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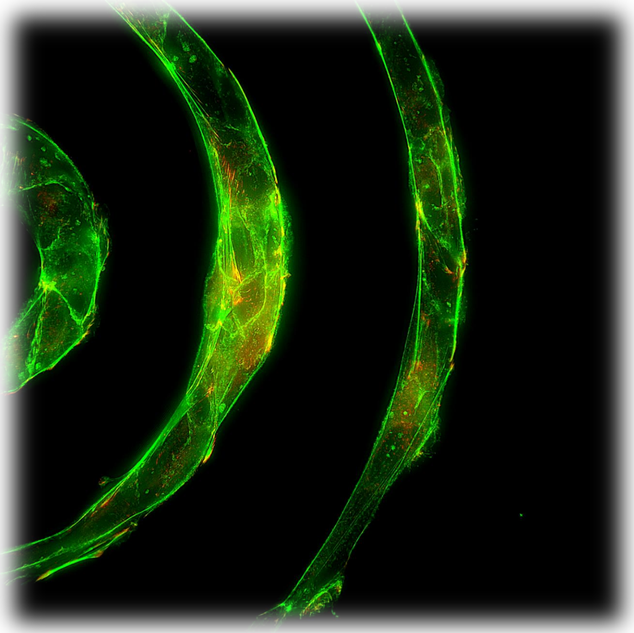
PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE

Cellular Basis of Mechanobiology:
Concepts and Tools



Andrea Ravasio
and.ravasio@gmail.com

Laboratory for Mechanobiology of Transforming Systems

Institute for Biological and Medical Engineering (IIBM)
Pontificia Universidad Católica de Chile

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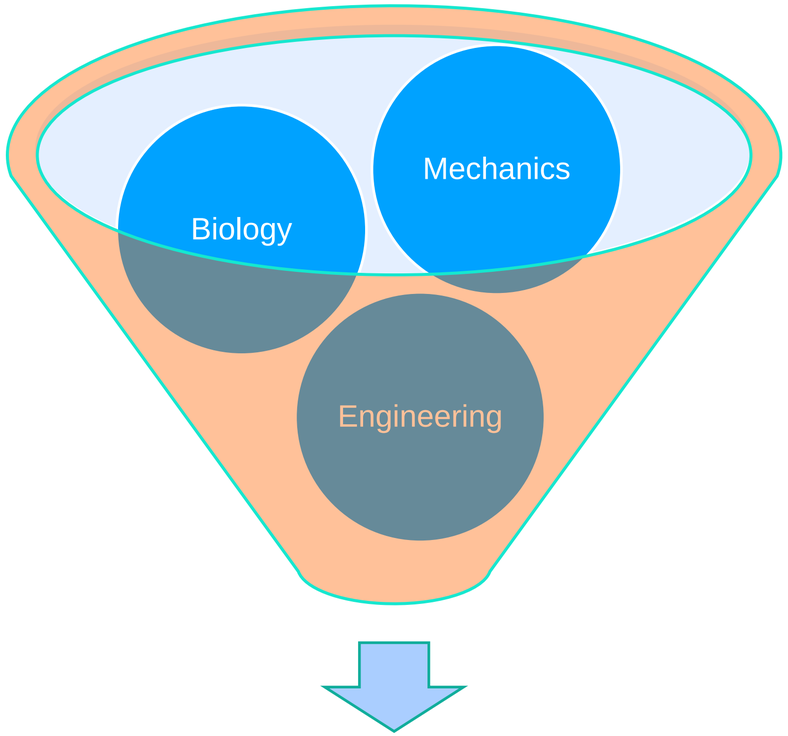
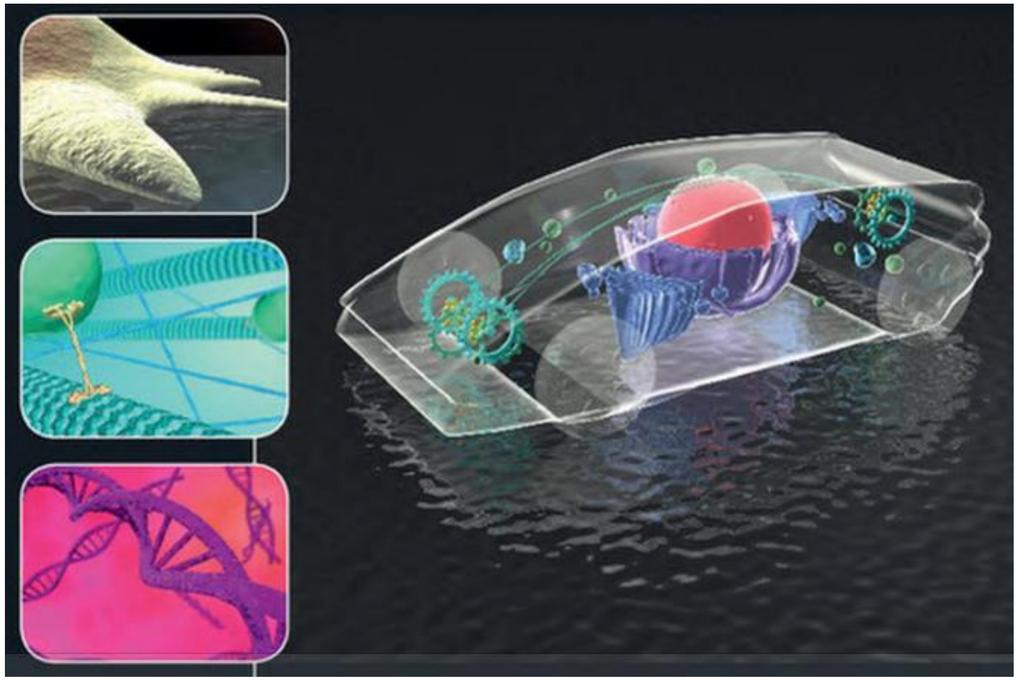
Content

- *What is mechanobiology*
- *Theoretical tools – reverse engineering*
- *Experimental tools – bio-microfabrication and bioengineering*

What is mechanobiology? A loose definition



Mechanobiology



Micheal Sheetz, Hanry Yu. **The Cell as a Machine** *Cambridge texts in Biomedical Engineering*

uses physics and engineering perspective to describe multiscale events occurring within the boundaries of living organisms.

What is mechanobiology? Hardware, applications and functions

Gaming

What is mechanobiology? Hardware, applications and functions

Applications

Hardware

Gaming

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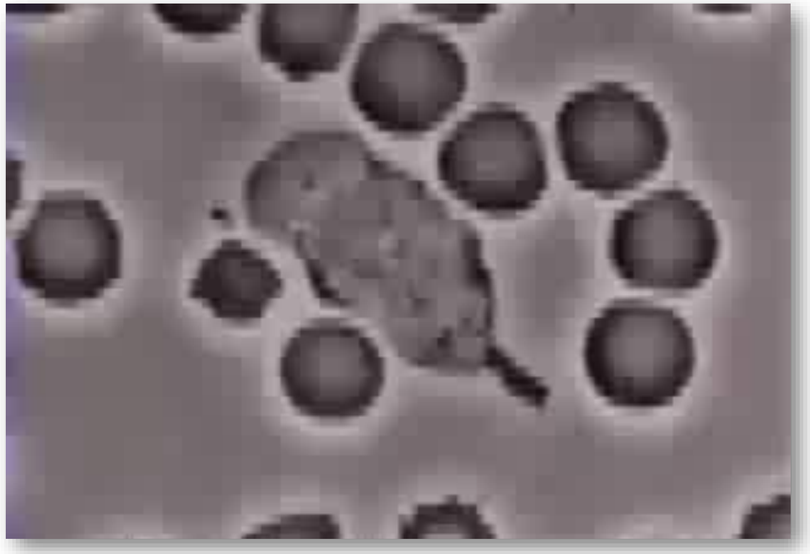
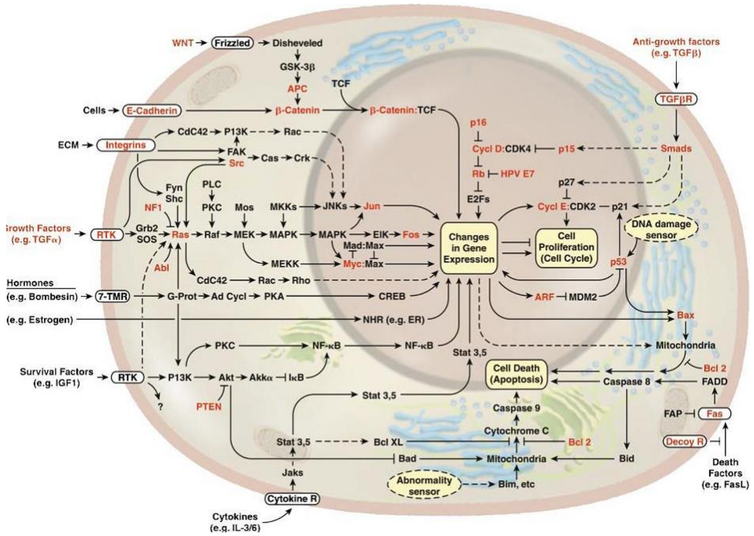
What is mechanobiology? Hardware, applications and functi

Functions



Immunosurveillance

Applications

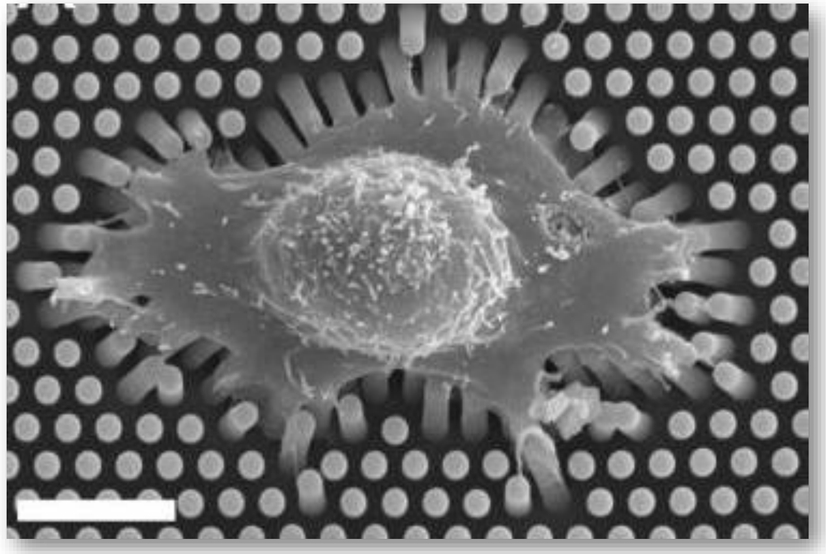


Cell signaling

(biochemistry/mol. biol.)

How the functions are controlled

Hardware



Mechanics of cell migration

(Mechanobiology)

How the system mechanically works

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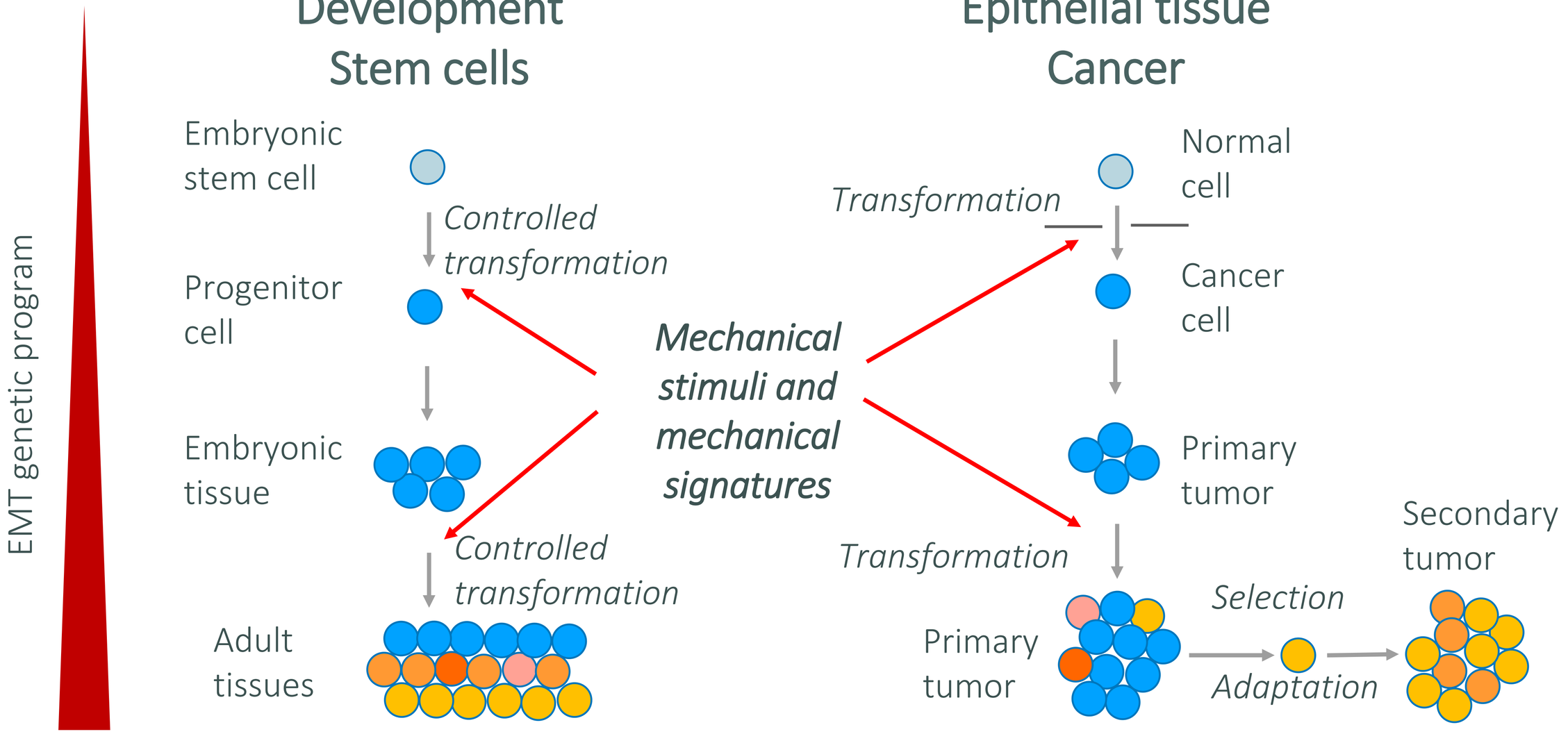
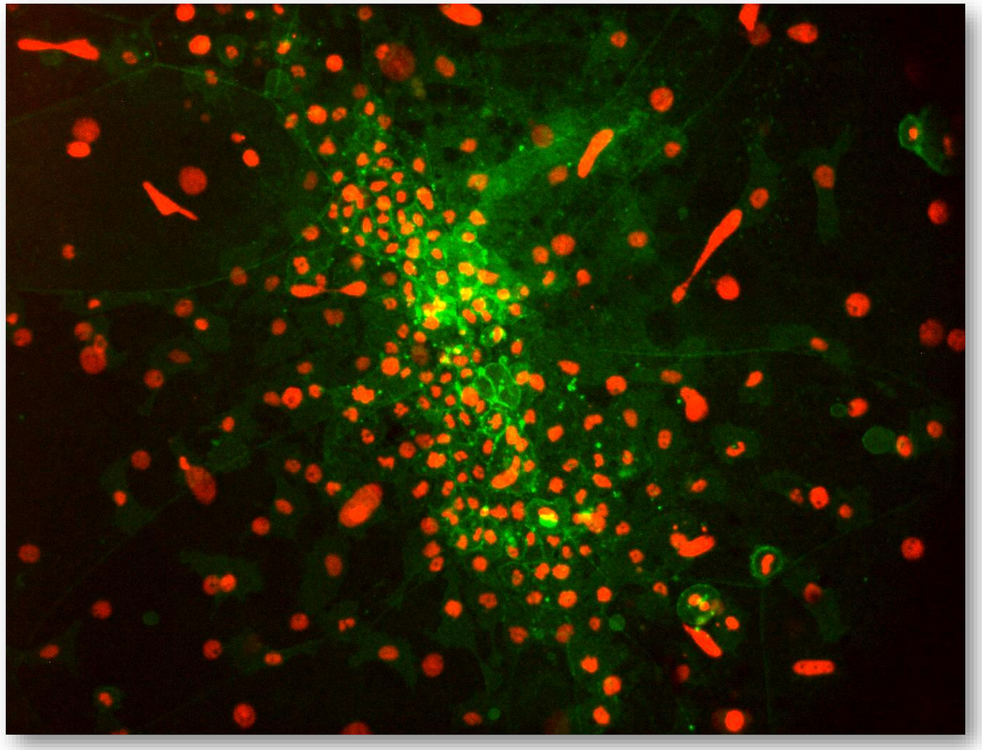
Mechanobiology of transforming systems



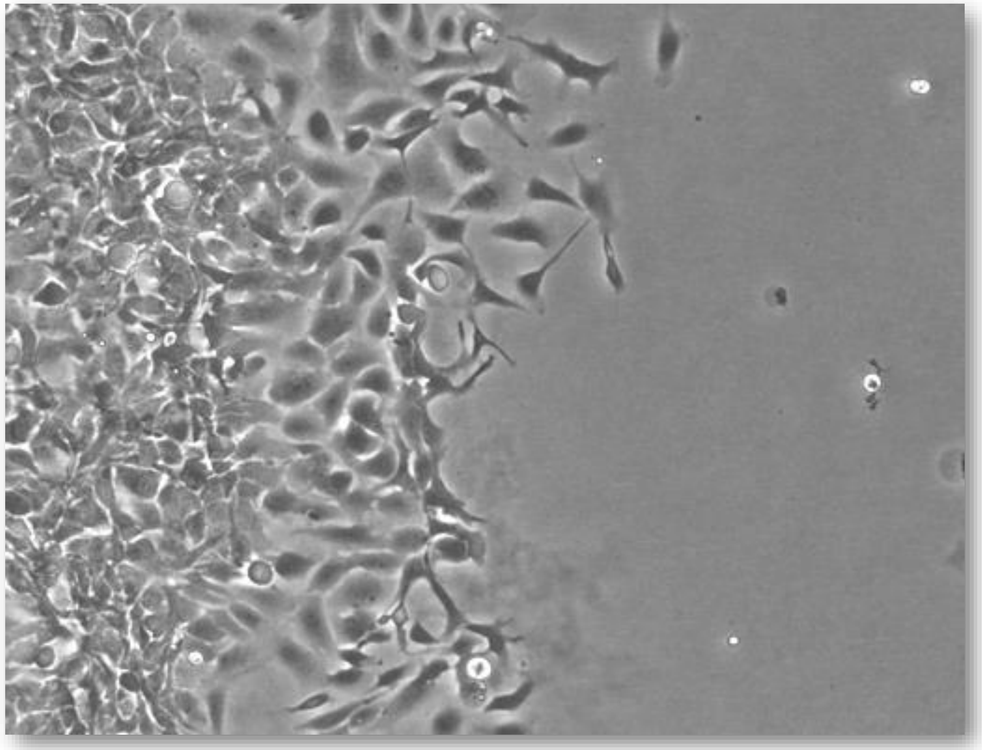
Mechanobiology of transforming systems

Aggregation of embryonic stem cells



Credits Mauricio Cerda, Miguel Concha

Disaggregation of cancer cells

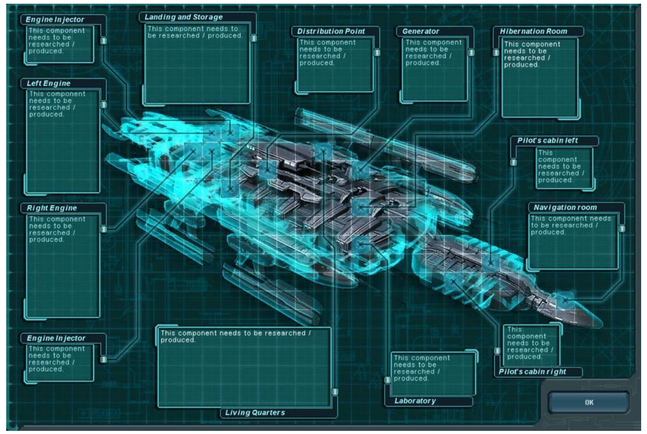


Theoretical tools - Reverse engineering

Reverse engineering an UFO

Discovery

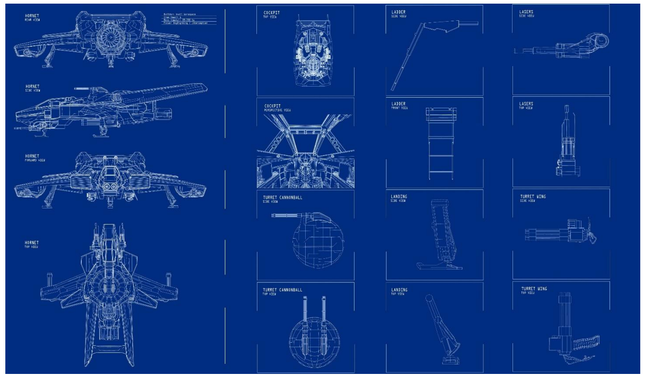
Integration of function



Description

List of known function and modules

Analysis of parts



Design of blueprints

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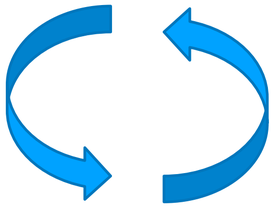
Engineering frameworks for biology

Thomas, Lee, Suh. **A Function-Based Framework for Undestanding Biological Systems.** *Annu. Rev. Biophys. Biomol. Struct.* 2004

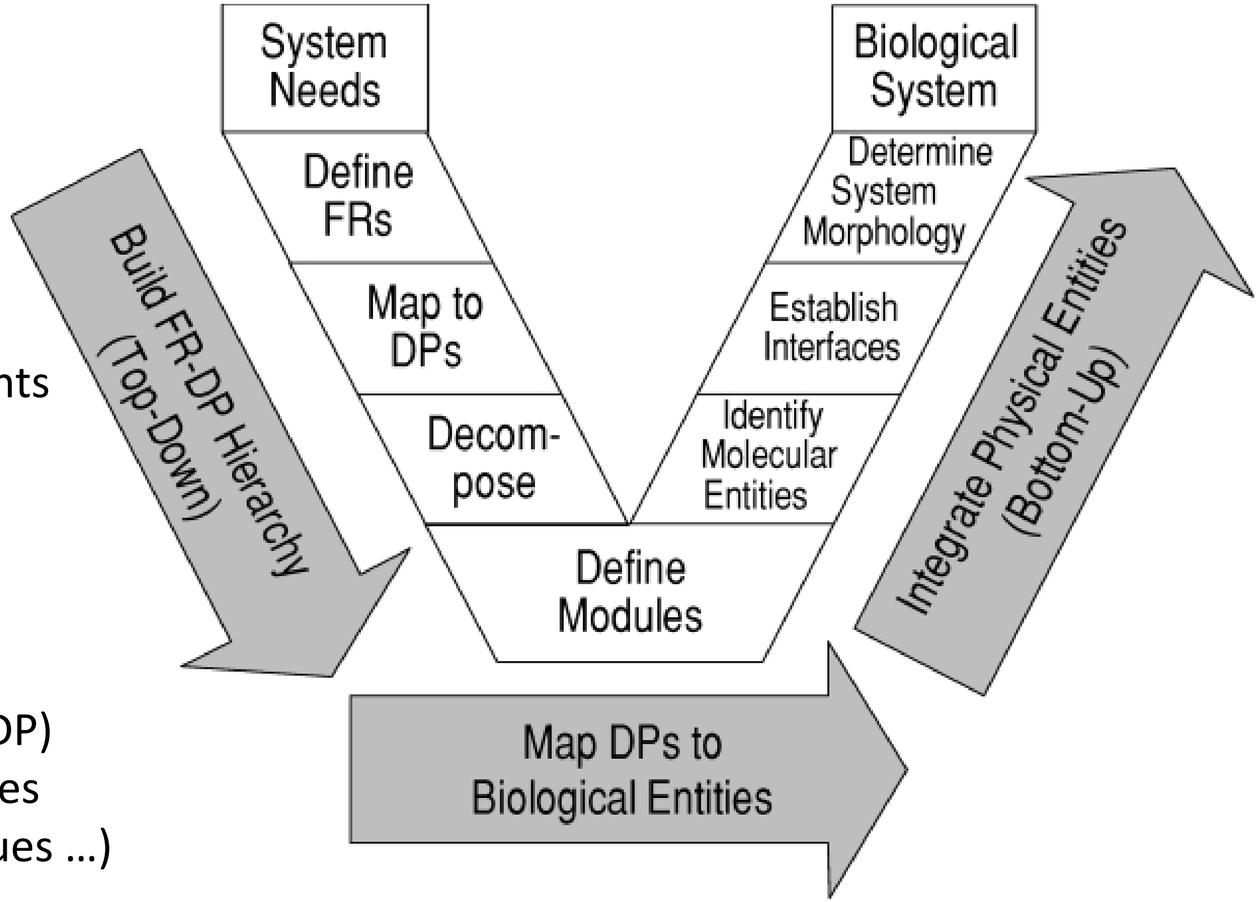
The Vmodel describes how the Design Matrix is used to study biological systems.

