The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific
and Cultural Organization

International Atomic
Energy Agency

World Year of PHYSICS 2005

SMR.1670 - 34

INTRODUCTION TO MICROFLUIDICS

8 - 26 August 2005

Cell Culturing on Chip and Monitoring of Cells

R. Luttge
University of Twente, Enschede, The Netherlands

Topics in this lecture

From cells in culture and single cells in geometric confinement
When approaching for reactor vessels the length scale of single cells care must be taken in the understanding of the interactions between cells and the reactor walls. New phenomena are studied in this context.

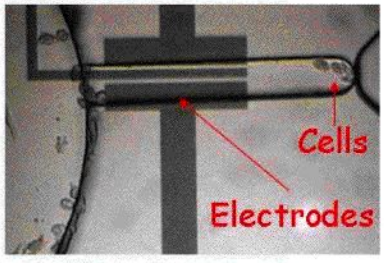
Biological cell culture experiments
Microchip technology brings also new methods to the biotechnological laboratory. These methods have to be evaluated against classical established biological experiments by statistic design of experiment.

9. Cell culturing on chip and monitoring of cells

- Introduction
- Strategic developments of cell culturing and cell analysis on chip
- Tackling integration
- Chip-based cellular systems to retrieve single-cell information
- Outlook: Future developments
- Summary

Topics in this section

Cells in microfluidics
Microfluidic-assisted cell capture and recording is one of the most powerful strategies of modern life-sciences.



Culture on chip
We will discuss the integration of cell culture plates and biotechnological processes. The devices may assist in understanding complex pathways by systems biology.

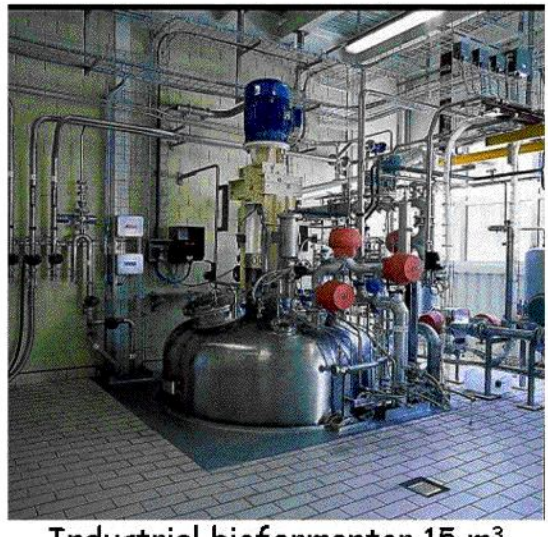
Introduction

- Bioreactor fermentation processes
- Important topic in drug development: How to achieve high-throughput screening (HTS)?
- Most new assays are designed to rapidly detect specific cellular effects reflecting action at various targets.
- Creating better artificial cell environments to build testing devices.

Spatial confinement of cells

- Microfabrication dimensions on the scale of cell dimension.
- Integration must mean something more to cellular investigation than patch clamp can do today.
- Single cell versus clusters of cells and tissue- influencing cell behavior. Isolation of cells as a new strategy?
- HTS assays using monolayer or suspension cultures still reflect a highly artificial cellular environment and may thus have limited predictive value for the clinical efficacy of a compound.

Large scale biofermentor



Industrial biofermentor 15 m^3

Bench-scale biofermentors

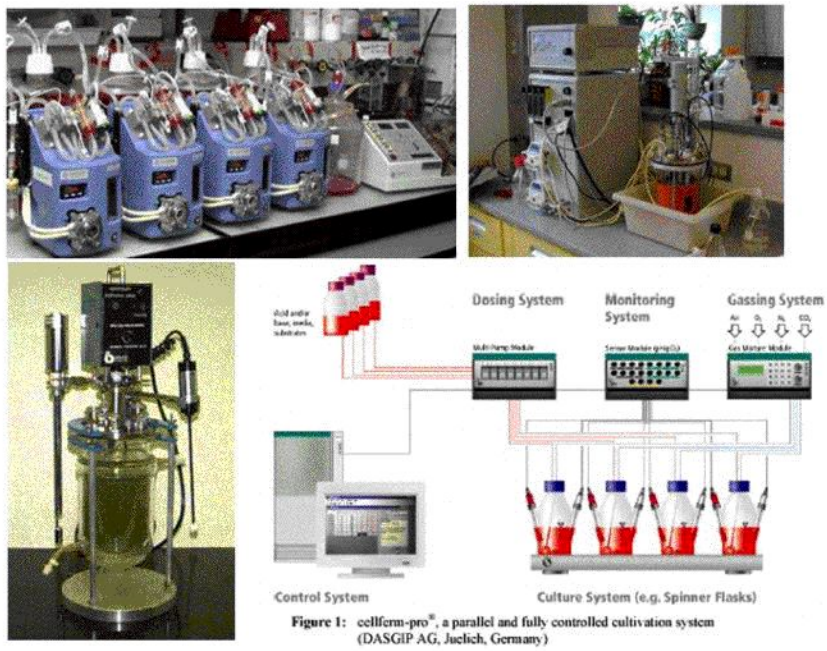


Figure 1: cellferm-pro®, a parallel and fully controlled cultivation system (DASGIP AG, Juelich, Germany)

www.spacebiol.ethz.ch/ research/bioreac_im.htm

Spactial restrictions

- *Space in space* ! (Small apparatus footprint required).
- Mix of conventional fabrication techniques and microtechnology, e.g., sensors for flow control.

Miniaturized hollow-fiber bioreactor

- Mammalian cell culture systems
  - Acordis Research, Germany

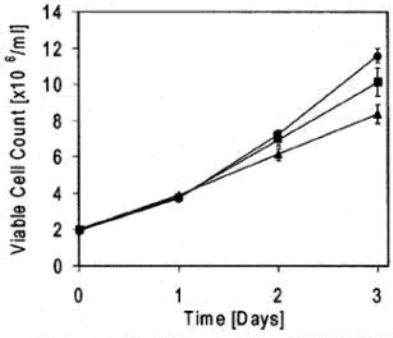
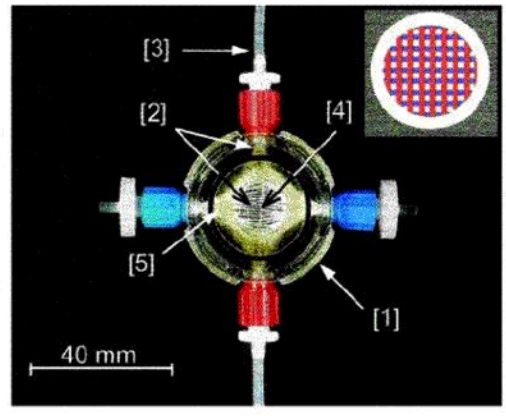


Figure 2. Growth of the leukemic cell lines CCRF-CEM, HL-60, and REH in hollow fiber bioreactors during 3 days of culture, starting with 2 × 10^6/mL (mean of $n = 3$ experiments ± SE).



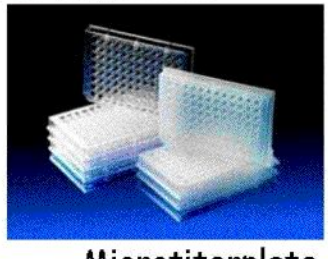
Gloeckner & Lemke, Biotechnol. Progress. 17 (2001), p. 828-831

Screening of micro-organisms

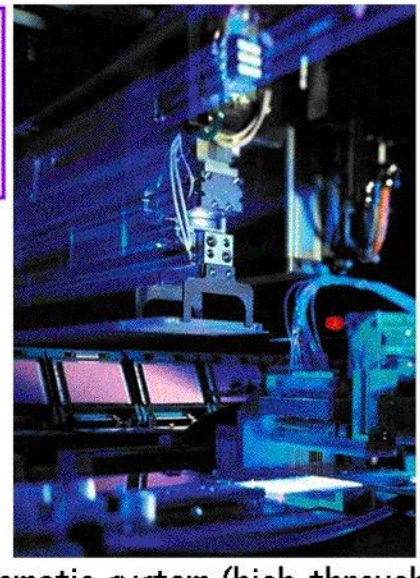
Smaller volumes!
Better handling!

Need for higher functionality!

Petri dish



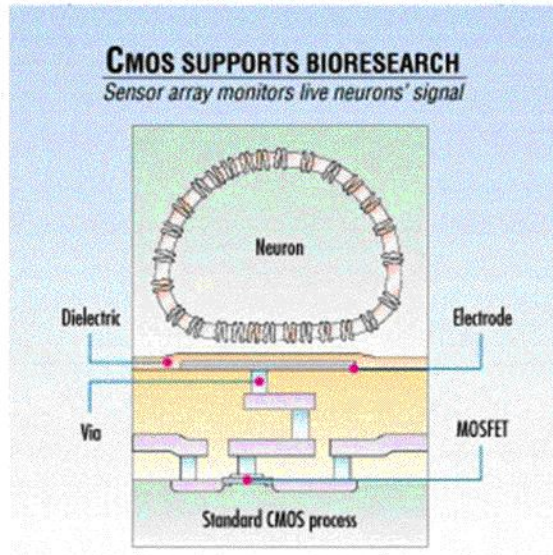
Microtiterplate



Automatic system (high-throughput)

Interfacing bioelectronic "cell reactor"

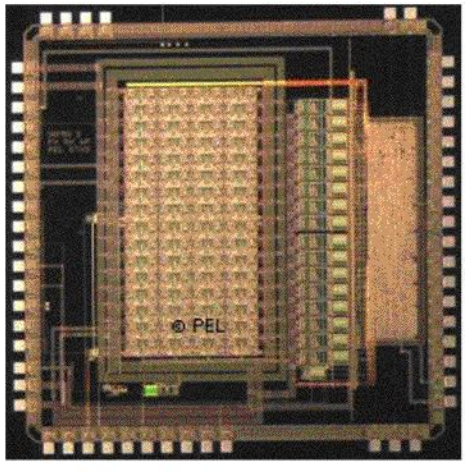
- Solid-state-based sensors for integration with medical diagnostic and analytical devices providing significant data relating to the bio-chemical and biophysical reactions, e.g., of therapeutic drugs.

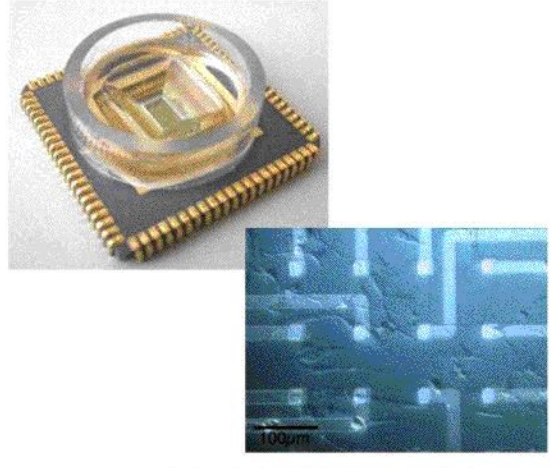


Source: Infineon

Neuro-Chip

- Neuronal networks, such as those found in the human brain, are flexible and adaptive. They are able to build complex networks and are able to process data very efficiently in a parallel manner. Bringing this functionality to an artificial environment?

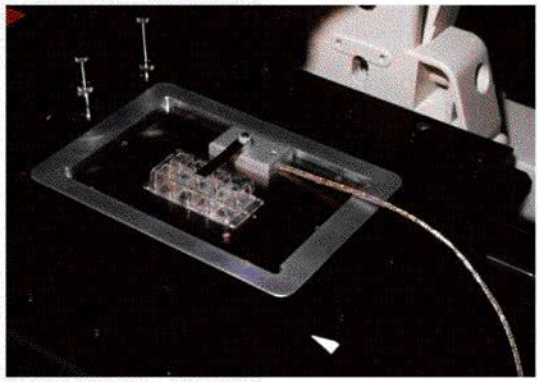




www.iqe.ethz.ch/pel/ highlights/neurochip.html

Strategic development

- Important research groups and their approaches.



