CELL ON CHIP

第一組

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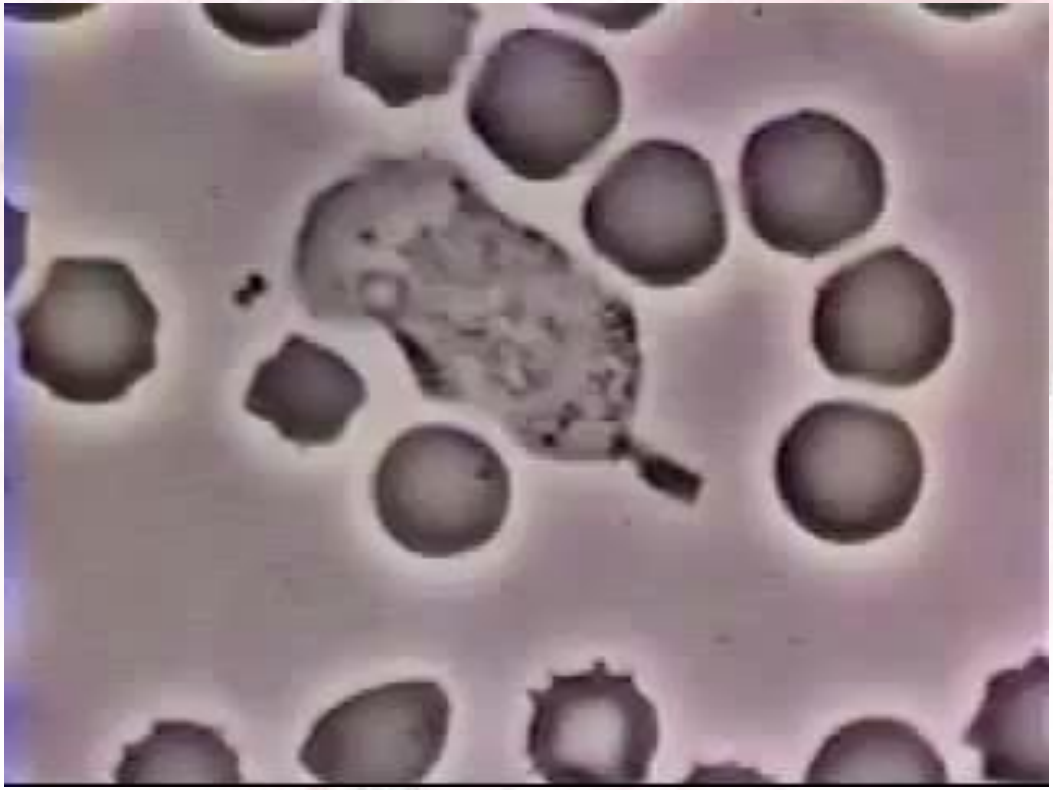
Before we start….



Think about…

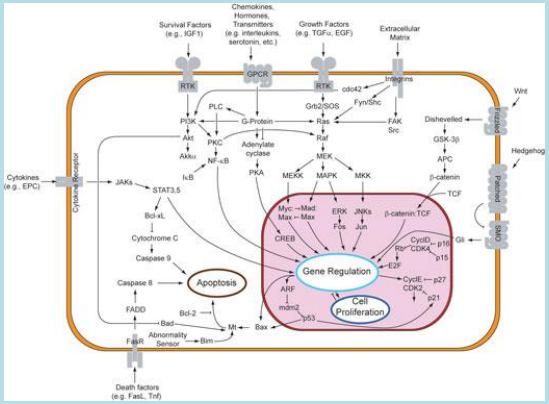
- 白血球細胞沒有大腦思考，如何辨認敵我?
- 如何追蹤細菌?
- 沒有腳如何快速移動?

