




Development of microfluidic devices – applications in cell culture, chemical gradient and electric field.

微流體晶片的發展 以及 化學與生物研究應用

BioMAPs Laboratory (BioMicrofluidic ApplicationS)

Ji-Yen Cheng (鄭 鄧言)

<http://rcas.sinica.edu.tw/faculty/jycheng.html>

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 Assistant Research Fellow, 2001-2007,
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 Department of Mechanical and Mechatronic Engineering, National Taiwan Ocean University, Taiwan
 Institute of Biophotonics, National Yang-Ming University, Taiwan ROT

For TIGP Advanced Analytical Chemistry, THU 09:10-12:00, 13/Mar/2014

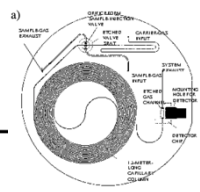




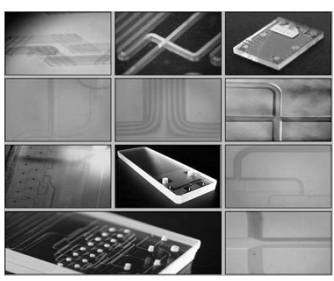


What is microfluidics?

- Deals with the behavior, precise control and manipulation of fluids that are geometrically constrained to sub-millimeter (micrometer, μm), scale.
- A multidisciplinary field intersecting engineering, physics, chemistry, microtechnology and biotechnology.
- Characteristic of liquids in microdomain
 - Laminar flow
 - Diffusion dominant when no mixing
 - Surface charge significant
 - Surface tension dominant
 - Fast thermal relaxation
- Benefits using microscale
 - Low sample/reagent volume
 - Low energy consumption
 - Reduced reaction time
 - Precisely controlled environment



First microfluidic GC in 2" silicon. Terry SC et al. (1979): A gas chromatographic air analyser fabricated on silicon wafer. IEEE Trans. Elect. Device, V26; No.12 pp. 880-1886.



Modified from Wikipedia



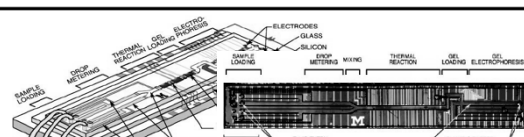

Chemical and Biological applications of microfluidic chip in our lab

- Rapid (500 sec) Microarray hybridization, 2005, Nucl. Acid. Res. 
- DNA amplification, 2005, Analyst, LOC highlight. 
- DNA microarray synthesis, 2009, Bioeng. Biotech. 
- Rare cell capturing (patent filed, 08/Jun/2011) 
- Microfluidic nanoslit sensor, 2013 Analyst 
- Sensor based on whole-cell array (Taiwan-Israel Nanoprogram) 
- Cell culturing in controlled microenvironment 
- Electrotaxis of lung cancer cell
 - Electrotaxis – short introduction 
 - Multi-field chip (MFC) for electrotaxis study (2009 Biosen. Bioelect., 2012 Biomicrofluidics) 
 - Electrotaxis in 3D scaffold 
- Wound healing mimicking 

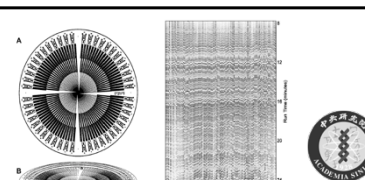
3 On-going works: 

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Microfluidic chip – some examples



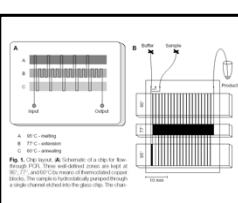
Metering/PCR/Electrophoresis/Detection,
M. Burns, et al., *SCIENCE*, 282, 1998, 484



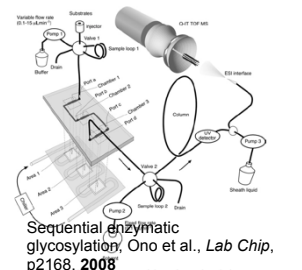
Sequencing, B. M. Paegel, C. A. Emrich, G.J. Wedemayer, J.R. Scherer, and R.A. Mathies, *PNAS*, 99, 574, 2002



Electrophoresis, Caliper Technologies,
<http://www.calipertech.com>



PCR, 90 sec/20 cycles, A. Manz et al., *Science*, 280, 1046, 1998



Sequential enzymatic glycosylation, Ono et al., *Lab Chip*, p2168, 2008

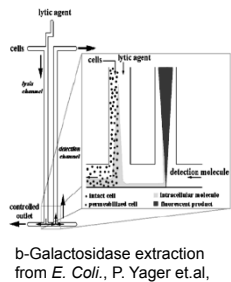
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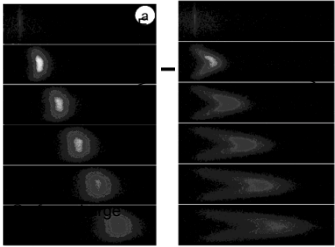
Flow characteristic in microdomain

Laminar flow
Diffusion dominant when no mixing
Surface charge significant
Surface tension dominant

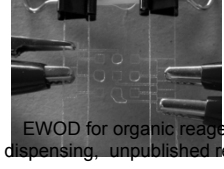




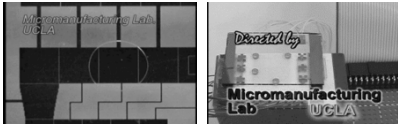
b-Galactosidase extraction from *E. Coli*, P. Yager et.al, *Anal. Chem.*, 2002



Electroosmosis flow vs. pressure flow, 300 μm x 80 μm , 50 ms/step, L.E. Locascio, et.al. *Anal. Chem.*, 2001, 73,2509-2515



EWOD for organic reagent dispensing, unpublished result



Droplet actuation by ElectroWetting On Dielectric (EWOD, electrical surface energy control), <http://cjmems.seas.ucla.edu/>

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Fabrication of microfluidic chip

- Semiconductor processing, soft lithography, and laser direct-writing





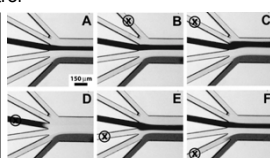


- Semiconductor processing
 - High setup cost
 - Parallel processing – long turnover - mass production
 - Clean room
 - Corrosive chemicals (HF,)
 - Deep etching difficult
 - Monotonous depth

- Laser direct-writing
 - Economical setup
 - Serial processing – short turnover - rapid development
 - Ordinary lab
 - No corrosive chemicals
 - Deep etching by accumulated ablation
 - Variable depth by power/speed control

Soft lithography (ref. G. Whitesides, Harvard Univ.)

- Economical raw material, economical instruments (office equipment)
- PDMS is used, easy for bonding
- Substrate not rigid, substrate easily contaminated
- Still need photolithography for making the first mould.
 - A modified LCD projector has been used cure PDMS pattern directly without photomask
- Substrate not compatible with aggressive organic solvents



TWIST valves
<http://gmwgroup.harvard.edu/>

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Semiconductor processing instruments for microfabrication



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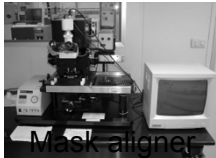
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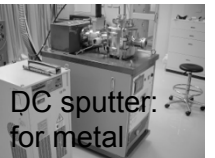

Cleanroom and processing instruments



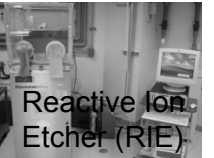
Spin coater



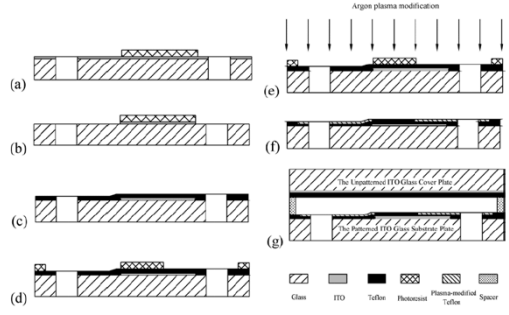
Mask aligner



DC sputter:
for metal



Reactive Ion
Etcher (RIE)



Argon plasma modification

The Unpatterned ITO Glass Cover Plate

The Patterned ITO Glass Substrate Plate

Glass
 ITO
 Teflon
 Photoresist
 Plasma-modified Teflon
 Sputter



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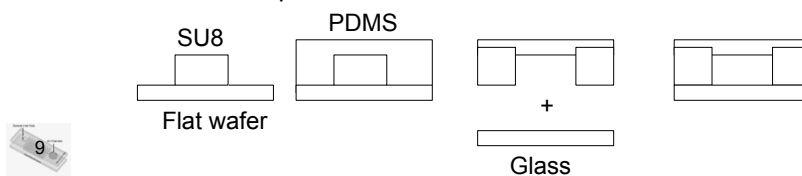
Fig. 3. Fabrication process of the EW-based valve and the hydrophilic microfluidic guidance.

Cheng and Hsiung, *Biomedical Microdevices*, 2004, V6, 341-347

Soft lithography – an example from youtube

Video: <http://www.youtube.com/watch?>

- Make mould (usually SU8, a negative tone PR)
 - Spin coating
 - UV Exposure for curing
 - Development
- Cast PDMS monomer and cure
- Peel off PDMS chip



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Microfluidic tools – Fabrication by laser direct-writing

- PMMA chip by CO₂ laser
- Glass chip by CO₂ laser -> by UV laser -> by green laser
- ITO micropatterning by green laser
- Photoresist structuring by UV laser



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PMMA by CO₂ laser

- Rapid prototyping of plastic microfluidic chip

Sens. Actuator. B:Chemistry, 2004, v99, 186-196

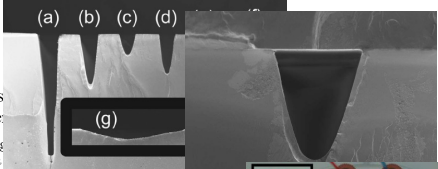
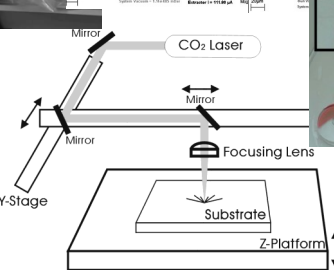
Direct-write laser micromachining and universal modification of PMMA for device development

Ji-Yen Cheng^{a,*}, Cheng-Wey Wei^{a,b}, Kai-Hsiung Hsu^a, Tai-Hong

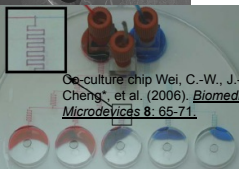
^a Institute for Applied Science and Engineering Research, 128 Sec. 2 Academia Road, Taipei 11529



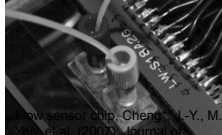
minimal feature $\approx 120\ \mu\text{m}$

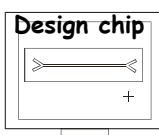
Co-culture chip Wei, C.-W., J.-Y. Cheng, et al. (2006). *Biomedical Microdevices* 8: 65-71.



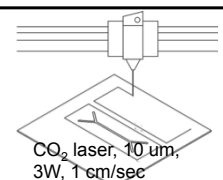
Flow sensor chip, Cheng, C.-Y., M.-H. Micromechanics and Microengineering



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Design chip



CO₂ laser, 10 μm , 3W, 1 cm/sec

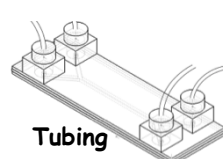
Laser cutting

Chip fabrication procedure

- 30 ~ 120 min

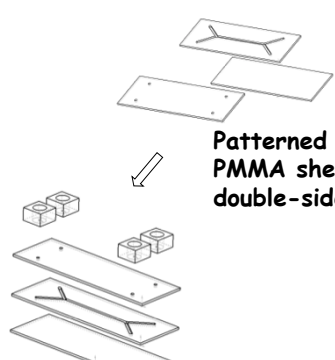
Culture chip fabrication

First step
PMMA chip fabricated by CO₂ laser machine



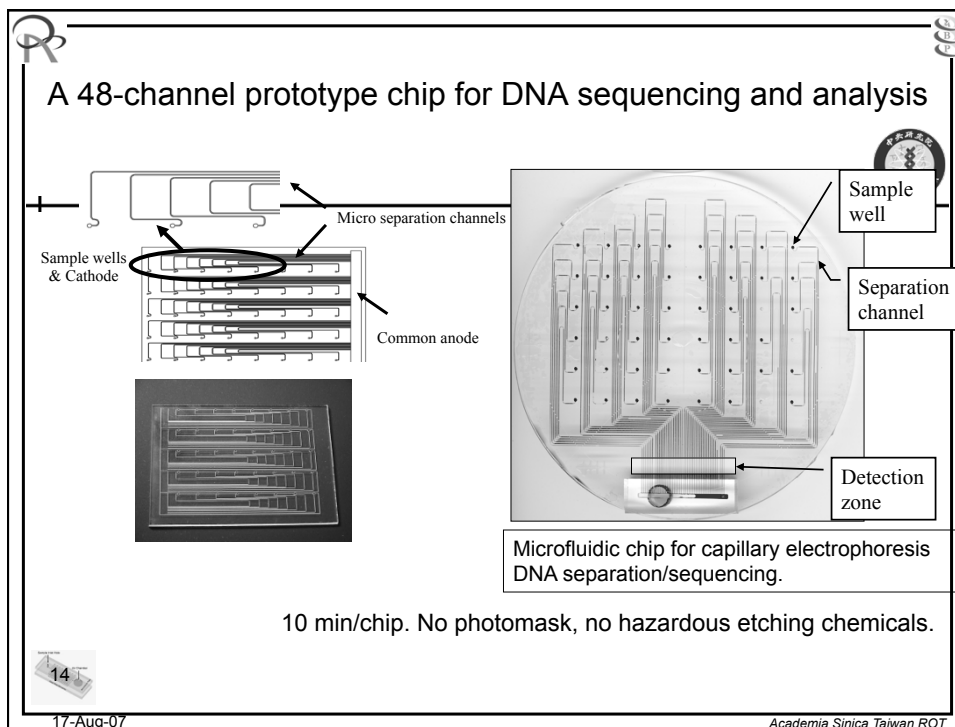
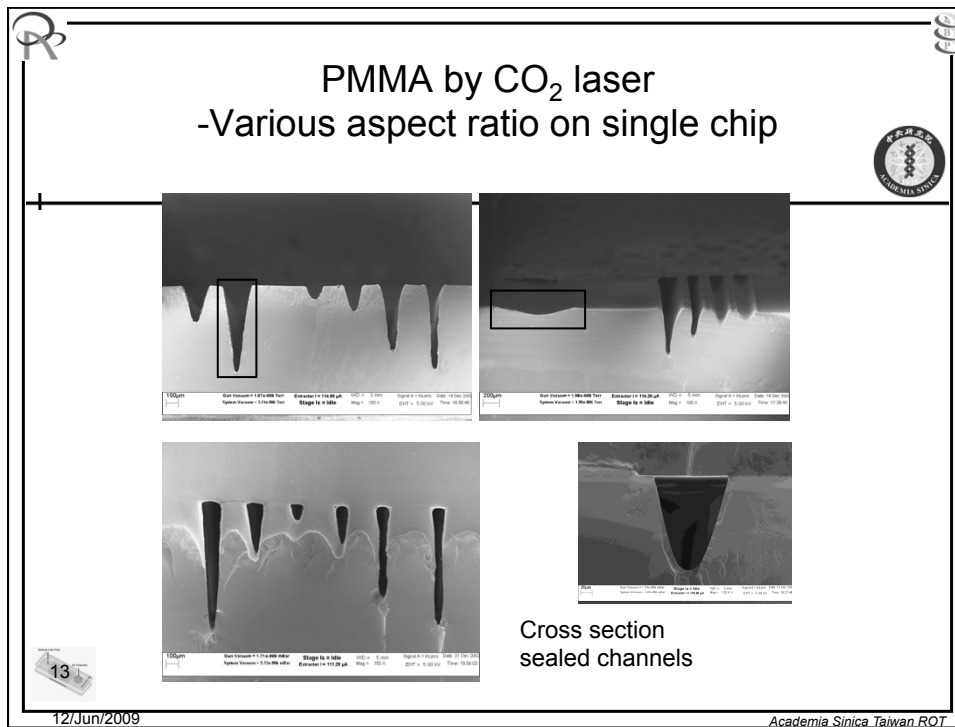
Tubing

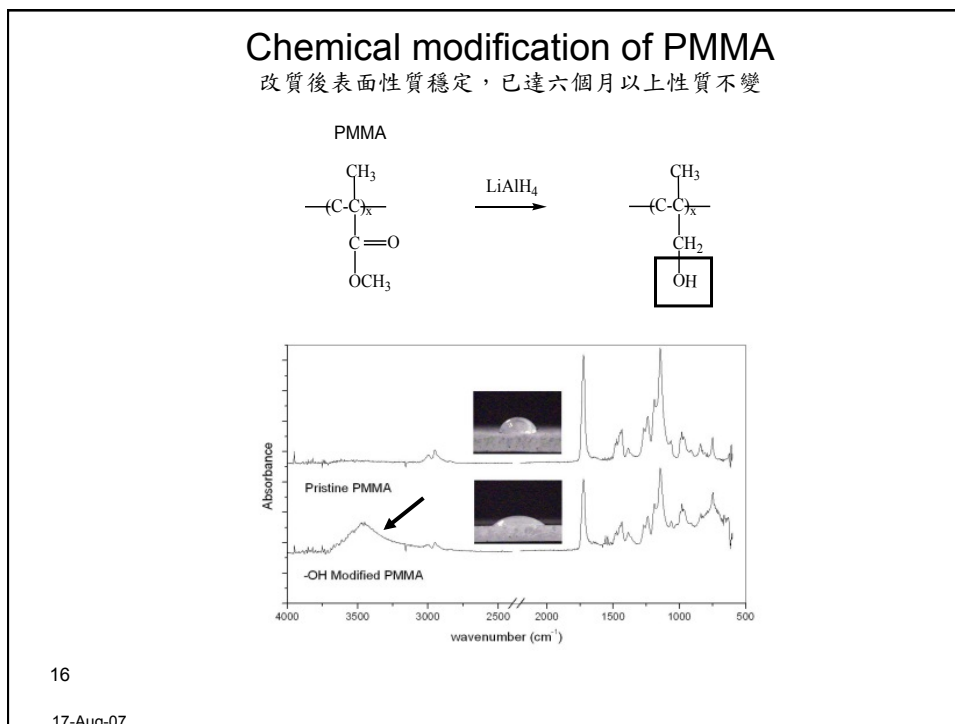
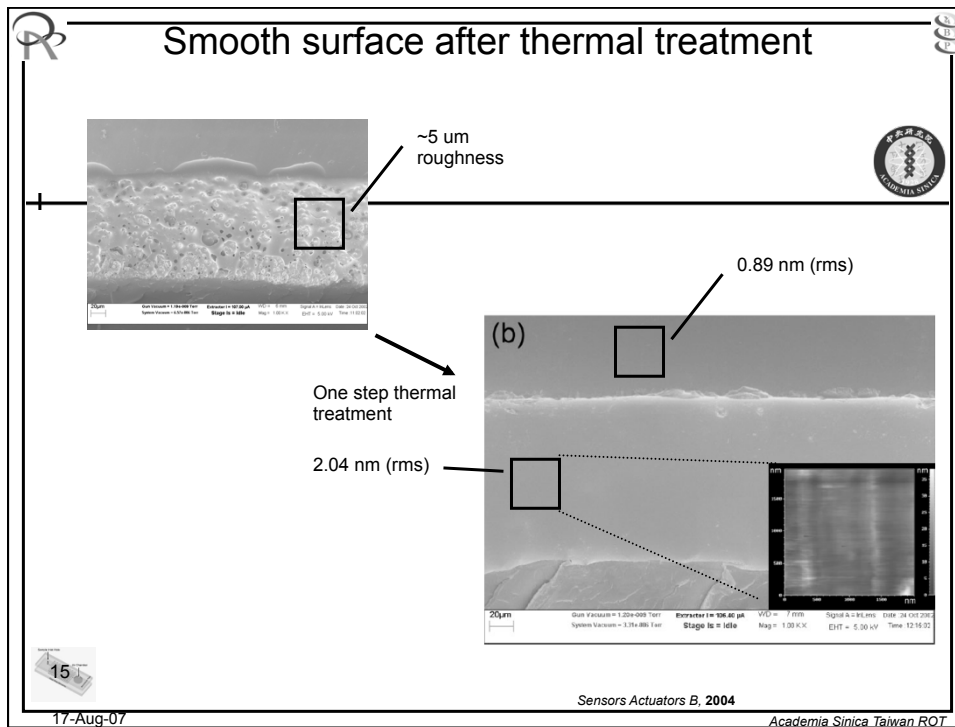
Patterned parts:
PMMA sheet or double-side tape

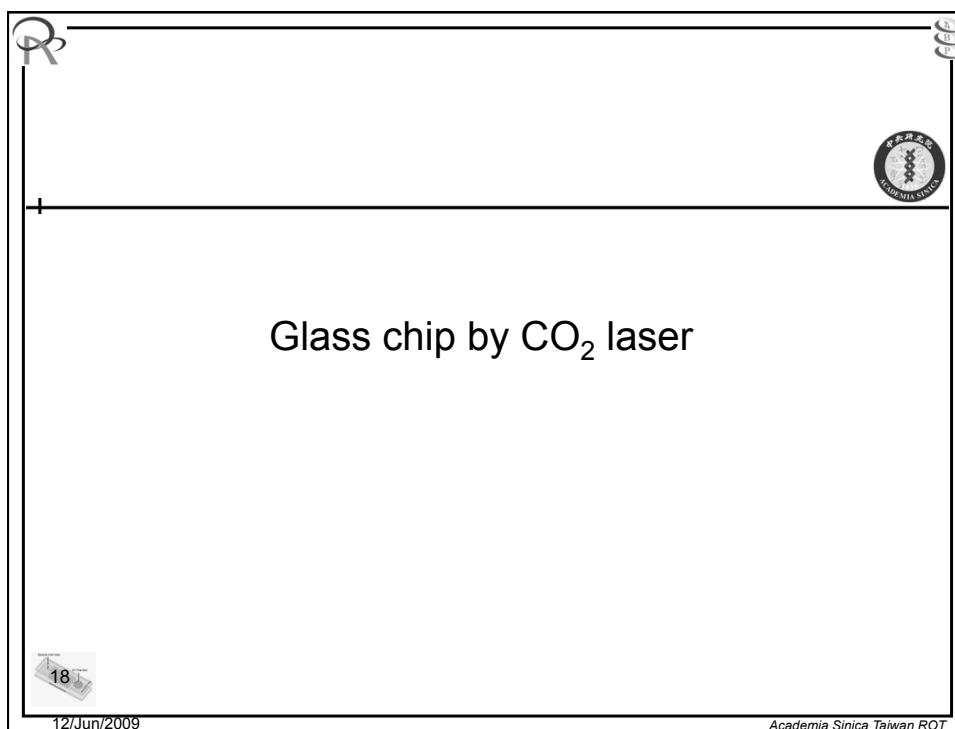
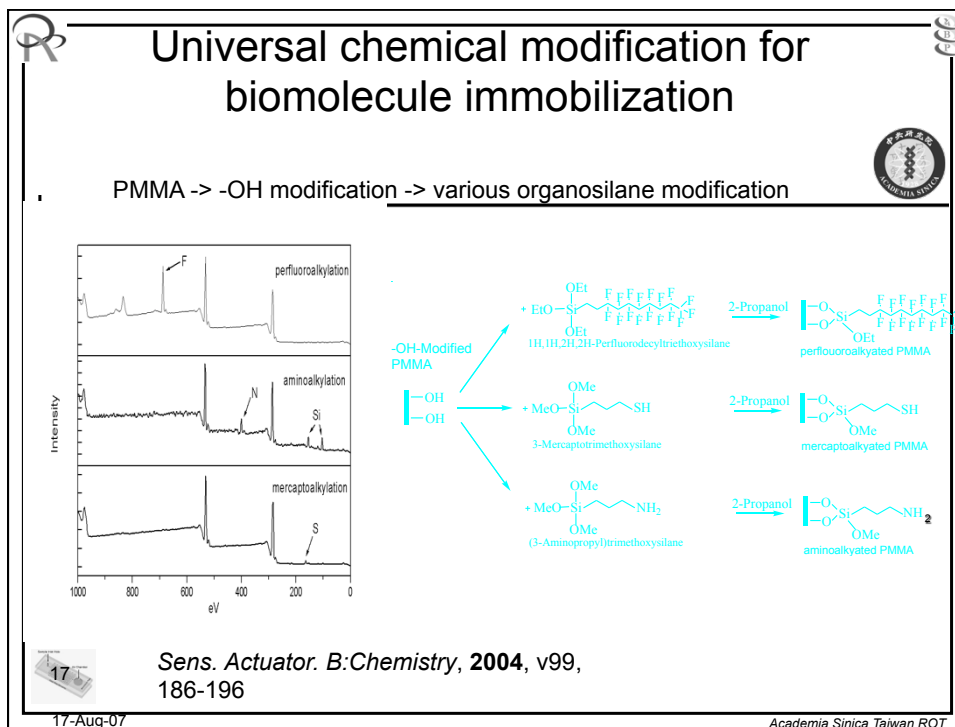


