

# Fluorescence microscopy techniques to investigate 3D cell culture and tissue regeneration

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SOCIETÀ ITALIANA DI FISICA  
Italian Physical Society



**POLITECNICO**  
MILANO 1863

# The mechanobiology lab@POLIMI



Horizon 2020  
European Union funding  
for Research & Innovation



**MT Raimondi**



**E Jacchetti**



**C Martinelli**



**C Conci**



**B Barzaghini**



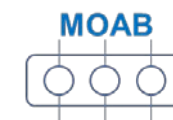
**A Nardini**



**A Bocconi**

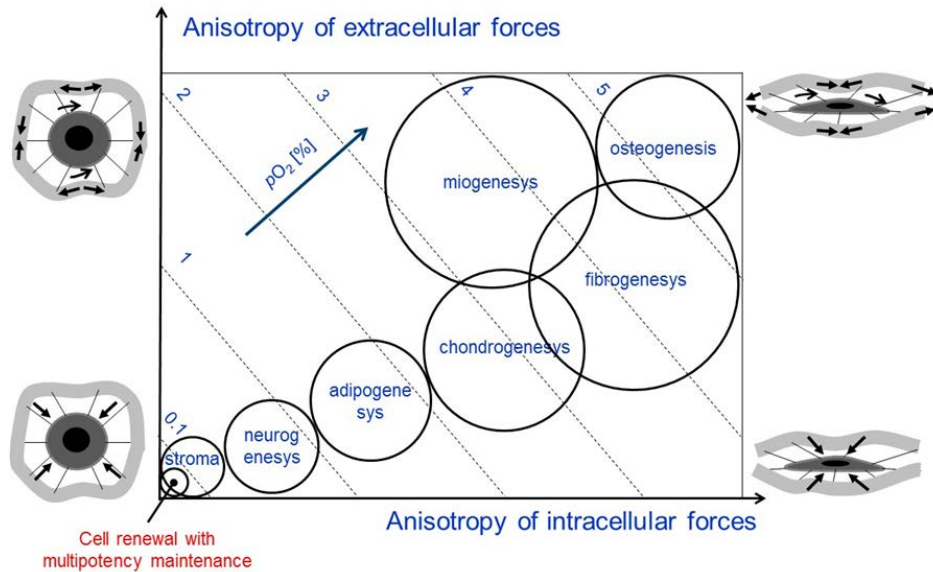
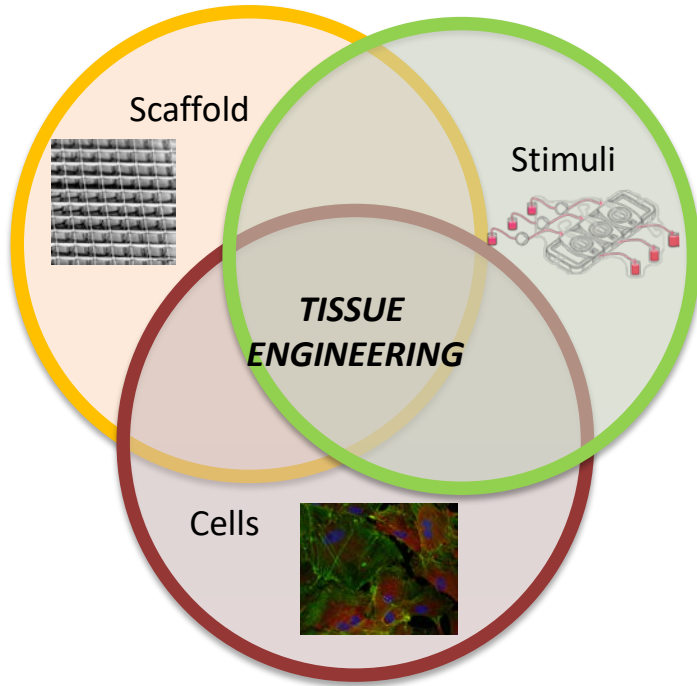


**C Testa**





# Tissue engineering



# Mesenchymal stem cells



**1. Mechanical properties** play an important role in cell fate and functionality

**2. 3D** cellular environments are more realistic models

**3.** It is extremely important to **visualize** investigated phenomena in real time

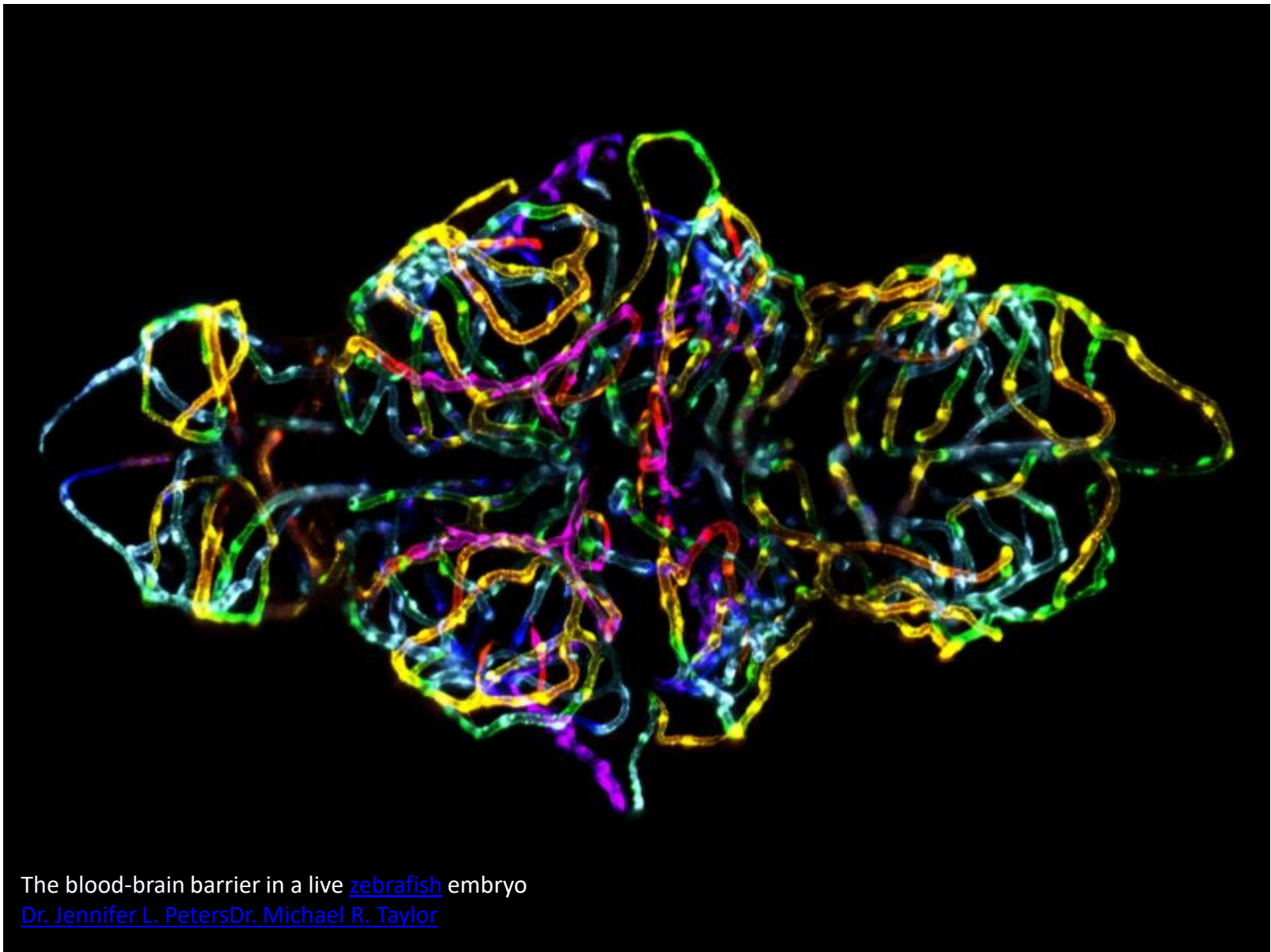
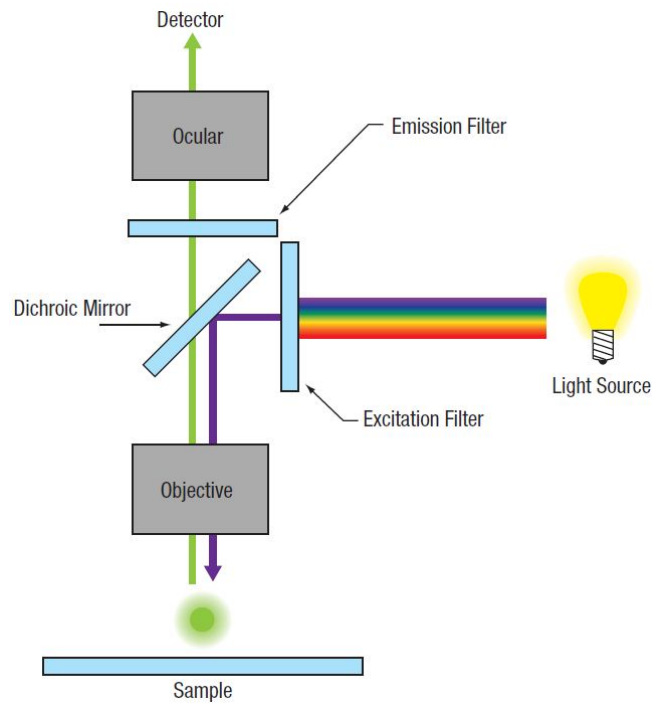
Sci Rep 2021; **11**, 3021.

Theranostics 2020; 10(16):7034-7052

Front Bioeng Biotechnol. 2020; 9:8:585363

Biophys Rev. 2019;11(5):817-831.

# Fluorescence microscopy



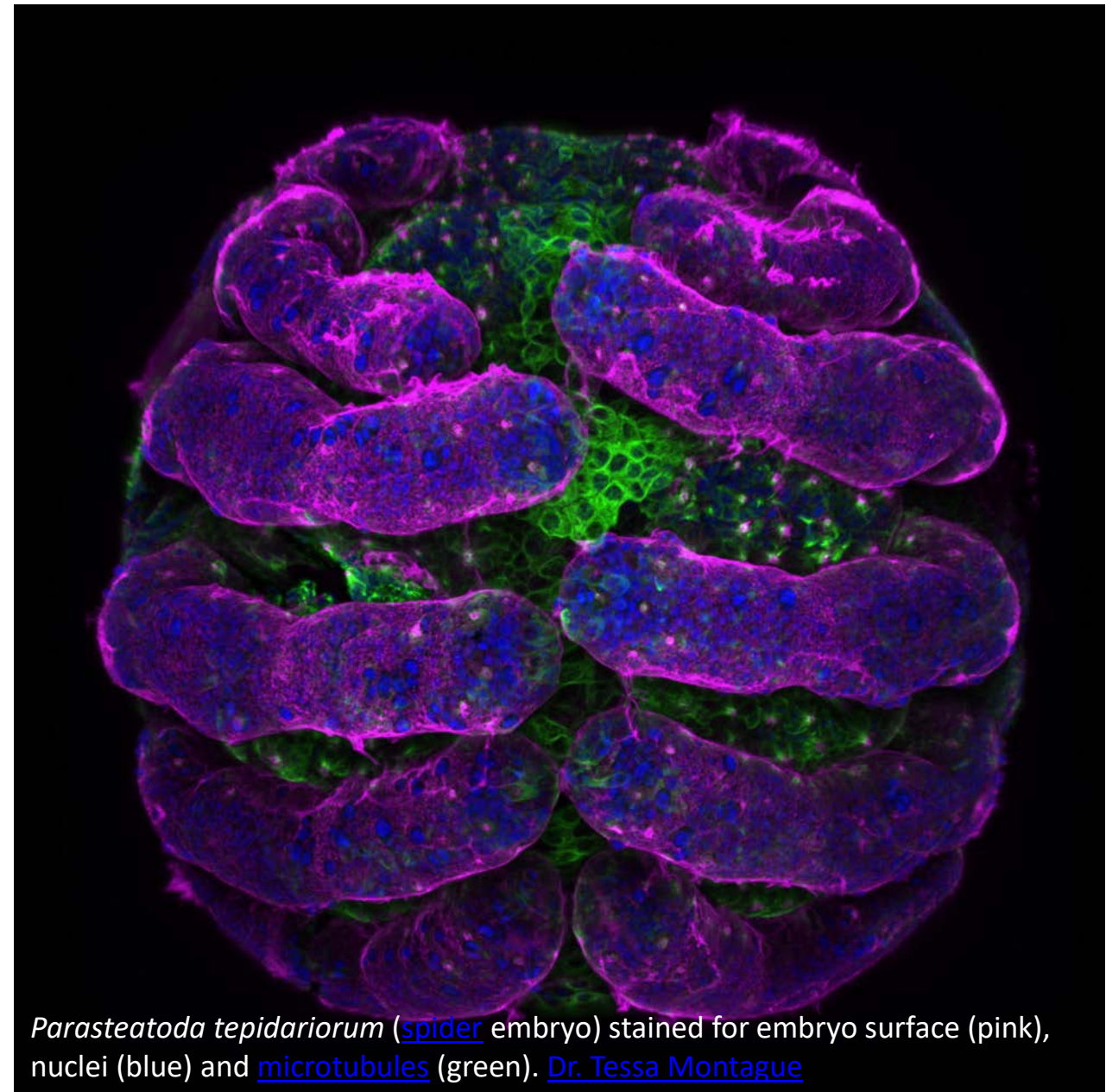
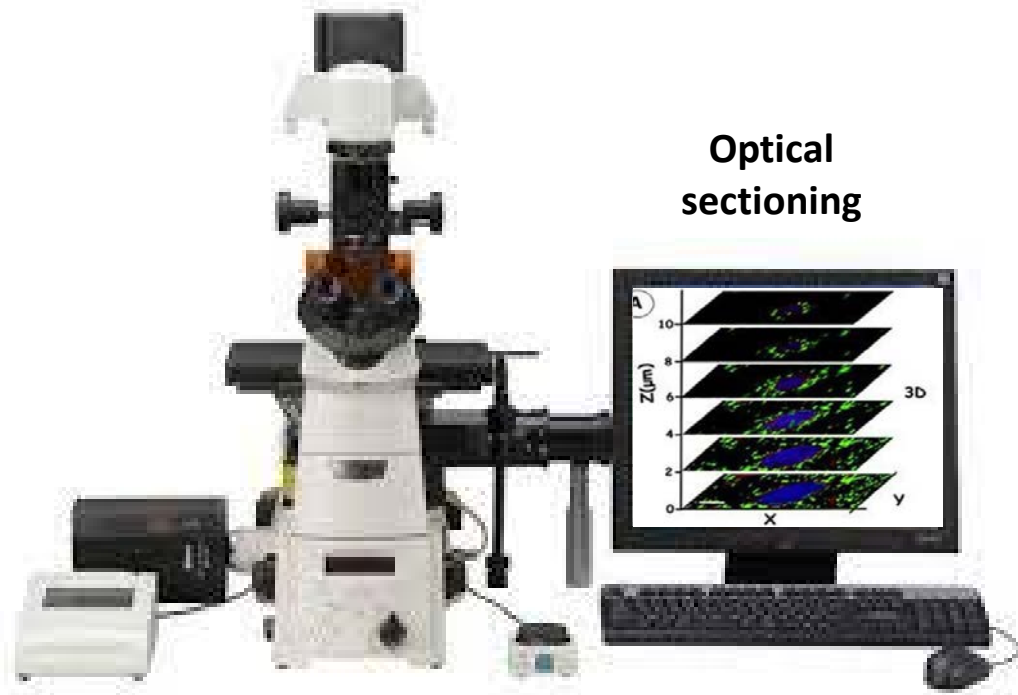
*Molecules* **2012**, *17*(4), 4047-4132

