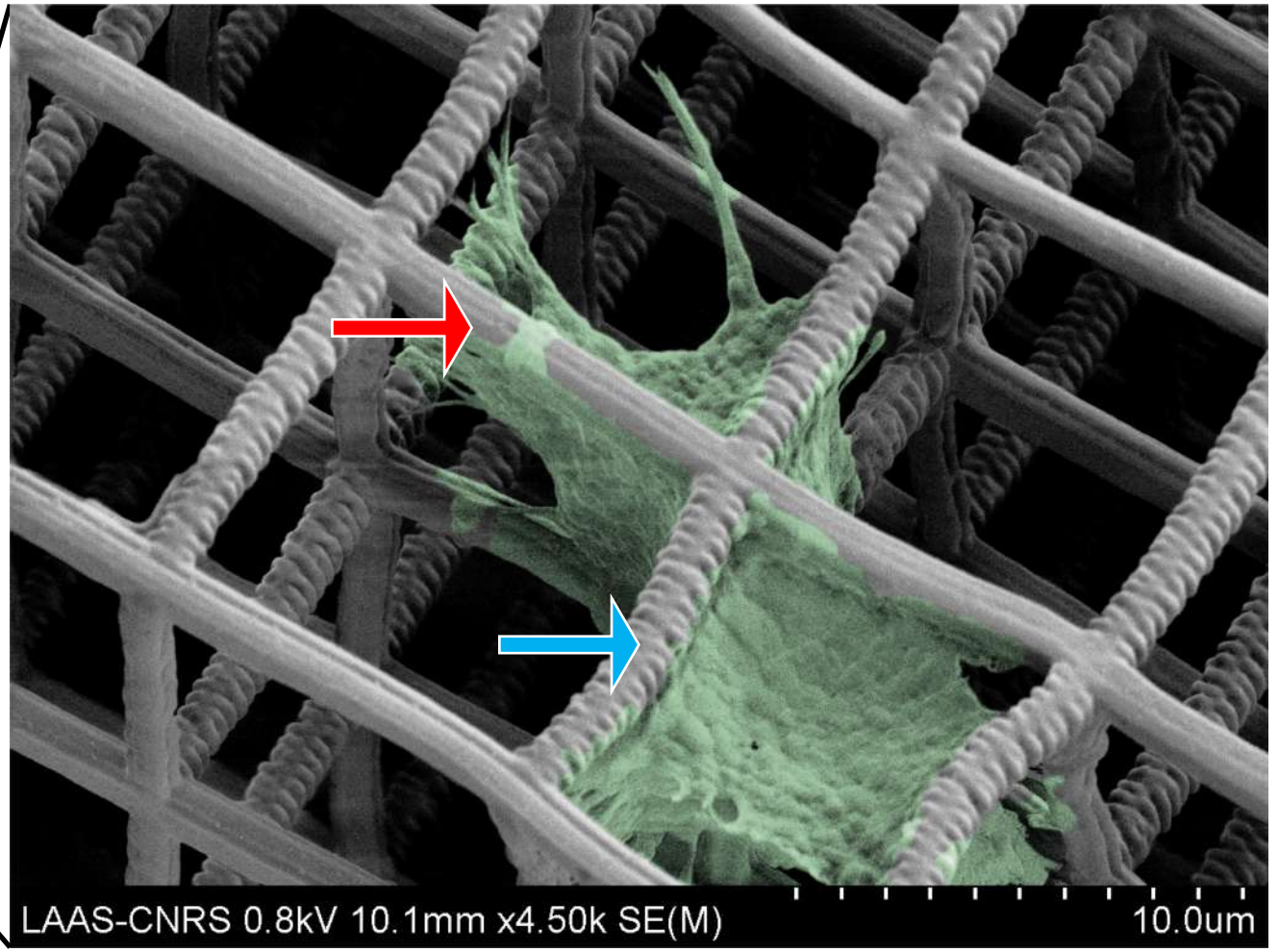
Macrophage/Device contacts



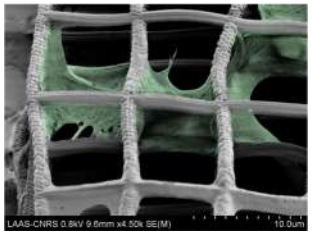


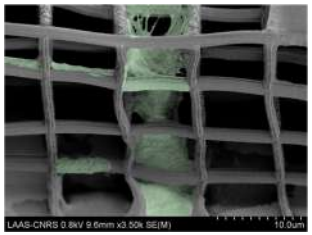


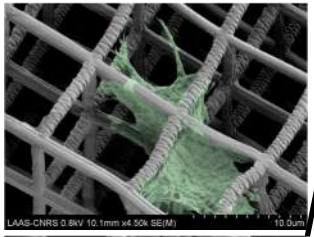


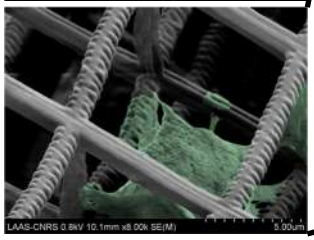


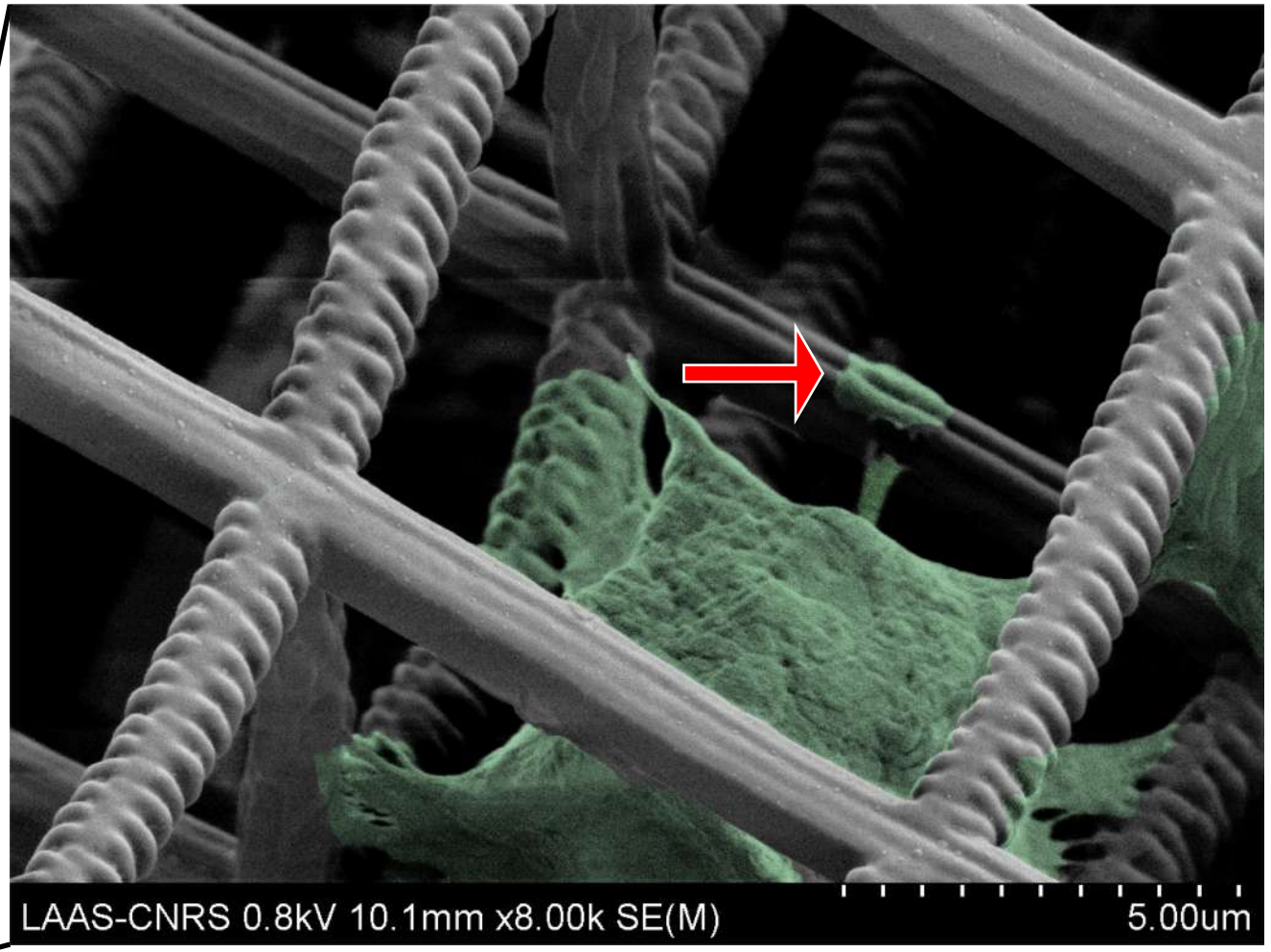
Macrophage/Device contacts