How does cell response to its environment

input

Signal molecule

Intracellular signaling network



output

energy metabolism

electric activity

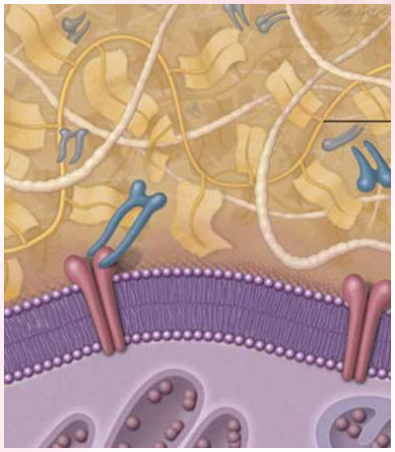
morphology

receptor

Cell-cell interaction

ECM

Cell-ECM interaction



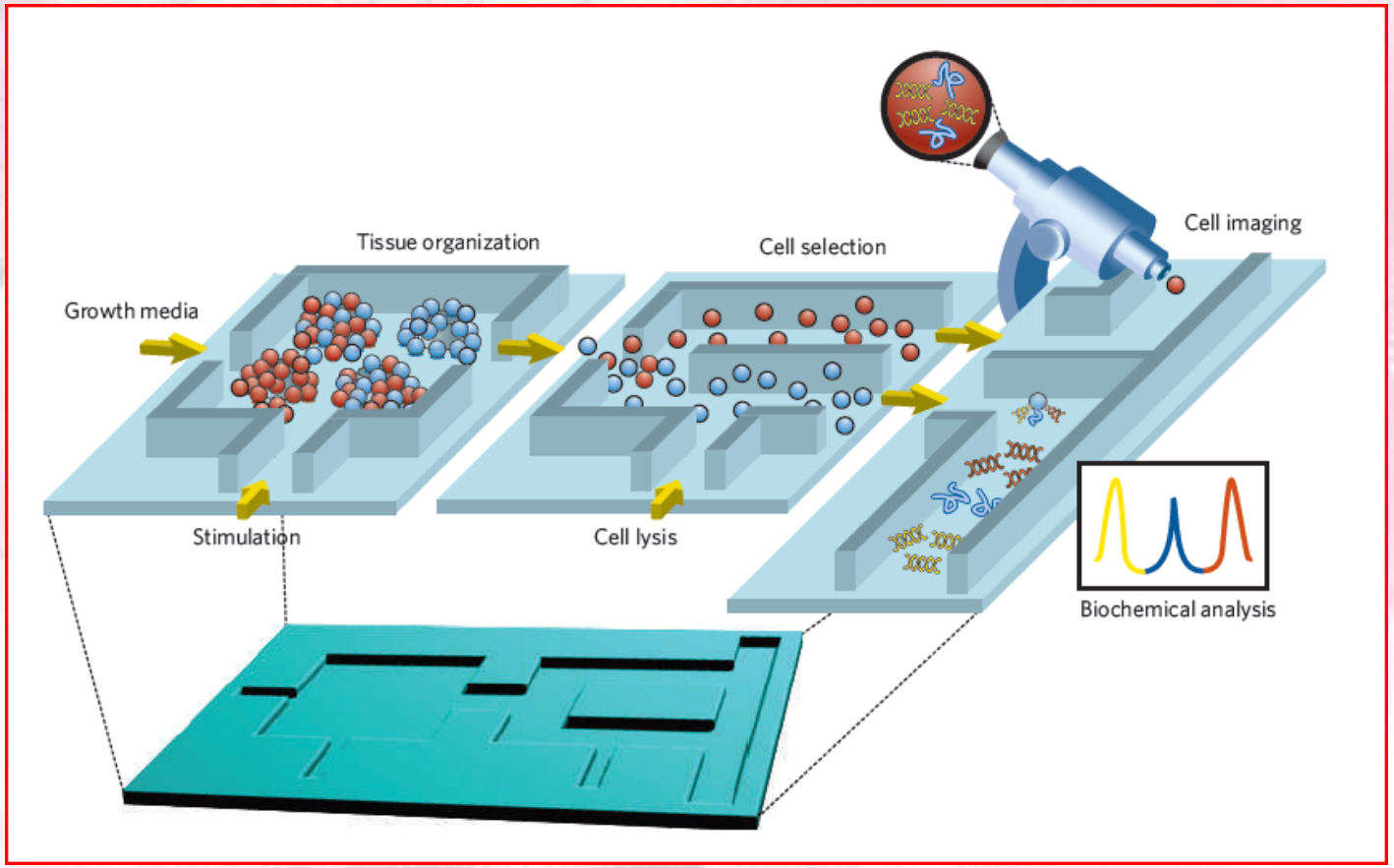
Cell on chip

- Provides spatial and temporal control of cell growth and stimuli :

  1. surfaces that mimic complex biochemistries and geometries of the extracellular matrix
  2. micro-fluidic channels that regulate transport of fluids and soluble factors

- Further integration with bio-analytic microsystems

  1. multifunctional platforms for basic biological insights into cells and tissues



