Fabrication and Application of Biochip - An Introduciton

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"We used to think our future was in the stars. Now we know it is in our genes."

- James Watson, Nobel Prize-winning geneticist and Human Genome Project developer.

- A story about overcoming "*the programming in his in-valid genes and competes against the best in the "valid" world.*"

The development goal for biochip:

Large capacity and tiny amount of sample

Biosensors - Definition by application

- Analyte is of biological origin.
  - DNA, RNA
  - Enzyme, Antibodies (Proteins)
  - Virus, Bacteria, Tissue
- Analyte is biologically important.
  - Ion
  - Drug molecule

Biochip: Biosensor or bioreactor in a format that fit in a small foot print by the integration, miniaturization, and parallel-processing techniques to practice

sample preparation (separation, concentration, labeling, transportation, ...),

analysis (mobility, molecular weight, ...),

detection (optical, magnetic, electromagnetic, electrostatic, mechanical, electrocehmical, ...),

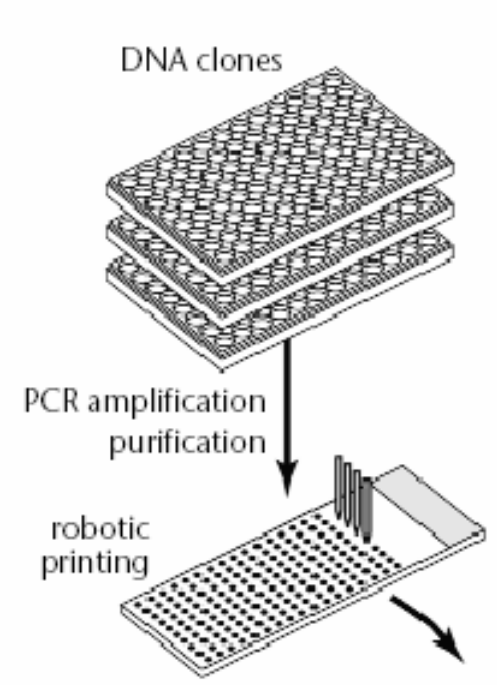
and (or) data acquisition (signal pick-up, noise reduction, interface, ...)

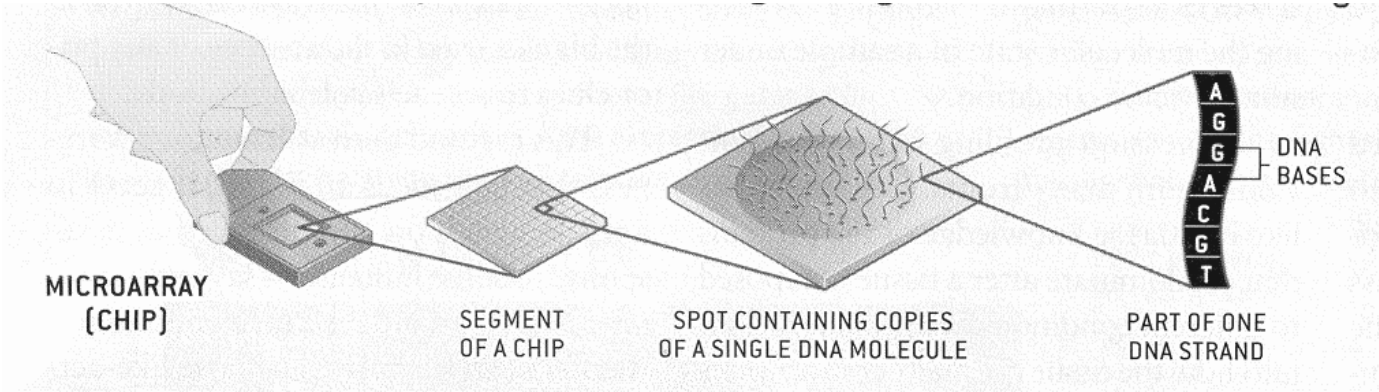
in aims of swift analysis, large throughput, high sensitivity, and (or) minimal sample consumption.

Various Type of Biochip

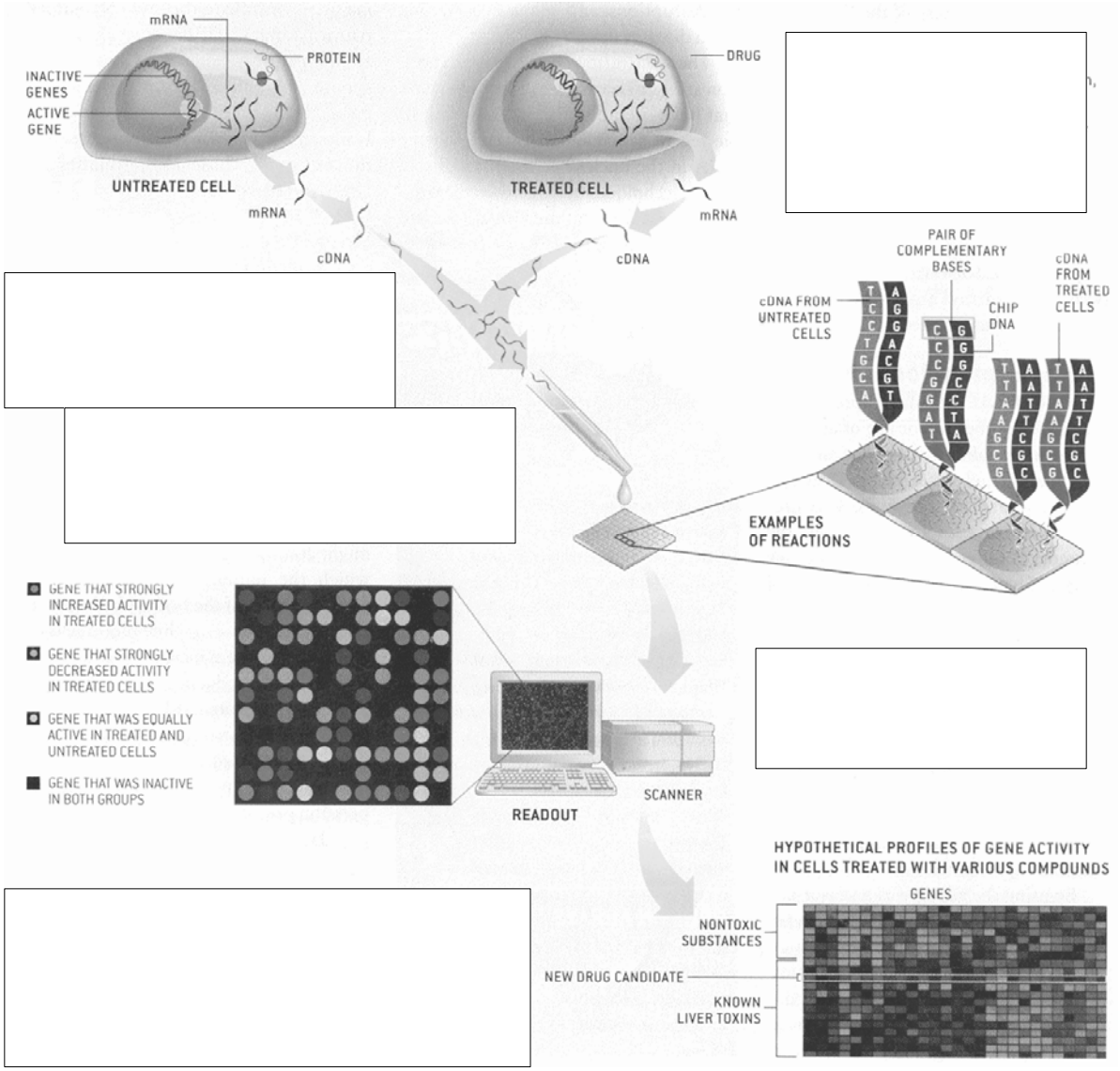
- DNA chip
  - cDNA microarray/chip
  - Oligonucleotide microarray/chip
- Protein microarray/chip
- Lab-on-a-chip

cDNA microarray Fabrication



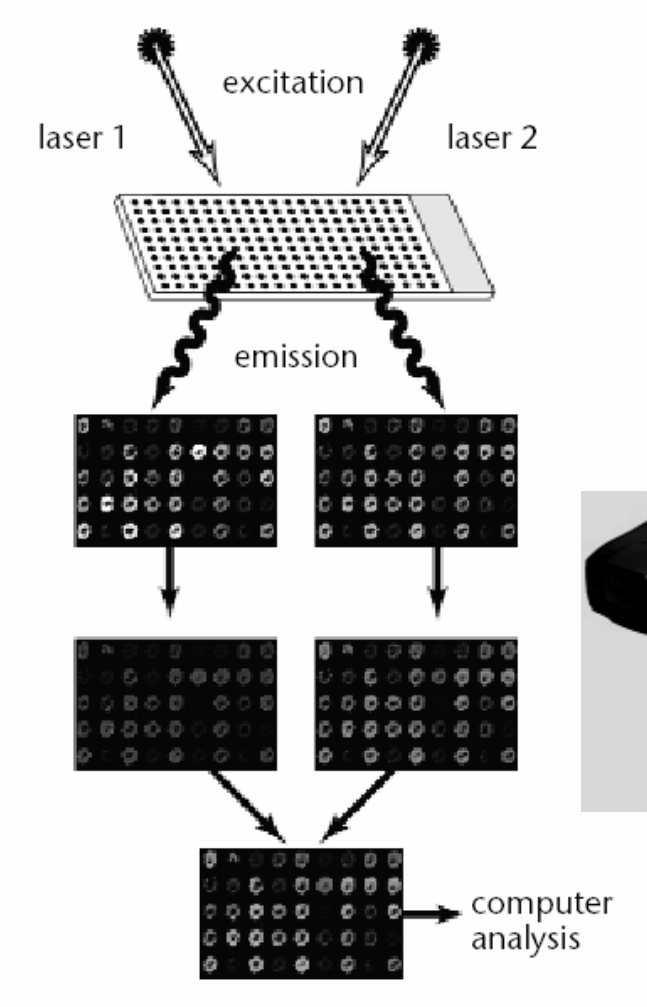


Gene Expression Profiling using DNA chip



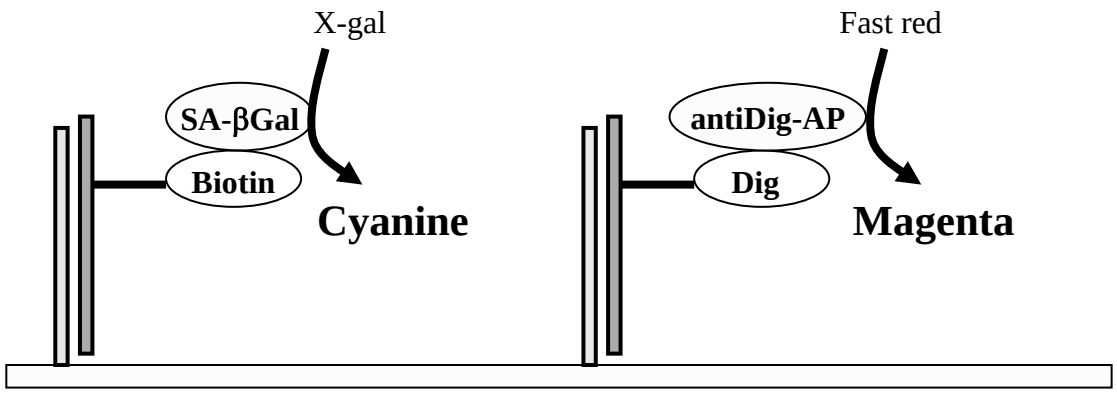
Excerpt from S. Friend, et.al., *Scientific Am. 2002, Feb,* p34

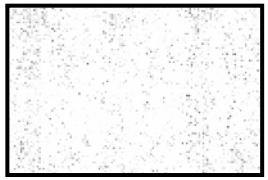
Fluorescence and Colorimetric Detection of DNA chip



P.O. Brown/Stanford U.

