Simple methods to confine cells in 1D, 2D and 3D: microfabrication for cell biology

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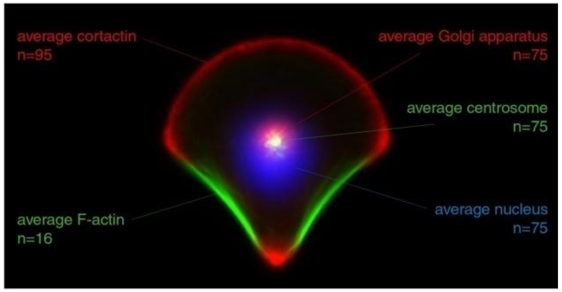
Microfabrication for cell biology

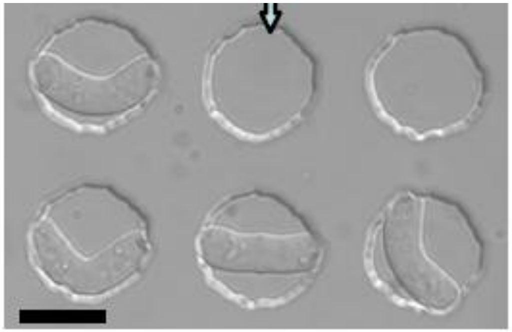
Advantage: the right scale to control the cell micro-environment
Disadvantage: too many possible alternatives and too complicated tools
Solution: the simplest is always the best – keep focused on the biological question you want to ask

I. Micro-patterning



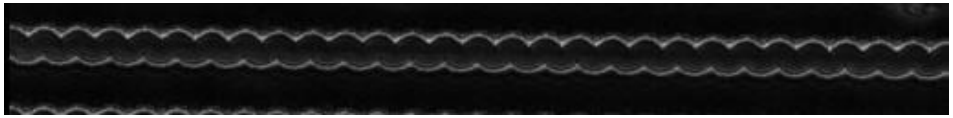
M. Théry

II. Micro-wells



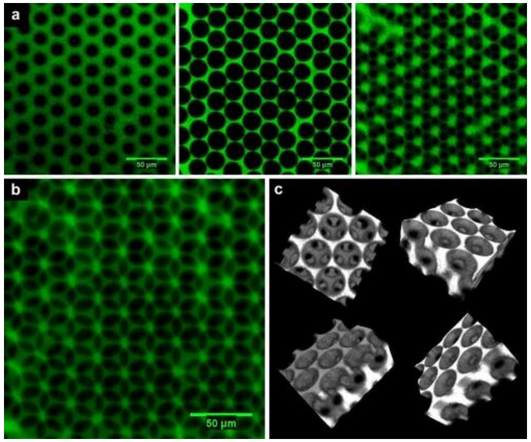
N. Minc

III. Micro-fluidics



T. Makushok

IV. 3D scaffolds



F. Lautenschlaeger

Spatial and temporal control of cell environment at the scale of the cell (0.1 to 100 μm, ms to days)

a) Control of growth medium (microfluidic):
- -chemostat, thermostat
- -drug delivery (serial dilutions, oscillations, etc…)
- -gradients

b) Surface control (micropatterning, micro-fabrication):
- -adhesion (stamping of adhesion molecules, contact guidance)
- -cell shape
- -cell positioning (for cell/cell communication)

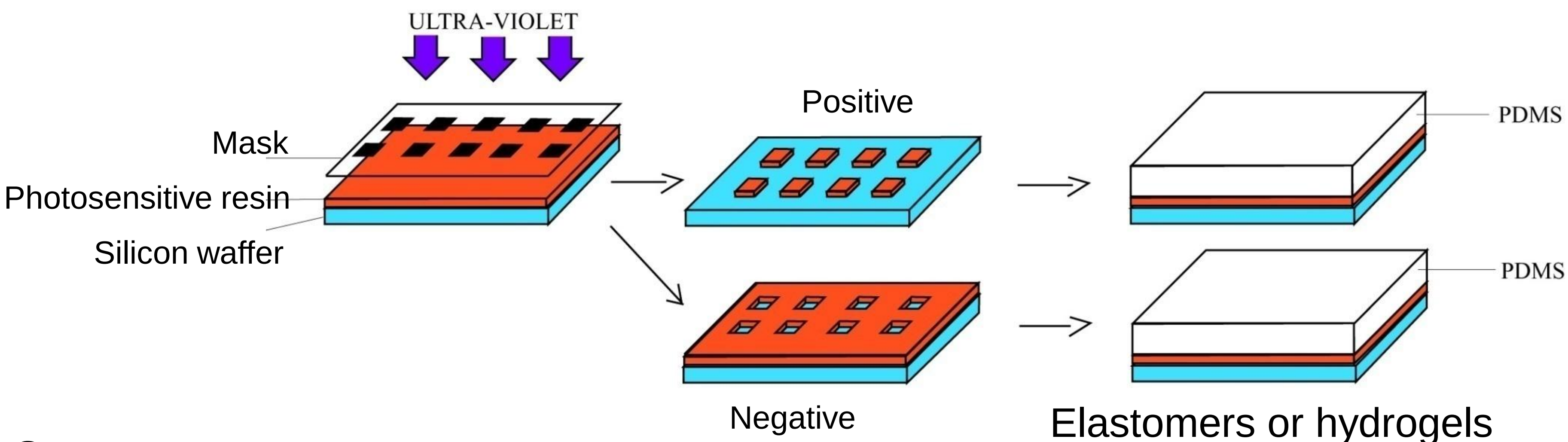
c) Substrate mechanical properties

d) Volume control for 3D confinement (microchambers for cells or in-vitro assays)

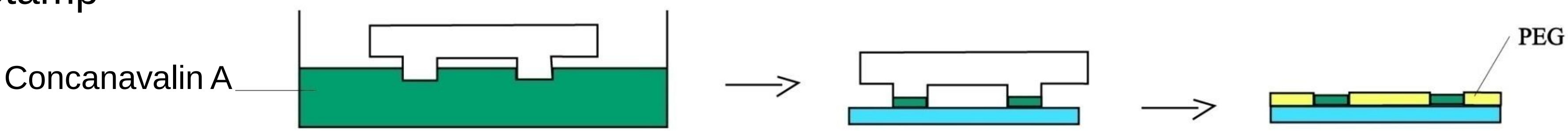
And all combinations…

Make your own tools:

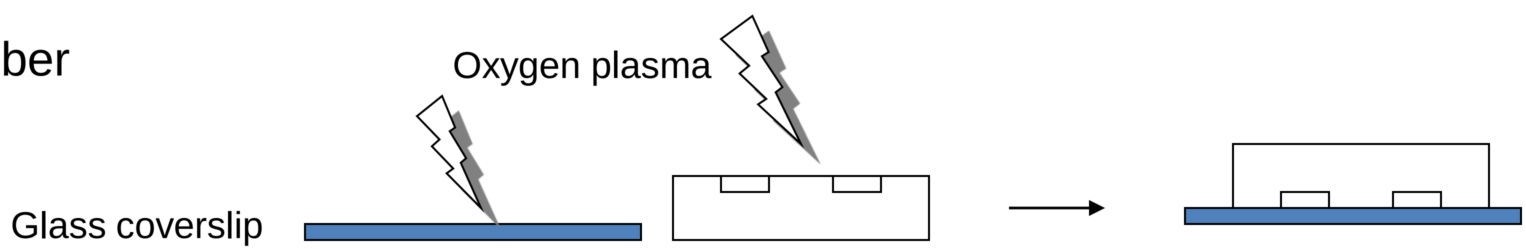
Soft lithography for microfluidics or micro-patterning



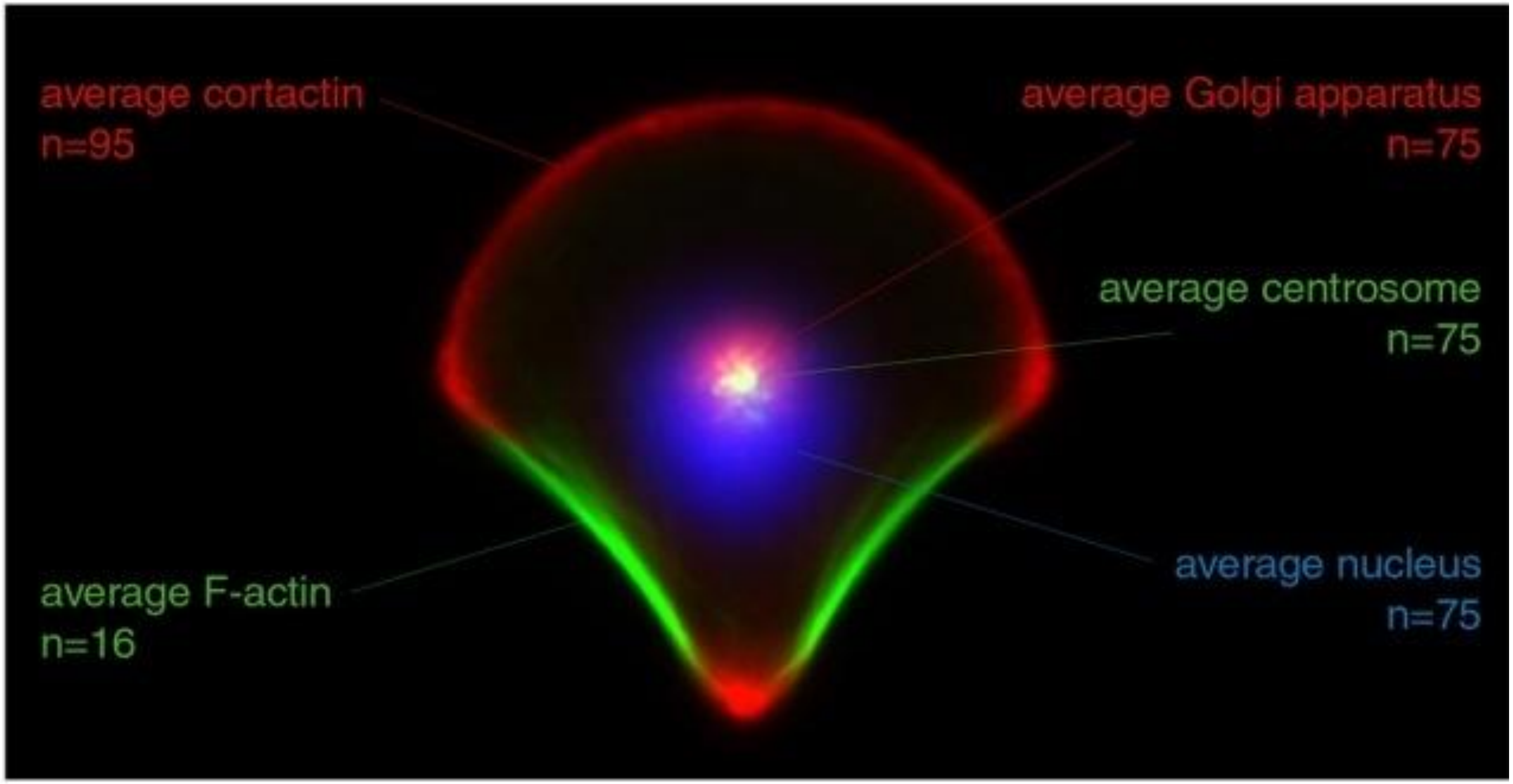
Stamp



Chamber

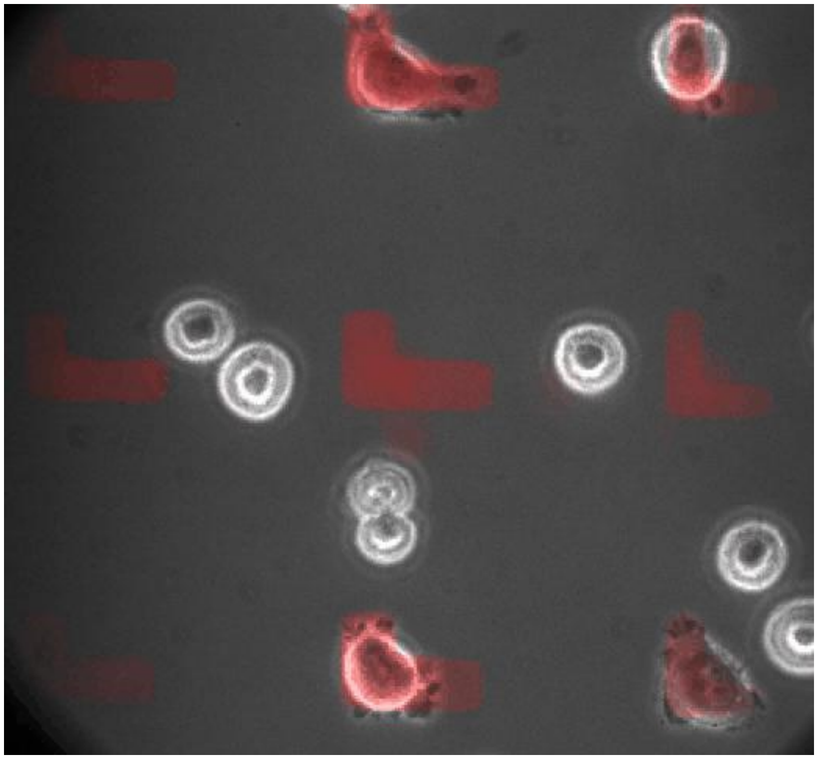


I. 2D micro-patterning: spatial and temporal control of surfaces

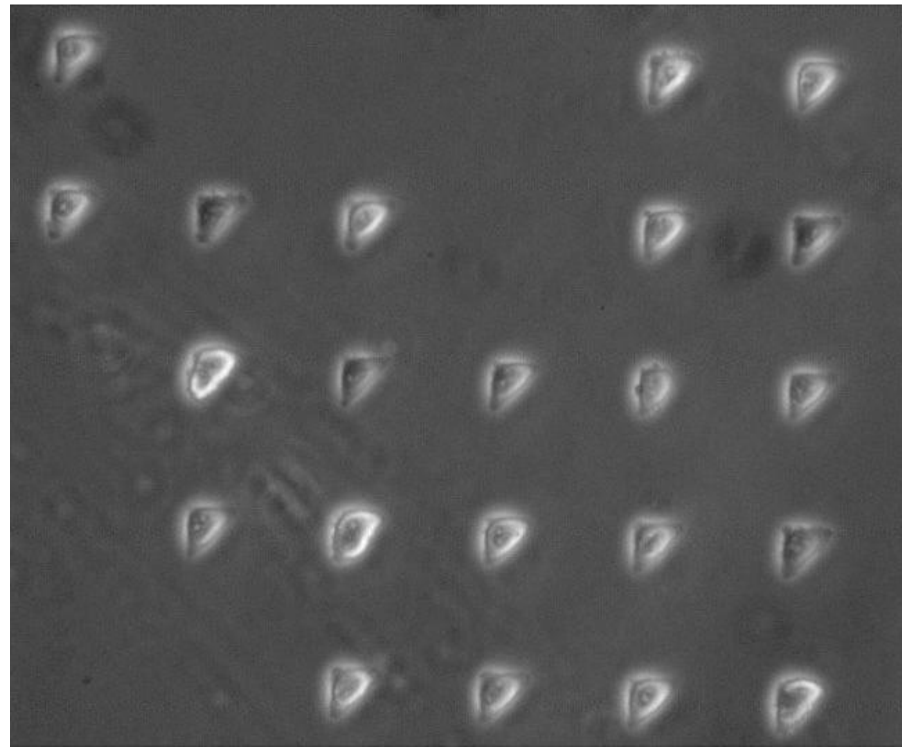


Théry et al., PNAS 2006

Micropatterning cell adhesion molecules



Mouse fibroblasts L929
on fibronectin islets (red)

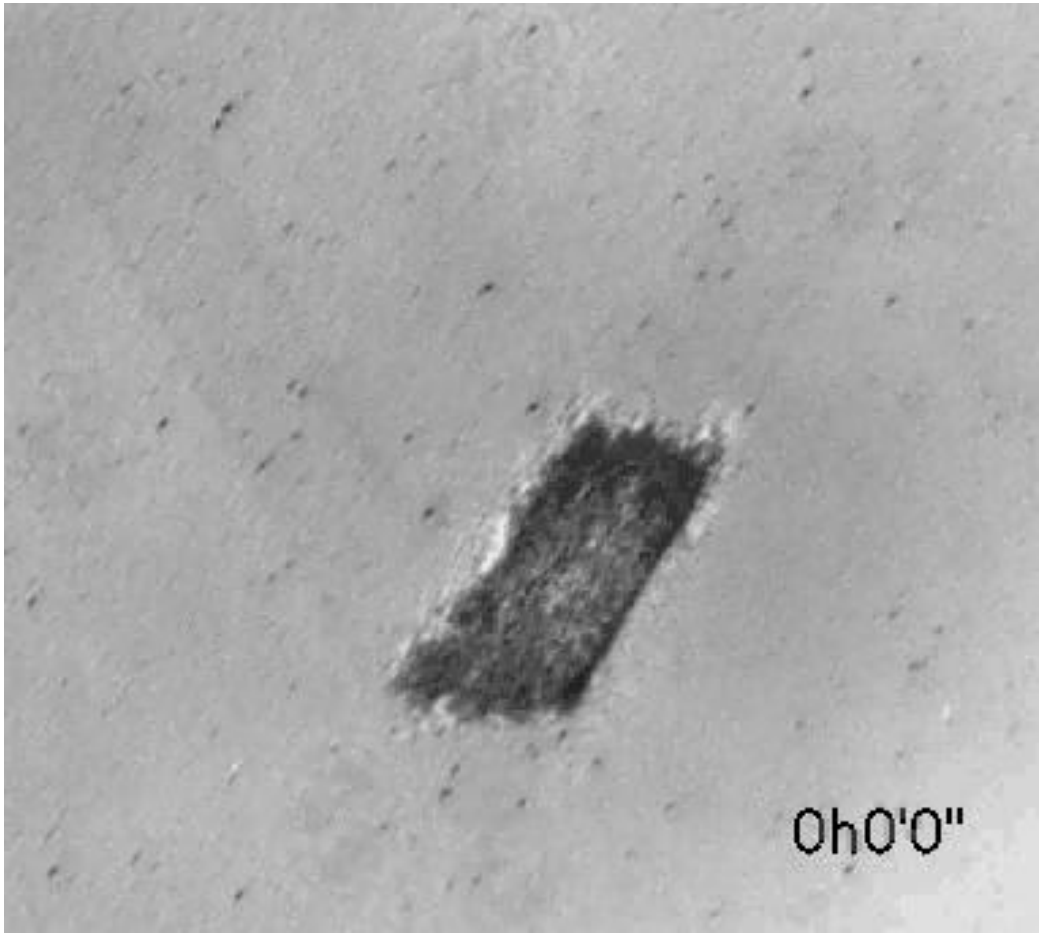


Hela cells

Images: Manuel Thery

Some applications of micro-patterning for cell biology

Control of internal cell organisation and cell division axis by adhesion geometry