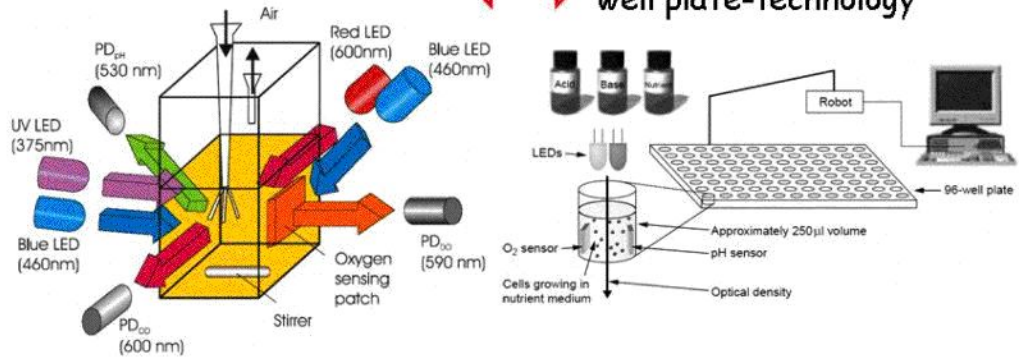
www.cyto-purdue.edu

9.2. Strategic developments

Group of G. Rao (University Maryland)

- Optidotes for pH, DO, CO_2
- Spin-off: Fluorometrix *www.fluorometrix.com*

Cuvette-based microbioreactor ⟺ Bioprocess monitoring using well plate-technology

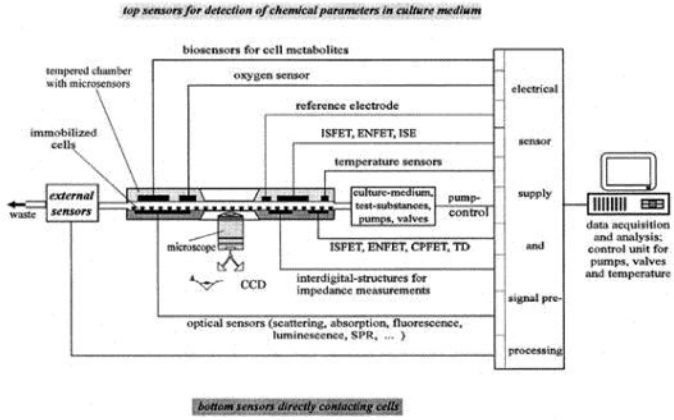


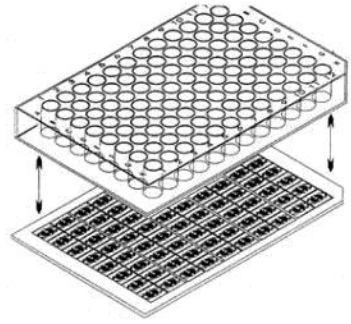
Harms et al.,Current Opinions in Biotechnology (2002), 13, p. 124-127
Kostov et al./ Biotechnology and Bioengineering (2001), 72, p. 346-352

Group of B. Wolf (Universität Rostock)

- Microwell format and flow-cell
- Single cell measurements
- Integrated sensors
- Multiwell plate, volume: 10 µl

Cell Monitoring System (CMS®)



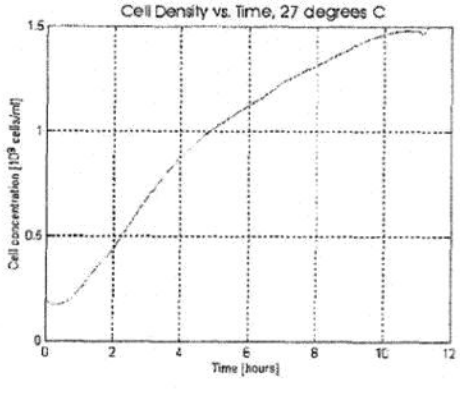


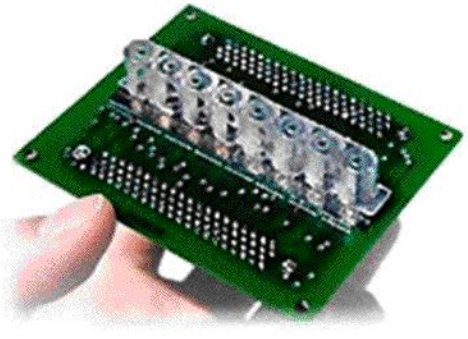
Baumann et al./ Sensors and Actuators B 55 (1999) 77–89
Lehmann et al./ Biosensors & Bioelectronics 15 (2000) 117–124

Group of Keasling (Berkeley)

- 8 well platform
- Sensing: Temp, OD
- Spin-off: Microreactor.com
- Biomass volume: 250 µl
- Electrochemical O_2 supply (generation rate: 10 µmol $O_2.h^{-1}$)

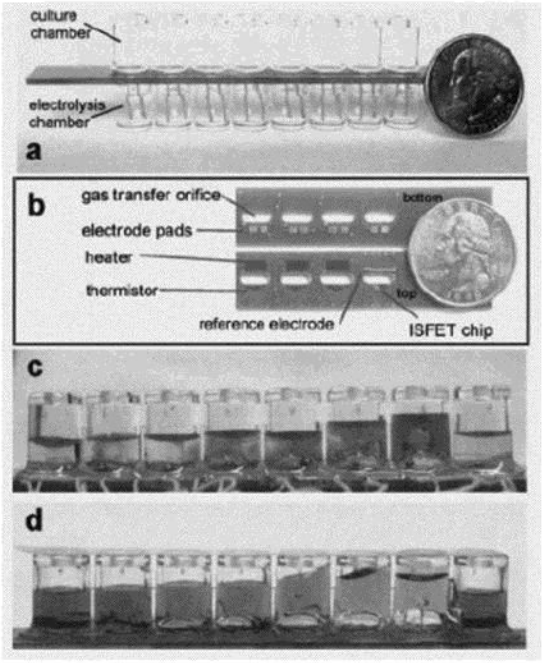
Silicon microbial bioreactor arrays





Maharbiz et al., in Proc. IEEE-EMBS conf. (2000), p. 165-170

μ-bioreactor array

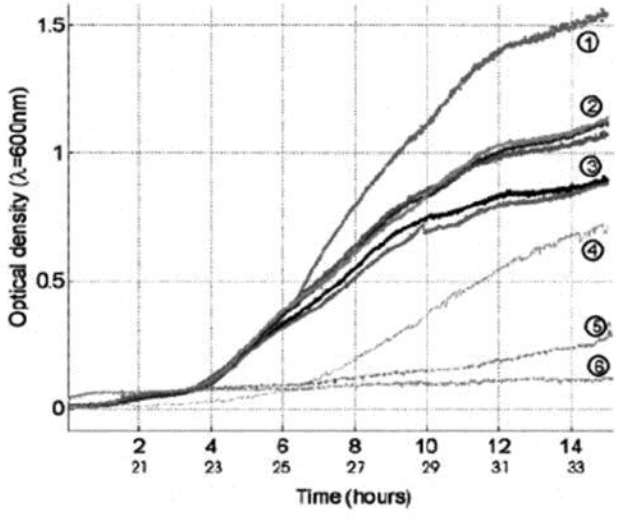


- PCB* technology
- Off-the-shelf components (modular assembly)
- Commercial Ion-selective Field Effect Transistor (ISFET) for pH
- E-coli growth
- Integrated gas dosing

* Printed Circuit Board

Maharbiz et al., Biotechnology and Bioengineering, vol. 85, no.4, 2004

Cell growth as a function of oxygen



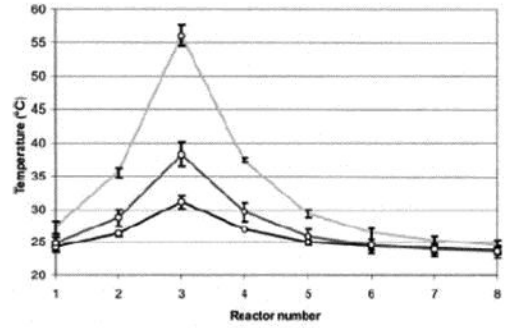
Oxygen supplied:
(1) 10 mmol O_2/h,
(2) 6 mmol O_2/h,
(3) 3 mmol O_2/h,
(4) 0 mmol O_2/h,
(5) 6 mmol O_2/h (lower time axis),
(6) 0 mmol O_2/h.

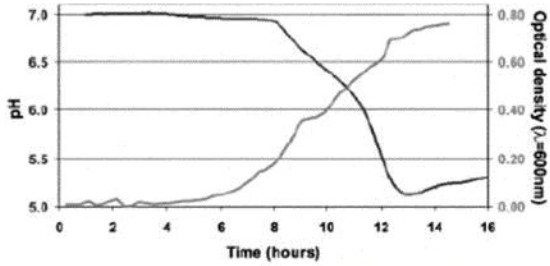
Optical density measurements were taken every 30 sec during fermentations.

Multiple curves are shown for curves (2) and (3) for reproducibility. 1 - 4 ("LB medium"). 5 - 6 ("M9 minimal medium" with 1% glycerol (lower time axis).

Maharbiz et al. / Biotechnology and Bioengineering, vol. 85, no.4, 2004

Temperature and pH control in fermentation

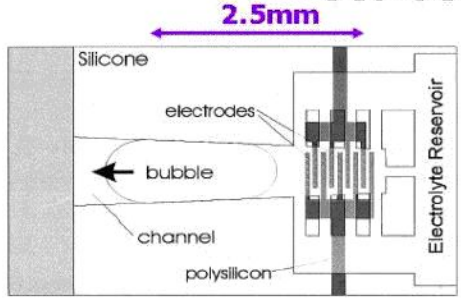


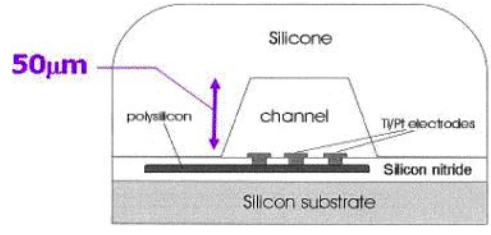


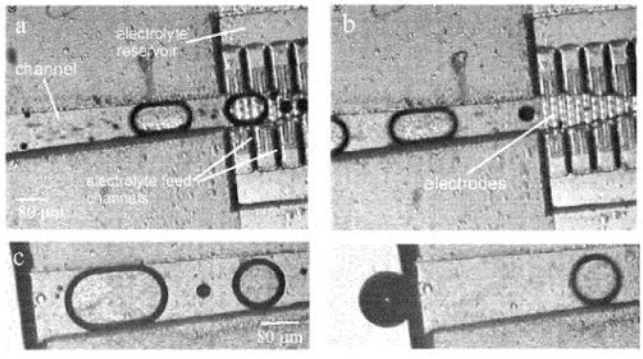
- Closed-loop buried resistor heater/thermistor at well 3 (gradient across the array).
  Error bars:
  T-variation/h,
  max. variation: < ±2°C.
- ISFET (Sentron Europe , E2310000, 0.5x1.5 mm) reference electrode assembled into the eight wells.
- pH monitored over 16-h of E. coli. fermentation in LB medium (1% glucose-enriched). OD_{600} from on-board optical system.

