

Glass Surfaces and Coatings for Biotechnology

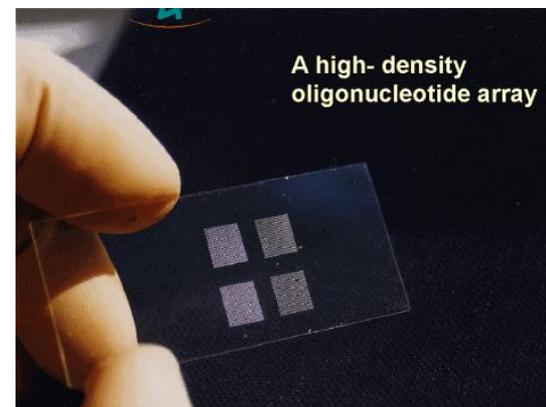
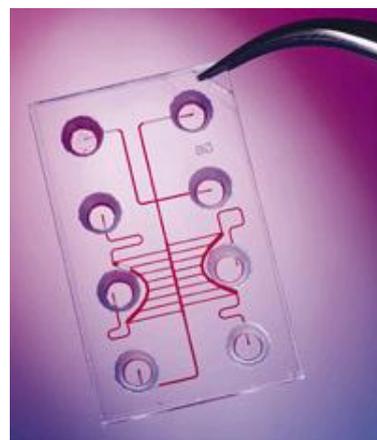
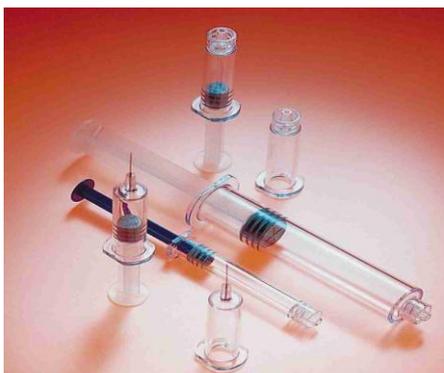
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PENNSTATE



IMI – NFG Winter School,
January 2008, Kyoto, Japan

Materials Research Institute

Center for Glass Surfaces, Interfaces and Coatings

Biomaterials and Bionanotechnology

lecture outline:

- relevant characteristics and properties of glass surfaces and coatings>>> **introduction**
- surface charge on flat glass substrates>>> **substrates for cell transfer assays**
- silane and hybrid sol/gel coatings>>> **DNA and other microarrays**
- carbon-doped “oxycarbide” glass>>> **blood contact materials**
- nanostructured coatings>>> **engineered surfaces for biology, biomedicine and biotechnology**

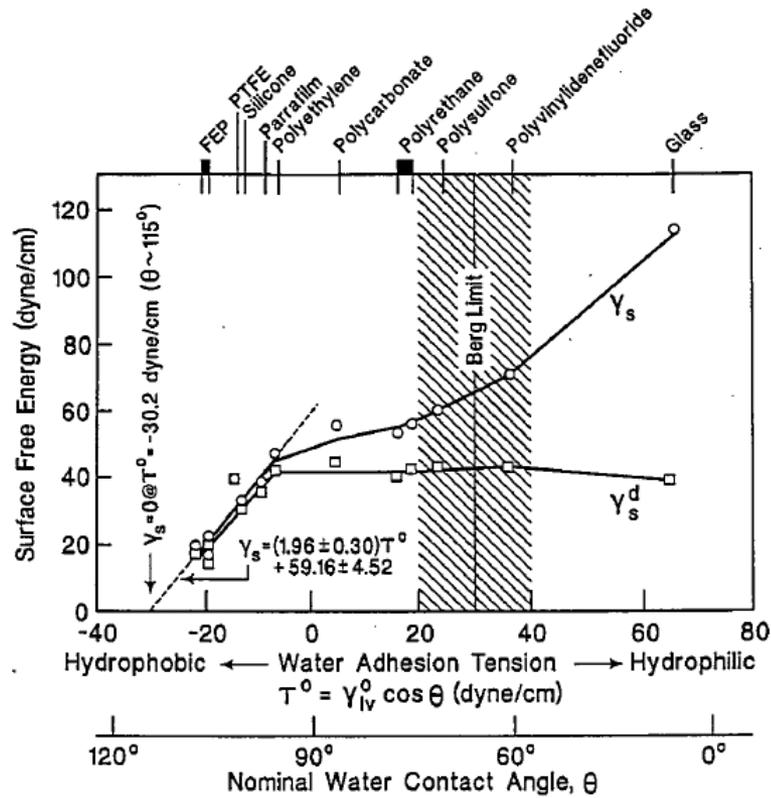
discussion and other applications:

- surfaces for pharmaceutical packaging
- superhydrophobic/superhydrophilic surfaces
- bio-active glasses and toxicity

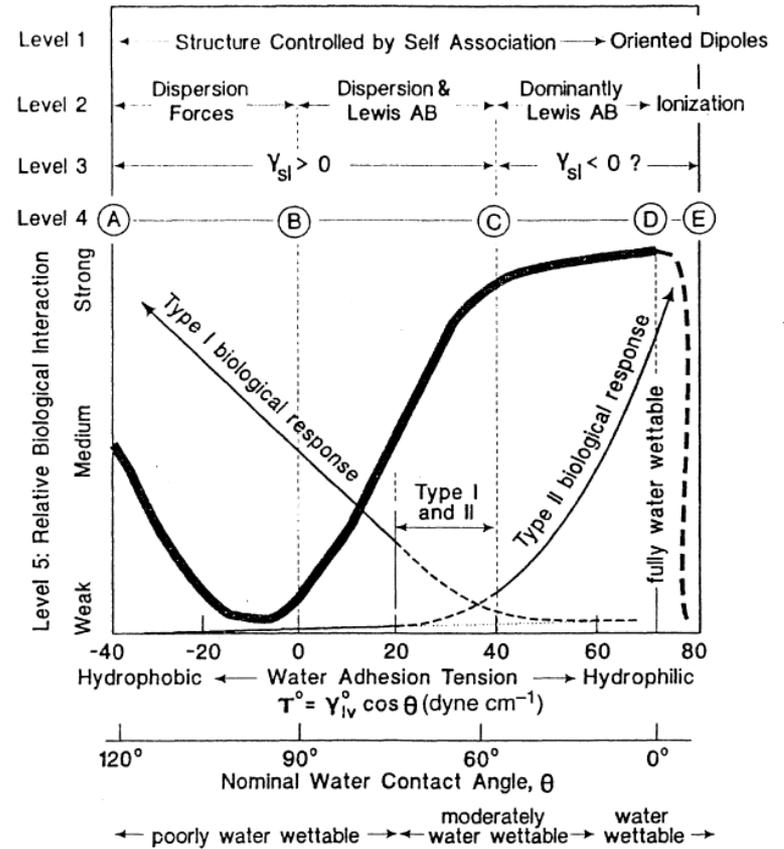
Biomaterials and Bionanotechnology

Characteristics and Properties of Glass Surfaces and Coatings

- composition
- chemical functionality
- contact angle/wettability
- surface charge and other surface forces
- porosity/roughness/specific surface
- cleanliness and chemical durability
- uniformity of ALL the above



Water in Biomaterials Surface Science



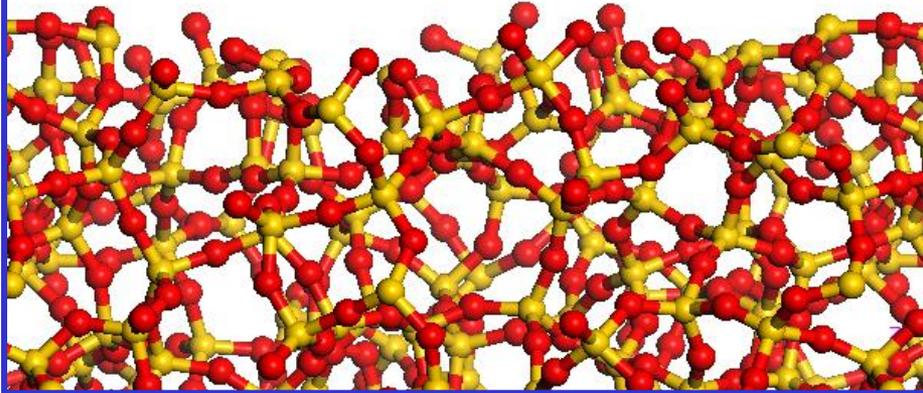
Biomaterials and Bionanotechnology

Methods of Characterization

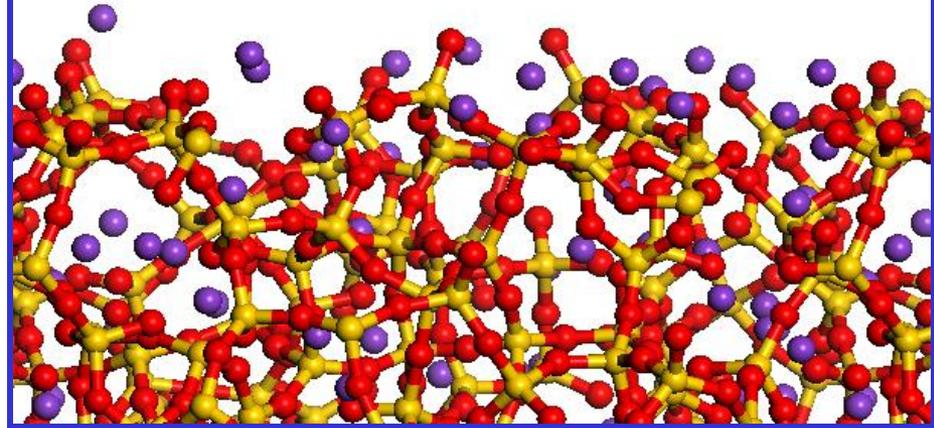
- surface composition (XPS)
- depth profiling (SIMS)
- surface roughness (AFM)
- organic adsorbates (FTIR/Raman)
- chemical structure (NMR)
- ellipsometry
- surface charge (streaming potential)
- contact angle tensiometry
- adhesion (CFM)

