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ICTP 40th Anniversary

**SMR 1564 - 23**

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# SPRING COLLEGE ON SCIENCE AT THE NANOSCALE

**(24 May - 11 June 2004)**

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## BIOCHIPS - Part I

**Rashid BASHIR**
School of Electrical & Computer Engineering, Purdue University
West Lafayette, IN, USA

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*These are preliminary lecture notes, intended only for distribution to participants.*

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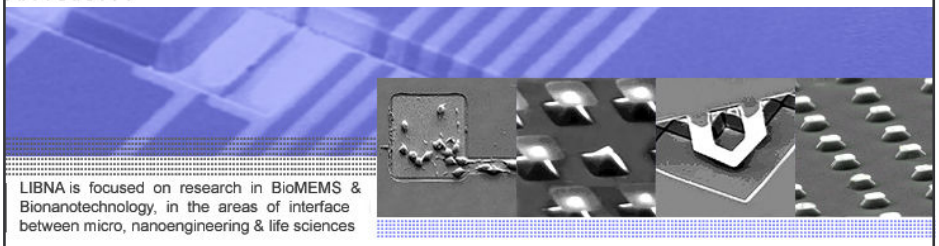
LIBNA




# Introduction to Bio-Chip, Biosensors, BioMEMS

**R. Bashir**
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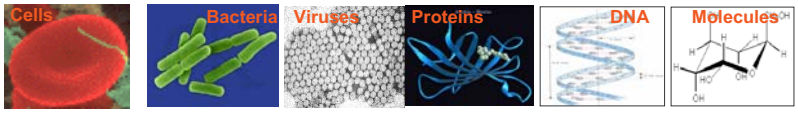
LIBNA

## Key Topics

- Biochips/Biosensors and Device Fabrication
- Cells, DNA, Proteins
- Micro-fluidics
- Biochip Sensors & Detection Methods
- Micro-arrays
- Lab-on-a-chip Devices



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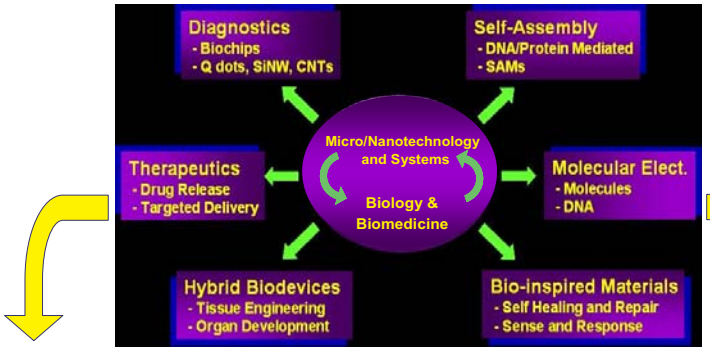
# BioMEMS and Bionanotechnology

**Apply micro/nano-technology to develop novel devices and systems that have a biomedical impact or are bio-inspired**




Novel Solutions for Frontiers in Medicine and Biology

Novel Solutions for Frontiers in Materials and Information Processing

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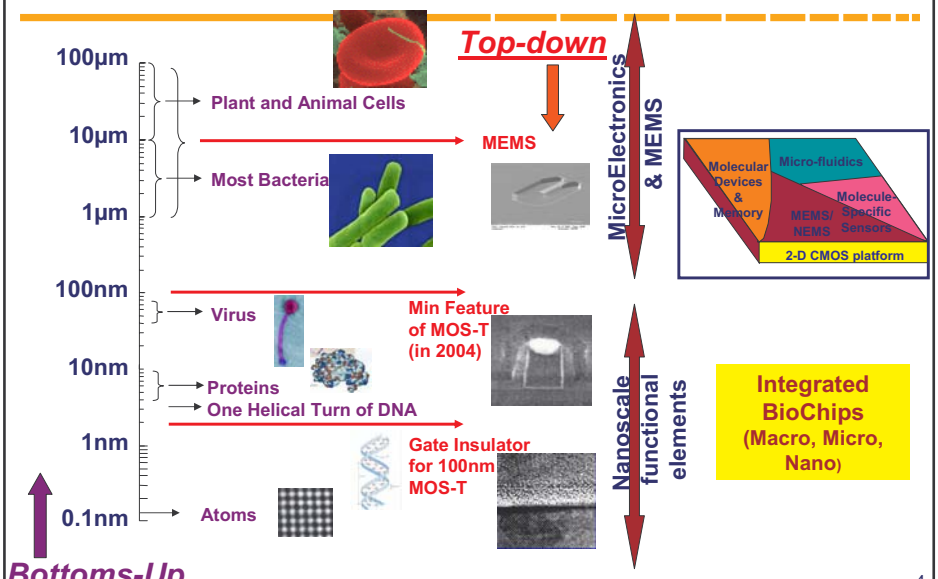
# On Size and Scale !

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# Definitions

- Biosensors are 'analytical devices that combine a biologically sensitive element with a physical or chemical transducer to selectively and quantitatively detect the presence of specific compounds in a given external environment' [Vo-Dinh and Cullum, 2000].
- Biochips can be defined as '*microelectronic-inspired* devices that are used for delivery, processing, analysis, or detection of biological molecules and species' [Bashir, 2004]. These devices are used to detect cells, microorganisms, viruses, proteins, DNA and related nucleic acids, and small molecules of biochemical importance and interest.