Maharbiz et al. / Biotechnology and Bioengineering, vol. 85, no.4, 2004

Electrochemical oxygen generator







- Biomass volume: 200 μl
- Electrochemical O_2 supply (rate: >10 μmol $O_2.h^{-1}$)
- Corrosion robustness investigated

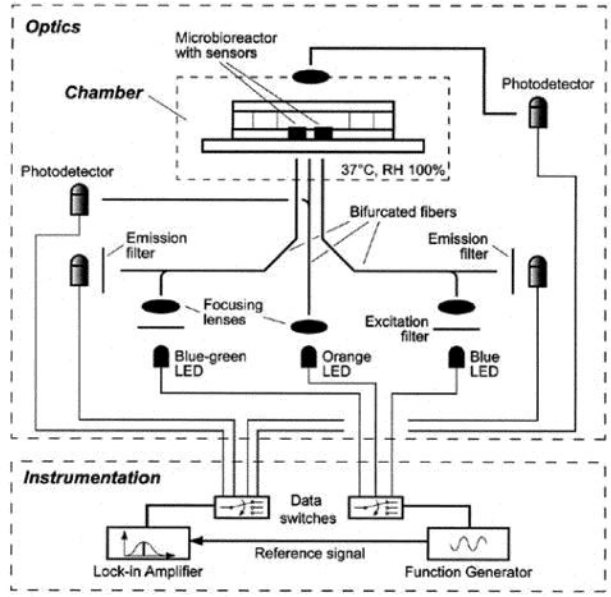
*Maharbiz et al. / J. Microel. Sys., (2003), **vol. 12, no. 5**, p. 590-599*

Group of K.F. Jensen, (MIT)

- Microscale biofermentor

Volume:
batch: 5 μl
fed-batch: 85 μl

Measurement:
pH, DO, OD



Zanzotto et al./ Biotechnology and Bioengineering, vol. 87, NO. 2, 2004

HT-Continuous perfusion culture array

Luke P. Lee's group (California)

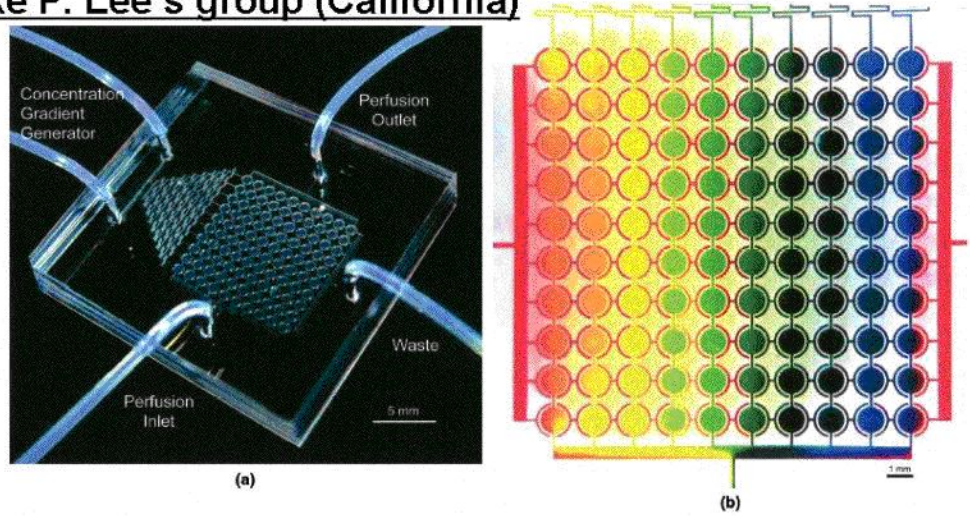


Figure 1. Microfluidic cell culture array for high-throughput cell-based assays. **a:** Photograph of the microfluidic cell culture array. A 10 × 10 array of microchambers was fabricated on a 2 × 2 cm device. The port at the left provided continuous perfusion of medium uniformly across the array. The port at the right was the outlet for the medium. Reagents and cells were loaded from the top and flow out through the bottom port. **b:** Concentration gradient across 10 columns. A concentration gradient generator was connected to the 10 columns at the top of the device. Red dye was initially perfused from left to right to fill all of the chambers. Blue and yellow dye was then loaded from the two separate ports at the top of the gradient generator, demonstrating the capability of conducting cell-based assays with multiple concentrations of reagents.

P.J. Hung et al., BIOTECHNOLOGY AND BIOENGINEERING, VOL. 89, NO. 1, JANUARY 5, 2005

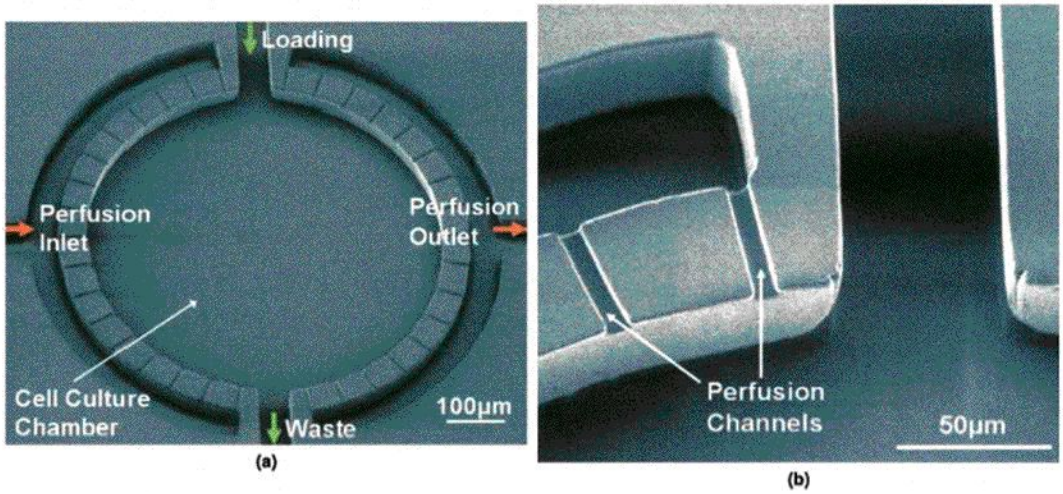
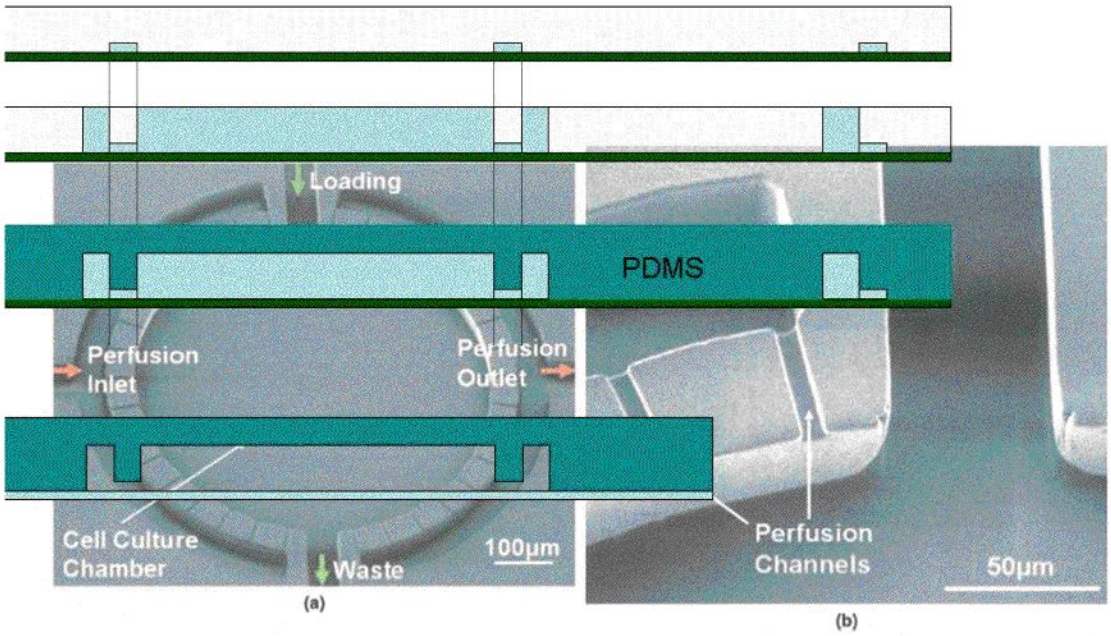


Figure 2. Single microfluidic culture unit. **a:** SEM picture of a single unit of the arrayed device before bonding to a coverglass. Multiple perfusion channels surround the main culture chamber. The microchamber was 40 µm in height with a diameter of 1 mm. Each culture unit had four fluidic access paths (left, right, top, and bottom) for "perfusion inlet," "perfusion outlet," "loading," and "waste," respectively. **b:** SEM image of perfusion channel dimensions. Each perfusion channel had a width of 5 µm and height of 2 µm, compared to the loading channel which had a width of 50 µm and height of 40 µm.

P.J. Hung et al., BIOTECHNOLOGY AND BIOENGINEERING, VOL. 89, NO. 1, JANUARY 5, 2005



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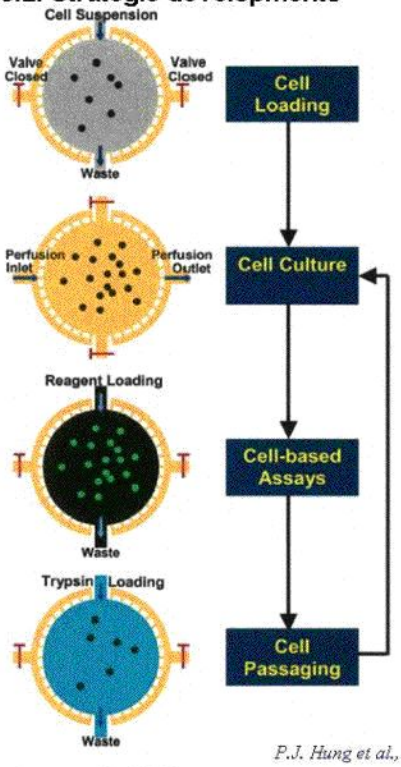
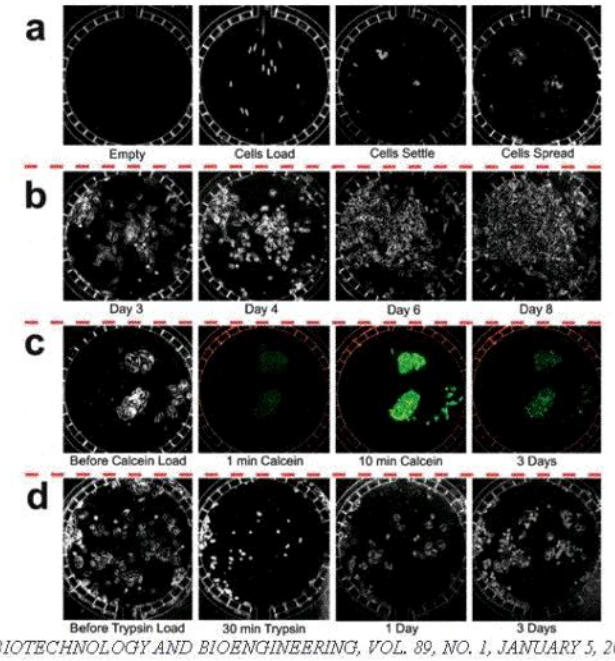
P.J. Hung et al., BIOTECHNOLOGY AND BIOENGINEERING, VOL. 89, NO. 1, JANUARY 5, 2005

Friday, August 19, 2005 Regina Luttge



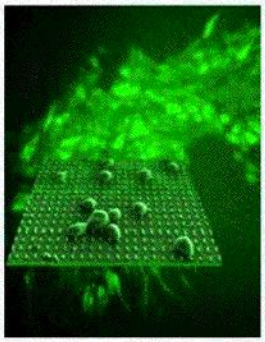
Operation principle & first results



P.J. Hung et al., BIOTECHNOLOGY AND BIOENGINEERING, VOL. 89, NO. 1, JANUARY 5, 2005

MESA+

Tackling integration for cell-based biosystems

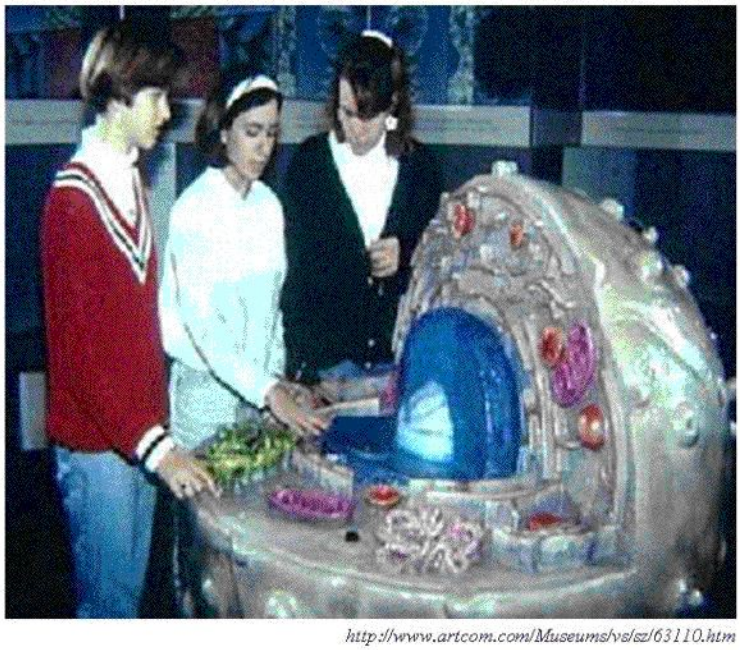


www.genomenewsnetwork.org

- Containing and detecting cells
  - Cell lysis for analytics (slides from Thursday session on sample preparation methods)
  - Cell reactors
- Following and carrying out reactions within the cell-chip environment.
- Stationary and flow-through microbioreactors