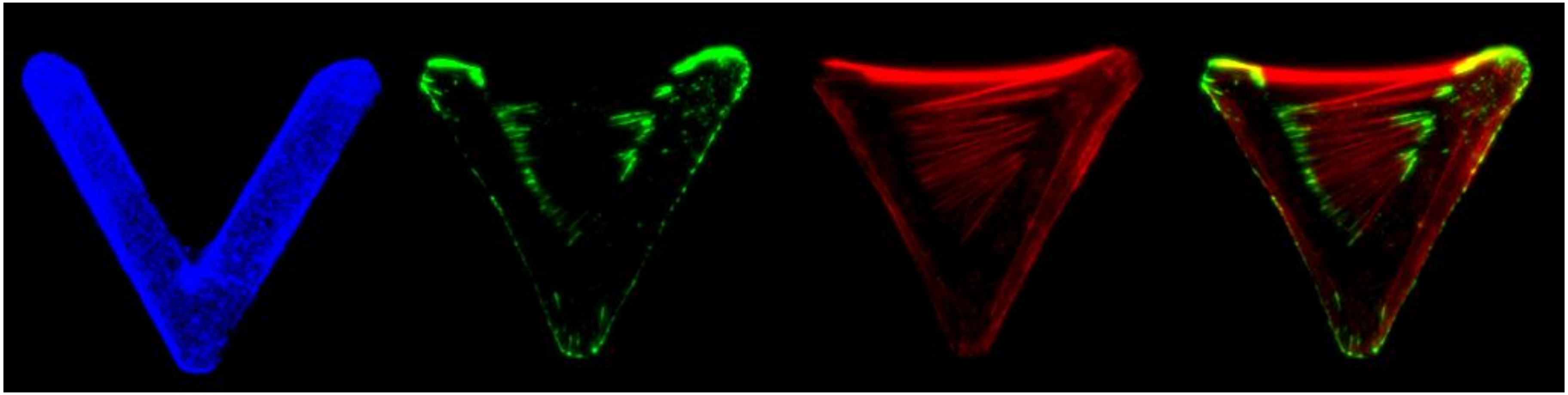


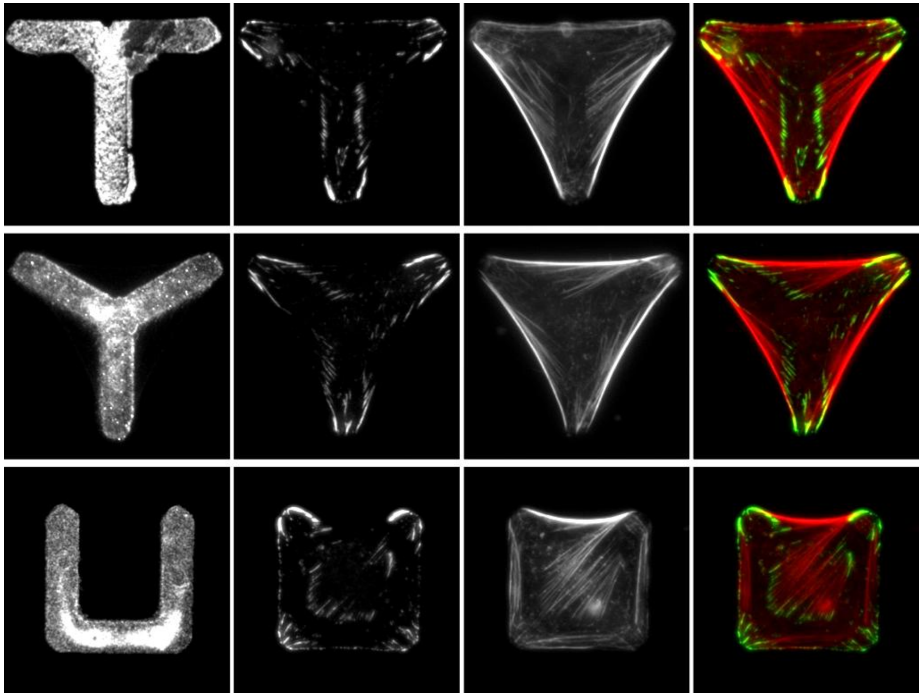
Thery et al., **CMC, 2006**

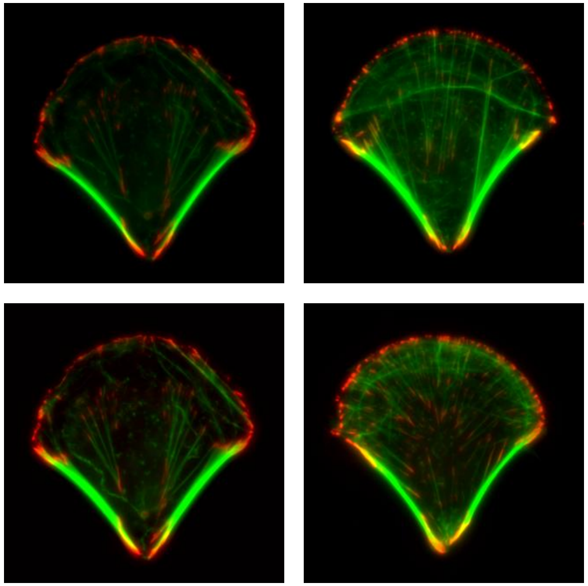
RICM

Cells form stress fibers over non adhesive edges

fibronectin vinculin actin



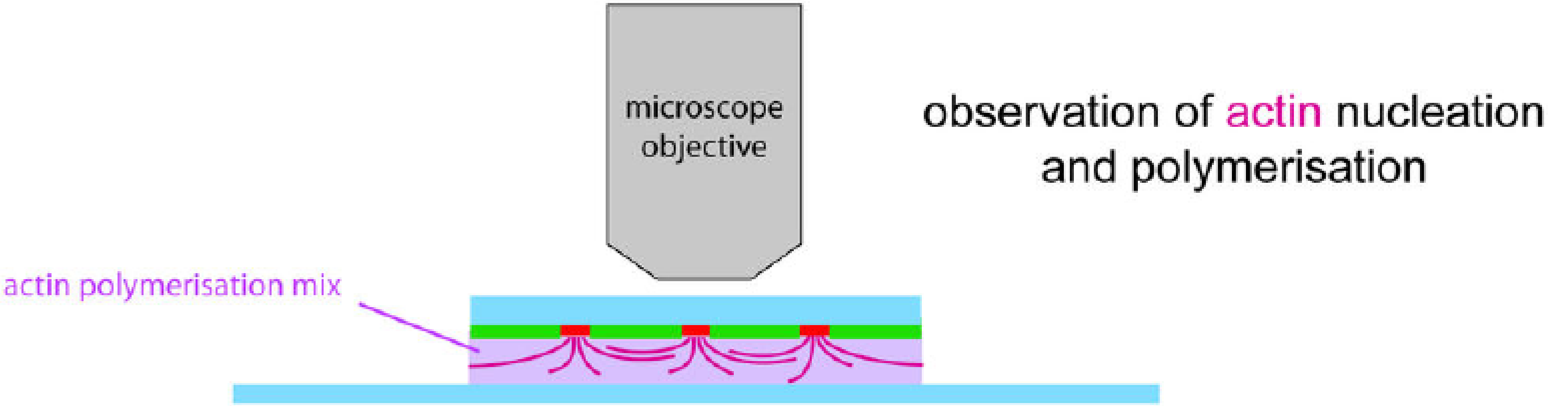


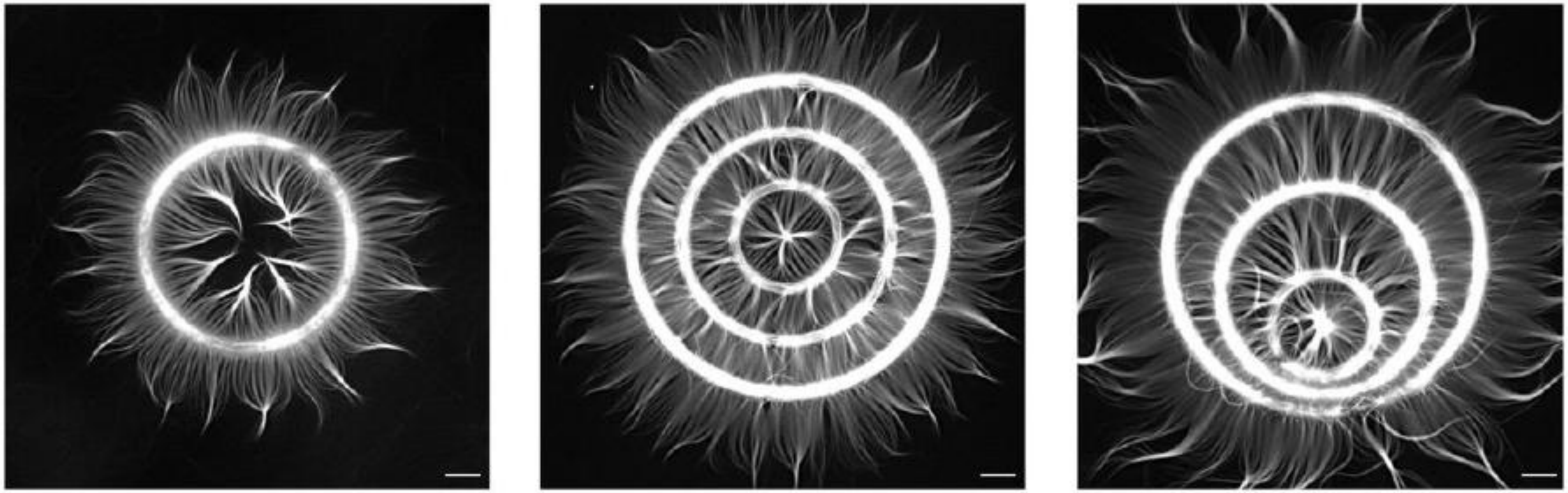


Thery et al., CMC and PNAS, 2006

Some applications of micro-patterning for cell biology

Control of actin nucleation pattern in vitro

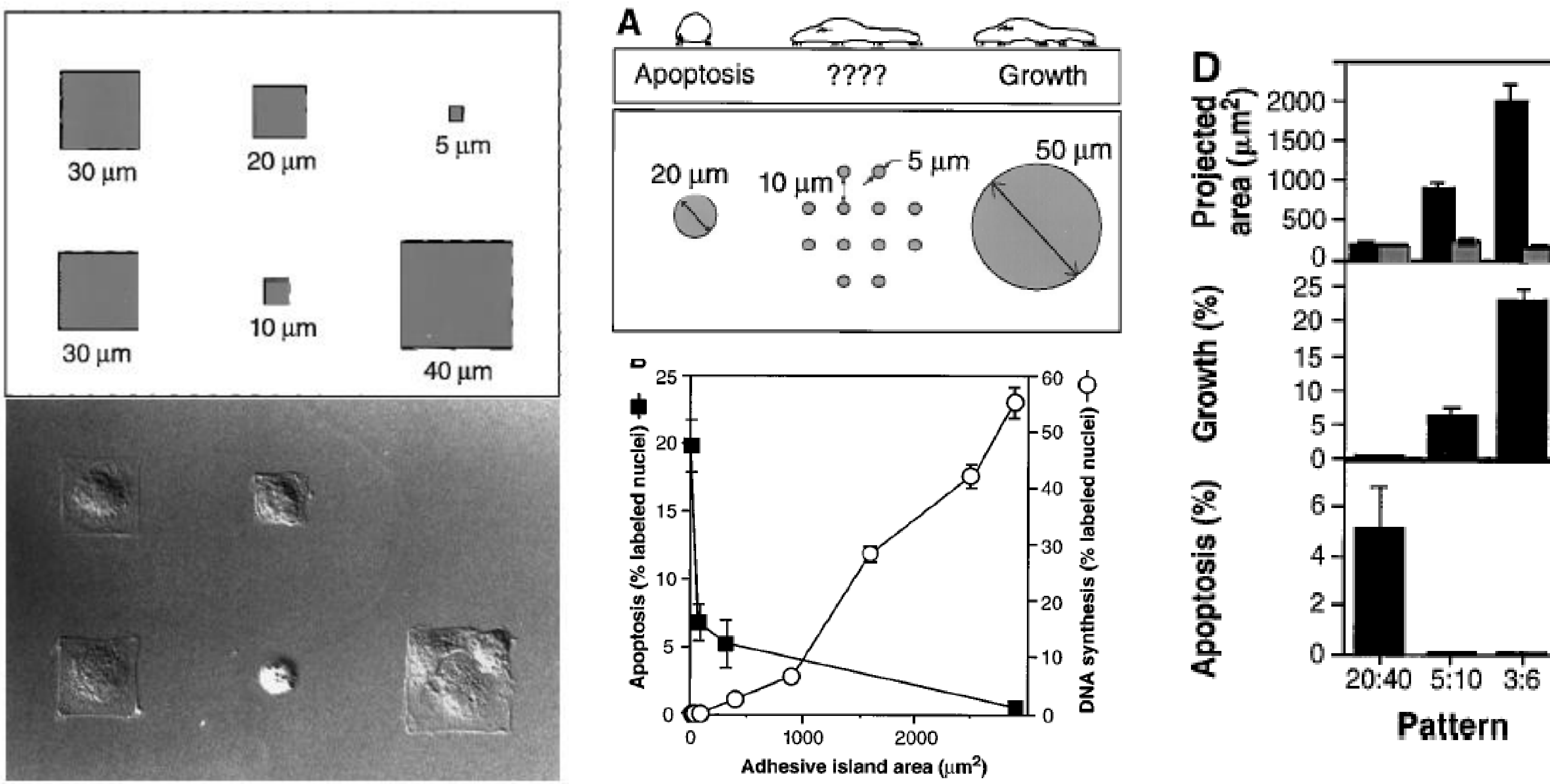




Raymann et al., Nat. Mat., 2010

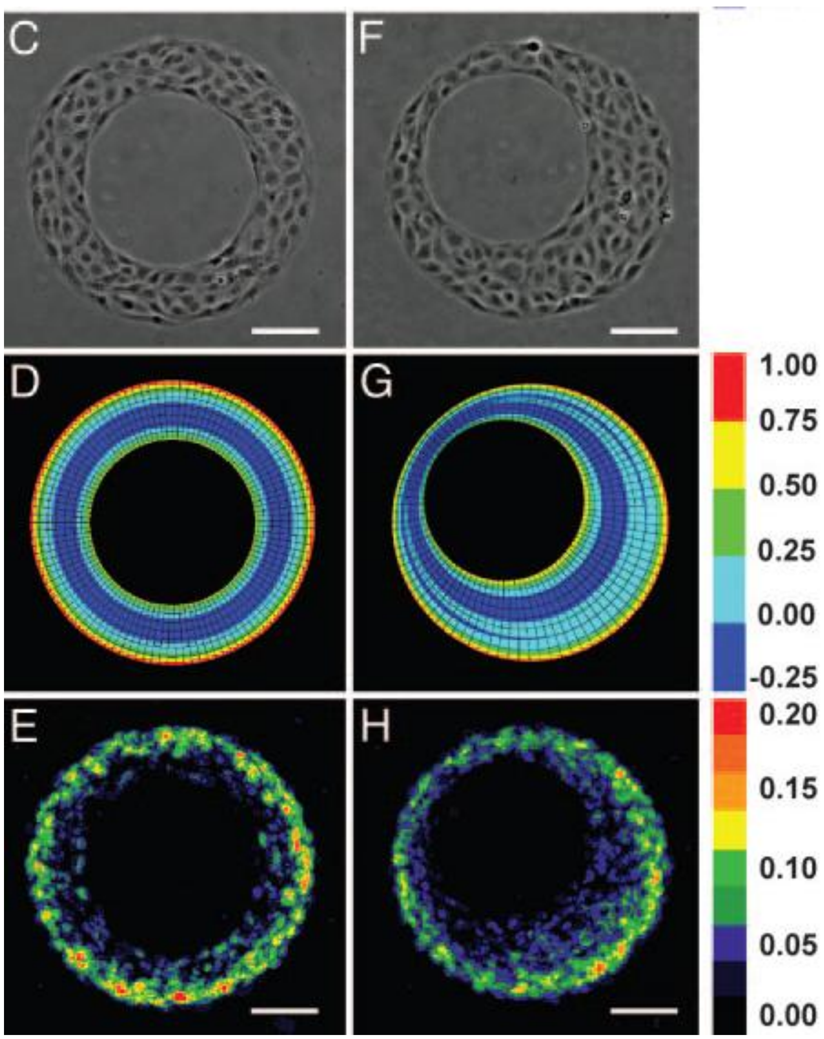
Some applications of micro-patterning for cell biology

Cell survival depends on cell spreading area, not adhesion area

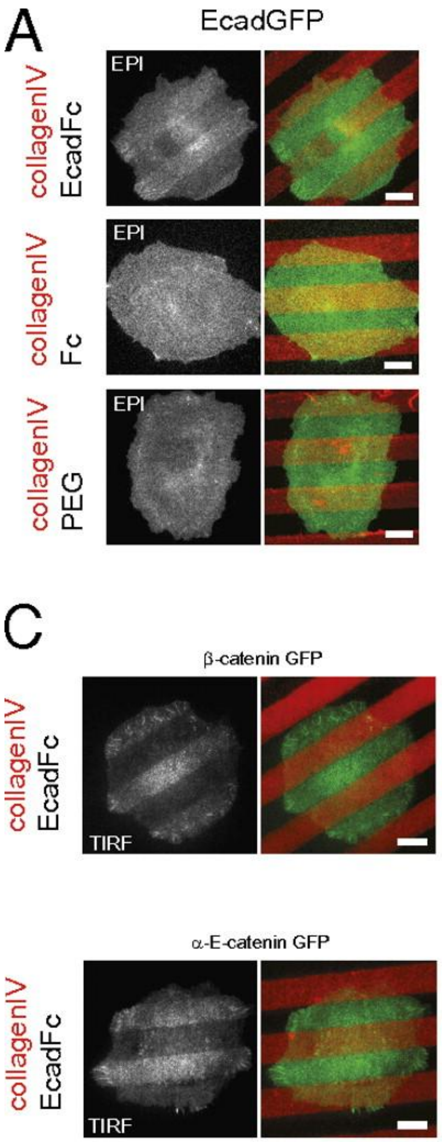


Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. Science. 1997 May 30;276(5317):1425-8

Some applications of micro-patterning for cell biology



Nelson et al., PNAS 2005

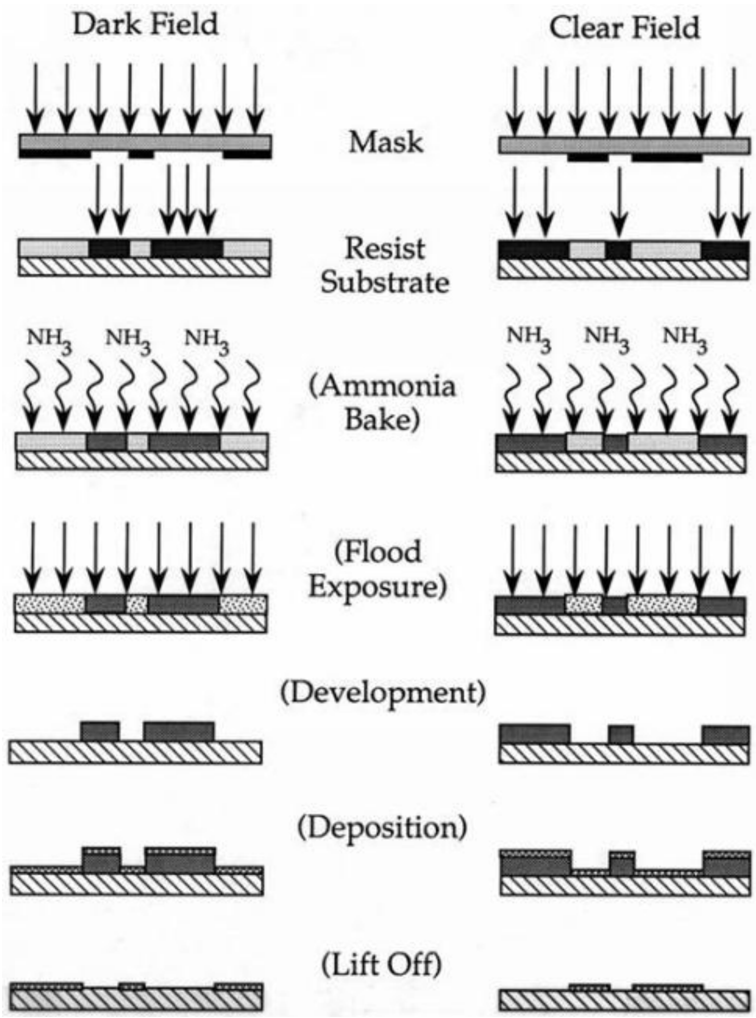


Borghi N et al. PNAS 2010

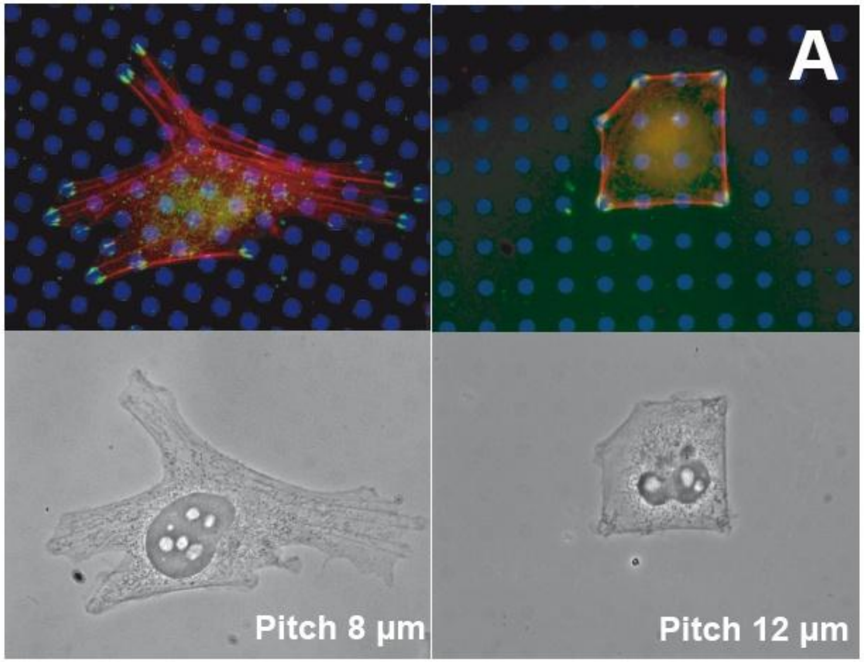
Making micro-patterns:
how to choose between a thousand and one technique?

Photolithography and lift-off

most robust but most painful



Exple: fibronectin dots of 4 µm

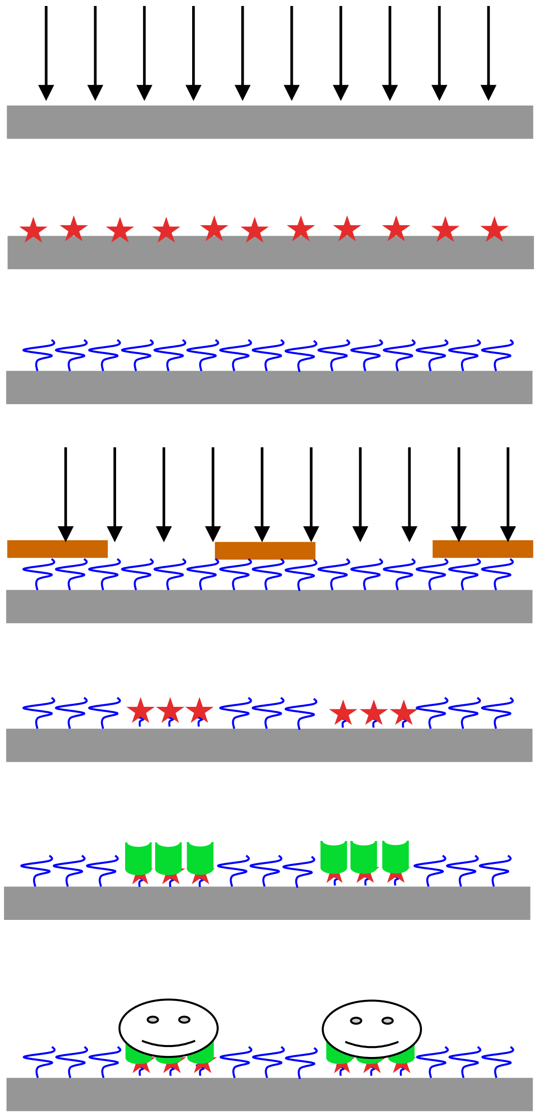


Guillou et al., Exp Cell Res.2008

To pattern proteins, see Sorribas et al, 2002, Biomaterials

UV-based patterning

Deep UV through a photo-mask



1. Deep UV irradiation of native PDMS or PACMD

Functional chemical groups on PDMS or PACMD surface

2. PLL-g-PEG-coated PDMS or PACMD surfaces

3. Deep UV irradiation through a mask of PLL-g-PEG-coated surfaces

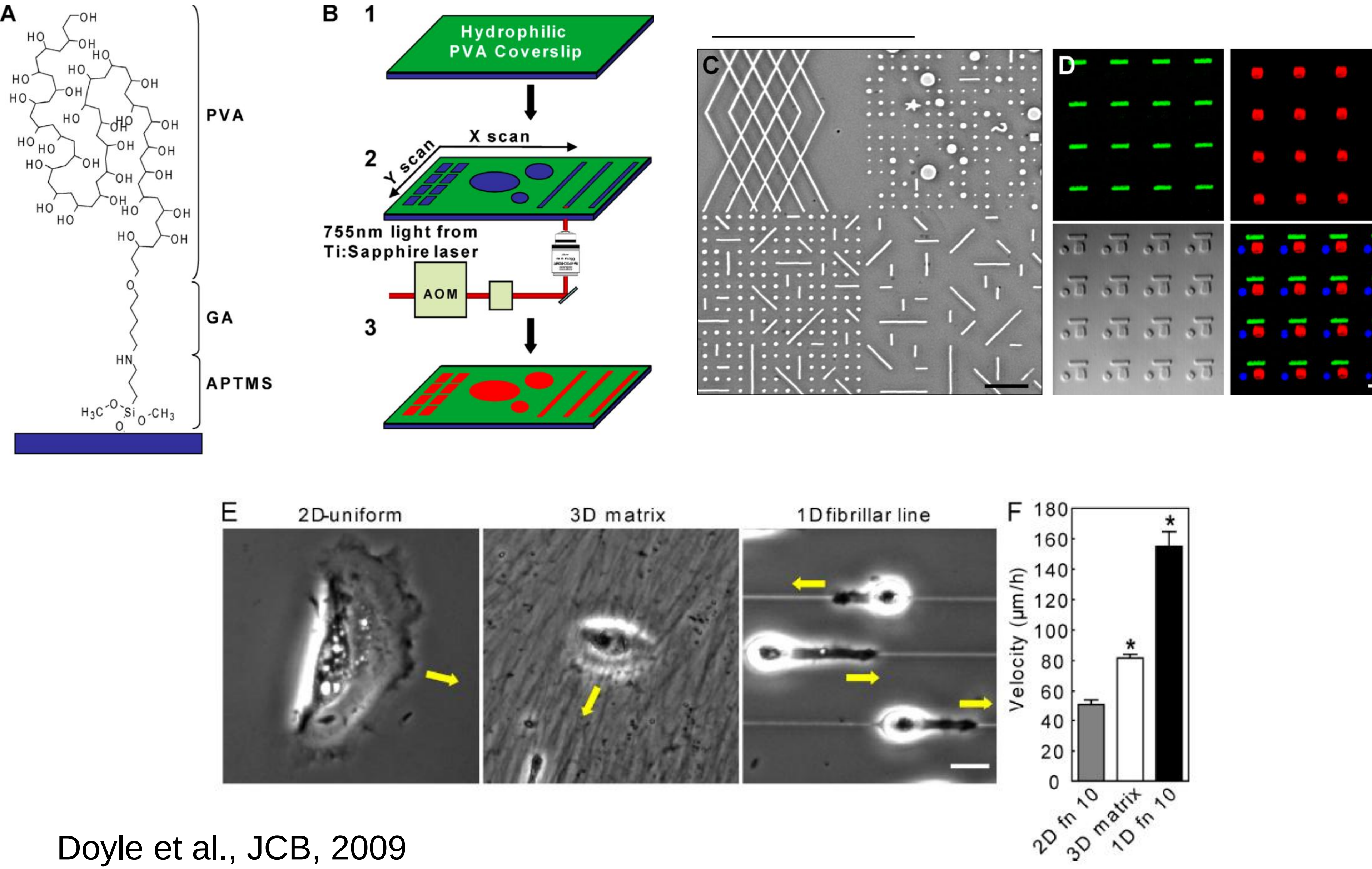
Functional chemical groups on the irradiated regions

