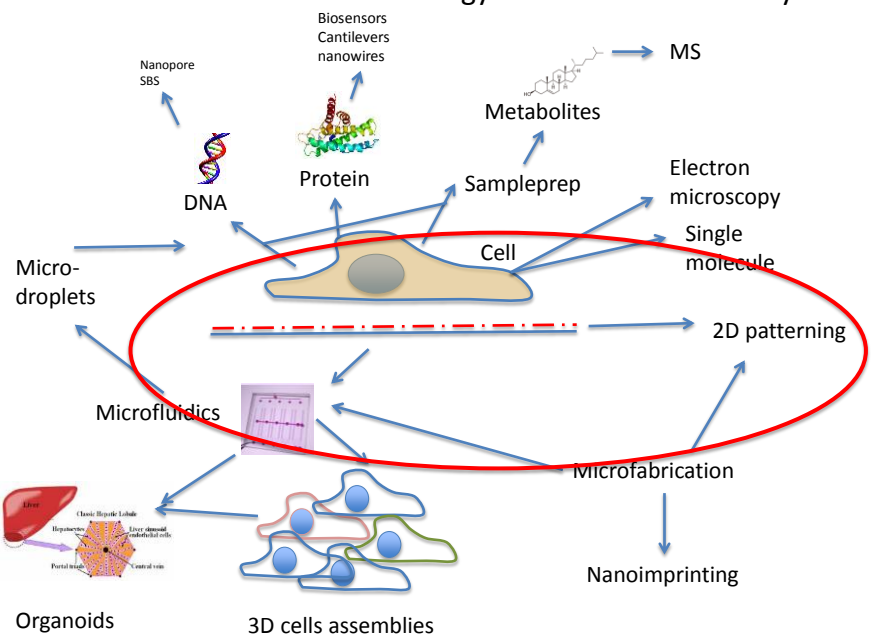
NTNU

Innovation and Creativity

Cells on chips

Mol3014, Øyvind Halaas, IKM

Course outline : nanotechnology for biomedicine : analysis

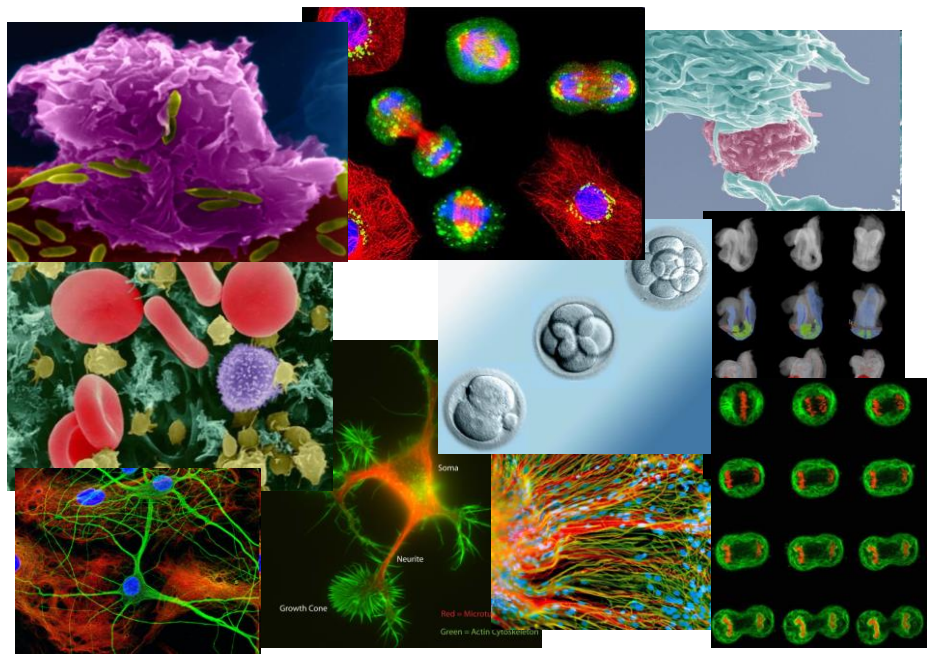


Learning goals

- Describe shortcoming of current methods of cell biology
- Elaborate on why single cell analysis is necessary
- Provide examples on cell standardization by micropatterning
- Provide examples of cell traps
- Show possibilities for cell-pairing
- Provide examples on cell sorting in microdevices

- Describe devices for migration in channels and on patterns

We are cells



Cells

The minimal unit of life

About 200 standard cell types

Neurons and immune cells are highly dynamic

Immune cells are highly diverse (many millions genotypes)

Cancer cells are changing and non-clonal (metastatic cancer cell very different from initial cancer stem cell)

Cell biology

The study of cells provide information about

Gene function (research, about 50% genes unknown function)
Development (embryology, tissue repair)
Stem cells (growth requirements, growth patterns)
Immune cells (vaccines, autoimmunity, cancer, allergies)
Neurons (cell signalling and networks)
Cancer cells (growth, aggression, metastates, drug targets)
Drug response (activity testing)

What can we learn from cell studies

Birth (proliferation)

- **cancer, tissue regeneration, immunology**

Food (metabolism, pinocytosis, endocytosis, phagocytosis)

- **disease, cancer, immunology**

Action (motility, movement)

- **immunology, cancer metastasis, tissue (re)generation**

Looks (morphology)

- **gene function, develoment, neuronal network**

Social networking (signalling)

- **gene/protein level alteration, internal body communication**

Death (apoptosis)

- **cancer, immunology, development, disease**

Readouts

Quantitation and Identification of

- **Metabolites (intracellular, extracellular, enzyme inhibitors)**
- **Nucleotide (DNA, mRNA, miRNA)**
- **Protein (eg cytokines, induced/repressed proteins)**
- **Cell (cell subtype, function, morphology, quality)**
- **Interactions between them**

Location, timing, levels, networks

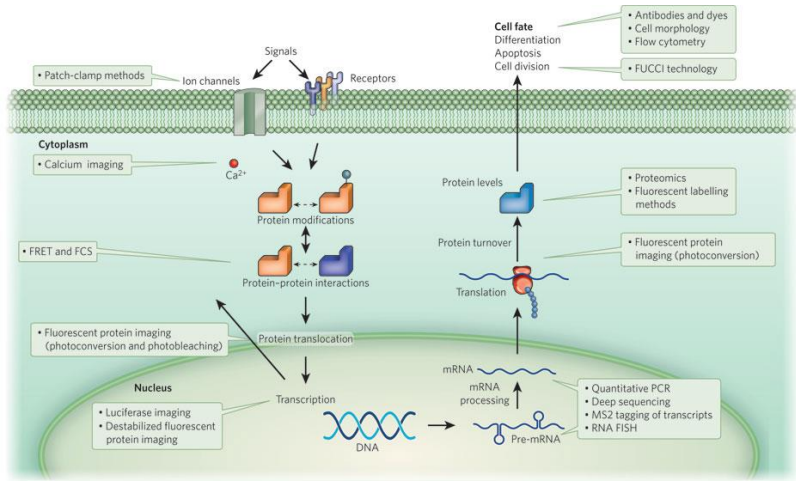
Methods

Imaging (morphology, localization)

Biochemical (identity and quantity)

Informatical (system)

What to analyze and how



Measurement of single-cell dynamics
David G. Spiller, Christopher D. Wood, David A. Rand & Michael R. H. White
Nature 465, 736–745 (10 June 2010) doi:10.1038/nature09232

Motivation for new approaches:

Todays bulk approaches have severe practical limitations and do not mimic tissues satisfactorily

Cells are unsynchronus, heterogenous and depend on ECM and cell:cell contact

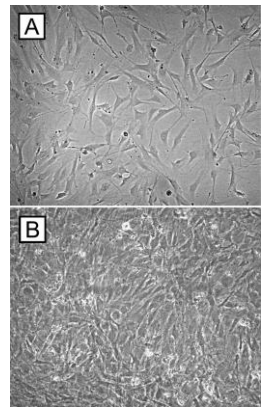
Today we have no spatial control and poor temporal control

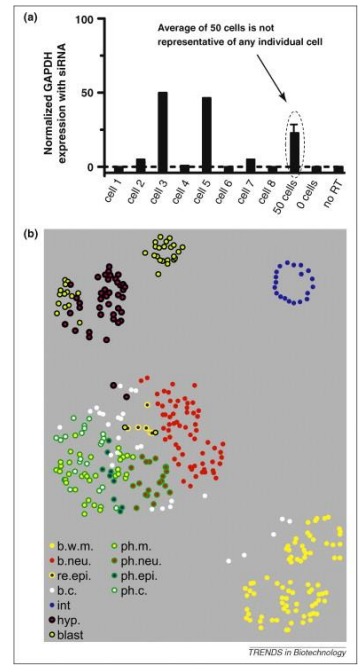
Single cell studies is today uncoupled from single cell analysis (standard microscopy exception)

Solutions:

Single cell analysis
Standardization of cells
Microfluidics
Microdroplets

Cells and even cell lines are heterogenous, this is averaged by bulk research, but represent problems for rare events and unsynchronized cells.