Glass Surface Structure Models

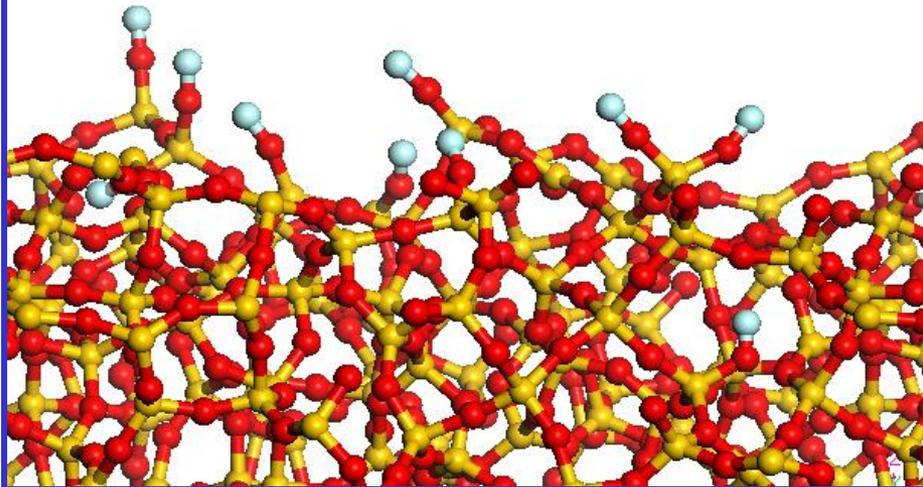
clean silica surface



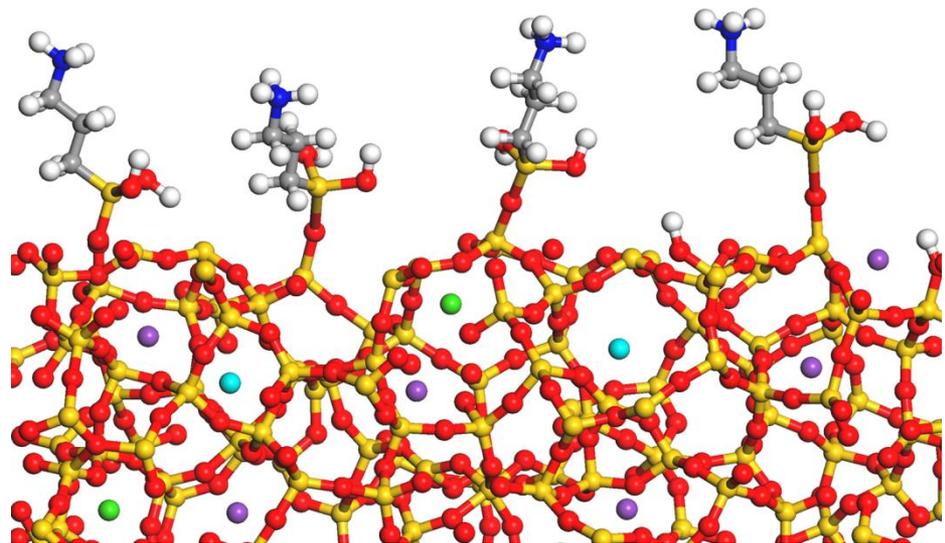
clean multi-component surface



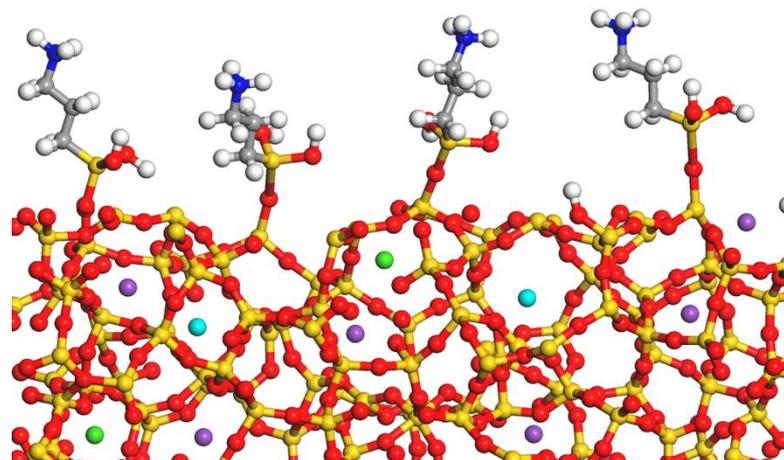
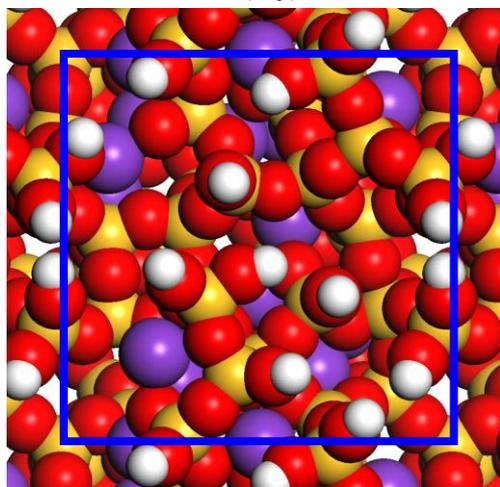
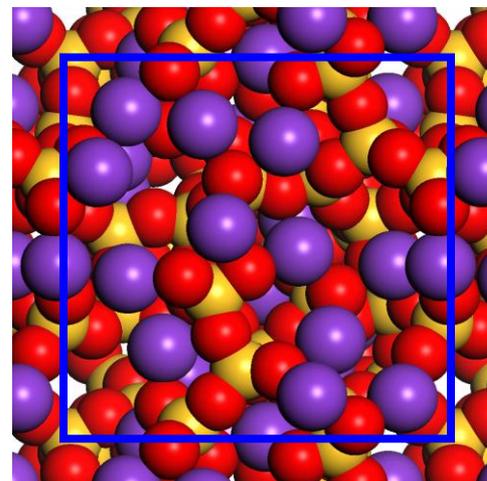
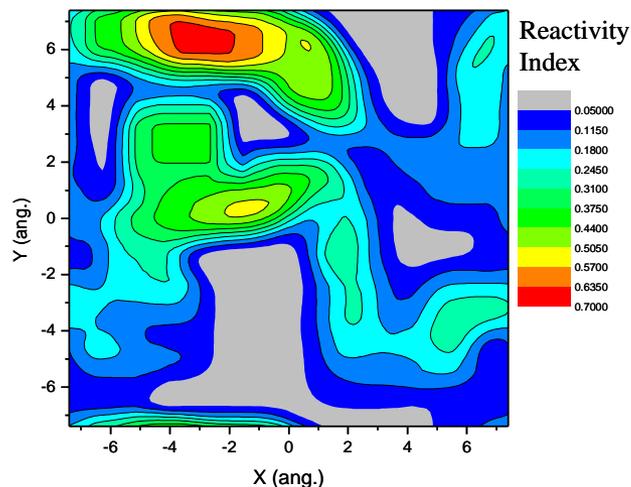
hydroxylated silica surface



functionalized multi-component surface



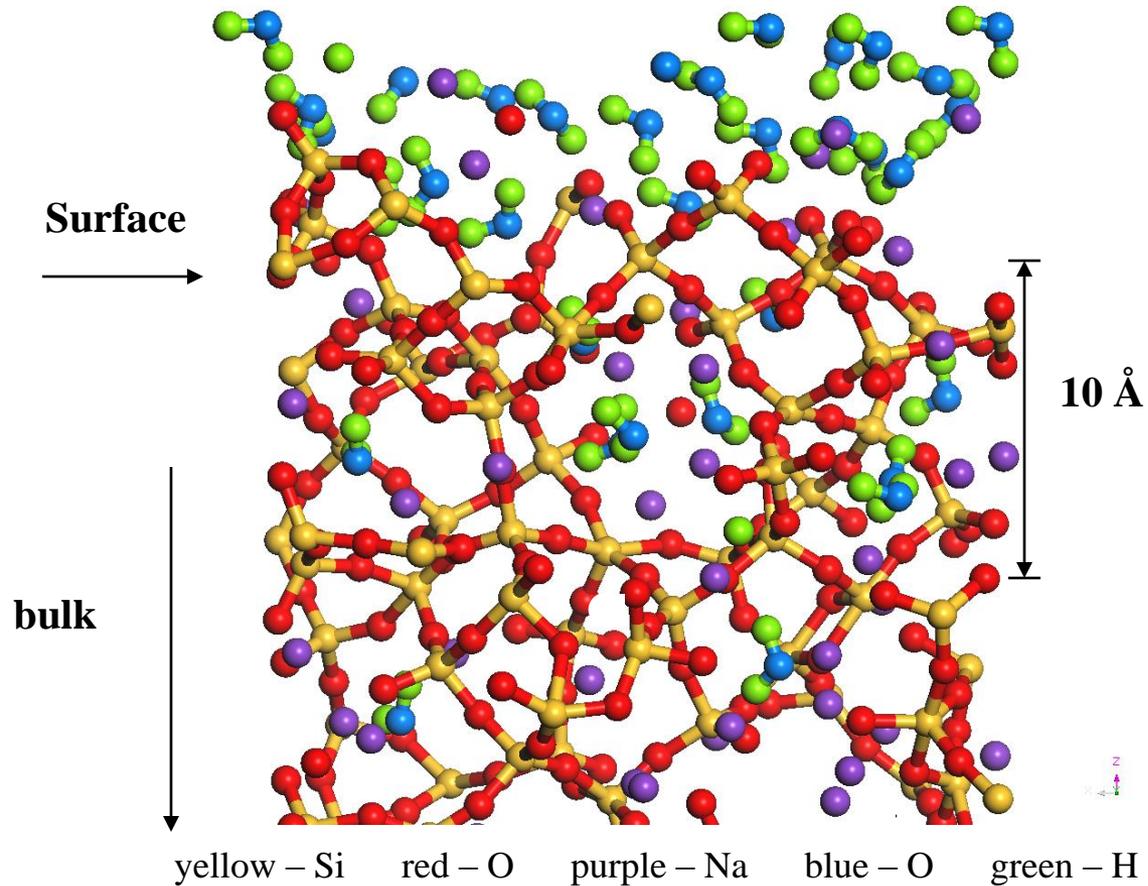
Computer simulation of glass surfaces: their atomic/nanoscale heterogeneity, hydroxylation and organo-functionalization



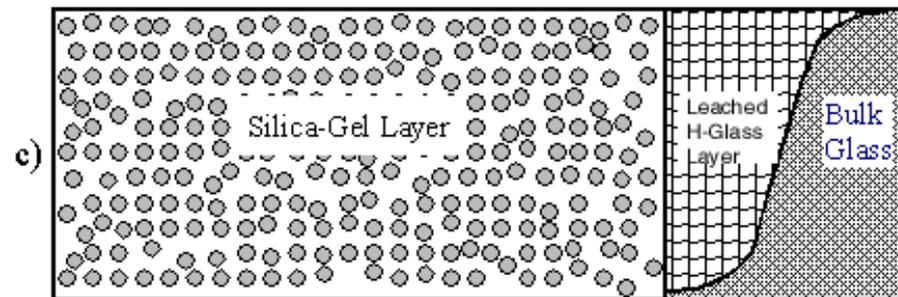
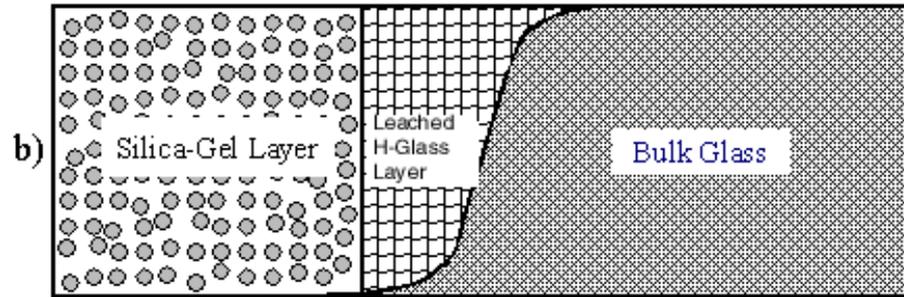
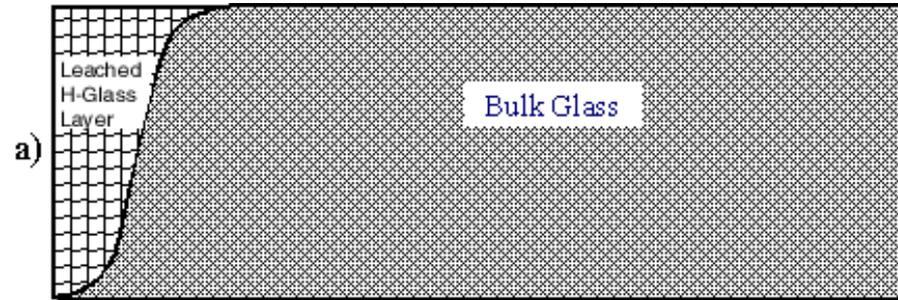
Water Molecules Adsorbing on a Simulated Sodium Silicate Glass Surface

Molecular Modeling of Water Interactions with Silica and Silicate Surfaces

Elam A. Leed and Carlo G. Pantano



Leaching and surface layer formation:



**Cubic Cell (22 Å)³
800 atoms**

↓ “relaxed” from 8000 K to 300 K
(in 500 ps)
↓ “relaxed” at 300 K (100 ps)

**1 Bulk Glass
Structure**

↓ Removal of above periodic
boundary condition.....
↓ “relaxed” at 300 K (200 ps)

**Glass
“Surface” Structure**

↓ Removal of: aluminum,
calcium, and sodium

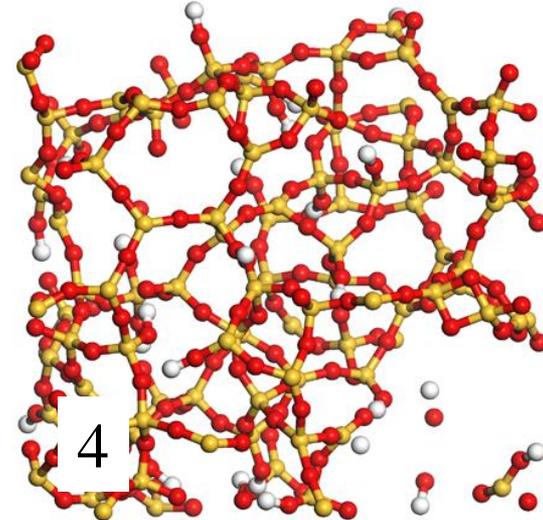
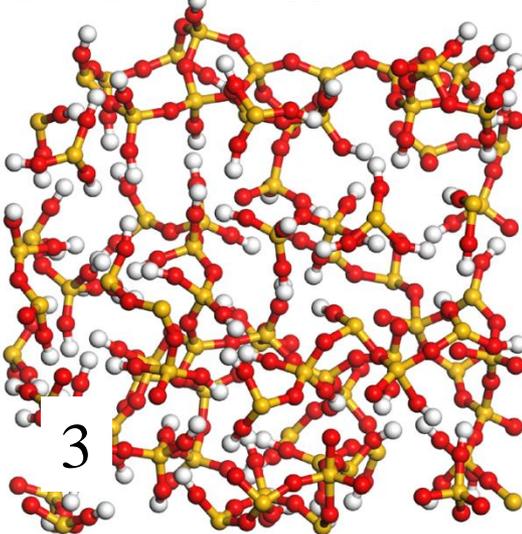
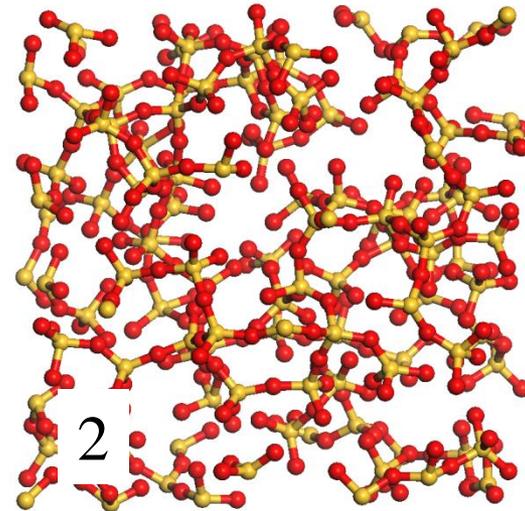
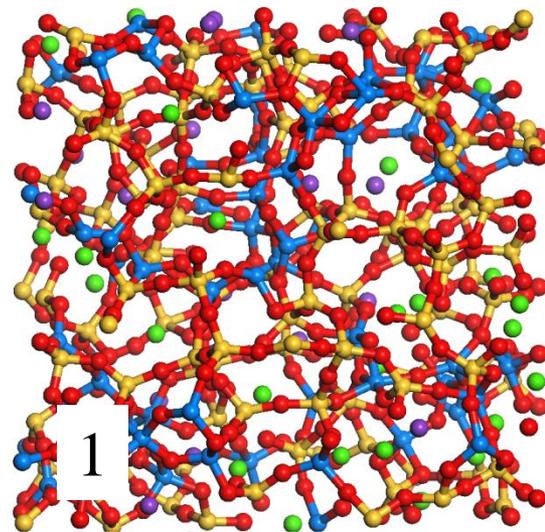
**2 Simulated Surface
Layer Structure**

→ Hydroxylation
(charge neut.)