[www.nikonsmallworld.com/](http://www.nikonsmallworld.com/)

<https://www.thorlabs.com/>

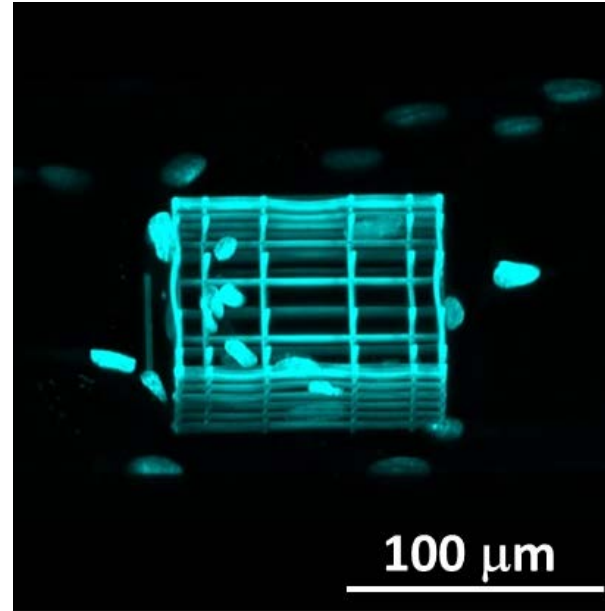
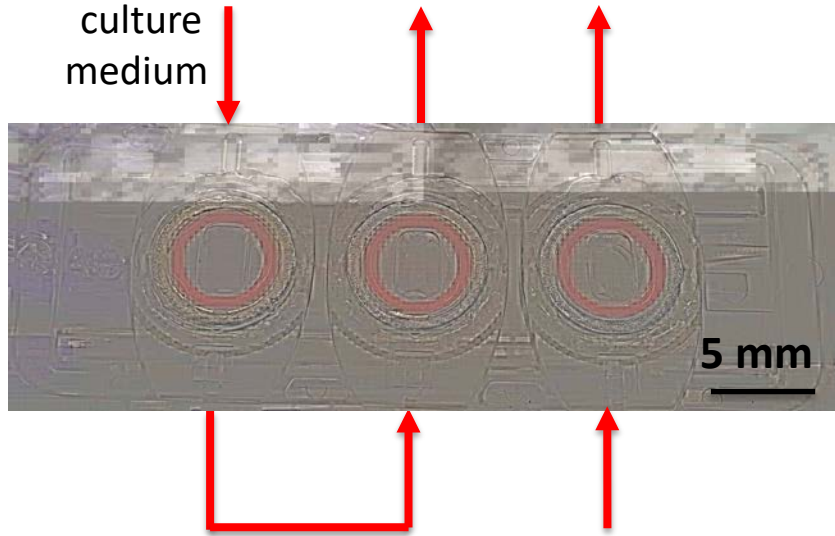


# Laser scanning confocal microscopy

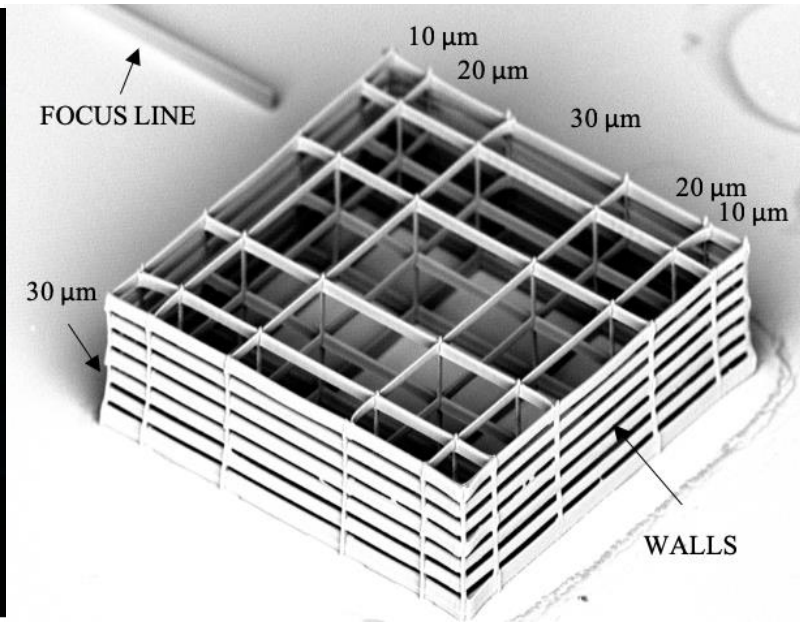


# Mechanobiology Lab. favorite devices

## Miniaturized optical accessible bioreactor (MOAB)

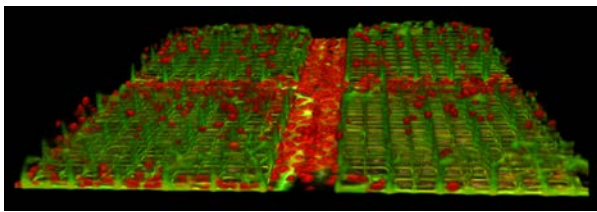


## Nichoid - scaffold

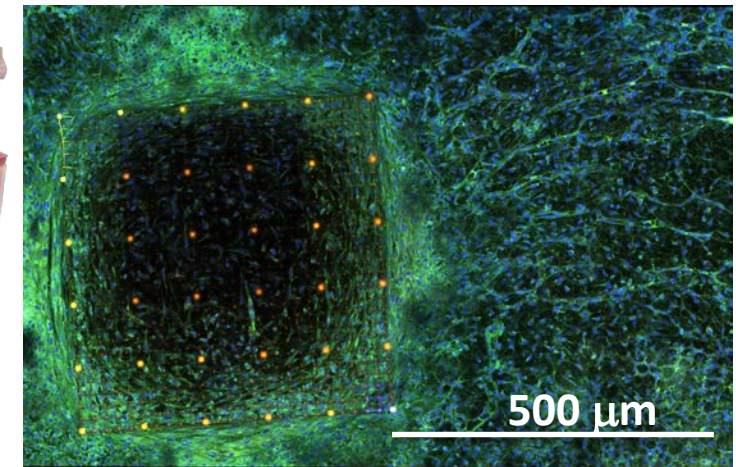
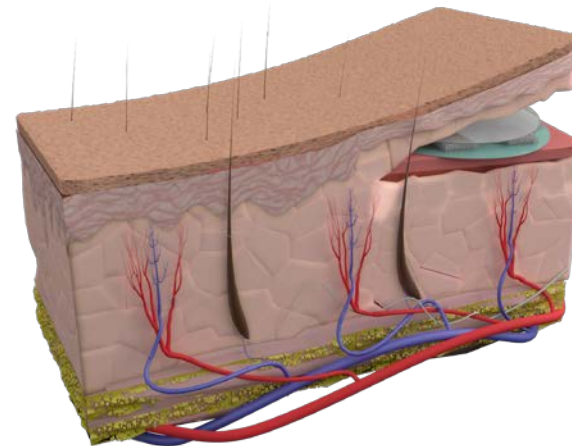
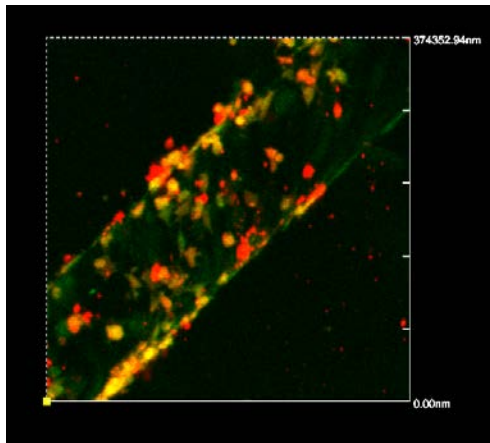