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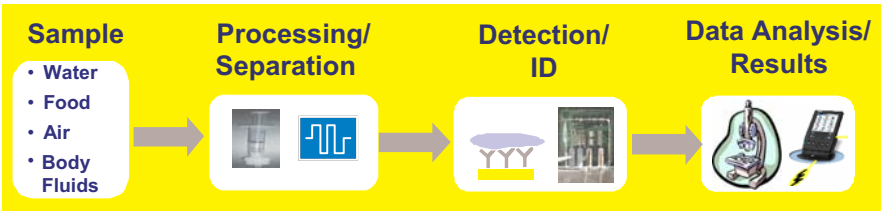
# Overview of Biosensor System

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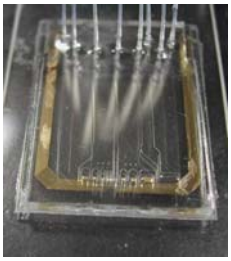
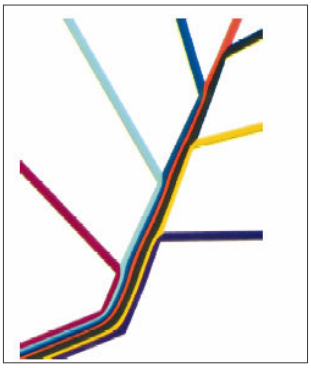
# Introduction

**Key Attributes of Biochips**

1. Small length scale
2. Small thermal mass
3. Laminar flow, Re < 1
4. High surface-to-volume ratio



W.J. Chang, Demir Akin, Miroslav Sedlek, Michael Ladisch, Rashid Bashir, *Biomedical Microdevices*, vol. 5, no. 4, pp. 281-290, 2003.



Whitesides Harvard University

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# Reasons for Miniaturization

- In general, the use of micro and nano-scale detection technologies is justified by,
  - (i) reducing the sensor element to the scale of the target species and hence providing a higher sensitivity → single entity/molecule
  - (ii) reduced reagent volumes and associated costs,
  - (iii) reduced time to result due to small volumes resulting in higher effective concentrations,
  - (iv) amenability of portability and miniaturization of the entire system
  - (v) point-of-care diagnostic,
  - (vi) Multi-agent detection capability
  - (vii) Potential for use *in vitro* as well as *in vivo*

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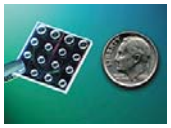
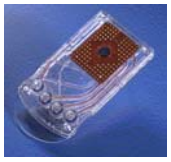
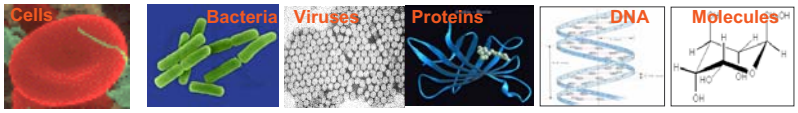
# Biochips for Detection

- Applications
  - Medicine
  - Pharmaceuticals
  - Food Safety
  - Homeland Security, etc.
- Integrated, Sensitive, Rapid, Cost x Performance
- Commercialized; Nanogen, Affymetrix, Caliper, Others….



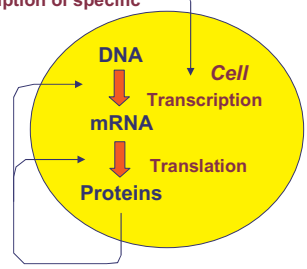


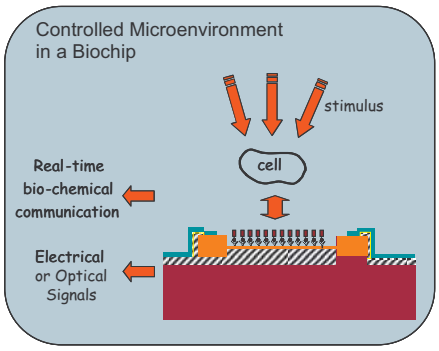





# Novel Tools for NanoBiology

**Transcription factors:**
**Proteins that control the transcription of specific genes**




- **Analysis of single cells and the study of their function in real time.**
- **Increase understanding of signaling pathways inside the cell.**
- **Basic cell functions such as differentiation, reproduction, apoptosis, etc. and their implications on various disease states.**
- **Focus of the post-genomic era and systems biology**

## BioChip/BioMEMS Materials

- Silicon and microelectronic materials
- Glass, Quartz
- Polymers
  - Poly (dimethylsiloxane) (PDMS)
  - Poly (methyl methacrylate) (PMMA)
  - Teflon, etc.
- Biological Entities
  - Cells, Proteins, DNA
  - Frontier of BioMEMS !

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## Introduction to Device Fabrication

- MEMS/NEMS Silicon Fabrication
  - Formation of structures that could be used to form sensors and actuators.
  - Processing of electrical or non-electrical signals.
  - Conventional and new semiconductor processing technology modules are used.
  - Etching, Deposition, Photolithography, Oxidation, Epitaxy, etc.
  - Deep RIE, Thick Plating, etc
- Bulk and Surface Micromachining

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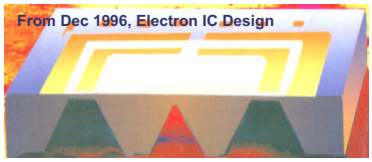
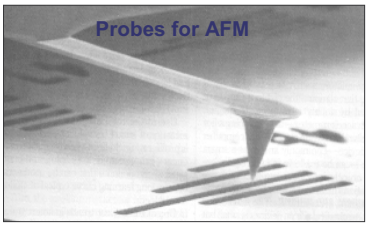
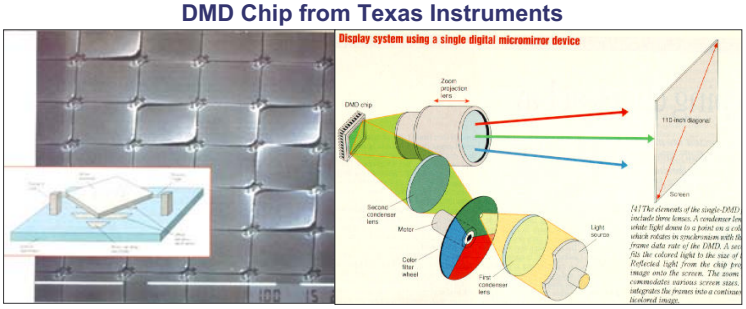
# MEMS Examples




Bulk Micromachined Accelerometer from Silicon Microstructures. Inc.