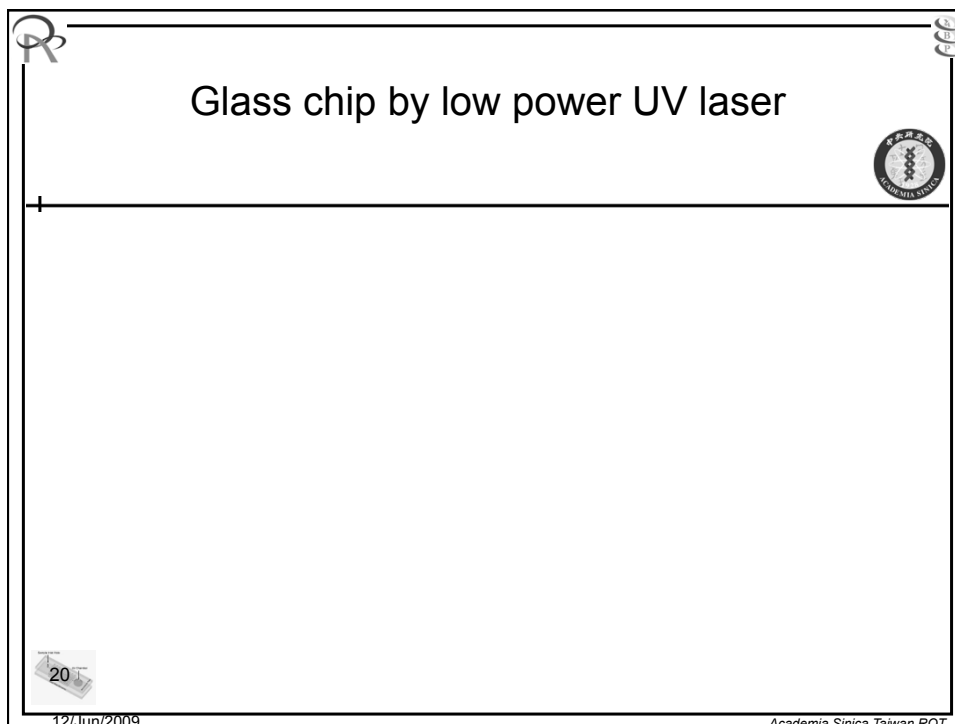
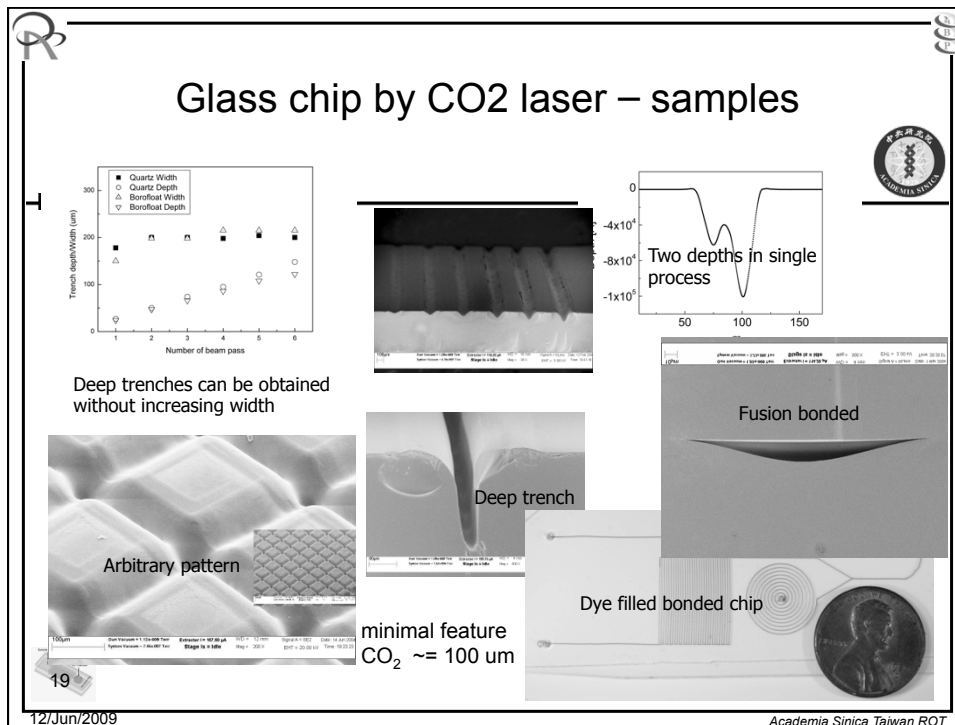
Chip assembly (bonding or adhesion)

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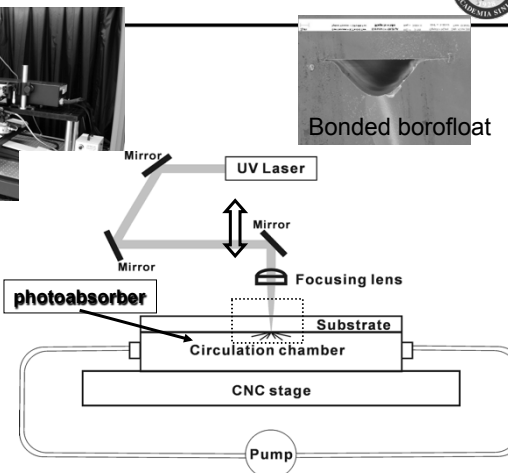


Glass chip by low power UV laser

- Laser Induced Backside Wet Etching (LIBWE) requires
1/40 laser power of direct etching

• The UV system

- Q-switched
- 266 nm
- 10-15 nsec
- 1.5 W @ 10kHz
- 250 uJ @ 6kHz
- 0.1 ~ 5 mm/sec
- Minimal feature 6 um



Bonded borofloat

photoabsorber

UV Laser

Mirror

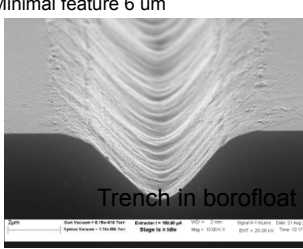
Focusing lens

Substrate

Circulation chamber

CNC stage

Pump



Trench in borofloat

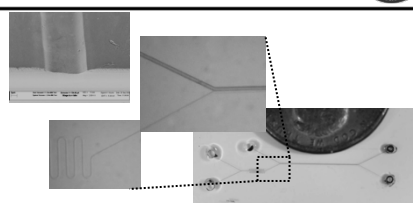
minimal feature
UV ~ 6 um, 20-35 mW, 2-4.5kHz

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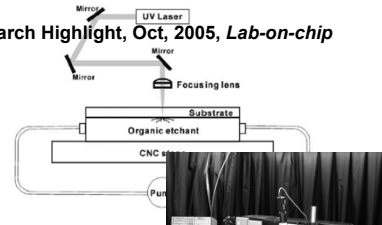
Glass chip by low power UV laser

- samples, 2005 and 2006, *J. Micromech. Microengin.*

✓ An interesting development in this area has been reported by Ji-Yen Cheng and associates at the Academia Sinica of the National Taiwan University in Taiwan.²² The authors describe a flexible approach for rapid prototyping of glass microstructures using a laser direct-writing method requiring no photomask generation. Specifically, the authors use a 266 nm diode-pumped solid state Nd:YAG laser operating at low power thresholds are obtained when using aromatic solvents with strong vibronic interactions. To demonstrate the ability of the technique to create complex feature structures, stepped trenches and transverse asymmetric trenches were fabricated in glass substrates and used to create an integrated liquid-phase flow reactor and concentrator. Such a technique should prove to be a useful tool for prototyping glass



Research Highlight, Oct, 2005, Lab-on-chip



UV Laser

Mirror

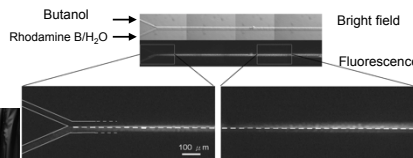
Focusing lens

Substrate

Organic etchant

CNC stage

Pump



Butanol →

Rhodamine B/H₂O →

Bright field

Fluorescence

minimal feature
CO₂ ~ 100 um
UV ~ 6 um

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Smooth surface by the laser backside etching - UV

Periodic ripple caused by CNC speed fluctuation

Optimized motion control parameters

Some structures made by the UV system

Deep, crack-free glass structures with ~6 um feature size

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A complex microfluidic chip –768 channels in a 4" glass chip for DNA separation - UV

Width ~ 20 um

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ITO micropatterning by green laser



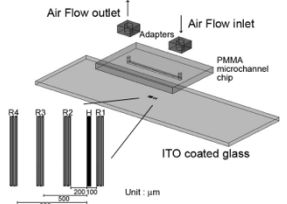


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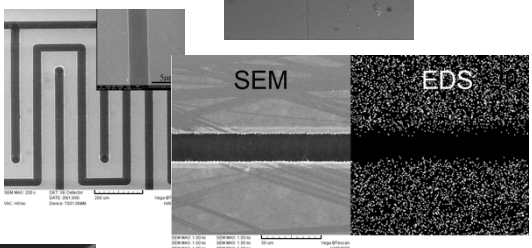
ITO micropatterning by green laser -transparent flow meter

J. Micromech. Microengin. 2007



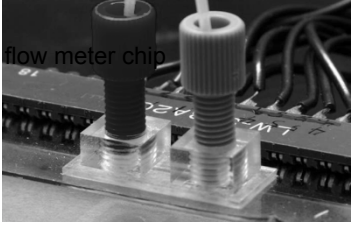


Unit : μm

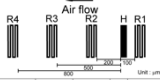


SEM EDS

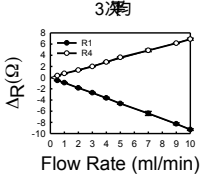
532nm, 15ns, 2kHz, 10mW, 3.5 μm



flow meter chip



(Ω)	R4	R3	R2	H	R1
R (Heated)	12377	12317	12757	N/A	12336
R (Flow)	12386	12326	12764		12328
ΔR	9	9	7		-8



3 濃度

$\Delta R(\Omega)$

Flow Rate (ml/min)

Temp/flow sensing by resistance change

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Photoresist structuring by UV laser -direct exposure



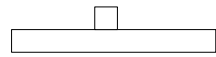
29

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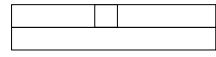
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Microstructures by laser direct-exposure -on-going

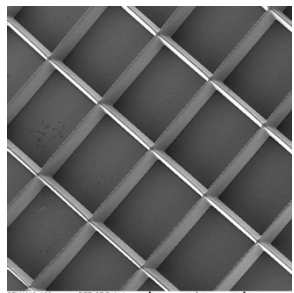


SU8

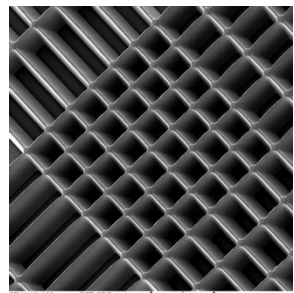


Laser curing





Bar 500 um



Bar 200 um

Patterning performed in average lab, no cleanroom facility needed!



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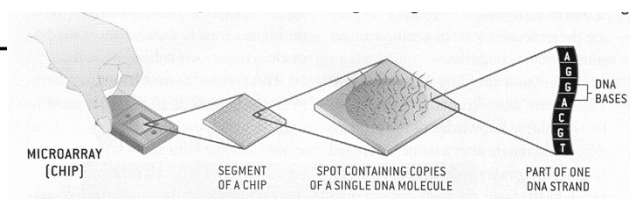
Chemical and Biological applications of microfluidic chip in our lab

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- DNA amplification, 2005, Analyst
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- Rare cell capturing
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Gene chip - Oligonucleotide Microarray as an example



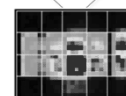
Short DNA on the chip



Expression of 13,500 genes

-4	-1	0	+1	+4
u1A	u1A	u1A	u1A	u1A
u1B	u1B	u1B	u1B	u1B
u1C	u1C	u1C	u1C	u1C
u1D	u1D	u1D	u1D	u1D

GGTGAATTATGACCTACTAT
CCACTAATACATGGATGATA
CCACTAATACATGGATGATA
CCACTAATACATGGATGATA



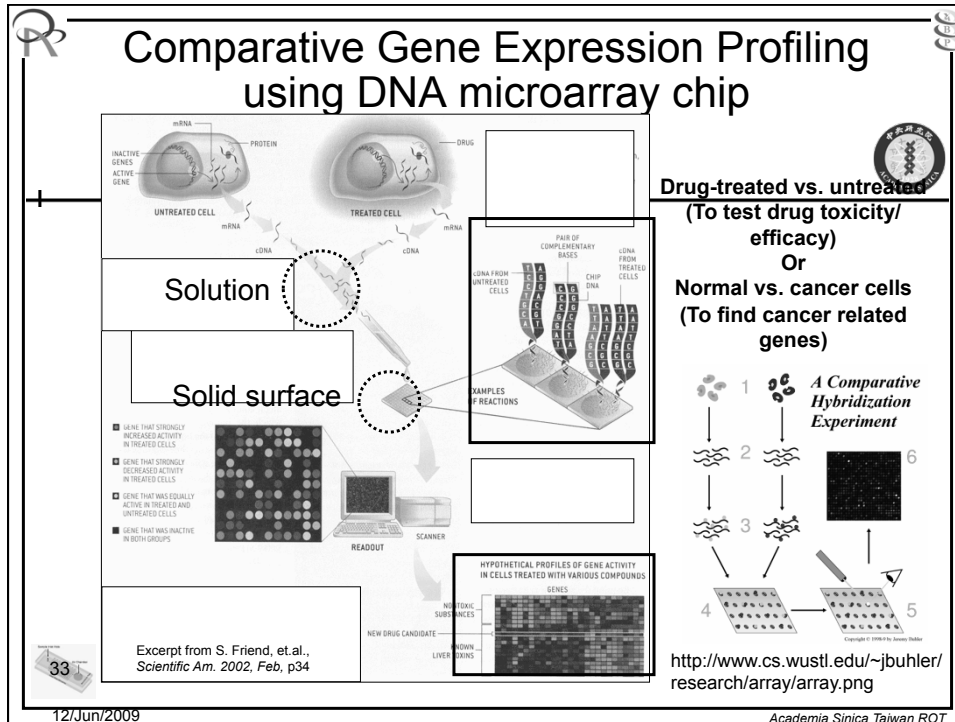
SNP detection



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www.affymetrix.com

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Drawbacks of current DNA microarray technology

- Array preparation has a high barrier by cost and/or time.
- The DNA-DNA hybridization time is long. Usually over night (more than 12 hours).
- The reaction cost is high. Usually US\$ 150 to 200 for each array.
- Concentration bias: ratio between high-level and low-level genes is exaggerated.

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Long reaction time and concentration bias of DNA hybridization

- Importance of equilibrium – theory and experiment:

Bhanot et al., (Princeton), *Biophys. J.*, 2003

C.R. Tomlinson et al., (U. Cincinnati), *Biotechnology*, 2005

Ratio of high abundant to low abundant gene is exaggerated when hybridization is not complete.

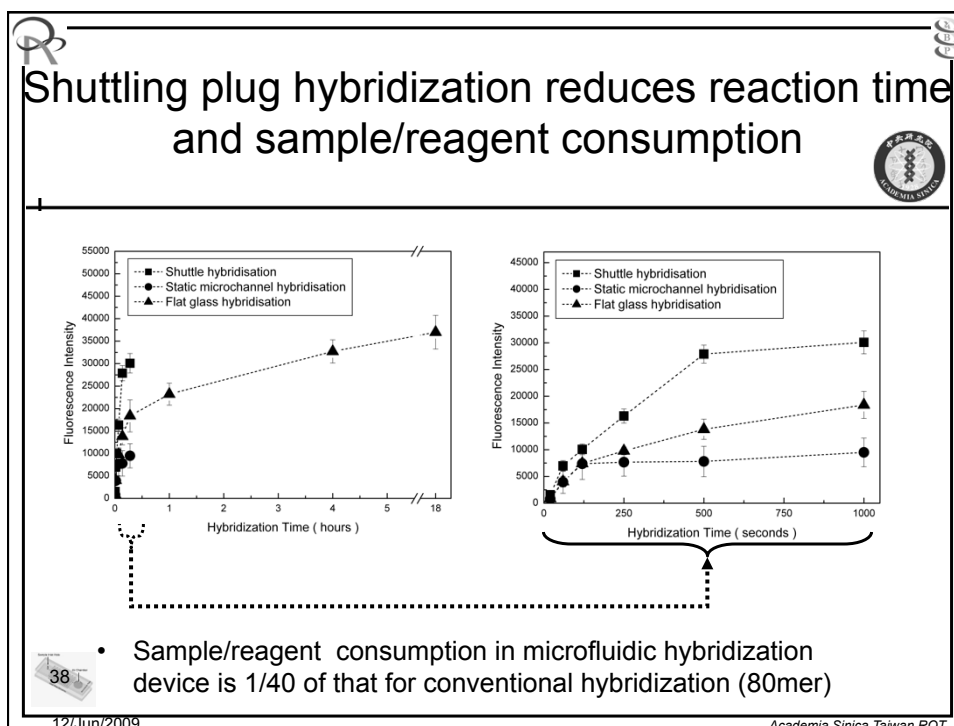
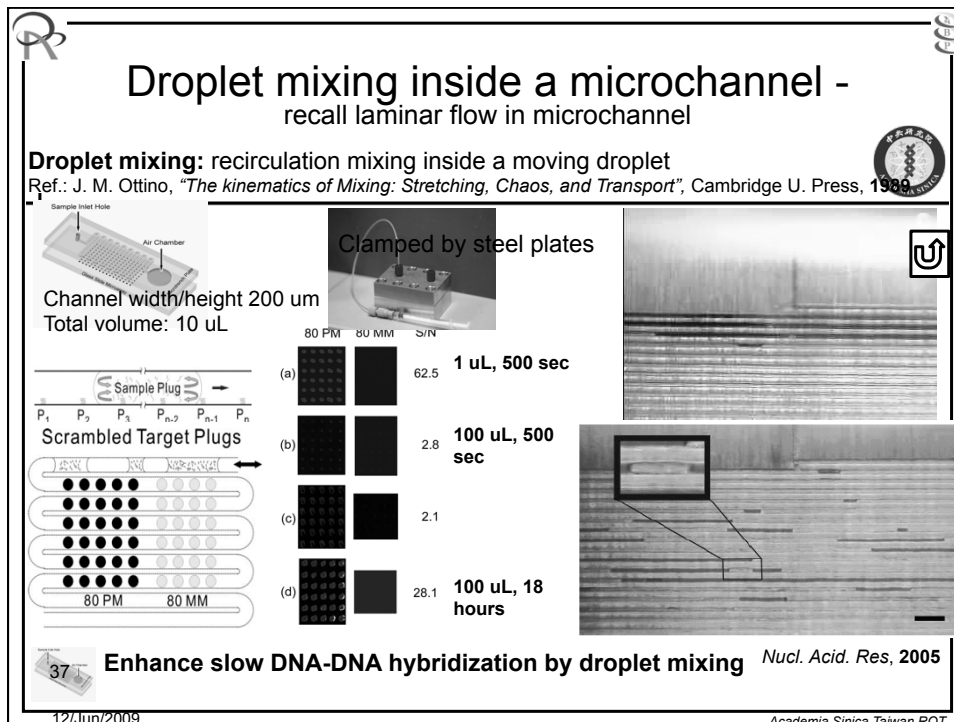
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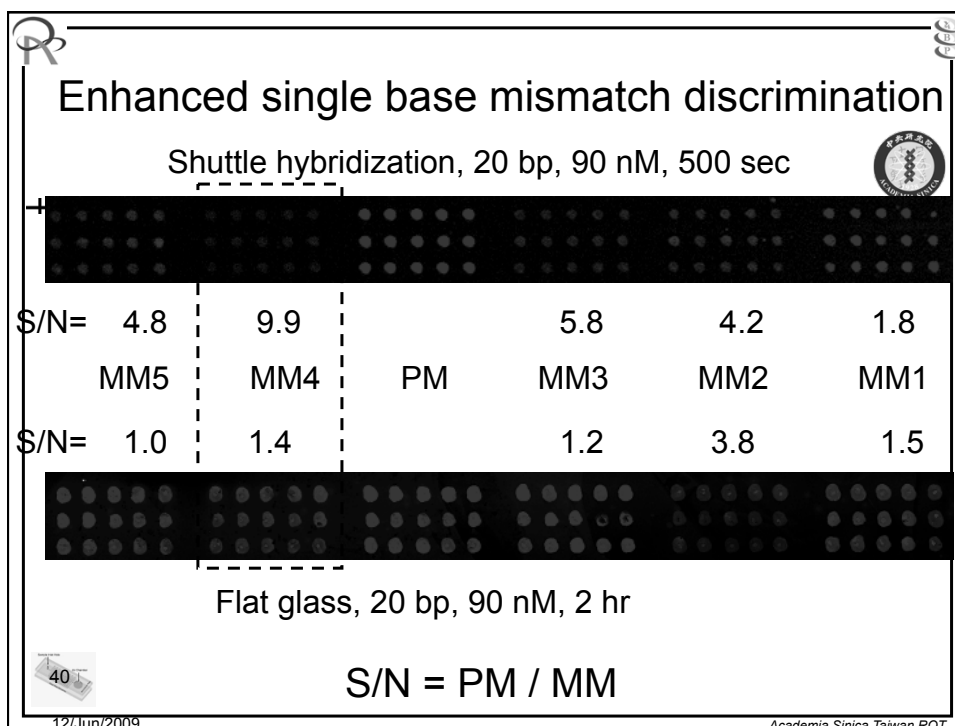
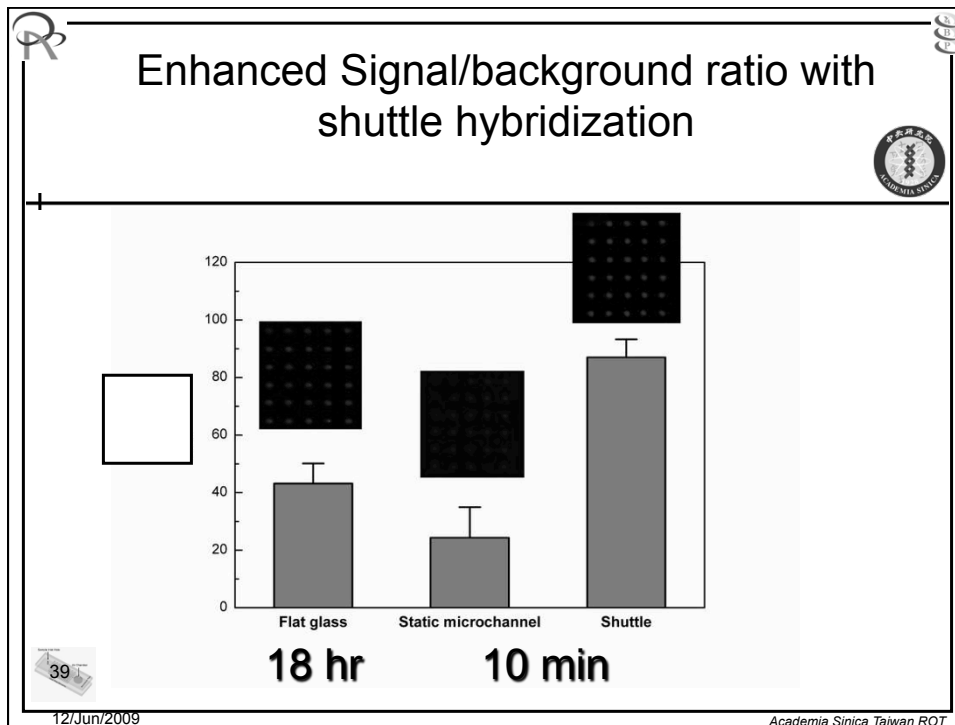
Simple hardware setup for the microarray/ microchannel integration

Nucl. Acid. Res, 2005

Clamped by steel plates

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Shuttle Hybridization

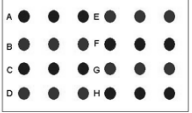
- RNA sample/cDNA probe spots

(In collaboration with Prof. 陳健尉/中興大學生醫所)

Target:
2.5 microgram
total RNA labeled
with Cy-5-dCTP.