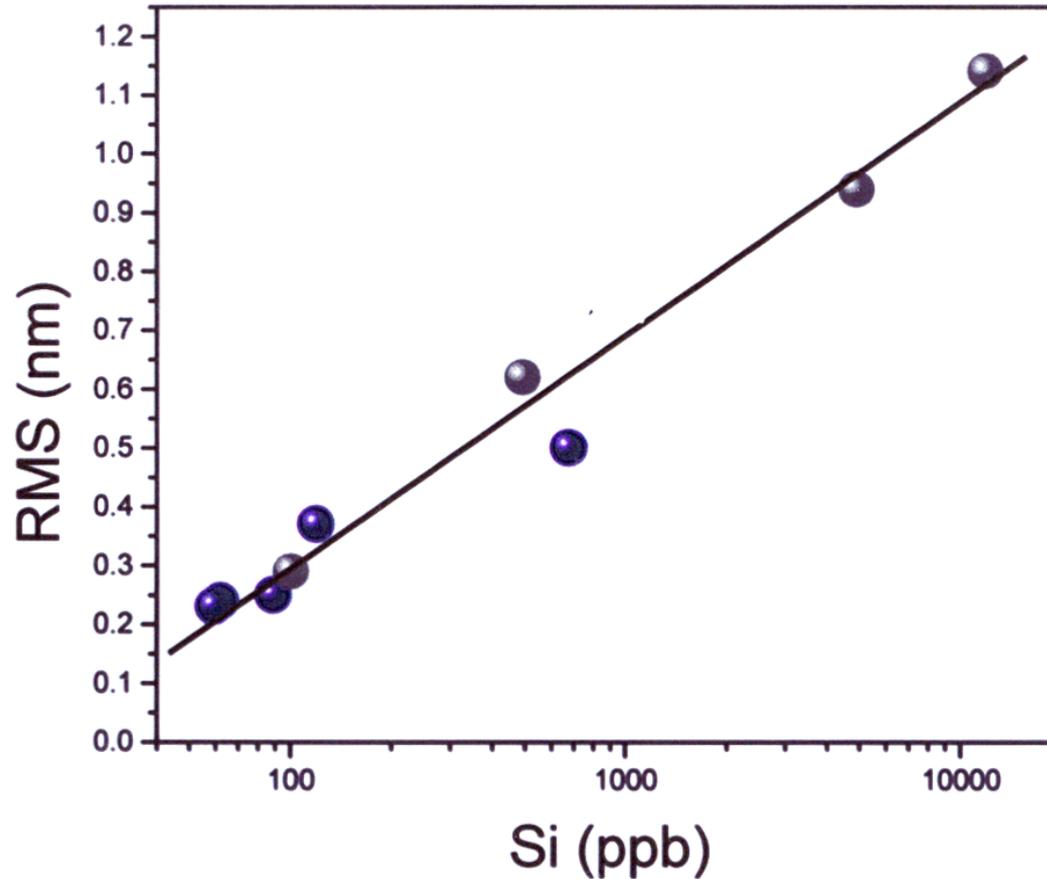
**3 Hydroxylated
Leached Glass Structure**

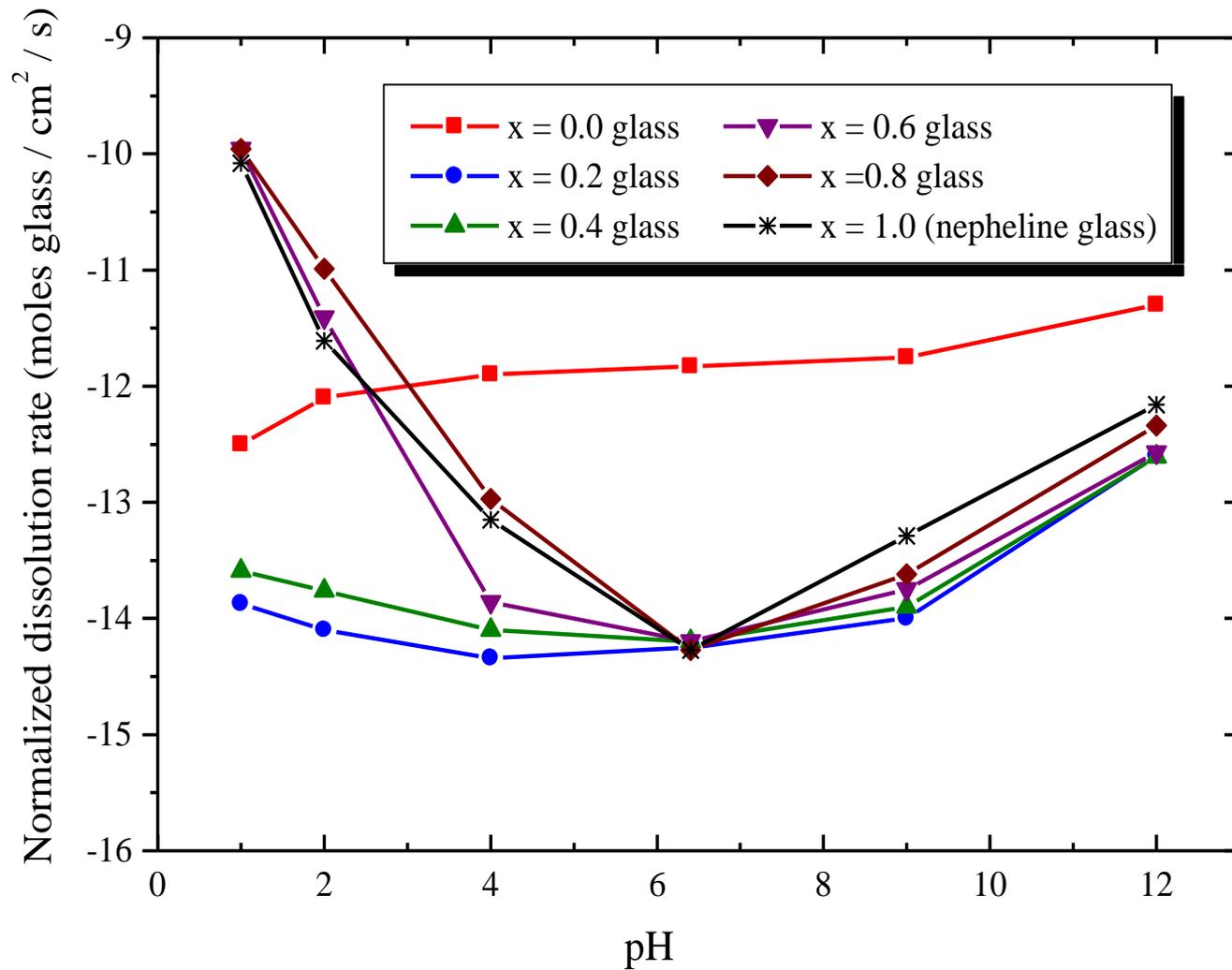
→ “condensed” at
300 K (200 ps)
(2 OH’s ~1.5 Å)