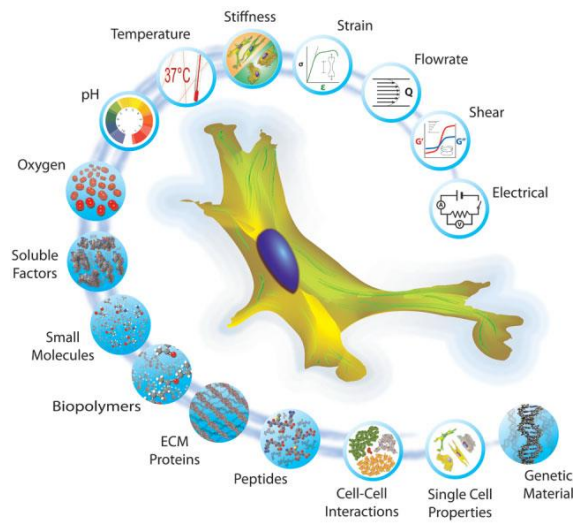




DOI: 10.1016/j.tibtech.2010.03.002

How to affect cell fate

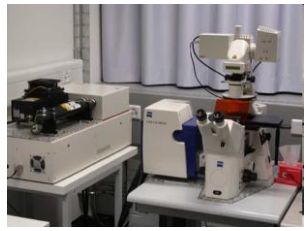
Microtechnologies offers the opportunity for highly localized cell perturbations

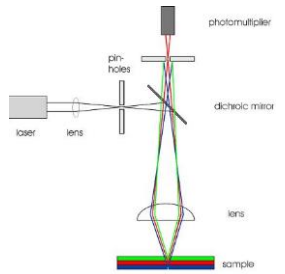


Biotechnol J. 2013 Feb;8(2):167-79. doi: 10.1002/biot.201200149. Epub 2012 Aug 14.
Arrayed cellular environments for stem cells and regenerative medicine.
Titmarsh DM, Chen H, Wolvetang EJ, Cooper-White JJ.

Single cell studies of today

A confocal only allow focus on a single layer determined by pinhole





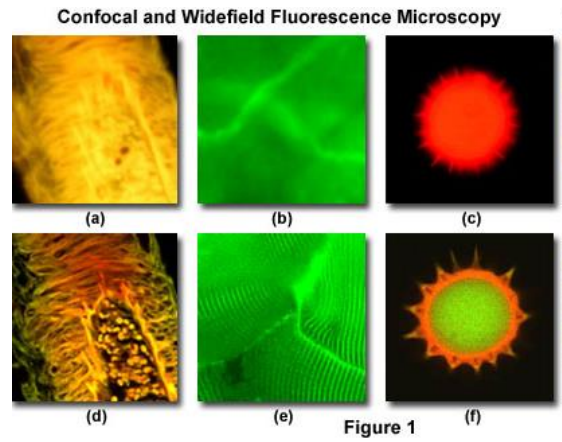
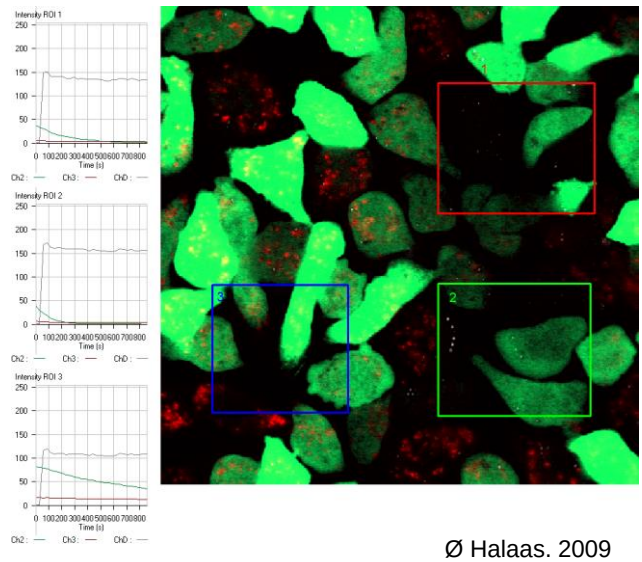


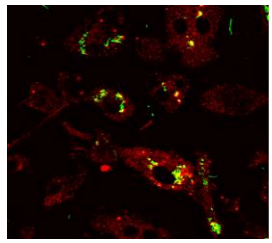
Figure 1

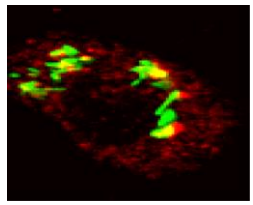
Cells are annoyingly hetergeneous

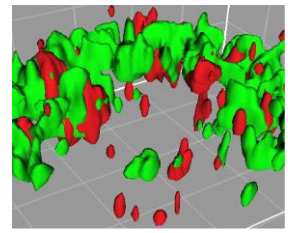


Ø Halaas. 2009

Cell imaging demands experienced researchers. Manuscript referees demand quantitative proof



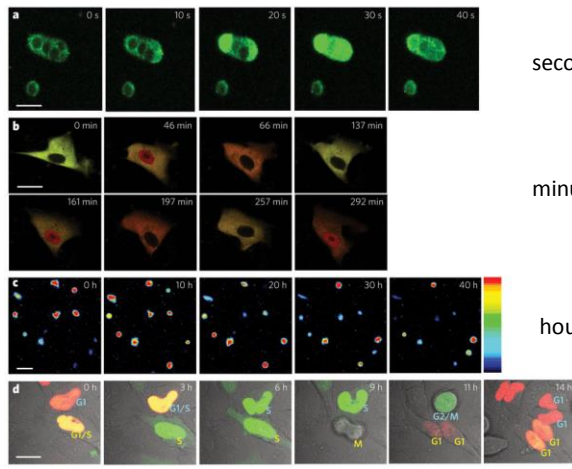




Question: How often does the red drug delivery system meet the green intracellular bacteria?

Optical imaging essential, but new tools are needed to become objective

Cells are dynamic structures



seconds

minutes

hours

Changes on many time scales

Microscopes

Good at high content (morphological detail) analysis
Good at high resolution (spatial) analysis
Good at time-resolved analysis

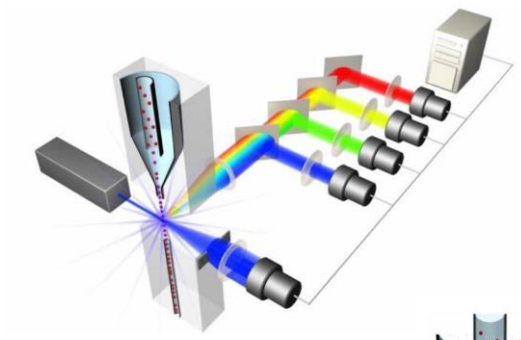
Expensive
Bad at analysing many cells
Information only for 3-4 different molecules

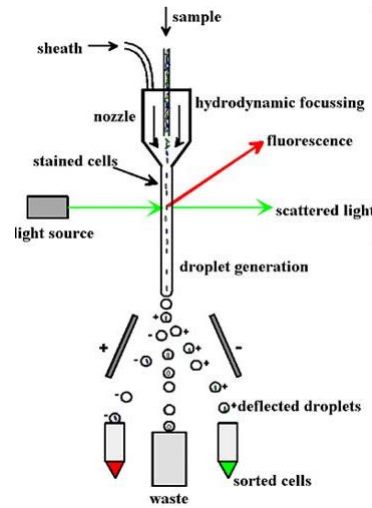
Single cell tools of today, the flow cytometer

A flow cytometric cell sorter (1990)

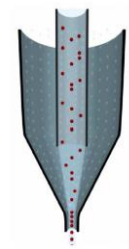
What you get when crossing an ink-jet printer with Coulter counter (particle size analyzer), Palo Alto 1965