- First, one describes the functions a system must achieve (system needs and then FRs).
- FRs are then mapped to processes, tissues, cells, or other components (DPs) that provide the given function.
- This is done iteratively, zigzagging between FRs and DPs, in order to decompose the FR-DP hierarchy to the necessary completeness.
- Once the FR-DP relationships are specified, one can then integrate physical entities into a model that is based on functions.

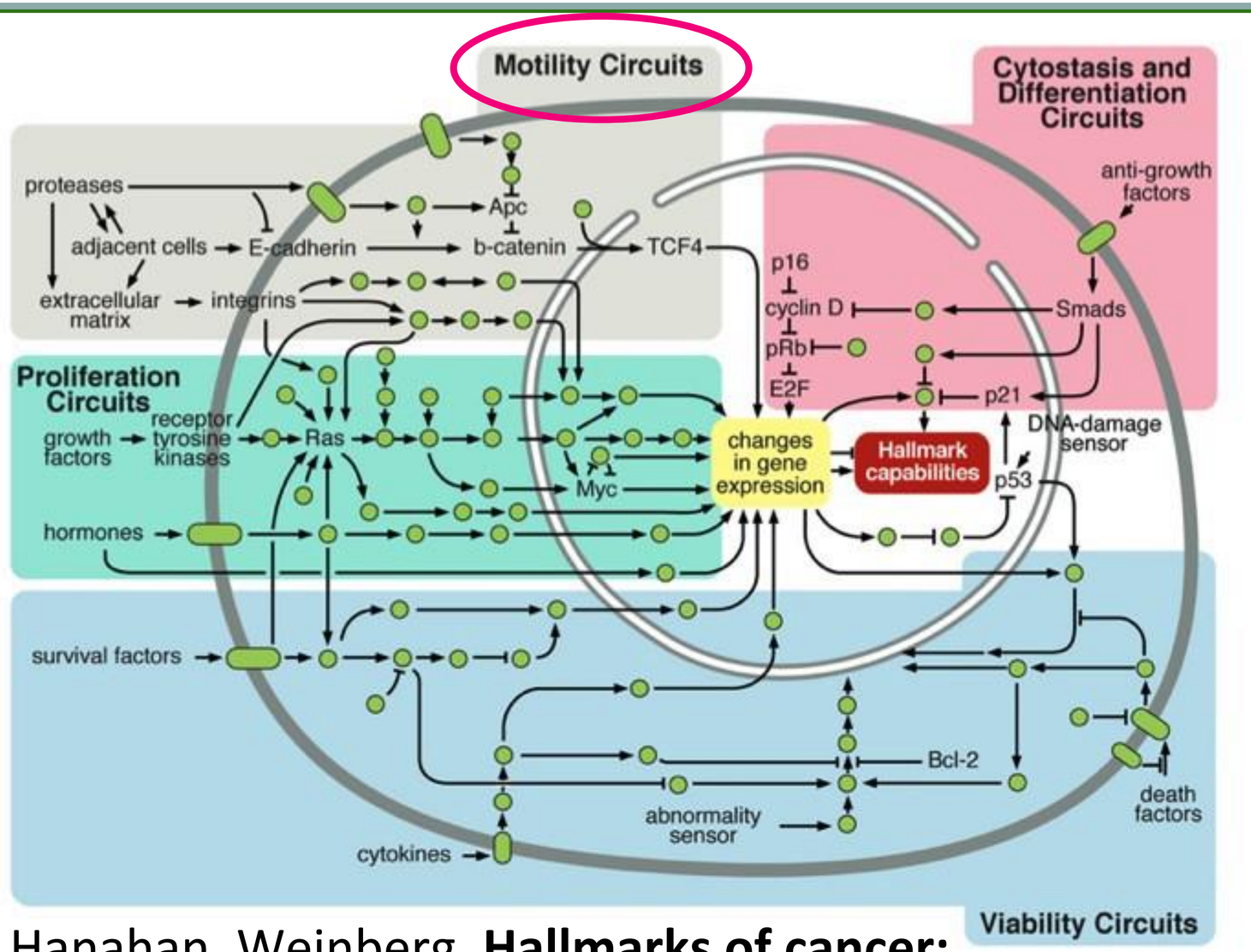
Function Requirements (FR)



Design Parameters (DP)
Or functional modules
(molecules – cells – tissues …)

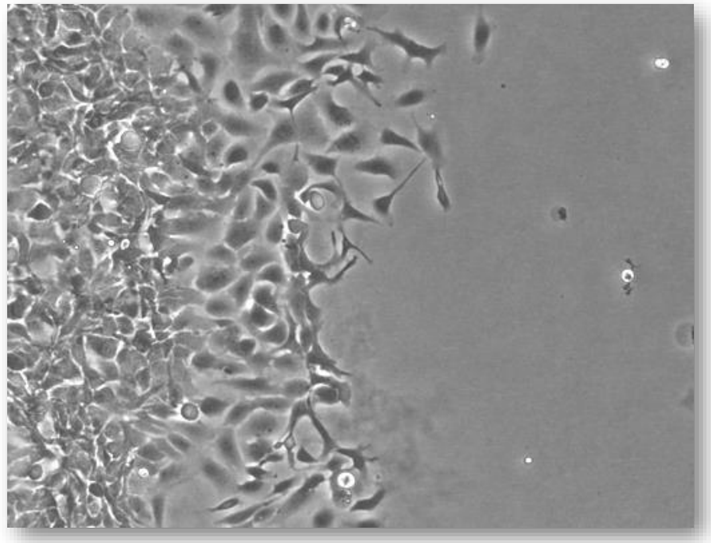


Reverse engineering of cancer transformation

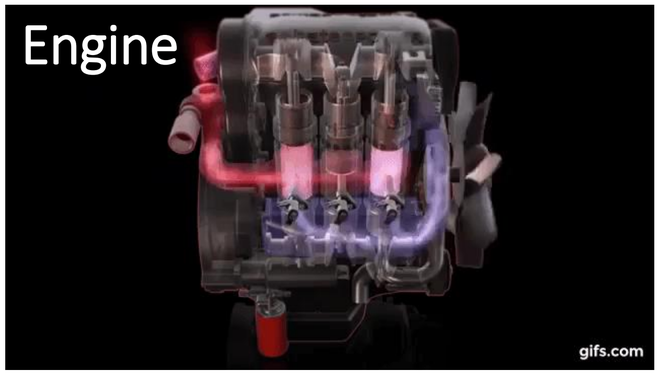


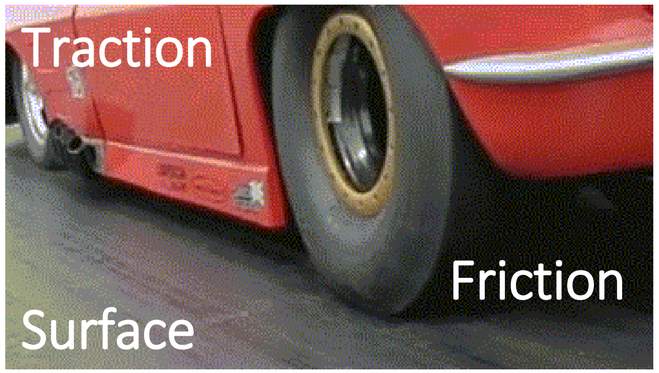
Hanahan, Weinberg. **Hallmarks of cancer: the next generation.** *Cell* 2011

Disaggregation of cancer cells



Collective dynamics in biology and engine

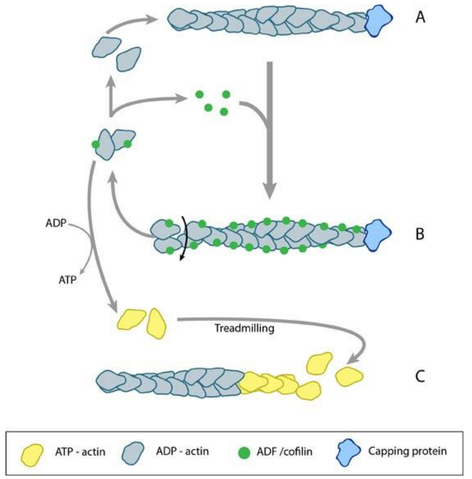


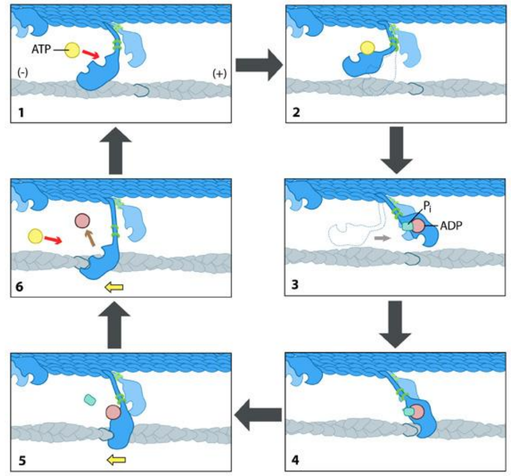


Cell engine

Actin polymerization
Actomyosin contractility

ATP → Forces

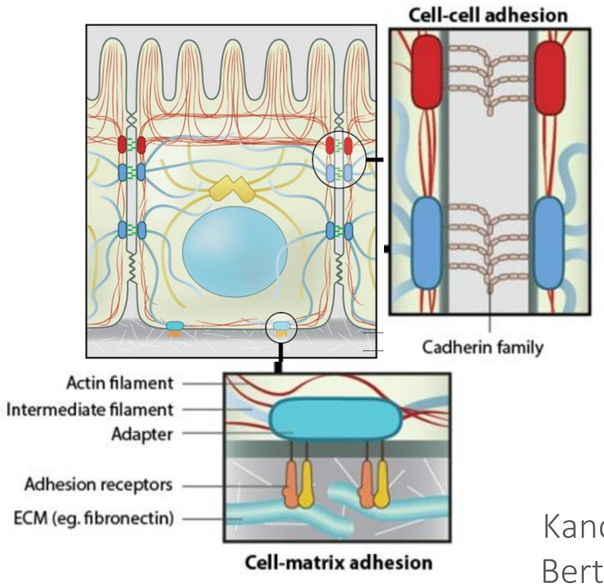


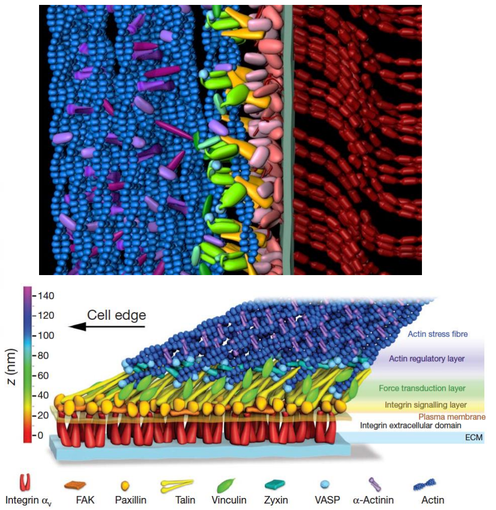


Cell ¨wheels and suspensions¨

Focal adhesion
Adherens junctions

Nanoscale mechanics of proteins





Kanchanawong P. et al. Nature *2010*
Bertocchi C. et al. Nat Cell Biol. *2017*

How do cells move?

Molecular scale *Cell scale*

Cell-cell adhesions

ATP

Actin dynamics

Engine always on

Focal adhesions

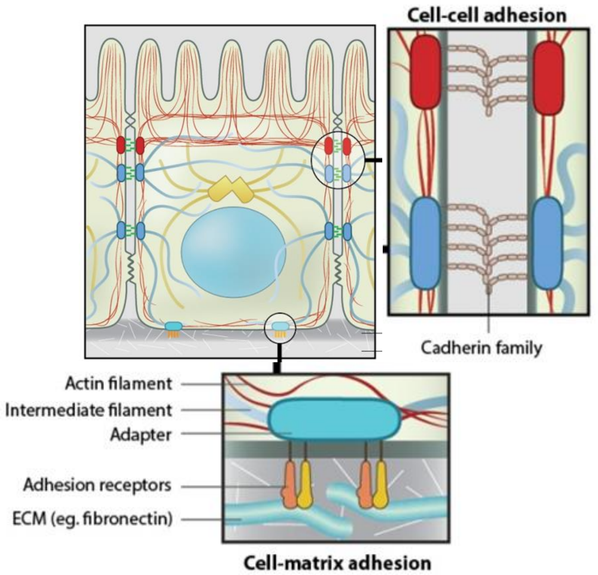
Differential engagement

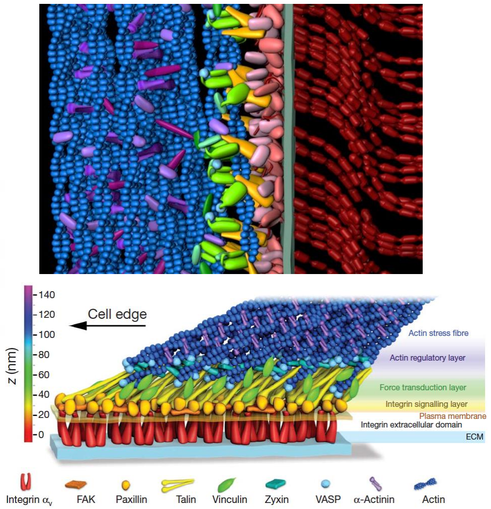
Cohesive forces

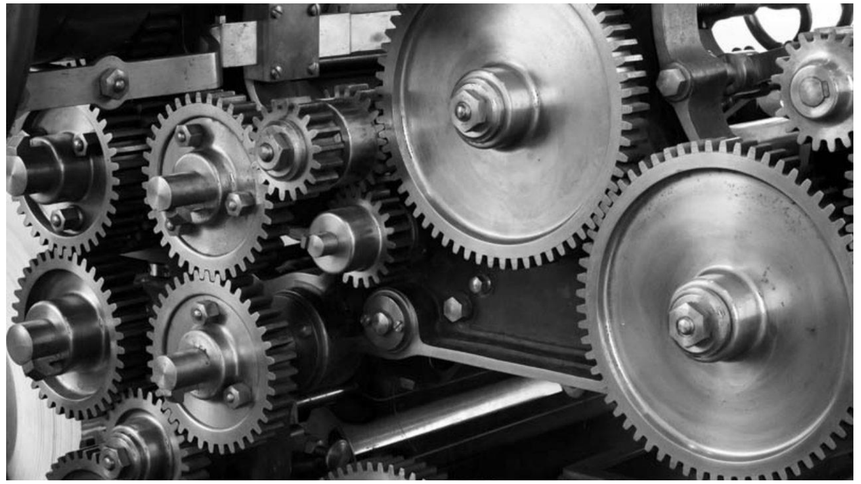
Cell contractility (*Cables*)
Cell protrusion (*Lamellipodia*)

Adhesive forces

Differential engagement







Basic multiscale model of collective cell migration

Molecular scale

Cell scale

Tissue scale

Cell-cell adhesions

ATP

Actin dynamics

Engine always on

Focal adhesions

Differential engagement

Differential engagement

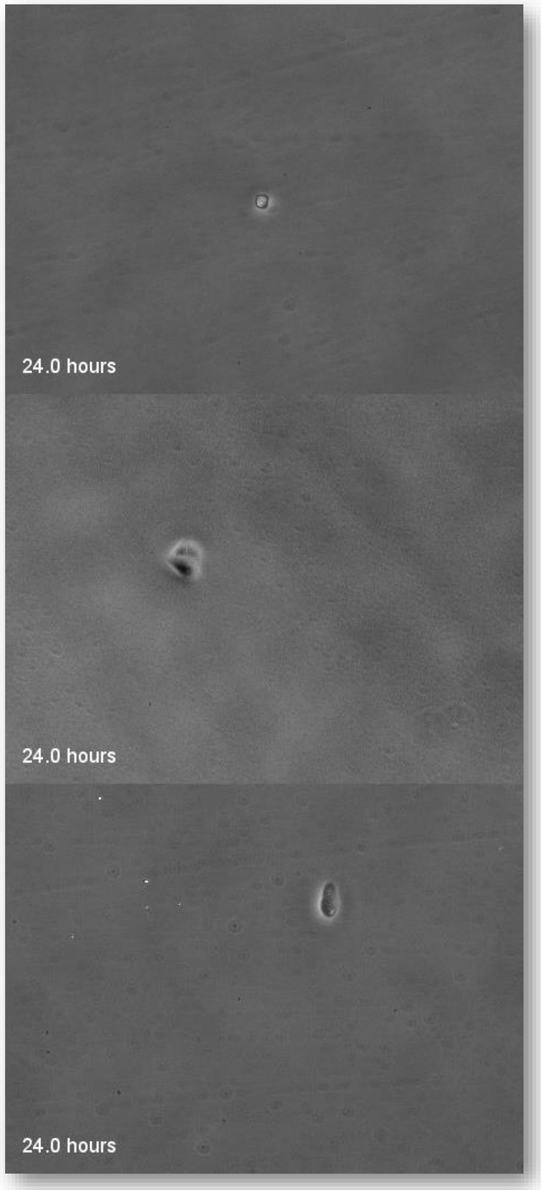
Cohesive forces

Cell contractility (*Cables*)
Cell protrusion (*Lamellipodia*)