**4 Leached Glass
Structure**



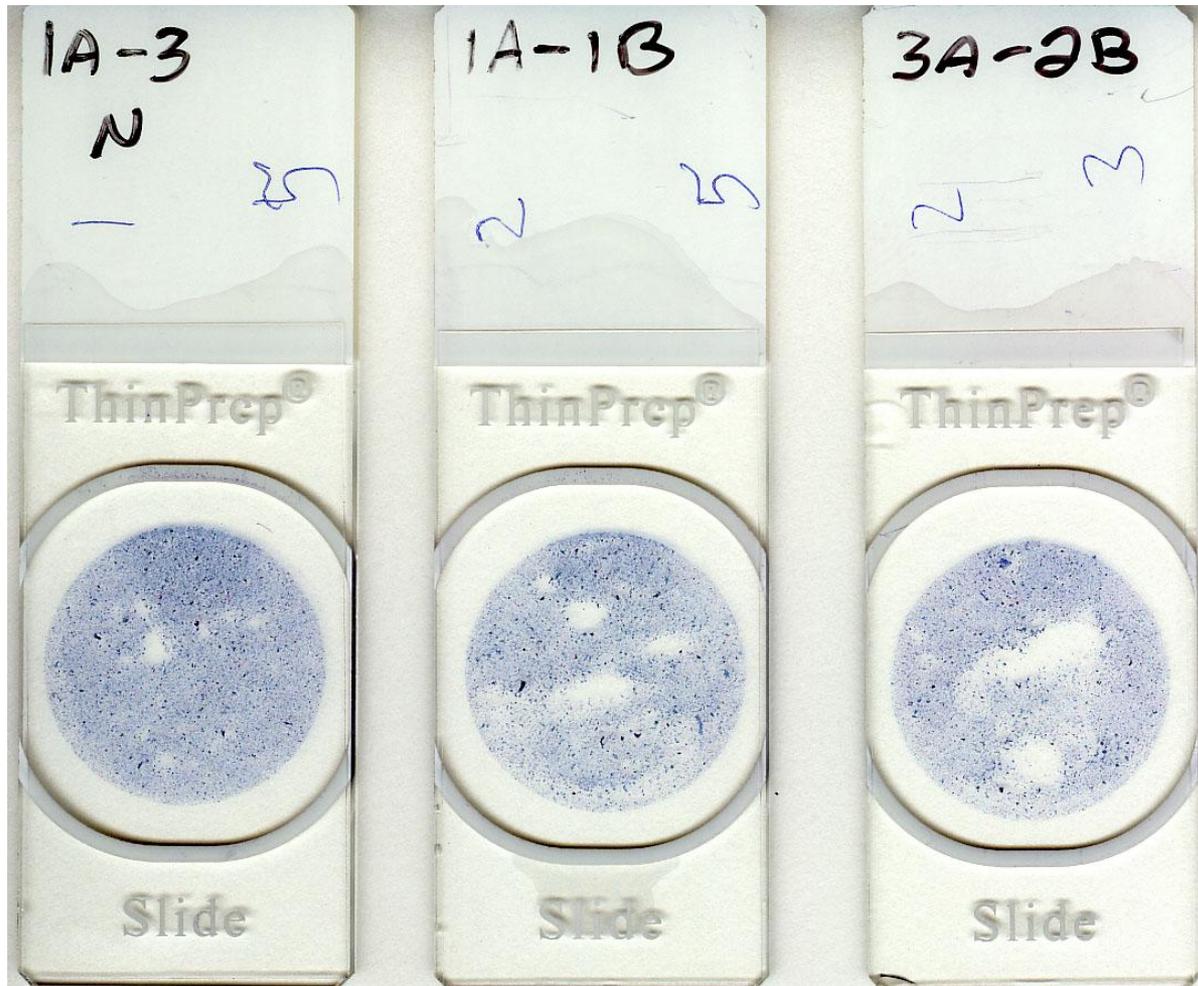
surface roughening by dissolution



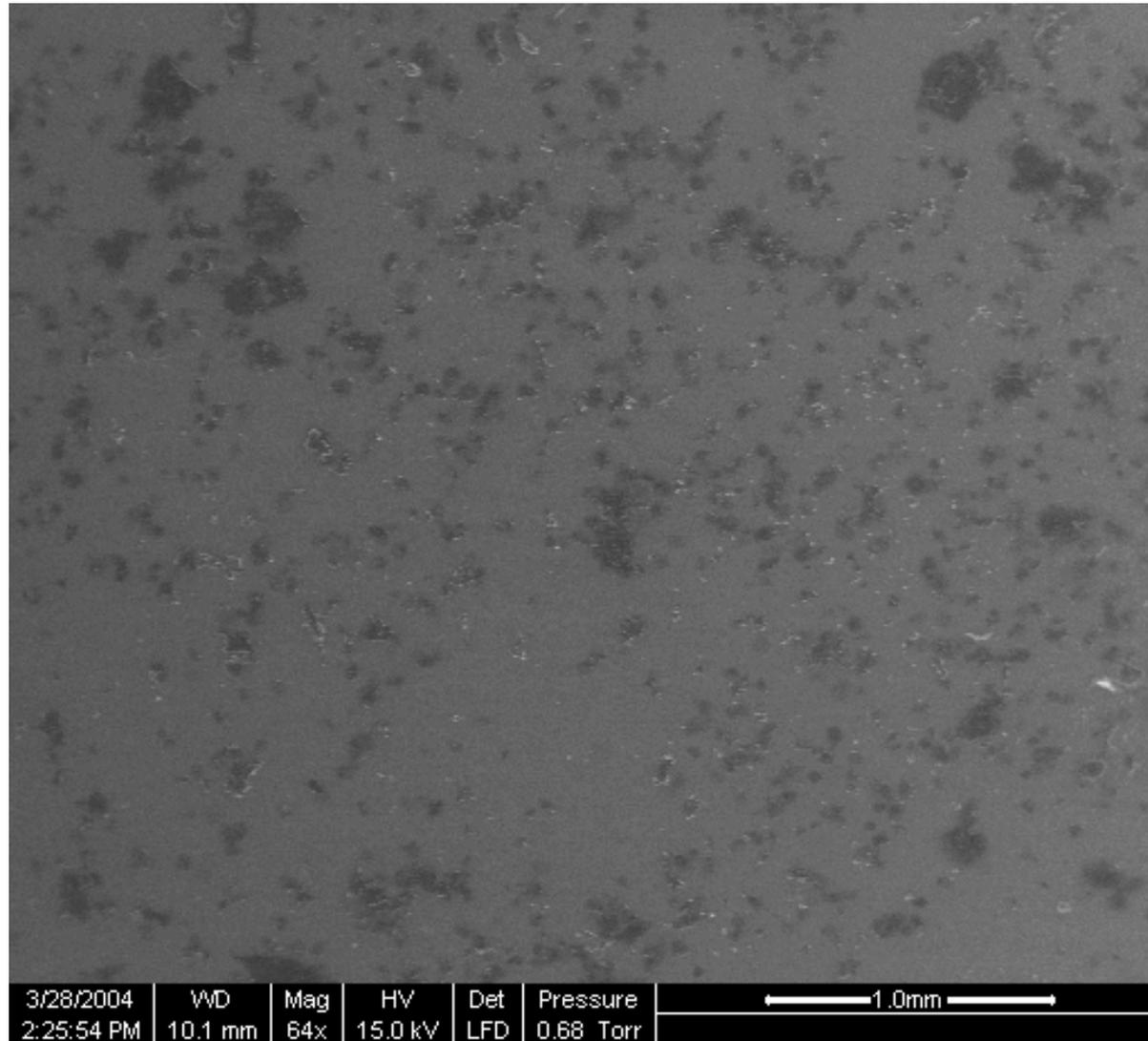


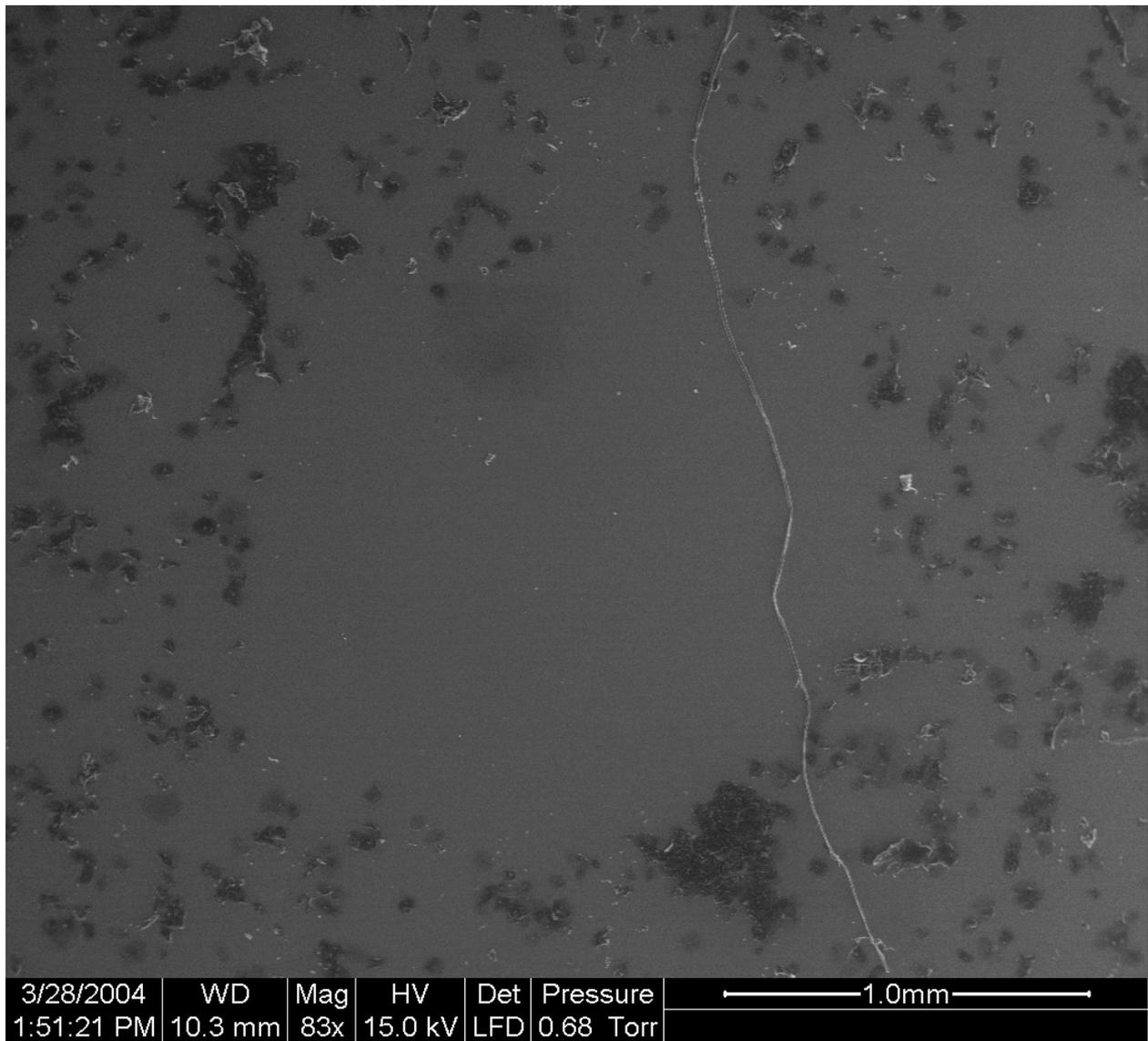
Normalized dissolution rates vs. pH for sodium-aluminosilicate glasses in the NBO glass series.