Probes:
8 genes (A to H)
with 3 repeats.

**Hybridization
time:**
10 minutes






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Circular PCR chip with radial temperature gradient

The Analyst, p931, V130, 2005
 April, 2006, 中研院研究成果發表記者會



43

BioMicrofluidic Applications, <http://biomicrofluidics.edu.tw/22/Mar/2010>

Academia Sinica Taiwan ROT

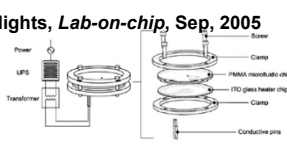


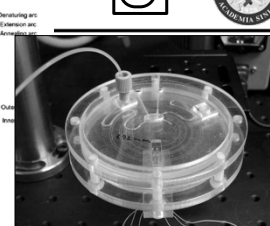

Circular PCR chip with radial temperature gradient

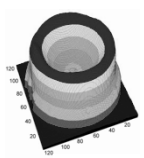
Ji-Yen Cheng*, Chien-Ju Hsieh, Yung-Chuan Chuang, Jing-Ru Hsieh, 2005, *The Analyst*, V130, p 931-940. Highlighted in Lab-on-chip, Sep, 2005.
 April, 2006, 中研院研究成果發表記者會

channel pattern is somewhat restrictive in terms of configurational flexibility (for example in the microdevice structure). To address this limitation, some researchers have utilized a 'reagent shunting' scheme, whereby reagents are moved backwards and forwards between regions held at a constant (and varying) temperature. Indeed, Ji-Yen Cheng and colleagues at the Research Center for Applied Sciences in Taiwan

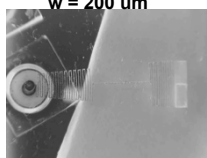
Research highlights, Lab-on-chip, Sep, 2005



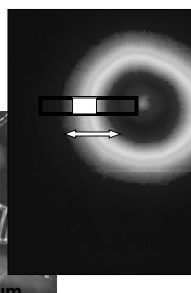




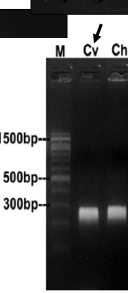
44



w = 200 um



w = 800 um



1500bp
500bp
300bp

新方法：1 小時 · 5 微升
電能消耗：3.5 W

V = 5 uL
30 cycles/1 hour

BioMicrofluidic Applications, <http://biomicrofluidics.edu.tw/22/Mar/2010>

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DNA 檢測晶片的應用與檢驗步驟



應用

1. 病原檢測，例如腸病毒，流感等病毒檢測
2. 基因型鑑定
3. 基因定序、、、等

檢驗步驟

生物檢體，
如：血液，
組織...等

樣品處理：
萃取、去雜
質、、、等。

**DNA 樣品
放大
- PCR**

特異序列檢
測



BioMicrofluidic Applications, <http://biomicrofluidics.sinica.edu.tw/22/Mex/2010>

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什麼是 PCR?

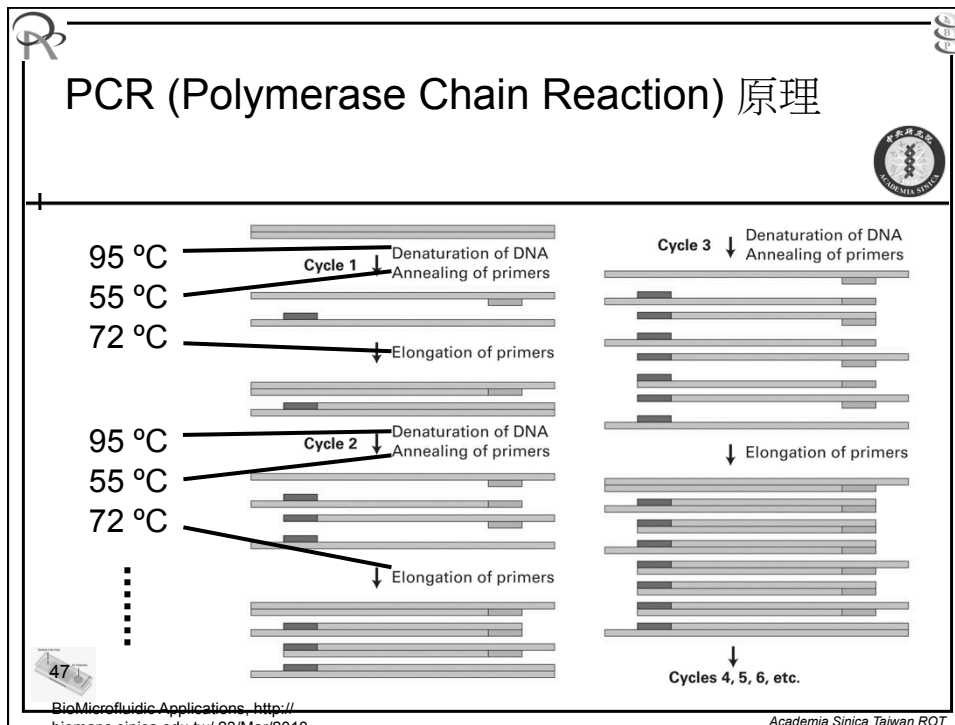


利用酵素進行反應，每次反應把 DNA 分子數目倍增，連續進行 升/降溫循環反應 之後，數目放大為 2, 4, 8, 16, ..., 2^n 倍。通常反應進行20-35次循環，數目可以放大一百萬倍。



BioMicrofluidic Applications, <http://biomicrofluidics.sinica.edu.tw/22/Mex/2010>

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進行 PCR 反應的條件：

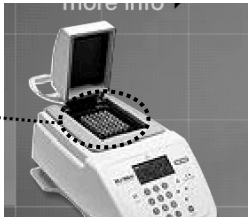
1. 試劑 (酵素 and 引子)
2. 反應槽
3. 溫度循環

BioMicrofluidic Applications, <http://biomicrofluidics.sinica.edu.tw/22/Mex/2010>

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市售的 PCR 反應器

溫度循環儀
PCR cycler ,
升溫速率 $\sim 3\text{ }^{\circ}\text{C/sec}$



升、降溫金屬塊，可裝 12 x 8 反應槽。

BioRad, <http://www.biorad.com>

反應槽
PCR tube ,
反應體積：20 ~ 100 微升 (μL)



<http://www.usascientific.com/0.2ml-seal-rite-pcr-tube-attached-dome-cap.aspx>

 49 BioMicrofluidic Applications, <http://biomicrofluidic.asia.edu.tw/22/Mex/2010>
Academia Sinica Taiwan ROT

使用晶片式反應器進行溫度循環

縮短時間
減少試劑量
減少能量消耗

 50 BioMicrofluidic Applications, <http://biomicrofluidic.asia.edu.tw/22/Mex/2010>
Academia Sinica Taiwan ROT

可攜式 DNA 放大晶片原理

微機電技術製成 環形加熱晶片 and 微流體反應槽晶片

微流體反應槽晶片

環形加熱晶片

直徑 85 mm

BioMicrofluidic Applications, <http://biomicrofluidics.asia.edu.tw/22/Mex/2010>
Academia Sinica Taiwan ROT

加熱晶片的溫度分佈

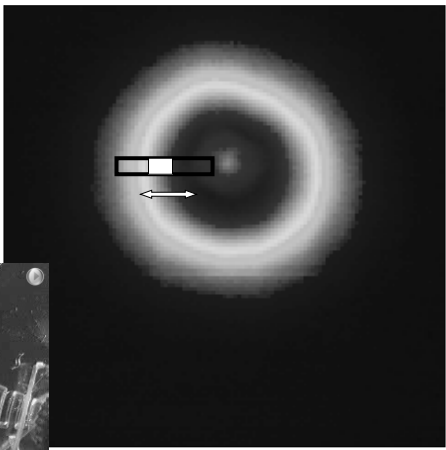
碗形 and 火山形

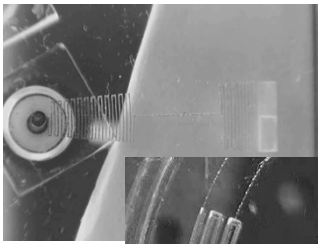
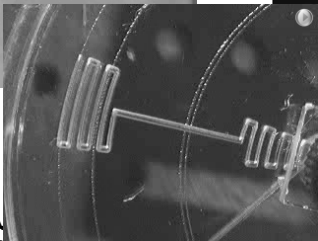
恆溫的溫度分佈

BioMicrofluidic Applications, <http://biomicrofluidics.asia.edu.tw/22/Mex/2010>
Academia Sinica Taiwan ROT

溫度循環反應的進行

- 以空間換取溫度循環
- 升溫速率：12 °C/sec
- 試劑體積：5 微升



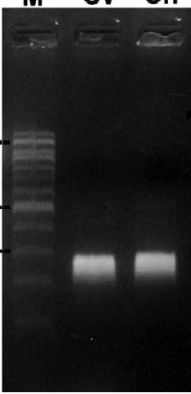





BioMicrofluidic Applications, <http://biomicrofluidics.asia.edu.tw/2004/09/04/04>
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比較傳統方法和晶片 DNA 放大 - DNA 產物分析

傳統方法：3.5 小時，100 微升



新方法：1 小時，5 微升
電能消耗：3.5 W

Suitable for utilization
in portable device

以上內容已發表於 *The Analyst*, 2005, V130, p931, 另刊載於 *Lab-on-a-Chip* 2005 年九月的 Research Highlight



BioMicrofluidic Applications, <http://biomicrofluidics.asia.edu.tw/2004/09/04/04>
Academia Sinica Taiwan ROT



More study on PCR chip?



Pipetting small volumes in microplates often leads to droplets at the sides or the rims of the wells. Advalytix uses advanced semiconductor lithography to structure the surface of the AmpliGrid slides in order to allow the specific positioning of one single microliter (1 μ L) on each of the 48 discrete reaction sites. Due to the hydrophilic nature of the reaction site, all aqueous reagents will be self-centered to the ideal position, even if not pipetted accurately, making sample handling easy and precise.

AmpliGrid AG480F

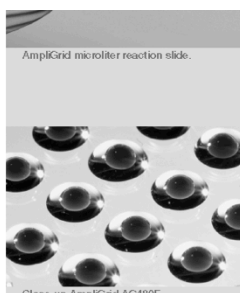
1 μ L reaction volumes can now be your 'standard', reducing your reagent and enzyme consumption by up to 25 times. Furthermore, reducing the reaction volume also increases the sensitivity and reaction efficiency by a factor of 10, at the same time reducing the required reaction time.

Pipetting errors will be a matter of the past as the flat surface provides precise optical control of sample deposition. Due to the high quality 2D-surface, any kind of optical control can be implemented to the AmpliGrid system.

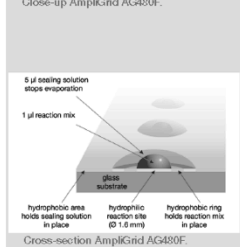
AmpliGrid slides are as easy to use for high throughput procedures, as they are for low sample numbers when working manually, since the technology is easy to integrate into most of the standard automated liquid handling systems for high throughput screening (HTS) applications.

To enable the full potential of the AmpliGrid system, Advalytix will be offering optimised kits for amplification and reverse transcription. These user-friendly kits are especially designed for the advanced, low-volume reaction geometry of the AmpliGrid slide.

<http://www.advalytix.com>



AmpliGrid microfluidic reaction slide.



Close-up AmpliGrid AG480F.



5 μ L sealing solution stops evaporation
1 μ L reaction mix
glass substrate
hydrophobic area holds sealing solution in place
hydrophilic reaction site (Ø 1.5 mm)
hydrophobic ring holds reaction mix in place
Cross-section AmpliGrid AG480F.

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BioMicrofluidic Applications, <http://biomicrofluidics.edu.tw/22/Mex/2010>

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Chemical and Biological applications of microfluidic chip in our lab



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- Rapid (500 sec) Microarray hybridization, 2005, Nucl. Acid. Res.
- DNA amplification, 2005, Analyst
- DNA microarray synthesis, 2009, Bioeng. Biotech.
- Rare cell capturing
- Microfluidic nanoslit sensor
- Sensor based on whole-cell array
- Cell culturing in controlled microenvironment
- Electrotaxis of lung cancer cell
 - Electrotaxis – short introduction
 - Multi-field chip (MFC) for electrotaxis study
 - Electrotaxis in 3D scaffold
- Wound healing mimicking

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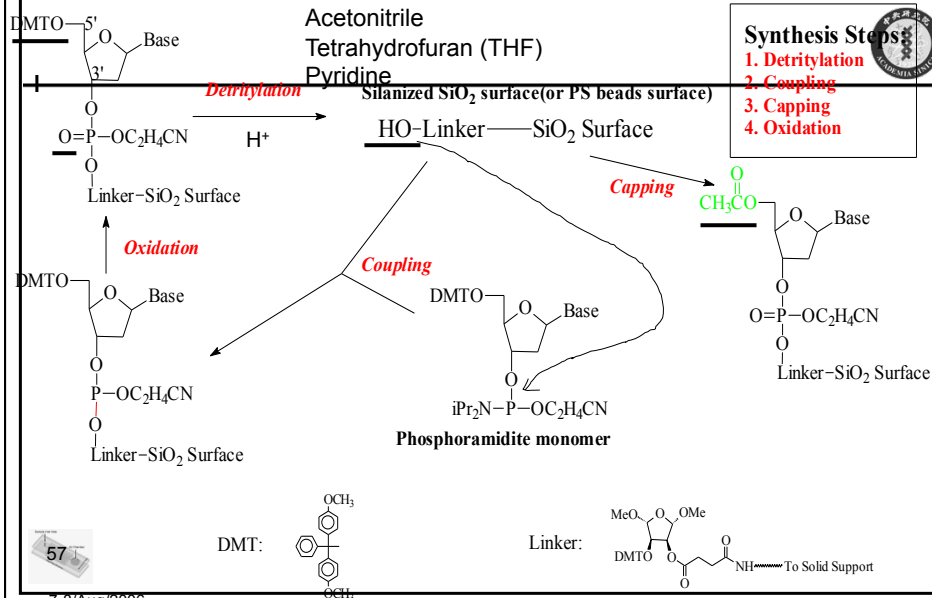
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Chemical synthesis of oligonucleotide: 3' -> 5'

Solvents:
Acetonitrile
Tetrahydrofuran (THF)
Pyridine

Synthesis Steps

1. Detritylation
2. Coupling
3. Capping
4. Oxidation



7-8/Aug/2006

Academia Sinica Taiwan ROT

Light-directed Synthesis of Oligonucleotide Array I: Photolithography

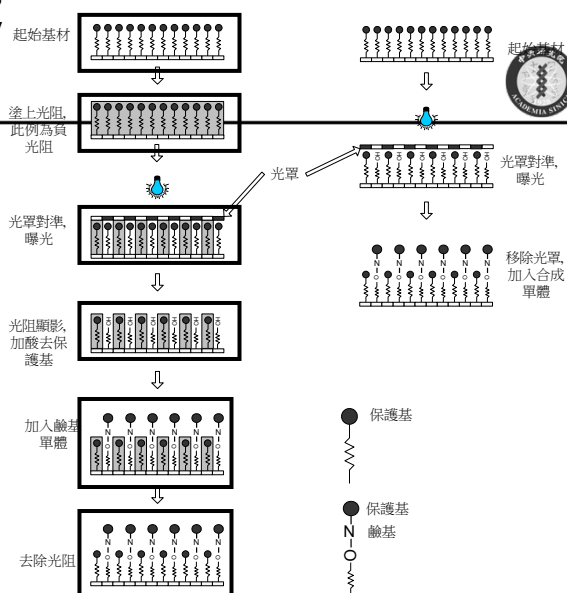
Glenn McGall et al. *Proc. Natl. Acad. Sci. USA*
v.93, p 13555, 1996.

郑邛言, 白果能, 科技新知第121期, p8, 2001

郑邛言, 白果能, 科技新知第121期, p8, 2001

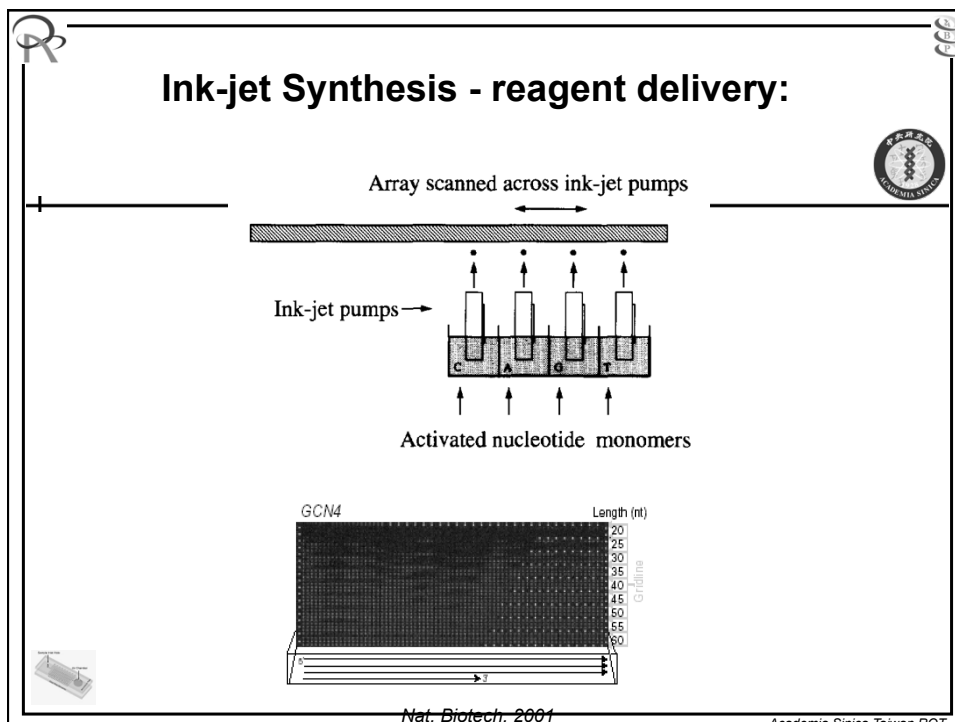
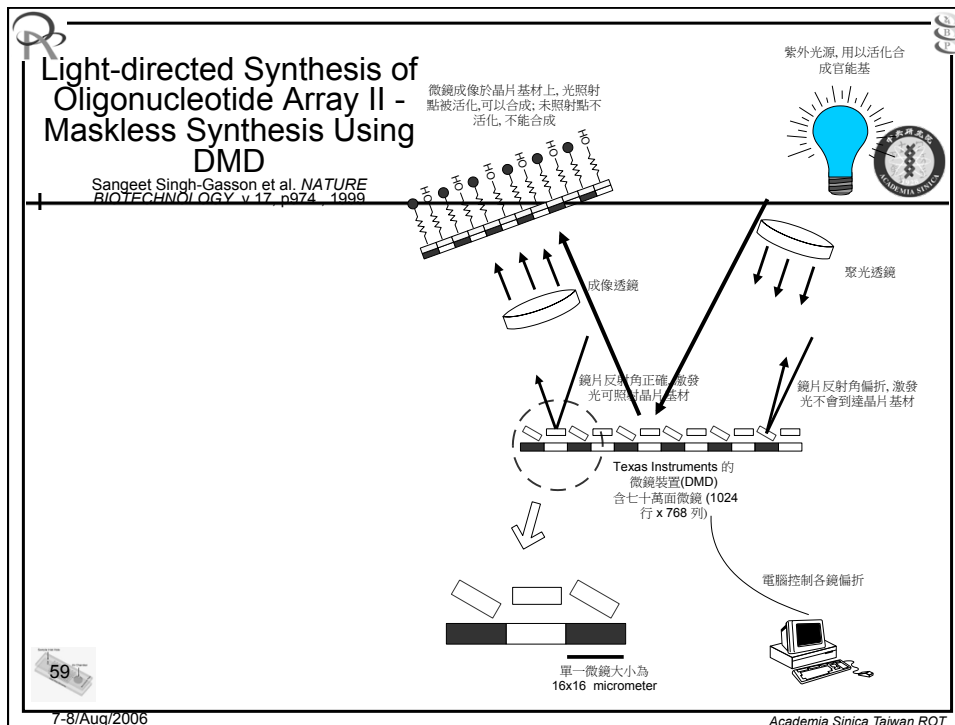
A. 有機酸去保護基

B. 光激發去保護基



7-8/Aug/2006

Academia Sinica Taiwan ROT

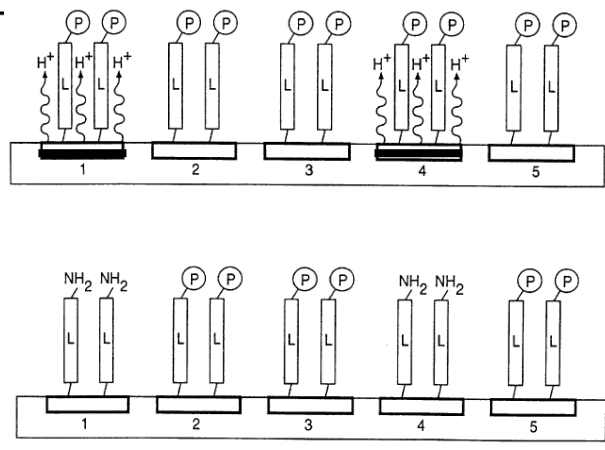




Electrochemical solid phase synthesis

D. D. Montgomery, U.S. Patent 6,093,302
<http://www.combimatrix.com/press.shtml>







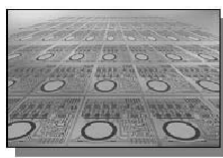
Academia Sinica Taiwan ROT



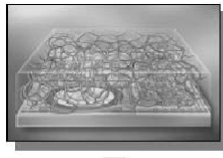
CombiMatrix's *in-situ* reactant generation

CombiMatrix





Lab-on-a-Chip.
Arrays of individually addressable microelectrodes permit synthesis of hundreds or thousands of different molecules in parallel.