US$ 55,000





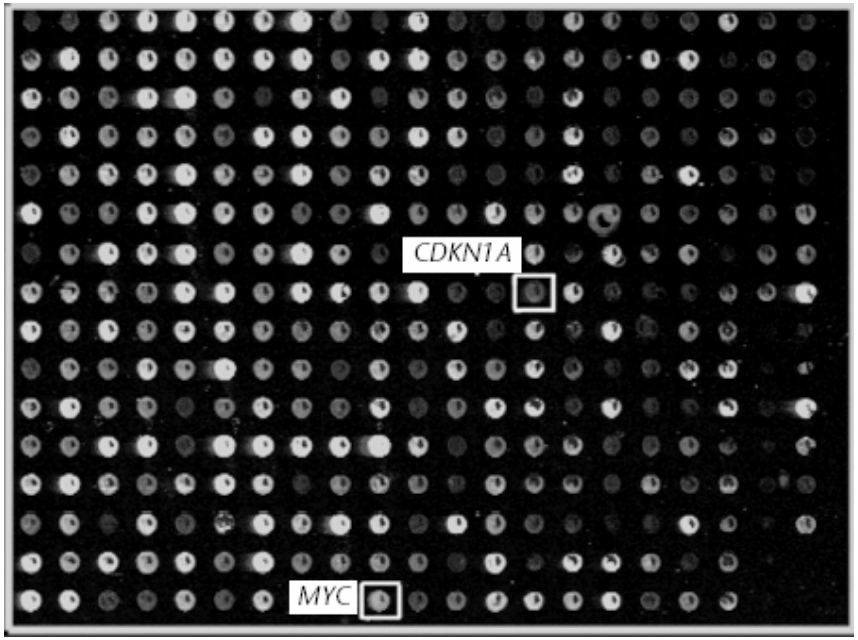
Dual Color Microarray

Image digitization

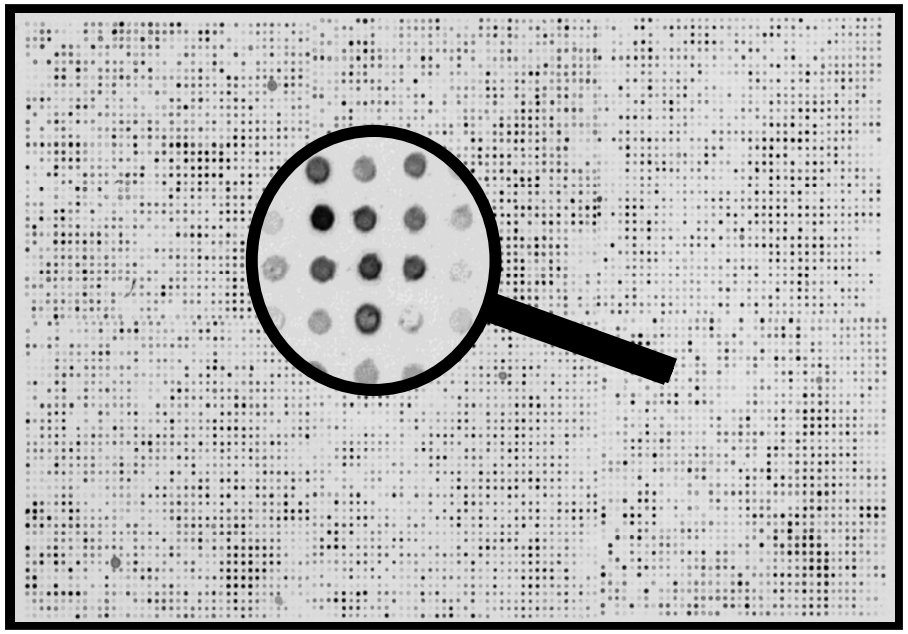
US$ 500~7,000

K. Peck (白果能)/IBMS Academia Sinica

Microarrays Images Using Different Detection Methods

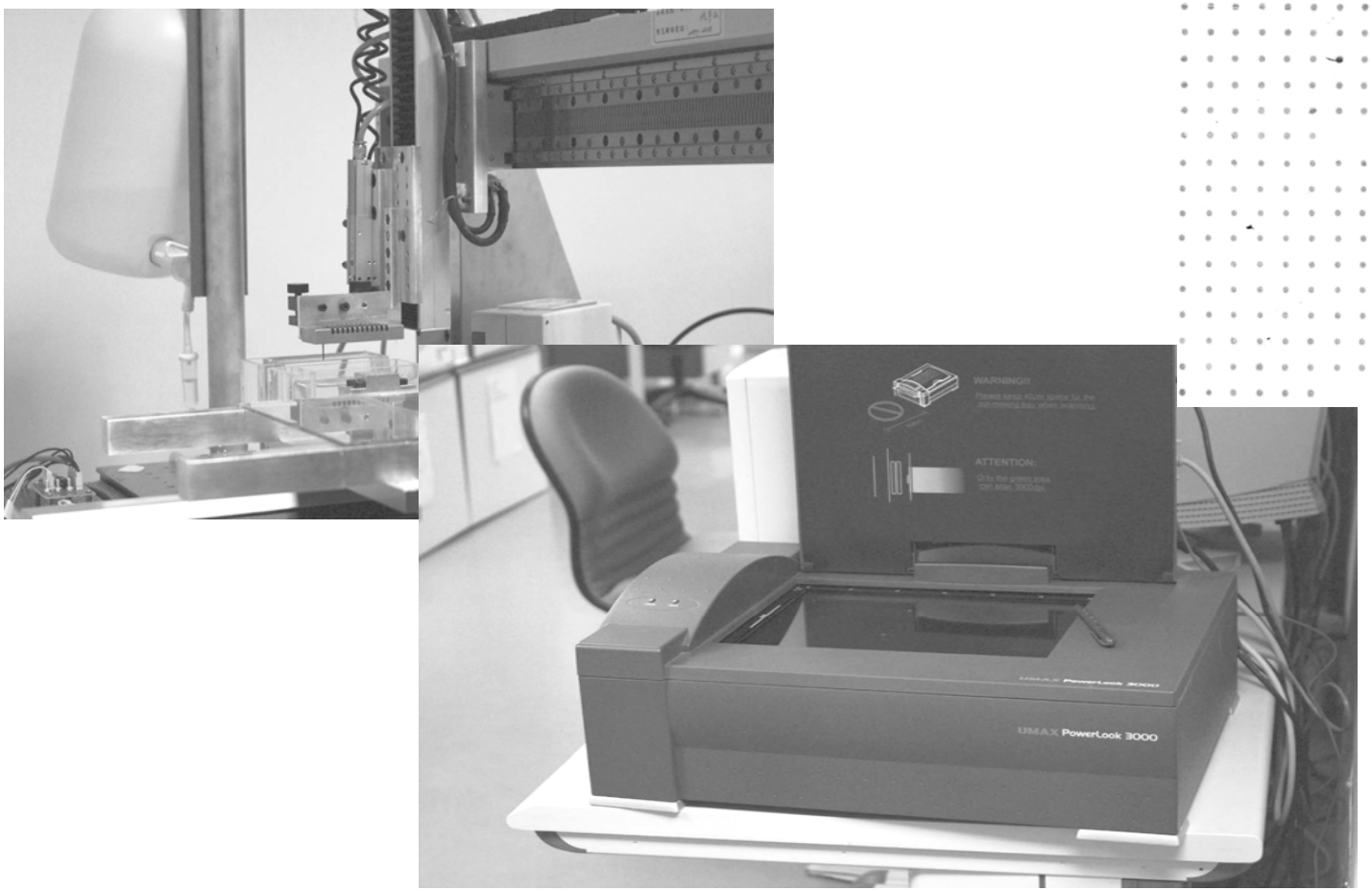


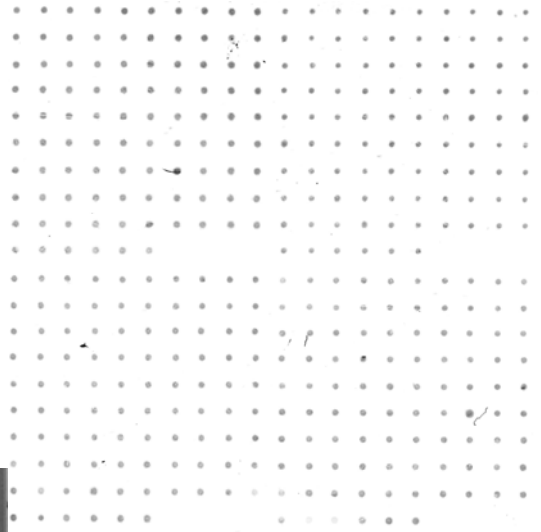
Pseudo color

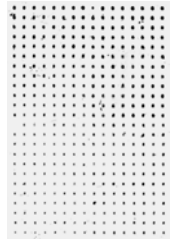


Real color

Arrayer, Scanner, and a 400-point Microarray







5 mm

cDNA microarray Applicat
genome as a biological pr

Drug discovery

Target gene

Herbal/traditional medicine studi

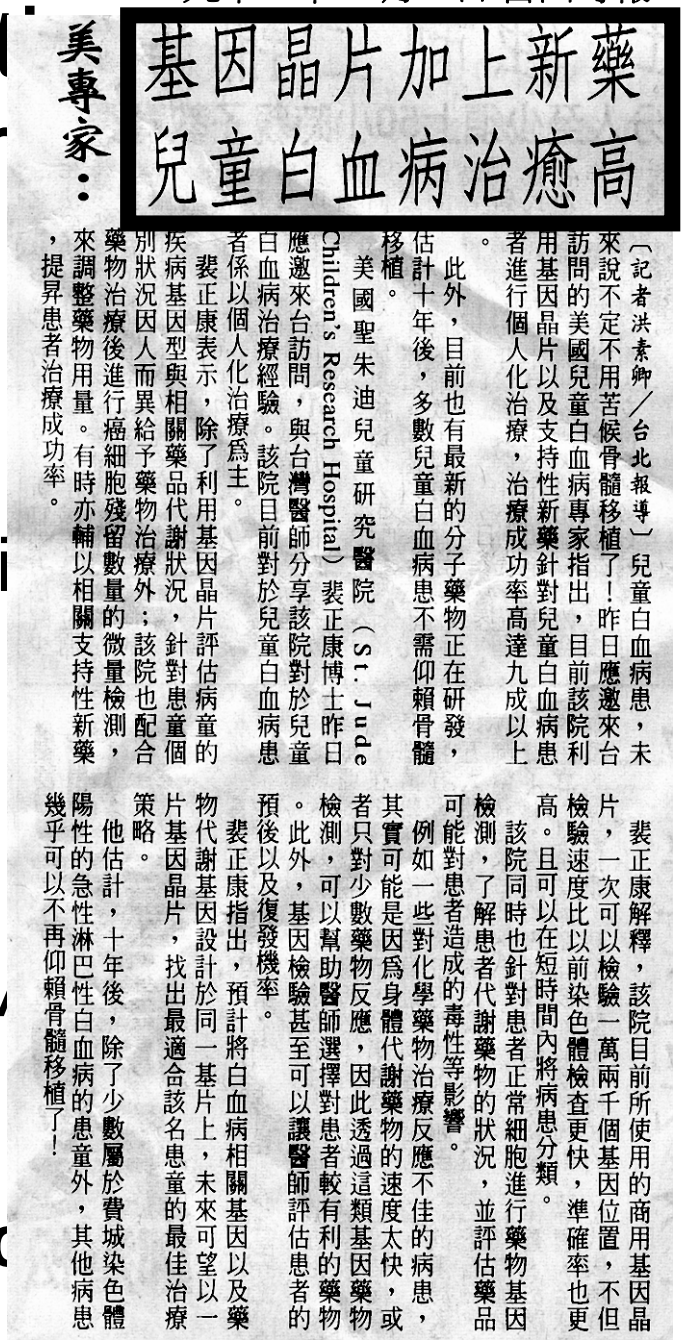
Disease categorization

Clinical outcome, drug response,

The example of acute leukemia, A

E. Lander, et.al., *Science,* 286, 531, **1999**

=> Toward personalized diagnc



九十一年 三月23日 自由時報

美專家：基因晶片加上新藥 兒童白血病治癒高

〔記者洪素卿／台北報導〕兒童白血病患，未來說不定不用苦候骨髓移植了！昨日應邀來台訪問的美國兒童白血病專家指出，目前該院利用基因晶片以及支持性新藥針對兒童白血病患者進行個人化治療，治療成功率高達九成以上。

此外，目前也有最新的分子藥物正在研發，估計十年後，多數兒童白血病患不需仰賴骨髓移植。

美國聖朱迪兒童研究醫院（St. Jude Children's Research Hospital）裴正康博士昨日應邀來台訪問，與台灣醫師分享該院對於兒童白血病治療經驗。該院目前對於兒童白血病患者係以個人化治療爲主。

裴正康表示，除了利用基因晶片評估病童的疾病基因型與相關藥品代謝狀況，針對患童個別狀況因人而異給予藥物治療外；該院也配合藥物治療後進行癌細胞殘留數量的微量檢測，來調整藥物用量。有時亦輔以相關支持性新藥，提昇患者治療成功率。

裴正康解釋，該院目前所使用的商用基因晶片，一次可以檢驗一萬兩千個基因位置，不但檢驗速度比以前染色體檢查更快，準確率也更高。且可以在短時間內將病患分類。

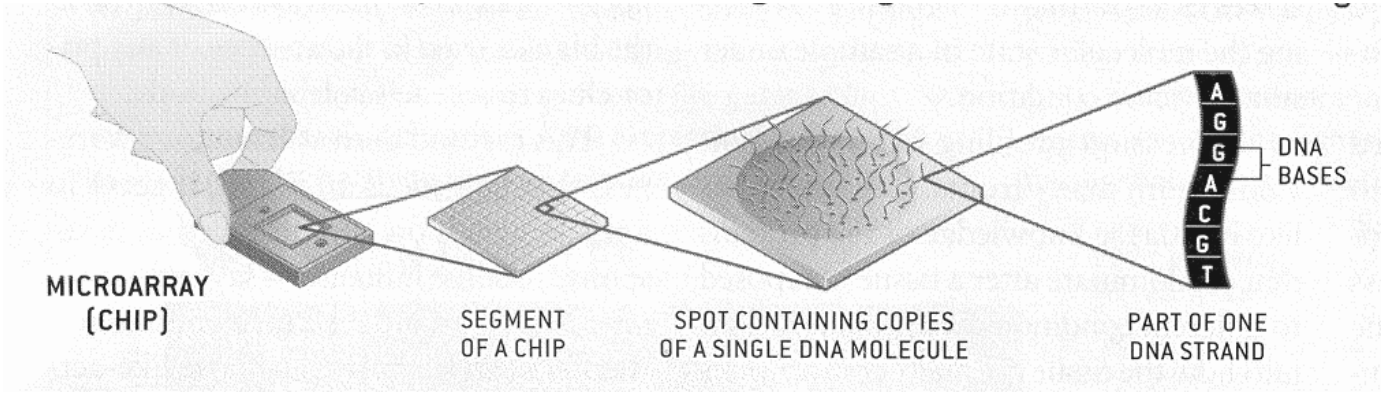
該院同時也針對患者正常細胞進行藥物基因檢測，了解患者代謝藥物的狀況，並評估藥品可能對患者造成的毒性等影響。

例如一些對化學藥物治療反應不佳的病患，其實可能是因爲身體代謝藥物的速度太快，或者只對少數藥物反應，因此透過這類基因藥物檢測，可以幫助醫師選擇對患者較有利的藥物。此外，基因檢驗甚至可以讓醫師評估患者的預後以及復發機率。

裴正康指出，預計將白血病相關基因以及藥物代謝基因設計於同一基片上，未來可望以一片基因晶片，找出最適合該名患童的最佳治療策略。

他估計，十年後，除了少數屬於費城染色體陽性的急性淋巴性白血病的患童外，其他病患幾乎可以不再仰賴骨髓移植了！

Oligonucleotide Microarray



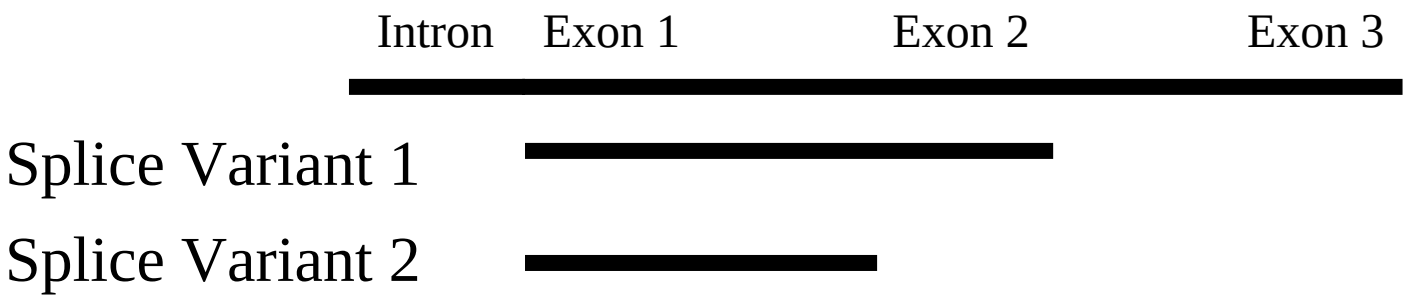
Short DNA on the chip

Expression of 13,500 genes

SNP detection

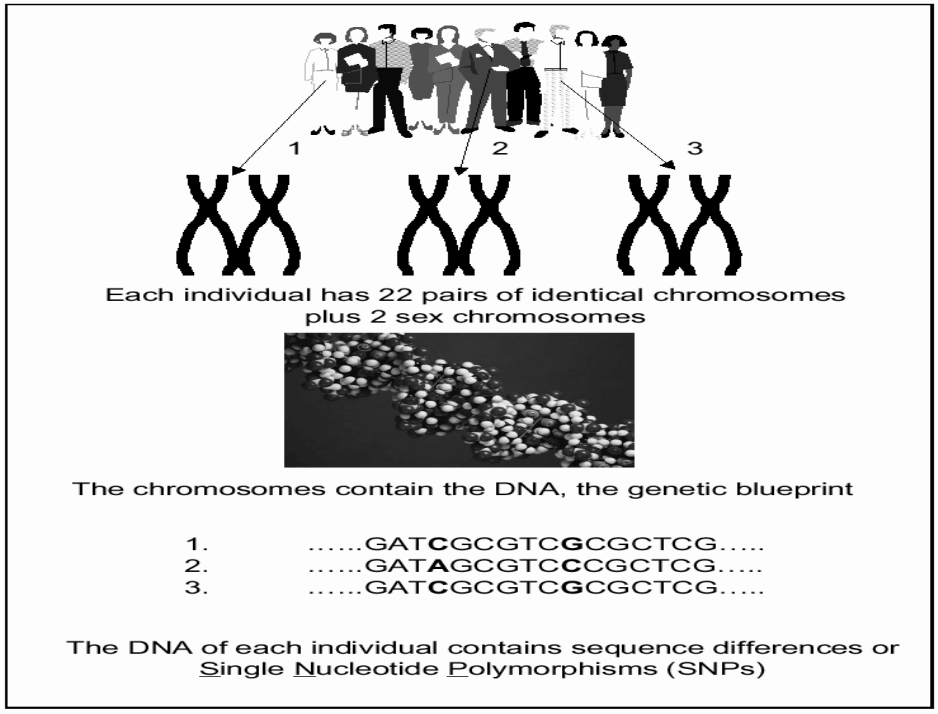
Applications of oligonucleotide microarray

- SNP study - not possible with clone-based cDNA array
- Gene expression study when short specific sequences are available. Performance compared to cDNA array is similar. *Nucleic Acids Res. 28,* 4552, **2000**
- Detection of splice variants.
- Protein:DNA and drug:DNA interaction - sequence specific interaction study using double-strand oligoarray. *Nat. Biotech., 17*, 573, **1999**; *Nucleic Acids Res. 27* ,4100 ,**1999**
- Loci-linkage detection - paired probe array. Utilizing cooperative hybridization. *Nucleic Acids Res. 26*, 1485 , **1999**
- Hybridization behavior: DNA paring with different lengths. *Nucleic Acids Res. 17* ,788 ,**1999**



Single Nucleotide Polymorphisms (SNPs) -- The 1/1000 differences between individuals

From “The SNP Consortium, http://snp.cshl.org/”



SNP genotype affects the drug response and disease susceptibility.

SNP is the key to unravel genotype-phenotype relationship

Examples of the polymorphism-efficacy relationship

New Eng. J. Med., 1999, v286, 487

- *Taq*IB polymorphism affects the efficacy of treatment with cholesterol-lowering drug. *New Eng. J. Med.*, **1998**, v338, 86
  - Gene: CETP
  - Therapy: pravastatin
  - Effective vs. in-effective allele: B1B1vs. B2B2

- SNP polymorphism affects the susceptibility to bronchodilator desensitization. *Lancet,* 1997, v350, 995
  - Gene: ADRB2
  - 1 mgqpgngsaf llapngshap dhdvtqerde vwvvgmgivm …Gly16, Glu27
  - r q … Arg16, Gln27

The SNP Consortium And Diversified Combination of Genotype

http://snp.cshl.org/

Published 1.42 million single nucleotide polymorphisms (SNPs) distributed throughout the human genome. *Nature*, **2001**, v409, 928 - 933

Imagine a genotype combination of

$$2^{1,420,000}$$

DNA sample in human body: 10^7 lymphocytes / ml in blood

Fabrication of oligonucleotide array

***in-situ* synthesized single-strand array**

Photolithograpy - Affymetrix.

