Biomedical Applications of MEMS

Dr. Bruce K. Gale
Fundamentals of Micromachining

Biochip Technology

with special thanks to
Xiaolian Gao, University of Houston

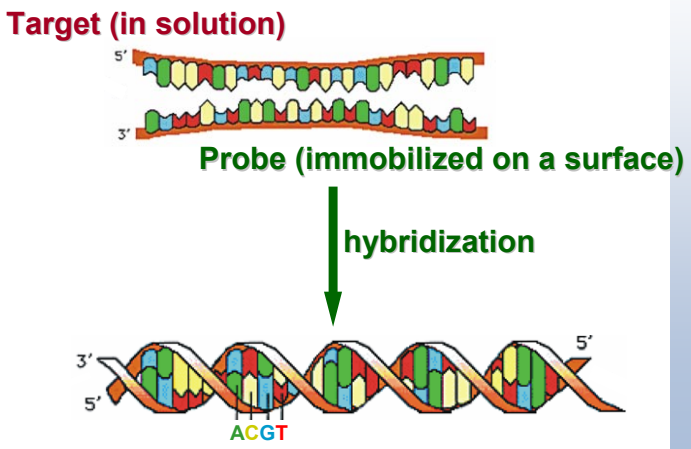
Genetic Database

- Challenges
  - Function must be assigned to gene (discovery)
  - Location of gene determined (mapping)
  - How often is gene used (expression)
  - How do these genes differ between individuals (genetic variation)

DNA Hybridization Arrays

- High density arrays of polynucleotide probes
- Used for genetic sequence analysis
- Why do we care?
  - New targets for drugs or other therapeutic intervention
  - Diagnostic markers for disease
  - Development of improved agricultural products

Basic Principles of DNA Microarrays



How Important is the Sequence ?

Biological relevance of SNPs (Single Nucleotide Polymorphism)

Hemoglobin B Sequence

```
  1 acatttgctt ctgacacaac tgtgttcact agcaacctca aacagacacc atggtgcacc
 61 tgactcctgX ggagaagtct gccgttactg ccctgtgggg caaggtgaac gtggatgaag
121 ttggtggtgg ggccctgggc aggctgctgg tggtctaccc ttggacccag aggttctttg
181 agtcctttgg ggatctgtcc actcctgatg ctgttatggg caaccctaag gtgaaggctc
241 atggcaagaa agtgctcggt gcctttagtg atggcctggc tcacctggac aacctcaagg
301 gcacctttgc cacactgagt gagctgcact gtgacaagct gcacgtggat cctgagaact
361 tcaggctcct gggcaacgtg ctggtctgtg tgctggccca tcactttggc aaagaattca
421 ccccaccagt gcaggctgcc tatcagaaag tggtggctgg tgtggctaat gccctggccc
481 acaagtatca ctagctcgct ttcttgctgt ccaatttcta ttaaaggttc ctttgttccc
541 taagtccaac tactaaactg ggggatatta tgaagggcct tgagcatctg gattctgcct
601 aataaaaaac atttattttc attgc
```

- Fatigue, paleness, and shortness of breath
- Pain
- Eye problems (can cause blindness)
- Delayed growth
- Infections
- Stroke
- Acute chest syndrome
- Hand-foot syndrome
- Yellowing of the skin and eyes.

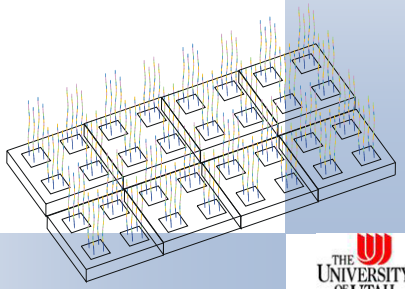
If X = A, normal

If X = T

Sickle Cell Anemia

Biochip - the beginning and terminology

- **Southern, Ekins, Drmanc,and Mirzabekov, Affymetrix pioneered (~1989)**
- **2-D array of DNA molecules (probes)**
- **Probes are anchored to a glass substrate**
- **When the array is exposed to other molecules (targets) carrying luminescence tags, the tags light up at the sites where binding occurs**
- **The emitted intensity provides qualitative and quantitative information - reporting what molecules are in a sample, etc.**



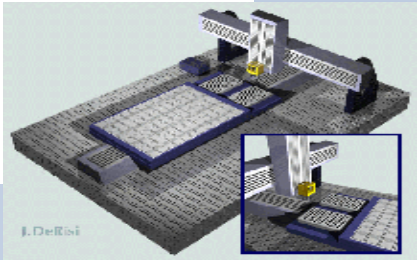
Manufacturing Methods

- Photolithography
  - Affymetrix chips
  - Advantages
    - Precise
    - Small spot size
    - Control
  - Disadvantages
    - Lower yield
    - Cost
- Mechanical printing (spotting)
  - Used by biologists
  - Soft lithography
  - Ink jet
  - Pins
  - Advantages
    - Cheap
    - Longer chains
  - Disadvantages
    - Less specificity
    - Lower density