A flow cytometric single cell sorter





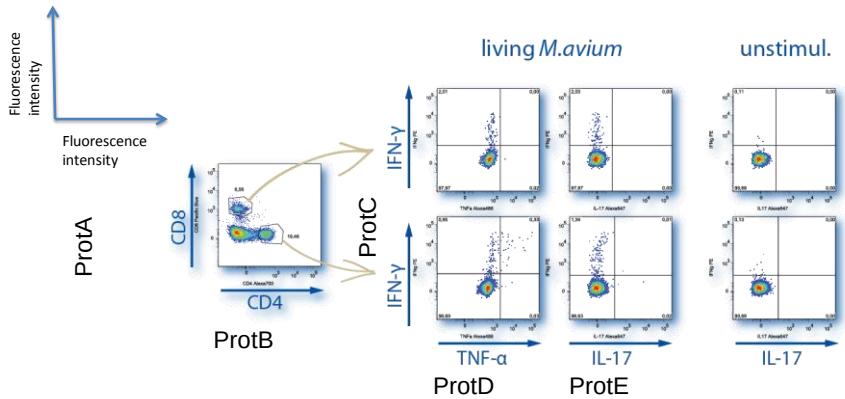
What happens if you merge ink-jet printer with coulter principle



http://www.helmholtz-muenchen.de/imi/zs/principle123.gif

Flow cytometry can detect rare cell types

One can analyze up to 8 protein levels in each cell subset

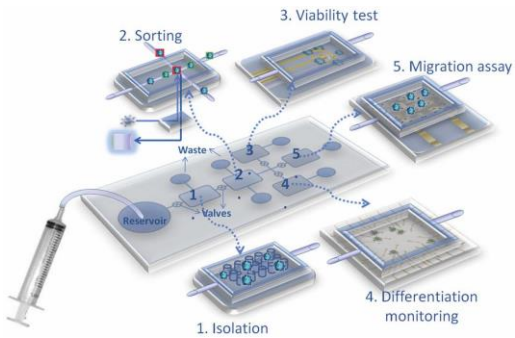


Flow cytometer

- Good at analysing many cells (millions)
- Fast instrument (20.000 cells/s)
- Can analyze up to 8 fluorescent parameters + size and optical density

- Bad at dynamic analysis (is mostly end-point analysis)
- Expensive
- No morphological information

Next generation cell biology: Cells on chips



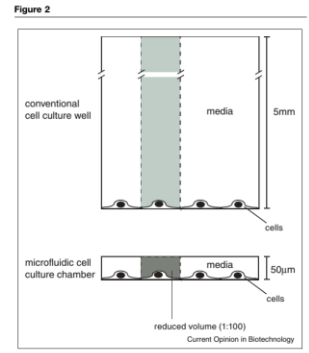
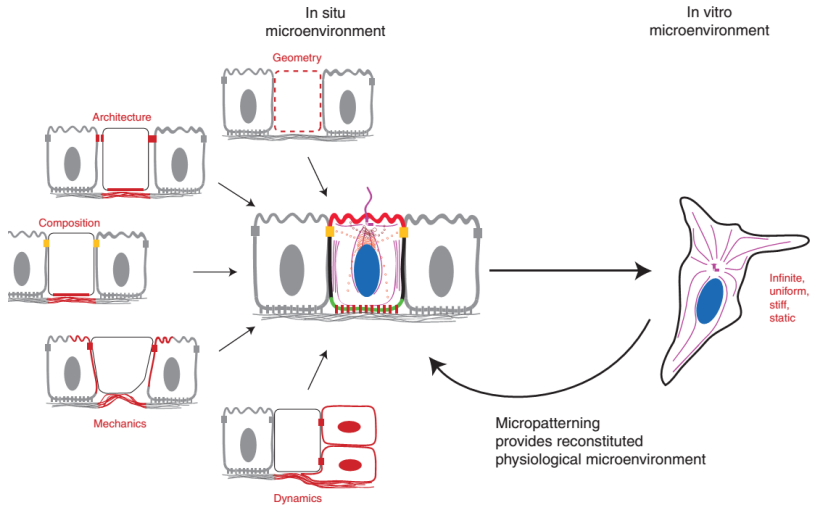


Illustration of '*small-volume effects*' in microfluidic cell culture devices. Smaller culture media volume for a given cell results in faster consumption of nutrients and increased concentration of metabolites or secreted molecules, similar to tightly packed tissues.

Lab Chip. 2013 Aug 27;13(19):3789-802. doi: 10.1039/c3lc50550b.
Cell chips as new tools for cell biology - results, perspectives and opportunities.
Primiceri E, Chiriacò MS, Rinaldi R, Maruccio G.

What is missing from conventional cell cultures?



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 confinment** (microchambers for cells or in-vitro assays)

And all combinations…

Table 1 Selected Studies on Stem Cells Using Lab-on-a-chip Devices

Stem Cell Application	Selected Studies
Control of Soluble Factors	Dynamic exposure to soluble drug
	Design for controlled-diffusive mixing
	Microfluidic based multi-injector method
	'Microbioreactor' system for human embryonic stem cell differentiation
	Paracrine and autocrine signaling control
Control of ECM Interactions	Combining soluble and insoluble factors in microarray
	3D microenvironment to study stem cell fate
Control of Cell-Cell Interactions	Design to control cell-cell interactions
	Design for cell pairing
	Design for co-culture of mES cells with other cell types
	Design for temporal control of cell-cell signaling
	Design for heterotypic cells with different shear sensitivities
Control of Mechanical Signals	Controlled elasticity geometry through PDMS patterns
	Control of elasticity using magnetic nanoposts
	Controlling nanoscale symmetry and disorder in cues
	Designs for surface pattern geometries
	Design for study of role of topography
	Control of 3D topography by electrospun nanowires
	Control of shear stress as a mechanical cue
	Shear control for long term culture
High Throughput Screening	High throughput integrated microfluidic systems
	'High-content screening' with combinatorial stimuli
	Design to study high throughput temporal responses
	Design to screen effects of drugs in long term cultures
Novel Studies for Potential Stem Cell Research	Slow perfusion by osmotic pump for long term culture
	Design for reversible bonding of microfluidic device to cell culture containing cover slip by vacuum
	Design for reversible bonding of microfluidic device by magnetic force

Table 1 Important differences between conventional (monolayer) and microfluidic *in vitro* cell culture systems

Conventional	Microfluidics
	Cell microenvironment
No confinement (open wells).	Confinement (closed systems).
Limited level of **spatial control** (*e.g.* only single-well or trans-well systems).	High level of **spatial control** (*e.g.* compartmentalization for co-culture, 3-dimensionality and sub-cellular resolution).
No **fluid control** (only static or chaotic).	High level of control over fluids (*e.g.* laminar flow, perfusion, and temporal control over fluid exchange).
Limited possibilities for creating **physical stimuli**.	Various **physical stimuli** possible (*e.g.* stiffness, shear, compression).
Low temporal and spatial control over **chemical stimuli** (only bulk addition).	Possibility to create highly defined spatial and temporal **chemical stimuli** (*e.g.* soluble or surface gradients).
Established and characterized culture **substrate materials**.	Limited characterization of applied **substrate materials** and limited use of biologically characterized materials.
	Biological read-out
Compatible with conventional standardized **biological assays**.	Compatibility issues with conventional standardized **biological assays**.
Compatible with established **read-out equipment**.	Compatibility issues with established **read-out equipment**.
Comparable to large amount of data from **historical experiments**.	Low number of available **historical experiments** limits comparison.
Limited possibilities for *in situ* **read-out of biological processes**.	Possibility to integrate sensors and assays for *in situ* **read-out of biological processes**.
	High-throughput screening (HTS)
High **reagent** and biological (cell) **material use** in HTS setting.	Reduced **reagent** and biological (cell) **material use** in HTS setting.
Limited possibilities to **parallelize and integrate** assays.	Highly applicable to **parallelization and integration** of assays.
Compatible to conventional high-throughput (robotics) **equipment**.	Not compatible with conventional high-throughput (robotics) **equipment**.

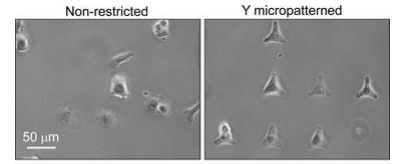
Single cell devices

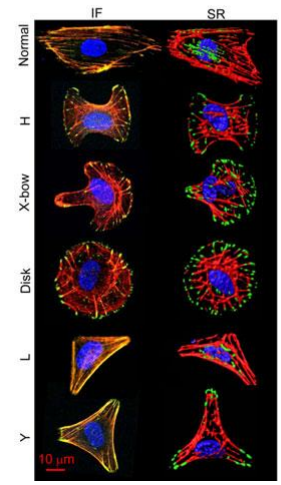
- Watching single event dynamics
- Relate to earlier lectures
- 1) Standardize single cell morphology
- 2) Isolate cells
- 3) Trap cell
- 4) Cell pairing
- 5) Select cells
- 6) Cell migration
- 7) Multiplexing
- 8) Cocultures

Table 1

Advantages of microfluidics for single cell analysis.