Maskless synthesis - NimbleGen. *Nat. Biotech., 17*, 974, **1999**

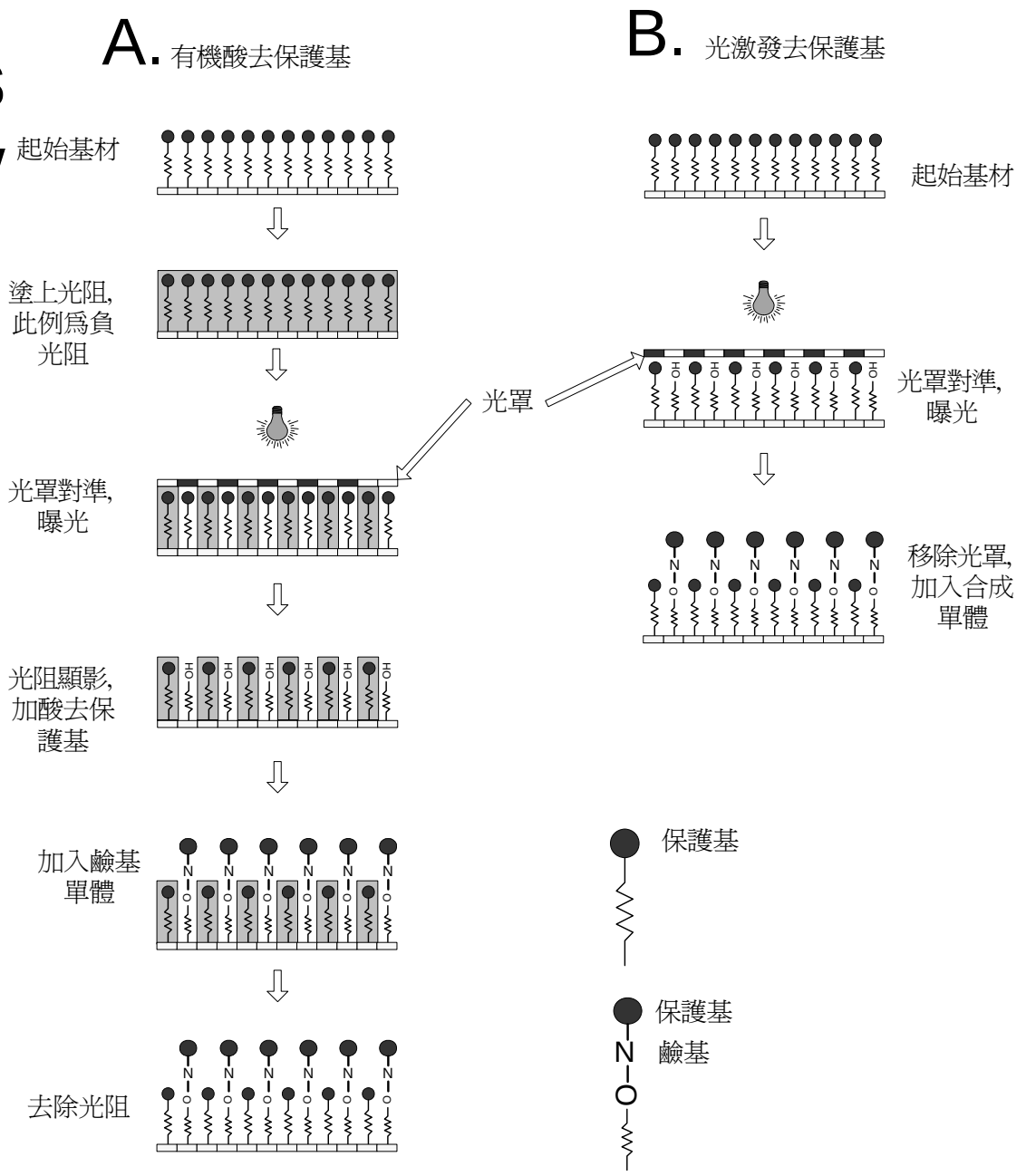
Ink-jet reagent delivery/Surface tension selection - Protogene; Rosetta, Agilent. *Biosensors and Bioelectronics, 11*, 687, **1996;** *Nature Biotech., 19*, 342, **2001**

Electochemical solid phase synthesis- Combimatrix

- Immobilization of Pre-synthesized ODN:
  - Spotting - Pick and Place approach
  - Electrically addressed immobilization. *Anal. Biochem., 255,* 188, **1998;** Nonogene: *Nature Biotech***. 1999**

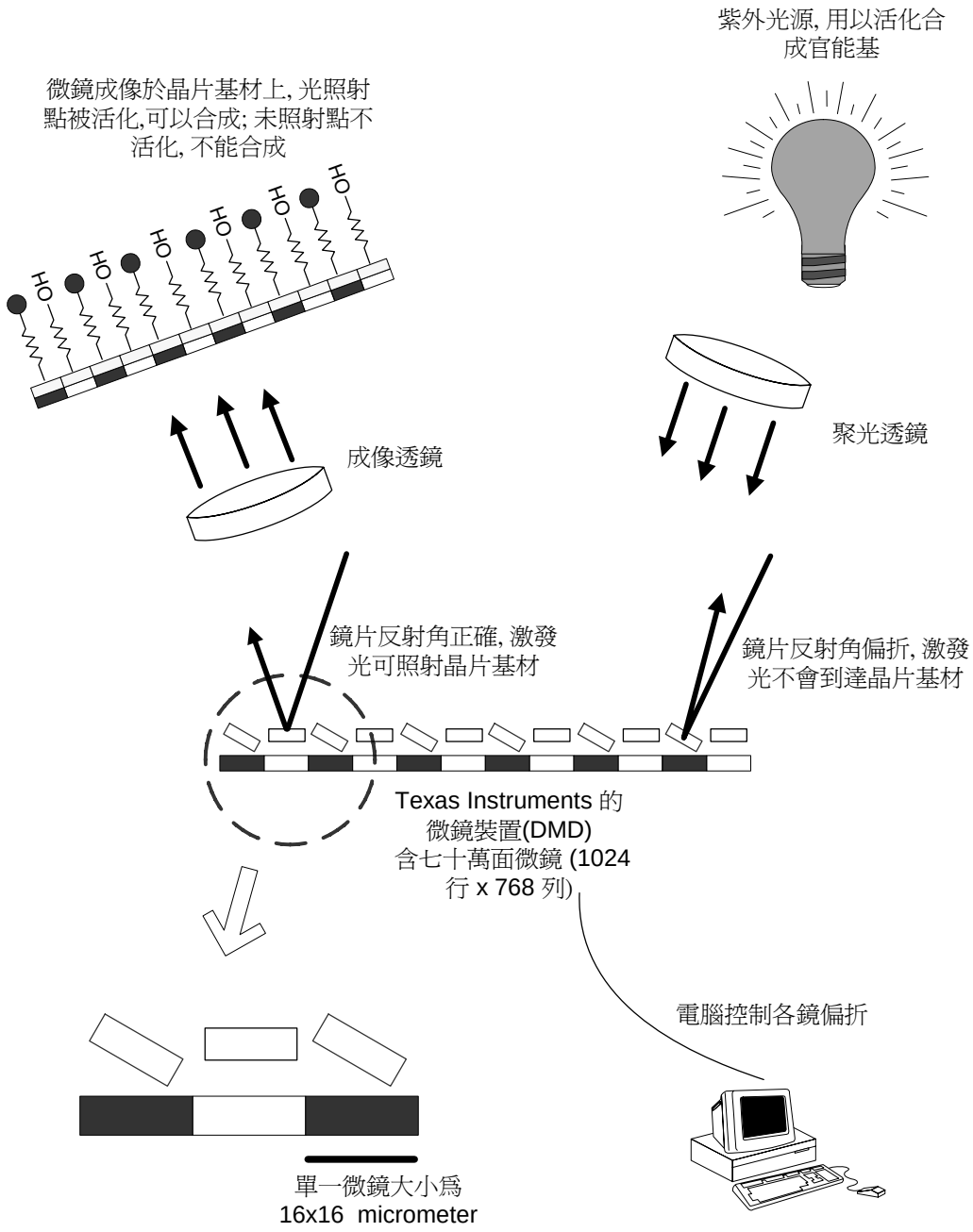
Light-directed Synthesis of Oligonucleotide Array I: Photolithography

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

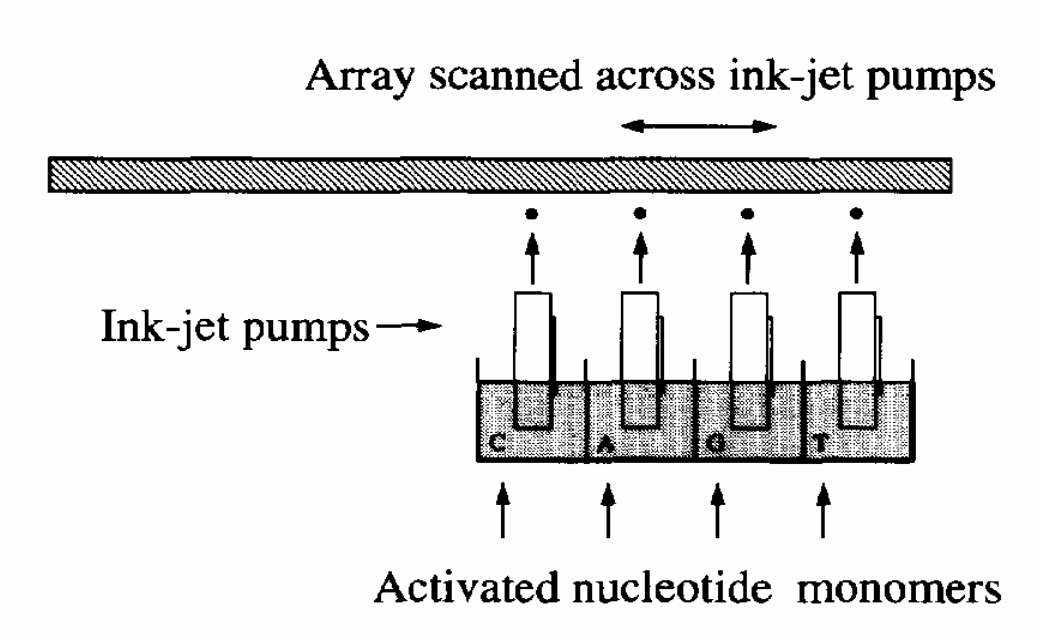


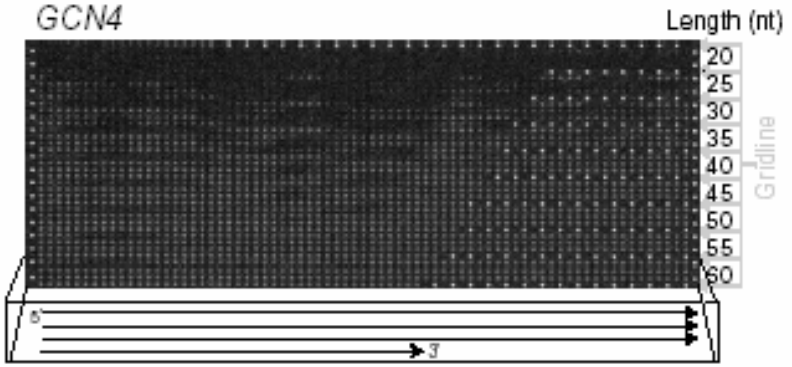
Light-directed Synthesis of Oligonucleotide Array II - Maskless Synthesis Using DMD

Sangeet Singh-Gasson et al. *NATURE BIOTECHNOLOGY* v.17, p974 , 1999



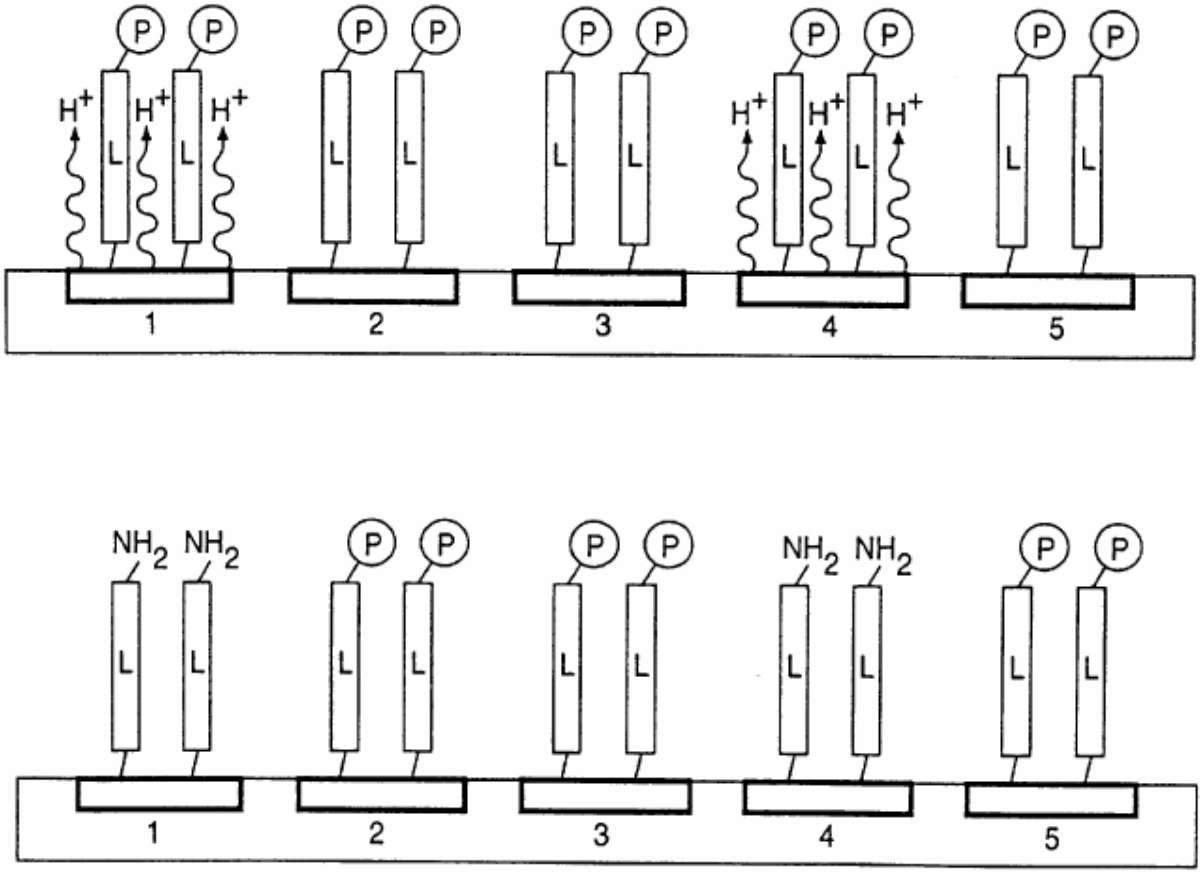
Ink-jet Synthesis - reagent delivery:





Electochemical solid phase synthesis

D. D. Montgomery, U.S. Patent 6,093,302



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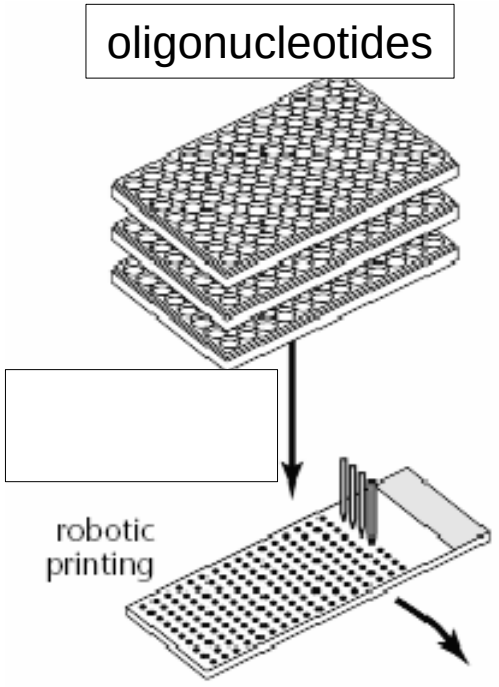
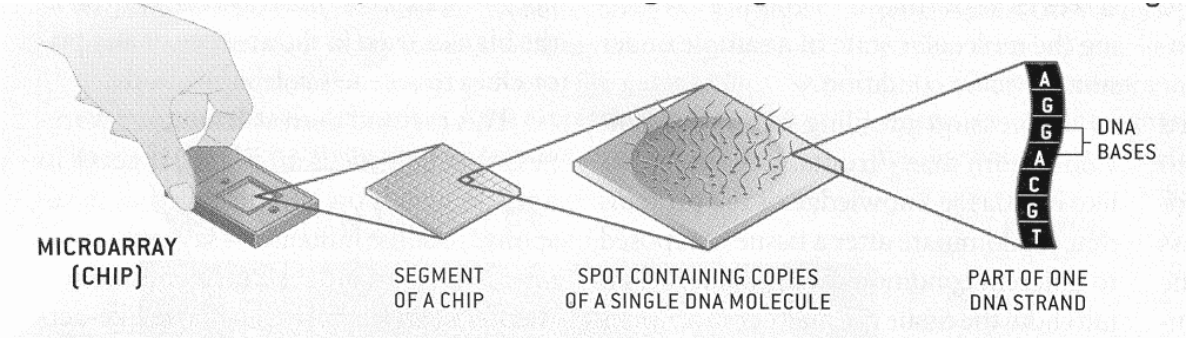
Procedure for oligonucleotide array preparation