DMD Chip from Texas Instruments

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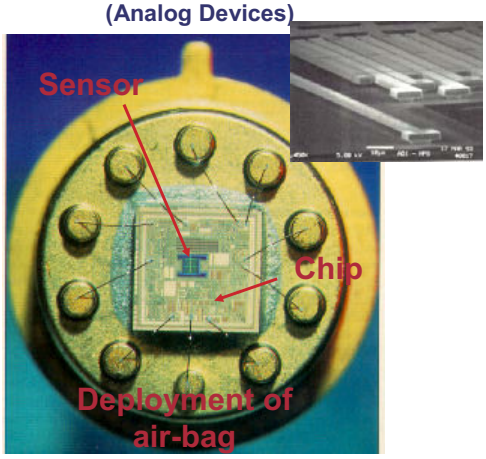
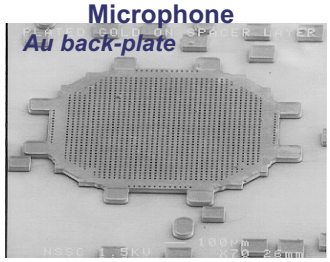
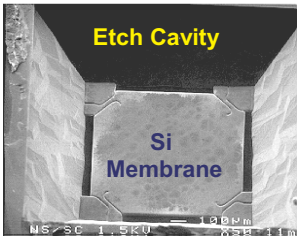
# MEMS Examples

**Single Chip Accelerometer**
(Analog Devices)




**Single Chip Microphone**




Draper Labs,
National Semiconductor, 1998

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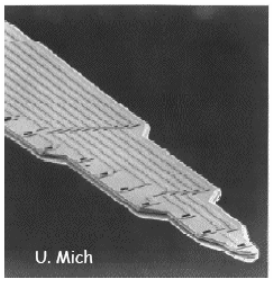
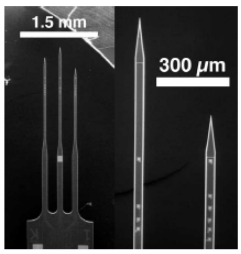
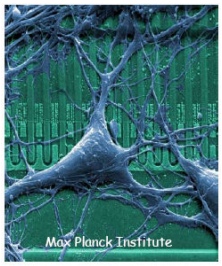
# Silicon BioMEMS Examples



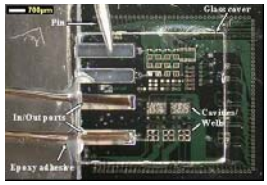




Stanford Neuro Probe

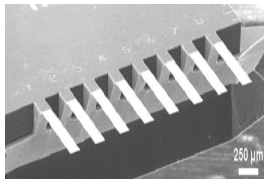




Kumetrix



Purdue Silicon BioChip




IBM Zurich Research

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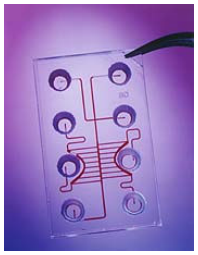
# BioMEMS/Biochip Fabrication

- In addition to Silicon….
- Biocompatibility, ideal for biomedical devices
- Transparent within the visible spectrum
- Rapid fabrication
- Photo-definable
- Chemically modifiable
- Possible choices
  - PDMS - polydimethylsiloxane,
  - Hydrogels – PMAA,
  - Teflon
  - SU-8, etc.

Immunochip (Aclara)



Lab on Chip (Caliper)

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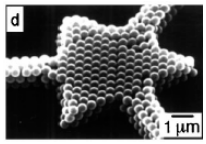
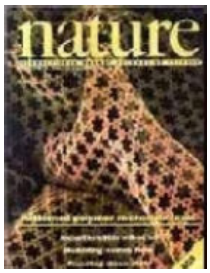
# Alternative Fabrication Methods

- Soft Lithography
  - Replication and molding
  - Micro-contact printing
  - Micro-molding in capillaries
  - Micro-transfer molding
  - Solvent assisted micro-molding
  - Dip Pen Lithography
- Compression Molding
  - Hot Embossing
  - Injection Molding
- Inkjet Printing

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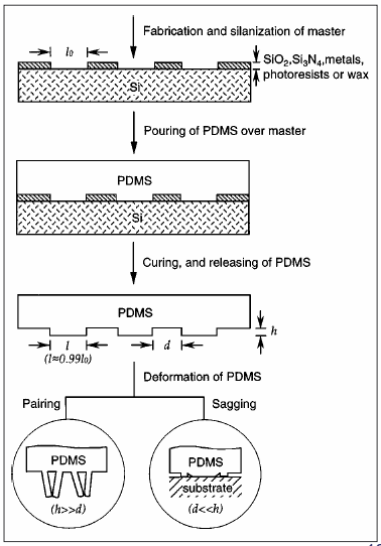
# Replication and Molding

- Master mold made from silicon, glass, metal, SU-8
- Surface treatment of master
- Pour PDMS (mix, oligomer, and CL agent)
- Cure (~60C, 1 hr)
- Peel off PDMS structure
- Mold can be used again

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Y. Gia, and G. M. Whitesides, Annu. Rev. Mater. Sci. 1998, 28, 153-84

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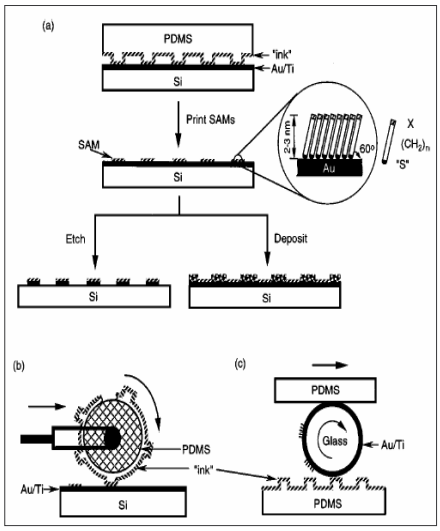
# μ-Contact Printing

- Ink the PDMS structure with molecules (alkylthiols, proteins, DNA, etc.)
- Transfer the layer through physical contact (optimize time)
- Inking is performed via covalent binding on substrate
- Can be performed on flat surface or curved surface