Application	Challenges with traditional methods	Advantages of microfluidics
Single-cell RT-qPCR	Limited abundance of starting template	Concentration enhancement in small volumes
	Cost and throughput required for analysis of large numbers of cells and target genes	Parallelization, automation, and economy of scale
Single-cell genomics	Amplification bias and sensitivity	Improved reaction bias and sensitivity in nL volumes and reduced contaminant DNA
	Isolating individual cells	Integrated microfluidic cell sorting and processing
Single-cell measurements of intracellular proteins	Movement of living cells	Confinement of live cells in microfluidic structures
	Low amount of signal	Integrated single-cell handling allows lysate analysis
Single-cell measurements of secreted proteins	Small amounts of secreted products from single cells	Concentration enhancement in small volumes
	Difficult to co-localize multiple cells in defined chemical environments	Ease of confinement in droplets or microchambers
Signaling studies	Mostly limited to static conditions	Easy temporal stimulation
	Inability to rapidly exchange conditions on suspension cells	Laminar flow and proper design enables cell sequestration
Live cell imaging	Difficulties of tracking cells through multiple frames	Confinement of clones facilitates cell tracking

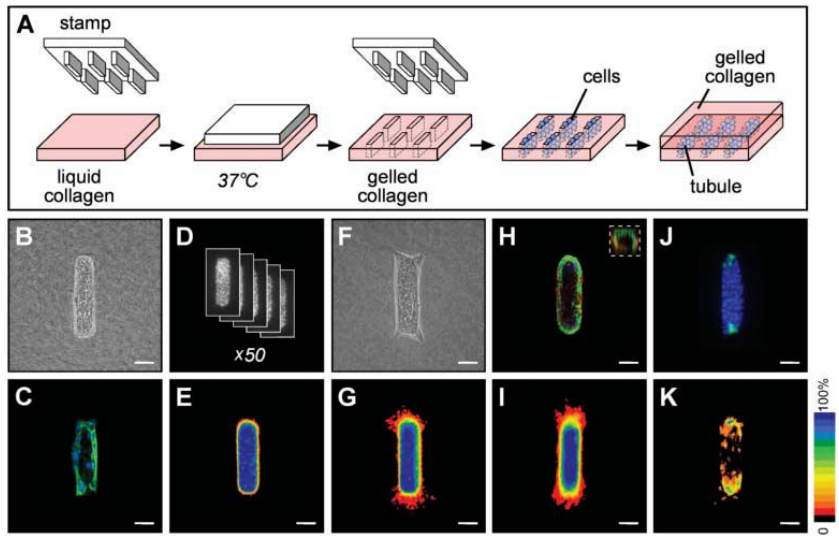




1) Standardization of single cell organization

http://cytoo.com/

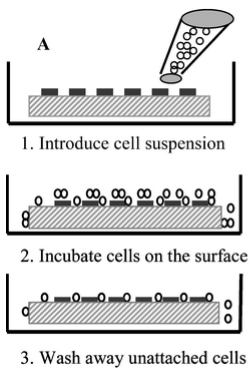
Standardizing cell cultures



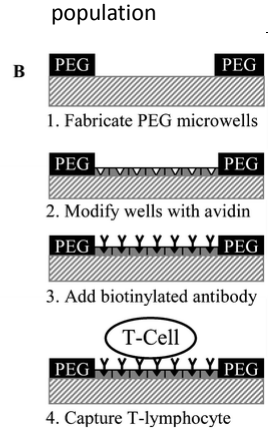
Nelson, C.M., Science, 2006

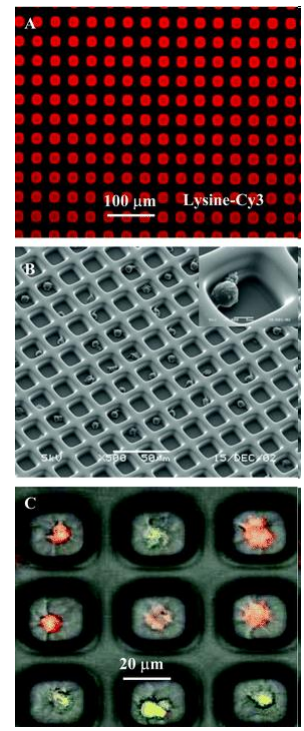
2) Single cell capture in microwells

From a pure cell population

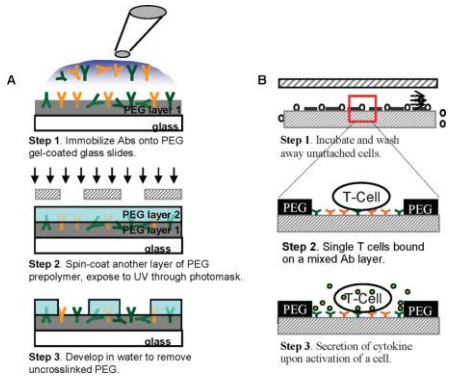


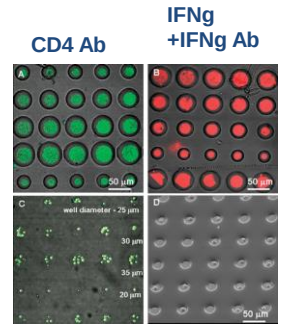
From a mixed cell population

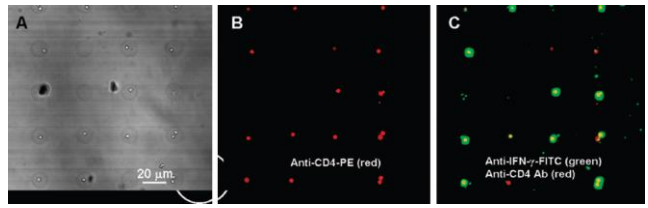




2) Microwells with detection modes

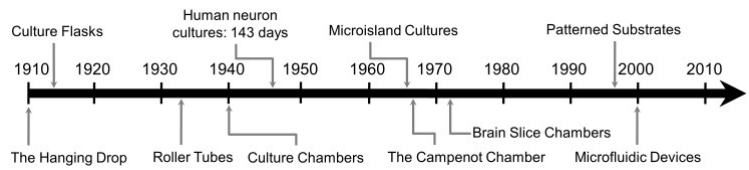




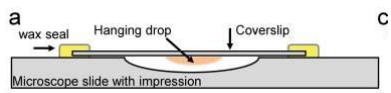


Detecting cytokine release from single T-cells.
Anal Chem. 2009 Oct 1;81(19):8150-6.
Zhu H, Stybayeva G, Silangcruz J, Yan J, Ramanculov E, Dandekar S, George MD, Revzin A.

Some cells are not adherent and dont want to be:
Alternative to adherent cells: Hanging drop cultures in microfluidcs



1909



2012

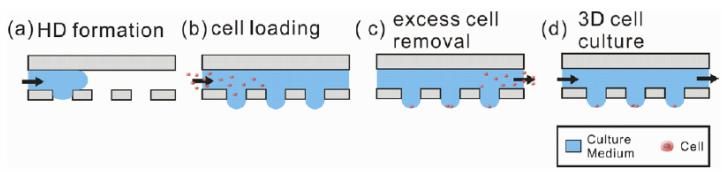
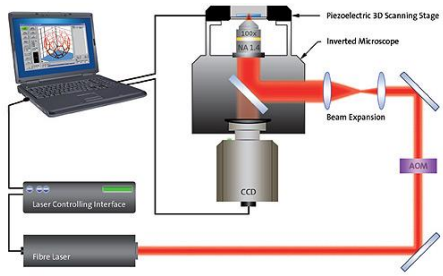
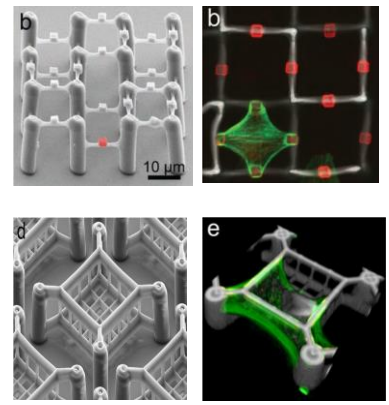


Figure 1: A cross-section of the operation of the microfluidic HD platform. (a) The microfluidic channel is filled and droplets are formed and hanging at the openings. (b) Cells are driven by the flow and docked in the HD. (c) Untrapped cells are rinsed off. (d) Cells are incubated and their extracellular solution can be modulated through the microchannel while gas can also be exchanged directly through the HD that is open to air.

