National
Center
for
High-performance
Computing

Octobor 25, 2009

Design and Analysis of Biochips

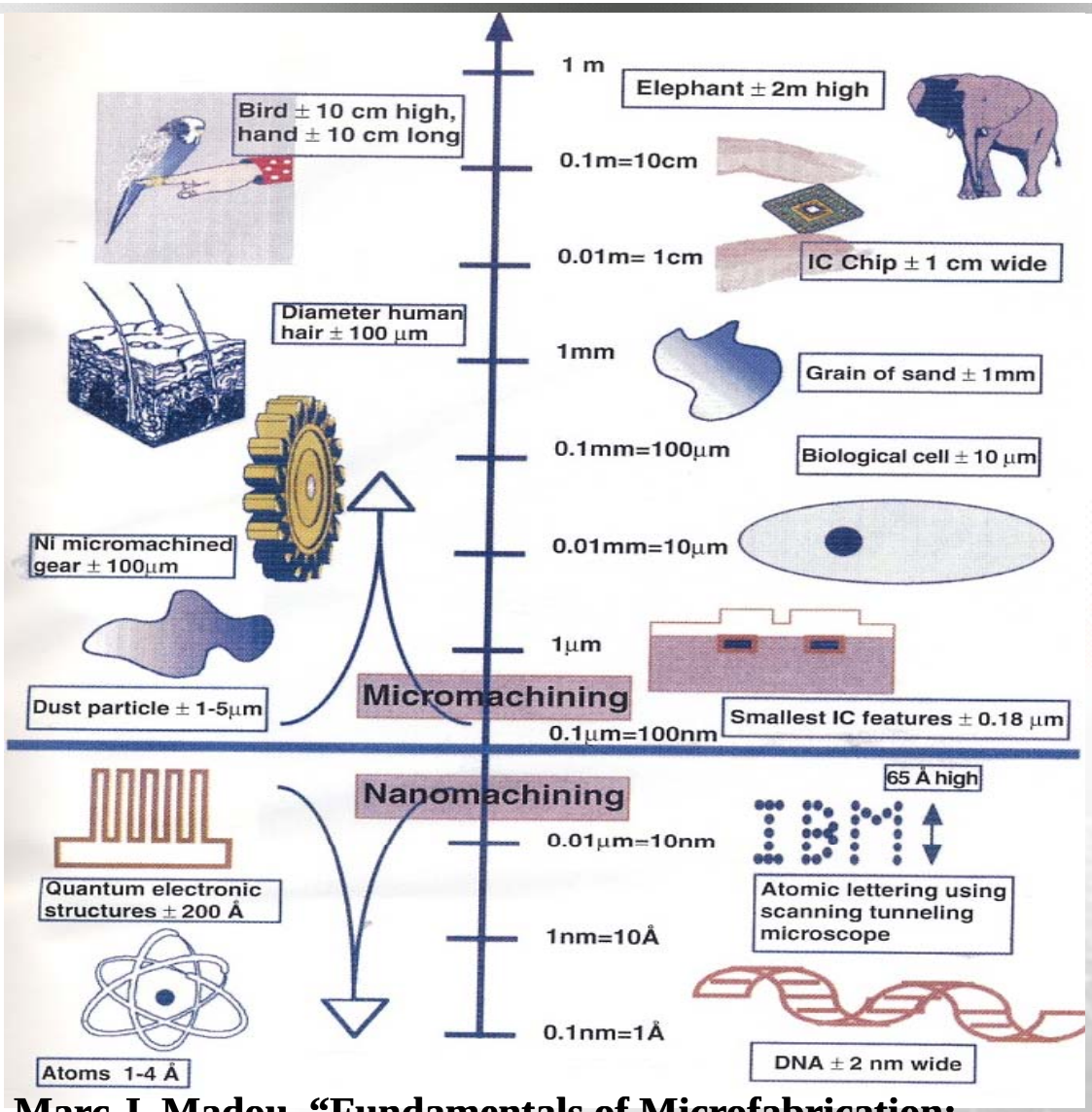
生物晶片設計與分析

Heng-Chuan KAN, Ph.D. (甘恆全博士)
研究員
國家高速網路與計算中心南部事業群

There’s Plenty of Room at the Bottom.

~ Richard P. Feynman, 1959

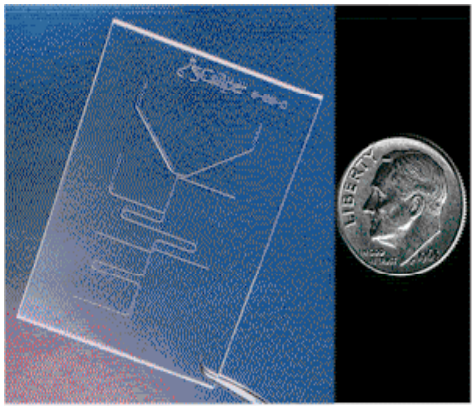
Length Scales: Where are We?

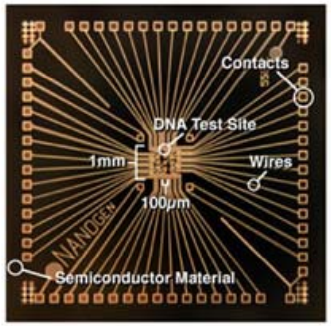


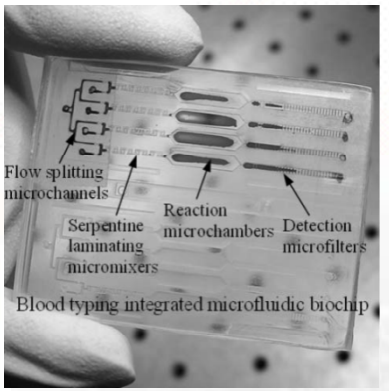
Marc J. Madou, "Fundamentals of Microfabrication: The Science of Miniaturization, 2nd Edition", 2002

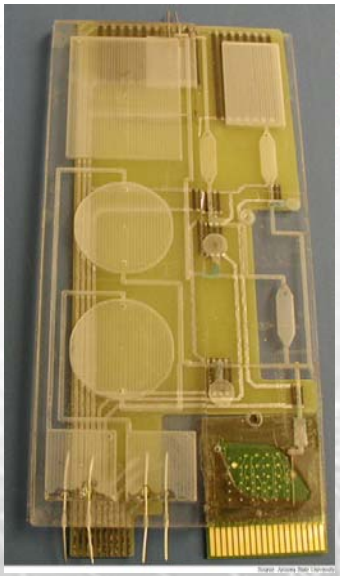
生物晶片

在玻璃、矽晶、塑膠等材質上，使用微機電、精密加工等工業技術來製成的微小化裝置，以從事生物性之反應與分析







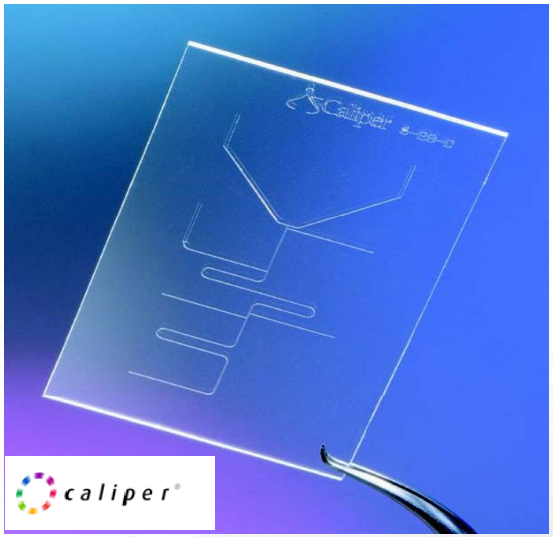


生物晶片之分類

- 微處理型生物晶片(Micro-processing Chips)
- 微陣列型晶片(Microarrays)

微處理型生物晶片之特色

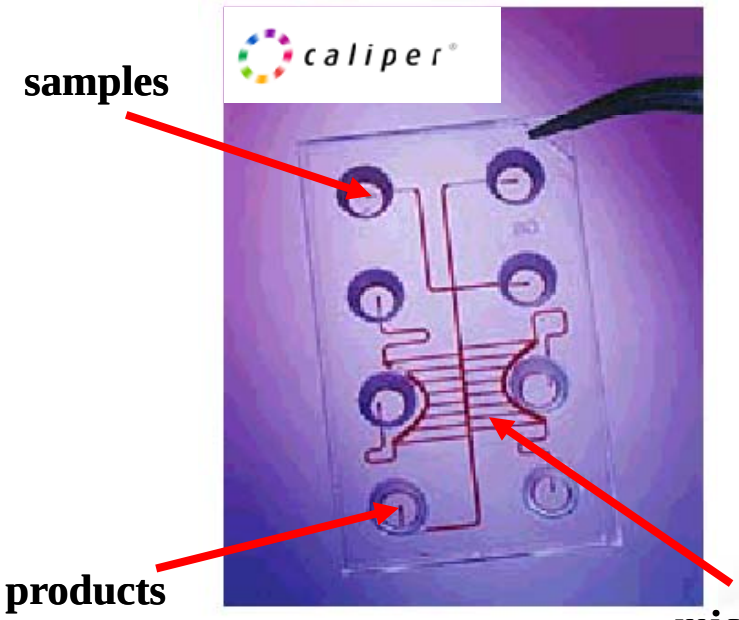
- 朝向微小化實驗室概念邁進，又稱為實驗室型晶片(Lab-On-A-Chip)
- 在晶片上從事生化反應、程序控制或分析
- 需應用微機電(MEMS)之製成技術，技術層次及複雜度較高
- 成本高，商業化較困難

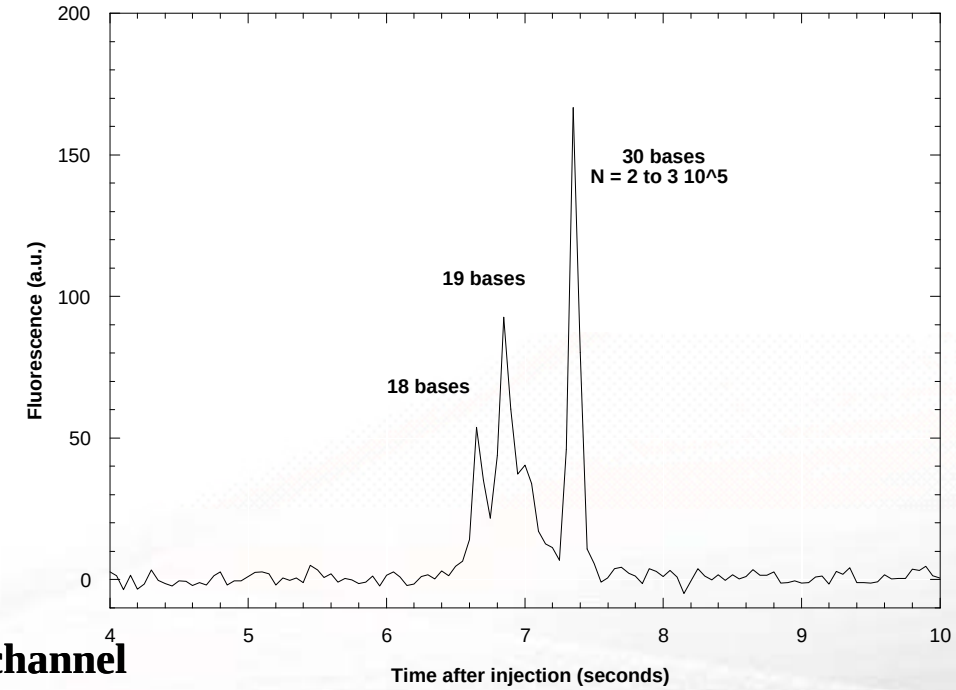


微處理型生物晶片之應用

- 樣品前處理晶片：例如處理血液(紅白血球分離)、組織、植物樣本的晶片
- 反應型晶片：從事微量化有機化學反應、生化反應、或聚合酵素鏈鎖反應(PCR)晶片等晶片(精子檢驗, 驗孕, 驗血糖)
- 分析型晶片：例如毛細管電泳(CE)晶片、高速篩檢晶片