Adhesive forces

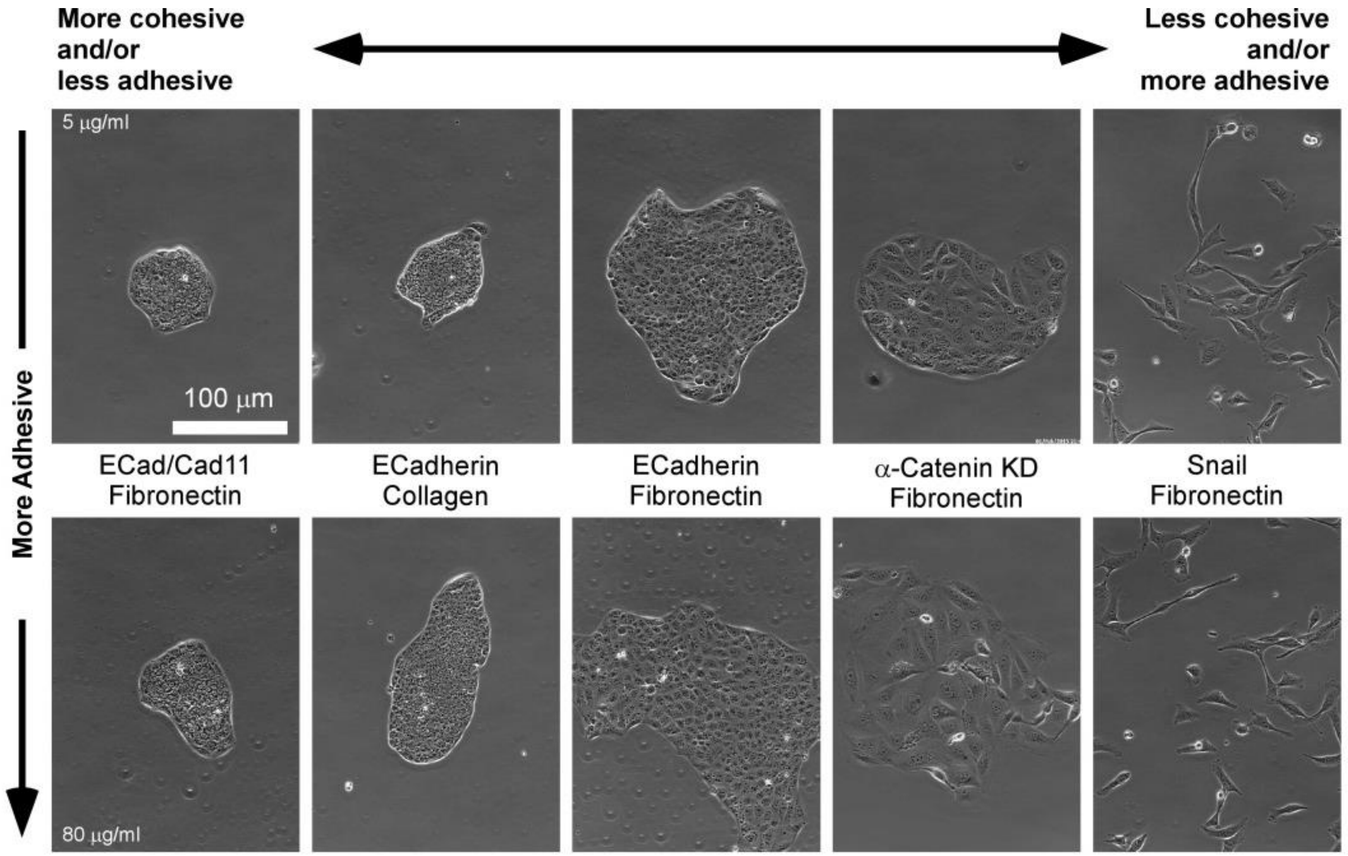
ECM protein coating

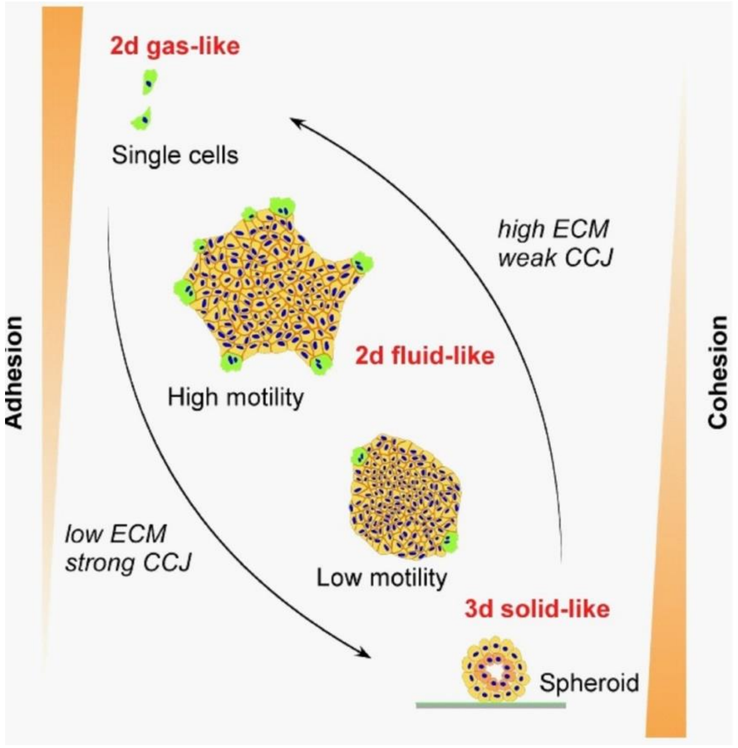
Active wetting

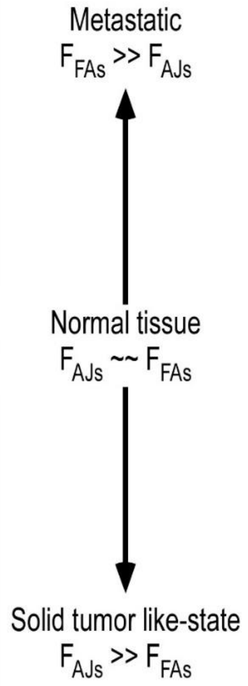


Ravasio A. et al. Int. Biol. 2015

Engineering cell transformation

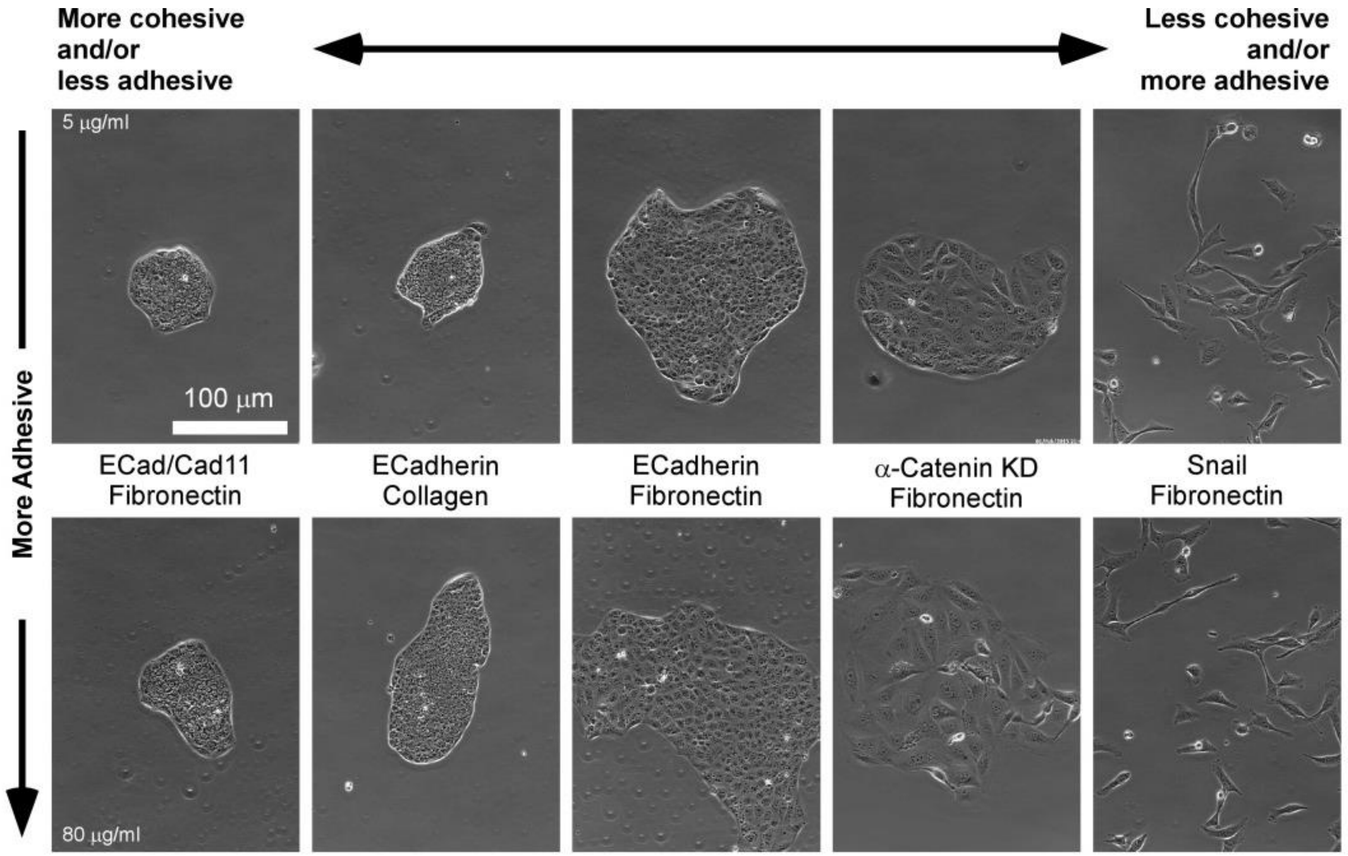


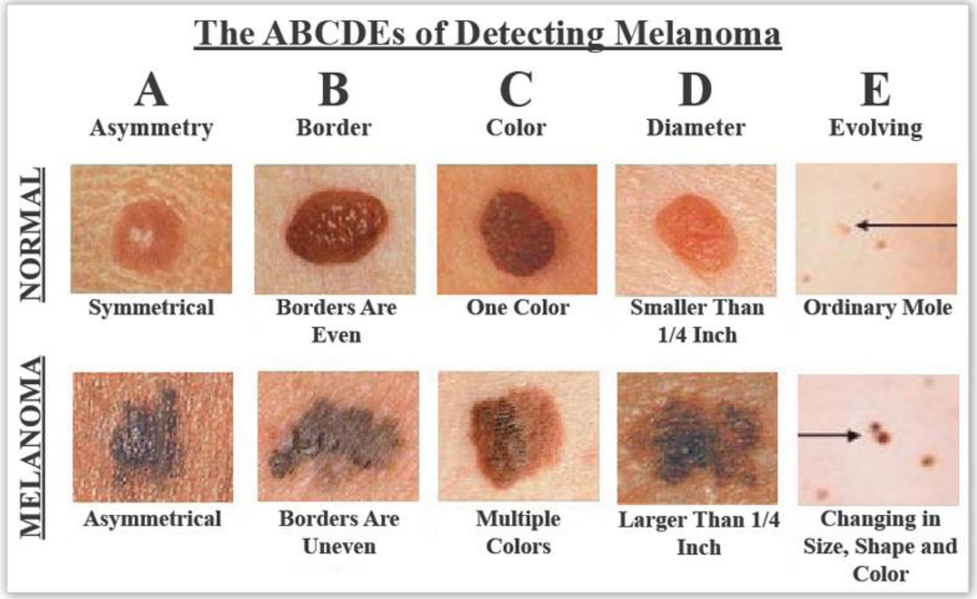




Ravasio A. et al. Int. Biol. 2015

Take home massage



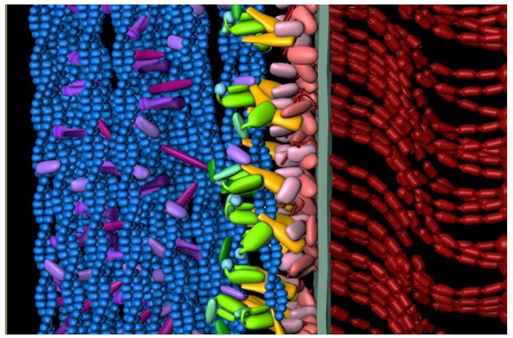


Ravasio A. et al. Int. Biol. 2015

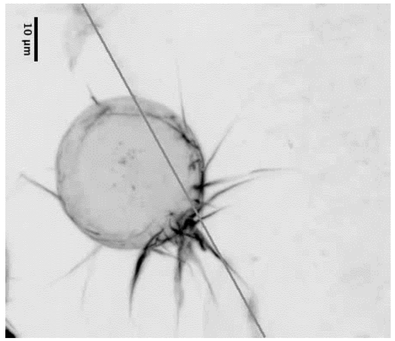
Experimental tools – Bio-microfab & bioengineering

Multiscale mechanics of Killifish development

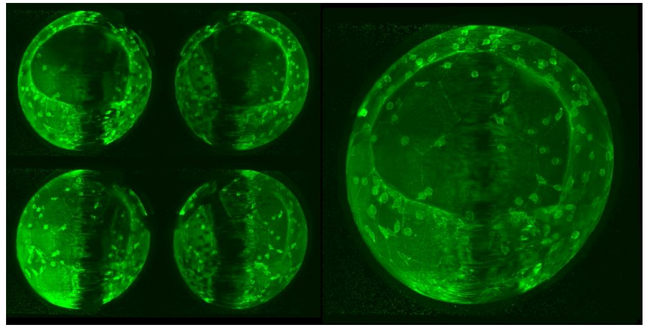
Molecular scale



Cell scale

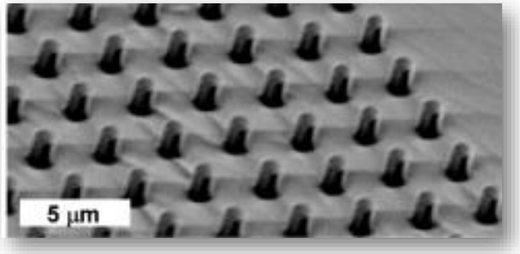


Embryo/tissue scale

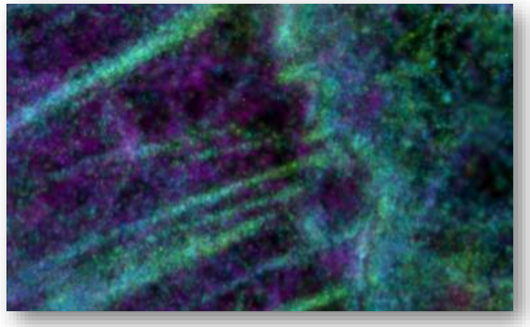


- In vitro
- In vivo
- In silico

Bioengineering



Microscopy



Cell biology

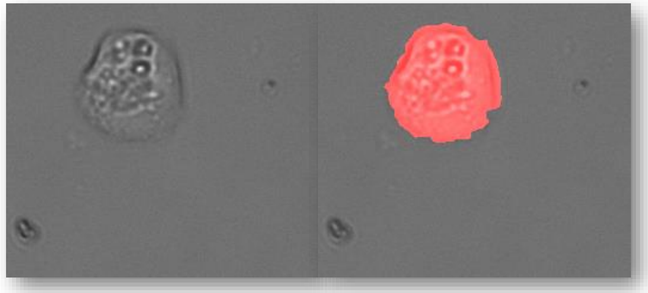
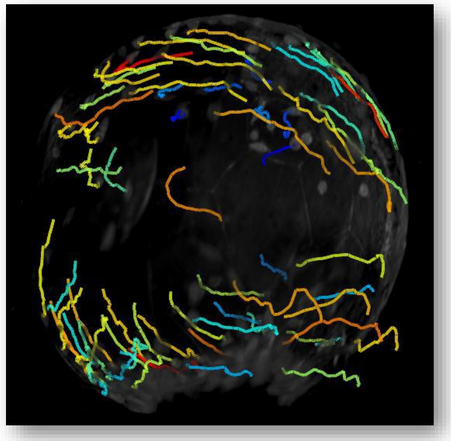
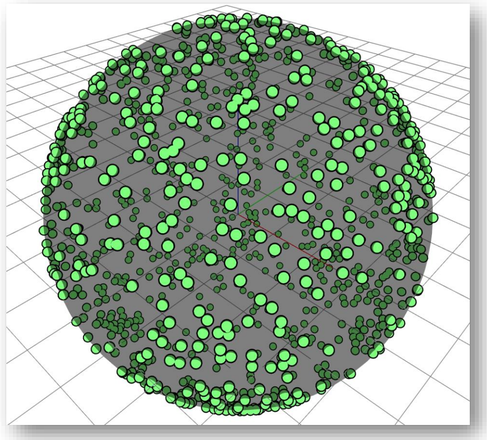


Image analysis



Mathematical modeling



Length, time and energy scales of life

Molecular scale **System scale**

Length

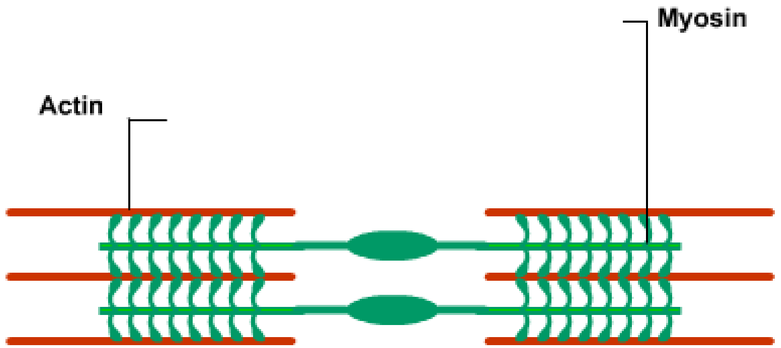
nm → *m*

Time

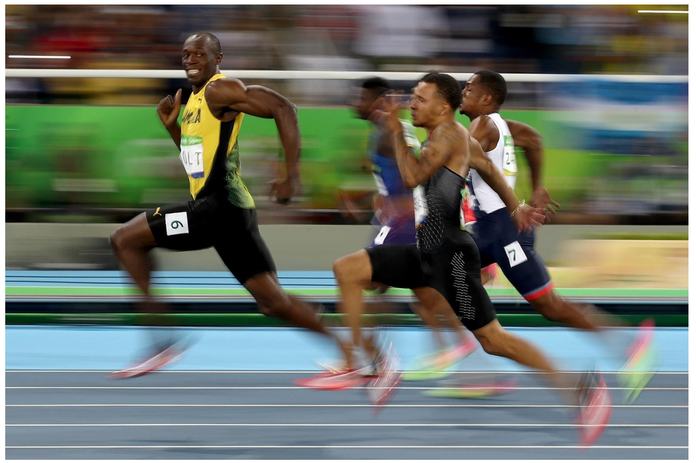
psec → *sec to centuries*

Energy

$4x10^{-21}$ *J* →



Myosin stroke (one ATP molecule)
4pN over few nm
$4(10^{-12})(10^{-9}) = 4x10^{-21}$ Nm (J)
Usain Bolt
100m in 9.52sec
10^{5} J

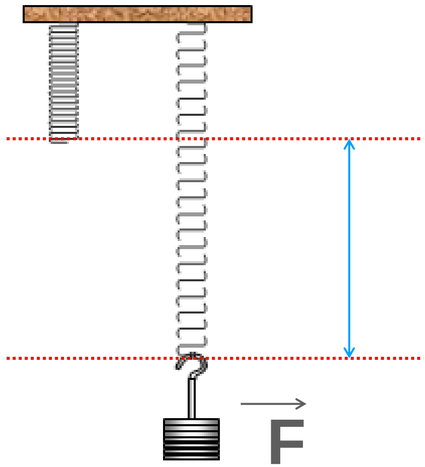


How to measure forces

$F = k\,X$

X → displacement

k → spring constant



Spring Scale

Use: measuring the amount of force generated by or acting on an object

Quantity being measured: force

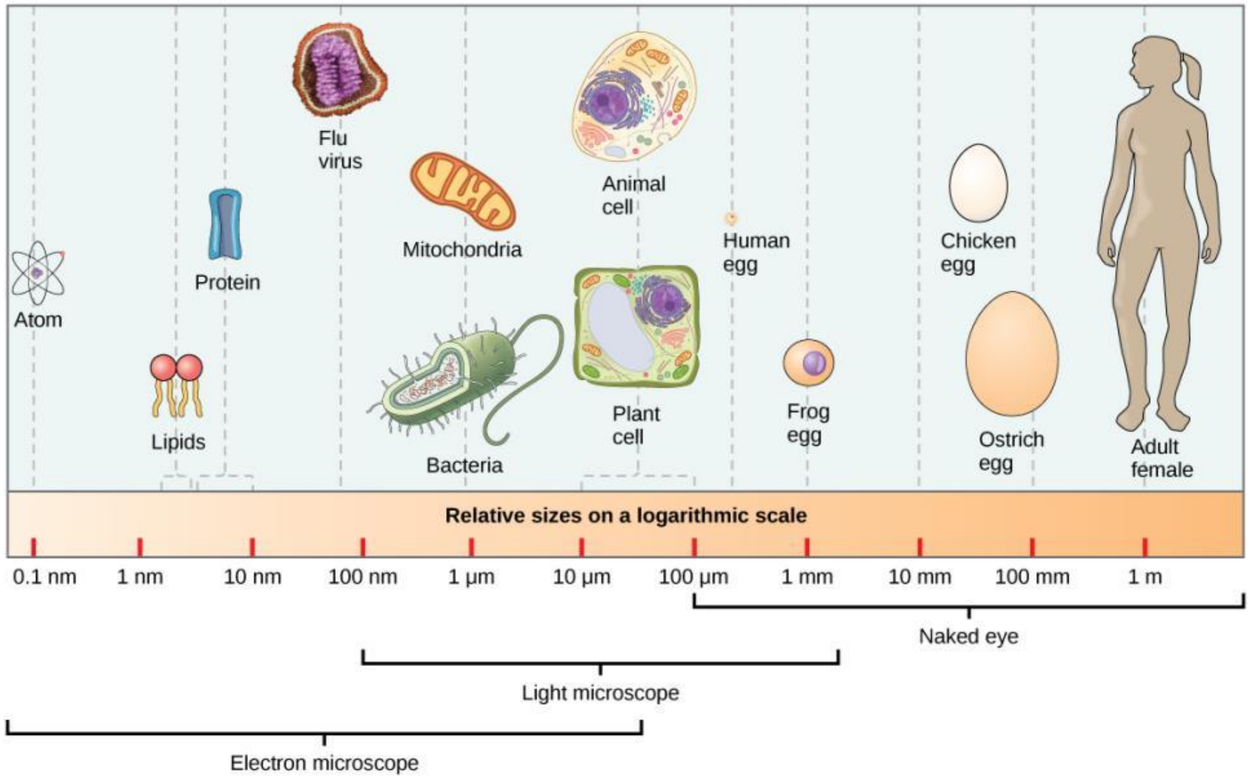
$$1\ \mathrm{N} = 1\ \mathrm{kg}\frac{\mathrm{m}}{\mathrm{s}^2}$$