## Microatlas- imaging windows

### Endothelial tissue model

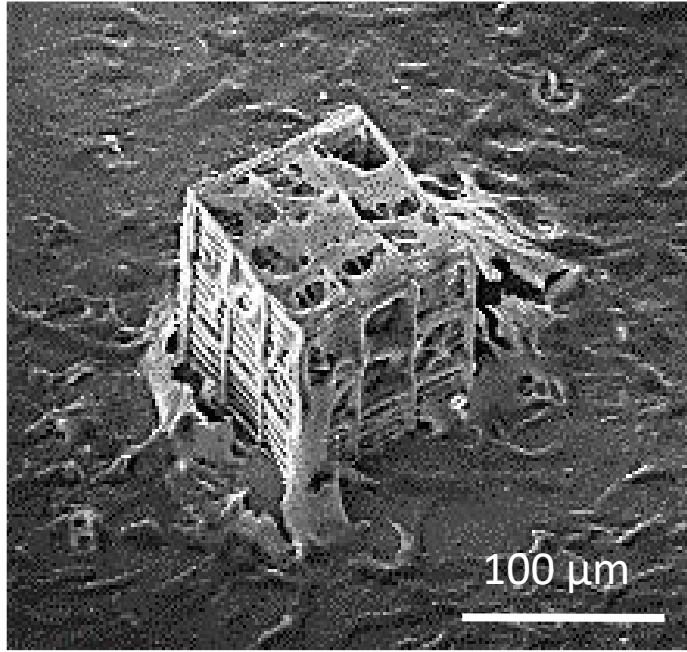


### Lymph node model



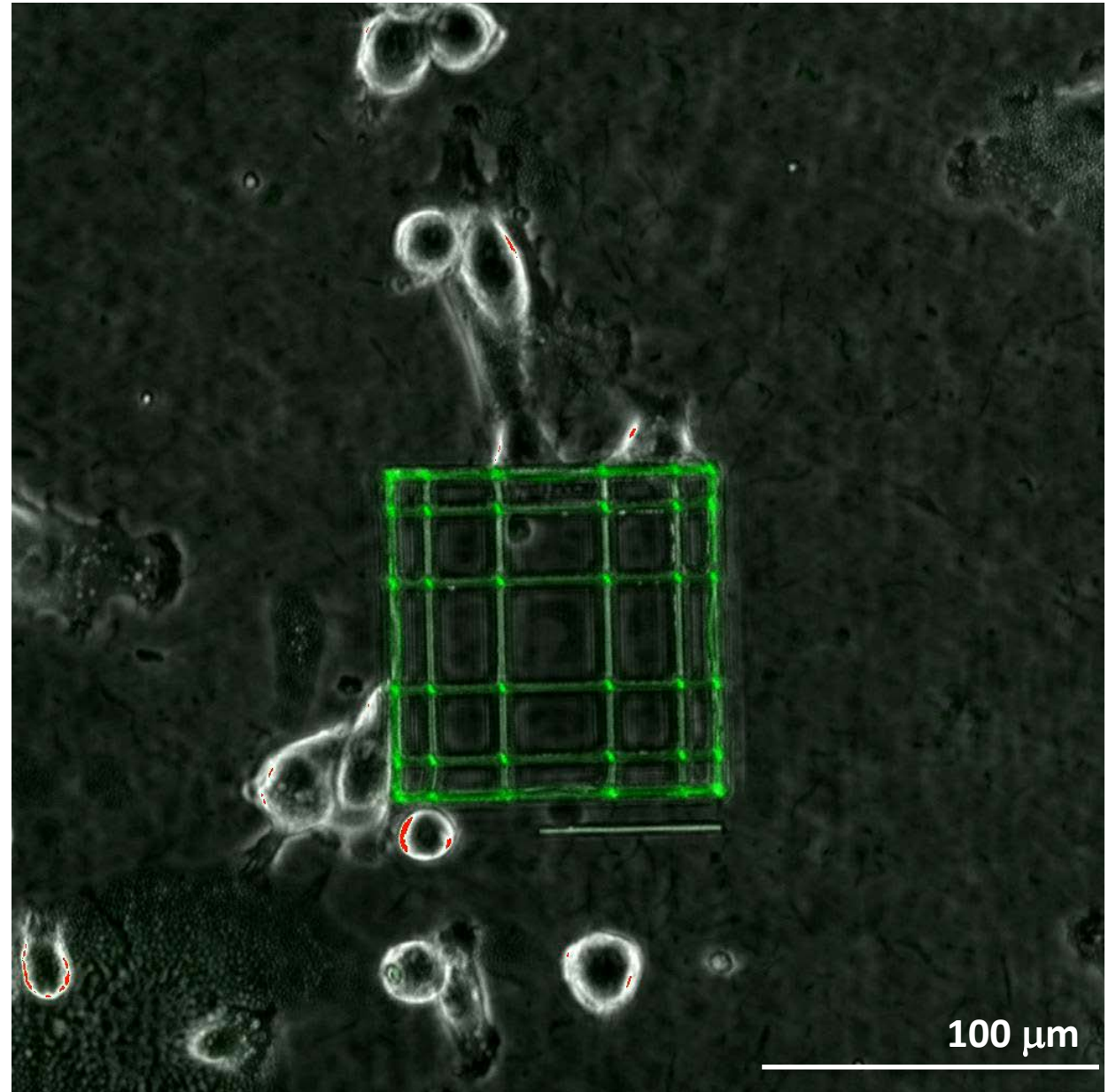


# Nichoid



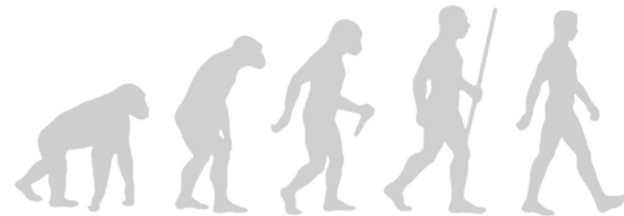
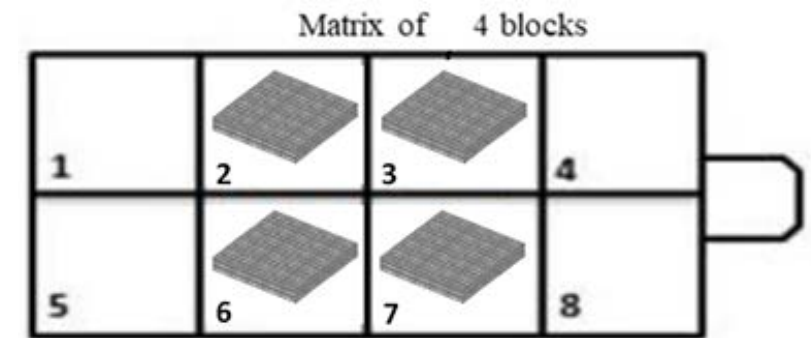
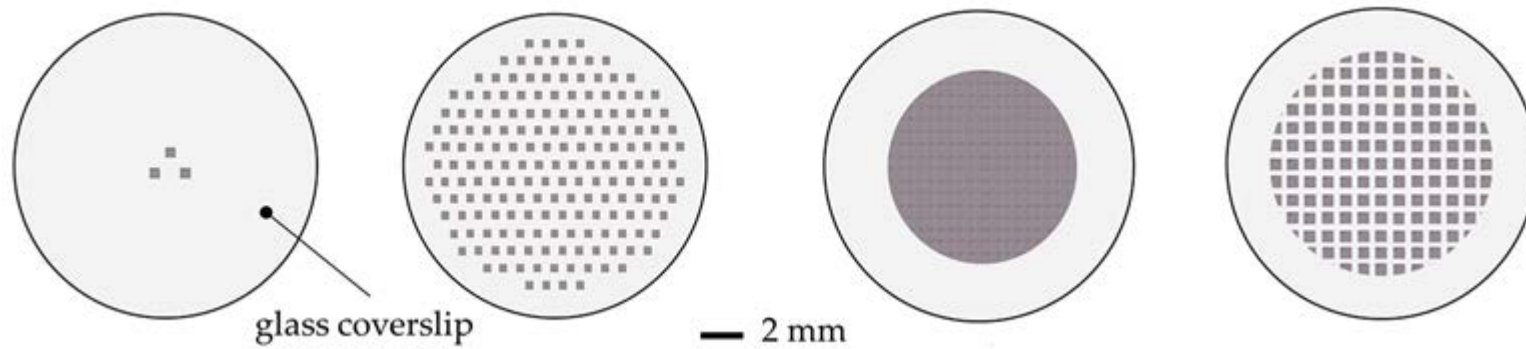
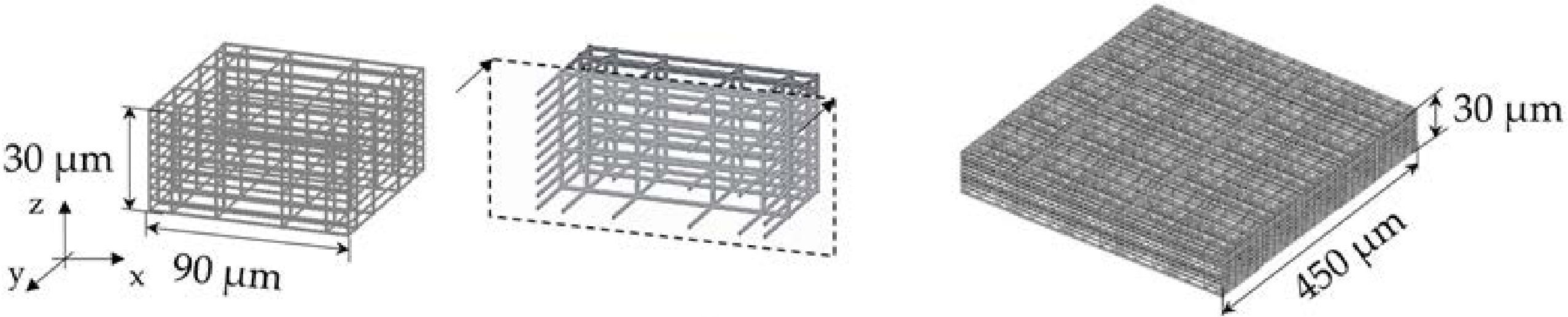
Scanning electron microscopy

# Epifluorescence microscopy

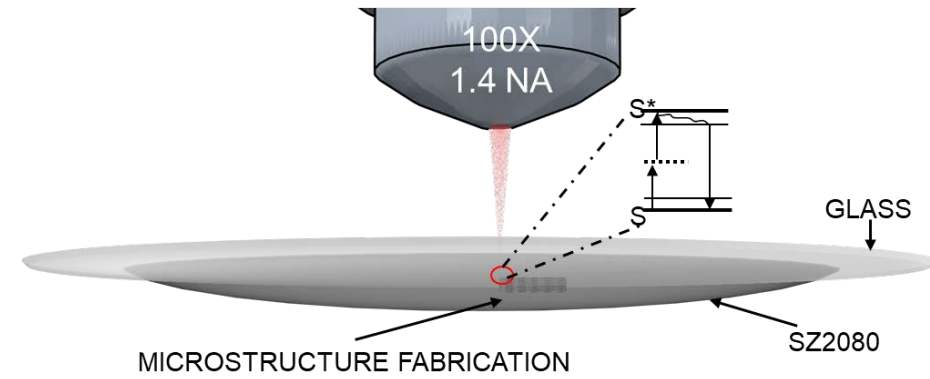
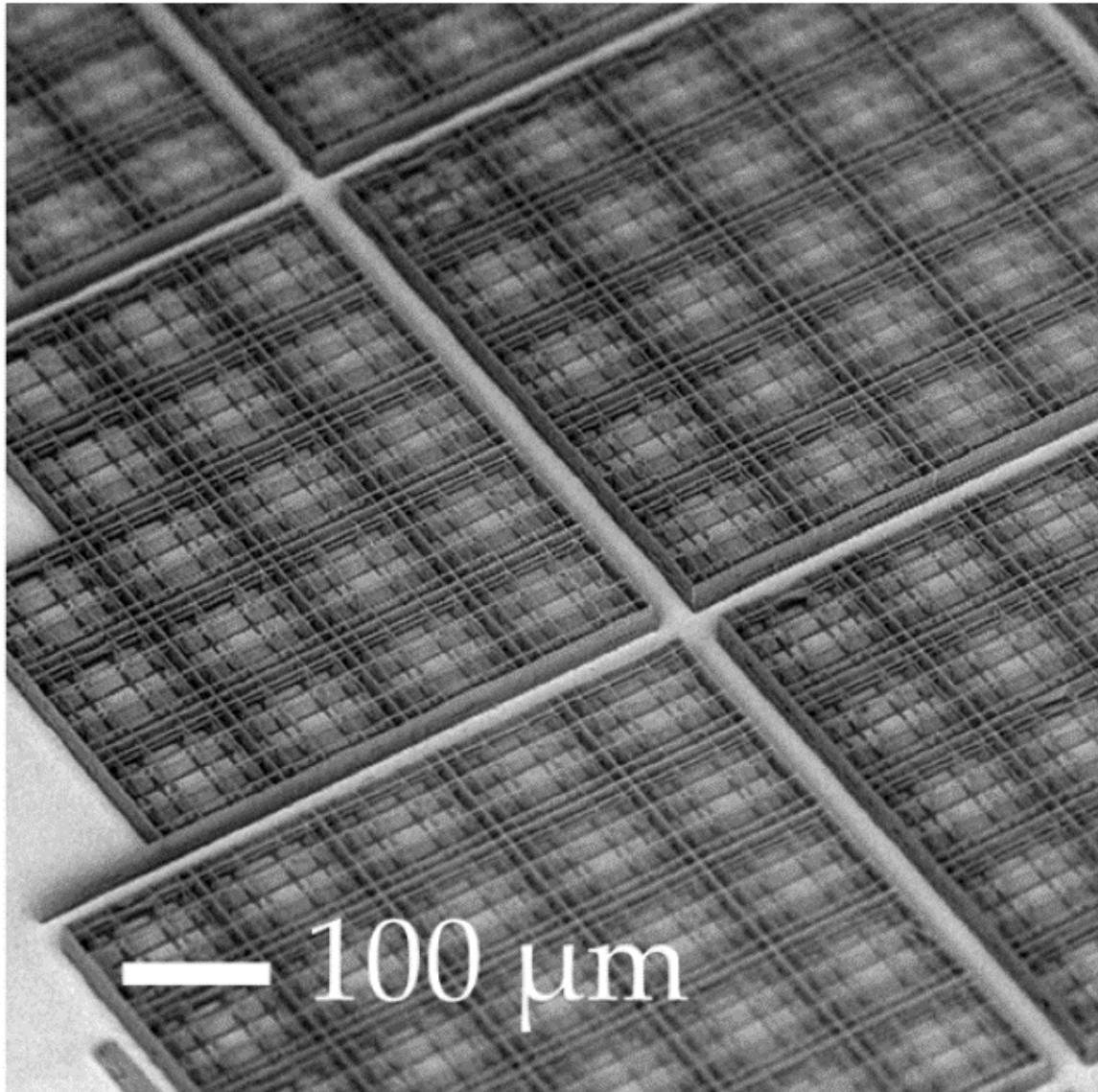




# Nichoid



# Two photon laser polymerization technique



Maximum Resolution:  
XY: 100 nm  
Z: 2 μm



R. Osellame

G. Cerullo

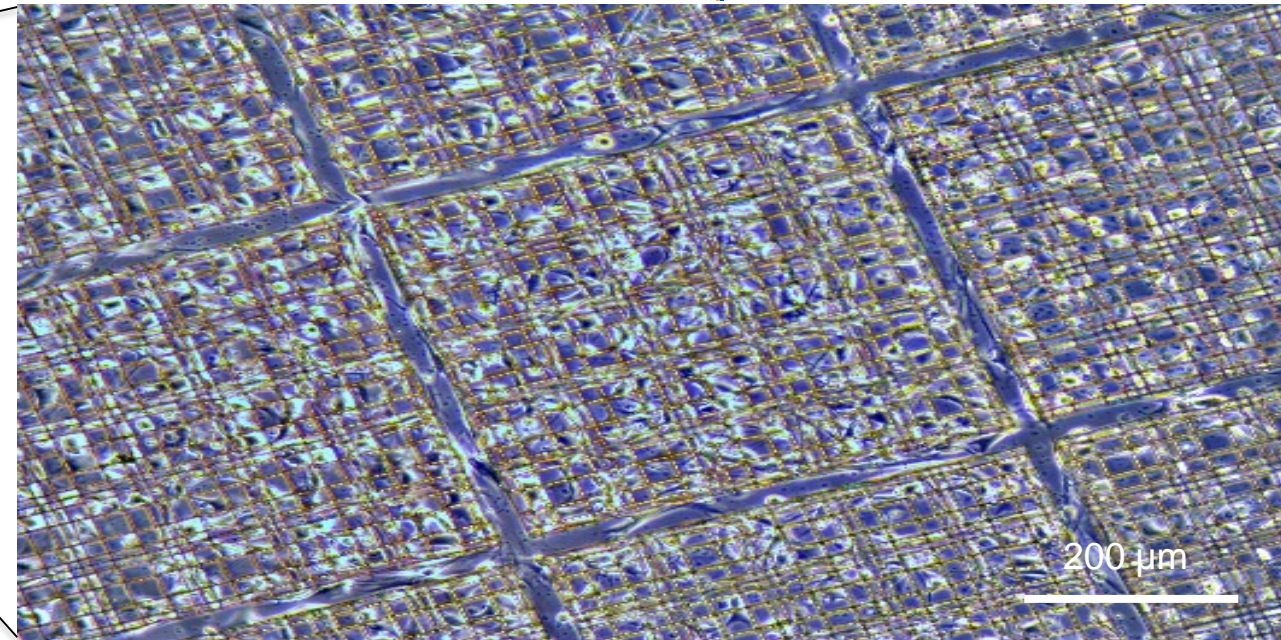
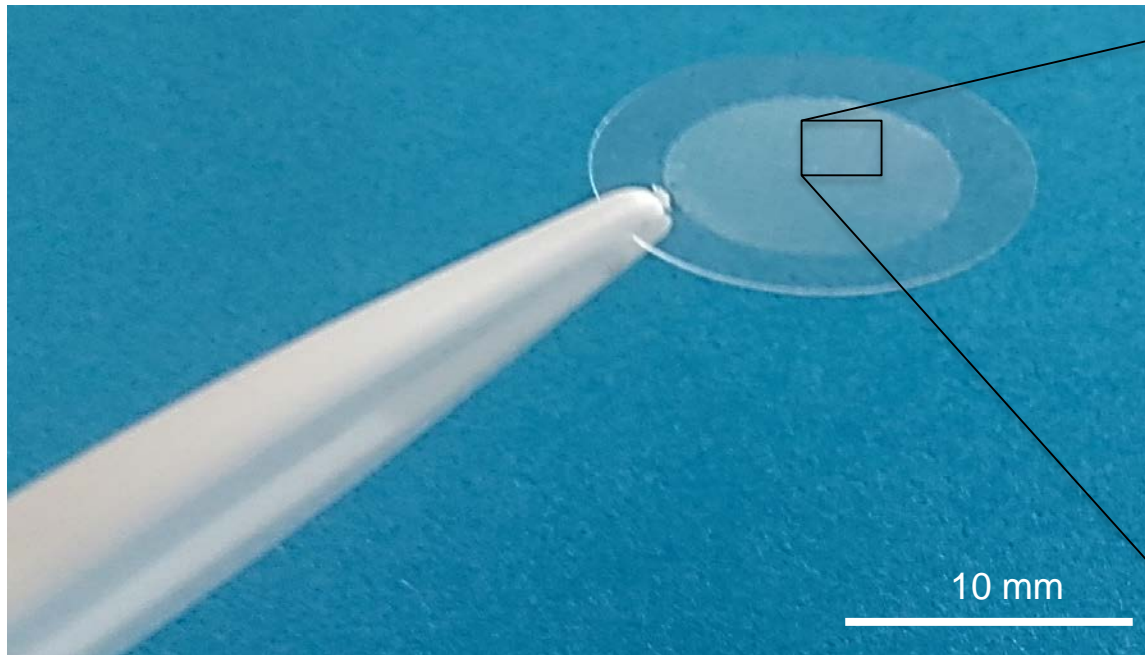
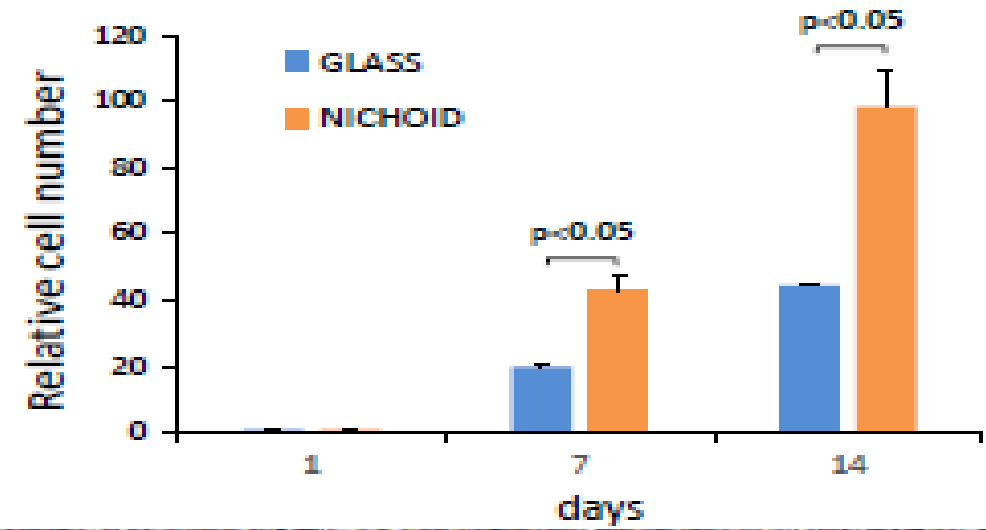
T. Zandini