Capillary Electrophoresis Separation

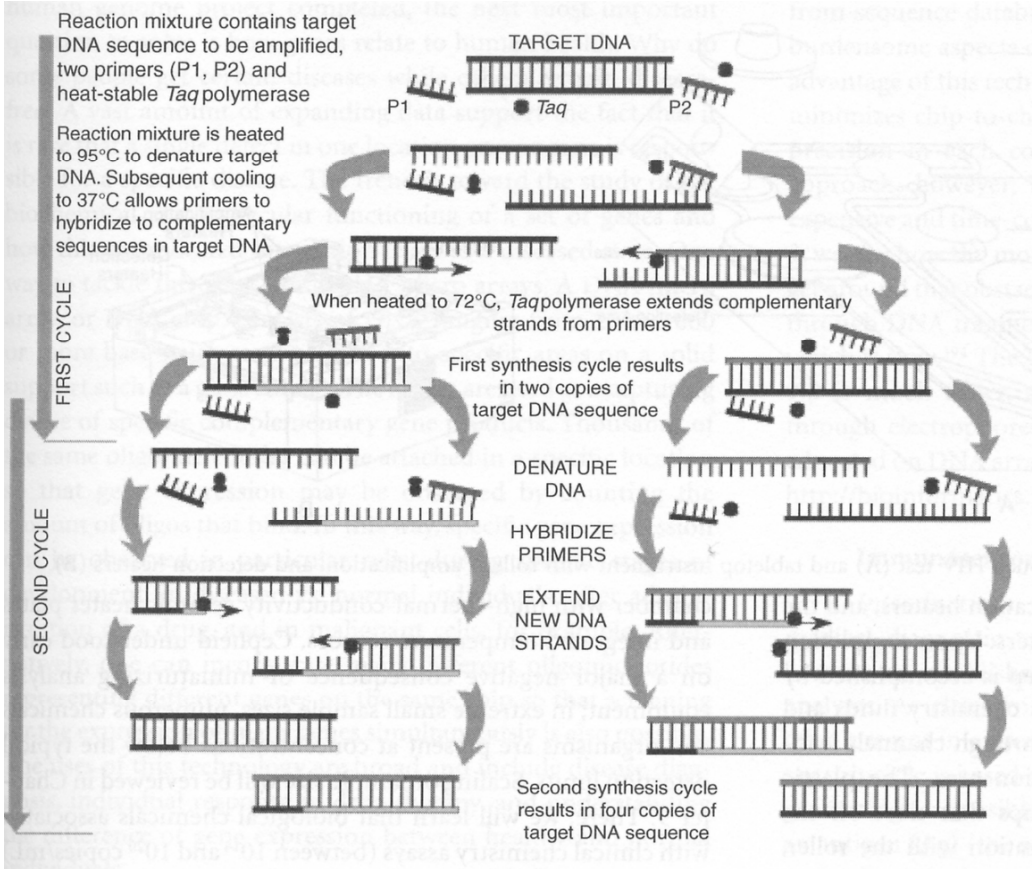




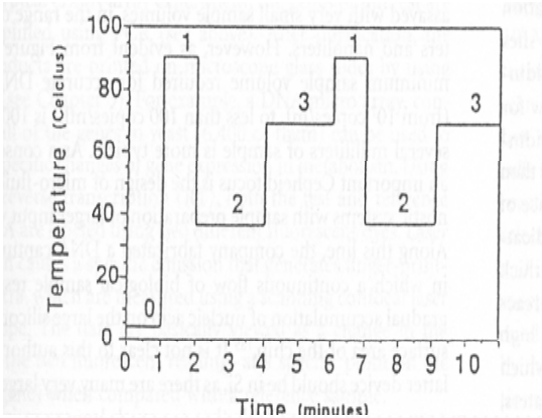
LabChip from Caliper Technologies

Polymerase Chain Reaction (PCR)

- PCR Principles



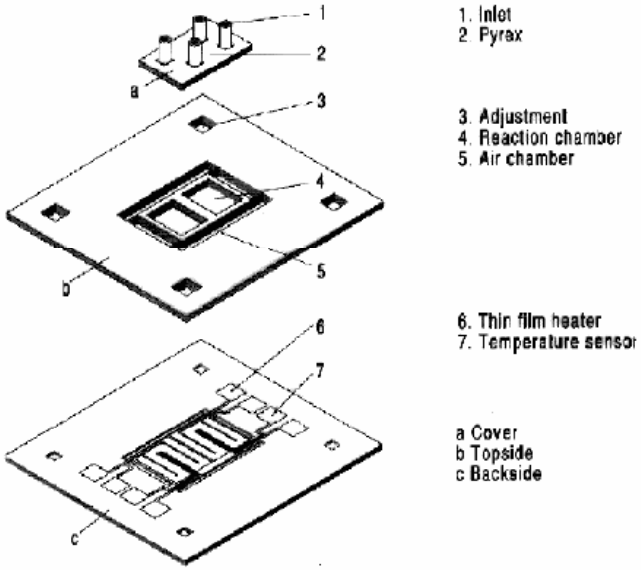
- Thermal Cycles

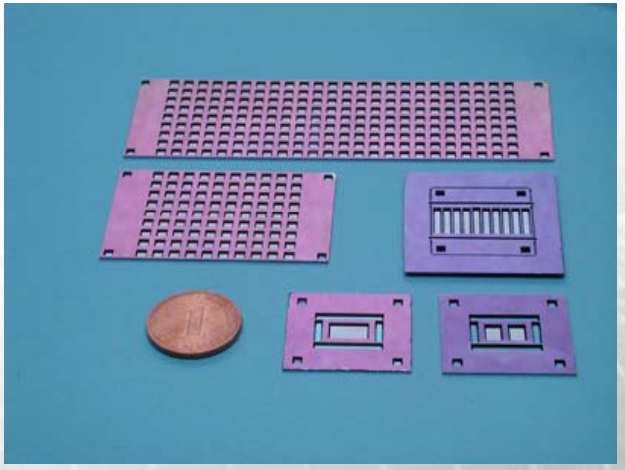


- Commercial PCR instrument from HYBAID

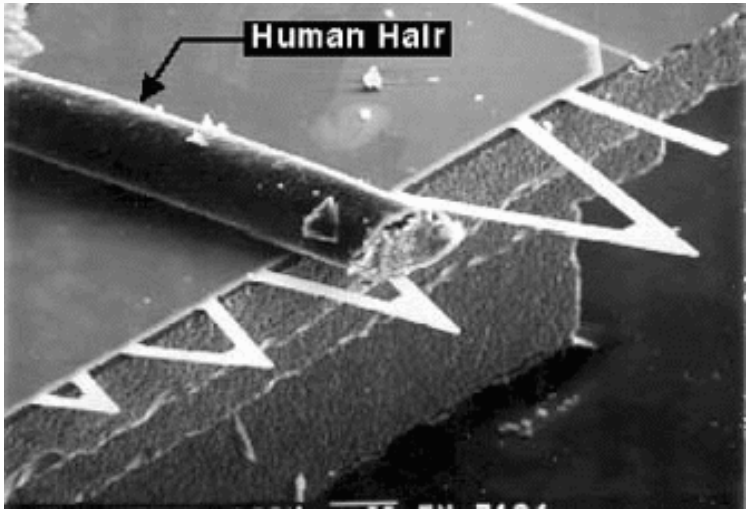
Single or Multiple PCR Chambers

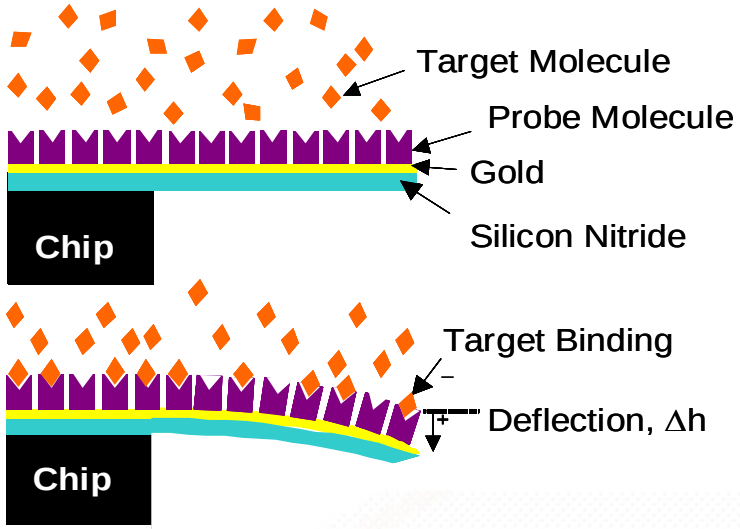
- PCR chamber (Dept. of Biological Microsystem, IPHT Jena)
  - Chamber volume: 3 ~ 10 μl
  - Heating/Cooling rate: 10 ~ 80 K/s
  - Temperature/Heating control: thin-film transducers
  - Thermal cycles: 30
  - PCR time: 10 ~ 30 mins