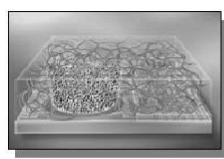


Porous Reaction Layer (PRL)
Biocompatible layer that supports the attachment of synthesized molecules.

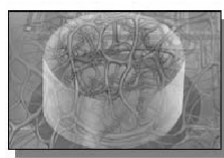


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Virtual Flask
A process that confines chemical reagents within a virtual flask over each microelectrode.



DNA Microarrays
Arrays of oligonucleotides capture molecules synthesized on the chip for use in genomics and proteomics applications.





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DLP Microfluidic Array Synthesizer (MArS)

- integrating synthesis and hybridization

Biotechnology and bioengineering, 2009

a

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BioMicrofluidic Applications, <http://biomicrofluidics.edu.tw/22/Mar/2010>

Academia Sinica Taiwan ROT

DLP Microfluidic Array Synthesizer (MArS)

Cheng*, J.-Y.; Chen, H.-Y., Microfluidic ARray Synthesizer (MArS) for Rapid Preparation and Hybridization of Custom DNA Microarray. *Biotechnology and Bioengineering* 2009, 104, 400-407

Existing technology:	Value:
1. DNA array photosynthesis by DMD (digital micromirror device).	1. The first to integrate synthesis and rapid hybridization
2. Robot for DNA microarray	2. The first to use commercial DLP for DNA synthesis
3. Expensive synthesis reagent (US\$ 650/g x 4g/day)	3. Reduce reagent by microfluidics (1 reagent for multiple slides)
4. Overnight (10-15 hrs) DNA hybridization	4. Reduce hybridization time to 15 minutes.
5. Fluorescence labeling	

b

c

Patterning by DLP

UV output from commercial projector

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BioMicrofluidic Applications, <http://biomicrofluidics.edu.tw/22/Mar/2010>

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Rare cell capturing





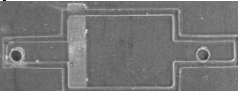
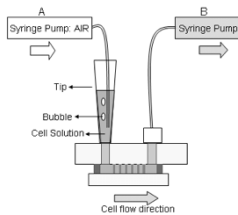
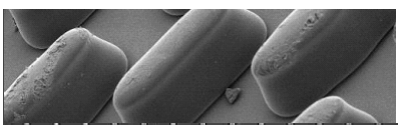
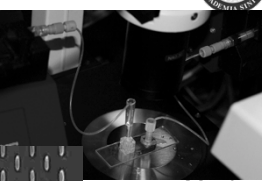
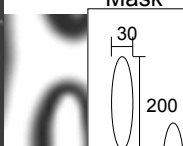
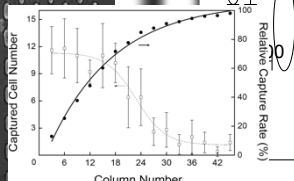
Academia Sinica Taiwan ROT



On-going: Sandwiched SU8 micropost for CL1-5 trapping -for CTC capturing project

in collaboration with Dr. 郭志禹 and 中興大學/台中榮總



height 75+/-5um
Anti-EGFR coated micropost

BioMicrofluidic Applications, Biol. Lab. 5-204, Academia Sinica

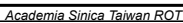
Academia Sinica Taiwan ROT



Ultra-sensitive, label-free detection of nucleic acid biomarkers and capturing of cell

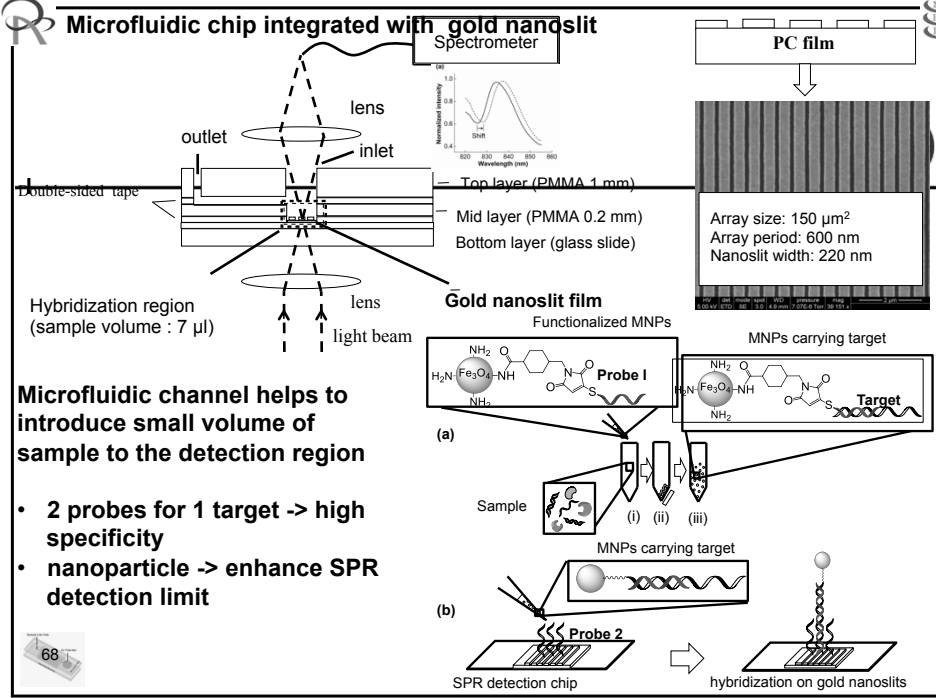
-
Applications of Magnetic nanoparticle-enhanced SPR on gold nanoslits

Mansoureh Mousavi (TIGP, 4th year)
Ji-Yen Cheng (鄭郅言), Pei-Kuen Wei (魏培坤)





Microfluidic chip integrated with gold nanoslit



Microfluidic channel helps to introduce small volume of sample to the detection region

- 2 probes for 1 target -> high specificity
- nanoparticle -> enhance SPR detection limit

Gold nanoslit film

Top layer (PMMA 1 mm)
Mid layer (PMMA 0.2 mm)
Bottom layer (glass slide)

Array size: 150 μm^2
Array period: 600 nm
Nanoslit width: 220 nm

Hybridization region (sample volume : 7 μl)

Functionalized MNPs

Probe I

Target

MNPs carrying target

Sample

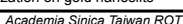
(i) (ii) (iii)

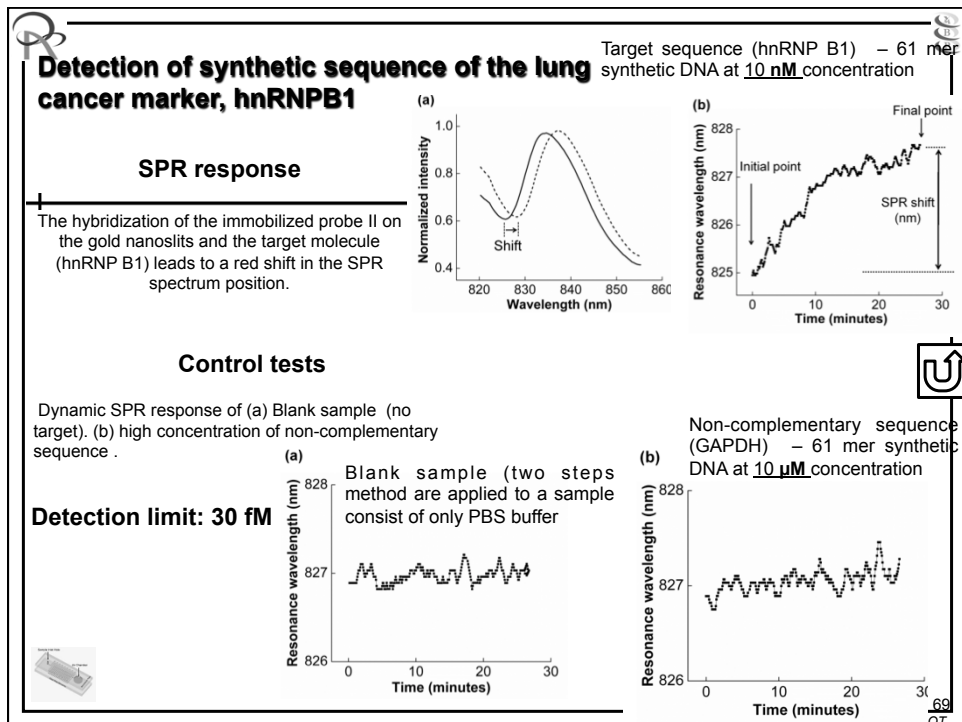
MNPs carrying target

Probe 2

SPR detection chip

hybridization on gold nanoslits





Chemical and Biological applications of microfluidic chip in our lab

- Rapid (500 sec) Microarray hybridization, 2005, Nucl. Acid. Res.
- DNA amplification, 2005, Analyst
- DNA microarray synthesis, 2009, Bioeng. Biotech.
- Rare cell capturing
- Microfluidic nanoslit sensor
- Sensor based on whole-cell array
- Cell culturing in controlled microenvironment
- Electrotaxis of lung cancer cell
 - Electrotaxis – short introduction
 - Multi-field chip (MFC) for electrotaxis study
 - Electrotaxis in 3D scaffold
- Wound healing mimicking

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Cancer metastasis and chemical/physical stimulation

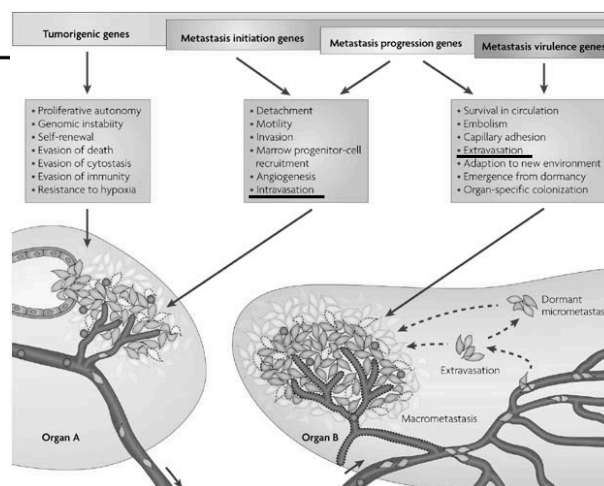


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Cancer metastasis

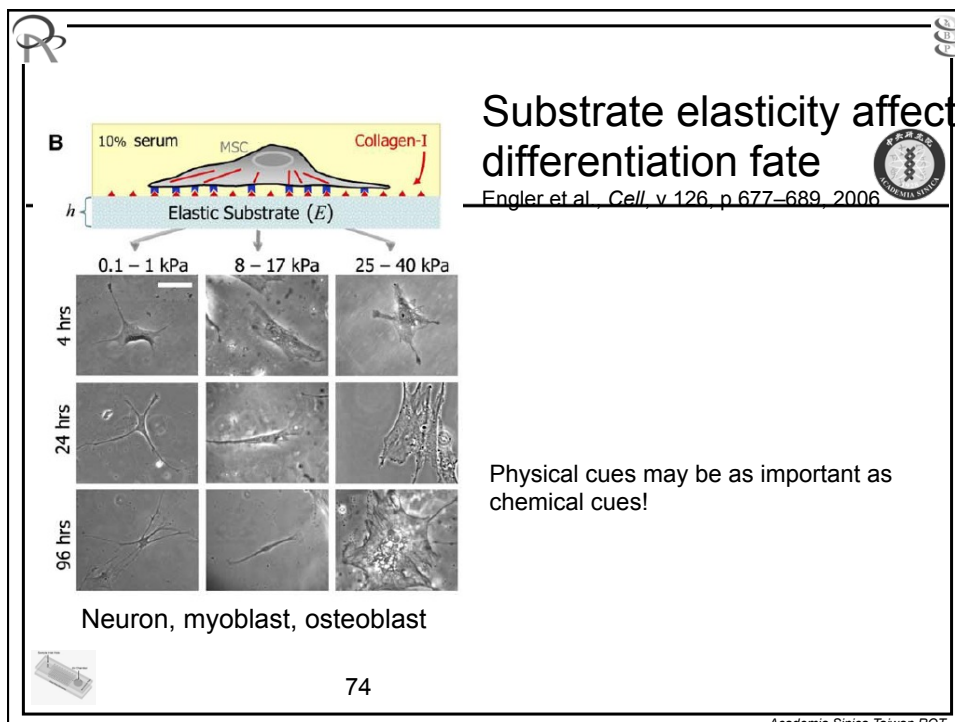
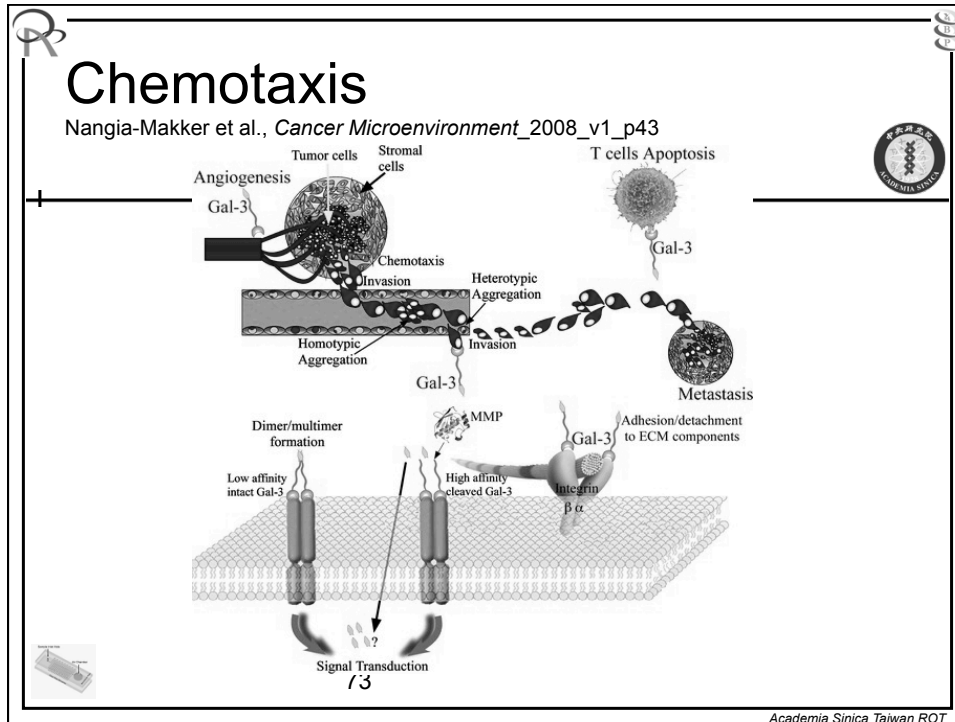
Don et al. *Nature Reviews Genetics*, v8, p341, 2007



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Nature Reviews | Genetics

Academia Sinica Taiwan ROT






Why do we study the effect of dcEF ?


75

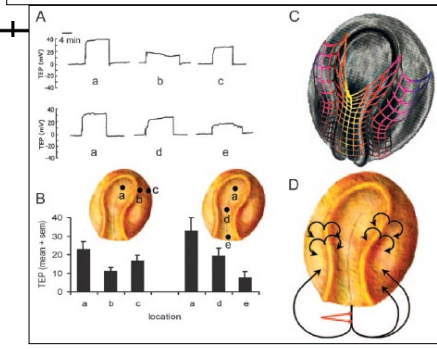

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Biological effect – *in vivo*



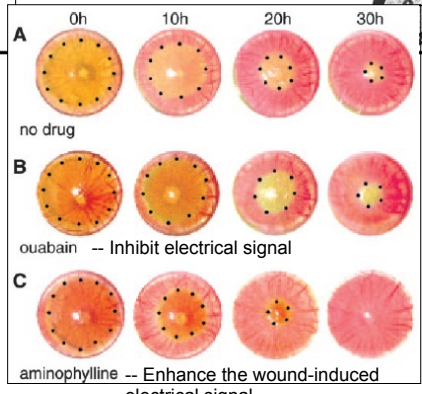
Extracellular EFs polarize the early vertebrate embryo and serve as cues for morphogenesis and pattern



Spatial differences in the transepithelial potential (TEP) generate electric fields within intact embryos.

R. Shi et al., Three-Dimensional Gradients of Voltage During Development of the Nervous System as Invisible Coordinates for the Establishment of Embryonic Pattern, *Dev Dyn.*, 202(2): 101-14, Feb. 1995

Closure of wounds is controlled by wound-induced electrical signals



Closure of wounds in rat cornea in 30hrs

B. Song et al., Electrical cues regulate the orientation and frequency of cell division and the rate of wound healing *in-vivo*, *Proc Natl Acad Sci USA* 99: 13577–13582, 2002.



C.D. MaCaig et al., Controlling Cell Behavior Electrically: Current Views and Future Potential, *Physiol Rev.*, 85(3): 943-978, July 2005



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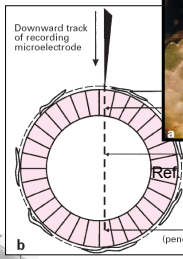
Electrical potential exists in/out a normal lumen (tube interior) in rat prostate.

Background

- TEP values vary depending on the physiological condition or the pathophysiological state of the tissue.
- The trans-lumen potential in normal rat prostate and has been measured to be ~4 mV, which corresponds to a gradient of ~400 mV/mm.
- Rat prostate cancer cell lines generally have more hyperpolarized potentials than normal cells.

Ref. Szatkowski M. et al., Electrophysiological recordings from the rat prostate gland in vitro: identified single-cell and transepithelial (lumen) potentials. *BJU Int.* 86, 1068-1075, 2000.

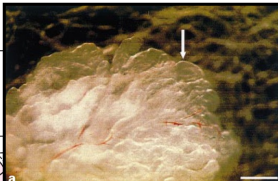


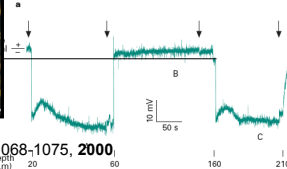


Downward track of recording microelectrode

Lumen

Epithelial cell (penetrated after lumen)





A typical profile of voltage recording from a prostatic duct.

Ref. Szatkowski M. et al., *BJU Int.* 86, 1068-1075, 2000.

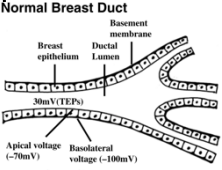
lumen potential (inside vs. outside) ~4 mV
Gradient : ~400 mV/mm

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Cancer Cell Electrotaxis under weak dcEF

Breast Adenocarcinoma

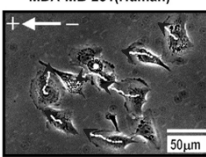
Normal Breast Duct



Apical voltage (~70mV)

Basolateral voltage (~100mV)

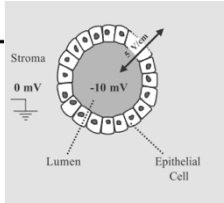
MDA-MB-231(Human)



50 μm

J. Pu et al., EGF receptor signalling is essential for electric-field-directed migration of breast cancer cells. *J. Cell Sci.*, 120(Pt 19), 3395-403, Oct. 2007

Prostate Adenocarcinoma



Stroma

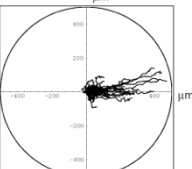
0 mV

-10 mV

Lumen

Epithelial Cell

Voltage gated sodium channel MAT-LyLu

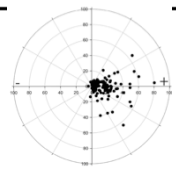


M.B.A. Djamozi, M. Mycielska, Z. Madeja, S.P. Fraser and W. Korohoda, *Journal of Cell Science*, 114, 2697-2705.(2001)

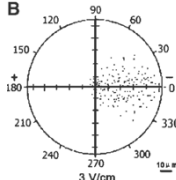
Lung Adenocarcinoma

CL1-5 (Our works)

CL1-5 300mV/mm



A549



3 V/cm

X. Yan, J. Han, Z. Zhang, J. Wang, Q. Cheng, K. Gao, Y. Ni and Y. Wang, *Bioelectromagnetics*, 30, 29-35. (2009)

Electrotaxis (galvanotaxis) is the induced directional movements of cells toward the cathode or the anode under weak DC EFs. Endogenous EF, which has strength of 10 to 100 mV/mm, has been known to play physiological roles in development and regeneration (ref. Colin D. McCaig, *Physiol Rev* 85:943-978, 2005)



Cathode ————— Anode

If cells migrate:

- toward anode, $\sum \cos \theta \rightarrow -1$
- toward cathode, $\sum \cos \theta \rightarrow 1$
- randomly, $\sum \cos \theta \rightarrow 0$

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Electrotaxis of cancer cell - literature

Cancer type	Cell line	Metastasis ability	Electrotaxis direction	Directedness $\cos\theta$	Ref.
Human breast cancer cell	MDH-MB-231	high	anode	-0.75 (300mV/mm)	
Rat breast cancer cell	MTLn3	high	anode	-0.72 (50mV/mm)	(2)
	MTC	weak	anode	-0.24 (50mV/mm)	
Rat prostate cancer cell	MAT-LyLu	high	cathode	0.82 (300mV/mm)	(3)
	AT2	weak	anode	-0.34 (300mV/mm)	
Human lung cancer cell	A549	high	cathode	0.76 (300mV/mm)	(1)

1. X. Yan, J. Han, Z. Zhang, J. Wang, Q. Cheng, K. Gao, Y. Ni and Y. Wang, "Lung cancer A549 cells migrate directionally in DC electric fields with polarized and activated EGFRs", *Bioelectromagnetics*, **30**, 29-35.(2009)

2. J. Pu, C.D. McCaig, L. Cao, Z.Q. Zhao, J.E. Segall and M. Zhao, "EGF receptor signalling is essential for electric-field-directed migration of breast cancer cells", *Journal of Cell Science*, **120**, 3395-3403.(2007)

3. M.B.A. Djamgoz, M. Mycielska, Z. Madeja, S.P. Fraser and W. Korohoda, "Directional movement of rat prostate cancer cells in direct-current electric field: involvement of voltage-gated Na⁺ channel activity", *Journal of Cell Science*, **114**, 2697-2705.(2001) – The 1st in cancer cell electrotaxis study.



If cells migrate:
toward anode, $\sum \cos\theta \rightarrow -1$
toward cathode, $\sum \cos\theta \rightarrow 1$
randomly, $\sum \cos\theta \rightarrow 0$


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Summary (our hypothesis):

Physical stimulation (dcEF) may also be a cue for cancer invasion




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Cell culturing in controlled microenvironment

M & M:
When Microfluidics meets Microscopy

Goal: long-term and real-time observation of cells under microcontrolled environment. Two specific topics:

1. chemical gradient
2. weak DC electric field

Both are closely related to cancer metastasis



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Chemical gradient in microfluidic chip



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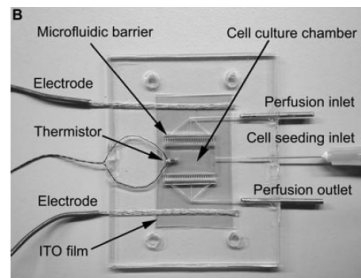
Real time observation of Cell culturing - existing technology

From: <http://www.tokaihit.com>



Stage-top incubator:
Can not manipulate microenvironment
-> microfluidic devices provide:

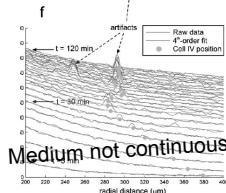
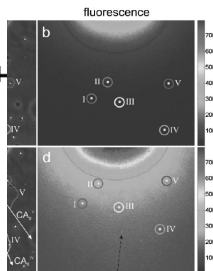
1. Chemical gradient
2. Electric field



BioTechniques v40, p368, 2006, Petronis et al.