Stanford Chips- Spotting

- Use robot to spot glass slides at precise points with complete gene sequences
- Used to measure qualitative relative expression levels of genes
  - Differential expression by means of simultaneous two-color hybridisation

www.genomics.stanford.edu



Photolithographic Design

- Smaller dimensions allow higher density analysis
- Signal drops with sample size
- Longer probes require more steps
  - 4 steps per layer
- Number of probes (and masks) goes up at 4n

Table 1. Combinatorial synthesis of polynucleotide probe arrays

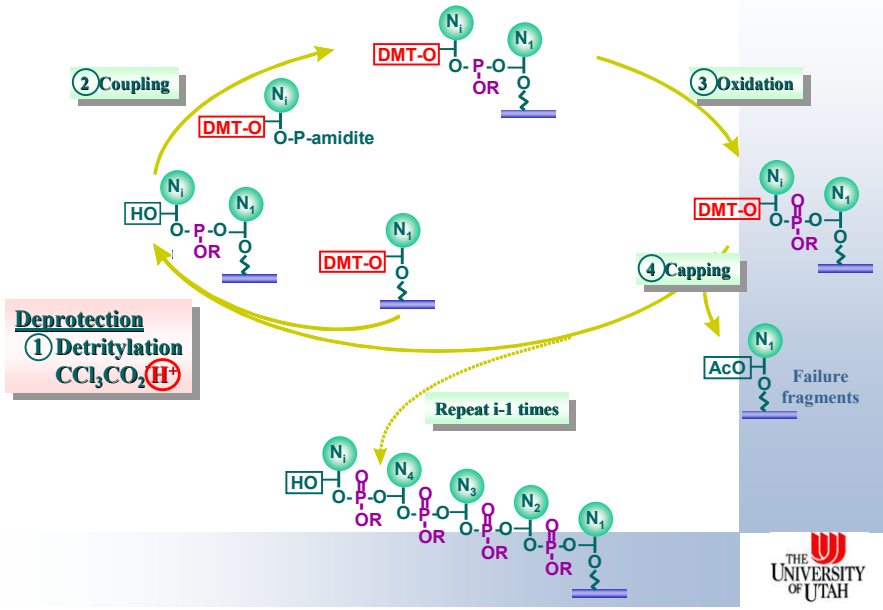
Probe Length	Chemical Steps	Number of
4	16	256
8	32	65 536
12	48	16 777 216
16	64	~4.3×10^9
20	80	~1.1×10^{12}

Table 2. Photolithographic resolution and maximum array density

Resolution (mm)	Array Density (sequences/cm^2)
500	400
200	2 500
100	10 000
50	40 000
10	1 000 000
1	100 000 000

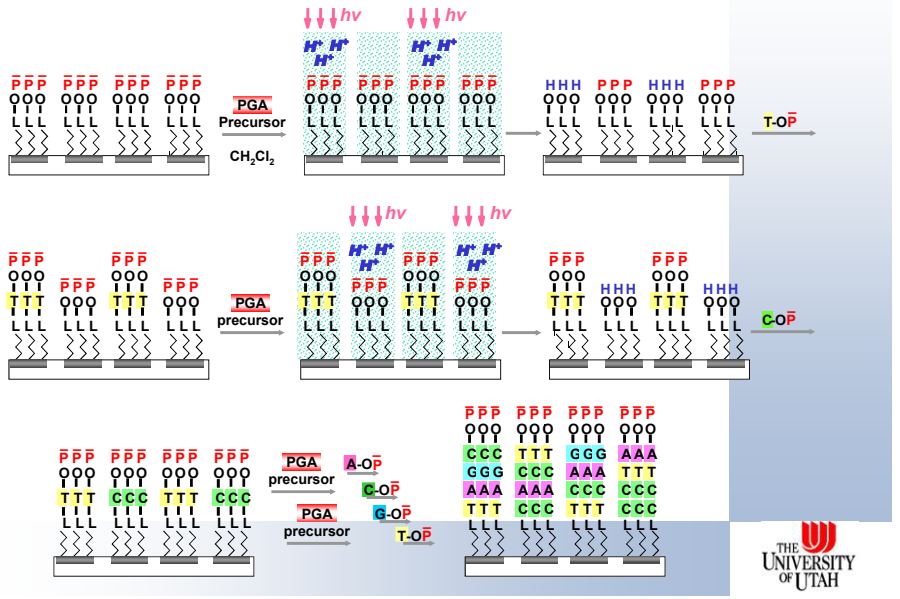
THE UNIVERSITY OF UTAH

Standard Conventional DNA Oligonucleotide Synthesis



THE UNIVERSITY OF UTAH

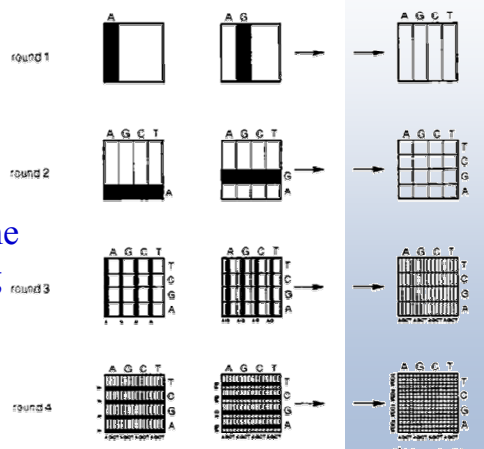
Light-directed Parallel Synthesis of oligo-DNA Using Acid-labile Groups Protected Phosphoramidites