  2. cell-based sensors

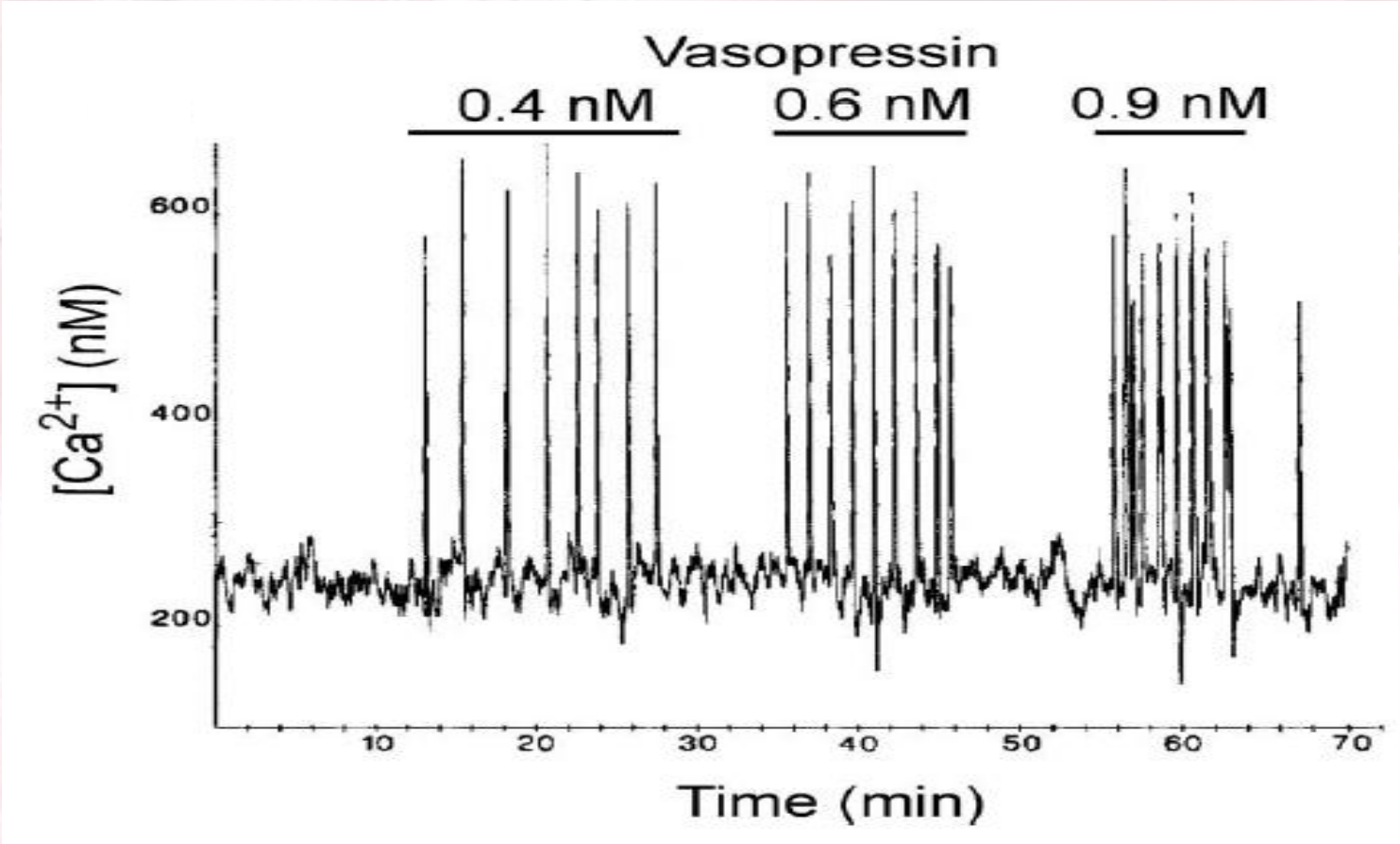
  -- sensing the cell response

single cell analysis

- Most cell-based biological assays yield data averaged across large groups of cells
- Individual cells, even those identical in appearance, differ in numerous characteristics.
- Traditional biochemical assays which analyze cells in bulk often overlook the rich information available when single cells are studied.

example

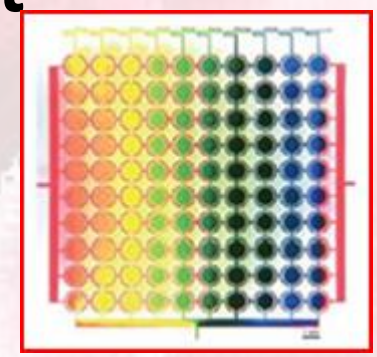
- Cell response after vasopressin stimuli



- Applications
  1. biological cell-level research
  2. basic biomedical and pharmaceutical research
  3. robust and portable point-of-care devices in clinical setting

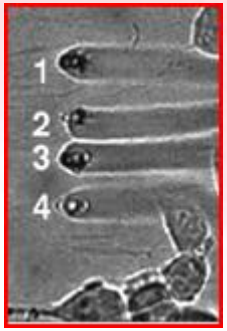
On-chip analysis of single cells in micro-engineered environments