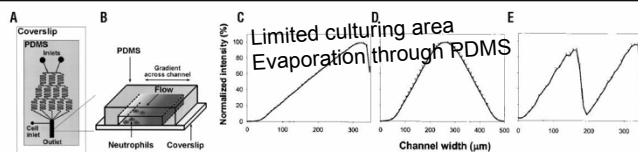
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Chemical gradient generation in microenvironment - literature works



Medium not continuously refreshed

Effusion between chambers
generates radial gradient, *Lab-on-
chip*, V6, p849, 2006, Frevert et
al.



Channel array generates complex gradient, *Nature Biotech*,
V20, p826, 2002, Jeon, Whitesides, Toner et al.

Limitations:

Diffusion dominated gradient distribution
Maximal gradient limited by diffusion
Small culturing area

Solution:

Combine diffusion with lateral flow

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Chip with chemical gradient generation for cell culturing - configuration

(a)

(b)

Temp. control heater: 16 ohm, 4.5 V, ~50 % cycle, ~0.65 W.

Major considerations:

1. Nutrition
2. Temperature
3. Chemical inlet

With DEP facilitated cell positioning

Legend:

- Cover glass
- Patterning ITO glass
- PET double-side tapes
- PMMA or glass

ITO: Indium Tin oxide

Shortcut to On-stage

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A device for chemical gradient generation and cell culturing – photo pictures

Biomechanics, 2008, V2, 024105, selected in *Virtual journal of Biological Physics Research*
Lab-on-chip, 2009, V9, p884 – selected as hot article

System for long-term cell culture on a microscope

CCD camera
 Microscope
 Cell images recorded
 Medium outlet
 Reagent inlet
 Medium inlet
 Thermal sensor
 Heater (ITO glass)
 Syringe pump
 PID temp. controller

(a)

CV=3%
 CV=1.7%

Successfully cultured

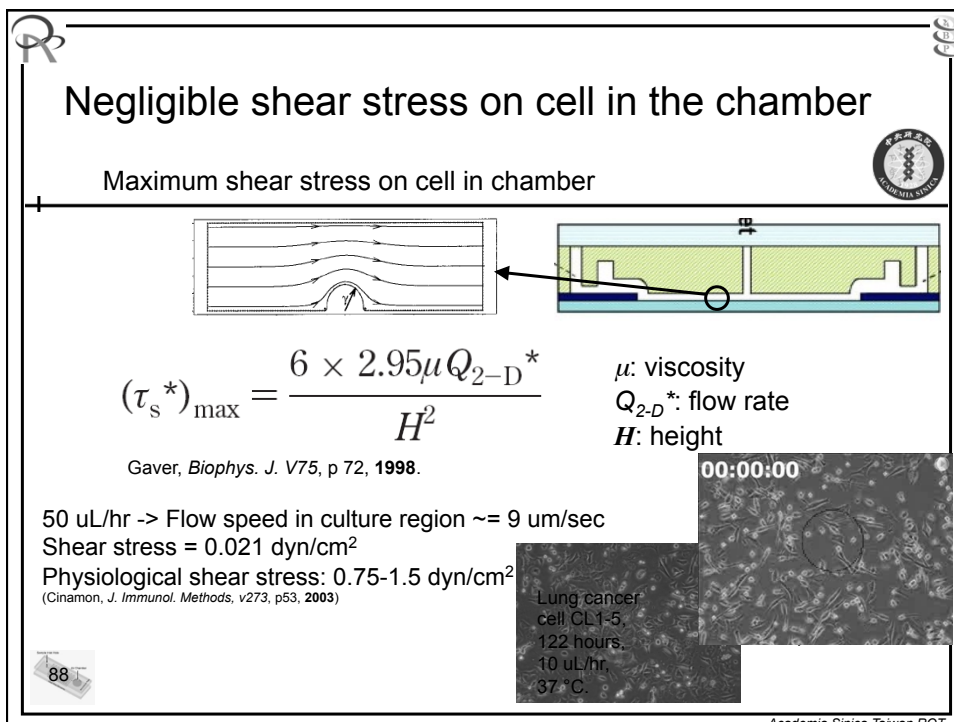
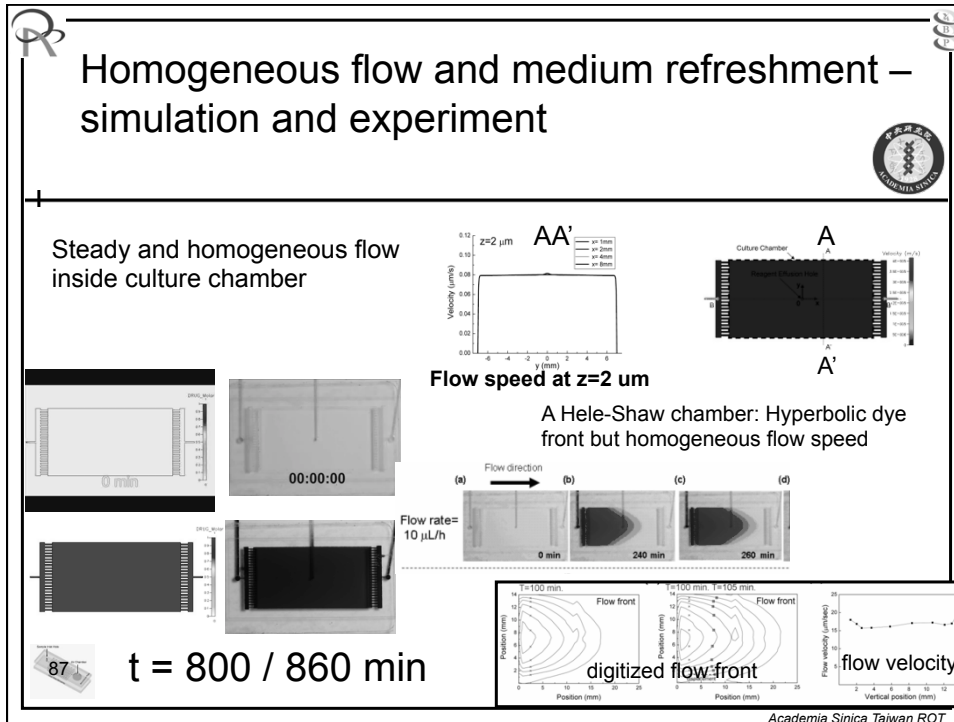
- CL 1-0
- CL1-0/GFP-actin
- CL 1-5
- CL1-5/GFP-actin
- SH-SY5Y
- MRC-5
- PC-12
- HSC-3
- HepG2
- A-549
- A-431
- HEK-293[293]
- NIH/3T3

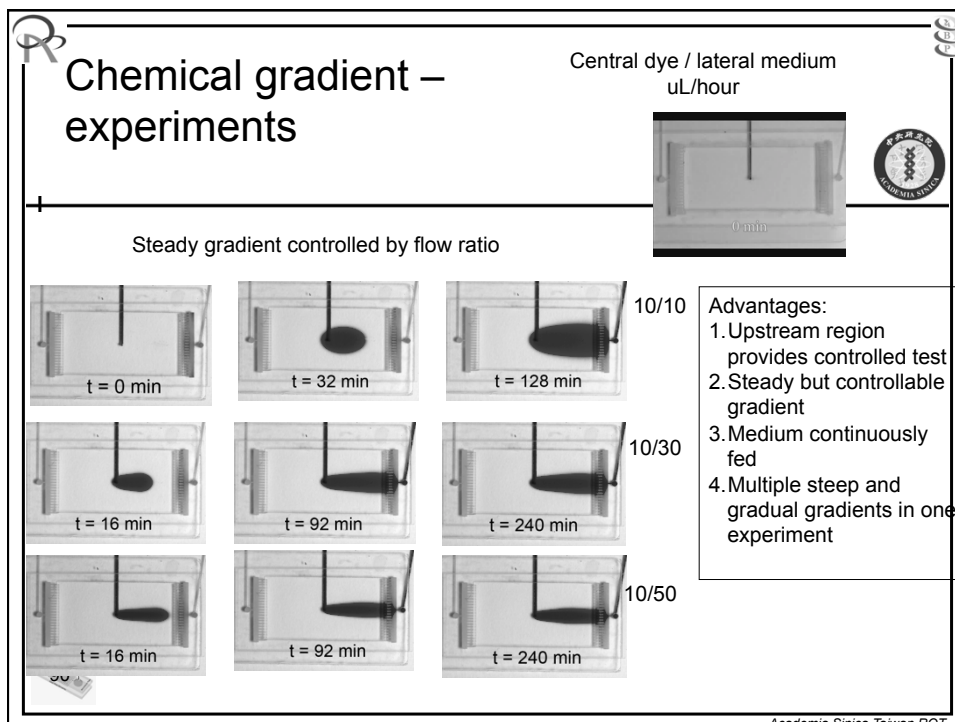
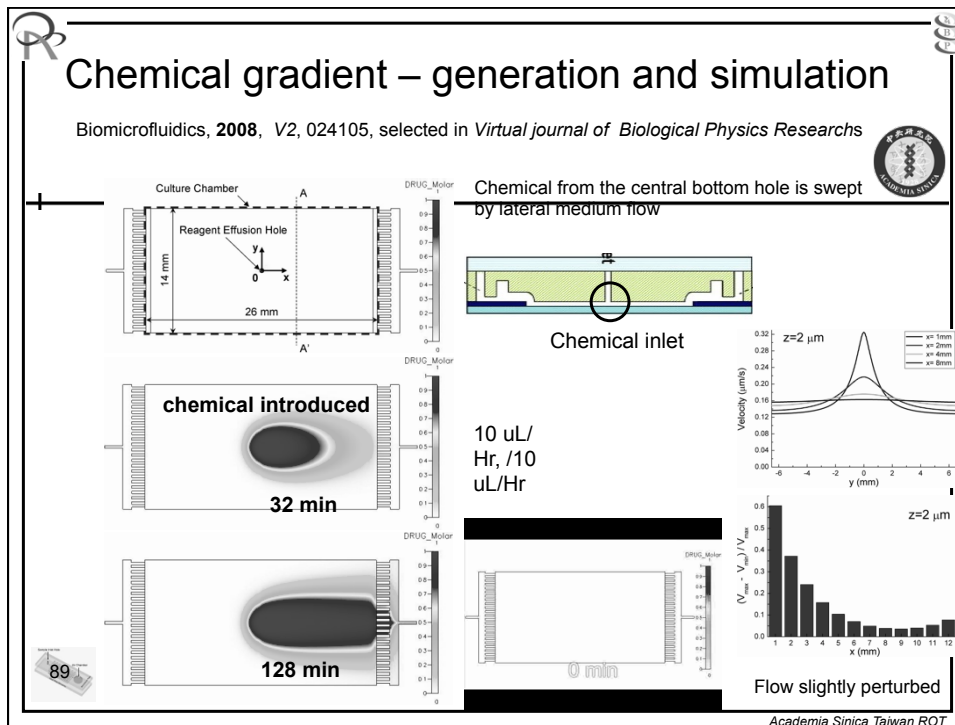
00:00:00

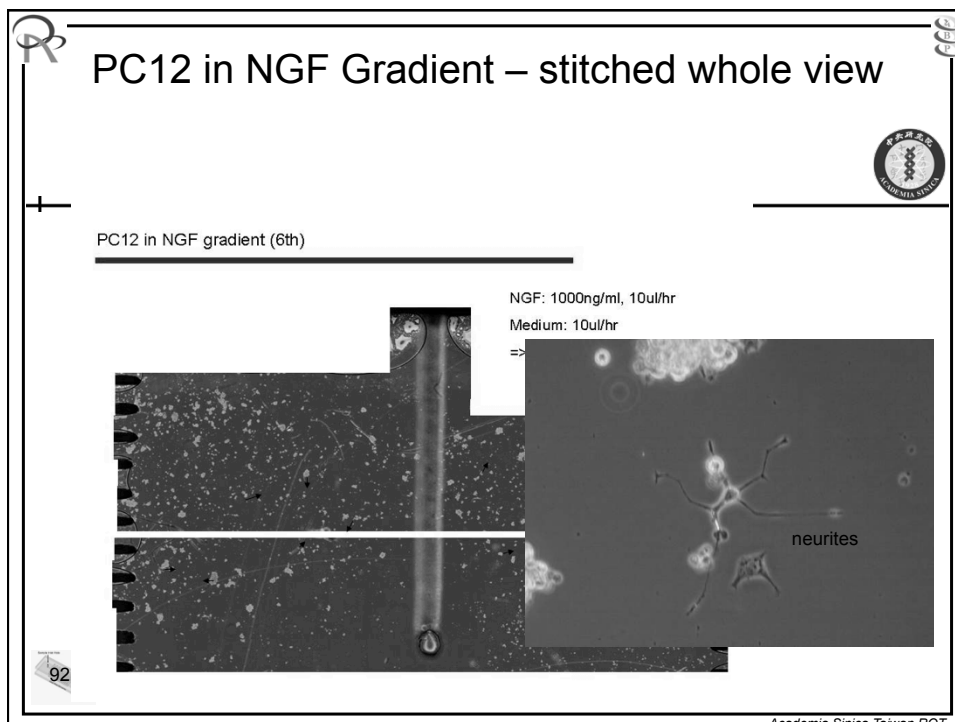
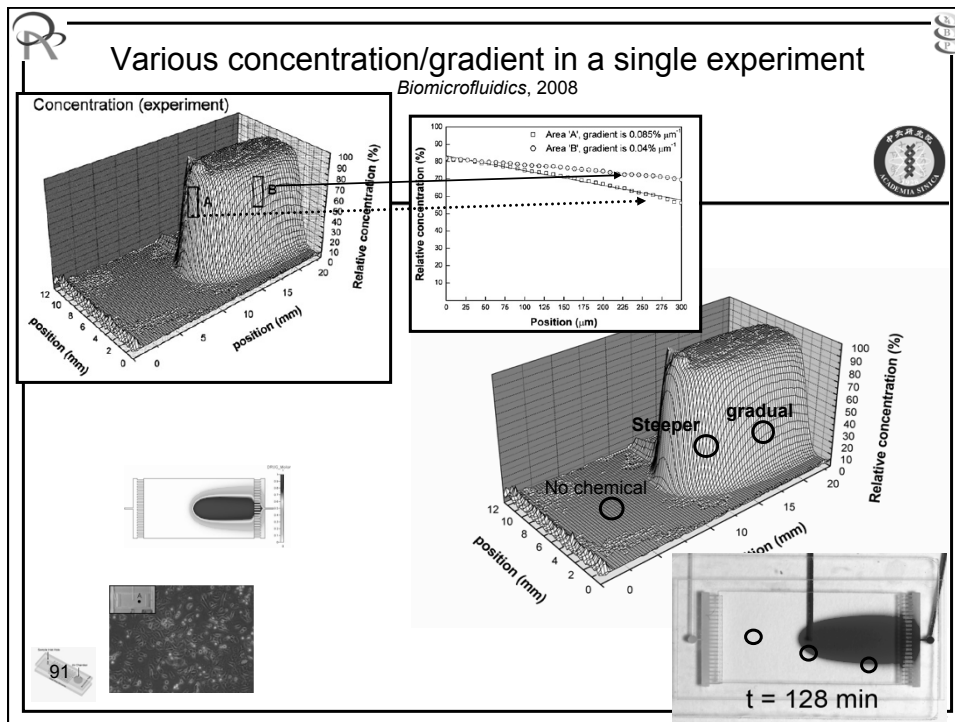
Lung cancer cell
 CL1-5,
 122 hours,
 10 uL/hr,
 37 °C.

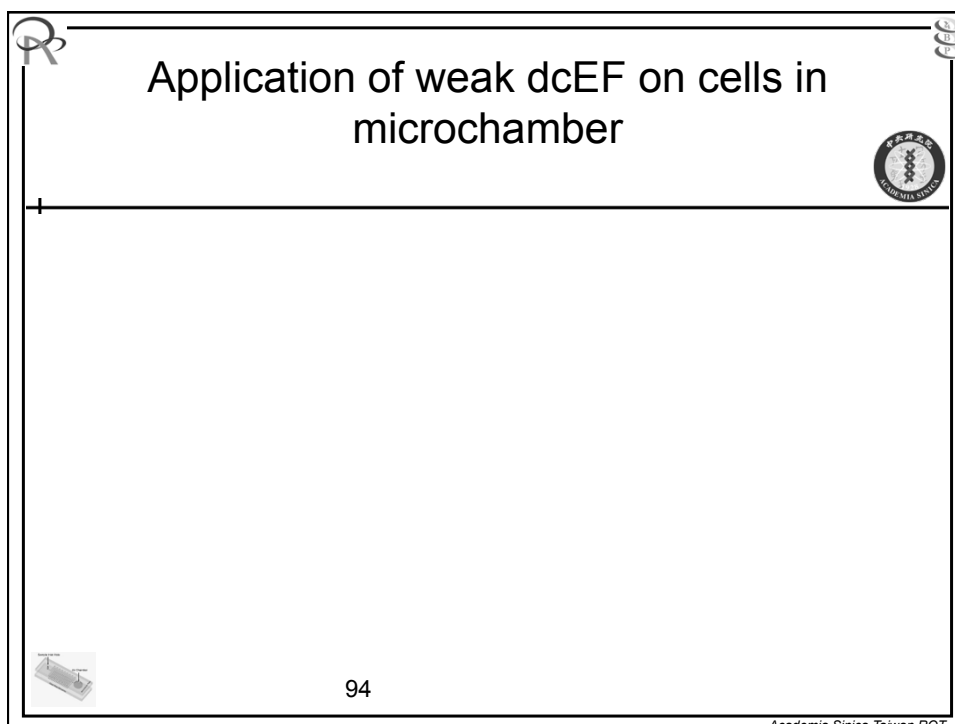
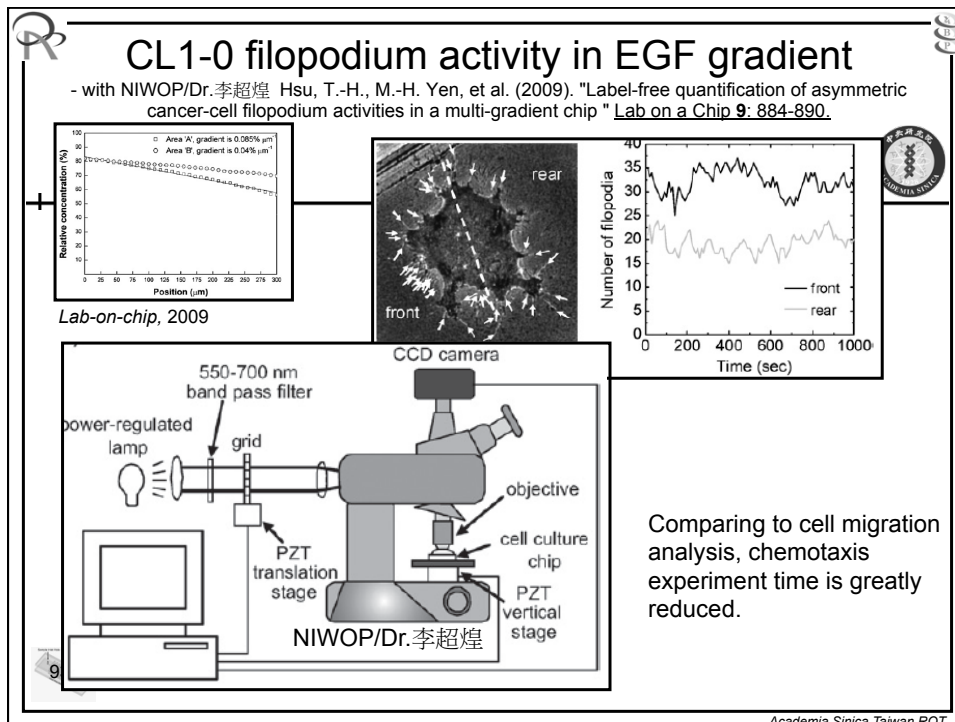
86

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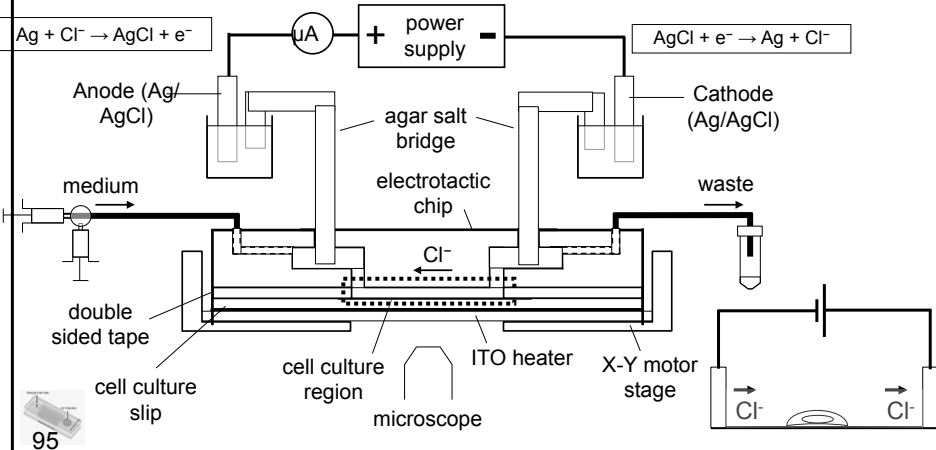






Our Electrotaxis Device – Ag/AgCl

electrodes is used to introduce constant current and EF
 C.-W. Huang, J.-Y. Cheng*, M.-H. Yen and T.-H. Young, "Electrotaxis of lung cancer cells in a multiple-electric-field chip", *Biosensors and Bioelectronics* **24**, 3510-3516.(2009)

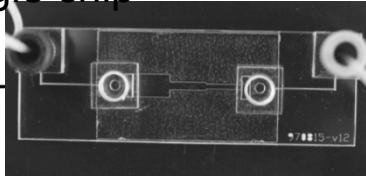
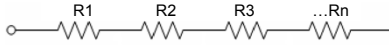
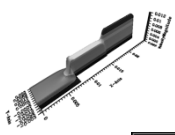
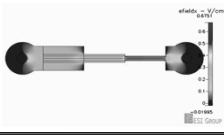
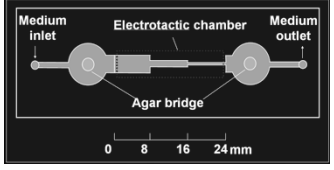



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Advantage of using Microfluidic – multiple EF in a single chip

Advantages:

1. airtight-sealed
2. negligible Joule heating
3. Multiple EF strengths

$$V_{R1} = \frac{V \times R1}{\sum Ri}$$

$$R = \frac{l}{\sigma \cdot W \cdot h}$$

Huang, C.-W., J.-Y. Cheng*, M.-H. Yen and T.-H. Young (2009). "Electrotaxis of lung cancer cells in a multiple-electric-field chip." *Biosensors and Bioelectronics* **24**: 3510-3516.

