Microfluidics in life sciences
Applications (I)

Joel S. Rossier
Table of content

- Why: Motivation for miniaturisation
- How: Design and Fabrication
- Where: Example of academic systems
- What: Example of industrial applications
What is micro? What is nano?
1. Why: Motivation for minaturisation

1. Small is beautiful?
2. Small is cheap?
3. Small is fashionable?
4. Small is flexible?
5. Small is …?
1. Smart, rapid, modular, portable, etc
2. Mimic the example of electronic evolution with Moore’s law.
Progress of a technology - Electronic chips

Jack Kilby (TI) Nobel Prize
Robert Noyce (Intel) $$$$!

INTEL 4004 MP
Pentium 4

Transistor


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Slides provided by
Larry Kricka
Chairman of AACC
Moore’s Law

- Miniaturisation = performance^2
- Each 18-24 months the performance is doubling and the miniaturisation
- Cost down
- Now: 40 nm chips
Progress of a new technology – Microchips: microarrays (Affymetrix)

- FDA approval
- Routine use
- Products
- Other labs

1st publication

1982 ~1990 ~2000 2004

- 1995~25
- 2002~1000

1995 2002

~25 ~1000

Number of publications

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1982 ~1990 e.g., GLC (Terry)

Roche
FDA

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Progress of a new technology - Bioelectronic chips (outside of Glucose sensing)

FDA approval /// just i-STAT /// Routine use
Products
Other labs
1st publication
Patents filed


i-STAT Nanogen AVIVA
Molecular Devices

DiagnoSwiss

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Chairman of AACC
Lab on a chip concept:

- Has existed as a concept for about 15 years
- Takes time to take off (Academic effort)
- Is now oriented towards classical applications
- Has the potential to be applied in many fields
Flux of molecules:

The flux $J_i$ of species $i$ is given by the Nernst Plank equation as follow:

$$J_i = -D_i \text{grad } c_i - \frac{z_i F}{RT} D_i c_i \text{grad } \phi + c_i \bar{v}$$  \hspace{1cm} (II.1)

where $D_i$ is the diffusion coefficient, $c_i$ the concentration, $z_i$ the charge and $\text{grad } c_i$ the concentration gradient of the species $i$, respectively and where $\text{grad } \phi$ is the potential gradient in the solution, and where $F$, $R$, $T$ and $\bar{v}$ are the Faraday constant, the gas constant, the temperature and the hydrodynamic flow respectively.
Fields of applications for microfluidics

- Standard technics
  - Capillary electrophoresis
  - HPLC
  - Dispensing
  - Mass spec. Infusion
  - Injection (mosquito skin)
  - Biosensor
  - ELISA
  - Ion channel detection
  - MicroSynthesis
  - ...

- Method only possible thanks to miniaturisation
  - Single cell readout/feeding
  - Power flow cell
  - Power bio cell
  - Neuron connection
Fundamentals of fluidic behaviour in microenvironment < 50 microns

- Large surface/volume ratio
- Surface treatment becomes fundamental
- Fluid viscosity is important
- Solid particles
  - Coagulation, suspension etc
- Gas handling (attention material permeation)
Fluid characteristics

- In microchannels:
  - Low Reynolds/Peclet number
  - Reduced natural convection
  - Electrokinetics pumping
  - Mixing only by diffusion
    - Mixing efficiency depends on the molecules parameters (size, Stokes/Einstein friction module)
Surface treatment of polymer

- Example with PET

- Contact angle measurement

http://web.mit.edu/nnf/people/jbico/drop.jpg

http://psii.kist.re.kr/Teams/psii/research/Con_4.jpg

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Super hydrophobic surface with or without 3-D surface


**Behaviour of water drops on different surfaces.**

a. Water drop wetting a normal surface forms a low internal contact angle.

b. Non-wetting, quasi-spherical drop forms a high internal contact angle on a superhydrophobic surface.

c. Drop on a solid surface decorated with pillars. The entire surface is coated with a water-repellent agent, and the space between the pillars is filled with air. Yoshimitsu et al. observe that the drop can sit happily on top of these pillars — the so-called 'fakir regime' — corresponding to an apparent contact angle larger than 150° (superhydrophobic behaviour). If the pillar height is shortened, the water contact angle decreases, because air is no longer trapped below the drop.
Surface based microfluidics