➢ **Control of the fluidic microenvironment**



- Gradient control

  控制某種可溶性分子在整個細胞大環境的濃度梯度分布

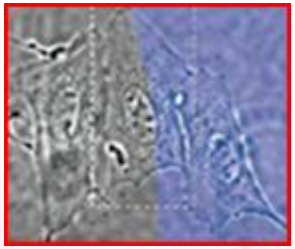


- Local control

  控制某種可溶性分子在單一細胞之周圍環境之濃度

- Sub-cellular control

  控制某種可溶性分子在單一細胞之不同部分的濃度分布

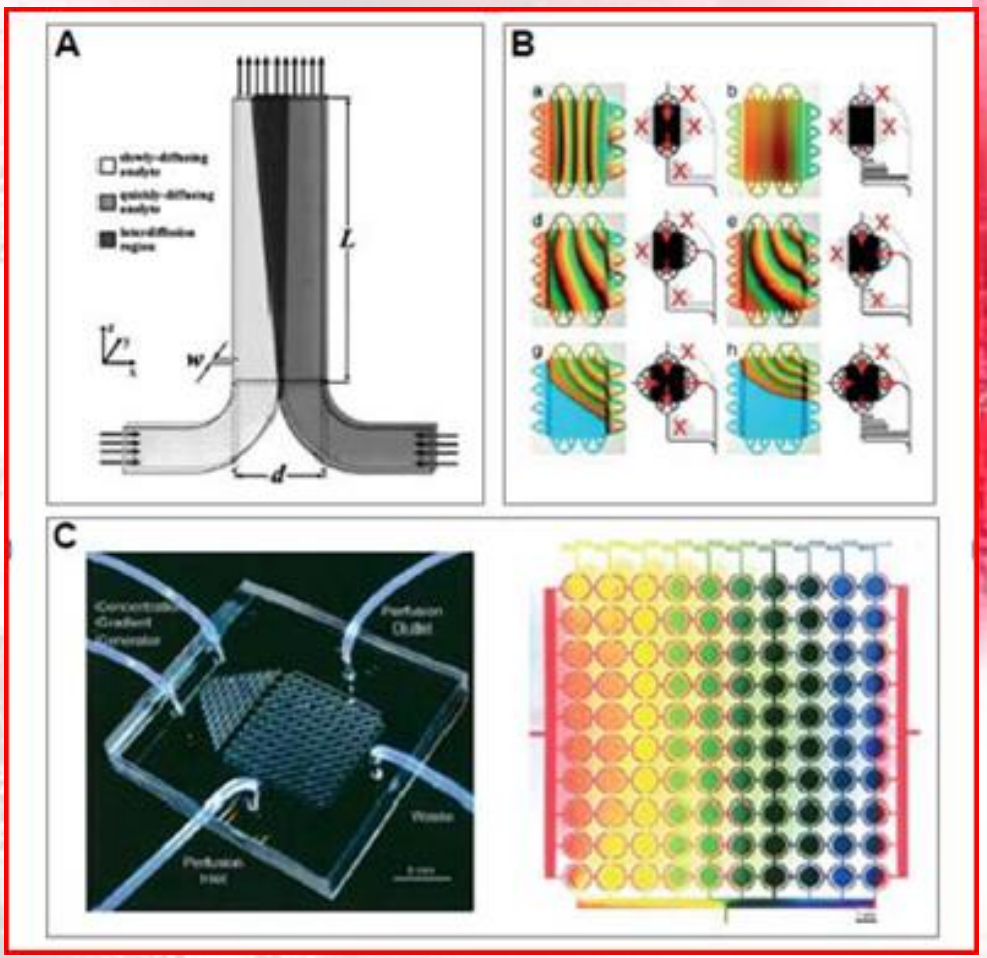


Control of the fluidic microenvironment

➢ **Gradients**

Traditional Methods

Utilizing laminar flow

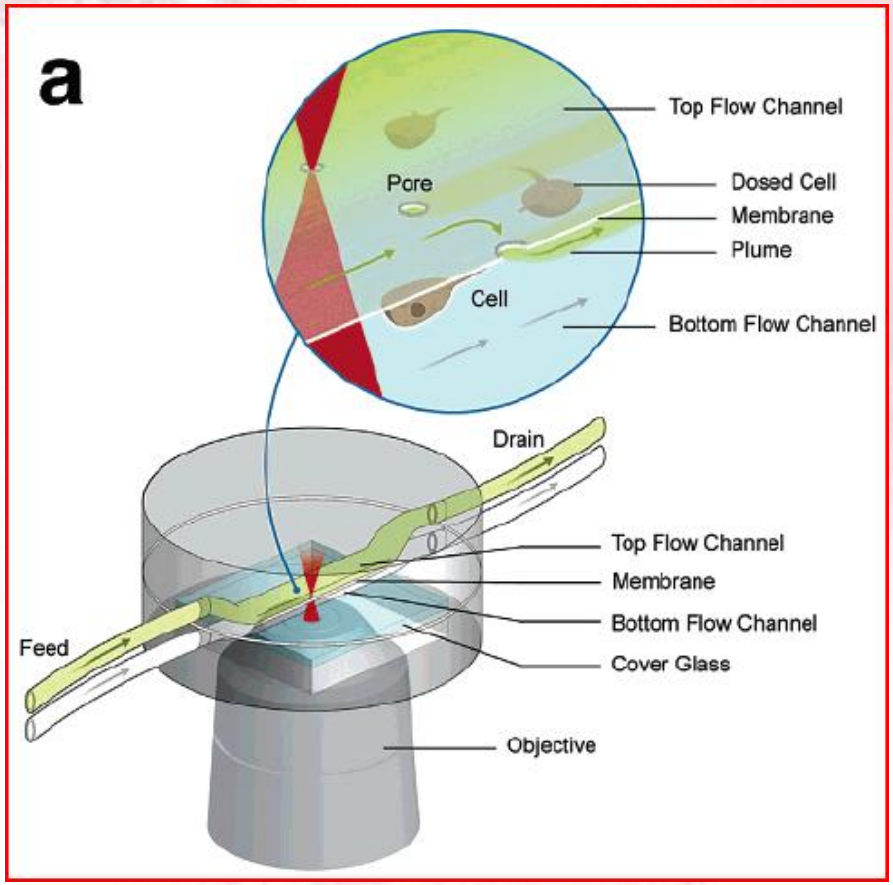


- Poor reproducibility and spatiotemporal control
- Cannot generate complex and stable gradients

