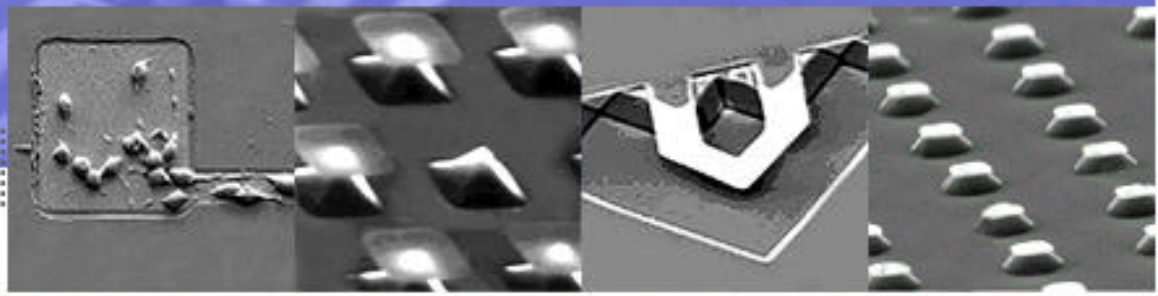
PURDUE UNIVERSITY

LIBNA

LIBNA is focused on research in BioMEMS & Bionanotechnology, in the areas of interface between micro, nanoengineering & life sciences



Introduction to BioMEMS & Bionanotechnology
Lecture 2

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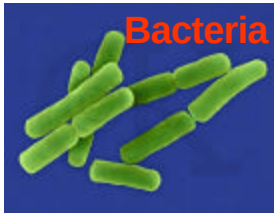
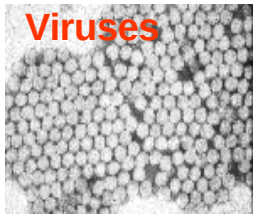
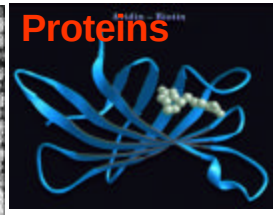
http://engineering.purdue.edu/LIBNA

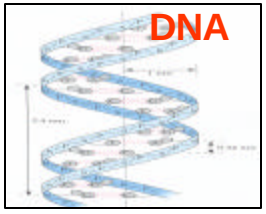
Key Topics

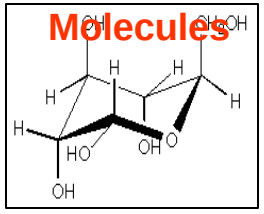
- **Biochips/Biosensors and Device Fabrication**
- **Cells, DNA, Proteins**
- **Micro-fluidics**
- **Biochip Sensors & Detection Methods**
- **Micro-arrays**
- **Lab-on-a-chip Devices**