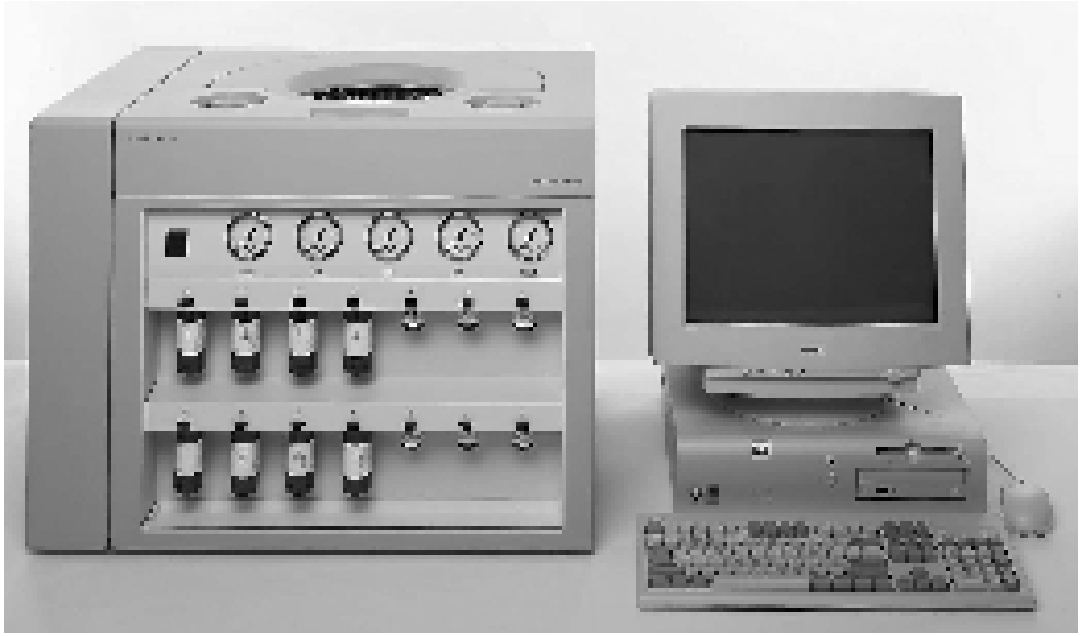
Oligonucleotide Synthesis ⇨





Length up to the synthesis capability of the synthesizer

A commercial oligonucleotide synthesizer



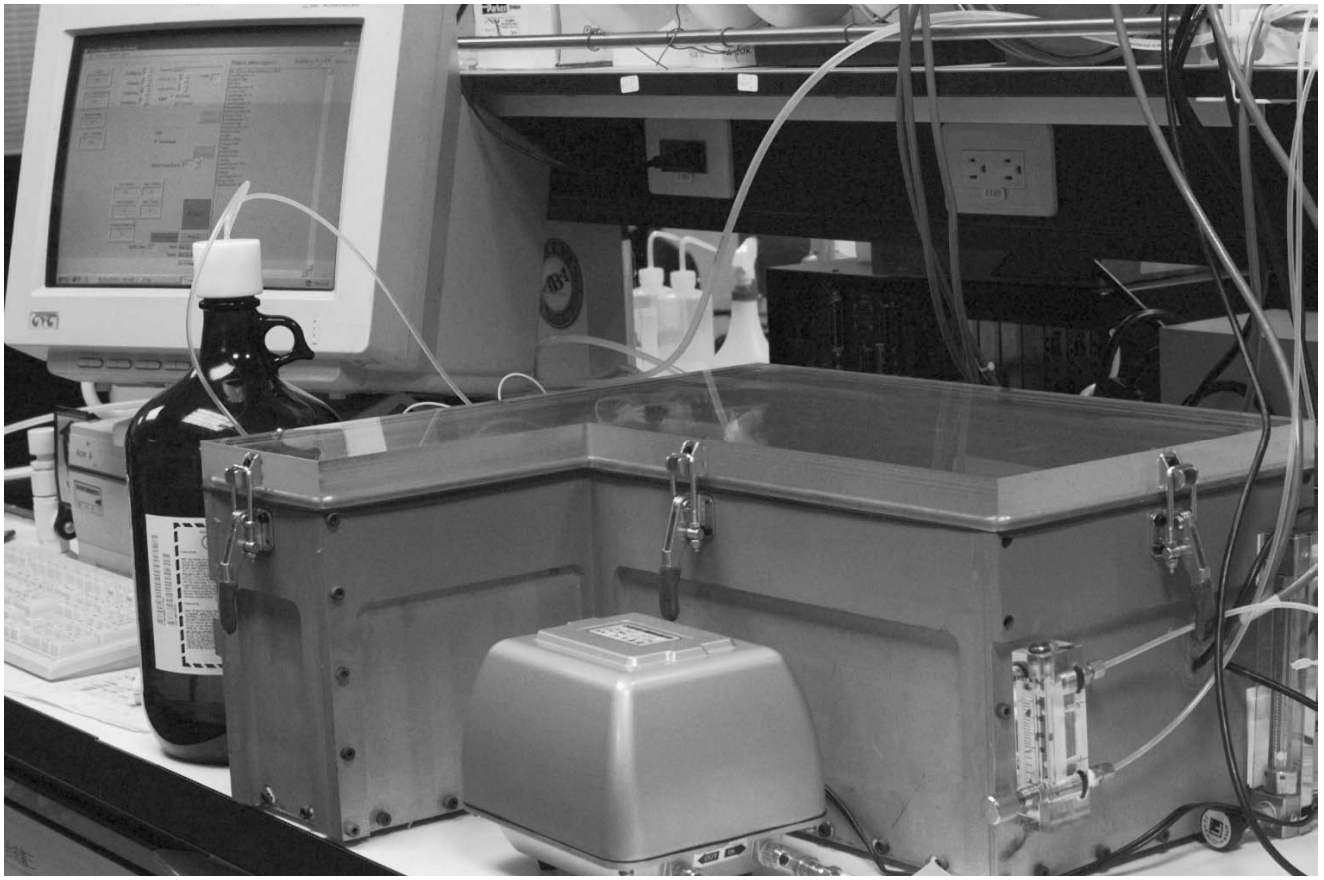
ABI 3900

48 x 6 = 288 oligonucleotides / 10 hours

NT$ 6,000,000

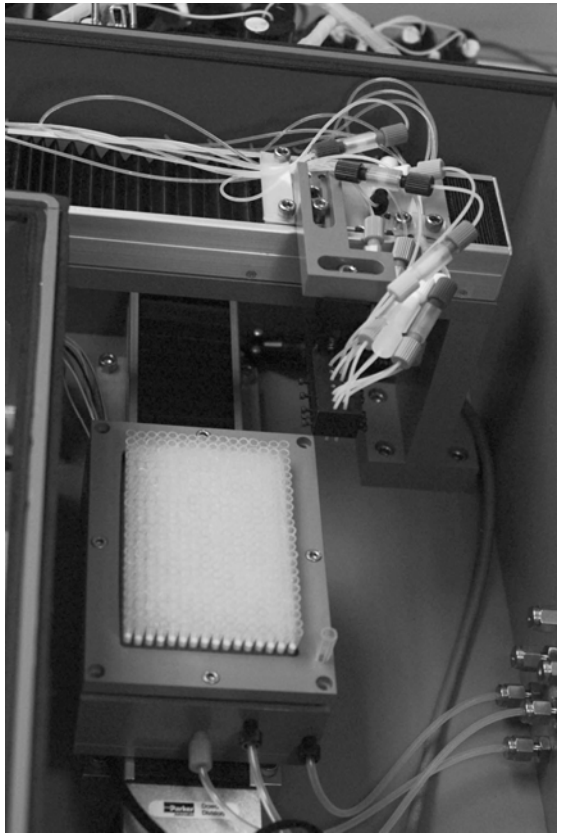
Picture of a 384 Channel Oligonucleotide synthesizer

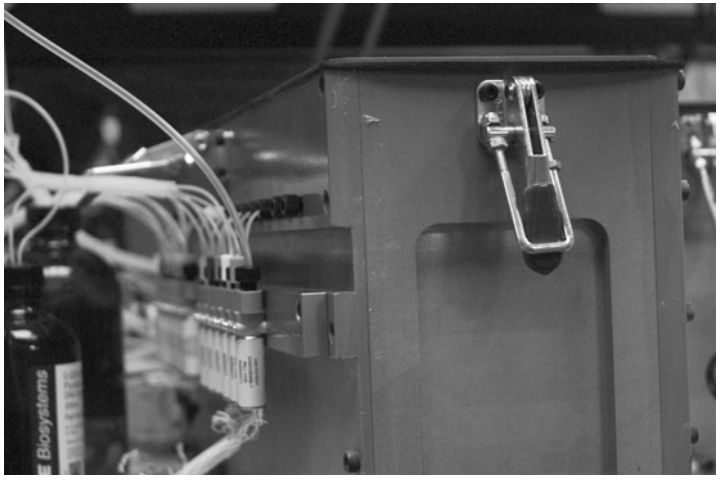
Whole view of the synthesizer



Pictures of a 384 Channel Oligonucleotide synthesizer

Reaction plate, XY table and injection nozzles





Reagent reservoir and valves

Valve driving circuit

384 oligonucleotides / 10 hours

Compatible to arrayer operation

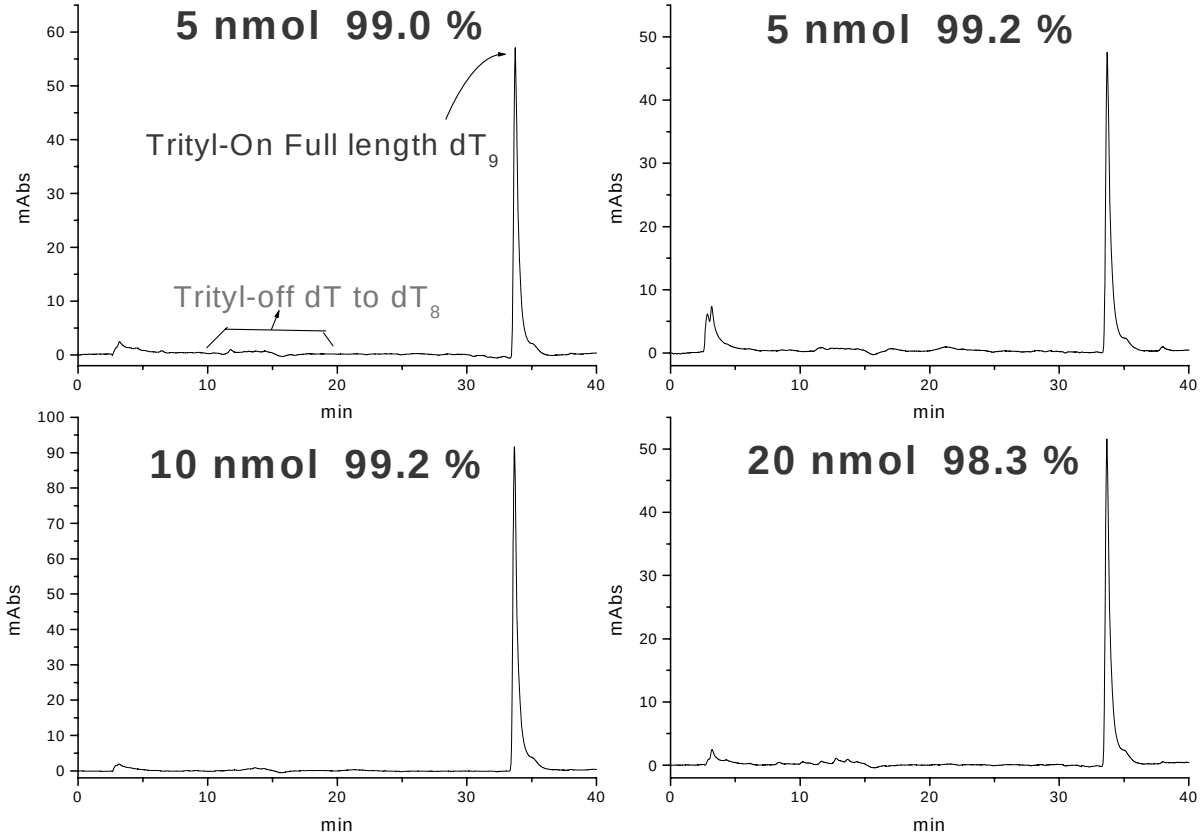
HPLC, CE Analysis of Product Purity and Step Yield

- Step yield =

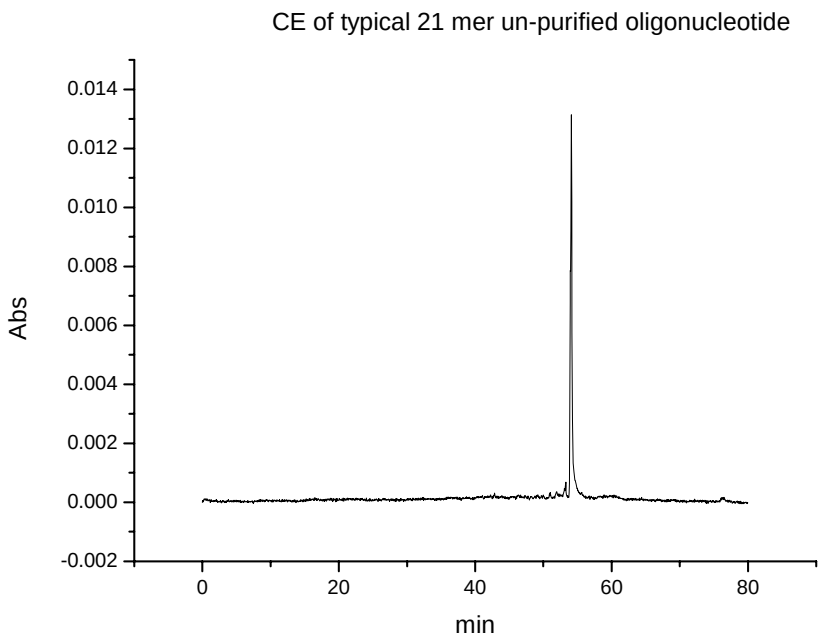
[Trityl-on product/Total product]$^{1/n}$

where n = length of oligonucleotide

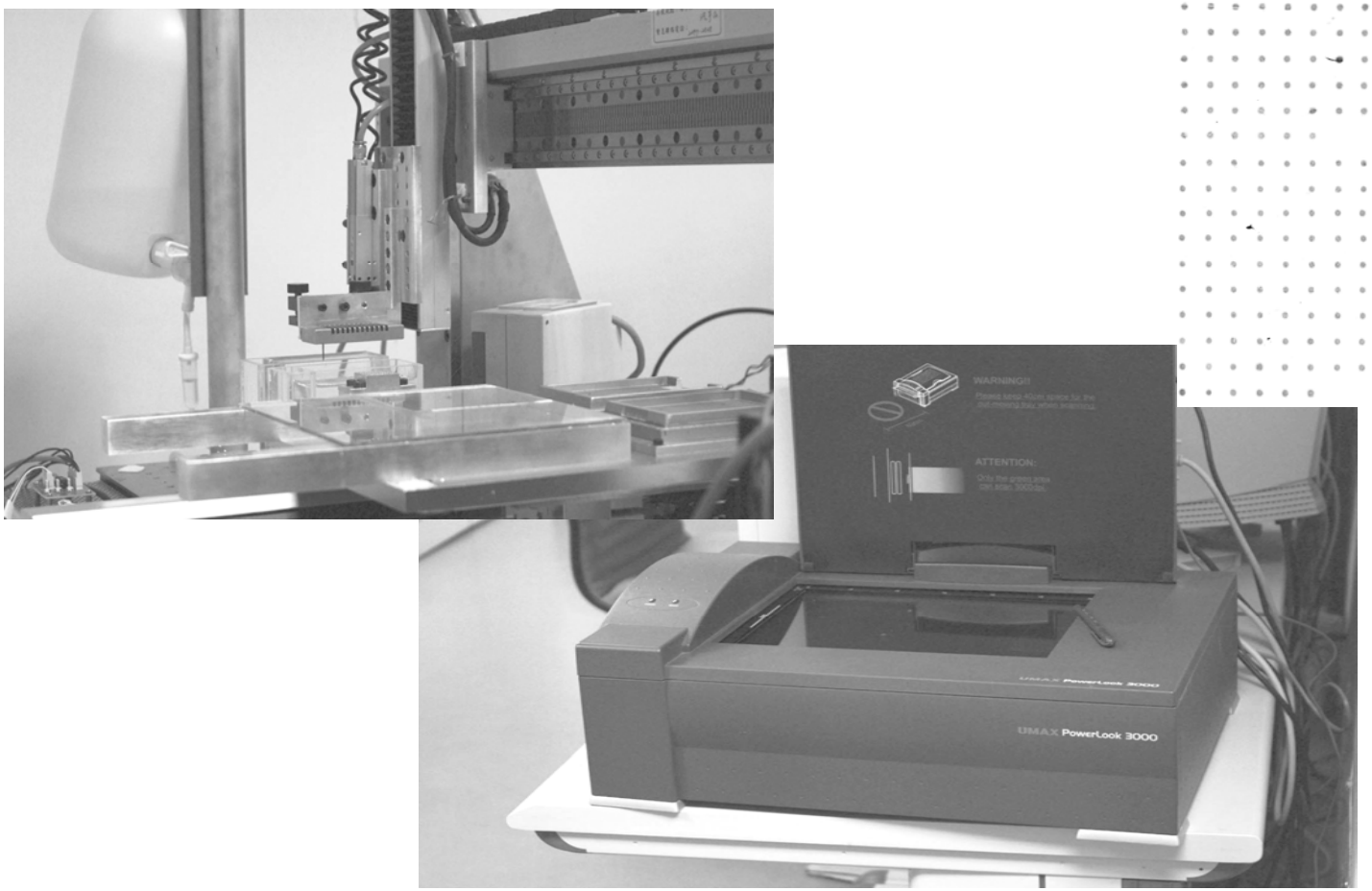
- HPLC chromatograph of dT_9.

