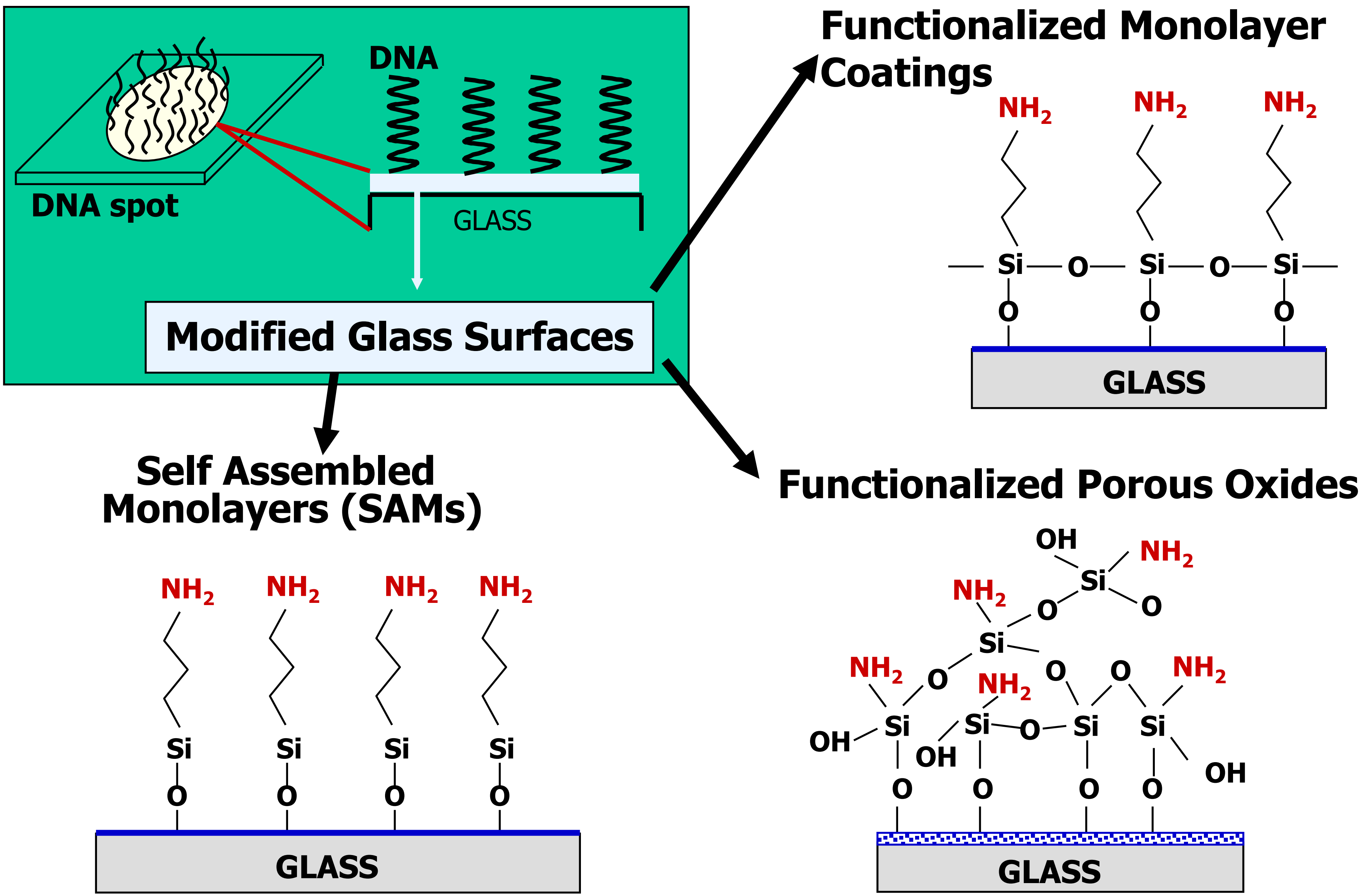
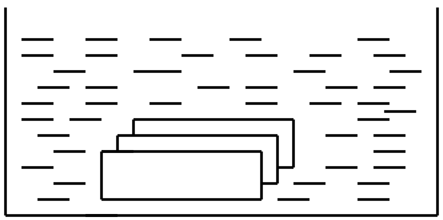
MODIFICATION of GLASS for DNA ATTACHMENT

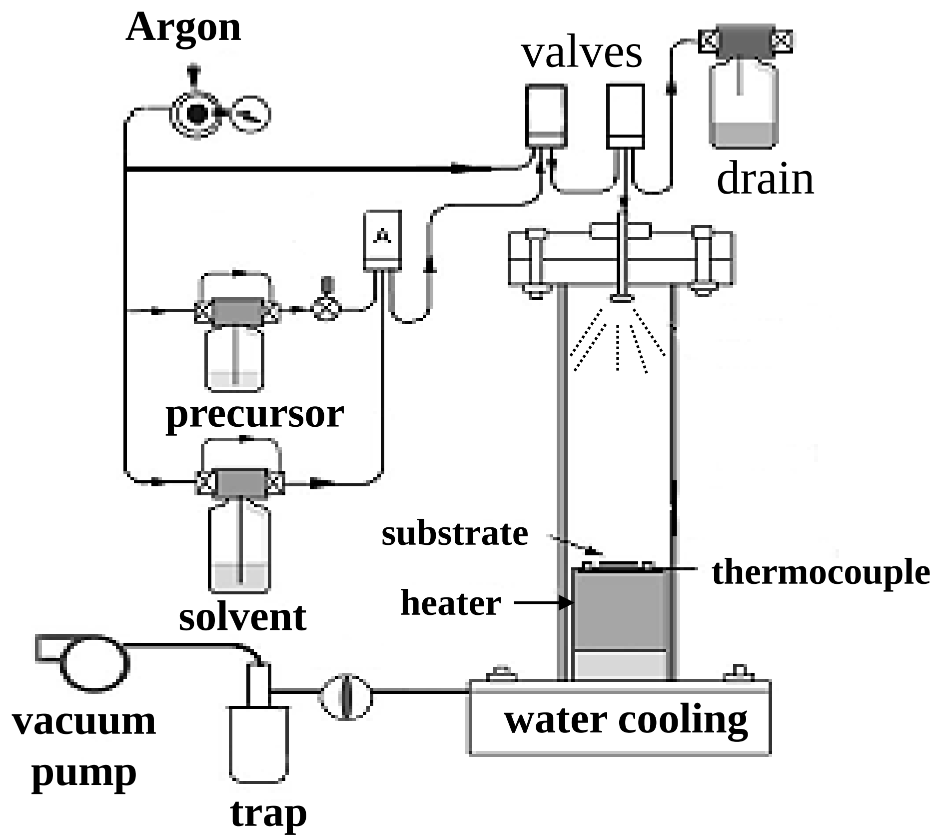


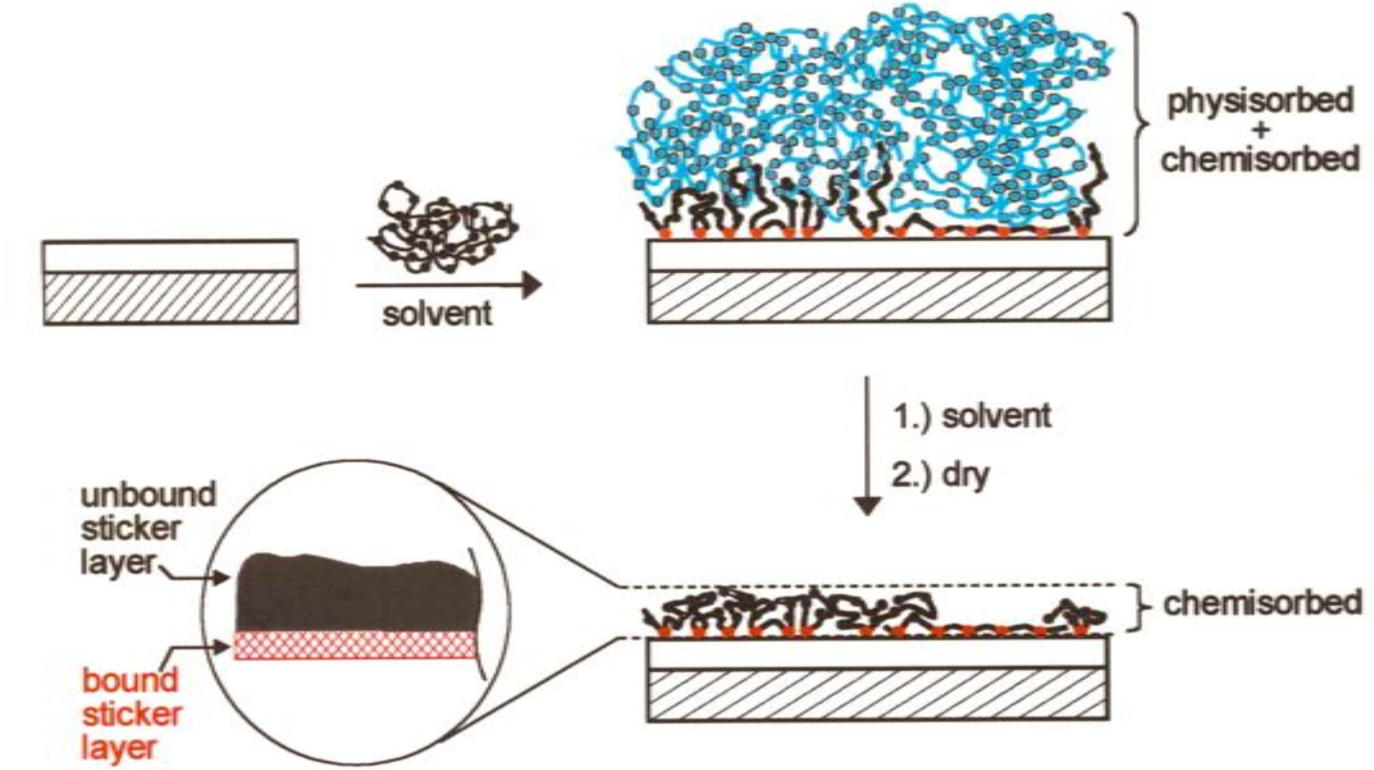
Silanization process:

Dipping



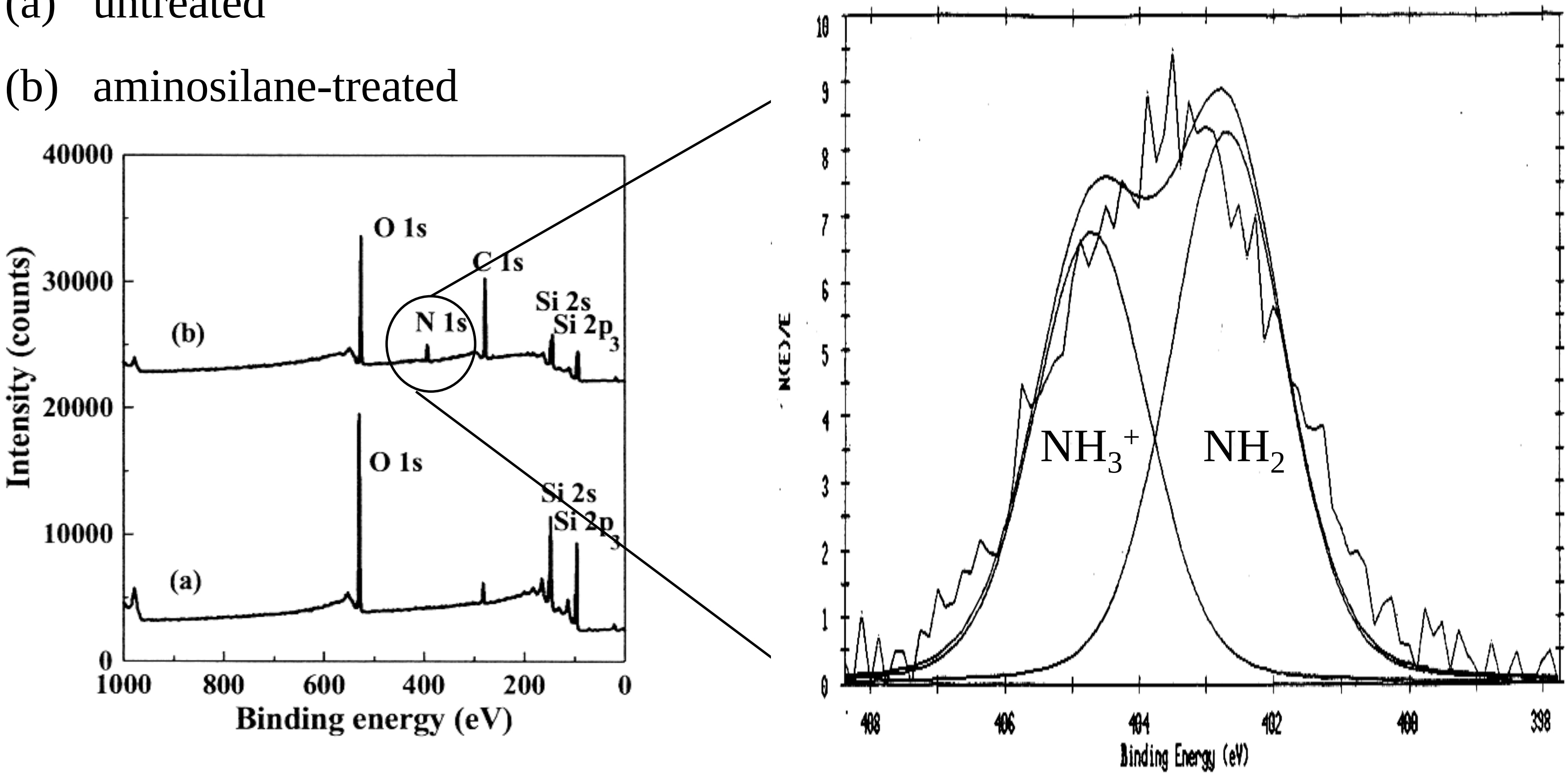
CVD




solvent
physisorbed
+
chemisorbed
1.) solvent
2.) dry
unbound
sticker
layer
bound
sticker
layer
chemisorbed