MESA+

Talking cell

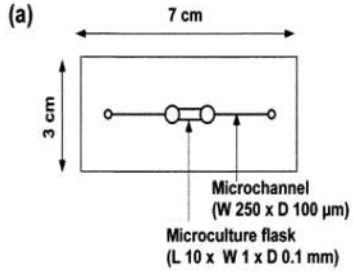


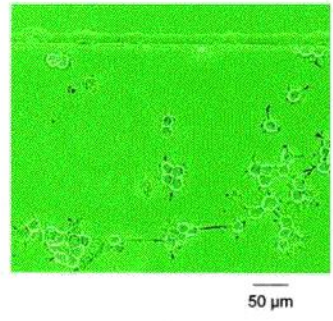
- Driving force: We want to know!

http://www.artcom.com/Museums/vs/sz/63110.htm

9.3. Tackling integration for cell-based biosystems

Cell cultivation microflask

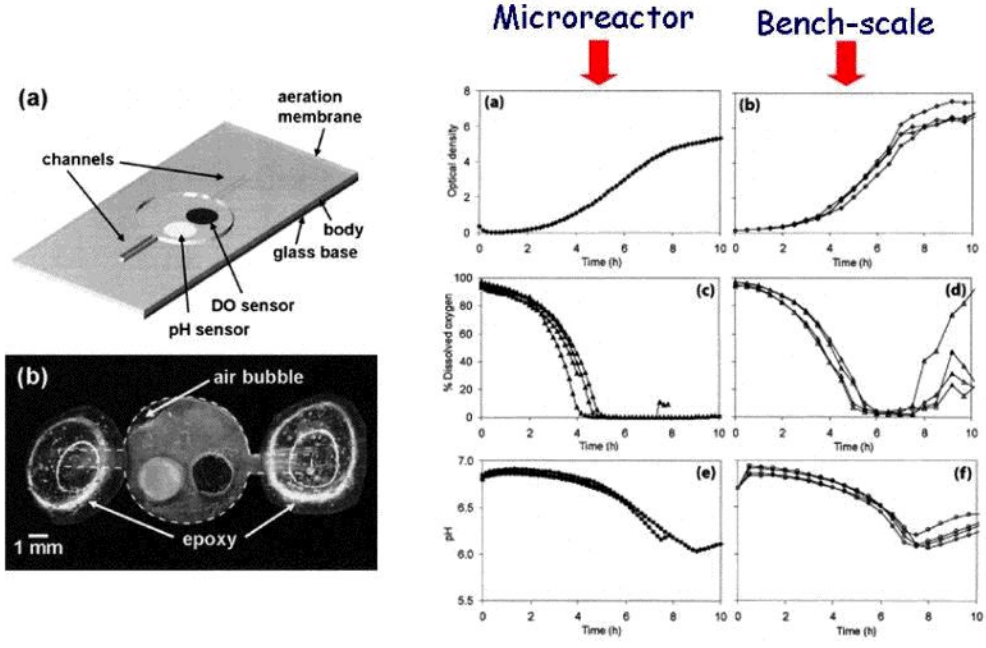




- Glass chip
- Good liquid control
- Secure cell stimulation
- Optical monitoring

E. Tamaki et. al, (2002) Analytical Chemistry, 74, 1560-1564.

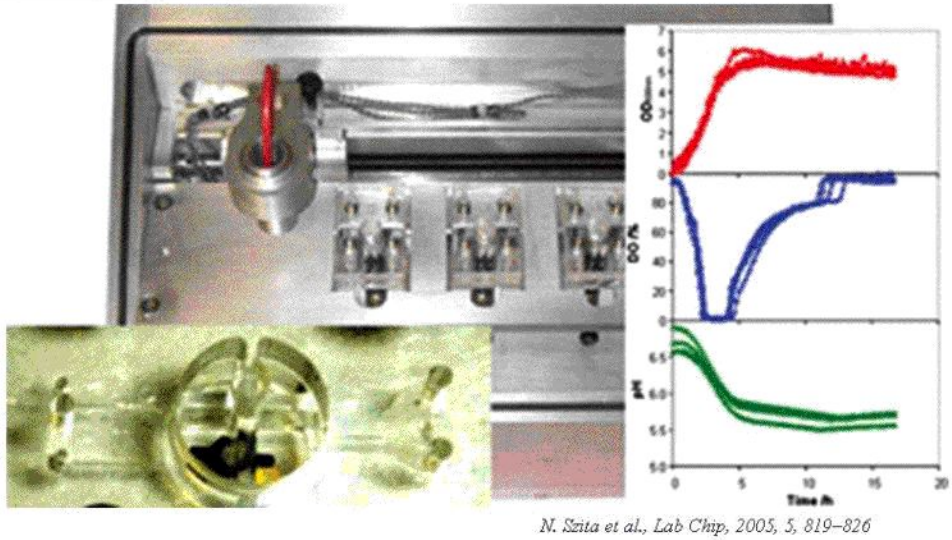
Bench-scale versus planar microreactor



Zanzotto et al./ Biotechnology and Bioengineering, vol. 87, no.. 2, 2004

Multiplexed microbioreactor system for high-throughput bioprocessing

Jensen's group



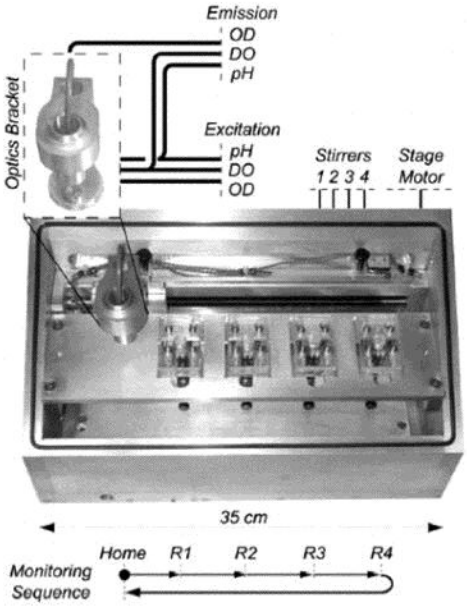
N. Szita et al., Lab Chip, 2005, 5, 819–826

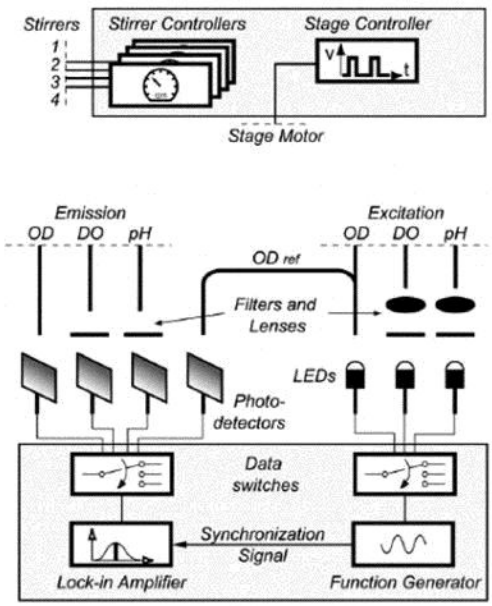
System components

- .....thus to demonstrate that high-throughput fermentation data can be obtained in real time from microbioreactors.
- Four stirred microbioreactors with a working volume of 150 μl and with integrated sensors for on-line monitoring of the fermentation growth parameters were used.
- The system includes miniaturized motors for magnetic stirring of the reactors, and optics for measuring the fermentation parameters.
- Optical density is determined with a transmittance measurement through the reactor chamber,
- and in-situ measurements of dissolved oxygen and pH are obtained with fluorescence lifetime sensors embedded in the bottom of the reactor chambers.

N. Szita et al., Lab Chip, 2005, 5, 819–826

Microbioreactors made out of PMMA and PDMS.





N. Szita et al., Lab Chip, 2005, 5, 819–826

Chip set-up

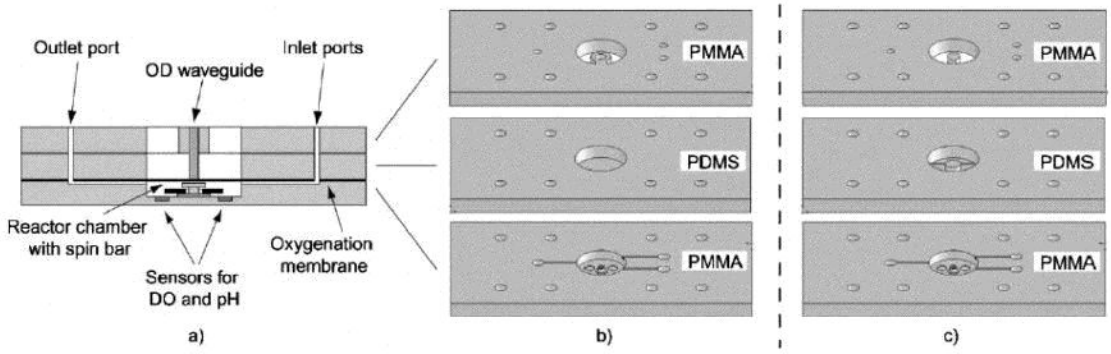
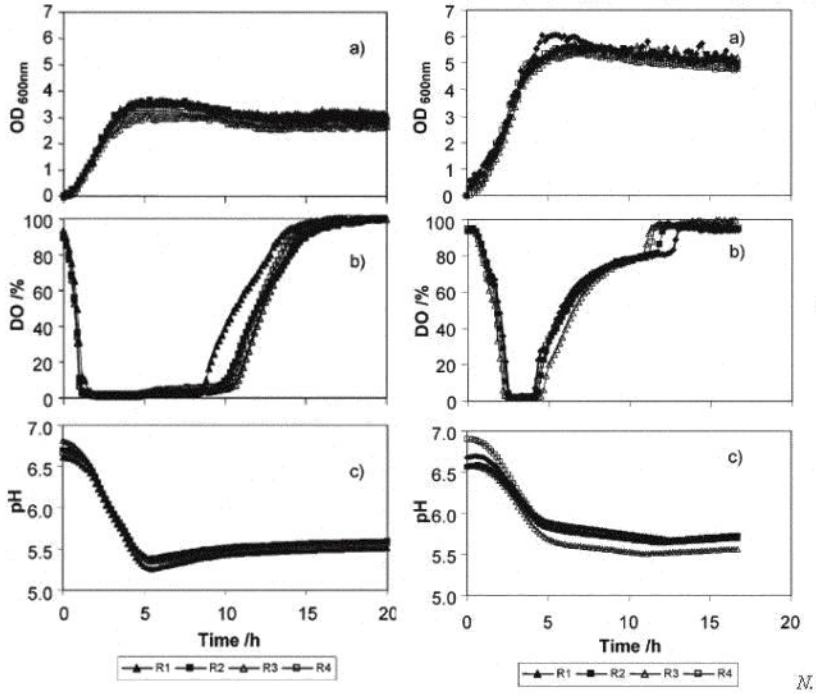


Fig. 2 (a) Schematic of longitudinal section of the microbioreactor with a PMMA fiber as OD waveguide. The cell culture in the microbioreactor is stirred with a magnetic spin bar, which rotates around a vertical post in the center of the reactor chamber. A cap at the top of the post and a shoulder at the bottom holds the spin bar at the desired height within the chamber. Oxygenation takes place through a thin PDMS membrane, indicated in the schematic by a thick line. (b) Solid models of the four layers for the microbioreactor design using a PMMA waveguide. The two bonded PDMS layers are shown as one layer. (c) Solid models of the three layers of the microbioreactor with the PDMS post as OD waveguide. (Solid models drawn to scale)

N. Szita et al., Lab Chip, 2005, 5, 819–826

9.3. Tackling integration for cell-based biosystems

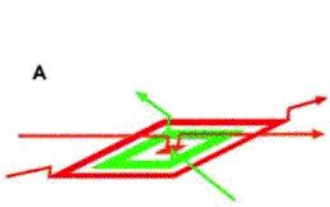
Parallel E. coli fermentations

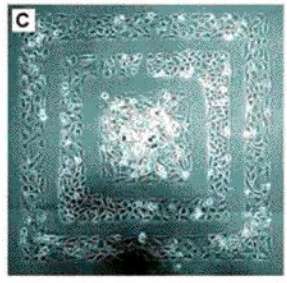


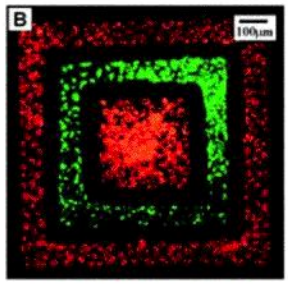
equipped with a PDMS post (left) and equipped with PMMA waveguide (right), both cases real-time measurements of:
(a) OD_{600},
(b) DO, and
(c) pH.

