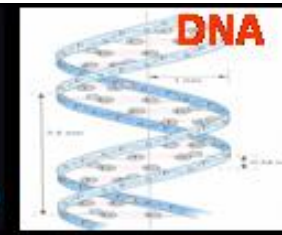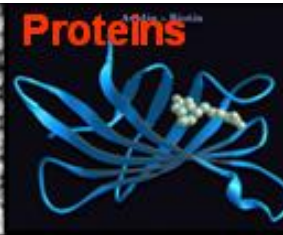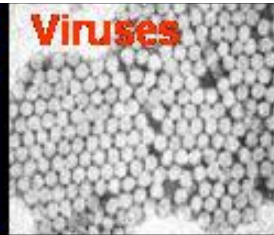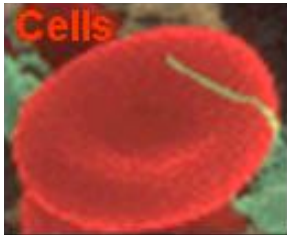# Key Topics

- **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)

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

Scale equation:

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

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

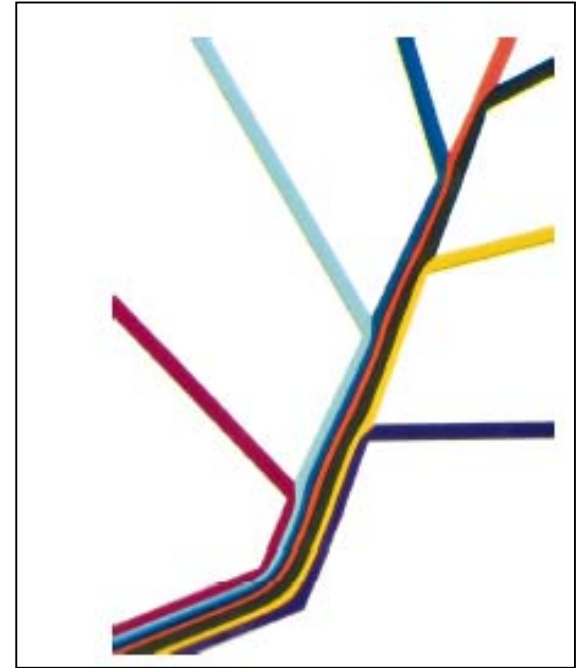$$\text{where } \mathrm{Re} = \frac{\rho u L}{\mu}, Fr^{-2} = \frac{gL}{u^2}$$

Wereley, et al. Purdue

Dimensionless Parameters

- Assume water flow;

$$\mu = 10^{-3} \text{ kg/(s-m)}, \quad \rho = 10^3 \text{ kg/m}^3$$

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

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

Wereley, et al. Purdue

Re in BioChips and Laminar Flow

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

4

Microfluidic Mixing

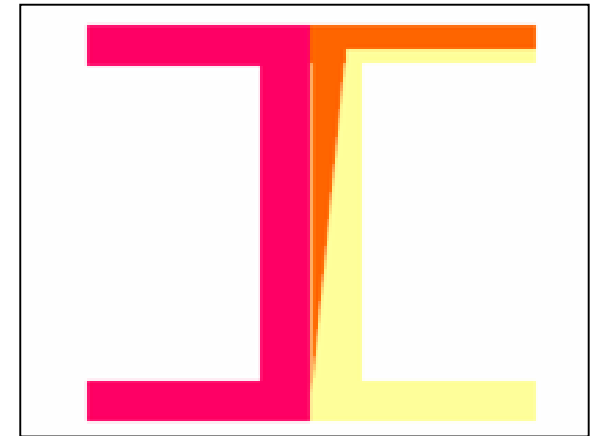- Mixing only by diffusion (or novel structures using hydrodynamics)
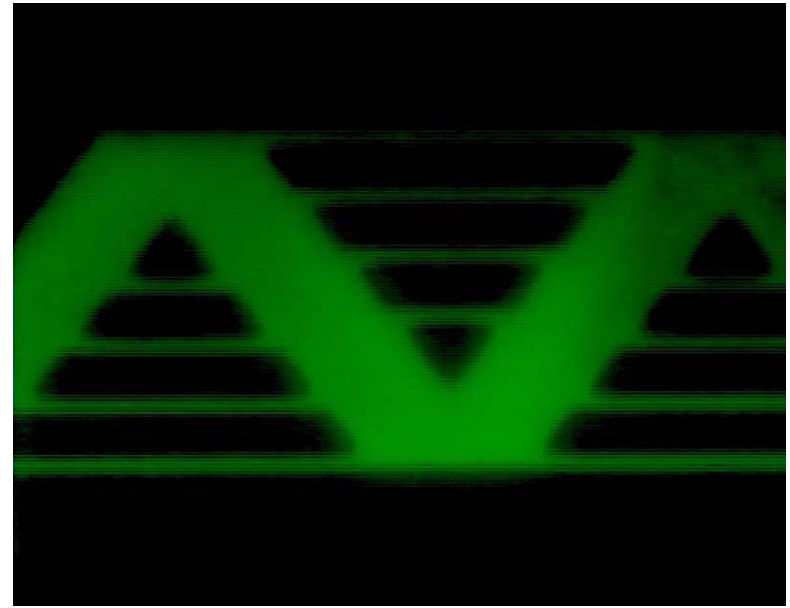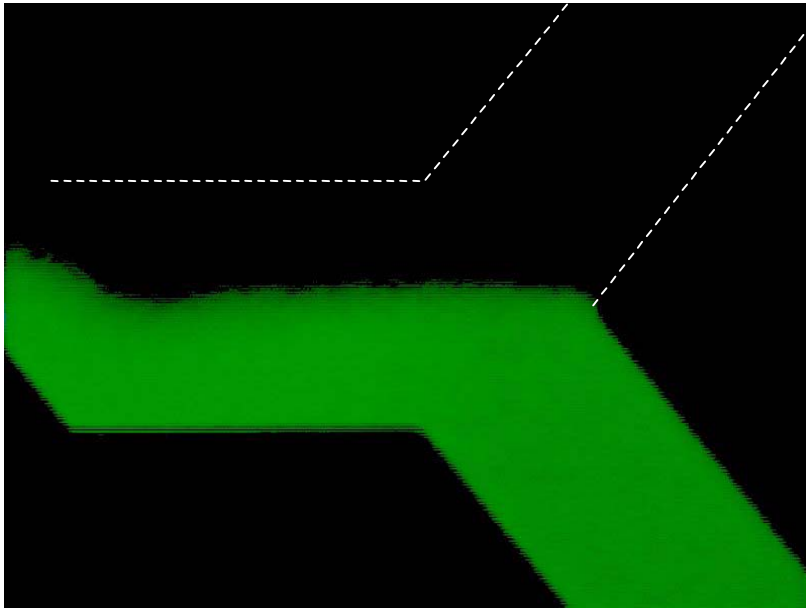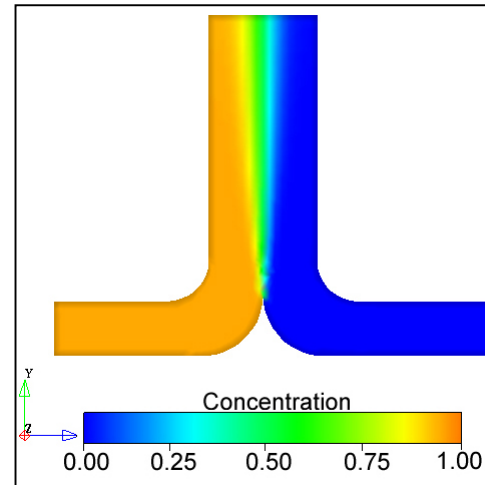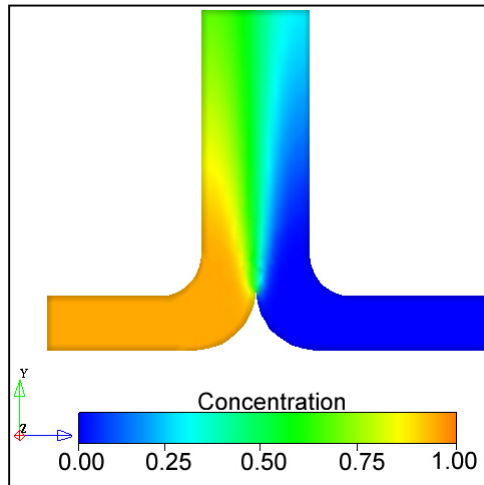

Regnier, et al. Purdue

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: $x^2 = 2Dt$ $D = \dfrac{k_b T}{6\pi\eta a}$

  - biotin (D ~ 350 µm^2/s)
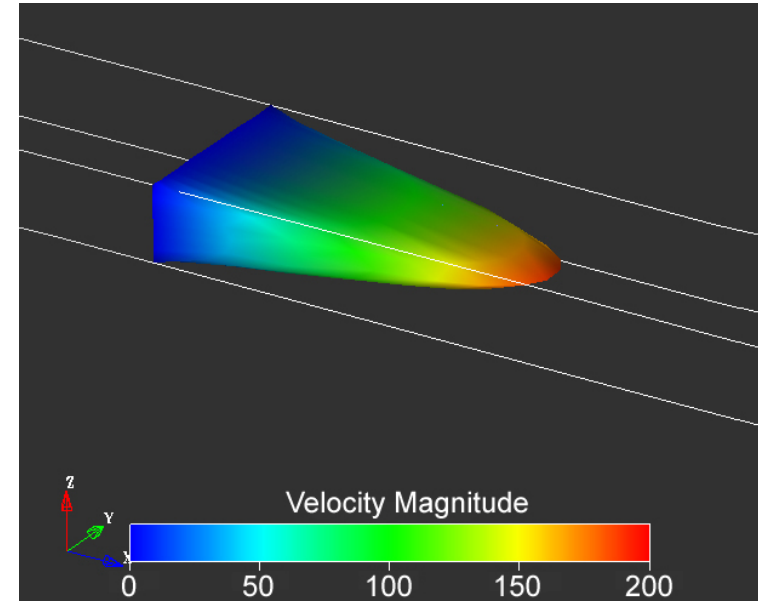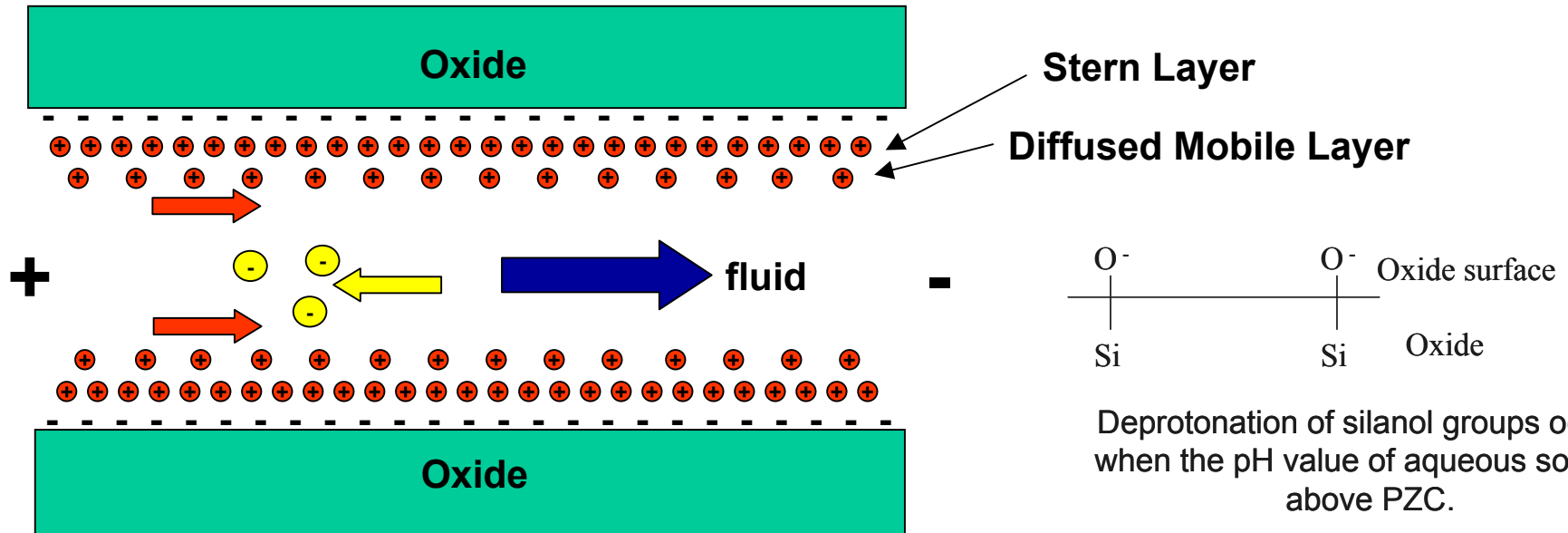 - albumin (D ~ 65 µm^2/s)

Yager (U. Washington)

Microfluidic Flow

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

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

Velocity Magnitude

Yager, et al. U. Washington

Electroosmotic Flow

Oxide

Stern Layer

Diffused Mobile Layer

+ ... **fluid** ... **-**

Oxide

O⁻ — O⁻ Oxide surface
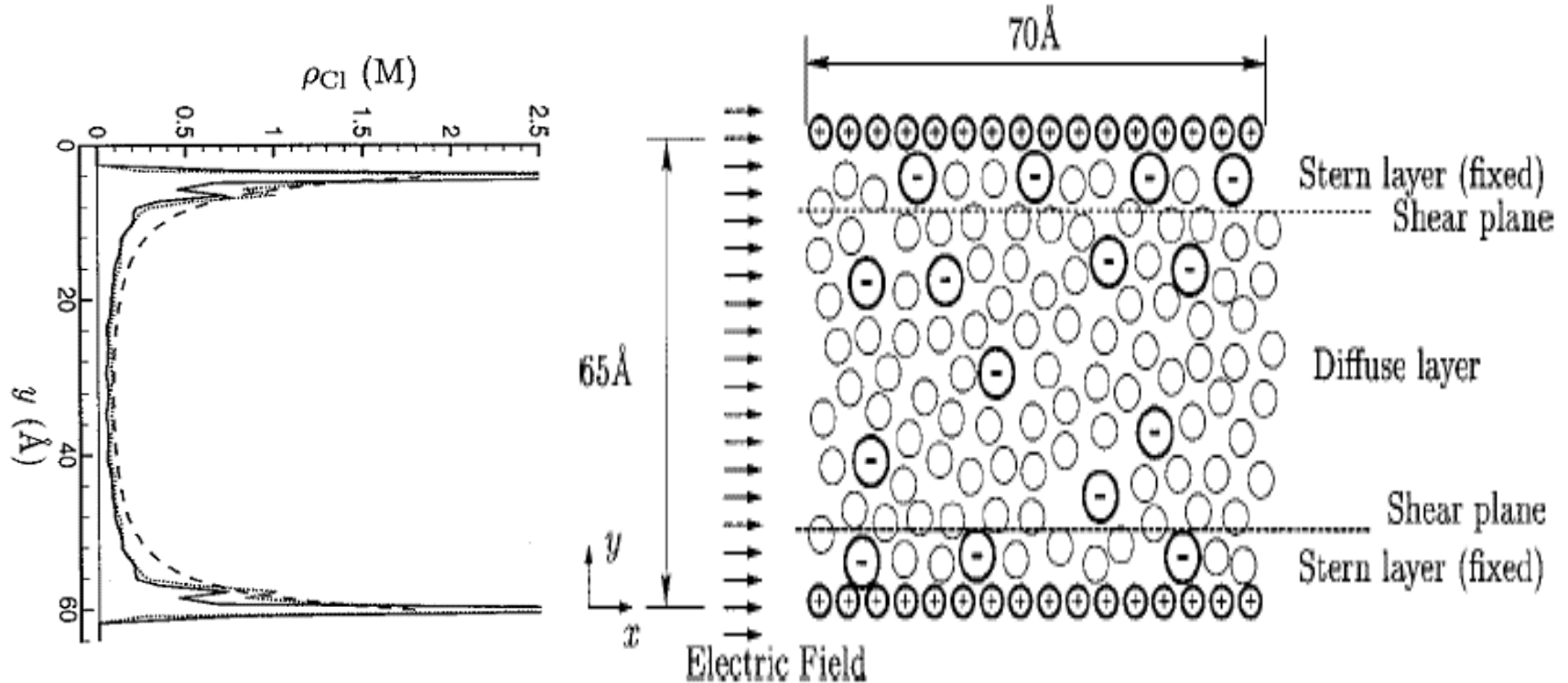
Si — Si Oxide

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

EOF
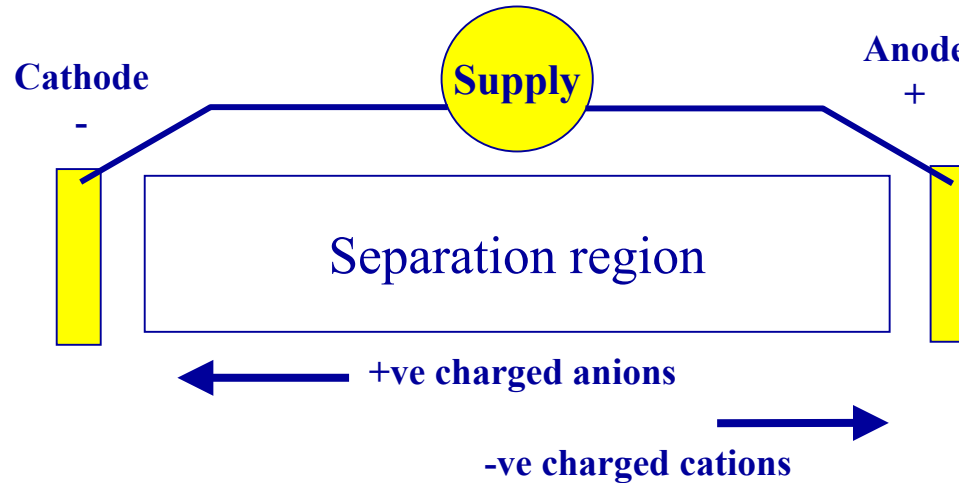
Pressure driven flow

8
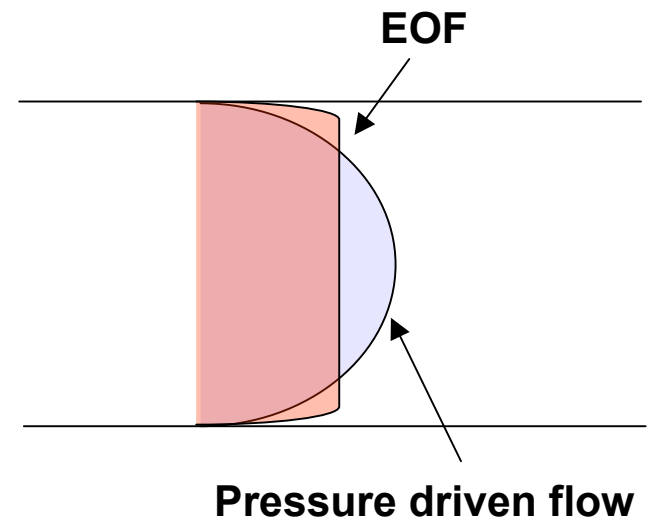
Electroosmotic Flow in Nano-channels

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*.
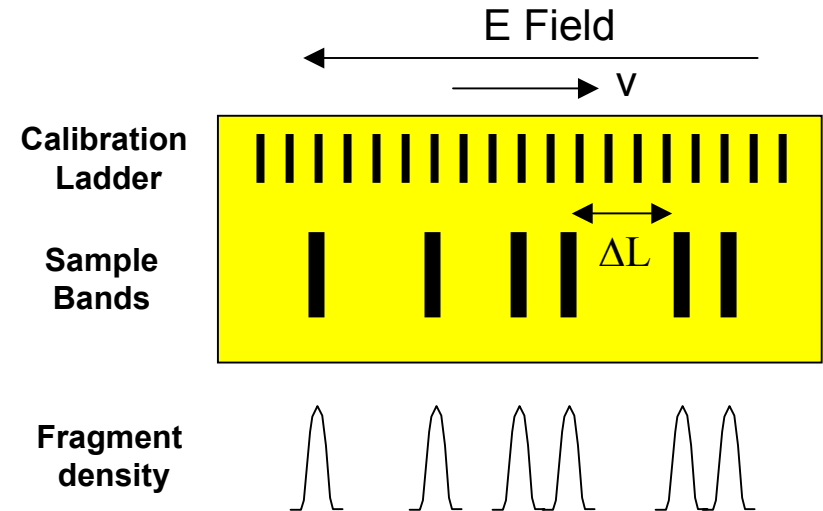
Electrophoresis

- Electrophoresis: charged species drift when placed under an electric field

- $v = -\mu \, dV/dx$
  - v - electrophoretic velocity
  - μ - electrophoretic mobility
  - dV/dx = applied electric field

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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= μV/2D
    - D is the diffusion coefficient