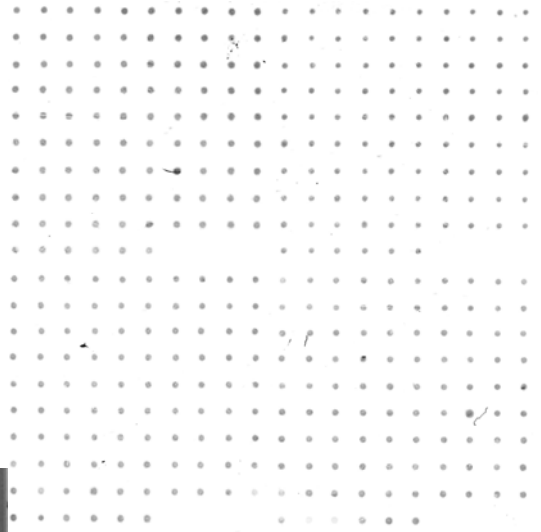


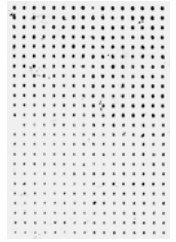
- CE
  - unpurified 21mer



Arrayer, Scanner, and a 400-point Microarray

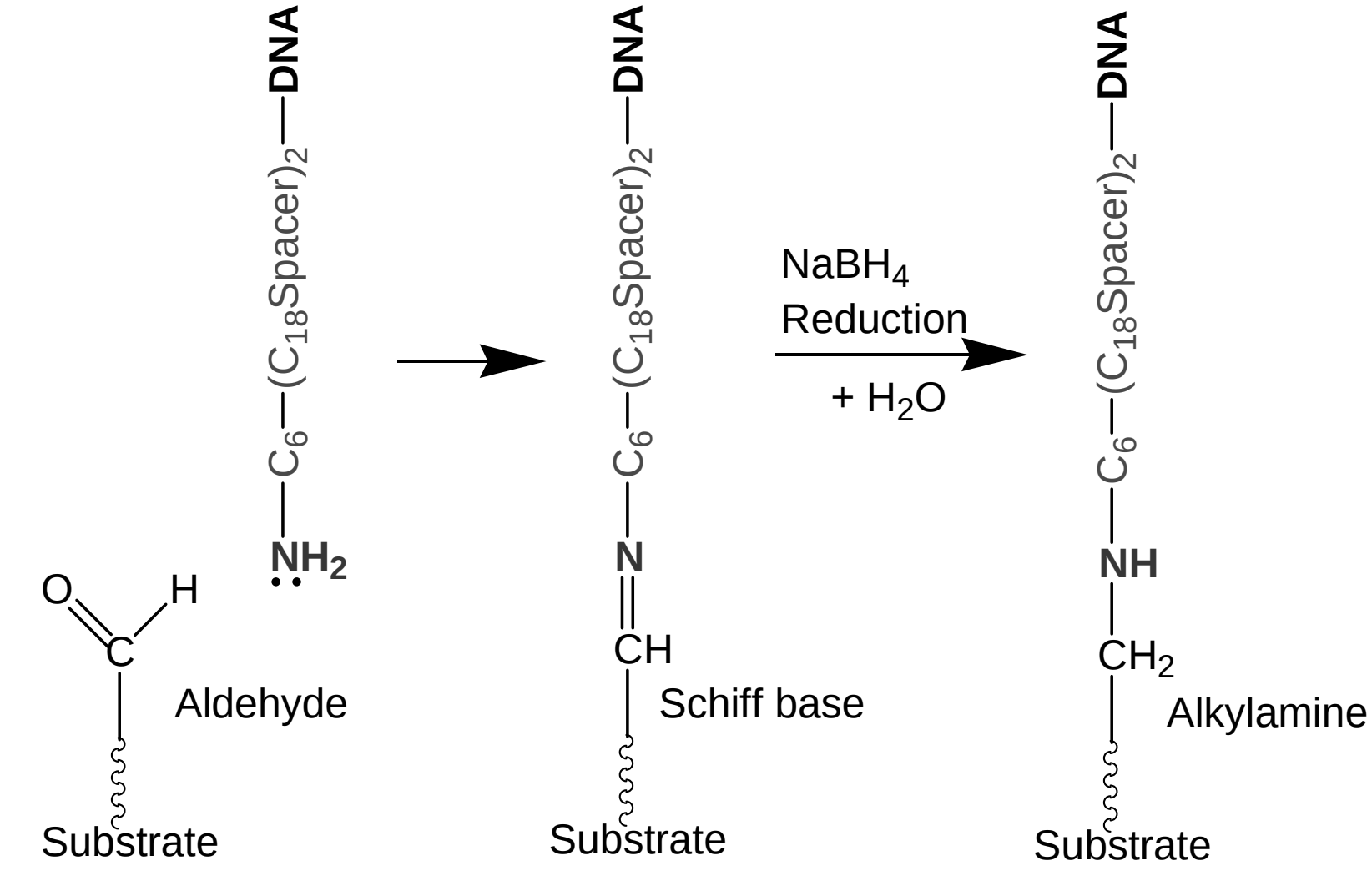






5 mm

DNA Immobilization Chemistry - Schiff base reaction



Genotyping Oligonucleotide Microarray

- SNP (single nucleotide polymorphism) is the most frequent variation found in individual DNA sequences. Currently 3,000,000 SNPs are estimated.
- High throughput, low cost genotyping technologies are required.
- Currently the most important limiting factor for routine SNP analysis is the DNA amplification: Simultaneous amplification of discrete exons is difficult
- We have proposed an approach to circumvent the aforementioned difficulty
- Oligonucleotide microarray is one of the most promising technology to detect tens of thousands of SNPs (genotyping) in one experiment. Throughput per day = spots per array x array per day.

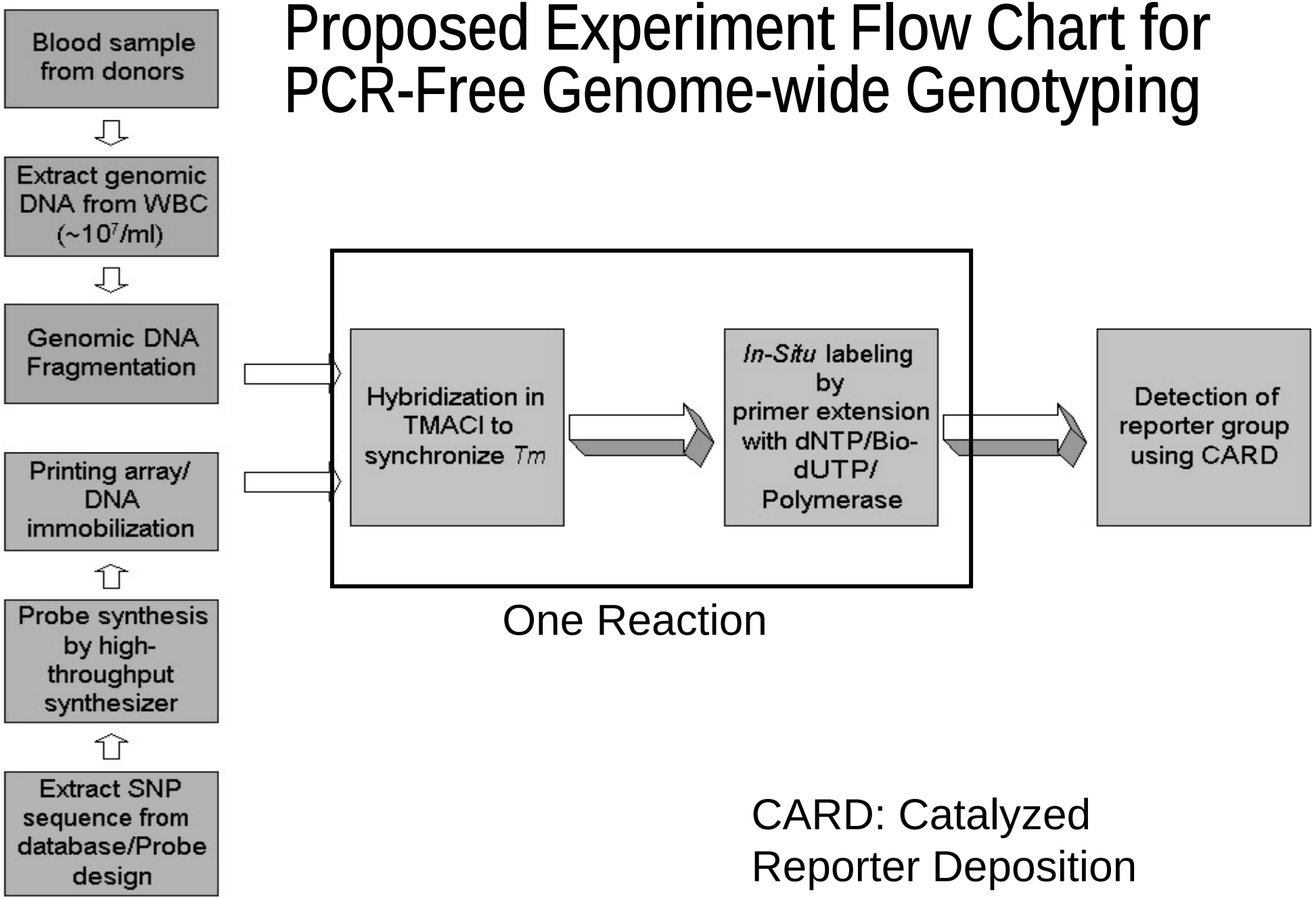
SNP distribution of some selected genes

- ~40 PCR and additional 80 primers are required if each exon is amplified separately for the following 5 genes.
- 3 million SNPs estimated in human genome => Genome wide genotyping require novel strategy

Gene Name	Gene Symbol	# of Exon	# of SNP in HGBase
Thrombomodulin	THBD	1	7
Coagulation factor V	F5	25	42
Low density lipoprotein receptor	LDLR	>=18	14
Parathyroid hormone-like hormone	PTHLH	>=6	16
Coagulation factor XIII, B polypeptide	F13B	NA	14

HGBase: Human Genic Bi-Allelic Sequences Database, *http://www.hgbase.com.*
~65,000 SNPs are collected and annotated.

Proposed Experiment Flow Chart for PCR-Free Genome-wide Genotyping

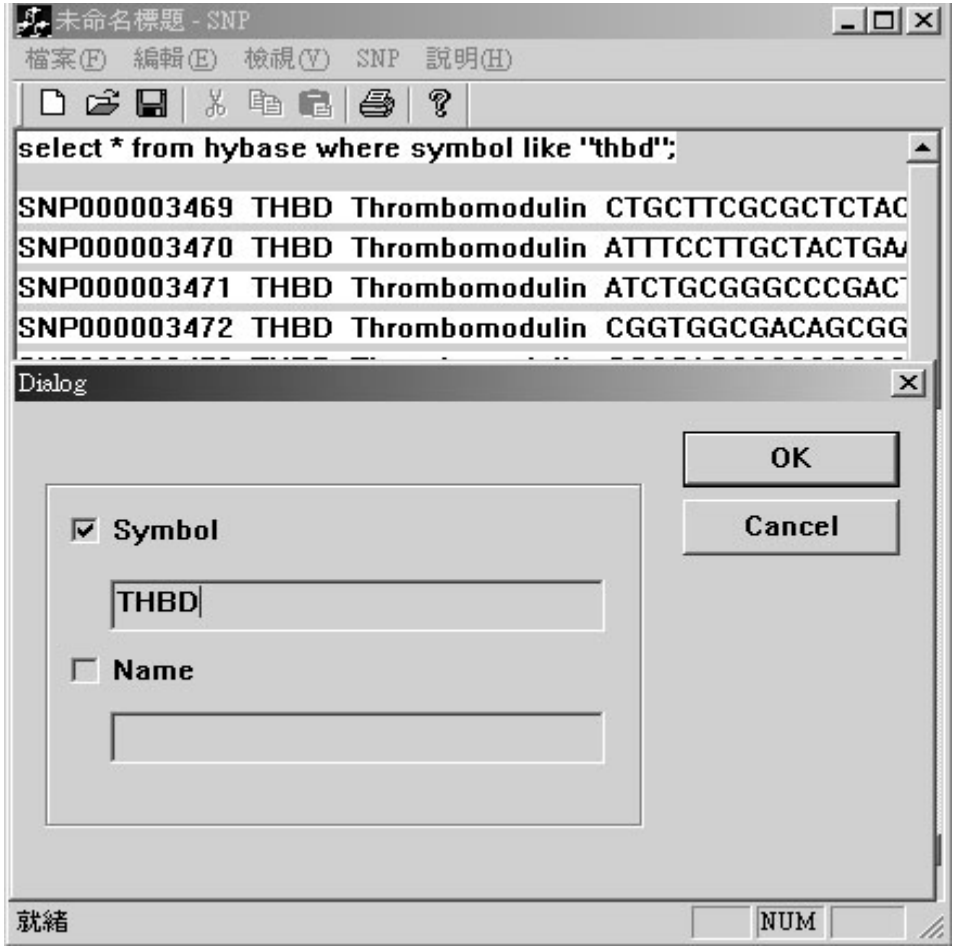


One Reaction

CARD: Catalyzed Reporter Deposition

SNP Sequence Extraction from HGBase

HGBase: Human Genic Bi-Allelic Sequences Database, *http://www.hgbase.com/*



Entry	Sequence	SNP ID
UP	CTGCTTCGCGCTCTACCCGGGCCCC	SNP000003469
Allele	G	SNP000003469
Allele	A	SNP000003469
Down	CGACCTTCCTCAATGCCAGTCAGAT	SNP000003469
	ATTTCCTTGCTACTGAACGGCGACG	SNP000003470
	G	SNP000003470
	C	SNP000003470
	CGGCGTTGGCCGCCGGCGCCTCTGG	SNP000003470
	ATCTGCGGGCCCGACTCGGCCCTTG	SNP000003471
	C	SNP000003471
	T	SNP000003471
	CCGCCACATTGGCACCGACTGTGAC	SNP000003471
	CGGTGGCGACAGCGGCTCTGGCGAG	SNP000003472
	T	SNP000003472
	C	SNP000003472
	CCCCGCCCAGCCCGACGCCCGGCTC	SNP000003472
	GGCGAGCCCCCGCCCAGCCCGACGC	SNP000003473
	C	SNP000003473
	T	SNP000003473
	CGGCTCCACCTTGACTCCTCCGGCC	SNP000003473
	ACCCCAGCACTTGTGTTGTCTGGTG	SNP000004296
	A	SNP000004296
	G	SNP000004296
	TGGCACCATCTCTGATTTTCAAAGC	SNP000004296
	AAACAAAGGTTGAGATGTAAAAGGT	SNP000012896
	A	SNP000012896
	G	SNP000012896
	TTAAATTGATGTTGCTGGACTGTCA	SNP000012896