(a) untreated

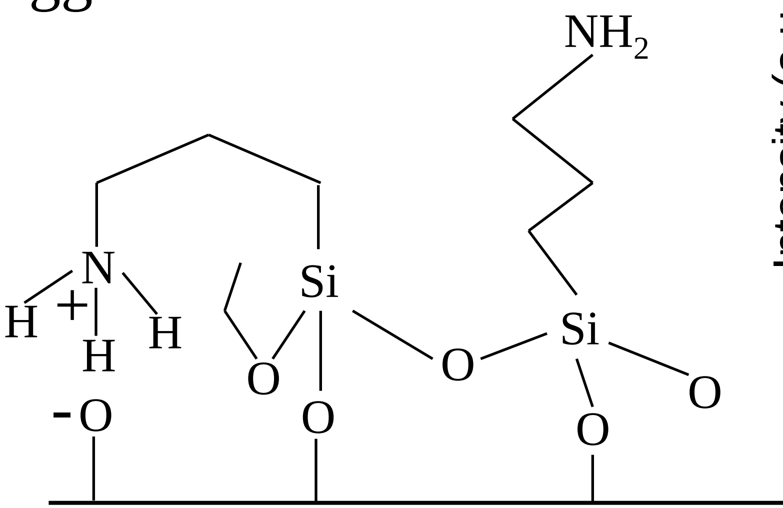
(b) aminosilane-treated

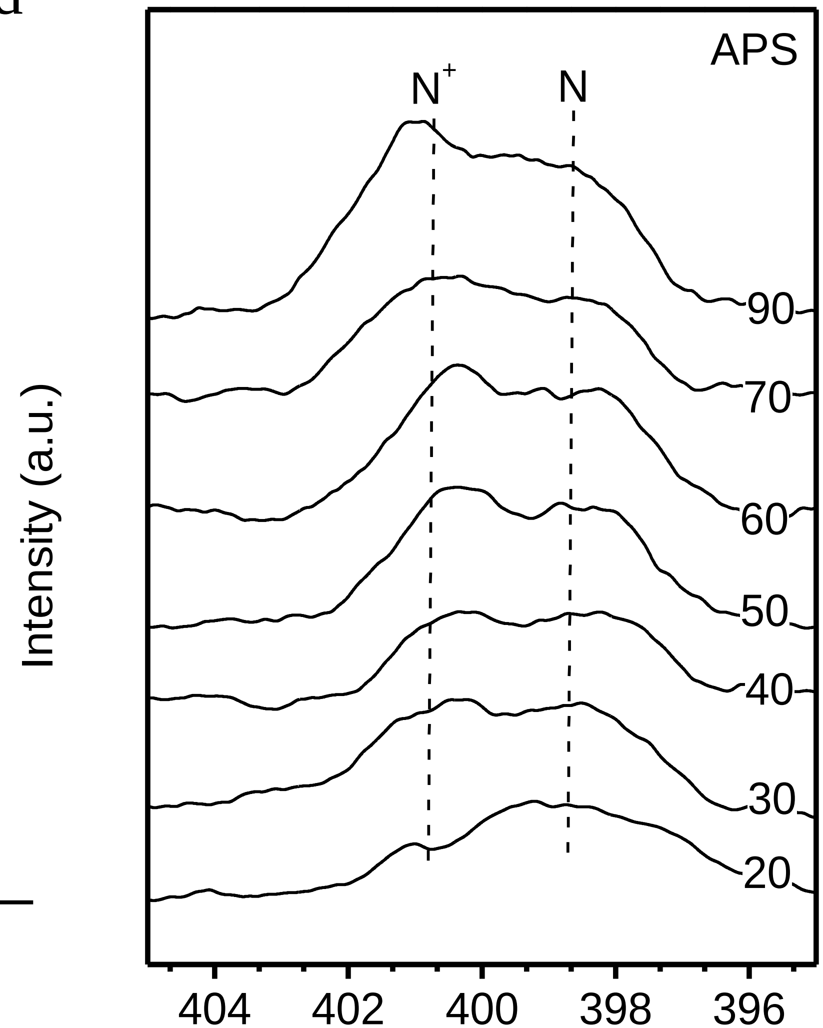


N1s high resolution XPS of APS treated glass.

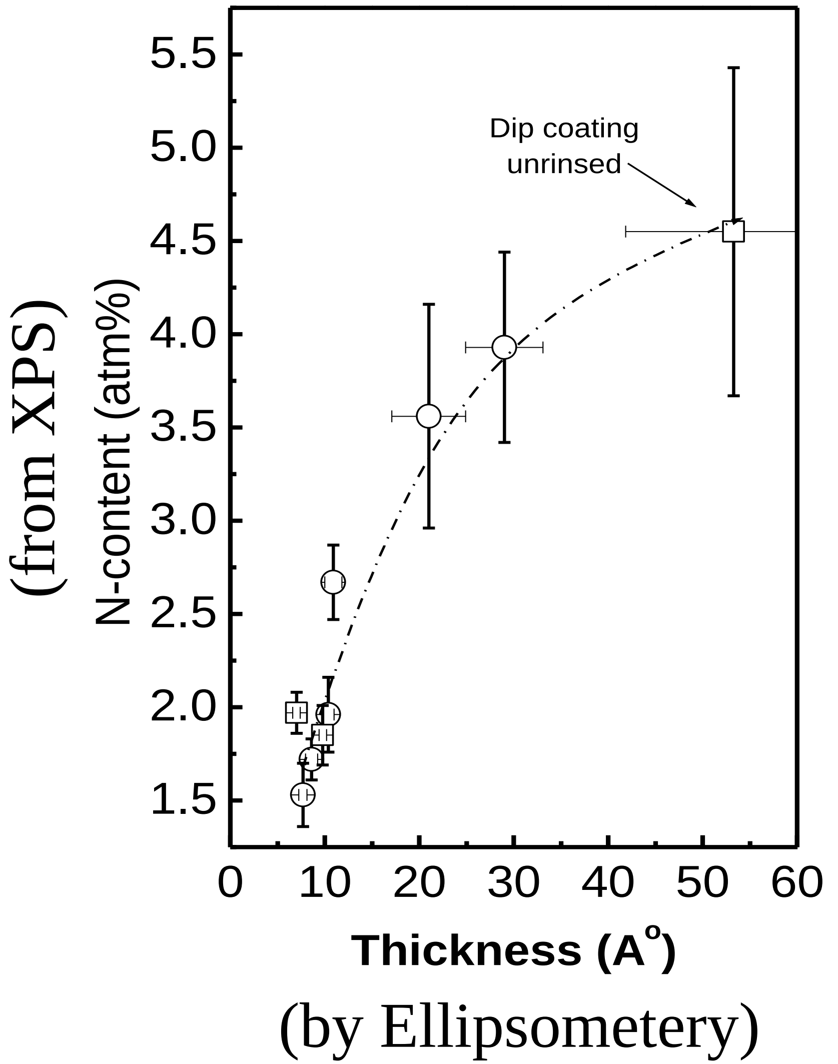
Angle dependent XPS results

A preference for the protonated amine to be oriented towards the glass surface and the non-protonated ones to be oriented away from the surface is suggested.





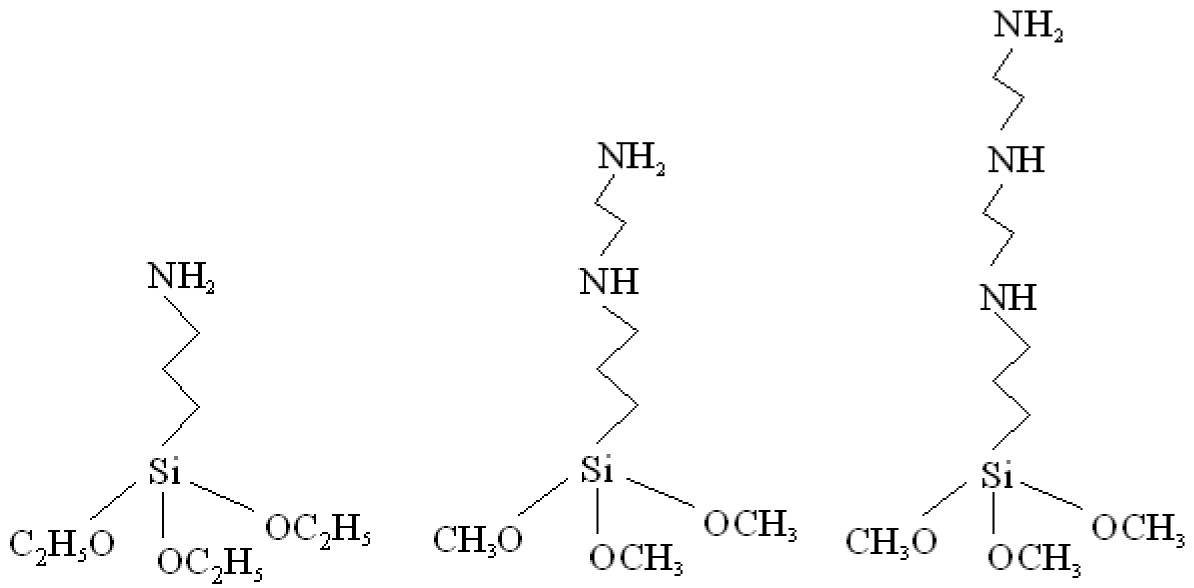
Thickness of silane layer



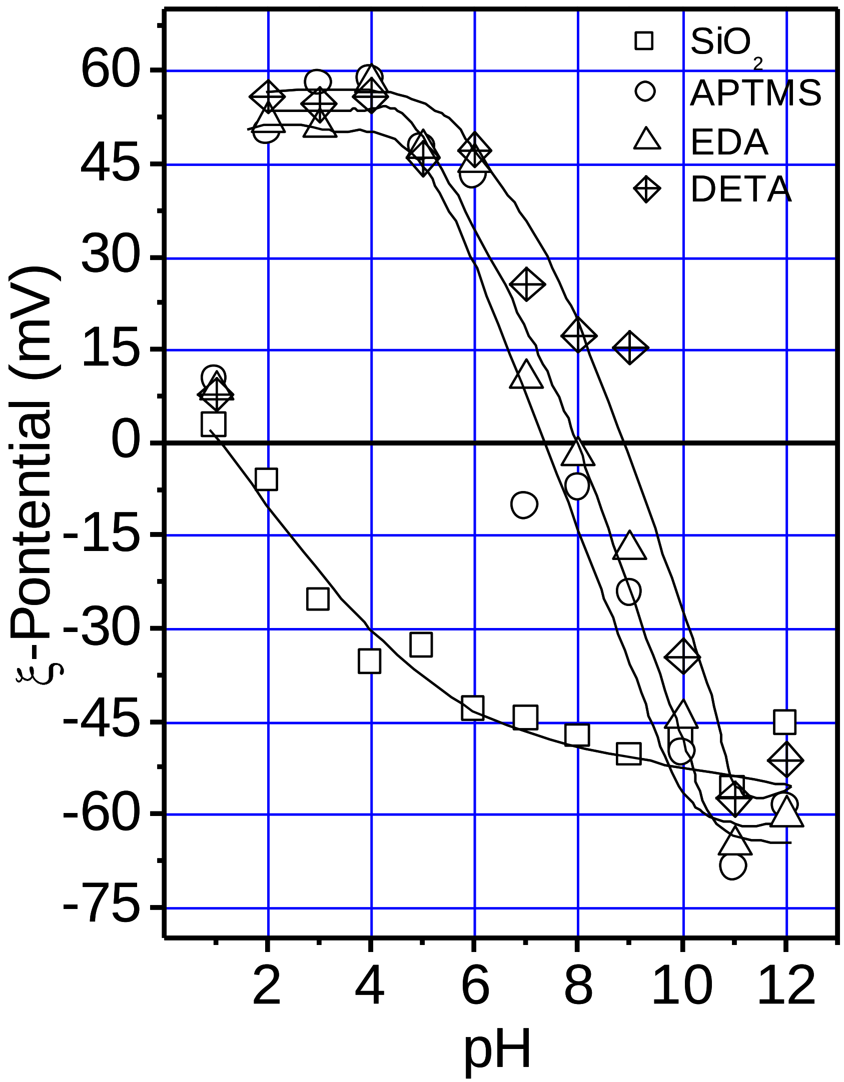
Using a pulsed-CVD technique, different silane thicknesses can be build up on clean, oxidized silicon wafer substrates.

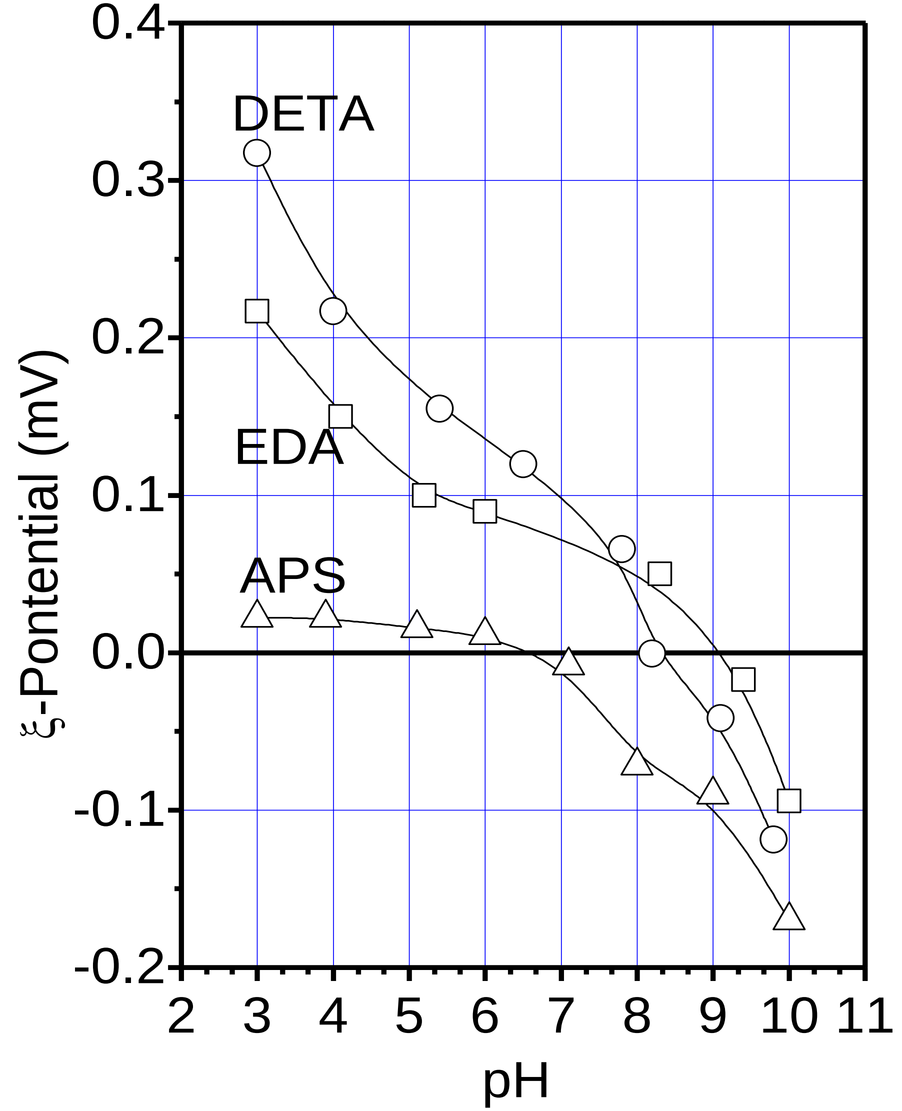
The length of fully stretched APS molecule is ~10Å.