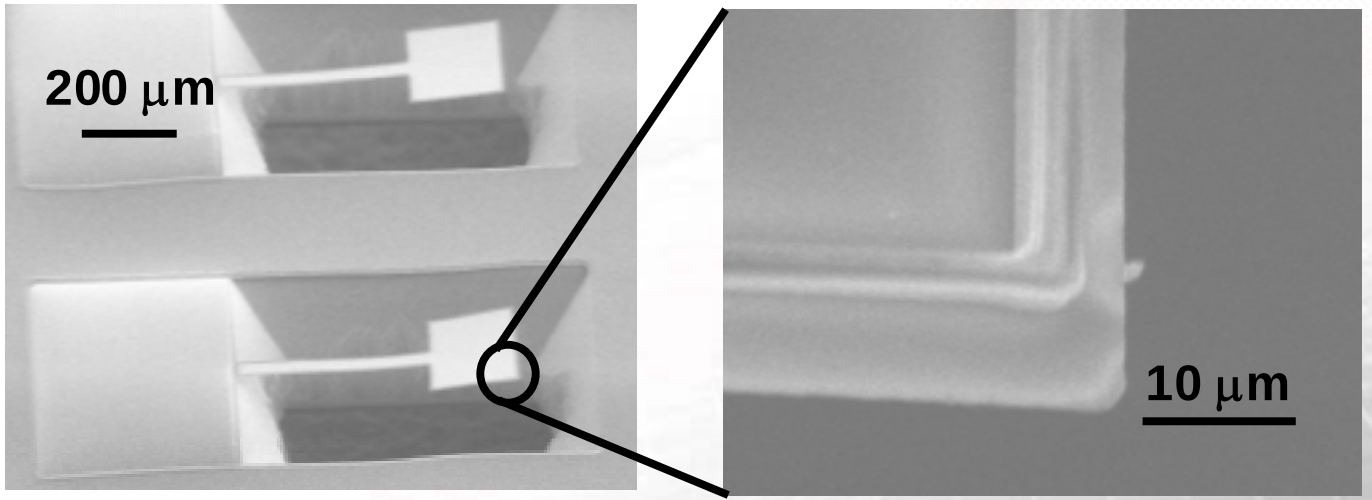




Biosensor Chips: Micro-Cantilevers

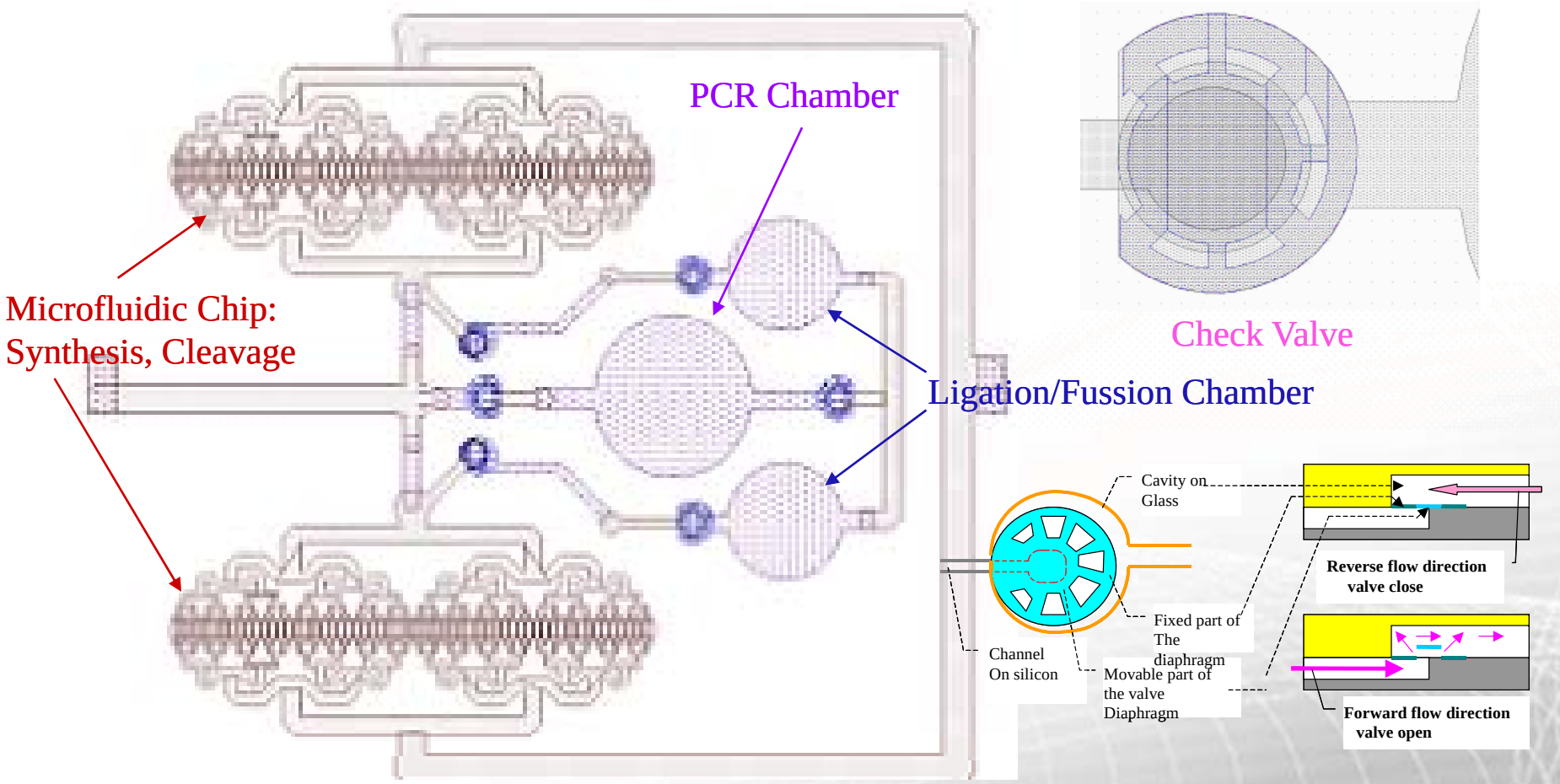






Synthesis Chips

- Objective: Assembly a large DNA (~10,000 bps) within 24 hours



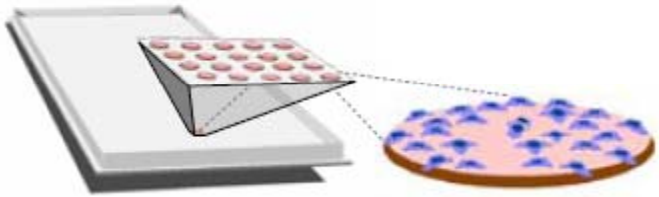
Integrated Biochips for Whole Blood Analysis

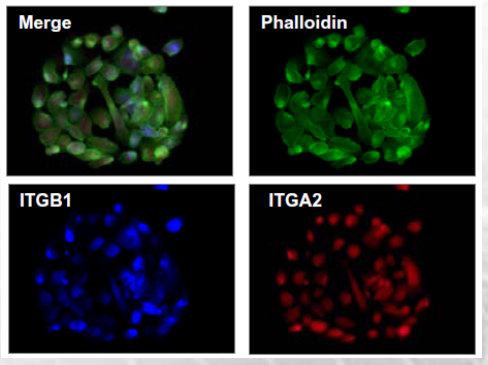
- Application: Detection of E. coli bacteria
- Functionalities
  - ✓ Target cell capture
  - ✓ Cell pre-concentration and purification
  - ✓ Cell lysis
  - ✓ DNA amplification (PCR) and detection (eSensor)
- Size: 10cm x 6cm x 0.2cm
- Fabrication: Plastic microfabrication

R. Liu et al., "Self-Contained, Fully Integrated Biochips for Sample Preparation, PCR Amplification, and DNA Microarray Detection" Anal. Chem., 2004

微陣列型晶片：Microarrays

- 基因晶片: 定序, 突變檢測
- 蛋白質晶片: 蛋白體篩檢
- 細胞晶片



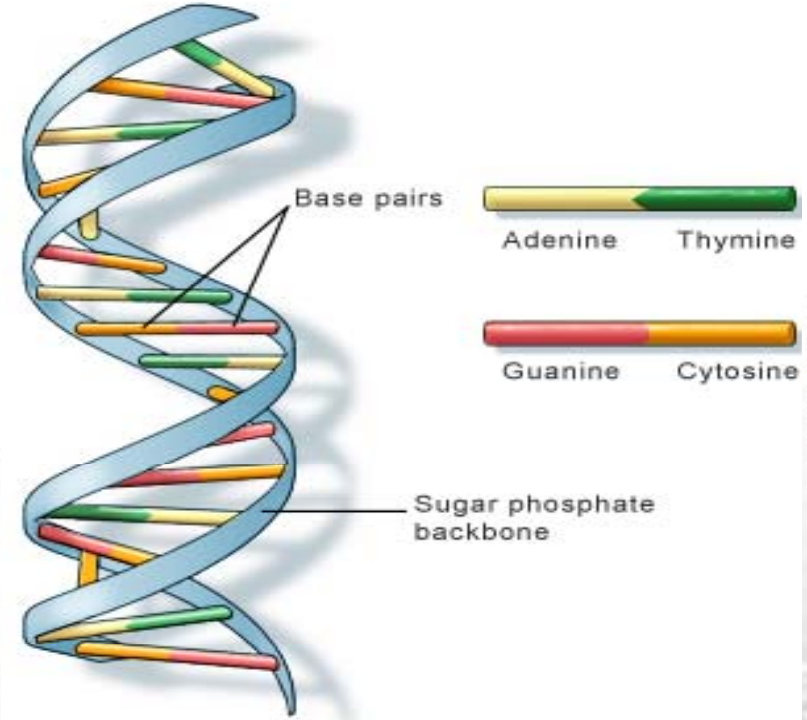


基因晶片之特色

- 體積小
- 所需樣品、使用試劑量少
- 快速分析
- 準確度高
- 可大量平行處理
  - 高密度 > 500 spots/cm^2 (Incyte, Affymetrix)
  - 中、低密度 < 500 spots/cm^2

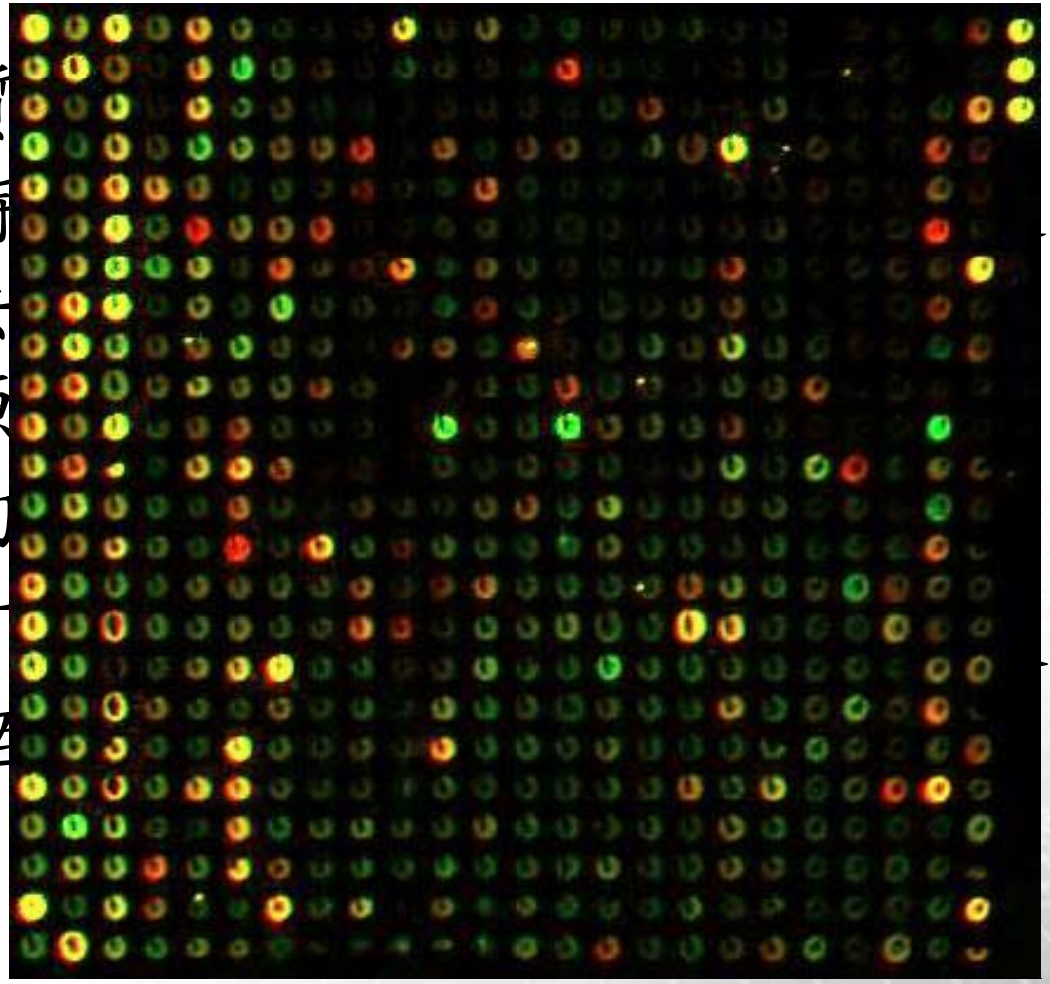
基因介紹

- 生物的組成為細胞
- 細胞組成：細胞核、細胞質、細胞膜/細胞壁（植物）
- 細胞核含有染色體
- 染色體由捲曲的核酸（DNA）序列組成
- DNA由ATCG鹼基組成，為雙螺旋結構
- 基因位於核酸序列上控制生物的表現



基因晶片之反應原理

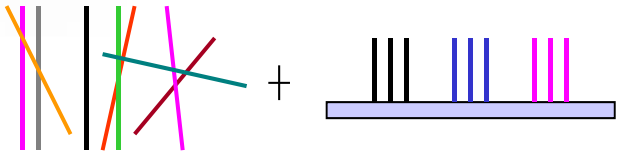
- 在晶片基質
  排列大量特
  過 DNA (去
  股配對的原
  標物的目的
- 生物探針可
  (oDNA)或互



Important Features of DNA/Gene Microarrays

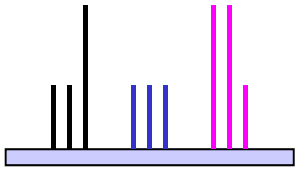
- ***Specificity*** (Accuracy): to ensure better hybridization efficiency
- ***Sensitivity*** (Dynamic Range): to detect gene expression differential levels
- ***Reproducibility*** (Spot-to-Spot & Array-to-Array CVs): to minimize technical errors associated with technology platforms

Specificity (Accuracy)

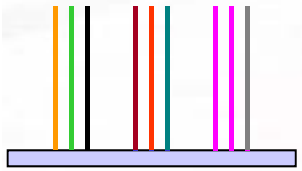


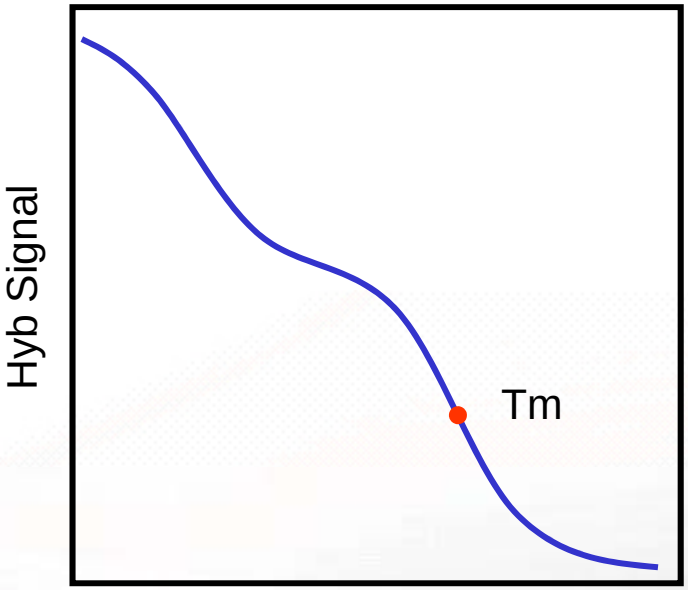
Temp. too high

Right Temp.