THE UNIVERSITY OF UTAH

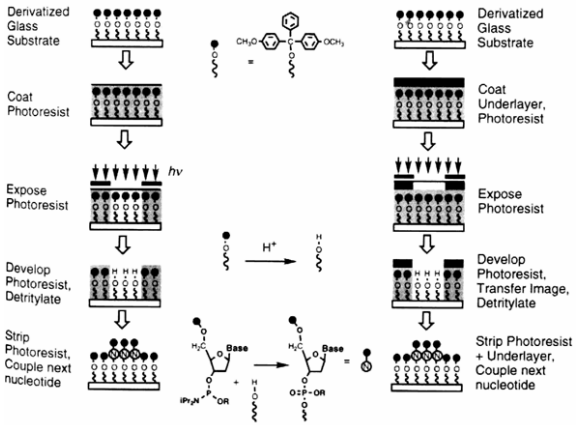
Basic Fabrication

- A, C, G, and T bases applied to each layer
  - Each has its own protective block
- Photolithography or printing used to define location by removing block
- Multiple methods

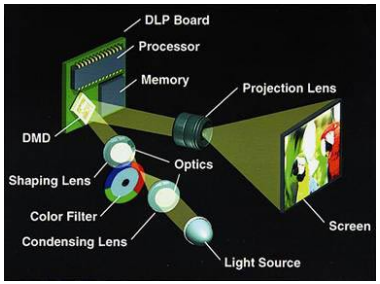


Photoresist Method

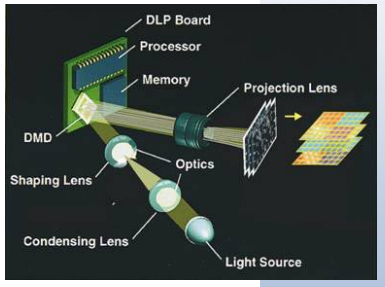
- Single layer and bilayer methods
- Bilayer method provides better chemistry for nucleotides



Digital Light Projection - A solution for flexibility, simplicity and reduced cost



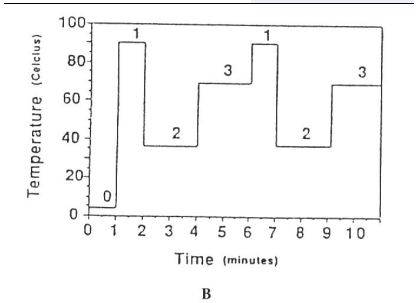
Digital Light Projector from Texas Instruments



Chip Projector at Xeotron

PCR

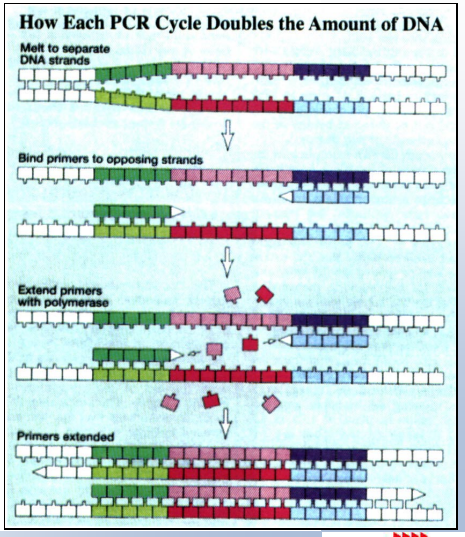
- Technique used to produce a large number of copies from a target DNA sequence
- Repetitive 3 step process
  - Denaturation (~95ºC)
  - Annealing (~55ºC)
  - Chain Extension (~ 72ºC)
- Creates 2n copies
  - Typically 30 cycles



- Typical molecular analysis problems require statistically significant quantities and must pass detection limits on the order of millions and billions of molecules

How PCR Works

- Basic PCR Reagents
  - Template DNA
  - Complementary Primers (~20 nucleotides)
  - Thermostable Polymerase Enzyme (TAQ)
  - Single nucleotides (A,C,G,T)
  - Buffers (pH and ionic concentrations)
- High temp to split strands
- Low temperature to anneal
- Medium temp to extend
- Repeat



Why Apply Micromachining?

- Small reagent costs
- Fast cycling time
  - Low thermal mass
  - High surface to volume ratio
- System integration
  - Electrophoresis
  - Point of care system
- Low cost

Design Considerations

- Biocompatibility
- Chamber volume
- Control system
- Bulk or surface micromachining
- Bonding method (if necessary)
- Move the fluid or cycle in position
- External equipment
- Components
  - Heater
  - Chamber
  - Reagent mixing
  - Temperature control
  - Feedback
  - Detection?

Temperature Control

- Heaters
  - Boron doped regions
  - Metallization
  - Other?
- Temperature measurement
  - Thermistor
  - Thermopile