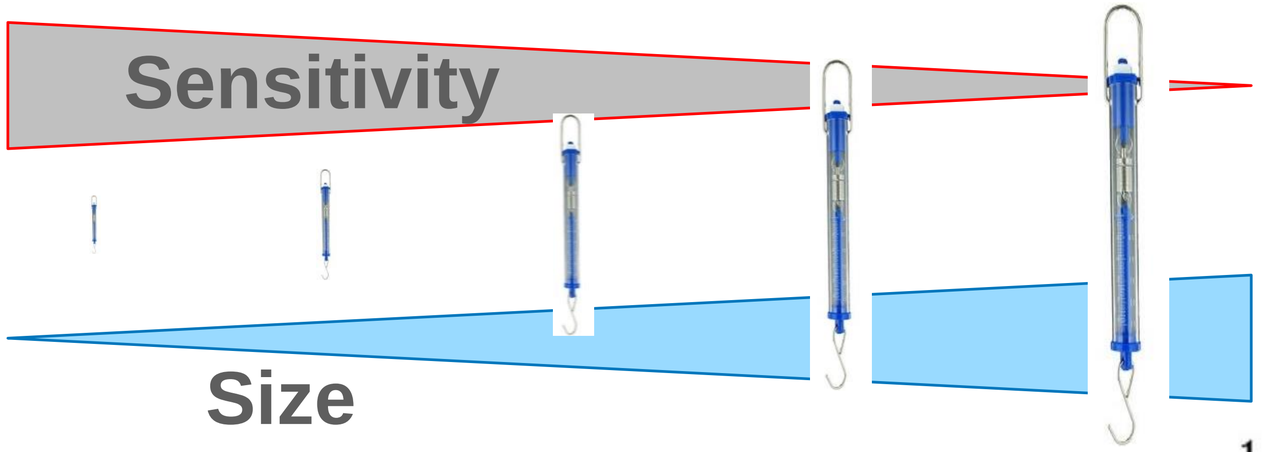
Traction forces in everyday life

Myosin size = 10^{-9} m
Myosin forces = 10^{-12} N

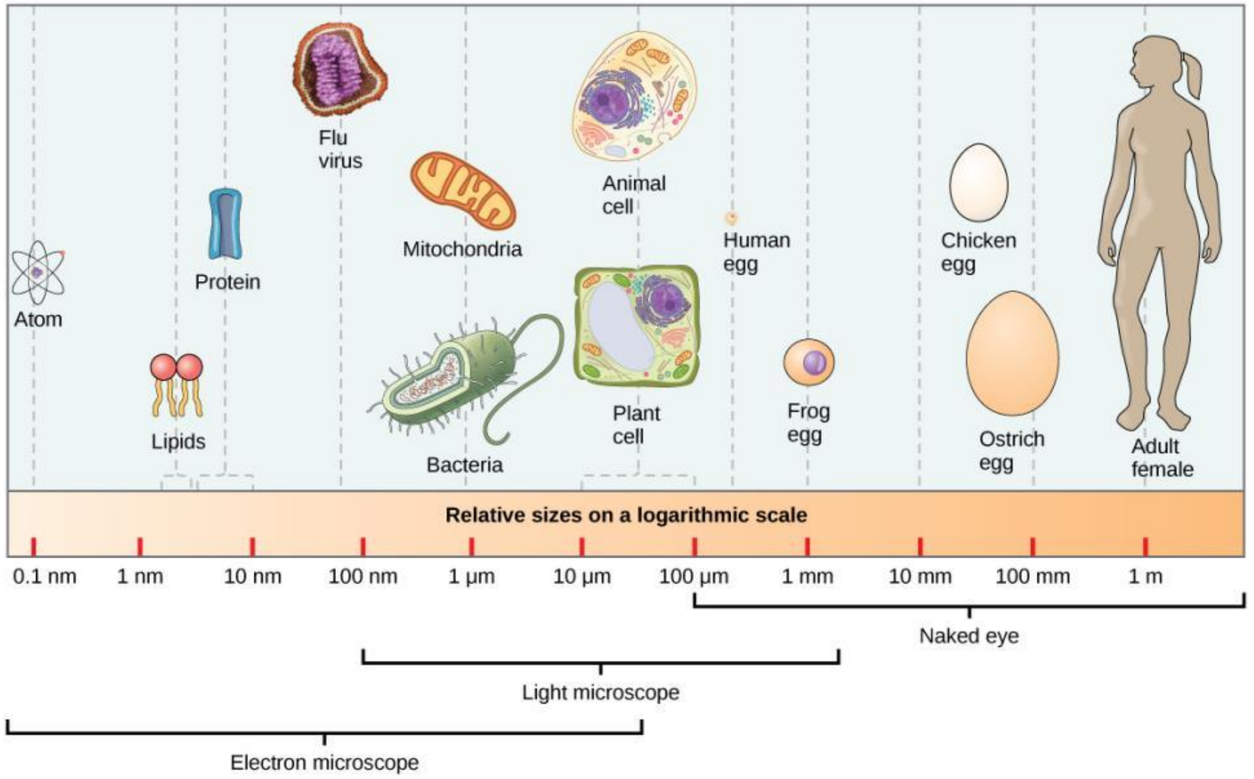
Cell size = 10^{-5} m
Cell forces = 10^{-9} N

$$1\ \mathrm{N} = 1\ \mathrm{kg}\frac{\mathrm{m}}{\mathrm{s}^2}$$

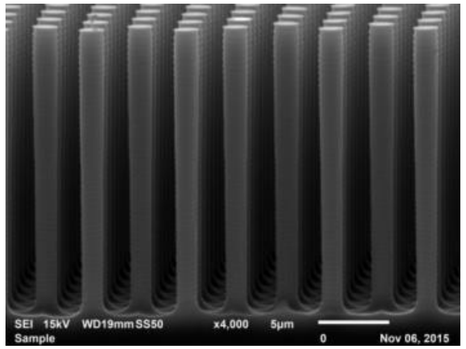


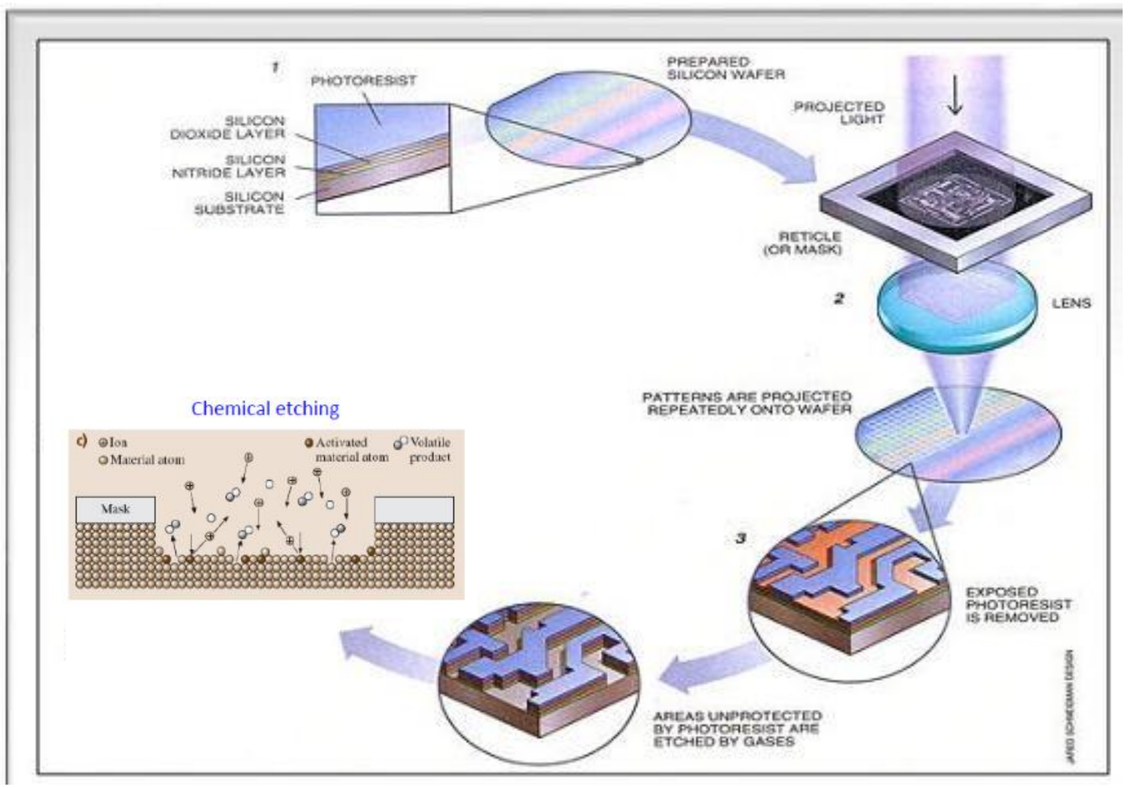


$$1\ \mathrm{N} = 1\ \mathrm{kg}\frac{\mathrm{m}}{\mathrm{s}^2}$$

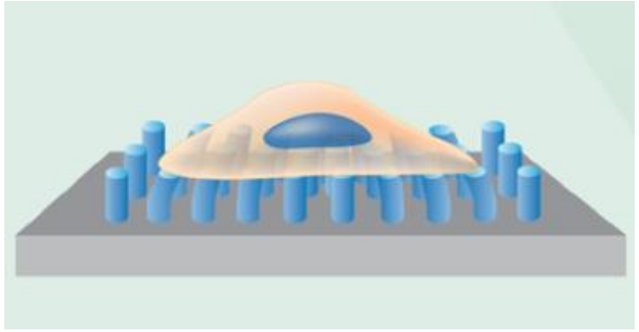


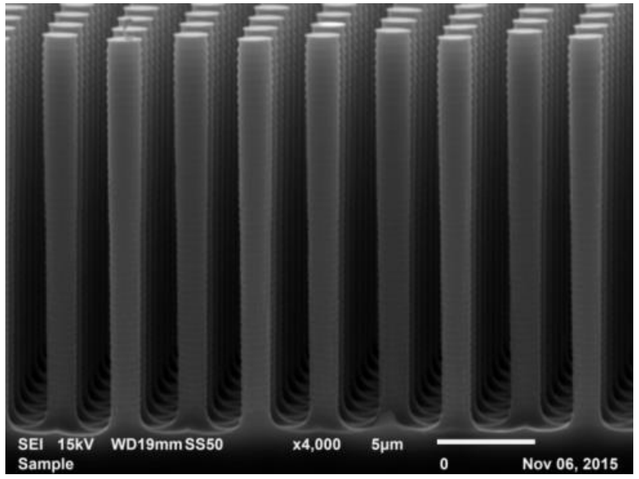
Microfabrication (the making of small “springs”)

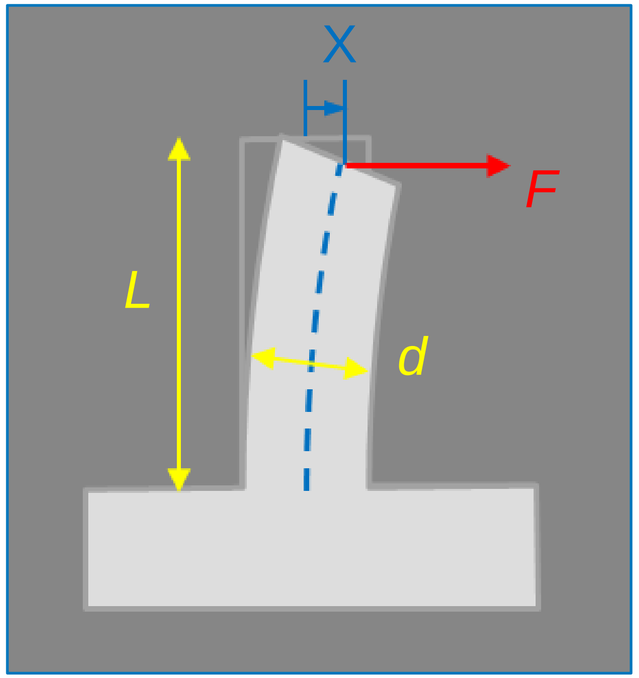




Microfabrication (the making of small “springs”)







$$F = k\,X \qquad k = \frac{3EI}{L^3}$$

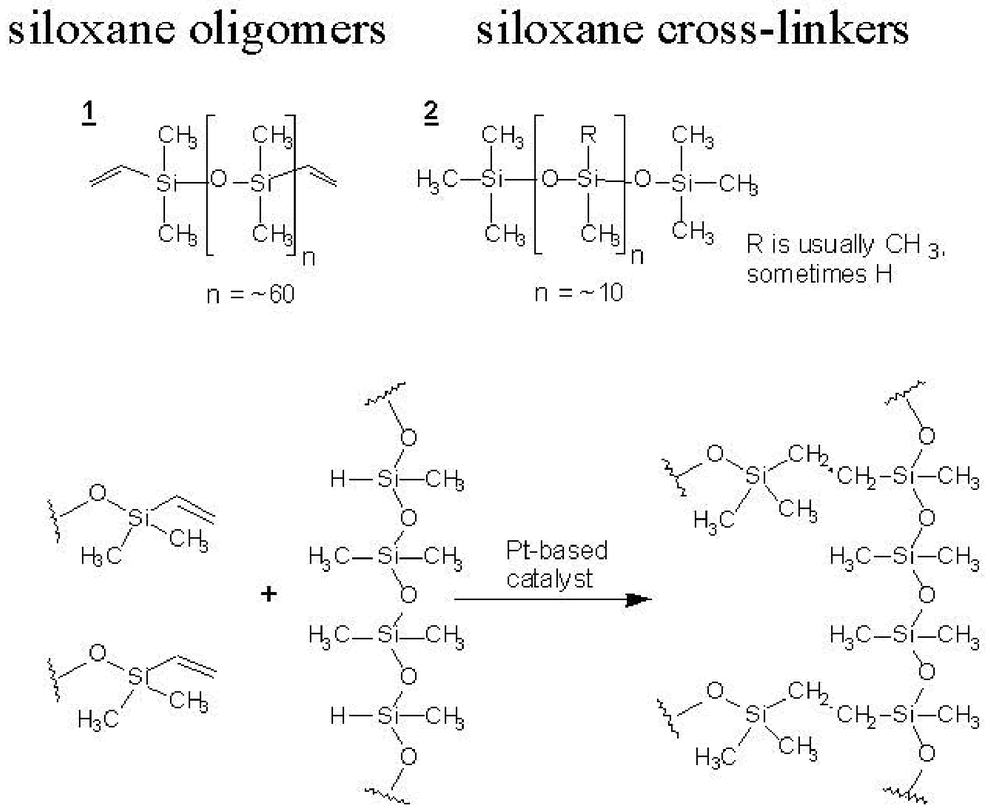
X → displacement

k → spring constant

$$I = \frac{\pi d^4}{64}$$

E (Young’s modulus) is a material property

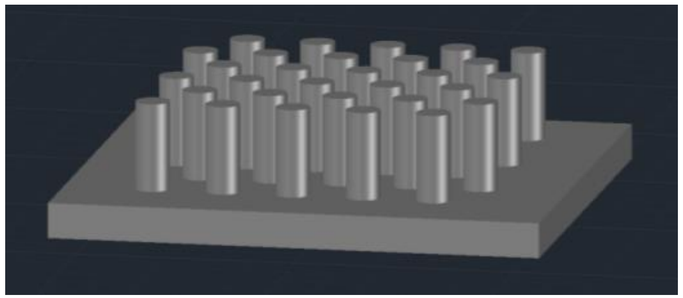
Microfabrication (the making of small “springs”)

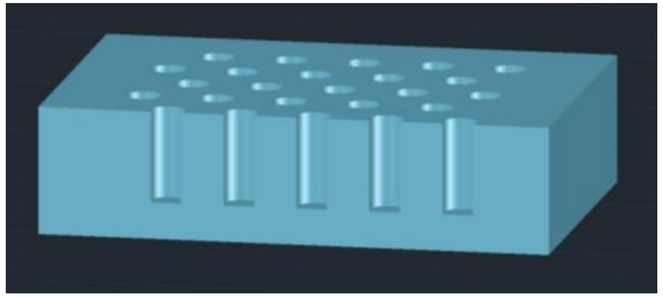


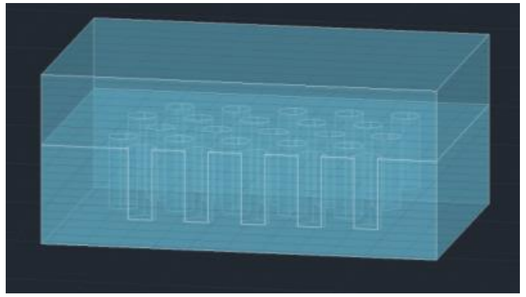
lydimethylsiloxane (PDMS) is an organosilicon

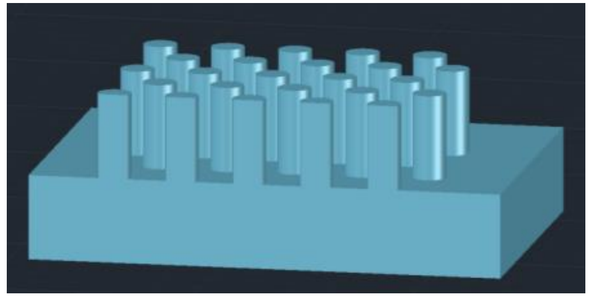
Standard formulation, is 10:1 ratio, which gives ~1 MPa Young’s modulus

Soft lithography (PDMS replica molding)







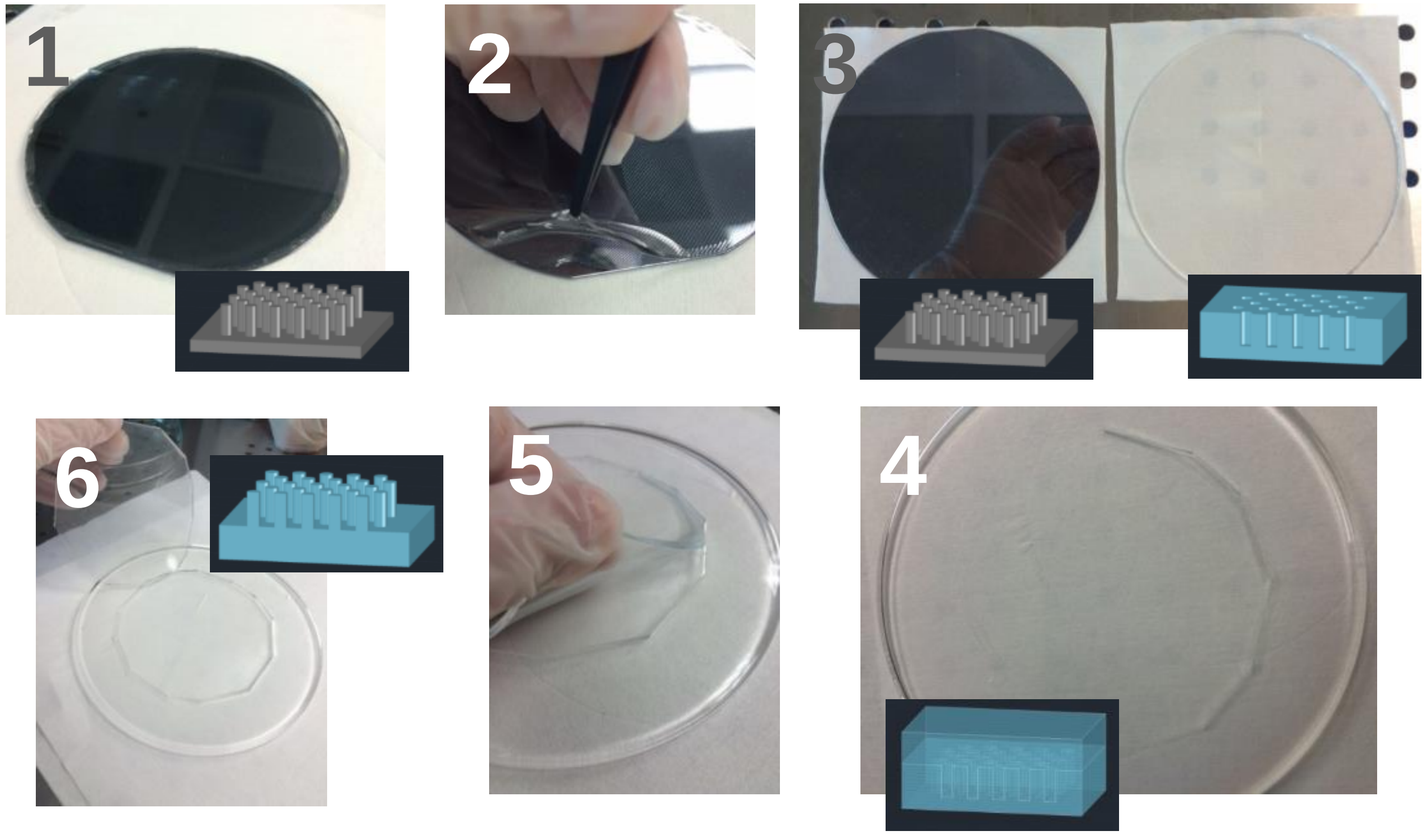


Casting and curing of PDMS onto PDMS

Final PDMS device

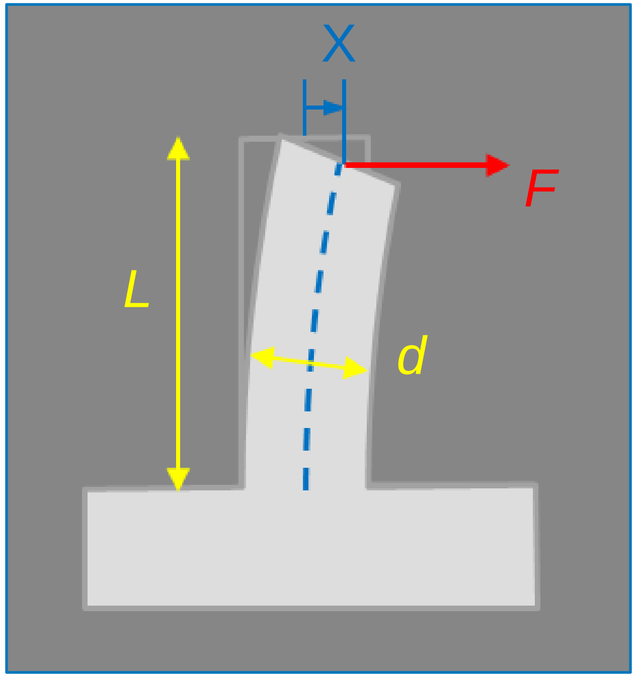
Images from Gianluca Grenci (MBI microfabrication facility manager)

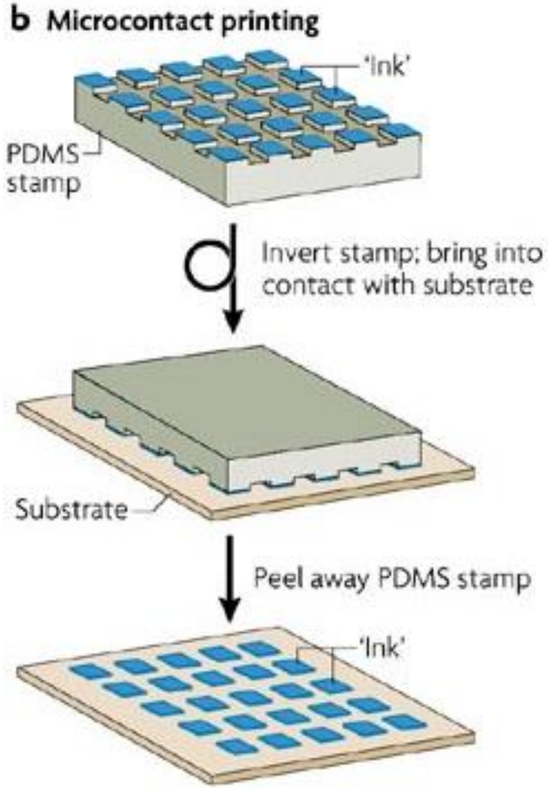
Soft lithography (PDMS replica molding)