Aminosilanes with variable alkyl-ligand functionality



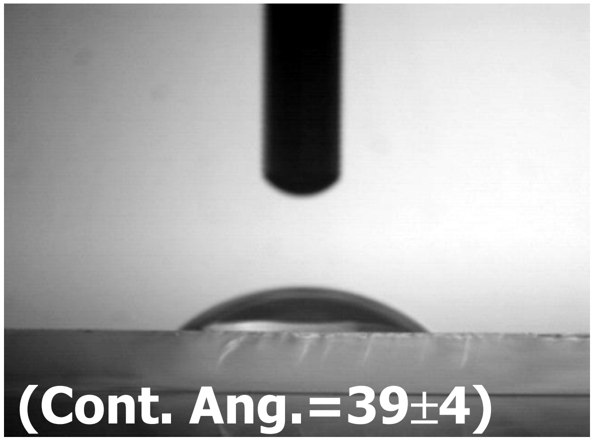
Surface charge on silane treated glass surfaces

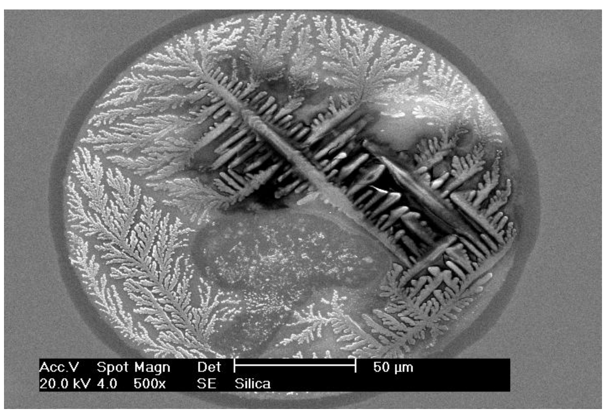




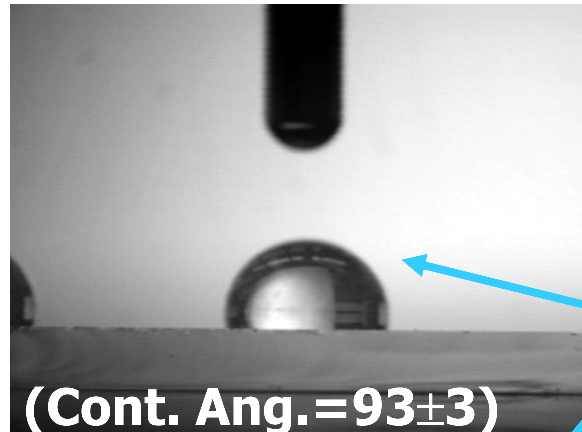
Microstructures and DNA Disributions in Spots

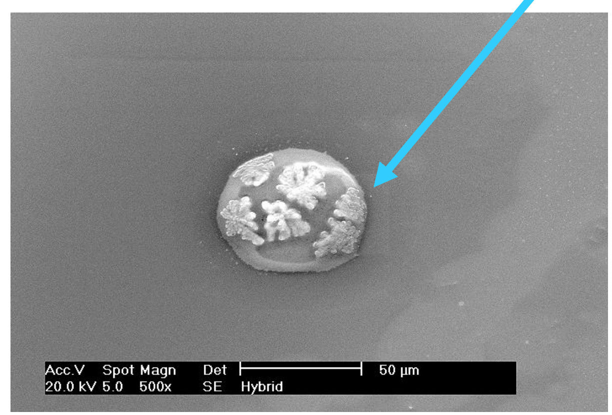
SILICA COATING (TEOS)





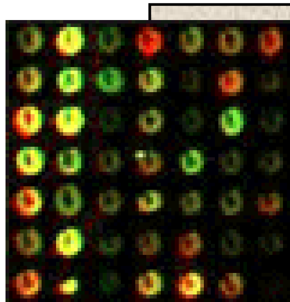
HYBRID SILICA COATING (TEOS:APS=75:25)





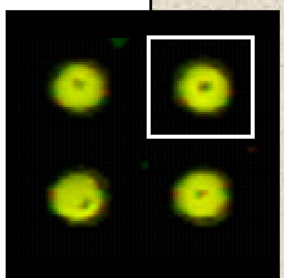
DNA solution (spot)

Hybridization of DNA on Sol/Gel-Derived Microarrays

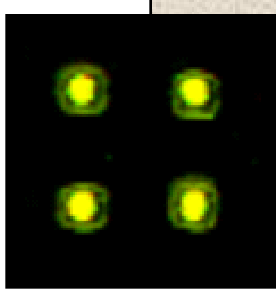


"DOUGHNUTS"

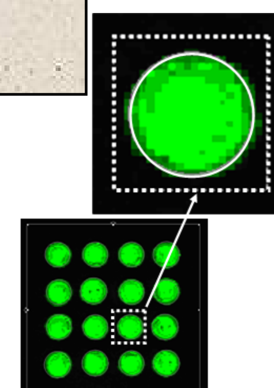
Annular pattern from irregular deposition of DNA



STANDARD APS

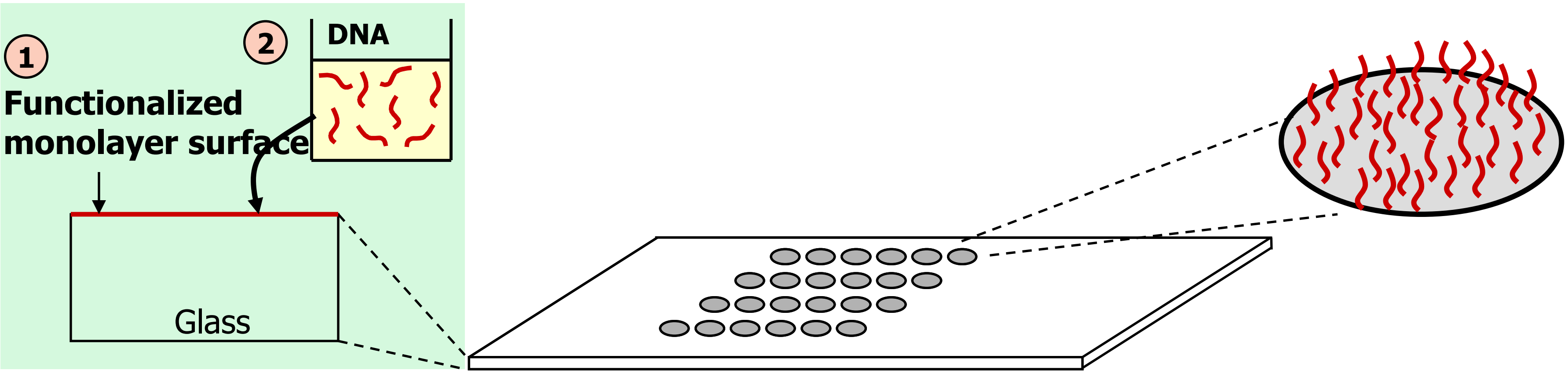


HYBRID Sol/Gel

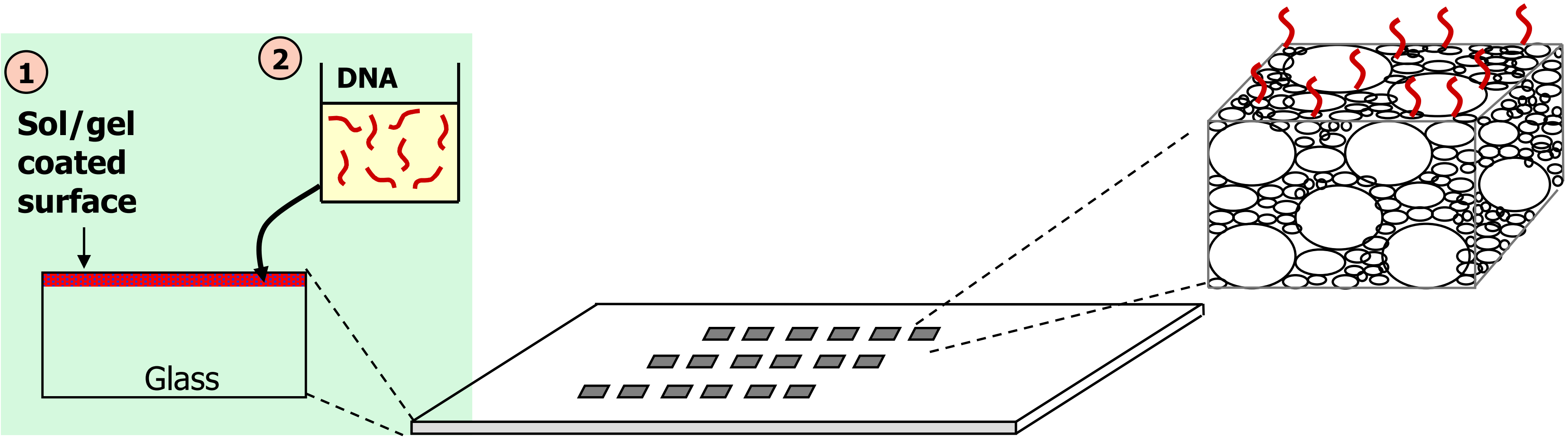


The DNA retention was determined by comparing the initial fluorescence intensit the spots with the intensity after vigorous washing steps to remove non-covalentl bound DNA. The absolute *before* and *after* fluorescence intensities for individual were evaluated by normalizing the intensity of the pixel points within the DNA spo (circular, gray area) to the surrounding region (background, dotted square) as sh here.

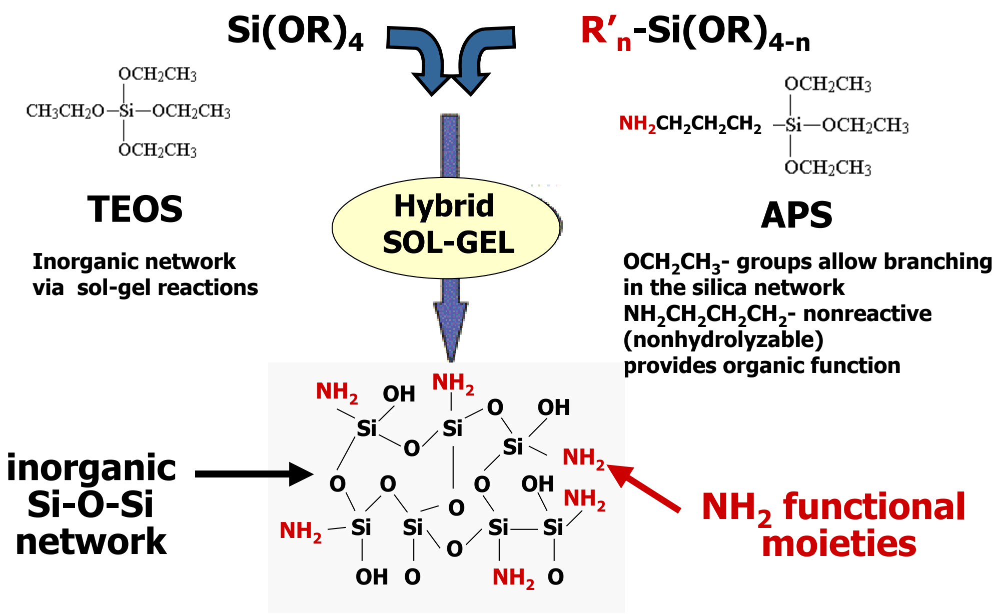
DNA probes immobilized on a glass surface



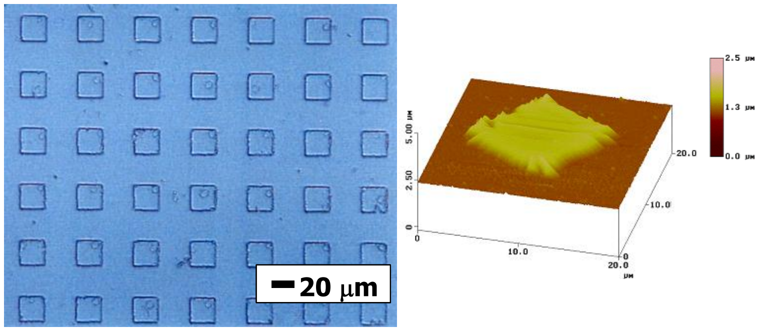
DNA probes immobilized on/in hybrid sol/gel film

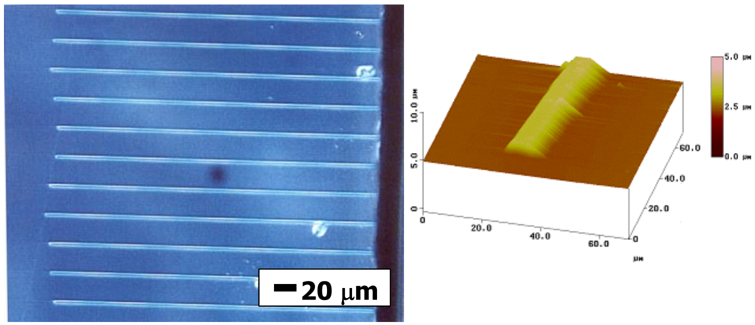


Sol/Gel Derived Porous Oxides & Hybrids

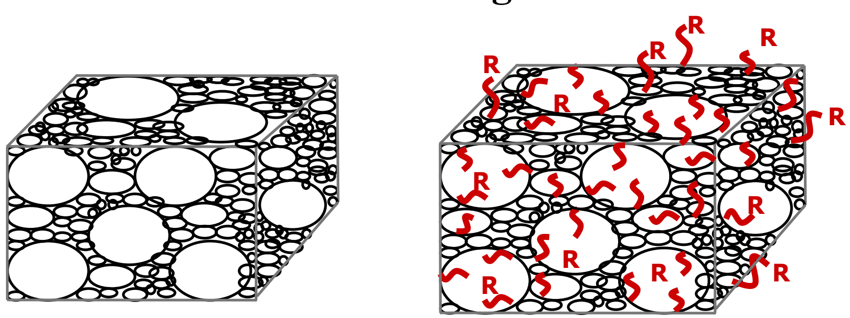


❑ Model Materials for DNA arrays
❑ Novel possibilities for biosensors and "lab-on-a-chip" applications





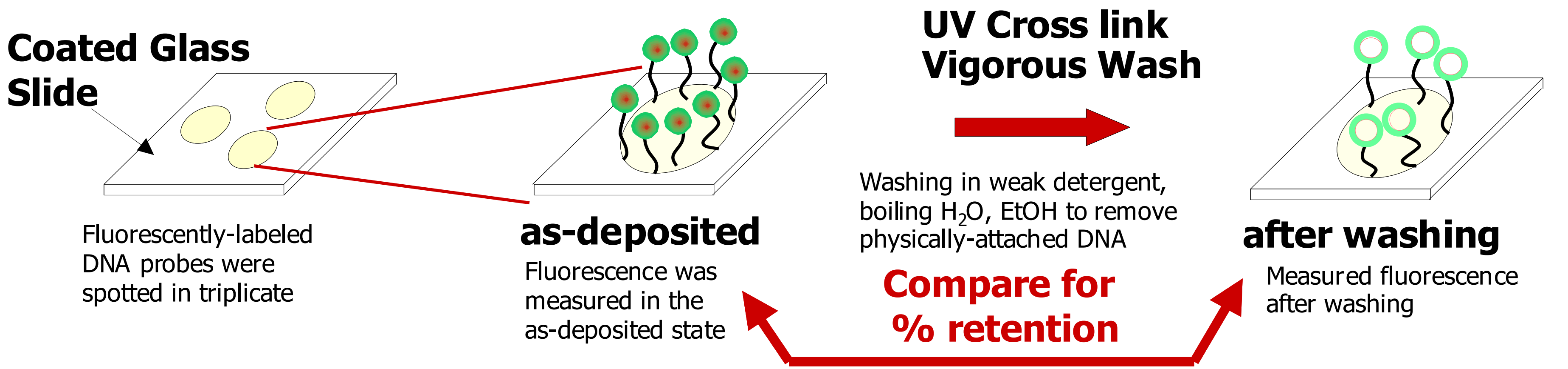
❑ Pore size
❑ Surface Chemistry
❑ Organofunctionality
❑ Surface Charge