Adsorption of Fibronectin onto irradiated regions

Cell adhesion on the Fibronectin patterns

Azioune et al., LOC, 2009 and see also papers by Welle and by Peterbauer

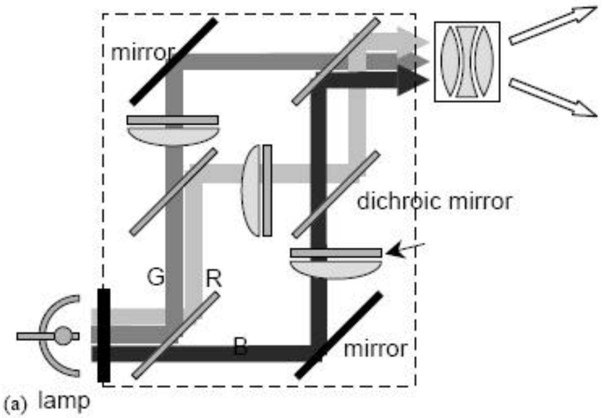
Laser beam writing

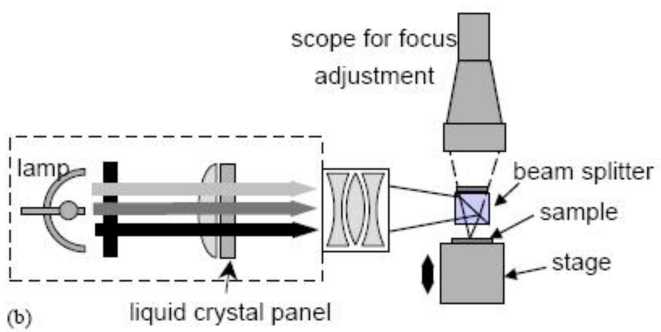


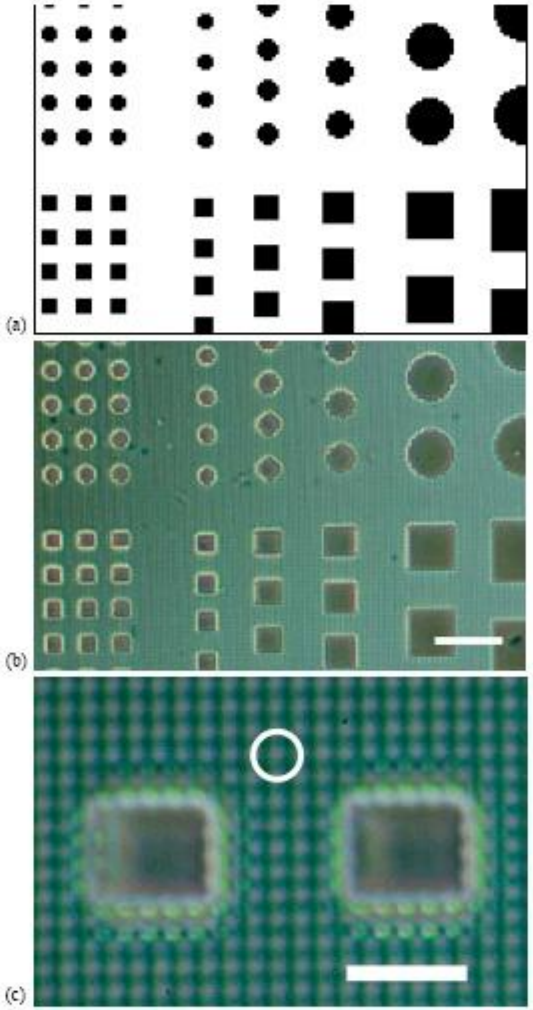
Doyle et al., JCB, 2009

UV-based patterning

with a video-projector



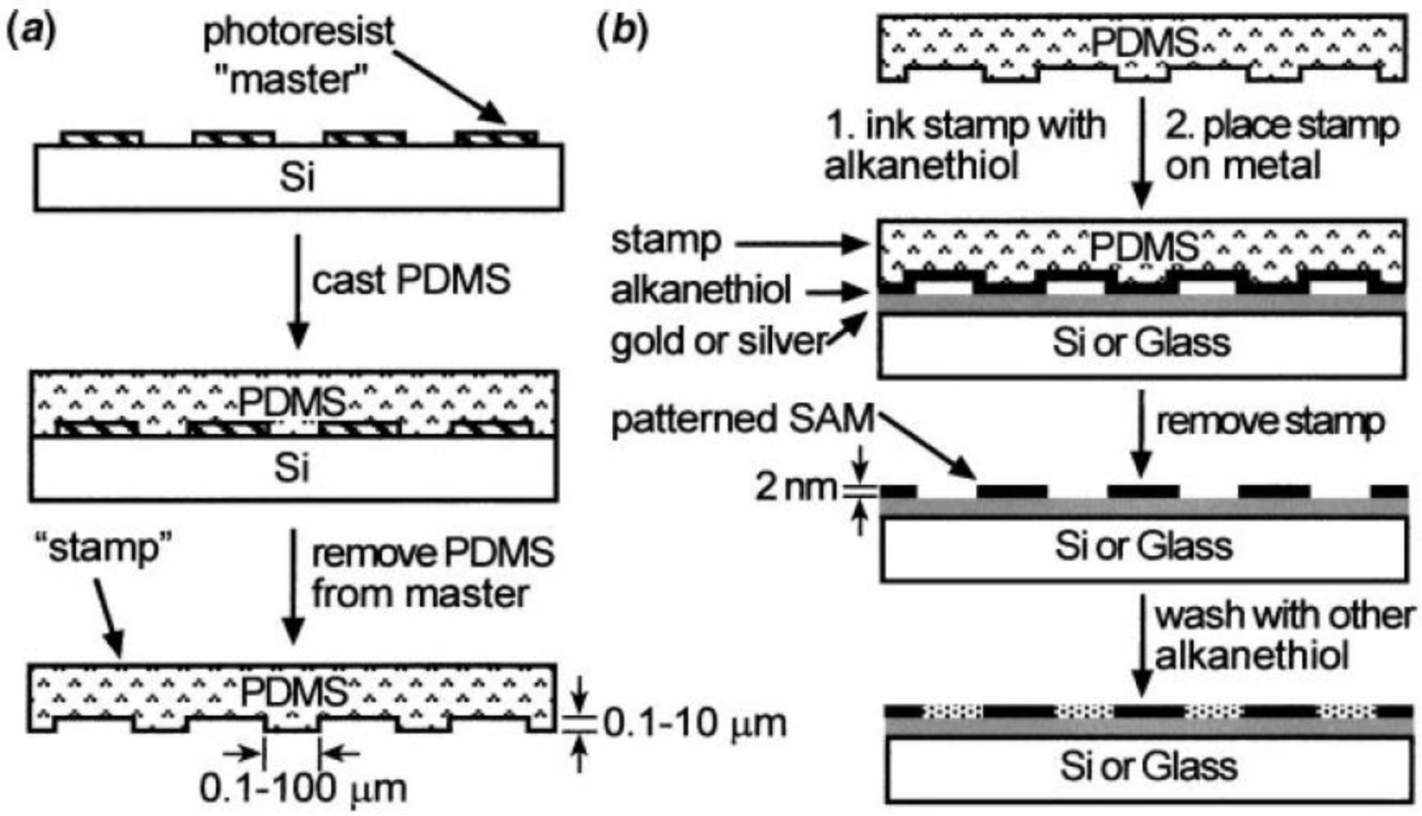




Itoga, Biomaterials , 2004

The most classic: micro-contact printing

SAMs on gold

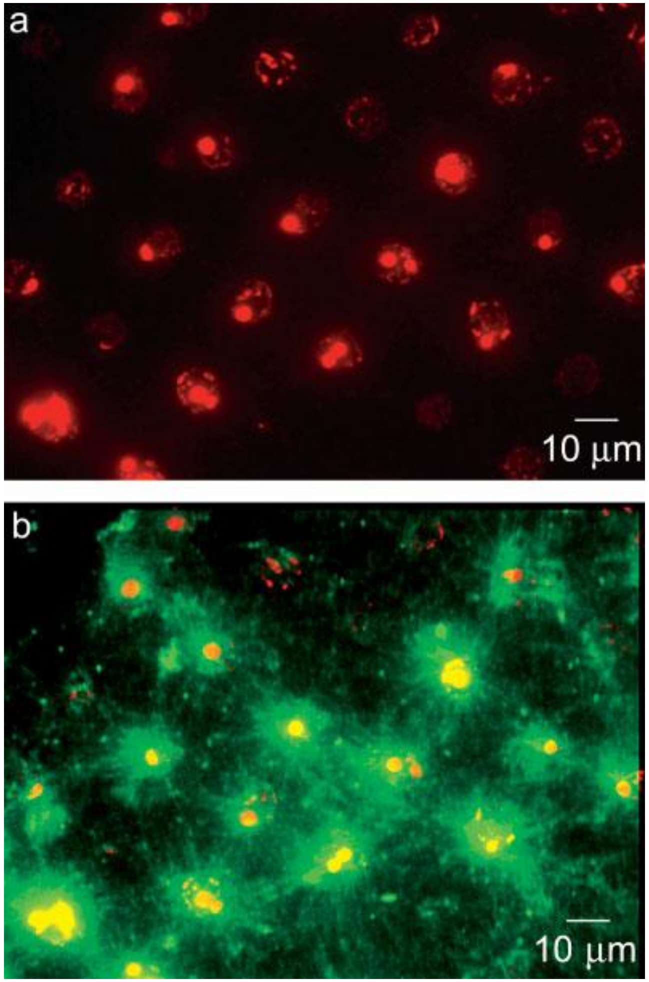


Whitesides, Ann. Rev. Biomed. Eng., 2001

Technique developed and extensively used by G. Whitesides and D. Ingber labs (see Otsuni, Meth. Mol. Biol., 2009)

The most classic: micro-contact printing

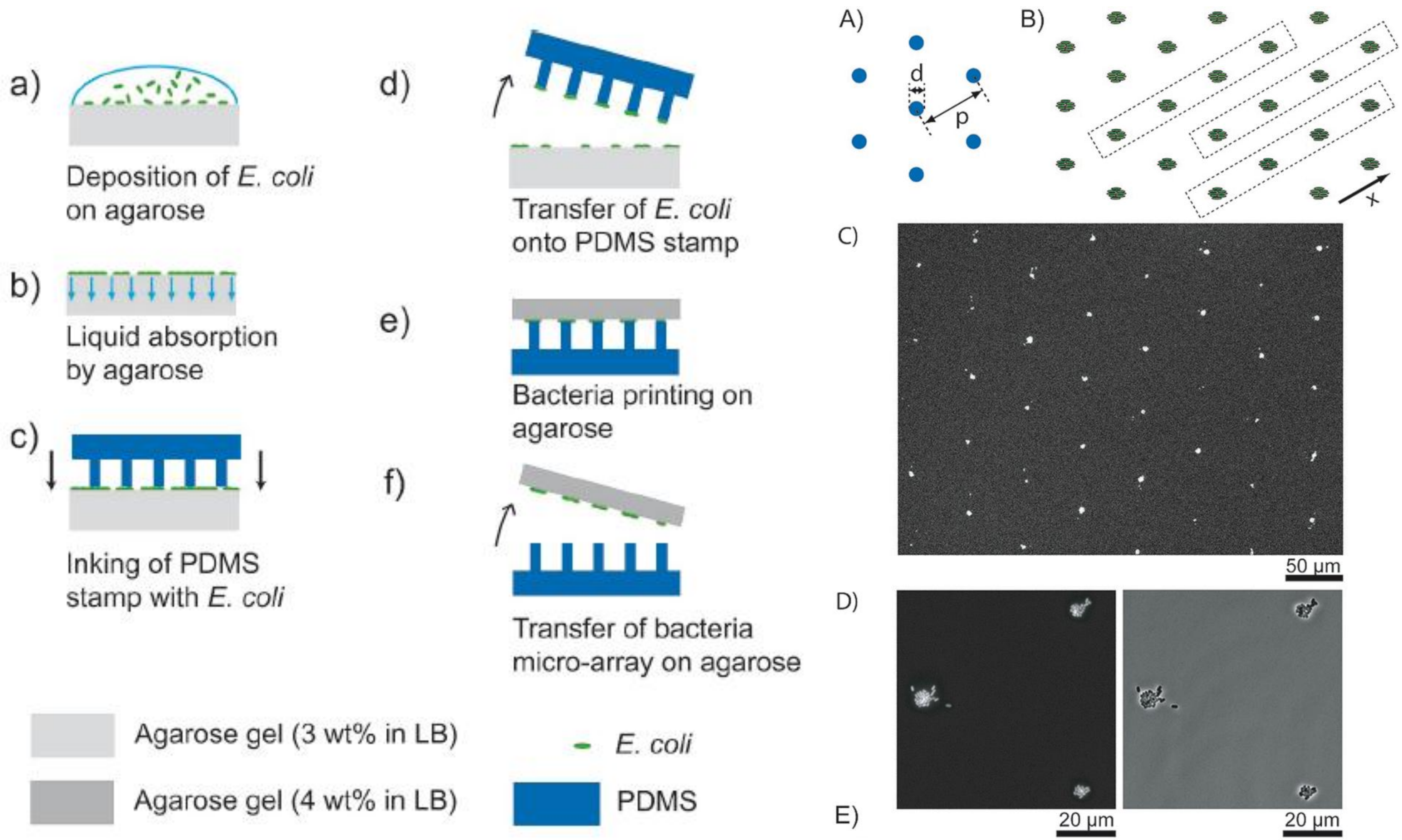
Printing centrosomes to make microtubule arrays



Shang W., Biotechnology and Bioengineering, 2005

The most classic: micro-contact printing

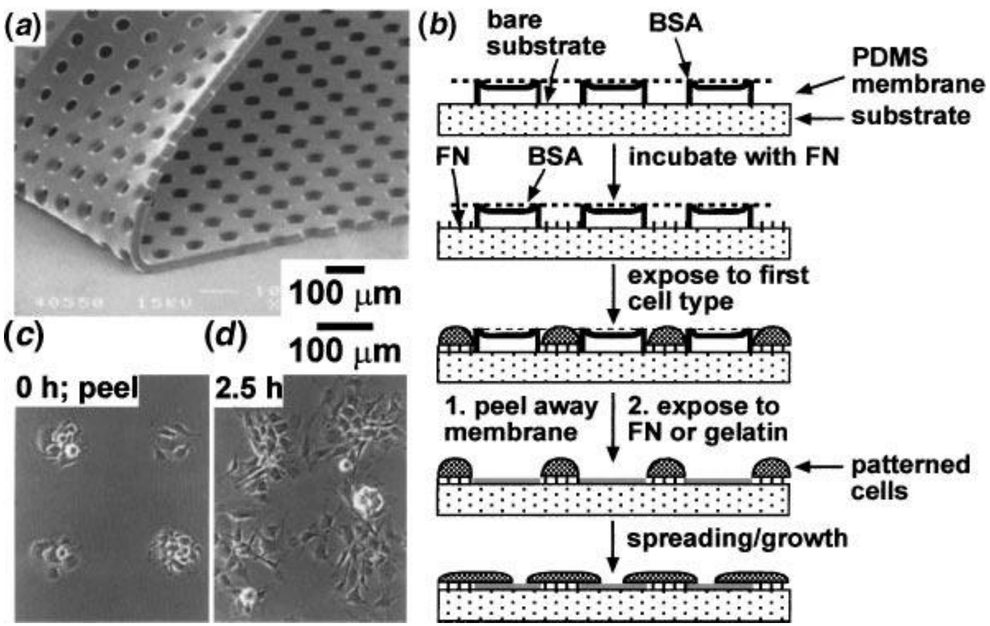
Printing bacteria



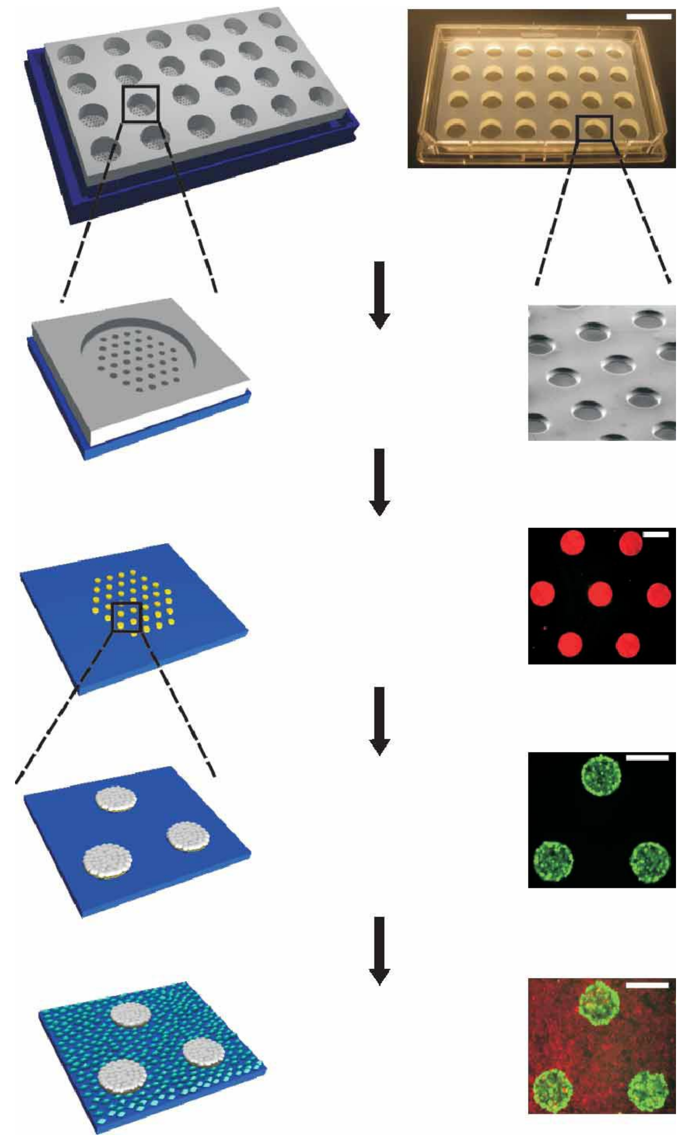
Xu L., NanoLet., 2007

Other techniques…

- micro-stencils (only for big patterns)



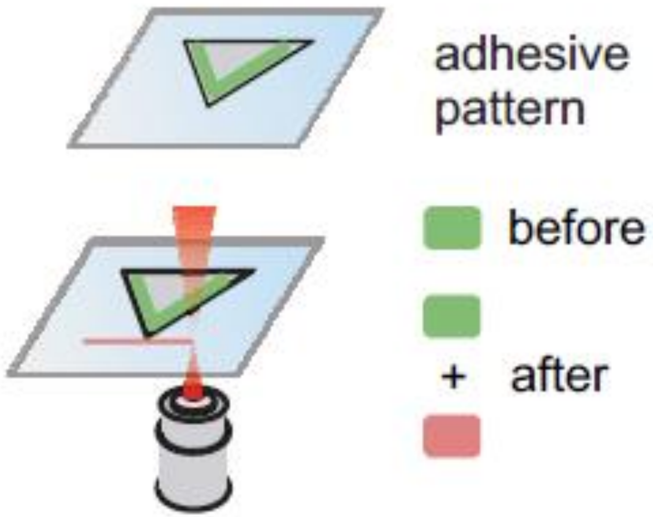
Jackman RJ, Langmuir, 1999

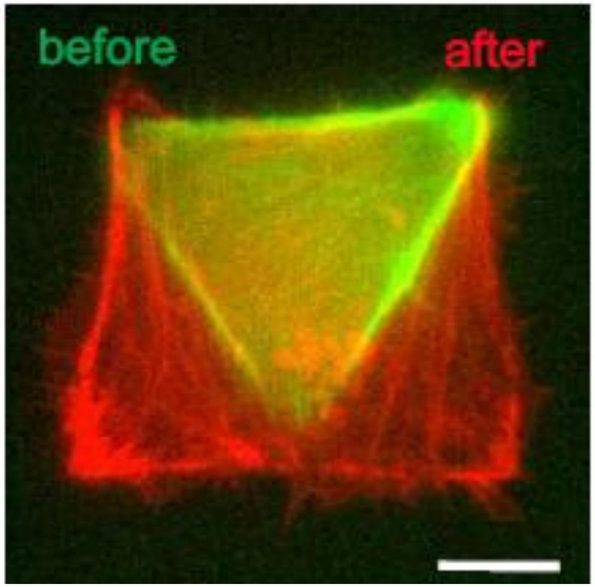


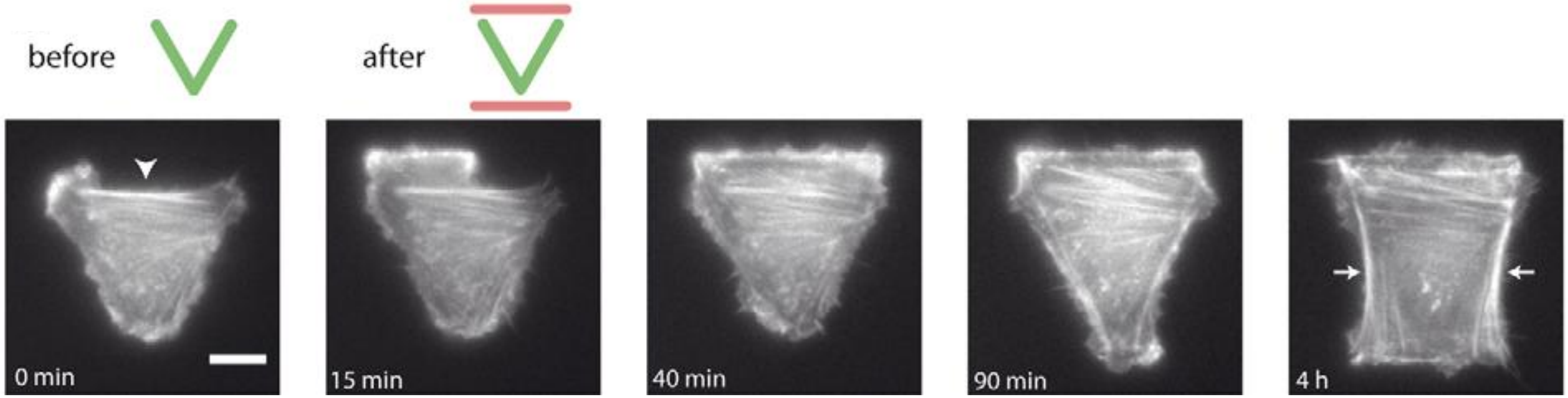
Khetani SR, Nat Biotech, 2008

Dynamic micro-patterns

Dynamic patterns: laser writting

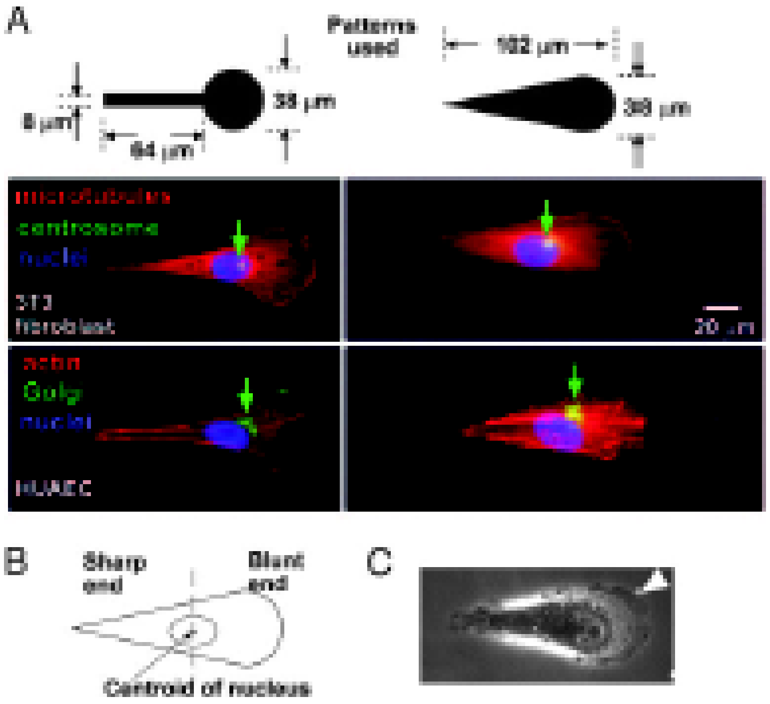


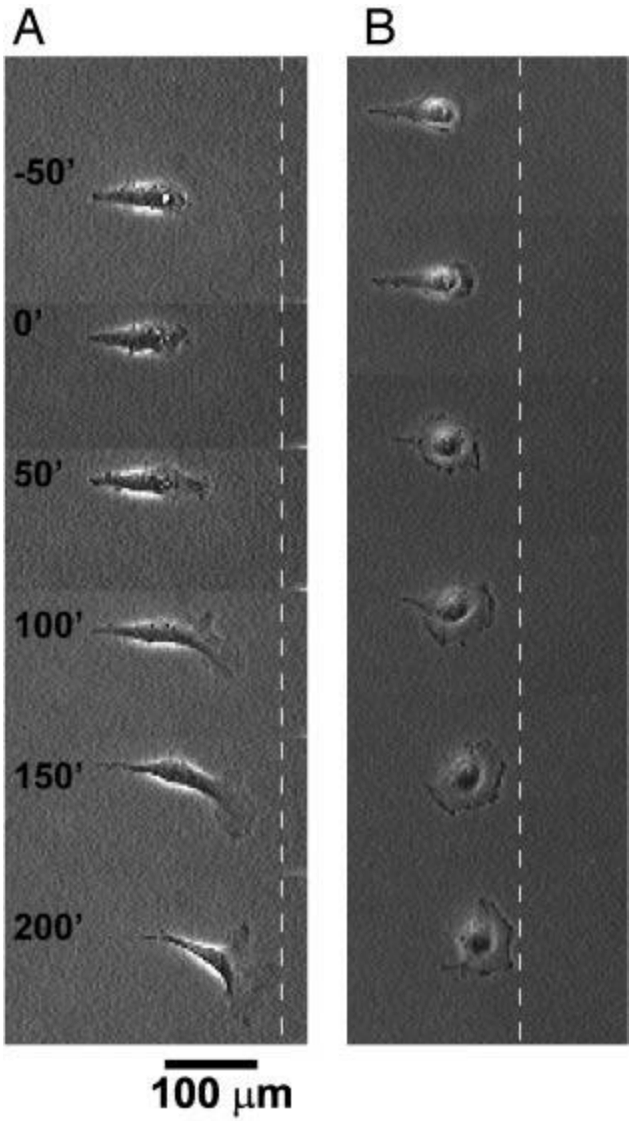




Vignault et al., JCS 2012

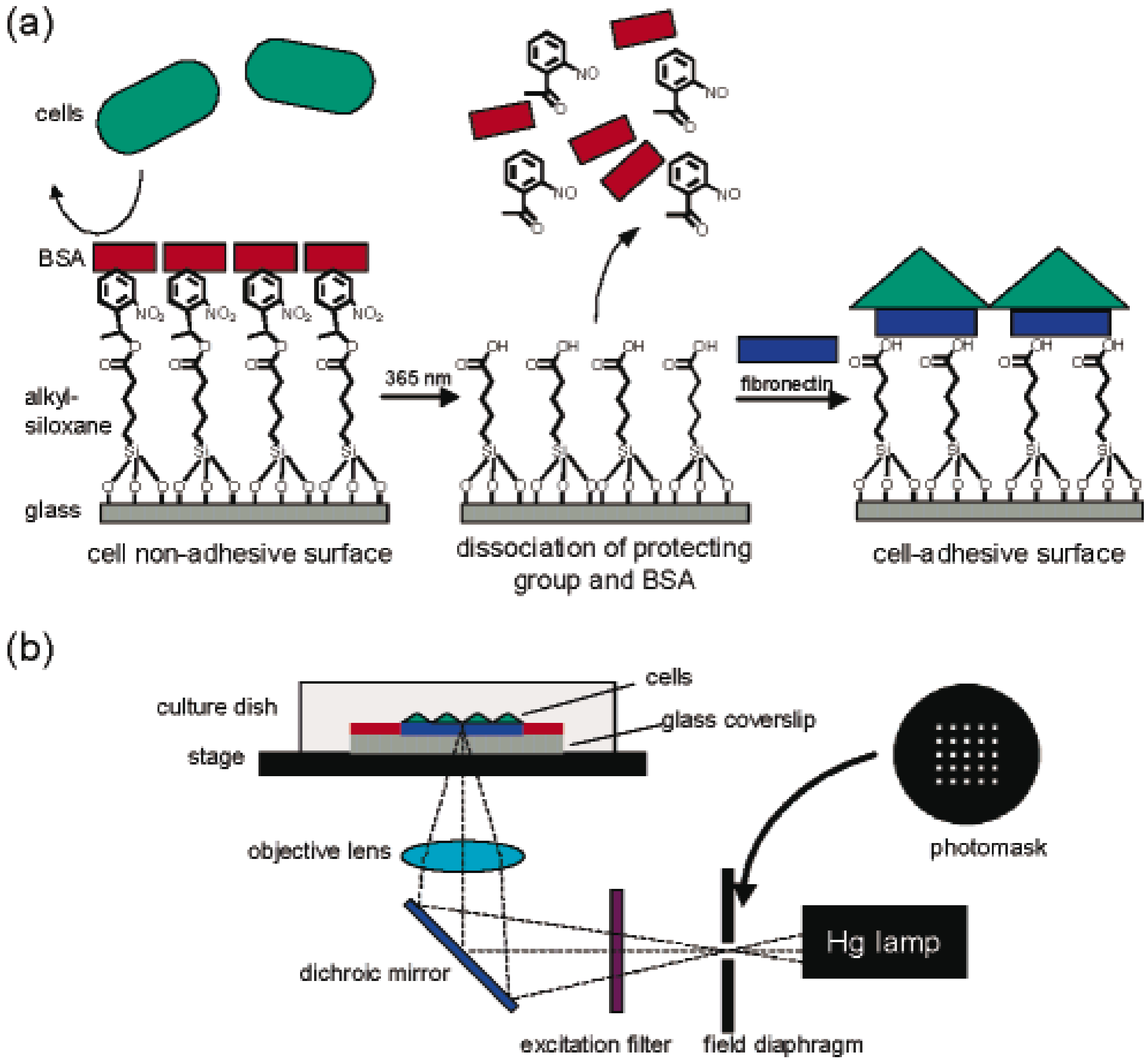
Dynamic patterns: electrochemical desorption