Most cells are not in their natural state on a hard surface
- Suspended single cell cultures



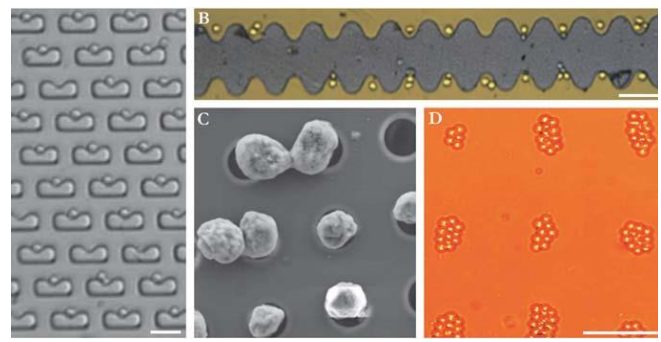


Nanoscribe, direct 3D writing

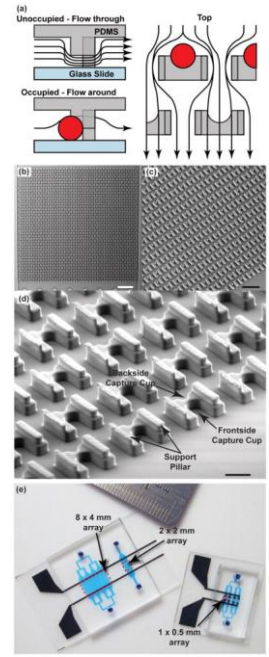
3) Cell traps

Alternative to printed biopatterns

Capture of non-adherent cells at specified locations exploiting fluid dynamics

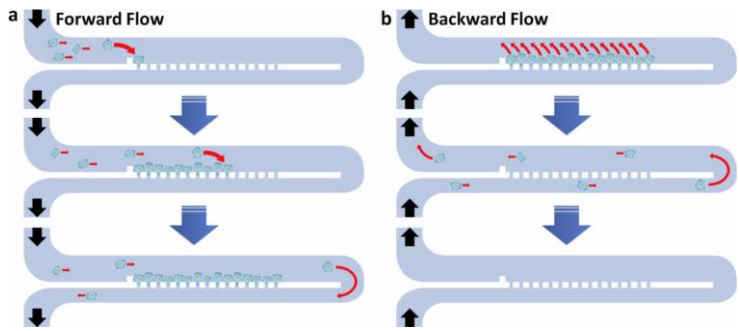


3) Cell traps



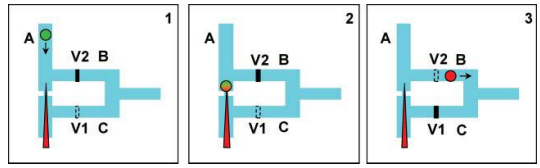
Nat Methods. 2009 Feb;6(2):147-52. doi: 10.1038/nmeth.1290. Epub 2009 Jan 4.
Microfluidic control of cell pairing and fusion.
Skelley AM, Kirak O, Suh H, Jaenisch R, Voldman J.

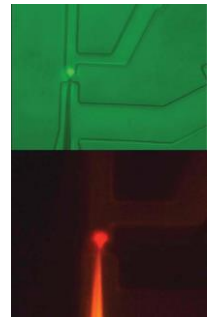
3) A resettable cell trap :
how to get cells out of the chip after trapping



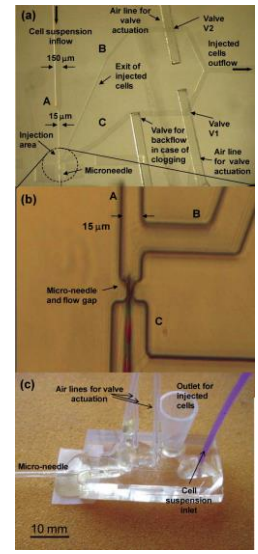
Ryan D. Sochol1*, Kosuke Iwai1, Adrienne T. Higa1, Joanne C. Lo2, Eric Zhou1, Liana Lo1, Chris Luong1, Megan Dueck1, Song Li1, Luke P. Lee1 and Liwei Lin1
Berkeley Sensor and Actuator Center
1University of California, Berkeley USA
2Sandia National Laboratories, Livermore USA

3) Using microfluidics for trapped single cell injections



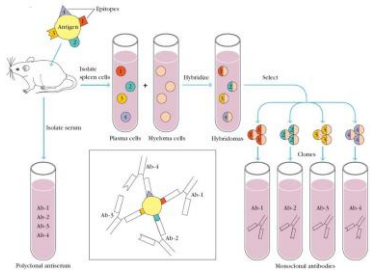


Microfluidic based single cell microinjection.
Lab Chip. 2008 Aug;8(8):1258-61.
Adamo A, Jensen KF.

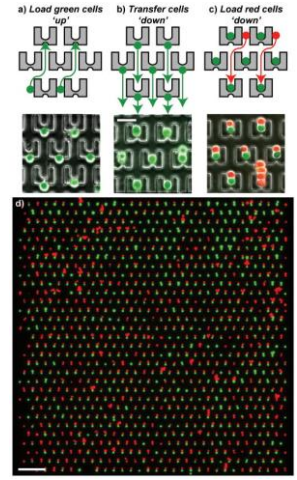


3,4) Cell trapping and pairing

example from hybridomas

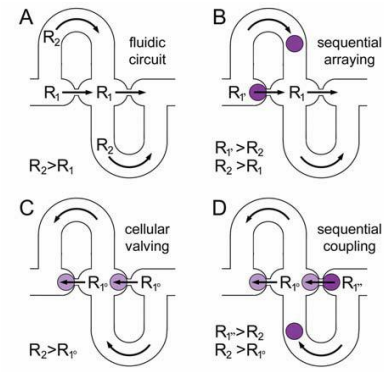


Hybridomas
Immune cells
Stem cells
Cancer cells
Neurons



http://www.nature.com/nmeth/journal/v6/n2/suppinfo/nmeth.1290_S1.html

4) Microfluidic cell pairing



Immune cells
Stem cells
Cancer cells
Neurons

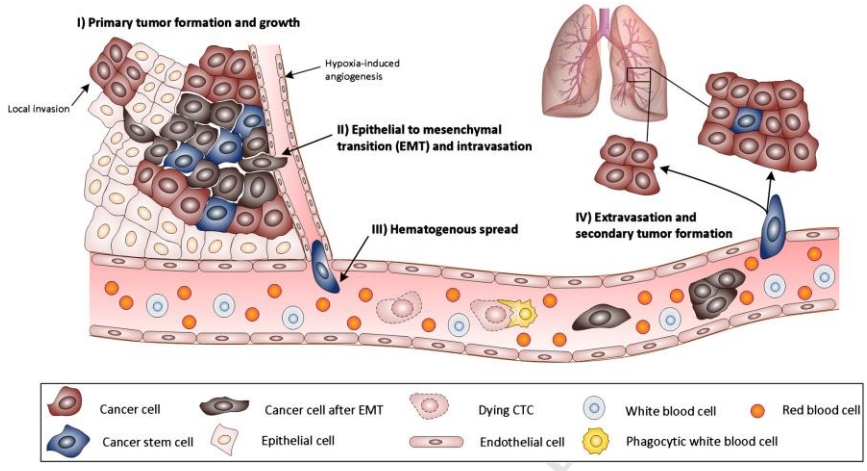
A microfluidic array with cellular valving for single cell co-culture
Jean-Philippe Frimat, Marco Becker, Ya-Yu Chiang, Ulrich Marggraf, Dirk Janasek, Jan G. Hengstler, Joachim Franzke and Jonathan West
Lab Chip, 2011, 11

DOI: 10.1039/C0LC00172D

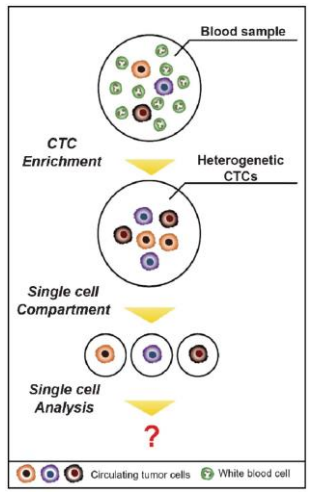
5) Cell isolation

- Adhesion
- Size filtering
- Dielectrophoresis
- Fluorescence

Circulating tumor cells



Liquid biopsies, circulating tumor cells

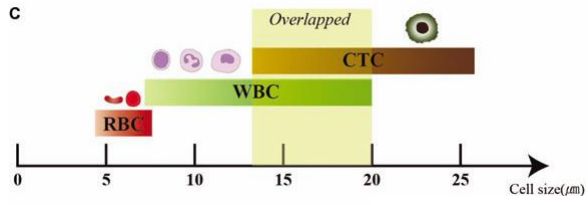


CTCs are few and heterogenous, needs to be studied at single cell level

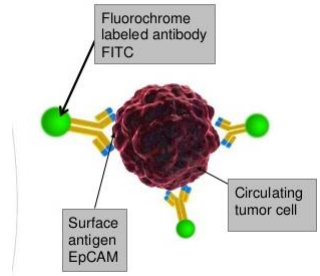
1) Enrichment
2) Single cell analysis

Lab Chip. 2013 Aug 28. [Epub ahead of print]
Advances and critical concerns with the microfluidic enrichments of circulating tumor cells.
Hyun KA, Jung HI.