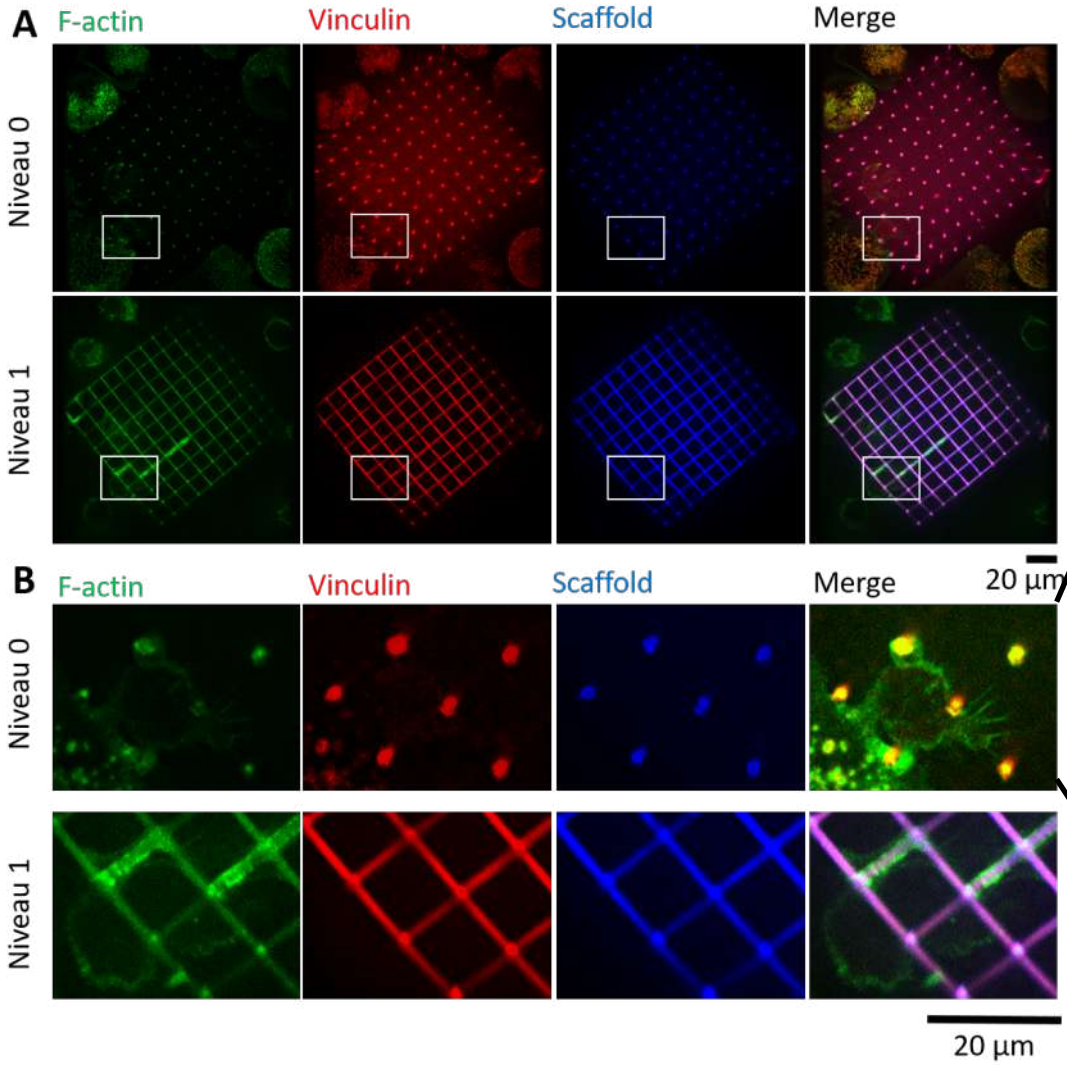




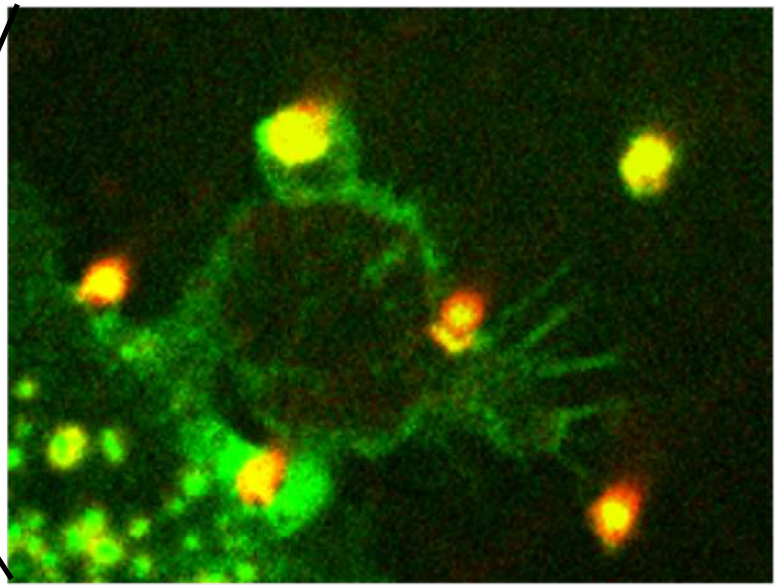




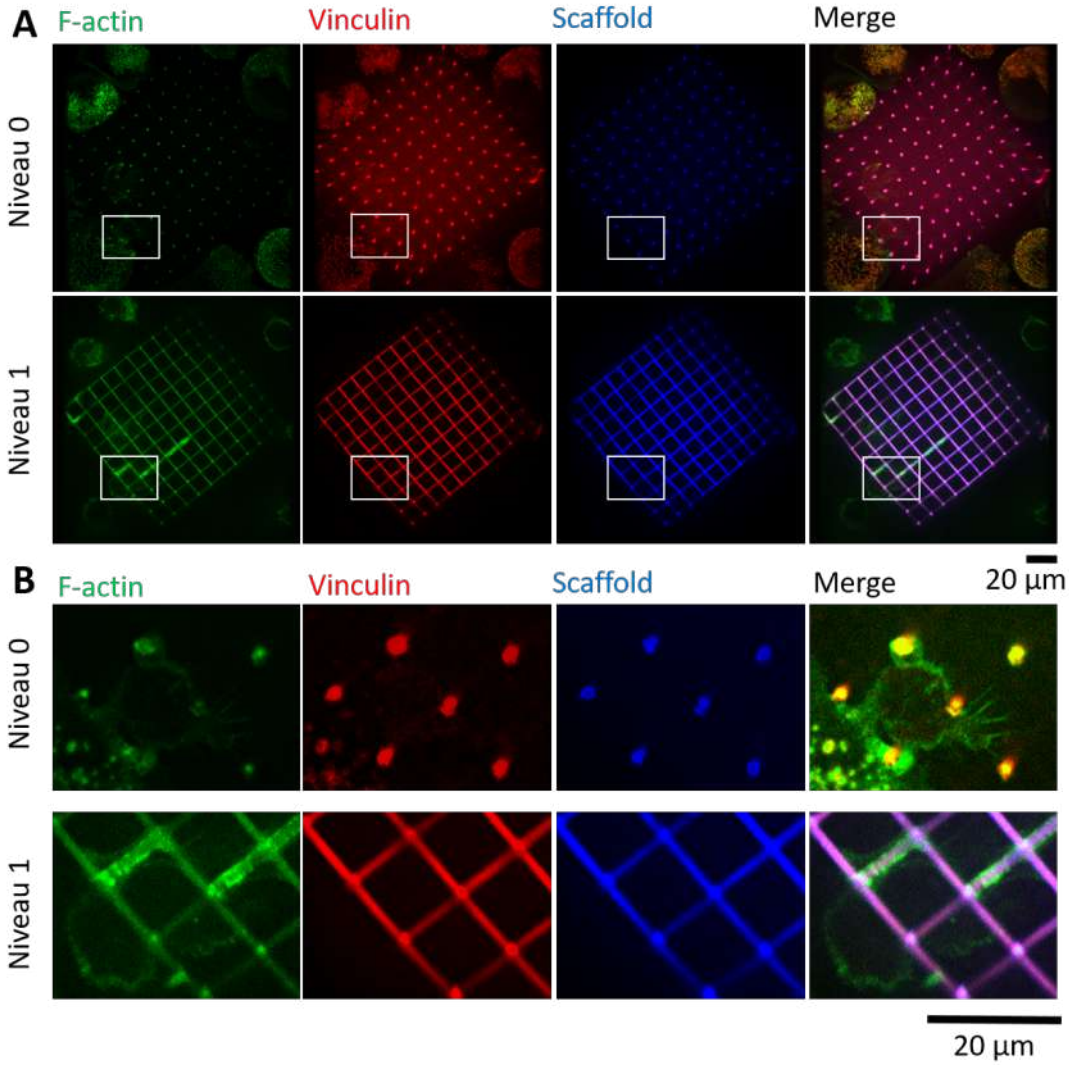
Macrophage/Device contacts



→ Cytoskeleton organisation is changed from 2D situation on glass (podosomes) to 3D resist scaffold

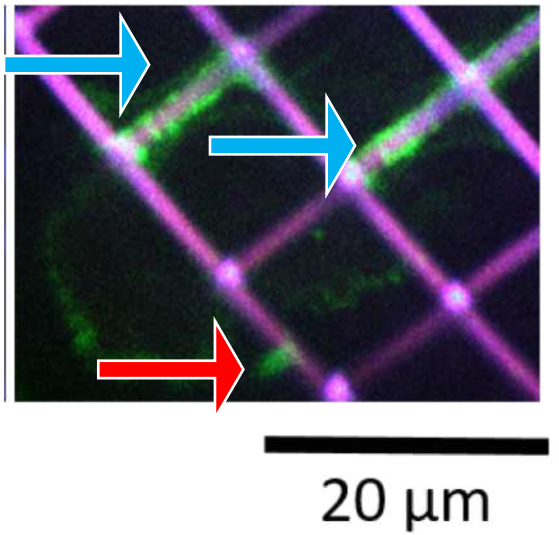