see Handbook of Sol-Gel Science
Vol 3, S. Sakka, Ed., 2005, pp.551-576

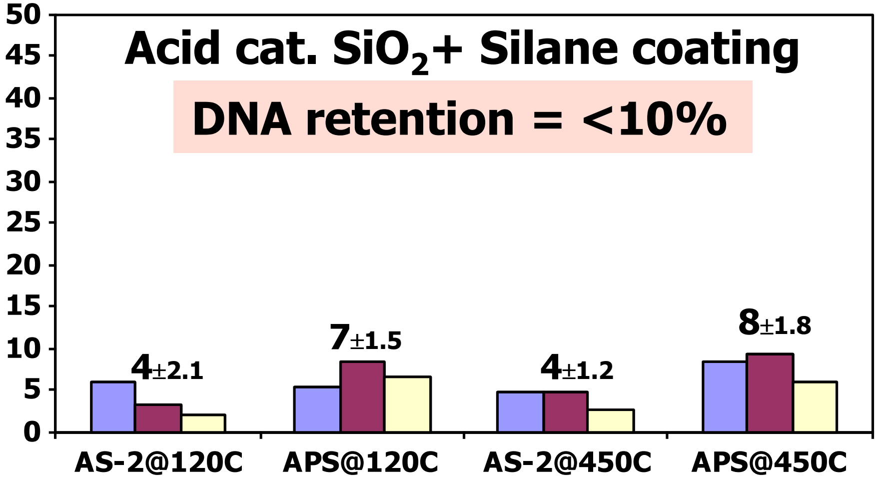
Characterization of Coatings

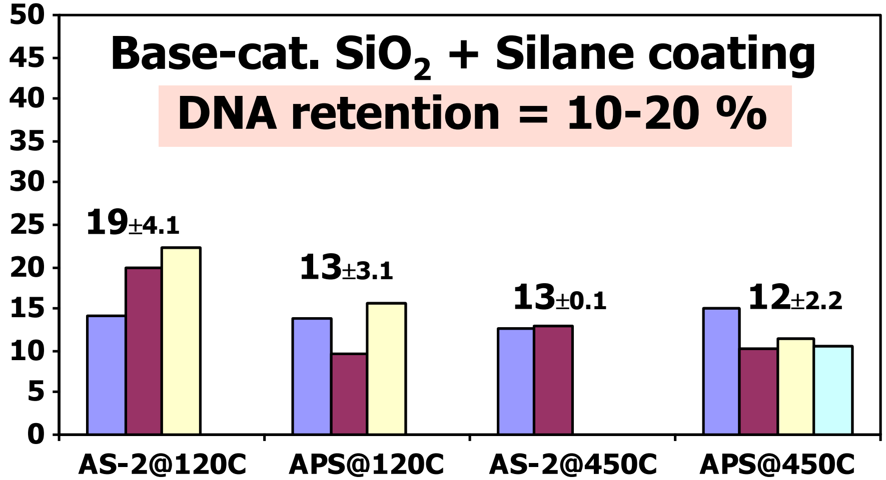
- **Coating Performance**: DNA Retention by Laser Confocal Scanning



- **Chemical Functionality**: XPS
- **Surface Morphology**: AFM
- **Pore Size and Distribution**: BET

DNA Attachment for Aminosilane Modified SiO_2 Coatings

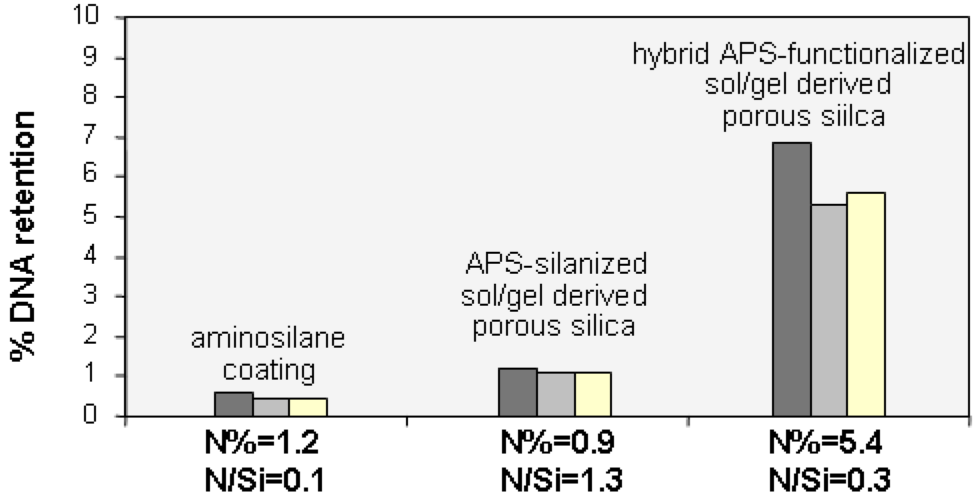




XPS-Surface Functionality (NH_2)

	N (at.%)	N/Si
ACID cat. SiO_2 + Silane coating		
APS treatment	**0.9**	**0.03**
AS-2 treatment	**1.3**	**0.05**
BASE-cat SiO_2 + Silane coating		
APS treatment	**0.8**	**0.03**
AS-2 treatment	**2.2**	**0.08**

NH_2 content of silane coatings on acid and base catalyzed SiO_2 is ≈ 1-2 at.%.

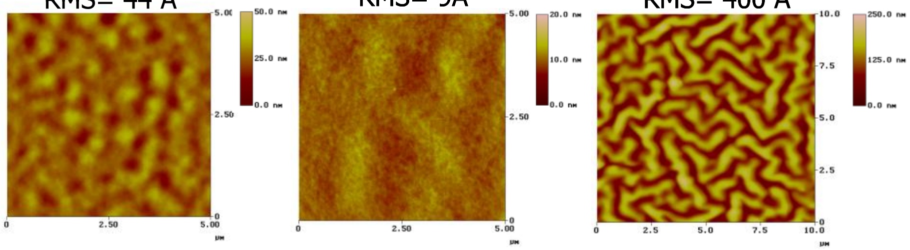


DNA retention and nitrogen (in atomic%) for glass slides with three different coating. DNA retention is determined based on confocal fluorescence measurements. The micro-spotted samples used in the analyses were produced by the same printing session using Cy3-tagged oligos. Each bar represents average retention value for a different slide, each containing six DNA micro-spots.

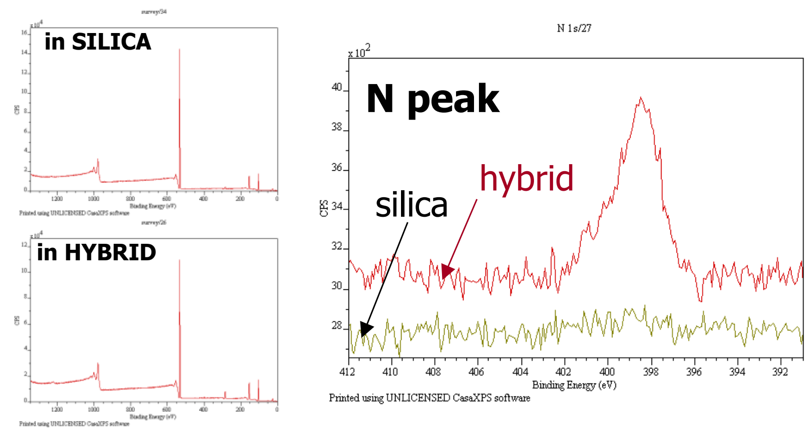
Characterization of DNA Substrates

Atomic Force Microscope (AFM)

Non-cat APS RMS= 44 Å | HCl-cat APS RMS= 9Å | NH_4OH-cat APS RMS= 400 Å

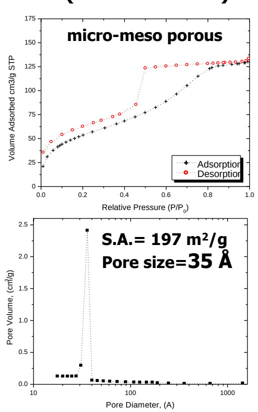


X-Ray Photoelectron Spectroscopy (XPS)

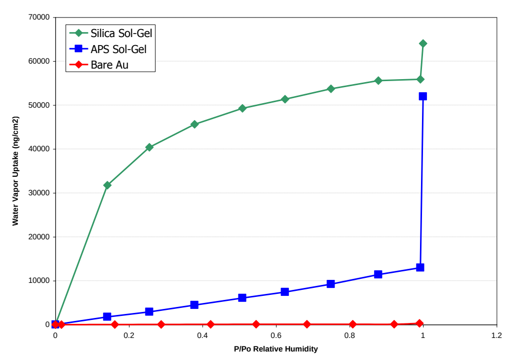


Gas and Liquid Adsorption

(TEOS:APS=3:1)

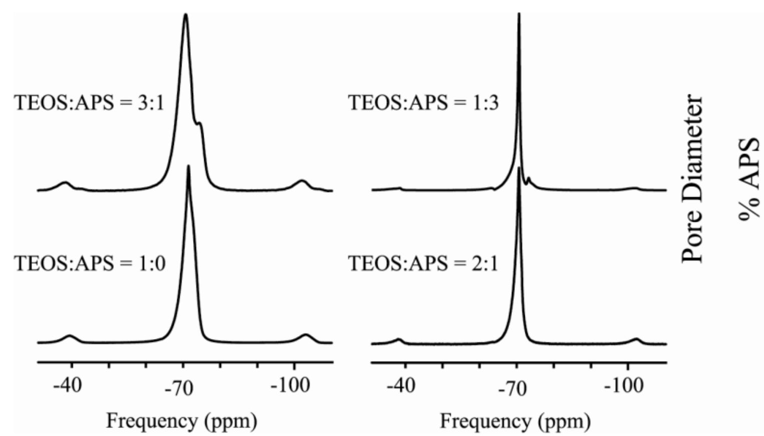


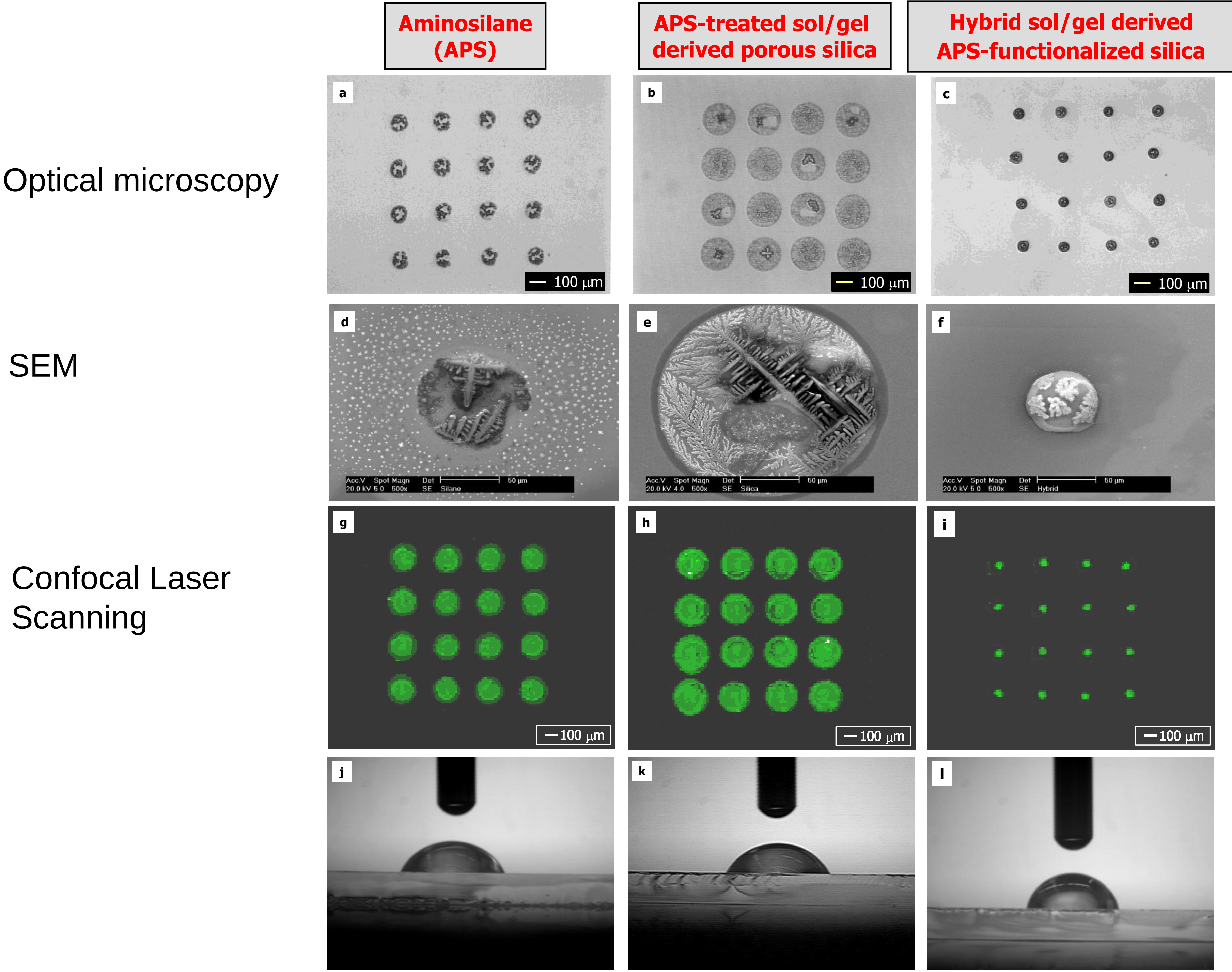
BET



Quartz Crystal Microbalance (QCM)

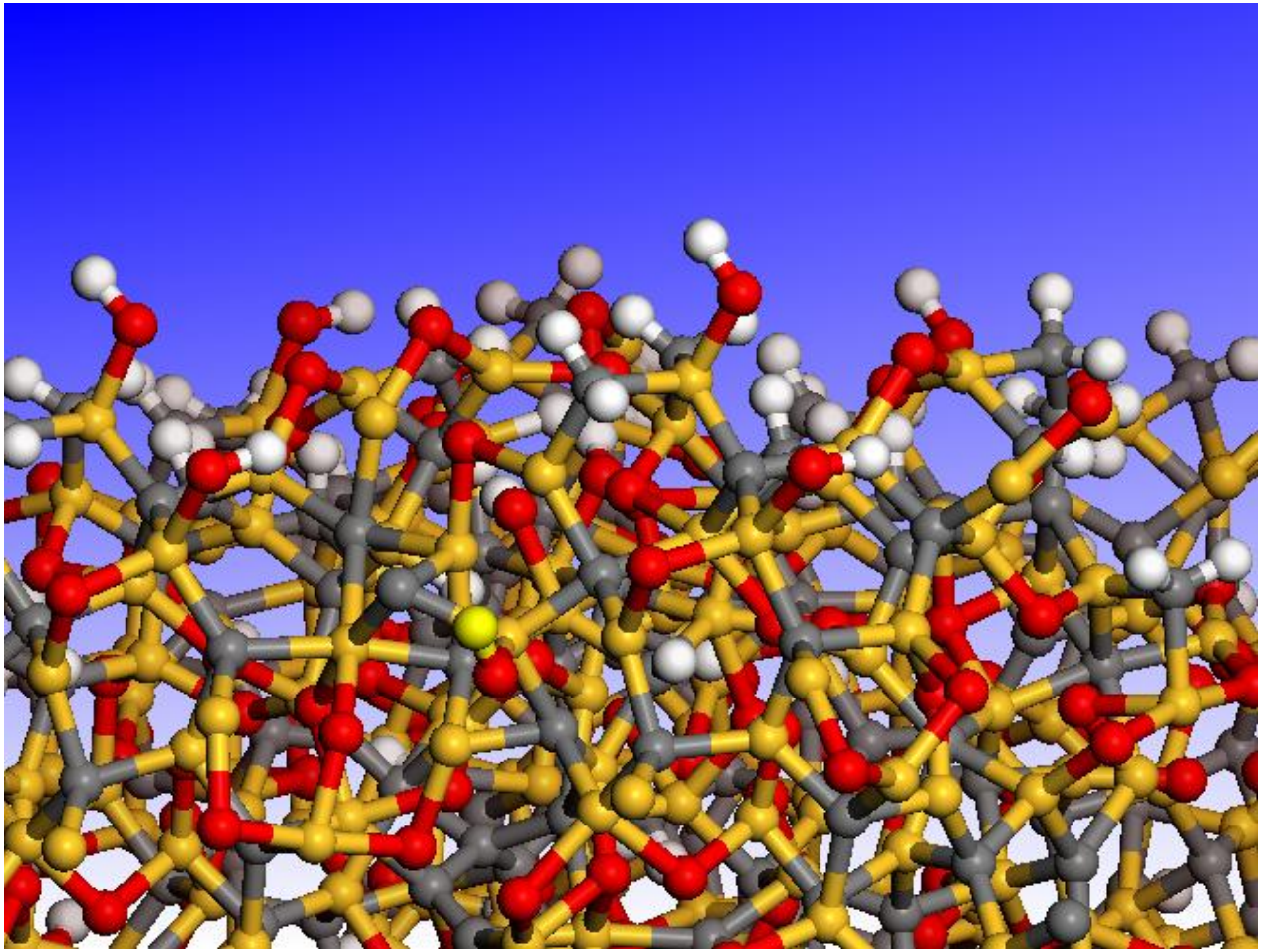
Nuclear Magnetic Resonance Spectroscopy (NMR)