E Field

v

Calibration Ladder
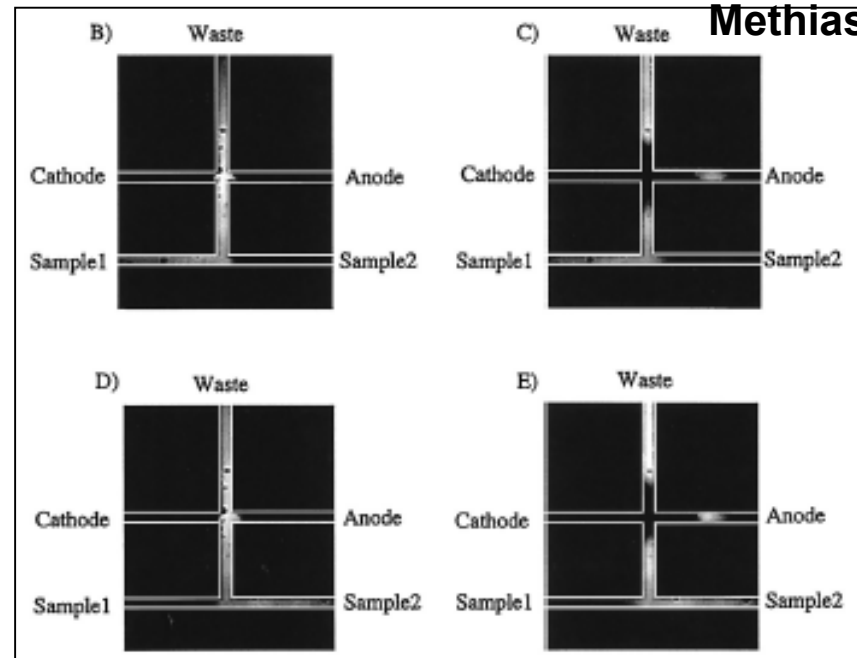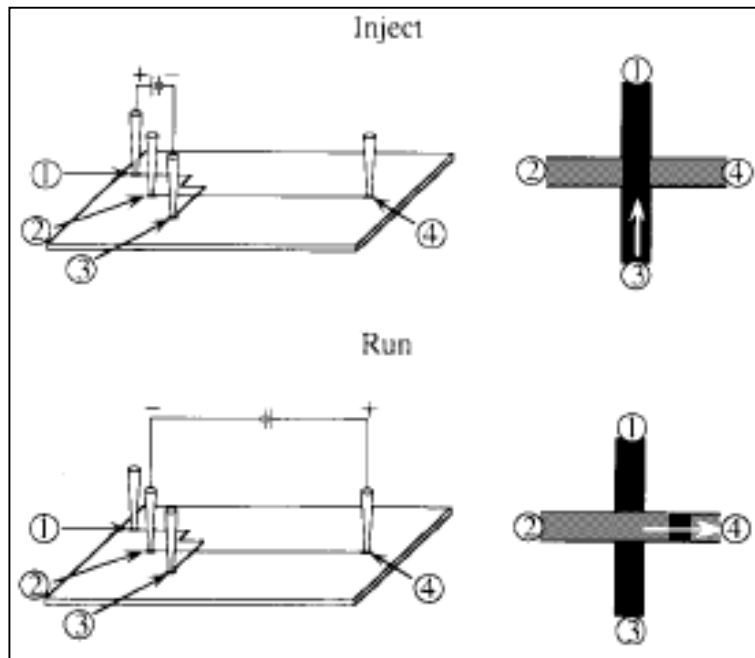
Sample Bands

ΔL

Fragment density

- 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
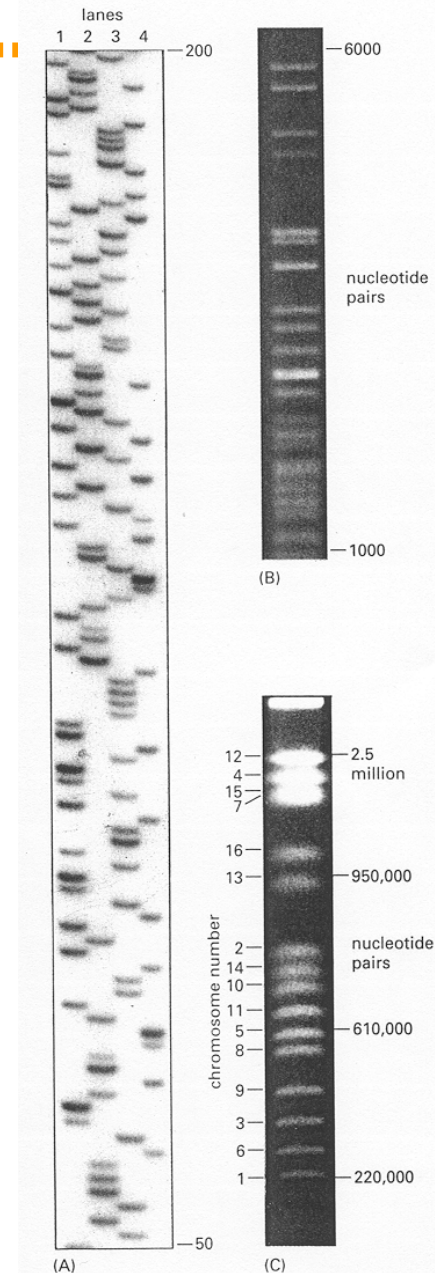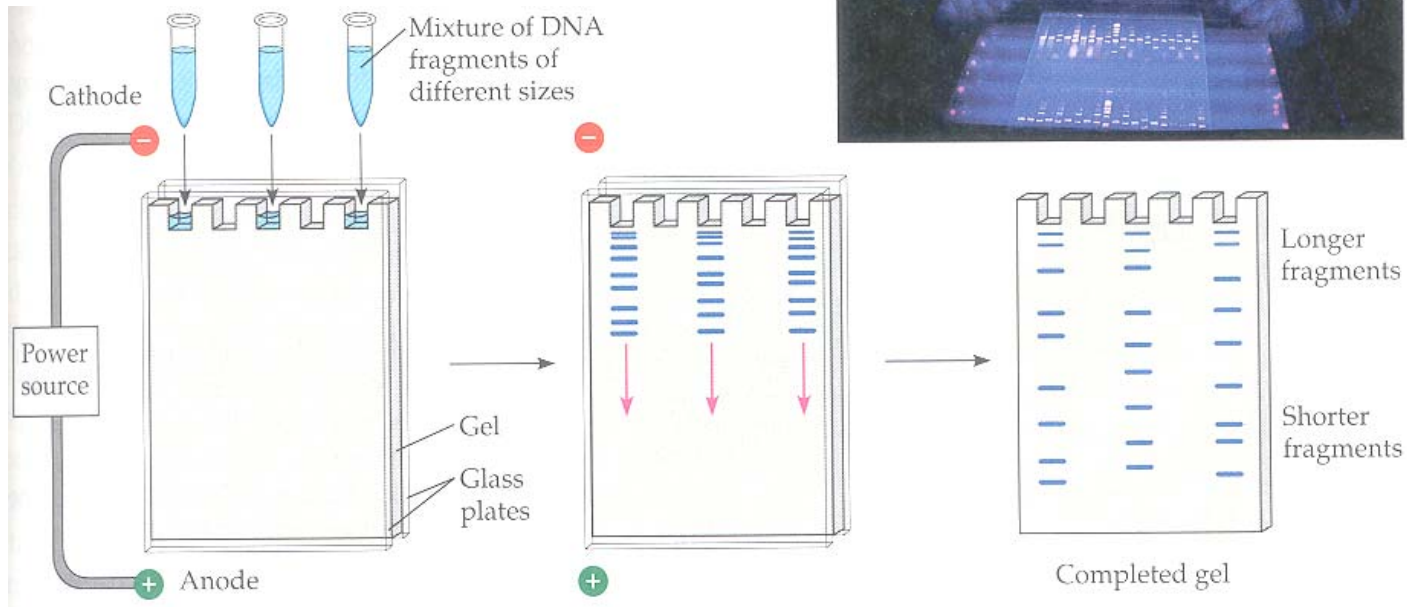
DNA Electrophoresis in a Chip

Methias, UCB

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

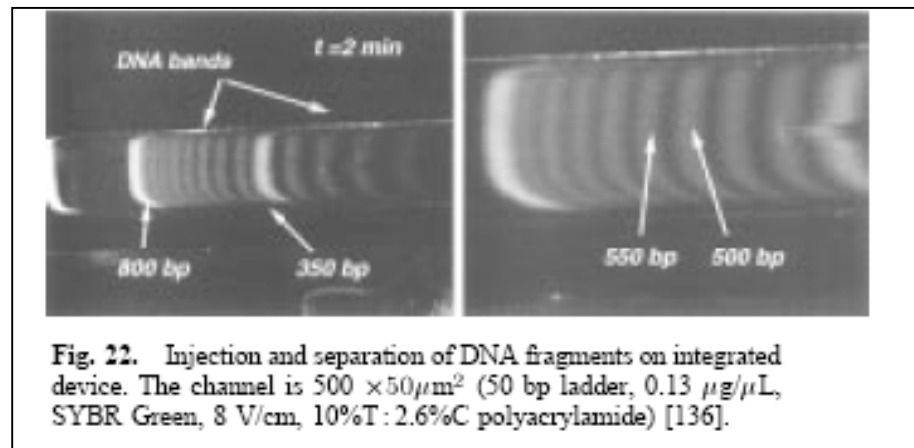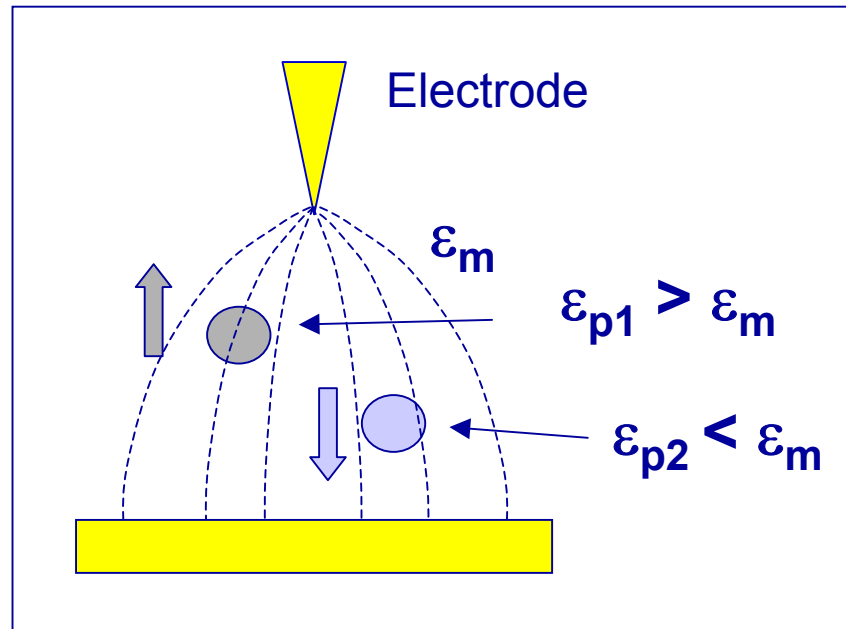Fig. 22. Injection and separation of DNA fragments on integrated device. The channel is $500 \times 50\mu m^2$ (50 bp ladder, 0.13 $\mu g/\mu L$, SYBR Green, 8 V/cm, 10%T : 2.6%C polyacrylamide) [136].

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Mastrangelo, Burns, Univ. of Michigan

Dielectrophoresis

Simplest approximation:

$$F = 2\pi\varepsilon_0\varepsilon_m r^3 \, \text{Re}\big[f_{CM}\big]\nabla\big|E_{RMS}\big|^2$$

$$f_{CM}\big(\varepsilon_p, \varepsilon_m\big) = \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m} \qquad \varepsilon_p = \varepsilon(\omega)$$

Dielectrophoresis on Interdigitated Electrodes
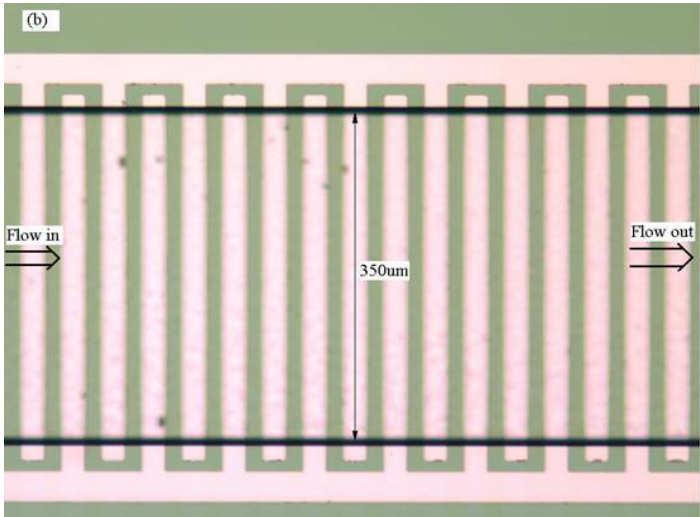
Interdigitated electrodes on a chip

Polystyrene beads : $\varepsilon_p < \varepsilon_m \rightarrow$ **negative DEP**

Cells : $\varepsilon_p < \varepsilon_m \rightarrow$ **Negative DEP**
Cells : $\varepsilon_p > \varepsilon_m \rightarrow$ **Positive DEP**

H. Li and R. Bashir, Sensors and Actuators, 2002, JMEMS, 2004
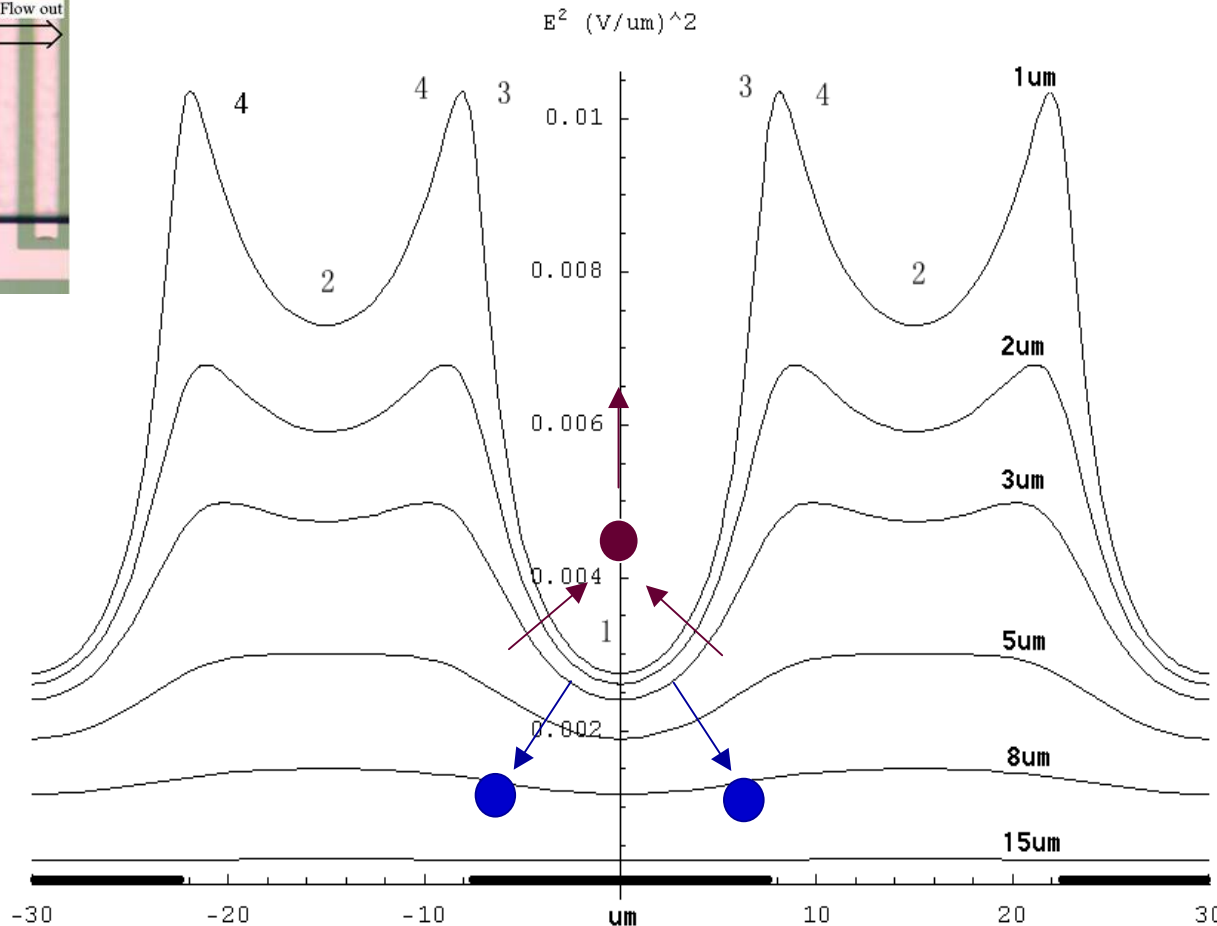
A Dielectrophoretic Filter

Beads and bacteria

Electrodes

Flow

Flow

Detection Chamber

Epoxy adhesive

Glass cover   12um

23um   17um

PTFE microbore tube
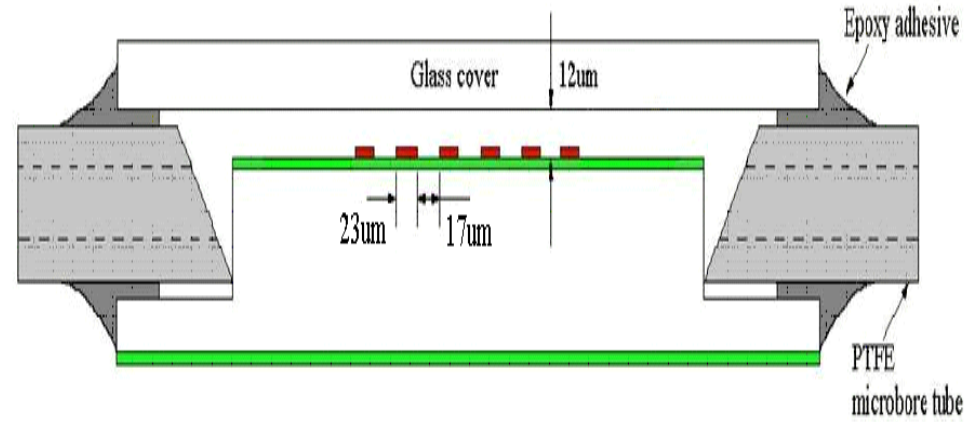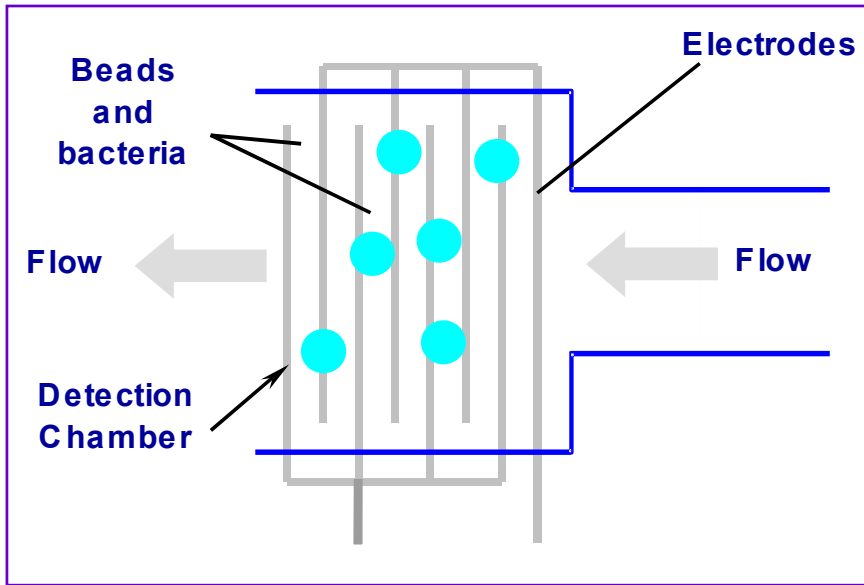
Schematic of the device cross-section

y direction (vertical)

x direction (horizontal)

Flow direction

Electrodes (cross-section)