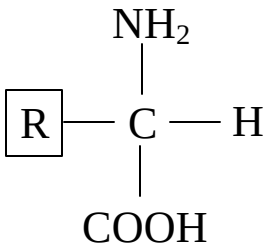


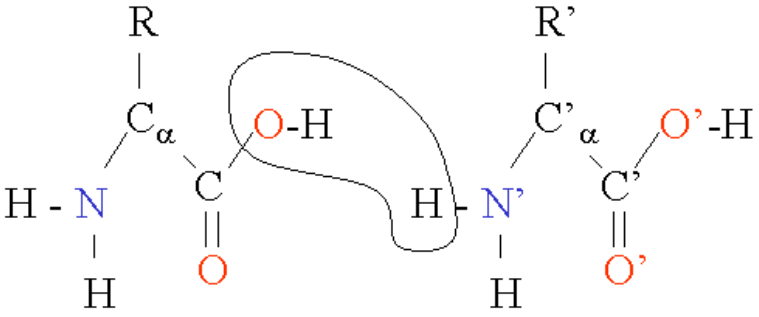


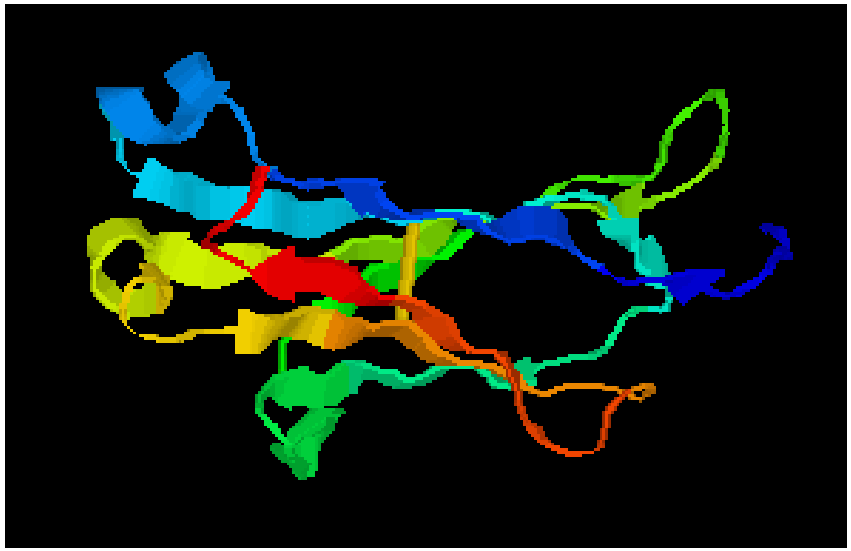


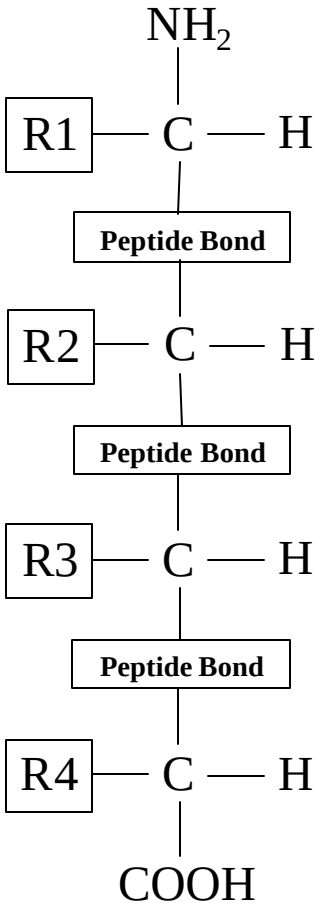


Protein Structure









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

http://www.umass.edu/microbio/chime/antibody/

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

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

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

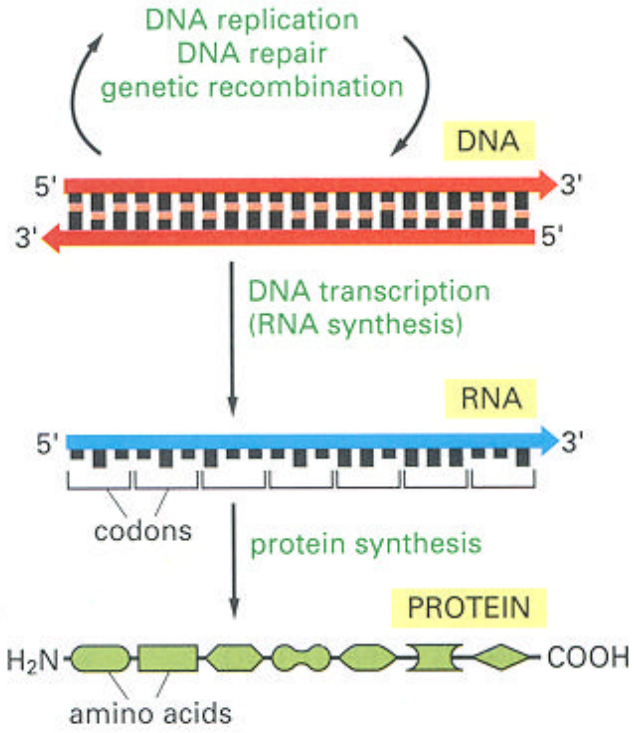


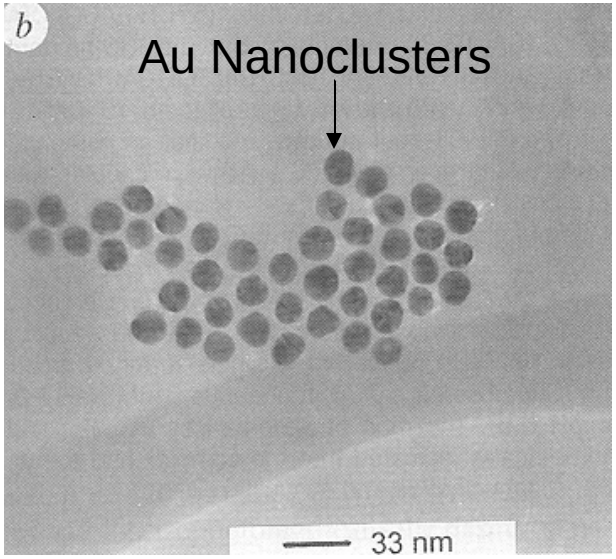
Figure 6–1 The basic genetic processes. The processes shown here are thought to occur in all present-day cells. Very early in the evolution of life, however, much simpler cells probably existed that lacked both DNA and proteins (see Figure 1–11). Note that a sequence of three nucleotides (a codon) in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

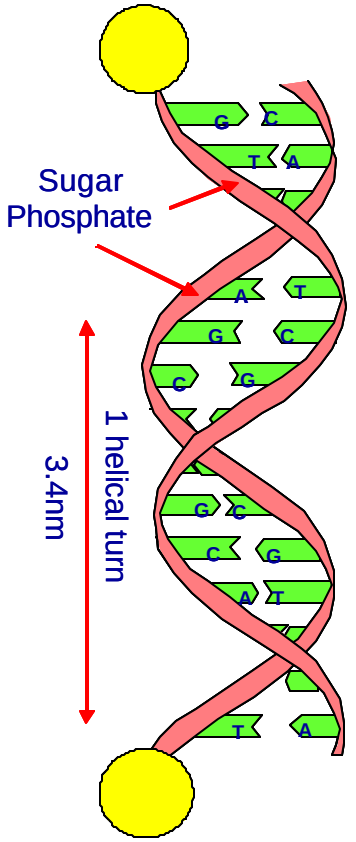
Summary

- 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.

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**

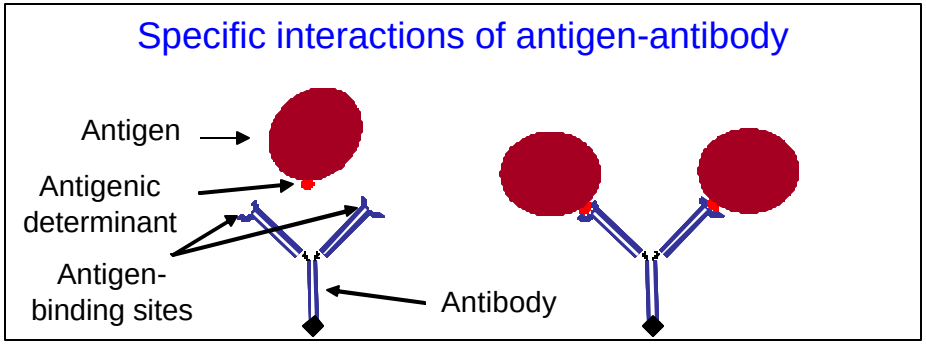




C. A. Mirkin, R. L. Letsinger, R. C. Mucic, and J. J. Storoff, "A DNA-based Method for Rationally Assembling Nanoparticles into Macroscopic Materials", Nature, Vol. 382, 15th August, 1996.
A. P. Alivisatos, K. P. Johnsson, X. Peng, T. E. Wilson, C. J. Loweth, M. P. Bruchez, and P. G. Schultz, "Organization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

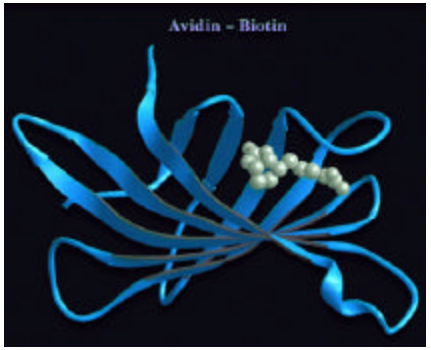
Bio-link 2: Protein Complex

- **Antigen/Antibodies**
  - **Complicated folded structures**
  - **Binding through hydrophobic, H bonds, ionic, van der Waals**

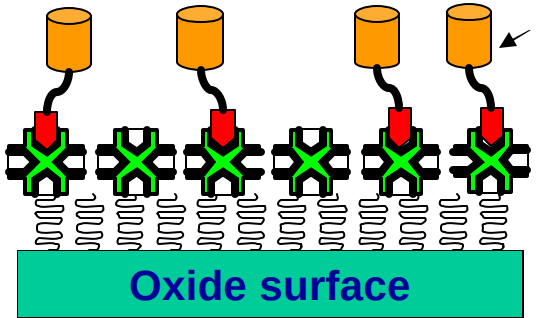


- **Ligand/Receptors**
  - **Avidin/Biotin**
    - **Commonly used in assays**
    - **Strong affinity (K_a=10^{15} M^{-1})**

Structure of one sub-unit of Avidin



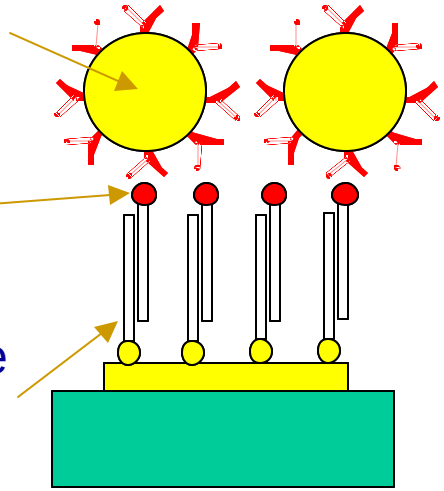
- **Attachment to surfaces is more challenging**
  - **e.g. BSA/avidin complex**
  - **Avidin maintains its activity when adsorbed on oxide through BSA**
  - **Covalent linkage on oxide through Silanes**