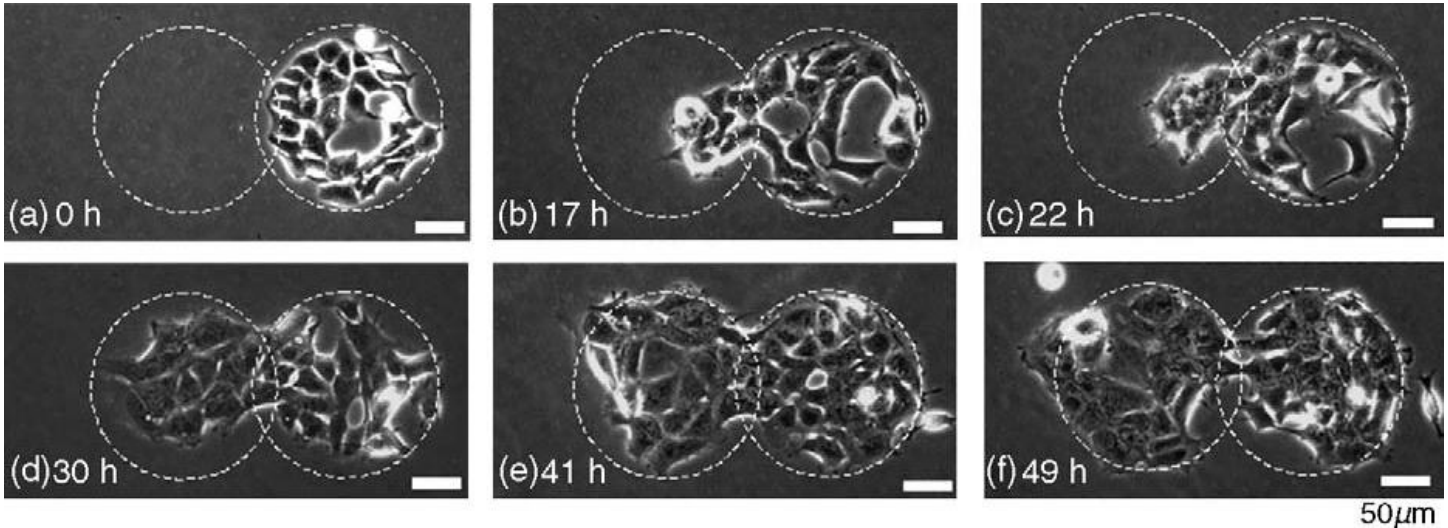


Jiang XJ et al., PNAS 2005

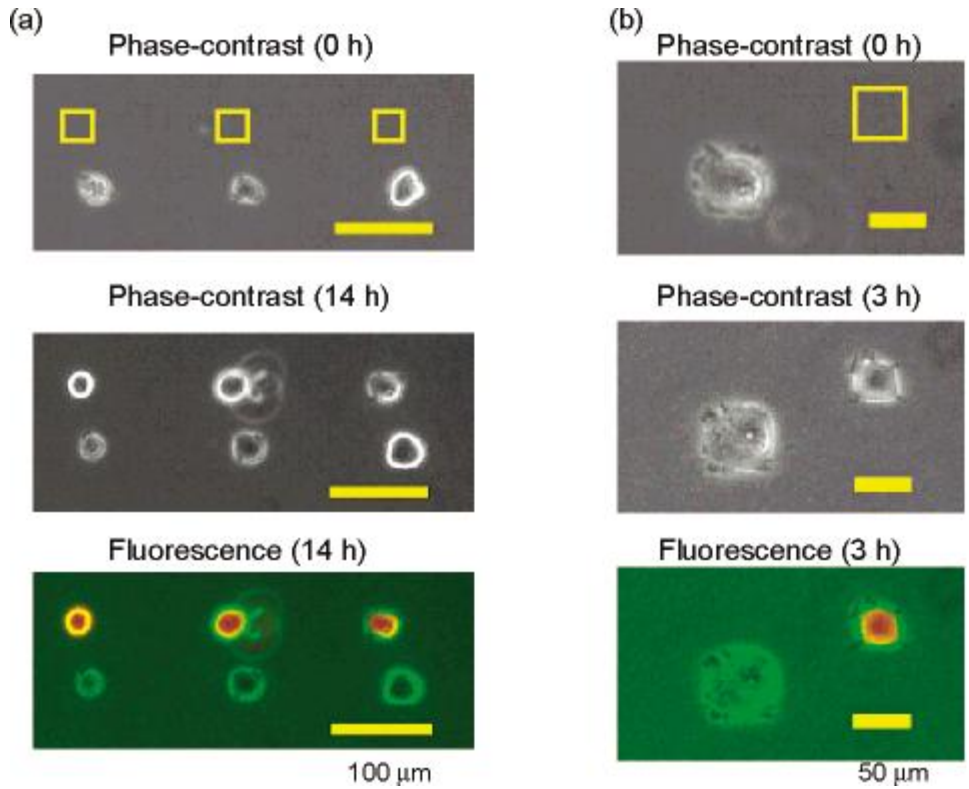
Dynamic patterns: photo-cleavable molecules



Nakanishi et al, JACS, 2004

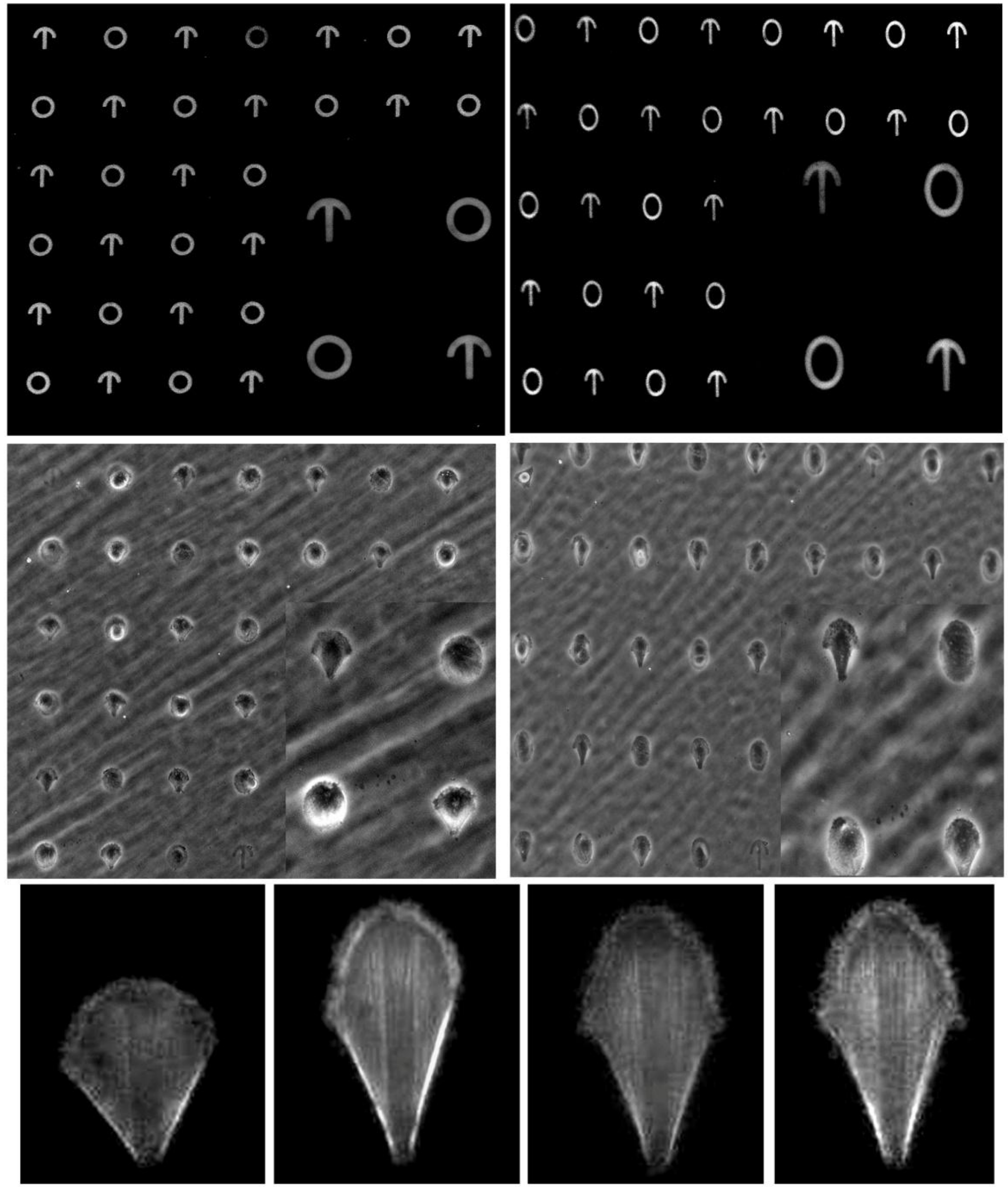


(a) 0 h
(b) 17 h
(c) 22 h
(d)30 h
(e)41 h
(f) 49 h
50 μm



(a)
Phase-contrast (0 h)
Phase-contrast (14 h)
Fluorescence (14 h)
100 μm
(b)
Phase-contrast (0 h)
Phase-contrast (3 h)
Fluorescence (3 h)
50 μm

Patterning on PDMS films for cell stretching: exerting forces



Fibrinogen

Phase contrast

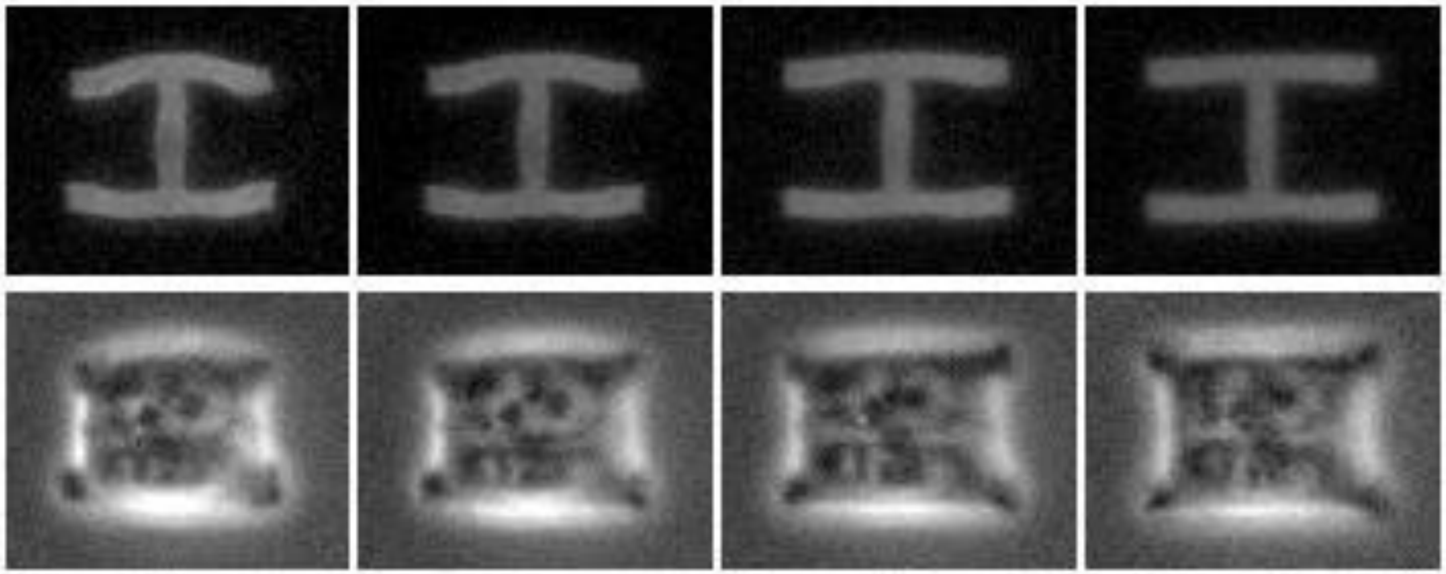
RPE1 cells
RLC-GFP

Before stretch after stretch

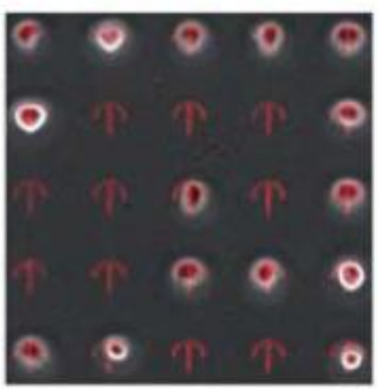
Azioune et al. Langmuir 2011

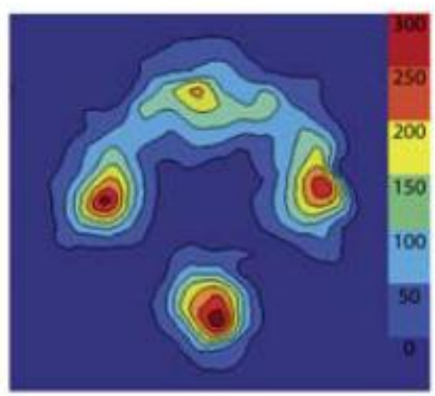
Deformable micropatterns for force measurement

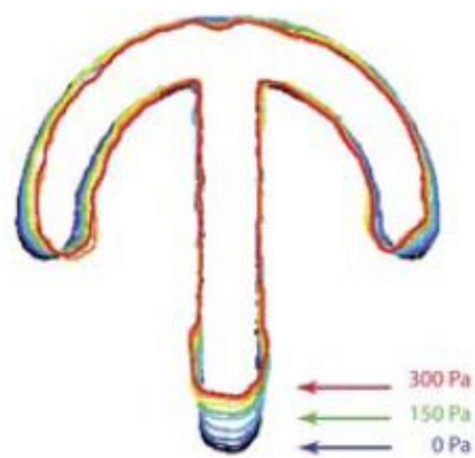
Addition of p160 ROCK inhibitor



MCF10A



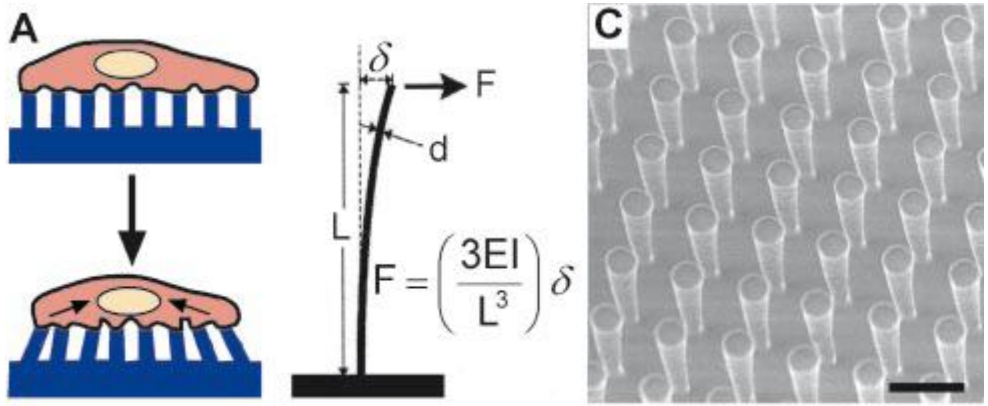




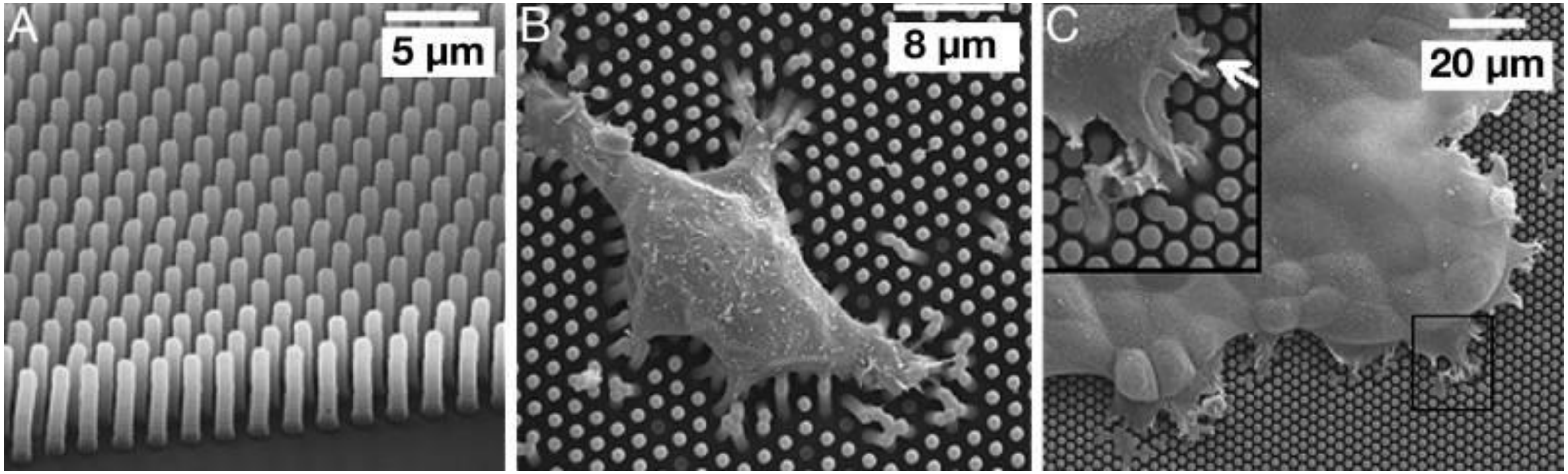
Tseng et al., LOC, 2011

Deformable microstructures for force measurement

- Micro-posts



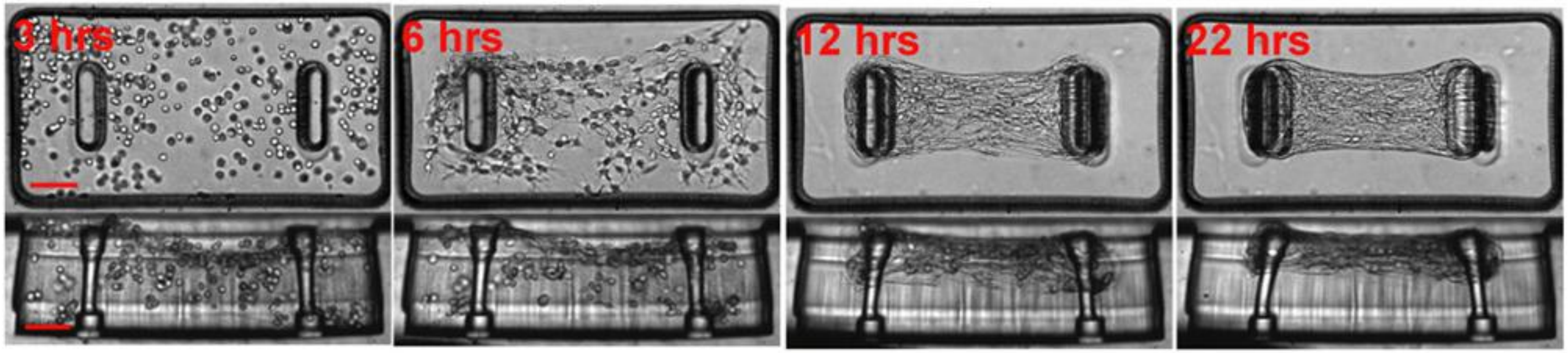
Tan JL, Tien J, Pirone DM, Gray DS, Bhadriraju K, Chen CS. Cells lying on a bed of microneedles: an approach to isolate mechanical force. Proc Natl Acad Sci U S A. 2003 Feb 18;100(4):1484-9

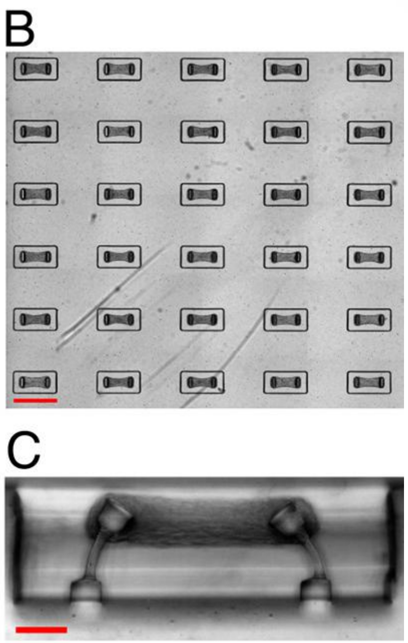


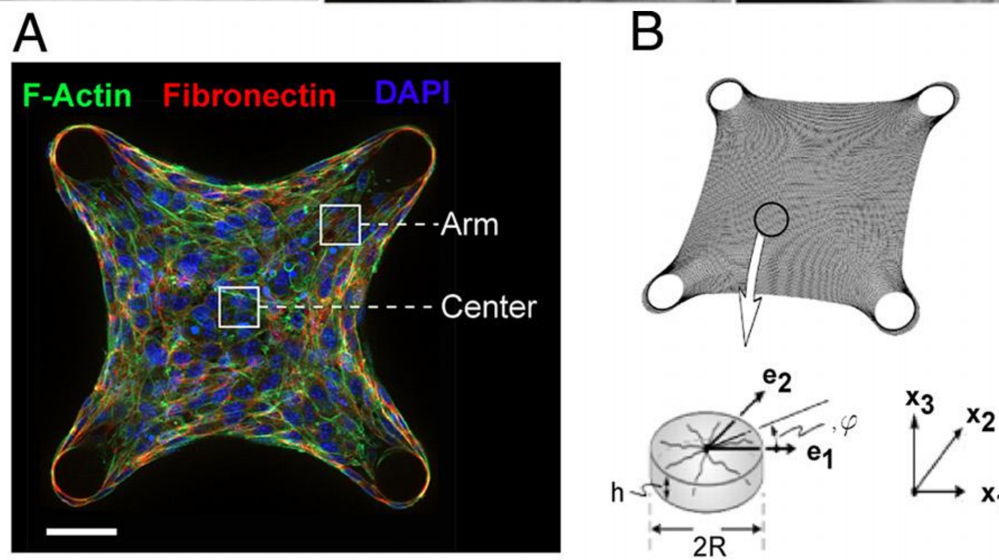
du Roure O, Saez A, Buguin A, Austin RH, Chavrier P, Silberzan P, Ladoux B. Force mapping in epithelial cell migration. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2390-5

Deformable microstructures for force measurement

- Macro-posts

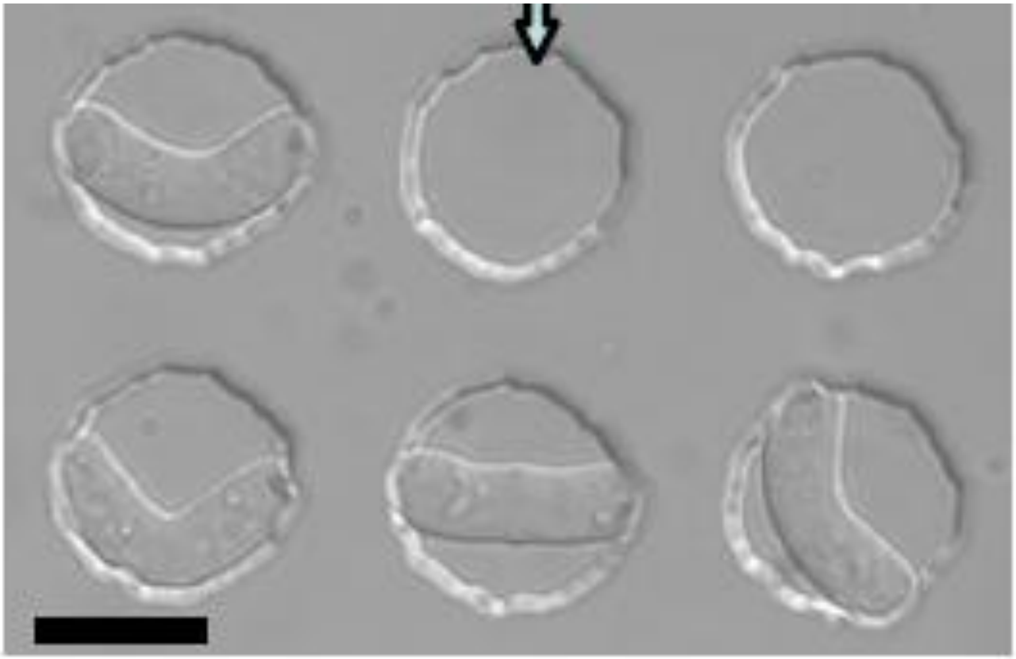




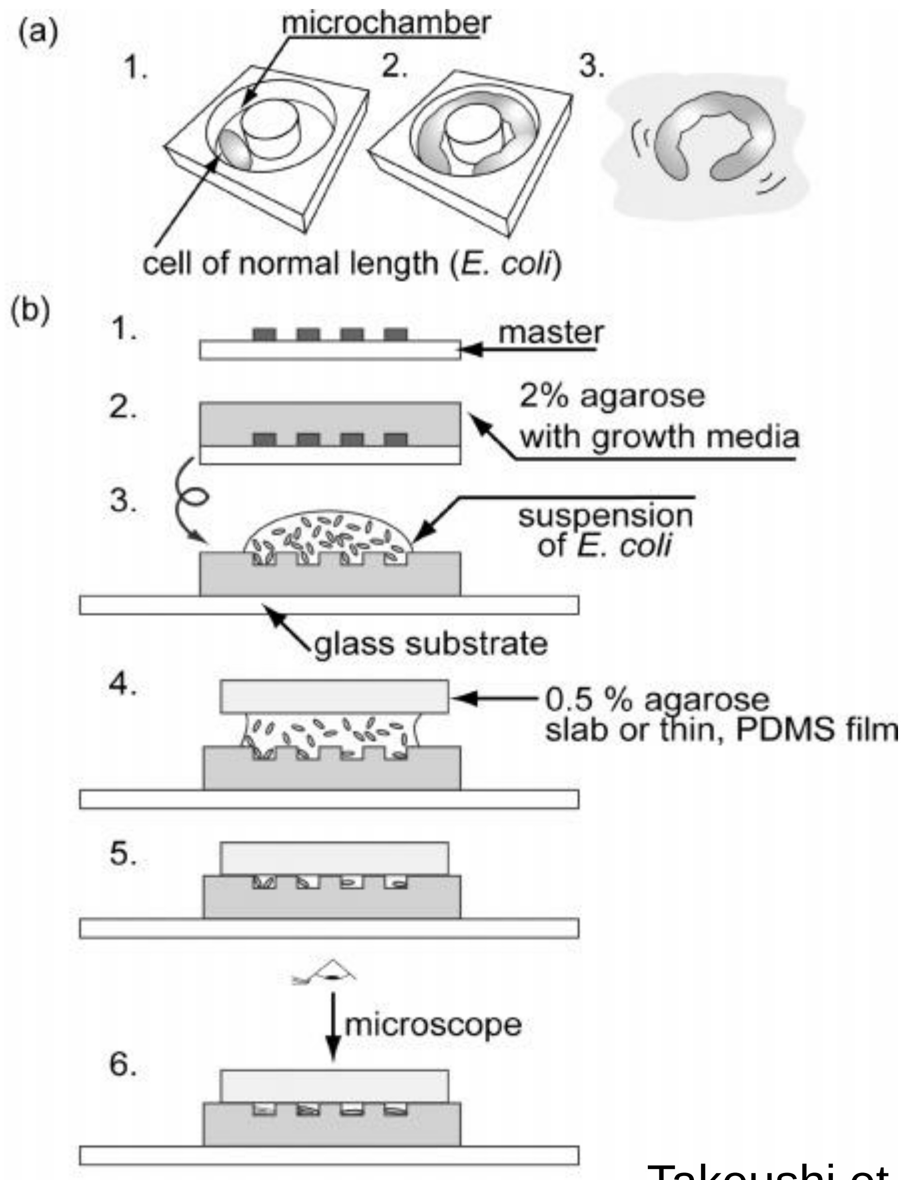


Legant WR, Pathak A, Yang MT, Deshpande VS, McMeeking RM, Chen CS. Microfabricated tissue gauges to measure and manipulate forces from 3D microtissues. Proc Natl Acad Sci U S A. 2009 Jun 23;106(25):10097-102

