生活與物理－奈米生技

周家復
中央研究院物理研究所
基因體研究中心
應用科學研究中心
(cfchou@phys.sinica.edu.tw)

NCCU
12/19/08
奈米生技 or 奈米生計？

PChome > 商店街首頁 > 保健 > 奈米保健

- 全部
- 超商
- 隔日

- 保健
- 奈米保健
  - 奈米保健食品 (30)
  - 奈米保健用品 (230)
  - 其他奈米商品 (93)

奈米能量煙嘴
肉桂精油奈米噴霧
奈米陶瓷吹風機
Red blood cells (~7-8 µm)
Fly ash ~ 10-20 µm
Ant ~ 5 mm
Dust mite ~ 200 µm
Human hair ~ 60-120 µm wide
Red blood cells (~7-8 µm)
Fly ash ~ 10-20 µm
Ant ~ 5 mm
Dust mite ~ 200 µm
Human hair ~ 60-120 µm wide

**The Scale of Things – Nanometers and More**

**Things Natural**

- Ant ~ 5 mm
- Dust mite ~ 200 µm
- Human hair ~ 60-120 µm wide
- Red blood cells (~7-8 µm)
- Fly ash ~ 10-20 µm

**Things Manmade**

- Head of a pin ~ 1-2 mm
- MicroElectroMechanical (MEMS) devices 10 - 100 µm wide
- Pollen grain
- Red blood cells
- Zone plate x-ray “lens” Outer ring spacing ~35 nm
- Self-assembled, Nature-inspired structure Many 10s of nm
- Nanotube electrode
- Carbon nanotube ~1.3 nm diameter
- Quantum corral of 48 iron atoms on copper surface positioned one at a time with an STM tip Corral diameter 14 nm

**The Challenge**

Fabricate and combine nanoscale building blocks to make useful devices, e.g., a photosynthetic reaction center with integral semiconductor storage.

- Red blood cells
- Pollen grain
- Head of a pin
- Nanotube electrode
- Carbon nanotube
- Quantum corral
- Self-assembled, Nature-inspired structure

**Microworld**

- 1 cm
- 10 mm
- 10^2 m
- 10^3 m
- 10^4 m
- 10^5 m
- 10^6 m
- 10^7 m
- 10^8 m
- 10^9 m
- 10^10 m

**Nanoworld**

- 1,000,000 nanometers = 1 millimeter (mm)
- 1,000 nanometers = 1 micrometer (µm)
- 0.1 nm
- 0.01 nm
- 0.001 nm
- 0.0001 nm
- 0.00001 nm
- 0.000001 nm
- 0.0000001 nm
- 0.00000001 nm
- 0.000000001 nm
- 0.0000000001 nm

**The Challenge**

Fabricate and combine nanoscale building blocks to make useful devices, e.g., a photosynthetic reaction center with integral semiconductor storage.
Nanotechnology’s Probable Business Impact
(CA, 2005)
Watson-Crick's DNA double helix model.

The Nobel Prize in Physiology or Medicine 1962

"for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material"
Cell and Molecules (細胞與分子)

1 bp = 3.4 Å (0.2 e⁻)
Dia. = 2 nm
1 helical pitch = 10 bp
**Reynolds number**

雷諾數 = 慣性力／黏滯力

\[
Re = \frac{\rho v_s^2}{\mu} = \frac{\rho v_s L}{\mu} = \frac{v_s L}{\nu} = \frac{\text{Inertial forces}}{\text{Viscous forces}}
\]

Typical values of Reynolds number

* Spermatozoa ~ 1\times10^{-2}
* Blood flow in brain ~ 1\times10^{2}
* Blood flow in aorta ~ 1\times10^{3}

Onset of turbulent flow ~ 2.3\times10^{3} for pipe flow to 10^{6} for boundary layers

* Typical pitch in Major League Baseball ~ 2\times10^{5}
* Person swimming ~ 4\times10^{6}
* Blue Whale ~ 3\times10^{8}
* A large ship (RMS Queen Elizabeth 2) ~ 5\times10^{9}

For water, \( \mu = 0.01 \text{ cm}^2/\text{s} \)

<table>
<thead>
<tr>
<th>( D_h ) (( \mu \text{m} ))</th>
<th>( v ) (cm/s)</th>
<th>( \text{Re} \ (D_h v/\mu) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.1</td>
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<tr>
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<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>500</td>
<td>0.1</td>
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For water, \( \mu = 0.01 \text{ cm}^2/\text{s} \)
Poiseuille flow