Circulating tumor cells are somewhat bigger than normal cells

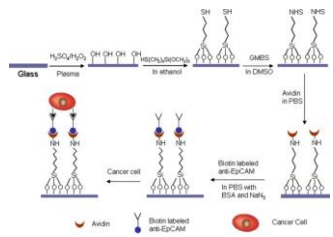


Circulating tumor cells are often EpCam-positive



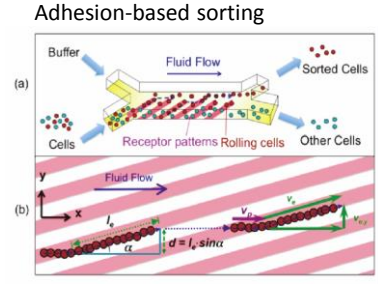
5) Adhesion based enrichment of CTC

Immobilizing anti-EpCam

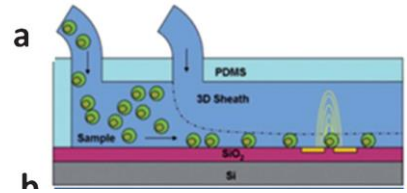


Lab Chip. 2013 Aug 27;13(19):3789-802. doi: 10.1039/c3lc50550b.
Cell chips as new tools for cell biology - results, perspectives and opportunities.
Primiceri E, Chiriacò MS, Rinaldi R, Maruccio G.

Adhesion-based sorting



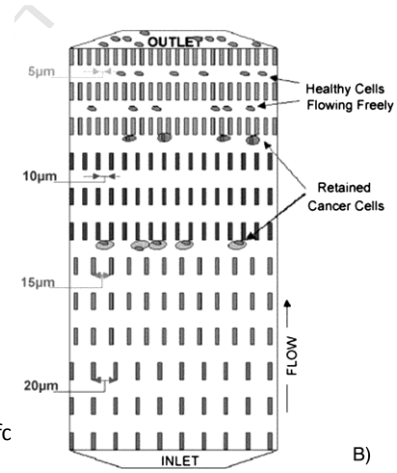
Forcing cells down on the pattern



5) Serial size-filtering of CTC

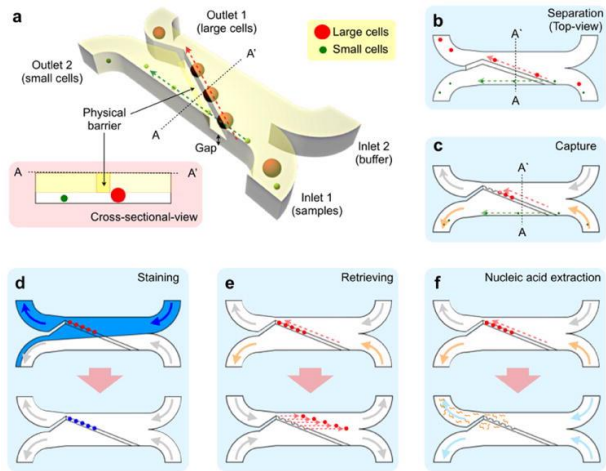
Detection and isolation of circulating tumor **cells**: Principles and methods.
Esmaeilsabzali H, Beischlag TV, Cox ME, Parameswaran AM, Park EJ. Biotechnol Adv. 2013 Aug 30. doi:pii: S0734-9750(13)00150-X. 10.1016/j.biotechadv.2013.08.016.

http://www.youtube.com/watch?v=xmgP8fBfcJU

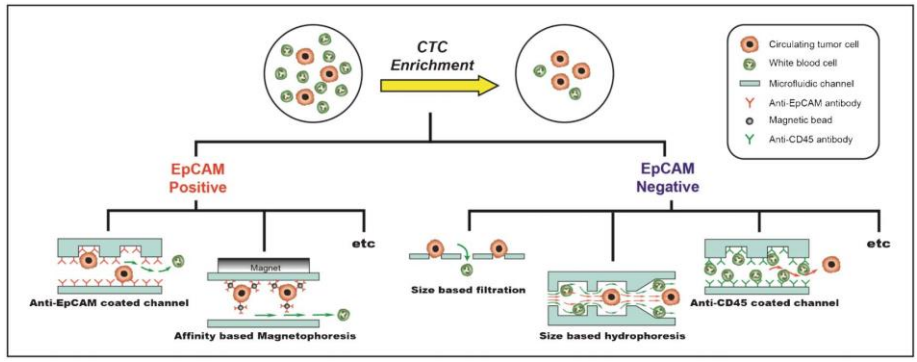


B)

5) On chip filtering capture and sample prep for larger cells



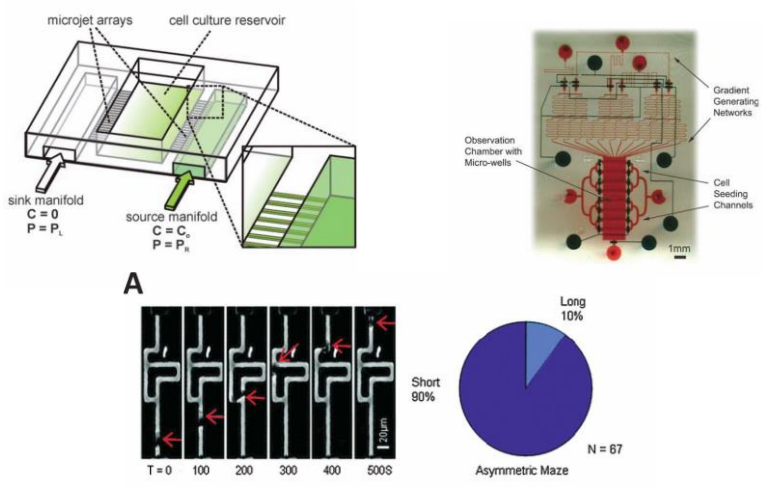
5) Micro/nanotechnological options for CTC enrichment



6) Migration

- Stem cell for tissue engineering (in vitro)
- Stem cells for tissue regeneration (in vivo)
- Tissue development and remodelling
- Immunity
- Cancer metastasis and tissue invasion
- Other diseases

6) Migration/Chemotaxis

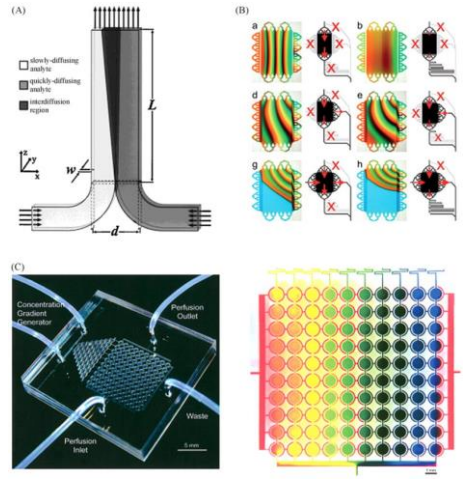


Lab Chip. 2013 Jul 7;13(13):2484-99. doi: 10.1039/c3lc50415h. Epub 2013 May 28.
Recent developments in microfluidics-based chemotaxis studies.
Wu J, Wu X, Lin F.

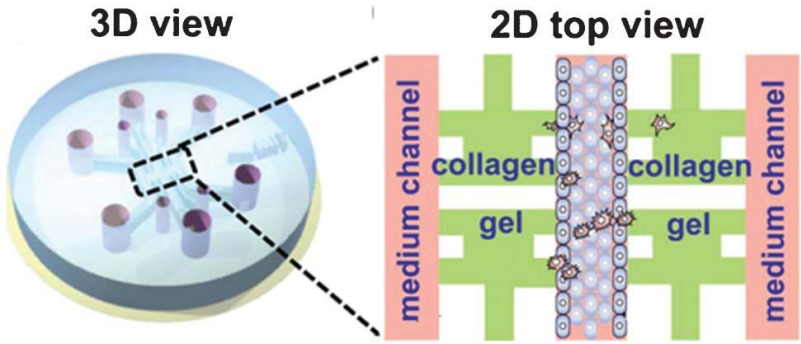
6) Soluble Gradients

Migration towards gradients

Constant flow or immobilize with photochemistry



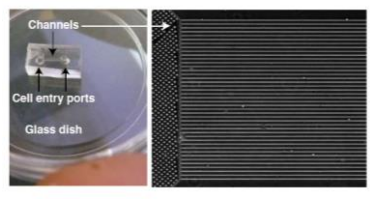
6) Migration

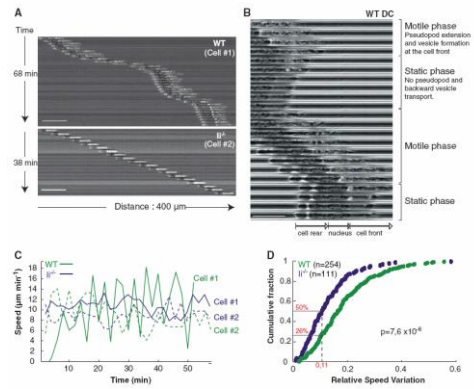


Lab Chip. 2013 Aug 27;13(19):3789-802. doi: 10.1039/c3lc50550b.
Cell chips as new tools for cell biology - results, perspectives and opportunities.
Primiceri E, Chiriacò MS, Rinaldi R, Maruccio G.