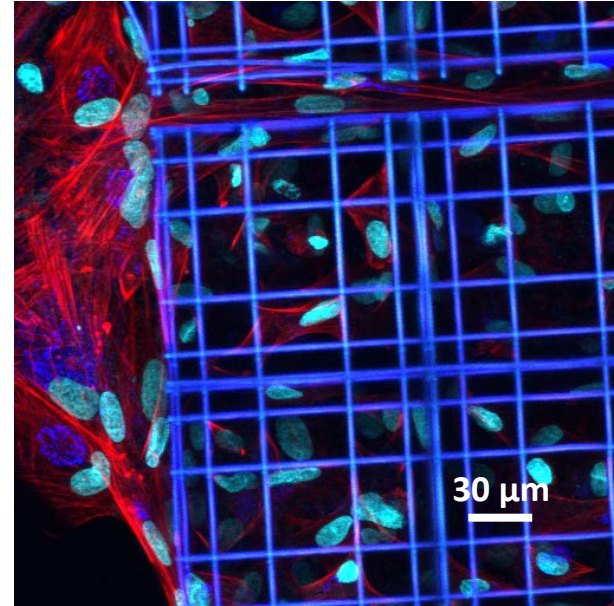
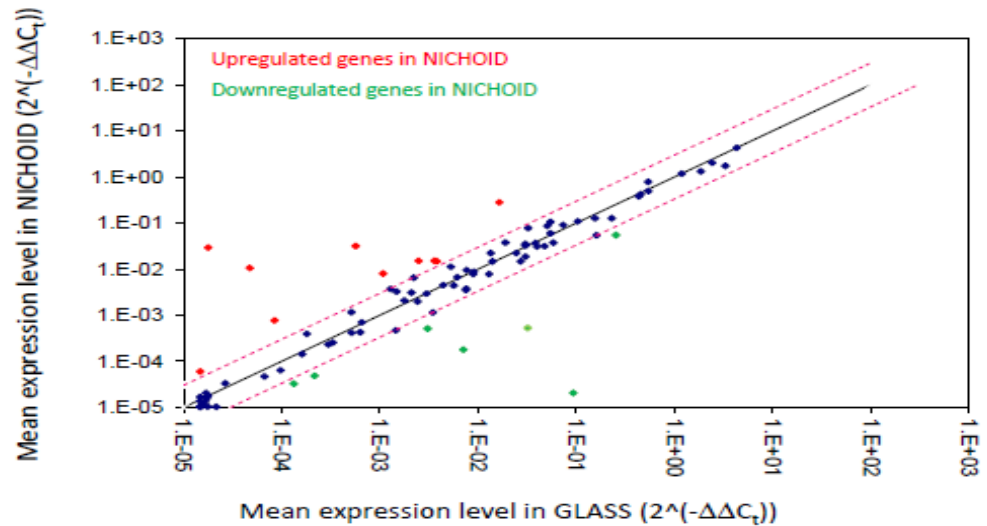
# Cell proliferation

Cell proliferation increased to nearly 60% within 14 days into the Nichoid



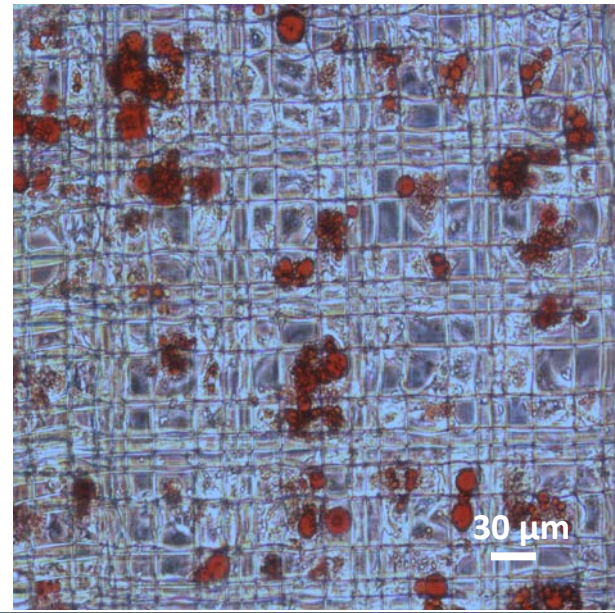
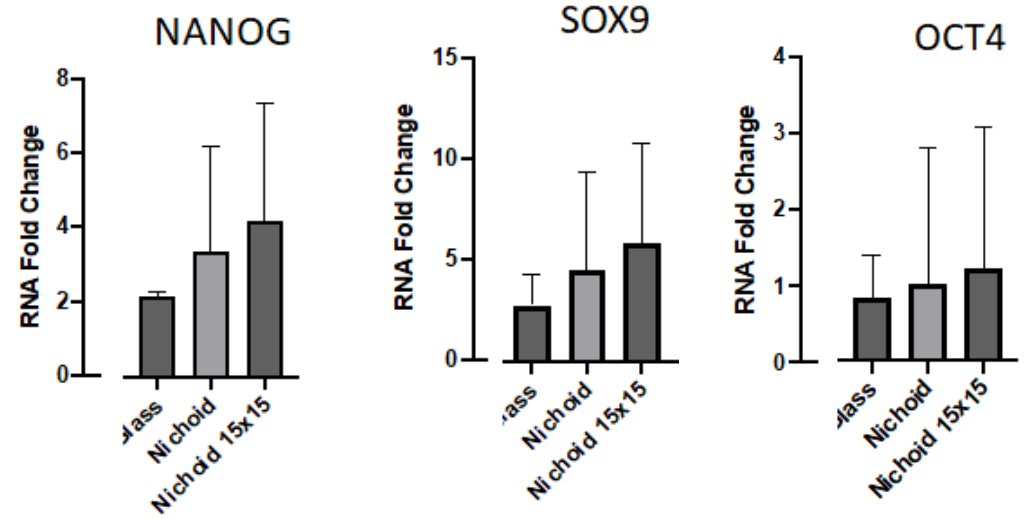


# MSC stemness and differentiation



Collagen/ scaffold/ nuclei

OIL RED



J Tissue Eng Regen Med 2017; 11: 2836–2845  
 Int. J. Mol. Sci. 2020, 21, 8498  
 Cells 9 (8):2020. 1873

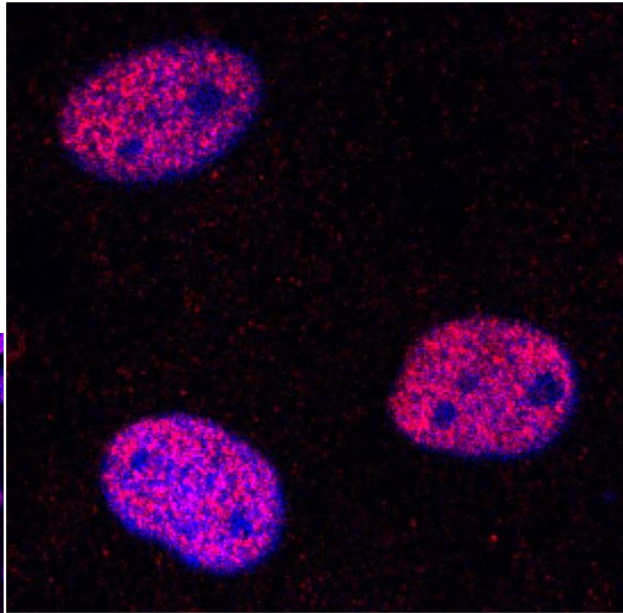


# Yap localization



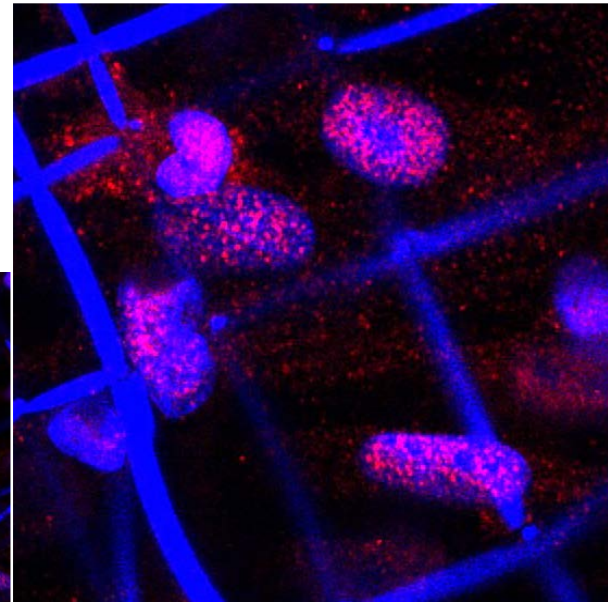
YAP localization is inside the cell nucleus in MSCs grown on FLAT substrate, while in the Nichoid it is mainly localized into the cytoplasm

Nuclear localization

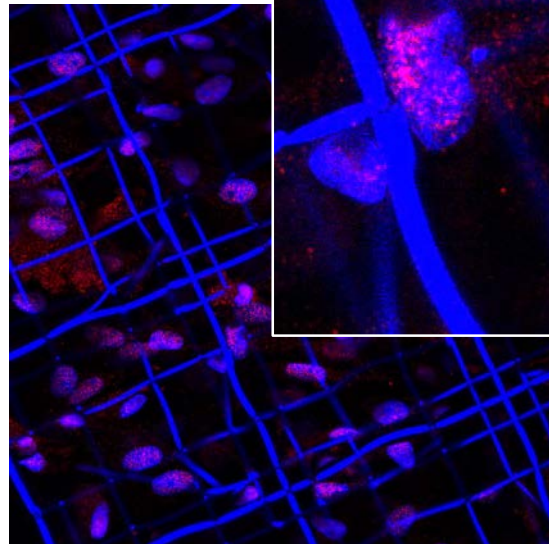
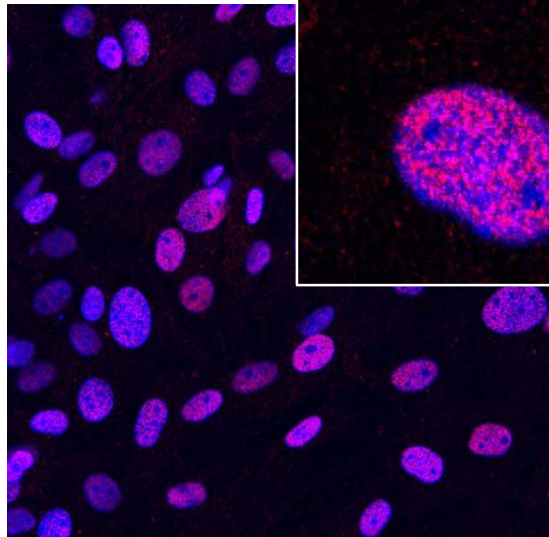
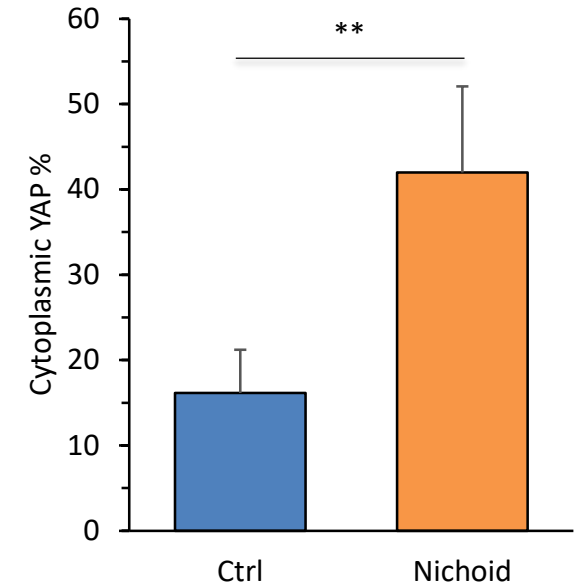