Macrophage/Device contacts

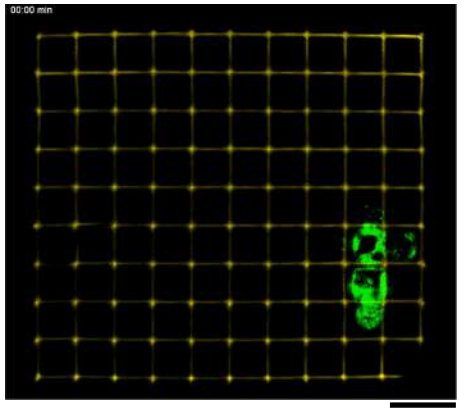


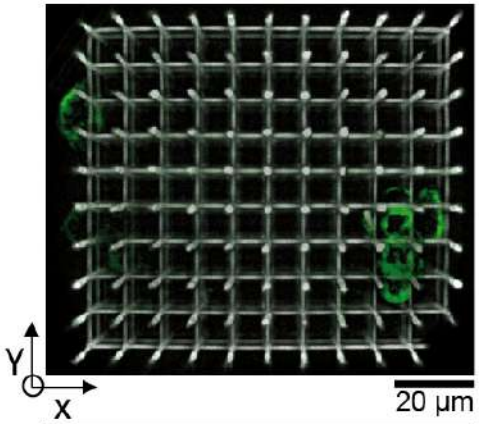
→ Filamentous actin all along a supporting beam: “Long contact”

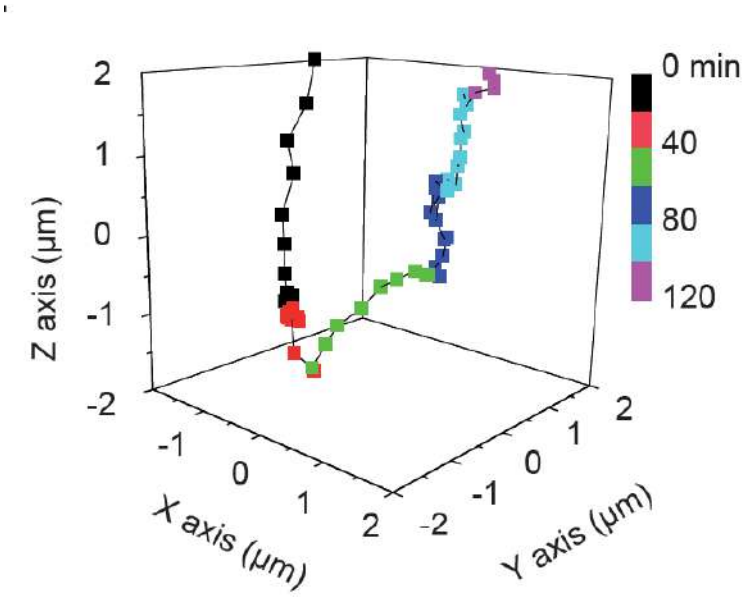
→ Filamentous actin around part of a beam : “wrapping contact”