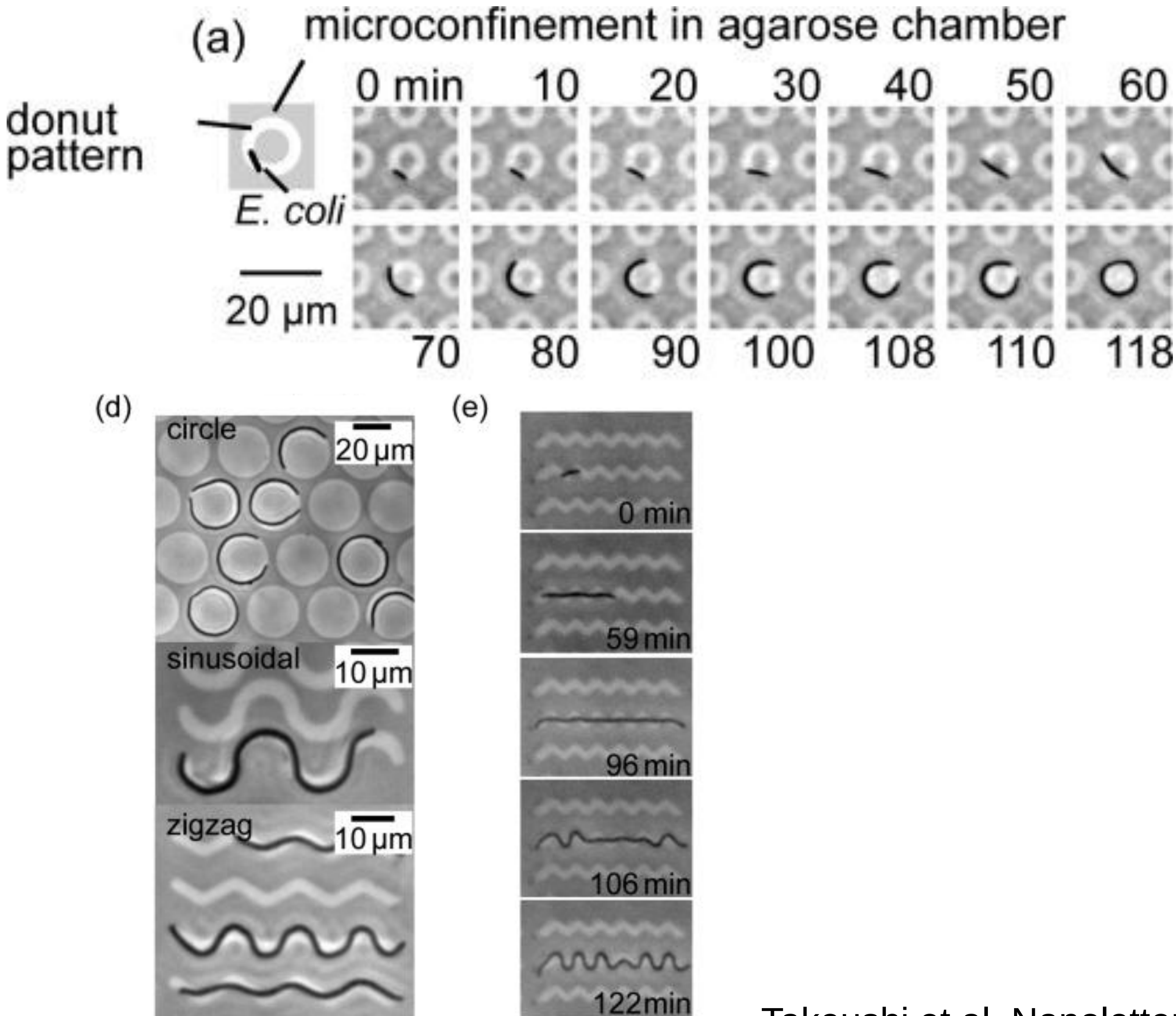
III. 3D Micro-patterning: micro-wells are the simplest '3D cell culture' system



Manipulating shape of bacteria

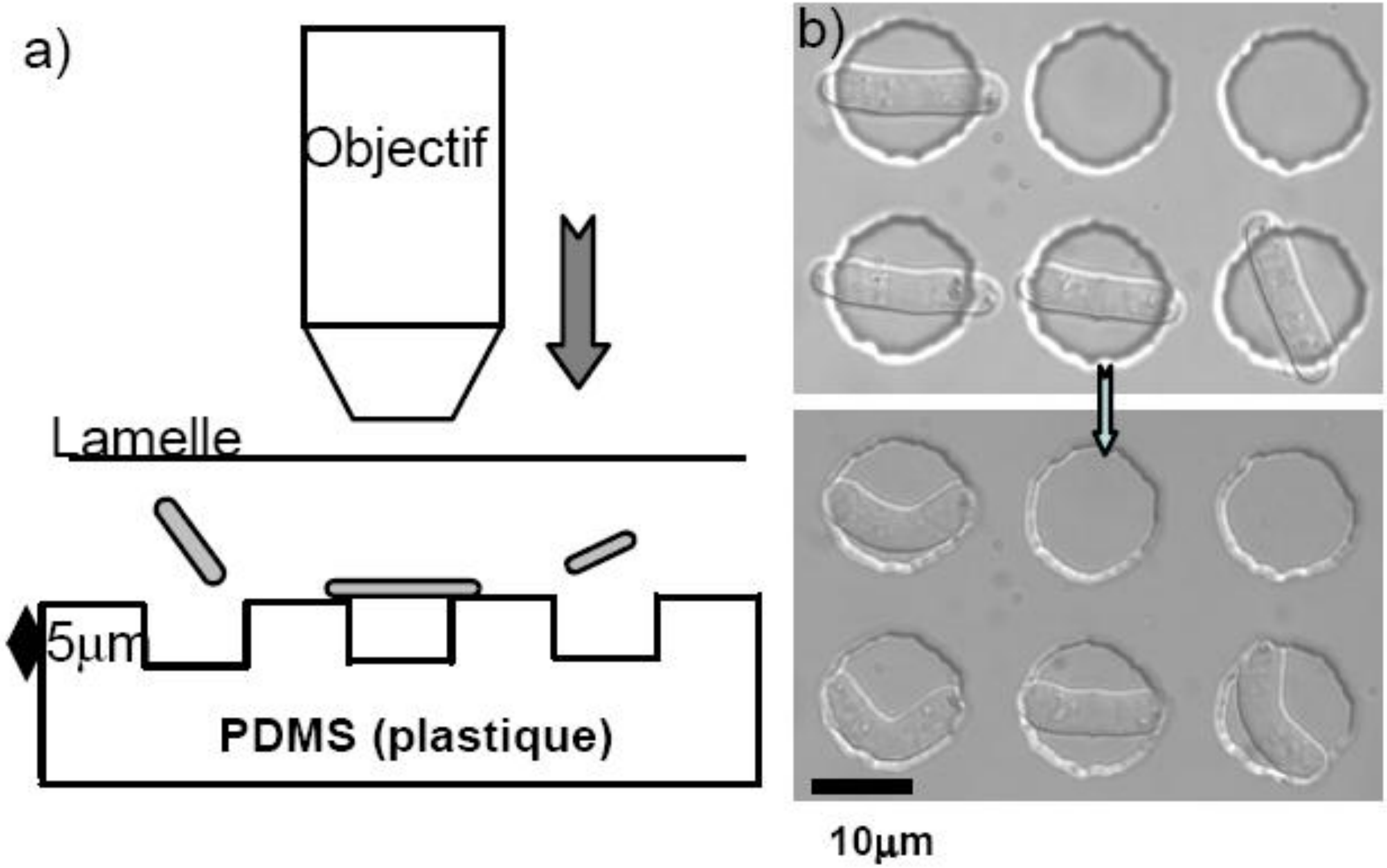


Takeushi et al. Nanoletters, 2005



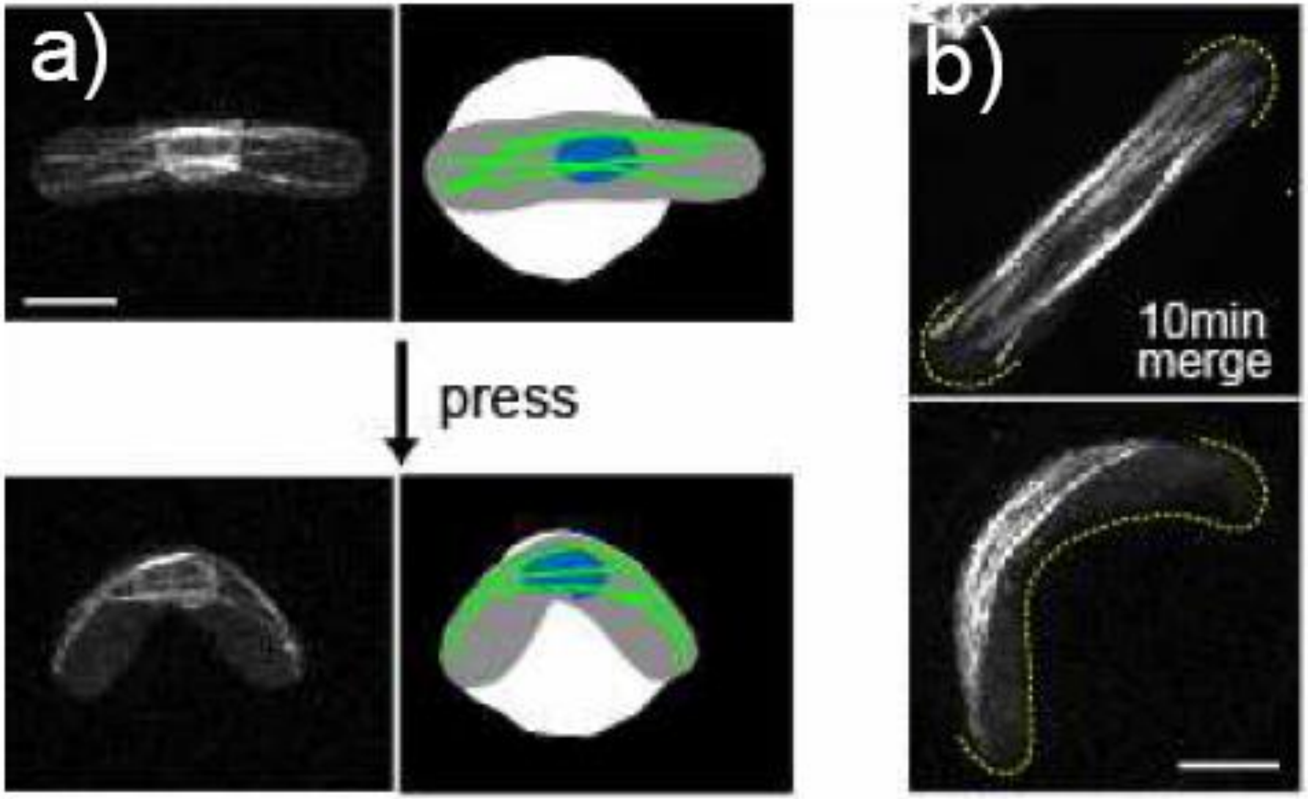
Takeushi et al. Nanoletters, 2005

Manipulating shape of yeast cells



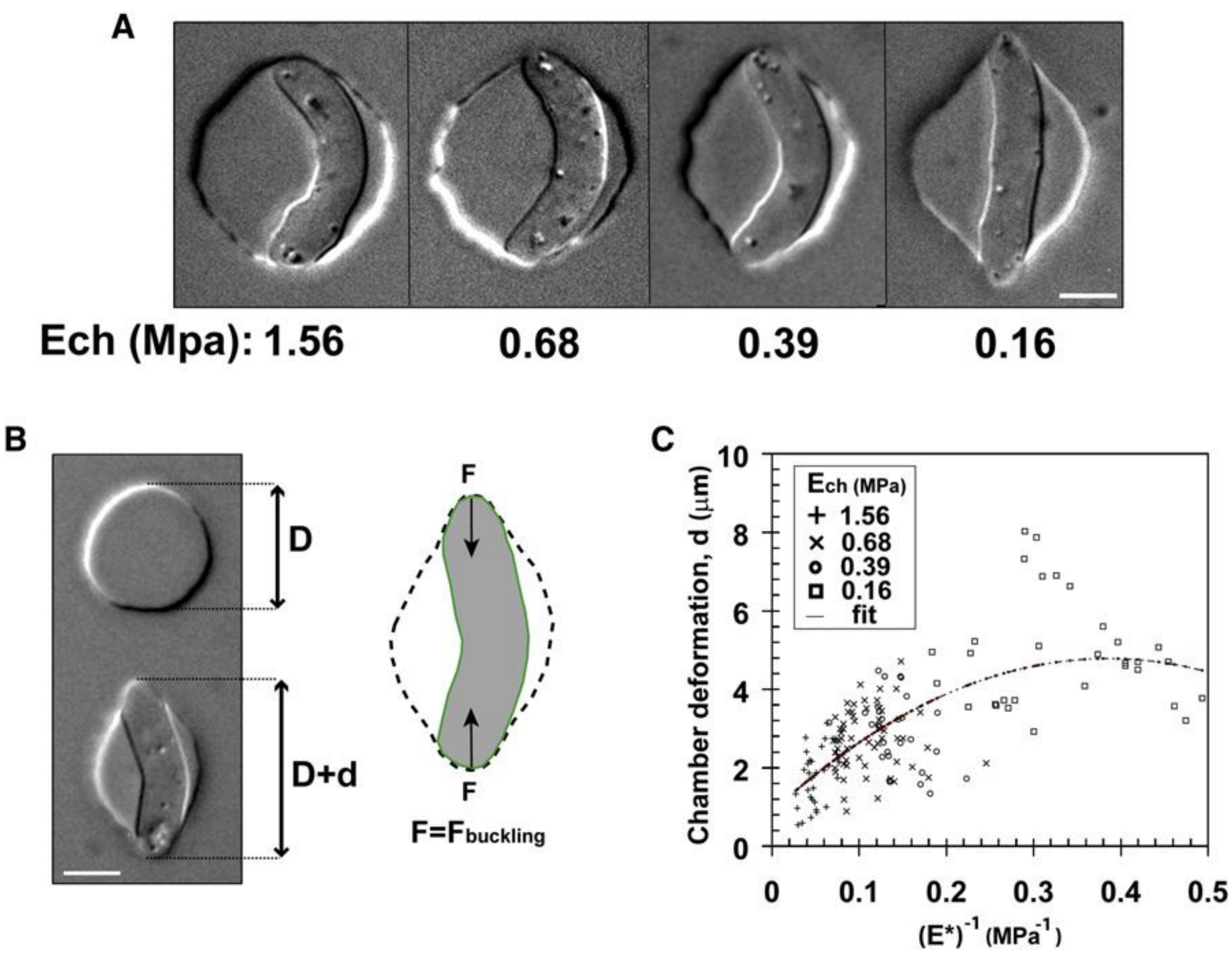
Minc N. et al., Curr. Biol., 2009

Manipulating shape of yeast cells



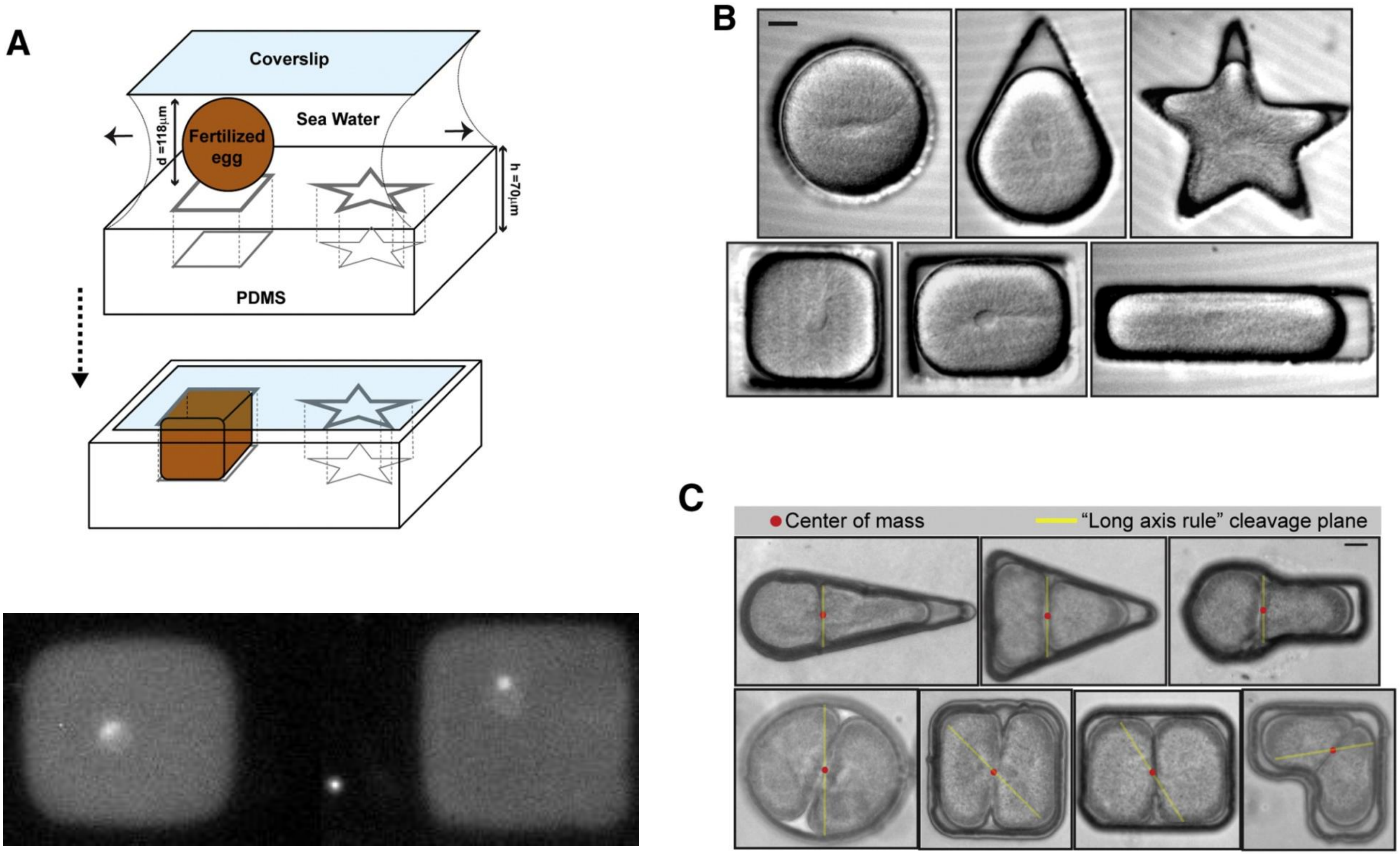
Minc N. et al., Curr. Biol., 2009

Measuring forces exerted by single yeast cells



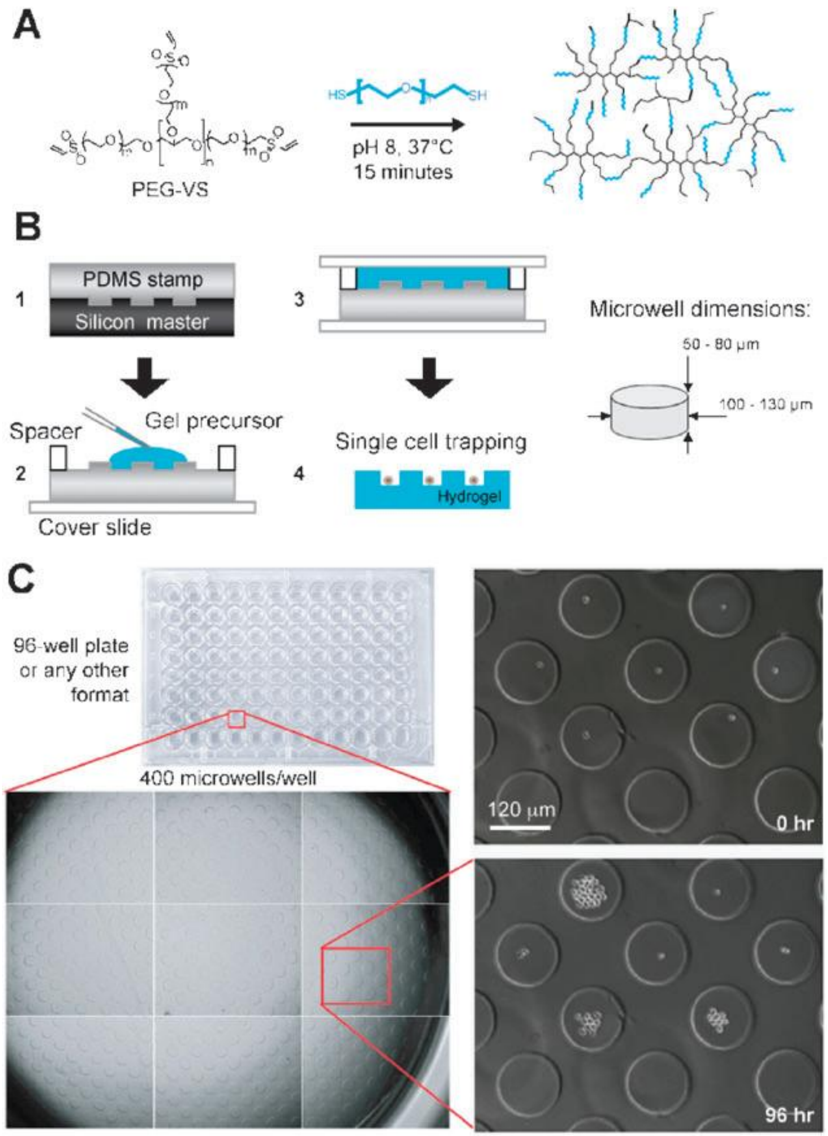
Minc N. et al., Curr. Biol., 2010

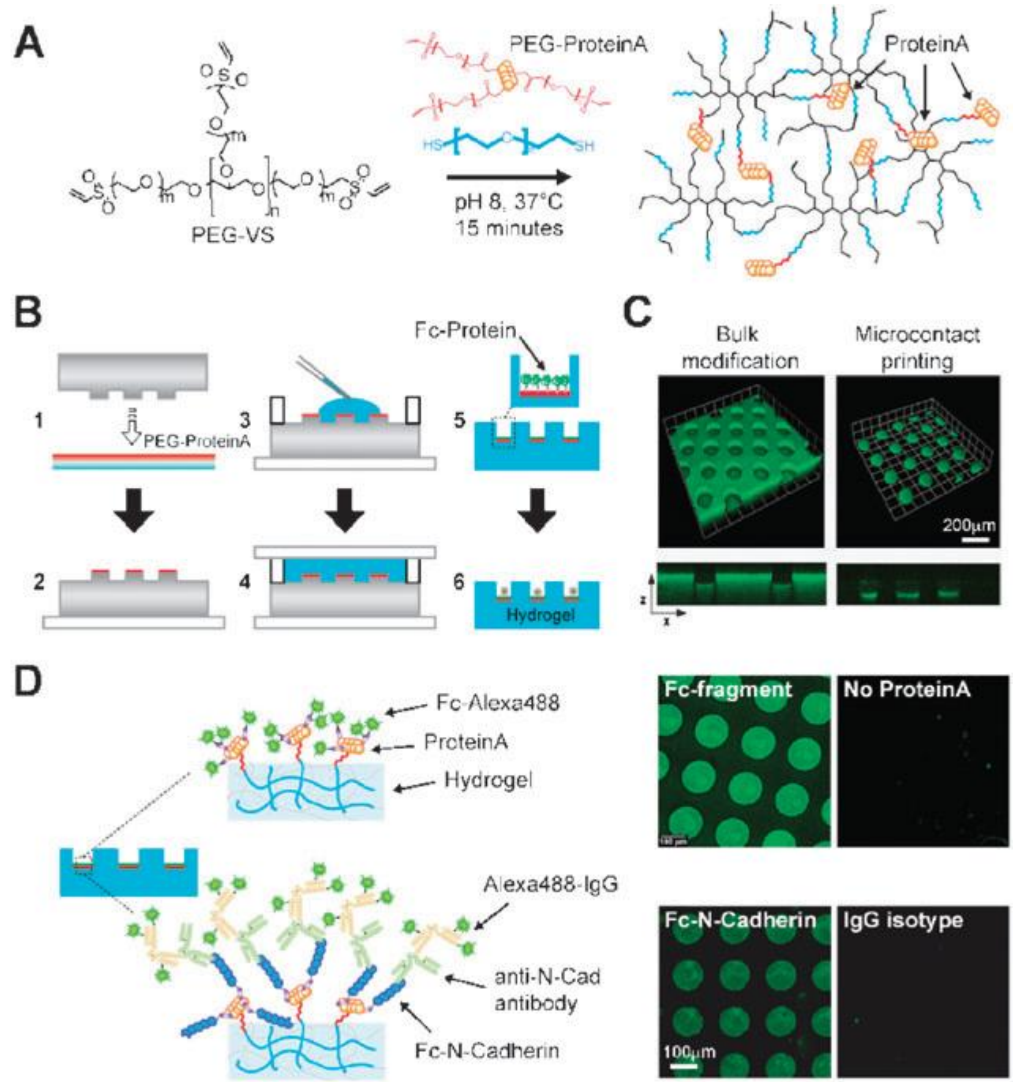
Controlling sea urchin embryo division axis