Temp. too low

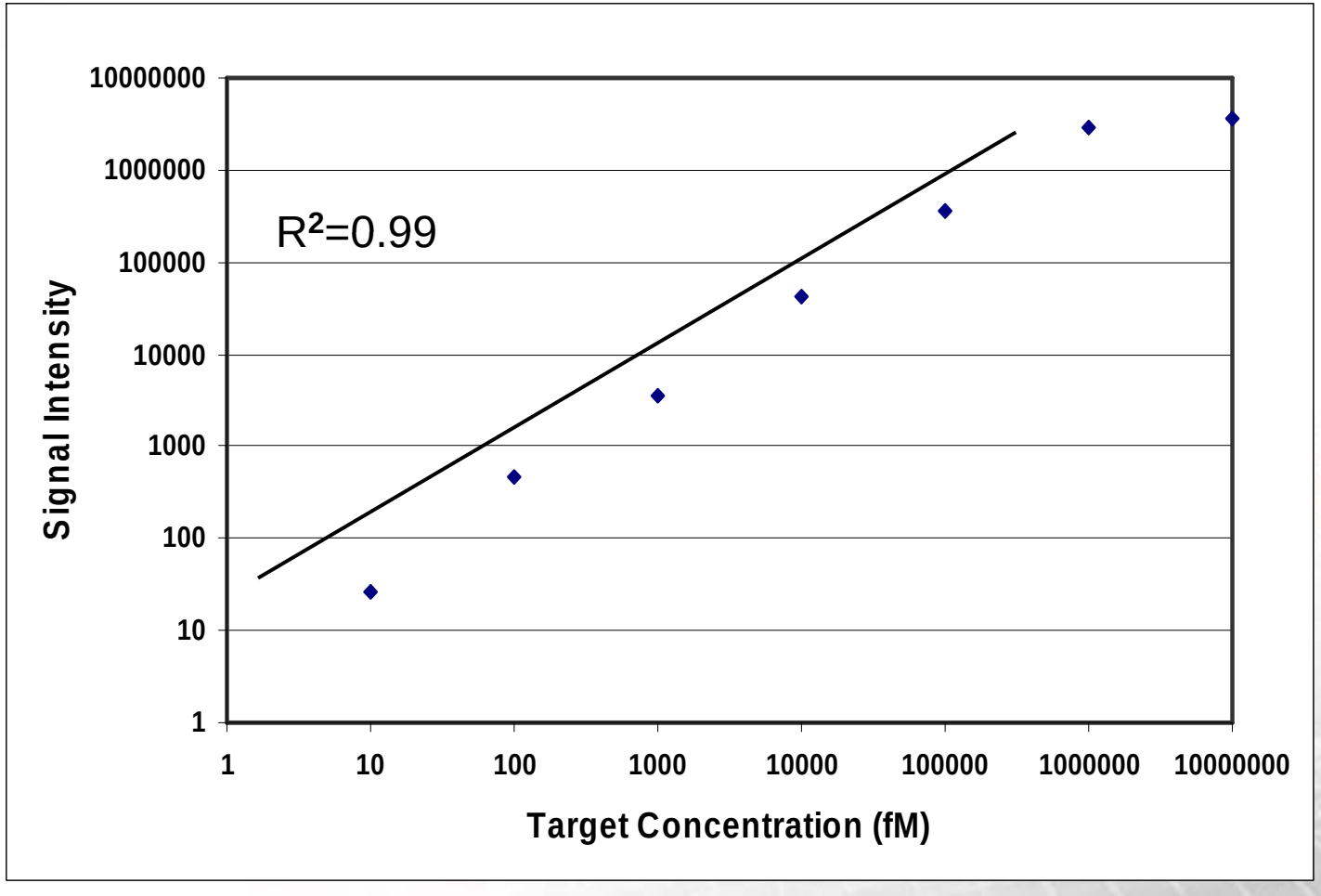




Important Features of DNA/Gene Microarrays (Conti.)

- ***Specificity*** (Accuracy): to ensure better hybridization efficiency
- ***Sensitivity*** (Dynamic Range): to detect gene expression differential levels

Sensitivity (Dynamic Range)



Linear dynamic range: 5 log

Important Features of DNA/Gene Microarrays

- ***Specificity*** (Accuracy): to ensure better hybridization efficiency
- ***Sensitivity*** (Dynamic Range): to detect gene expression differential levels
- ***Reproducibility*** (Spot-to-Spot & Array-to-Array CVs): to minimize technical errors associated with technology platforms

Spot-to-Spot Uniformity

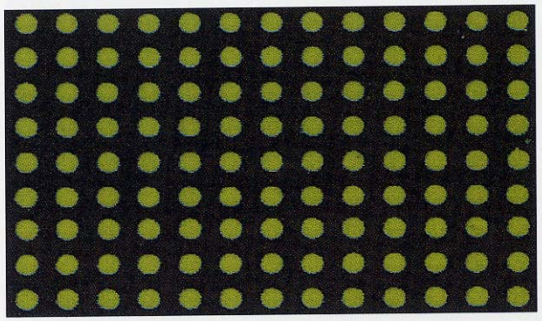


Figure 6. Microarray printed with the Pin and Ring technology. Plant DNA printed as 150-µm spots on polylysine-coated slides stained with eosin. (Data courtesy of Ray Samaha, EOS Biotechnology.) (See Chapter 3.)

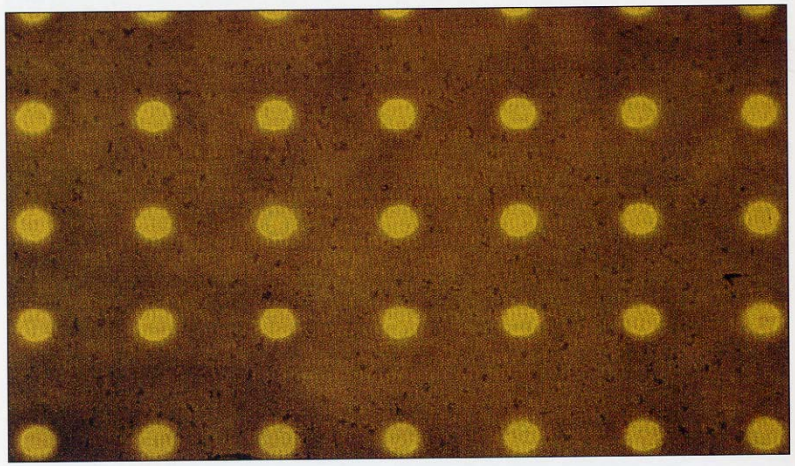
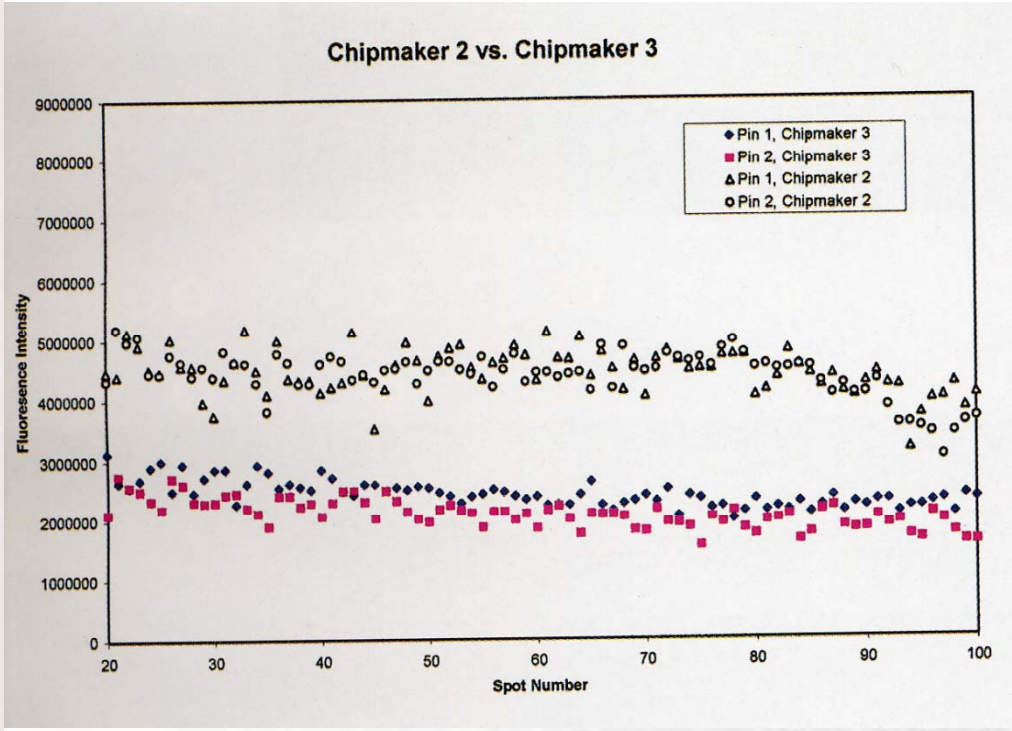
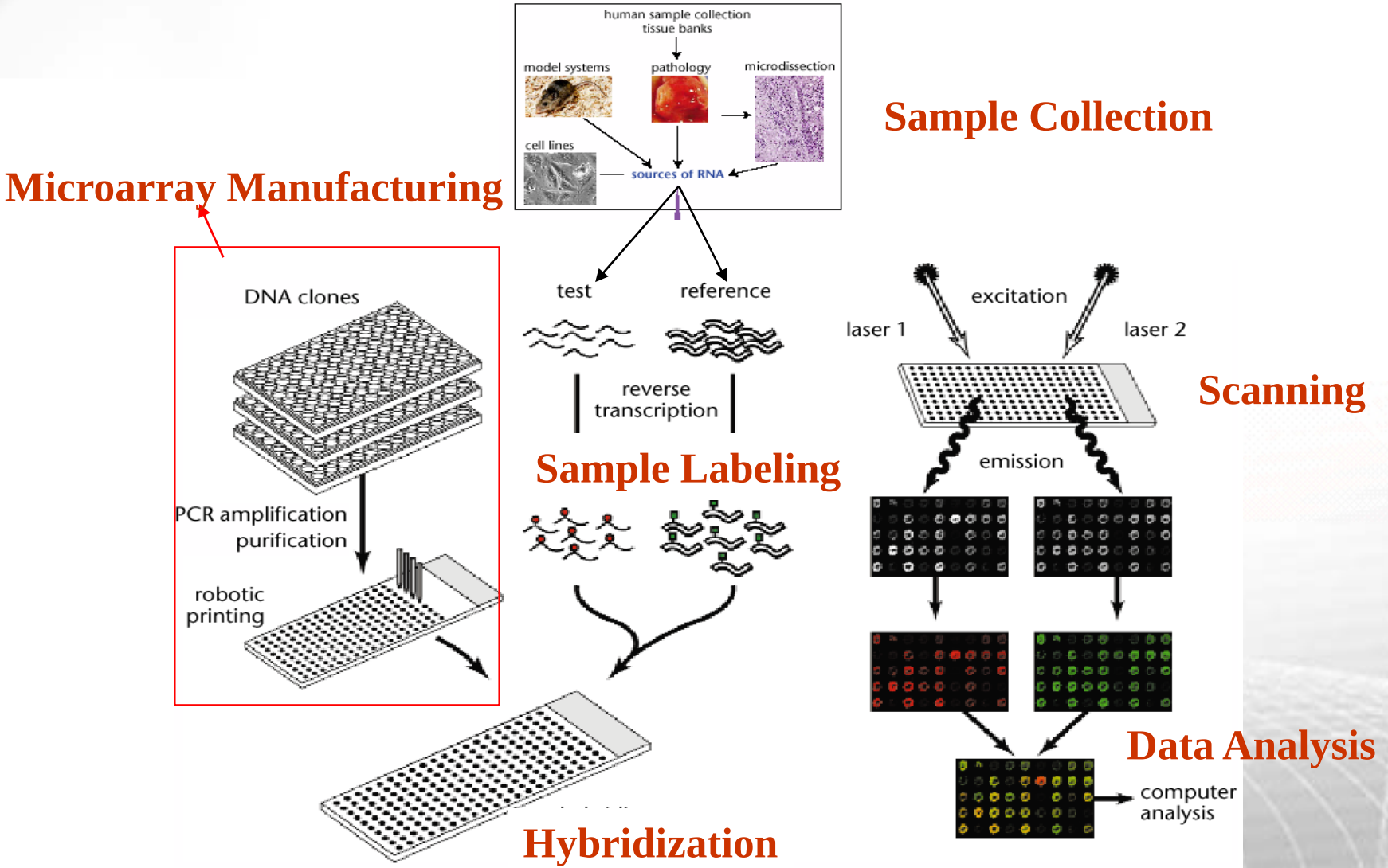


Figure 7. Pin and Ring printing on nitrocellulose. Shown is a transmission light micrograph of DNA spotted directly onto a nitrocellulose membrane. (Data courtesy of Dr. Bertrand Jordan.) (See Chapter 3.)