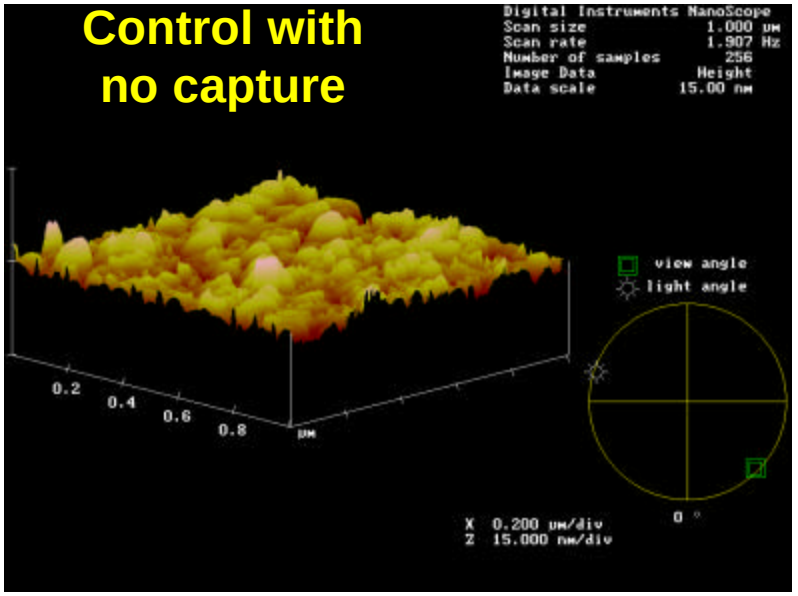


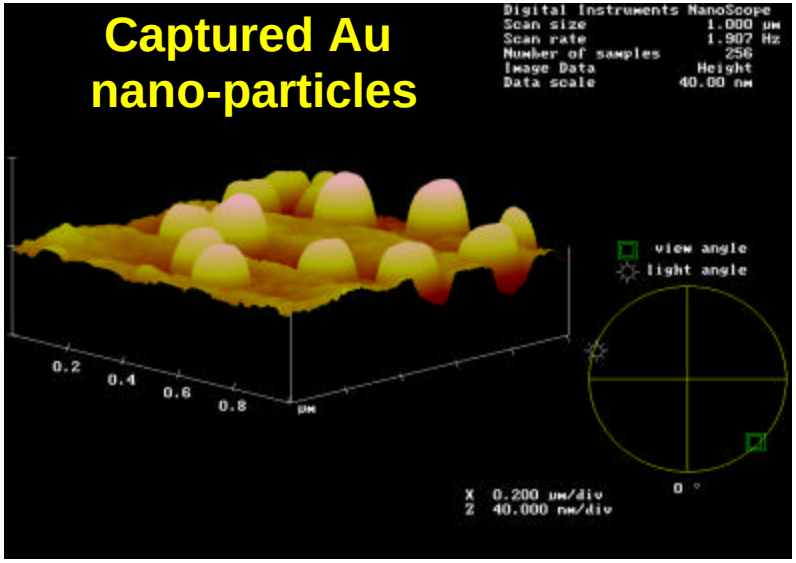
http://www.rcsb.org/pdb/
http://step.sdsc.edu/projects95/Protein.lesson/avidin-biotin.html

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.

Basis for Genomic Detection



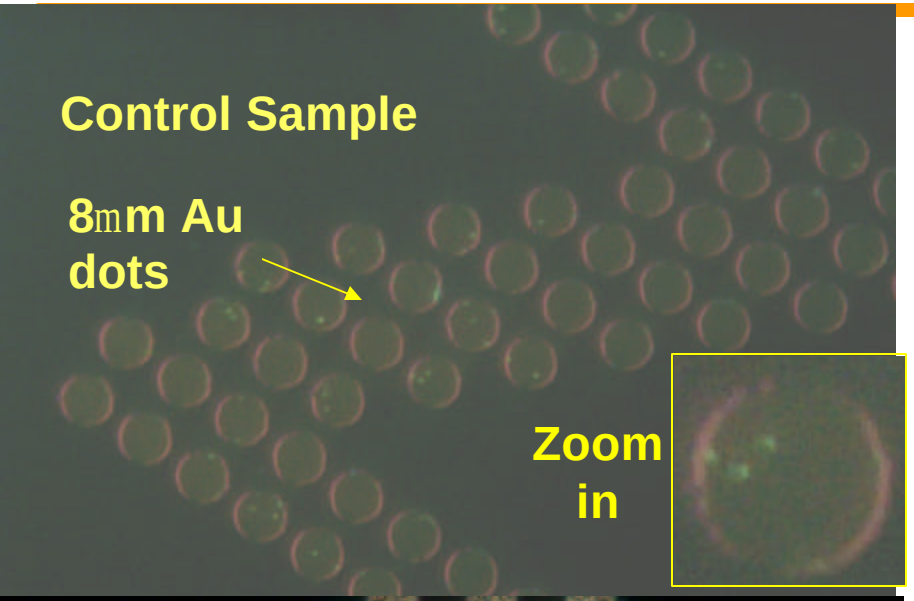


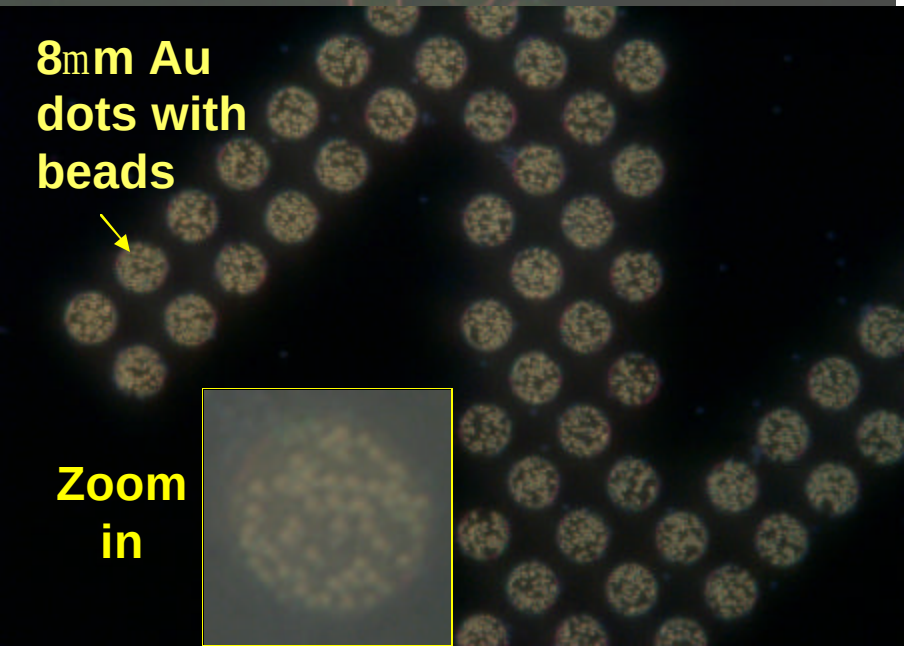


- Thiolated DNA 1
- DNA 2 + Biotin
- Avidin coated PS beads

→ **bead capture on the Au pads**

DNA Capture Probes on Au Surface



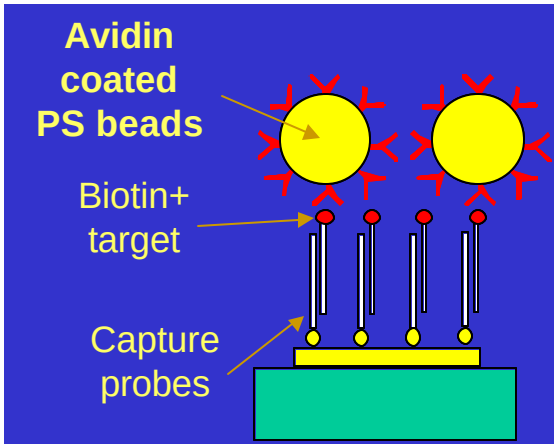


Controls:

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

Avidin coated PS beads

→ **No bead capture**



Thiolated attachment

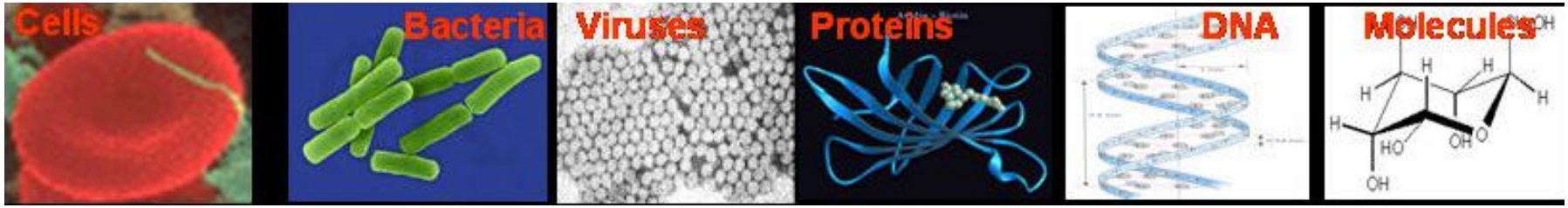
Complimentary hybridization w/ biotin

Avidin coated PS beads

→ **bead capture on patterned Au**

Key Topics

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



Continuous Fluid Flows

Navier Stokes Equation (dimensional form)

$$r\frac{DV}{Dt} = r\frac{\partial \vec{V}}{\partial t} + r\left(\vec{V}\bullet\nabla\right)\vec{V} = r\,\vec{g} - \nabla p + m\nabla^2 V$$

Scale equation:

$$V = uV';\ \vec{x} = Lx';\ p = \frac{mu}{L}p';\ t = \frac{L}{u}t'$$

$$\mathrm{Re}\frac{D\vec{V}}{Dt} = \mathrm{Re}\left(\frac{\partial V}{\partial t} + \left(V\bullet\nabla\right)\vec{V}\right) = \mathrm{Re}\cdot Fr^{-2}\frac{\vec{g}}{|\vec{g}|} - \nabla p + \nabla^2 V$$

$$\text{where } \mathrm{Re} = \frac{r\,uL}{m},\ Fr^{-2} = \frac{gL}{u^2}$$

Wereley, et al. Purdue

Dimensionless Parameters

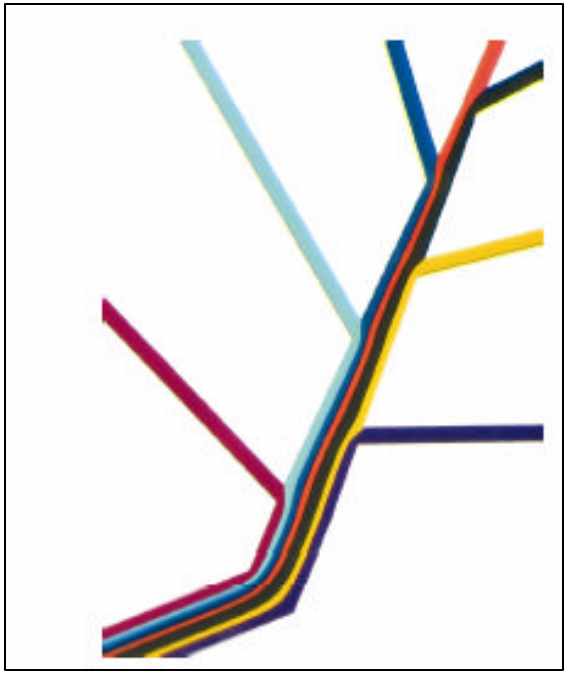
- Assume water flow;

  $\mu=10^{-3}$ kg/(s-m), $\rho=10^{3}$ kg/m^3
- Length ~ 10 μm=10^{-5} m
- Velocity ~ 1 mm/s=10^{-3}m/s
- Then: $Re=10^{-2}$, $Fr^{-2}=100$,
- N-S equation becomes Poisson Eqn

$$0 = -\nabla p + \nabla^2 V$$

Re in BioChips and Laminar Flow

- **Reynolds number, Re= $LV_{avg}\,r\,/\,m$**
- **Re=inertial forces/viscous forces implies inertia relatively important**
  - ***L* is the most relevant length scale,**
  - **μ is the viscosity, r is the fluid density,**
  - **V_{avg} is the average velocity of the flow.**
- **Reduced Re**
  - **Higher μ (molasses)**
  - **Reduce flow rate (traffic in NY!)**
  - **Reduce L (i.e. micro devices)**