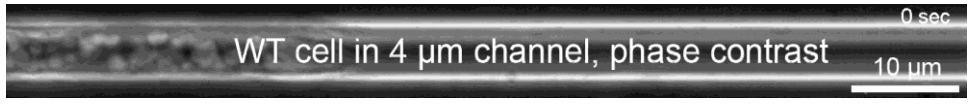
6) Migration in microchannels:

microchannels with a 4µm by 5µm section



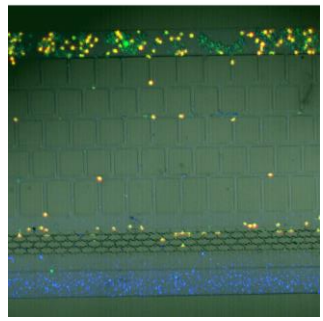


Science. 2008 Dec 12;322(5908):1705-10.
Regulation of dendritic cell migration by CD74, the MHC class II-associated invariant chain.
Faure-André G, Vargas P, Yuseff MI, Heuzé M, Diaz J, Lankar D, Steri V, Manry J, Hugues S, Vascotto F, Boulanger J, Raposo G, Bono MR, Rosemblatt M, Piel M, Lennon-Duménil AM.



http://www.sciencemag.org/cgi/content/full/sci;322/5908/1705/DC1
Faure-André et al., Science, 2008

Own research: Recreating infections on a chip
Understanding recruitment toward tuberculosis granuloma

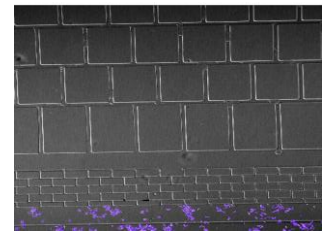


Cell mixtures

Cell-sized network

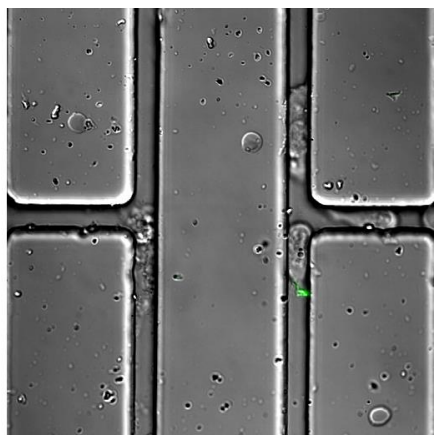
Bacterial filter

Bacteria/cells

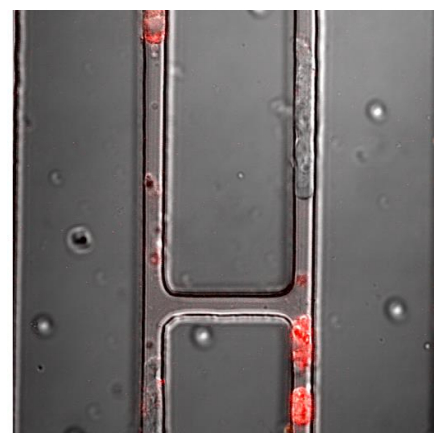


Phagocytic cells stop moving when dealing with substrates

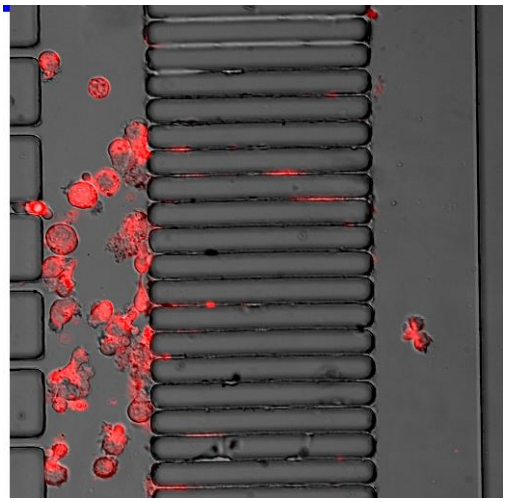
Mycobacteria are resilient.



Eating dead cells=cross presentation

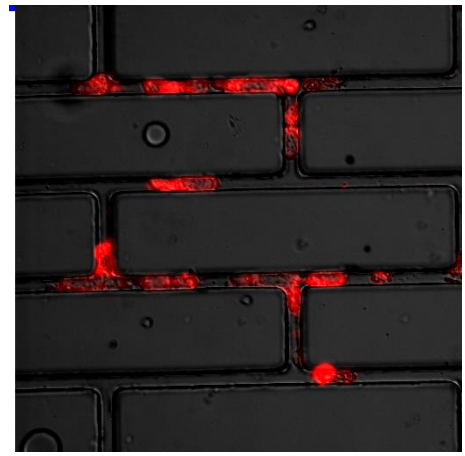


Cell retention filters segregates compartments



Moving in and out of the artificial tissue

Forcing cells to make choices where we control input



6) Migration on tracks (not tunnels)

J Cell Biol. 2009 February 23; 184(4): 481–490.
doi: 10.1083/jcb.200810041

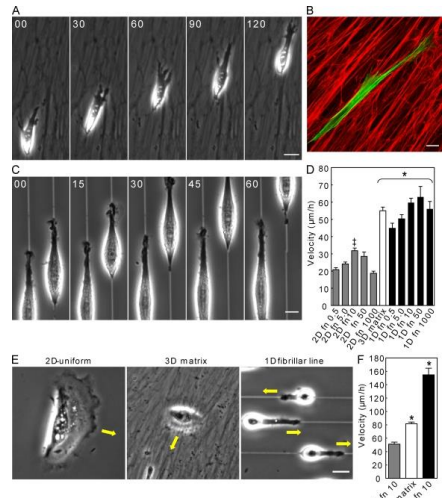
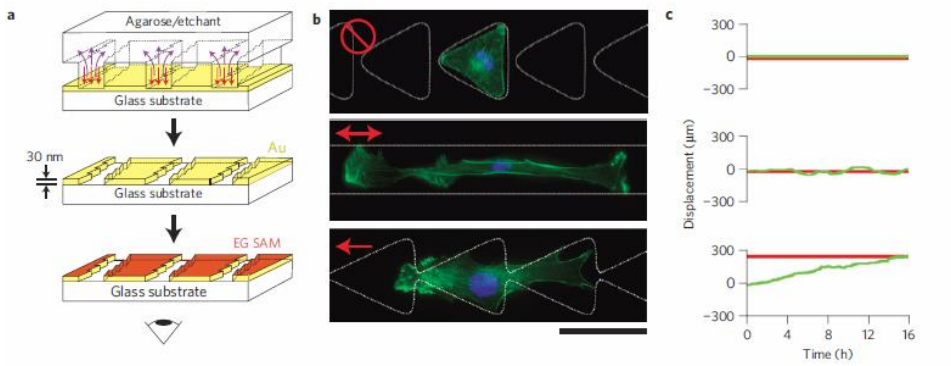
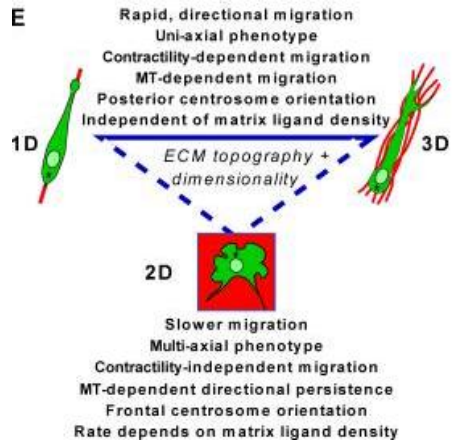


Figure 2.
1D topography functionally mimics 3D fibrillar matrix. (A) Rapid migration of NIH-3T3 fibroblasts through a 3D cell-derived matrix. (B) Cytoskeletal alignment (green, actin) along oriented FN fibers (red). (C) Fibroblasts plated on single ~1.5-μm lines mimic this 3D phenotype and migrate continuously. (D) Cell migration rates on 2D surfaces and 1D fibrillar lines at different FN ligand densities versus 3D cell-derived matrix. (E) HK migration over 2D, 3D matrix, and 1D fibrillar lines. (F) HK migration rates on substrates in E. Bars: (A, B, and E) 20 μm; and (C) 10 μm. *, P < 0.01 versus 2D; ‡, P < 0.05 versus 0.5 and 1,000 FN. Error bars indicate SEM.