Images from Gianluca Grenci (Head of MBI microfabrication)

PDMS functionalization (µcontact printing)





Pillars to measure cell traction forces

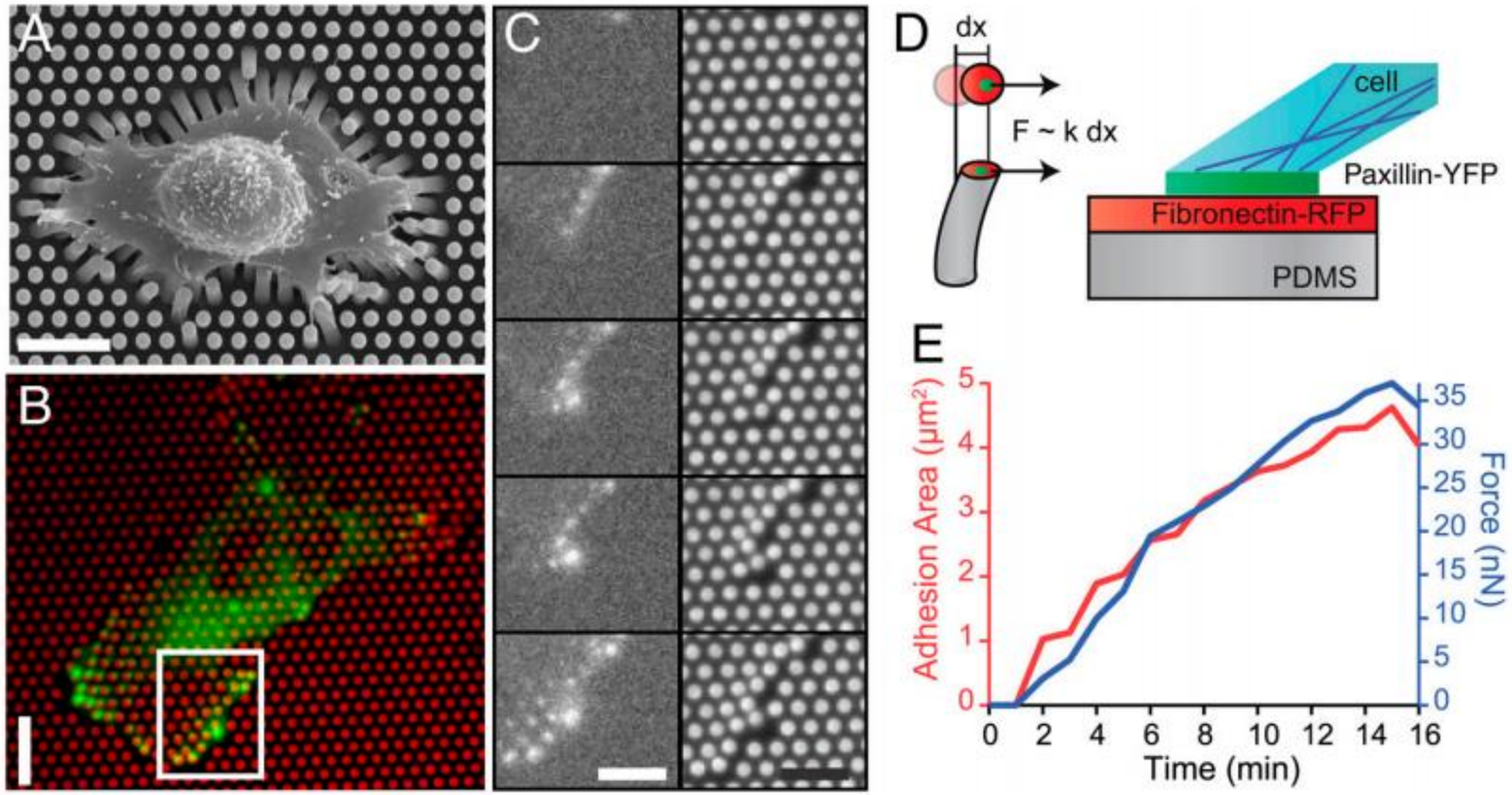
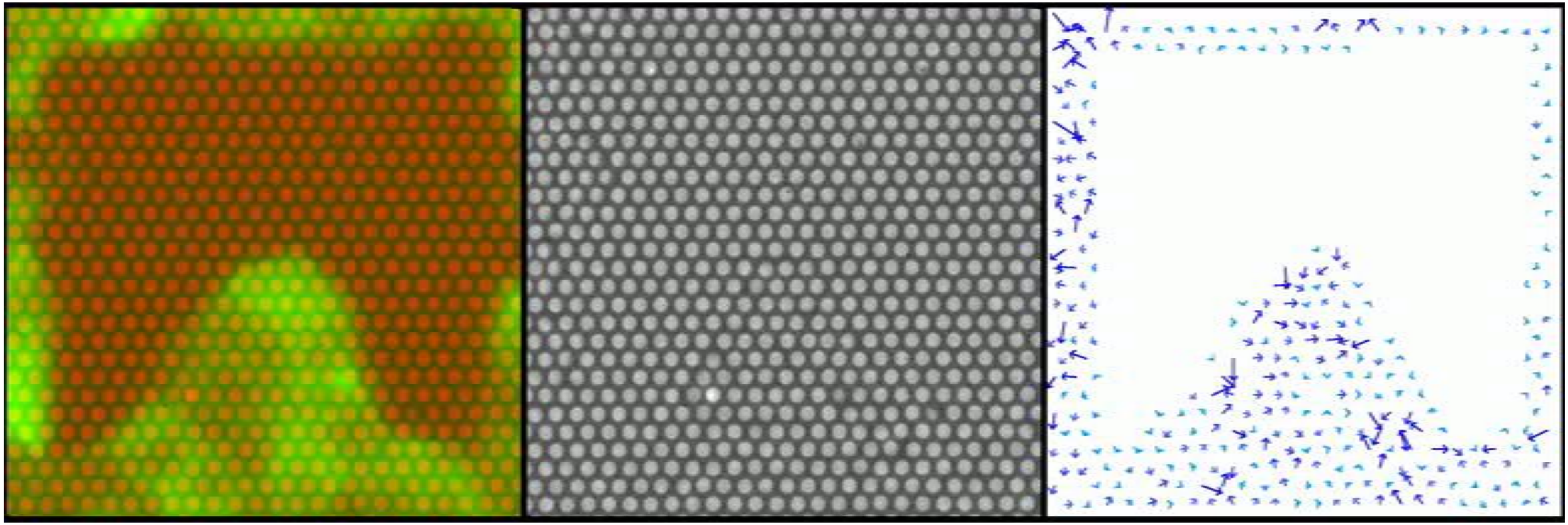


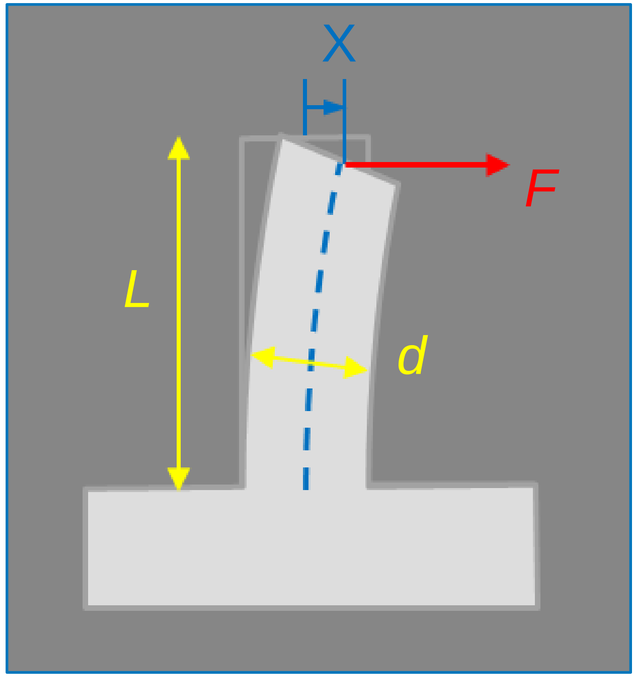
Fig. 1. Cell adhesion and traction forces developed by REF52 fibroblasts expressing YFP-paxillin on micropillar substrates. (*A*) Scanning electron micrograph image of a typical REF52 cell on a micropillar substrate. (Scale bar, 15 μm.) (*B*) Epifluorescent image of a single cell deforming the micropillar substrate (here of spring constant $k = 34$ nN/μm). Micropillars are labeled by Cy3-fibronectin (red), and YFP-paxillin-rich patches are in green. (Scale bar, 15 μm.) (*C*) Sequential images of the insert area of *B* showing the dynamics of FA growth and micropillar displacements. (Scale bar, 10 μm.) (*D*) Schematic representation of the experimental setup showing the formation of FAs on the top of a PDMS micropillar. (*E*) Typical example of the formation of an FA area (red) and the buildup of force (blue) as a function of time (on a substrate of 34 nN/μm).

Pillars to measure tissue traction forces



Ravasio A, et al “Mechanical coupling between purse-string and cell crawling regulates epithelial gap closure.”
Nature Communications. 2015 Jul 9;6:7683. doi: 10.1038/ncomms8683.

Pillars to change the rheology of the microenvironment



$F = k\,X$

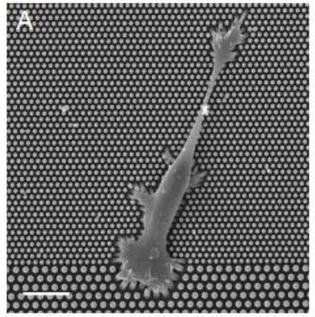
$k = \dfrac{3EI}{L^3}$

$I = \dfrac{\pi d^4}{64}$

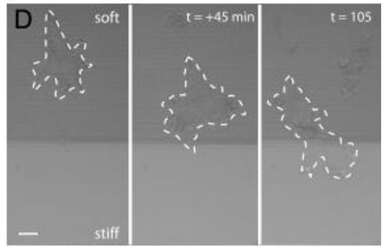
X → displacement

k → spring constant

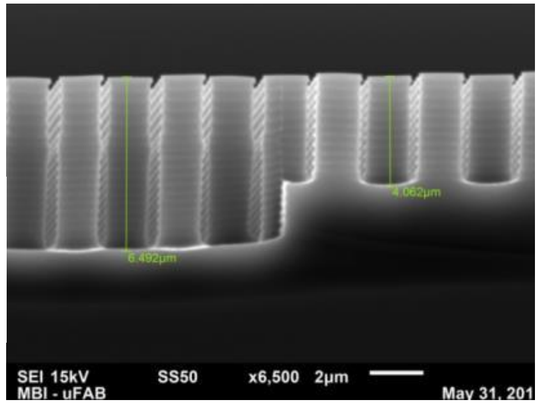
change *d*



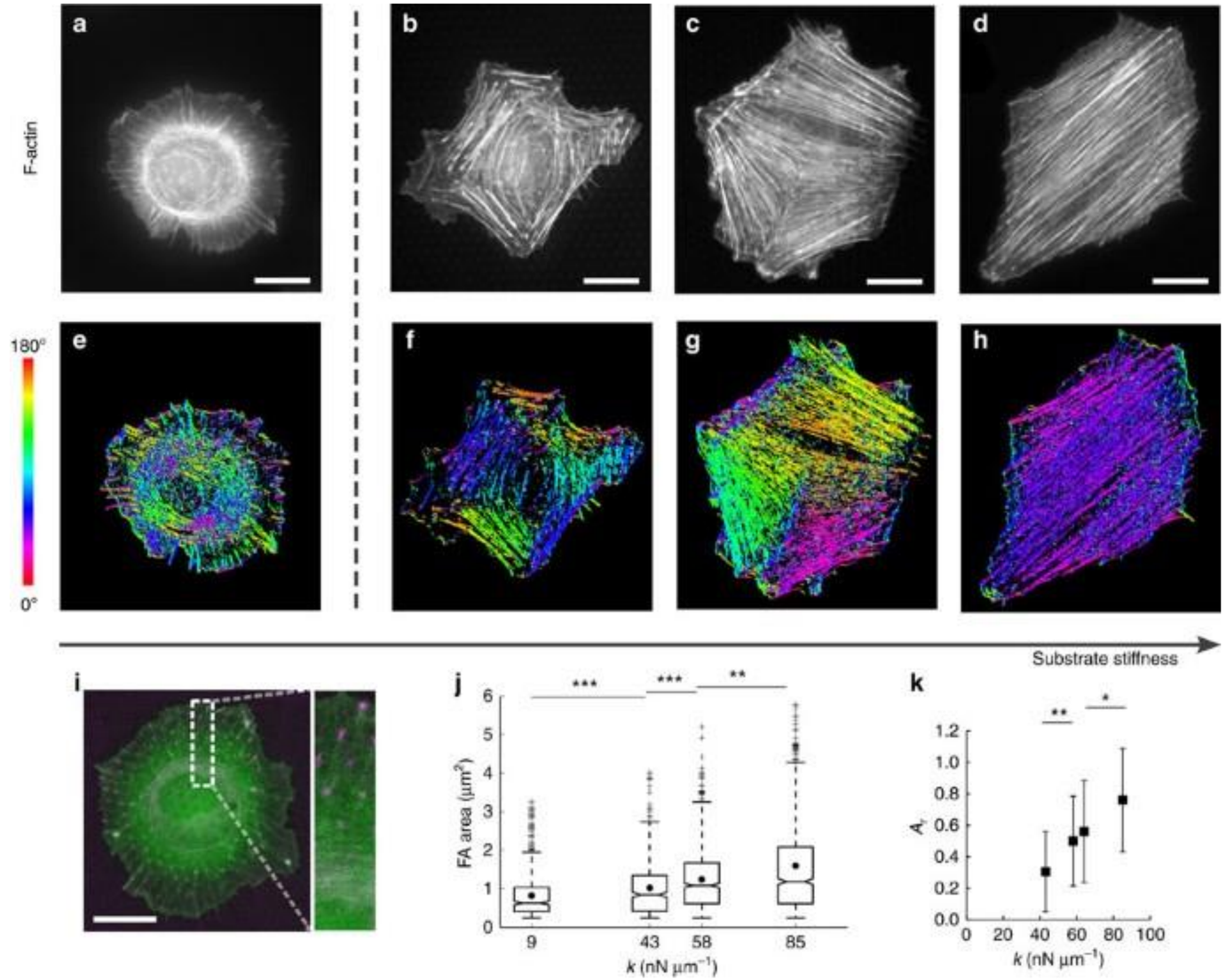
Durotaxis



change *L*

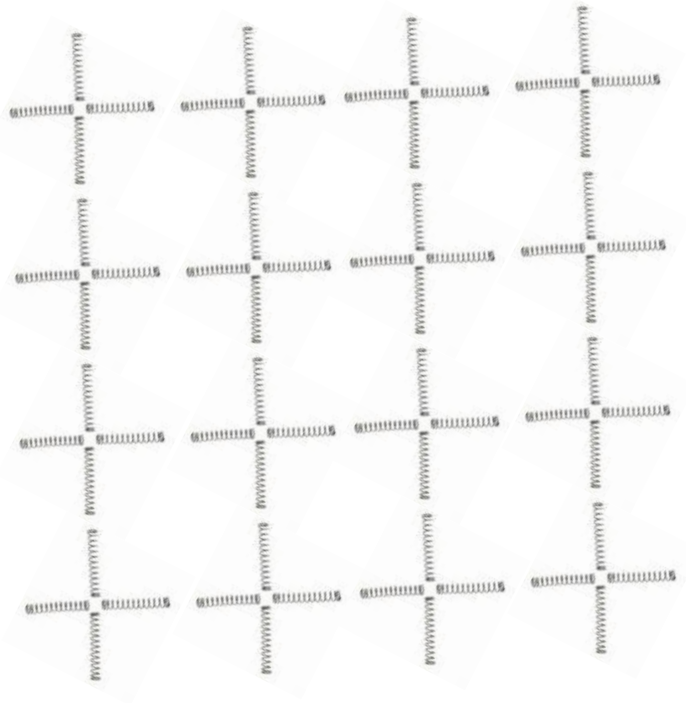


Pillars to change the rheology of the microenvironment



Gupta M.. et al. Nat. Comm. 2015

TFM using continuous elastic substrates



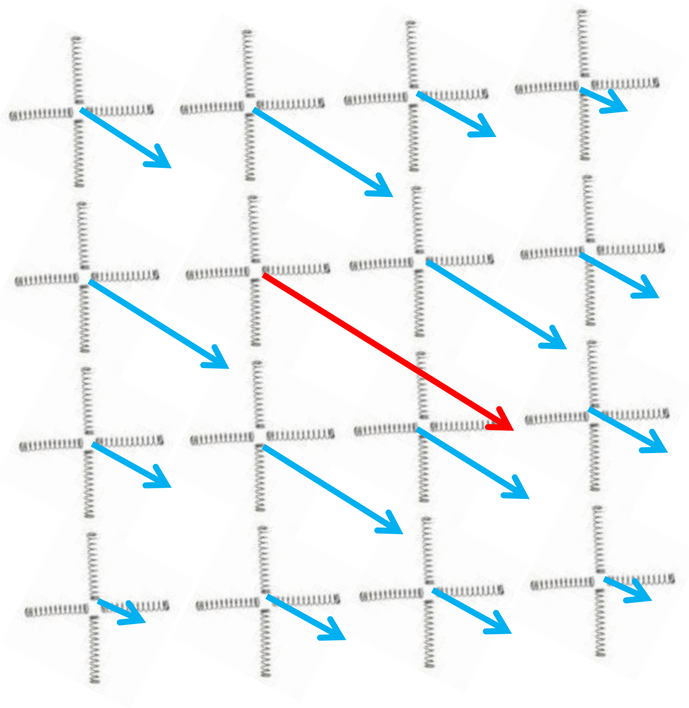
TFM using continuous elastic substrates

$$F = k\,X$$

X → displacement
k → spring constant

$$M = k^{-1}\,\mathrm{F}$$

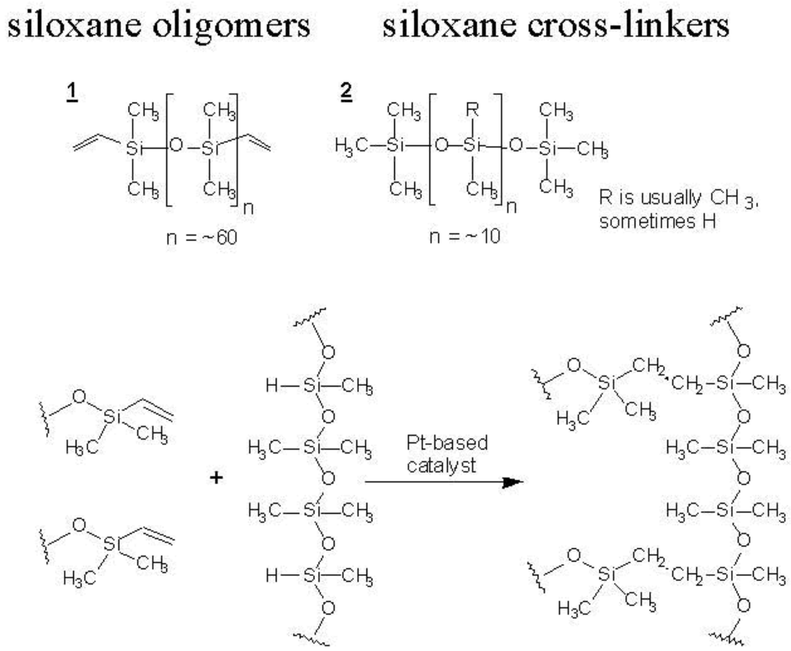
M → displacements vector
k → elasticity matrix



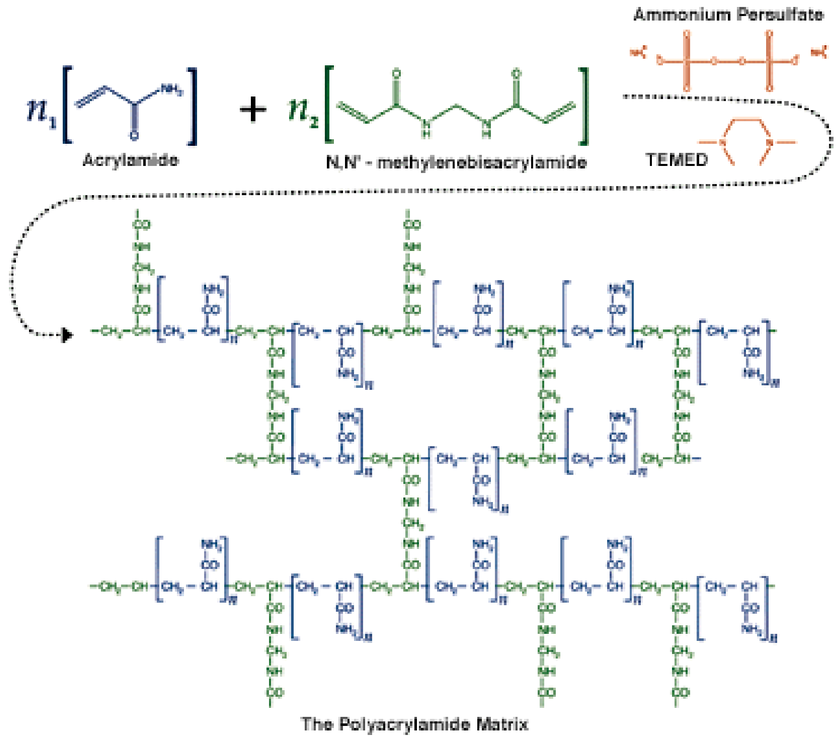
Analytical solution of the matrix k^{-1} using the elastic theory
Inversion of k^{-1} to calculate F (Force vector)