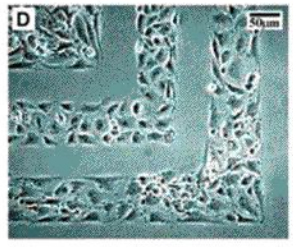
N. Szita et al., Lab Chip, 2005, 5, 819–826

Cell patterning using microfluidics







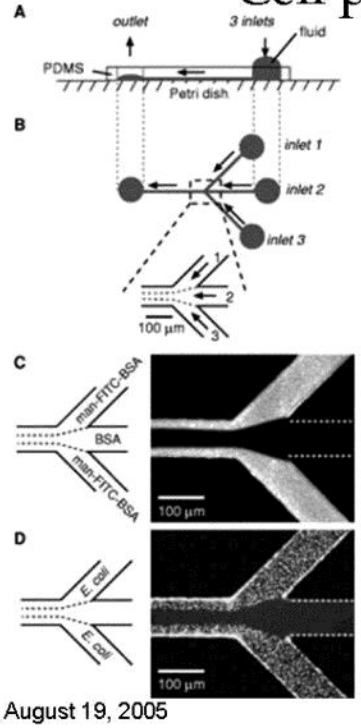


- Channel structures formed by a PDMS stamp when it is in contact with the surface of the substrate limits the migration and growth of the cells.
- Cells cultivated 24 h
- Stamp passivated by BSA to prevent cell growth.

J. Chiu et al., (2000) Proc. Natl. Acad. Sci., 97, 2408

MESA+

Cell patterning using microfluidics



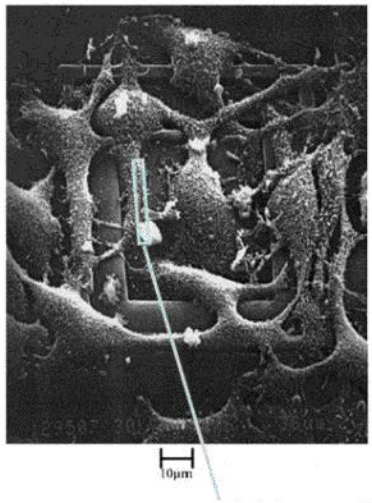
- Usage of laminar flow of multiple parallel liquid streams in microchannels to pattern cells:

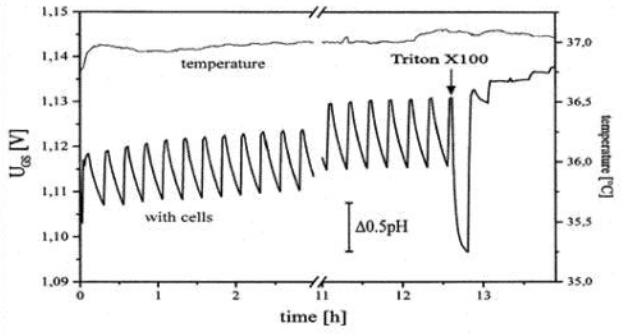
  1) Pattern with adhesion promotors and inhibitors
  2) Deliver cells
  3) Localize chemicals (nutrients, drugs etc.)

Takayama, et al., (1999) Proc. Natl. Acad. Sci., 96, 5545

MESA+

Cells cultured on integrated ISFET



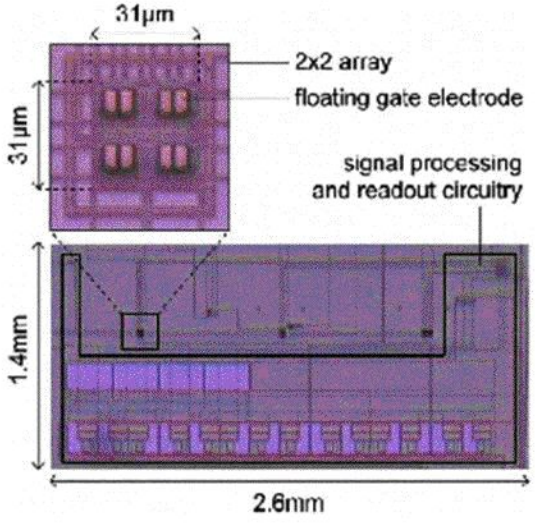


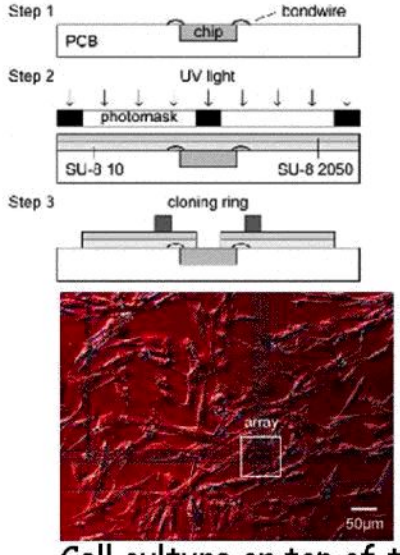
Acid production versus time, during pump cycles (5 min on, 10 min off)

HeLa cells on ISFET; gate area = 20 x 2 µm²

W.H. Baumann e.a., Sens. Act. B 55, 1999, 77-89

Cell culturing on potentiometric sensor chip





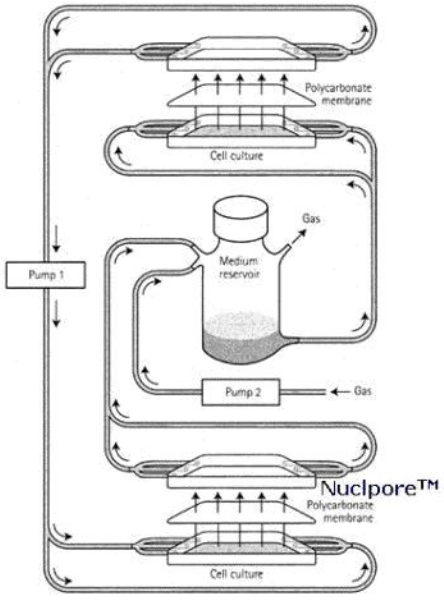
Cell culture on top of the electrode array

M.J. Milgrew et al., Sens. & Act. B, March, 2004, in press

Case study I:
"Intermediate small"

9.3. Tackling integration for cell-based biosystems

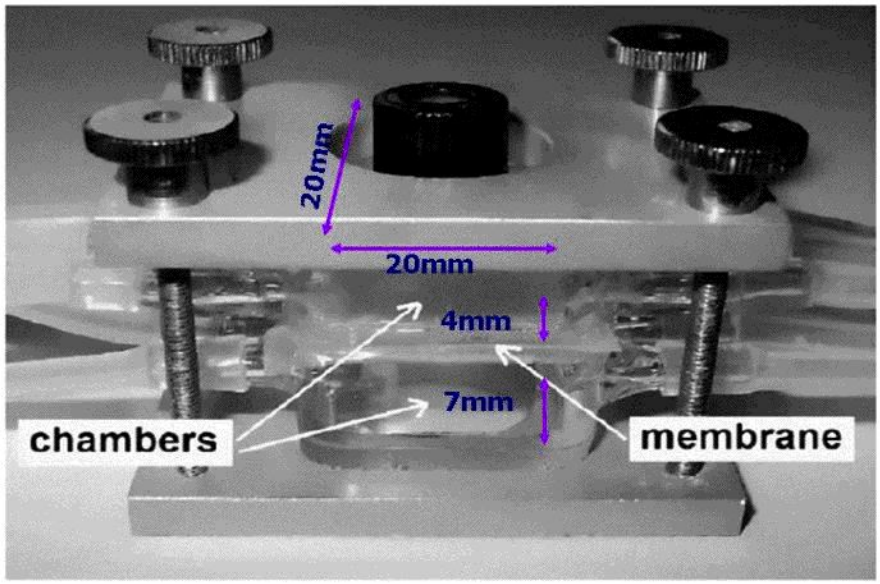
Bioreactor for three-dimensional cell (co)-culture



- Simple lab-scale minaturized dynamic reactor design.

A. Lichtenberg et al., Biomaterials 26 (2005) 555–562

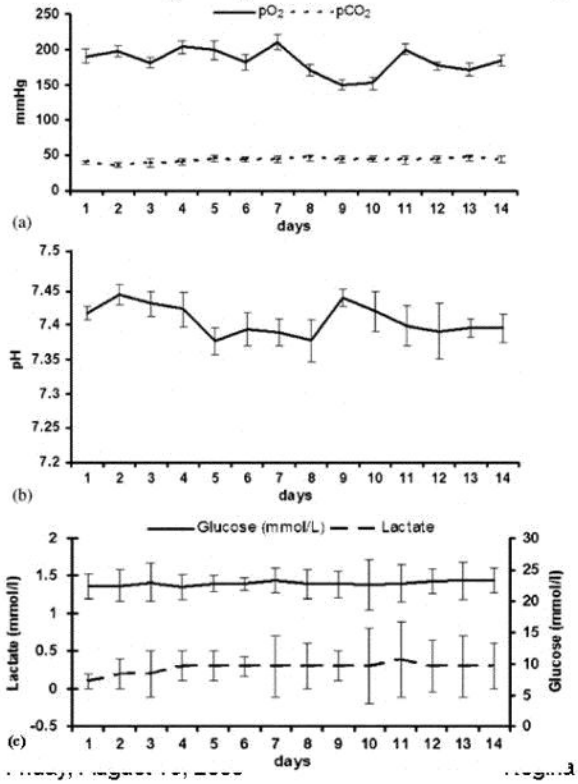
Culture chamber



A. Lichtenberg et al., Biomaterials 26 (2005) 555–562

MESA+

Metabolic parameters



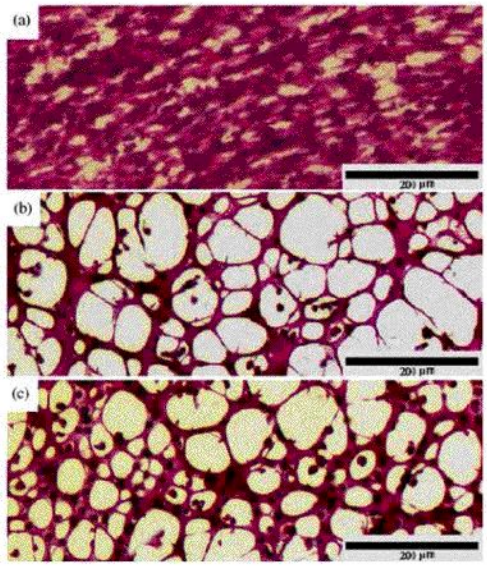
(a) Medium pO_2 and pCO_2
(b) Medium pH
(c) Release of lactate and glucose concentration in medium

➡ Physiological stable!
➡ Low lactate release!