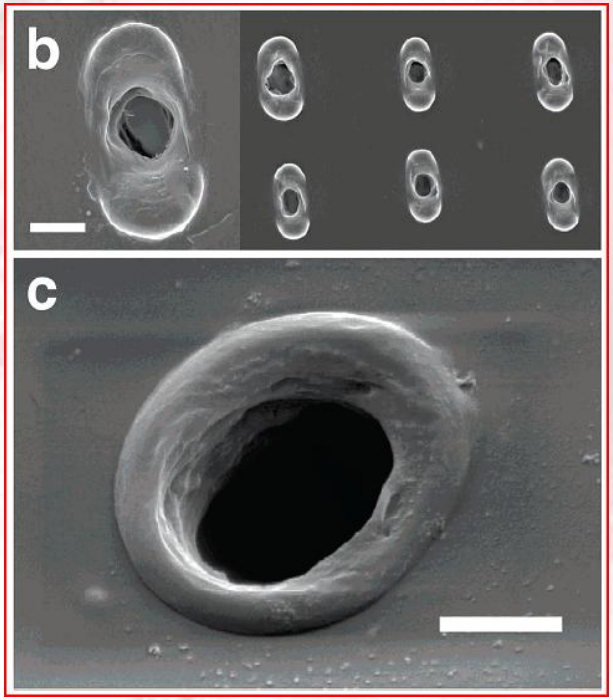
Micro-fluidic gradient generator

➢ Local control

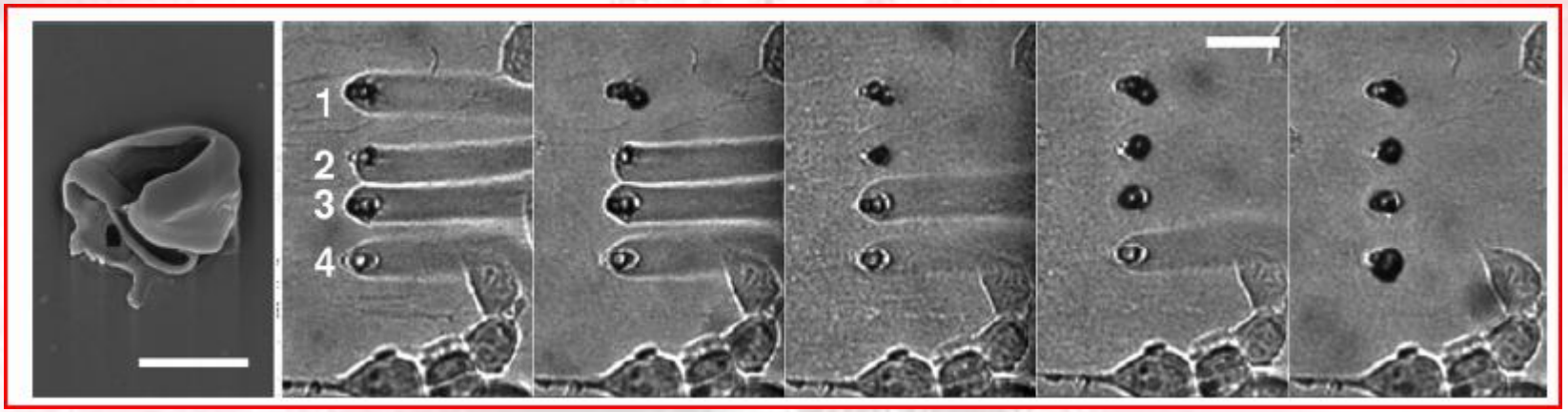
Method



- A ultrathin polymer membrane that separates two stacked laminar-flow chambers
- A train of focused femtosecond laser pulses
- Create pores in the membrane producing laminar flow effector stream that enters the lower pressure cell culture chamber.



Closure of pores using protein photo-cross-linking.



➢Subcellular control - laminar flow

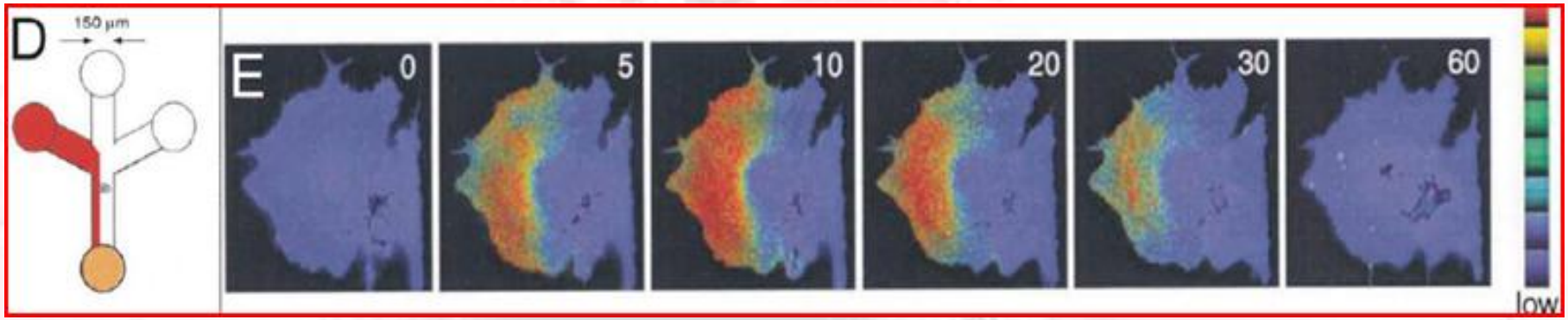
Method

- Utilizing two merging fluid streams
- Expose selective regions of a cell to the soluble hormone epidermal growth factor (critical to cell growth and survival)

Result

- Normal cells : receptor signaling remained localized
- Cells overexpressing the hormone's receptor : the signal spread throughout the cell

ex: a number of tumors



Micro-engineered, three-dimensional substrates

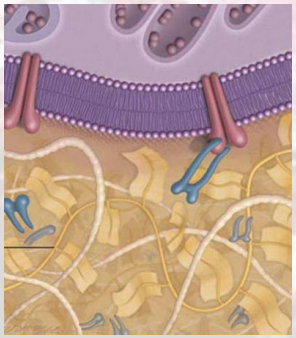
- Cell motility

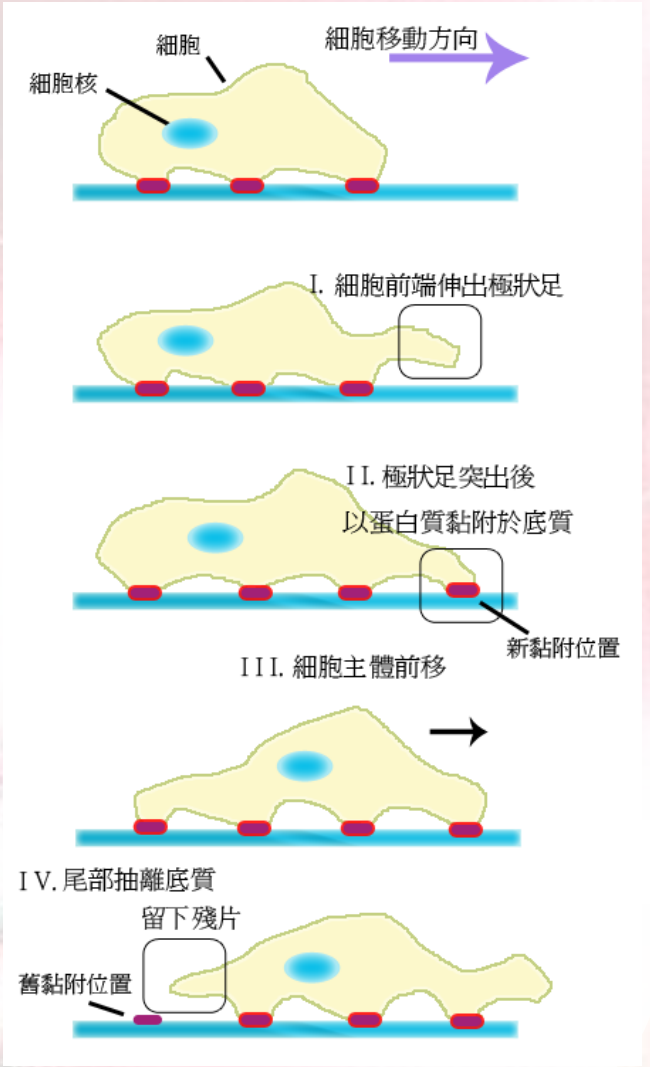
1. Change the arrangement of cytoskeleton with the help of myosin

   ⇨ morphology change

2. Interaction with ECM

   ⇨ movement





Micro-engineered, three-dimensional substrates

- Method I : **Photolithography**

- Using UV light to expose a mask containing the desired patterns onto photosensitive resists.
- Transfer biomolecules of interest by etching or lift-off onto the resist patterns .