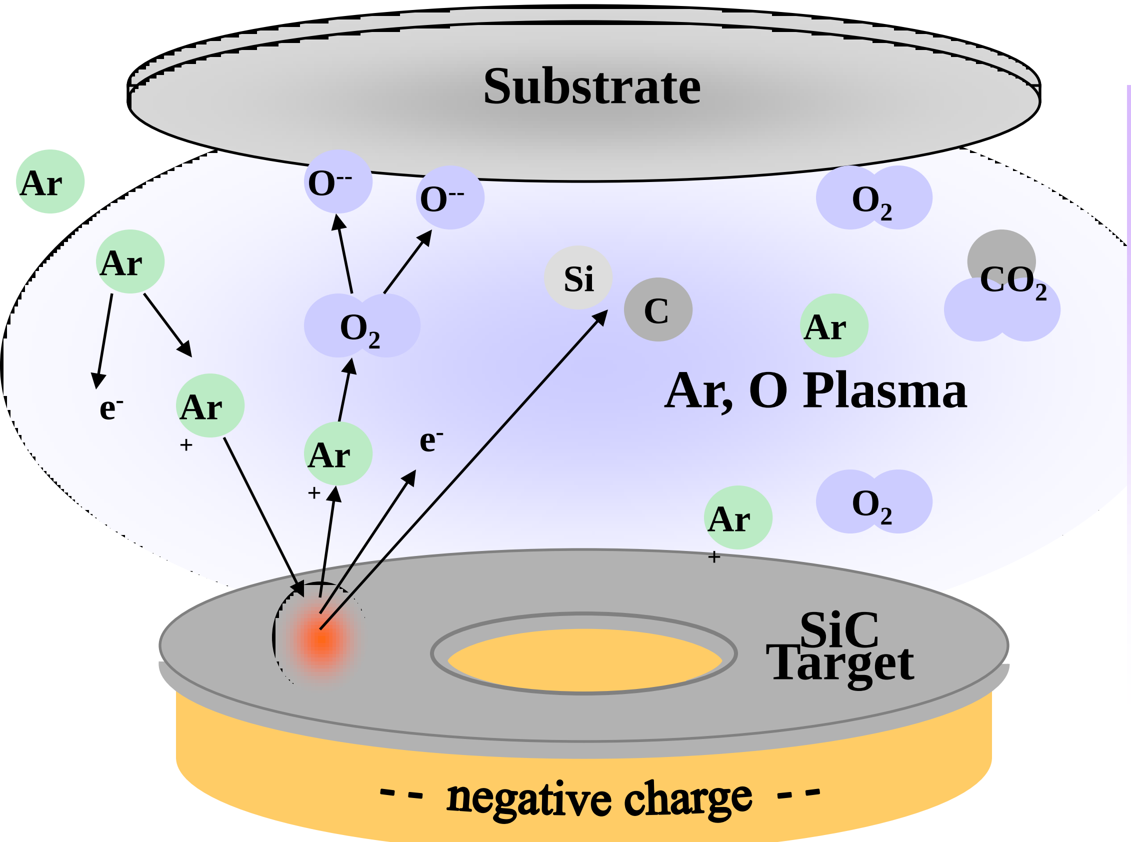


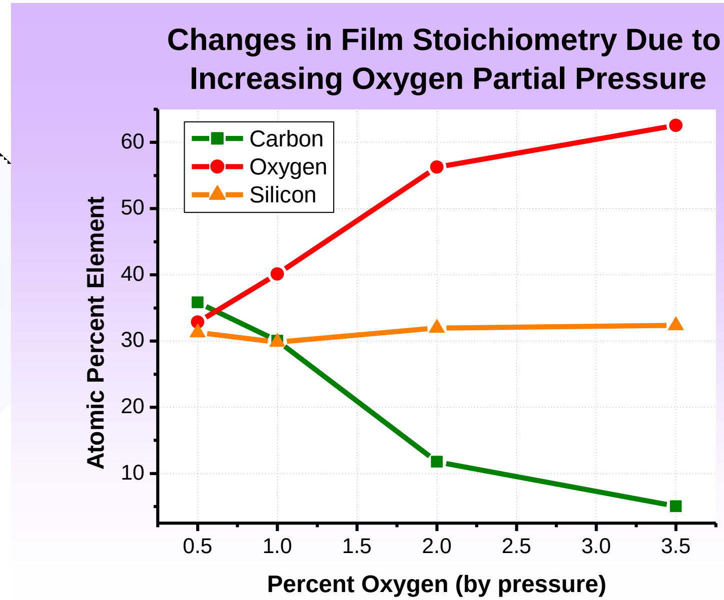
Aminosilane (APS)
APS-treated sol/gel derived porous silica
Hybrid sol/gel derived APS-functionalized silica
Optical microscopy
SEM
Confocal Laser Scanning
a
b
c
100 µm
100 µm
100 µm
d
e
f
Acc.V Spot Magn Det 50 µm
20.0 kV 5.0 500x SE Silane
Acc.V Spot Magn Det 50 µm
20.0 kV 4.0 500x SE Silica
Acc.V Spot Magn Det 50 µm
20.0 kV 5.0 500x SE Hybrid
g
h
i
100 µm
100 µm
100 µm
j
k
l

Silicon Oxycarbide Glass

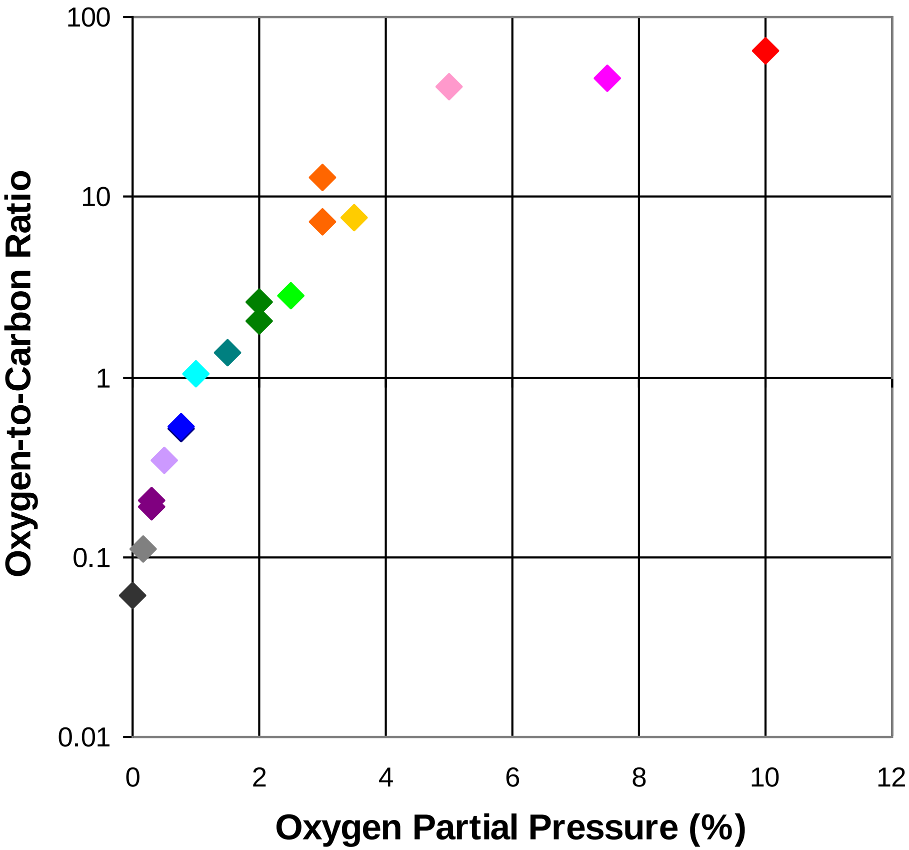


Reactive sputter deposition of Si-oxycarbide from a Si-carbide target





Varying oxygen partial pressure yields compositions from SiO_2 to SiC



XPS Atomic Percentage			Color	O/C Ratio
Si 2p	O 1s	C 1s		
42.8	3.3	53.9		**0.061**
43.3	5.7	51.0		**0.11**
41.3	9.4	49.3		**0.19**
41.5	10.1	48.4		**0.209**
39.0	15.6	45.5		**0.343**
38.4	20.9	40.8		**0.512**
38.4	21.4	40.2		**0.533**
36.3	32.3	31.4		**1.03**
35.6	37.0	27.3		**1.36**
36.3	42.8	20.9		**2.05**
34.4	47.4	18.3		**2.60**
34.9	48.2	16.9		**2.85**
37.0	55.4	7.6		**7.3**
36.0	56.6	7.4		**7.7**
36.5	58.9	4.6		**12.7**
35.3	63.2	1.5		**40.9**
35.5	63.1	1.4		**45.2**
35.4	63.6	1.0		**64**

Blood Coagulation versus Surface Composition

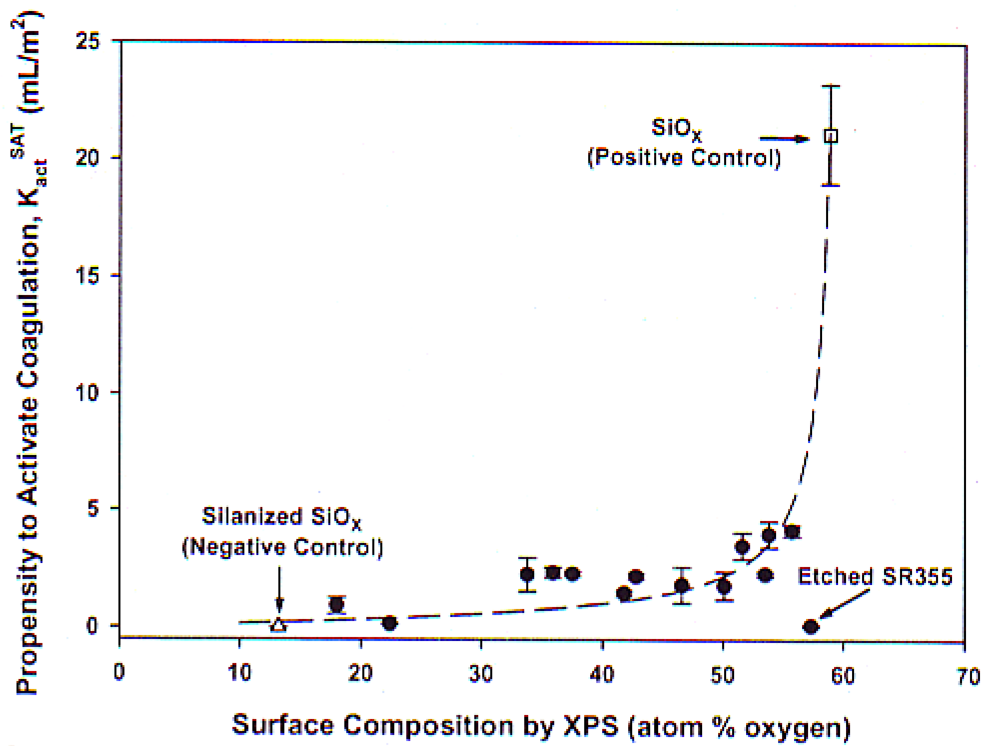
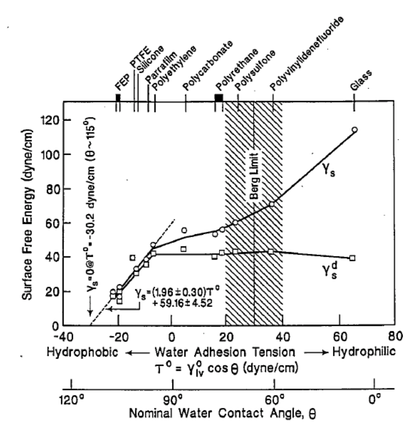


Fig. 3. K_{act}^{SAT} versus (surface) oxygen content for the various SiO_xC_y and reference samples. ((●) SiO_xC_y glass samples, (△) OTS treated glass, (□) clean glass). Uncertainty indicated by error bars represent standard deviation of mean for $N = 3$.

see Acta Biomaterialia, 1, 583 (2005)

E.A. Vogler / Adv. Colloid Interface Sci. 74 (1998) 69–117



PENNSTATE

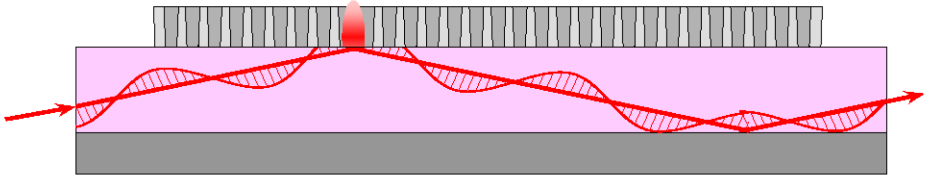
Materials Research Institute

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

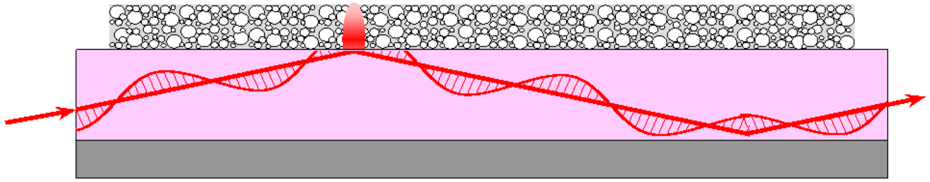
Center for Glass Surfaces, Interfaces and Coatings

Nanoporous IR Transparent Amorphous Coatings for Chem-Bio Sensors: Functionalization and Biomolecule Immobilization