2D Control

Cytoplasmic localization

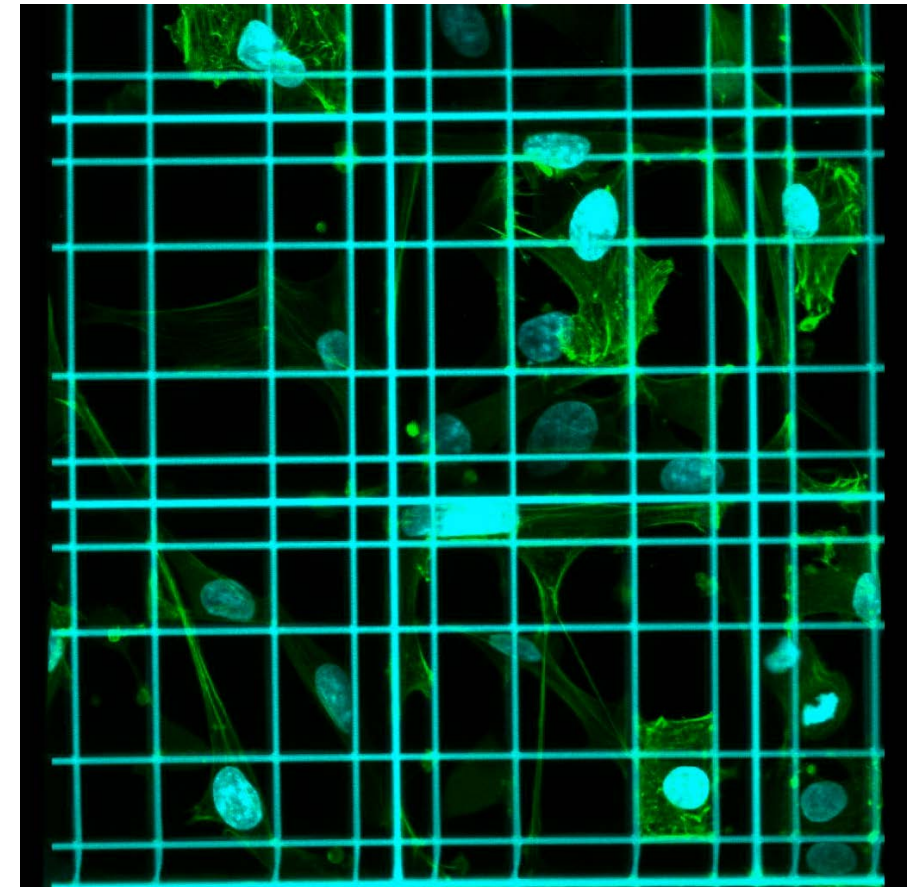
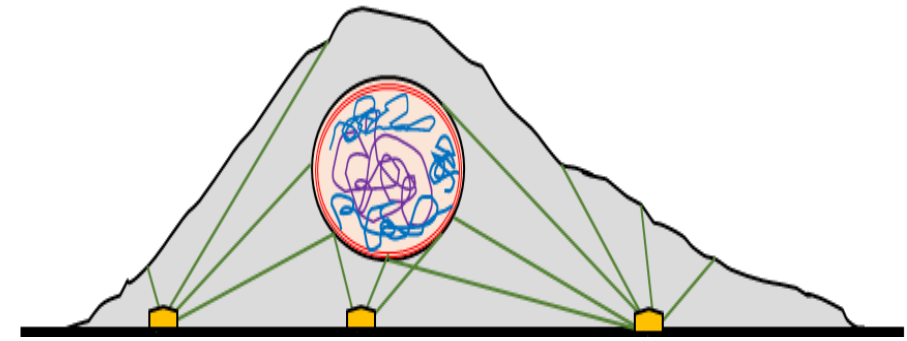
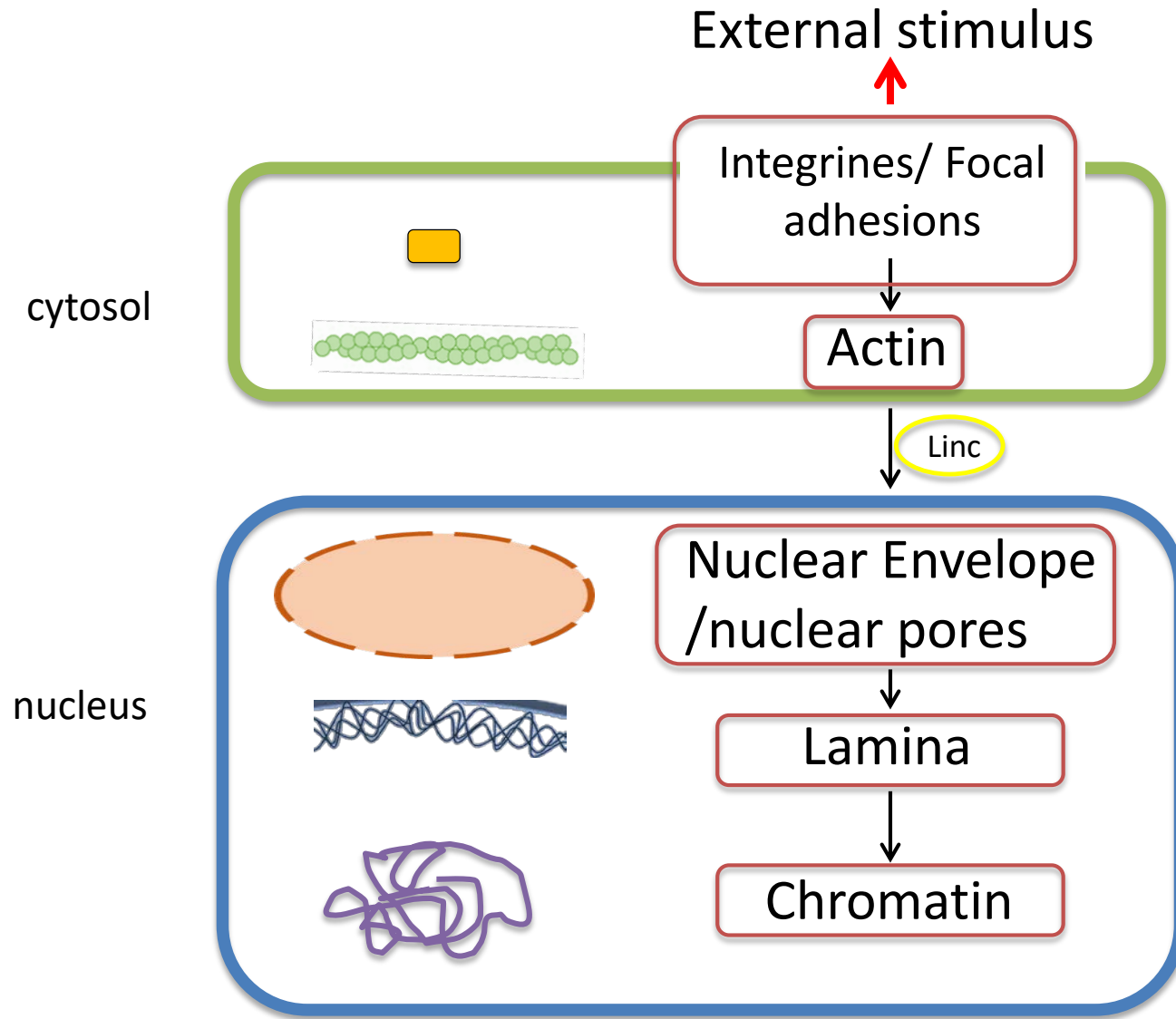