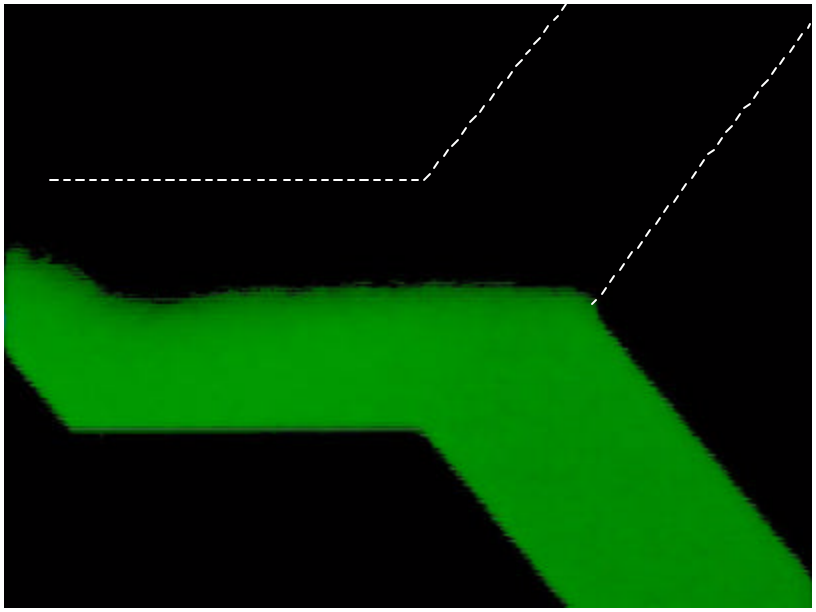


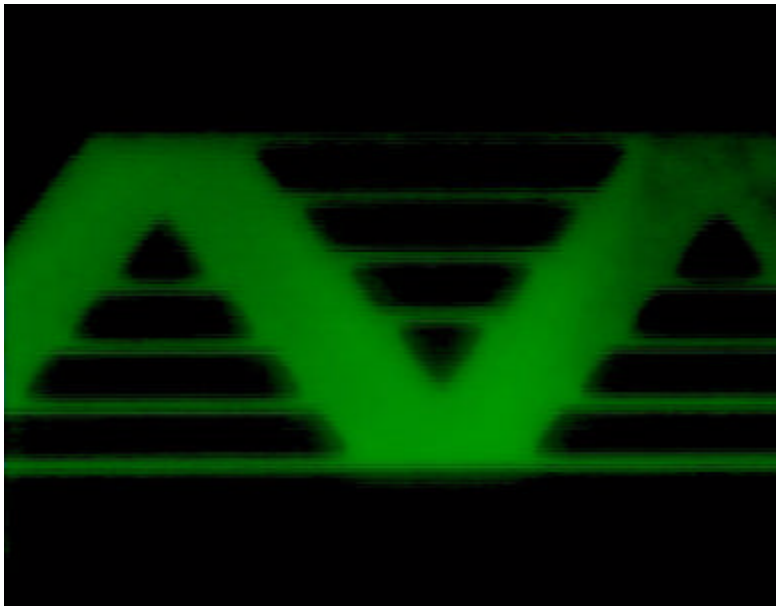
Whitesides et al., (Harvard)

- **Re is usually much less than 100, often less than 1.0 in micro devices**
- **Flow is completely laminar and no turbulence occurs.**

Microfluidic Mixing

- Mixing only by diffusion (or novel structures using hydrodynamics)

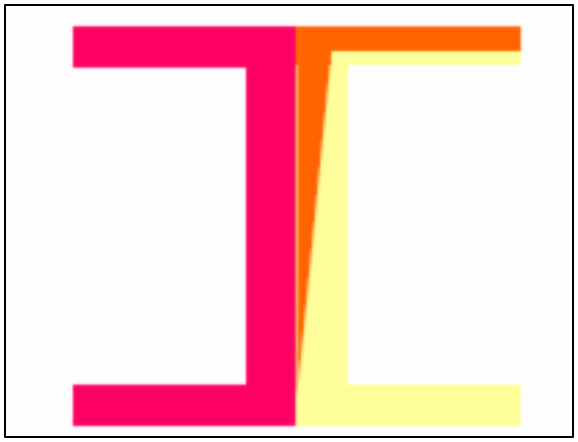




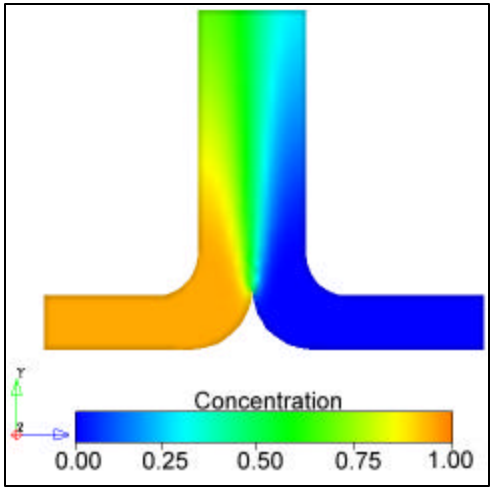
Regnier, et al. Purdue

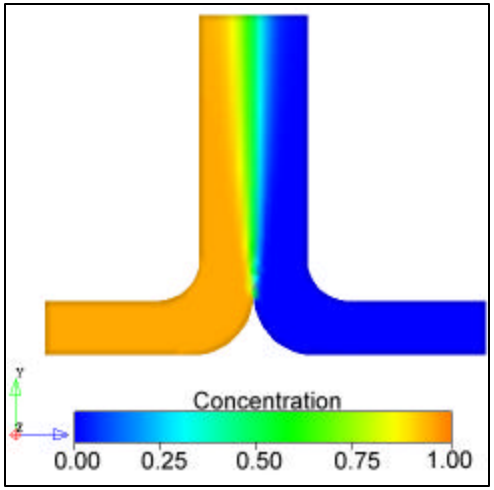
Particle Separation

- Particle separation/filter in micro-fluidic devices - without a membrane
- Smaller particles will diffuse farther and will get separated from the flow
- Diffusion distance: $x^2 = 2Dt$ $\quad D = \frac{k_b T}{6\pi\eta a}$
  - biotin (D ~ 350 μm²/s)
  - albumin (D ~ 65 μm²/s)



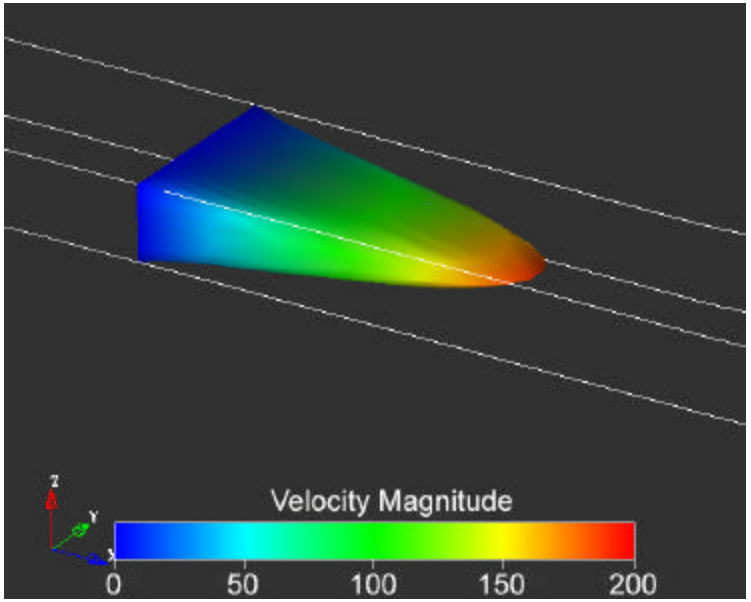
Yager (U. Washington)





Microfluidic Flow

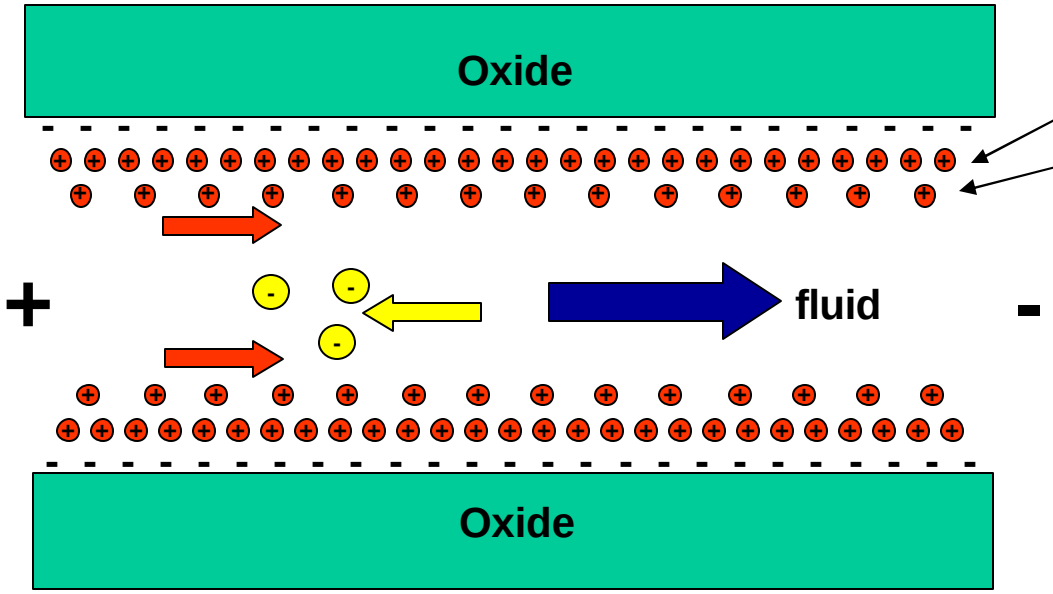
- Pressure driven flow
  - Parabolic profile
  - No-slip boundary condition (Velocity at interface is zero)