Minc et al., Cell, 2011

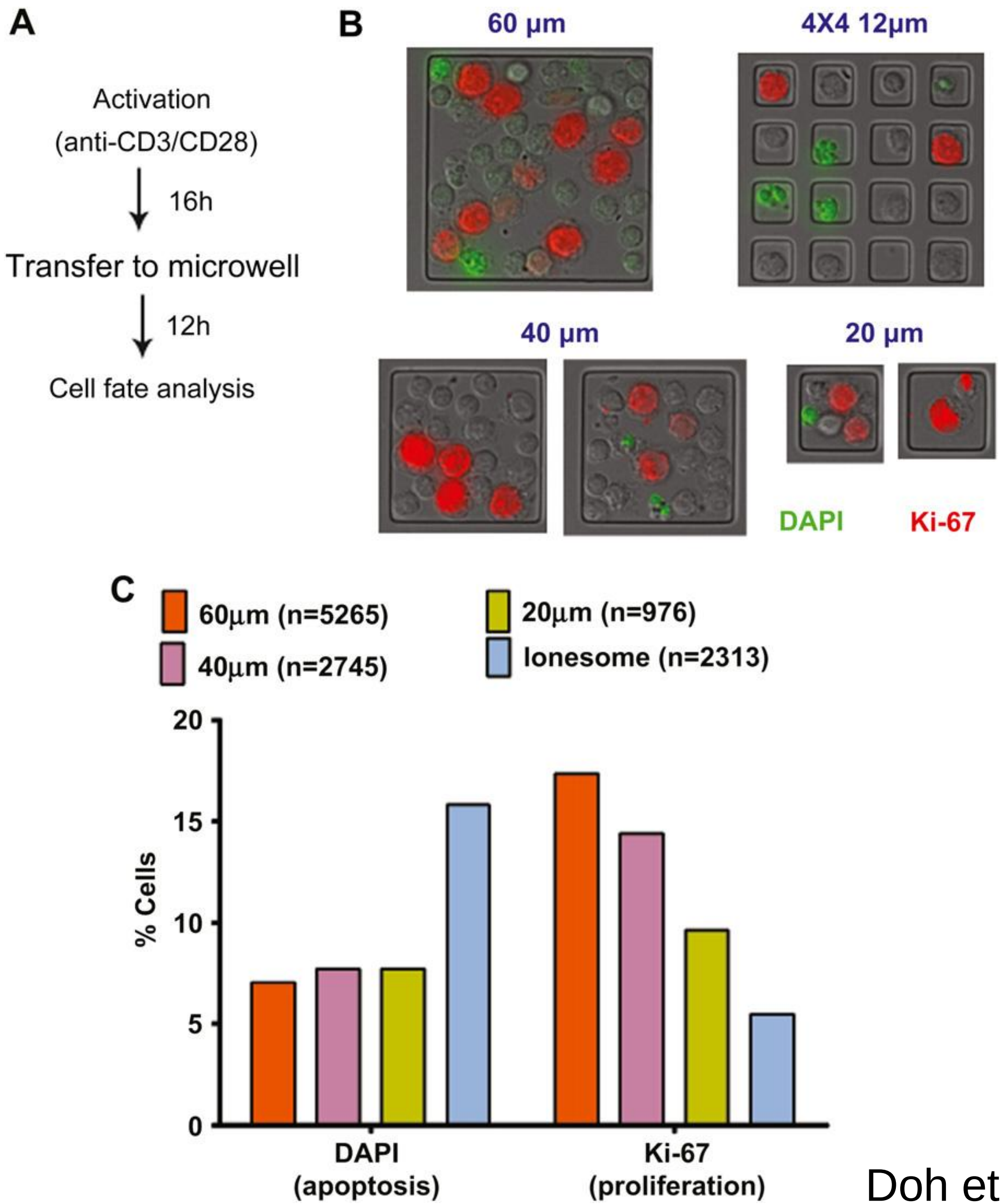
Wells for stem cells





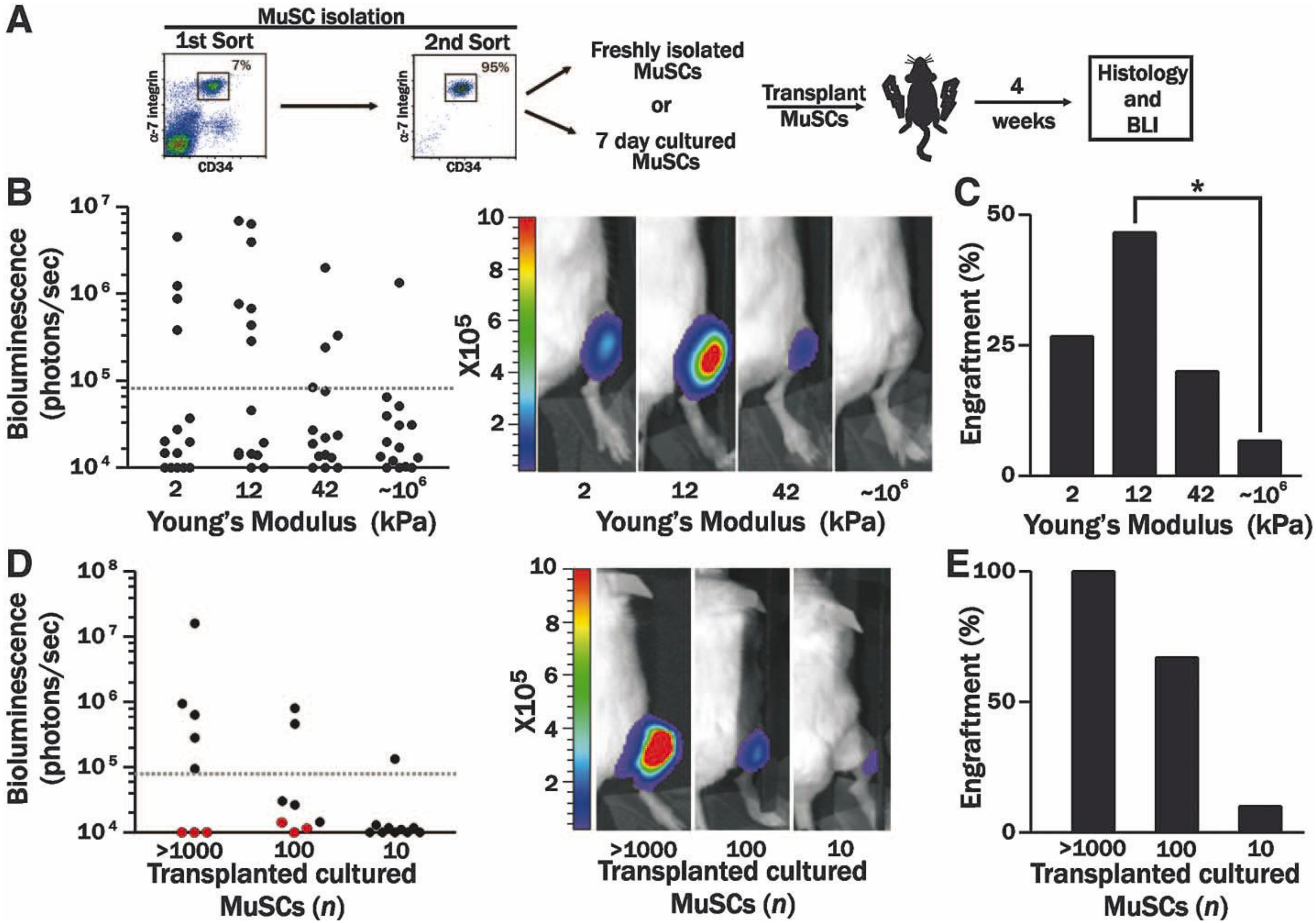
Lutholf M. et al., Integrative Biol., 2008

Differenciating cells inside micro-wells: cell density effects



Doh et al, Biomaterials, 2010

Differenciating cells inside hydrogel micro-wells: substrate rigidity effects



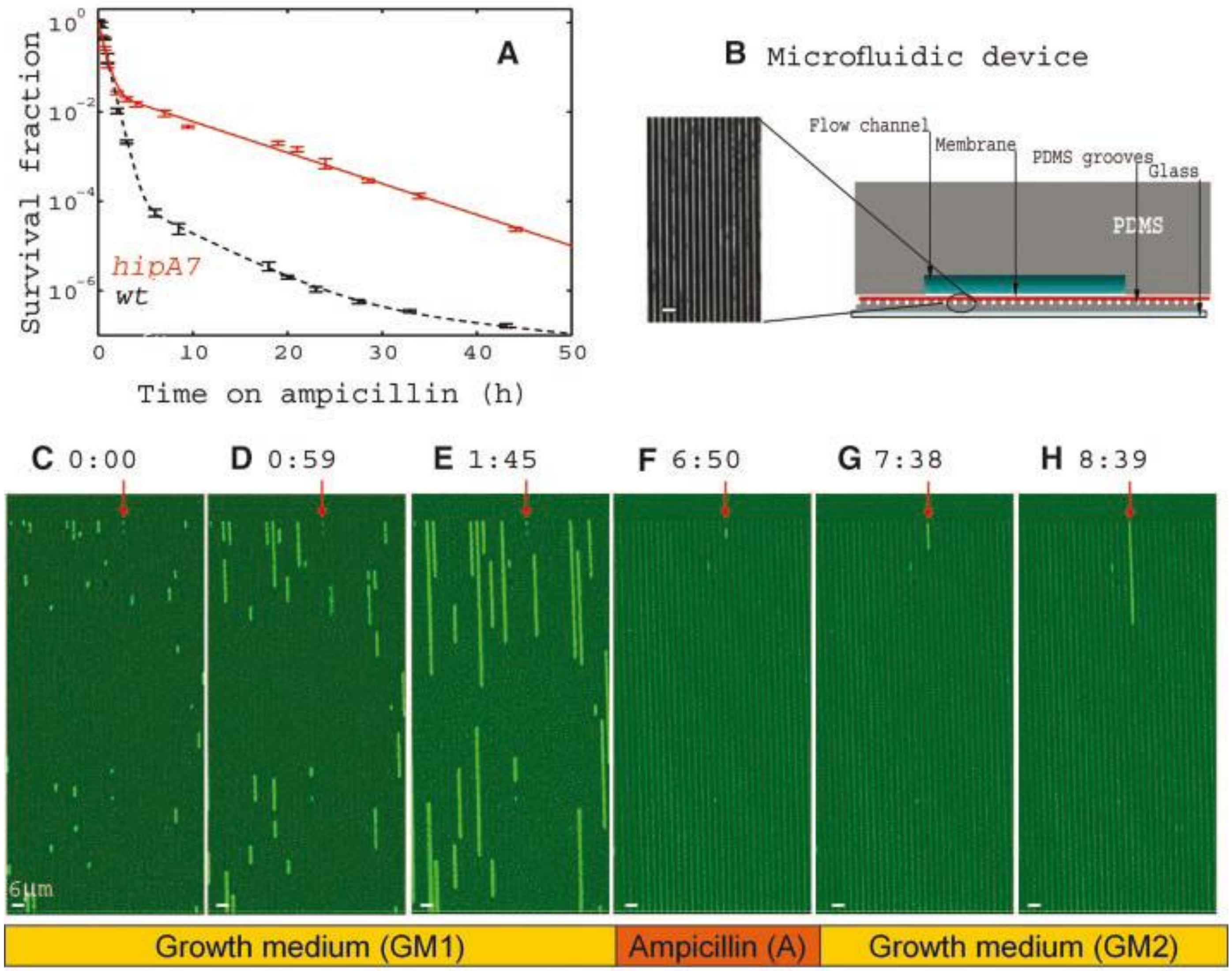
Gilbert et a., Science 2010

IV. Combining cell culture and micro-fluidics

Micro-fluidics for microbiology

Confining cells under a diffusible layer

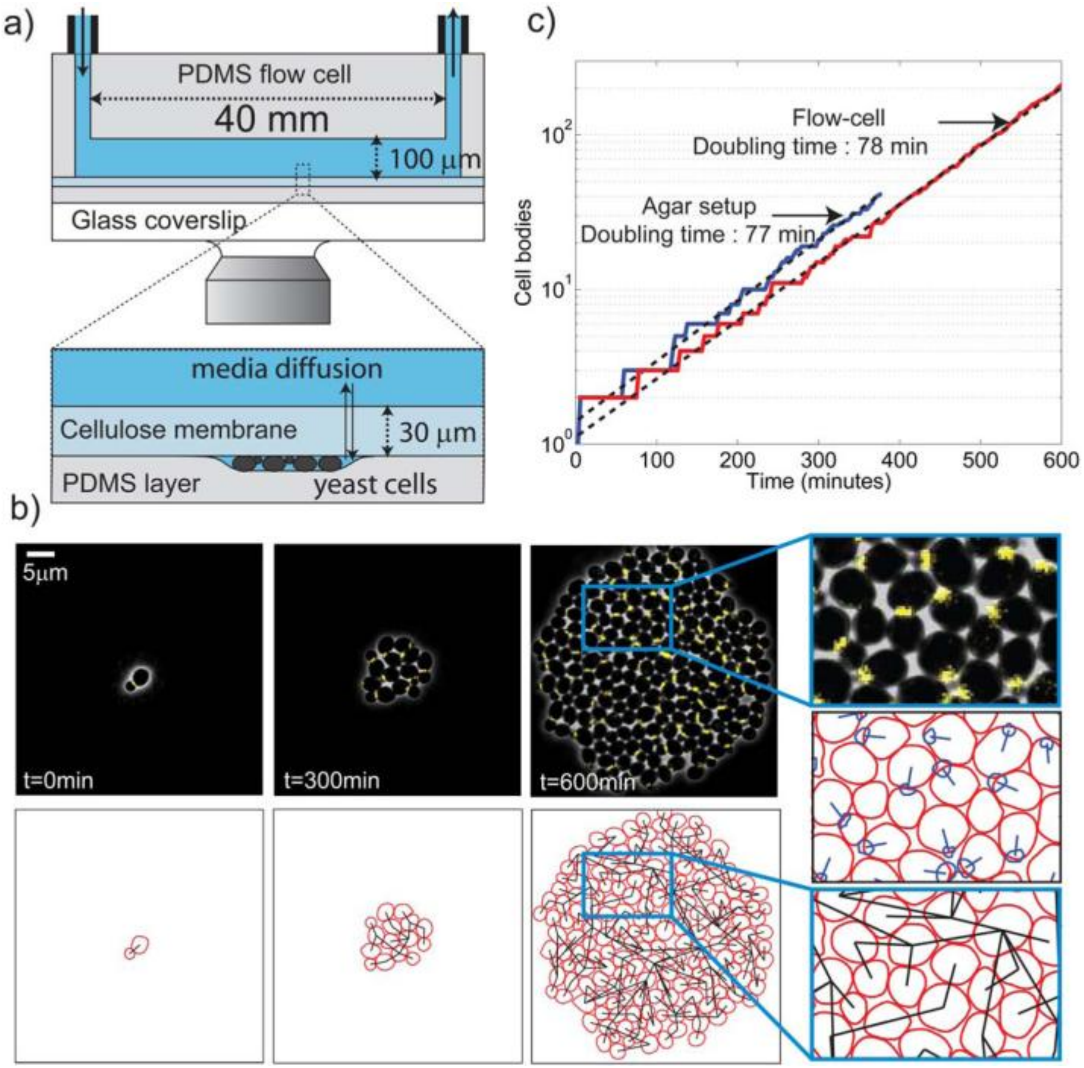
Following growth rate of bacteria in micron-sized lines



Balaban Science 2004

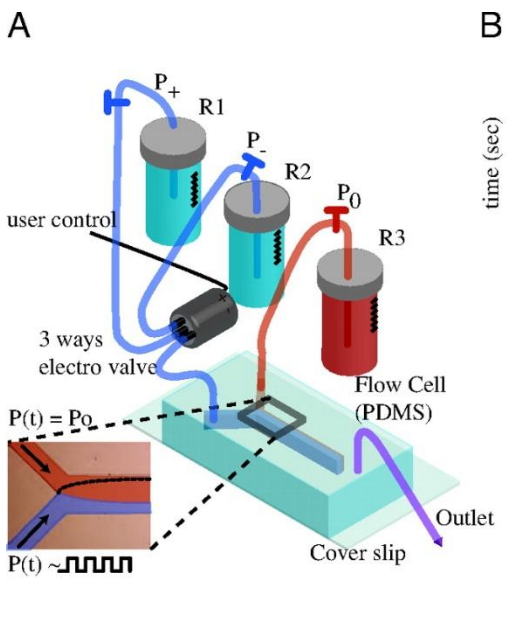
Confining cells under a diffusible layer

Yeast colony growth

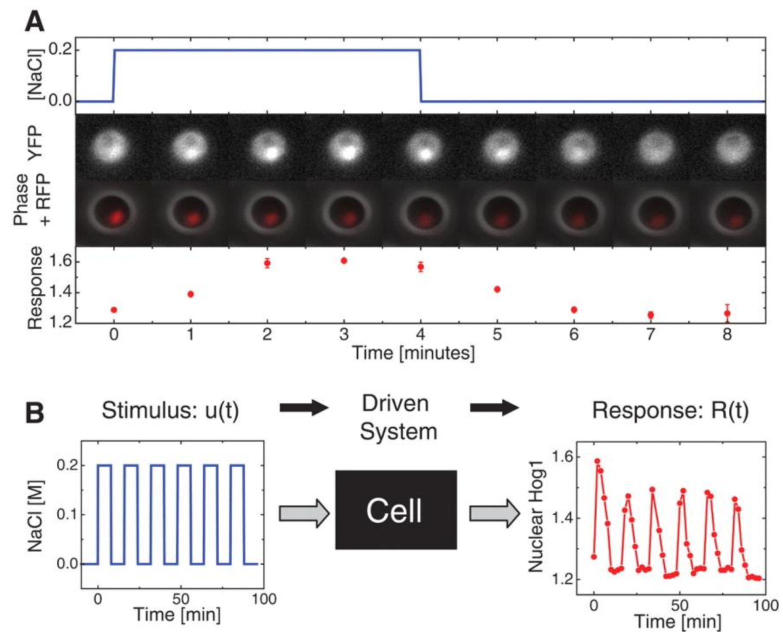


Charvin PLOS One 2008, PNAS 2009, PLOS Biol 2010

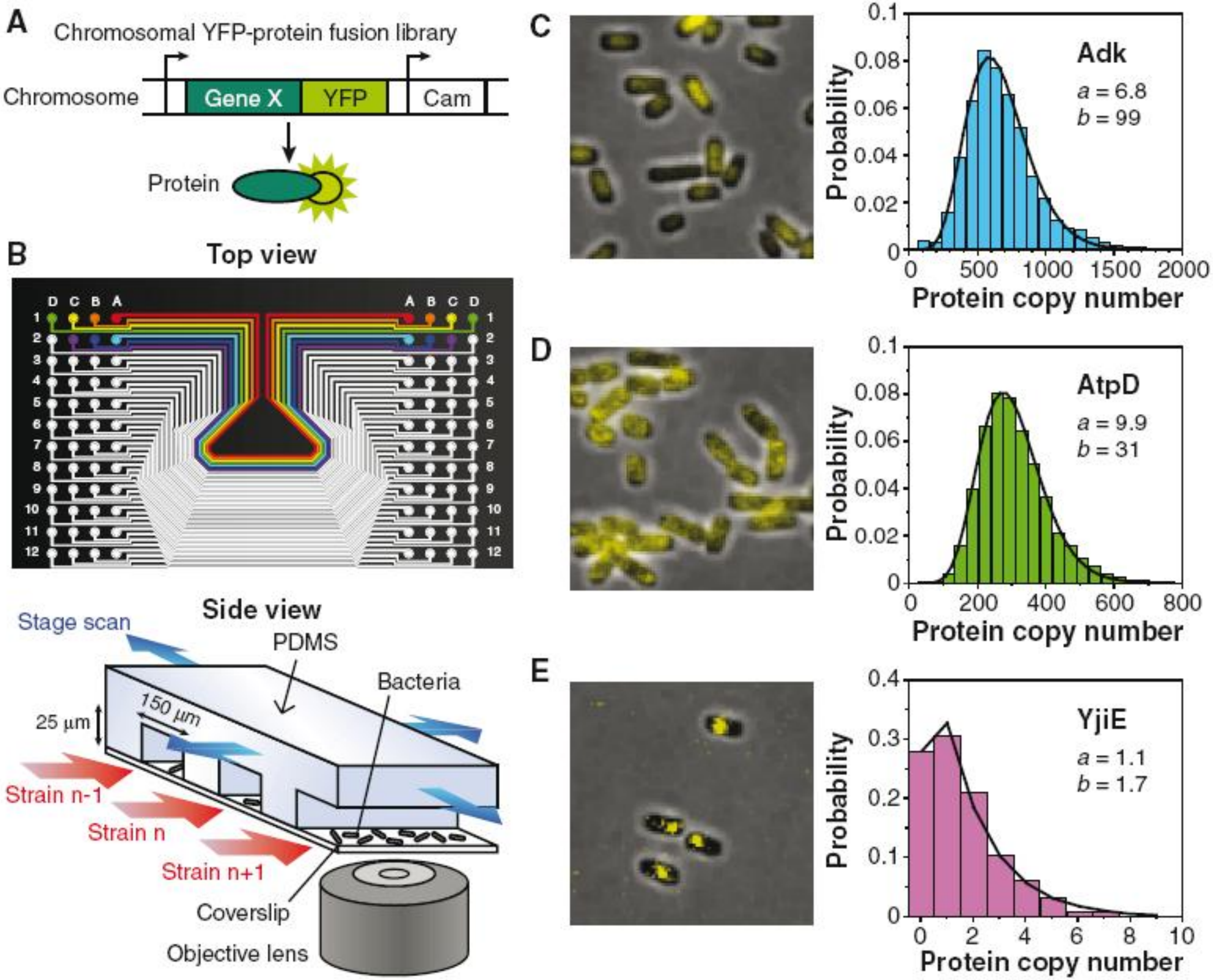
Microfluidic device to study frequency response of single cells



Hersen P. et.al. PNAS 2008



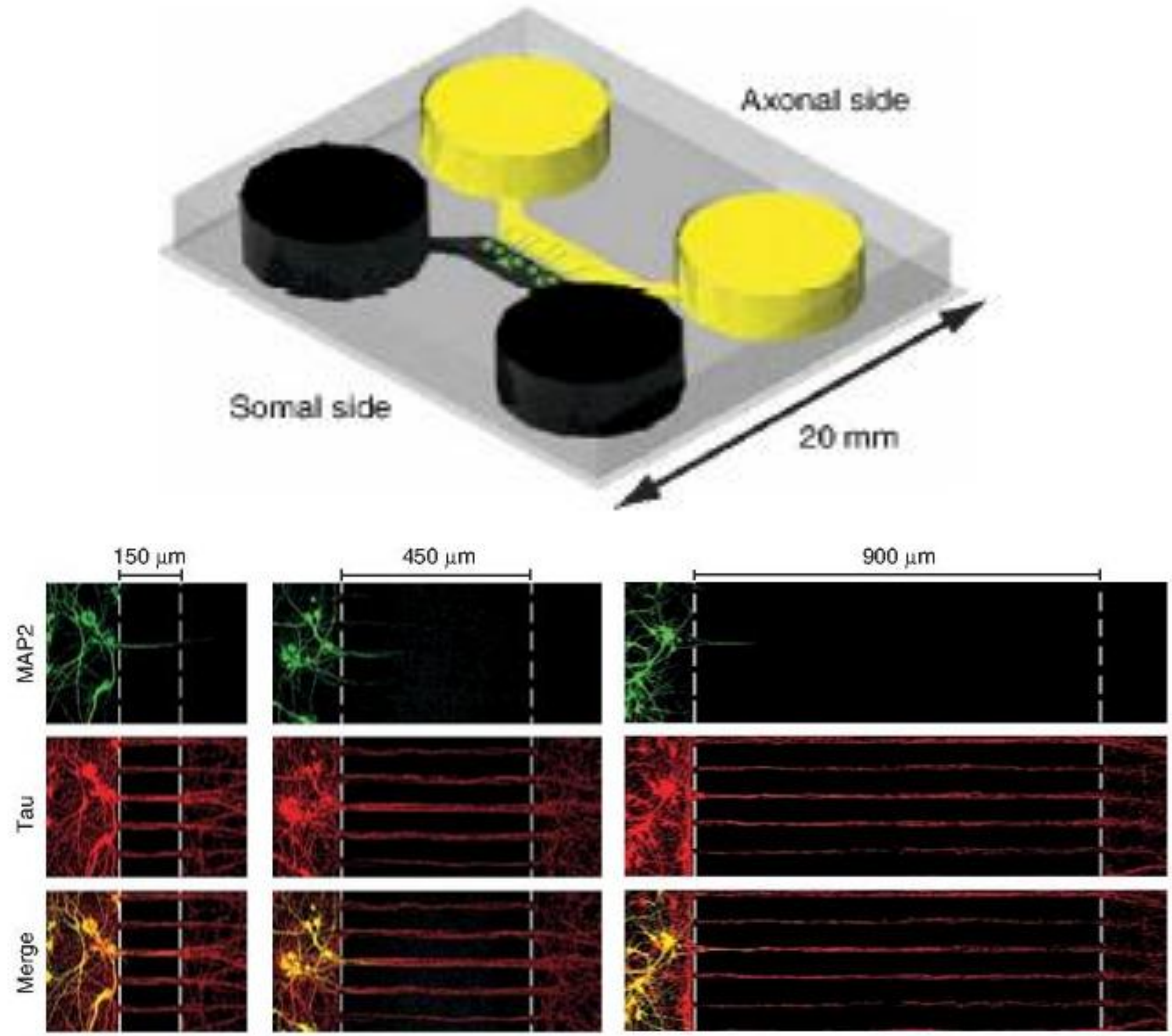
Mettetal et al., Science, 2008



Xie Science 2010

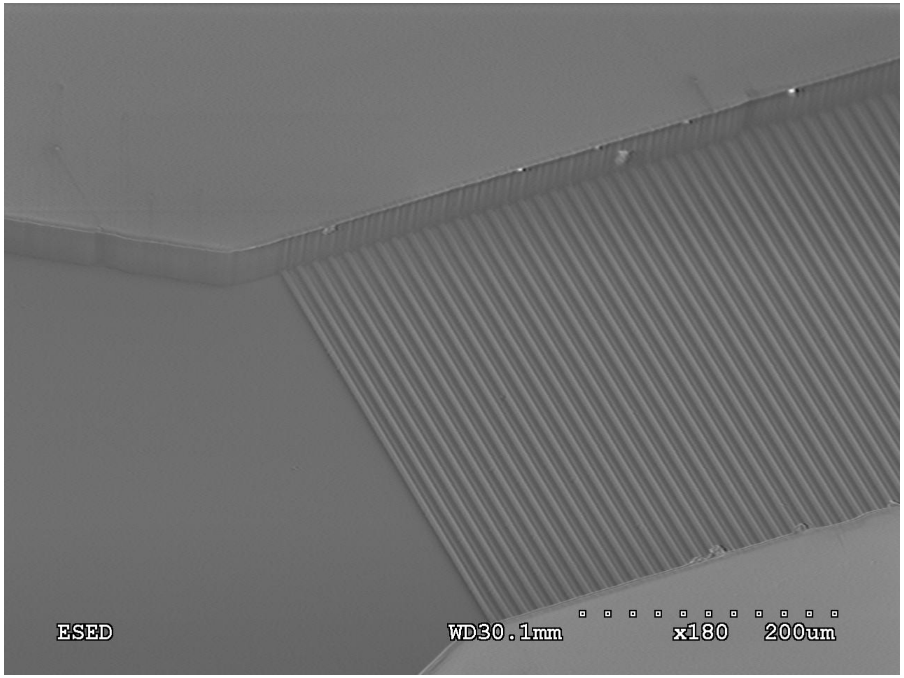
Micro-fluidics for in vitro cell biology