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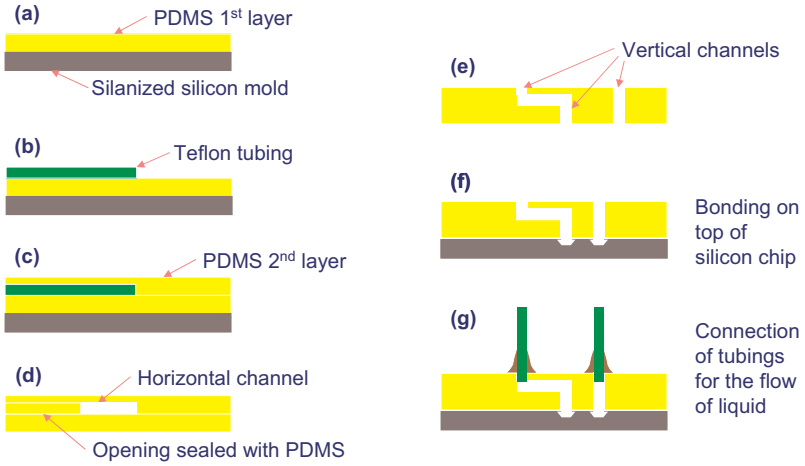
# PDMS/Glass (Silicon) Hybrid Biochip



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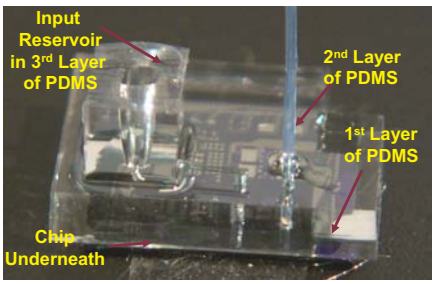
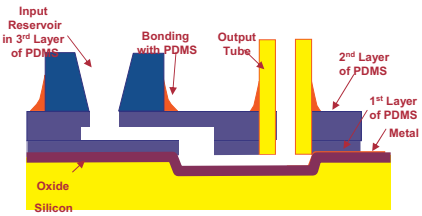
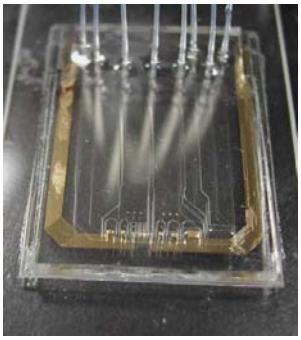
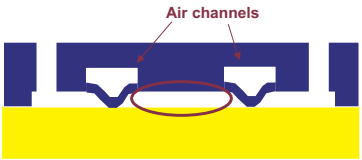
## Silicon Base, 3 PDMS layers, Top I/O port




## Glass Base, 3 PDMS layers, Top I/O port, Valves



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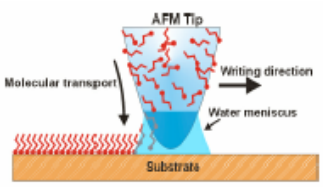
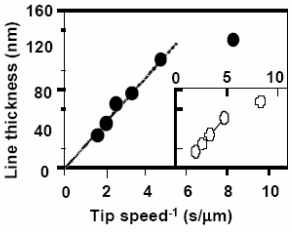
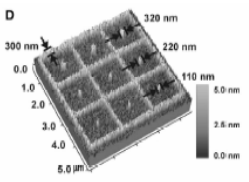
# Dip Pen Lithography

- AFM Tip used to 'write' molecules
- Being commercialized by Nanoink, Inc.
- SAMs, DNA, Proteins, etc.
- Serial (need array of cantilevers for parallel writing)
- Continuous source of molecules – microfluidics !




C. S. Mirkin, et. al, Science, **283**, 661 (1999); Science **286**, 523 (1999); **288**, 1808 (2000).

Lee, K.B.; Park, S.J.; Mirkin , C.A. ; Smith, J.C.; Mrksich, M. Protein nanoarrays generated by dip-pen nanolithography Science 2002 , 295, 1702-1705

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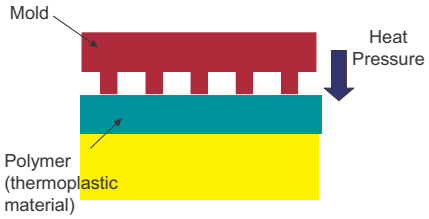
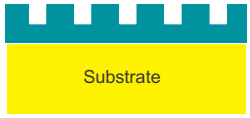
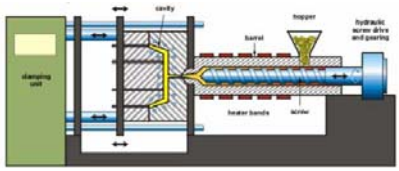
# Compression Molding

## Hot Embossing




## Precision Injection Molding



**Features down to 0.1um deep and 0.6um wide (for CD-R)**

Immunochip (Aclara)



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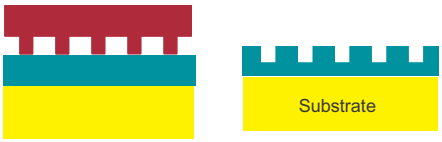
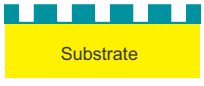
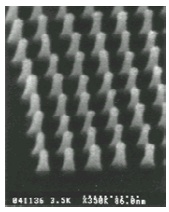
# Nano-Imprint Lithography



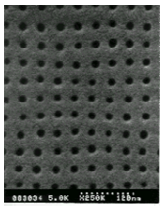




- Nano-scale extension of hot embossing
- Need a nano-scale master mold
- Added to ITRS Roadmap

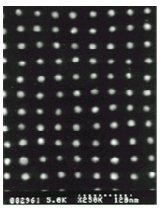
Imprint mold with 10nm diameter pillars



10nm diameter holes imprinted in PMMA



10nm diameter metal dots



**Steve Chou, Princeton U.**

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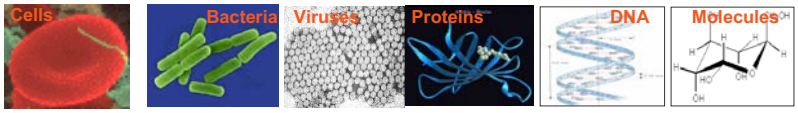
# Key Topics