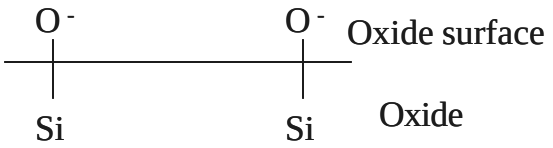


Yager, et al. U. Washington

- Electrokinetic flow
  1. Electroosmosis (EOF)
  2. Electrophoresis (EP)
  3. Dielectrophoresis (DEP)

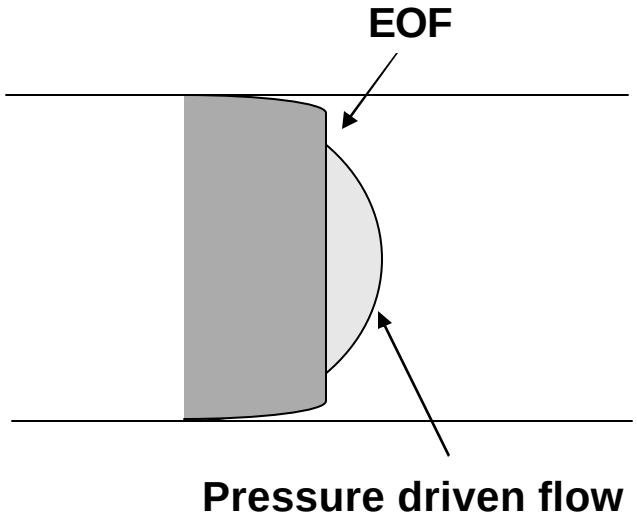
Electroosmotic Flow



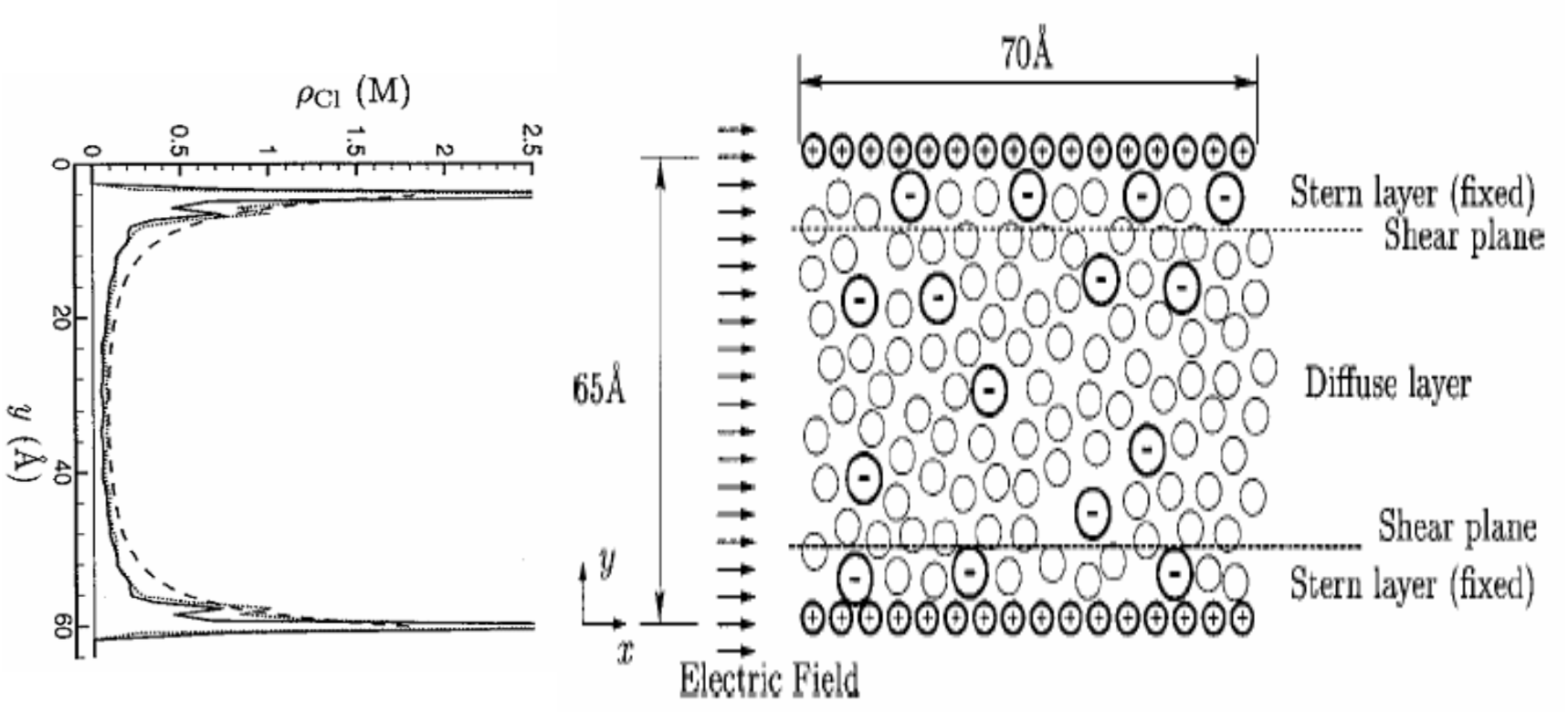


Deprotonation of silanol groups occurs when the pH value of aqueous solution above PZC.