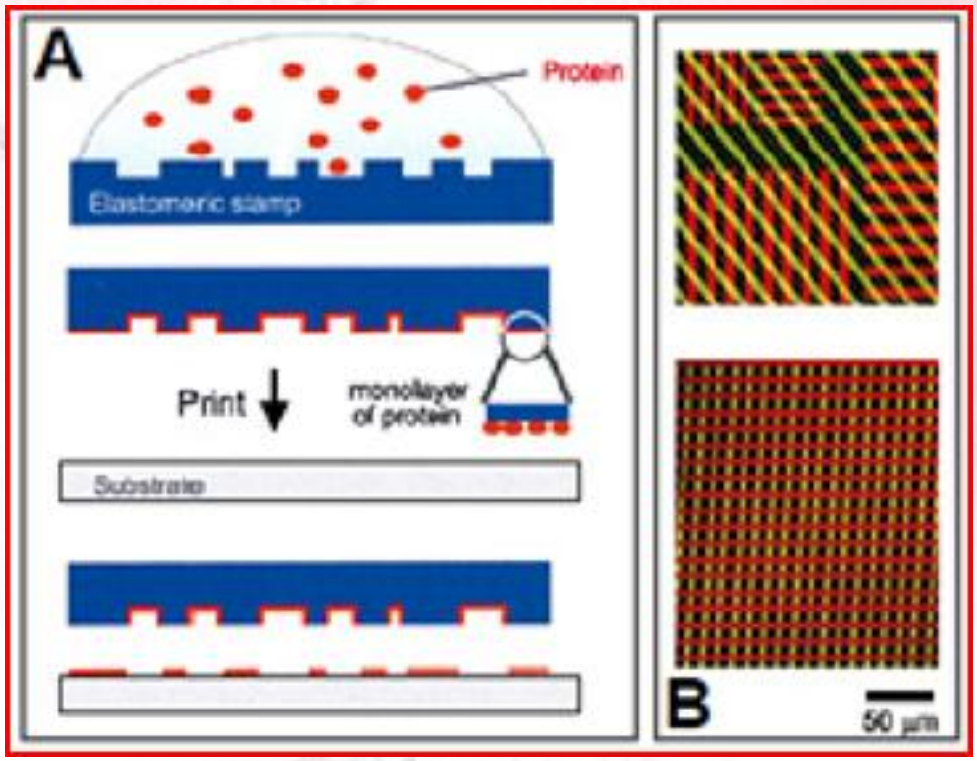
- Pros:

  pattern quality and patterns with high resolution

- Cons:

1. requires cleanroom facilities
2. have to be modified for each new substrate

➢ Method II : **Microcontact printing (µCP)**



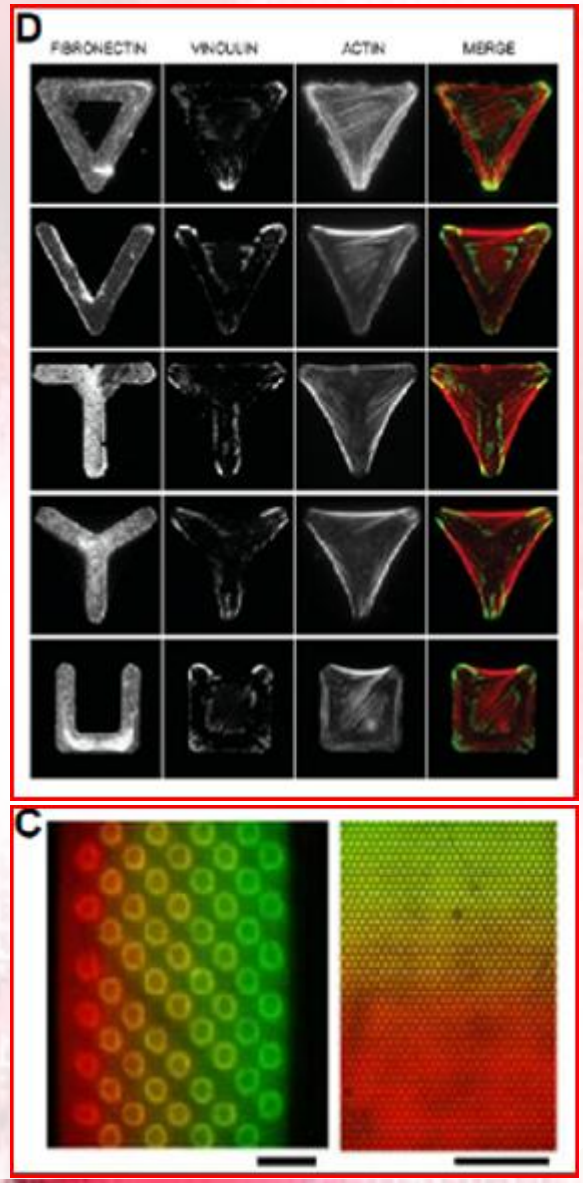
- Microstructured stamp (fabricated in PDMS by molding).
- Coating stamp with molecule of interest by dipping in solution.
- Put the stamp in contact with the substrate to allow transfer.
- Nonprinted adjacent surface can be made passive with another molecule to prevent cell spreading beyond the printed areas.

Microcontact printing (µCP)

- Pros :
- Easy and convenient
- Can be used ~100 times over a period of several months without noticeable degradation of the quality of the printing
- Can achieve resolution below 500 nm

- Cons:
- Typically is limited to patterns containing only one or two types of molecules.