Macrophage 3D path

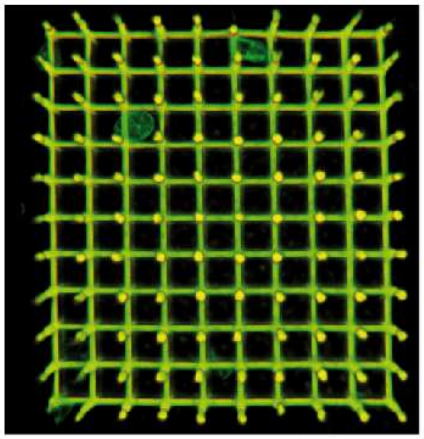


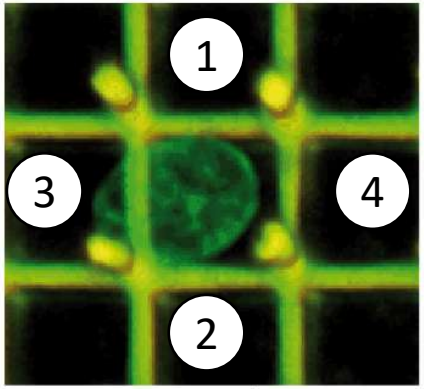


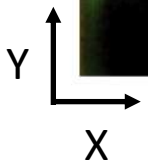


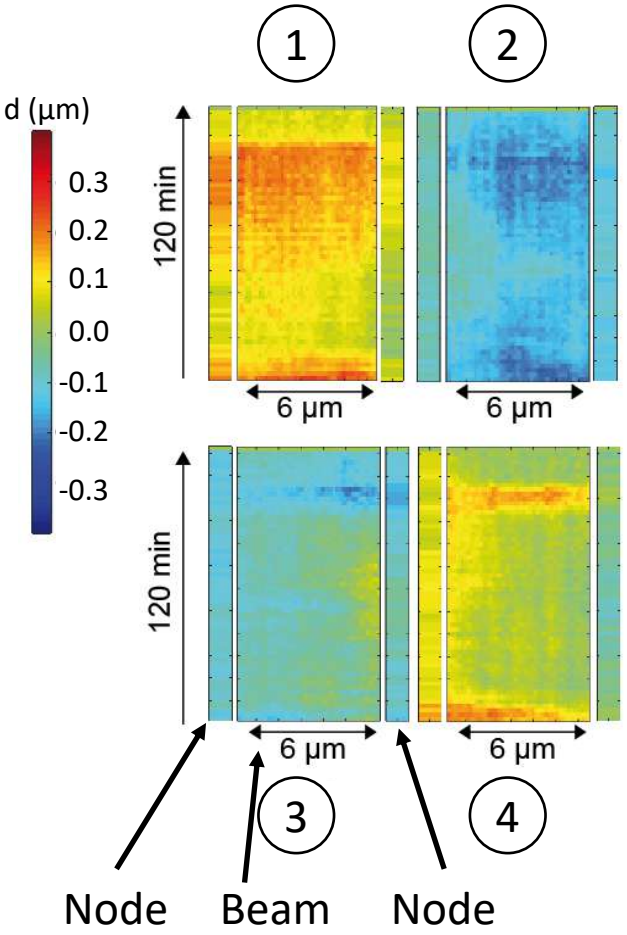
V = 7.2 µm/h

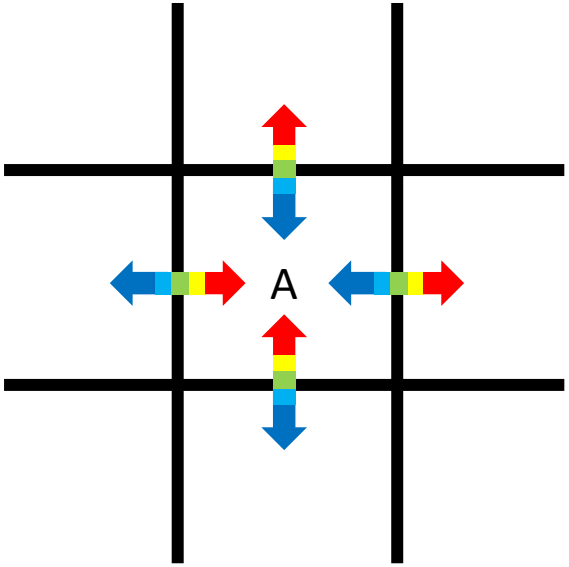
Beam displacement (in-plane)