We'll start with the flow of a viscous fluid in a channel. The channel has a width in the $y$-direction of $a$, a length in the $z$-direction of $l_z$, and a length in the $x$-direction, the direction of flow, of $l_x$. There is a pressure drop along the length of the channel, so that the constant pressure gradient is (such a pressure gradient could be supplied by gravity, for instance). Assuming the flow to be steady, $\partial v_x / \partial t = 0$. Also, we'll assume that the flow is of the form, then. The no-slip boundary condition at the top and bottom edges of the channel reads $v_x(y = \pm a/2) = 0$. The Navier-Stokes equation then becomes

$$\eta \frac{\partial^2 v_x}{\partial y^2} + \frac{\Delta P}{l_x} = 0. \quad [3.14]$$

Integrating twice, we obtain

$$v_x(y) = \frac{1}{2\eta} \frac{\Delta P}{l_x} y^2 + C_1 y + C_2, \quad [3.15]$$

where $C_1$ and $C_2$ are integration constants. To determine these, we impose the boundary conditions to obtain

$$v_x(y) = \frac{1}{2\eta} \frac{\Delta P}{l_x} \left[ (a/2)^2 - y^2 \right]. \quad [3.16]$$

We see that the velocity profile is a parabola, with the fluid in the center of the channel having the greatest speed. Once we know the velocity profile we can determine the flow rate $Q$, defined as the volume of fluid which passes a cross section of the channel per unit time. This is obtained by integrating the velocity profile over the cross sectional area of the channel:

$$Q = \int_0^{l_z} \int_{-a/2}^{a/2} dy v_x(y) dz$$

$$= \frac{l_z a^3 \Delta P}{12\eta l_x}. \quad [3.17]$$

The analogous result for flow through a pipe of radius $a$ and length $l$ in the presence of a uniform pressure gradient $\Delta P/l$ is

$$Q = \frac{\pi a^4 \Delta P}{8\eta l}. \quad [3.18]$$

The important feature of both of these results is the sensitive dependence upon either the channel width $a$ or the pipe radius $a$. For instance, for a pipe with a fixed pressure gradient, a 20% reduction in the pipe radius leads to a 60% reduction in the flow rate! This clearly has important physiological implications -- small amounts of plaque accumulation in arteries can lead to very large reductions in the rate of blood flow.
低雷諾數的生命世界

Life at Low Reynolds Number
E.M. Purcell
Lyman Laboratory, Harvard University, Cambridge, Mass 02138
June 1976


\[ \eta = 1 \text{ centipoise} \]
\[ \nu = 10^{-2} \text{ cm}^2/\text{sec} \]
\[ R = 3 \times 10^{-5} \]

\[ \text{coasting distance} = 0.1 \text{ Å} \]
\[ \text{coasting time} = 0.3 \text{ microsec} \]

Figure 4
Laminar Flow (層流)-movie
Cavitation Microstreaming

- Flow streaming around bubbles in an acoustic field
- Optimized mixing conditions (waveform, amplitude, etc.)

\[
f = \frac{\sqrt{3\gamma P_0}}{\rho} \frac{1}{2\pi a}
\]

R. Liu et al., Anal. Chem. 2003

Now (10 sec for 100 uL)
5 kHz, 40 Vpp, square wave
Manufacturing and prototyping

Fabrication methods
- Molding, embossing, NanoImprinting

Device components
- Valves
- Pumps
- Mixers
- NIL Mask, 70 nm

Assays
- Channel hyb
- Amplification - PCR
- Cell and DNA capture
- Amplification-Detection On Chip

Integrated solutions
- Multi-layer devices
- Multi-functional cartridges
Objective: Integrate whole sample prep with microarray for low abundance bacteria detection from blood (1 mL)

Successfully demonstrated cell capture + purification + lysis + PCR + detection of 1000 E. coli K12 cells / 1mL sheep blood

DNA Microarray (晶片)

Scan with laser scanner.