Nichoid



DAPI – YAP

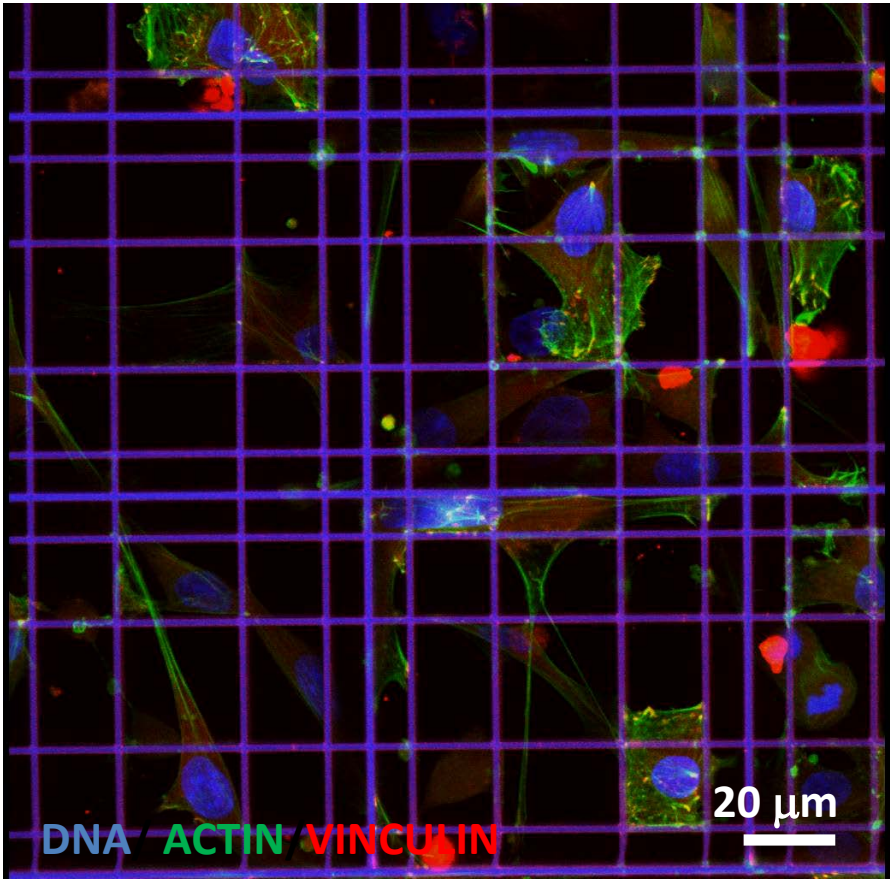
# Mechanotransduction pathway





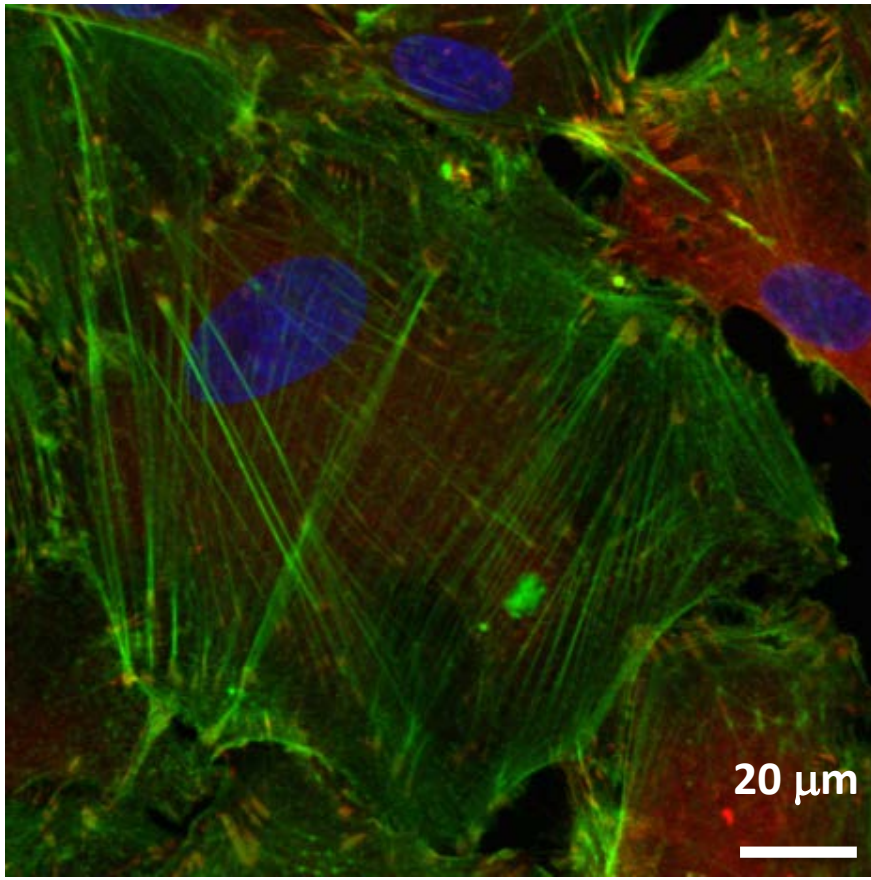
**Focal adhesion**

**3D structure (Nichoid)**

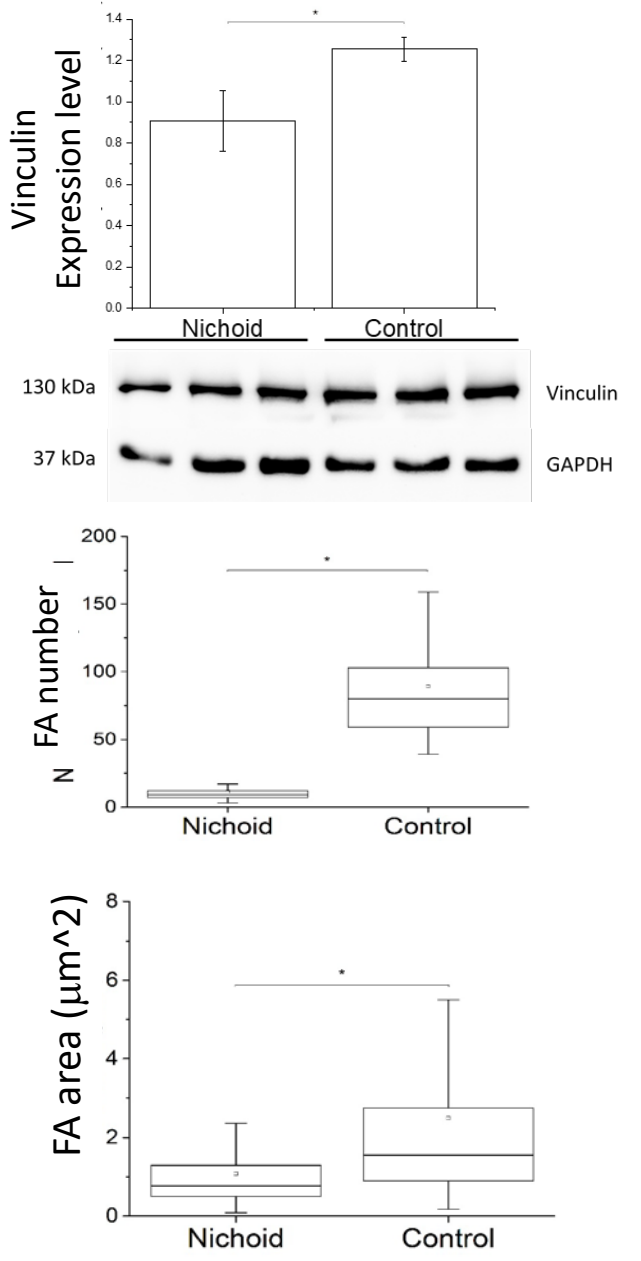


3D cell growth affects cell adhesion properties

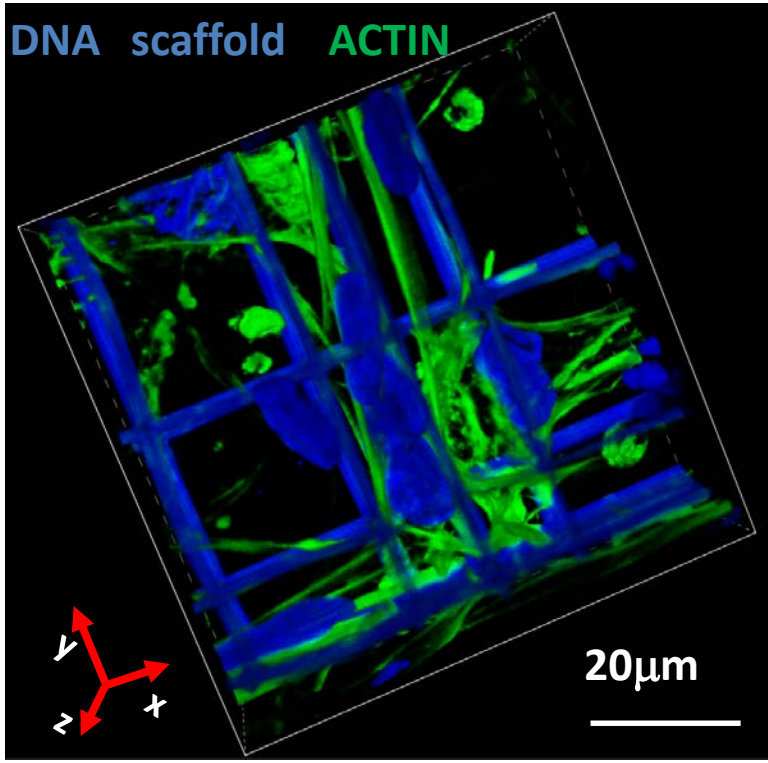
**Flat-2D Control**



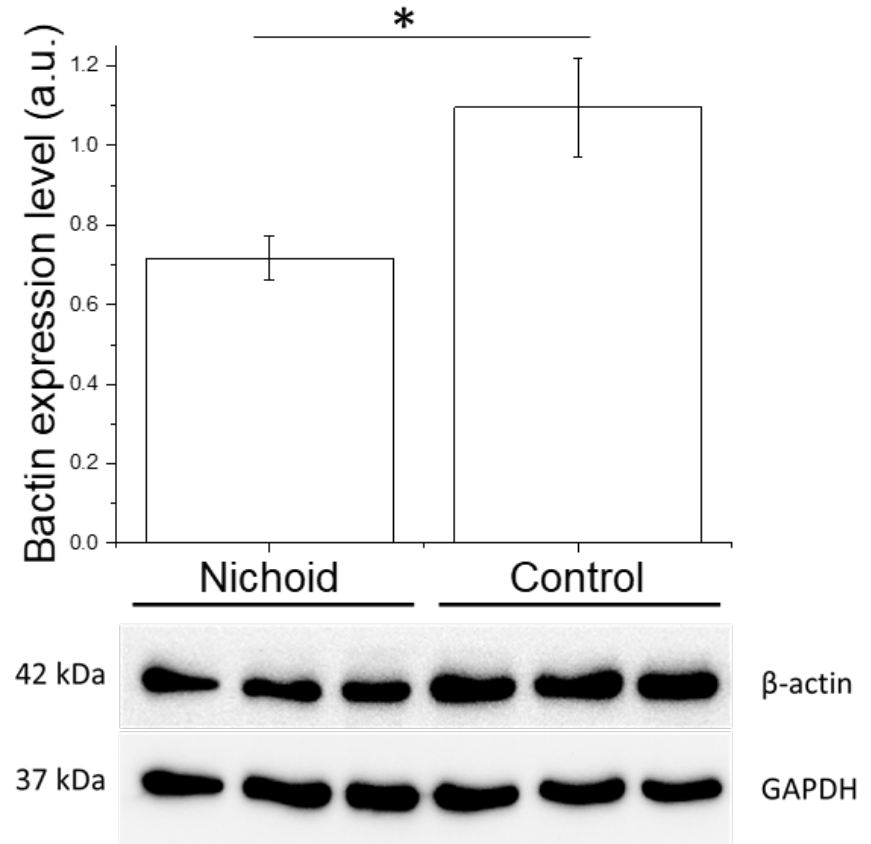
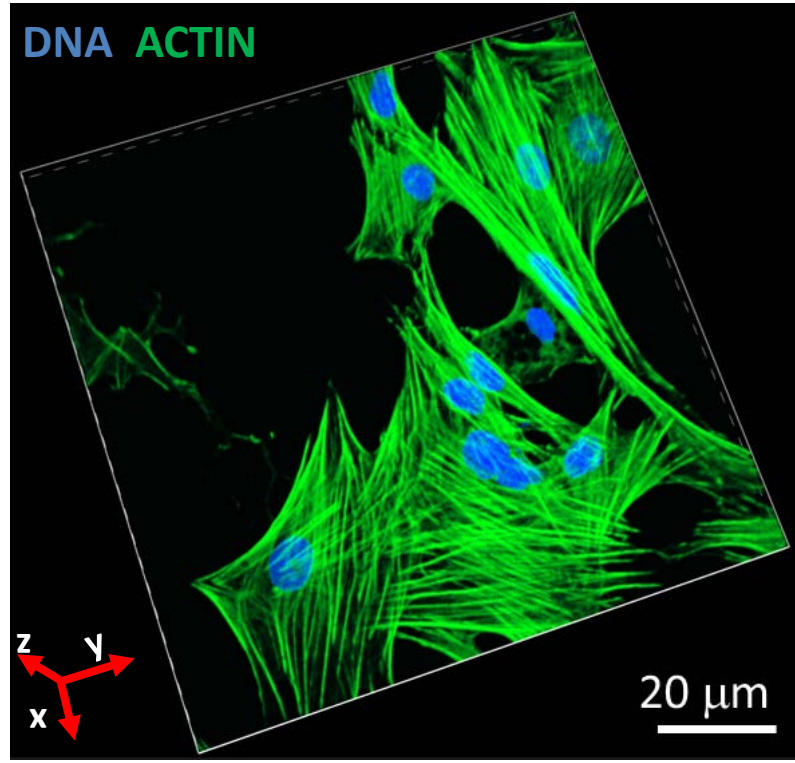
Low expression of vinculin  
Few and no mature FAs



### Nichoid



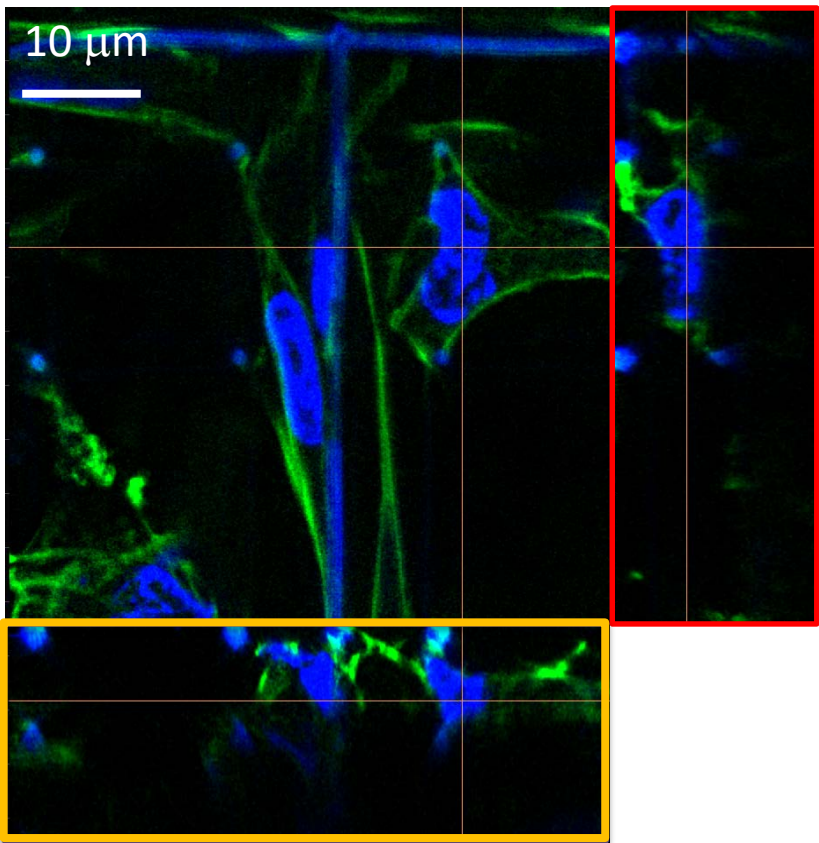
### Control



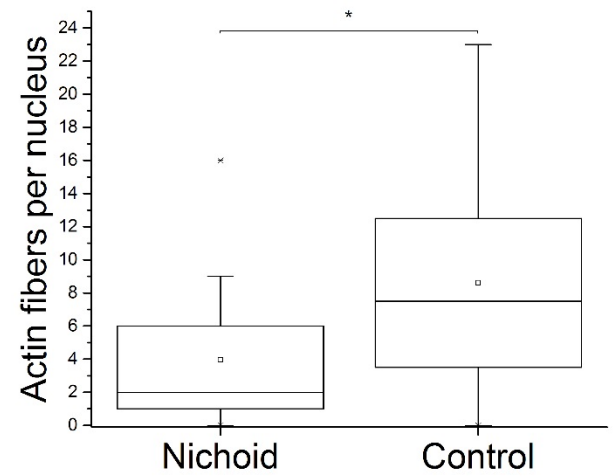
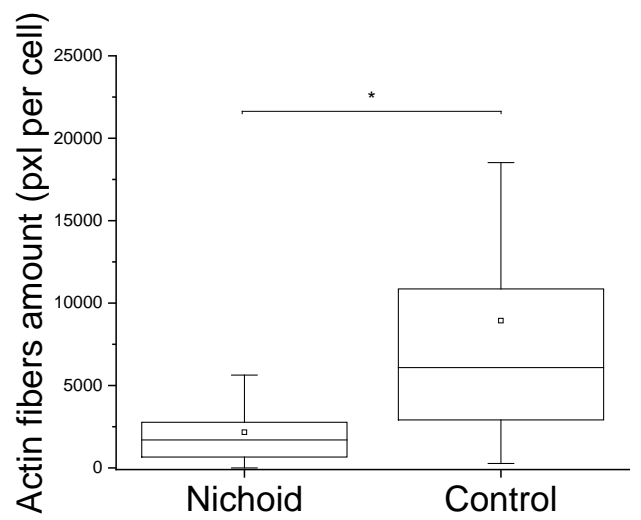
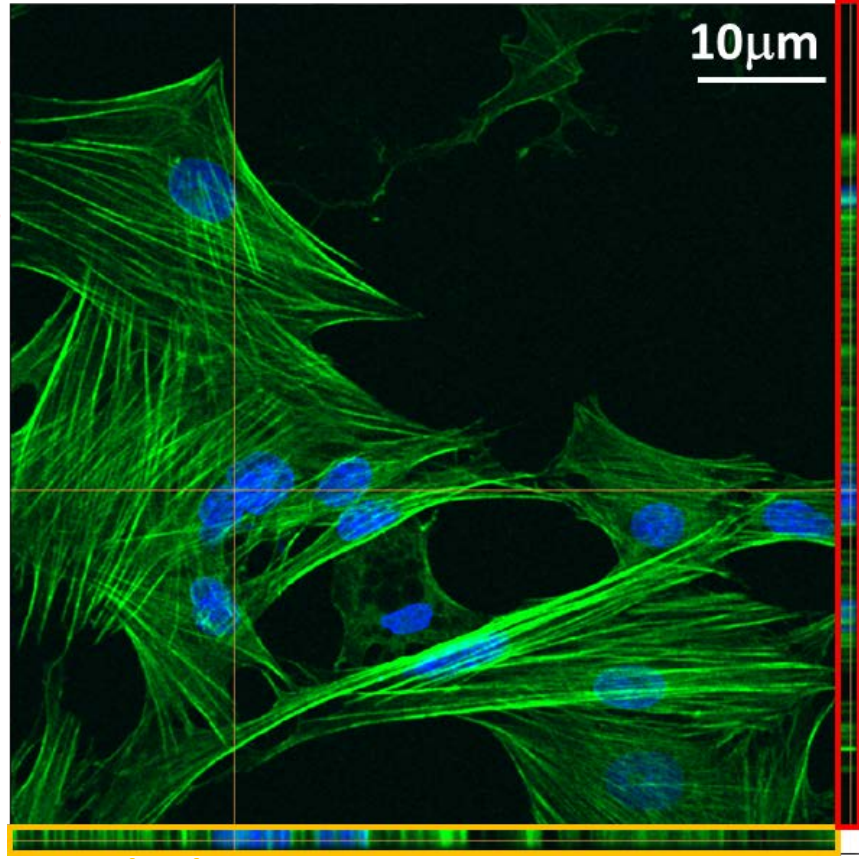
3D cell growth reduce internal force transmission by remodeling actin cytoskeleton



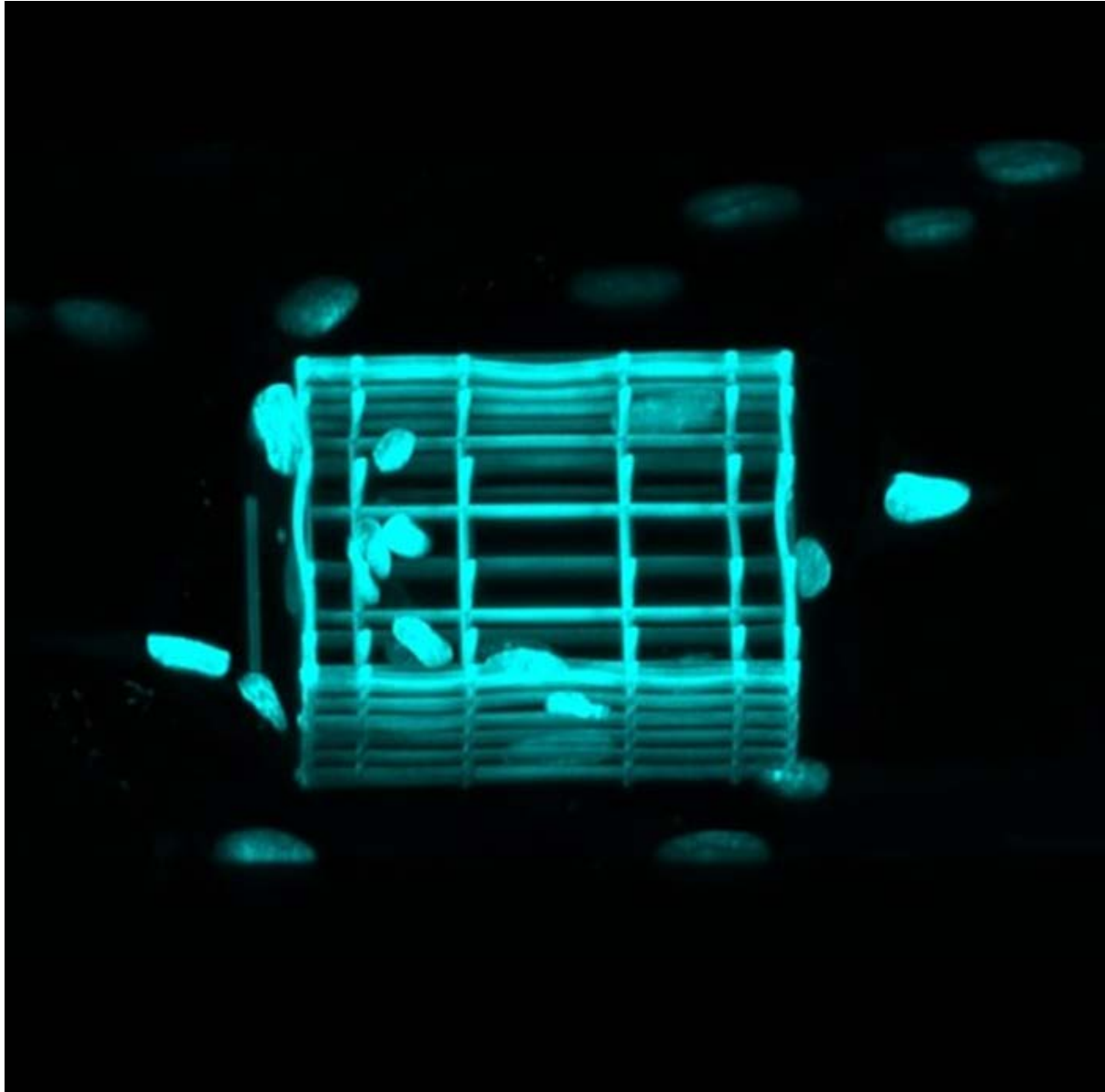
### Nichoid



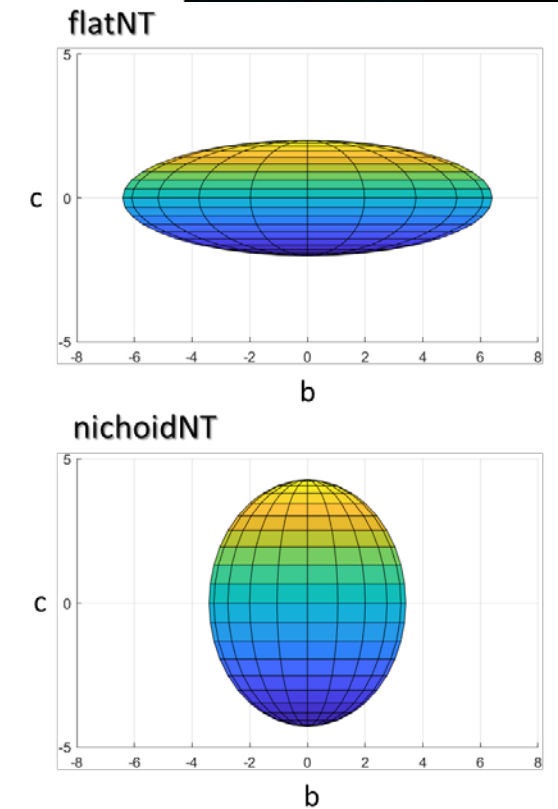
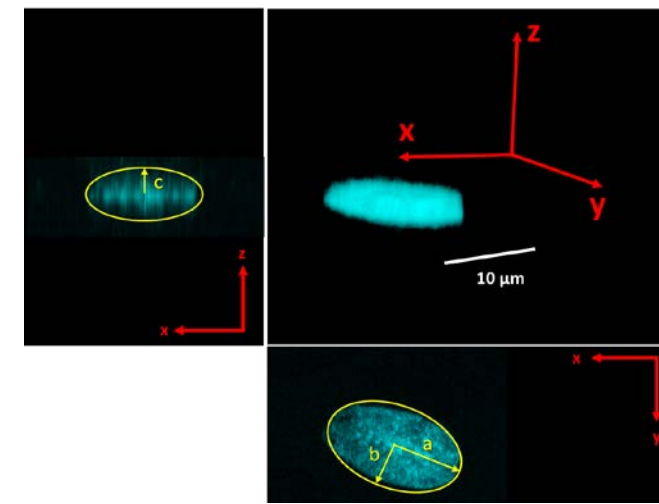
### Control