- **Electrowetting**

- **For example**
  - Advanced Liquid Logics

*Fig. 1 Envisioned digital microfluidic circuit and the four fundamental droplet operations necessary*

Mechanical and Aerospace Engineering Department, University of California, Los Angeles (UCLA) Los Angeles, CA 90095, U.S.A.
Electrowetting

- Surface tension modulation by application of an electric field

http://www.liquavista.com
Electrowetting theory

Electrowetting Theory

The simplest derivation of electrowetting behavior is given by considering its thermodynamic model. While it is possible to obtain a detailed numerical model of electrowetting by considering the precise shape of the electrical fringing field and how it affects the local droplet curvature, such solutions are mathematically and computationally complex. The thermodynamic derivation proceeds as follows. Defining the relevant surface tensions as:

- $\gamma_{ws}$ - The total, electrical and chemical, surface tension between the electrolyte and the conductor
- $\gamma_{w0}$ - The surface tension between the electrolyte and the conductor at zero electric field
- $\gamma_s$ - The surface tension between the conductor and the external ambient
- $\gamma_w$ - The surface tension between the electrolyte and the ambient
- $\theta$ - The macroscopic contact angle between the electrolyte and the dielectric
- $C$ - The capacitance of the interface, $\varepsilon_0 \varepsilon_r / d$, for a uniform dielectric of thickness $d$ and permittivity $\varepsilon_r$
- $V$ - The effective applied voltage, integral of the electric field from the electrolyte to the conductor

Relating the total surface tension to its chemical and electrical components gives:

$$\gamma_{ws} = \gamma_{w0} - \frac{CV^2}{2}$$

The contact angle is given by the Young-Dupre equation, with the only complication being that the total surface energy $\gamma_{ws}$ is used:

$$\gamma_{ws} = \gamma_s + \gamma_w \cos(\theta)$$

Combining the two equations gives the dependence of $\theta$ on the effective applied voltage as:

$$\theta = \cos^{-1} \left( \frac{\gamma_{w0} - \gamma_s - \frac{CV^2}{2}}{\gamma_w} \right)$$

An additional complication is that liquids also exhibit a saturation phenomena; after certain voltage, the saturation voltage, the further increase of voltage will not change the contact angle, and with extreme voltages the interface will only show instabilities. The ultimate and complete explanation of electrowetting, mainly because of this effect, is still missing.

http://en.wikipedia.org/wiki/Electrowetting
Fabrication of electrowetting support hardware

Printed circuit board used to pattern electrodes under dielectrics

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Electrowetting

Electrostatic charges: hydrophilic

Dielectric

Voltage

Electrode

Hydrophobic coating
Electrowetting

Dielectric

Hydrophobic coating

Voltage

Electrode

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Electrowetting

- Dielectric
- Electrode
- Hydrophobic coating
Electrowetting
Electrowetting: different designs are possible

Figure 1. Two-plane DMF devices formed by using photolithography. a) A schematic of the one-plane configuration b-g) Video sequences (top-to-bottom) depicting droplet movement, merging, splitting, and dispersing, respectively. In (e), droplets containing HCl (120 nM) and methyl red (0.5 mg mL⁻¹) were added to each on-chip reactor. Devices in (b), (d), and (f) were formed from flexible substrates; devices in (c) and (g) were formed from silicon wafers. The devices in (b) and (d) were covered with 8 μm PDMS; devices in (c) and (g) were covered with 3 μm polyimide and 40 μm PDMS. All devices were covered with 55 nm TeflonAF.

Figure 2. One-plane DMF devices formed by using photolithography. a) A schematic of the one-plane configuration. b-g) Video sequences (top-to-bottom) depicting droplet movement and merging. The devices were formed from flexible substrates covered with 9 μm PDMS and 50 nm TeflonAF.