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Morphological response of lung cancer cell under dcEF



- Migration
- Orientation
- Assymetric filopodia activity (with Dr. 李超煌)



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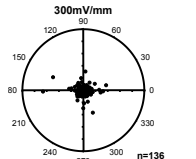
Cell response - migration

- response of cells with different metastasis potential

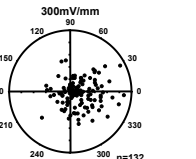


Huang, C.-W., J.-Y. Cheng*, M.-H. Yen and T.-H. Young (2009).
 "Electrotaxis of lung cancer cells in a multiple-electric-field chip."
Biosensors and Bioelectronics 24: 3510-3516.

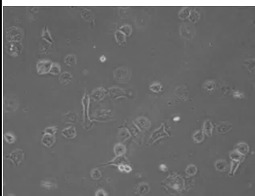
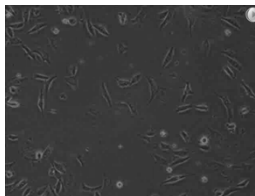
← - + → , two hours



CL1-0



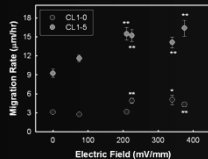
CL1-5

300 mV/mm, 7 hours

Response 1: Cell Migration under EF

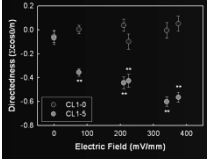
When the applied EF increases, migration rate of CL1-5 trends up while CL1-0 shows no obvious correlation.



Migration rate = cell displacement (μm) / EF treating time (hr)

Response 1: Cell Migration under EF

Under the applied EF, CL1-5 migrate toward the anode while CL1-0 have no obvious response.

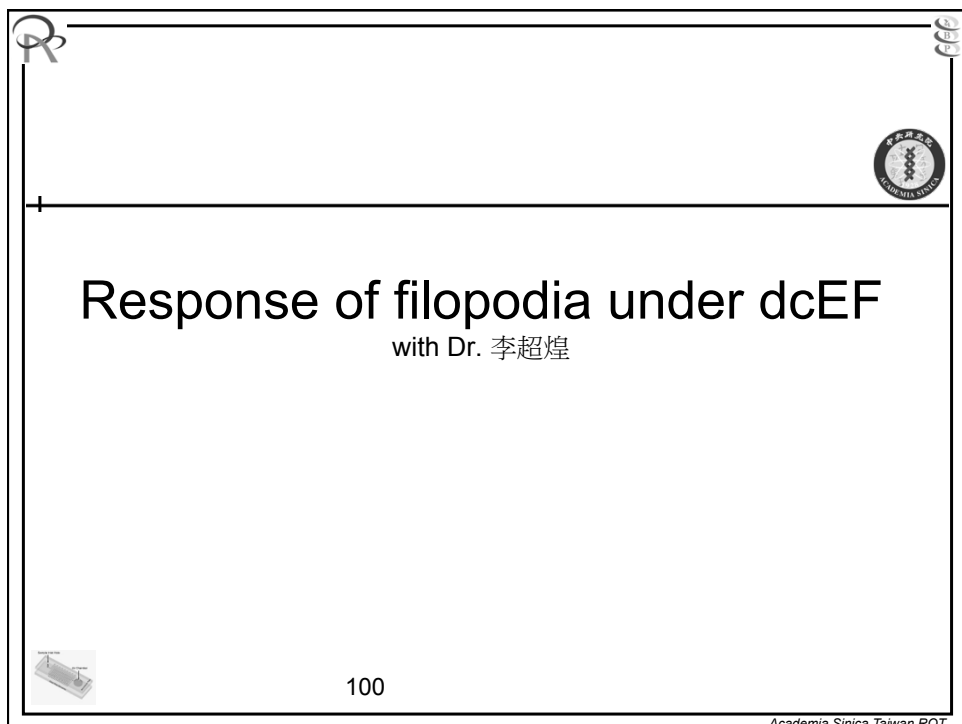
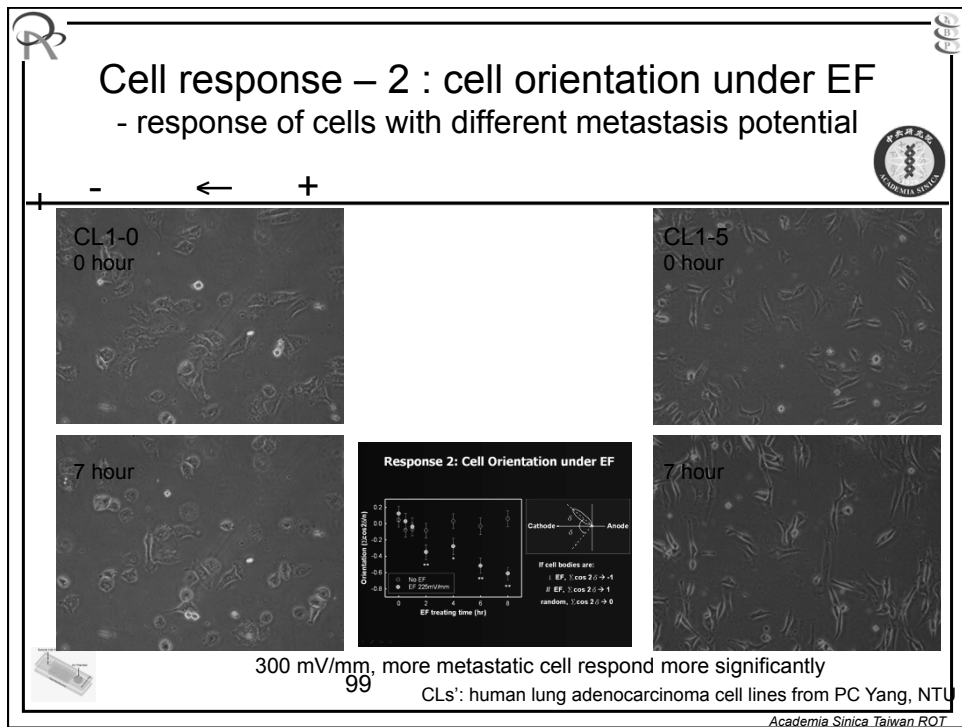




If cells migrate:
 toward anode, $\gamma \cos \theta \rightarrow -1$
 toward cathode, $\gamma \cos \theta \rightarrow 1$
 randomly, $\gamma \cos \theta \rightarrow 0$



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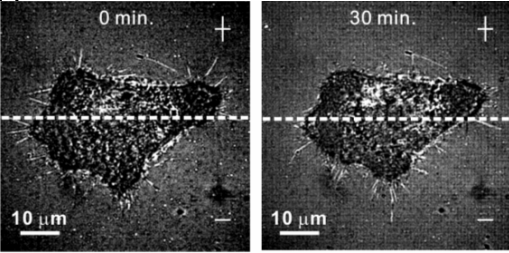




Asymmetric distribution of filopodia under dcEF

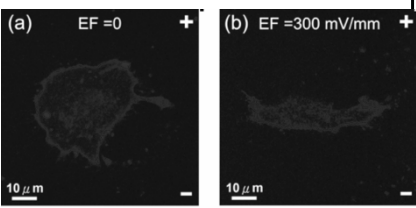
Wang, C.-C., Y.-C. Ka, P.-Y. Chi, C.-W. Huang, J.-Y. Lin, C.-F. Chou, J.-Y. Cheng* and C.-H. Lee* (2011). "Asymmetric cancer-cell filopodium growth induced by electric-fields in a microfluidic culture chip." *Lab on a Chip* 11: 695–699.





0 min. 30 min.

10 μ m



(a) EF = 0 (b) EF = 300 mV/mm

10 μ m

Super-resolution SINAP images of a CL1-5 lung cancer cell. (a) Before the treatment of EFs. (b) After the treatment of a 180 mV/mm EF for 30 minutes.

Distributions of EGFRs labeled by Cy5 of two CL1-5 cells (a) before and (b) after the treatment of a 300 mV/mm dcEF.

Distribution of protein IS involved

?? Migration toward +, filopodia/EGFR polarized toward -



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Improvement of MFC chip

Hsieh-Fu Tsai, Shih-Wei Peng, Chun-Ying Wu, Hui-Fang Chang, and Ji-Yen Cheng
Biomechanics 6, 034116 (2012); doi: 10.1063/1.4749826



- In MFC, multiple electric fields were created by manipulating cross-sectional area
- Drawbacks:
 1. Flow velocity increases with increasing electric field
 2. Inhomogenous cell distribution
 3. Require separate control experiments (EF = 0)

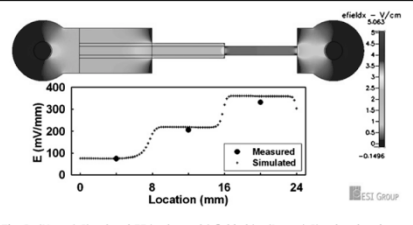
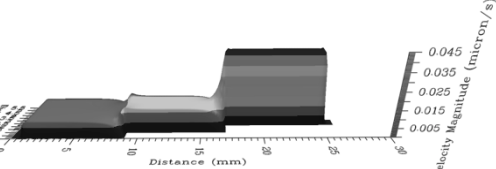


Fig. 3. (Upper) Simulated EF in the multi-field chip. (Lower) Simulated and measured E along the channel. The measured EF strength is 74 ± 0.1 , 205 ± 0.8 and 331 ± 1.3 mV/mm. The error bars are too small to be seen in the figure.



Velocity Magnitude (micron/s)

Distance (mm)



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Aims

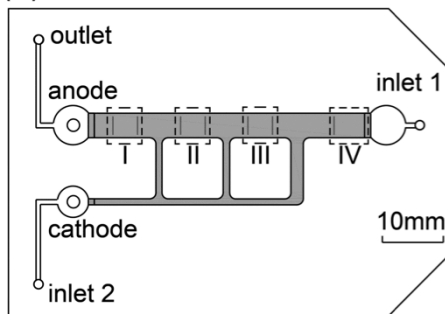
- Network circuit design to decouple the flow field and the electric field.
- Improve experimental throughput



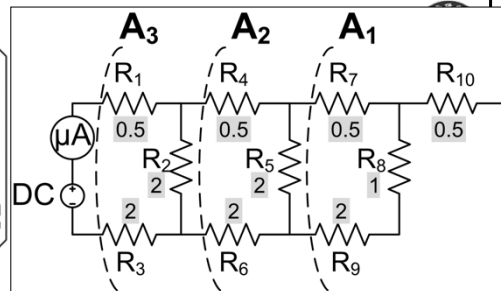
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Chip design

(a)



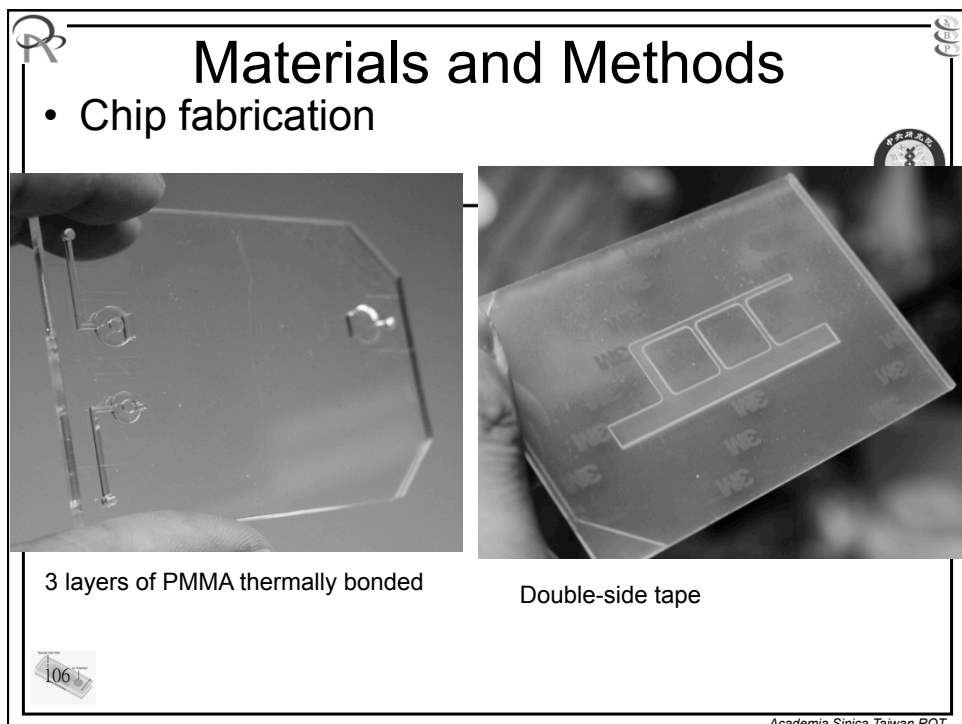
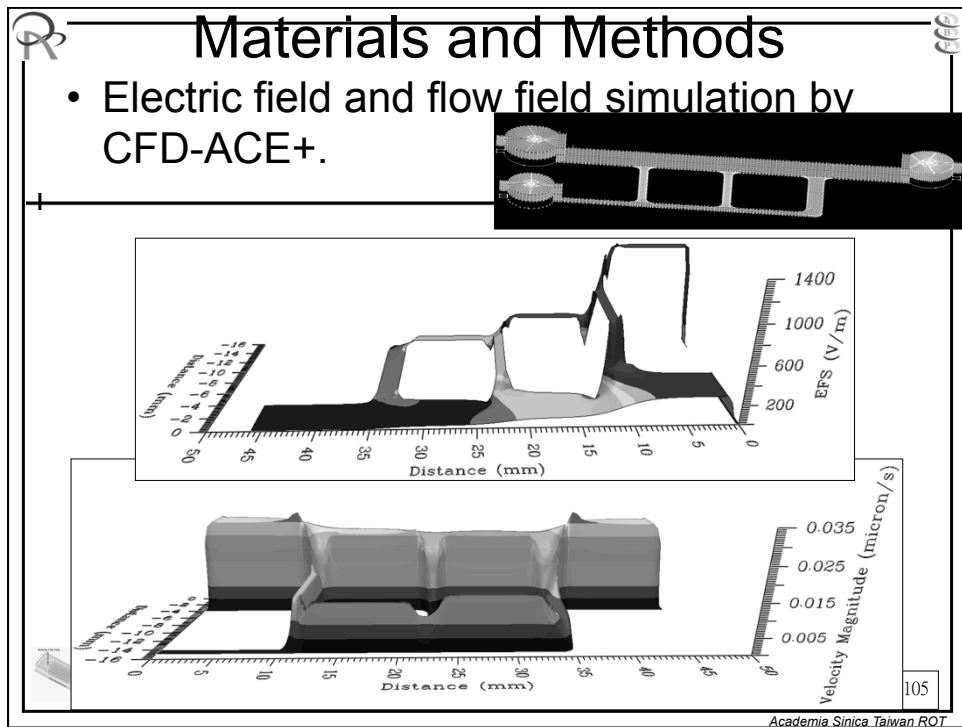
■ cell culture area
□ observation region

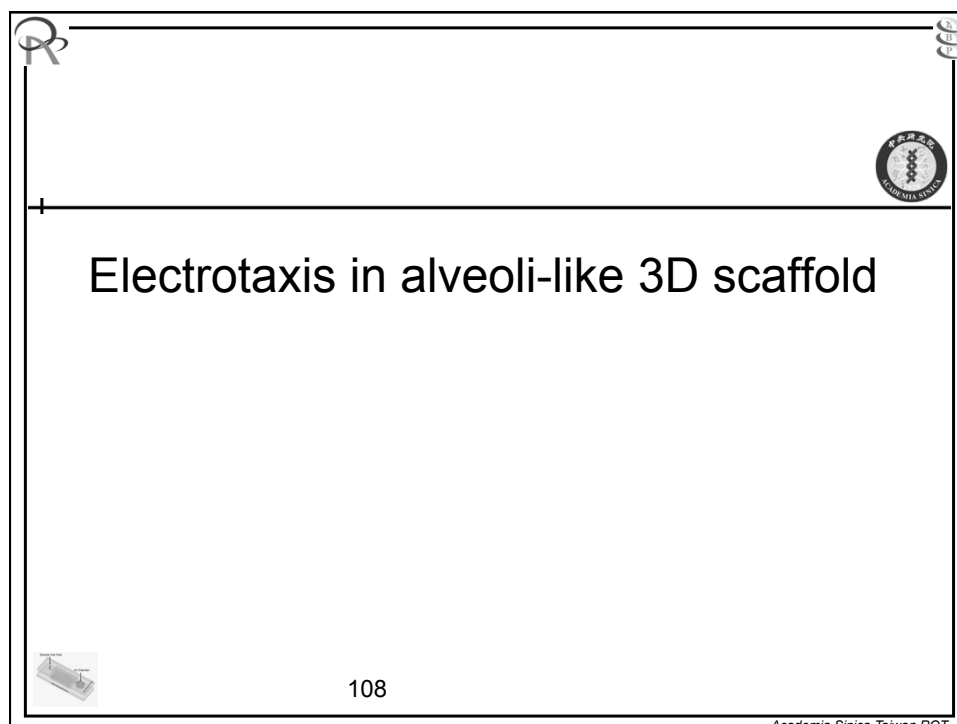
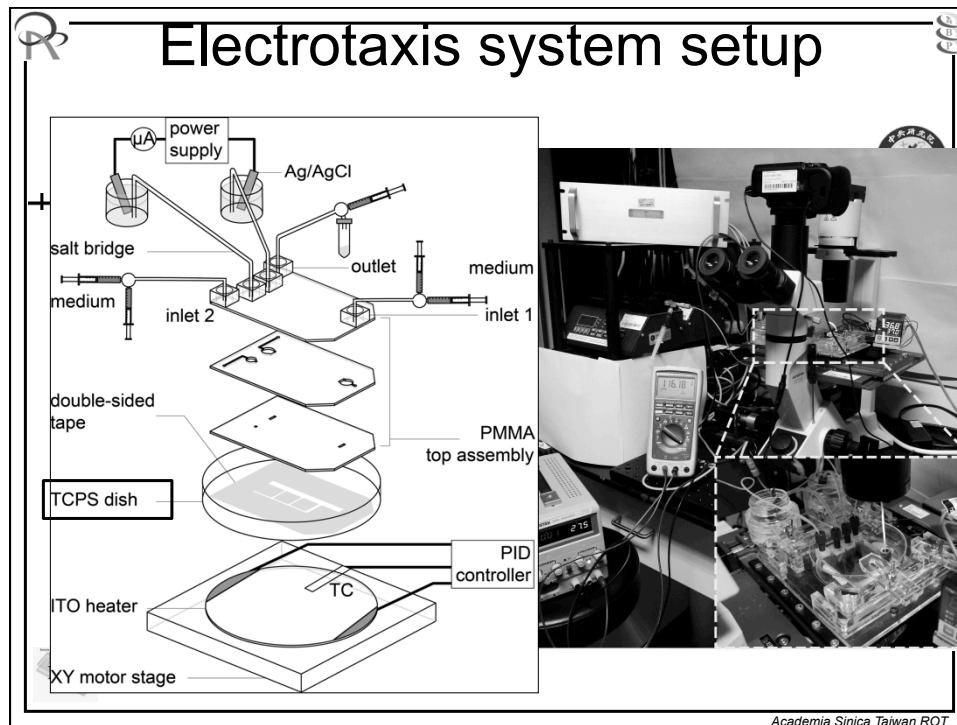


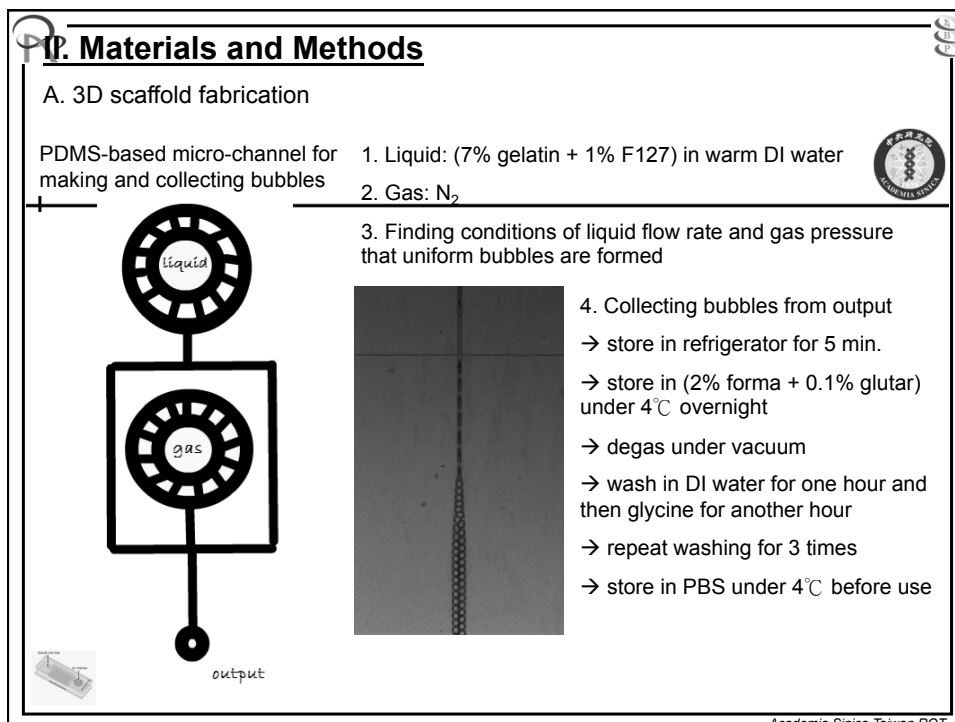
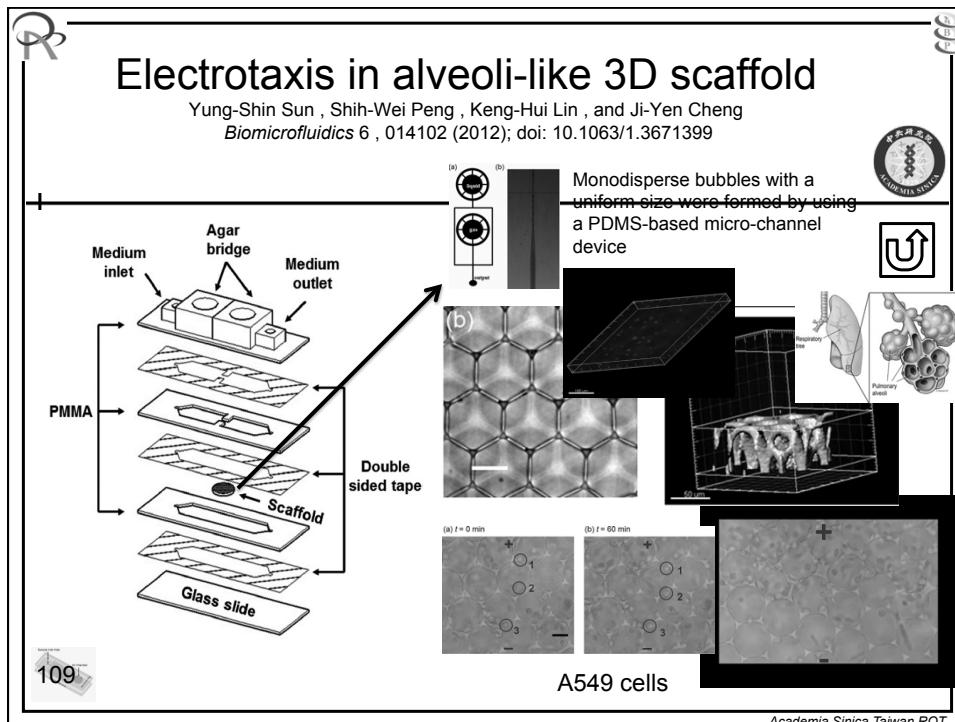
$$V_{R_1} : V_{R_4} : V_{R_7} : V_{R_{10}} = \left(\frac{A_1 + R_5}{R_5} \right) \left(\frac{A_2 + R_2}{R_2} \right) \left(\frac{R_1}{R_7} \right) : \left(\frac{A_1 + R_5}{R_5} \right) \left(\frac{R_4}{R_7} \right) : 1 : 0 = 7.93 : 2.75 : 1 : 0$$



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B. Fluidic chamber fabrication

- PMMA substrates: thickness = 1 mm
- Double sided tapes: thickness = 70 μm .
- Two protrudent pieces (3 mm $\times$ 4.5 mm each): Used to clip the scaffold and prevent it from sliding away during observation.
- Empty channel of the bottom PMMA: 50 mm $\times$ 12 mm in dimension.
- PMMA have 4 holes on it: middle two glued to bigger adapters for agar salt bridges and the outside two glued to smaller adapters for medium flow

The diagram illustrates the components and assembly of a fluidic chamber. On the left, a 3D perspective view shows the assembled chamber with labels: 'Medium inlet', 'Agar bridge', 'Medium outlet', and 'Medium inlet'. On the right, an exploded view shows the layers: 'PMMA' (two plates with holes), 'Double sided tape' (two sheets), 'Scaffold' (a circular mesh), and 'Glass slide' (a flat plate). Arrows indicate the assembly sequence from the glass slide at the bottom up to the PMMA top plate.