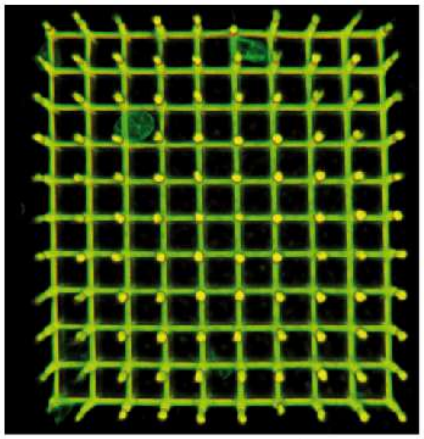


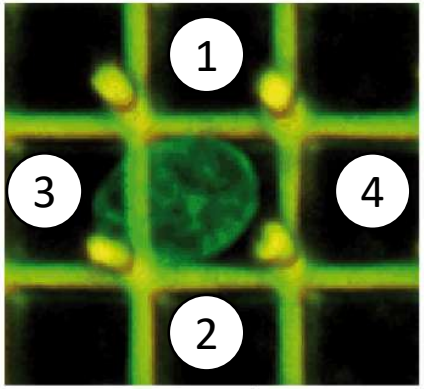


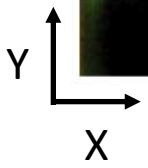


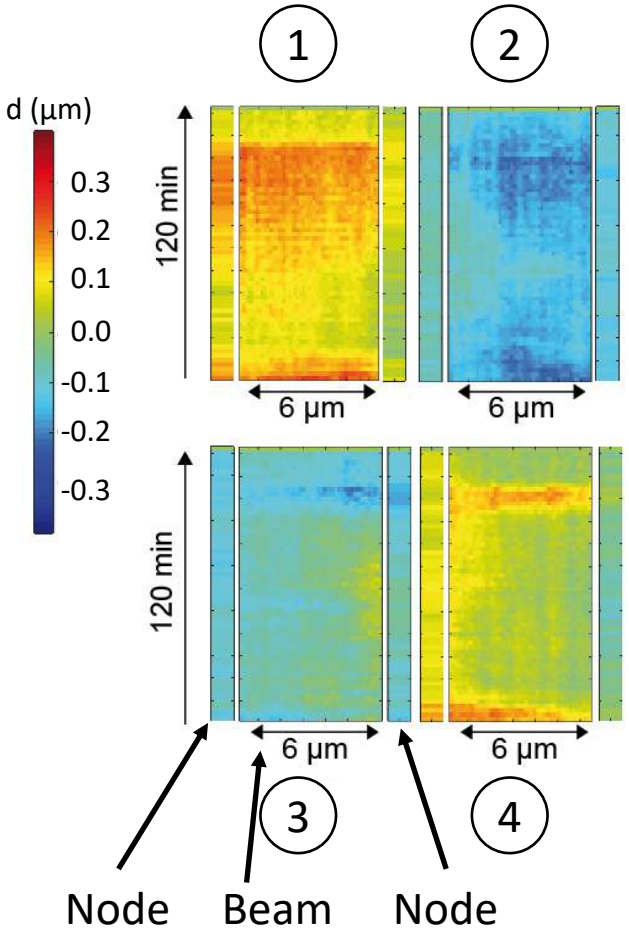


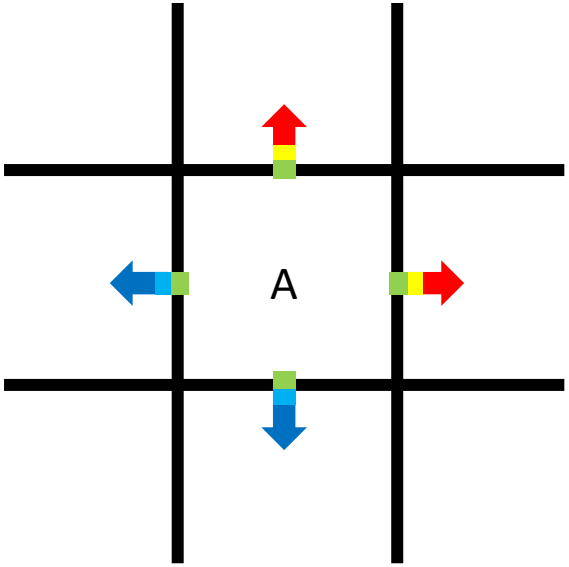
Beam displacement (in-plane)



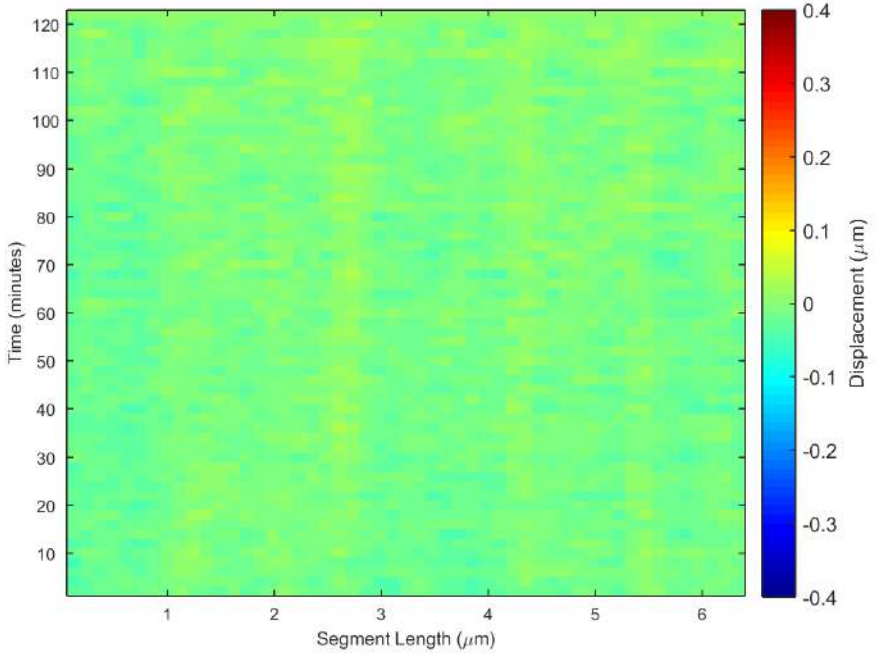


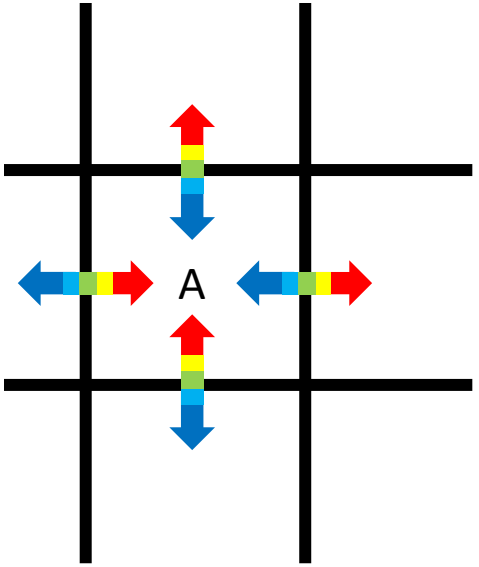




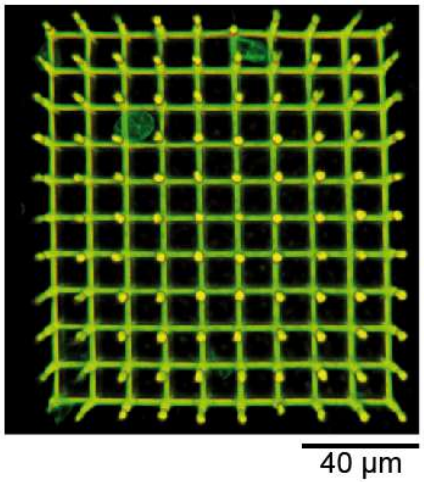


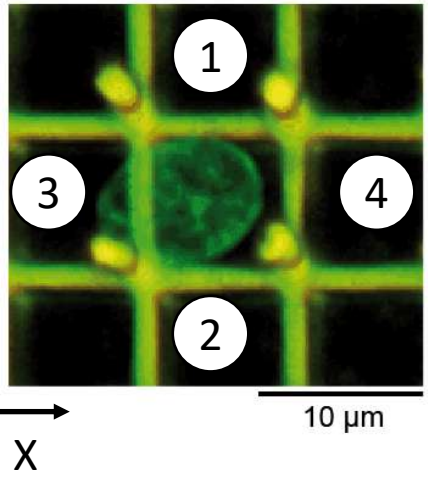
Beam displacement (in-plane) without cell