X-component of DEP force at different heights
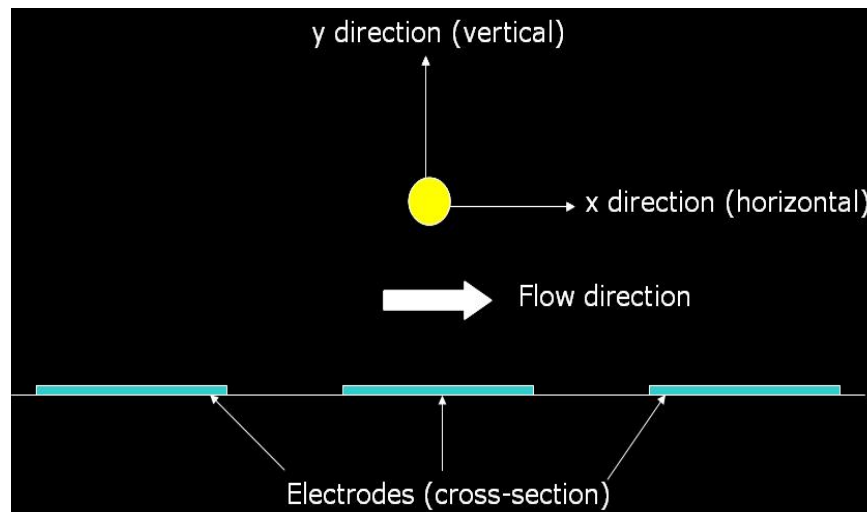
x-component of DEP force at different heights
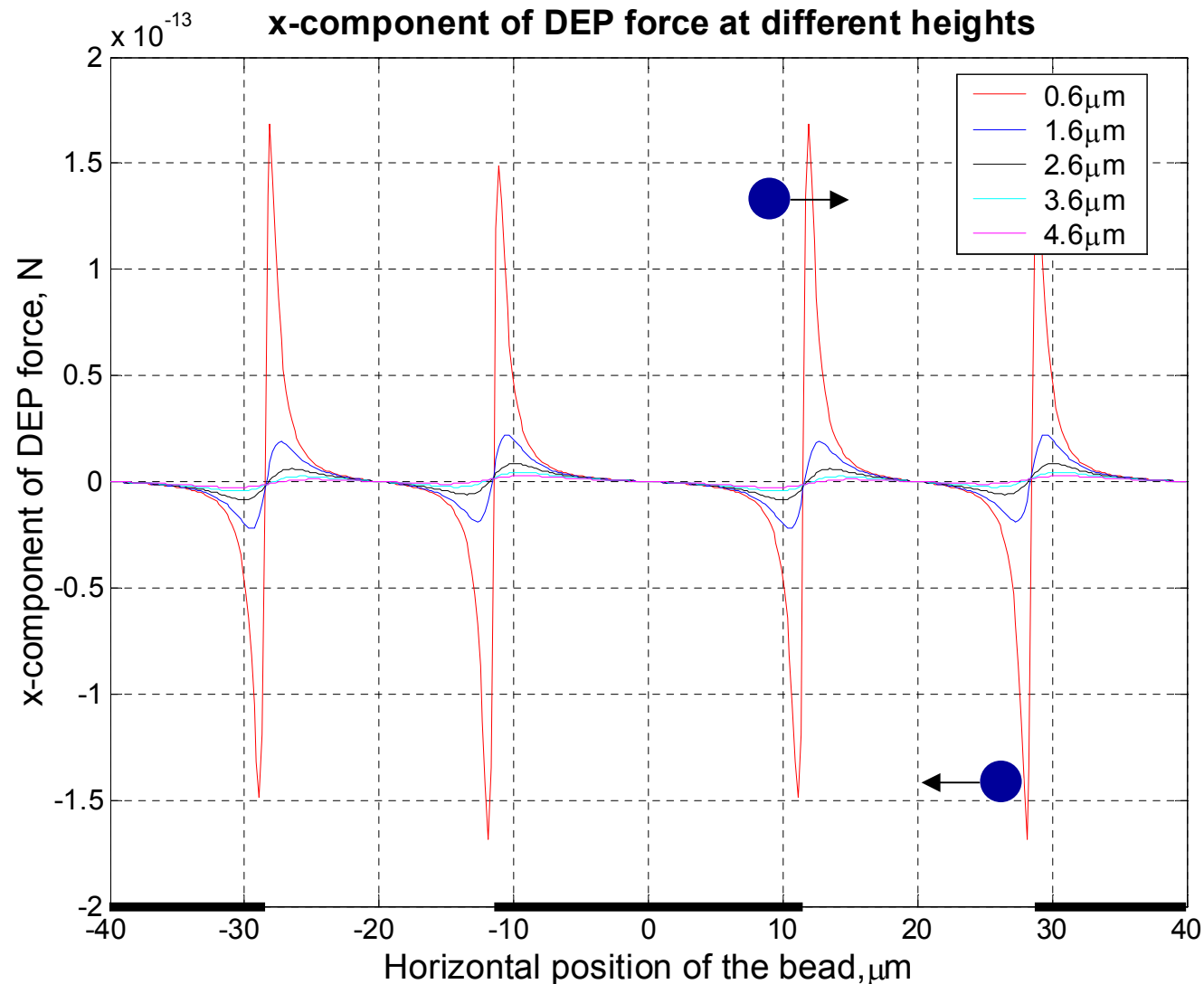
- **Bead diameter: 0.7μm**
- **Bead conductivity: 2e-4 S/m**
- **Relative permittivity of bead: 2.6**
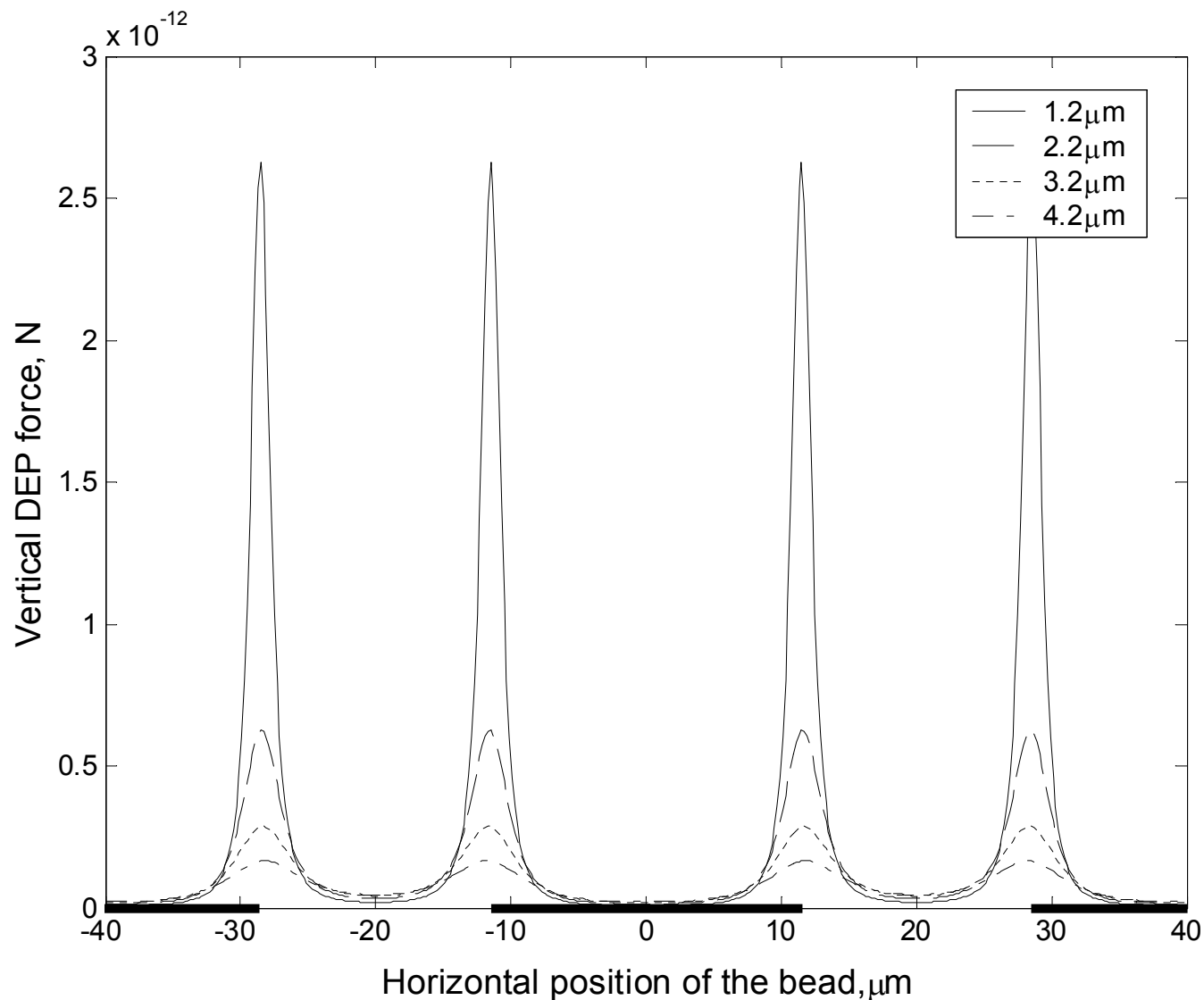- **Bead density: 1.05 g/cm3**
- **Medium (DI water) conductivity: 2.5 S/m**
- **Relative permittivity of medium: 80**
- **Medium density: 1.0 g/cm3**
- **Voltage: 1Vrms**
- **Frequency: 580KHz**

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Forces on a particle in a micro-fluidic flow

- **1. DEP Force**

- **2. Sedimentation Force**

$$F_{sedi} = \frac{4}{3}\pi R^3 (\rho_p - \rho_m) g$$
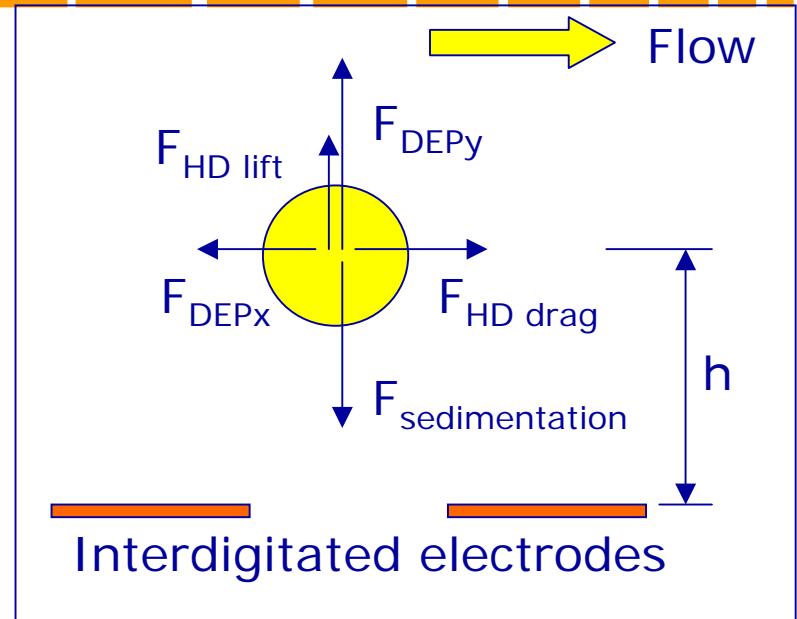
- **3. Hydrodynamic Drag Force:**

$$F_{HD-drag} \approx 6\pi k R \eta (\upsilon_m - \upsilon_p)$$

- **Assume a parabolic laminar flow profile:**

$$\upsilon = 6\langle\upsilon\rangle \frac{x}{h}\left(1 - \frac{x}{h}\right)$$

$$\langle\upsilon\rangle = \frac{U}{wh}$$

U: flow rate in µl/min

- **4. Hydrodynamic lifting force**

$$F_{HD-lift} \approx 0.153 R^2 \eta \frac{1}{(x-R)} \cdot \left.\frac{d\upsilon_m}{dx}\right|_{x=0}$$

- **Two orders of magnitude smaller than typical DEP lifting force**
- **Neglected here**

Flow

$F_{HD\ lift}$ F_{DEPy}

F_{DEPx} $F_{HD\ drag}$

$F_{sedimentation}$

h

Interdigitated electrodes

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Holding voltage of the negative DEP traps on interdigitated electrodes versus flow rate
for polystyrene beads with different diameters in DI water (conductivity ~1.5μS/cm) at 1MHz

— Simulation
□ Experiments

2.38μm bead

5.44μm bead

Voltage^2 (V^{2}p-p)

Flow rate (μl/min)

yeast cells

Vaccinia virus

B. Cereus spores

Listeria innocua bacteria

Voltage^2 (V^{2}p-p)

Flow rate (μl/min)

21
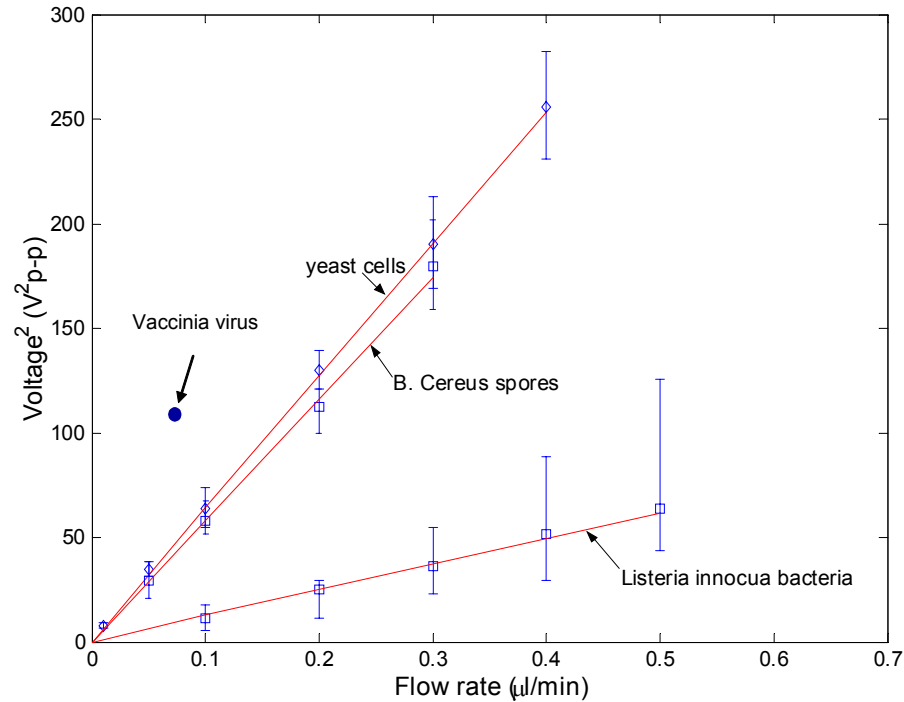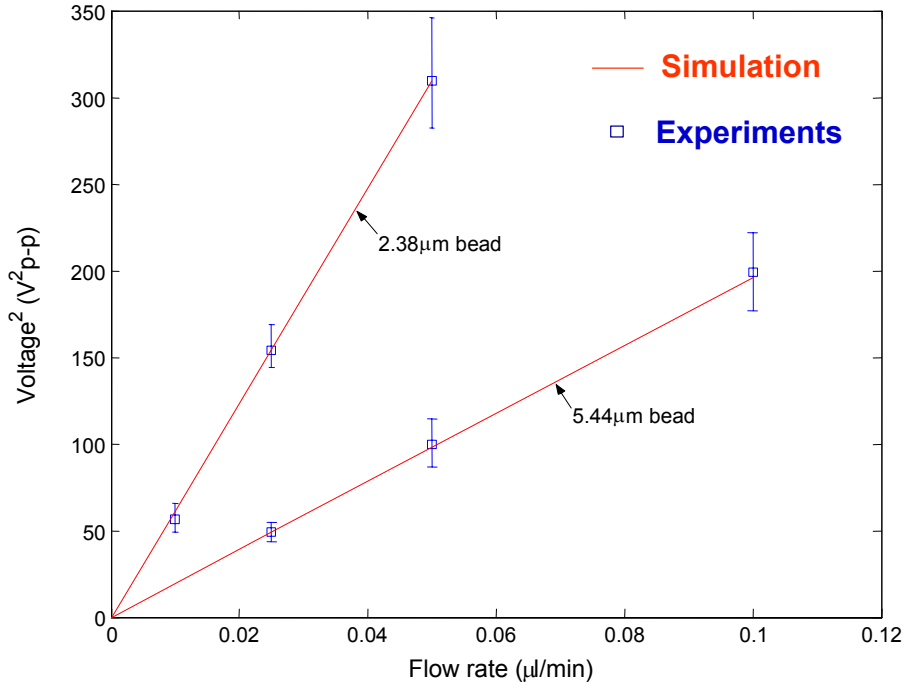
Dielectrophoretic Trapping of *Vaccinia* virus (positive DEP)

- **Fluorescent imaging of nano-scale virus particles (Vaccinia virus and Human Corona Virus)**
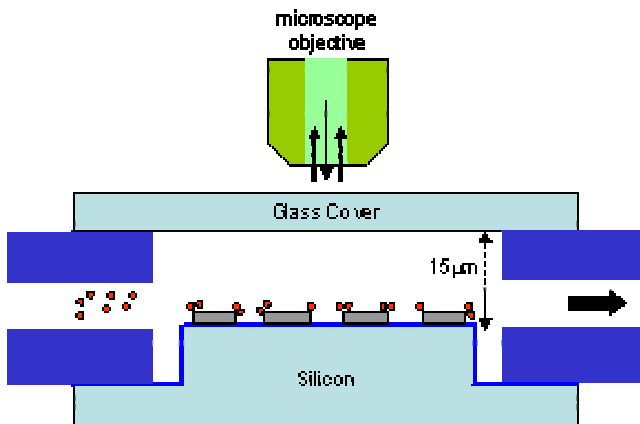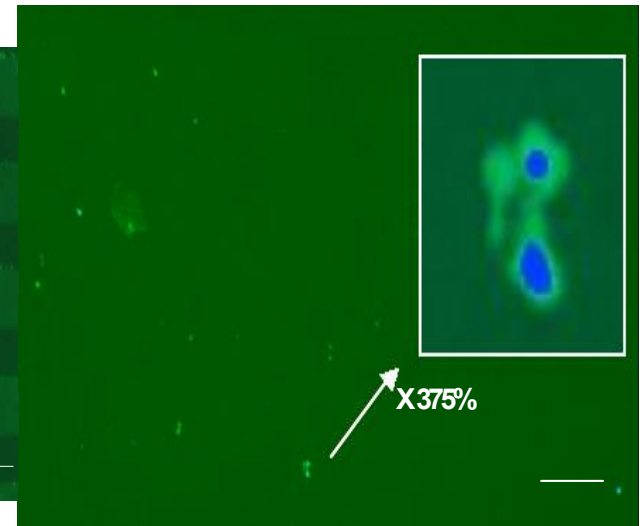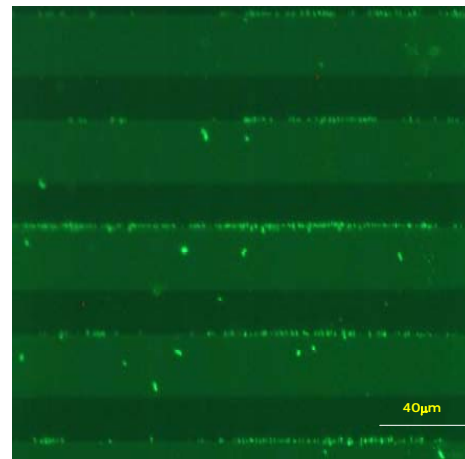
- **Trapping of viruses in DEP filters**

- **Dual labeling of viruses with fluorescent dyes**

10µm Virus particles

Electrode Edges

0.50 u
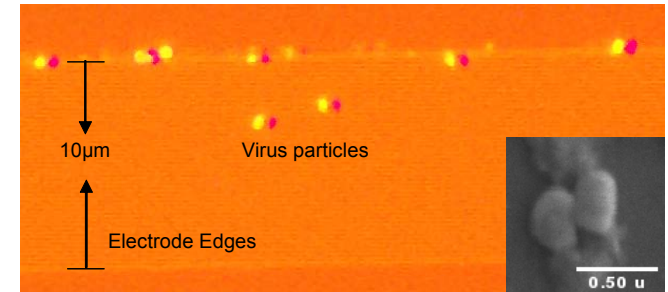
The dual (DiOC63, green and DiL, red) labelled viral particles

microscope objective

Glass Cover

15µm

Silicon

40µm

X375%

Virus Size ~ 250x350nm
Picture taken at: 10Vpp, 1MHz, DI water
~1.5µS/cm, flow rate ~0.1µl/min

400x magnification: viral surface lipid membrane labeled green (DiOC63) and viral nucleic acids were stained blue (Hoechst 33342 stain)

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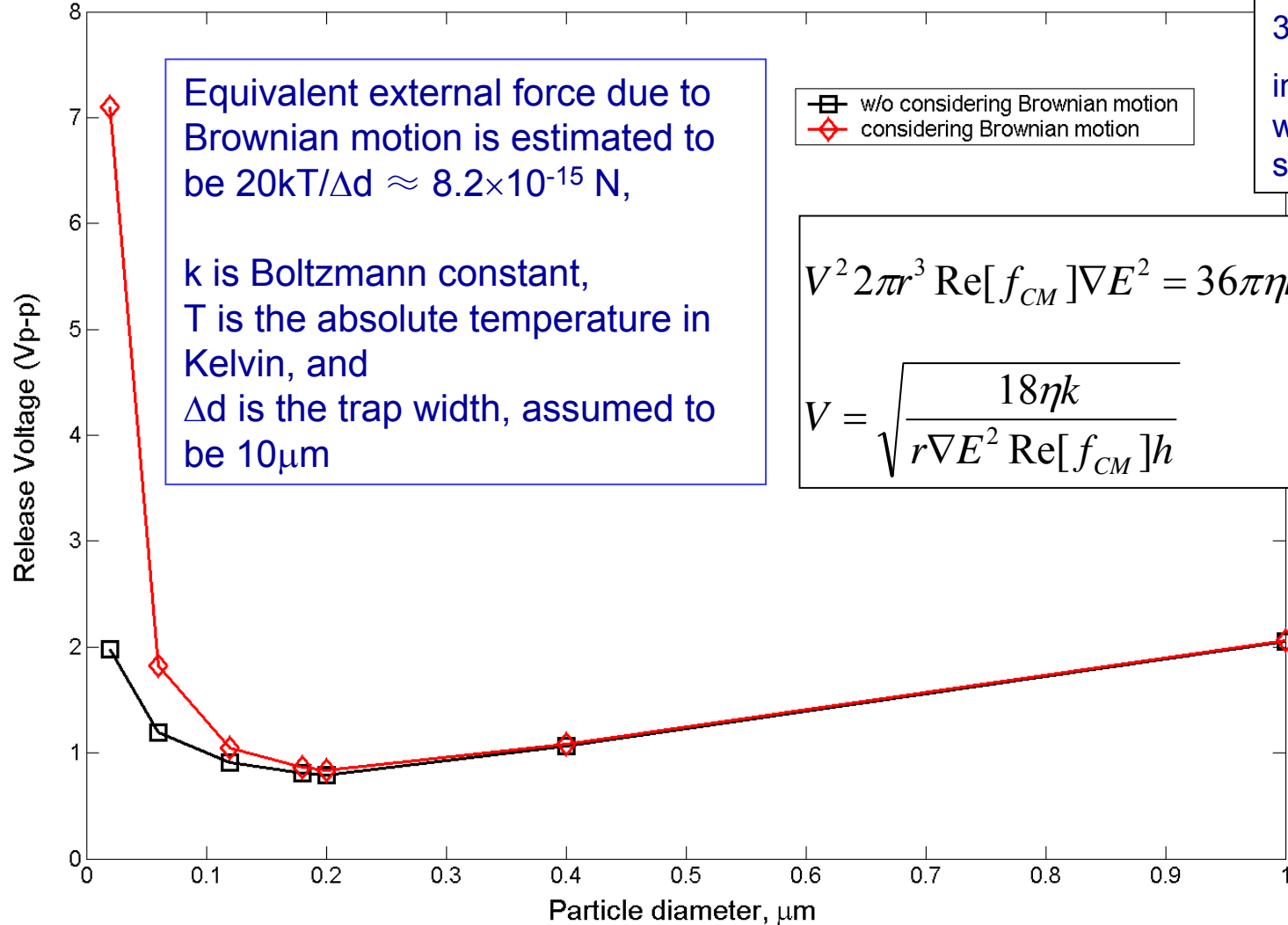D. Akin, H. Li, R. Bashir, *Nano Letters*, 4, pp. 257 -259, 2004