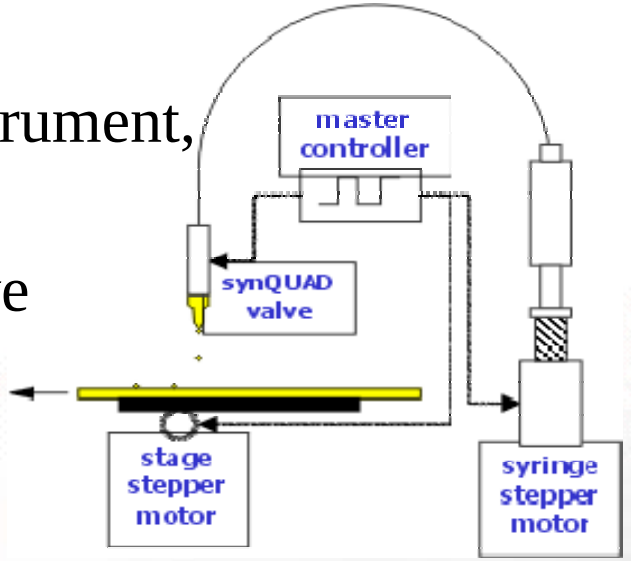


微陣列型基因晶片處理流程



Manufacturing/Technology Platforms

- Spotted Microarrays
  - Non-contact printing
    - Piezoelectric pump (Packard Instrument, Incyte Pharmaceuticals)
    - Syringe pump with solenoid valve (Cartesian Tech)



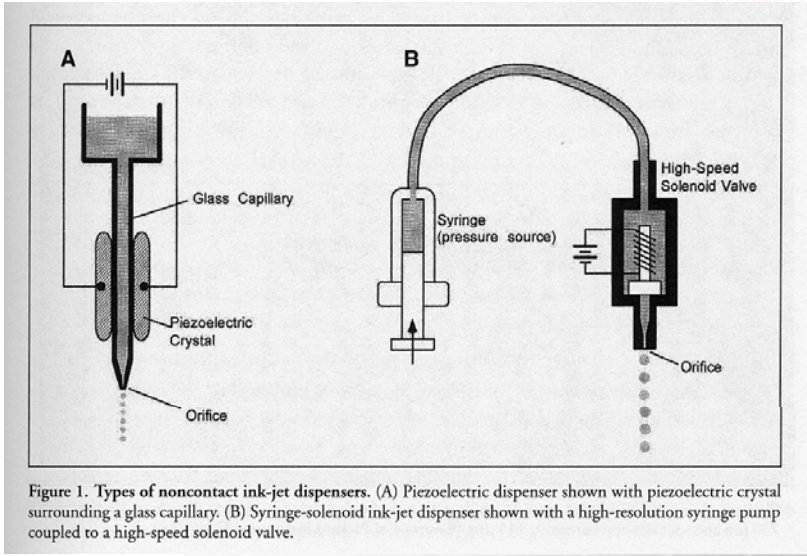
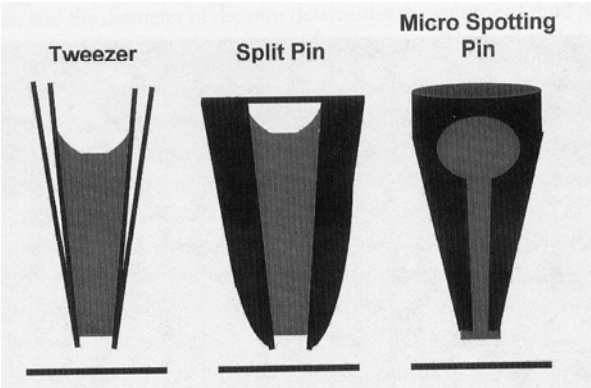
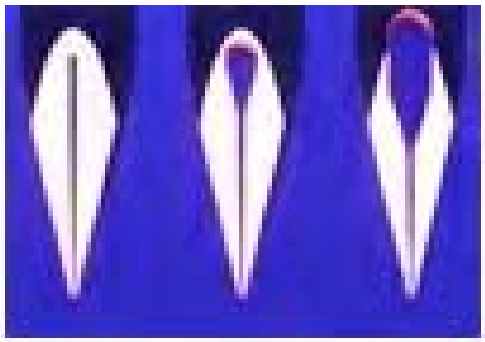


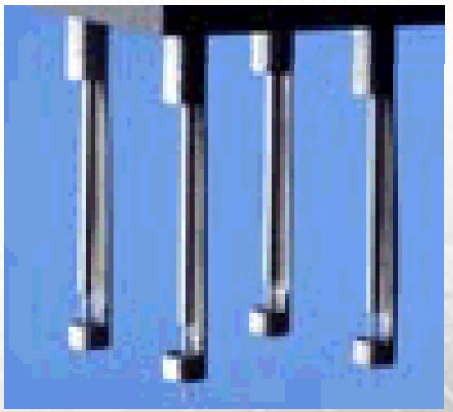
Figure 1. Types of noncontact ink-jet dispensers. (A) Piezoelectric dispenser shown with piezoelectric crystal surrounding a glass capillary. (B) Syringe-solenoid ink-jet dispenser shown with a high-resolution syringe pump coupled to a high-speed solenoid valve.

Manufacturing/Technology Platforms (Conti.)

- Spotted Microarrays
  - Contact printing
    - Split-pin (TeleChem Intl.)
    - Pin-And-Ring (Genetic Microsystems)
    - Capillaries (Genomatrix)



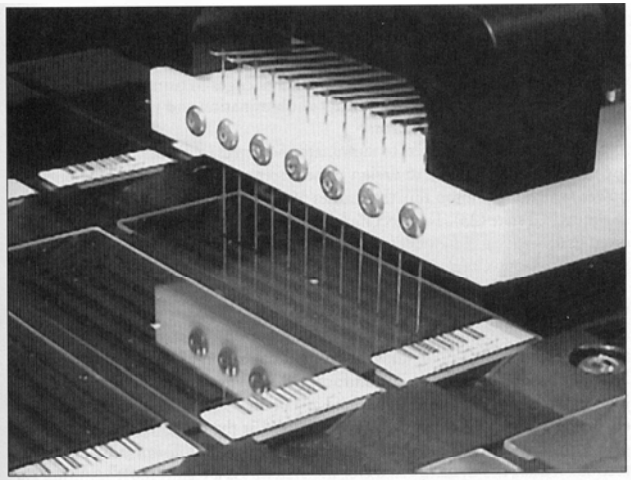


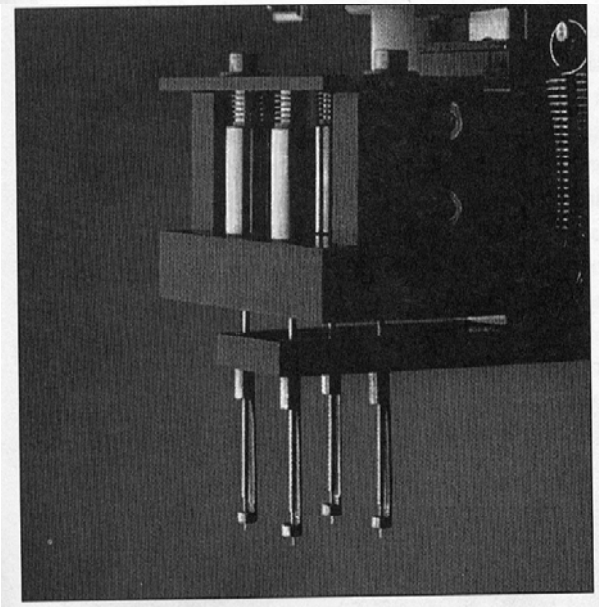


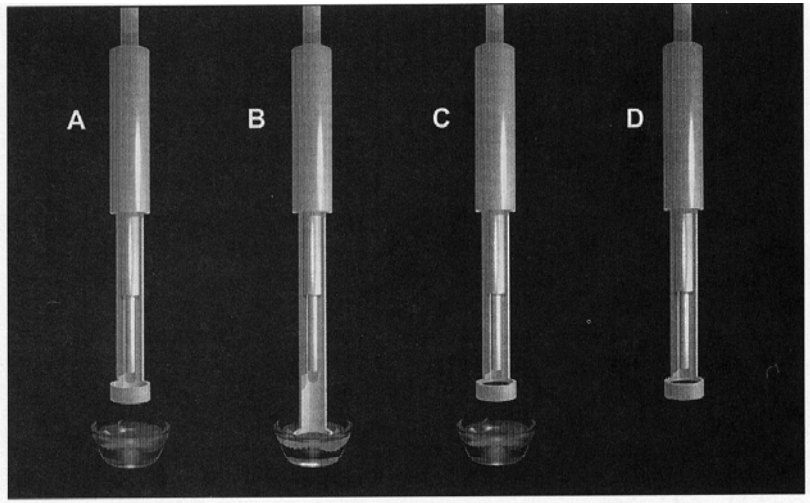
Microarray Printing System

- Hardware
  - Print head
  - Plate and substrate handling
  - Positioning stage
- Environmental Control
  - Humidity (65% ~ 75%)
  - Dust (from ceiling tiles, ventilation systems, etc)
- Instrument Control Software
  - Flexibility

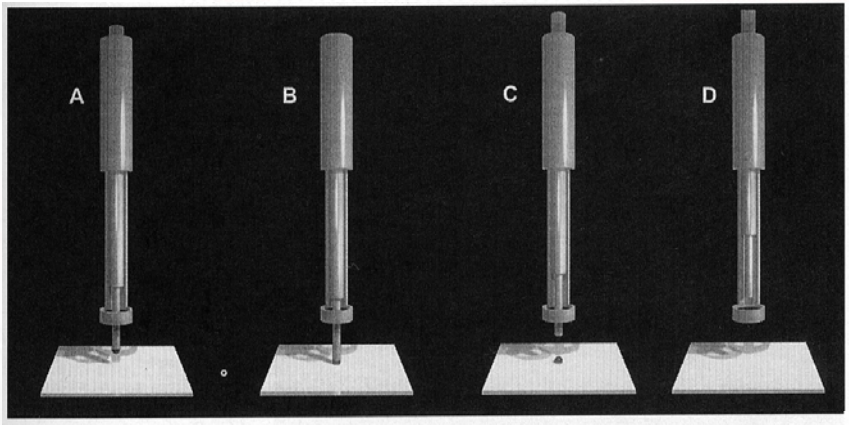
Pin-And-Ring (PAR)