Cell Transfer for Cervical Cancer Diagnosis

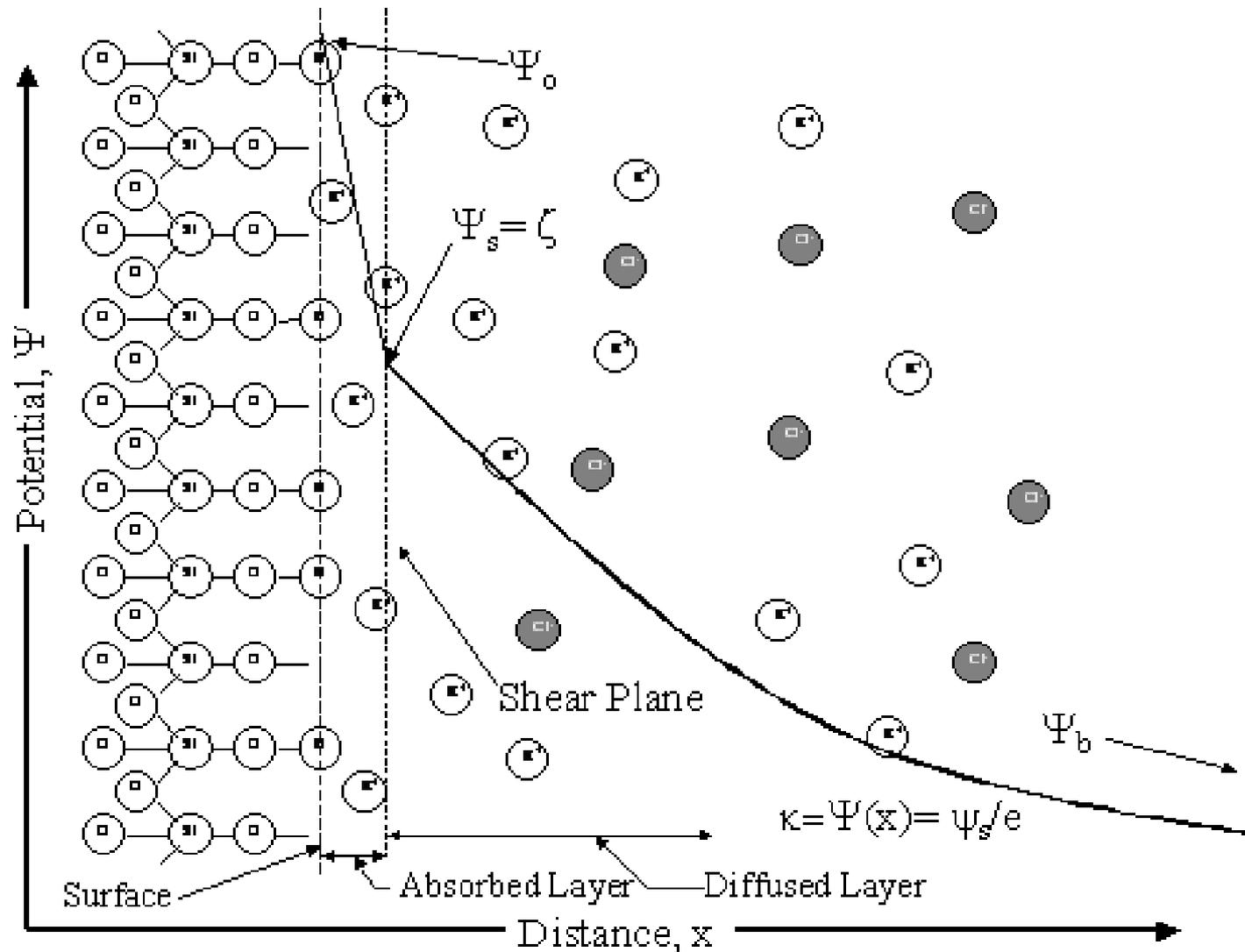


GYN cell transfer layer by SEM



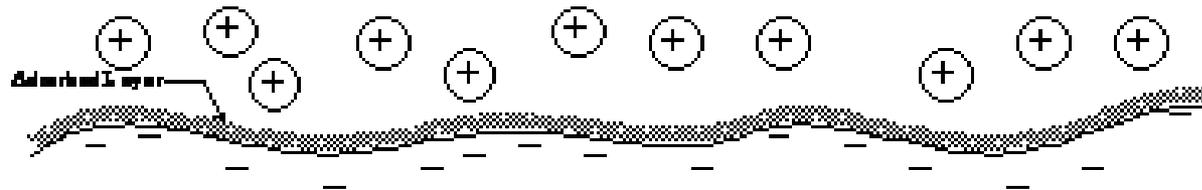


Electrical double layer at the glass-water interface

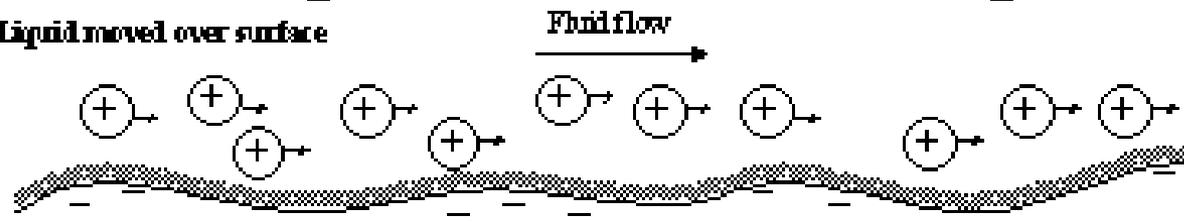


Streaming potential determination of surface charge

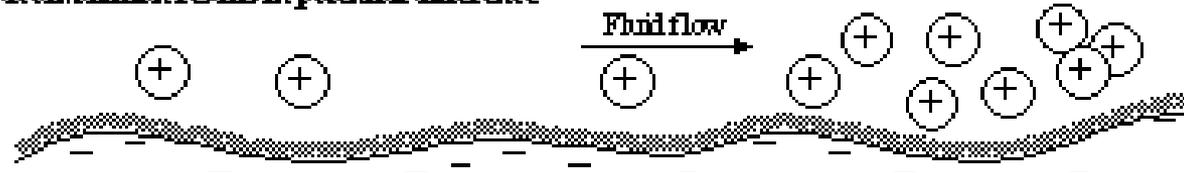
Electric double layer at rest



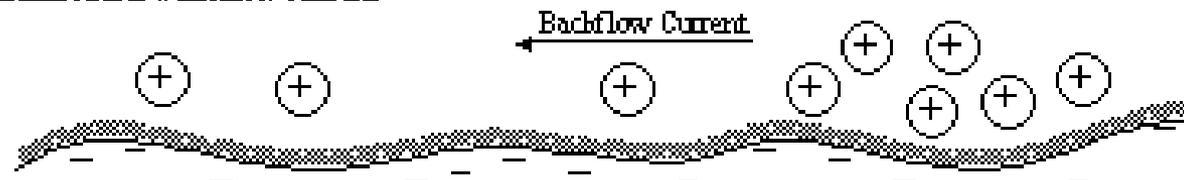
Liquid moved over surface



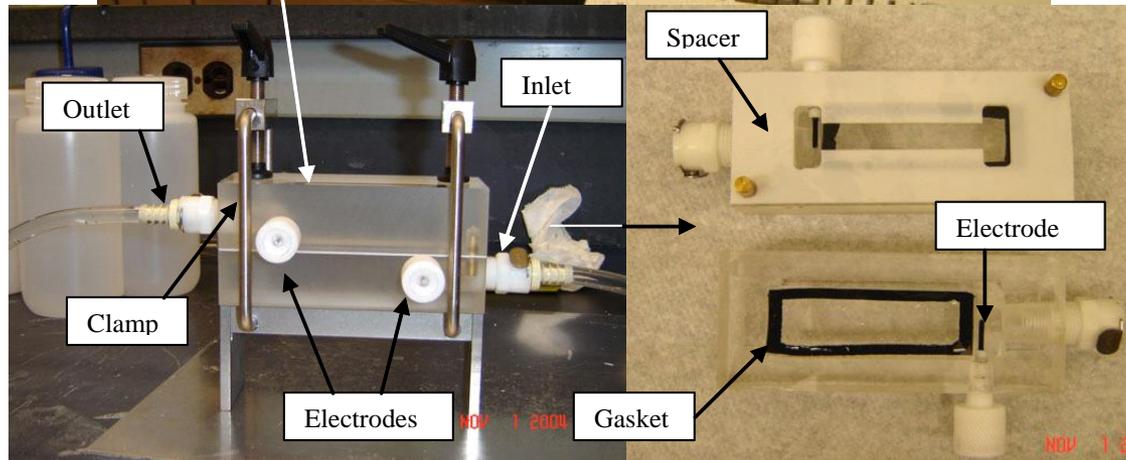
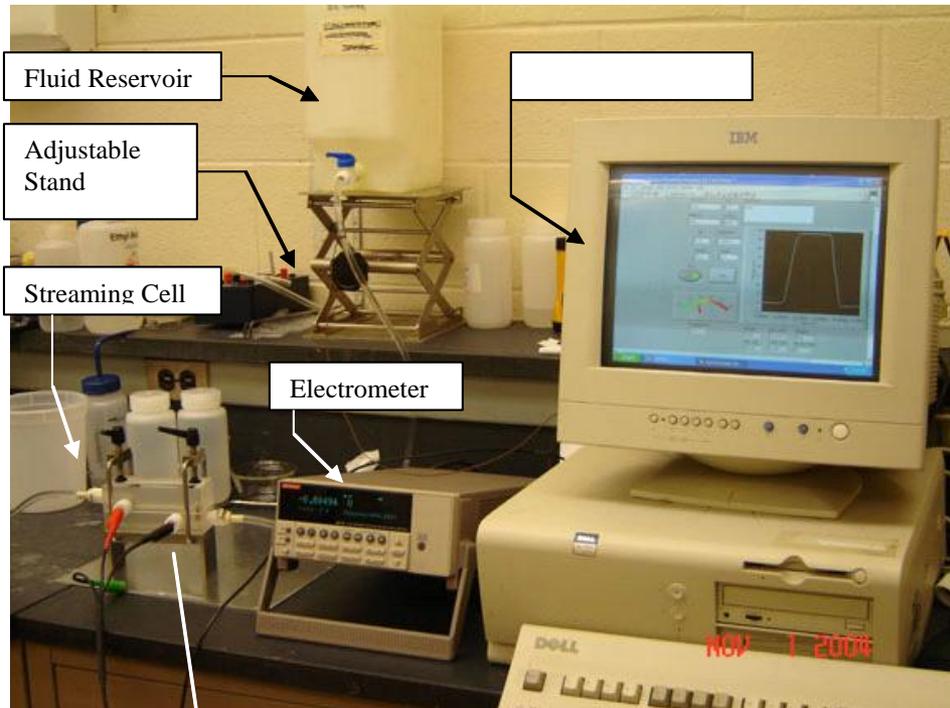
Ion accumulation results in potential difference