- $Q_{EOF} = \varepsilon E \zeta A/\eta$
  ζ = zeta potential, η = viscosity
- Charges at interface
- Counter ion accumulation at interface
- Results in plug flow
- Electrophoresis also takes place

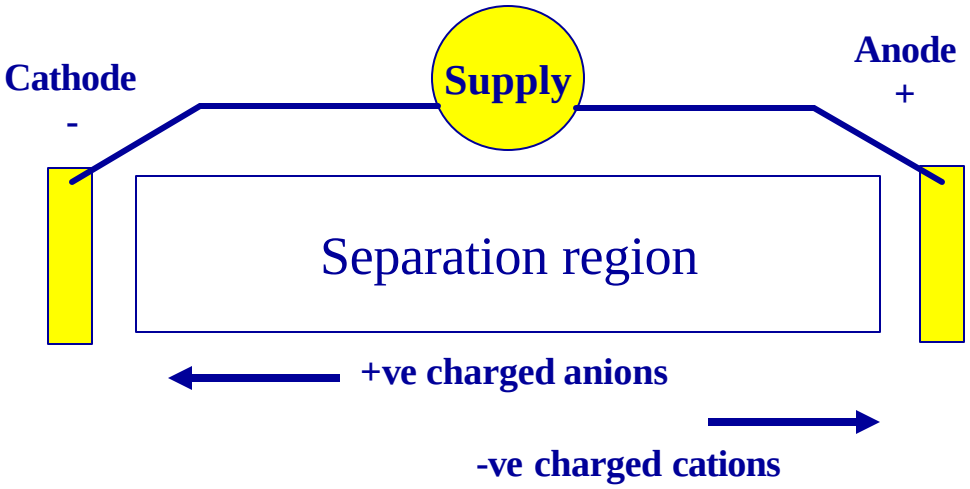


Electroosmotic Flow in Nano-channels



Surface was assumed positively charged. Concentration of Cl ions in bulk is 0.01 M. Concentrations near surface and at middle of channel are 3.21 M and 0.2 M, respectively. ———simulation with uniformly charged wall atoms; ------ simulation with discrete wall atom charges. From *Freund 2002*.

Electrophoresis



- Electrophoresis: charged species drift when placed under an electric field
- $v = -m\, dV/dx$
  - v - electrophoretic velocity
  - μ - electrophoretic mobility
  - dV/dx = applied electric field

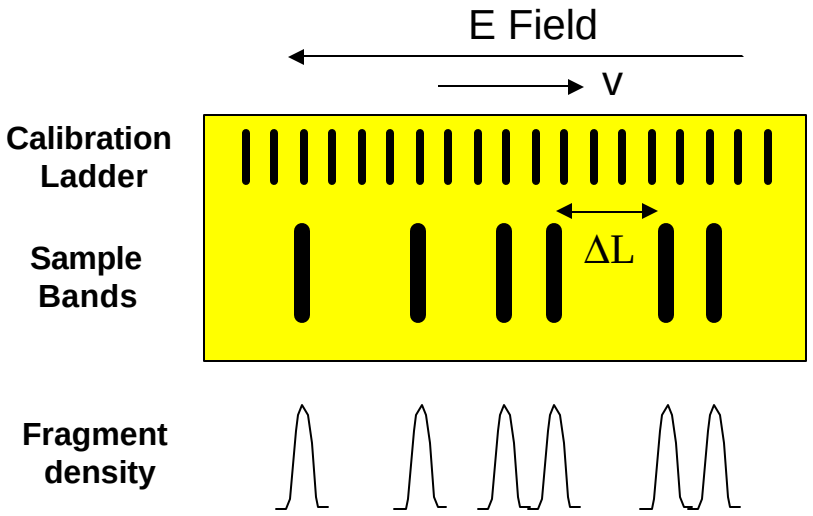
DNA Gel Electrophoresis

- DNA has phosphate backbone which is negatively charged - hence DNA drifts in an E-field
- The charge/mass (e/m) ratio is constant hence electrophoretic mobility is independent of size in liquid medium.
- Thus, another sieving medium is needed where separation can take place due to difference in length.
- The separation region is filled with a gel - sieving matrix with pores through which the DNA molecules can traverse.
- The field stretches the molecules and they move in a snake-like fashion through the pores of the gel.

  μ in gels is inversely proportional to log of fragment size (sieving effect)
- Polyacrylamide gel is used to separate DNA molecules of 10-500 bases - pores are small
- Agarose gel is used to separate larger molecules (300-10,000 base pairs)

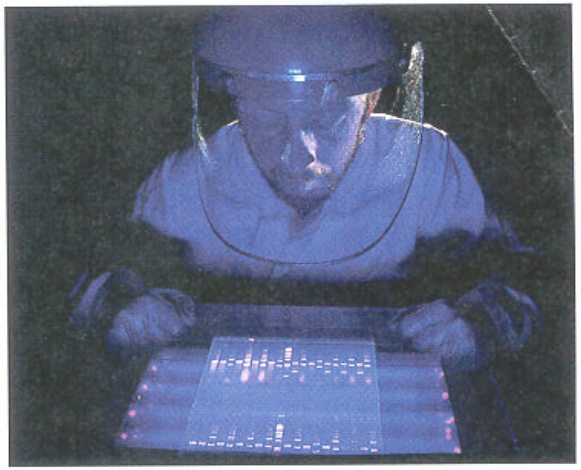
DNA Electrophoresis

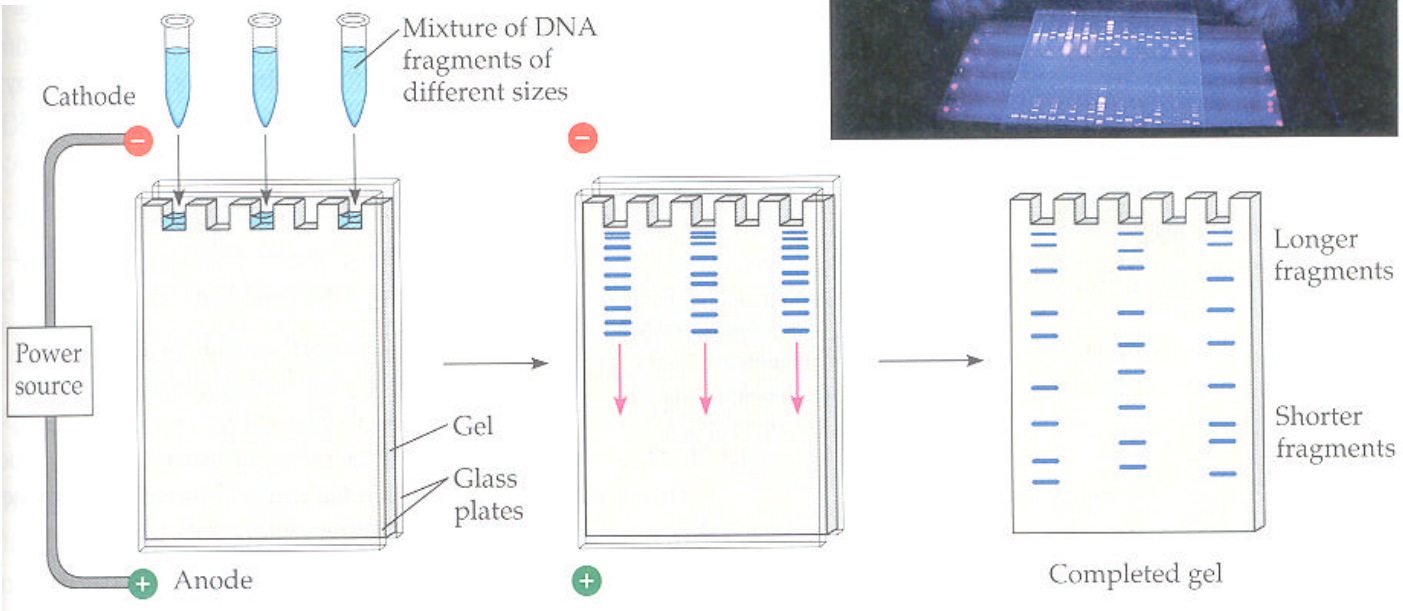
- Separation $\Delta L = \Delta\mu\ E\ t$
- Resolution of separation is measured by planes N,
  - N = (# of distinguishable bands within the length of the gel)^2
  - $N = \mu V/2D$
    - D is the diffusion coefficient