Polarization factor=0.5,

flow rate 0.1μm/min, in the channel with cross-section 350x11.6μm2,

interdigitated electrodes with 23μm width and17μm spacing

Equivalent external force due to Brownian motion is estimated to be 20kT/Δd $\approx$ 8.2$\times$10^{-15} N,

k is Boltzmann constant,
T is the absolute temperature in Kelvin, and
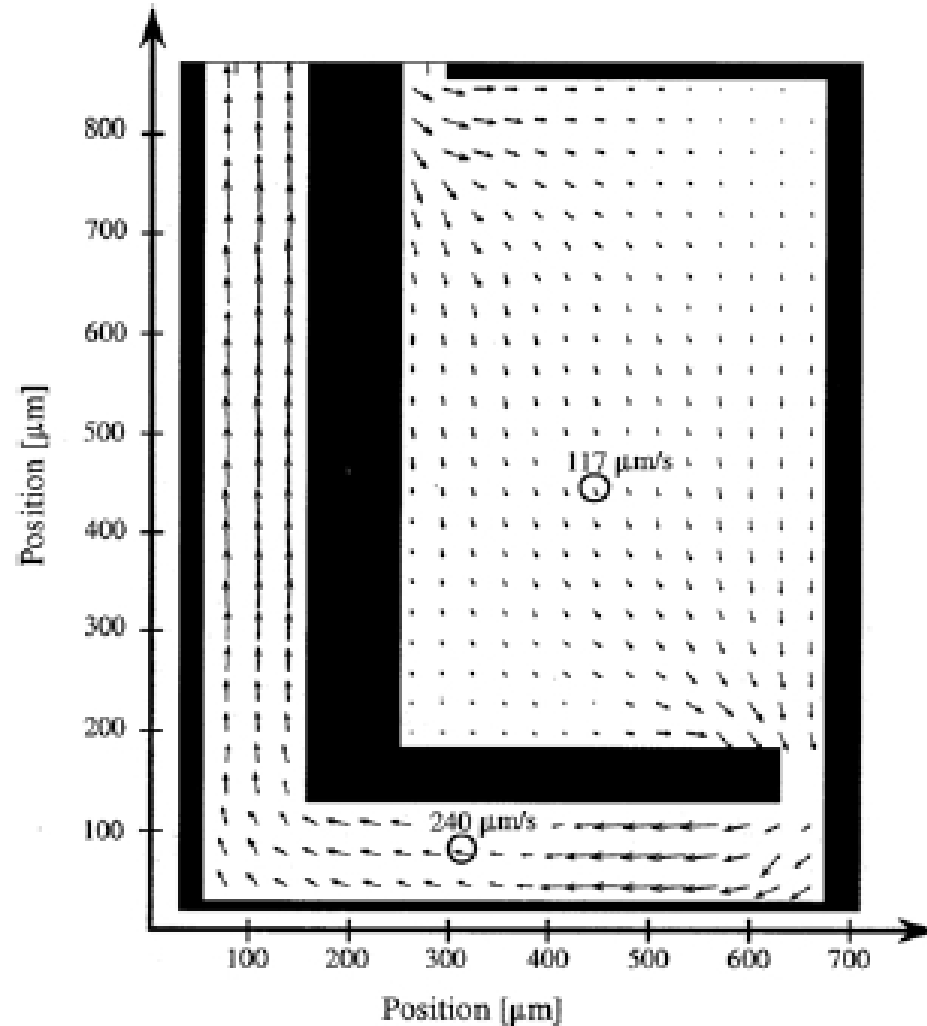Δd is the trap width, assumed to be 10μm

$$V^2 2\pi r^3 \mathrm{Re}[f_{CM}]\nabla E^2 = 36\pi\eta kr\left(\frac{r}{h} - \frac{r^2}{h^2}\right) \approx 36\pi\eta k \frac{r^2}{h}$$

$$V = \sqrt{\frac{18\eta k}{r\nabla E^2 \mathrm{Re}[f_{CM}]h}}$$

Release Voltage (Vp-p) vs Particle diameter, μm

- w/o considering Brownian motion
- considering Brownian motion

Micro-fluidic Characterization

- Micro-Particle Imaging Velocimetry (μPIV)
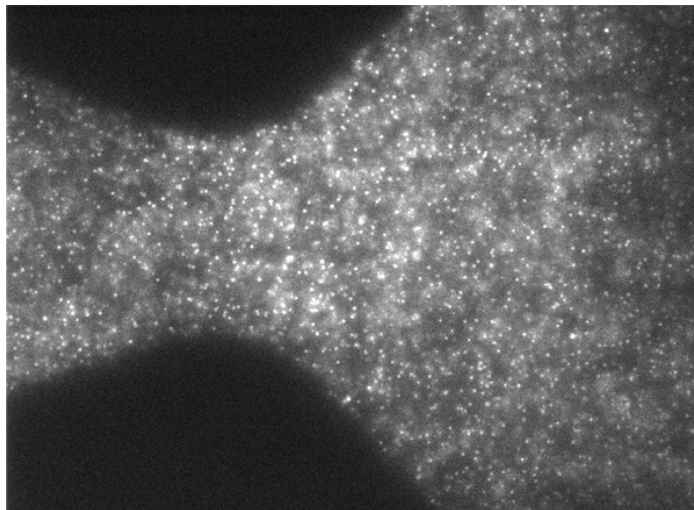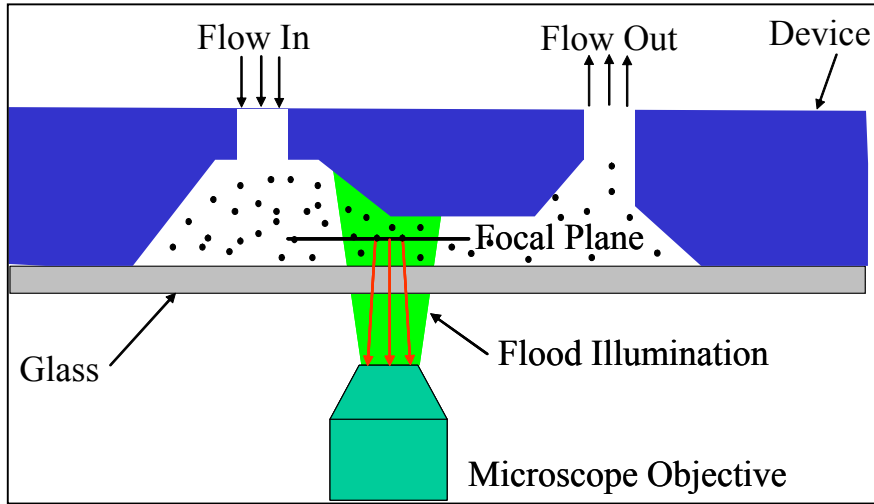
Flow In Flow Out Device
Focal Plane
Glass Flood Illumination
Microscope Objective

Wereley, et al. Purdue

Gomez, et al. 2001

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Key Topics

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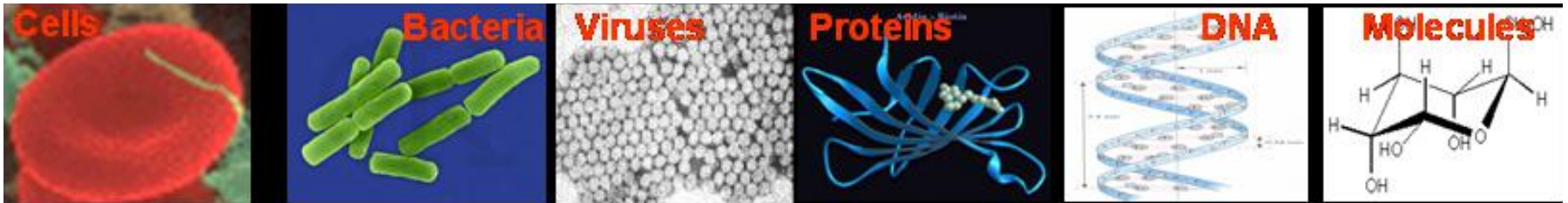
Biochip Sensors

- Detect cells (mammalian, plant, etc.), microorganisms (bacteria, etc.), viruses, proteins, DNA, small molecules
- Use optical, electrical, mechanical approaches at the micro and nanoscale in biochip sensors
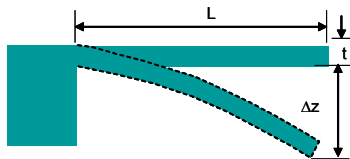
Sensing Methods in BioChips

Mechanical Detection

Surface Stress Change Detection

$$\Delta z = 4\left(\frac{l}{t}\right)^2 \frac{(1-\nu)}{E}(\Delta\sigma_1 - \Delta\sigma_2)$$

- Δz = deflection of the free end of the cantilever
- L = cantilever length
- t = cantilever thickness
- E = Young's modulus
- ν = poison's ratio
- $\Delta\sigma_1$ change in surface stress on top surface
- $\Delta\sigma_2$ change in surface stress on bottom surface

Mass Change Detection

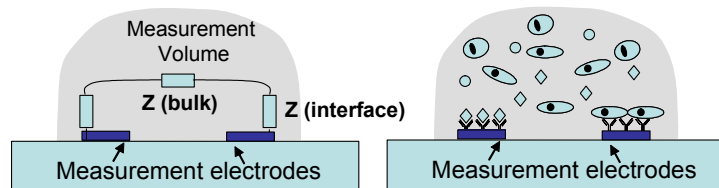$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

$$\Delta m = \frac{k}{4\pi^2}\left(\frac{1}{f_1^2} - \frac{1}{f_o^2}\right)$$

- k = spring constant
- m = mass of cantilever
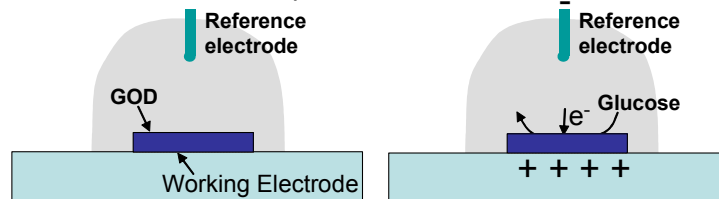- f_0 = unloaded resonant frequency
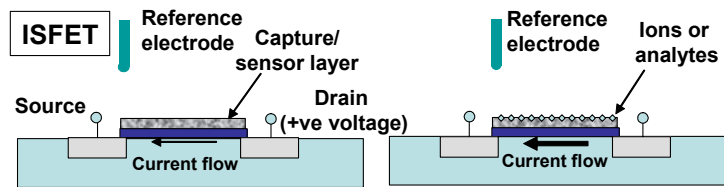- f_1 = loaded resonant frequency

(a)

Electrical Detection

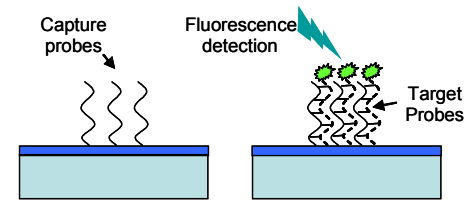### Conductometric Detection

Measurement Volume

Z (bulk)   Z (interface)

Measurement electrodes

Measurement electrodes

Amperometer Detection

Reference electrode

GOD

Working Electrode

Reference electrode

$|e^-$ Glucose

+ + + +

Potentiometric Detection

ISFET   Reference electrode   Capture/ sensor layer

Source   Drain (+ve voltage)

Current flow

Reference electrode   Ions or analytes

Current flow

(b)

Optical Detection

Capture probes   Fluorescence detection   Target Probes

DNA detection on chip surfaces

Capture probes   Fluorescence detection   Target Probes
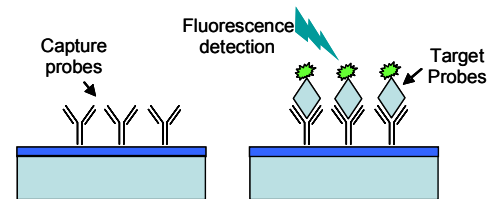
Protein detection on chip surfaces
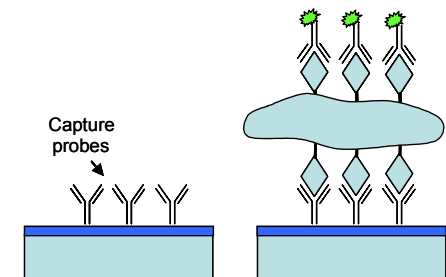
Capture probes
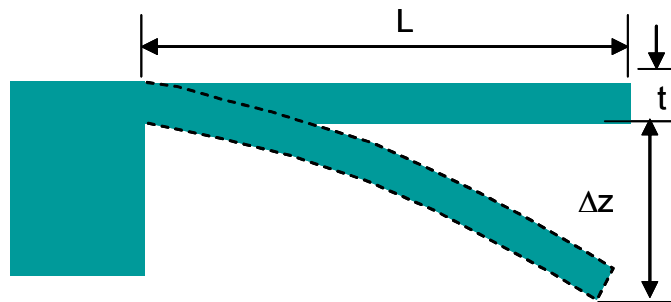
Cell detection on chip surfaces

(c)

Mechanical Detection

Surface Stress Change Detection

$$\Delta z = 4\left(\frac{l}{t}\right)^{2}\frac{(1-\nu)}{E}\left(\Delta\sigma_{1} - \Delta\sigma_{2}\right)$$

- Δz = deflection of the free end of the cantilever
- L = cantilever length
- t = cantilever thickness
- E = Young's modulus
- ν = poison's ratio
- $\Delta\sigma_{1}$ change in surface stress on top surface
- $\Delta\sigma_{2}$ change in surface stress on bottom surface

0.2μm thick, 100μm long, silicon cantilevers
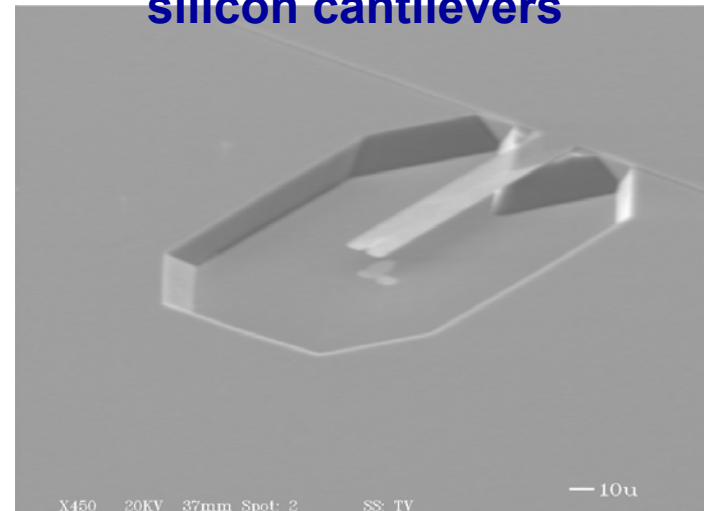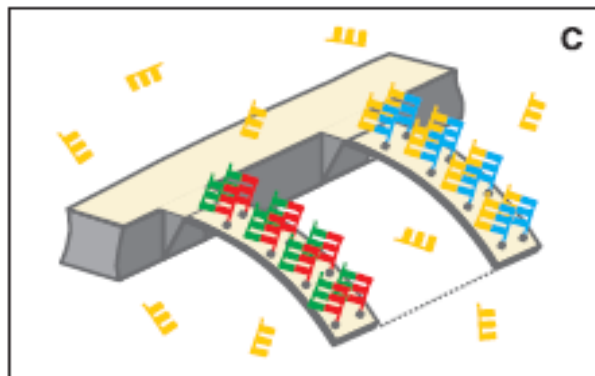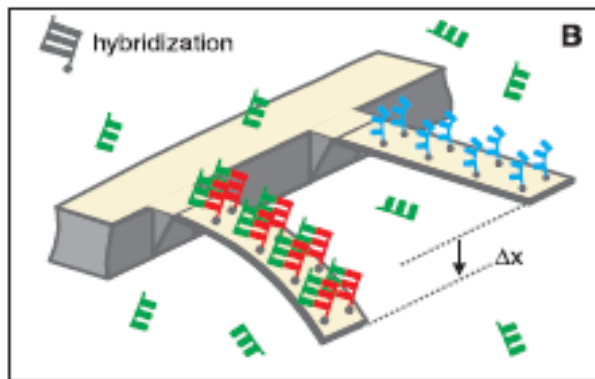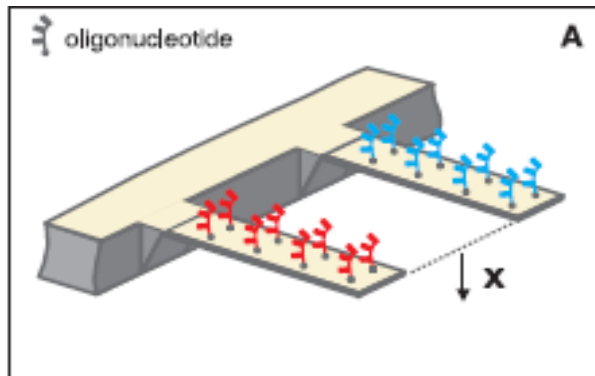
Microcantilever Stress Sensors
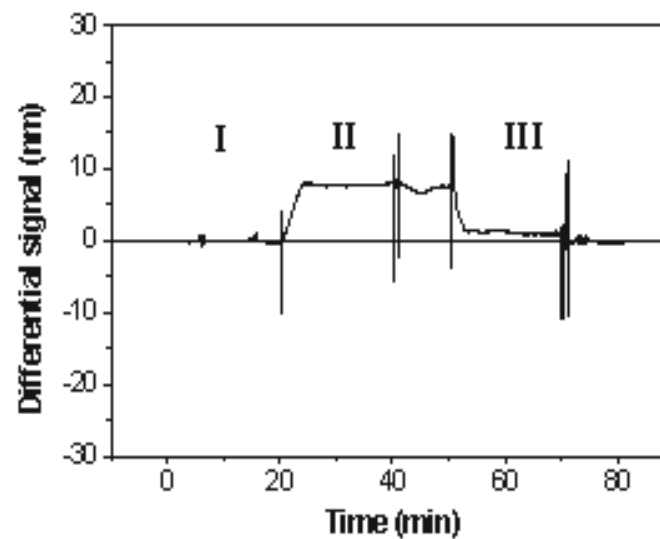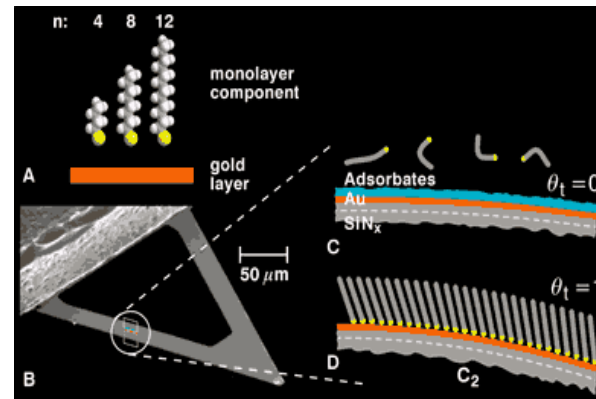
DNA Detection