TFM using continuous elastic substrates

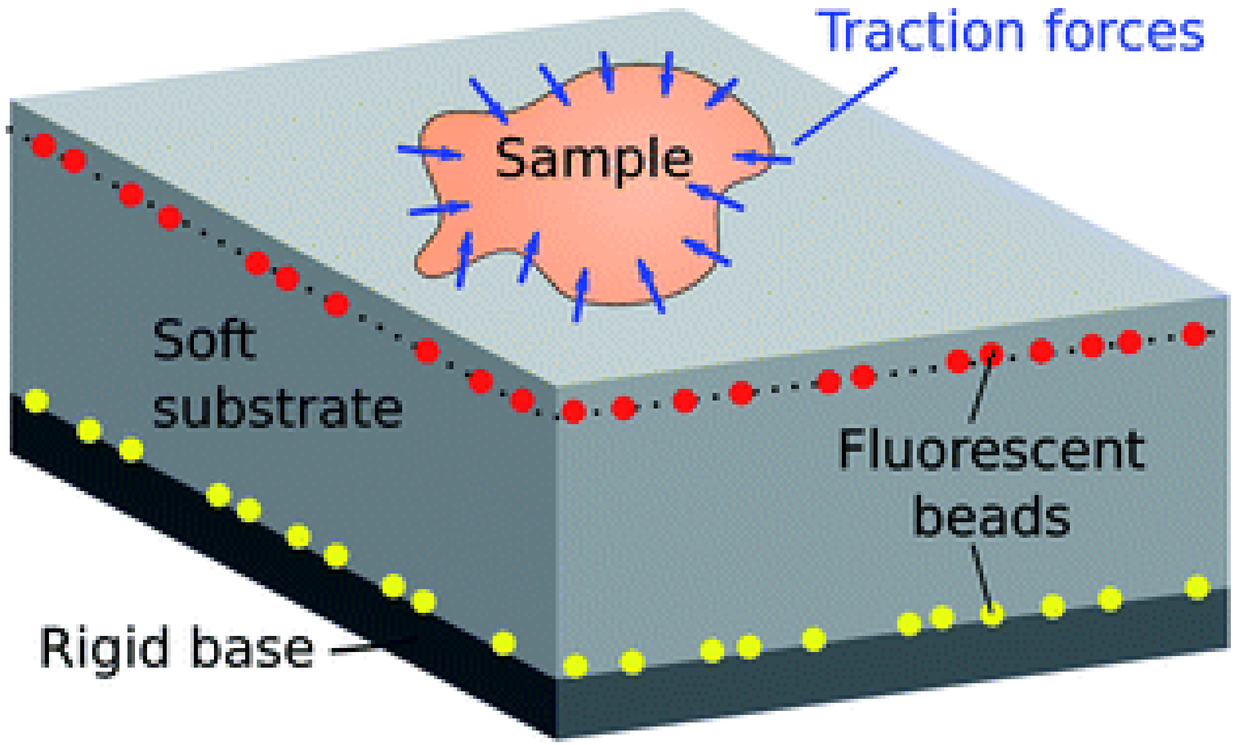
Soft silicon gels



Hydrogels (polyacrylamide)



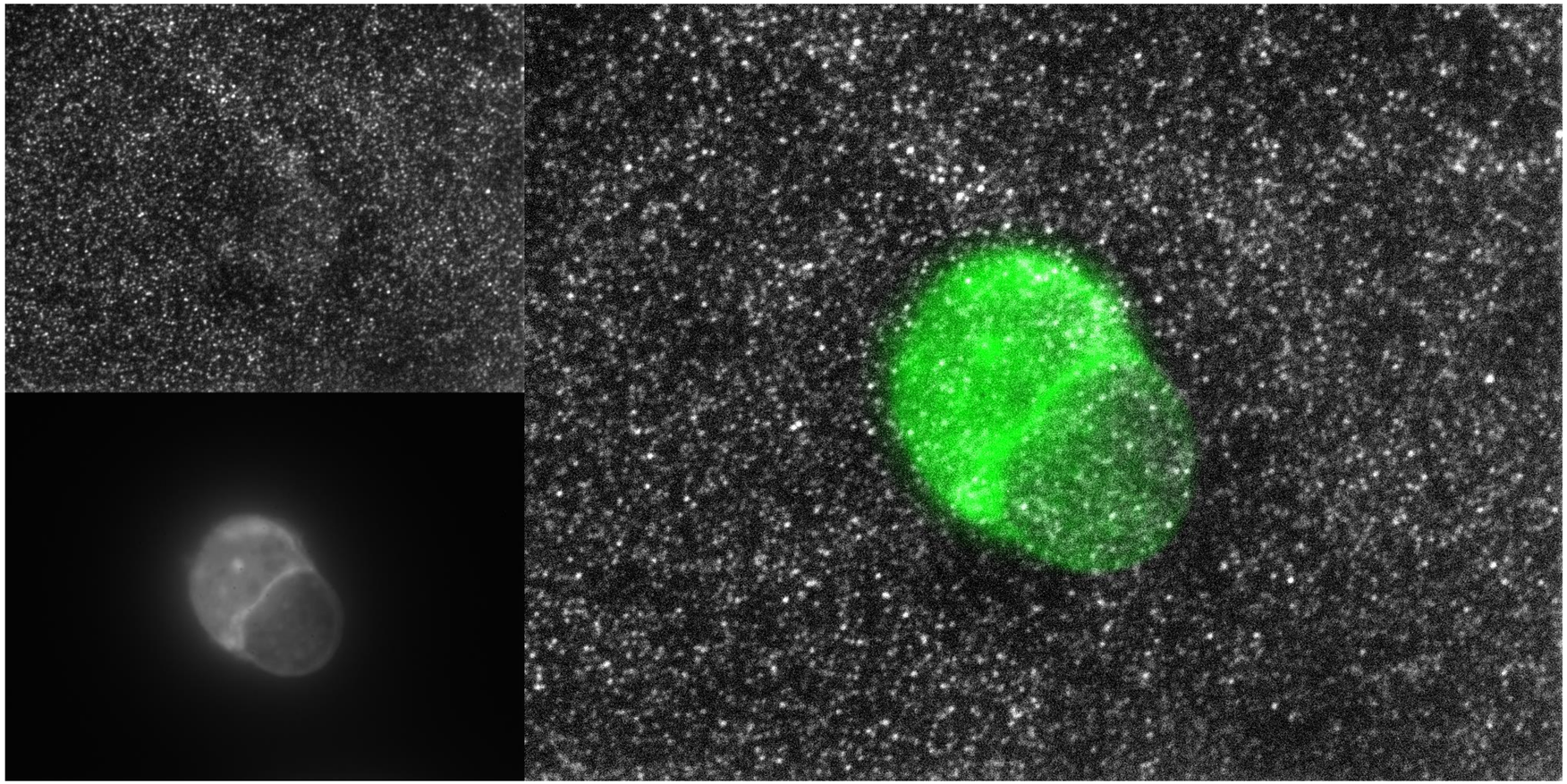
TFM using continuous elastic substrates



Style et al. Traction force microscopy in physics and biology. Soft Matter 2014

20:10

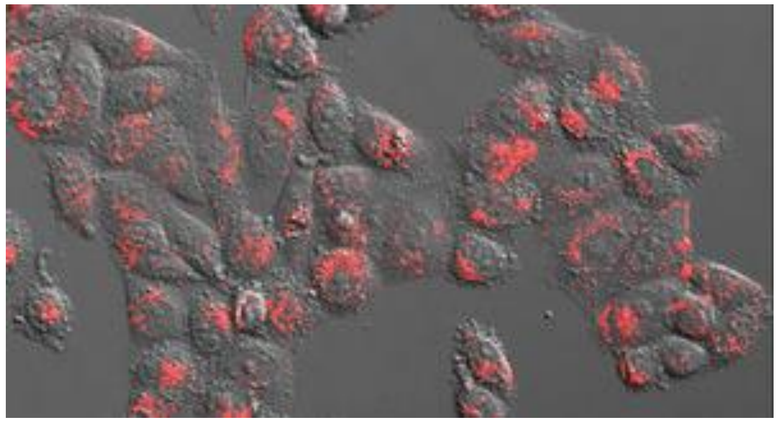
TFM using continuous elastic substrates



Displacement filed (M) obtained by
particle tracking
or Particle Image Velocitometry (PIV)

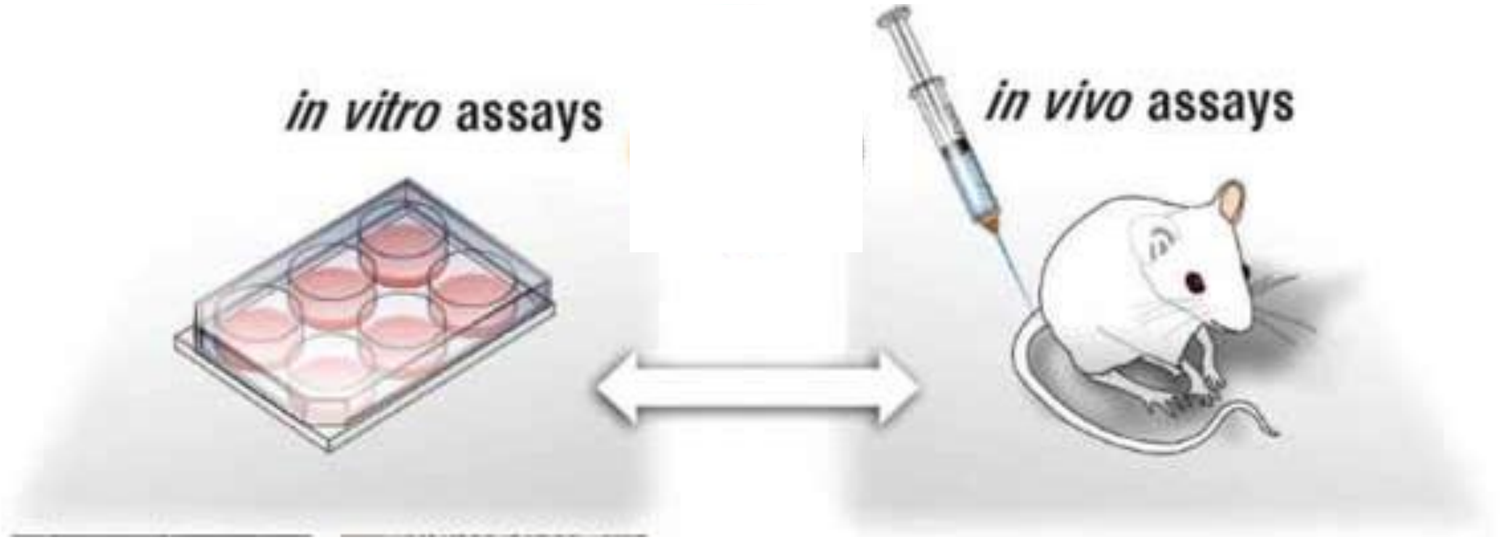
20:10

in vitro systems



- Non-physiological microenvironment (flat, unstructured),
- Non-physiological microrheology (GPa)
- Non-physiological chemical composition (commercial growth media, buildup of unstored layers),
- Non-physiological mechanical environment, etc
- Simple biological composition (typically one cell type per experiment)

in vitro-in vivo dichotomy



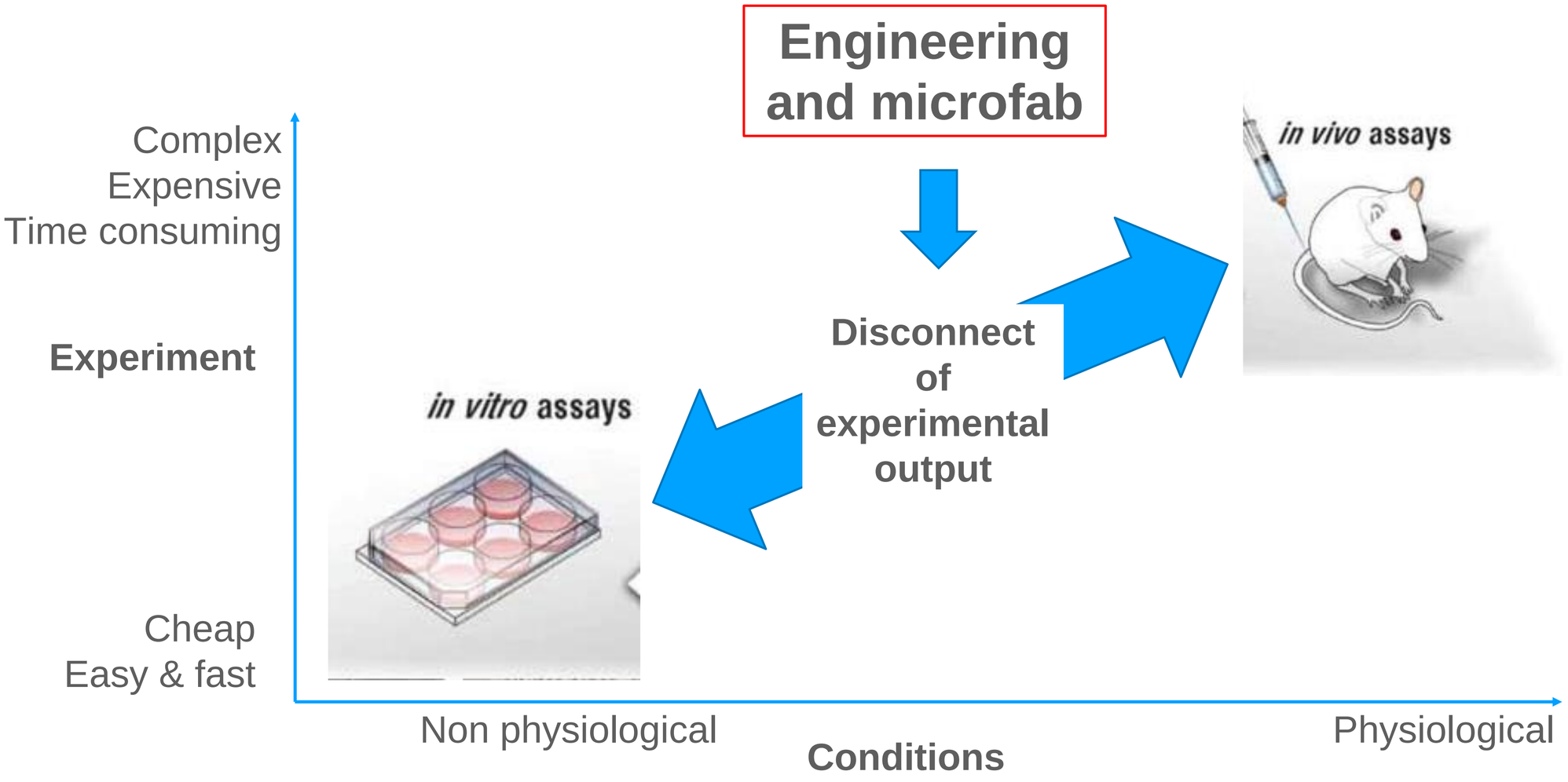
Adv:
Easy to use and cheap
High reproducibility
Amiable for molecular and cellular exploration

Disadvantages:
Low physiological relevance - simplistic model that do not capture in-vivo condition and complexity

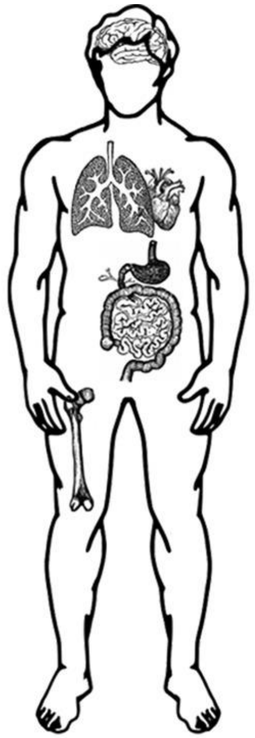
Adv:
Physiologically relevant - informative of real condition and capture biological and medical complexity

Disadvantages:
Expensive
Time consuming
Use of non-compatible animal models
Ethical concerns

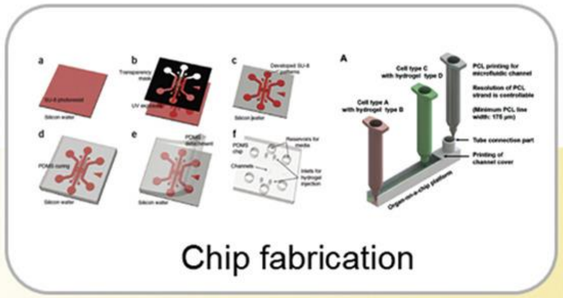
Bridging in vitro-in vivo dichotomy



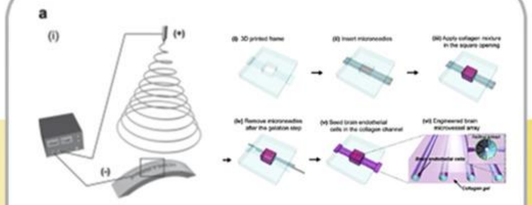
Engineering of biomimetic environments



***In vivo* cellular microenvironment**

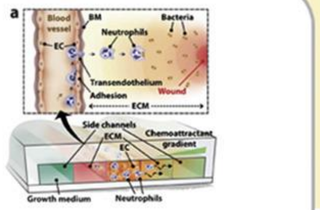


Chip fabrication

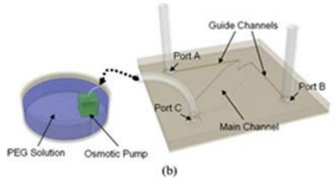


Compartmentalization

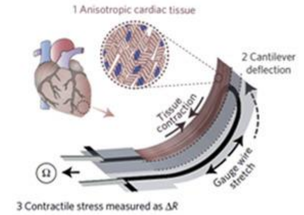
***In vivo*-like structural environment**



Biochemical gradient

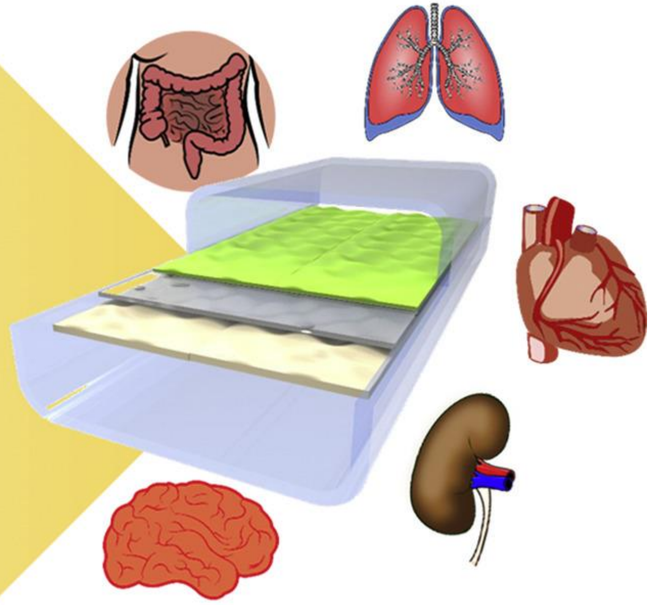


Fluid shear stress



Mechanical strain

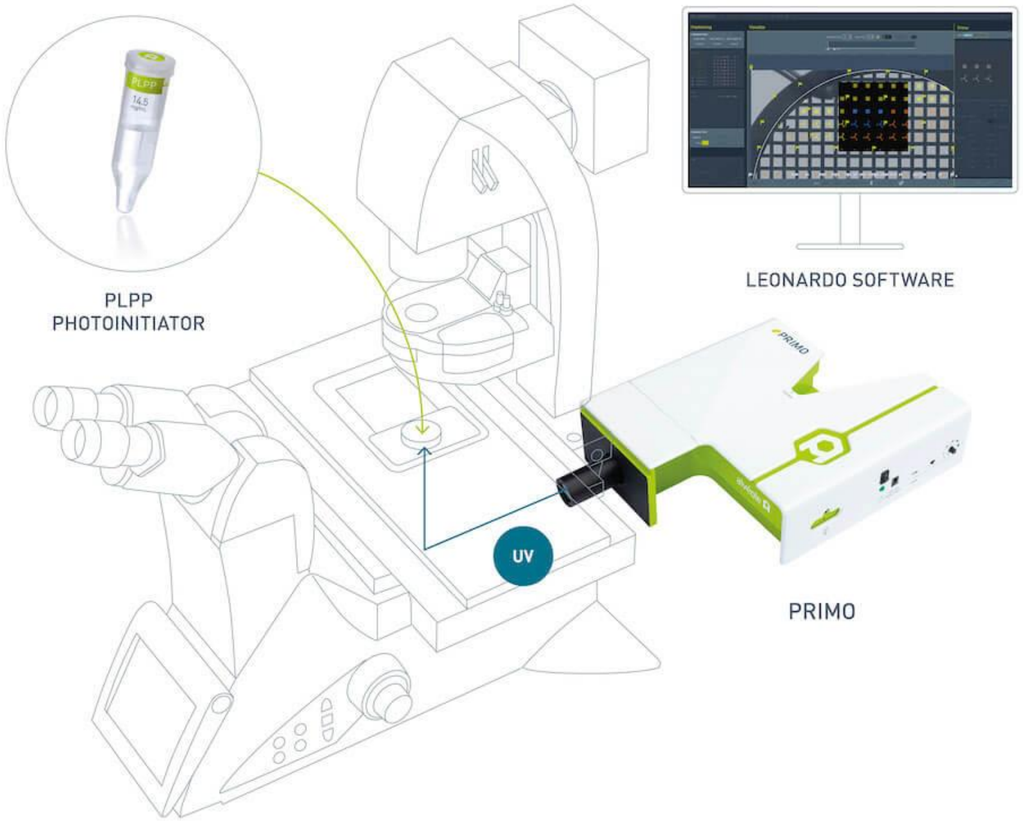
***In vivo*-like dynamic biochemical/ mechanical stimulus**