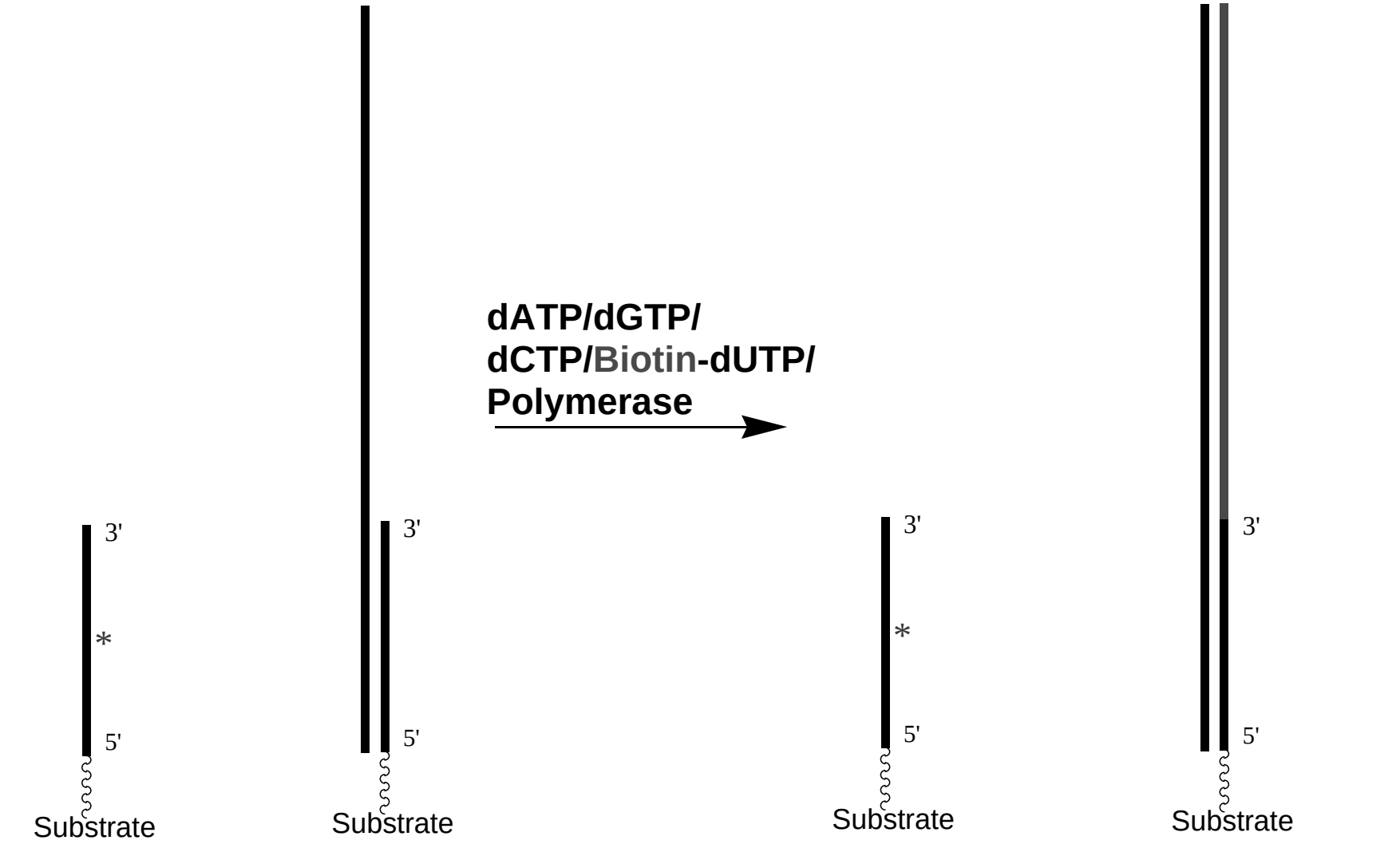
Automated probe design

Hot Spot (SNP position) at 3’-terminal

			Up Stream Length	Dn Stream Length	
	Up/Allele/Down	**SNP ID**	20	0	**Probe Sequence**
UpStream	CTGCTTCGCGCTCTACCCGGGCCCC	SNP000003469	TCGCGCTCTACCCGGGCCCC		TCGCGCTCTACCCGGGCCCCa
Allele	G				TCGCGCTCTACCCGGGCCCCg
Allele	A				TCGCGCTCTACCCGGGCCCCc
DnStream	CGACCTTCCTCAATGCCAGTCAGAT				TCGCGCTCTACCCGGGCCCCt
UpStream	ATTTCCTTGCTACTGAACGGCGACG	SNP000003470	CTTGCTACTGAACGGCGACG		CTTGCTACTGAACGGCGACGa
Allele	G				CTTGCTACTGAACGGCGACGg
Allele	C				CTTGCTACTGAACGGCGACGc
DnStream	CGGCGTTGGCCGCCGGCGCCTCTGG				CTTGCTACTGAACGGCGACGt

⋮

Primer *in-situ* extension labeling



Mismatched probe/ No target annealed

Matched probe/ Target annealed

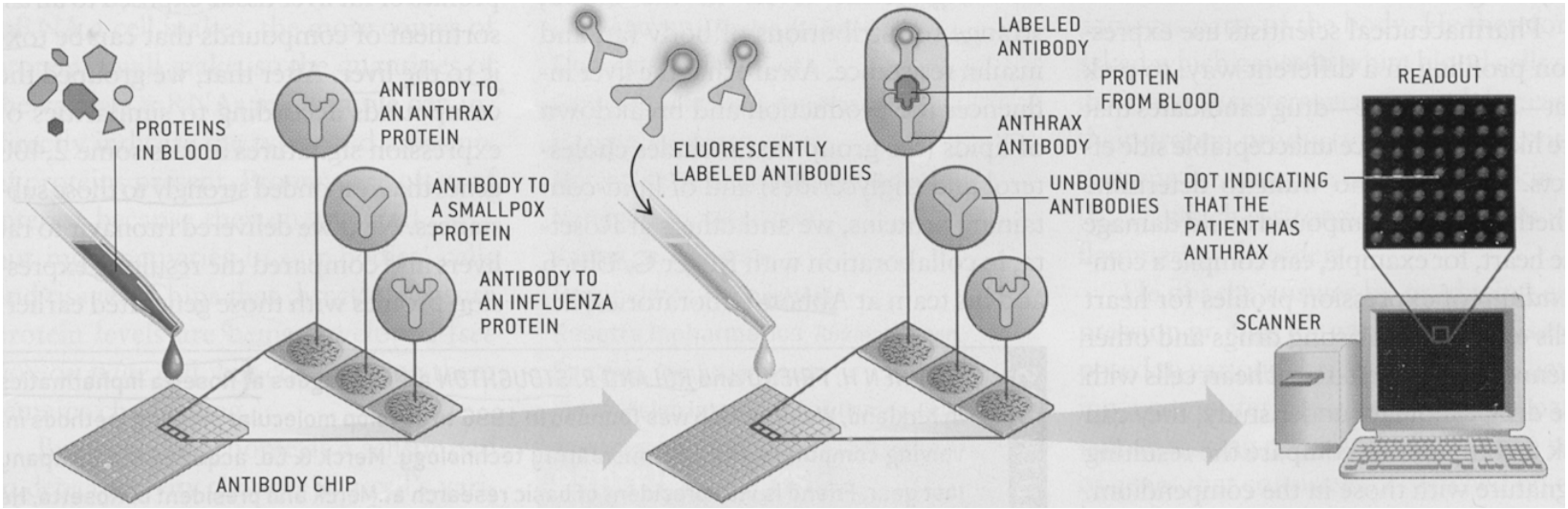
No extension. See below

Extended probe with reporter group

Protein Microarray

Advantages in use:

- Sample availability
- Specificity



Obstacles:

- Available antibodies
- Target antigen idenification

Protein Microarray Applications

Swift and sensitive antigen (pathogen) detection

Very suitable for diagnostic purposes

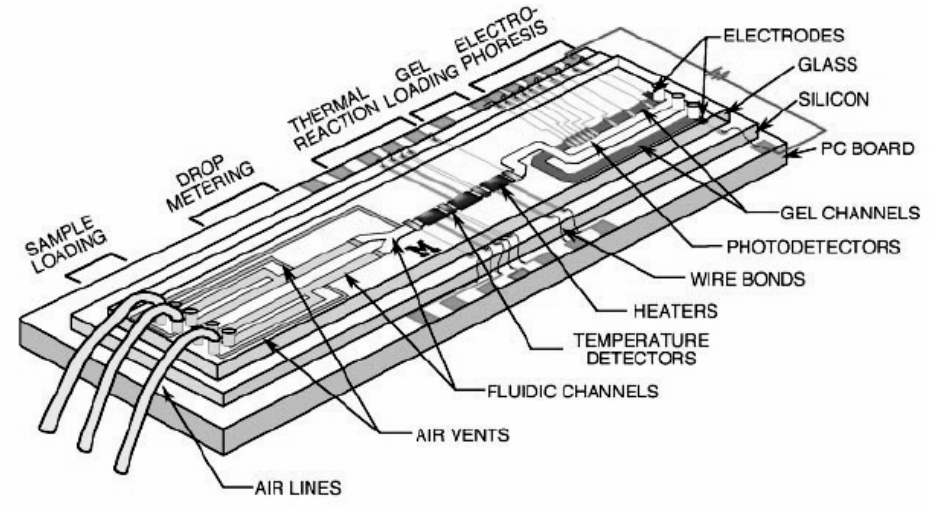
Protein-protein interaction

Drug target identification

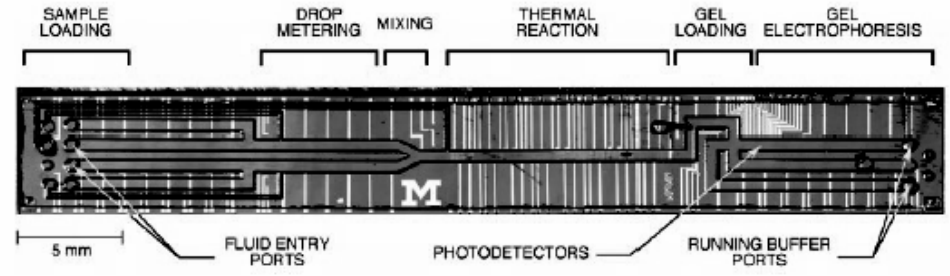
DNA and Protein array:

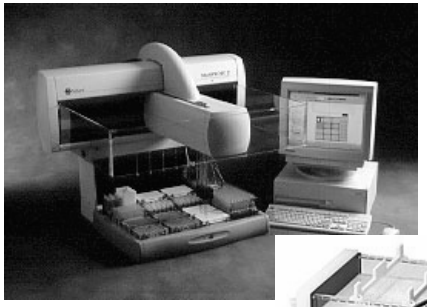
Only analysis is performed on chip.

Lab-On-a-Chip: Integration of Microfluidics and Diverse Components



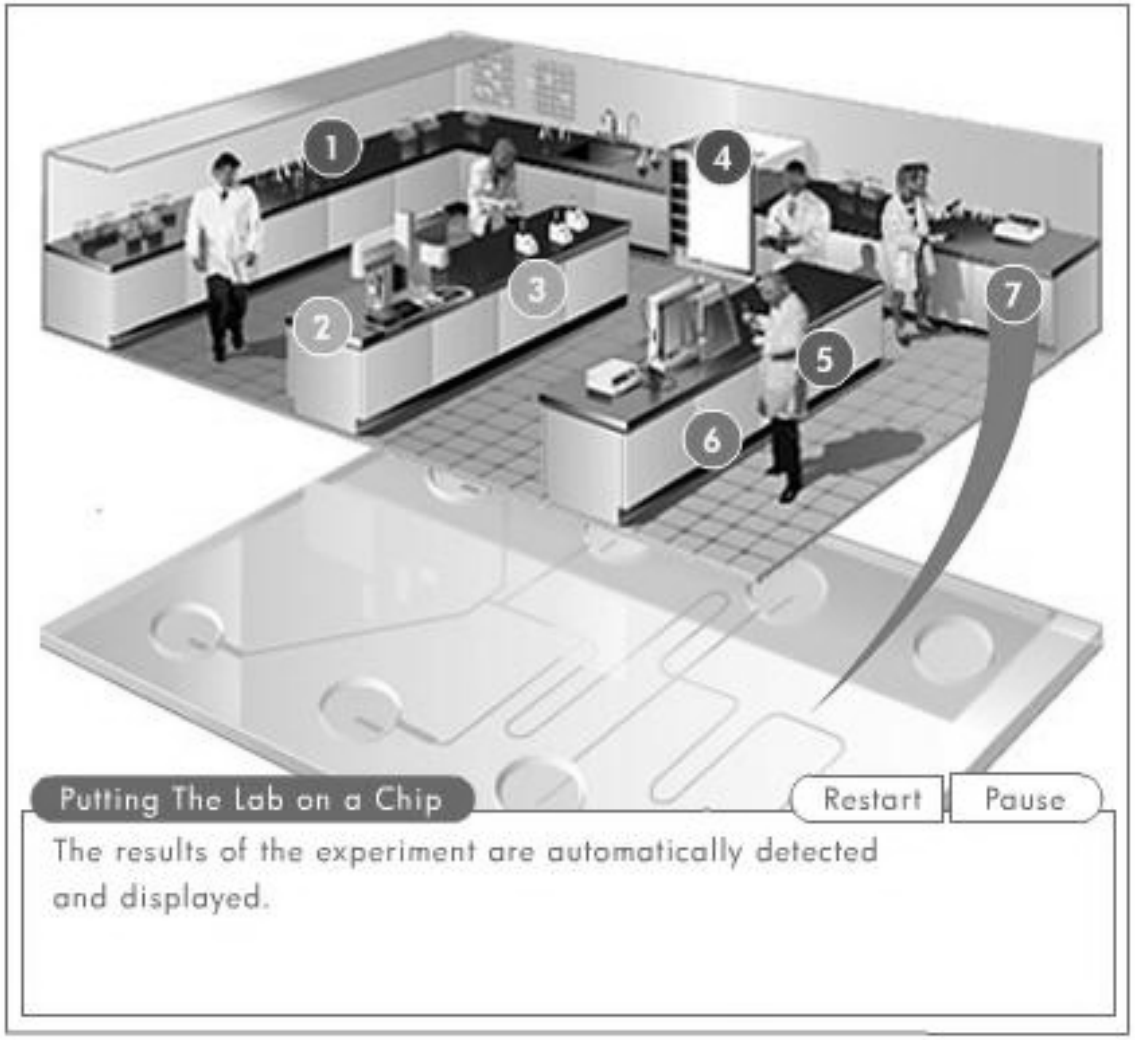
M. Burns, et.al., *SCIENCE, 282,* **1998,** 484

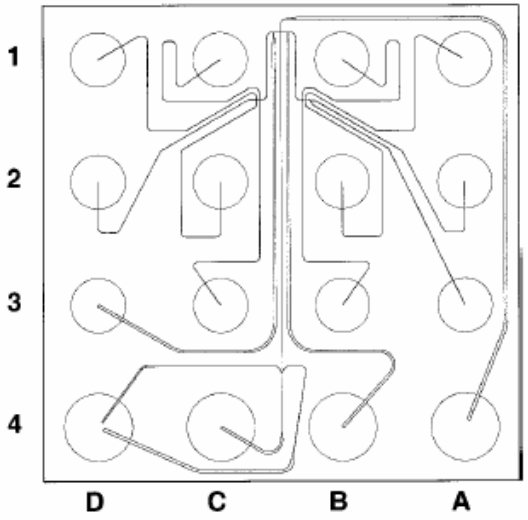


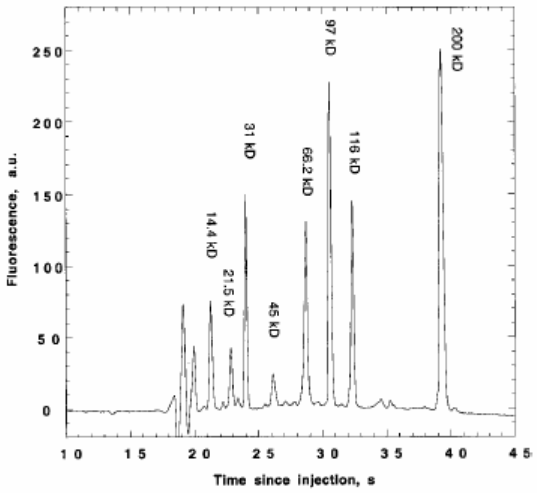


Sample metering	Pipetting machine
DNA amplification	PCR temperature cycler
Mixing	Shaker, Vortex
Digesting	Thermostat
Separation	Slab Gel, CE station
Detection	Fluorescence microscope, spectrometer