Fritz et al, *Science*, **288,** April 2000

Microcantilever Stress Sensors

**Detection of PSA,
Prostate Specific Antigen
(cancer marker protein in blood)**
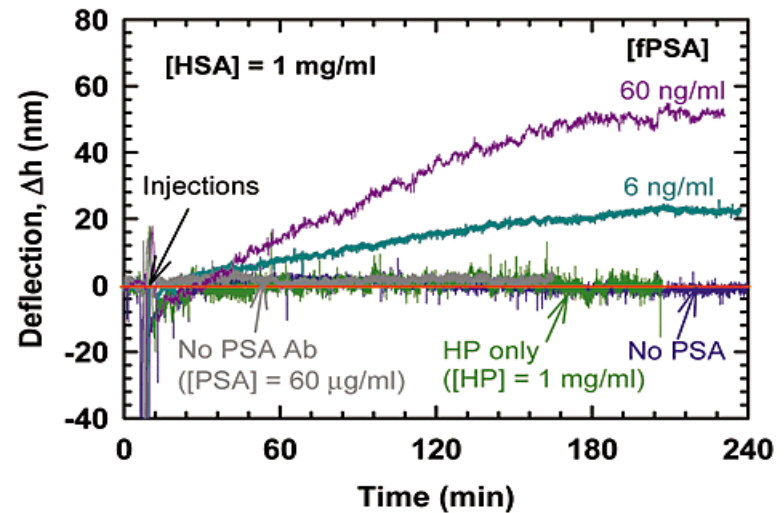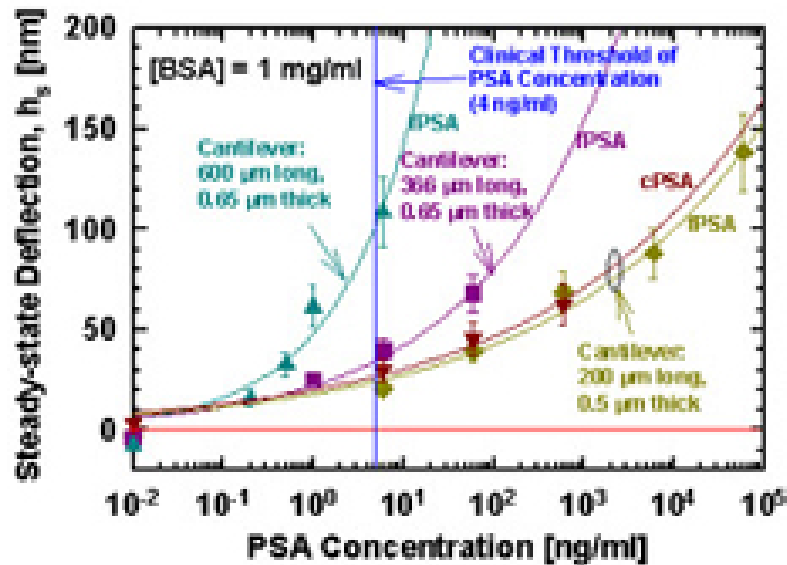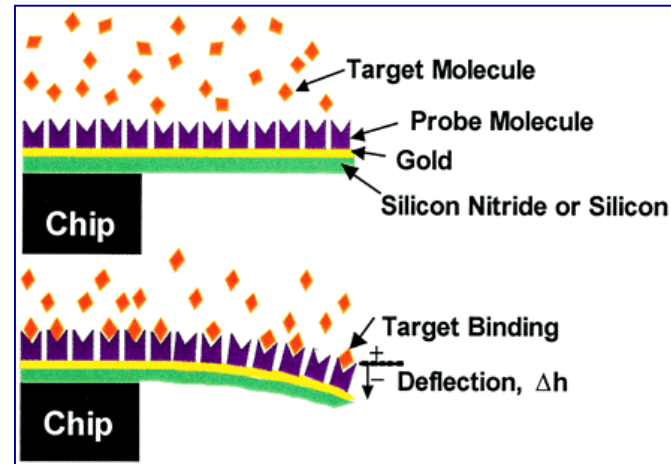
- PSA ~ 30kDa ~ 30 x 1e3 x 1.66e-24gm
- In 1ng/ml ~ 2e10 molecules/ml
- Area of 20um x 60um, each protein 10nm x 10nm → ~1e8 proteins

Deflections have been measured with a resolution of **0.4x10-12 m.***

Polymer/Silicon Cantilever Sensors

- **Environmentally sensitive micro-patterned polymer structures on cantilevers**

- **Hydrogel patterned on cantilever and then exposed to varying pH**

Cantilever over a well

PMMA-based Hydrogel Polymer

50 μm

Deflection at the tip (μm) vs pH of Fluid Around the Cantilever

Experiment — Model

Cantilevers touched the bottom

slope = 18.3 mm/pH (=1nm/5e-5△pH)
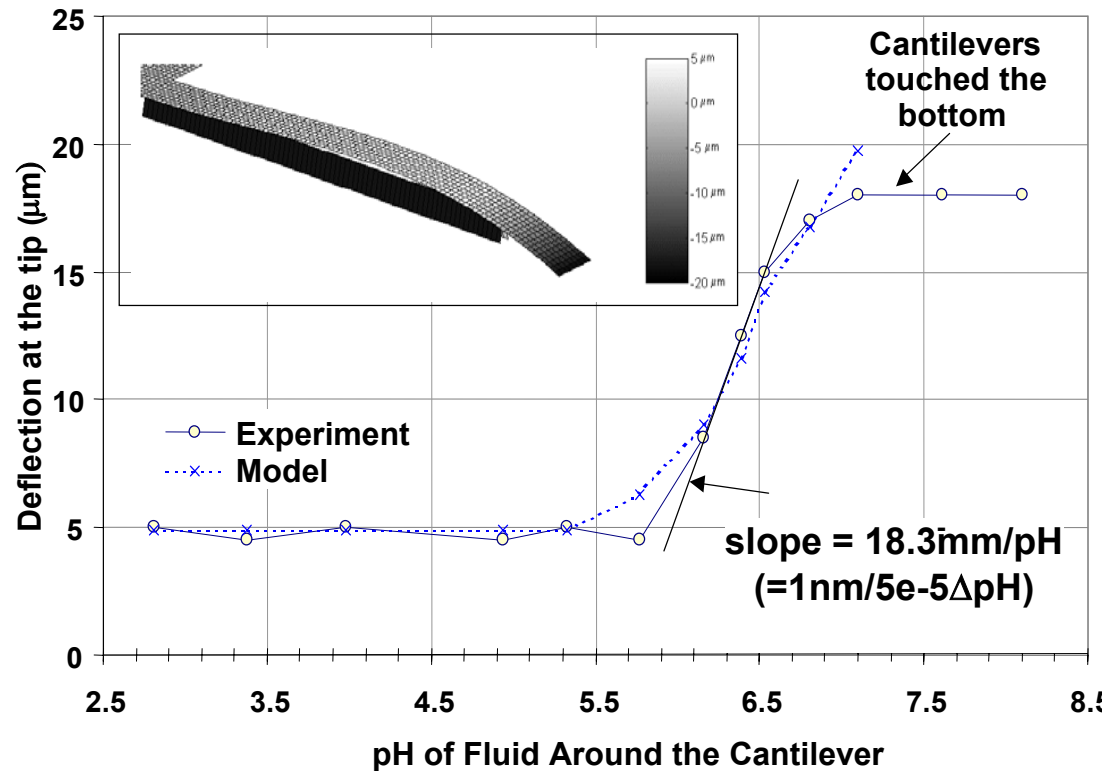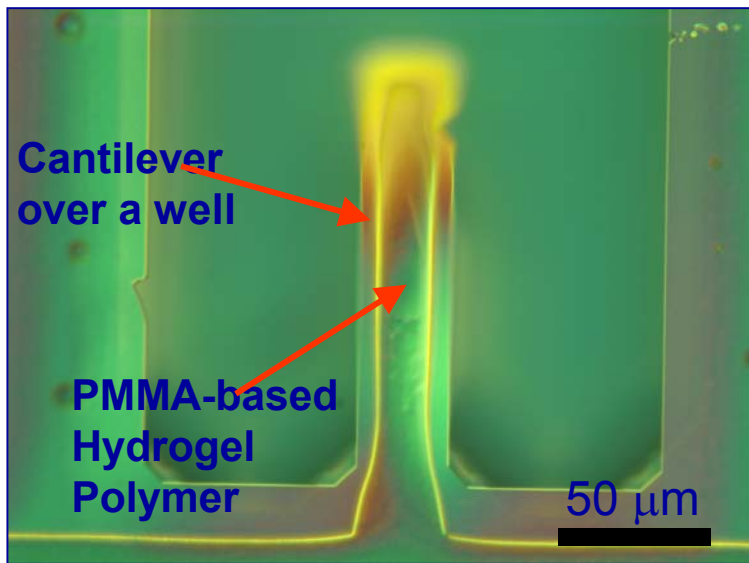
- △pH = 1-10e-5
- pH = 6.5 → ~ 1.9e5 H$^+$ in 1000μm^3
- △pH = 5e-4 → change of ~ 150 H$^+$

R. Bashir, J.Z. Hilt, A. Gupta, O. Elibol, and N.A. Peppas, Applied Physics Letters, Oct 14th, 2002;
J. Zachary Hilt, Amit K. Gupta, Rashid Bashir, Nicholas A. Peppas Biomedical Microdevices, September 2003, Volume 5, Issue 3, 177-184

Unloaded Resonant Frequency :

$$f_0 = \frac{1}{2\pi}\sqrt{\frac{k}{m*}}$$

Spring constant for a rectangular shaped cantilever beam: $k = \dfrac{Et^3 w}{4l^3}$

Loaded Resonant frequency : $f_1 = \dfrac{1}{2\pi}\sqrt{\dfrac{k}{m*+\delta m}}$

δm is the added mass

$$\Delta m = \frac{k}{4\pi^2}\left(\frac{1}{f_1^2} - \frac{1}{f_o^2}\right)$$

• k = spring constant
• m = mass of cantilever
• f_0 = *unloaded resonant frequency*
• f_1 = *loaded resonant frequency*

Mass Change Detection

PSD comparing measurements done using thermal and piezo driven

- Thermal Noise
- Piezo driven resonance

Cantilever Beam
Length = 10 µm
Thickness = 30 nm

f_0 = 270 kHz

Piezoelectric Driven

Thermal Noise Spectra

Amplitude (units)
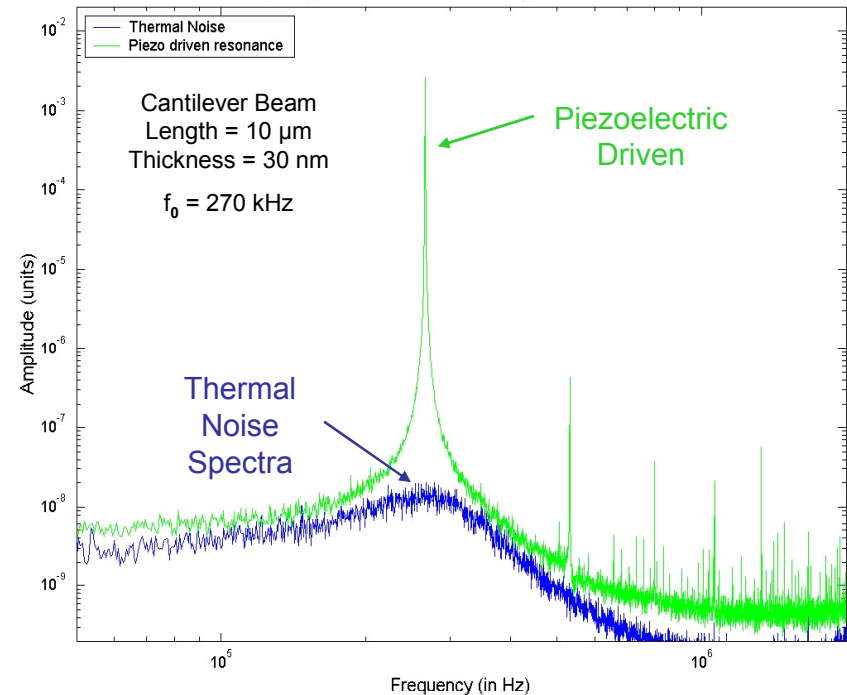
Frequency (in Hz)

Detection of Bacterial Mass

$$\omega_o{}^2 = \frac{k}{m}$$

E-coli Cells

33
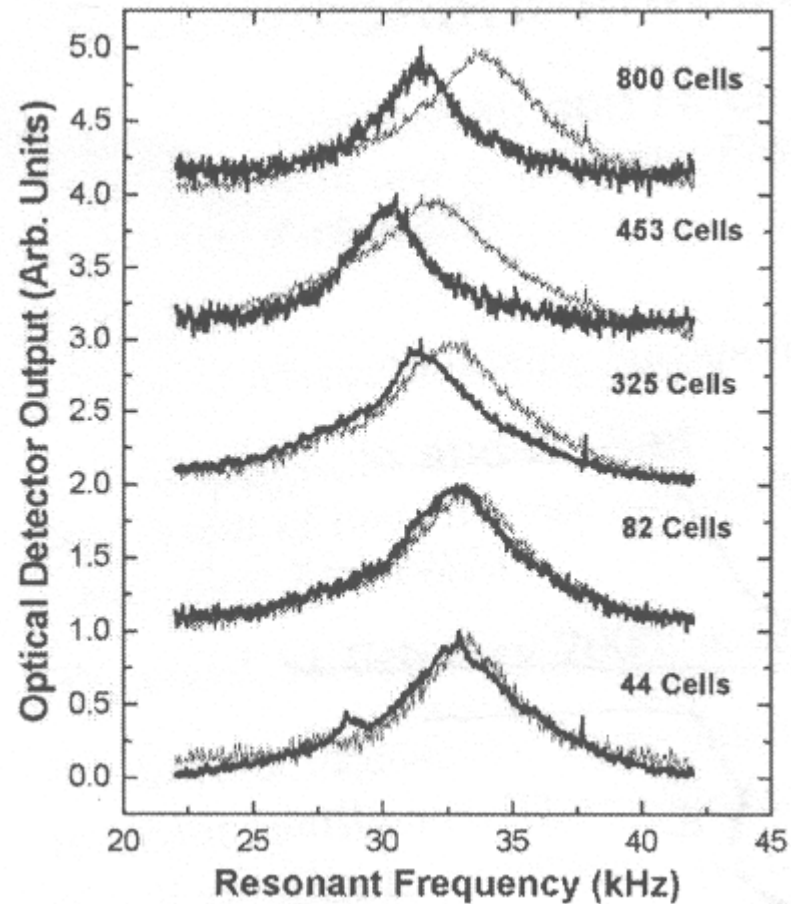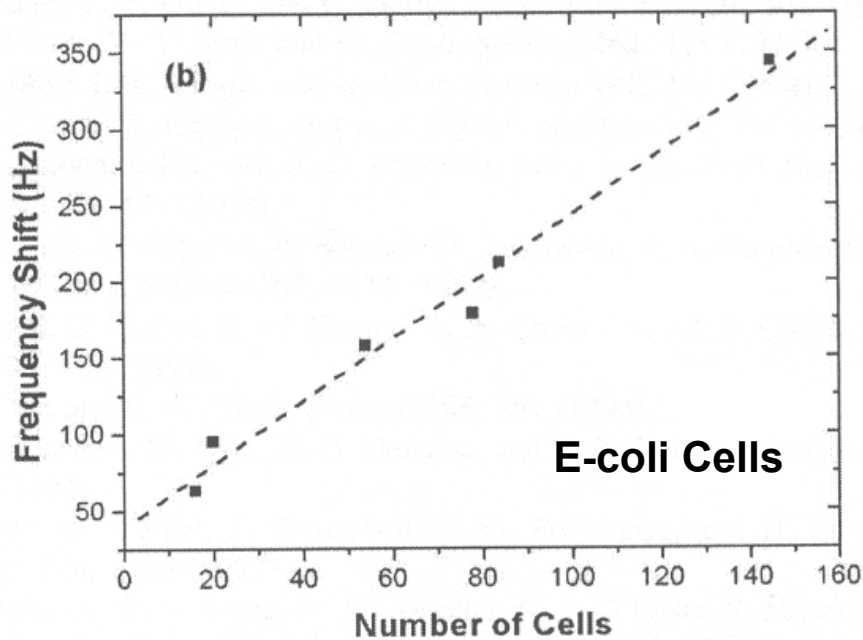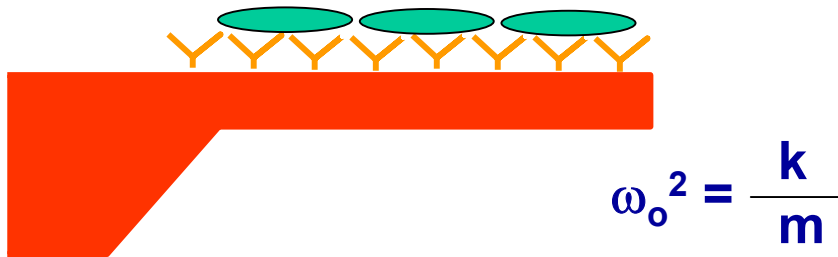
Detection of Listeria Cell Mass

Non-specific binding of *Listeria innocua* bacterial cells to a cantilever beam

1 μm

10 μm

Frequency Shift Vs. No. of Cells

$Y = 4.65x + 13.32$

$R^2 = 0.99$

Frequency Shift (Hz)

Effective no. of cells

$m^* = (x/L)m$

x

L

Frequency change after binding of 180 dry bacteria cells

Loaded Resonant Frequency (84.7 kHz)

Unloaded Resonant Frequency (85.6 kHz)

Amplitude (units)

Frequency (Hz)

x 10^4

A. Gupta, D. Akin, R. Bashir, SPIE, 2003.

Minimum Detectable Mass
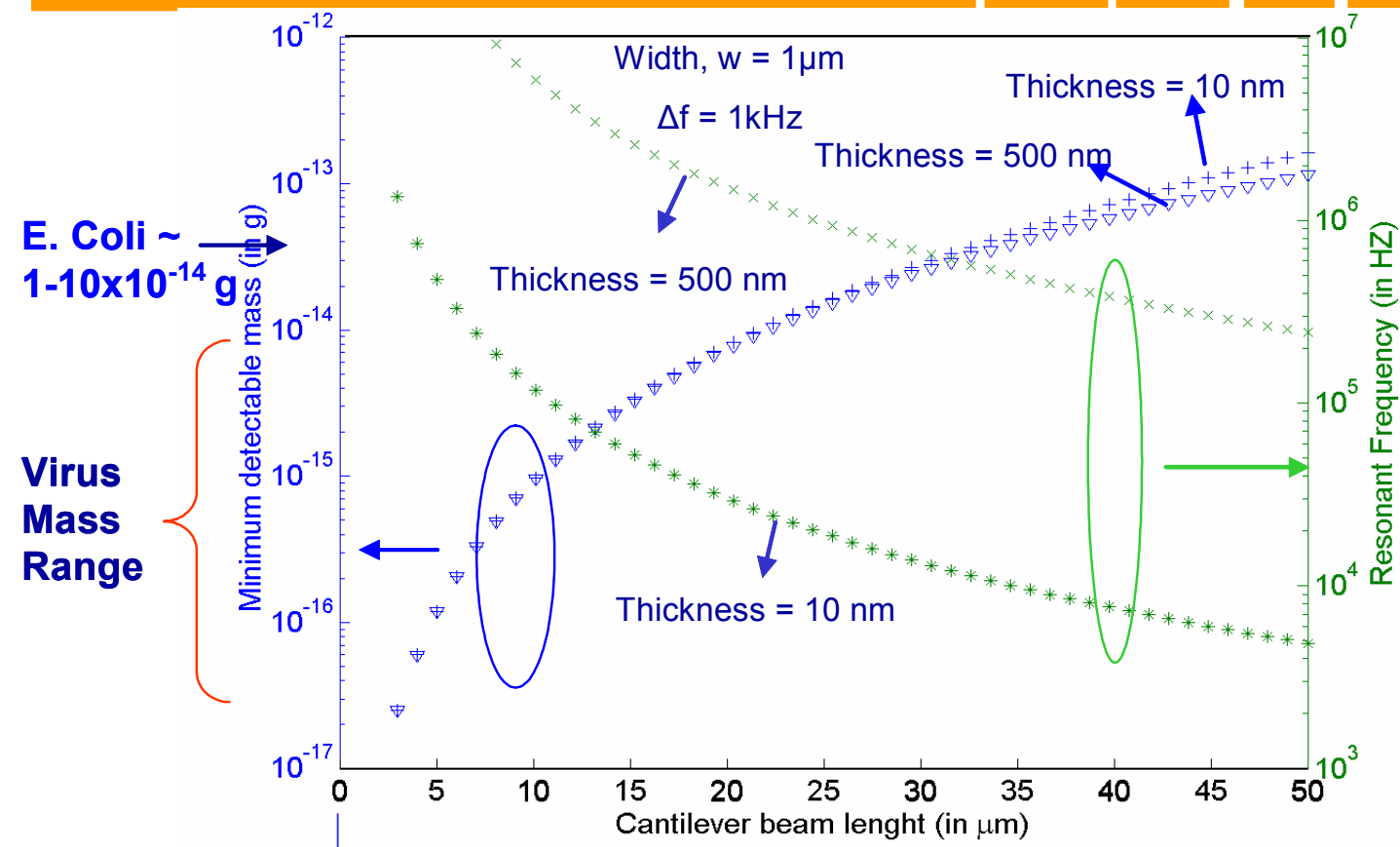
E. Coli ~
1-10x10^{-14} g

Virus
Mass
Range

Width, w = 1μm

Δf = 1kHz

Thickness = 10 nm

Thickness = 500 nm

Thickness = 500 nm

Thickness = 10 nm

Minimum detectable mass (in g)

Resonant Frequency (in HZ)

Cantilever beam lenght (in μm)

100kDa
Protein

DNA bp ~ 10^{-21} g

$$\delta m = 0.24 \cdot l \cdot w \cdot t \cdot \rho \cdot \left(\frac{1}{\left(1 - \frac{2\pi\delta f (0.98)l^2}{t} \sqrt{\frac{\rho}{E}} \right)^2} - 1 \right)$$

Minimum Detectable Mass

- **The frequency measurement is limited by thermo-mechanical noise on the cantilever beam.**

- **Minimum Detectable Frequency, Δf,min =**

$$\frac{1}{A}\sqrt{\frac{f_0\,k_B\,TB}{2\,\pi kQ}}$$

- **Minimum Detectable Mass, Δm,min =**

$$\frac{1}{A}\sqrt{\frac{4\,k_B\,TB}{Q}}\,\frac{m_{eff}^{\,5/4}}{k^{\,3/4}}$$

- **k_B = Boltzmann constant**

- **T = Temperature in Kelvin**

- **B = Bandwidth measurement, (~ 1 kHz)**

- **Q can increase by 100X by driving the cantilevers**

Minimum Detectable Mass for Silicon Cantilever Beams

- Q=5
- Q=500

Width of cantilever beam = 1 μm

Thickness of cantilever beam = 10 nm

Minimum Detectable Mass (g)

Length of cantilever beam (μm)