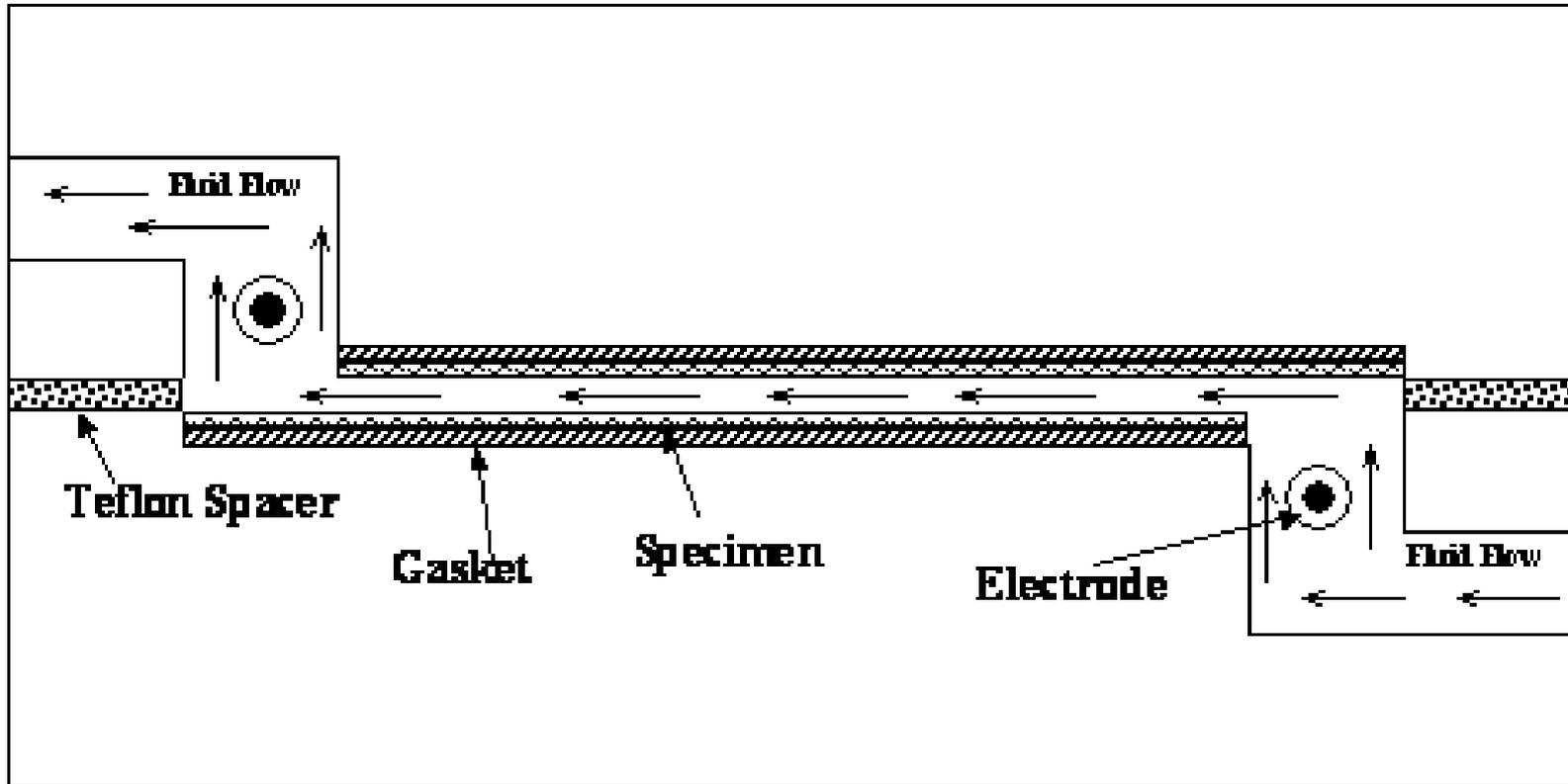


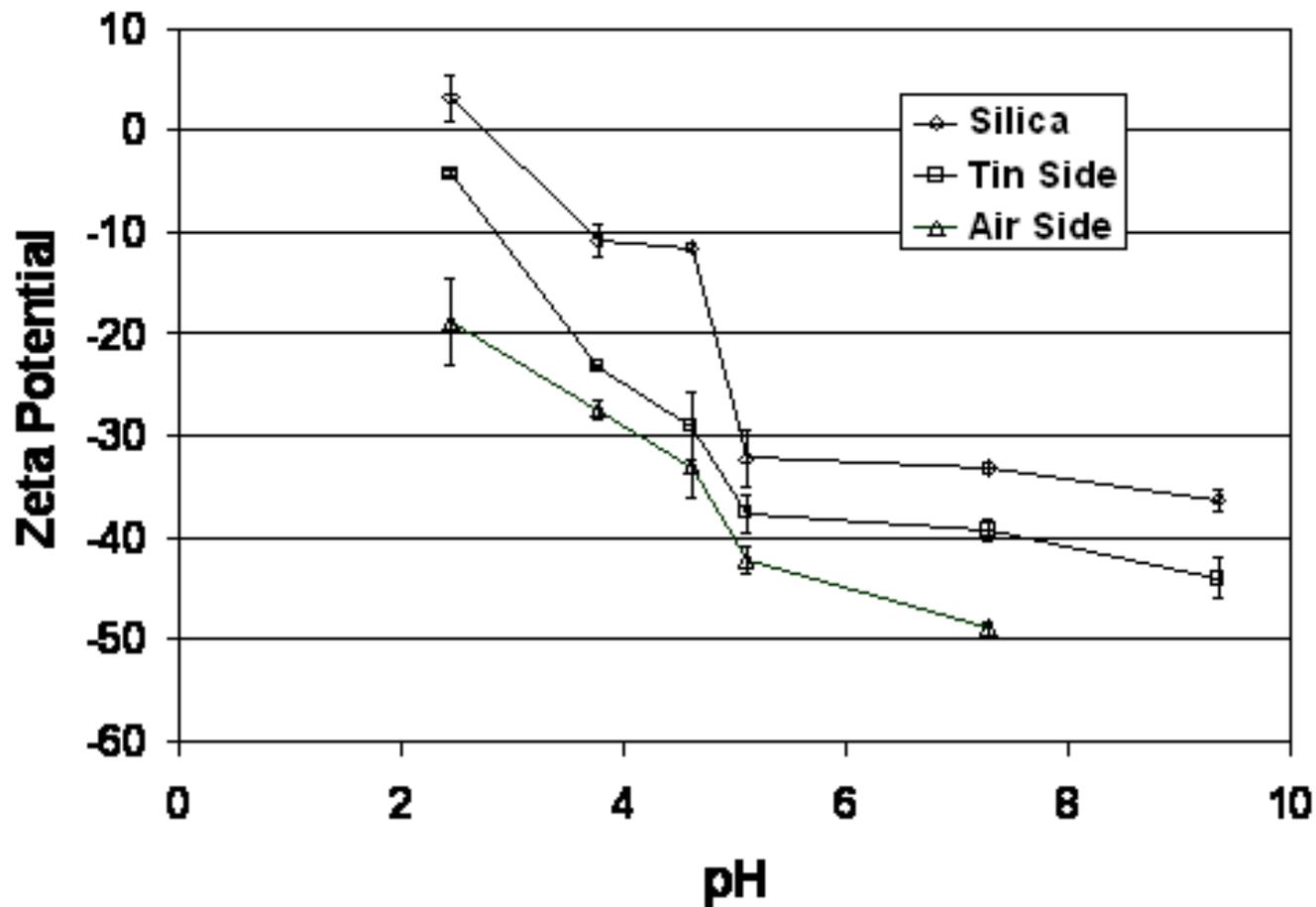
Potential causes backflow current

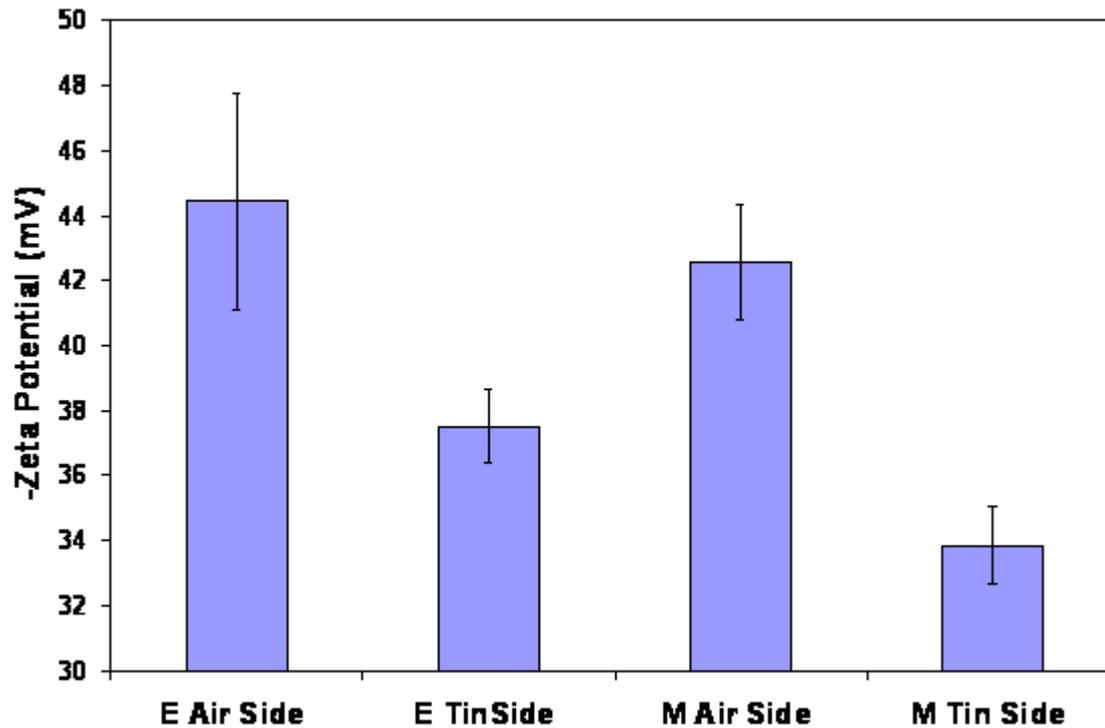


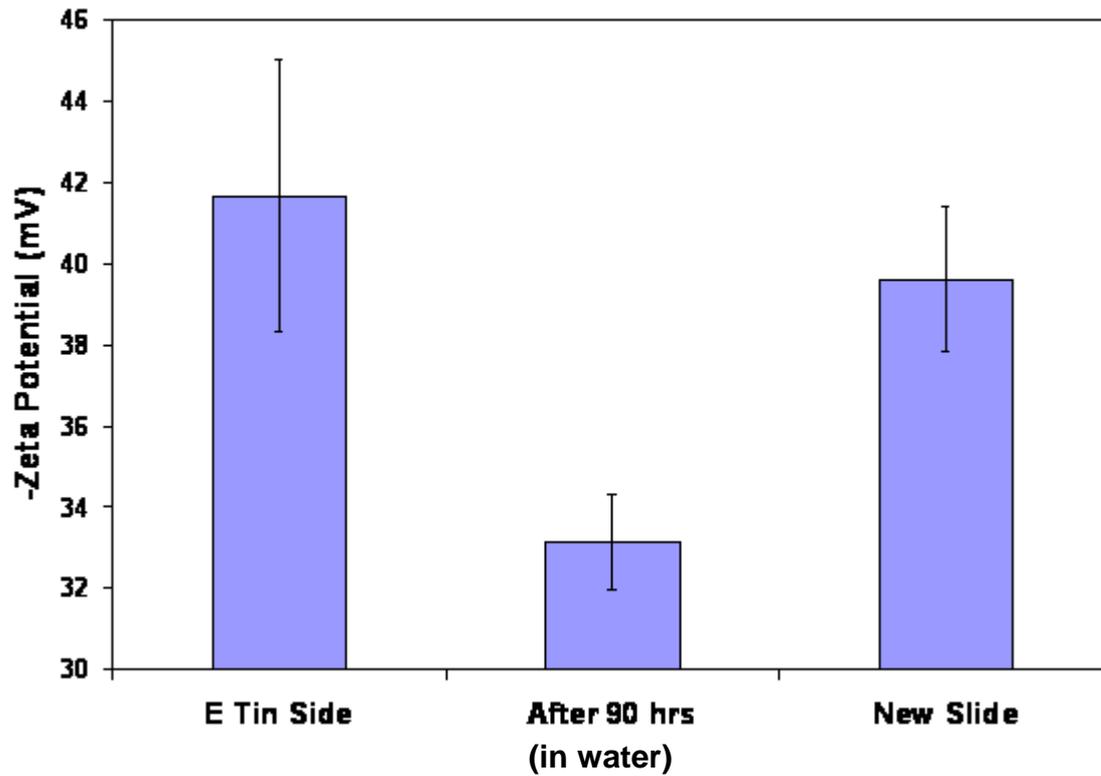
Streaming Potential System for Flat Glass

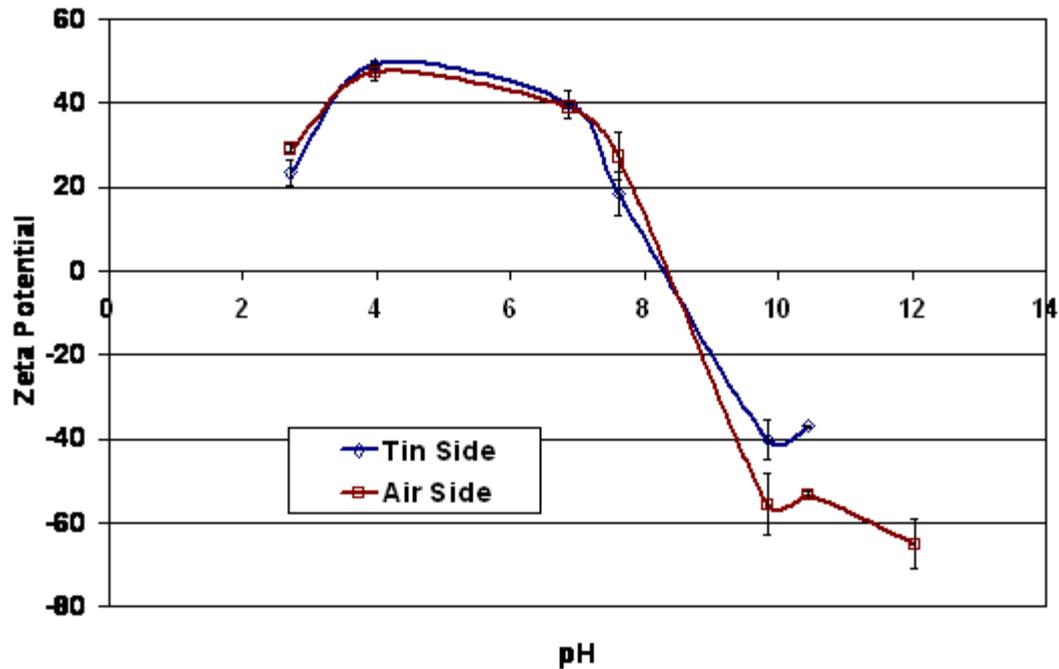








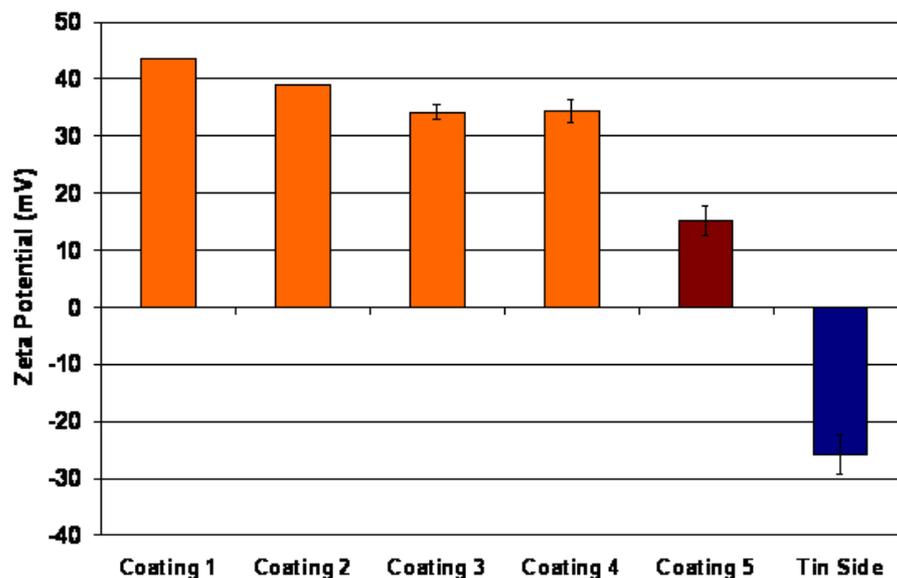




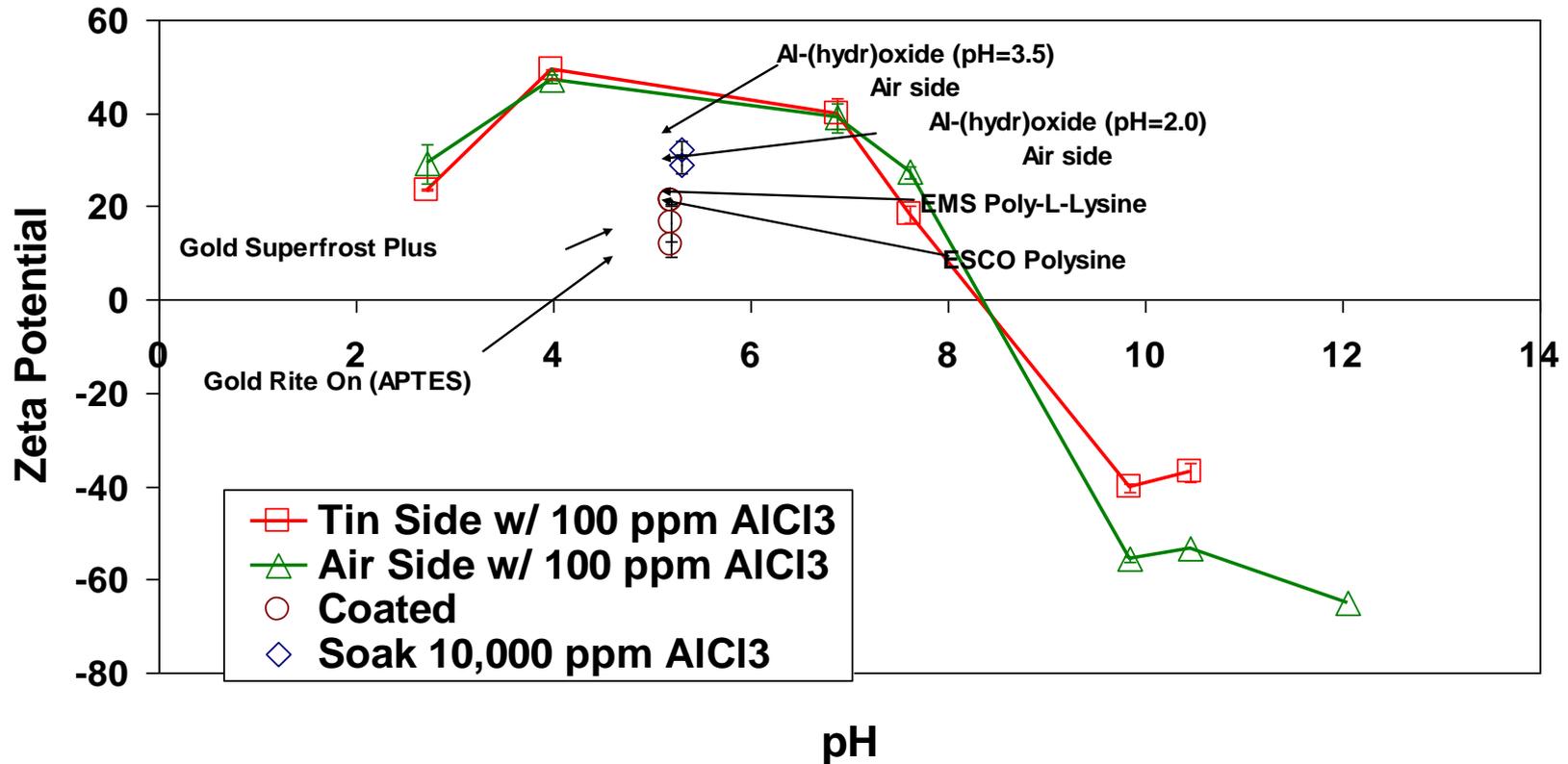
Zeta potentials determined for the air and tin surfaces of soda-lime-silicate glass slides for 10^{-3} KCl solutions containing 100 ppm of $AlCl_3$ at different pH's.

Surface compositions (by XPS) for the **aluminum-hydroxide sol/gel coated slides**, and the tin surface of an uncoated E slide for reference; Coating 5 was rinsed before the heat treatment.

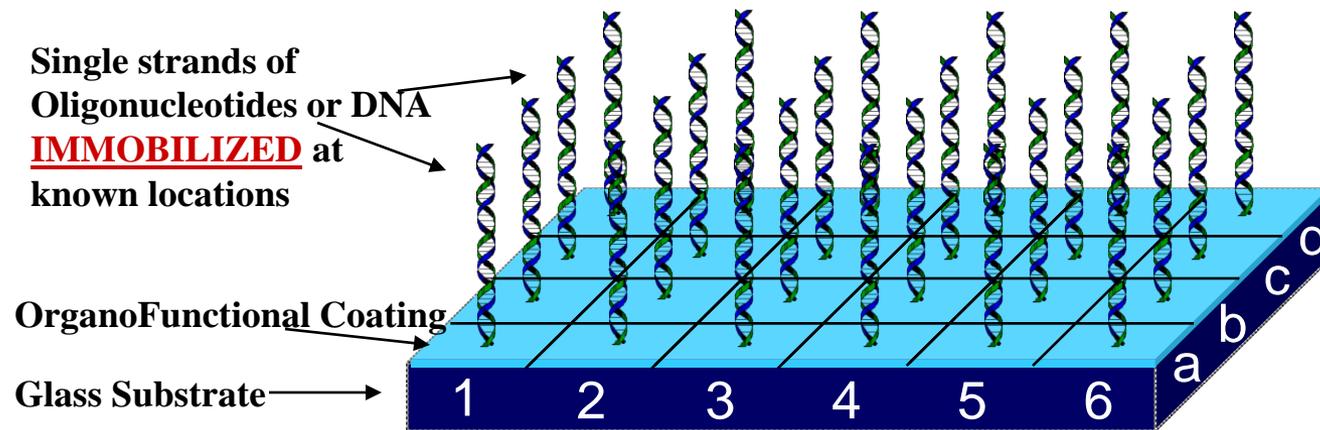
	Na (at%)	O (at%)	Sn (at%)	N (at%)	Ca (at%)	Mg (at%)	C (at%)	Cl (at%)	B (at%)	Si (at%)	Al (at%)
Coating 1	3.0	45.8	0.0	0.2	0.0	0.1	17.4	6.7	1.2	3.9	21.7
Coating 2	3.3	49.0	0.0	0.3	0.0	0.1	13.5	6.6	0.8	0.6	25.8
Coating 3	3.5	49.1	0.0	0.5	0.0	0.1	12.6	6.7	0.3	0.9	26.2
Coating 4	4.8	47.9	0.0	0.5	0.0	0.1	11.7	8.5	0.8	0.7	25.0
Coating 5	6.2	54.2	2.2	0.2	1.4	1.5	10.9	1.3	0.3	16.1	5.5
Tin Side	4.8	59.6	4.0	0.2	2.9	2.7	4.0	0.4	0.2	21.1	0.1



Coated Glass Slides– inorganic and (commercial) organic coatings



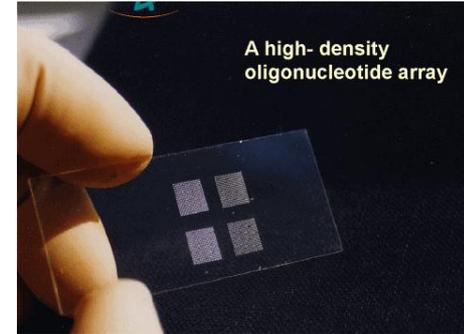
DNA Microarray: a glass-based biological sensor



glass substrates provide: chemical inertness
optical platform
low fluorescence background
flatness and smoothness
low cost!