- **RED**: RNA expressed in HSV infected cells
- **GREEN**: RNA expressed in uninfected cells
- **YELLOW**: RNA expressed in both infected and uninfected cells

Intensity is a measure of amount
DNA 分子検測原理

Probe DNA

Substrate

Optical/E-chem Labeling

Signal DNA

Target DNA
Introduction to the F1-ATPase 分子馬達

- ATPase: Produce and hydrolyze ATP
- F1 Complex: $\alpha_3\beta_3$ hexamer and $\gamma$ subunit
- $\gamma$ subunit (> 12nm) rotates due to the conversion of chemical to mechanical energy (efficiency 80-100%, torque 120-150 pN·nm).

Soong et al., Science 290, 1555 (2000)
Biomotor-based single molecular sensors

C.F. Chou et al.,
Electrodeless Dielectrophoresis of DNA

無電極式介電泳技術在 DNA 上的應用
DEP for Manipulation of Biomolecules
利用介電泳技術來操控生物分子

- Dielectrophoresis (DEP) is a phenomenon in which a force is exerted on a dielectric particle in a non-uniform electric field – 介電泳現象是來自於一介電粒子因感受到非均勻電場所產生的現象，其粒子的極化現象可由下面公式及圖表來解釋：

\[ F_{DEP} = 2\pi r^3 \varepsilon_\text{m} \text{Re} \left[ \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \right] \nabla |\vec{E}|^2 \]

Sphere of radius \( r \), \( \varepsilon_m = 80 \) for water

- DEP is a technique which can be used to separate cells or molecules based on their difference in polarizability – 所以介電泳技術可利用細胞或分子的受極化的程度不同來分離不同物質

Our Approach: Electrodeless DEP
(無電極式介電泳技術)

Advantages (優點):
1) Simpler fabrication process (製程相對電極式介電泳技術簡便)
2) Structure chemically inert (結構不易受化學物質損壞)
3) High E fields without risk of electrolysis (可接受高電場施加)
Molecular Trap (分子捕捉阱)

Field focusing mostly occurs at the tips of constriction.

→ Increase of molecule concentration in the trap.

在無電極式介電泳技術設計結構內所產生的電場分佈圖
在中間部份可以捕捉到最多量的分子
無電極式介電泳之DNA捕捉

368 bp DNA

EDEP device

交流電場未開

交流電場開啓

C.F. Chou et al., Biophys. J. 83: 2170 (2002);
無電極式介電泳之DNA捕捉 - movie
EDEP Array for Cell Trapping

E. coli

4 µm constriction,
10 µm deep
50-200 MHz, 100 Vpp/cm
Buffer salt concentration:
up to 100 mM

Enrichment ~ $10^{3-4}$

EDEP Array for Cell Trapping/Separation

*E. coli (†DEP)*

*RBC (−DEP)*

Stretching of DNA with buffer flow
DNA 伸展

Hydrodynamic flow

Elongation flow

Molecular combing
Bensimon, Science (1997)

Optical tweezers
S. Chu; S. Block (1994-5)

Electrohydrodynamic flow
Bakajin et al. PRL (1998)
用奈米流道作DNA伸展

175°C and 50 kg/cm² (4.9×10⁶ Pa), 5 min

<table>
<thead>
<tr>
<th>Channel dimension</th>
<th>Stretched DNA length</th>
<th>Percentage of stretching</th>
</tr>
</thead>
<tbody>
<tr>
<td>300nm x 700nm</td>
<td>6.2 ± 1.3 μm</td>
<td>15%</td>
</tr>
<tr>
<td>300nm x 500nm</td>
<td>12.7 ± 4.5 μm</td>
<td>30%</td>
</tr>
<tr>
<td>75nm x 120nm</td>
<td>39.8 ± 7.7 μm</td>
<td>95%</td>
</tr>
</tbody>
</table>

T5 phage DNA 103 kb (35 μm)

DNA伸展後爬坡

Gu, Gupta, Chou, Wei, Lab Chip, 7, 1198 (2007)

DNA strands going up the ramp in the interfaced area (movie)
一端點固定之DNA伸展

前提說明：線型的DNA，在無外力情形下，捲縮成一團。(圖一)

實驗設計：將DNA的一端固定在玻片上，水流沖刷，DNA會被水流拉扯而伸展開。(圖二)