36

Materials Legend

Cross-sectional view   Top view

(a)

Silicon

(b)

Silicon dioxide

(c)

Etch Window
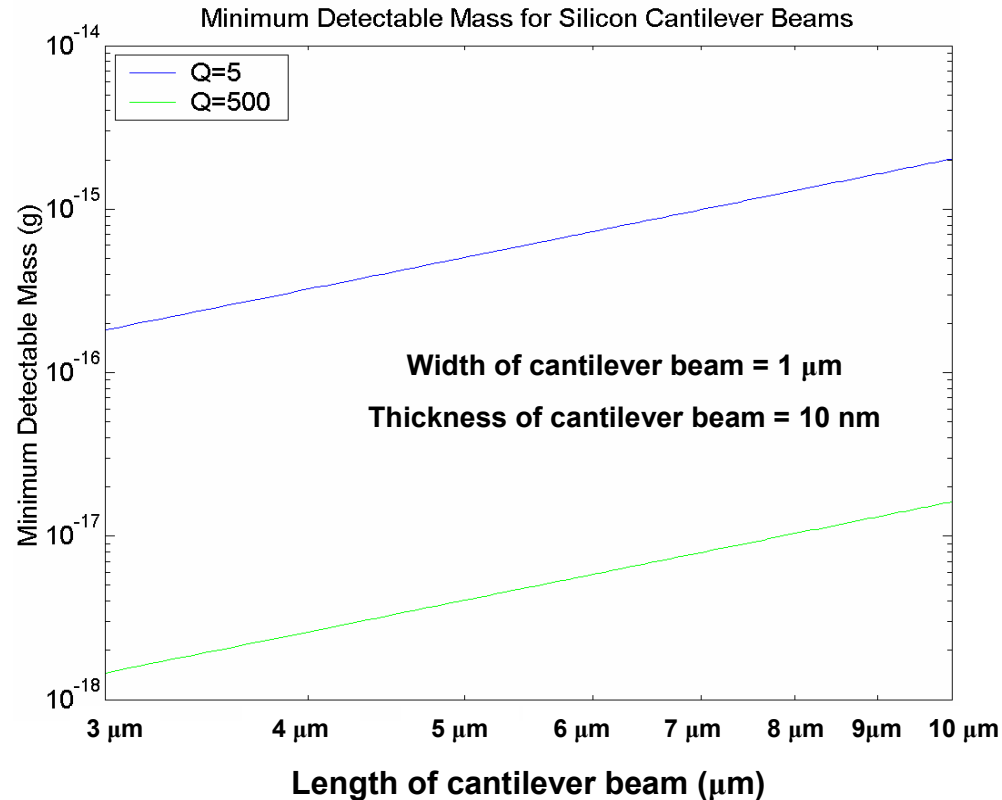
PECVD Silicon dioxide

(d)

Bottom of channel

(e)
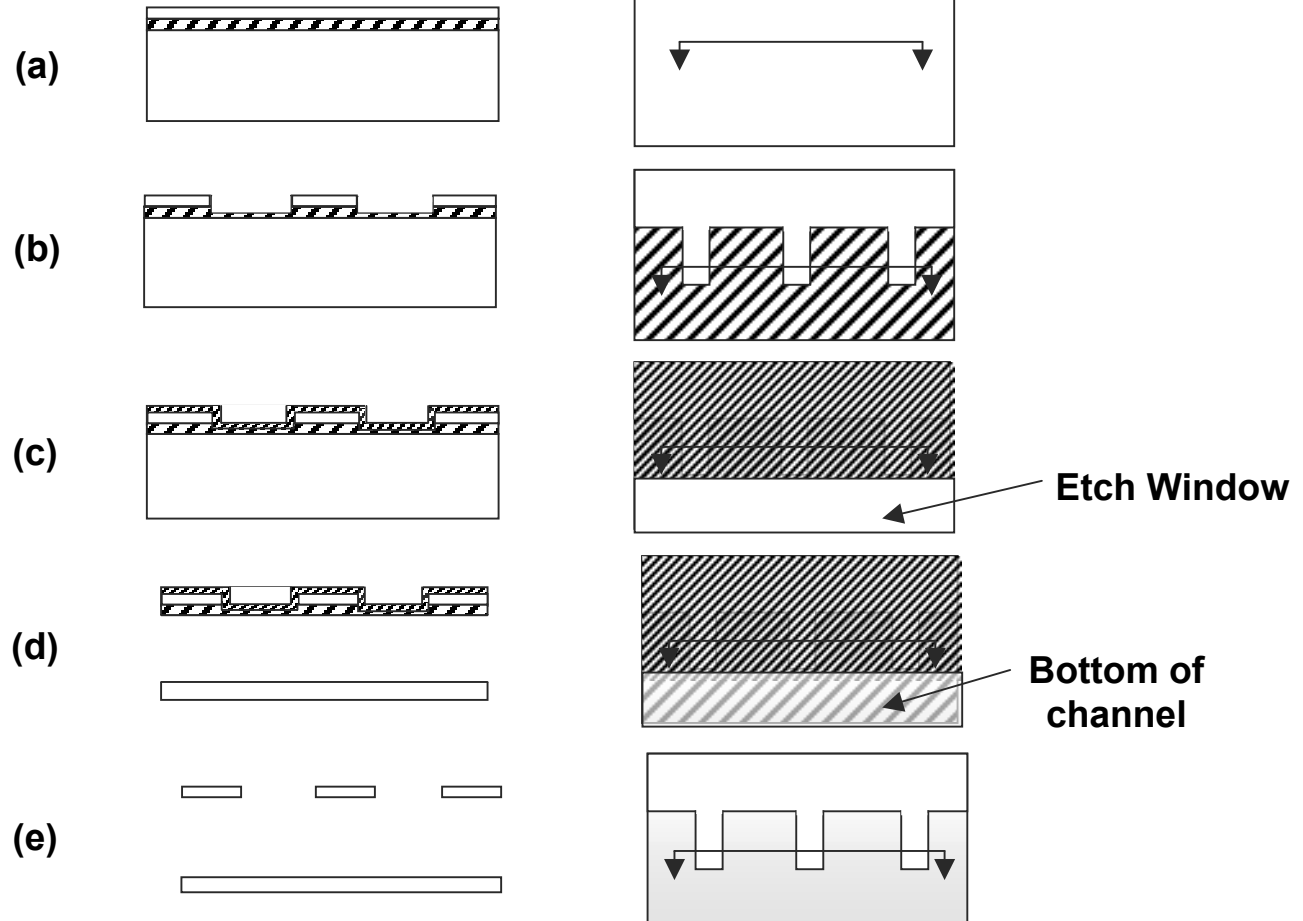
SEM Pictures of Cantilevers

3 Vaccinia Virus Particles

1μm

Vaccinia Virus Particles

Cantilever Beam

1μm

38

A. Gupta, D. Akin, R. Bashir, *Applied Physics Letters***, March 15, 2004.**
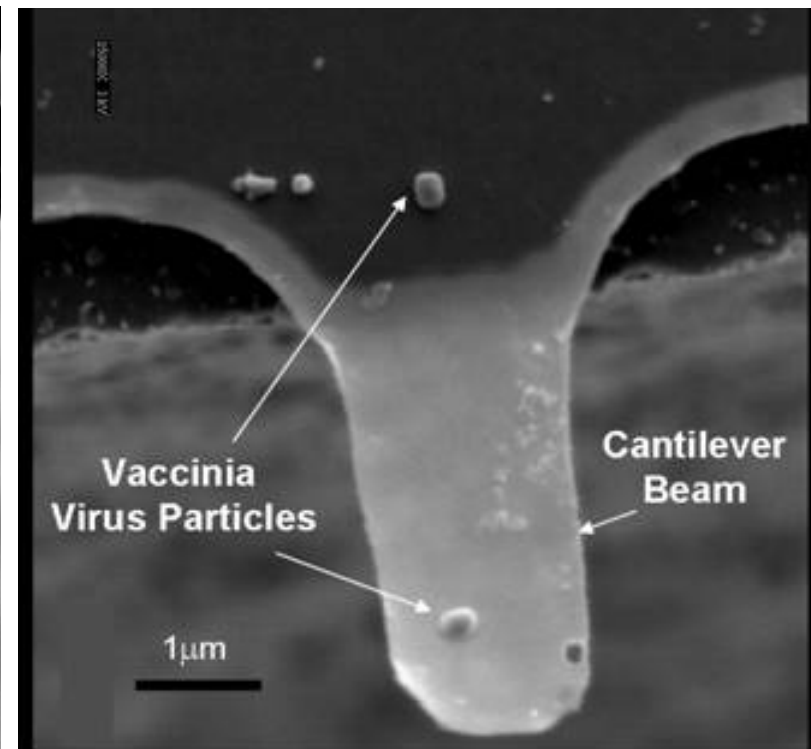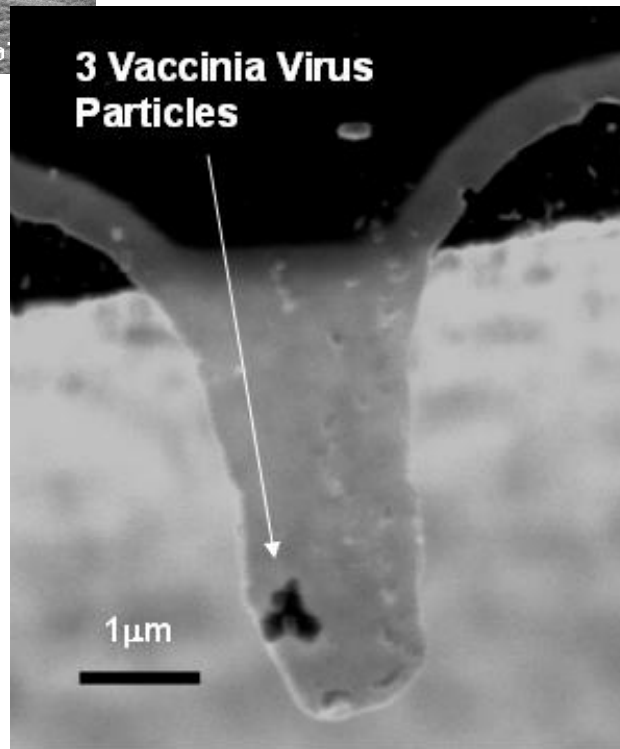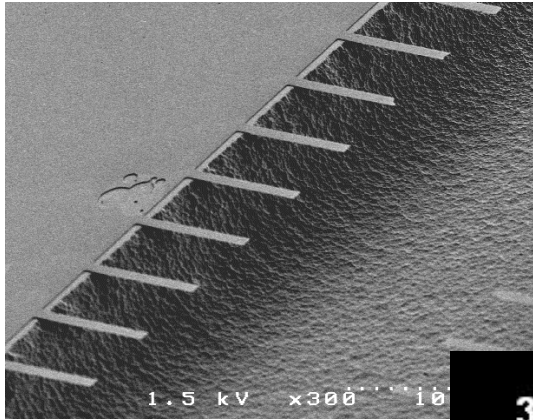
Frequency Shift vs. No. of Particles

$\Delta f = 60 \text{kHz}$

$f_1 = 1.21$ MHz

$f_0 = 1.27$ MHz

Q ~ 5
k = 0.006 N/m

Amplitude (a.u.)

10^{-9}

10^{-10}

Cantilever beam with virus particle
Unloaded cantilever beam

Frequency (in Hz)   x 10^6

0.6   0.8   1   1.2   1.4   1.6   1.8   2

$y = 33.6x + 25.4$

$R^2 = 0.9$

Resonant Frequency Shift (kHz)

- 1 kHz frequency shift for 160 ag
- Sensitivity ~ 6.3 Hz/ag

Effective No. of Virus Particles

0   1   2   3   4   5   6
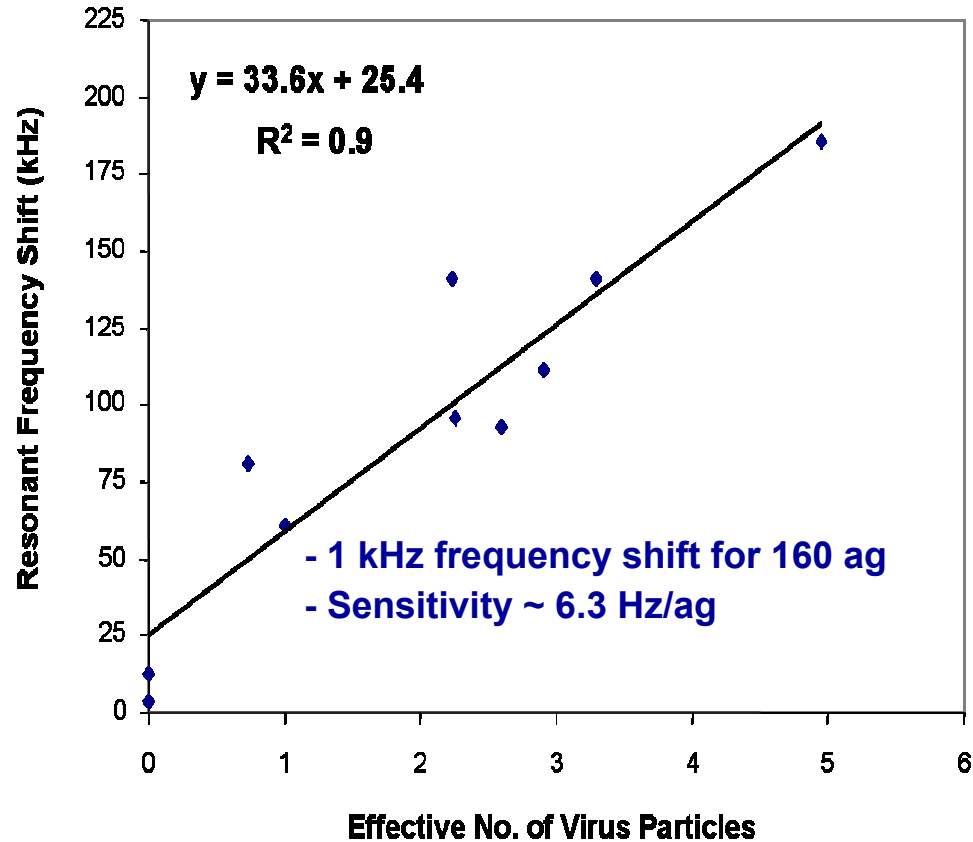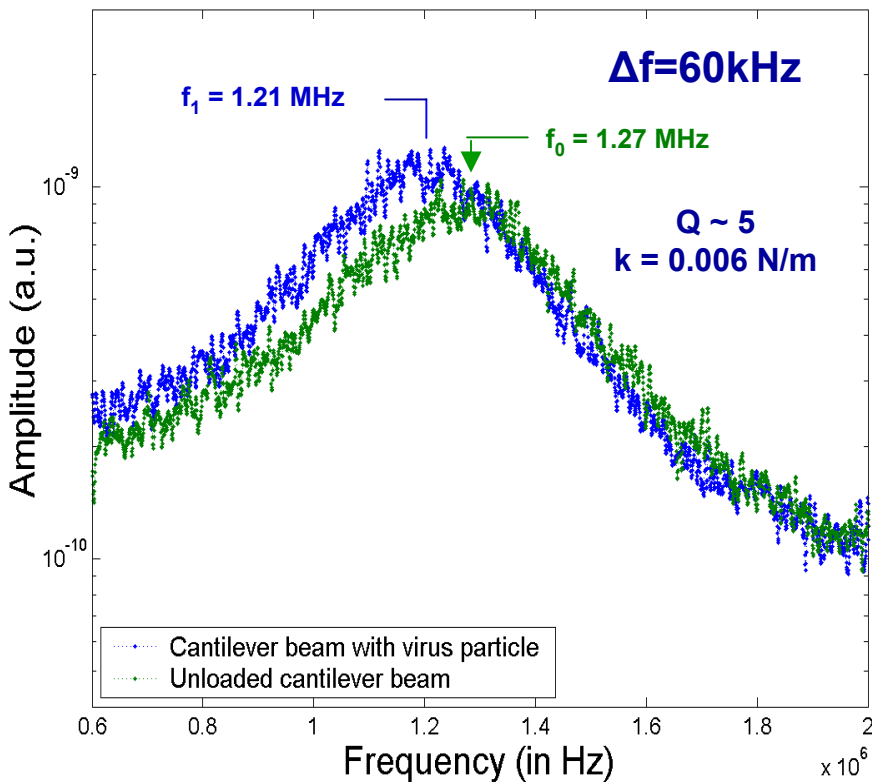
- Average mass of Vaccinia Virus ~ 9.5fg
- Work on going to integrated concentration elements
- Integrated Abs on cantilevers

A. Gupta, D. Akin, R. Bashir, *Applied Physics Letters*, March 15, 2004.

Mass of Molecules

(a) device layer / SiO₂ / Si

(c) Cr/Au

(b)

(d)

(f)
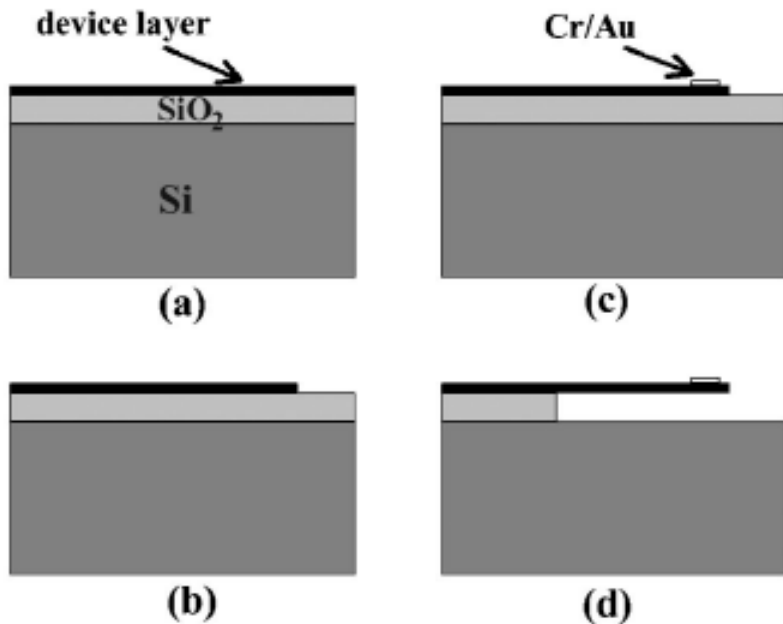
To probe the amount of thiolate binding to the Au contacts, we have measured the frequency spectra before and after the thiolate self-assembly. Figure 14 shows the measured shift in the resonant frequency for DNP-PEG4-C11thiol binding on 50- and 400-nm-diam Au contacts. The measured frequency shifts were 125 Hz and 1.10 kHz, corresponding to calculated masses of 6.3 and 213.1 ag, respectively.
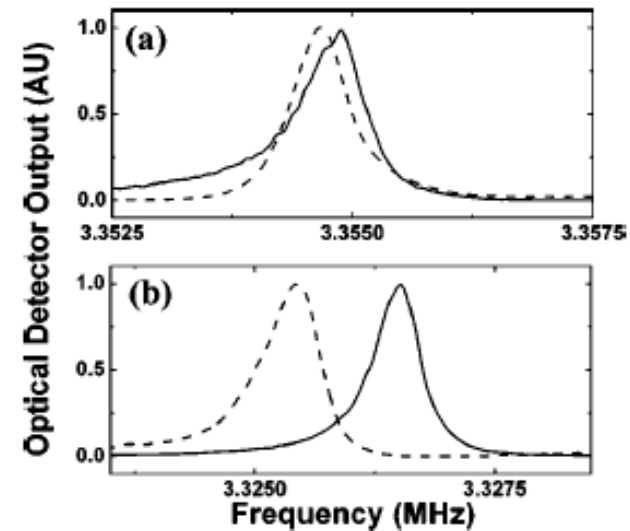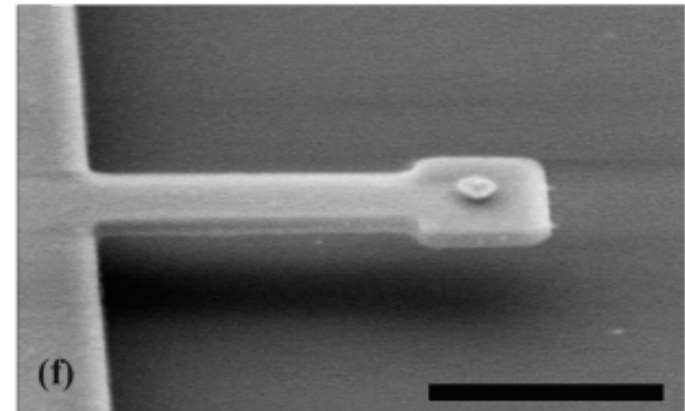
FIG. 14. Experimentally measured frequency spectra before (solid line) and after (dashed line) the adsorption of the thiolate on (a) 50- and (b) 400-nm-diam Au contact. Rectangular beam dimensions were $l = 10\ \mu m$, $w = 1\ \mu m$, and $t = 250$ nm.

Electrical/Electrochemical Detection

1. amperometric biochips, which involves the electric current associated with the electrons involved in redox processes,

2. potentiometric biochips, which measure a change in potential at electrodes due to ions or chemical reactions at an electrode (such as an ion Sensitive FET), and

3. conductometric biochips, which measure conductance changes associated with changes in the overall ionic medium between the two electrodes.