DNA Microarrays

A planar device comprised of an array of DNA single strands immobilized on the surface of an insoluble solid support.

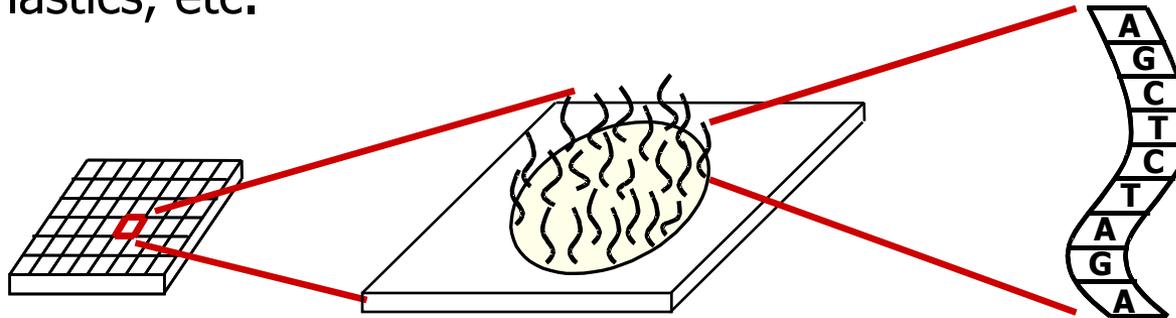


Solid support:

Glass slides
Silicon
Plastics, etc.

Molecules:

oligonucleotides, proteins, cells or tissues



SEGMENT OF THE MICROARRAY

1x2 cm²

of spots = 100-500,000

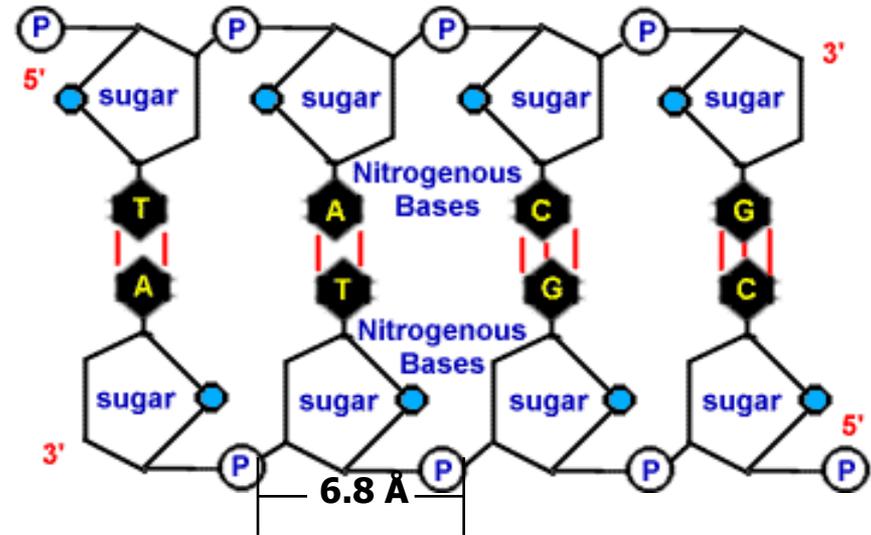
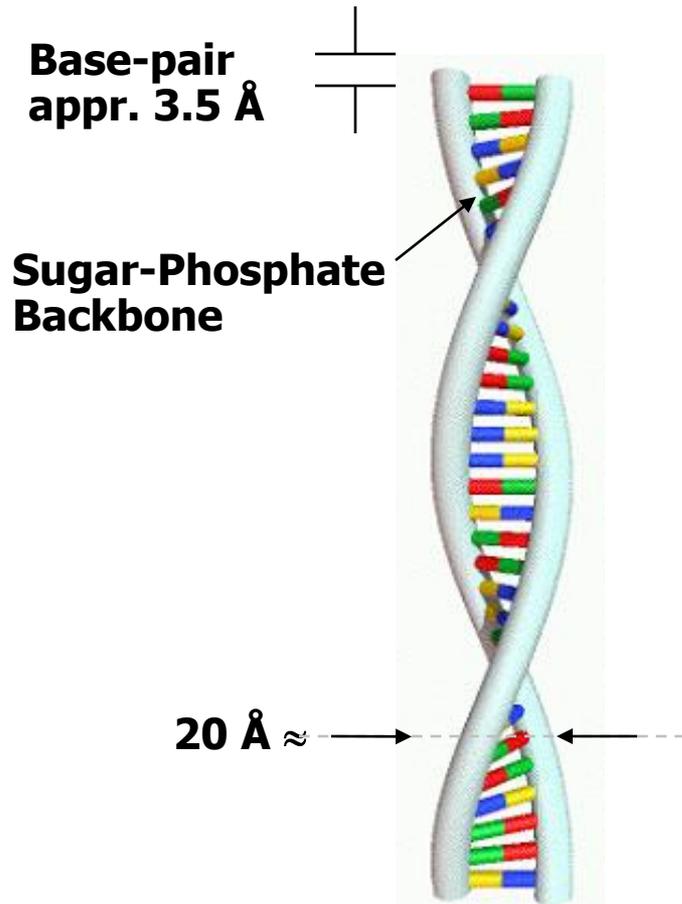
SPOT CONTAINING DNA Probes

(10-250 μ m)

Immobilized DNA Probe

For DNA arrays:
Each spot contains
10⁶ to 10⁹ of
identical DNA fragments.

DNA Structure: The Fundamentals

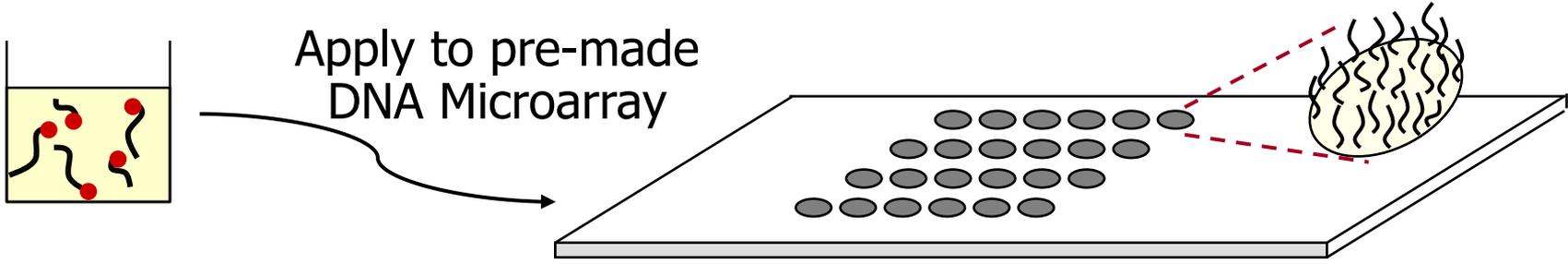


- DNA is a linear polymer made up of a sugar and phosphate backbone with variable side groups of different nitrogen bases. (A, C, G, T)
- DNA may be *single* or *double* stranded.

- **COMPLEMENTARY BASE PAIRING:**
Weak H-bonding between the base pairs
G ↔ C and T ↔ A
(HYBRIDIZATION)

T-C-A-G-G-T-T
A-G-T-C-C-A-A

DNA Microarrays (Gene Chips for sequencing)

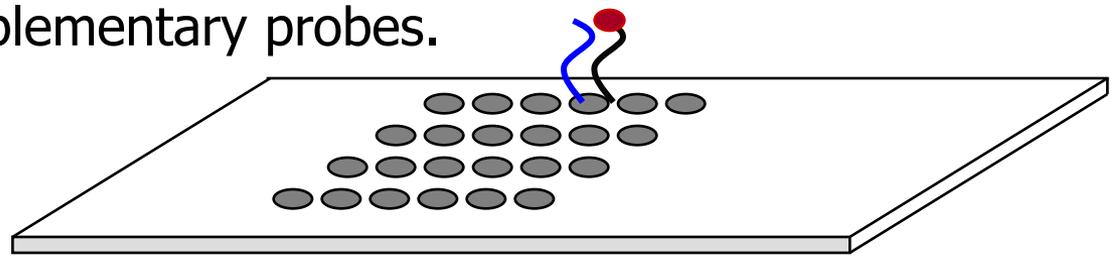


Unknown DNA solution with fluorescent dyes.

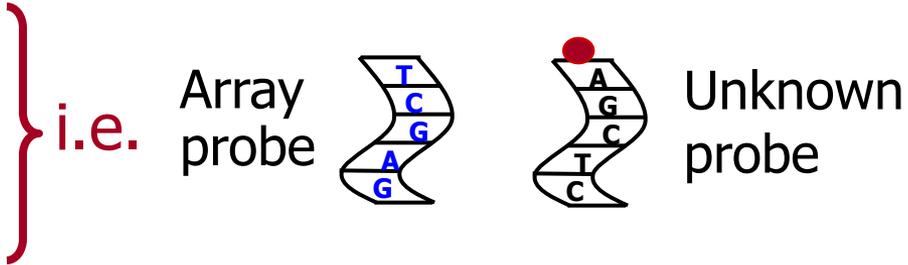
Each spot contains identical DNA probes of different known sequence.

Laser Confocal Scan

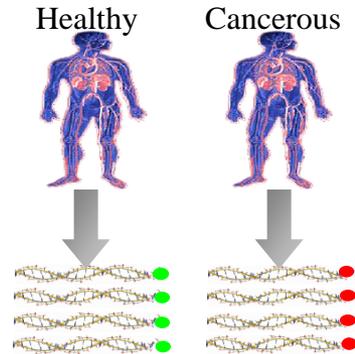
Unknown DNA molecules attach to their complementary probes.



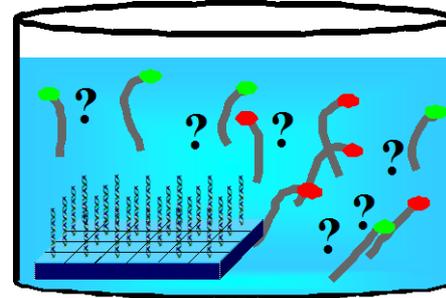
The sequence of the unknown strand is now determined.