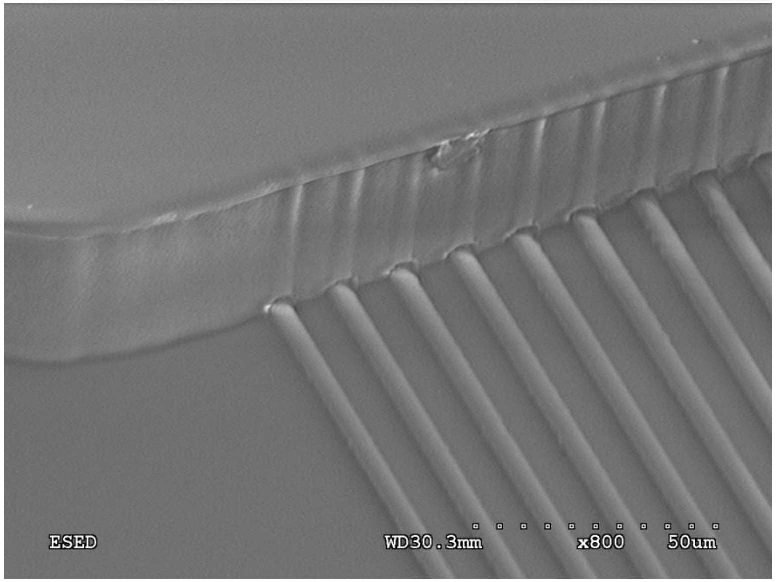
Organizing neuron cultures



Taylor et al., 2005

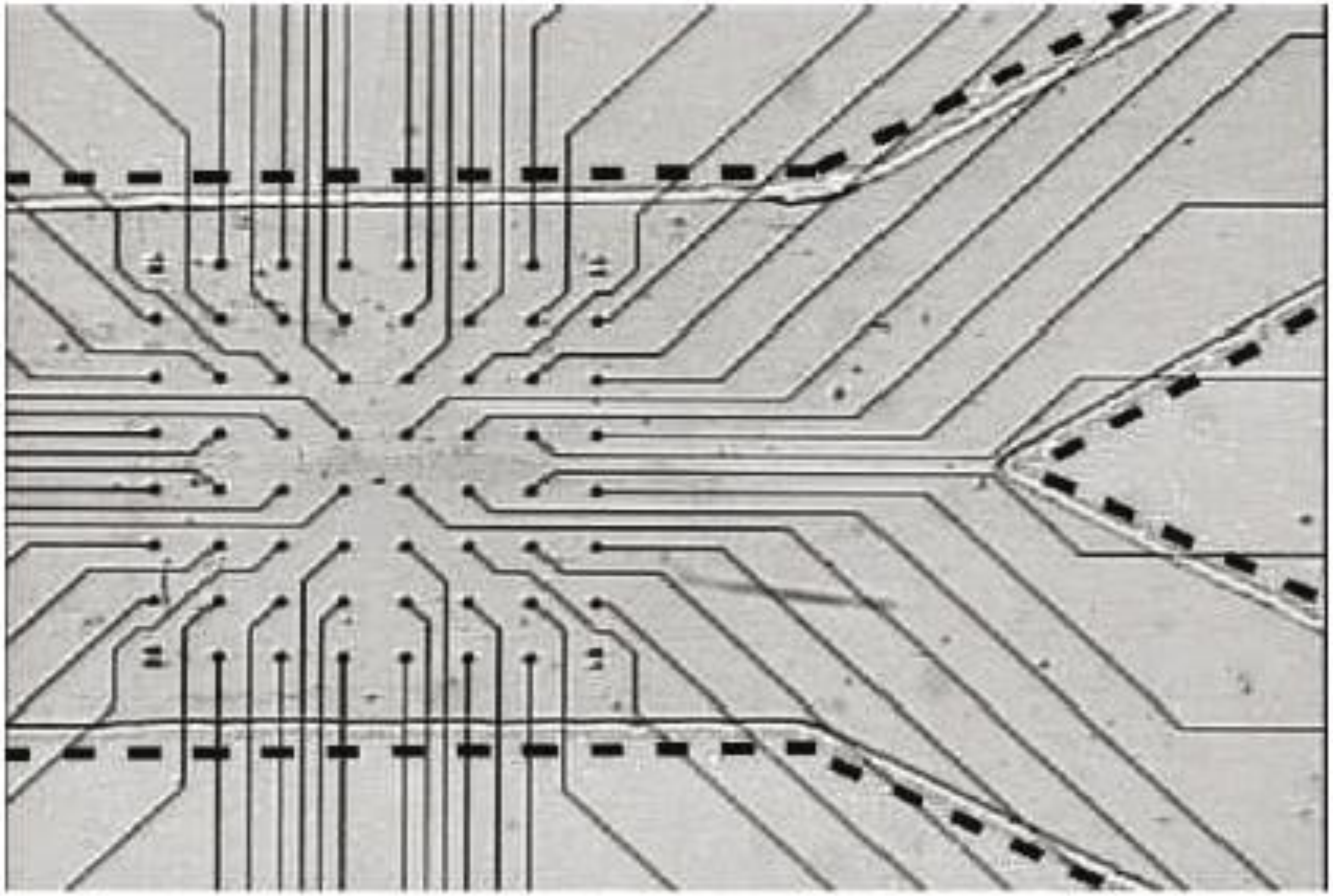
A general design for confinement plus flow control





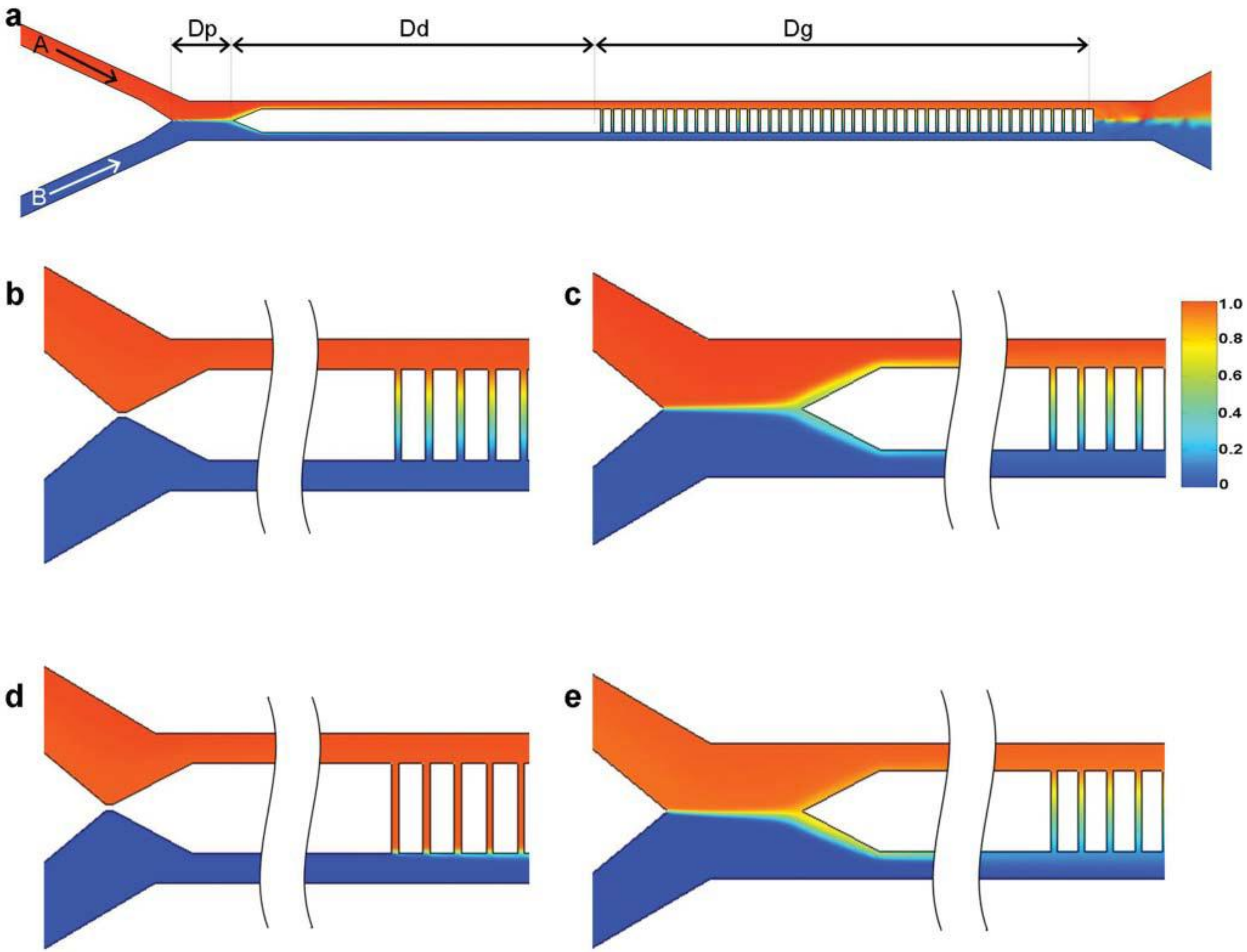
Géraldine Liot, group of Frédéric Saudou, Institut Curie

Microfluidics and micro-electrods…



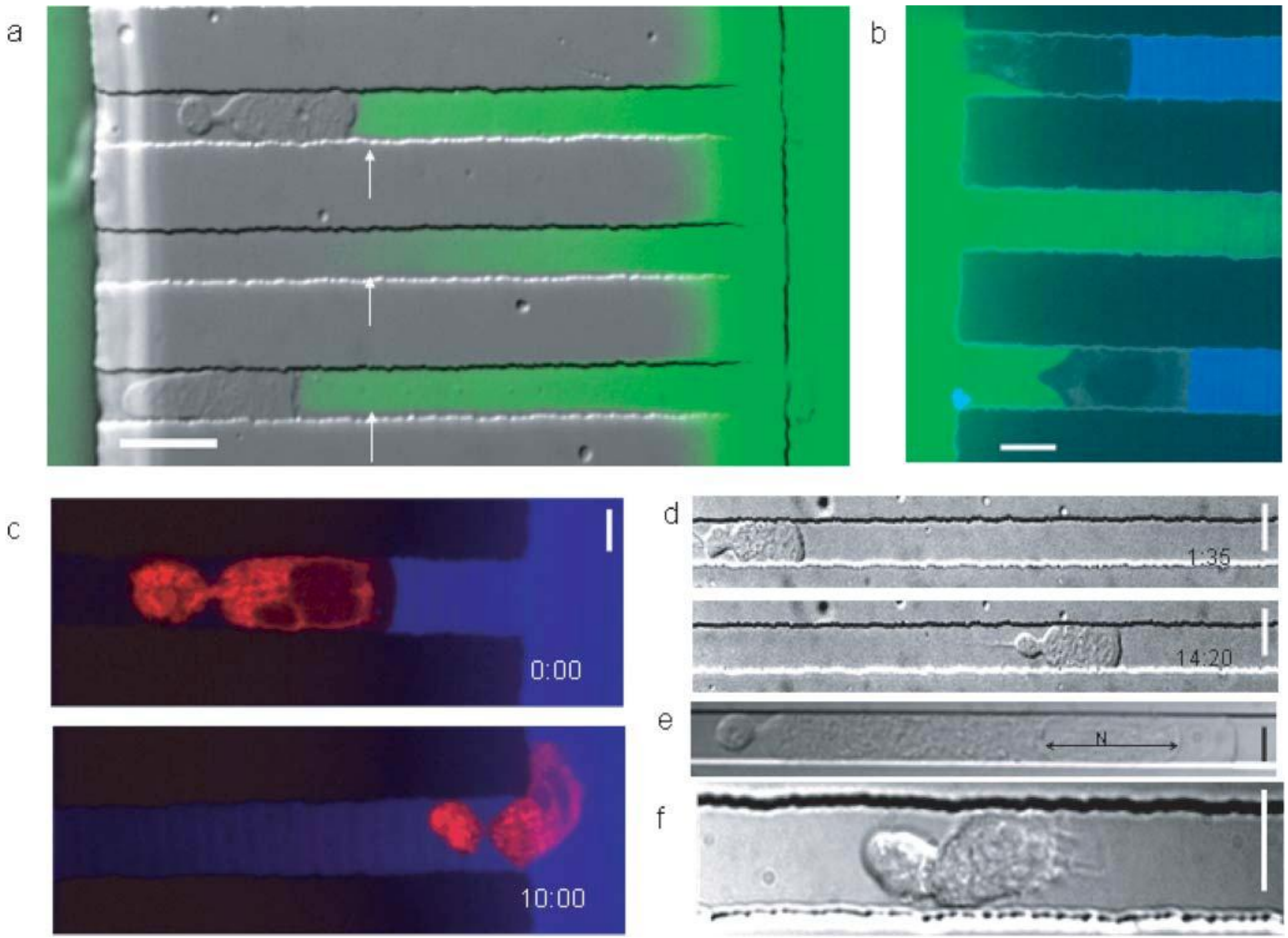
Pearce, T. M et al. *Lab Chip (2005)*

Improving the design by equilibrating pressure to produce gradients



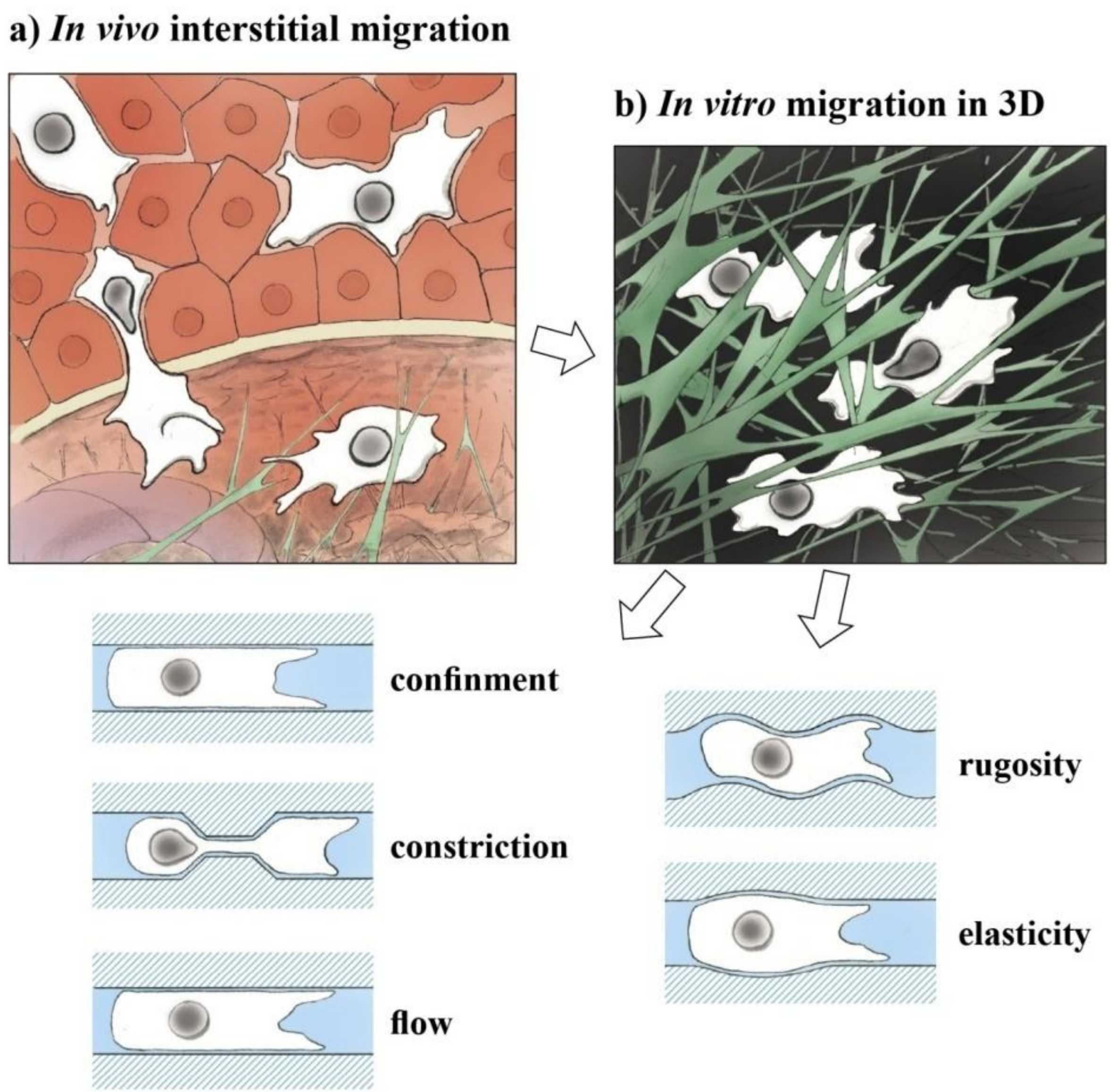
Irimia D. et al., 2007, LOC

For neutrophile migration

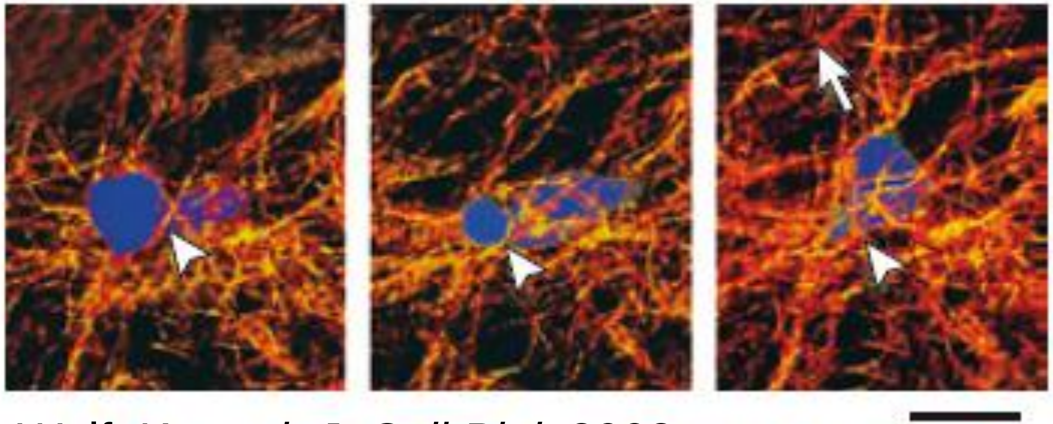


Irimia D. et al., 2007, LOC

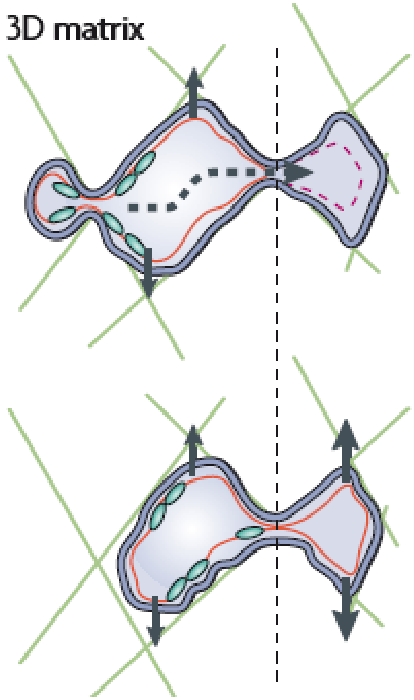
A ‘reductionist’ appraoch to ‘3D’ cell migration



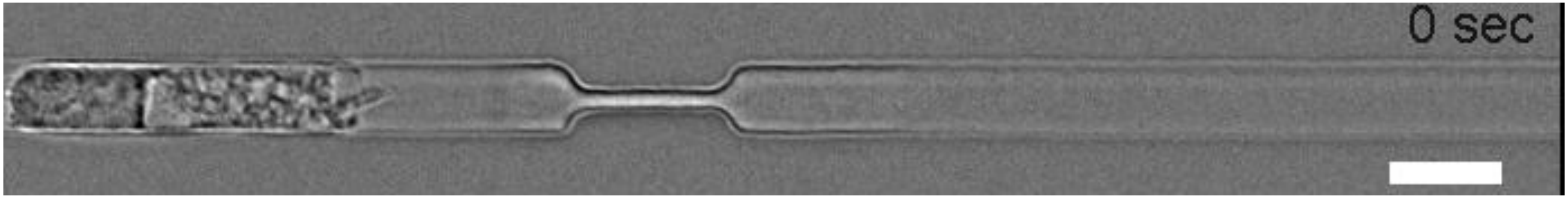
Passing through a small hole
(Olivier Collin, Yana Touré, Hawa Thiam)