Adv. Mater. 2007, 19, 133–137
Beads or cells can be transported

Digital microfluidics for cell-based assays

Irena Barbulovic-Nad, Hao Yang, Philip S. Park and Aaron R. Wheeler

We introduce a new method for implementing cell-based assays. The method is based on digital microfluidics (DMF) which is used to actuate nanolitre droplets of reagents and cells on a planar array of electrodes. We demonstrate that this method is advantageous for cell-based assays because of automated manipulation of multiple reagents in addition to reduced reagent use and analysis time. No adverse effects of actuation by DMF were observed in assays for cell viability, proliferation, and biochemistry. A cytotoxicity assay using Jurkat T-cells was performed using the new method, which had ~20 times higher sensitivity than a conventional well plate assay. These results suggest that DMF has great potential as a simple yet versatile analytical tool for implementing cell-based assays on the microscale.
Advance Liquid Logics

http://www.liquid-logic.com/applications.html
Surface Acoustic wave as a pumping means

Pressure transfered to the liquid

Tsunami effect
Rayleigh Wave

www.advalytics.com
Advalytics: surface acoustic wave
Nanodrop: optical cell of 1 microL

- Optical cell held by surface tension
- 1 drop of solution (1 microL)
- Spectroscopy in a drop
Nanodrop

Meet NanoDrop
How It Works
UV-Vis
Fluorescence
Applications
Our Products

Say hello to your new lab partner.
Pressure driven flow on a chip

- Fluidics without Electrical field
- Mechanical pressure in the chip
- Open channel or packed channel for affinity or chromatography separation
Fast flow control: microfluidic multiplex pressostat


(Equivalent to divider bridge potentiostat in electronics)

- Two stages pressure regulation
- Calibrated leak
- Dynamic pressure feedback: Automated compensation of Bubbles, environmental pressure Changes, viscosity changes:
Pressure control with Fluigent system

Plug and play (USB)
Time constant <20 ms
Stability: several hours
Large pressure range
  (-> 1 atm)
High sensitivity
  (~0.1 mm H2O)
Complex protocols can be easily automated

www.Fluigent.com

Jean-Louis Violy, Institut Curie
Comparison of Syringe driven flow and Fluigent system

Controlling flows with FASTAB technology leads to a completely different behavior. The time response is no longer dependent on the compliance of the tubing and the microsystem; as soon as the dynamic pressure is applied, the effect is instantaneous in the whole device. Besides, in many cases, calculating flows in a microfluidic network controlled by the MFCS flow sequencer is easier and more accurate than using volume-based pumps.
Flow control by Fluigent

- Oil drop formation
- Block flow
- Injection

Experiment performed in Laboratoire de Biologie Chimique // ISIS - ULP: [http://www.isis.u-strasbg.fr/lbc/]

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Microfluidic separation

- Capillary Electrophoresis
- Chip Electrophoresis
- MS sampling (nanoelectrospray)
- HPLC MS
Capillary electrophoresis

- Separation method occurring in a capillary
Electro-induced convection

- Electroosmotic flow
- Characteristics: almost flat front in microchannels

- In nanochannel: again a sort of parabolic profile!
Migration + electroosmotic flow separation
Microfluidics vs Microvalving

- Remote valving
  - Electrokinetic injection
  - Pressure control
- In situ valving
  - Local material deformation
  - Material shifts

Microvalving is a challenge in microfluidics
How to inject a small plug?

- Injection pattern
Microchip Structures for Submillisecond Electrophoresis

- Figure 1 Schematic of microchip used for high-speed electrophoretic separations. (Inset) Enlargement of the injection valve and separation channel.

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Figure 3 High-speed electropherogram of rhodamine B and di-chlorofluorescein resolved in 0.8 ms using a separation field strength of 53 kV cm\(^{-1}\) and a separation length of 200 m. The start time is marked with an arrow at 0 ms.

_{Anal. Chem., 70 (16), 3476 -3480, 1998_}

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1D electrophoresis

- Biopolymer Sizing
- Sieving through a polymer matrix
- 1D-gel electrophoresis to be replaced by Chip electrophoresis
Caliper-Agilent technology Lab on a chip
Separation with Bioanalyser

- View as a Gel or Electropherogram
Fully Automatic LOC

Agilent 5100 ALP
Fast & Reliable Sample Analysis

Agilent Technologies
ScreenTape: rapid electrophoresis

ScreenTape is a patented credit card sized tape containing 16 microgels. Analyse up to 16 DNA, RNA or protein samples in less than one minute per sample, with no gel preparation or chip priming!

Similar but not identical!!! to Caliper/Agilent technology:
Older microfluidics patents are going to expire (first work in late 80’s!) leading to More similar products.