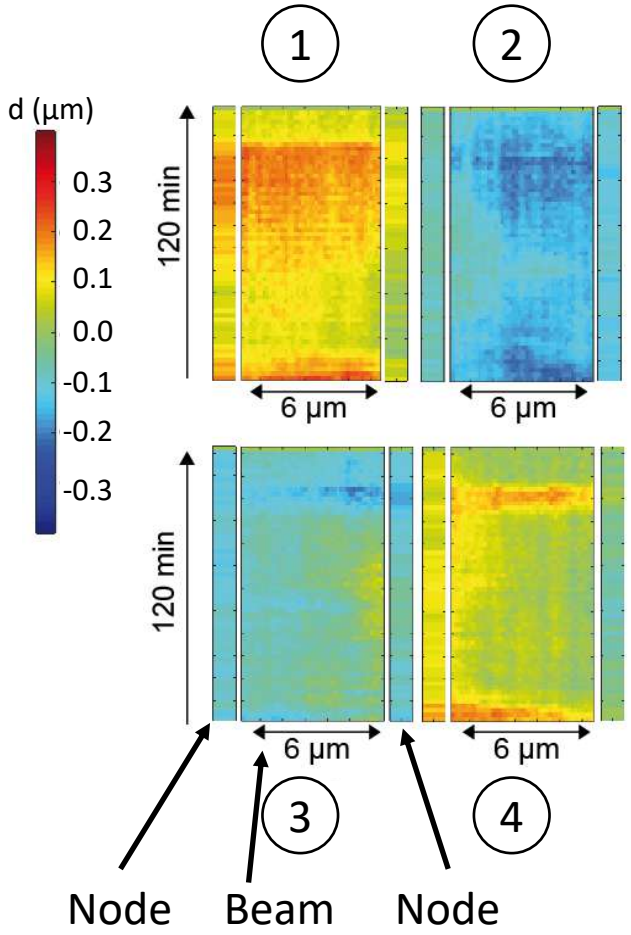


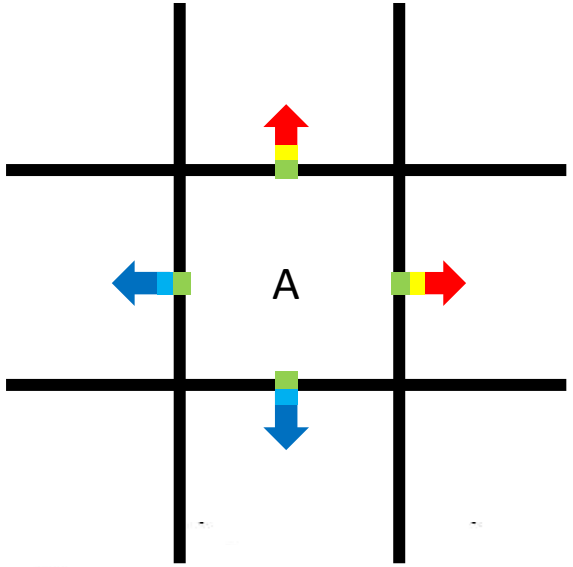


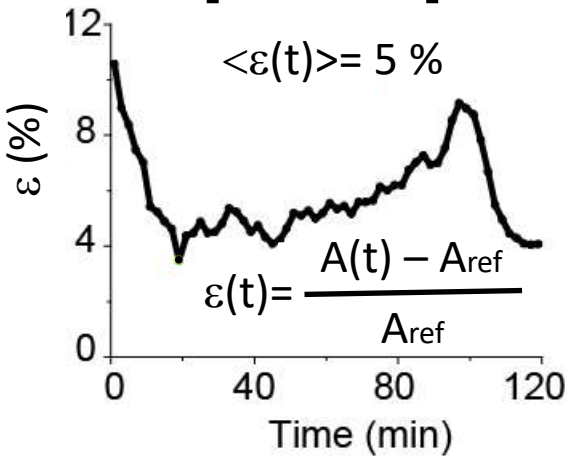
Beam displacement (in-plane)











Mechanical properties of the scaffold : E*, ν*.

Material properties

IP-DIP Resist

$\rho = 1280$ kg/m^3

E= 880 Mpa*

$\nu = 0.45$

Jayne et al, 2018, Advanced materials technologies.

Mesh deformation

Finite Element Simulation

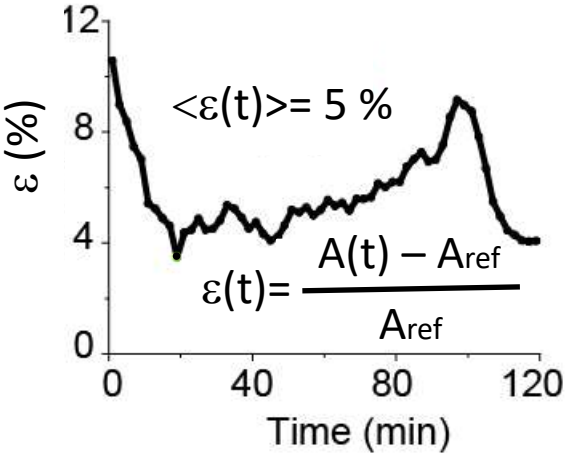
E*= 12 MPa

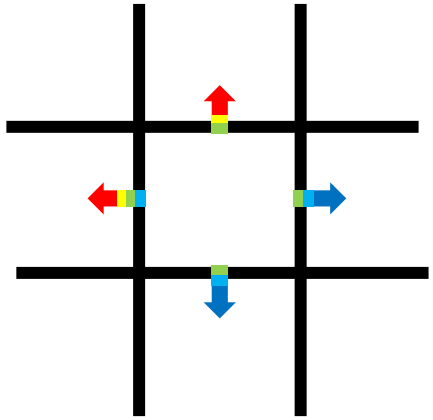
ν* = 0.37

Description of the scaffold as a continuous elasctic isotropic material

Mechanical Stress induced by Macrophage 3D migration

Strain variation over time



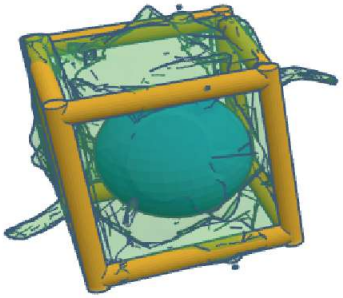


Elastic theory of an isotropic material

(i) Isotropic Stress :

$$\sigma = \frac{E^*}{3(1-2v^*)}\frac{dV}{V}$$

σ = 600 kPa



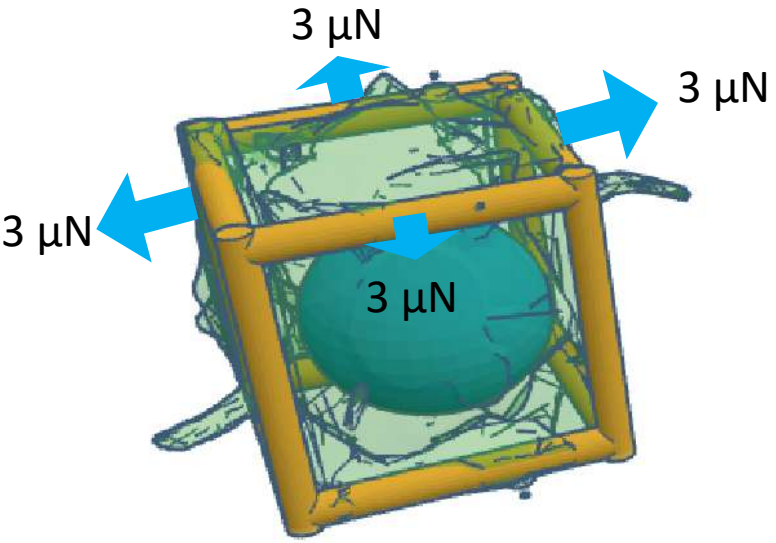
(ii) Biaxial stress :

$$\sigma = \frac{E^*}{2(1-v^*)}\frac{dS}{S}$$

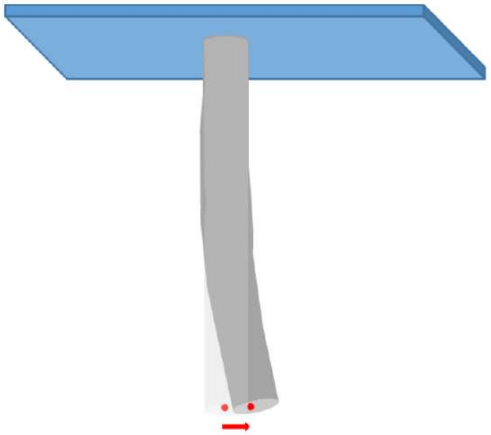
σ = 500 kPa

General Conclusion on the two approaches

σ = 500 kPa → 3 µN applied on the beams



F = 0.3 nN on the pillars



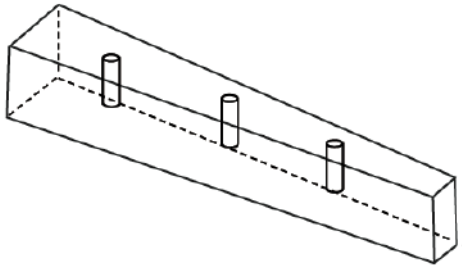
Global migration force in 3D

Local migration force in a confining channel

Spatial confinement induces a
« pushing » pattern of forces

Perspective : Create Gradients !