1. Amperometric Detection

Reference electrode

GOD

Reference electrode

Glucose

$\downarrow e^-$

Working Electrode

+ + + +

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{D-gluconic acid} + H_2O_2$$
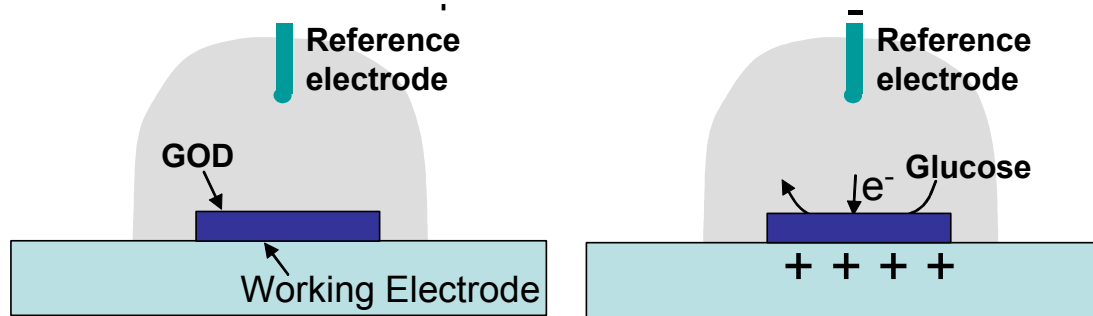
hydrogen peroxide is reduced at -600mV at Ag/AgCl anode reference electrode.

- Detection of Glucose, Lactate, Urea, etc.
- Enzyme entrapped in a gel
- Surface regeneration and sensor reusability

Lactate sensor

Glucose sensor

Perdomo, et al., 2000

Sensor current / nA — Concentration / mM
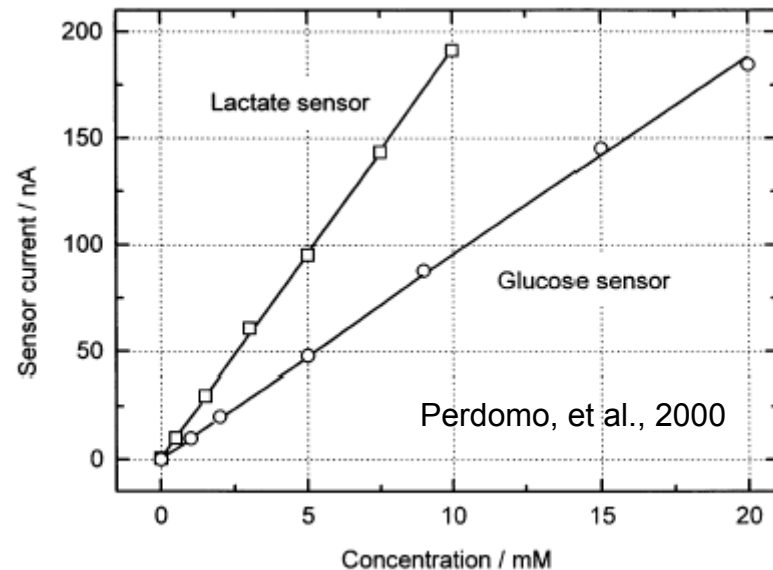
Detection of DNA Hybridization

- Capture probes are attached to electrodes.
- Target DNA binds to complementary probes
- DNA sequences, called signaling probes, with electronic labels attach to them (ferrocene-modified DNA oligonucleotides, E1/2 of 0.120 V vs. Ag/AgCl, act as signaling probes).
- Binding of the target sequence to both the capture probe and the signaling probe connects the electronic labels to the surface.
- The labels transfer electrons to the electrode surface, producing a characteristic signal.
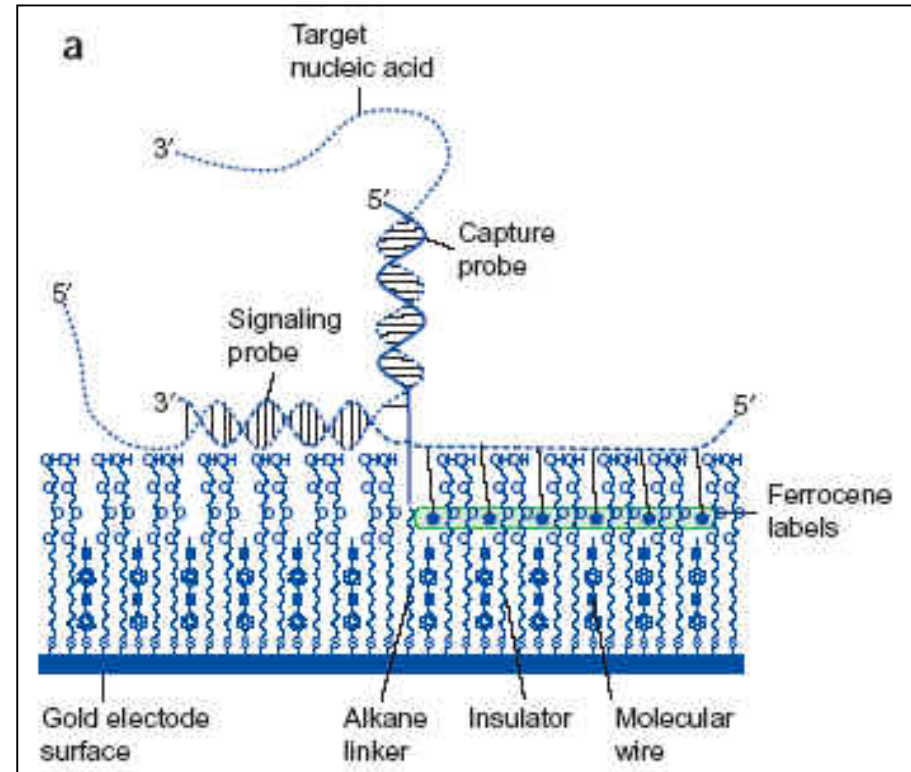
Umek, et al. J. Molecular Diagnostics, 3, 74-84, 2001
Drummond, Hill, Barton, Nature Biotech, v21, n10, Oct 2003, p1192
http://www.motorola.com/lifesciences/esensor/tech_bioelectronics.html

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2. Potentiometric Sensors
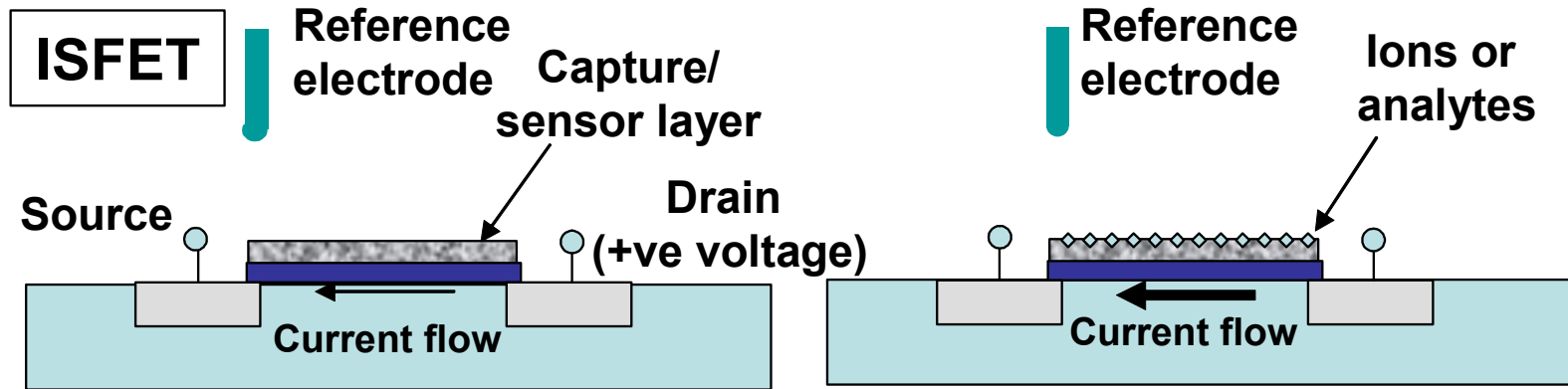
- ISFETs, ChemFETs, etc.
- Potential difference between the gate and the reference electrode in the solution
- Change in potential converted to a change in current by a FET or to a change in capacitance in low doped silicon
- Gate material is sensitive to specific targets
- pH, Ions, Charges

Nanoscale pH Sensors

- Label Free !!
- Detection of pH change
- Detection of protein binding
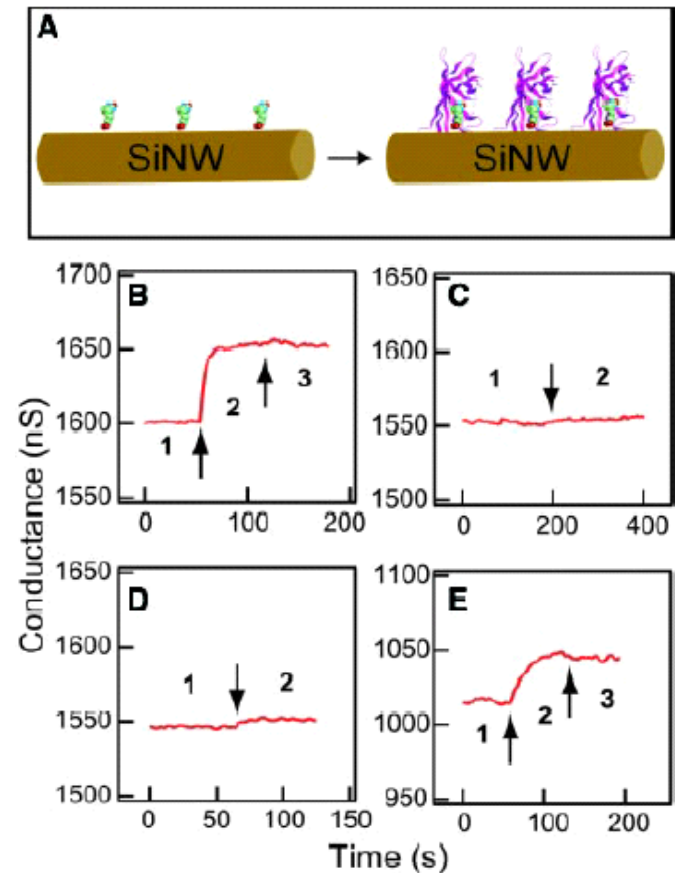
- Streptavidin binding detection down to at least 10 pM.
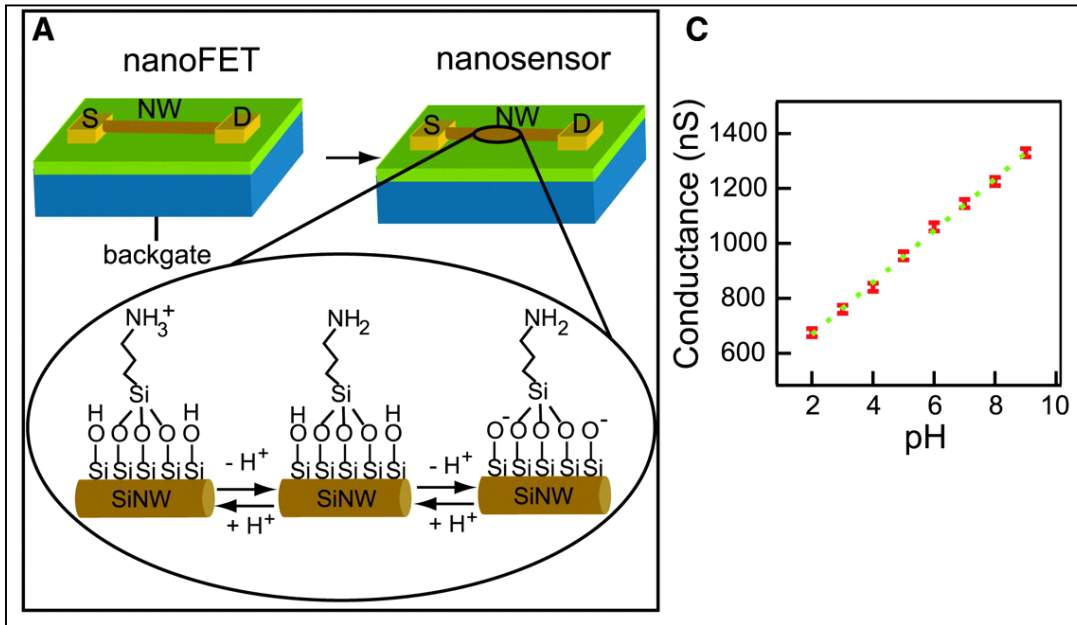- Substantially lower than the nanomolar range demonstrated by other procedures.

Y. Cui, Q. Wei, H. Park, and C.M. Lieber. Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. *Science*, 293:1289–1292, 2001.

Integrated Silicon Nanowire Sensors

Objectives:

- **Bio-sensors with electronic output**

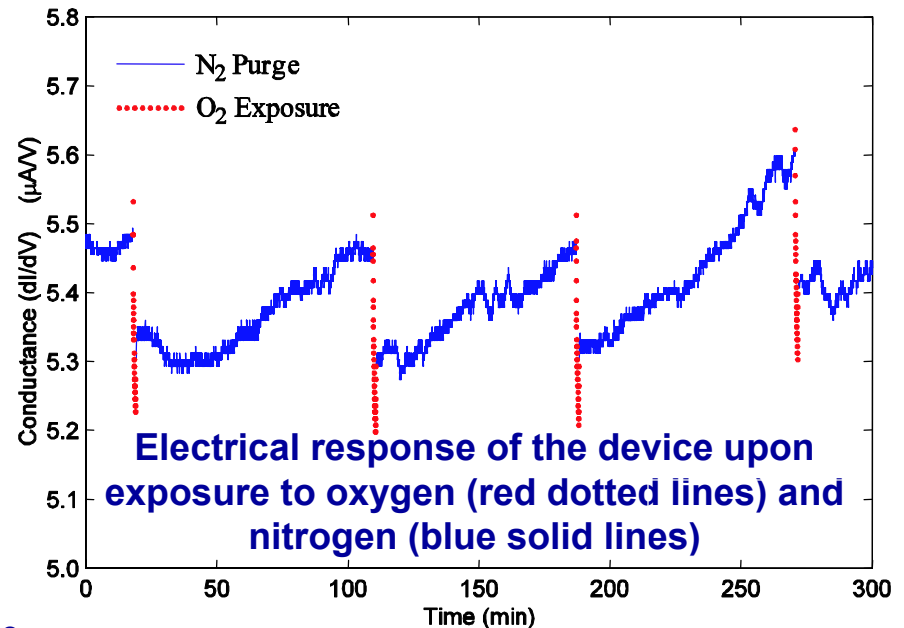- **Capability of dense arrays integrated with ULSI silicon**

- **Direct Label Free Detection of DNA and Proteins**

- Plate Size ~ 20nm X 1μm X 3μm
- Wire Size ~ 20nm X 20nm X 3μm

Capture Molecules

Target Molecules

Metal

N+   N+

oxide

N-/P-

Integrated Bottom Gate

P type Silicon

Electrical response of the device upon exposure to oxygen (red dotted lines) and nitrogen (blue solid lines)
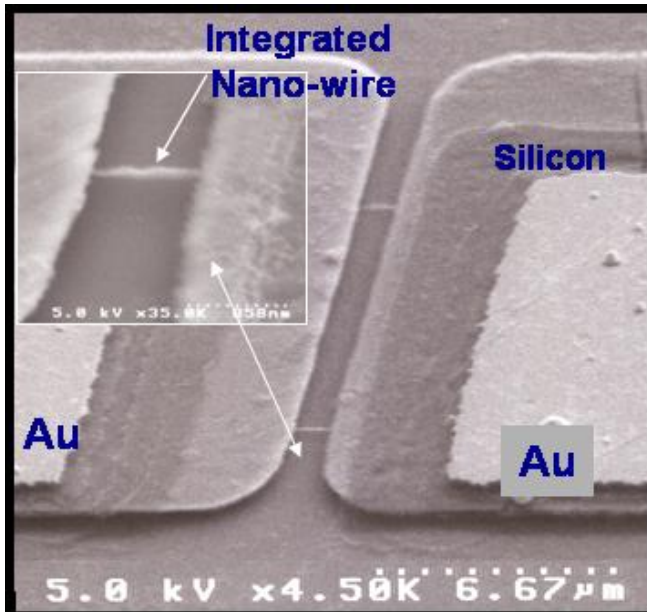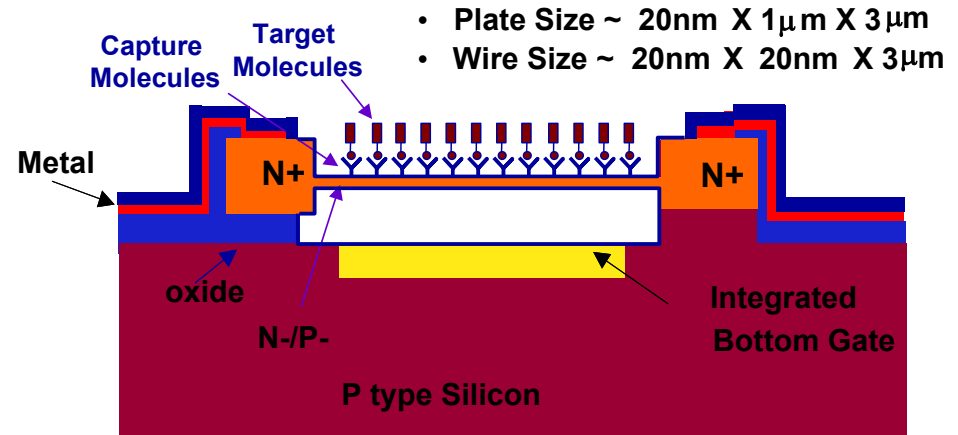
Field Effect Sensing of DNA

J. Fritz, Emily B. Cooper, Suzanne Gaudet, Peter K. Sorger, and Scott R. Manalis, Electronic detection of DNA by its intrinsic molecular charge, PNAS 2002 99: 14142-14146.

47
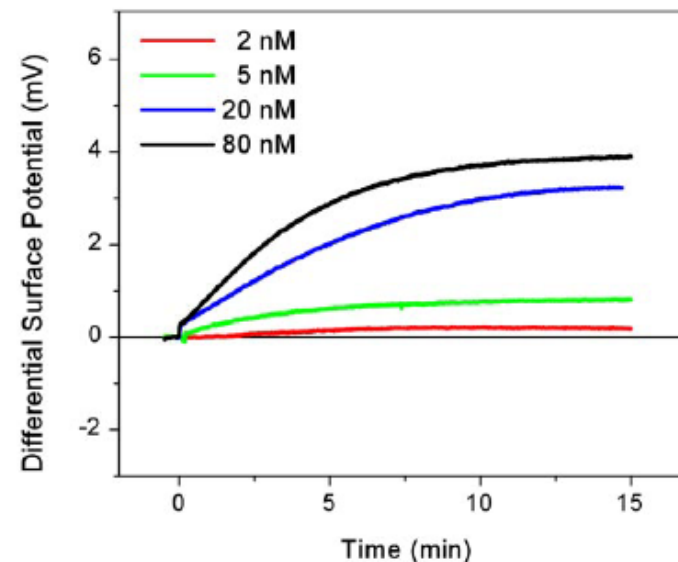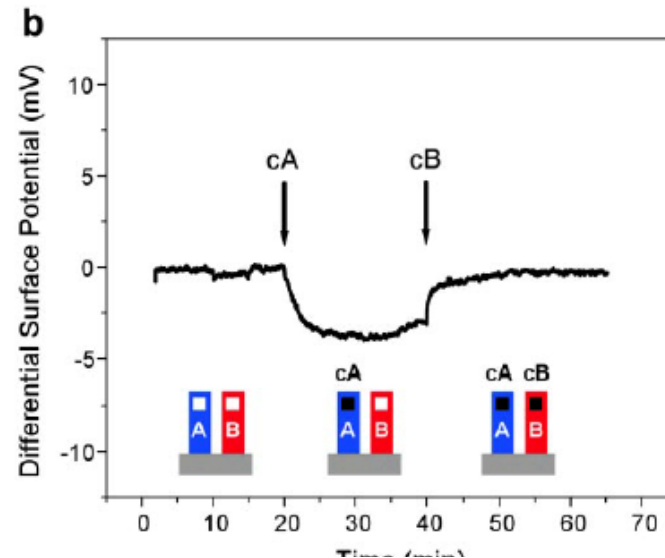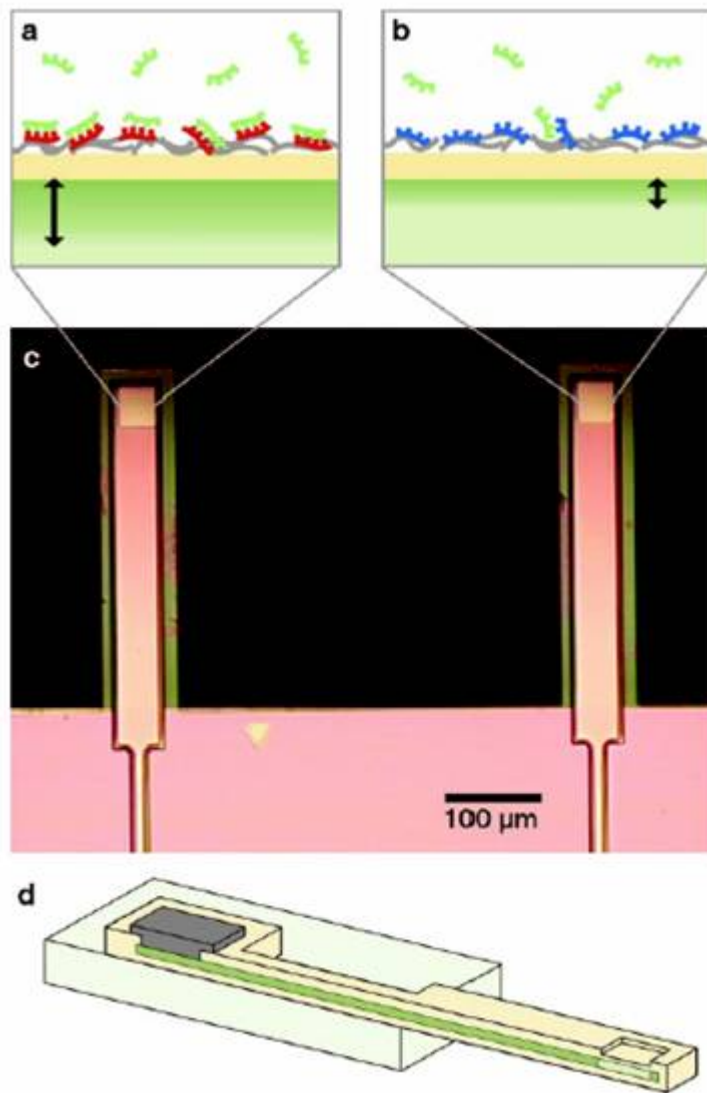
3. Conductometric Biochips

- Conductometric sensors measure the changes in the electrical impedance between two electrodes, where the changes can be at an interface or in the bulk region and can be used to indicate biomolecular reaction between DNA, Proteins, and antigen/antibody reaction, or excretion of cellular metabolic products.