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ScreeTape, Lab 901:

<table>
<thead>
<tr>
<th>Features</th>
<th>Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Versatile</td>
<td>ScreenTape can separate complex mixtures of DNA, RNA and proteins</td>
</tr>
<tr>
<td>Ready to use</td>
<td>Each ScreenTape contains a gel matrix, running buffer and electrode and is ready to use straight from the box</td>
</tr>
<tr>
<td>Safe</td>
<td>No reagent preparation means you avoid exposure to hazardous reagents and stains</td>
</tr>
<tr>
<td>Rapid results</td>
<td>Samples are separated and analysed in around one minute per sample</td>
</tr>
<tr>
<td>Preserve valuable samples</td>
<td>Only 1 ul of sample required</td>
</tr>
<tr>
<td>Full traceability</td>
<td>Bar-coding on the ScreenTape provides full traceability of results</td>
</tr>
<tr>
<td>Use tape multiple times</td>
<td>If you only have a few samples, run the remaining lanes next time</td>
</tr>
</tbody>
</table>

1. Gel matrix
2. Running buffer
3. Barcode
4. Description, lot number and expiry date
5. Electrodes
Fluidigm: valving on a chip: system fully integrated

Application: qPCR
Fluidigm: valving with elastomer

Nanoflex Valve
How to inject into a microfluidic chip network

Pressure pinched injection

Fig. 2: Schematic diagram of a two-way micro-8-port valve connected to a micro-channel network as shown in Fig. 1, which describes the movement of a sample and an elution solution as a function of the position of the valve. This figure illustrates how a sample can be injected into a micro-channel network: a) the standby state before sample loading; b and c) configuration of the pressure pinched injection, where the sample solution is loaded into the cross and where the loaded plug is pressed by two countercurrent flows. A symmetrical triangle (b) or a non-symmetrical (c) shape of the loaded plug can be obtained using the same pinch pressures or different ones at B and D connections. d) Configuration of the separation position where the sample plug is injected under pressure and evolves in a parabolic shape.

Lab Chip, 2002, 2, 45–49
Plug with parabolic shape

Fig. 3 Photographs of the micro-channel intersection during the pressure-driven injection of a solution of 2 mM sodium chloride + 10 mM Eosin B in water under the configuration presented in Fig. 2. The photographs were taken at the following times after positioning of the multi-port valve in the configuration of Fig. 2b: A, 0 s; B, 0.6 s; C, 1 s; and D, 3 s. The feature of Fig. 3A remained stable during several minutes and injection can be operated at any time after first stabilisation of the flows.

Fig. 4 Conductimetry chromatogram obtained for the separation of a 10 mM Eosin B + 2 mM sodium chloride solution after injection of this mixture in the conditions described in Fig. 3. The flow rate of syringe pump driving the sample solution is 3.33 µL min⁻¹, while the flow rate for buffer driving is 0.9 µL min⁻¹.
Nanochromatography

- Packing or monolithic
  - Packing
    - Easy to choose the surface of interest
    - Tricky to immobilised in the nanocolumn
    - Use of frit or size restriction in the column
  - Monolithic
    - In situ synthesis
    - Lower choice of surface material
    - Easier to adapt to any form
3-D organisation

- Optimisation of surface by nanopillars
- Microfabricated organised surface

Microlithography:
Micro-Nanopillar Arrays

Monolithic pillar for chromatography or CEC

Microfluidic enrichment for low concentration (pM)

5. Jemere AB, Oleschuk RD, Ouchen F, Fajuyigbe F, Harrison DJ:
An integrated solid-phase extraction system for sub-picomolar

SPE micro-device for protein digest analysis. (a) Isometric projection of
the packed bed used for SPE and capillary electrophromatography
(CEC) illustrating the 1 μm deep wells used to trap the bead bed.
The volume of the chamber is 330 pl, which enables preconcentration
of extremely small sample volumes. (b) Elution traces of 1 μM of a
fluorescent dye, BODIPY (S/N ~ 45) and 10 pM BODIPY (S/N = 434),
determined after concentration for 3 min from pH 9.3 aqueous buffer from
the SPE bed depicted in (a). Elution was performed with 60% acetonitrile/60% pH 8.3 buffer. (Figures reproduced from [5] with
permission.)
Monolithic column

Monolithic column can be fabricating by polymerisation of monomer by UV activation.