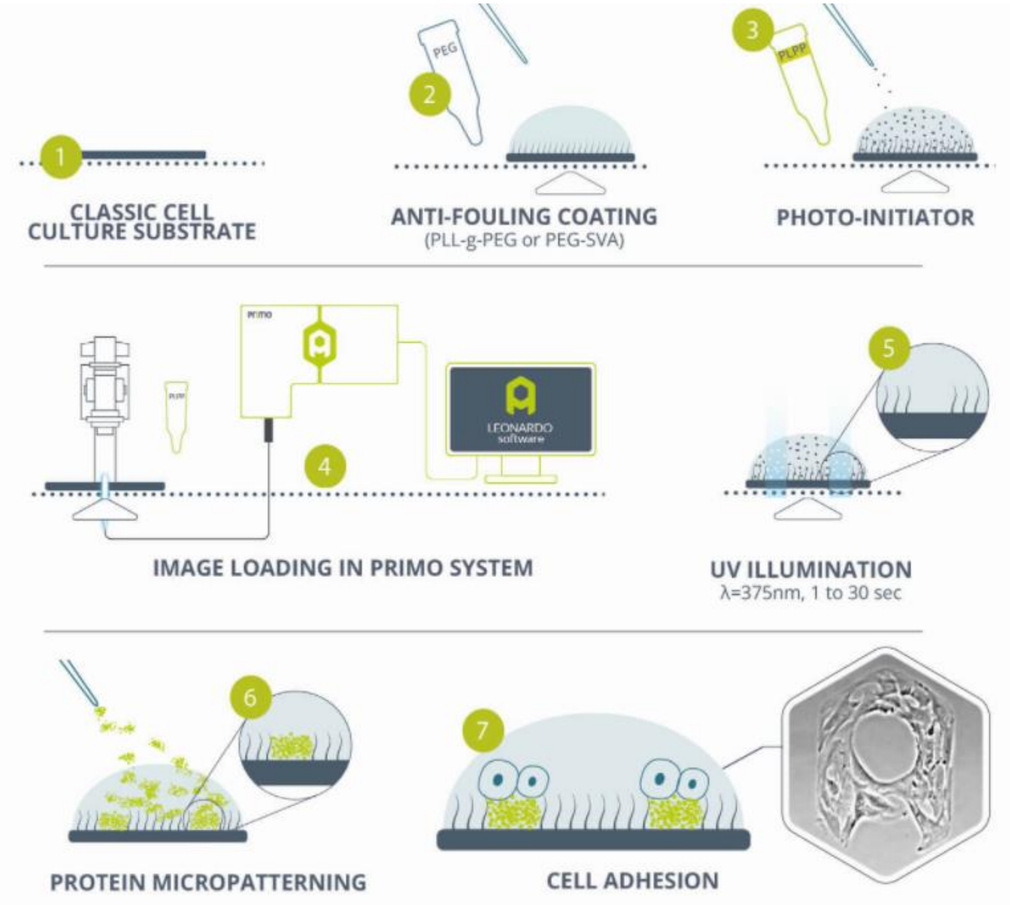


***In vivo*-like *In vitro* cell culture platform**

Bioengineered devices to mimicry in vivo biochemical, biological, and mechanical characteristics and recapitulate System dynamics

BioPhotolithography - Primo

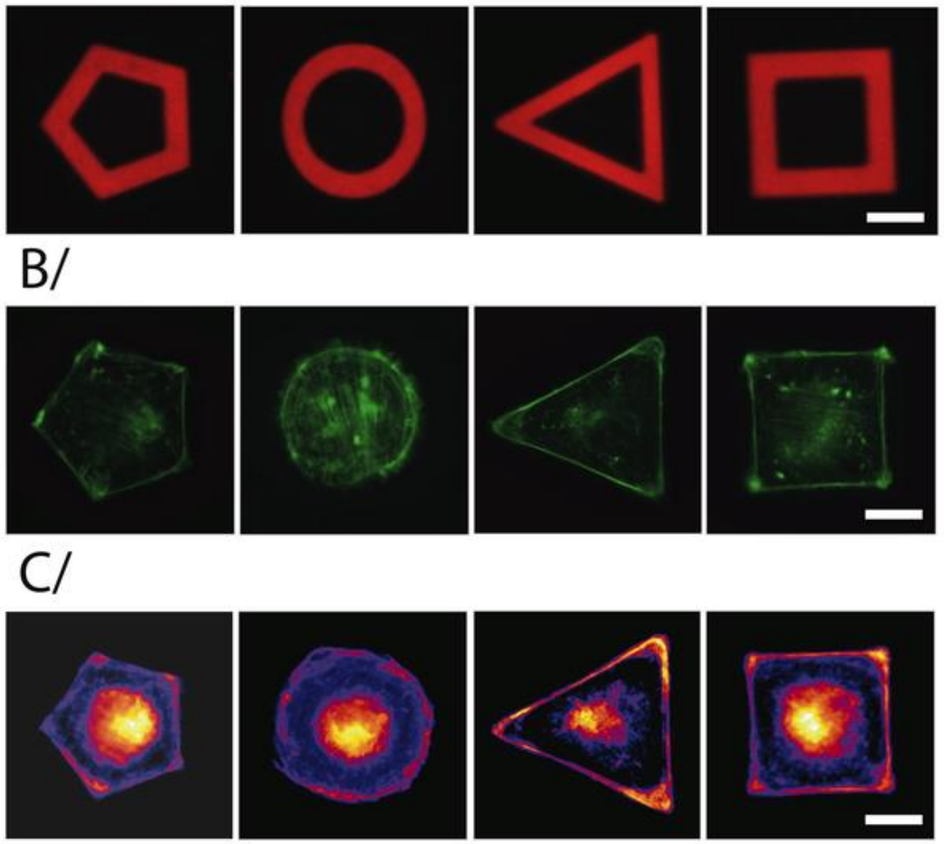




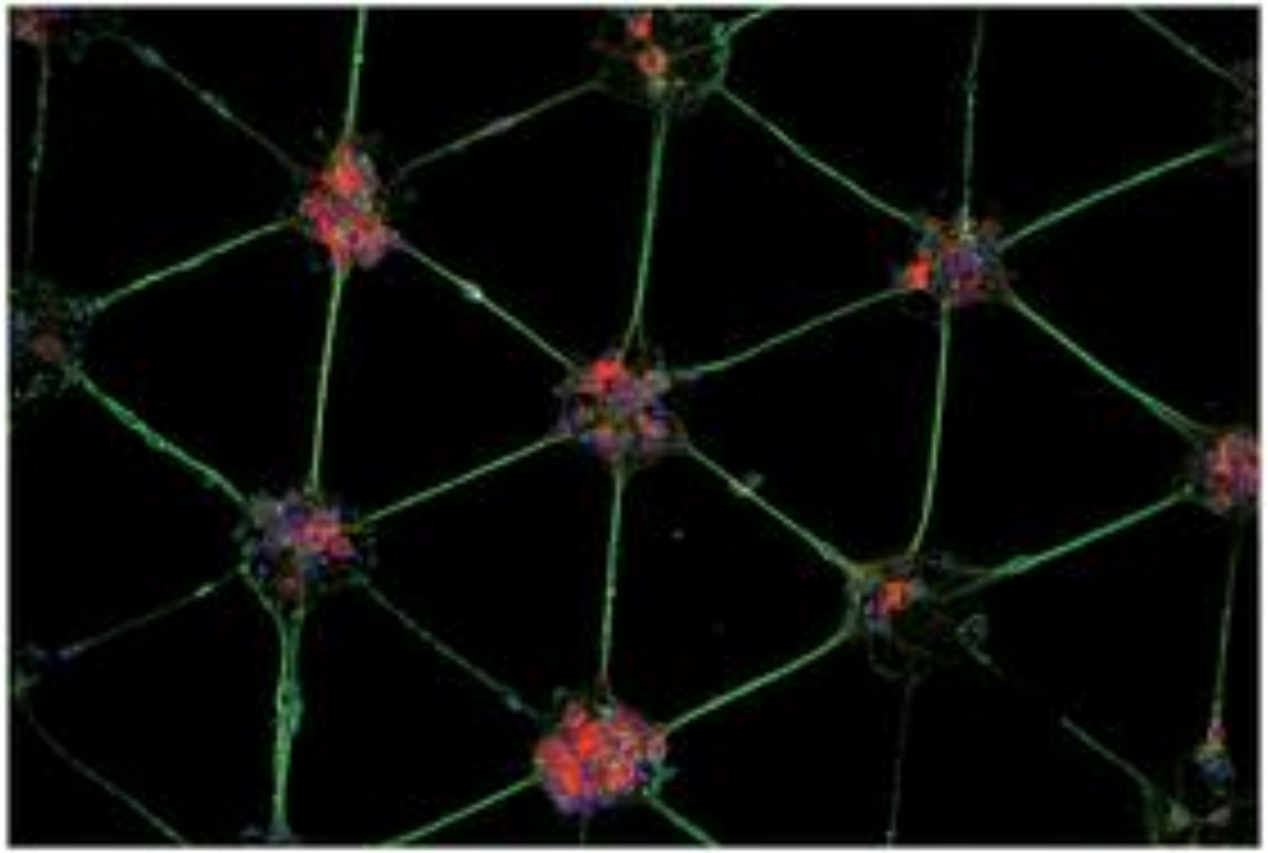
Primo Alveole – Fondequip @ Ravasio

Tissue Geometry 2D

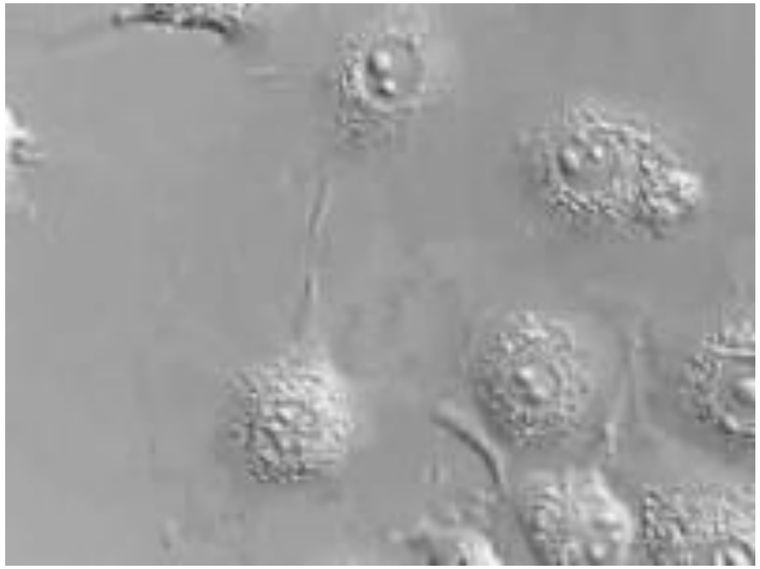
Investigation of tissue architecture



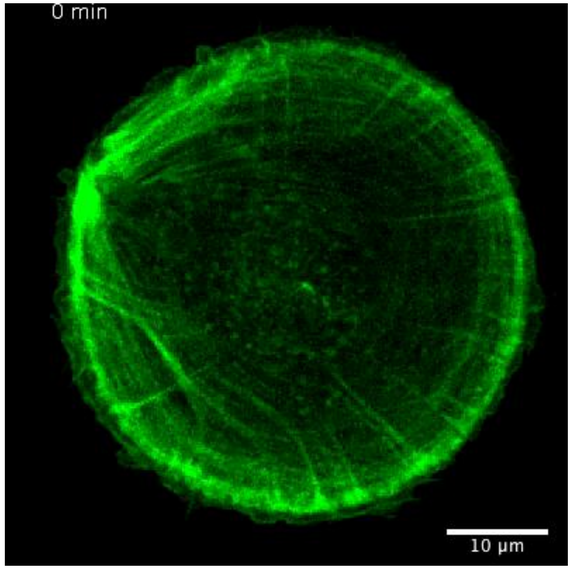
Printing of natural Neuronal Networks



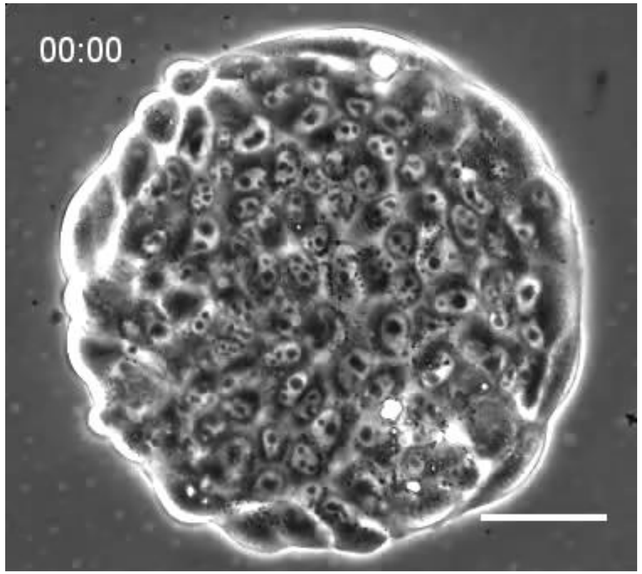
Tissue Geometry 2D



Unconfined cell migration



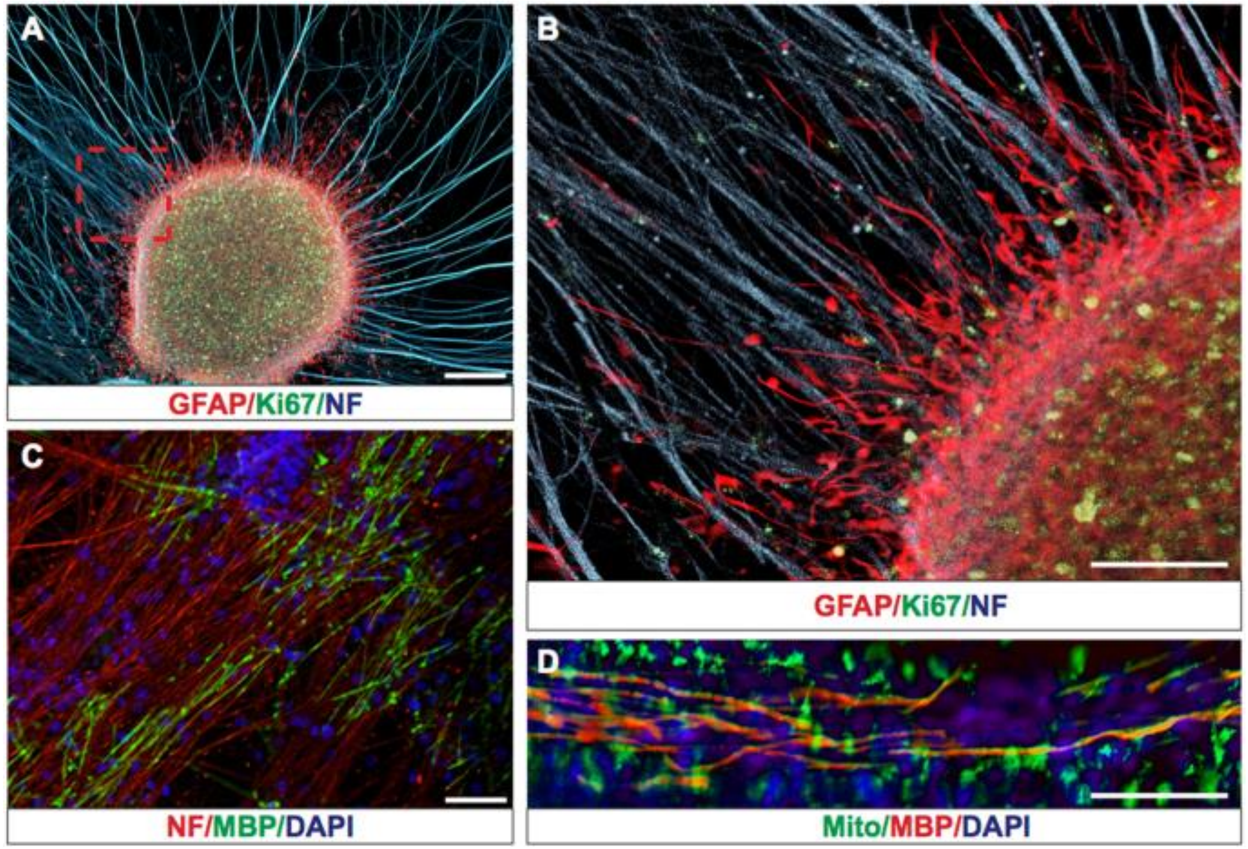
Tee YA et al. 2015. Nat Cell Biology



Doxzen K et al. 2013. Integrative Biology

Tissue Geometry 2D

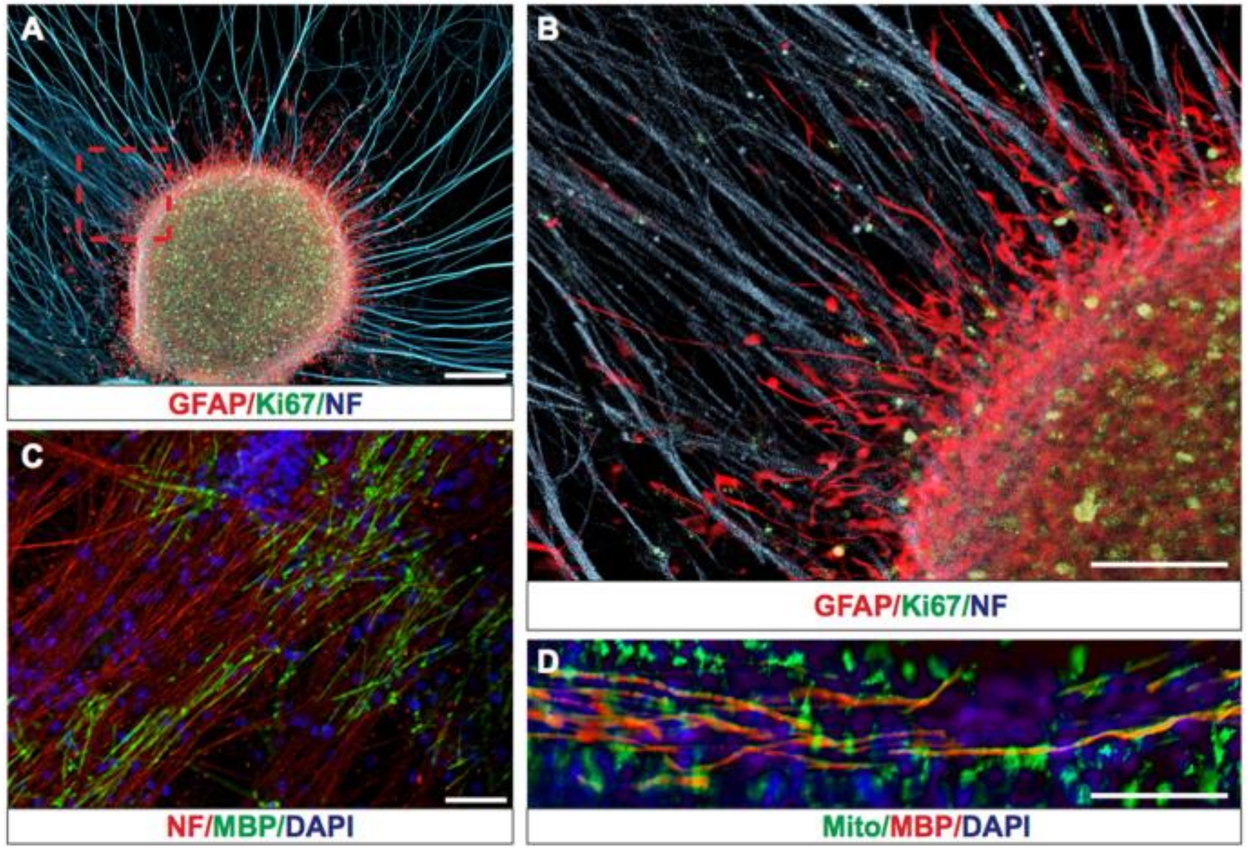
Glioblastoma cells migrate on neurons and capillaries