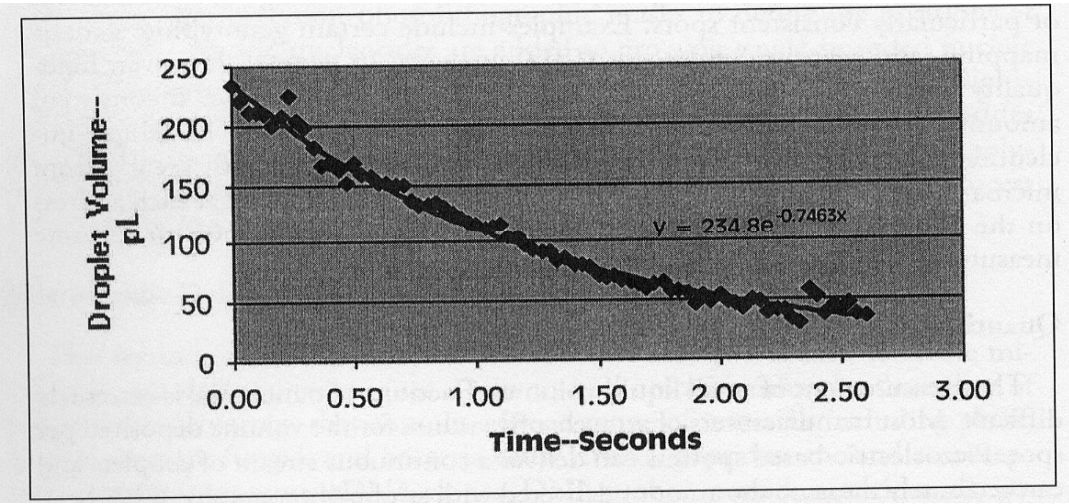
Sample loading



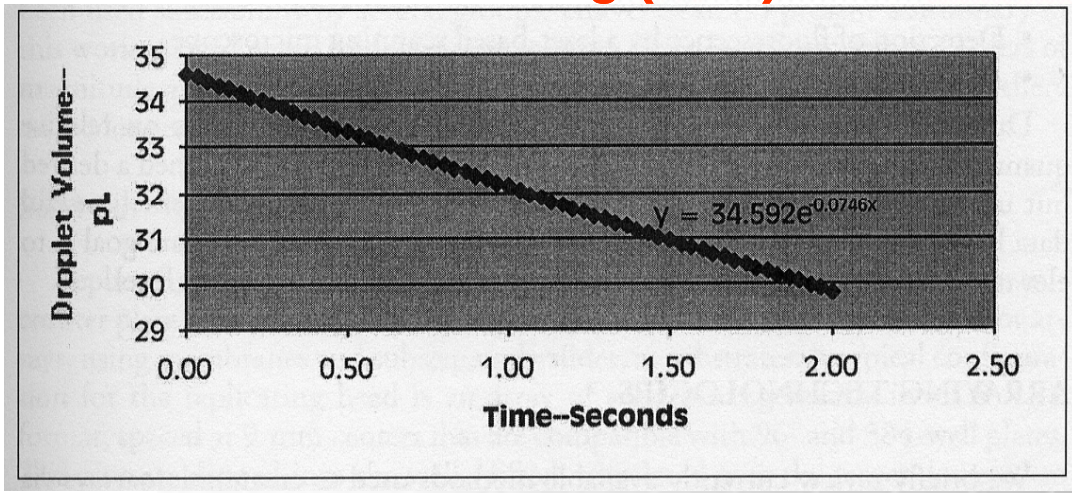
Printing on substrate

Sample Evaporation

Solid Pin



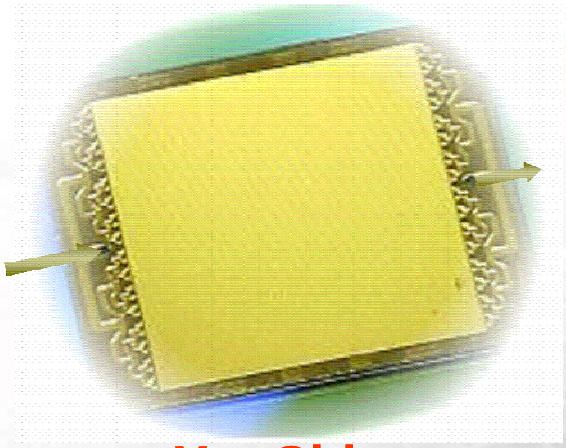
Pin-And-Ring (PAR)



Manufacturing/Technology Platforms (Conti.)

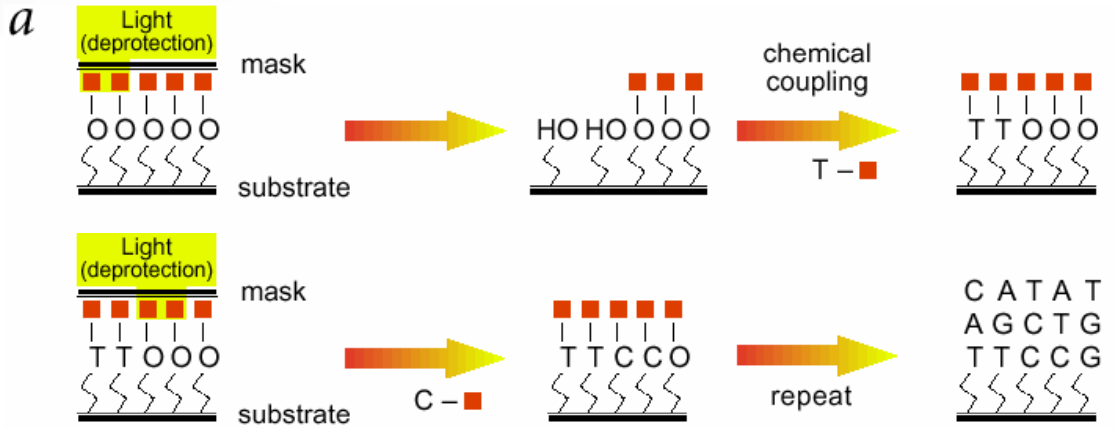
- In Situ Oligo Synthesis
  - Photosynthesis
    - Affymetrix (photolithography)
    - Xeotron (digital photolithography)

GeneChip



XeoChip

AFFYMETRIX


a
Light (deprotection)
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chemical coupling
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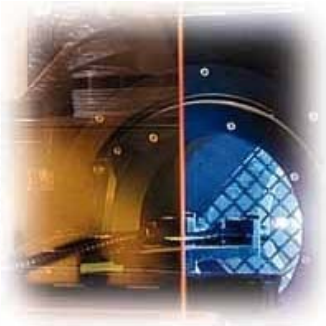

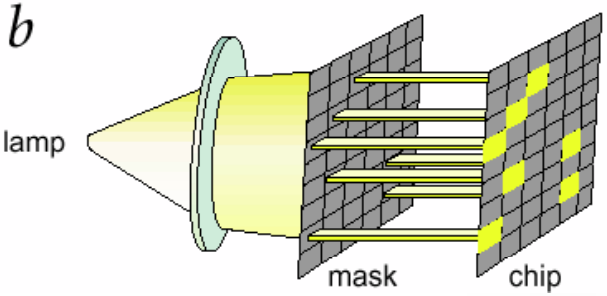
b
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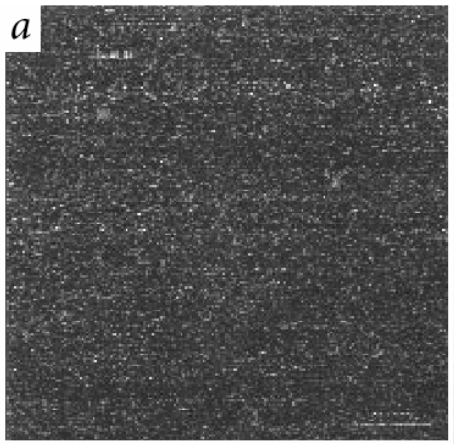
chip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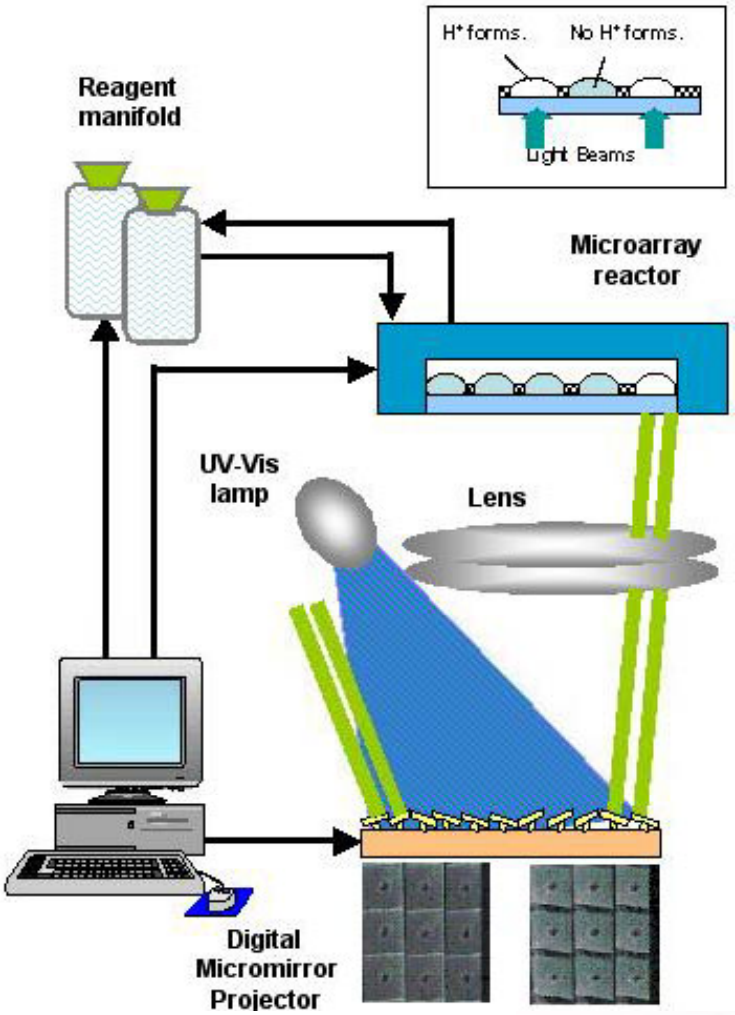
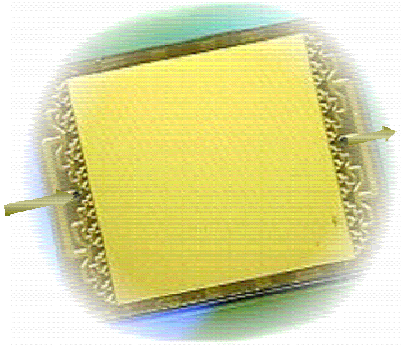
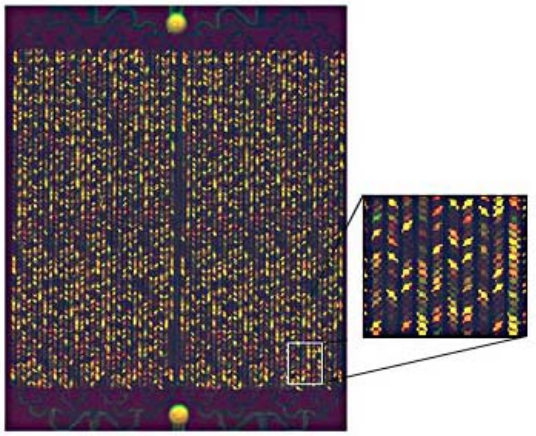
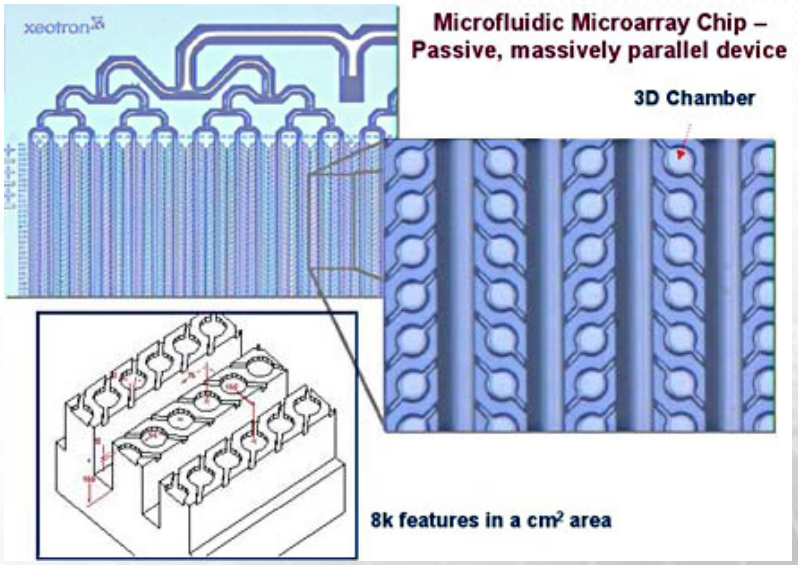
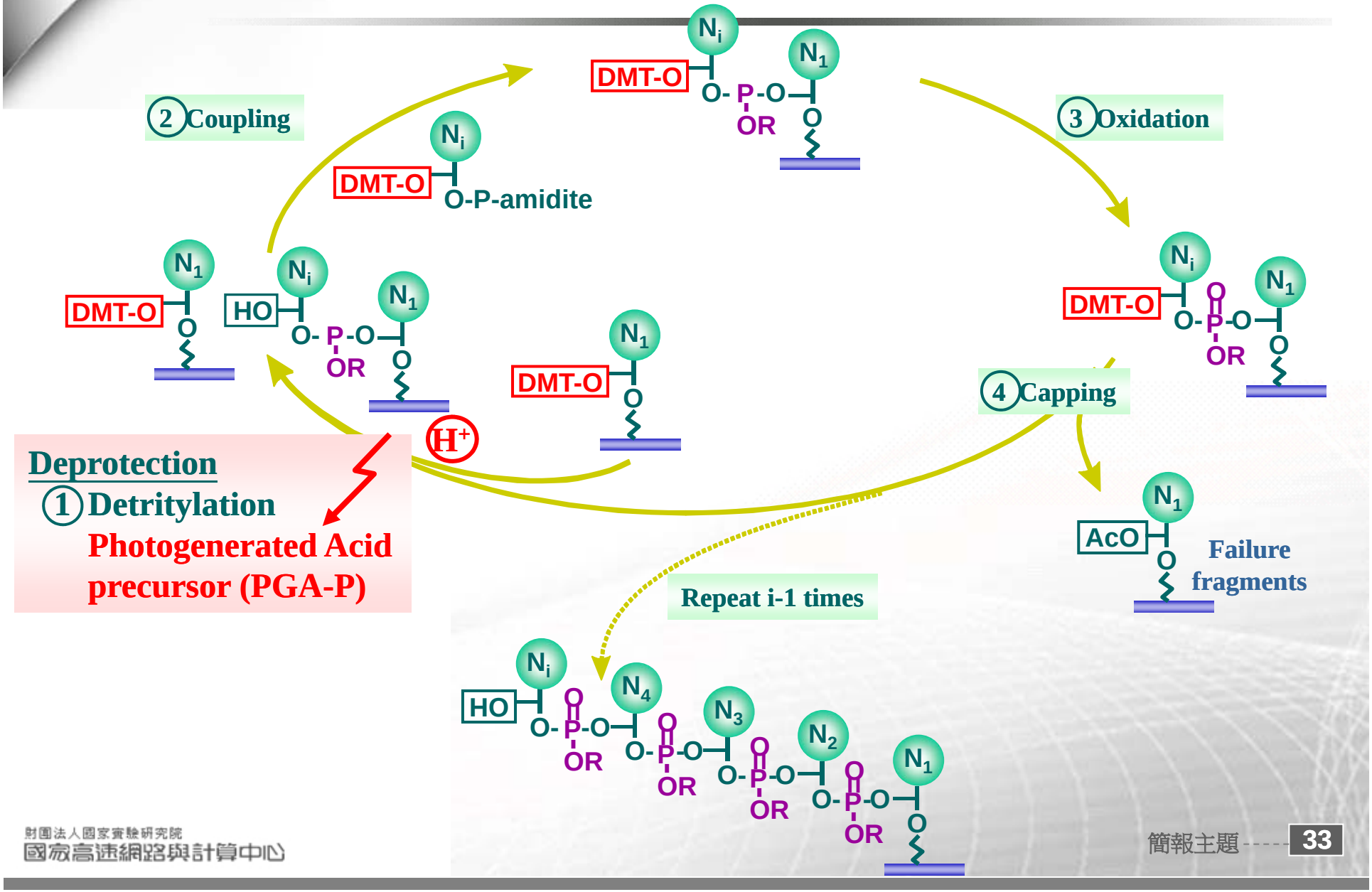