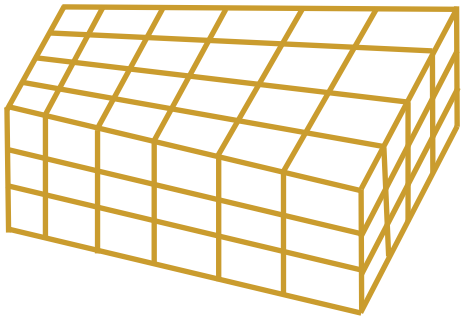
Micro channel equipped with micro pillars force sensor



Channel width variations

- **GRADIENT OF SPATIAL CONFINEMENT**

3D Micro scaffold



Scaffold period or exposure parameters variations

- **GRADIENT OF SPATIAL CONFINEMENT**
- **GRADIENT OF RIGIDITY**

Acknowledgements : Emma Desvignes

→ L'équipe ELiA
Julie Foncy
Aurore Esteve
Justine Creff
Kayum Jimenez
Hélène Cayron
Maxime Sahun
Emmanuelle Trevisiol
Etienne Dague
Laurent Malaquin
Aline Cerf
Mariel Cano Jorge
Lin Yang

→ Moran Mirabal Group
Jose Moran-Mirabal
Mouhanad Babi

T

→ L'équipe MDP
Marion Portes
Natasha Escallier
Solène Accarias
Myriam Ben Neji
Karine Pingris
& toute l'équipe

→ Plateforme CBI
Thomas Mangeat
Amsha Proag

→ TEAM
Adrian Laborde
Franck Carcenac
Laurent Mazenq
Fabien Mesnilgrente
Lucie Seveno

→ Plateforme Caractérisation
Charline Blatché
Sandrine Assié-Souleille

→ Ma famille
Florence Desvignes
André Desvignes
Lisa Desvignes
Martin Desvignes
Françoise Gravil
Jean-Pierre Gravil

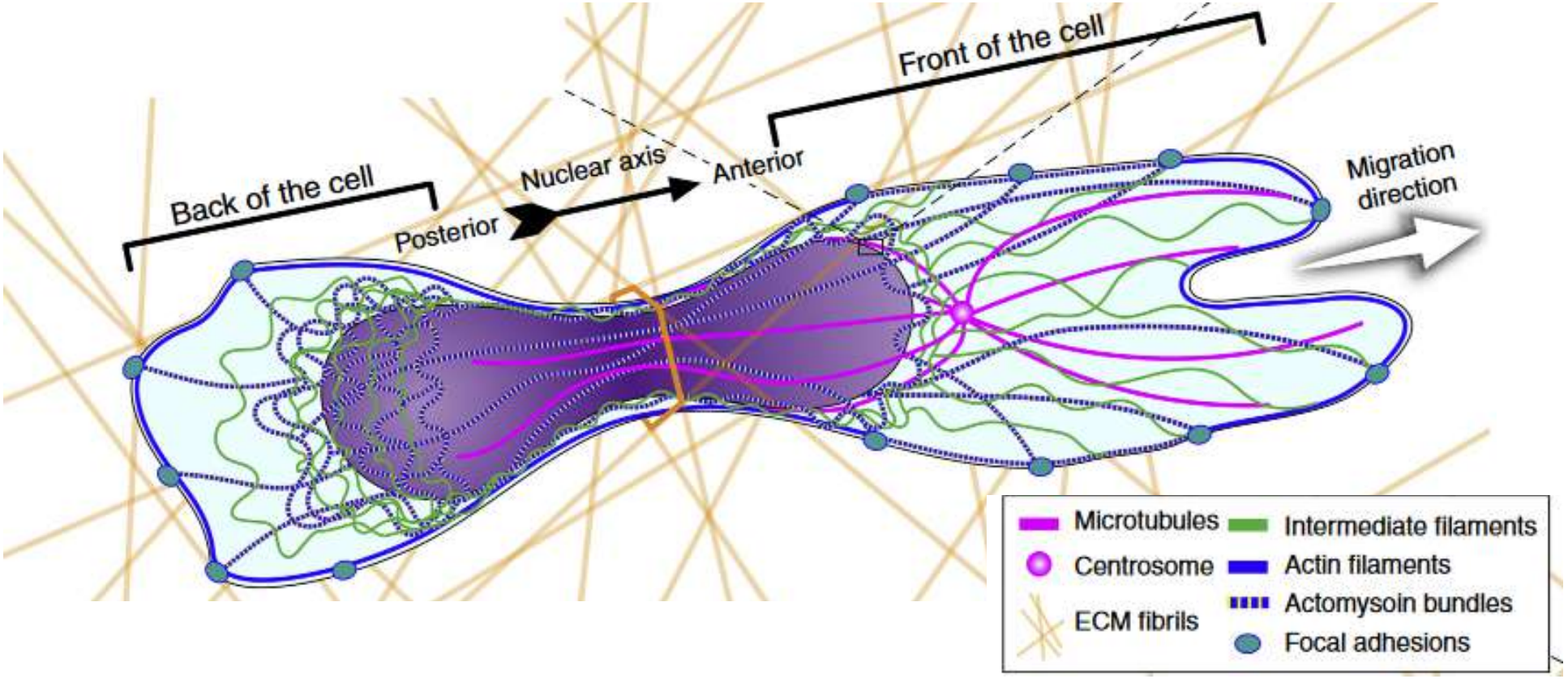
→ Mon conjoint
Sébastien Gravil

With the support of

ANR

Université Fédérale
Toulouse Midi-Pyrénées

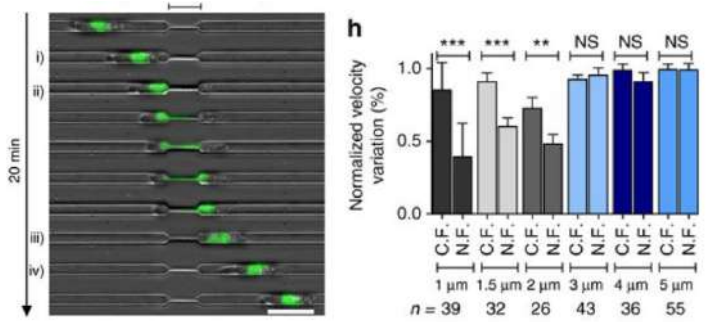
Spatial confinement



Mcgregor, AL et al, 2016, *Current Opinion cell biology*

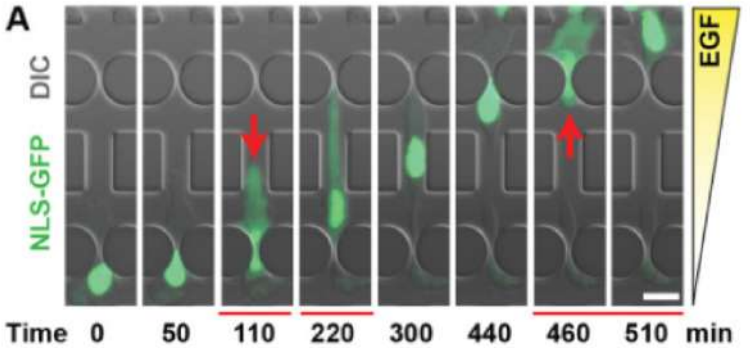
Role of the nucleus under spatial confinement