A. Lichtenberg et al., Biomaterials 26 (2005) 555–562

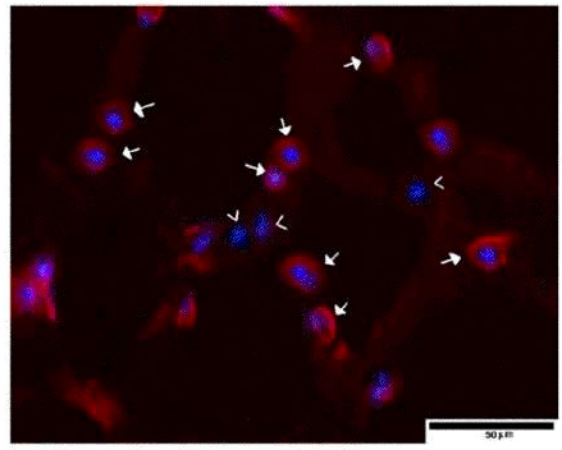
MESA+

Morphology



(a) Native neonatal ventricular cardiac rat tissue;

(b) fibrin matrix with seeding of $1x10^7$ cells/2ml total matrix;

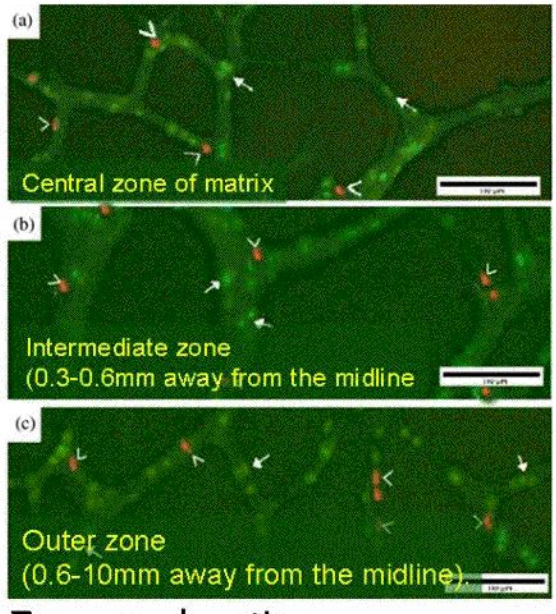


MF20 cardiomyocytes (blue dye) and non-cardiomyocytes (marked with "<)"

(fibrin matrix with seeding of 2x107 cells/2ml total matrix.

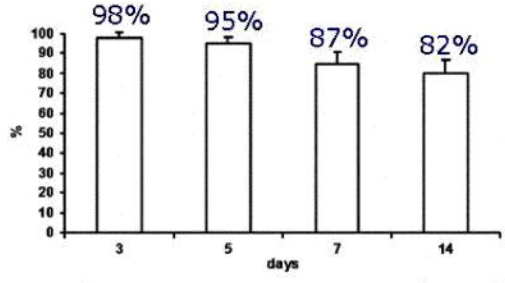
A. Lichtenberg et al., Biomaterials 26 (2005) 555–562

Immunostaining of cells



Transversal sections:
viable cells (green)
dead cells (red)

- LIVE/DEAD® viability/ cytotoxicity assay*
  (* Molecular Probe Inc.)



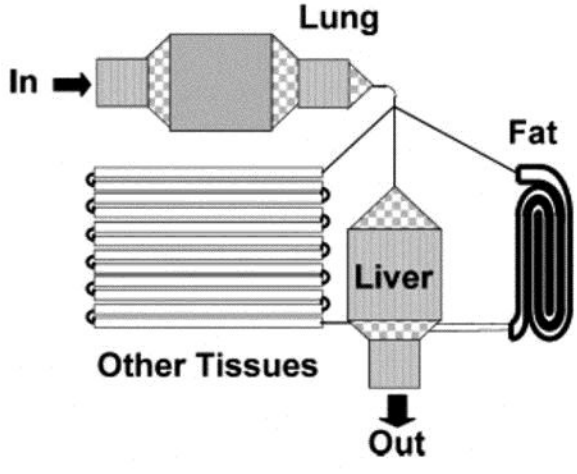
The mean percentage value of the live cells (day 0) and after 3, 7, 14 days of culture.

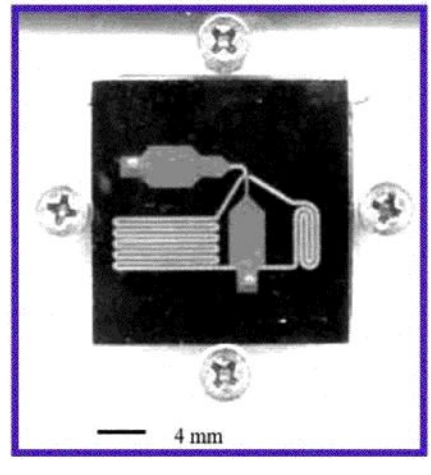
A. Lichtenberg et al. / Biomaterials 26 (2005) 555–562

Case study II:
A "complete" biosystem on chip

Microscale device for toxicity studies

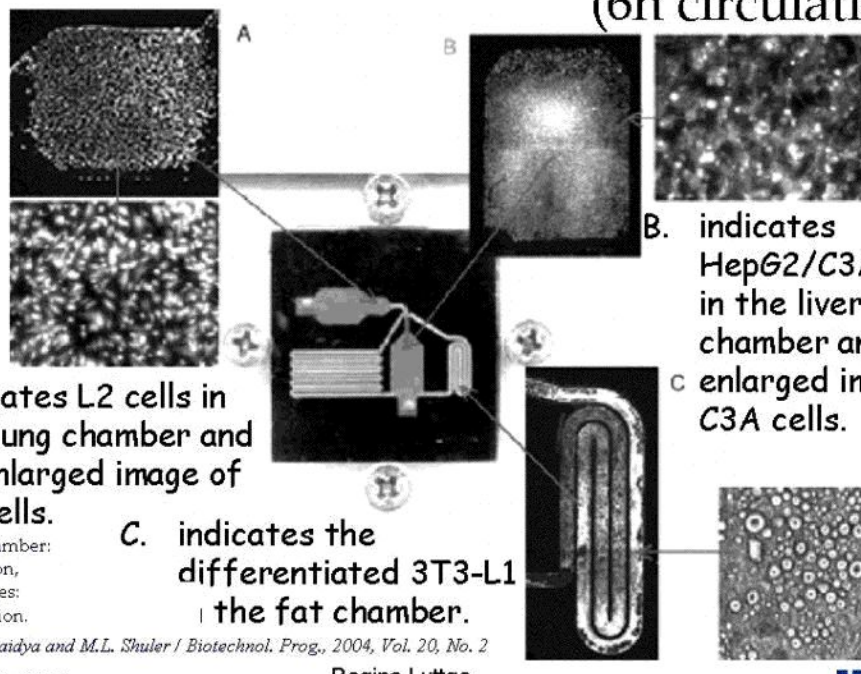
- Incorporation of 3T3-L1 cells to mimic bio-accumulation in a Cell Culture Analog device.





K.Viravaidya and M.L. Shuler / Biotechnol. Prog., 2004, Vol. 20, No. 2

Stained cells on the μ-CCA chip (6h circulation)



A. indicates L2 cells in the lung chamber and an enlarged image of L2 cells.

B. indicates HepG2/C3A in the liver chamber and enlarged image of C3A cells.

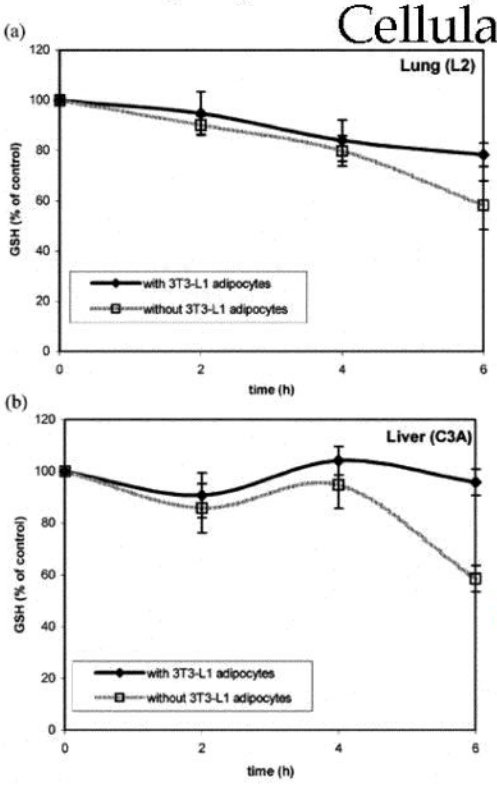
C. indicates the differentiated 3T3-L1 in the fat chamber.

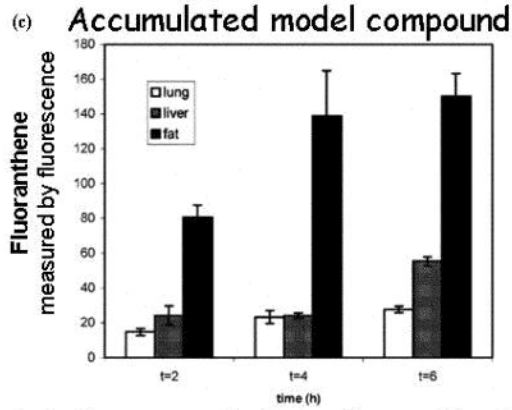
Cells in the chamber: 4x magnification, Enlarged images: 20x magnification.

K.Viravaidya and M.L. Shuler / Biotechnol. Prog., 2004, Vol. 20, No. 2

9.3. Tackling integration for cell-based biosystems

Cellular defense mechanism and bioaccumulation





Naphthalene metabolites formed in liver deplete the glutathione (GSH) in lung

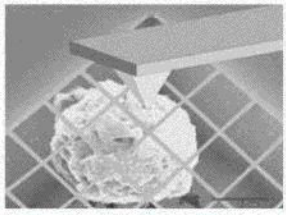
➡ toxicity test for:

(a) L2 cells, and

(b) HepG2/C3A.

K.Viravaidya and M.L. Shuler / Biotechnol. Prog., 2004, Vol. 20, No. 2

Chip-based cellular systems to retrieve single-cell information



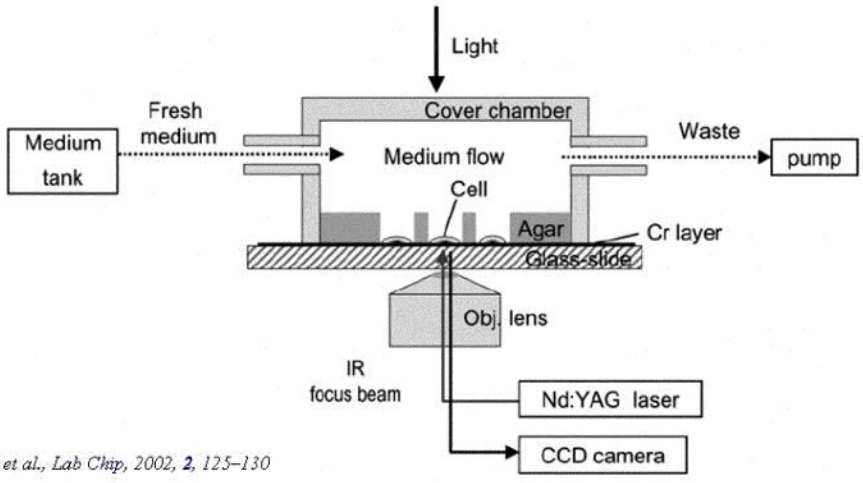
AFM-tip, cell manipulation
IOM, Leipzig, Germany

- Cell confinement in experimental set-up
- Recording from single cells
  - Optical analysis
  - Electrical analysis

Case study III: "Relaxed micro-patterning" using combined rapid replication & laser direct write methods

Agar-microchamber cell-cultivation system

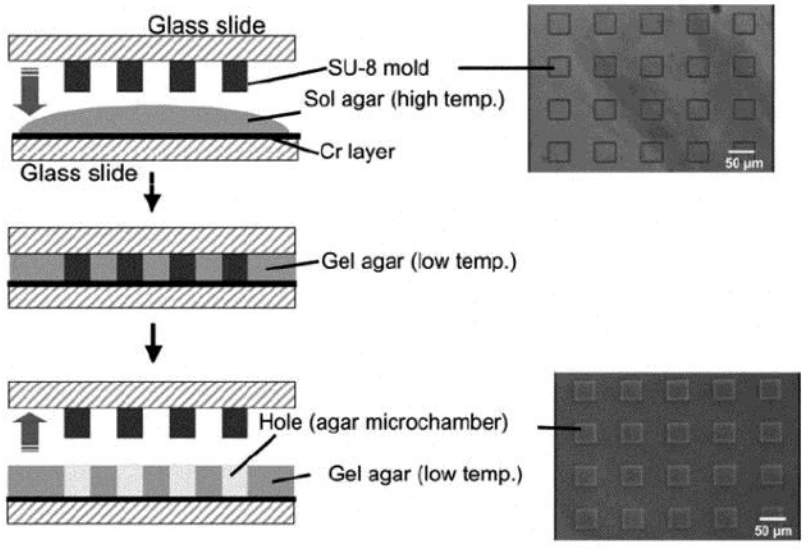
- flexible change of microchamber shapes during cultivation by photo-thermal etching