H⁺ forms.
No H⁺ forms.
Light Beams
Reagent manifold
Microarray reactor
UV-Vis lamp
Lens
Digital Micromirror Projector






xeotron
Microfluidic Microarray Chip –
Passive, massively parallel device
3D Chamber
8k features in a cm² area

Xeotron DNA Oligonucleotide Synthesis



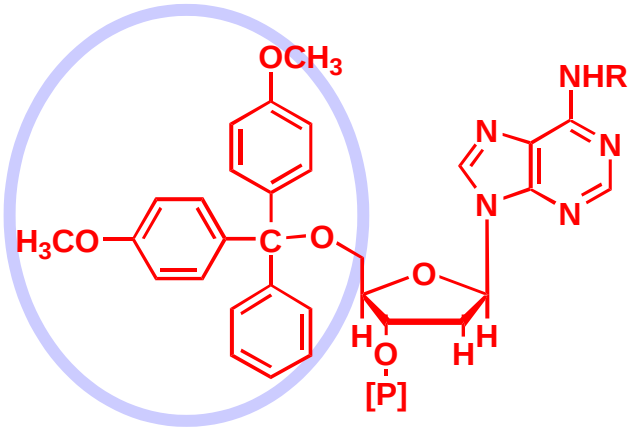
Parallel DNA Synthesis – Chemistry Distinct

XEOTRON

Conventional

Nucleotide Monomer

Standard protecting group



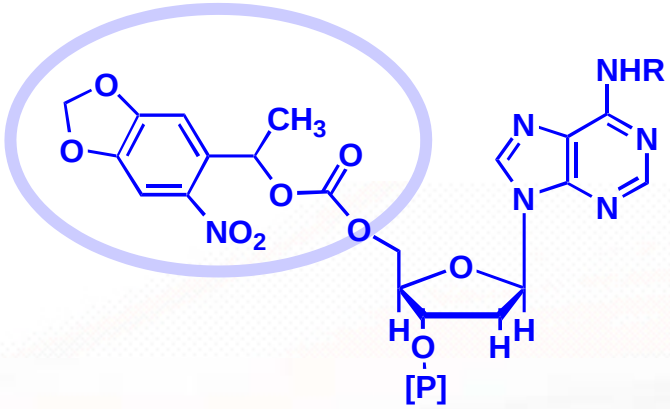
- **Readily available** (DNA, RNA, nucleoside analogue, peptide monomers, etc)
- **High Yield**
- **Possible for analog sequences**

OTHERS

Photolabile Group Protected

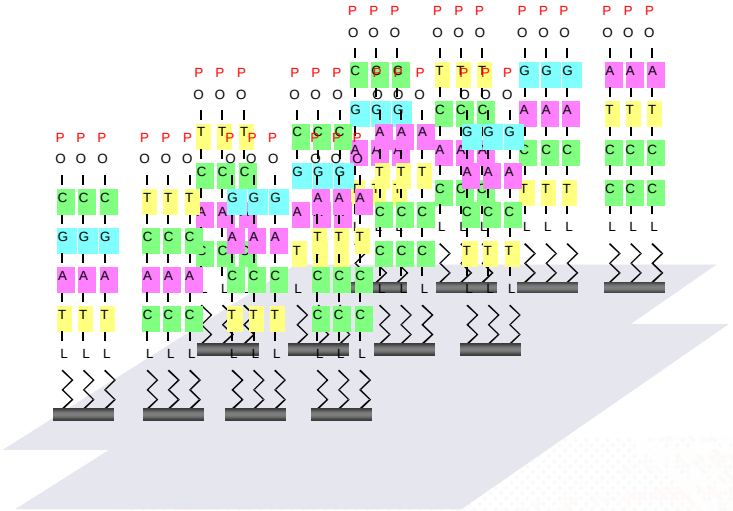
Nucleotide Monomer

Photolabile protecting group



- **Very limited availability**
- **Chemistry not ideal**
- **Very difficult for analogs**

Automated Microarray Synthesis

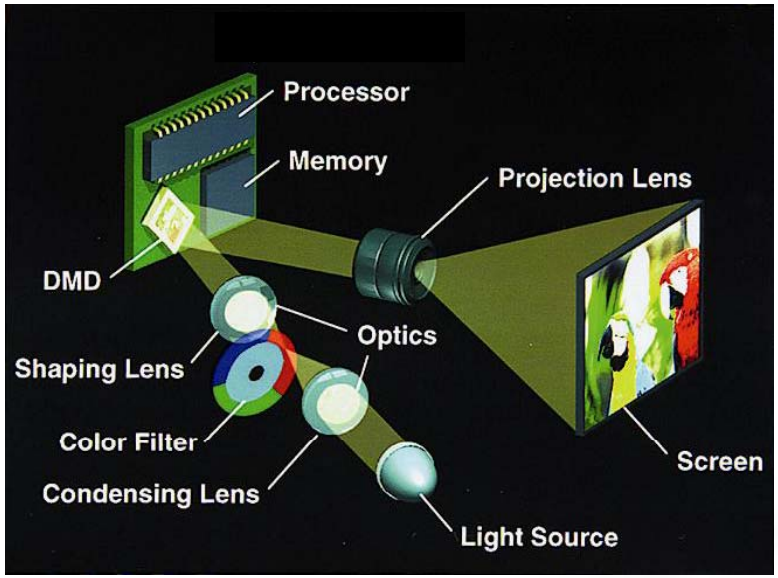


In a stepwise synthesis, one type of monomer is coupled at a time to the predetermined sites.

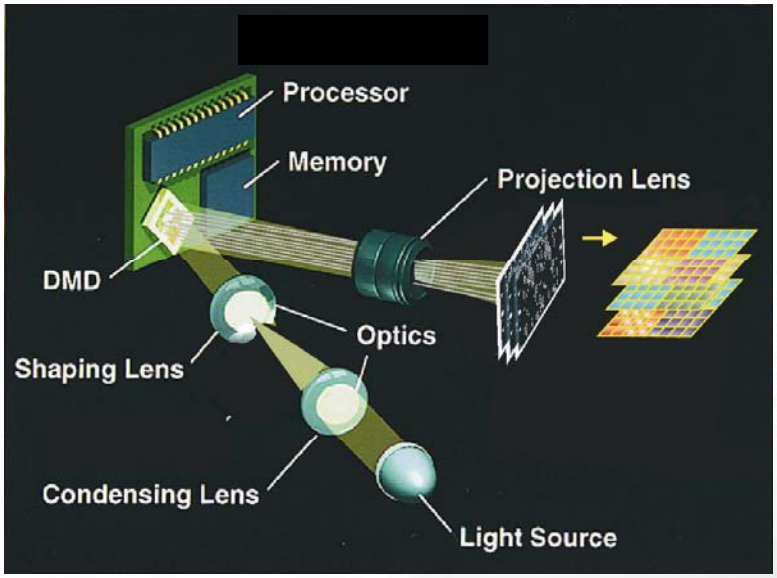
If the synthesis is done using photomask approach:

A 20-mer oligo array could require 20x4 photomasks!