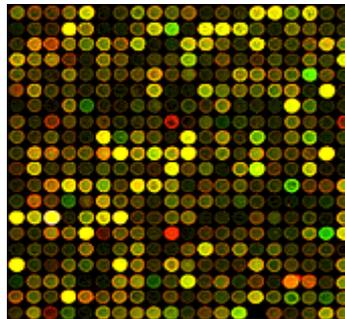
1) Targets are isolated and labeled



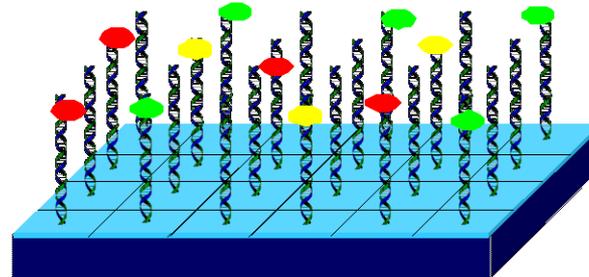
2) Labeled targets combined with array



4) Hybridized array is scanned



3) Array is washed after hybridization*



Use of Microarrays: Gene Expression Experiment

Use of Microarrays: Drug Discovery

Untreated cells



Drug-applied cells



Extract RNA

Reverse transcript to cDNA

Fluorescent labeling



Apply to array

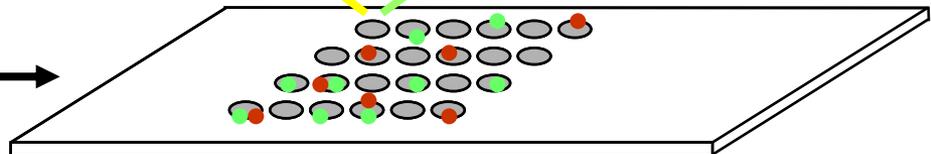
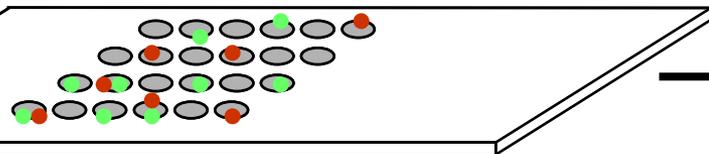
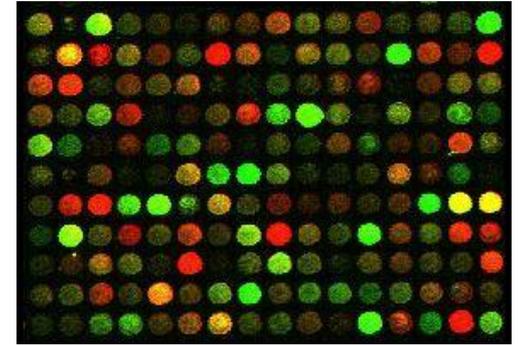
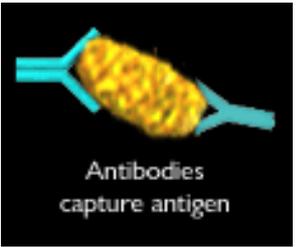
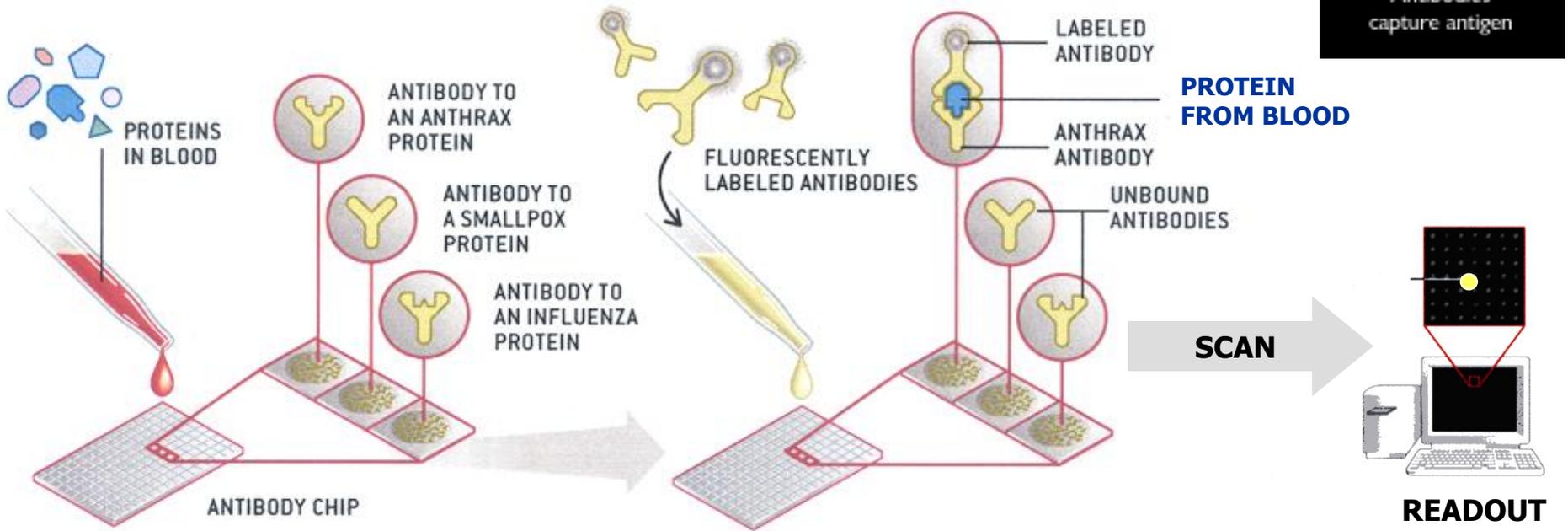


Image Analysis for Quantification



Protein Arrays: Diagnostic Analysis



PROTEIN FROM BLOOD

SCAN

READOUT

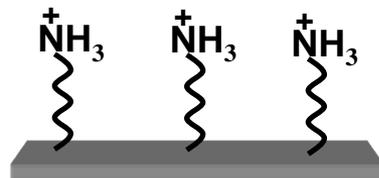
- Squares of antibodies able to bind a specific protein representing a disease-causing agent.
- Apply blood to the array of antibodies → proteins from blood attach
- Apply fluor-labeled antibodies recognizable by the attached proteins, forming a antibody "sandwich"
- Dot indicating that the patient has anthrax.

Multiple Surface Chemistries Provide Opportunities for Immobilization of Various Probes

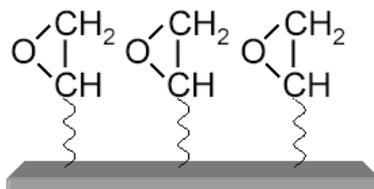
and in addition to
DNA arrays/probes:

ELISA's
Protein arrays
Carbohydrate arrays
Chem-Bio Sensors

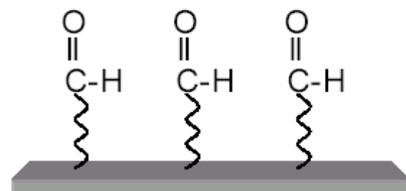
Surface Coatings



Amino



Epoxy



Aldehyde

Recommended Probes

- PCR products
- Long oligos (size ≥ 50 mers)

- Short and long oligos
- PCR products

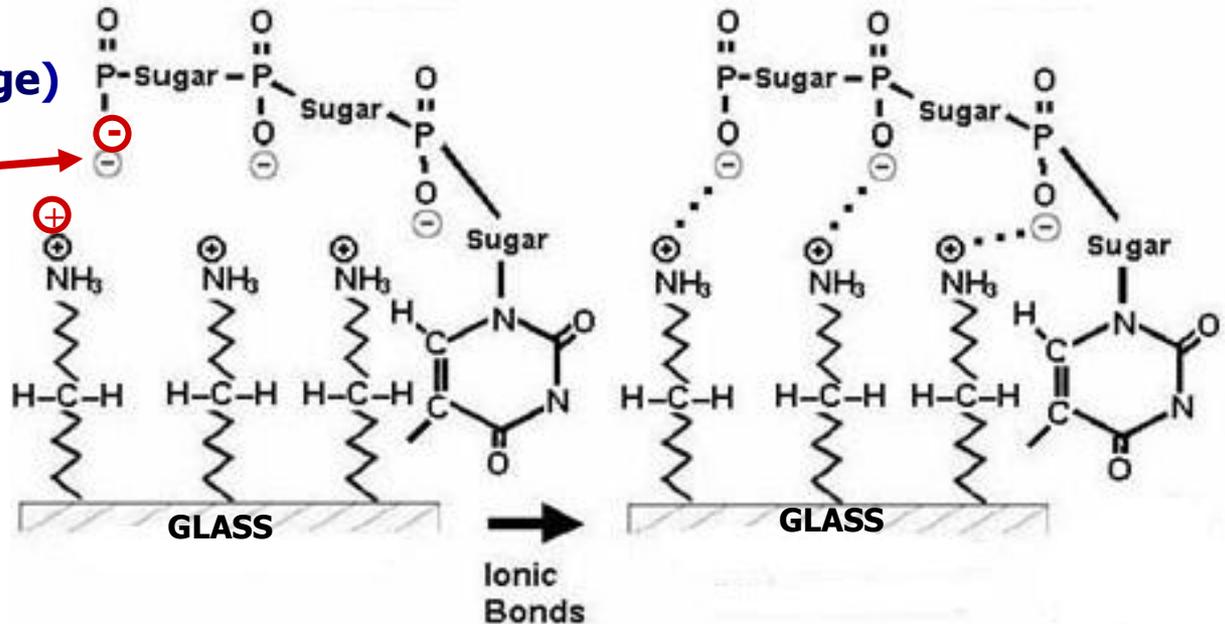
- Peptides
- Short and long NH_2 -modified oligos
- NH_2 -modified PCR products
- Antibodies

Immobilization of Unmodified DNA to glass substrates

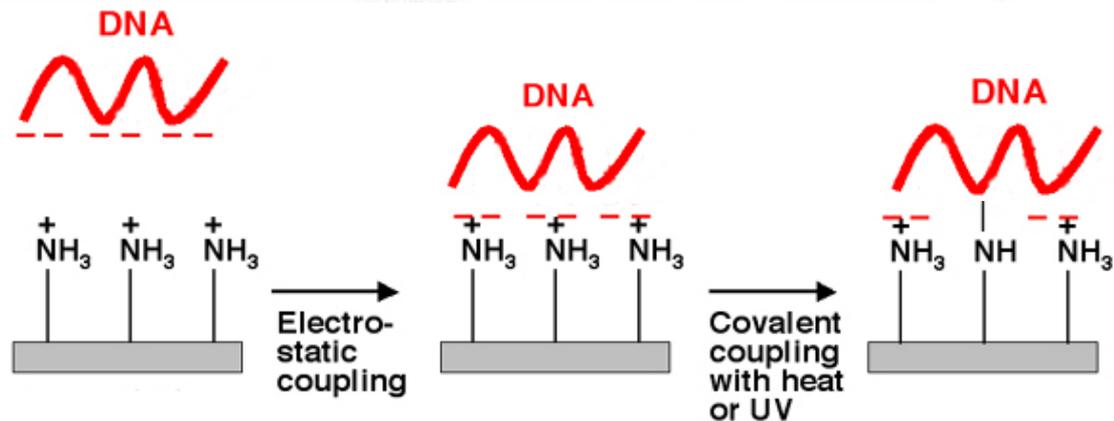
Phosphate-Sugar
DNA backbone
(carries negative charge)

electrostatic
attraction

functional amine
group-NH₃⁺

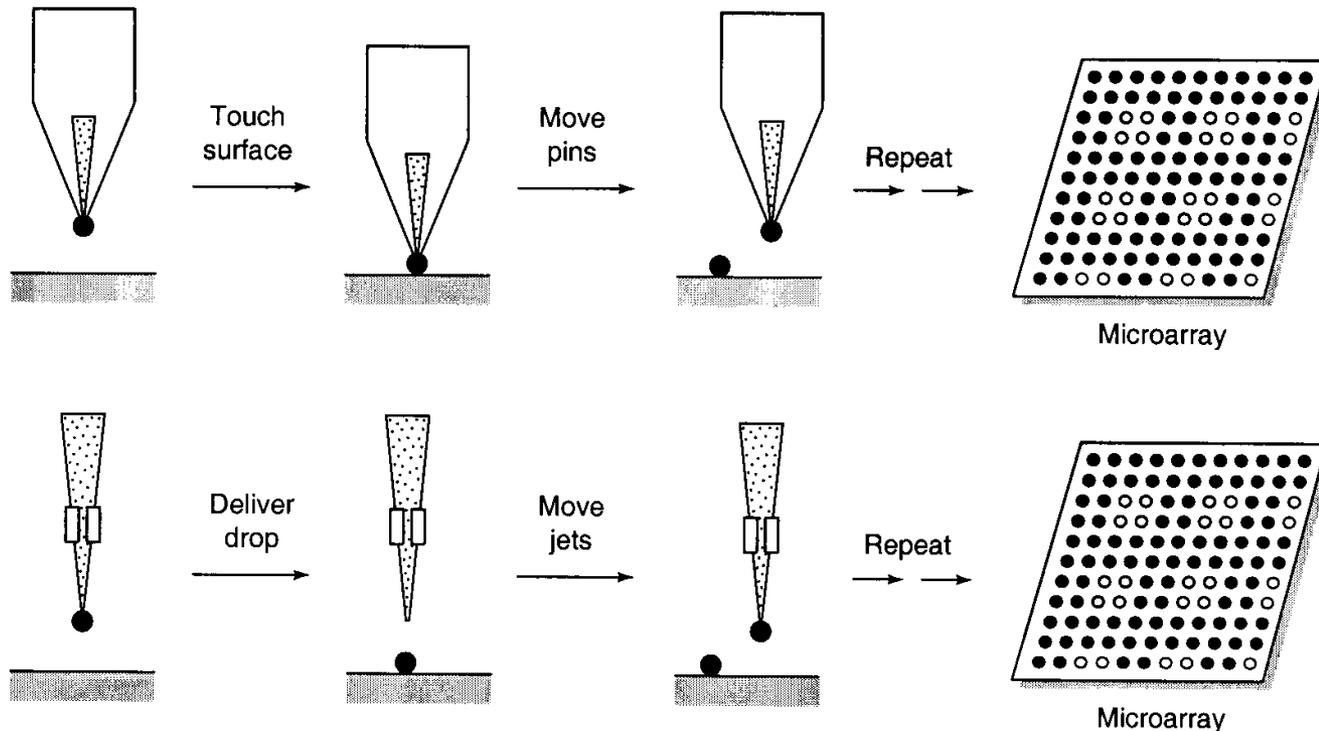


Unmodified DNA strands carry intrinsic (PO₄)³⁻ groups; glass surfaces functionalized with protonated amino groups (NH₂) can be used for their initial immobilization.



Physical Deposition of Modified and Unmodified DNA

Microspotting (Shalon and Brown, Stanford, 1995)



Because of the ease of use and affordability, microspotting has become the most common microarray technology for basic research.

Physical Chemistry/Engineering of the Microspotting Process