*Moriguchi et al., Lab Chip, 2002, **2**, 125–130*

9.4. Chip-based cellular systems to retrieve single-cell information

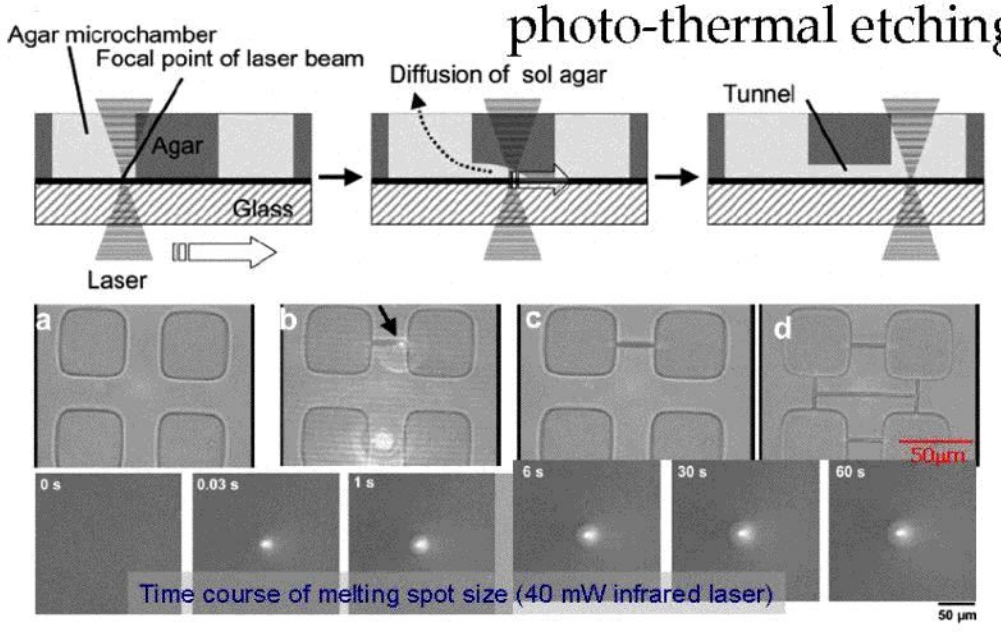
Fabrication of agar cultivation chambers



*Moriguchi et al., Lab Chip, 2002, **2**, 125–130*

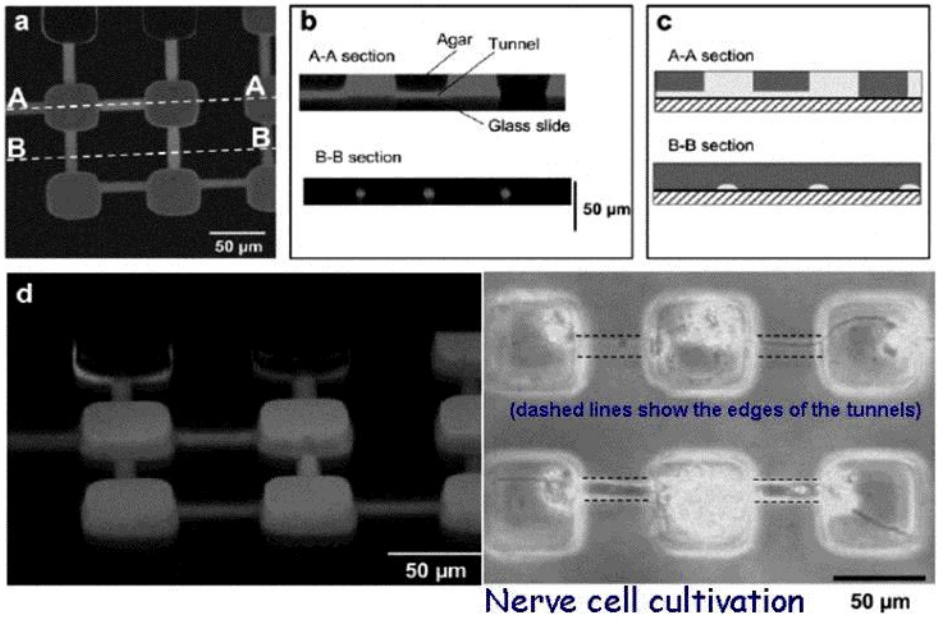
Friday, August 19, 2005 Regina Luttge

Conecting chambers by photo-thermal etching



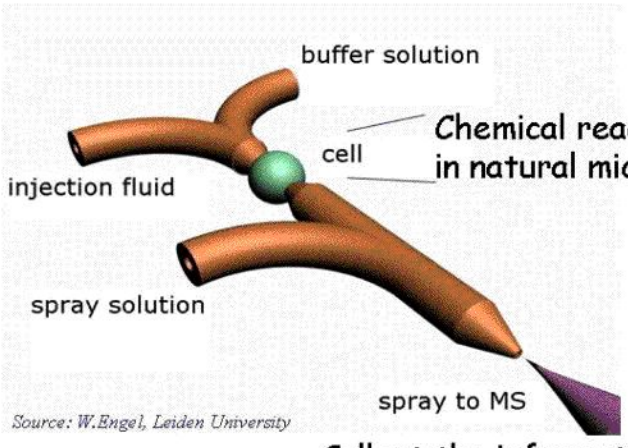
*Moriguchi et al., Lab Chip, 2002, **2**, 125–130*

Agar microchamber array

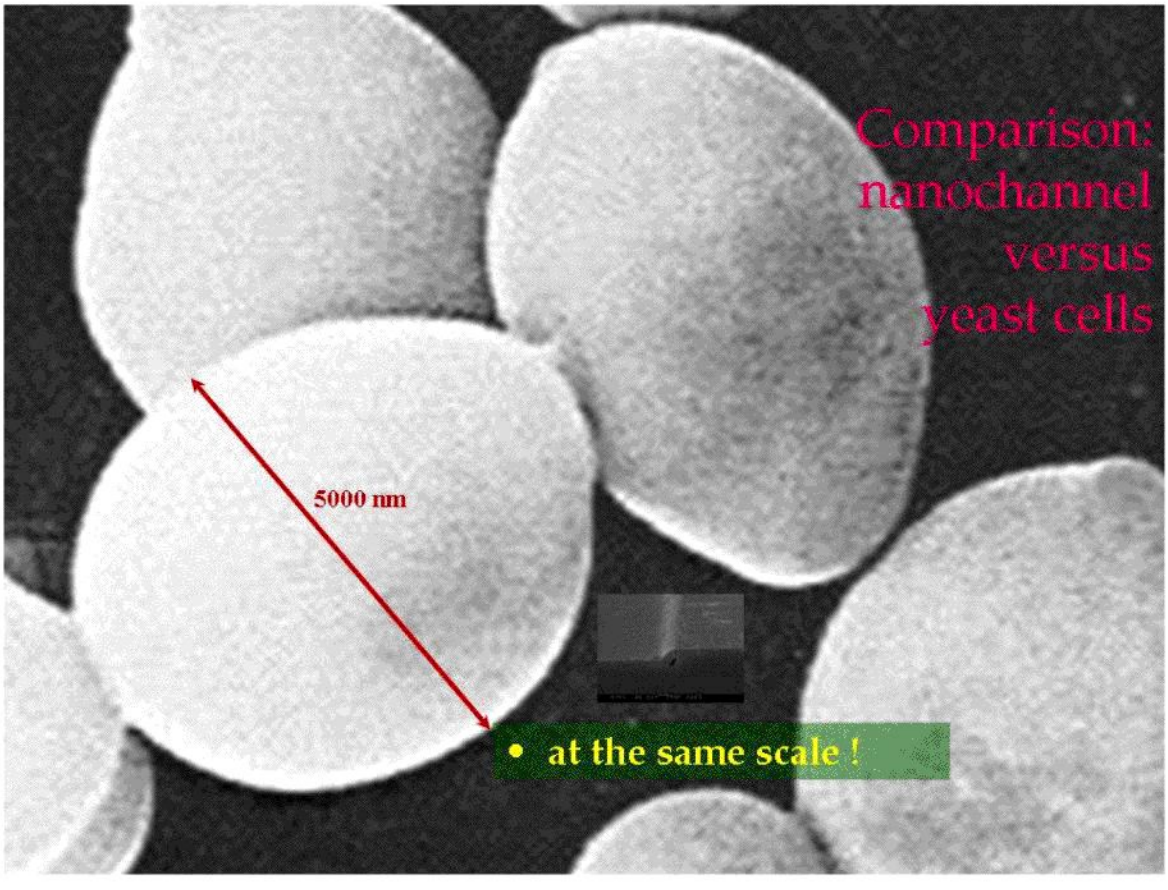


*Moriguchi et al., Lab Chip, 2002, **2**, 125–130*

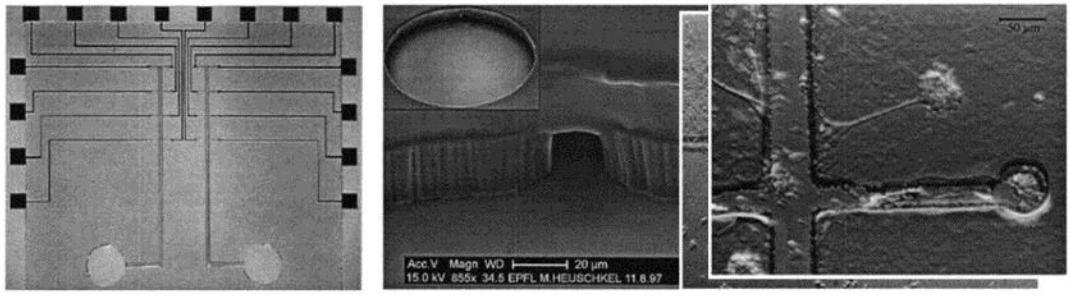
Laboratory-in-a-cell



Collect the information by applying established analytical chemsitry (e.g., mass spectrometry)



Cell analysis system with electrodes and buried channels

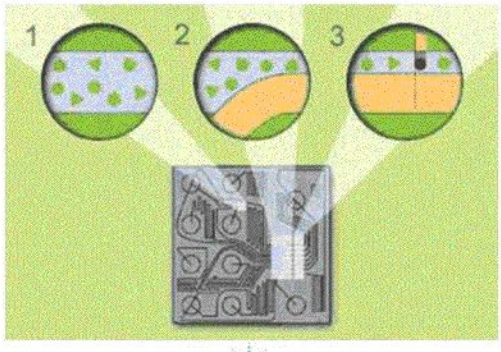


For culture, stimulation, and recording of neural cell arrays

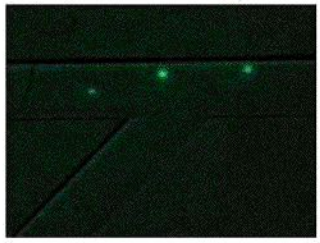
- Allows for continuous monitoring of electrical activity while delivering very locally different conditions to the cells
- Indium-tin electrodes
- Channels are sealed with multi-layer resists (Riston and SU-8)

M.L. Heuschkel et al., (1998), Sensors and actuators B, 48, 356-361

Hydrodynamic focusing of cells



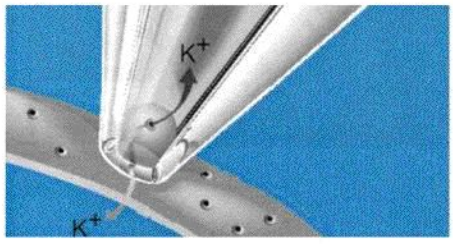
Agilent Technologies

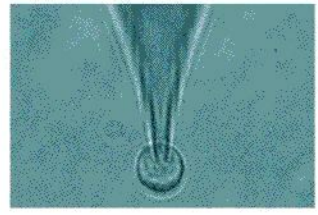


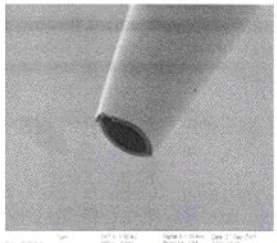
Principle of the analysis of cell parameters (cell assays). The micro-channels of the chip are filled with cell buffer.