Digital Light Projector (DLP) for Microarray Synthesis

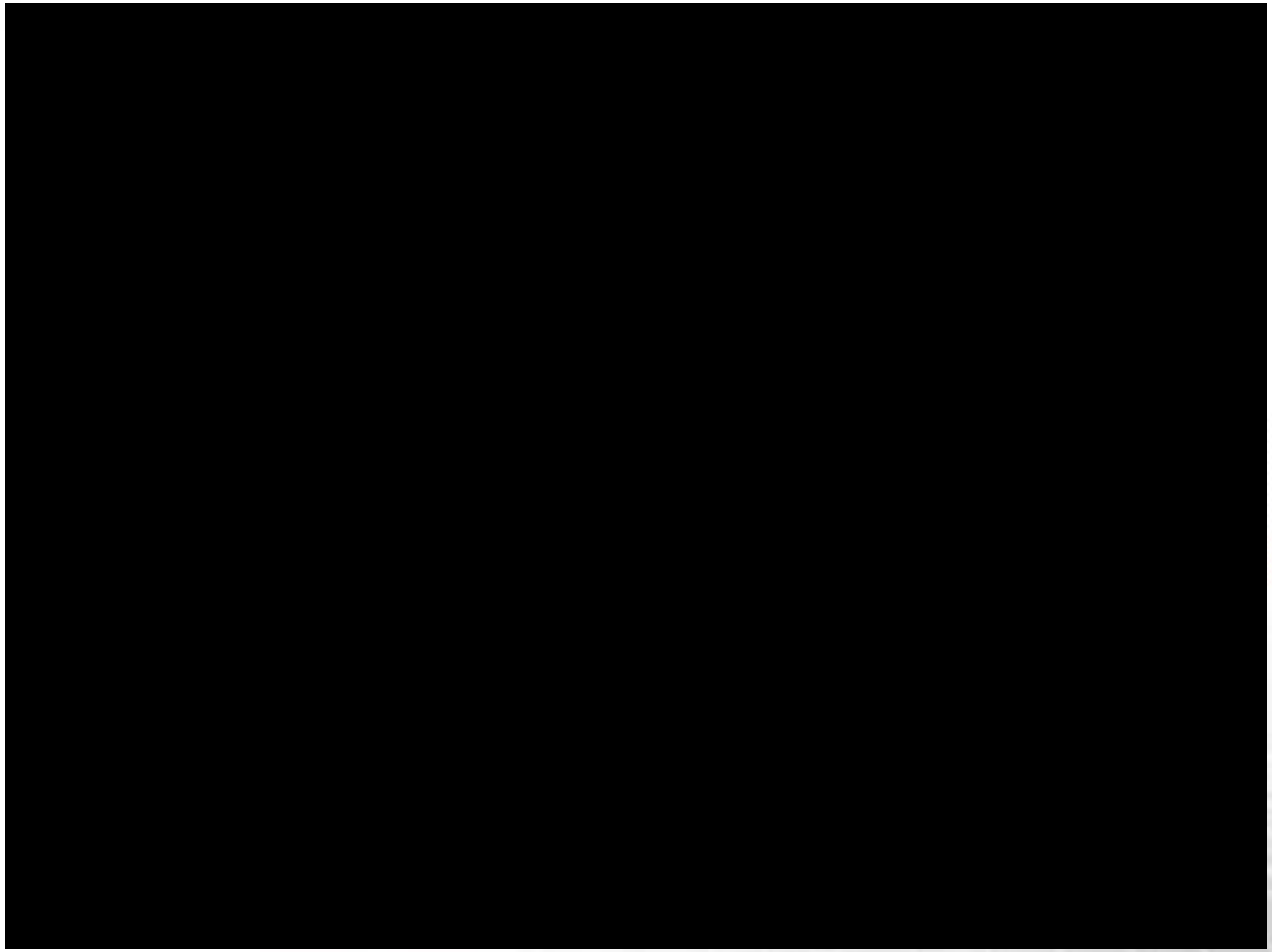


Texas Instruments DLP

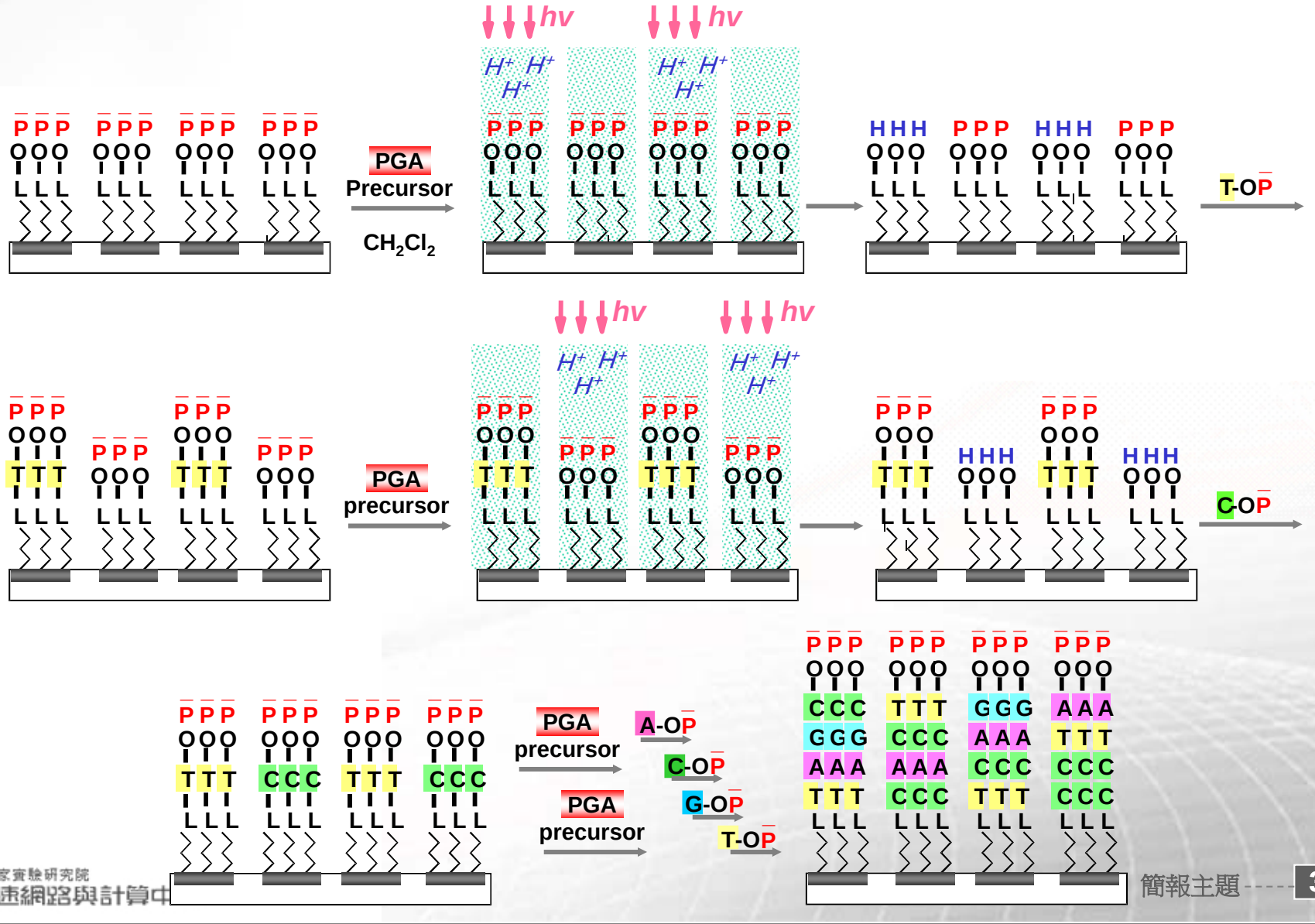


Xeotron DLP Process

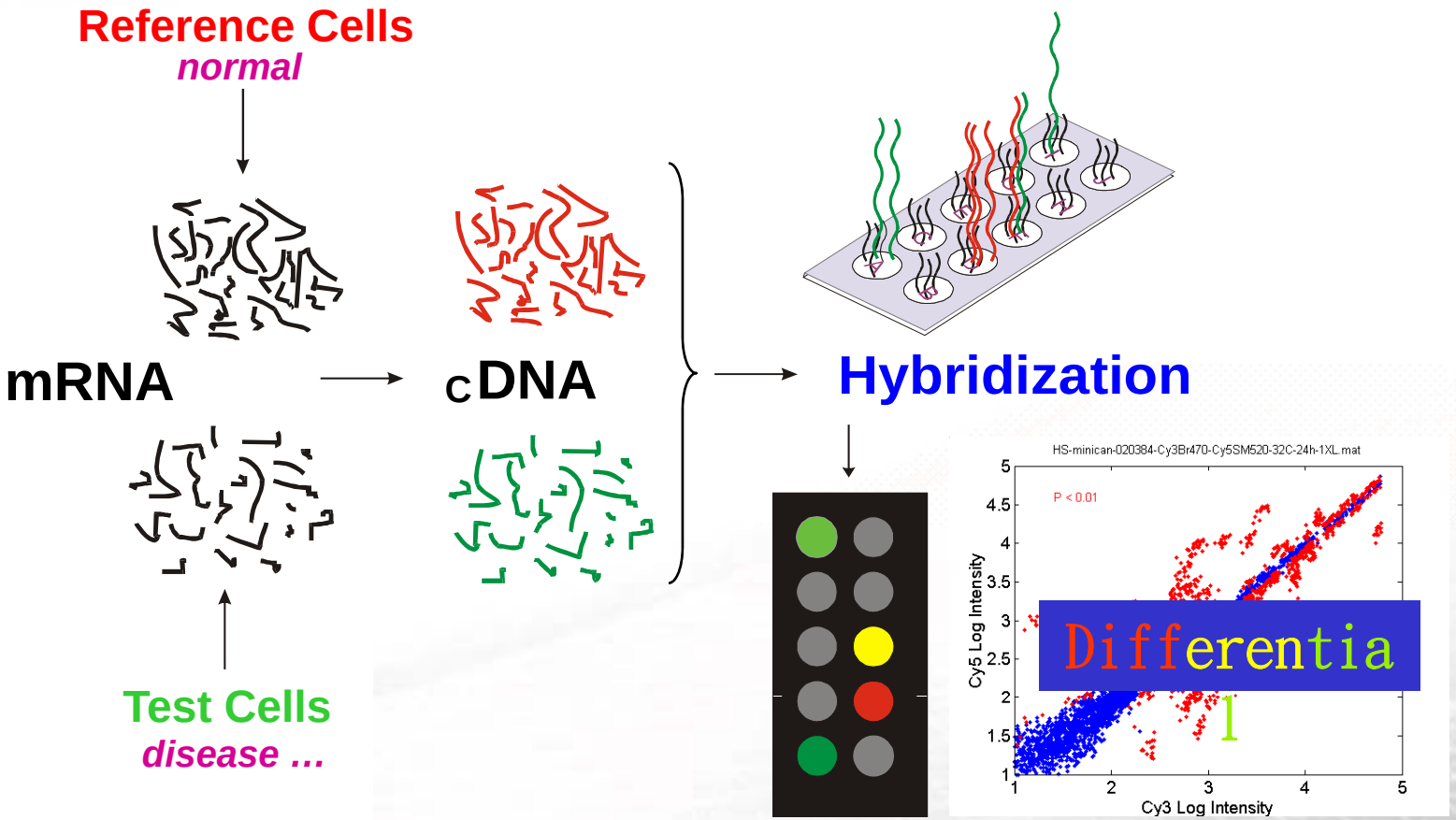
Xeotron's Microfluidic Chip – How it Works



Parallel Synthesis of Oligo-DNA

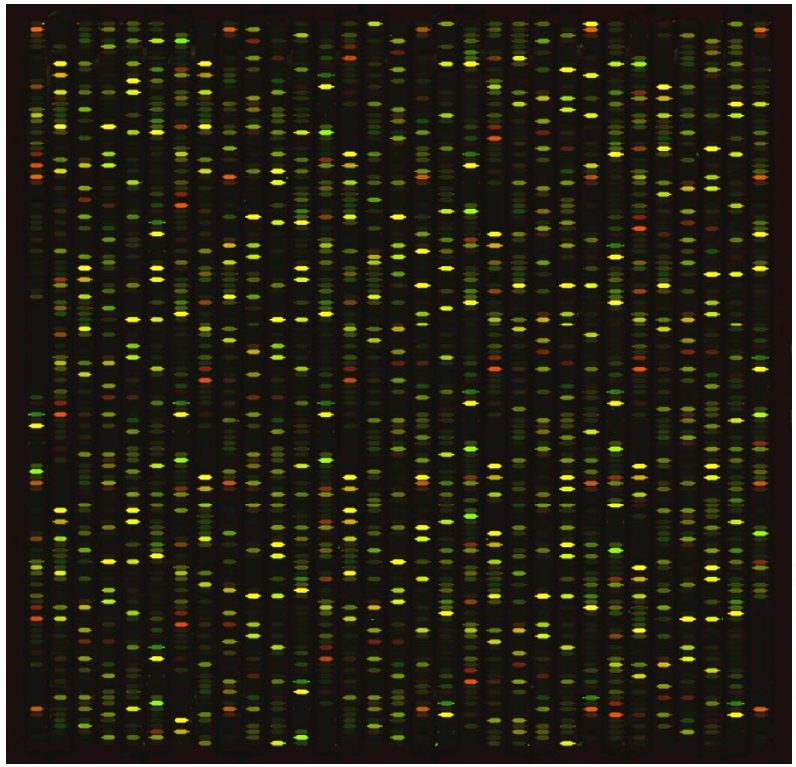


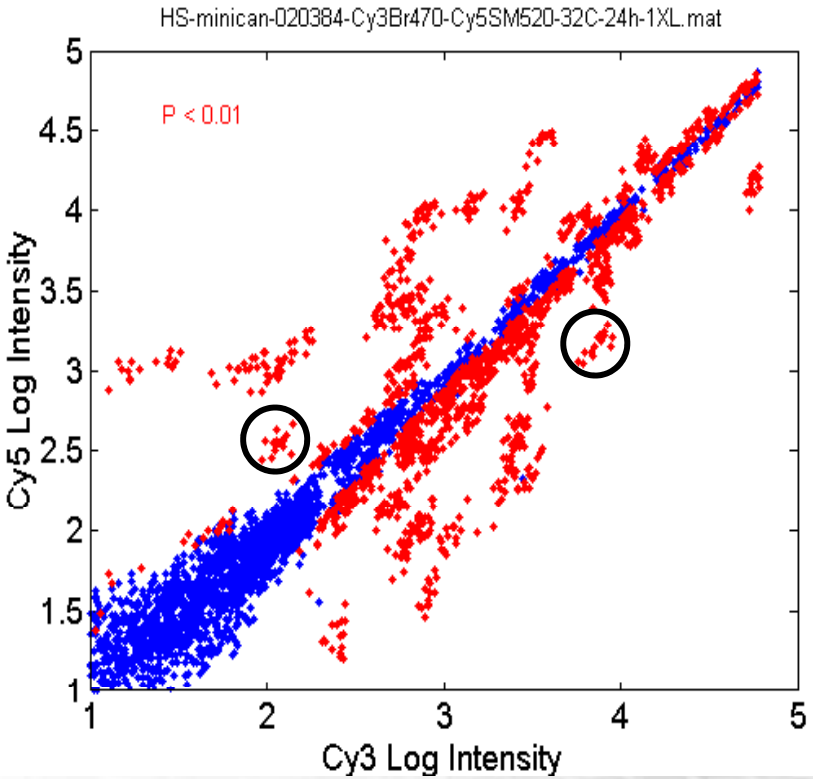
Gene Expression Analysis



Gene Expression Data

Sample A (Cy5) vs. Sample B (Cy3)





Cluster --- A Landmark of Gene Expression Study

"You don't look anything like the long haired, skinny kid I married 25 years ago. I need a DNA sample to make sure it's still you."

Thank you for your attention!!!