用處：DNA伸展後，方便於顯微鏡觀察。可以用作觀察DNA和蛋白質的交互作用。

圖一、靜止狀態

DNA
玻片

圖二、水流拉扯伸展

DNA
玻片
水流
影片說明 - 以水流伸展DNA
DNA在拔河

高分子奈米通道裡的特異行為
1. 觀察DNA單分子經由奈米通道的拔河行為，～兩邊力的來源是由“entropy”熵的不同造成

2. 經由研究DNA單分子在奈米通道的運動，探討熵力的大小
DNA 定序

- Year 1977
- Maxem and Gilbert – “Chemical Cleavage Protocol”
- Sanger – Dideoxy sequencing or chain termination method.
- Gilbert and Sanger shared 1/2 Nobel Prize in 1980.
- Sanger method is used widely because of its practicality.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Genome size</th>
<th>Size relative to human</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>9,750</td>
<td>0.0003%</td>
<td>complete 1985, Wain-Hobson, et al.</td>
</tr>
<tr>
<td>Mycoplasma genitalium</td>
<td>580,070</td>
<td>0.0171%</td>
<td>complete 1995, TIGR</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>816,394</td>
<td>0.0240%</td>
<td>complete 1995, Univ. of Heidelberg</td>
</tr>
<tr>
<td>Lyme disease spirochete</td>
<td>946,000</td>
<td>0.0278%</td>
<td>complete 1997, TIGR</td>
</tr>
<tr>
<td>Methanococcus jannaschii</td>
<td>1,664,974</td>
<td>0.0490%</td>
<td>complete 1996, TIGR</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>1,830,137</td>
<td>0.0538%</td>
<td>complete 1995, TIGR</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>4,397,000</td>
<td>0.1293%</td>
<td>complete 1998, Sanger Centre</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4,639,221</td>
<td>0.1364%</td>
<td>complete 1997, Univ. of Wisc. + others</td>
</tr>
<tr>
<td>Yeast, Baker's</td>
<td>12,067,280</td>
<td>0.3549%</td>
<td>complete, 1996, Stanford Genome Center</td>
</tr>
<tr>
<td>C. elegans</td>
<td>100,000,000</td>
<td>3%</td>
<td>complete, 1999 Washington Univ. and Sanger Ctr.</td>
</tr>
<tr>
<td>Fruit fly</td>
<td>180,000,000</td>
<td>5%</td>
<td>complete, 2000 European and US groups</td>
</tr>
<tr>
<td>Pufferfish, Japanese (Takifugu rubripes)</td>
<td>400,000,000</td>
<td>12%</td>
<td>90% complete, 2005</td>
</tr>
<tr>
<td>Rice</td>
<td>400,000,000</td>
<td>12%</td>
<td>complete, 2002 International Rice Genome Seq. Proj.</td>
</tr>
<tr>
<td>Pig</td>
<td>2,700,000,000</td>
<td>79%</td>
<td>Mapping complete - sequencing mid stages</td>
</tr>
<tr>
<td>Rat</td>
<td>2,900,000,000</td>
<td>85%</td>
<td>Draft assembly</td>
</tr>
<tr>
<td>Cattle</td>
<td>3,000,000,000</td>
<td>88%</td>
<td>Begun in April, 2003, draft assembly</td>
</tr>
<tr>
<td>Mouse</td>
<td>3,000,000,000</td>
<td>88%</td>
<td>Complete, 2002</td>
</tr>
<tr>
<td>Rabbit</td>
<td>3,000,000,000</td>
<td>88%</td>
<td></td>
</tr>
<tr>
<td><strong>Human</strong></td>
<td><strong>3,400,000,000</strong></td>
<td>1.0</td>
<td><strong>Complete, 2001, 2002, 2003</strong></td>
</tr>
<tr>
<td>Onion</td>
<td>18,000,000,000</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>Marbled lungfish</td>
<td>139,000,000,000</td>
<td>40.9</td>
<td></td>
</tr>
<tr>
<td>Fern</td>
<td>160,000,000,000</td>
<td>47.1</td>
<td></td>
</tr>
<tr>
<td>Amoeba proteus</td>
<td>290,000,000,000</td>
<td>85.3</td>
<td></td>
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DNA 定序
DNA 定序－毛細管電泳

Capillary (Array) Electrophoresis
基因组定序的目标

Cost:
~ $1M/genome
Time: months

Goal

Cost: $1,000/
genome
Time: hours
A single α-hemolysin channel (Ø =2.5 nm) embedded in a lipid bilayer
Dekker group, Nat. Mater. 2003

D. Branton, J. Golovchenko, Harvard