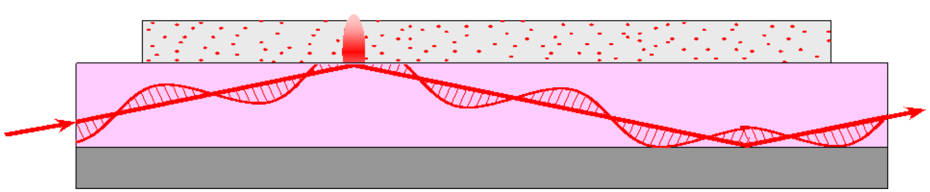
Sculptured Thin Films

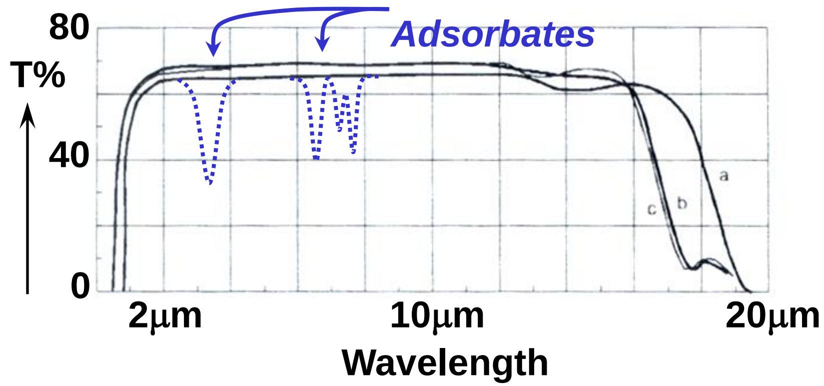


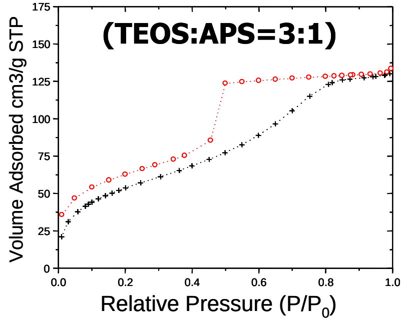
Hybrid Sol/Gel

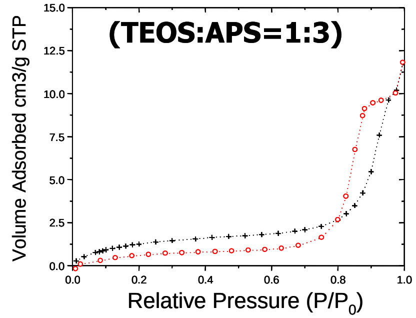


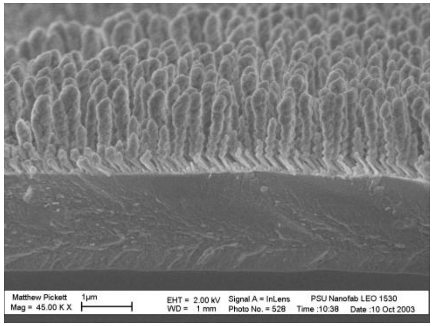
Nanoparticle Coating

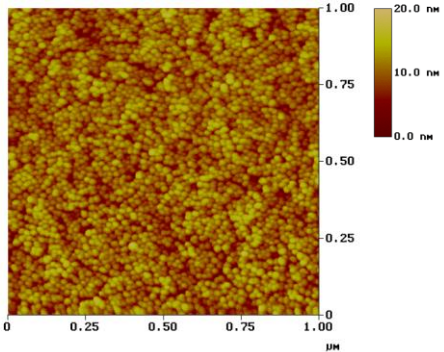










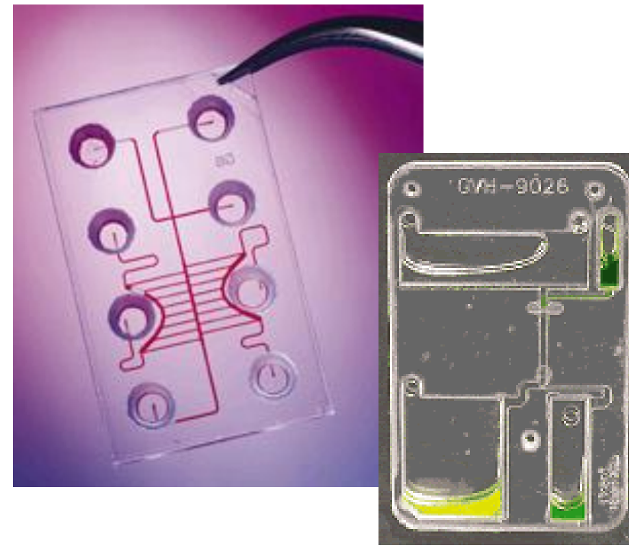


Micro-Systems for Bioanalytical Applications

“Lab-on-a-chip systems”

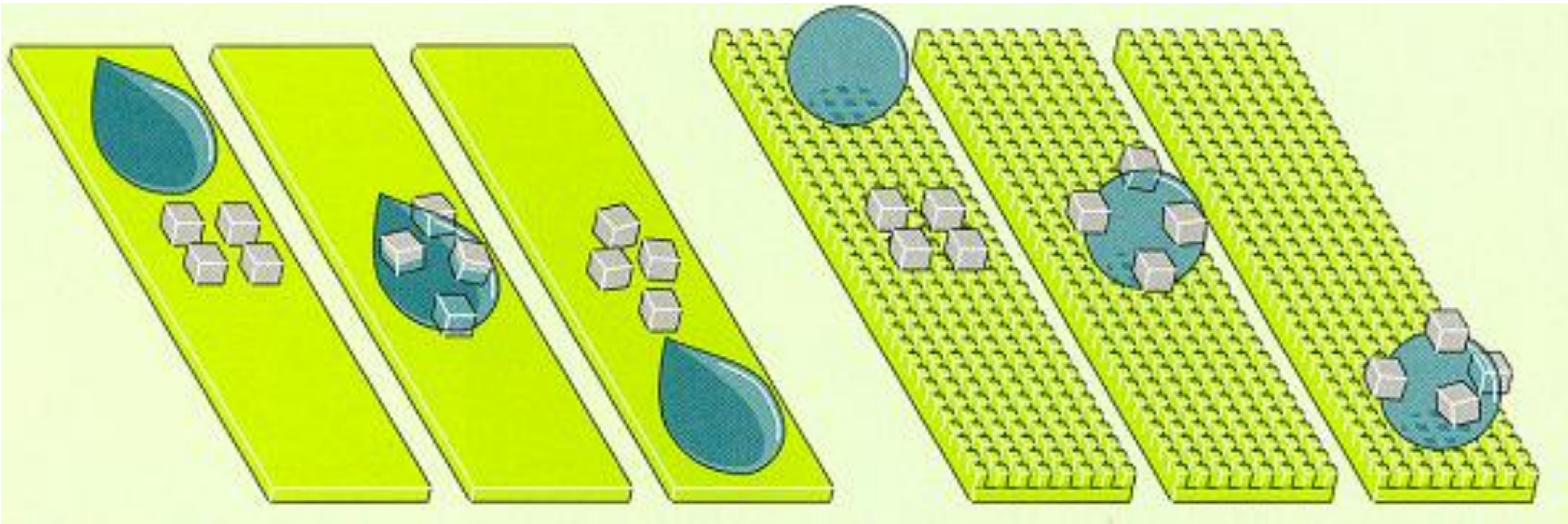
Applications

- ❑ Biological analysis and assays
- ❑ Biological or industrial sensors
- ❑ Chemical analysis and synthesis
- ❑ Bio-reactors
- ❑ Medical diagnosis
- ❑ Drug discovery-delivery



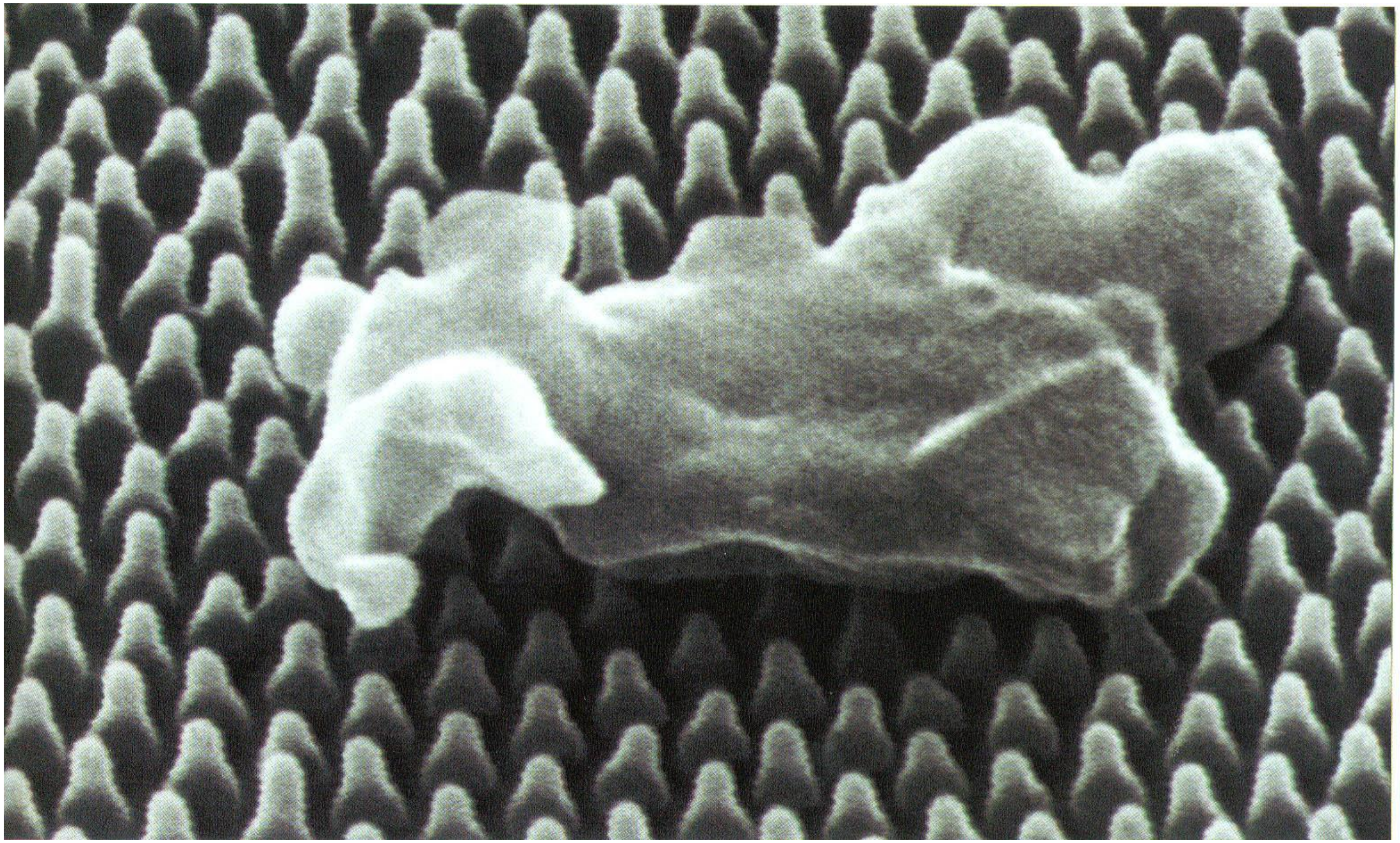
Advantages of Microsystems

- ❑ Smaller reagent (biological samples) volumes
- ❑ Improved selectivity and sensing (high surface area/volume)
- ❑ Increased reaction/assay speed
- ❑ Parallel and simultaneous analyses of large number of assays