- Applications :
- axon guidance
- cell culture on defined geometry



Applications

Tremendous utility in almost all areas of single cell biology

➢Examples:

- stem cell differentiation
- neuronal regeneration
- cancer metastasis

Cell-based sensor

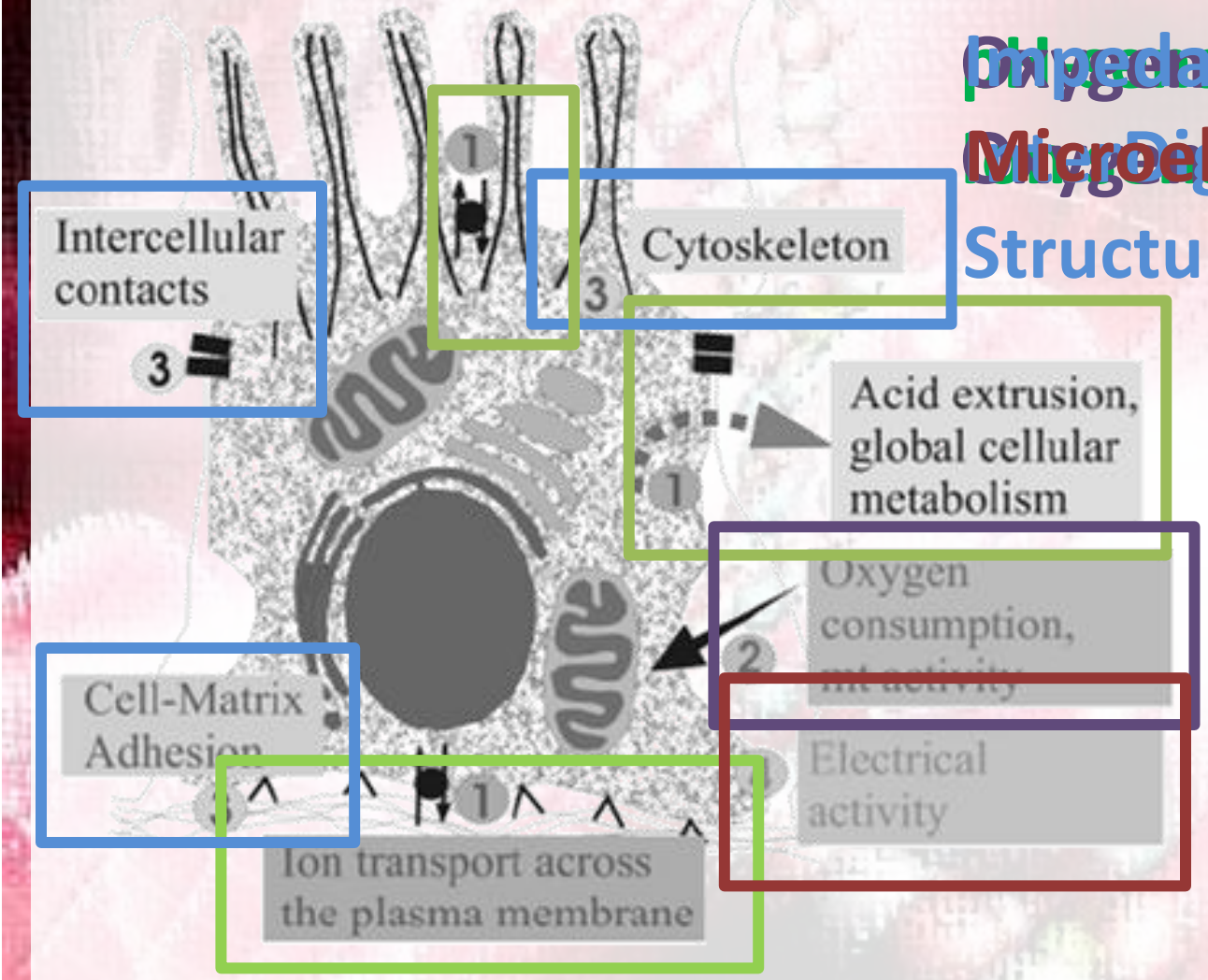
- Ways
  1. In real time and non-invasively.
  2. Growing cells directly on the surface sensor chips
  3. Materials which are non-toxic and accepted by most cells as substrate
- Metrics
  1. Potentiometric (V)
  2. Amperometric (I)
  3. Impedimetric (R)
- Usages
  1. Pharmaceutical drug discovery
  2. Individualized clinical testing
  3. Whole-cell-based biosensors.

Traditional ways to analysis of living cells - Optical screening with dyes

- Toxic or phototoxic properties
- Less suitable for long term monitoring when many dyes applied
- No appropriate dyes for time-resolved studies
  - cell adhesion
  - cell metabolic activity
  - electric activity

  (which is the domain of sensor based functional monitoring of cells)

Parameters accessible to microsensor



Impedance sensor:

Microelectrode Array

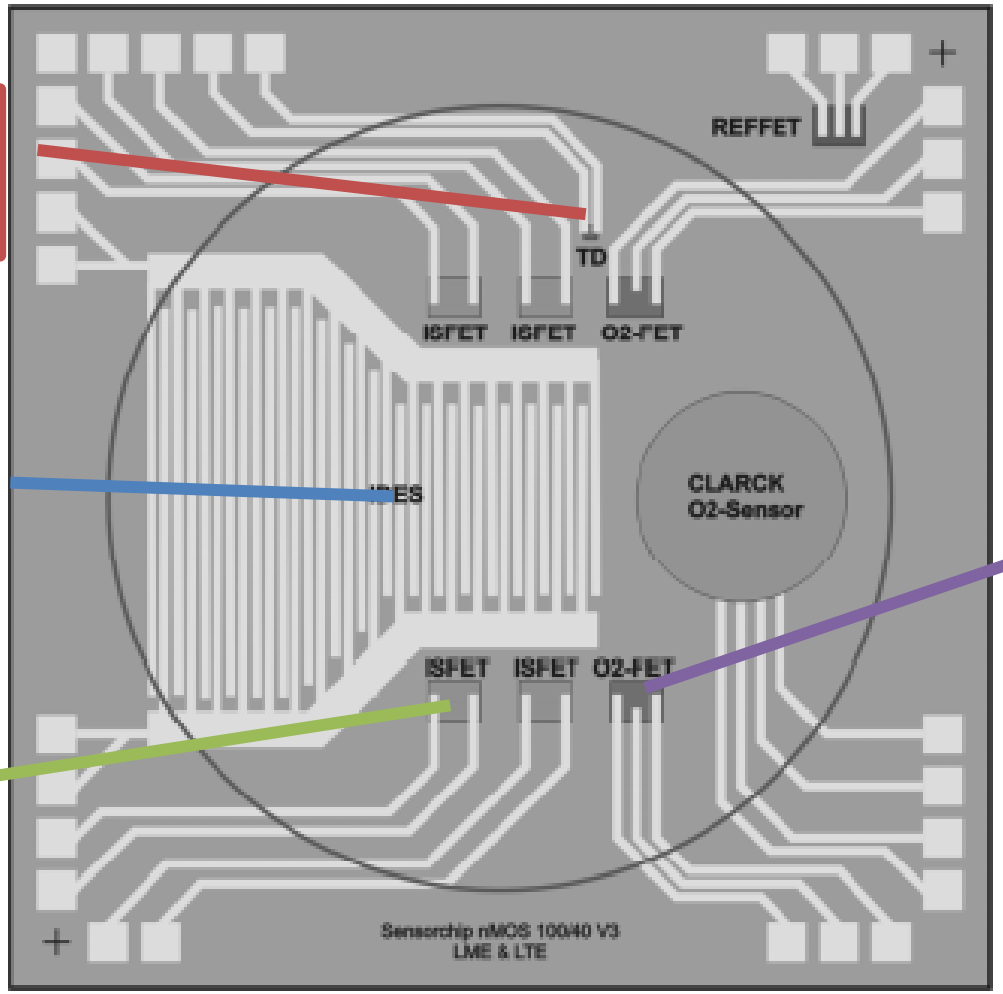
Structure (IDES)

chip with different types of sensors

Temperature

Adhesion & Morphology

pH (Ion)



Oxygen

Figure 3: Silicon chip layout

Sensors Directed Adhesion & Morphology

- changes in cell adhesion or cell morphology result in changes in impedance.

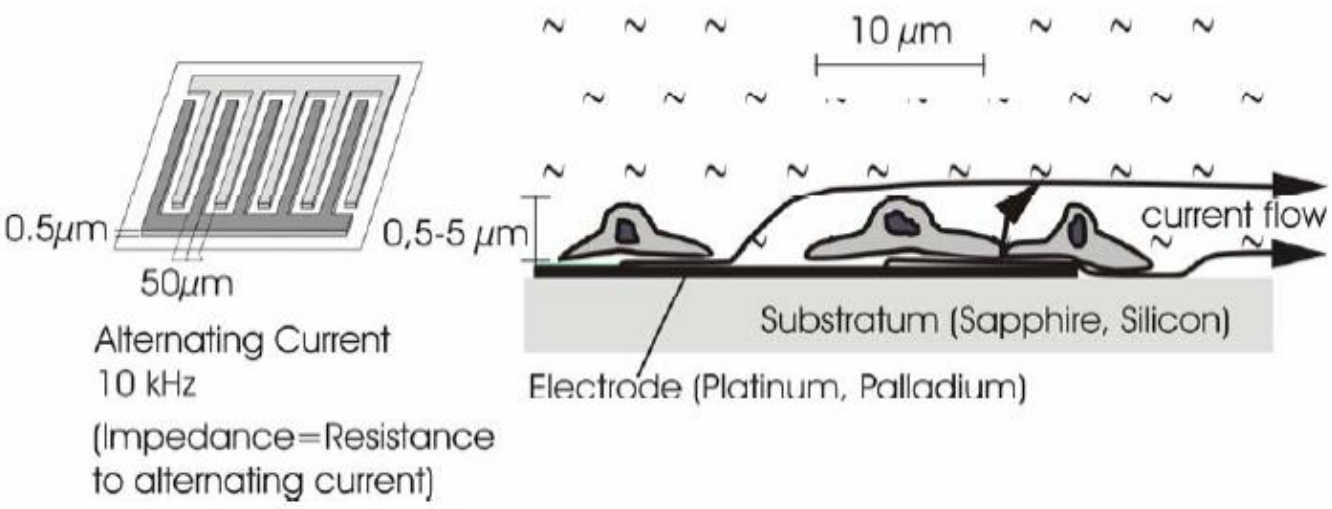


Figure 11: Geometry of IDES and IDES-based impedance measurement on cell cultures

Challenges for Single-cell Analyses on LOC

- The small absolute amount and low concentrations of the cellular species of interest

  →hard to detection.
- Purification or separation strategies prior to analysis

  →loss or dilution
- the mixed hydrophobic–hydrophilic nature of biological macromolecules

  →adsorption to surfaces
- living cells tend to be perturbed by manipulation

  →stringent requirements in performing experiment

Summary

- Cell on chip provides spatial and temporal control of cell growth and stimuli
- As a result, It provides biologists with unprecedented opportunities for cell handling and investigation on a single cell basis
- Electronic microstructures on sensor chips can analyze cellular responses by recording properties of cell metabolism and morphology

Reference

- “Cells on chips,” Jamil El-Ali , Peter K. Sorg and Klavs F. Jensen , *NATURE*, Vol. 442,2006
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- “Analysis of single mammalian cells on-chip,” C. E. Sims and N. L. Allbritton, Lab Chip, 2007, 7, 423–440.