3D cell growth reduces actin-nucleus interactions

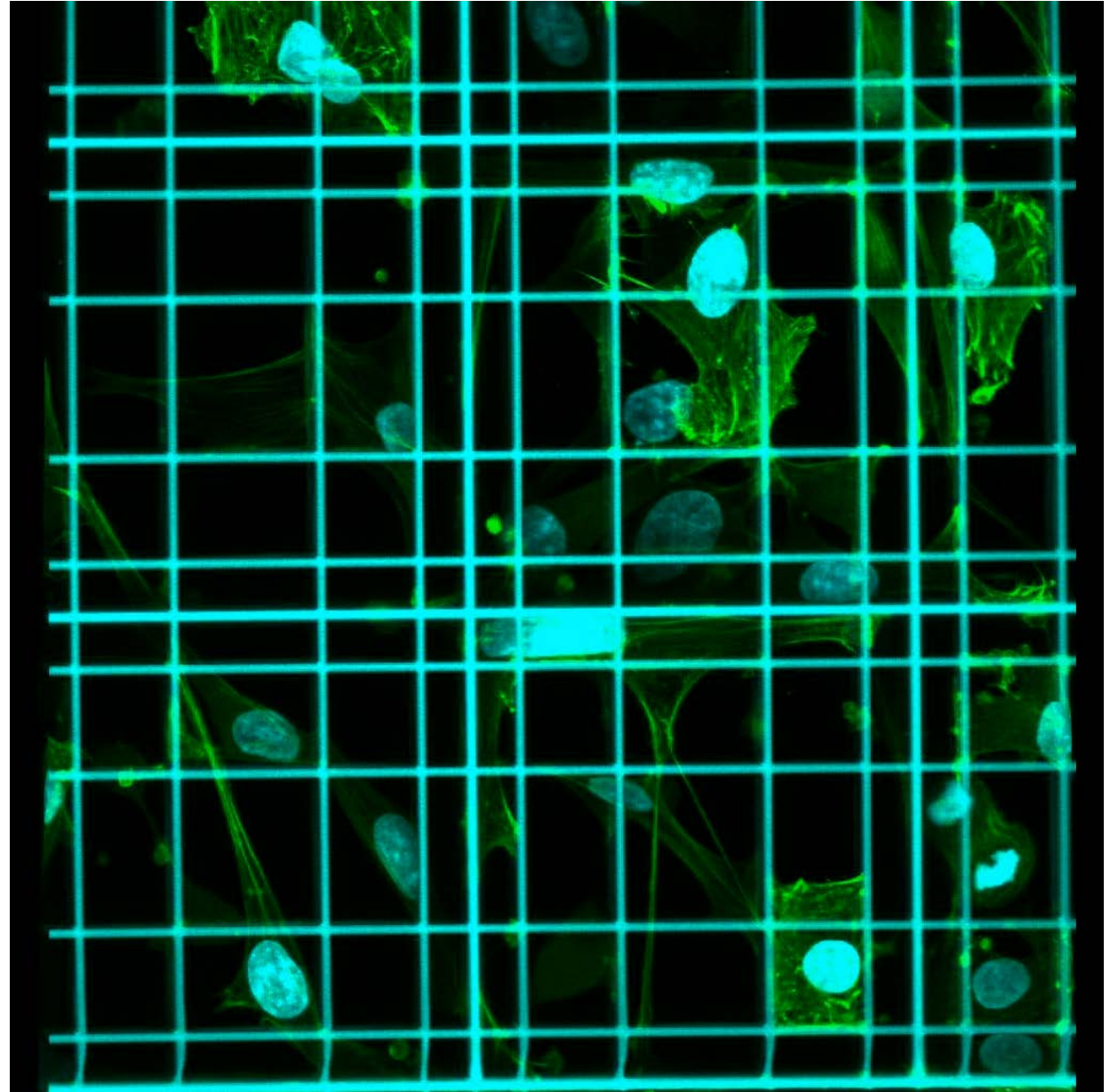


3D cell growth  
affects nuclear  
morphology



## Nichoid-cell interaction:

Nichoid can influence cell fate by reorganizing specific proteins.



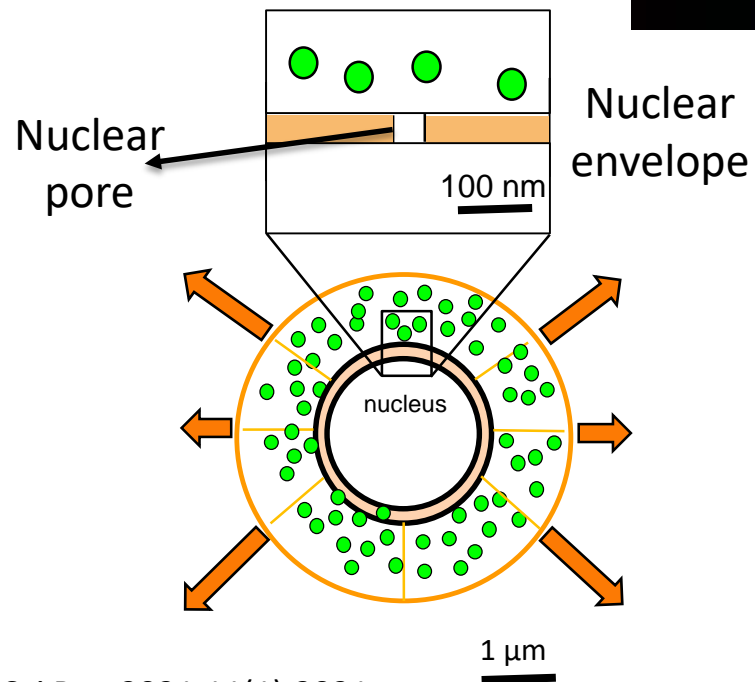


# Stress-strained nuclear envelope permeability

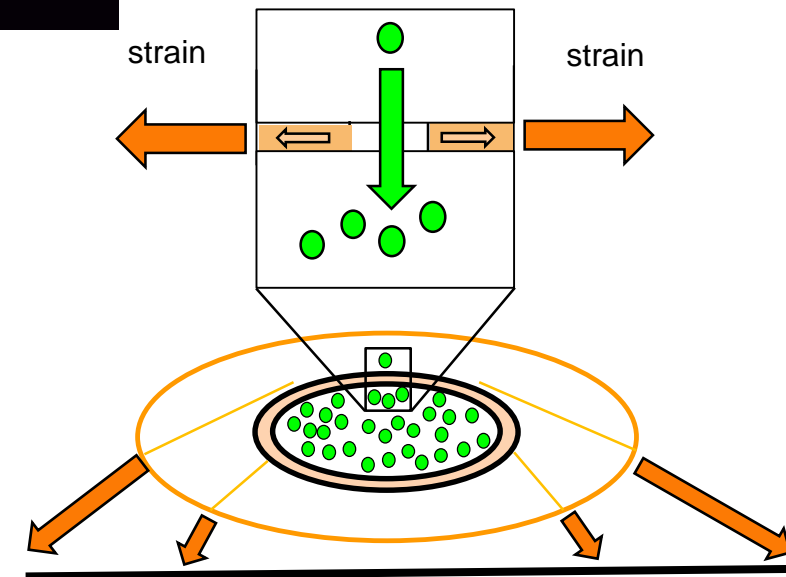
## Fluorescence recovery after photobleaching



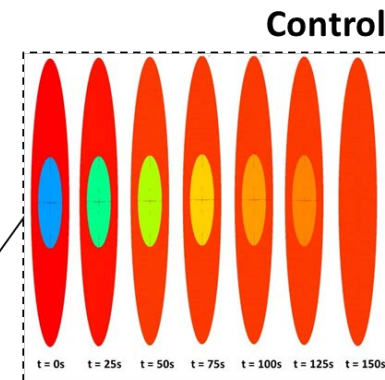
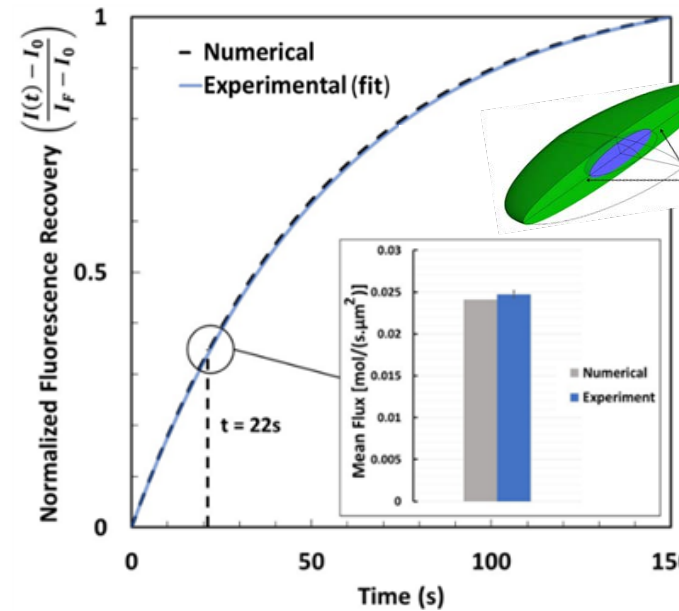
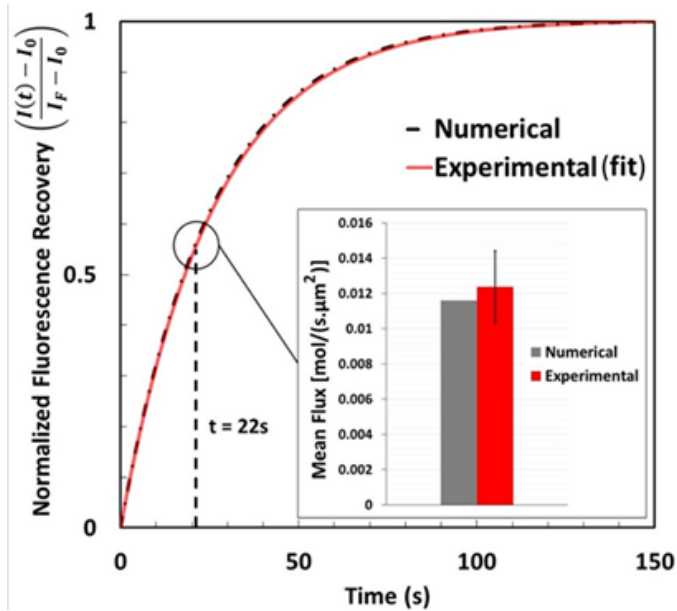
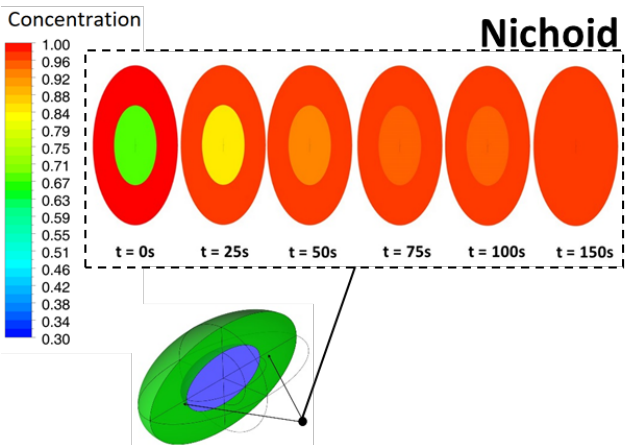
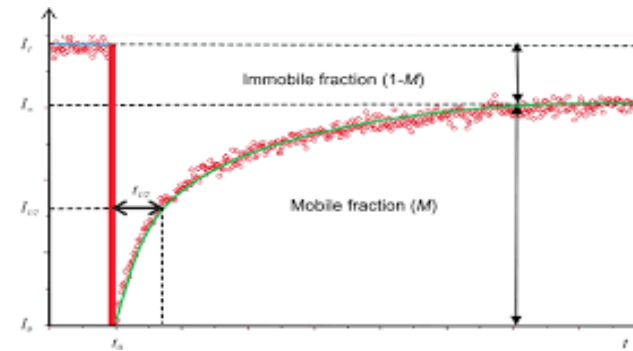
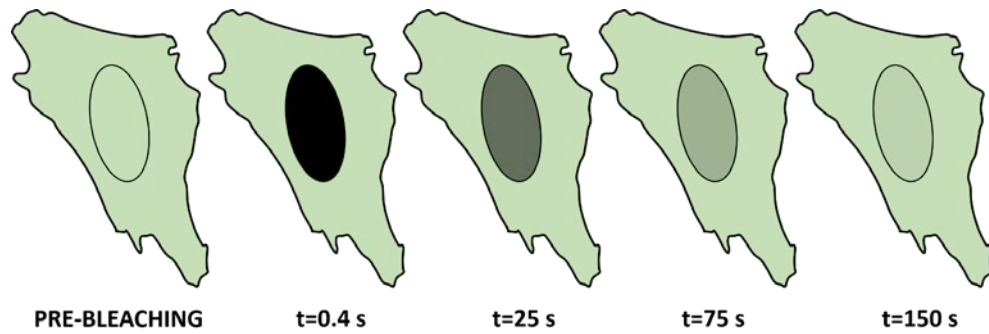
### Roundish nuclei



### Spread nuclei



# Protein nucleocytoplasmic translocation

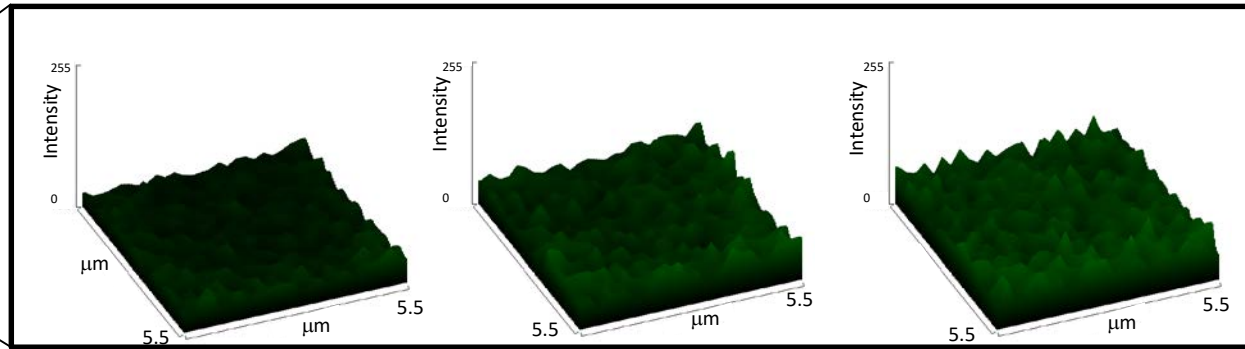
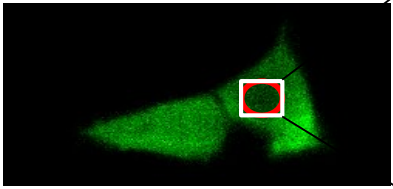