- Biochips/Biosensors and Device Fabrication
- **Cells, DNA, Proteins**
- Micro-fluidics
- Biochip Sensors & Detection Methods
- Micro-arrays
- Lab-on-a-chip Devices

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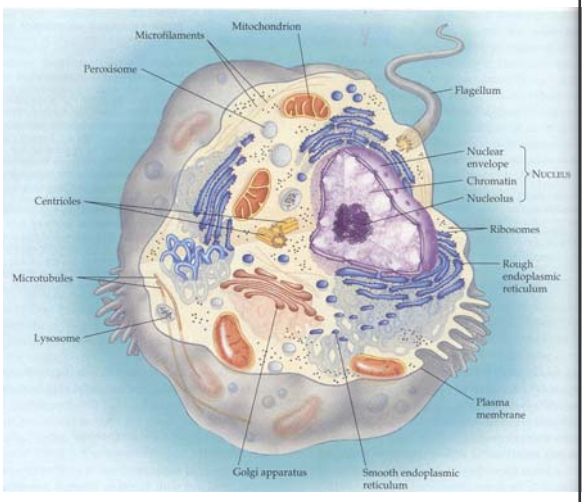
# Cells – Brief Overview

- Genetic information is contained in chromatin (a diffused mass which distinguishes to a chromosome when cell is ready to divide)
- Humans have 46 chromosomes in each cell (except in reproductive cells)
- Chromosomes are long, uninterrupted, packed, super-coiled linear polymer strands of DNA (deoxyribonucleic acid) - 6 cm long when extended
- In humans, each chromosome is 50-400 x $10^6$ units long

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From: Biology, 4th Edition by Campbell

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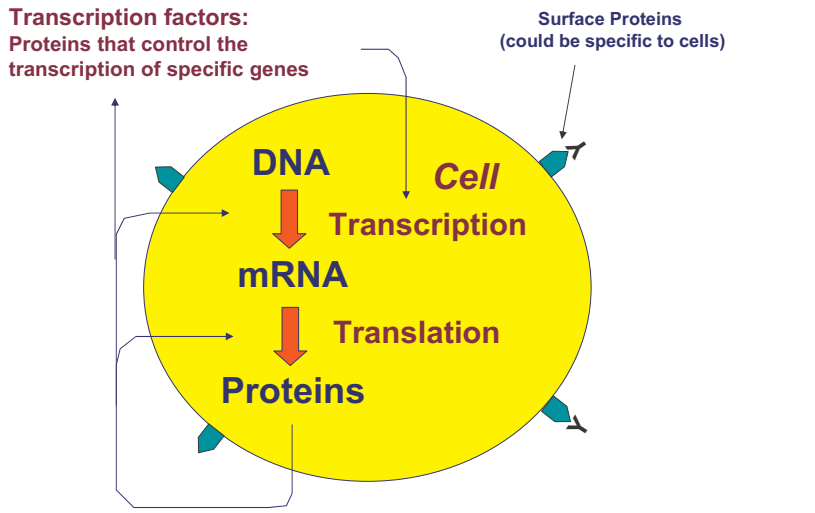
# Cells – Brief Overview

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# DNA to Proteins

- Transcription
  - double stranded DNA is converted to a single stranded mRNA
  - RNA polymerase synthesizes the mRNA
- Translation
  - Ribosomes 'translate' the sequence of bases in the mRNA to proteins.
  - These proteins than perform various functions inside and outside the cell

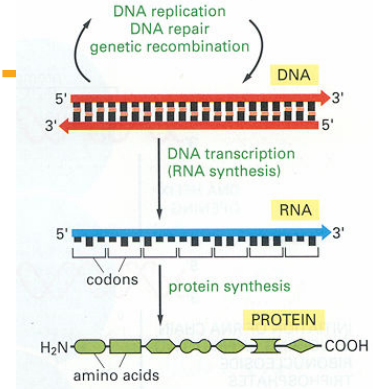


**Figure 6–1 The basic genetic processes.** The processes shown here are thought to occur in all present-day cells. Very early in the evolution of life, however, much simpler cells probably existed that lacked both DNA and proteins (see Figure 1–11). Note that a sequence of three nucleotides (a codon) in an RNA molecule codes for a specific amino acid in a protein.

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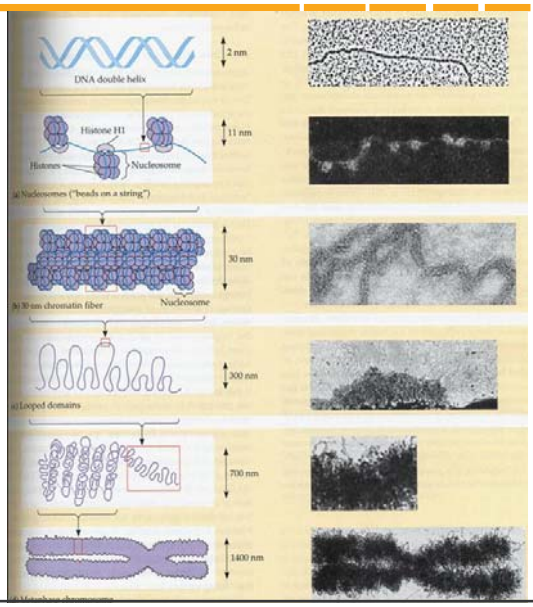
# Chromosomes → DNA