The diagram illustrates the experimental setup for studying *C. elegans* electrotaxis. A power supply (labeled "power supply" and "μA") is connected to two electrodes. These electrodes are part of a system that includes an agar bridge, PBS (phosphate-buffered saline), and a medium inlet/outlet. The setup is housed within a PMMA (poly(methyl methacrylate)) chamber. A scaffold is placed within the chamber, and an electric field (EF) is applied across it, as indicated by a large arrow. The chamber is supported by an ITO heater and a glass slide. A microscope is positioned below the chamber to observe the *C. elegans* (represented by a small worm icon) as they migrate in response to the electric field. A logo for "SHANGHAI JIAO TONG UNIVERSITY" is visible in the top right corner.

III. Results and Discussion

A. 3D scaffolds with uniform pore sizes

A. Picture of a 3D scaffold.
Dimension: 1 mm in thickness and 6 mm in diameter.

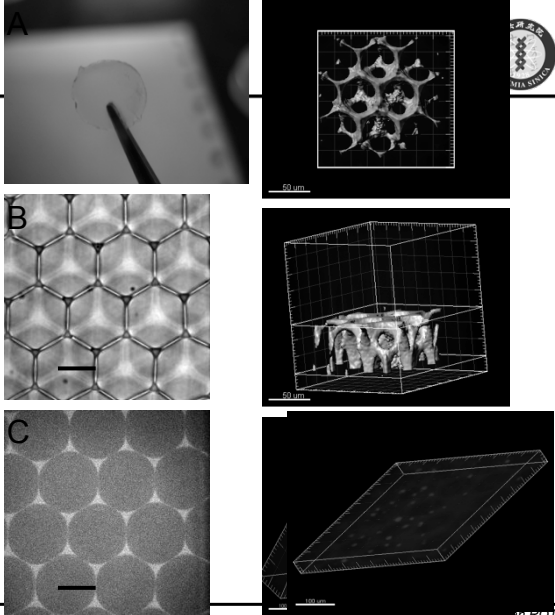
B. Bright-field confocal image of a 3D scaffold. Scale bar = 50 μm .

C. Fluorescent confocal image of a 3D scaffold. Scale bar = 50 μm .

D. Re-constructed 3D top-view image of a 3D scaffold. Top-to-bottom connected pores is around 50 μm .

E. Re-constructed 3D side-view image of a 3D scaffold. Side-to-side connected pores is about 20 ~ 30 μm .

F. Re-constructed 3D image of CL1-0 cells (stained with CellVue®) inside a 3D scaffold.



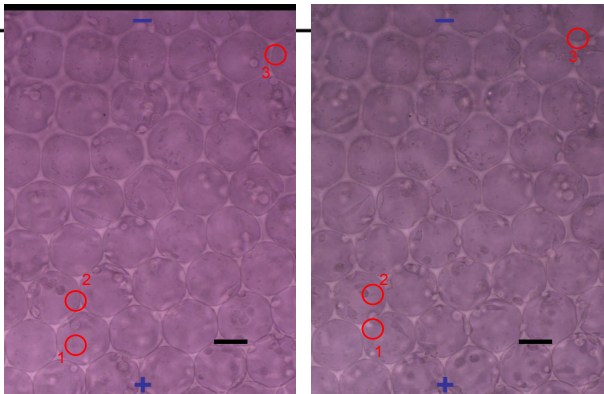
Cell migration under dcEF inside 3D scaffolds

-- Cells were able to move across neighboring bubbles through inter-connected pores

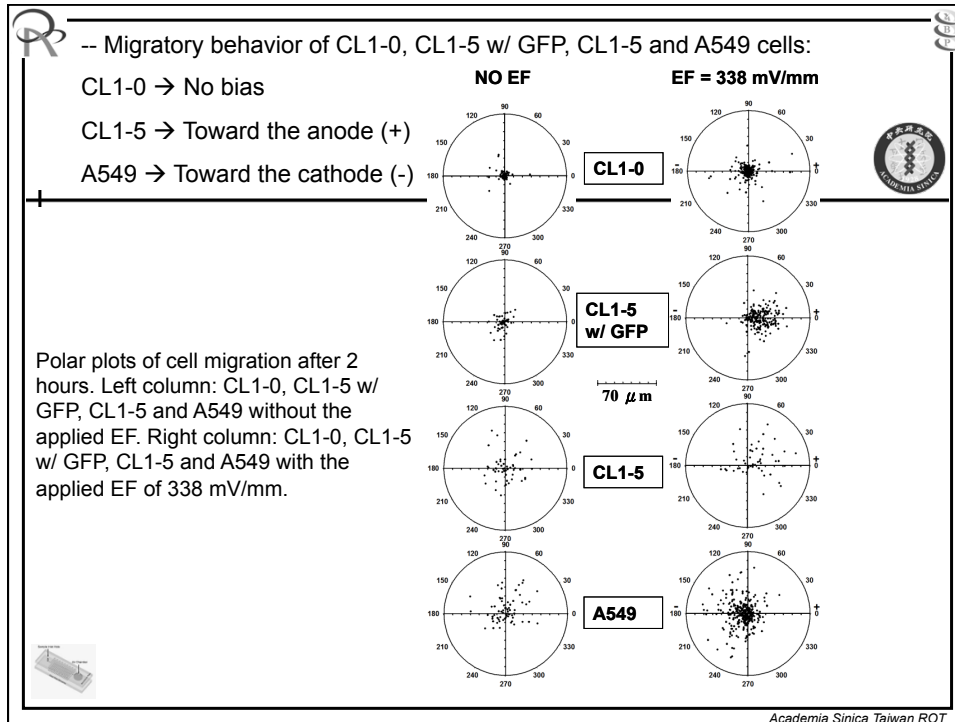
A. $t = 0$ min

B. $t = 60$ min

A549 cells inside a 3D scaffold under an applied EF of 338 mV/mm at A. $t = 0$ min and B. $t = 60$ min. Clearly some cells migrated through inter-connected pores.



1. Cancer cells inside *in vivo* human body can move and penetrate through a variety of surrounding biomaterials.
2. A potential application of such scaffolds in tissue engineering with great biocompatibility.



Comparison of cell migration under dcEF in 2D and 3D environments

Cell Type (stimulation time = 2 hr)	Directedness ± SEM	Speed ± SEM (μm/hr)
CL1-0 (2D, EF = 375 mV/mm) [1]	-0.005 ± 0.065	5.04 ± 0.74
CL1-0 (3D, EF = 338 mV/mm)	0.06 ± 0.05	3.63 ± 0.27
CL1-5 (2D, EF = 375 mV/mm) [1]	-0.60 ± 0.04	14.11 ± 0.86
CL1-5 (3D, EF = 338 mV/mm)	-0.33 ± 0.10	11.56 ± 1.11
A549 (2D, EF = 300 mV/mm) [2]	0.76 ± 0.12	13.0 ± 2.50
A549 (3D, EF = 375 mV/mm)	0.25 ± 0.04	7.73 ± 0.36

[1] Huang, C.W., et al., Biosensors & Bioelectronics, 2009. **24**(12): p. 3510-3516.
 [2] Yan, X.L., et al., Bioelectromagnetics, 2009. **30**(1): p. 29-35.

- Cells in both environments showed identical migration directions. However, for CL1-5 and A549 cells, the absolute values of directedness in 3D scaffolds are smaller than those in 2D substrates.
- Cells in 3D scaffolds moved slower than those on 2D substrates.

→ Difference in environmental **stiffness**:

Gelatin-made scaffolds: stiffness = 3 ~ 10 kPa
 Glass substrates: stiffness = 50 ~ 90 GPa; PMMA substrates: stiffness = 1.8 ~ 3.1 GPa

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~~BioMicrofluidic Applications, BioMAps Laboratory Oct/2010~~

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教育研究



FIG. 8. Dependence of wound-healing rate on different concentrations of β -lapachone in the absence of EP.

FIG. 9. Dependence of wound-healing rate on different concentrations of β -lanathone in the presence of E.

FIG. 8. Dependence of wound-healing rate on diff.

ACADEMIA SINICA, TAIWAN, R.O.C.

Mechanism of electrotaxis

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Pathways reported before

- EGFR
- Mitogen Activated Protein Kinase/ERK
- PI3K/Akt/PTEN
- Voltage sensitive sodium channel
- Calcium channel

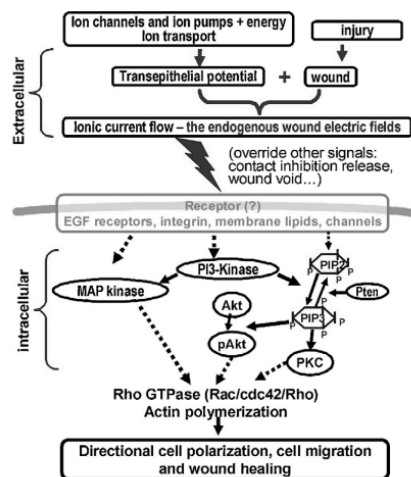


Fig. 6. Electrical signaling in cell migration and wound healing. Schematic diagram shows the generation of the endogenous wound electric signals and its integration into some important signaling pathways for cell polarization, migration and wound healing.

Seminars in Cell & Developmental Biology 20 (2009) 674–682

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Role of charged molecules on cell surface

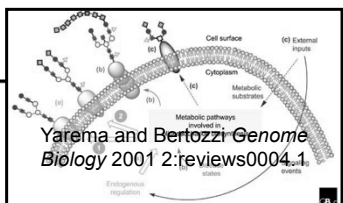
Cell Motility and the Cytoskeleton 64: 833-846 (2007)

Electric Field-Induced Polarization of Charged Cell Surface Proteins Does Not Determine the Direction of Galvanotaxis

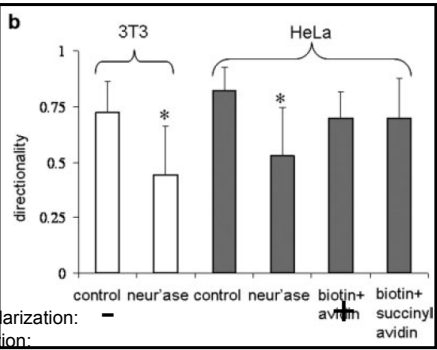
Erik I. Finkelstein,¹ Pen-Hsiu Grace Chao,² Clark T. Hung,² and Jacqueline Choe¹ [PMID: 17500777](#)

- neuraminidases (i.e. sialidases) to remove negative charges (sialic acid)
- avidin to add positive charges
- "...polarization of charged surface molecules, as measured by WGA, is induced by the EF, but is not functionally coupled to EF induction of directional motility."

Ref. Finkelstein, 2007, *Cell Motility and Cytoskeleton*, v64, p833



Yarema and Bertozzi *Genome Biology* 2001 2:reviews00041



b

Cell Type	Condition	Directionality
3T3	control	~0.75
	neur'ase	~0.45*
HeLa	control	~0.85
	neur'ase	~0.55*
	biotin+ avidin	~0.70
	biotin+ succinyl avidin	~0.70

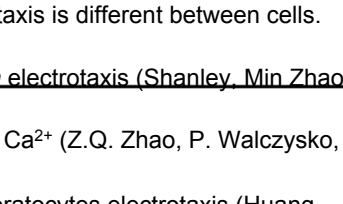
Sial. Polarization: -
Migration: -

121

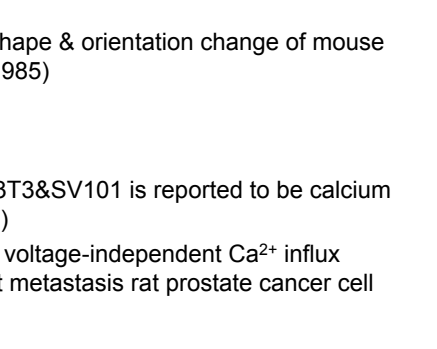
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Controversy in Calcium dependency

- Requirement of calcium for electrotaxis is different between cells. Arguments exist.
- Ca^{2+} is necessary for *Dictyostelium* electrotaxis (Shanley, Min Zhao, et al, 2006)
- HUVEC reendothelization requires Ca^{2+} (Z.Q. Zhao, P. Walczysko, Min Zhao, 2008)
- Involvement of Ca^{2+} in zebrafish keratocytes electrotaxis (Huang, Robinson et al., 2008)
- Requirement of Calcium for cell shape & orientation change of mouse embryo fibroblast (Onuma&Hui, 1985)
- **But**
- murine fibroblastic cell lines, NIH3T3&SV101 is reported to be calcium independent (Brown&Leow, 1994)
- (Djamgoz et al., 2006) reported a voltage-independent Ca^{2+} influx mechanism between two different metastasis rat prostate cancer cell lines.



Yarema and Bertozzi *Genome Biology* 2001 2:reviews00041



b

Cell Type	Condition	Directionality
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Sial. Polarization: -
Migration: -

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From morphological study to molecular level mechanism:



Gene expression change under dcEF of human lung cancer cell line CL1-5

Purpose: to predict EF sensing proteins



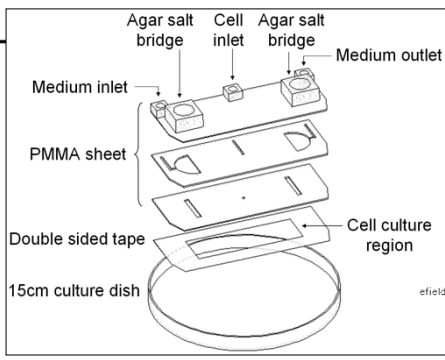
123

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Large Electric-Field Chip (LEFC) is required for DNA microarray experiment





Cell culture region:

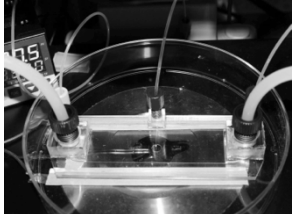
SFC: 3x15 mm²

LEFC: 75x24 mm² ↻ 40-fold

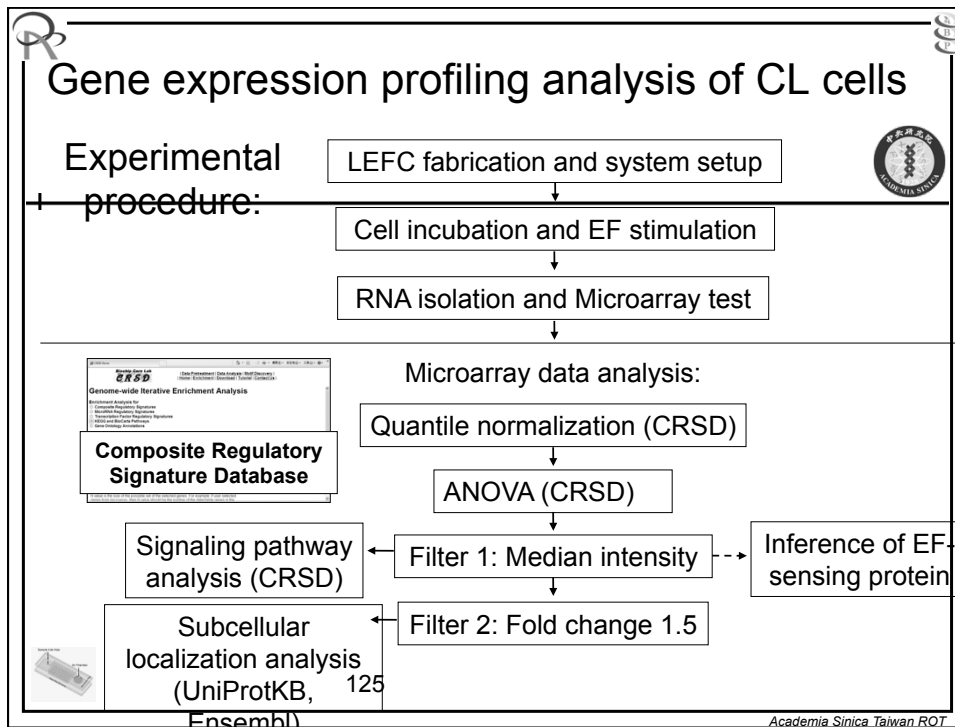
⇒ at most 1x10⁶ cells can be harvested from one LEFC



Huang, C.-W., H.-Y. Chen, et al. (2011). "Gene Expression of Human Lung Cancer Cell Line CL1-5 in Response to a 0.124 Current Electric Field." *PLoS ONE* 6(10): e25928



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Summary on gene expression of CL cells

- EF-regulated gene expression change

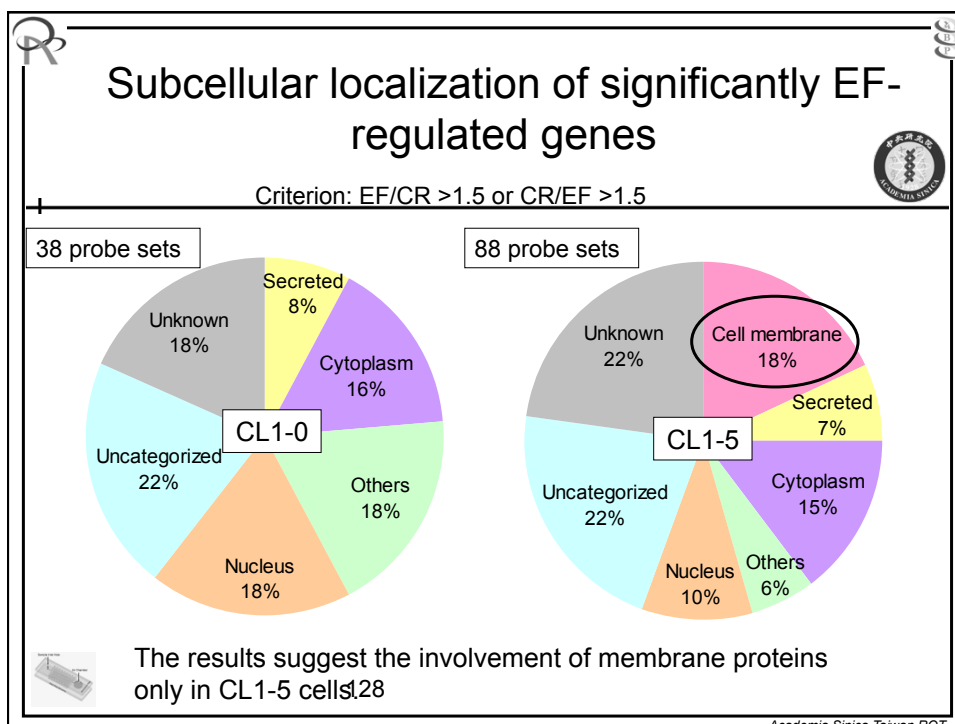
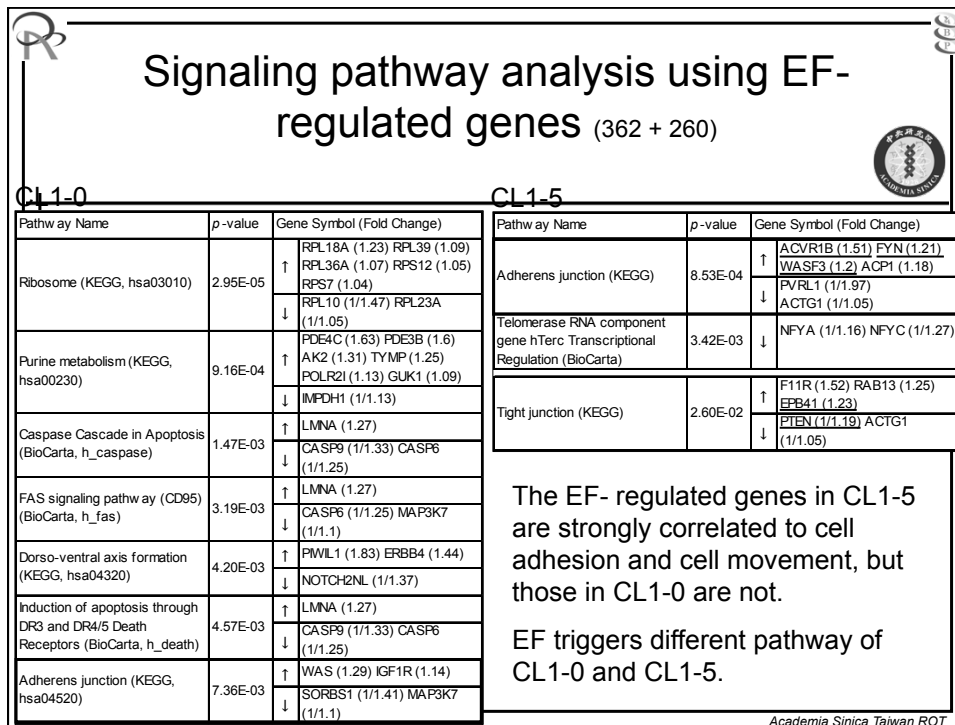
Affymetrix HG U133 Plus 2.0 array (20722 genes, 54675 probes)
 EF: 300mV/mm, 2hr
 n = 3 for each condition, each sample from 1-3 exp.

Probe set number	CL1-5	CL1-0
CR v.s. EF, ANOVA (p<0.05)	1631	1469
All signal strengths > median (65.5)	432	292
Fold change (EF/CR) > 1.5 or < 1/1.5	88	38
	126	

	CL1-5	CL1-0
Total gene #	362	260
Up-regulated	238	135
Down-regulated	124	125

Number of EF affected genes are low!

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Approach for EF-sensing protein prediction

1. Intrinsic difference of CL1-0 and CL1-5 may be the origin of EF sensing

CL1-5 (YYYYYY) v.s CL1-0 (Y) ⇒ 670 genes (encoding membrane protein) express differently

2. Genes of EF sensing proteins may not be directly regulated by EF but should participate in pathways related to EF regulated genes.

interaction network

Q: EF-regulated gene

⇒ Membrane proteins in CL1-5 network of EF-regulated genes.