1. Pressure driven flow is used to move cells in a controlled manner through the micro-channels of the chip.
2. Cells are hydrodynamically focused to a portion of the channel by a side stream of buffer.
3. Cells pass the fluorescence detector in single file.

Ion channel analysis



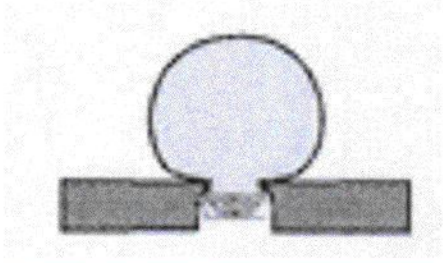


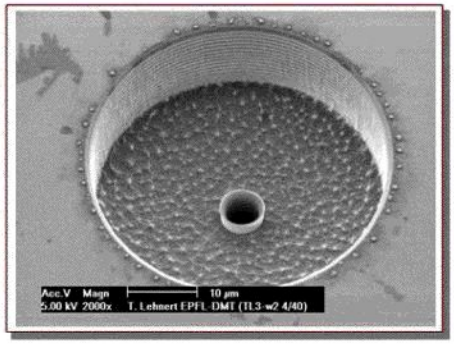


- Conventional patch clamp nozzle and cell attached.

T. Lehnert, et al., (2002), Appl. Phys. Lett.,(81)24

Planar ion channel analysis nozzel

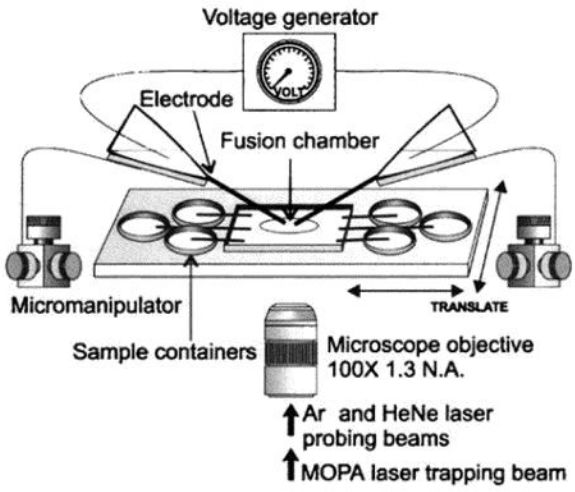




- Planar Si-based patch clamp nozzle.
- Chip placed between two glass slides with microfluidic channels.
- Nozzle ID=2.5 µm.
- Megaohm seal resistances obtained.

T. Lehnert, et al., (2002), Appl. Phys. Lett.,(81)24

Liposome and cell fusion chip

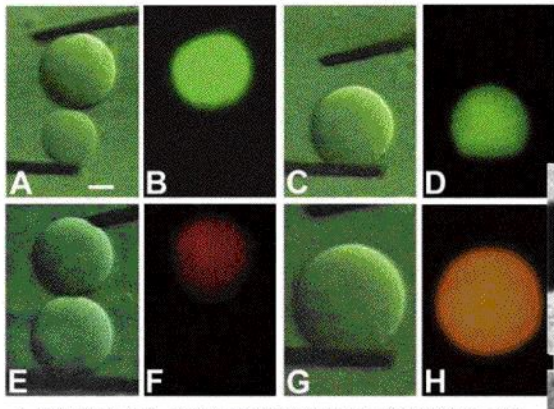


- Microdevice consisting of sample wells, channels and fusion chamber
- Cells/liposomes are sorted optically in sample containers and transported using optical trapping to fusion chamber
- Red blood cells were sucessfully fused using microelectrode-assisted electrofusion in the fusion chamber

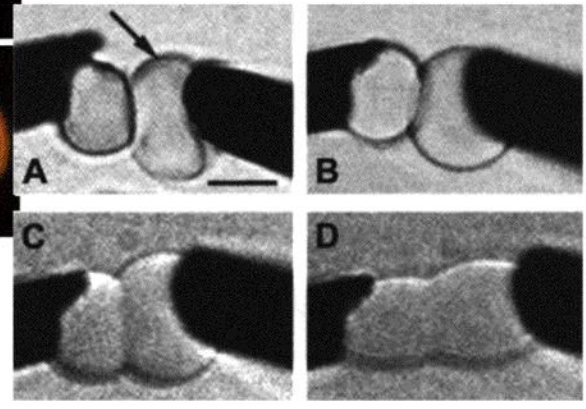
A. Strömberg et al., (2001) Microfluidic device for combinatorial fusion of liposomes and cells, Analytical Chemistry, 73, 126-130
D. Chiu, (2001) A microfluidics platform for cell fusion,Current opinion in chemical biological, 5, 609-612

9.4. Chip-based cellular systems to retrieve single-cell information

Liposome and cell fusion chip



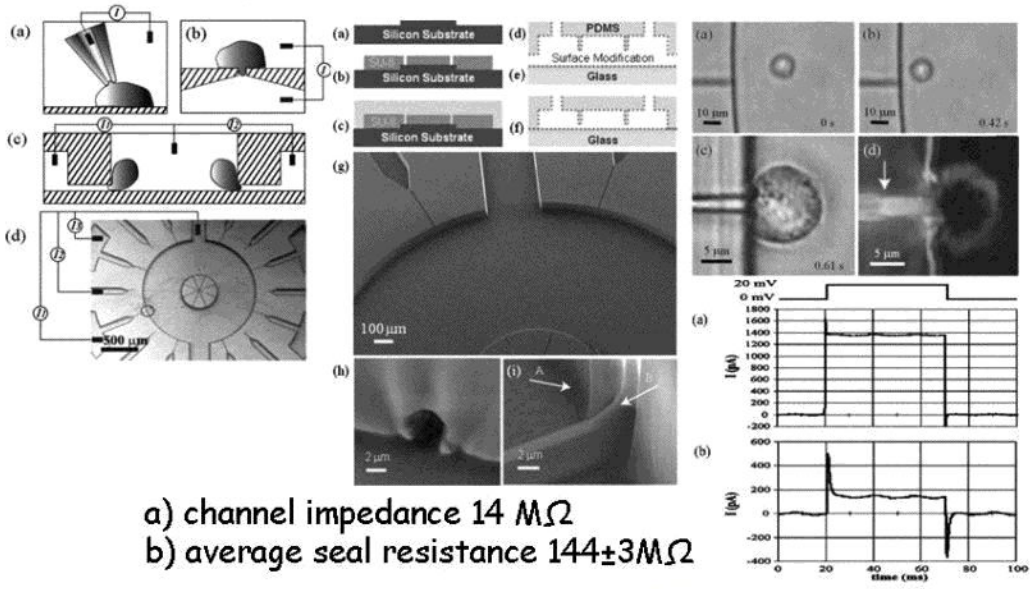
A. Strömberg et al., (2001) Microfluidic device for combinatorial fusion of liposomes and cells, Analytical Chemistry, 73, 126-130



Chiu, D. (2001) A microfluidics platform for cell fusion, Current opinion in chemical biological, 5, 609-612

Multiple patch-clamp array chip

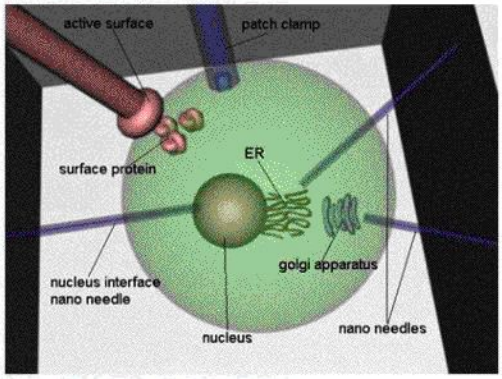
- Patch clamping of HeLa cells



a) channel impedance 14 MΩ
b) average seal resistance 144±3MΩ

Seo et al. / Appl. Phys. Lett., Vol. 84, No. 11, 15 March 2004

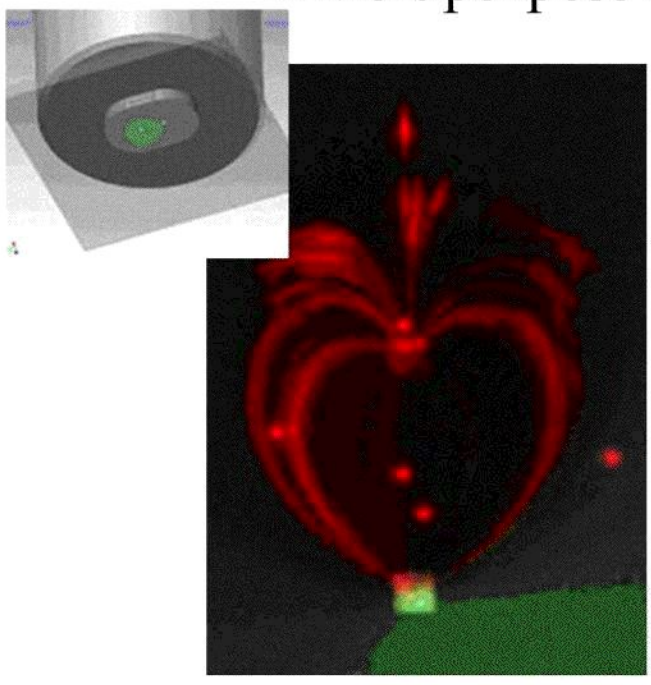
Outlook: Future developments



Source: W.Engel, Leiden University

- Trend towards nano-bioreactor.
- Highly parallel single cell arrays for drug and biomarker screening.
- Systems biology to identify all cellular signaling pathways.

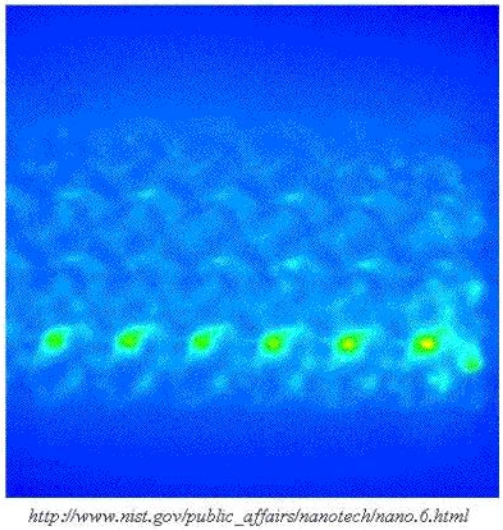
A Multipurpose Microfluidic Probe



IBM researchers David Juncker, Heinz Schmid, and Emmanuel Delamarche have overcome these limitations by developing a novel technology that offers unprecedented functionality and, most notably, the ability to manipulate single cells. The device is called a "microfluidic probe" (MFP) in reference to the fact that it is moved over a surface like a scanning probe, similar to scanning tunneling (STM) or atomic force microscopes (AFM), both invented by researchers at IBM's Zurich Research Laboratory.

Nature Materials Vol.4, No. 8, pp 622-628 (2005)

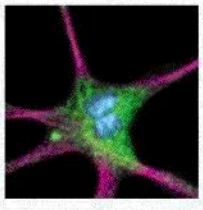
Nanochemistry on a biochip @NIST



http://www.nist.gov/public_affairs/nanotech/nano.6.html

- The stained bacterial cells are part of a ***prototype*** "lab on a chip" sensor system.
- The cells adhere to posts constructed within microscopic channels of a plastic sensor device.
- The blue, green and yellow colors reflect increasing densities of cells, which eject potassium in the presence of certain chemicals.

Summary



- Bioreactor smaller, and smaller
  - Bringing complete biosystems on screening platform into reach of a technical system.
- Lab-on-a-Chip ⇒ Lab-in-a-Cell
  - The toolbox for biologists is rapidly growing, enabling them to carry out all kinds of sophisticated experiments on the cellular level.
  - Precise control of biochemical cellular environment and possibility to analyze composition of single cells could lead to artificial Lab-in-a-Cell processes.