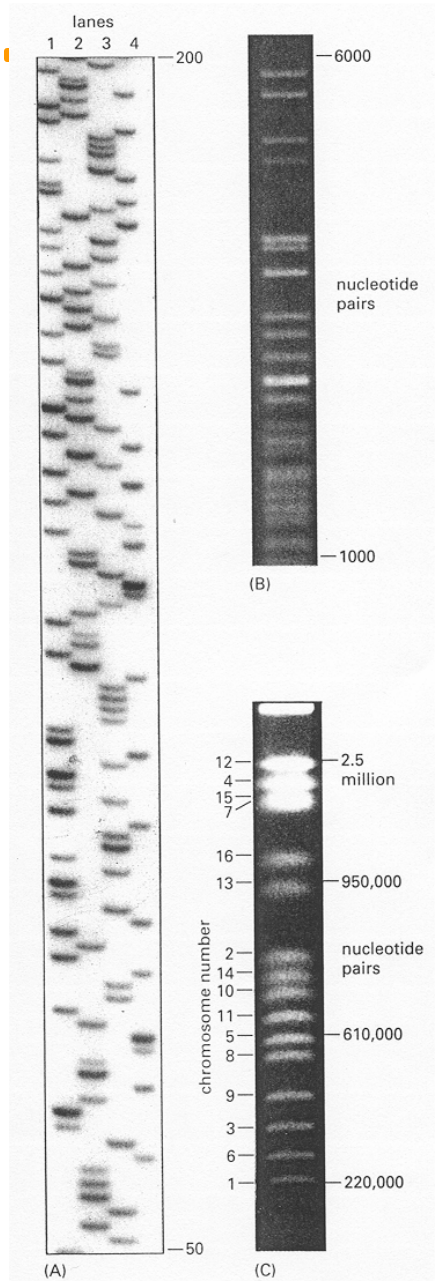


- Higher voltages increase resolution but Joule heating is an issue and needs to be considered
- Separation can also be done in capillaries since higher fields can be used (higher velocities and shorter times)

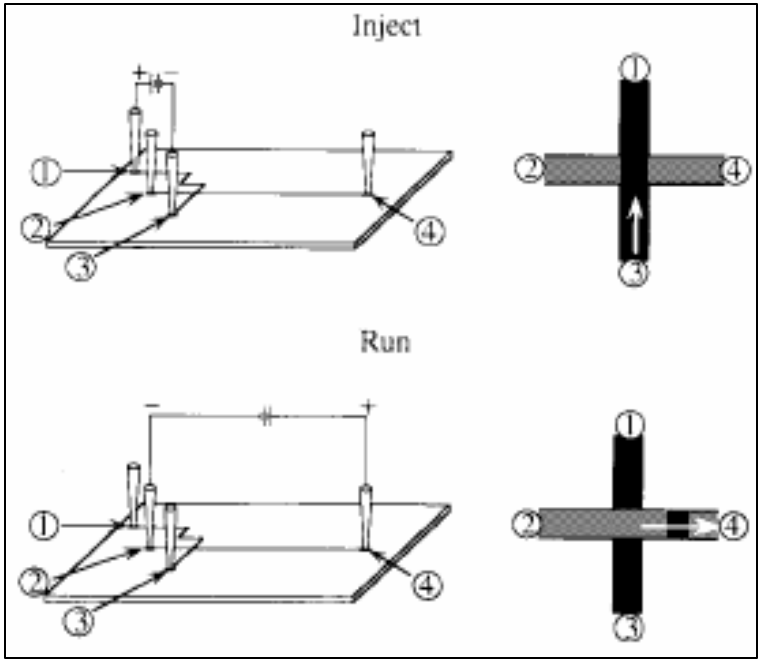
DNA Electrophoresis







DNA Electrophoresis in a Chip



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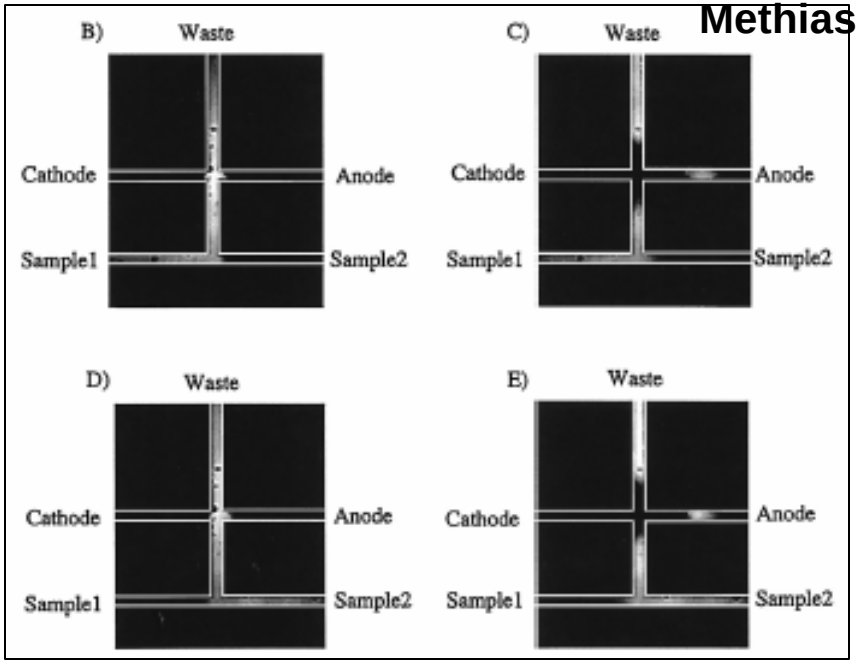

- Small sample size
- Higher fields, higher velocities
- Faster results

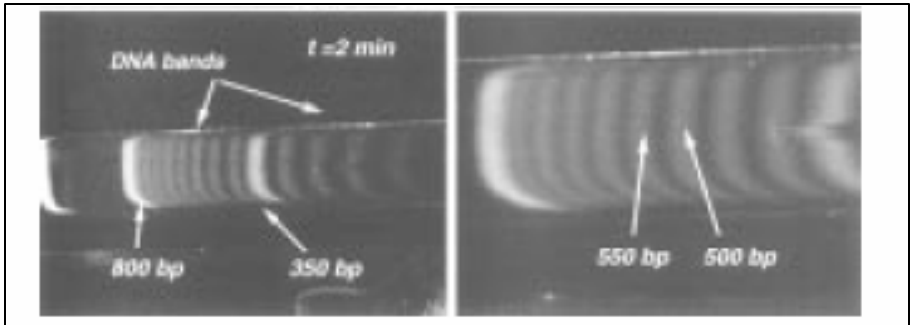

Fig. 22. Injection and separation of DNA fragments on integrated device. The channel is 500 $\times 50 \mu m^2$ (50 bp ladder, 0.13 $\mu g/\mu L$, SYBR Green, 8 V/cm, 10%T : 2.6%C polyacrylamide) [136].

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