Lab-On-a-Chip
Miniaturized Laboratory

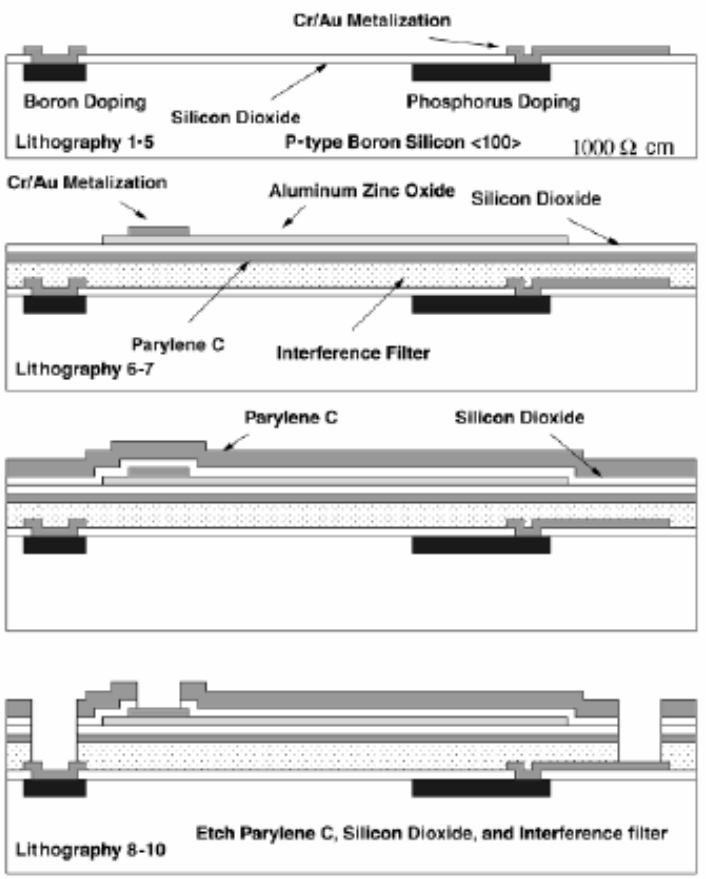


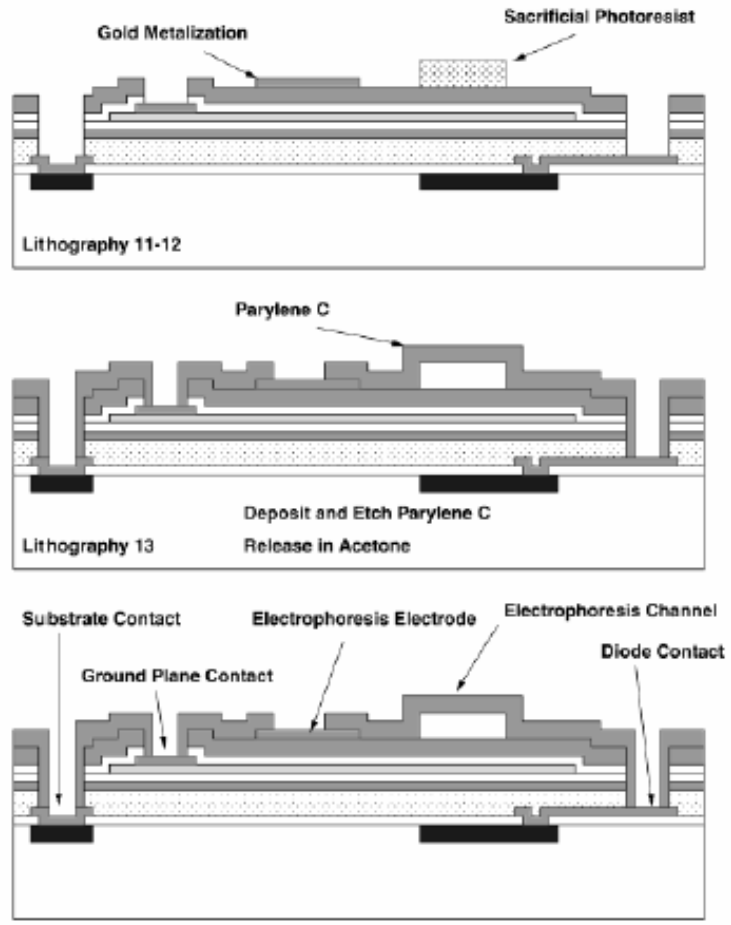




Caliper Technologies, *http://www.calipertech.com*

Lab-On-a-Chip Fabrication Methods I -- Procedures used in semiconductor industries

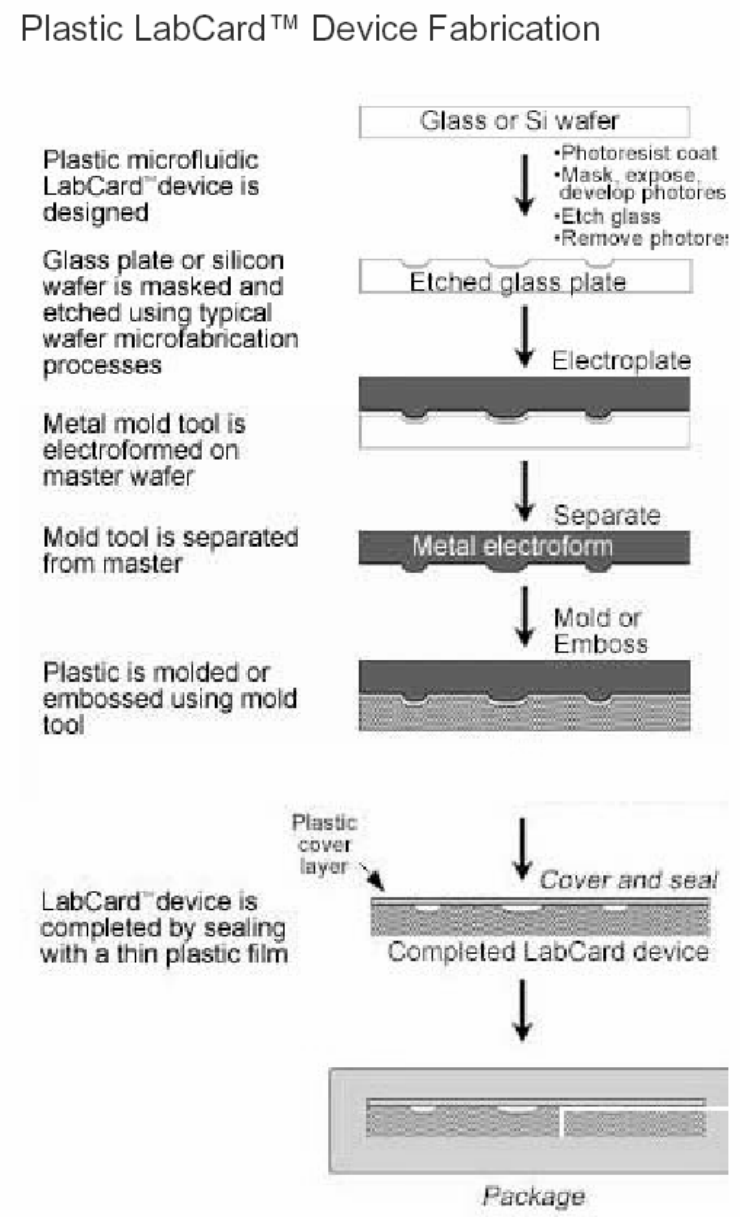




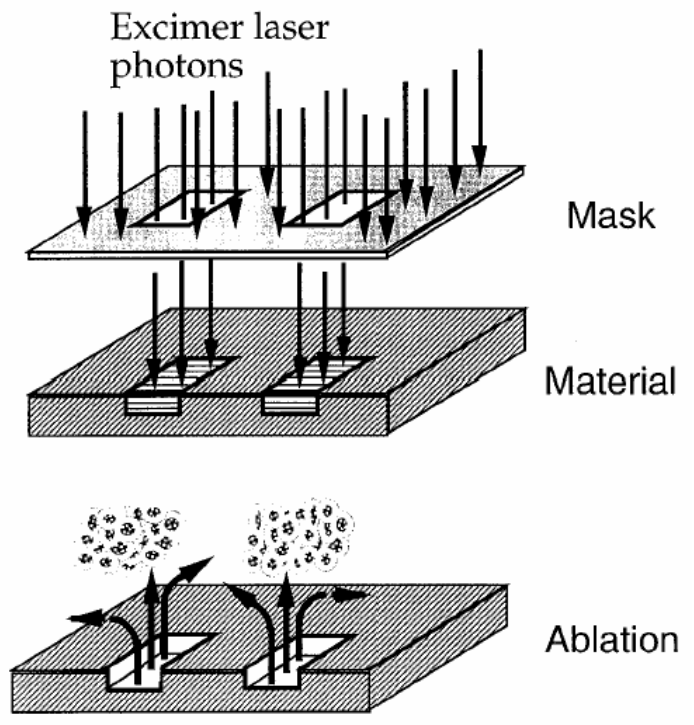
J. Webster, M. Burns, et.al., *Anal. Chem. 73,* 1622, **2001**

Lab-On-a-Chip Fabrication Methods II

Embossing/Molding of Plastic microfluidic chip

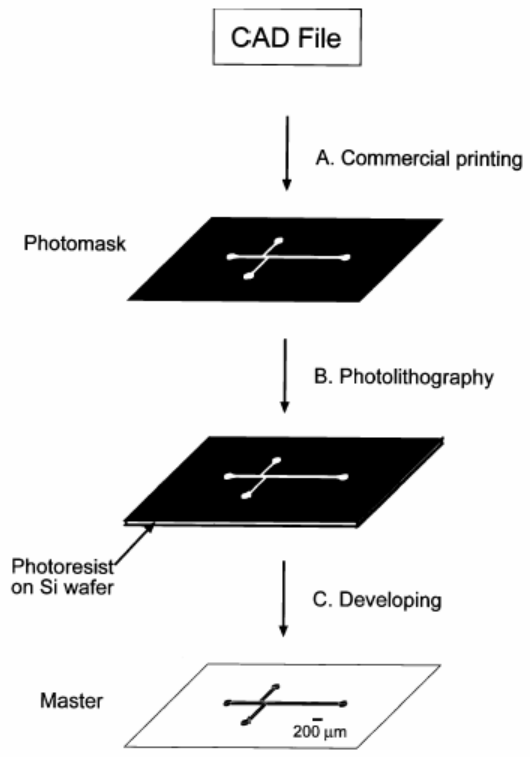


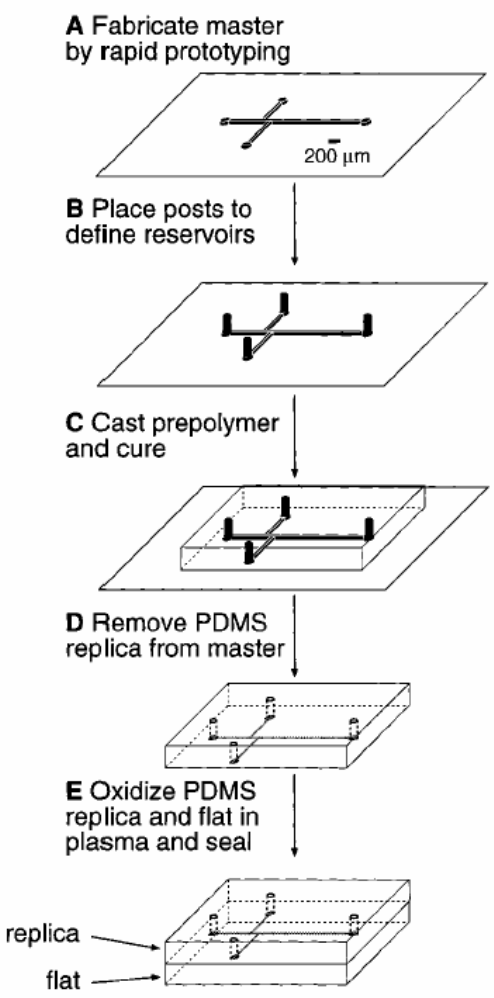
Lab-On-a-Chip Fabrication Methods III



Laser ablation

H. Girault, *Anal. Chem., 69,* 2035, **1997**

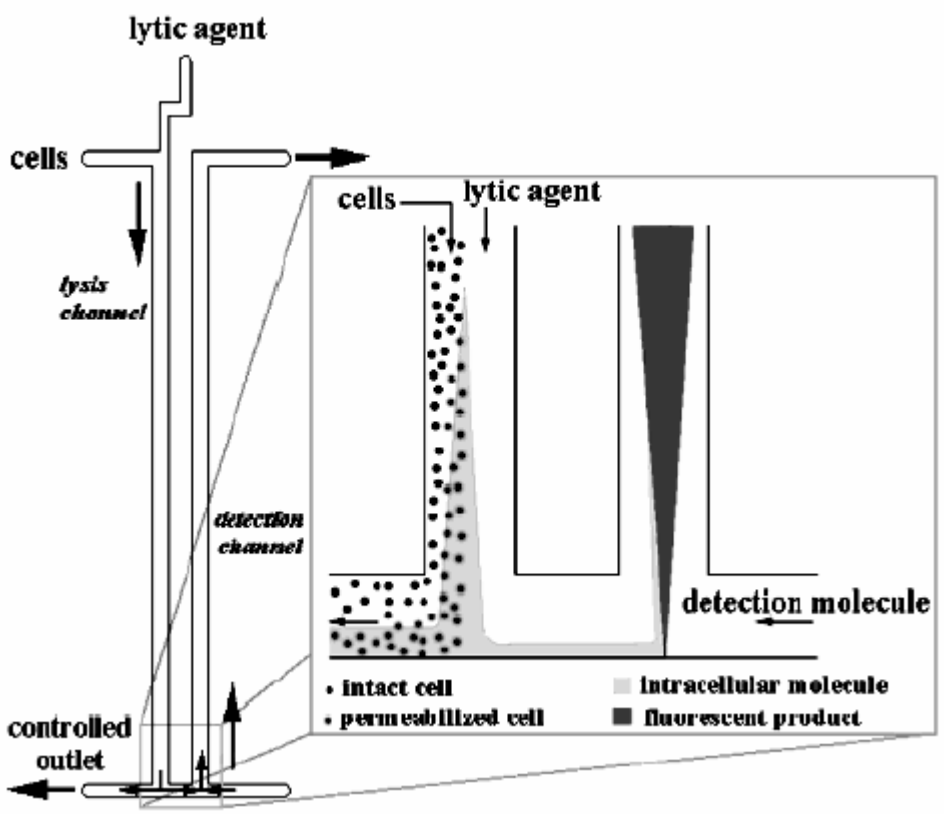


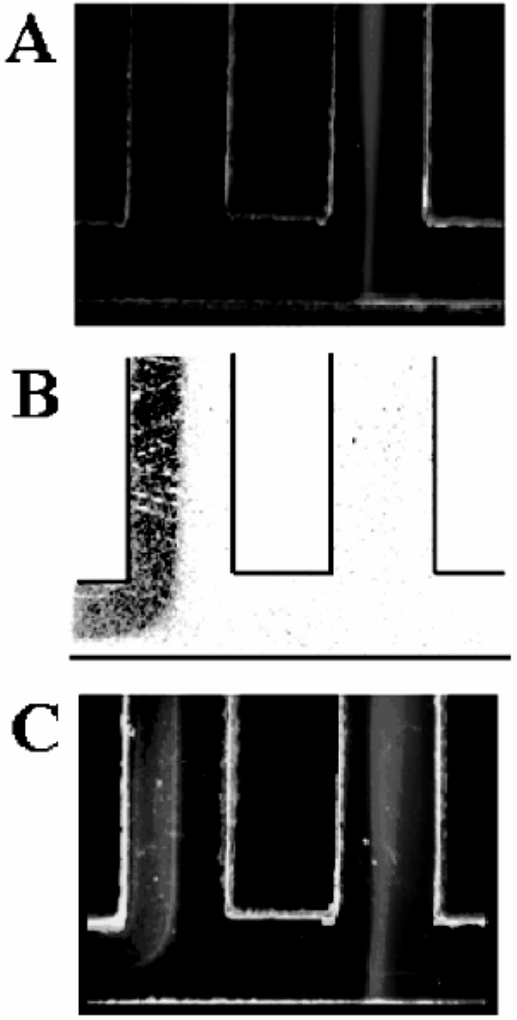


Soft lithography using PDMS, G.M. Whitesides, et.al., *Electrophoresis, 21,* 27, **2000**

Simultaneous Cell Lysis And Protein Extraction

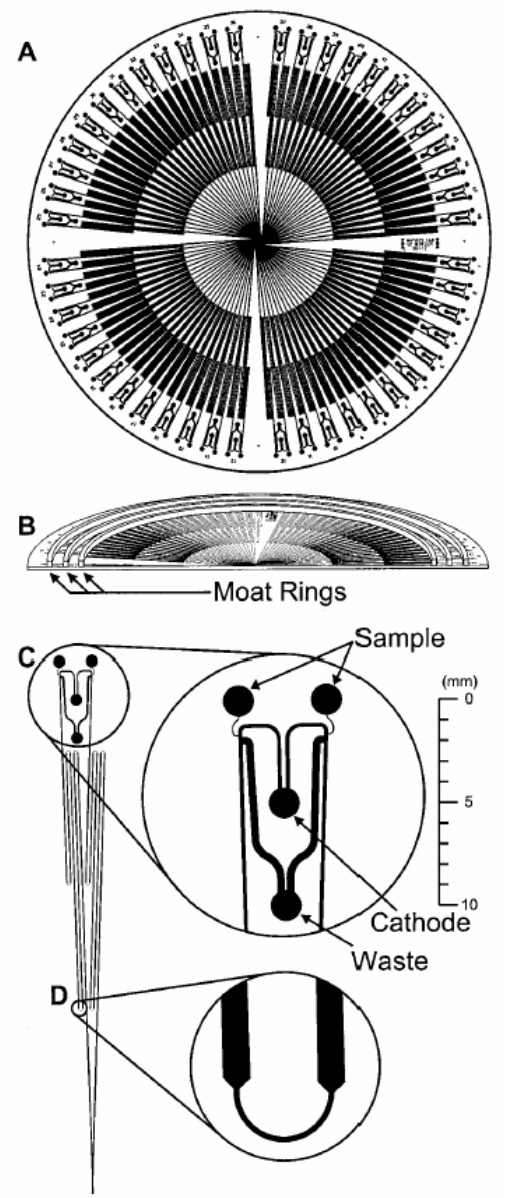
β-Galactosidase extraction from *E. Coli.*,
Channel width: 1mm