Decreasing complexity

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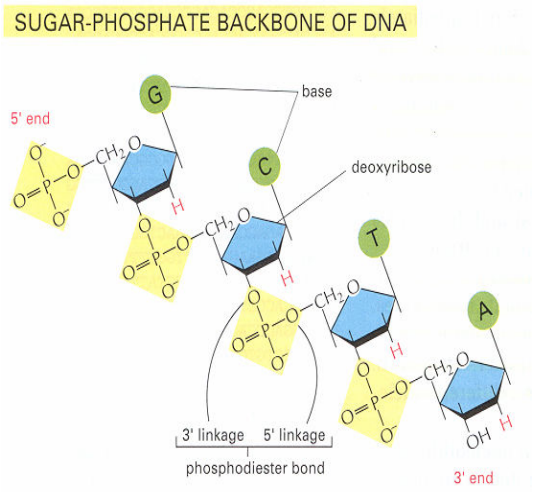
# Structure of DNA

- DNA is composed of;
  - a phosphate back-bone where each phosphate radical has a negative charge
  - a Deoxyribose (D in DNA) sugar
  - 4 types of bases or nucleotides. These are adenine (A), thymine (T), cytosine (C), Guanine (G)
- A binds to T and G binds to C - complementary base pairs

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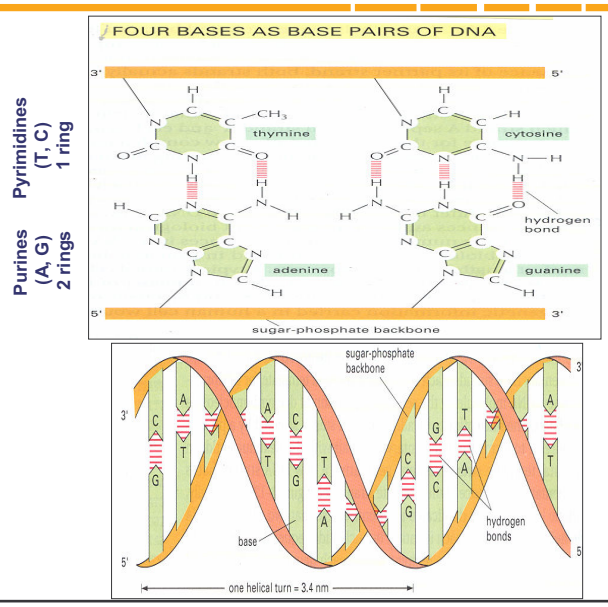
# Structure of DNA

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# DNA Hybridization

- When DNA is heated to a temperature (~>90°C) or exposed to pH > ~12, the complementary strands dissociates - *DNA denaturation*
- Process is reversible (exposure to a melting temperature $T_m$ > 65°C) and 2 complementary ssDNA will *hybridize* to each other and join to form dsDNA
- Hybridization can happen between any two complementary single stranded molecules (DNA/DNA, DNA/RNA, RNA/RNA)
- Can provide a very sensitive means to detect specific nucleotide sequences
- Factors affecting hybridizaton : temperature, Salt and buffer concentration, G & C content - $T_m$ can be calculated
- Rate of hybridization is proportional to concentration of target and probe and limited by the lower concentration material

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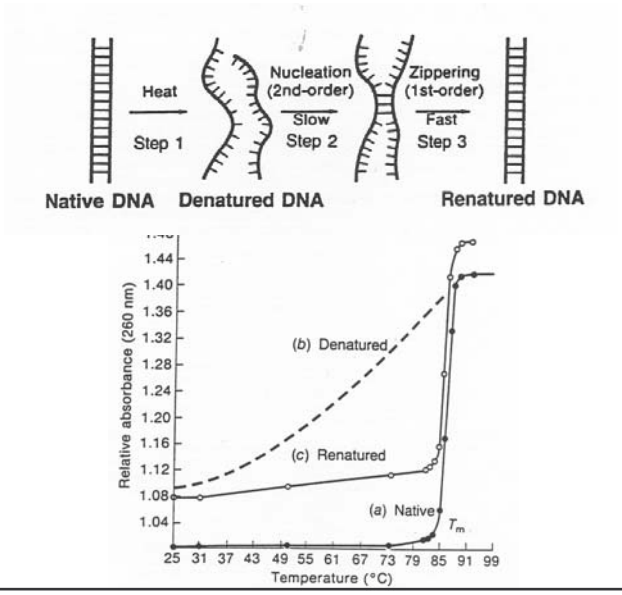
# DNA Hybridization

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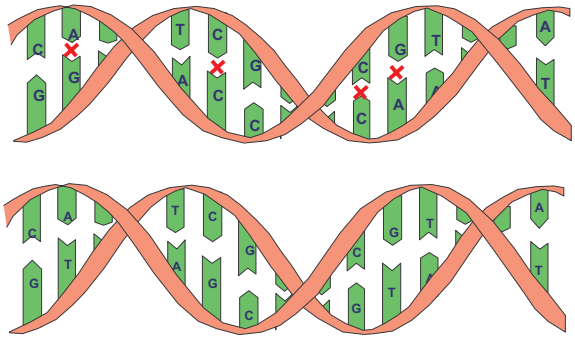
# DNA Hybridization

## Stringency

**Reduced Stringency Hybridization**

**Stringent Hybridization**



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# PCR - Polymerase Chain Reaction

- **Technique to amplify (make multiple copies) of known DNA molecules - invented in 1985**
- **Use enzyme called DNA polymerase and primers (short ssDNA strands)**
- **Billions of copies can be made within hours in laboratory**
- **Very useful in research, diagnosis, forensics, etc where large samples are required from very small concentrations.**

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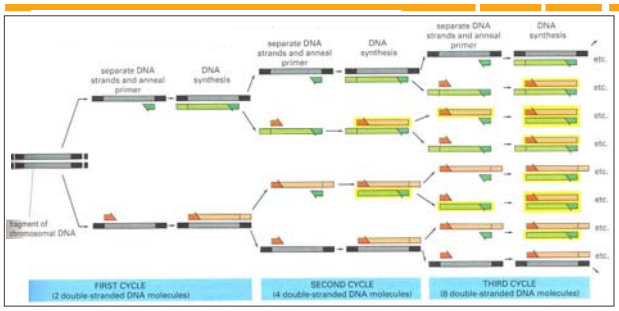
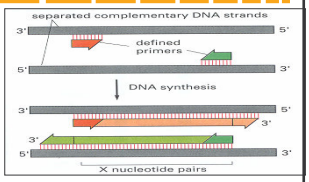
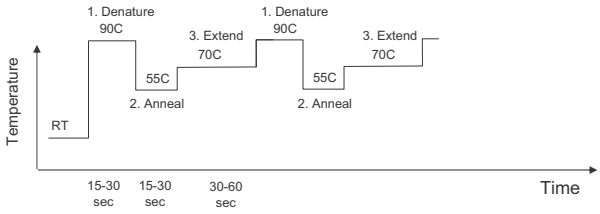
# PCR Sequence