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EF-sensing protein prediction

Predicted EF-sensing proteins based on the two criteria in the hypothesis

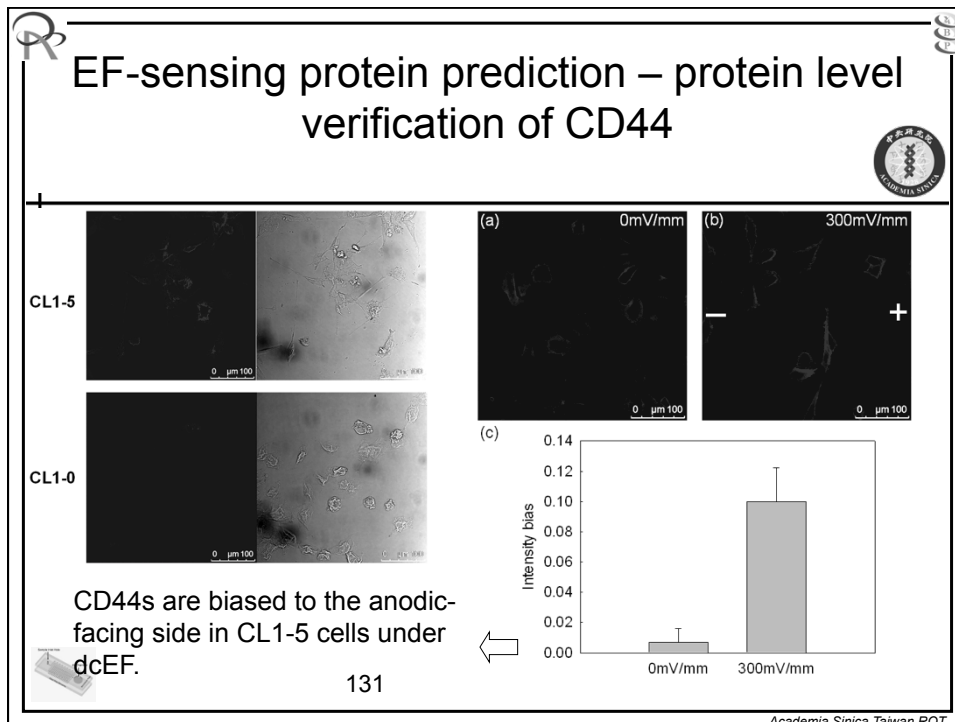
In CL1-5 only

Probe Set ID	Gene Symbol	Gene Name	Object type (GeneGo)	Control		1-5/1-0 ratio	EF stimulation		1-5/1-0 ratio
				signal intensity CL1-0	signal intensity CL1-5		signal intensity CL1-0	signal intensity CL1-5	
212063_at	CD44	CD44 molecule (Indian blood group)	Generic receptor	44	7682.7	174.61	127.1	6830.9	53.74
228083_at	CACNA2D4	calcium channel, voltage-dependent, alpha 2/delta subunit 4	Voltage-gated ion channel	11.6	893.7	77.04	3.78	1139	301.31
225407_at	MBP	myelin basic protein	Generic binding protein	27.5	1088.4	39.58	18.07	1381.4	76.45
203987_at	FZD6	frizzled homolog 6 (Drosophila)	GPCR	111.7	3351.1	30	128.29	2625.2	20.46
201286_at	SDC1	syndecan 1	Generic receptor	258.5	2315.3	8.96	244.06	2204.2	9.03
203028_s_at	CYBA	cytochrome b-245, alpha polypeptide	Generic enzyme	400.4	3292.4	8.22	452.42	4200.1	9.28
202668_at	EFNB2	ephrin-B2	Receptor ligand	176.3	954.5	5.41	283.45	982.81	3.47
204379_s_at	FGFR3	fibroblast growth factor receptor 3	Receptor with enzyme activity	1718.2	150.2	1/11.44	1797.7	164.19	1/10.95

GPCR: G protein-coupled receptor

Criterion: ratio > 4 and signal intensity > 500 at least in one cell line

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For acquiring cells for protein analysis - Exp design

- Aim: investigate the activation of RTKs in CL1 electrotaxis. Because *in situ* electrophoresis is the most widely adapted model
- Use XLEFC for electrical stimulation and cell collection.
- XLEFC
 - Uniform electric field
 - Air-tight sealing
 - Avoid contamination
 - Avoid medium evaporation
 - Easily disassembled after EF stimulation
- PathScan RTK signaling antibody array kit for screening

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On-going works



- Cell behavior under dcEF stimulation:
 - Molecular mechanism of EF sensing (protein phosphorylation) 
 - Effect of EF on neuron cell differentiation
 - Diagnostic tool on cancer cell malignancy based on electrotaxis
 - Electrotaxis in vivo using model organism
- Biosensing using nano/micro structures
 - microRNA detection in urine sample for diagnosis of kidney injury
 - Whole-cell biosensor (for detecting toxicity of non-specific source)
- Chemical applications – extraction chip 



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BioMicrofluidic Applications, BioMAps Laboratory Oct/
2010

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microRNA as urinal marker and therapeutic agent for acute kidney injury



以微型核醣核酸作為急性腎衰竭之尿液診斷標記以及治療劑

- A collaboration of:
 - Ji-Yen Cheng (鄭鄧言 應科 副研究員)(nanoslit SPR microfluidic chip for miRNA detection)
 - Heng Lin (林恆 台北醫學大學生理科 副教授) (mouse model of AKI and CIN)
 - His-Hsien Chen (陳錫賢 台北醫學大學附設醫院 腎臟內科主任/助理教授) (Select patient and collect samples based on clinical case histories)
- Background
 - 顯影劑腎病變已成為醫源性急性腎衰竭的重要原因,占住院中急性腎衰竭原因的第三位。顯影劑腎病治療多為支持性療法,在嚴重的患者必要時給則須要透析治療。(內科學誌 2009 ; 20, p171-180)
 - 需要透析治療的病人預期有30%的住院率以及80%的兩年死亡率。
 - Early stage diagnosis is very important for the treatment of acute kidney injury (AKI) or contrast medium induced nephropathy (CIN).
 - Current standard marker (serum creatinine) does not reveal the injury until 48 to 72 hours, which is too late for effective treatment.



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中央研究院
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האוניברסיטה העברית בירושלים
The Hebrew University of Jerusalem



Bacterial Whole Cell Sensor on a Chip for Environmental Monitoring

2014.2.23

Tori Yi-Ching Tsai & Paul Hsieh-Fu Tsai
Principal Investigator: Dr. Ji-Yen Cheng, Shimshon Belkin



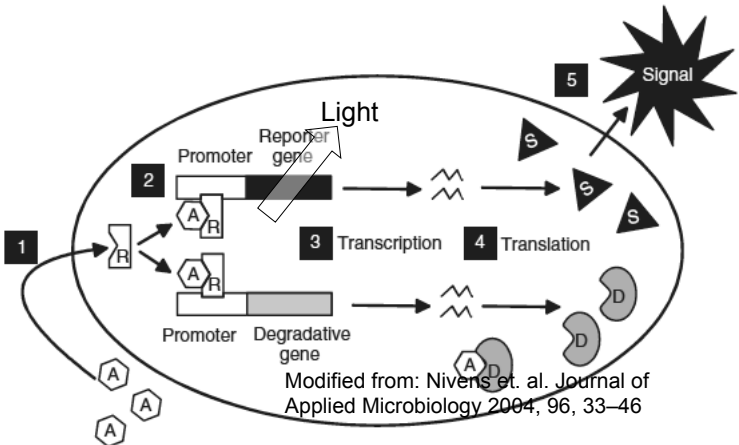
135

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Principle of using whole-cell as sensor for environment monitoring





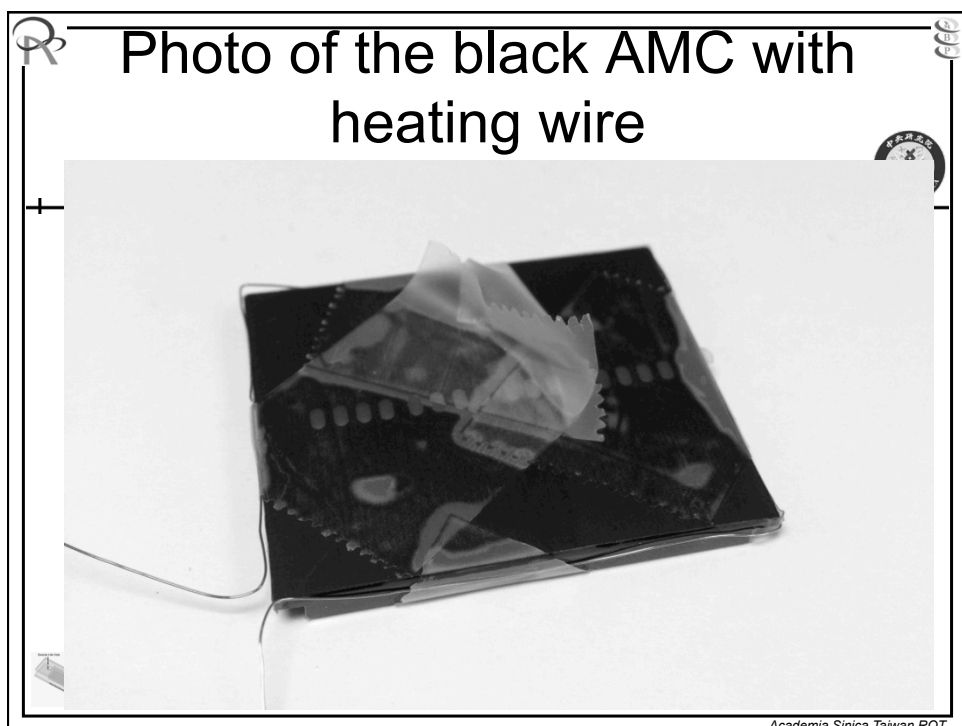
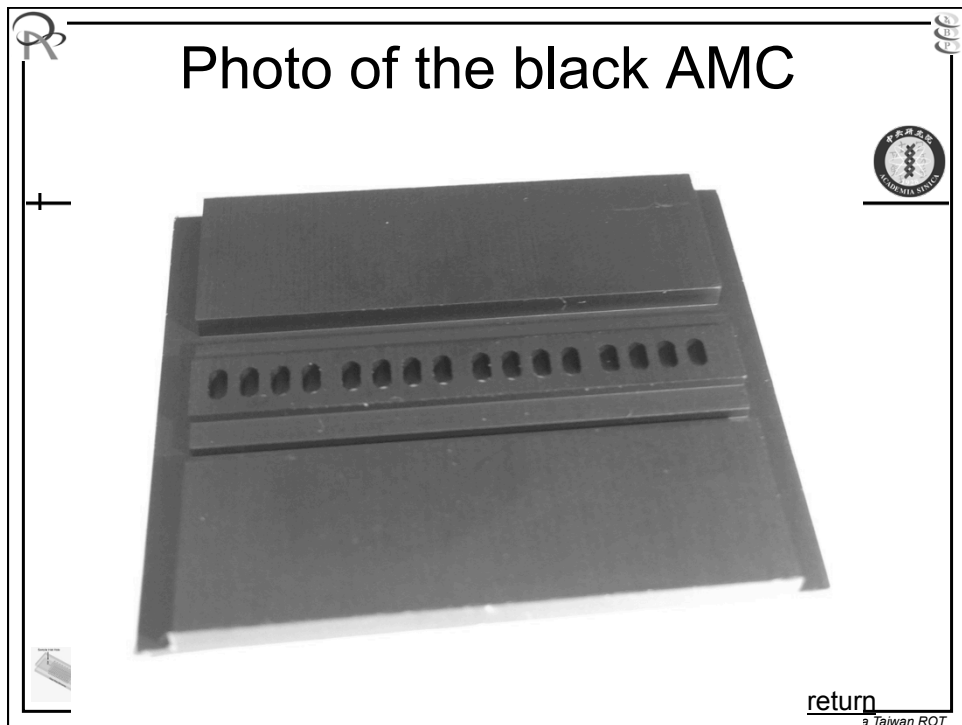
Modified from: Nivens et. al. Journal of Applied Microbiology 2004, 96, 33–46

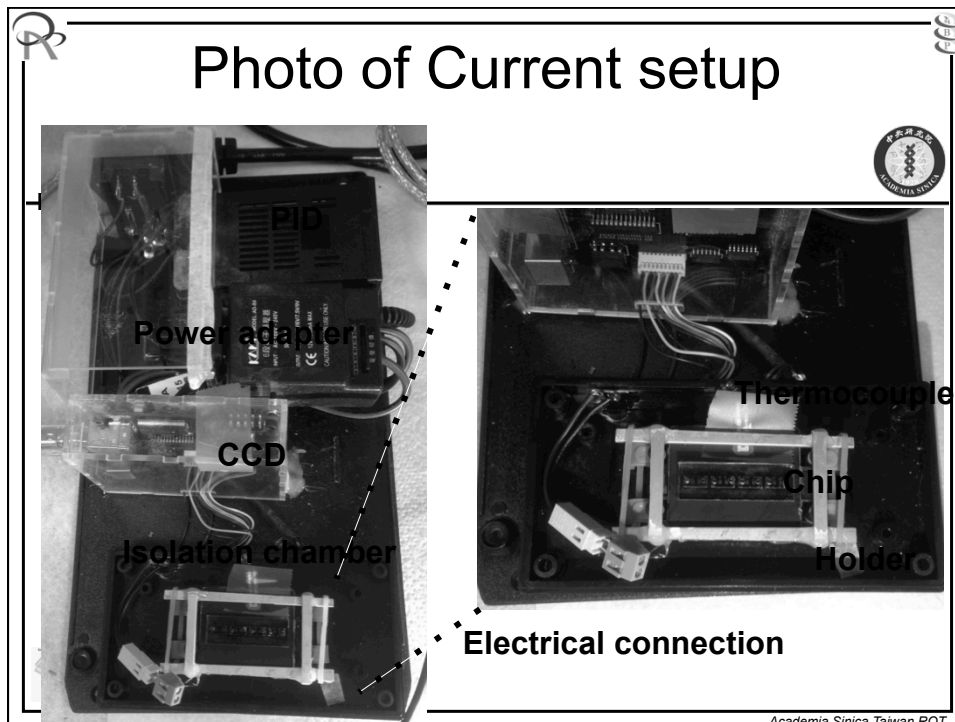


Disadvantage and Advantage: Non-specific, biological effect

BioMicrofluidic Applications, BioMApe Laboratory Oct/2010

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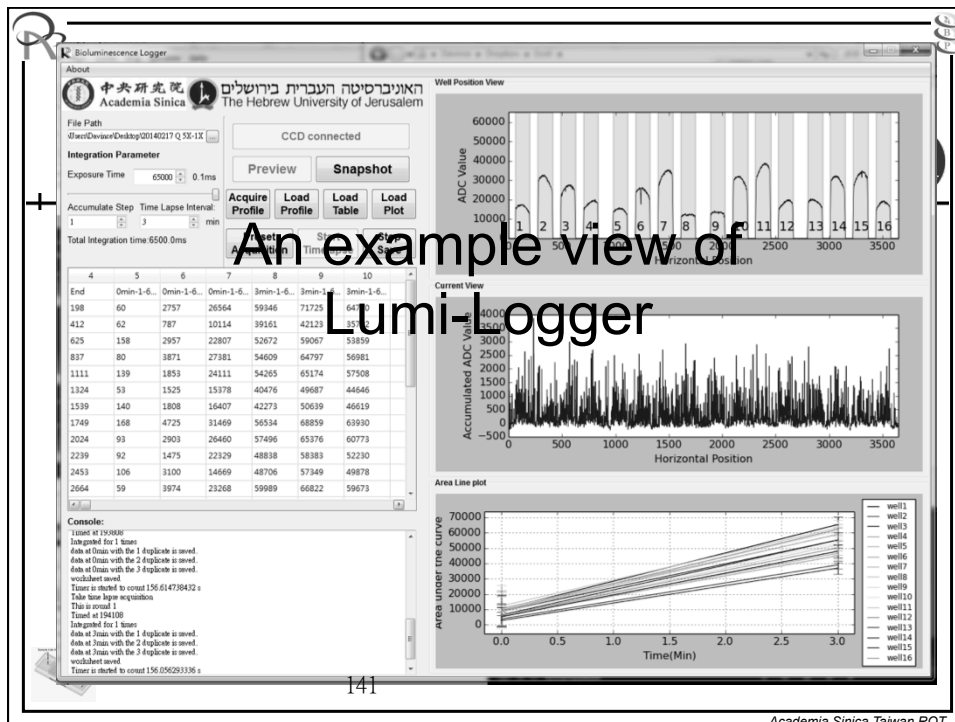
A software for monitoring luminescence signals: Lumi-Logger

- Access the Mightex TCD1304 Linear CCD module through libusb library and communicate through the USB protocol.
- Utilize Python, PyQT, Matplotlib, Numpy, Scipy
- Support automatic identification of well location and numbering.
- Support time lapse and single acquisition of data and automatically integrate the area under the curve for each well.
- Automatically save filtered data and raw data.
- Save all the data of each time point into a .csv file.
- Able to load the .csv file and load each data set and plot it when selected.
- Executable by Pyinstaller
- Automatically triplicate the measurement and save the data.
- Real-time plotting of area-under-the-curve for each peak.



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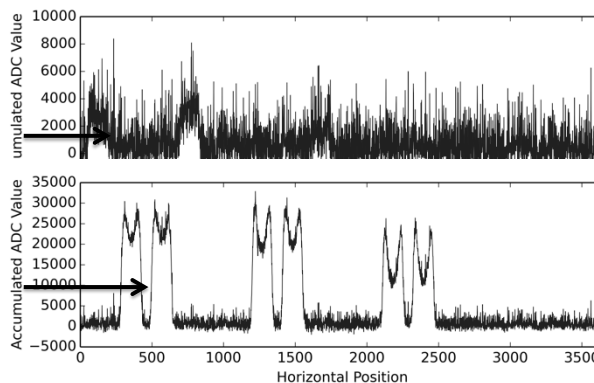


An example of Data processing – peak identification

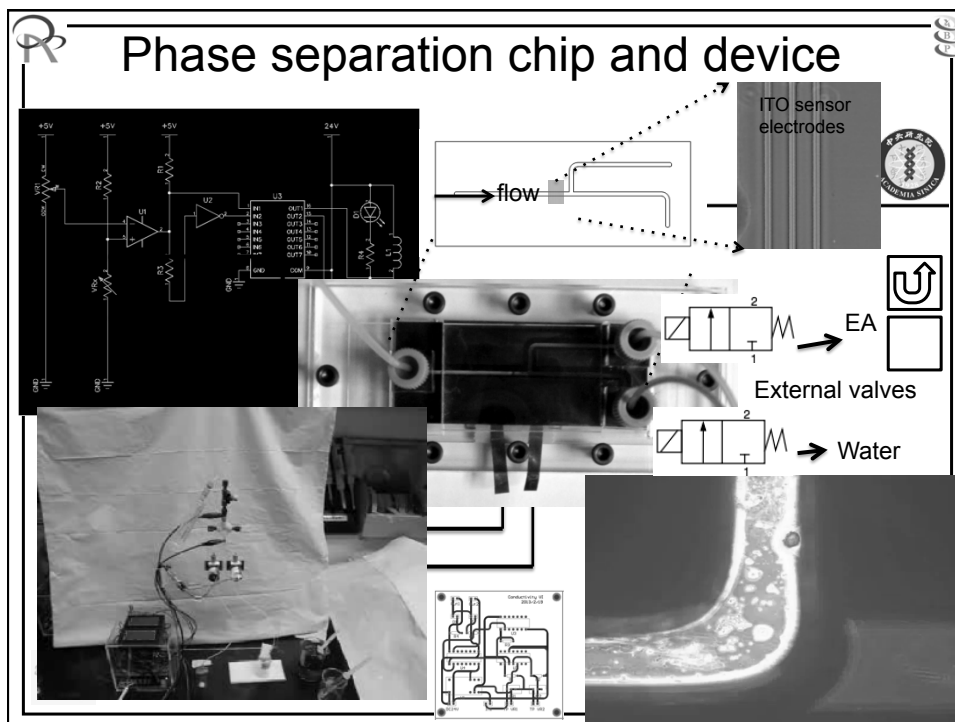
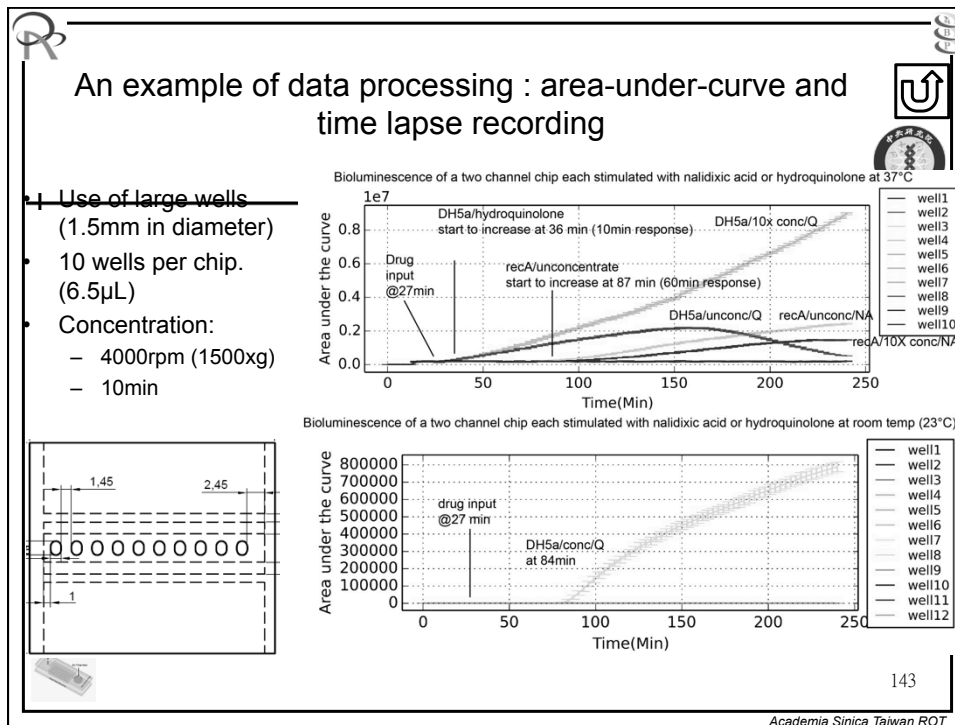
- The program uses the Scipy's built-in Simpson's numerical function to integrate the area under the curve.
- From ADC value of the CCD, the recA stimulated with 0.0 mg/mL NA for 2 hours (upper panel of the figure) is about 2500 a.u. while calculated area is 374557.
- From ADC value of the CCD, the DH5a stimulated with 0.2mg/mL hydroquinolone for 2 hours is about 20000 a.u. while calculated area is 3269755.

recA strain (upper panel)
is stimulated by an
antibiotic, Nalidixihyc acid

DH5a strain is stimulated
by hydroquinolone



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Conclusion:
 Microfluidic devices really help to speed up chemical and biological studies.

But
 Microfluidics along is merely an enabling technology.
 It is the problem that is important.

- Rapid (500 sec) Microarray hybridization, 2005, Nucl. Acid. Res.
- DNA amplification, 2005, Analyst
- DNA microarray synthesis, 2009, Bioeng. Biotech.
- Rare cell capturing
- Microfluidic nanoslit sensor
- Sensor based on whole-cell array
- Cell culturing in controlled microenvironment
- Electrotaxis of lung cancer cell
 - Electrotaxis – short introduction
 - Multi-field chip (MFC) for electrotaxis study
 - Electrotaxis in 3D scaffold
- Wound healing mimicking 2012, Biomicrofluidics



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Questions

\$\$: NSC Taiwan, contract no.: 100-2113-M-001-014-MY3
 NSC 102-2923-M-001-004-MY2



Macrofluidic, $Re = 1 \times 10^7$ at the bottom of
 Taroko gorge, Taiwan

22/01/2006



Contact: Ji-Yen Cheng, jycheng@gate.sinica.edu.tw
 RCAS, Academia Sinica Taiwan

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