Limiting role



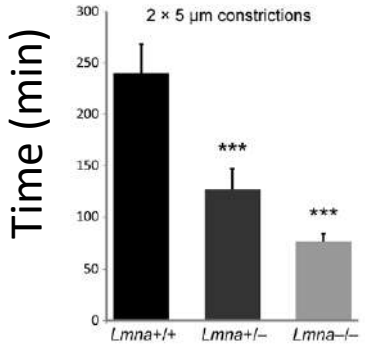
Thiam HR et al, 2016, *Nature Communications*

Nuclear rupture during confined migration



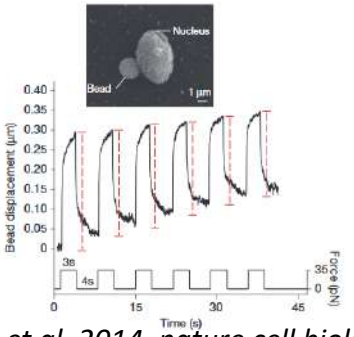
Denais, CM et al, Science, 2016

Influence of Nuclear rigidity

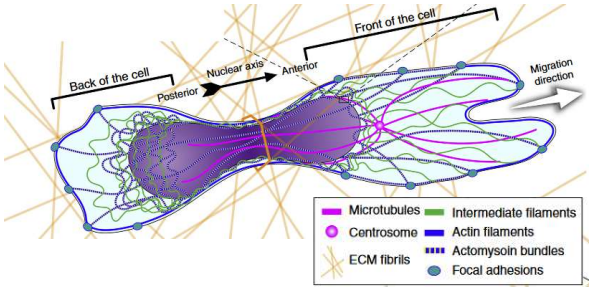


Davidson et al, 2014, *Cellular and Molecular Bioenginering.*

Nuclear mechanosensing

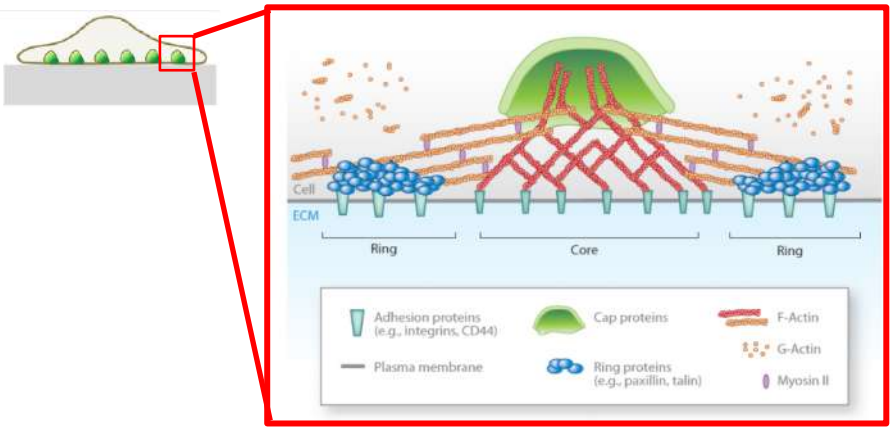


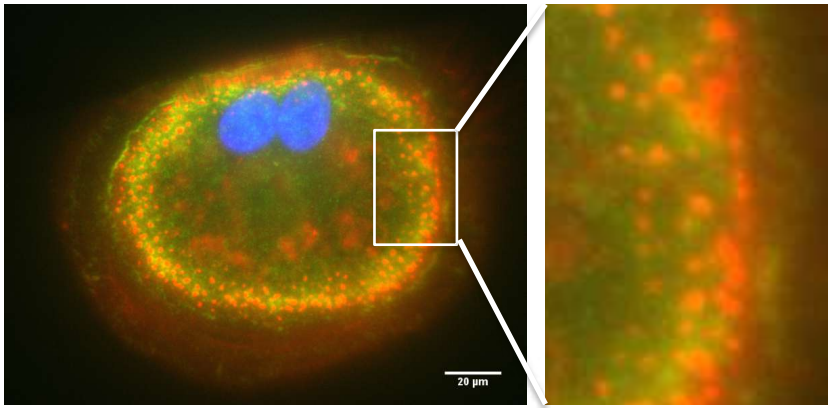
Guillut C et al, 2014, nature cell biology

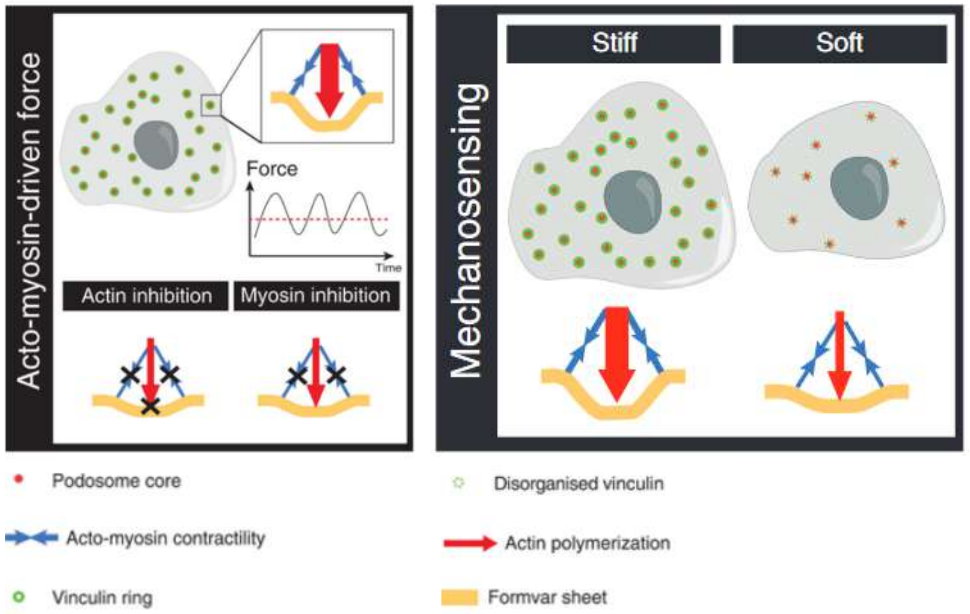


Mcgregor, AL et al, 2016, *Current Opinion cell biology*

Podosome 2D : Structure subcellular of the macrophage







→ Adhesion

→ Degradation

→ Mecano sensor

→ Involved in migration