Fig. 1 Schematic graph of the SPE microchip. (a) Hot embossing procedure; (b) channel arrangement on the microchip; (c) micrograph for the frit and packings in one channel; (d) SEM image for the monolithic frit.
Scheme 1. Procedure to immobilize carbohydrates onto monolith beds: a) Original polymer bed with DATD as cross linker; b) 1,2-Diol group of DAD was cleaved using sodium per-iodate & c) Sugar with amino spacer was immobilized via reductive amination.

Figure 1. Optical microscope images:
   a) Empty column b) Monolith capillary column

Figure 2. Scanning electron microscope images:
   a) Empty capillary b) Monolith capillary column

http://www.orc.wur.nl/UK/Events/PhD+trip+2007/Abstracts/Kishore+Tetala/
CEC with monolithic polymerised column

HPLC (High performance liquid chromatography)

- Standard system:
Agilent HPLC on a chip
Nanostream: parallel HPLC on each cartridges

Nanostream CL™ System for high-throughput chemical analysis
With a 24-fold increase in analytical throughput, the Nanostream CL System is ideally suited for a range of analytical applications including physiochemical property profiling, pre-formulation and formulation studies and amino acid analysis. UV absorbance detection is standard; options for fluorescence detection and an integrated plate changing system are available.
Use of nano(micro-) particles

- Location of the beads
  - Random organisation
  - 3-D organisation

- Magnetic vs non magnetic beads
  - Different surface materials
  - Different compositions
Magnetic nanoparticles

- Different features
  - Size dispersion
    - Monodisperse
  - Magnetic core
    - Different magnetic core diameter/volume ratio

Magnetic nanoparticles vs microparticles
- Higher surface to volume ratio
- Lower sedimentation
Magnetic particles can contain fluorescent properties

Magnetic Nanoparticles

- **Monodisperse**
  - (Ademtech)
  
  - Particle Size Distribution
    - Transmission Electronic Microscopy, Light Scattering

![TEM micrograph of 300 nm magnetic particles]

Regular network of particles

High monodispersity

![Graph showing particle size distribution with peaks at 200 nm, 300 nm, and 600 nm]
Magnetic nanoparticle arrays

The “lazy” way: “EPHESIA”
Self Assembled matrices
superparamagnetic nanoparticles

Experimental Setup

Classical « SOFT LITHOGRAPHY »
SU8 Photoresist master
PDMS micromolding
Whitesides et al. 1994-2000,
PMMA interface
3-D organisation of bead column with magnetic field

Magnetic field organised column
- Renewable chromatography phase
- DNA separation
- Enzymatic digestion

Micrograph of 600 nm magnetic particles (magnification x100)

Jean-Louis Viovy, Institut Curie

Organization in a microfluidic cell
DNA Separation by sieving

- Long DNA strains separation

![Images of DNA separation at different time points with a graph showing the center of mass position over time.]

Average Collision Times:
- T4 = 2.9 s
- \( \lambda = 0.8 \) s

Jean-Louis Violy, Institut Curie

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Application of microfluidics for affinity assay

- Immunoassay
- ELISA (enzyme linked immunosorbent assay)
- DNA Hybridisation
- etc
Diffusion Immunoassay:

- Dilution by diffusion
Diffusion based assays
H-filter

Figure 2.5. 2D solution for the diffusion of biotin ($D = 340 \, \mu m^2/s$, left image) and albumin ($D = 65 \, \mu m^2/s$, right image) through a T-sensor. A normalized concentration of 1.0 enters at the left inlet where the velocity flow rate is $125 \, \mu m/s$. Buffer enters at the right inlet at the same velocity.

Paul Yager’s Group

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Affinity assay: Immunoassay

- **Standard Immunoassay technology**
  - ELISA (Enzyme Linked ImmunoSorbent Assay)
  - ELFIA (Enzyme Linked Fluorescent ImmunoAssay)
Diffusion Immunoassay in microfluidics

Figure 7. (A) Scheme of the T-sensor microdevice with the two different inlets and the interdiffusion zone. (B) Dose response curve for the small drug phenytoin from different blood samples and standards. Reprinted from [91], with permission.

Diffusion Immunoassay II

Rapid diffusing antigen

Slow diffusing antibody
Diffusion Immunoassay III
Diffusion Immunoassay IV
Diffusion Immunoassay

Figure 7. (A) Scheme of the T-sensor microdevice with the two different inlets and the interdiffusion zone. (B) Dose response curve for the small drug phenytoin from different blood samples and standards. Reprinted from [91], with permission.