Wolf, K. *et al. J. Cell Biol.* 2003



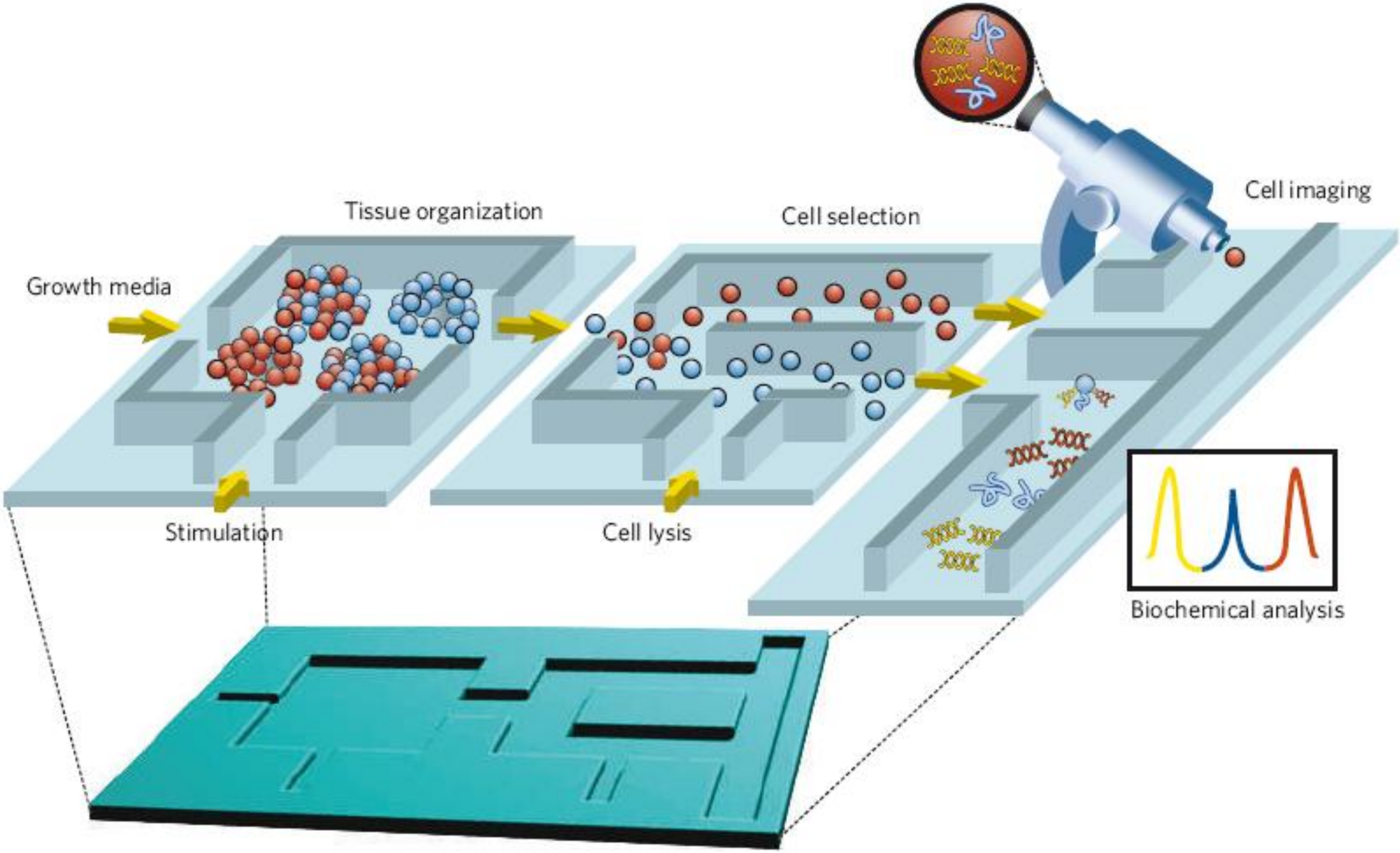
Paluch and Charras, NRMCB, 2008



Yana Touré

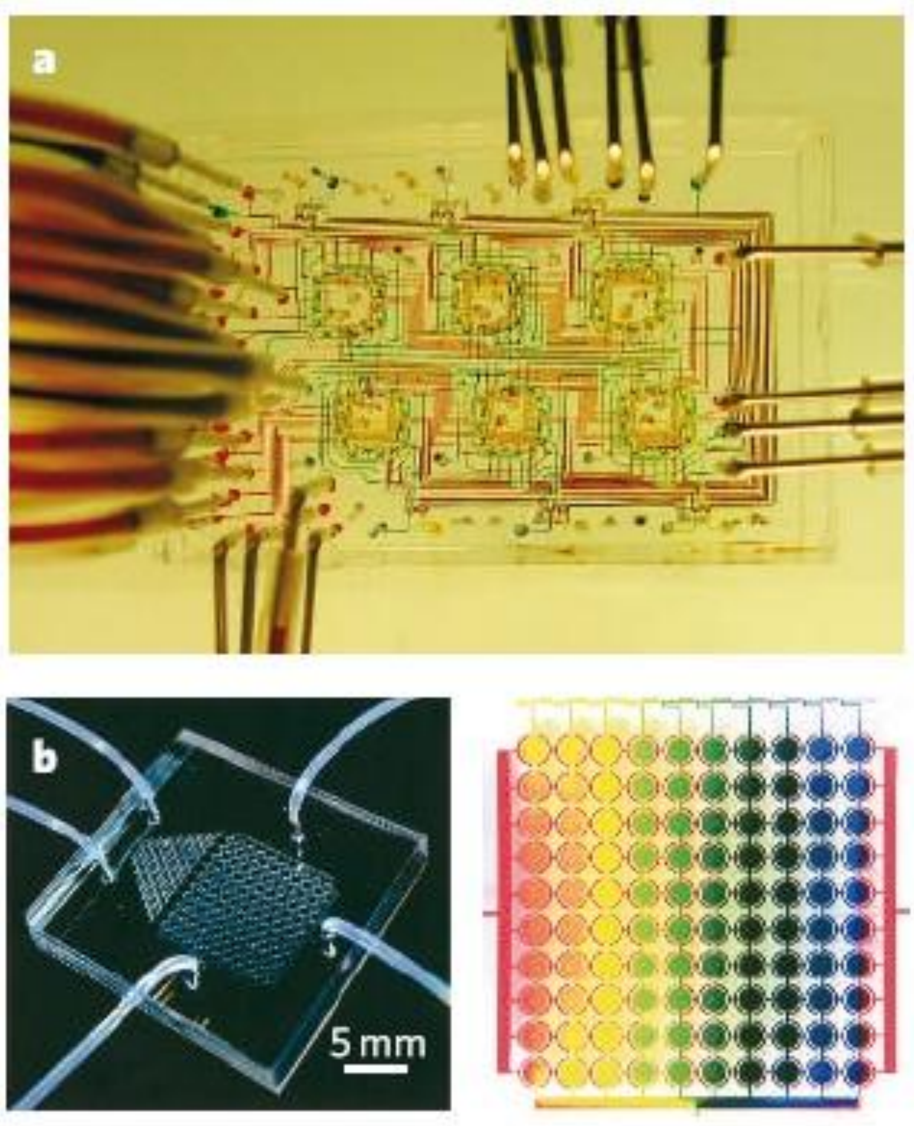
Integrated systems

Integrated cell culture systems

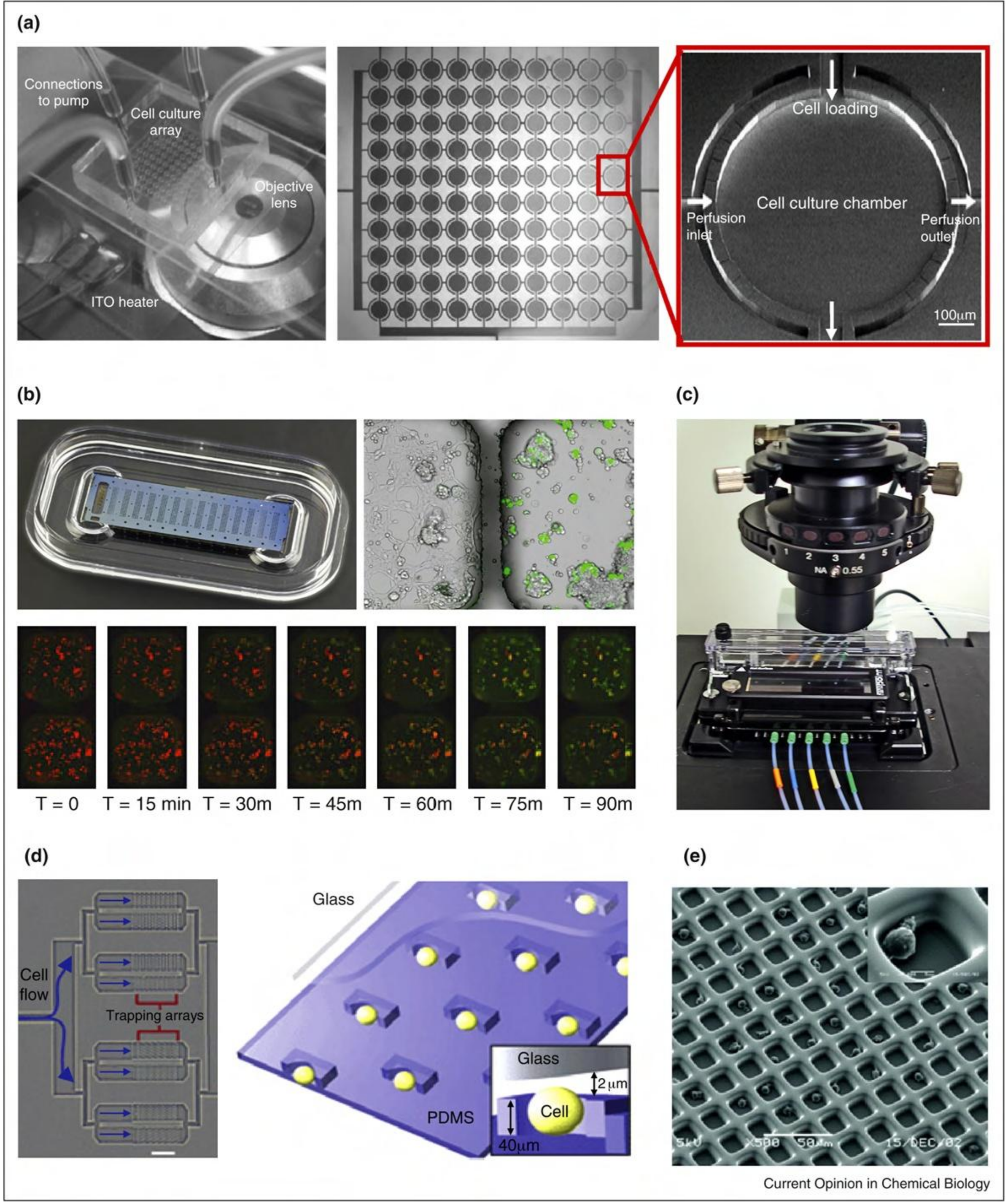


El-Ali et al. , Nature, 2006

Integrated cell culture systems



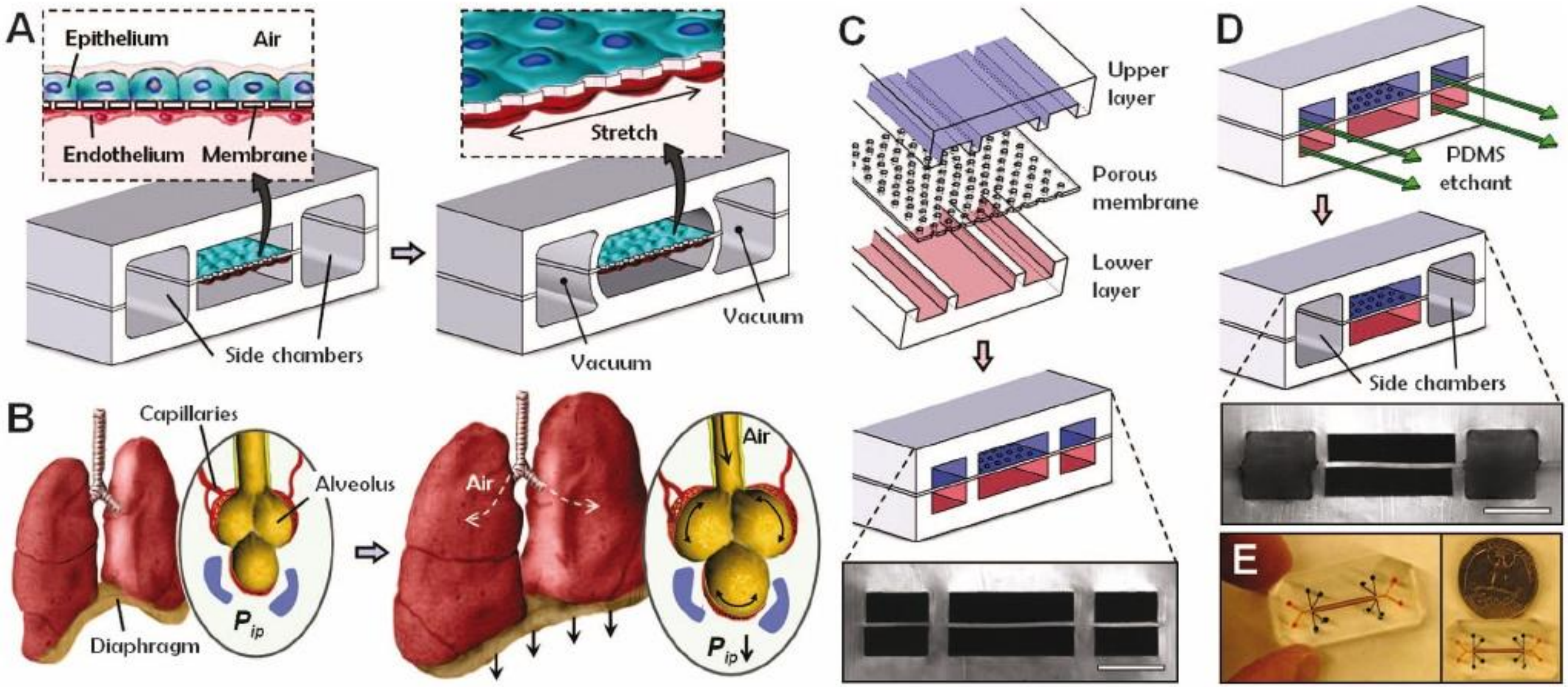
Balagadde et al. , Science, 2005



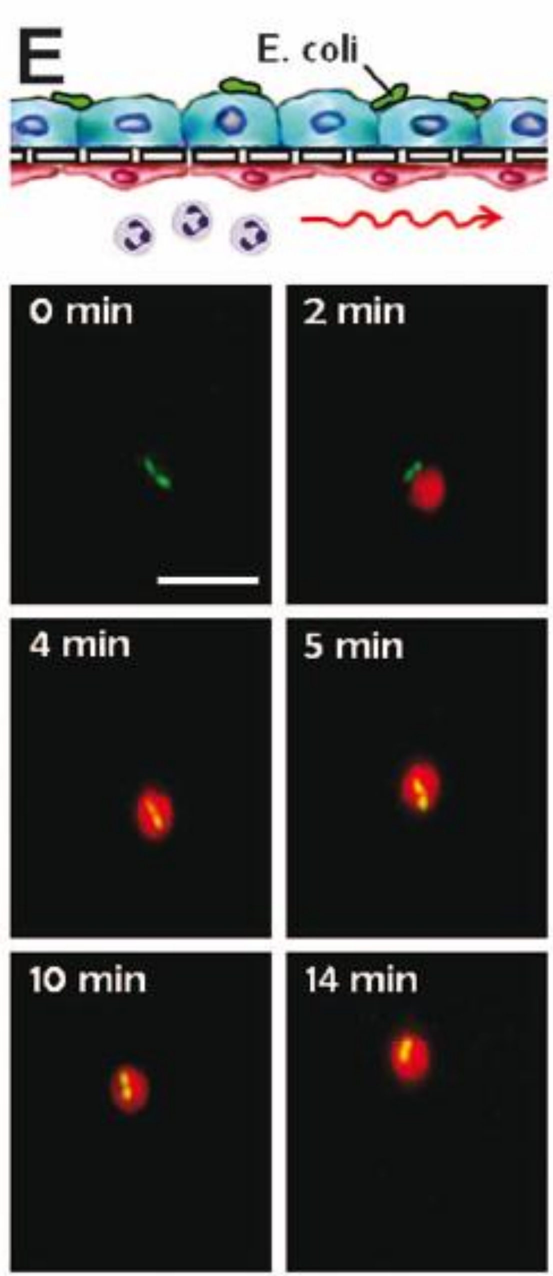
Wlodkowic, 2010

Micro-fluidics for tissue engineering

Artificial organ

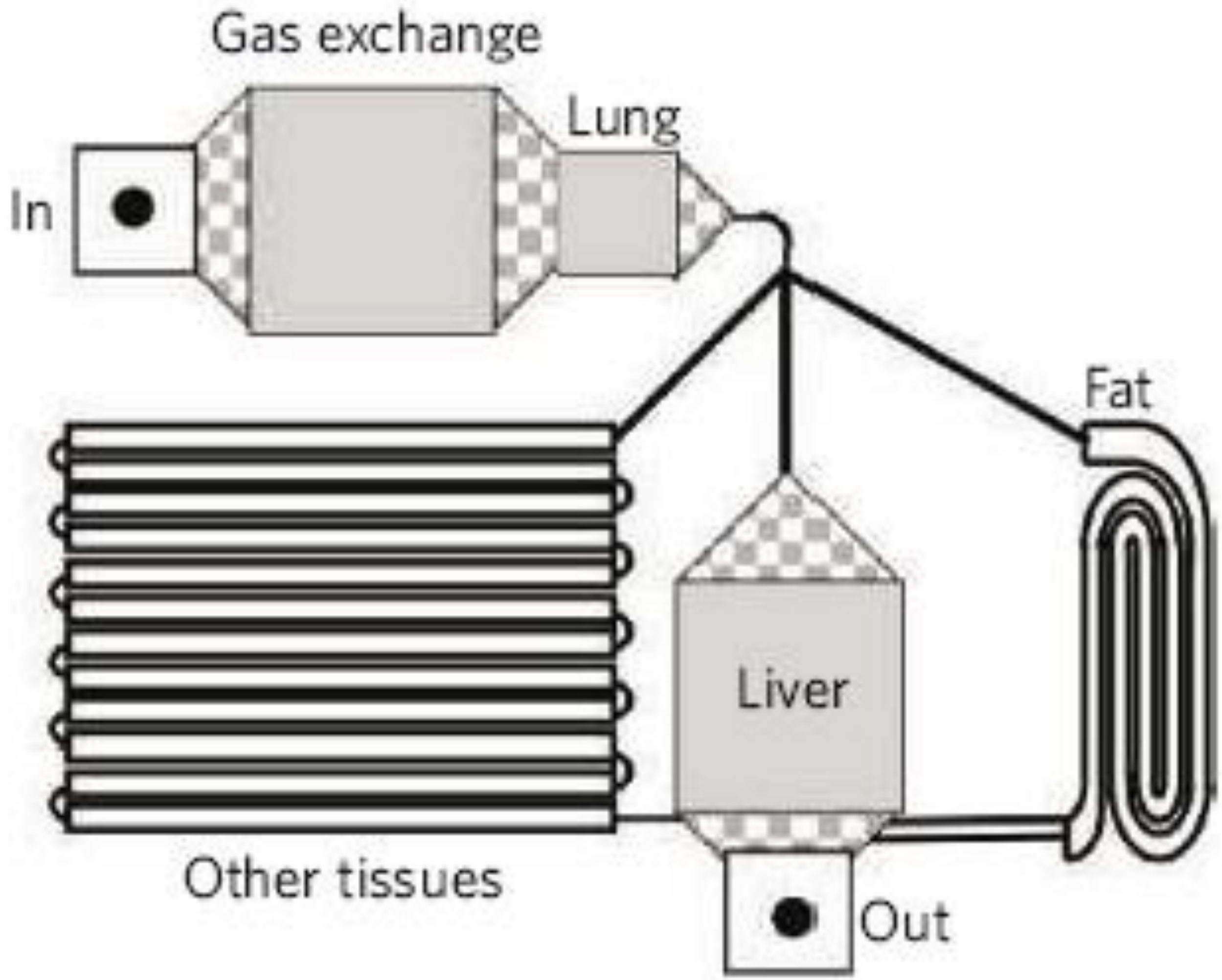


Huh et al., Science, 2010



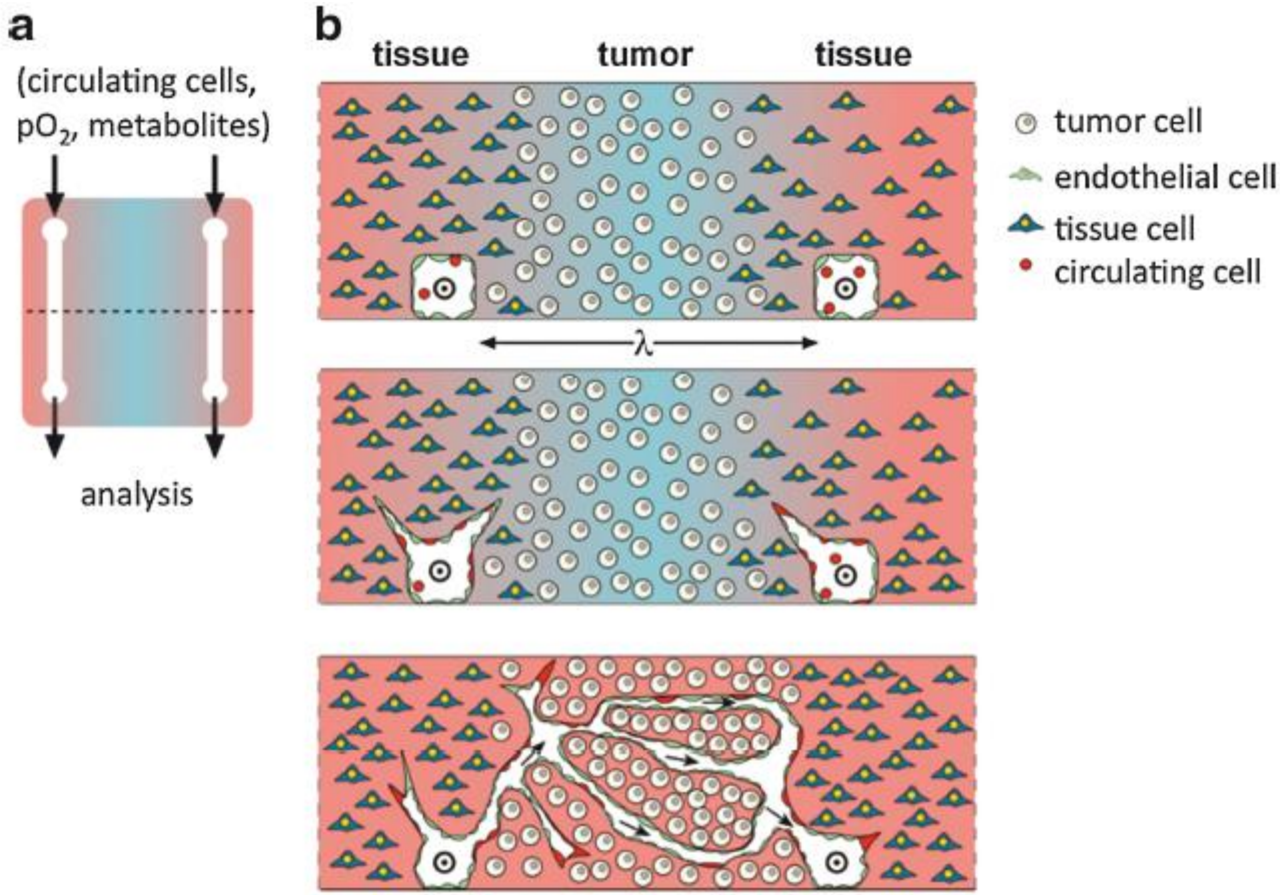
Huh et al., Science, 2010

Organism on a chip…



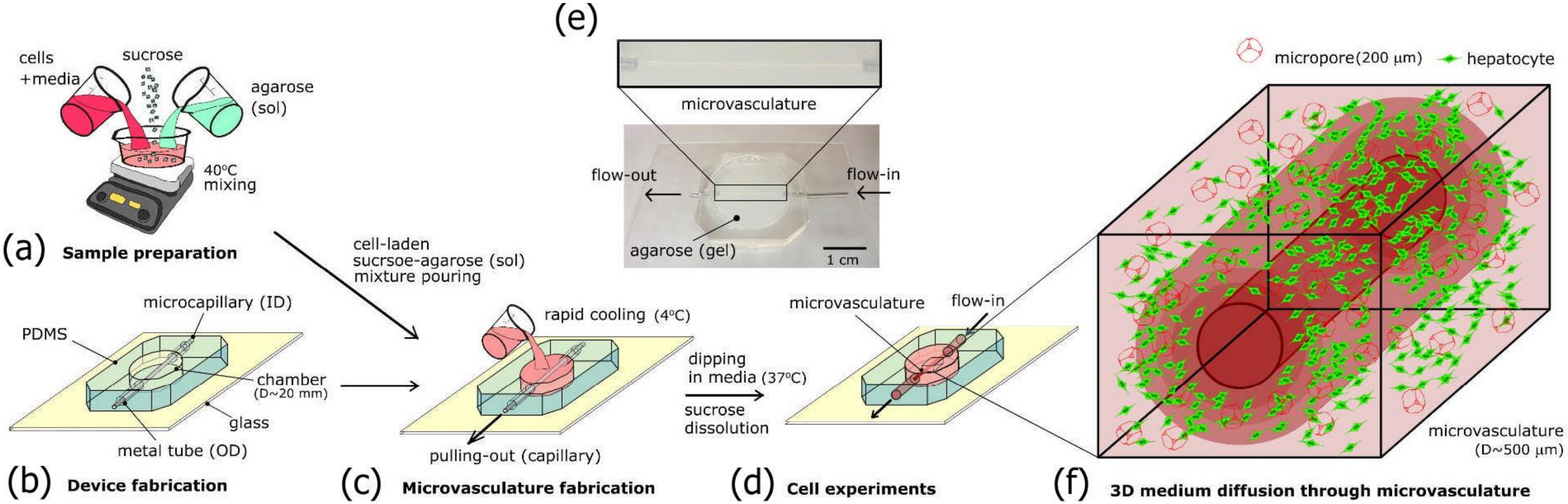
Viravaidya, *Biotechnol. Prog. (2004).*

Micro-fluidics in hydrogels



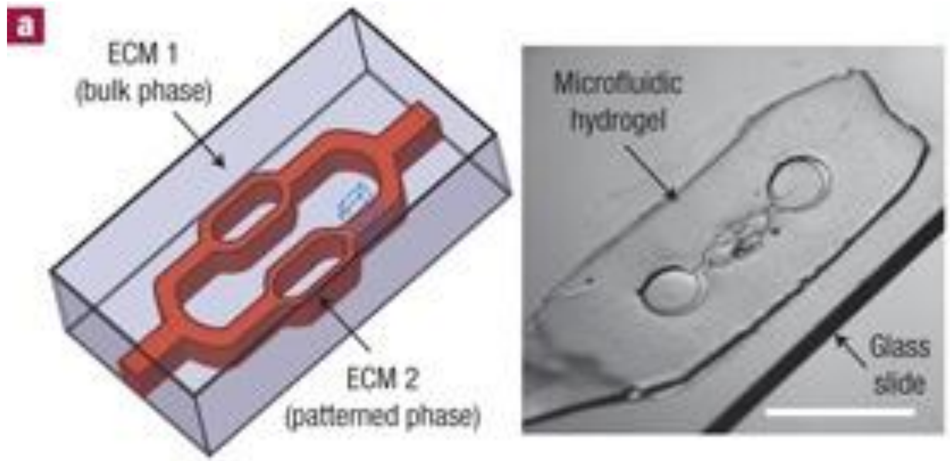
Stroock, Tissue Engineering, 2010

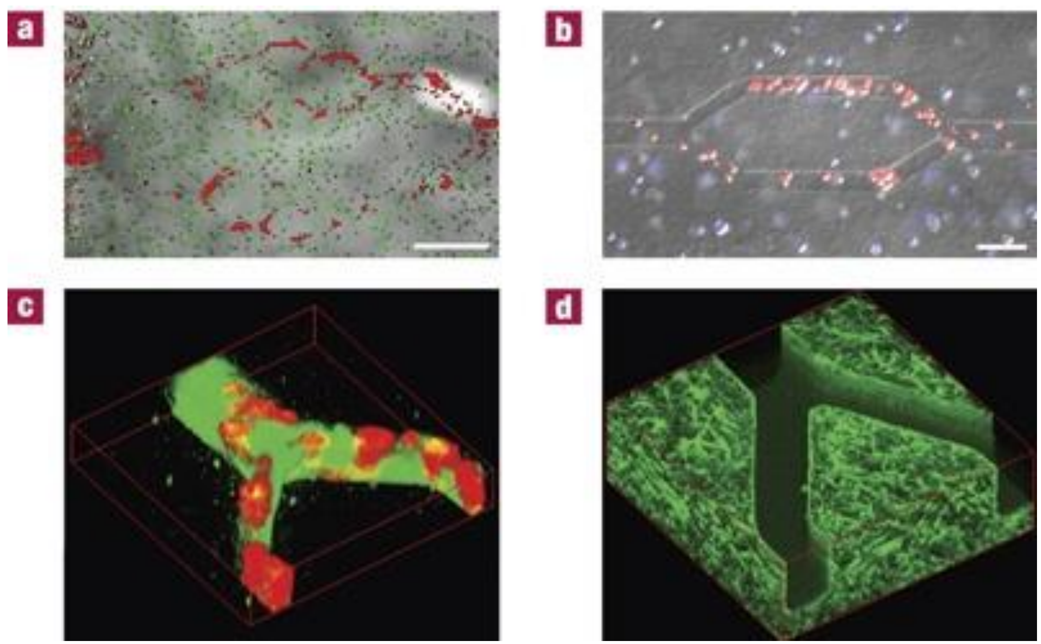
Micro-fluidics in hydrogels



Park JH et al., Biotech Bioingen. 2010

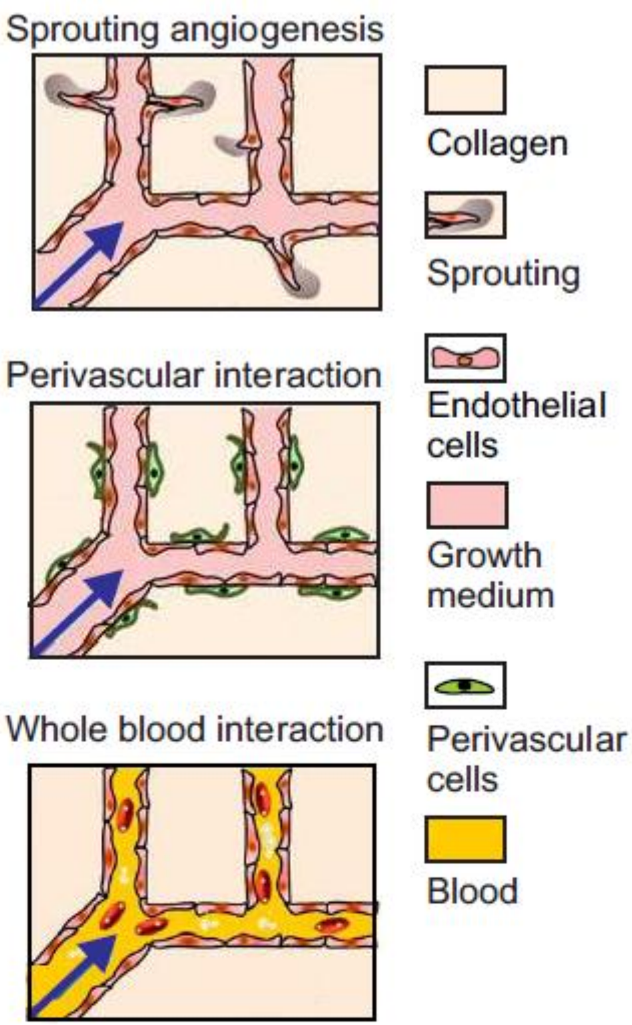
Micro-fluidics in hydrogels

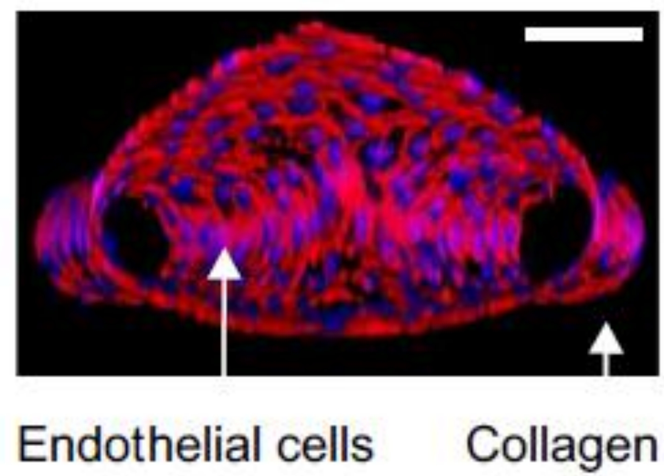




Gilette et al., Nat. Mat. 2008

Blood vessels on a chip





Zheng Y et al. PNAS 2012