Zepecki et al, 2019 Oncogene

Tissue Geometry 2D

Glioblastoma cells migrate on neurons and capillaries

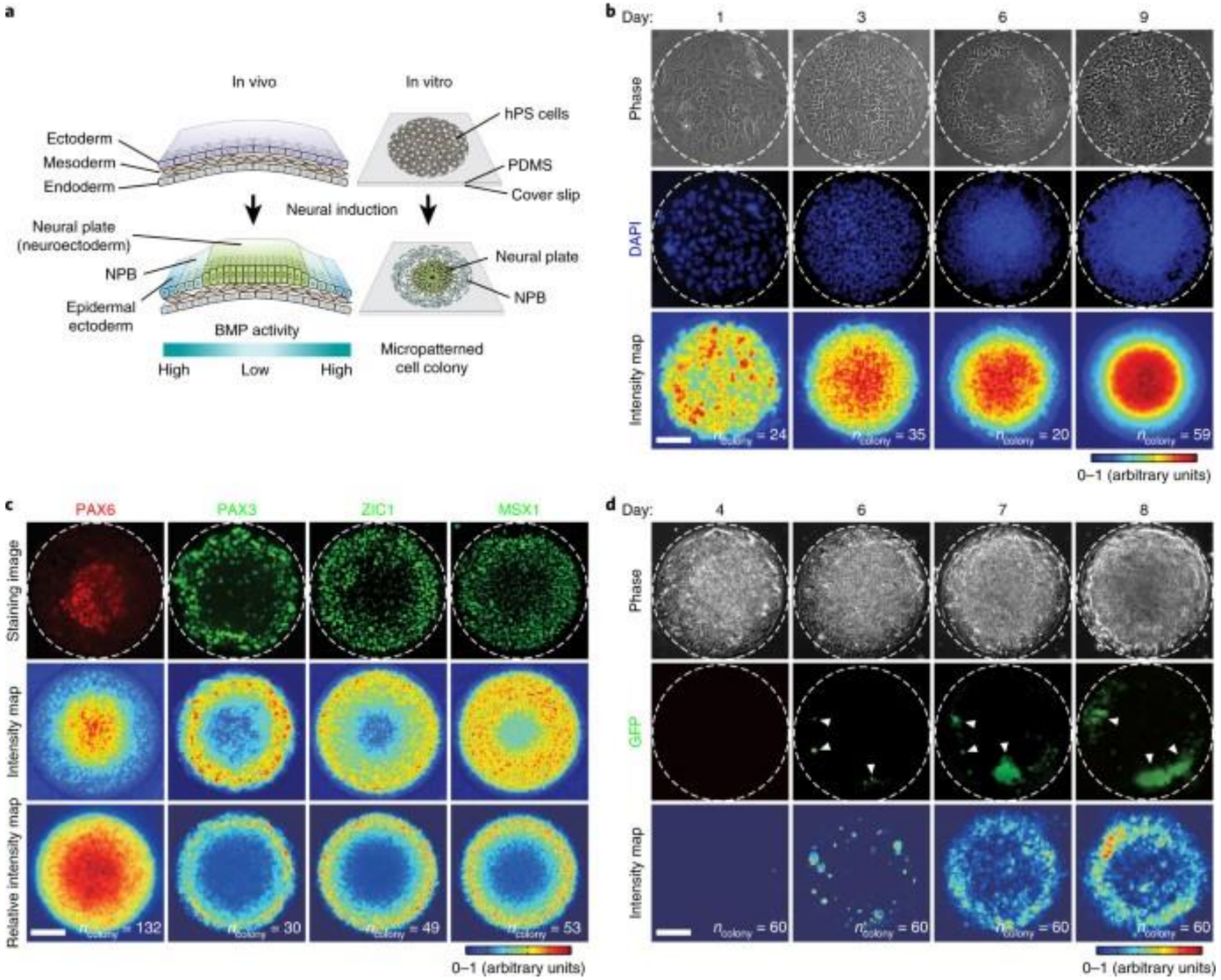


Zepecki et al, 2019 Oncogene

1D migration of glioblastoma to mimic cancer cell migration in vivo

Credits Pascalle Monzo, Nils Gauthier

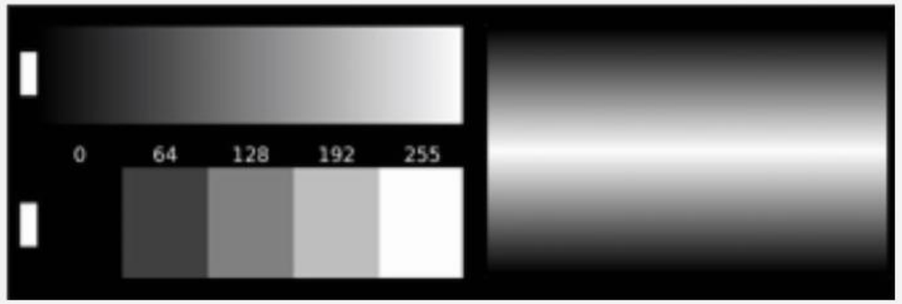
Tissue Geometry 2D



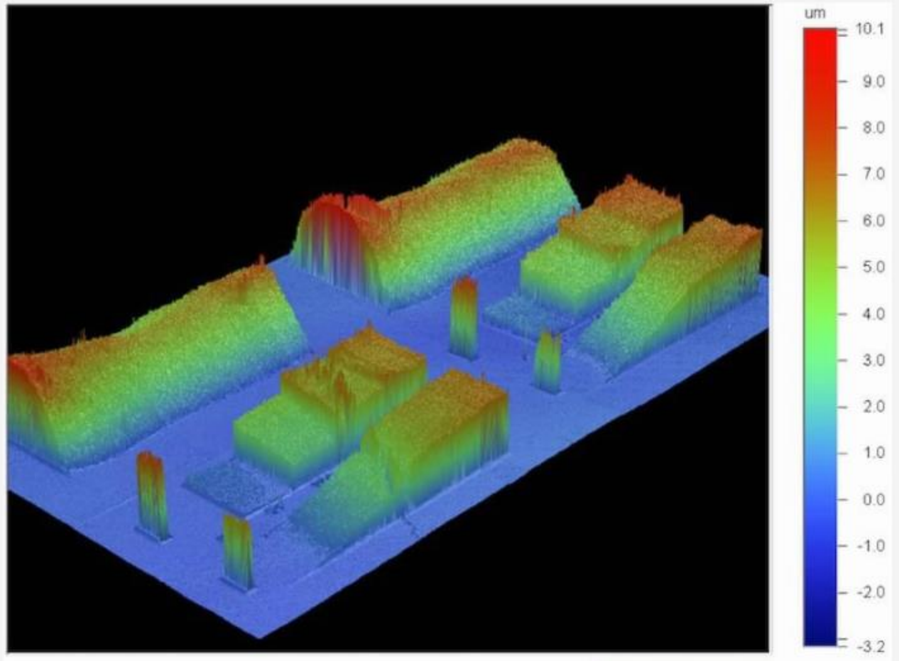
Mechanics-guided embryonic patterning of neuroectoderm tissue from human pluripotent stem cells

Xue er al. 2018 Nature Materials

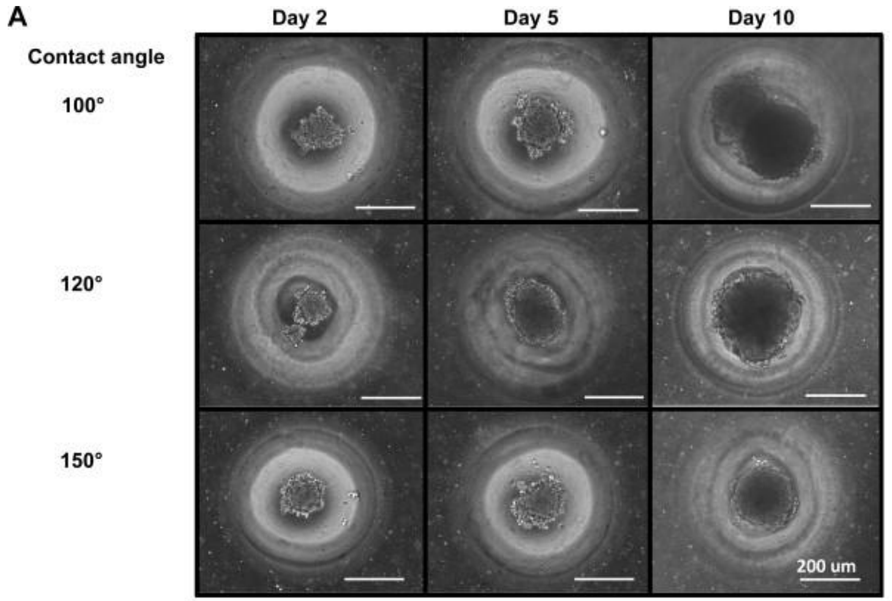
Tissue Geometry 3D



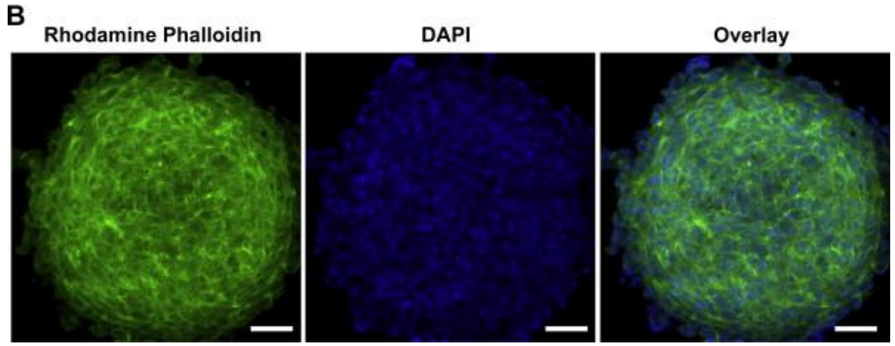
Pattern loaded into Leonardo software to do microfabrication in gray levels.



Grayscale Lithography

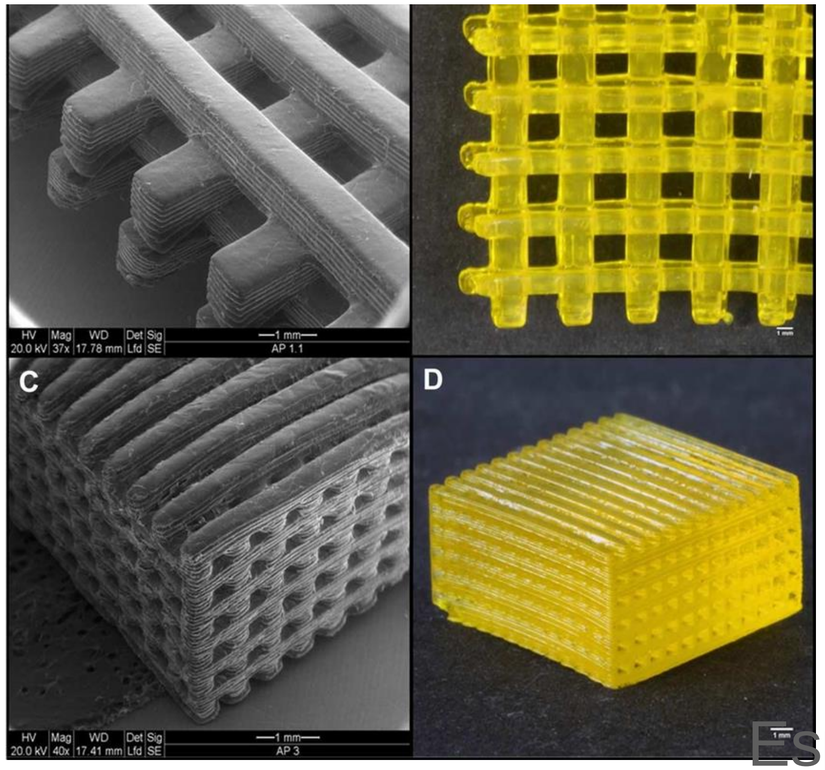


Microspheres to generate sheroids

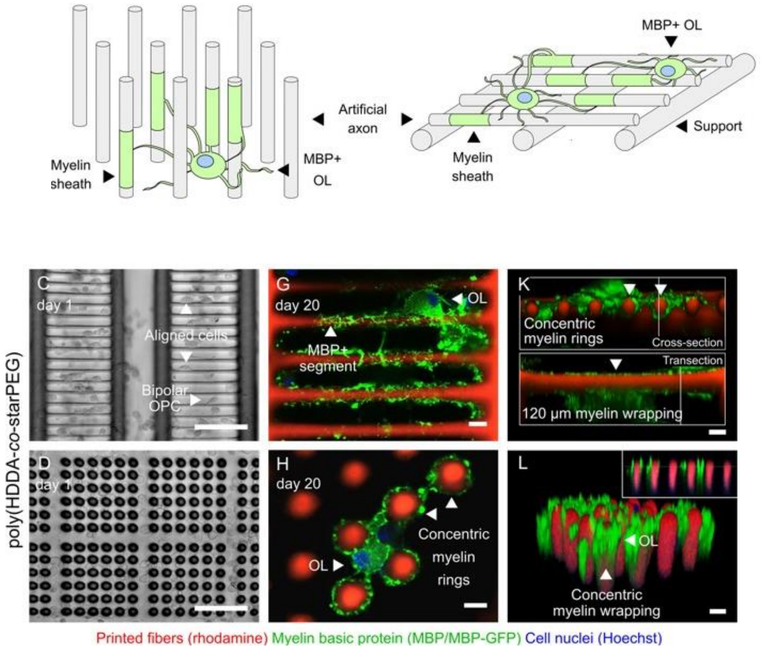


Tissue Geometry 3D

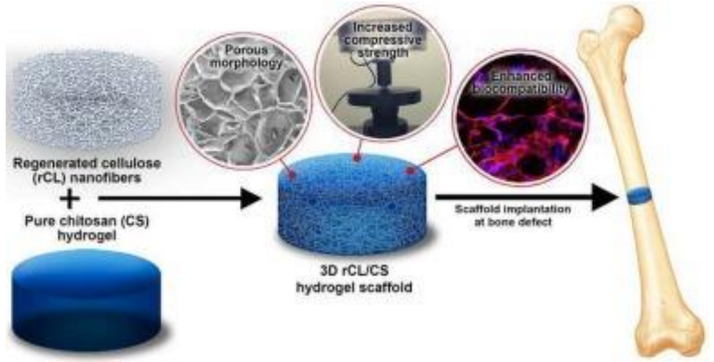
Sterolithography using photocurable hydrogels – Electron beam lithography, 2 Photon, UV lithography (Primo)

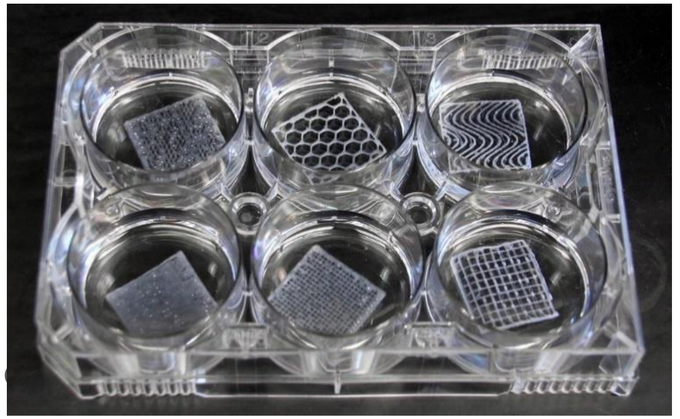


Micrometer size scaffolds (e.g. artificial axons)



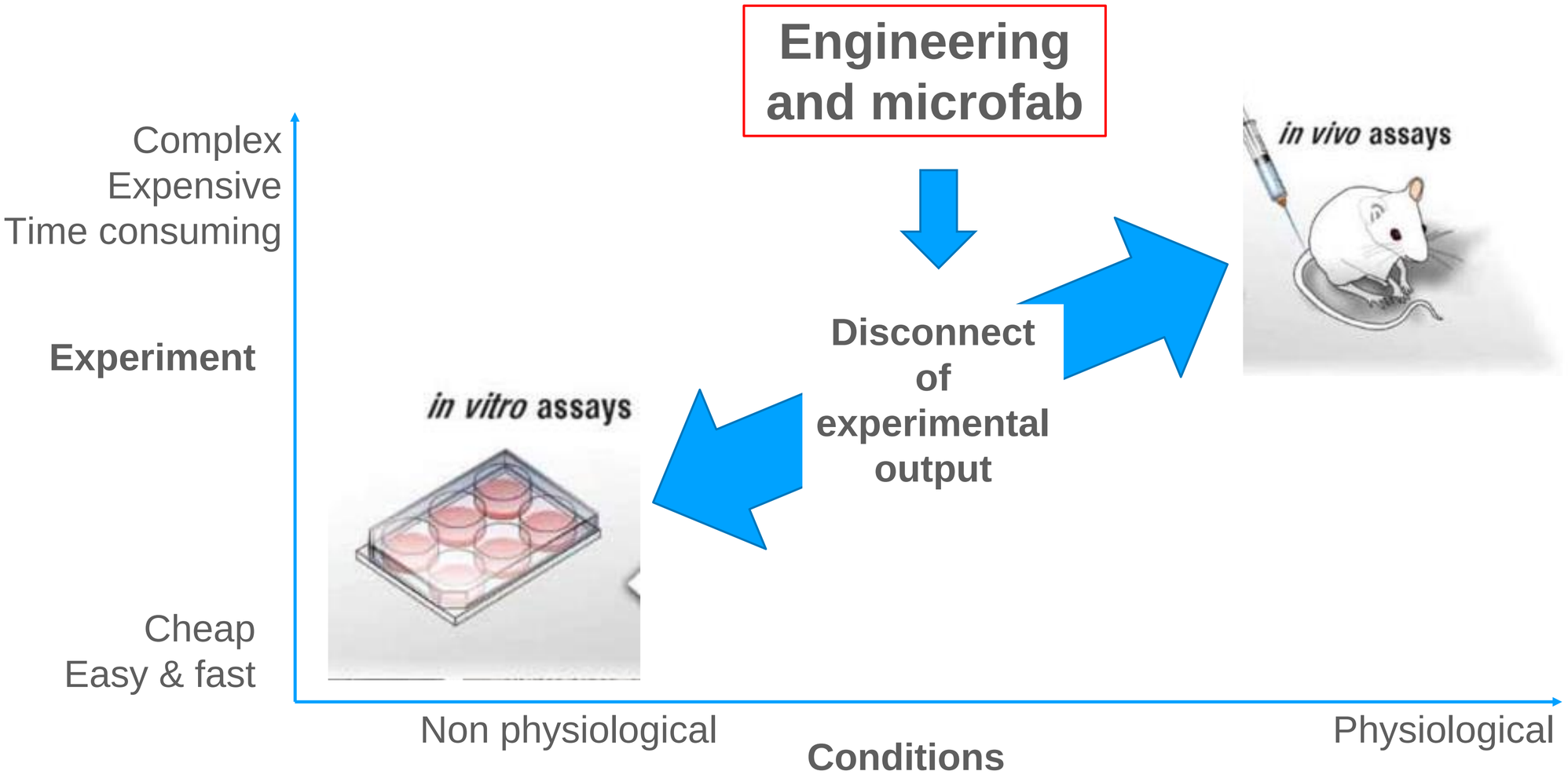
Microscale scaffolds (e.g. trabeculae orientation in bones)



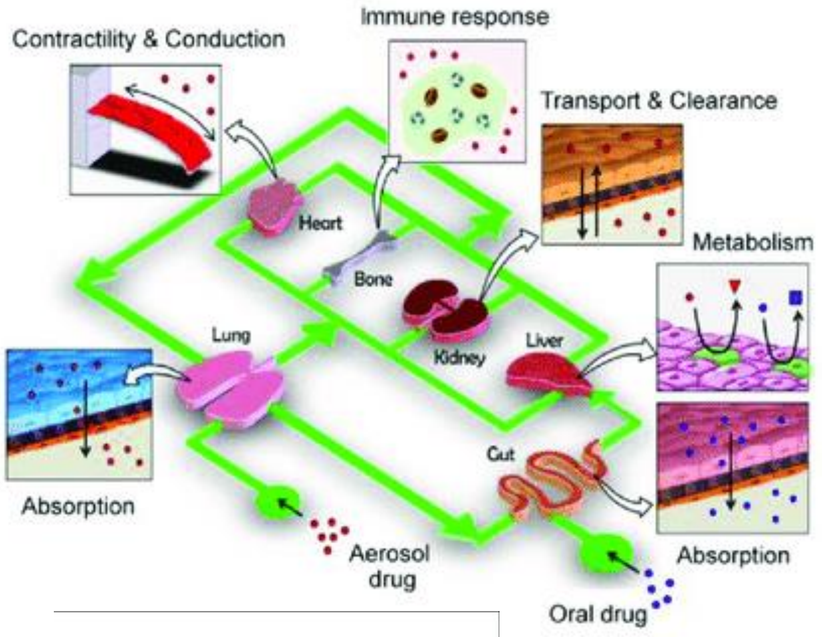


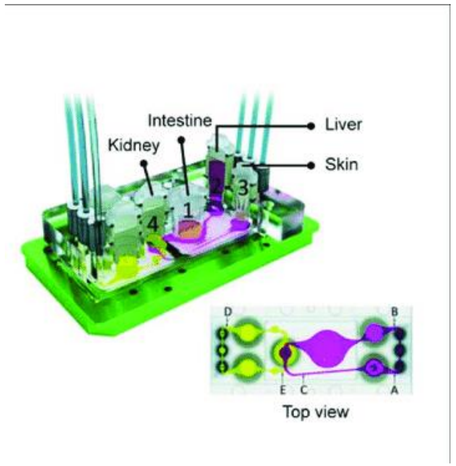
Espinosa-Hoyos et al Sci. Rep *201*

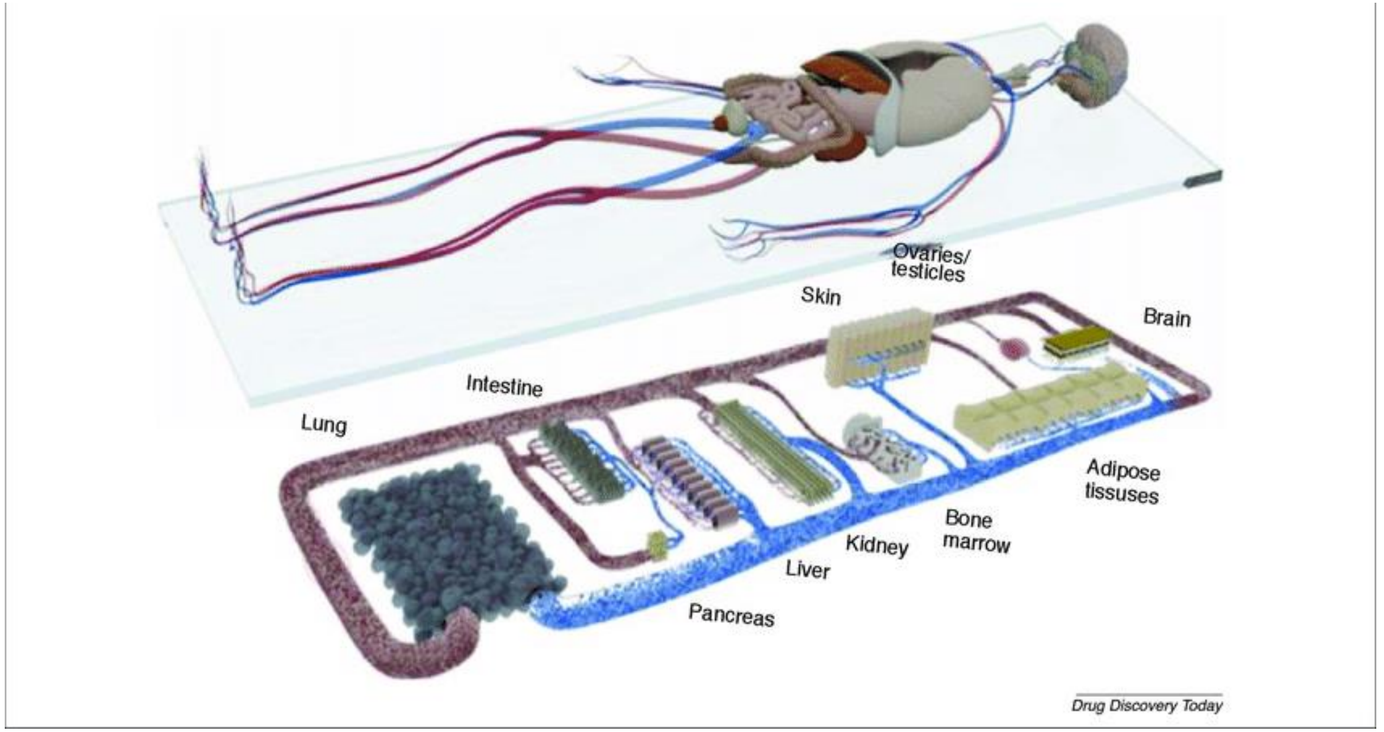
Bridging in vitro-in vivo dichotomy



Human- and patient-on-a-chip







Benam et al 2019 Drug Discovery Today

Q&A

Undergrad and Phd thesis available

Last - Slide