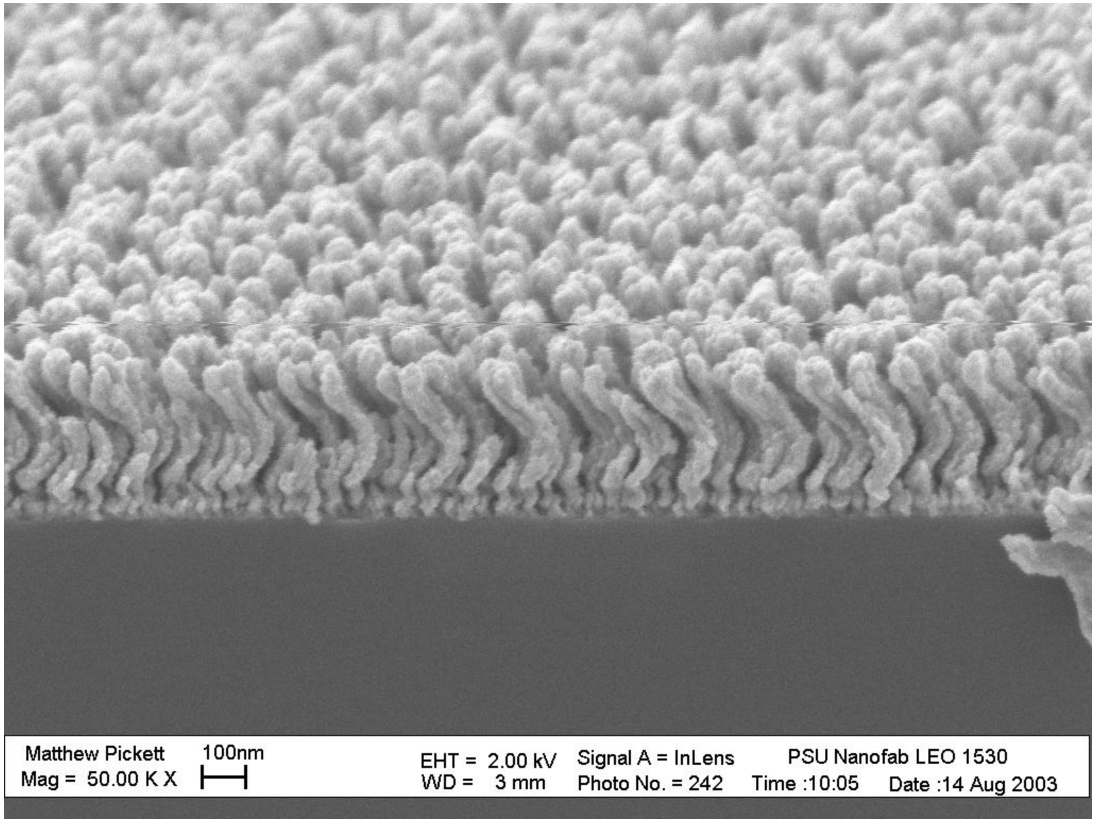


smooth hydrophobic surface

super-hydrophobic surface

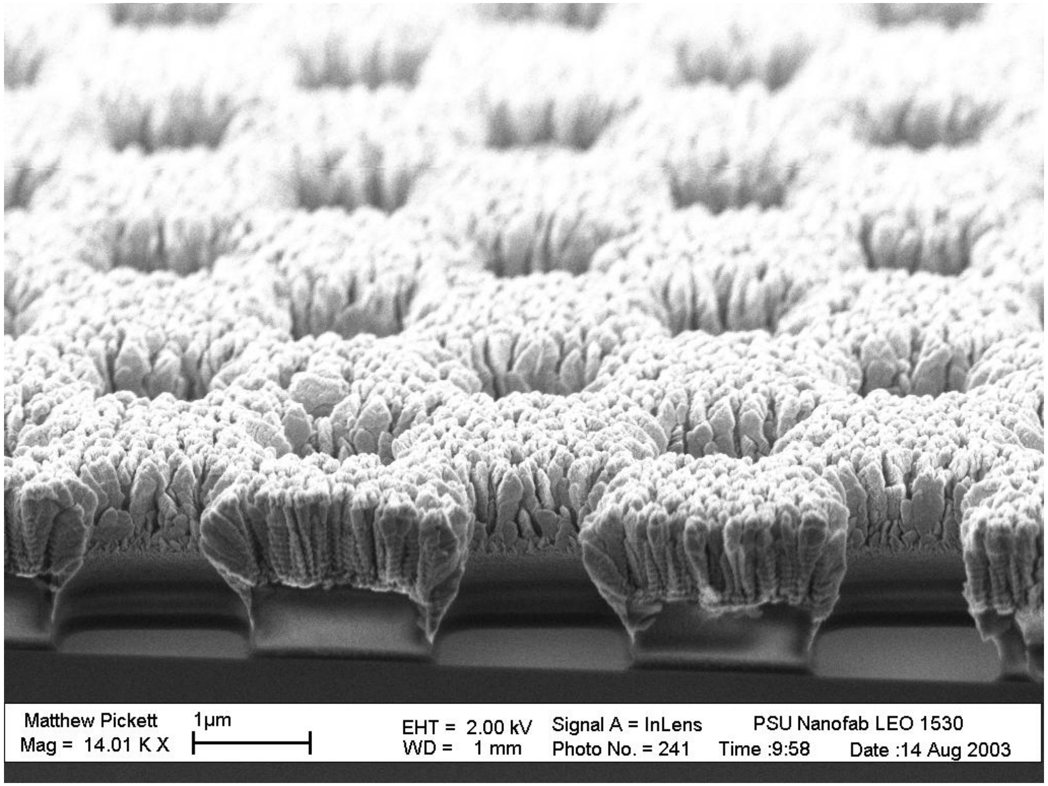


Sculptured Thin Films

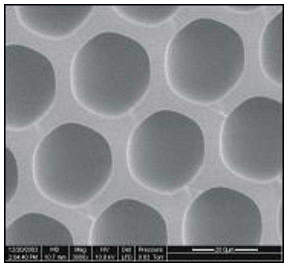


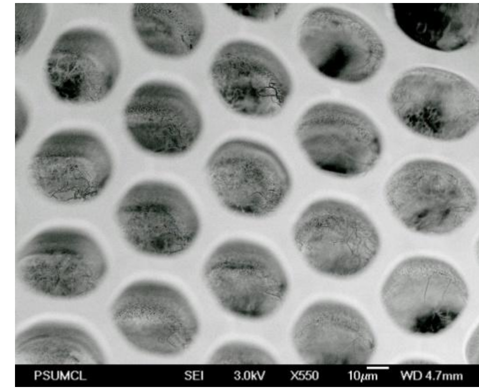
- Metals, Semiconductors and Oxides

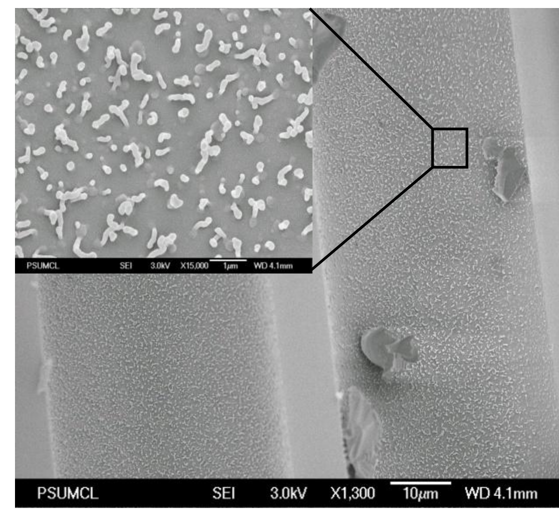
Sculptured Thin Films



MULTICAPILLARY FRACTIONATING COLUMNS

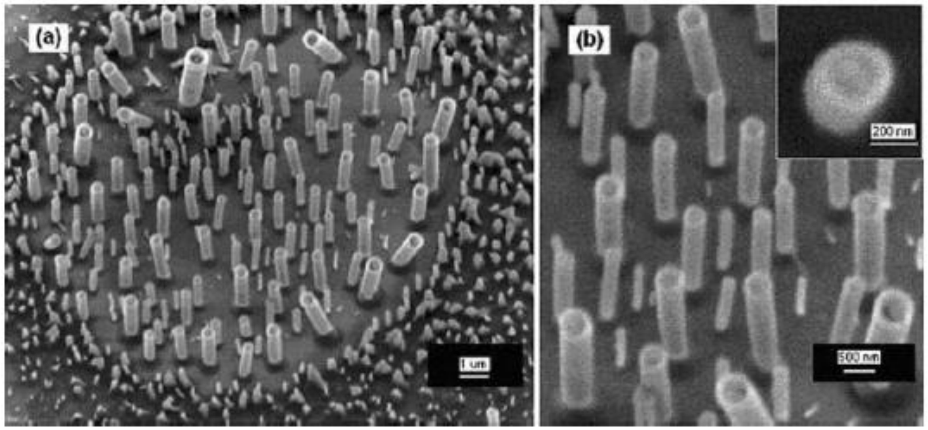


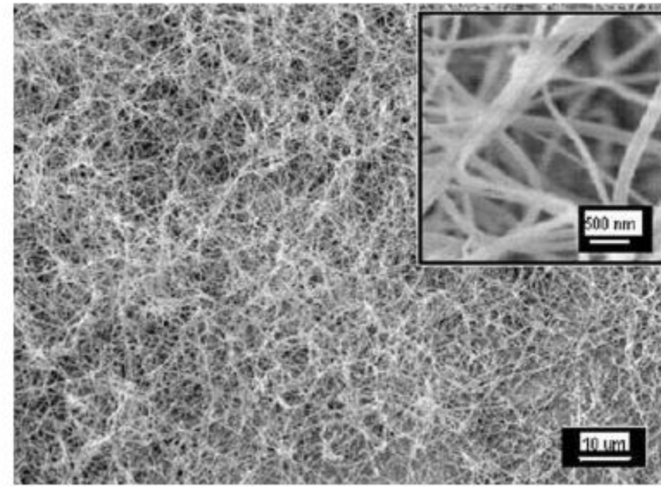




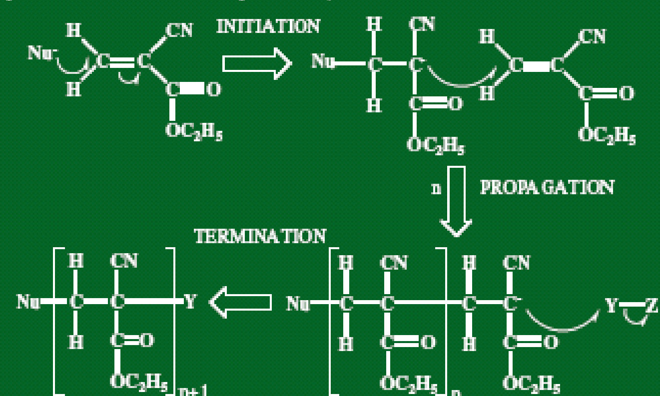
ChromBA
THE PINNACLE IN CHROMATOGRAPHY

glass surface-catalyzed growth of cyanoacrylate nanofibers



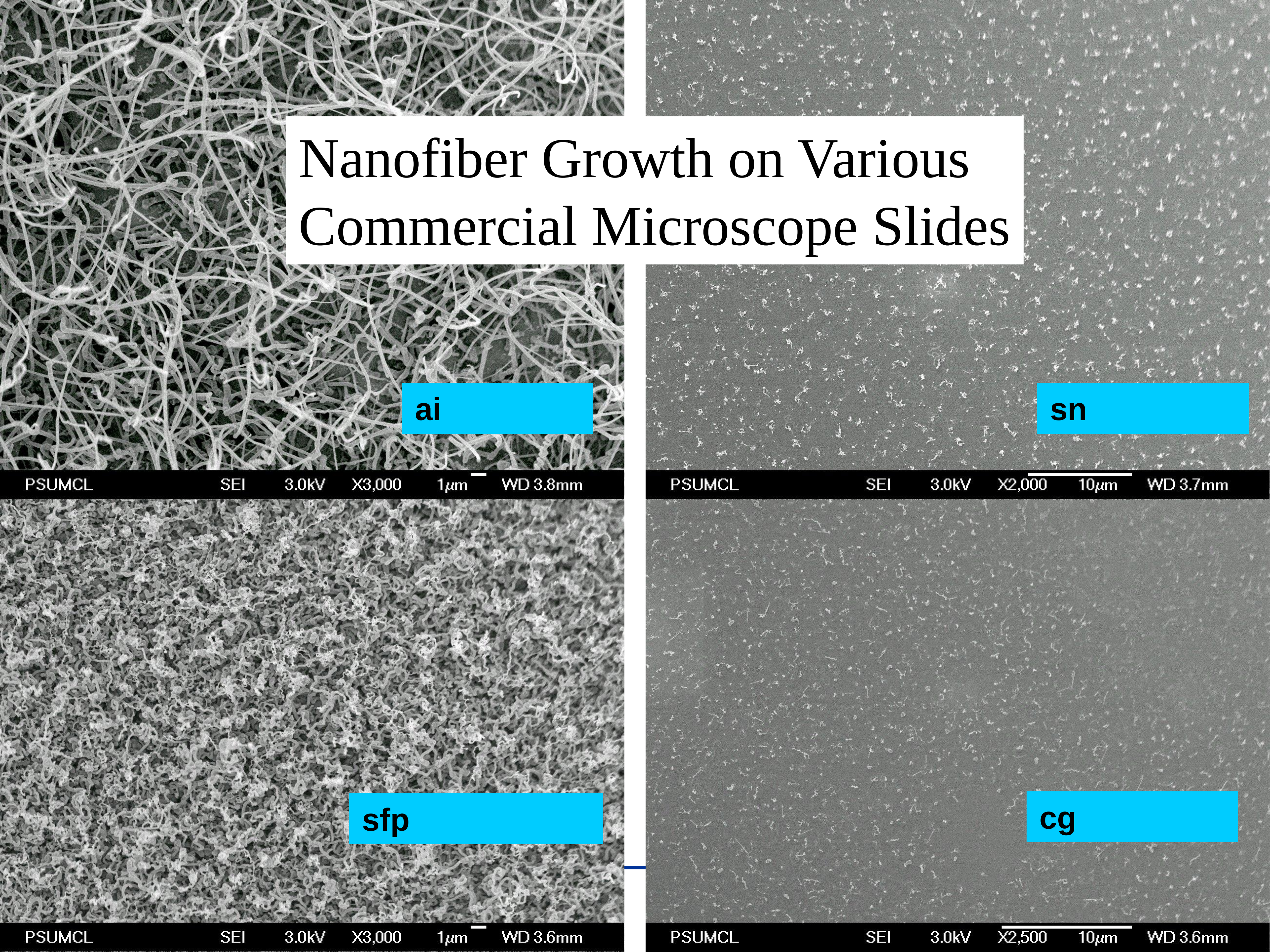


- Vapors of Ethyl 2-cyanoacrylate (ECA) undergo rapid anionic polymerization initiated by nucleophilic attack as follows:

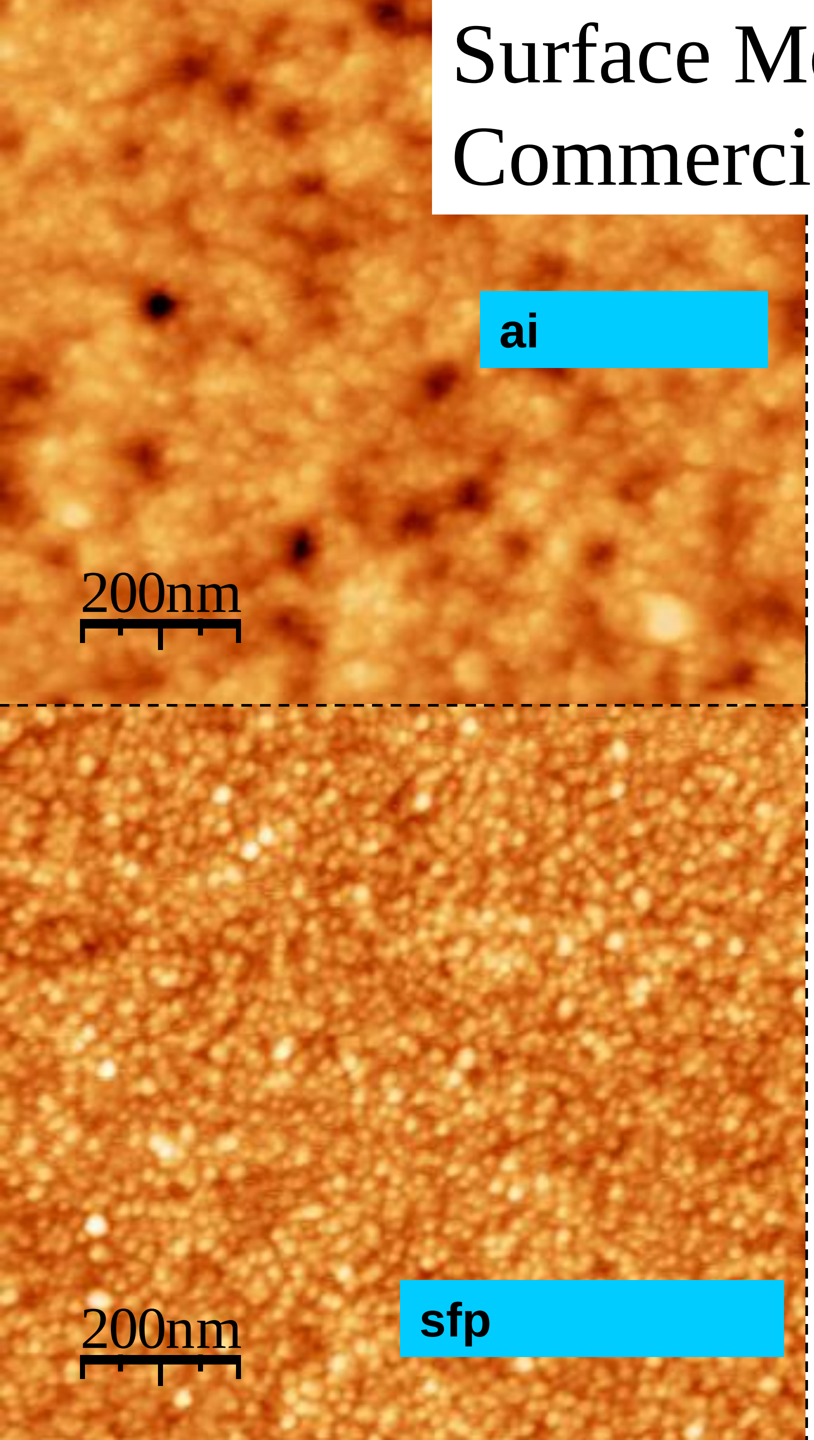


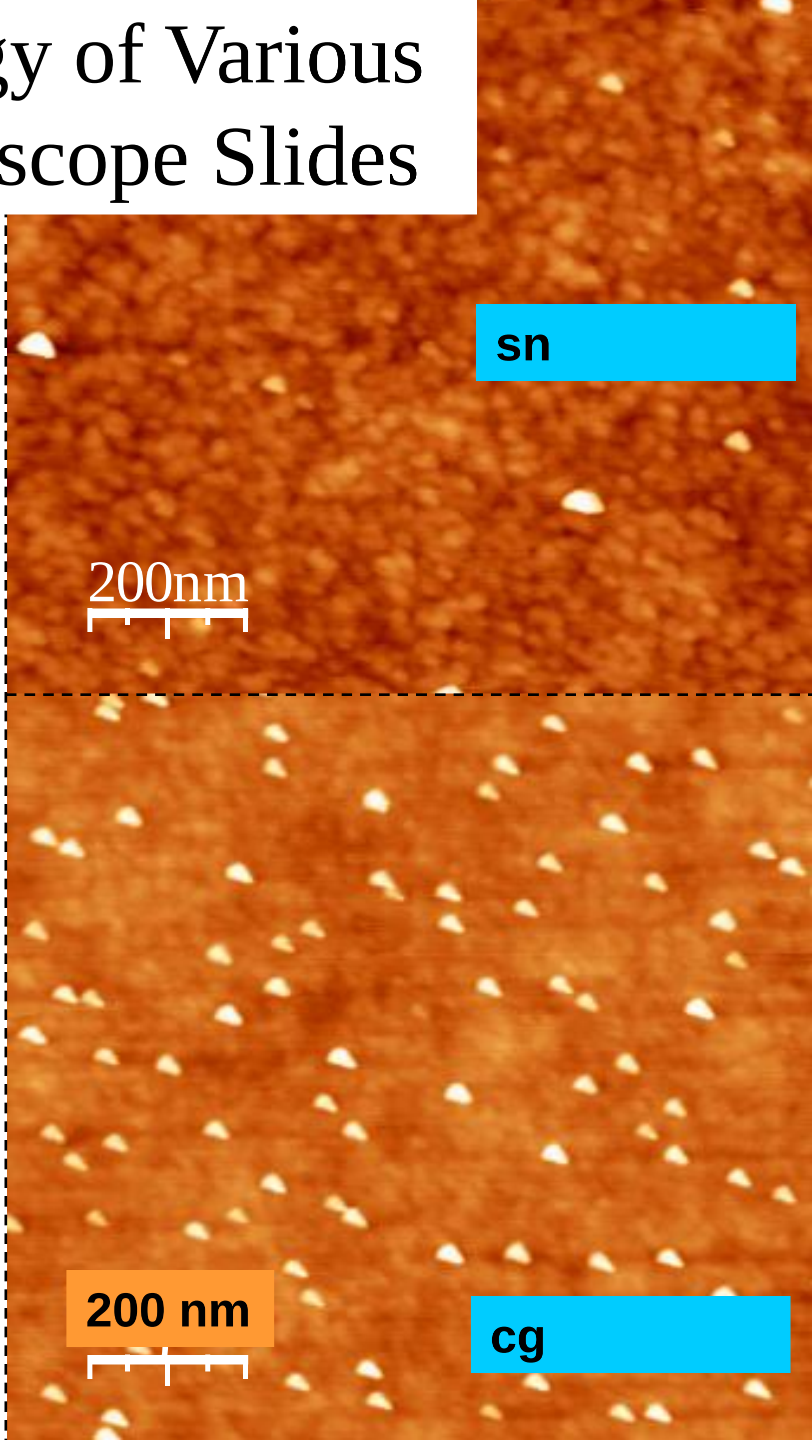
see PJ Mankidy, et al
- Chem Comm, 2006
- Nanoletters, 2006

Nanofiber Growth on Various Commercial Microscope Slides



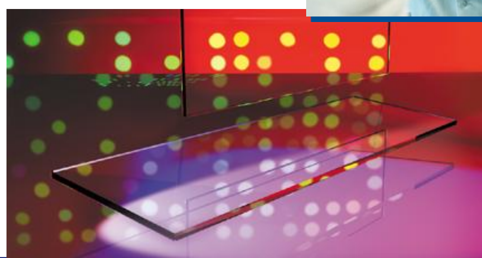
Surface Morphology of Various Commercial Microscope Slides





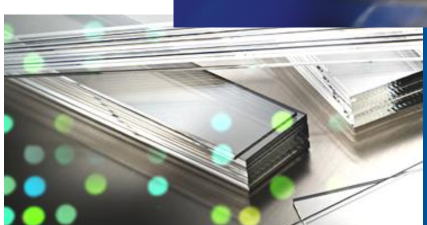
The value chain to various areas of commercial and developmental interest

Drug Discovery
Diagnostics
Research



Microarrays

Coatings

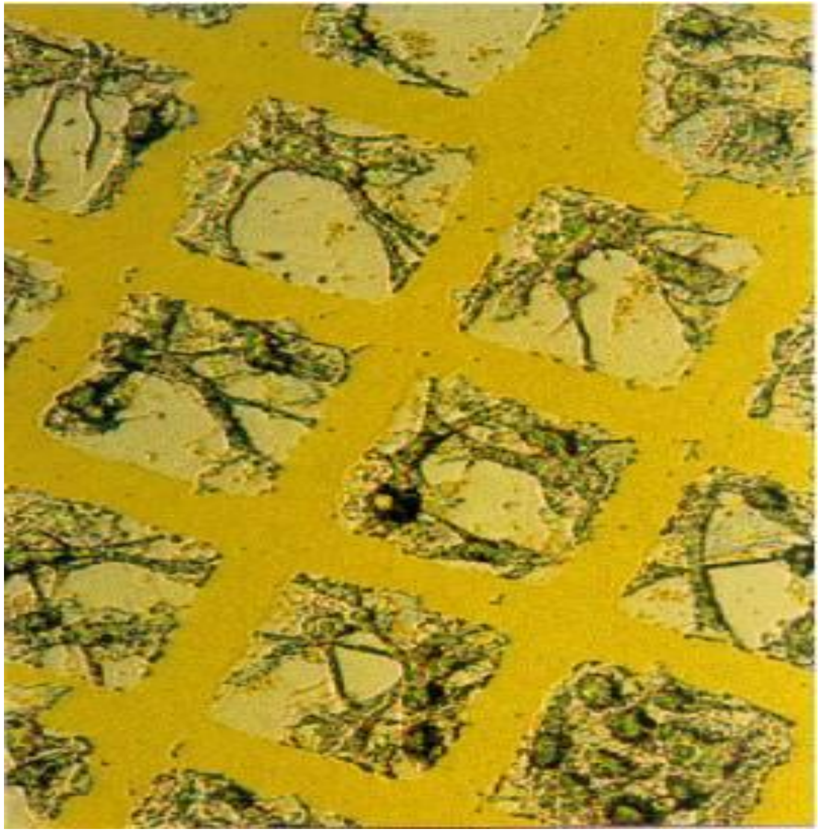


Materials

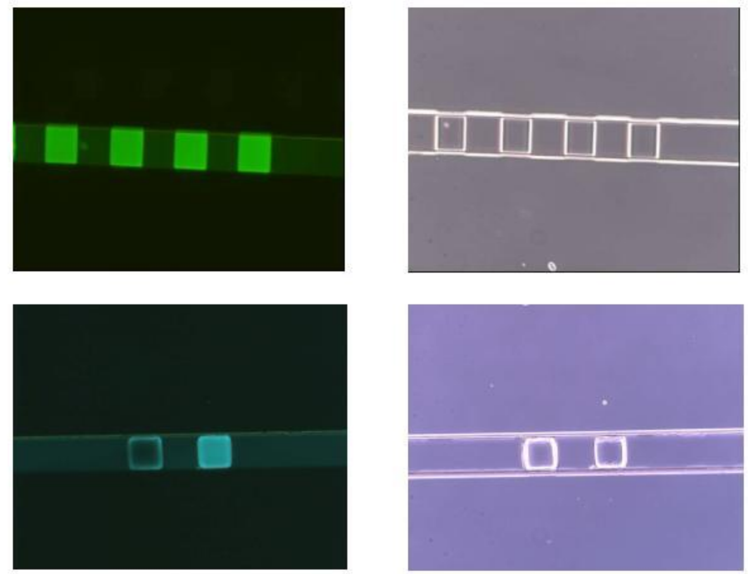
Glass Surfaces and Coatings for Biotechnology

- Glass is a low cost material that keeps on giving through value added compositional tailoring, surface treatment and coating.
- Glass surface composition, organofunctionalization and other monolayer coatings can be used to control (surface) reactivity from passive to active.
- Sol/Gel coatings and other nanostructures offer a way to control (surface) reactivity through nanoporosity
- Glass surfaces and sol/gel coatings can be readily hydrated and/or functionalized…. biology likes water!
- Glass surfaces and coatings can be patterned for arrays, microfluidics, biomolecule immobilization, cell transfers, (living) cell encapsulation and lab-on-a-chip, in general.

Patterned Hydrogels for Sensors

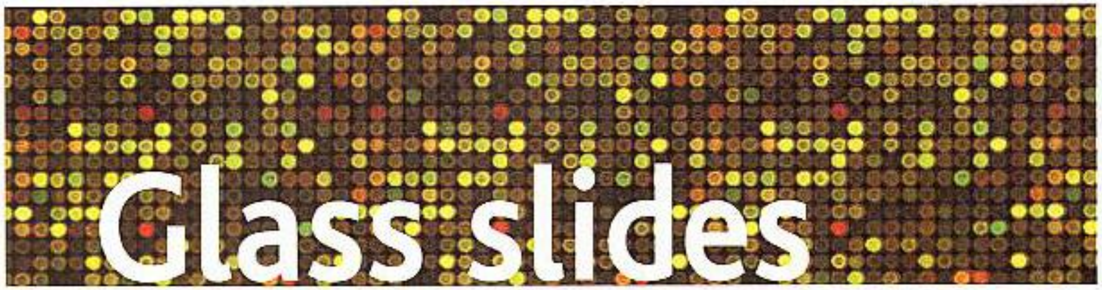


cell and protein based biosensors using patternable hydrogel materials



Hydrogel polymerizaton is initiated by UV light and can support functional proteins and cell growth

M. Pishko



Glass slides to DNA microarrays

by Samuel D. Conzone* and Carlo G. Pantano†

A tremendous interest in deoxyribonucleic acid (DNA) characterization tools was spurred by the mapping and sequencing of the human genome. New tools were needed, beginning in the early 1990s, to cope with the unprecedented amount of genomic information that was being discovered. Such needs lead to the development of DNA microarrays; tiny gene-based sensors traditionally prepared on coated glass microscope slides. The following review is intended to provide historical insight into the advent of the DNA microarray, followed by a description of the technology from both the application and fabrication points of view. Finally, a description of the unmet challenges and needs associated with DNA microarrays will be described to define areas of potential future developments for the materials researcher.

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Most individuals, outside of academic circles focused on genomics, became aware of the potential commercial, technical, and social importance of the human genome project during the late 1990s. The human genome project was formally initiated in 1990[1] and was expected to last 15 years. It had the major goals of identifying all of the genes in human DNA, determining the sequences of those genes, and storing the information in public databases. However, the project moved quickly from the onset and, by 1998, the Department of Energy (DOE) and the National Institutes of Health (NIH) predicted that the human genome project would be completed by 2003.

The big buzz about biotech

The tremendous success in rapidly mapping and sequencing the human genome (a working draft sequence of the human genome was completed in 2000), has lead many commentators to predict that similar achievements would follow on the applications side, leading to unprecedented discoveries related to human health[2,3]. Gaudy promises of high-tech clinics with the ability to prescribe drugs based on the genetic make-up of the patient were well ahead of their time. This normal lag from discovery (the sequenced human genome) to true applications (genetically engineered drugs) is partially attributable to the lack of tools, which could enable researchers to utilize effectively the tremendous amount of information that was generated during the human genome project.

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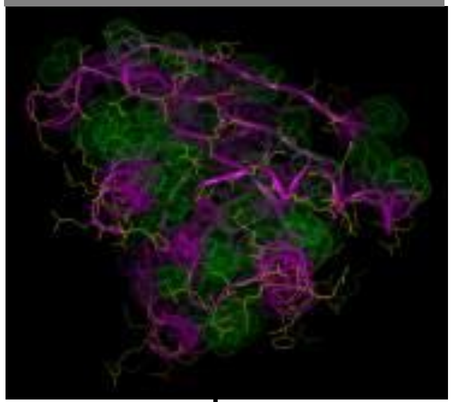
These high-level technological advancements and major markets eventually percolate to the „glass scientist“

How do I package this fragile, "sticky," complex, liquid-formulated drug in a glass container, while ensuring stability, low cost?

Biotherapeutic



- Complex, unstable "protein"
- Expensive
- Liquid formulated
  (infinite chem, viscosities)
- High/low concentration
  (1 to >1000 μg/ml)

Packaging

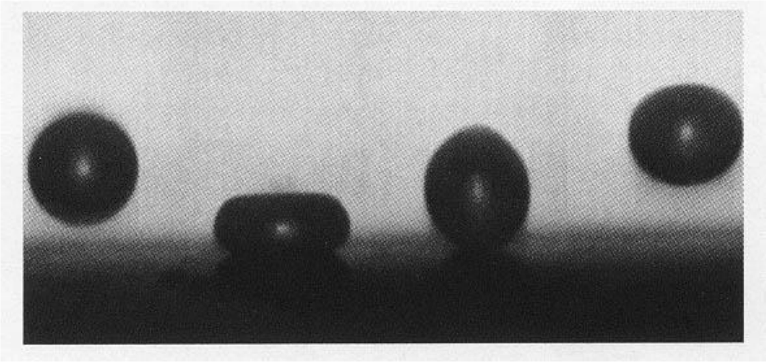
- Borosilicate glass
  (NOT inert and only part of system)
- Sterilize-able
- With, w/o lubricant
- Multiple formats
- Must be low cost

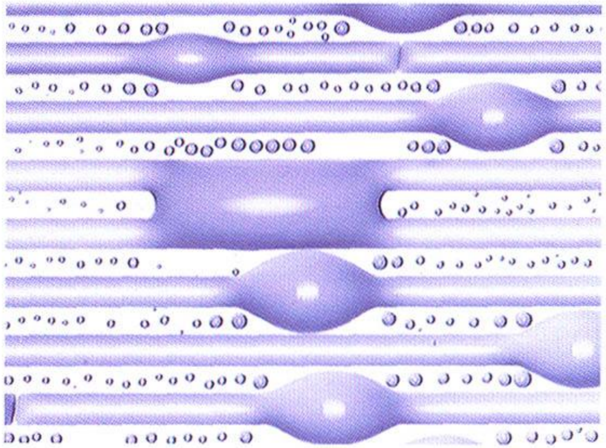
Packaged Drug

- Stable (~2 yrs)
- Economical
- FDA compliant
- Mass produce-able

superhydrophobic/superhydrophilic surfaces and coatings

- substrates for biotechnology
- patternable for microfluidics
- easy-clean surfaces





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PerioGlas™

(Bioglass® Synthetic Bone Graft Particulate)

A *chemically* bonded implant-tissue interface . . .the consequence of which is more rapid filling of defects than is produced by materials such as hydroxylapatite, which is merely osteoconductive.

- ☐ Rapidly fills bony defects by osteoproduction
- ☐ Effective in repairing and restoring the periodontium
- ☐ Suction placed adjacent to site does not disturb the material
- ☐ Developes bond to both bone and certain soft tissue
- ☐ Initiates a *rapid* chemical bond which inhibits epithelial downgrowth
- ☐ Easily mixed, transferred and contained in site

Note: Wilson, J. Low S. Bioactive Ceramics for Periodontal Treatment... *Journal Of Applied Biomaterials*, Vol 3, 123-129 (1992)

PerioGlas™ (Bioglass® Synthetic Bone Graft Particulate) is shipped sterile in its own mixing cup – six (6) 0.5 cc units to a box (Pk6). Instructions accompany each box.

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Pre-operative x-ray of a patient with a six (6) millimeter periodontal lesion and a three (3) wall defect.

Post-operative x-ray showing placement of radiopaque PerioGlas™/Bioglass® *which is clearly visible at the gingival margin.*

Five year post-operative x-ray now showing *normal* bone height. *No* evidence of a recurrence of the periodontal disease.

PerioGlas™ in the surgical site of a patient with an 8mm-3 wall defect. Note how PerioGlas™ does not migrate and adapts easily to the defect.