• Primers are short strands of nucleotides which are complementary to specific regions of the target DNA to the amplified - hence the 'end' sequence of short regions of the target DNA to be copied is needed
• DNA Polymerase is an enzyme which takes nucleotides from the ambient solution and starts to construct the complementary sequence
• An adequate supply of nucleotides are needed (dNTPs - deoxyribonucleose triphosphates - dATP, dCTP, dGTP, dTTP)

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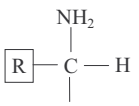
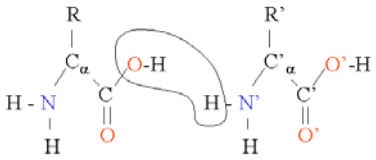
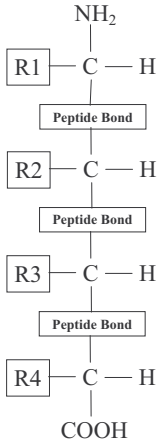
# Protein Structure




http://www.umass.edu/microbio/rasmol/rotating.htm
http://www.umass.edu/microbio/chime/antibody/

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# Protein Structure

- There are 20 different amino acids that can make an infinite number of proteins.
- 3 bases within the mRNA are called a 'codon'.
- 4 different bases in combination of 3 results in 64 possible codons.
- 3 of these are 'stop codons'
- 61 specify the 20 amino acids - hence there is degeneracy

- alanine - ala - A
- arginine - arg - R
- asparagine - asn - N
- aspartic acid - asp - D
- cysteine - cys - C
- glutamine - gln - Q
- glutamic acid - glu - E
- glycine - gly - G
- histidine - his - H
- isoleucine - ile - I
- leucine - leu - L
- lysine - lys - K
- methionine - met - M
- phenylalanine - phe - F
- proline - pro - P
- serine - ser - S
- threonine - thr - T
- tryptophan - trp - W
- tyrosine - tyr - Y
- valine - val - V

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| Amino Acid | DNA Base Triplets | M-RNA Codons |
|---|---|---|
| alanine | CGA, CGG, CGT, CGC | GCU, GCC, GCA, GCG |
| arginine | GCA, GCG, GCT, GCC TCT, TCC | CGU, CGC, CGA, CGG AGA, AGG |
| asparagine | TTA, TTG | AAU, AAC |
| aspartate | CTA, CTG | GAU, GAC |
| cysteine | ACA, ACG | UGA, UGC |
| glutamate | CTT, CTC | GAA, GAG |
| glutamine | GTT, GTC | CAA, CAG |
| glycine | CCA, CCG, CCT, CCC | GGU, GGC, GGA, GGG |
| histidine | GTA, GTG | CAU, CAC |
| isoleucine | TAA, TAG, TAT | AUU, AUC, AUA |
| leucine | AAT, AAC, GAA, GAG GAT, GAC | UUA, UUG, CUU, CUC CUA, CUG |
| lysine | TTT, TTC | AAA, AAG |
| methionine | TAC | AUG |
| phenylalanine | AAA, AAG | UUU, UUC |
| proline | GGA, GGG, GGT, GGC | CCU, CCC, CCA, CCG |
| serine | AGA, AGG, AGT, AGC TCA, TCG | UCU, UCC, UCA, UCG AGU, AGC |
| stop | ATG, ATT, ACT | UAA, UAG, UGA |
| threonine | TGA, TGG, TGT, TGC | ACU, ACC, ACA, ACG |
| tryptophan | ACC | UGG |
| tyrosine | ATA, ATG | UAU, UAC |
| valine | CAA, CAG, CAT, CAC | GUU, GUC, GUA, GUG |

| DNA | Computer |
|---|---|
| Chromosome | Floppy Disk |
| Gene | File |
| Codon (3 bases) | Byte (8 bit character) |
| Base (A,T,C or G) | Bit (0 or 1) |
| Mutation | Corrupted File |

http://waynesword.palomar.edu/codons.htm

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# Summary

- **Hereditary information is encoded in the chemical language of DNA and reproduced in the cells of all living organisms.**
- **DNA is composed of a string of four basic nucleotides referred to as Adenine, Guanine, Cytosine, and Thymine.**
- **In all living cells, double-stranded DNA undergoes the process of 'transcription' to form single-stranded mRNA (messenger RNA).**
- **The mRNA is composed of a string of four basic nucleotides (Adenine, Guanine, Cytosine, and Uracil).**
- **mRNA's undergo the process of 'translation' by the ribosomes to form various proteins which then perform and enable the critical functions of life.**

- DNA - deoxyribonucleic acid (ACGT)
- RNA - ribonucleic acid (ACGU)
- Bases - nucleotides, AGTCU
- Proteins made of 20 amino acids
- RNA polymerase synthesizes the mRNA
- Ribosomes synthesize the proteins

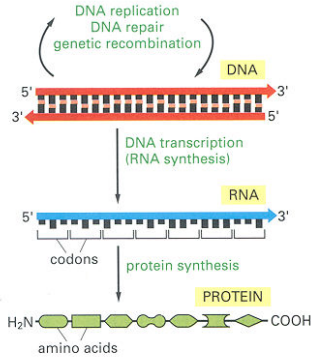


**Figure 6–1 The basic genetic processes.** The processes shown here are thought to occur in all present-day cells. Very early in the evolution of life, however, much simpler cells probably existed that lacked both DNA and proteins (see Figure 1–11). Note that a sequence of three nucleotides (a codon) in an RNA molecule codes for a specific amino acid in a protein.