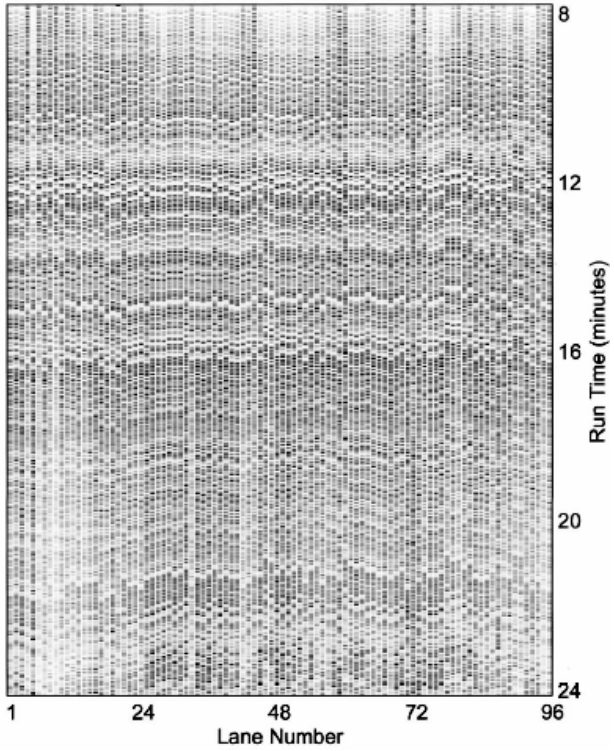


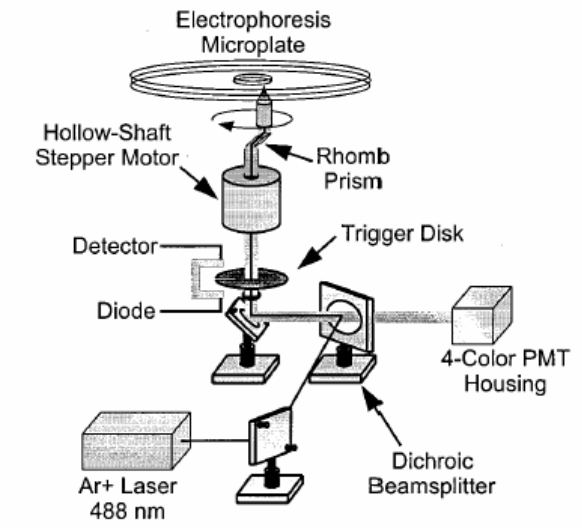


P. Yager et.al, *Anal. Chem.*, **2002**

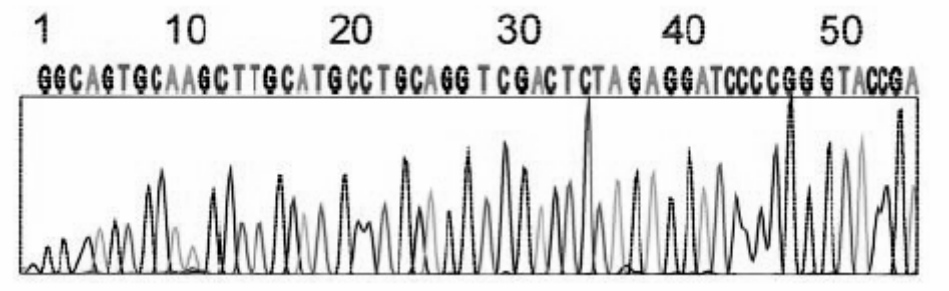
DNA Sequencing Chip





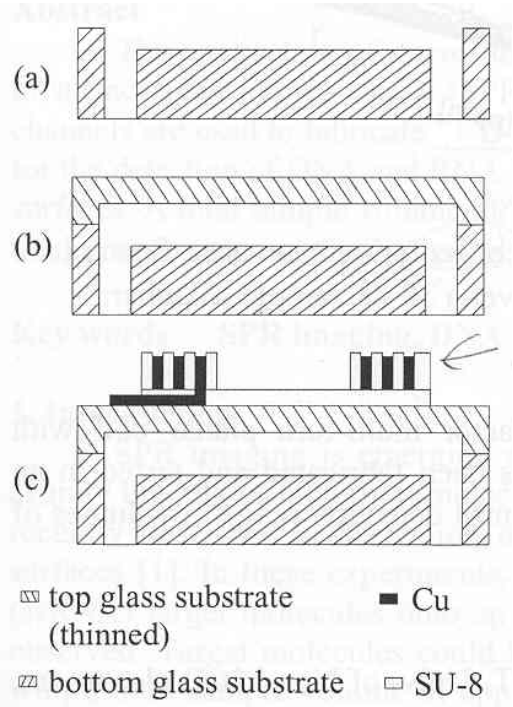


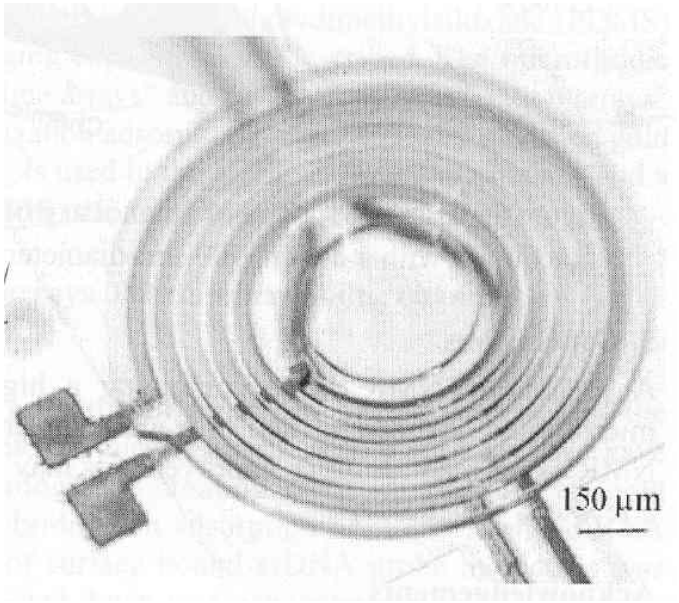
Detection system



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

On-Chip NMR Detection

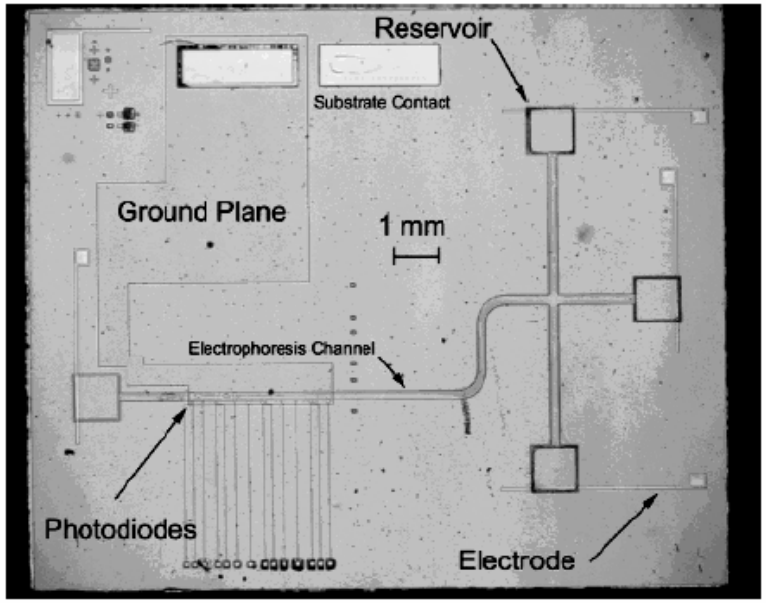


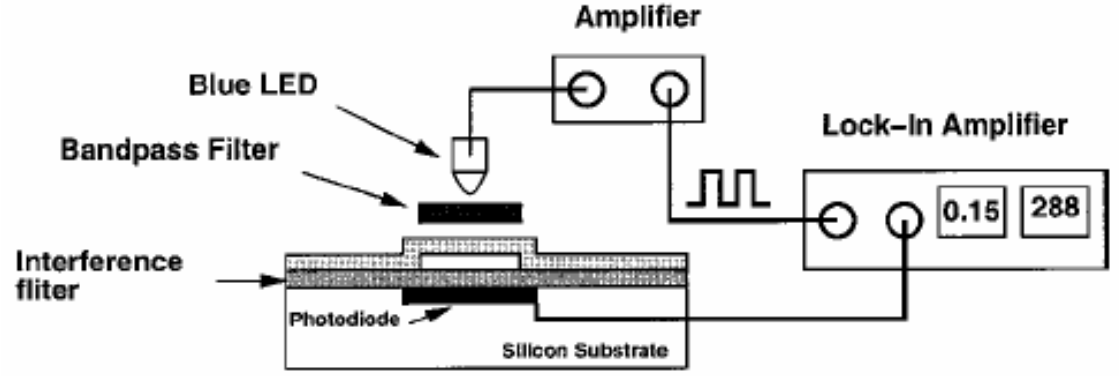


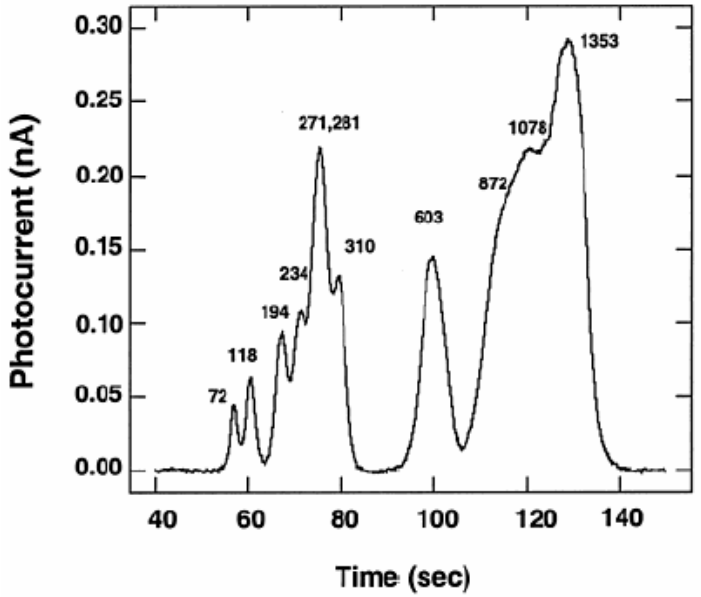
Sample consumption: 30 nL.
Analysis is miniaturized.

C. Massin, A. Daridon, et.al.
"*Proceeding of the μTAS, 2001",* p438

Capillary Electrophoresis Chip With Fluorescence Detection

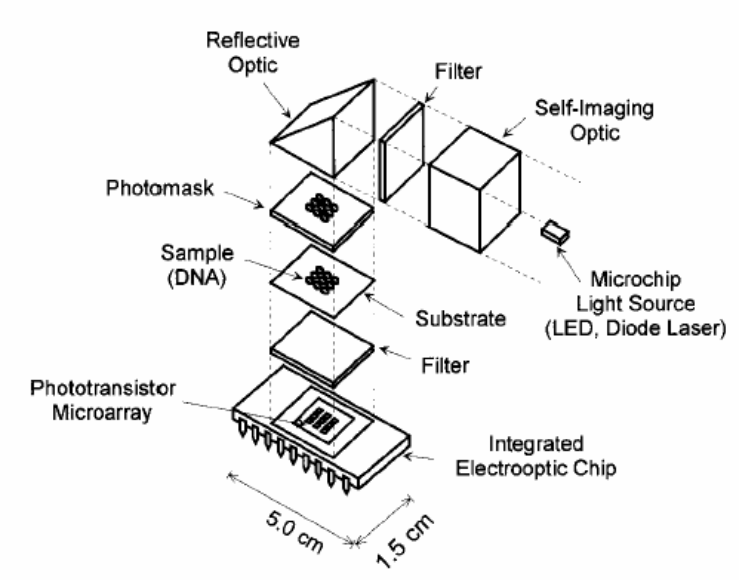


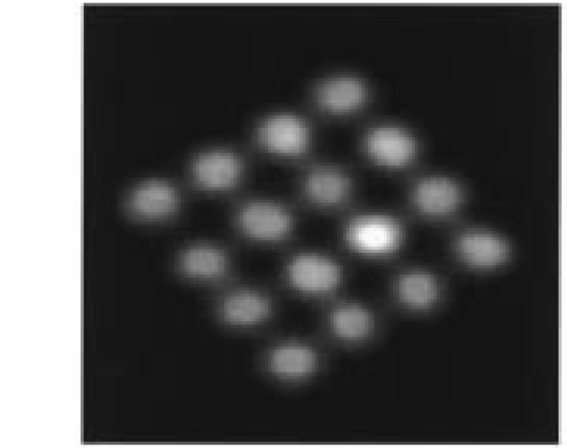


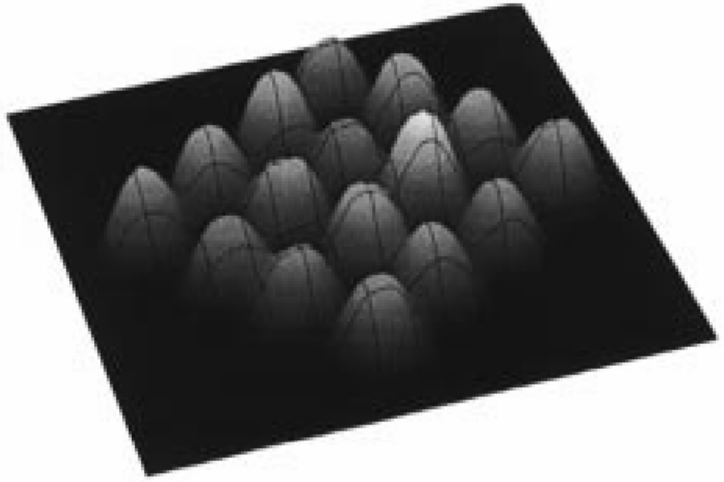


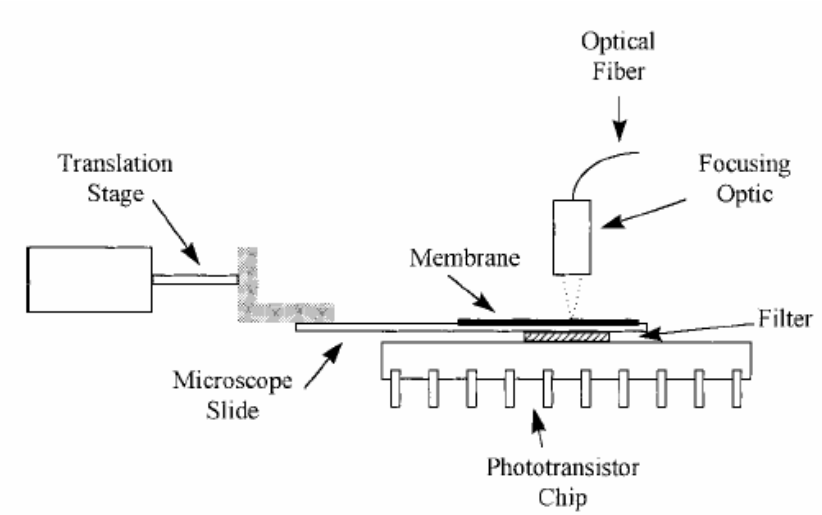
J. R. Webster, et.al., *Anal. Chem.*, *73*,1622, **2001**

DNA Hybridization Detection Integrated on IC Chip









T. Vo-Dinh, et.al., *Anal. Chem.*, *71*,358, **1999**

Summary

- Central theme:
  - Deciphering the genome and proteome, the internal book of life, by detection biologically active molecules.
- The DNA microarray and CE chip are currently the most mature yet being improved.
- The designs and applications of biochip still remain to be explored.

- The major challenges and goal
  - Huge number of analyte
  - Small amount of sample
  - Merge all the advantages

swiftness sensitivity

throughput sample consumption

=> Total Analysis

Thank you!

Acknowledgements:

Dr. Konan Peck 白果能 博士

陳鴻輝
高偉程
高玉書
蕭自宏