6) Also adhesive patterns in the right shape can induce motility

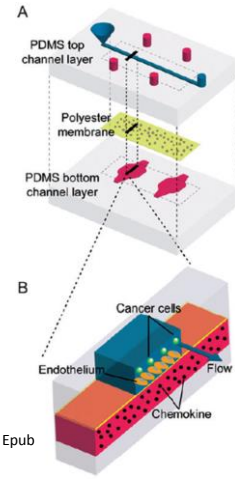


Conclusions from extensive review 1D-2D-3D



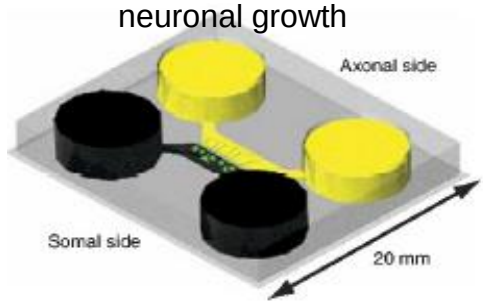
Vertical compartmentalization

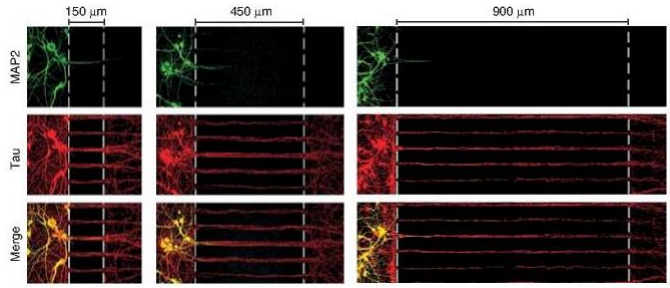
A tool for looking at migration in blood-model



Chem Soc Rev. 2010 Mar;39(3):1036-48. doi: 10.1039/b909900j. Epub 2010 Feb 1.
Fundamentals of microfluidic cell culture in controlled microenvironments.
Young EW, Beebe DJ.

Combining confinement and micro-fluidics: dual chambers for neuronal growth



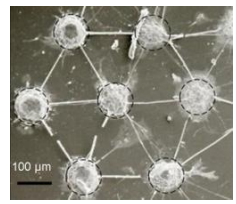


Taylor et al., 2005

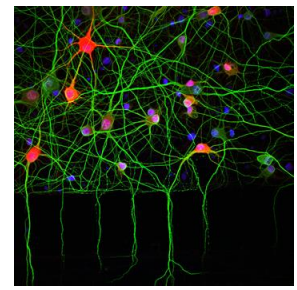
Neurons on chips

Add electrodes

Create circuits



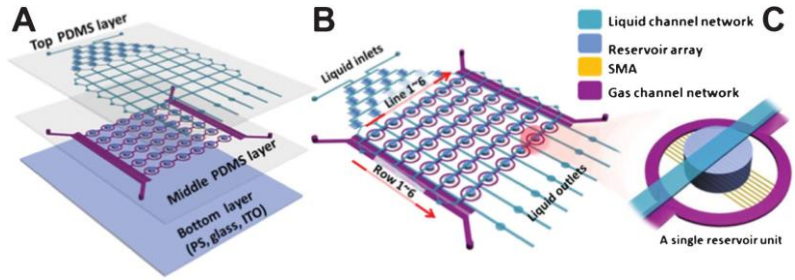
Detain soma, let axons grow



7) Multiplexing cell cultures

Giving different mixtures of different biologicals to different cells,

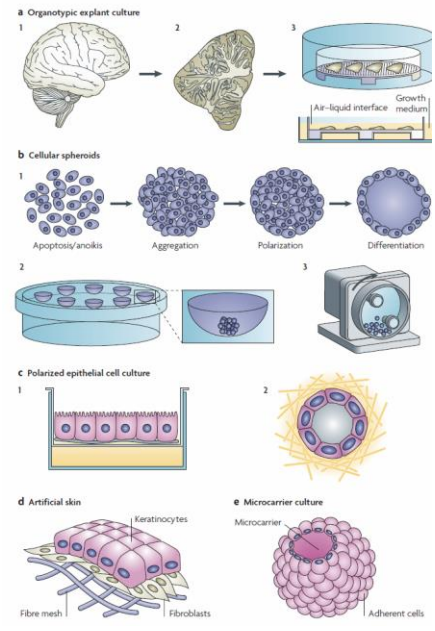
Robotless- High throughput screening on a chip



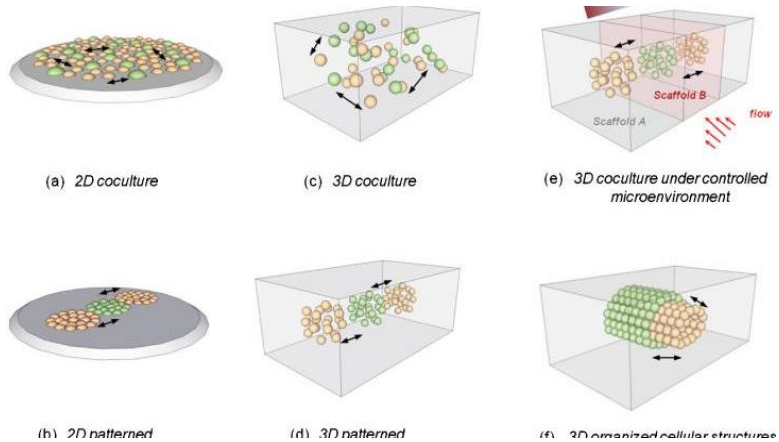
Scheme 1 Chip structure. A: Illustration for the three layered components of the chip: the top two are PDMS layers, and the bottom one is the cell culture substrate layer. B: Illustration for the structure of integrated chip. C: Illustration for the single reservoir unit of the chip.

The third dimension gaps cell culture with tissues (more next lecture «organs on chips»)

http://www.ncbi.nlm.nih.gov/pubmed/22797912

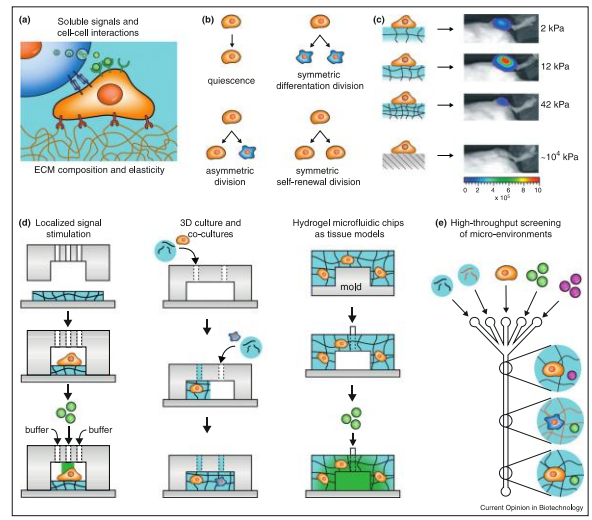


8) Options for in vitro co culture cell growth



8) New approaches for cell cultures

Curr Opin Biotechnol. 2011 Oct;22(5):690-7. doi: 10.1016/j.copbio.2011.07.00 1. Epub 2011 Aug 5.
Biomaterials meet microfluidics: building the next generation of artificial niches.
Kobel S, Lutolf MP.



General conclusions

A very large variety of techniques: how to chose the one you need?

1.Cell type: imposes the type of protein, and the type of protein binding

2.Length of the experiment: imposes the type of anti-adhesive molecule and the binding method

3. Main parameters to modulate
-Size of the patterns (bigger are easier, threshold is around few tens of microns)
-Cell culture substrate (glass is the best for optics and gold is the best for chemistry)
-Anti-adhesive backfilling (Silanes, PLL-PEG, Pluronic, BSA, etc…)
-Protein binding to the substrate (covalent, electrostatic or adsorption)

Some methods, like photolithography and lift-off work in almost all cases, but are very heavy for a biology lab, while others are very easy but can apply only to very specific cases

pensum
Analyst. 2016 Jan 21;141(2):504-24. doi: 10.1039/c5an00382b.

Microfluidics for research and applications in oncology.

Chaudhuri PK, Ebrahimi Warkiani M, Jing T, Kenry, Lim CT.

Overview of single-cell analyses: microdevices and applications
Sara Lindström*a and
Helene Andersson-Svahn
Lab Chip, 2010,10, 3363-3372

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