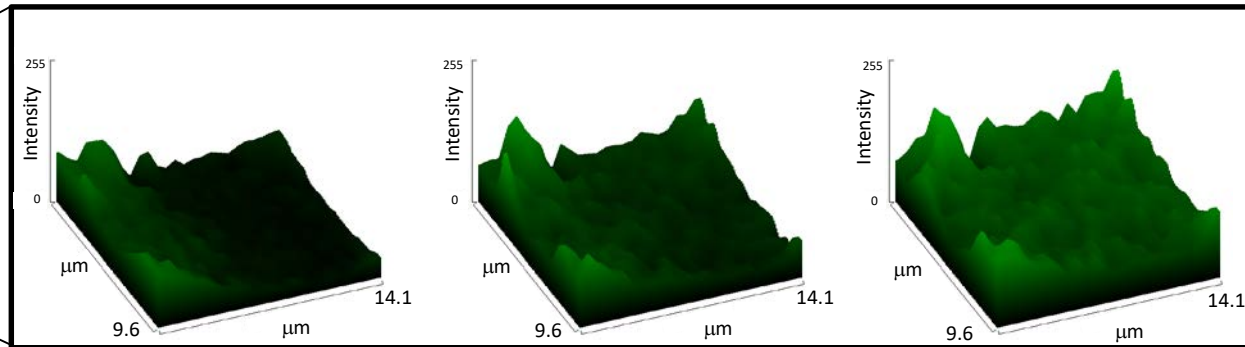
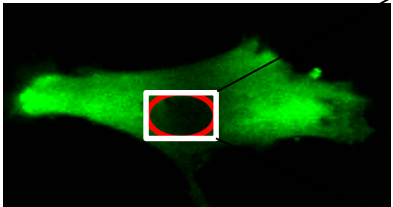
# Protein influx into the nucleus

equatorial plane fluorescence recovery

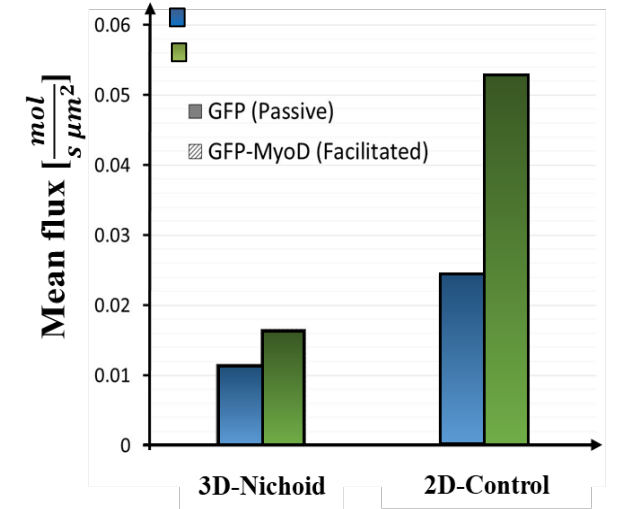
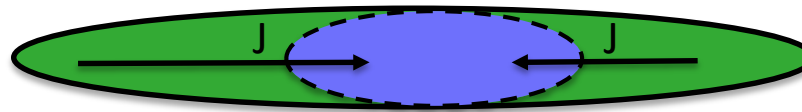
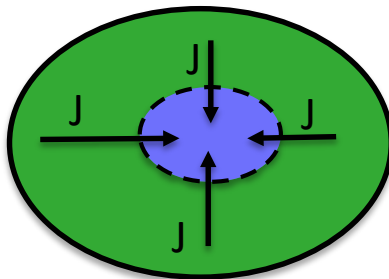
Nichoid



Control



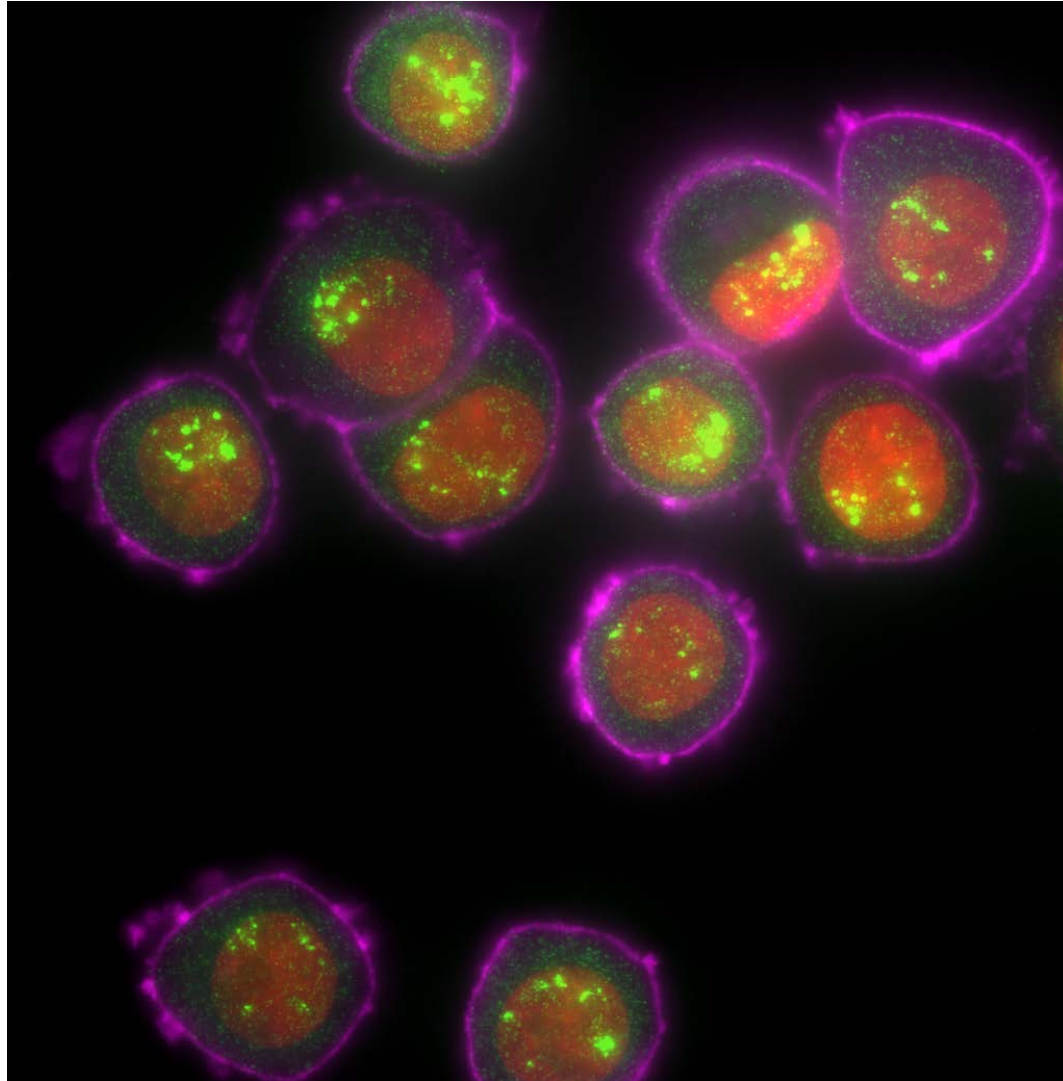
t 200 ms 4 s 35 s



GFP reduction ~ 50%  
MyoD-GFP reduction ~ 70%

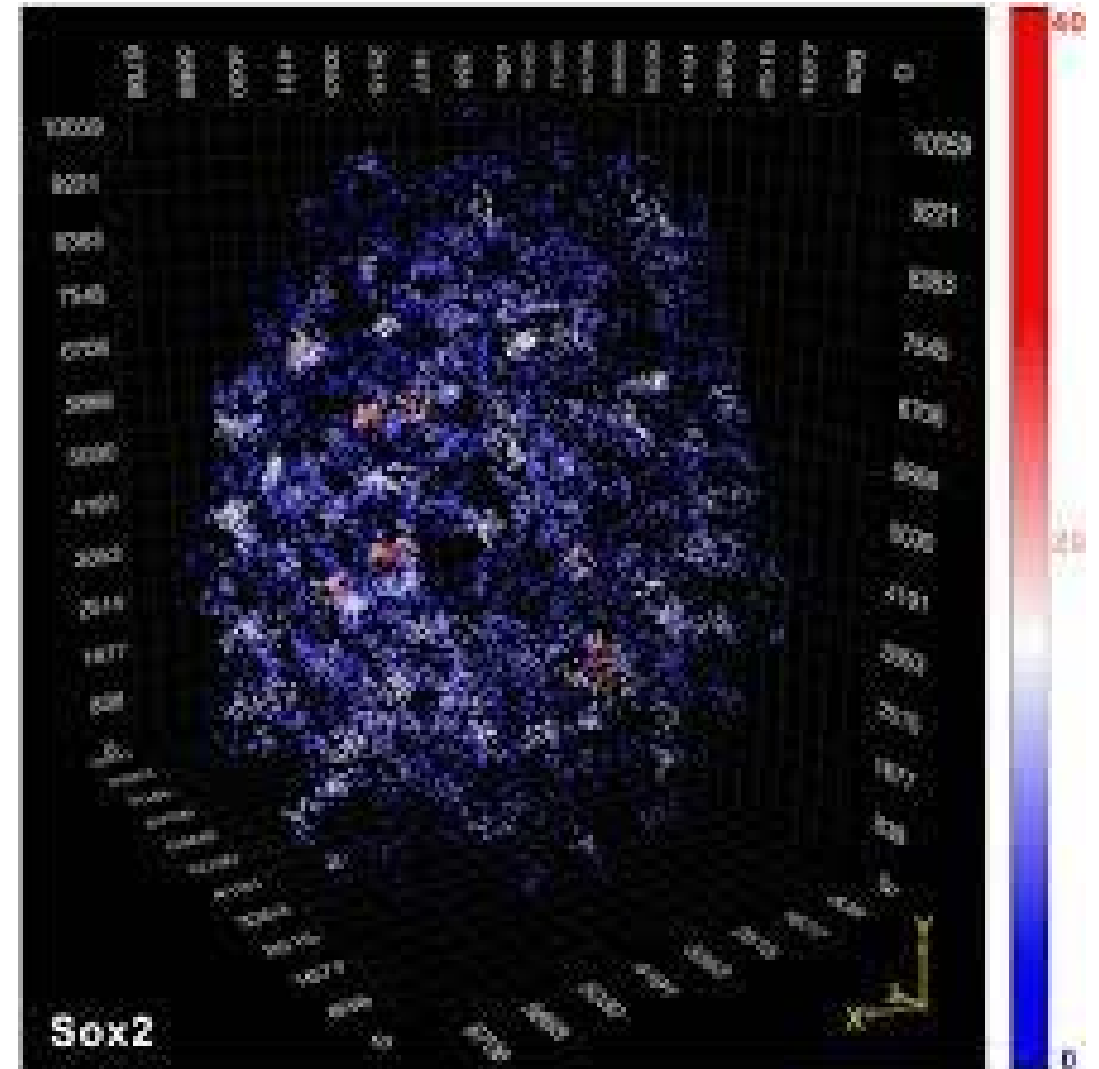
# Perspective

## FISH microscopy



<https://www.biosearchtech.com/>  
<https://www.janelia.org/lab/liu-lab>

## Single particle tracking



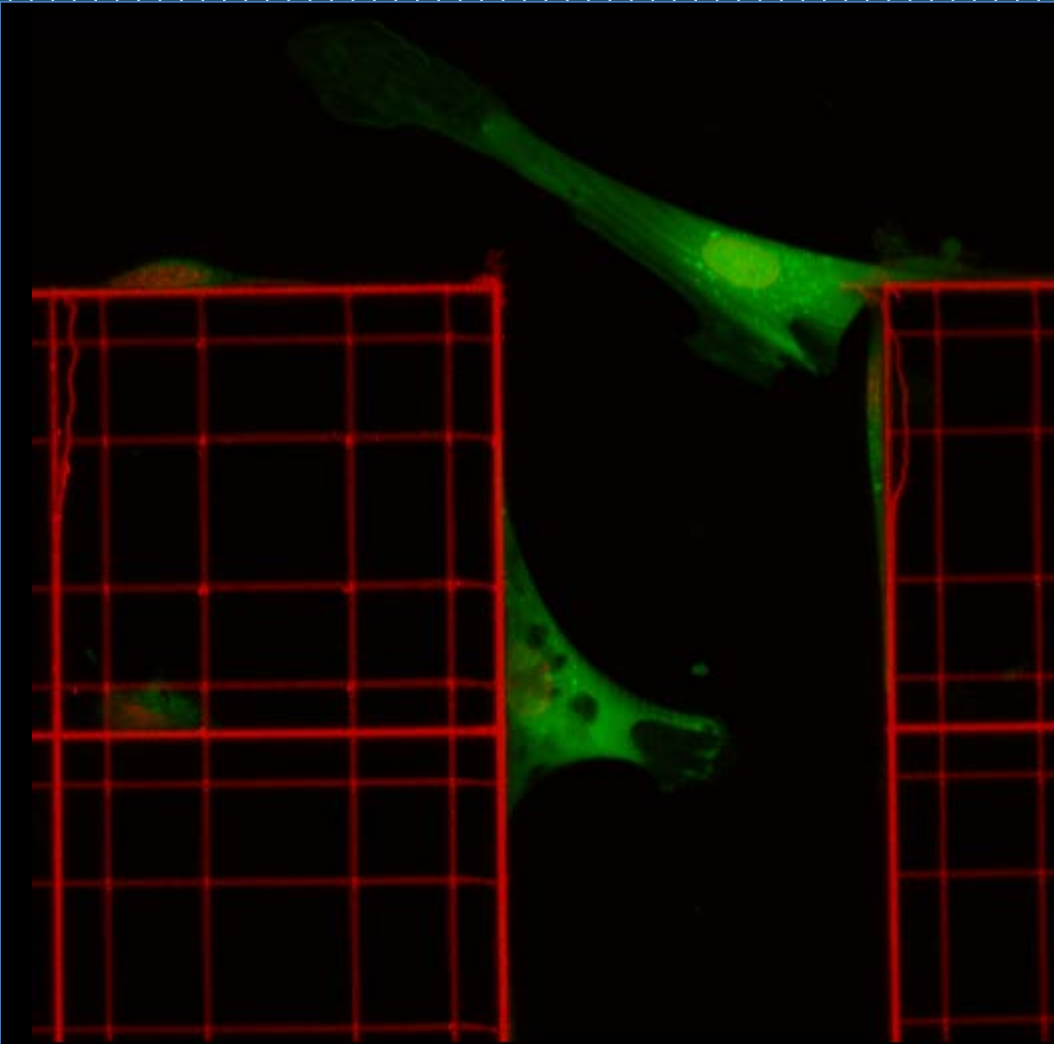




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POLITECNICO  
MILANO 1863



*Thanks for your  
kind attention*

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