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# Summary

- The nucleotide sequence of DNA and its expression in various cells is of utmost importance to life scientists because every disease state or biological function could be traced back to a single or a group of genes (DNA sequences).
- Determination of signaling pathways of proteins is vital to understanding the functions of cells
- Information in DNA is static, transcription and translation processes are dynamic
- Genomics and proteomics have wide applications in biotechnology, medicine, agriculture, biology, etc.



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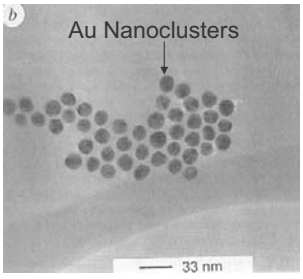
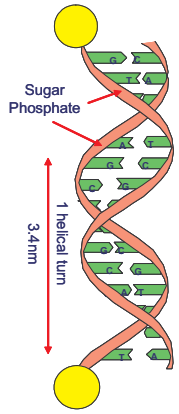
# Bio-link 1: DNA

- **A DNA strand is specific to its complement ⇒ Use DNA as an "address" label and attachment system to assemble objects**
- **DNA can be attached to gold-coated objects via thiol (SH)**
  - **SH forms metal thiolate bond**

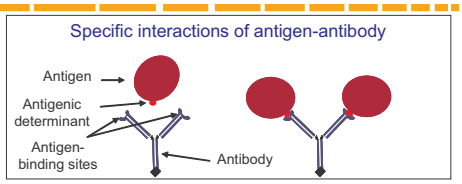


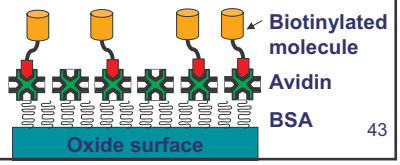

C. A. Mirkin, R. L. Letsinger, R. C. Mucic, and J. J. Storoff, "A DNA-based Method for Rationally Assembling Nanoparticles into Macroscopic Materials", Nature, Vol. 382, 15th August, 1996.
A. P. Alivisatos, K. P. Johnsson, X. Peng, T. E. Wilson, C. J. Loweth, M. P. Bruchez, and P. G. Schultz, "Organization of Nanocrystal Molecules Using DNA", Nature, Vol. 382, 15th August, 1996.

# Bio-link 2: Protein Complex

- **Antigen/Antibodies**
  - **Complicated folded structures**
  - **Binding through hydrophobic, H bonds, ionic, van der Waals**
- **Ligand/Receptors**
  - **Avidin/Biotin**
    - **Commonly used in assays**
    - **Strong affinity ($K_a$=$10^{15}$ $M^{-1}$)**
- **Attachment to surfaces is more challenging**
  - **e.g. BSA/avidin complex**
  - **Avidin maintains its activity when adsorbed on oxide through BSA**
  - **Covalent linkage on oxide through Silanes**




**Structure of one sub-unit of Avidin**




http://www.rcsb.org/pdb/
http://step.sdsc.edu/projects95/Protein.lesson/avidin-biotin.html

R. Bashir, R. Gomez et al. , "Adsorption of Avidin on Micro-Fabricated Surfaces for Protein Biochip Applications", Biotechnology and Bioengineering, Volume 73, Issue 4, May 2001, pp. 324-328.

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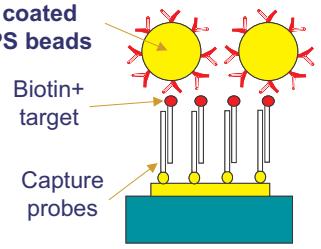
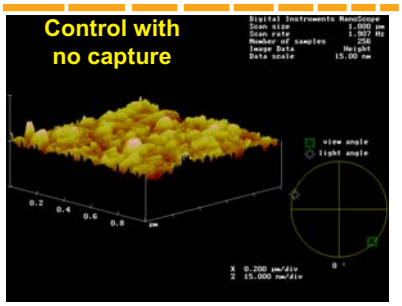
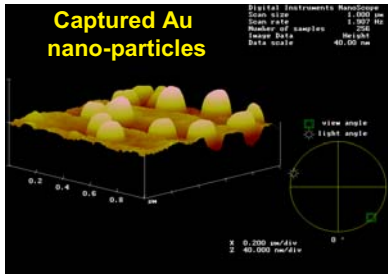
# Basis for Genomic Detection

**Avidin coated PS beads**




- Thiolated DNA 1
- DNA 2 + Biotin
- Avidin coated PS beads

→ **bead capture on the Au pads**

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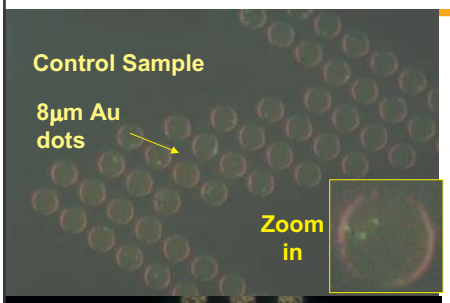
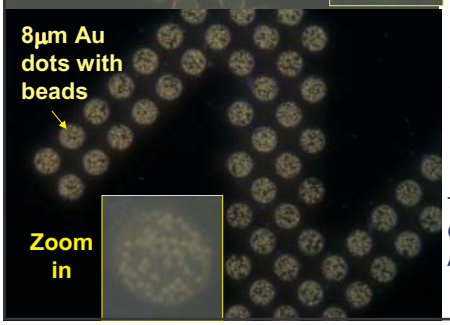
# DNA Capture Probes on Au Surface



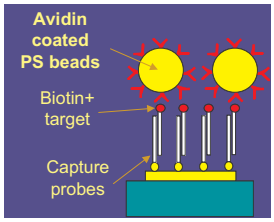




Controls:

1) Non-thiolated attachment w/ hybridization
2) Thiolated Attachment w/ non-complimentary hybridization

Avidin coated PS beads

→ **No bead capture**




Thiolated attachment
Complimentary hybridization w/ biotin
Avidin coated PS beads

→ **bead capture on patterned Au** 45