Measurement Volume

Z (bulk)

Z (interface)

Measurement electrodes

Measurement electrodes
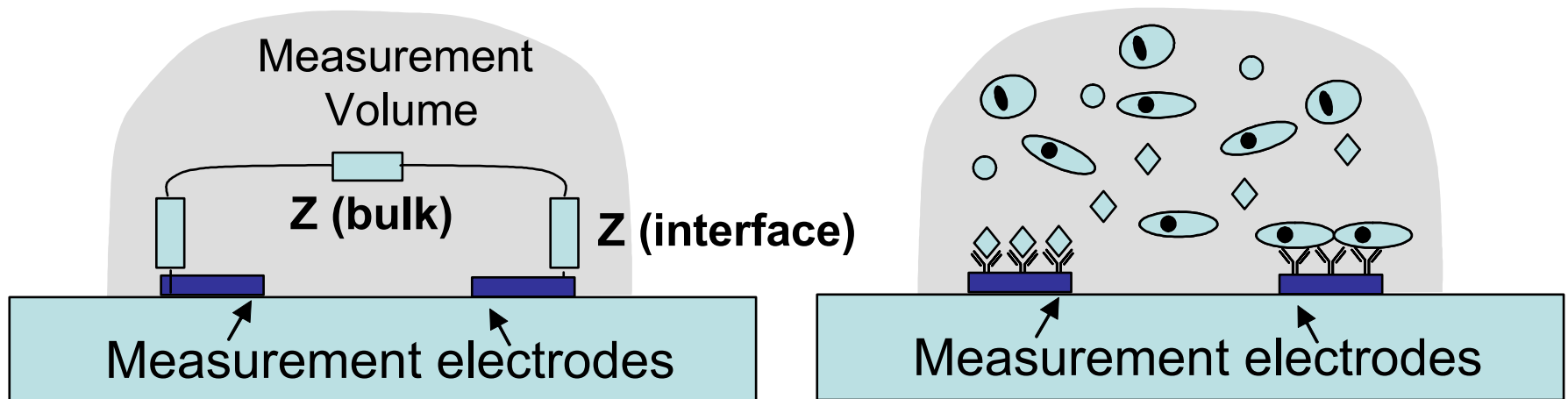
Nanoparticle Mediated DNA Detection
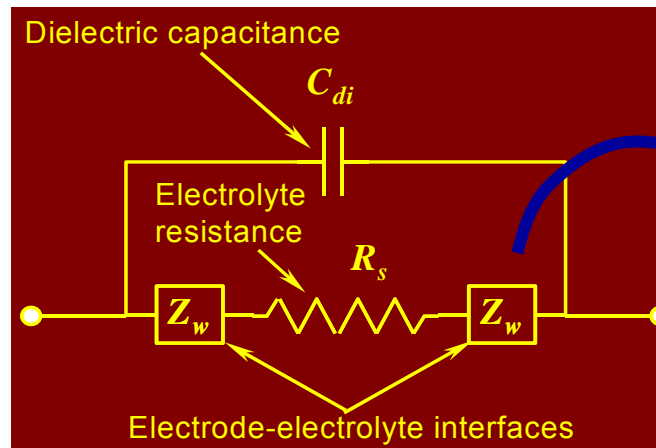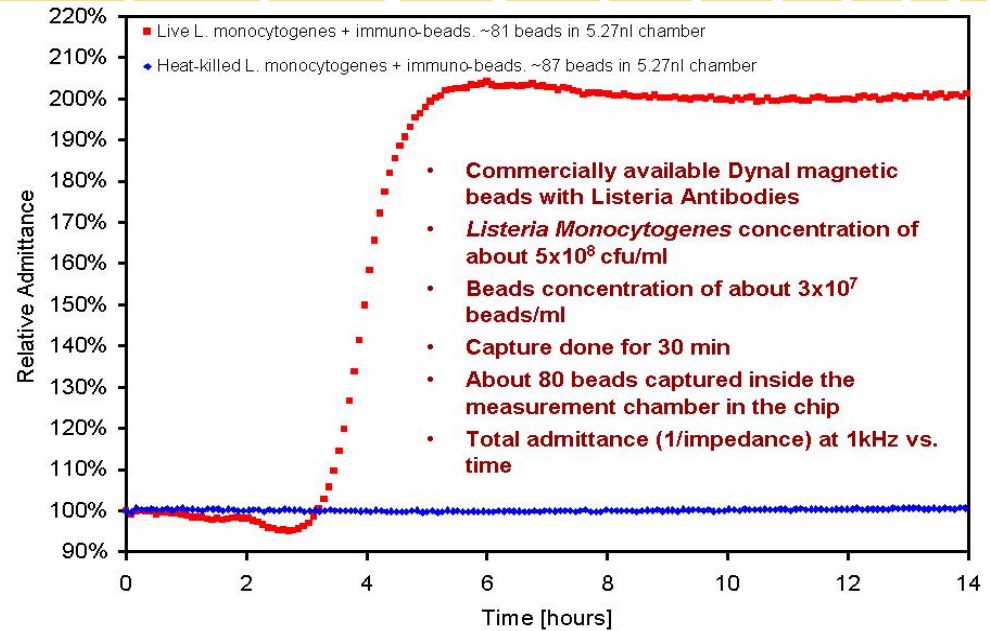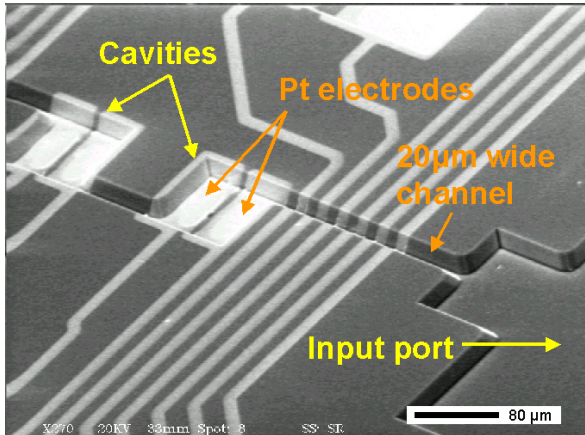
- Au nanoparticles assemble between two electrodes if DNA is hybridized

- Silver staining of the Au nanoparticles

- Conductance changes between micro-scale electrodes indicate DNA hybridization

- Sensitivity of 5×10^{-13} M shown

Park, S.-J.; Taton, T. A.; Mirkin, C. A. Array-Based Electrical Detection of DNA Using Nanoparticle Probes, Science, 2002, 295, 1503-1506.
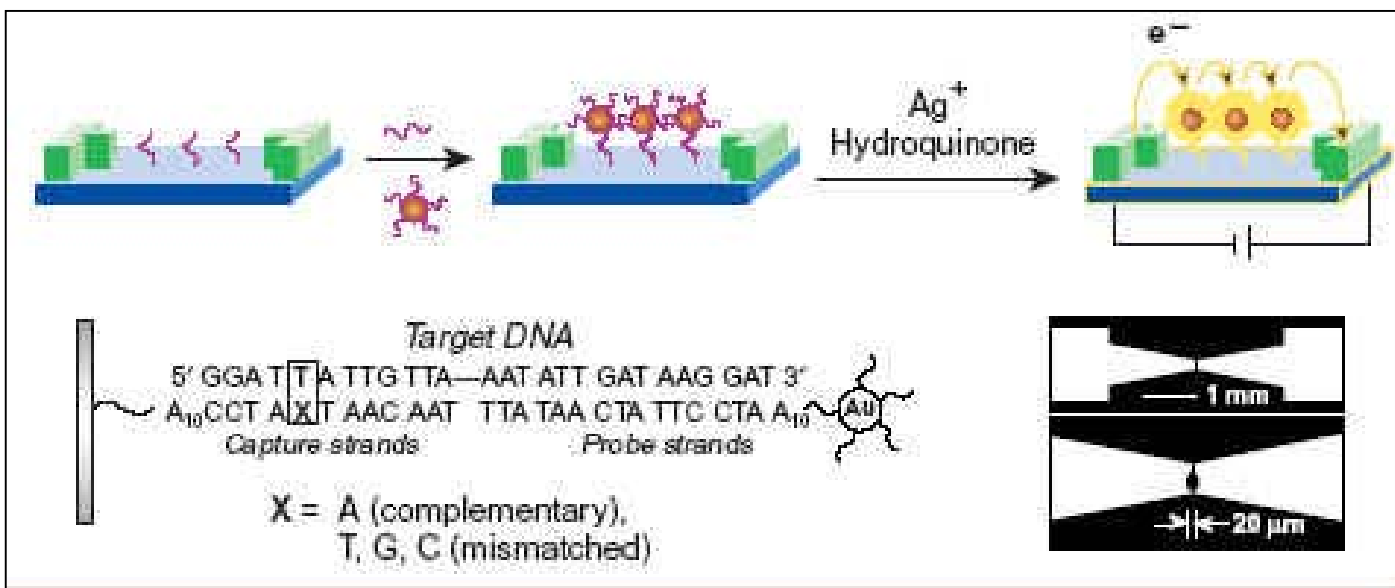
Micro-fluidic Devices for Conductance Detection of Bacterial Metabolism

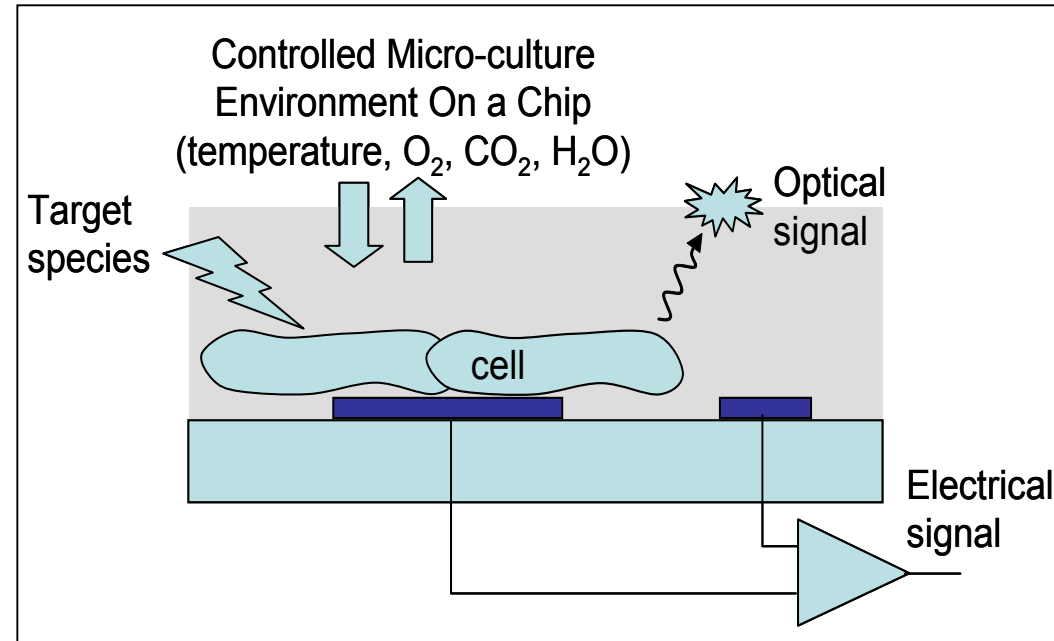- **Detection of Cell Growth by measuring their metabolic activity in micro-fluidic devices**

Cavities
Pt electrodes
20µm wide channel
Input port
80 µm

Live L. monocytogenes + immuno-beads. ~81 beads in 5.27nl chamber
Heat-killed L. monocytogenes + immuno-beads. ~87 beads in 5.27nl chamber

- Commercially available Dynal magnetic beads with Listeria Antibodies
- *Listeria Monocytogenes* concentration of about 5×10^8 cfu/ml
- Beads concentration of about 3×10^7 beads/ml
- Capture done for 30 min
- About 80 beads captured inside the measurement chamber in the chip
- Total admittance (1/impedance) at 1kHz vs. time

Dielectric capacitance
C_{di}
Electrolyte resistance
R_s
Z_w Z_w
Electrode-electrolyte interfaces

Electrode-Electrolyte Interface Model:

$$Z_w = \frac{1}{(j\omega)^n B}$$

Constant-angle impedance

R. Gomez, et al., Biomedical Micro-Devices, vol. 3, no. 3, p. 201-209, 2001.
R. Gomez, et al., Sensors and Actuators, B, 86, 198-208, 2002.

- The transductions of the cell sensor signals maybe achieved by:
  - the measurement of transmembrane and cellular potentials,
  - impedance changes,
  - metabolic activity,
  - analyte inducible emission of genetically engineered reporter signals, and
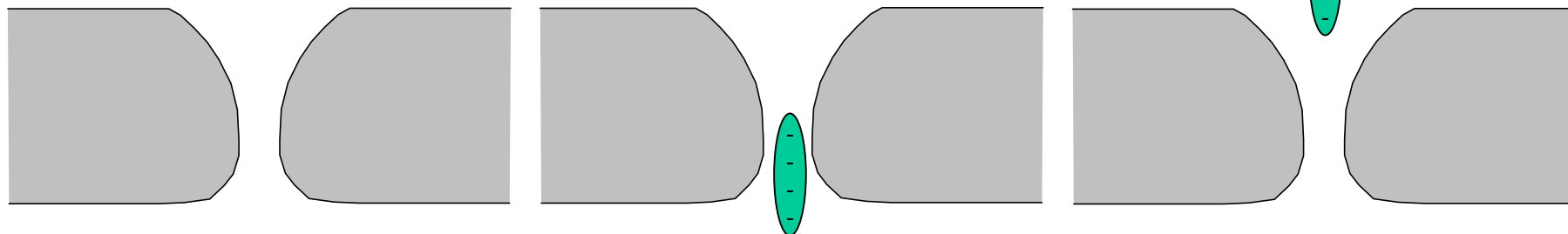 - optically by means of fluorescence or luminescence.

Controlled Micro-culture Environment On a Chip (temperature, O_2, CO_2, H_2O)

Target species

cell

Optical signal

Electrical signal

L. Bousse, Whole cell biosensors, Sensors and Actuators B (Chemical), Vol. B34, No. 1-3, August 1996, pp. 270-5.

J.J. Pancrazio, J.P. Whelan, D.A. Borkholder, W. Ma, D.A. Stenger, Development and application of cell-based biosensors, Annals of Biomedical Engineering, Vol. 27, No. 6, November 1999, pp. 697-711.

D.A. Stenger, G.W. Gross, E.W. Keefer, K.M. Shaffer, J.D, Andreadis, W. Ma, J.J. Pancrazio, Detection of physiologically active compounds using cell-based biosensors, Trends in Biotechnology, Vol. 19, No. 8, August 1, 2001, pp. 304-309.
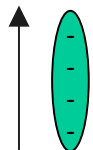
+

***Trans* chamber**

-

***Cis* chamber**
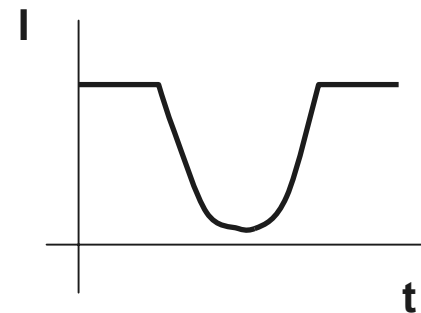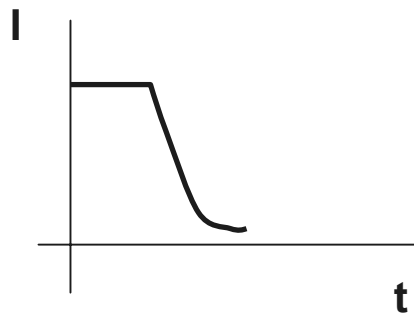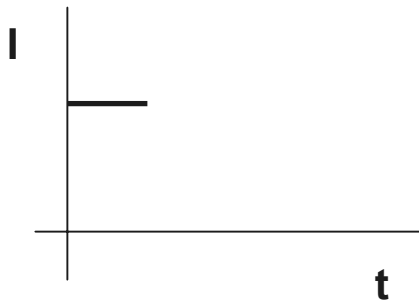
Micro-pore for cellular studies

Negative charges

plasmid

peptidoglycan cell wall

DNA

ribosome

Micro-pore

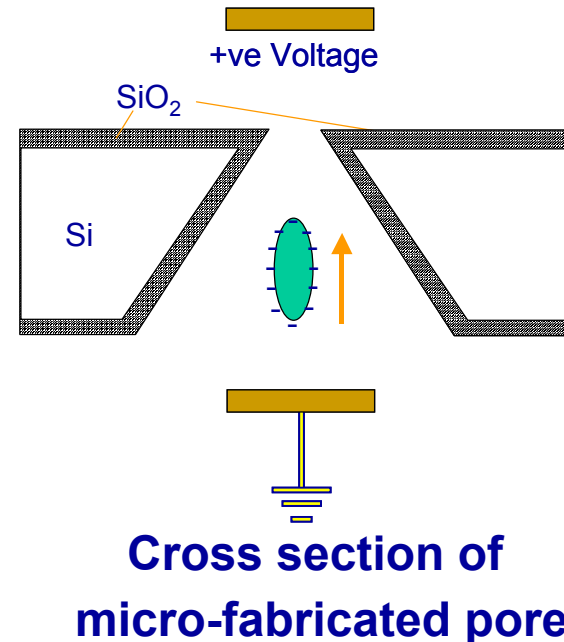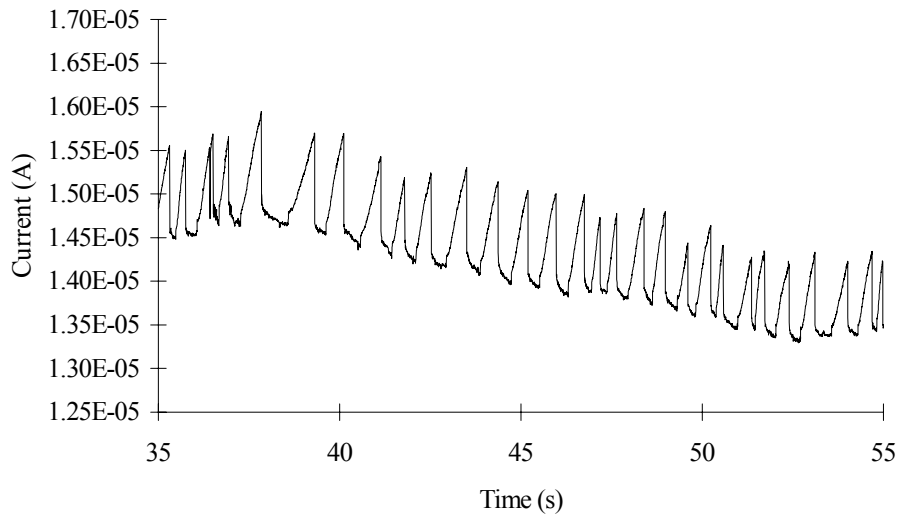**Optical Picture of a Pore in a micro-fabricated filter**
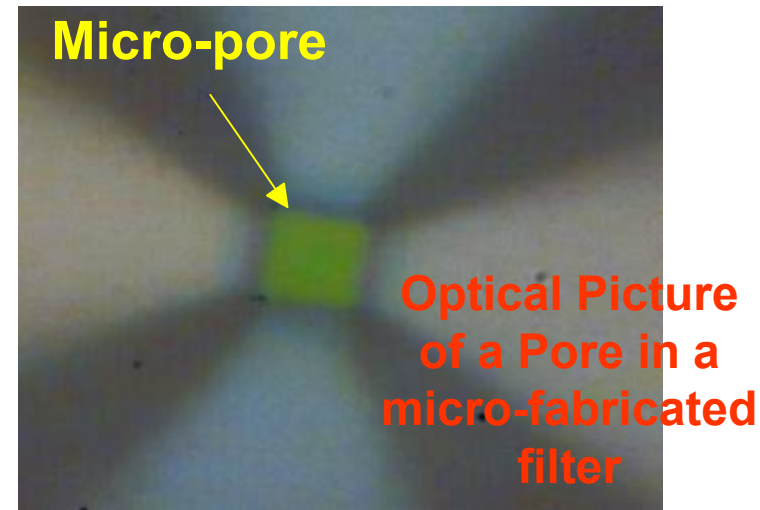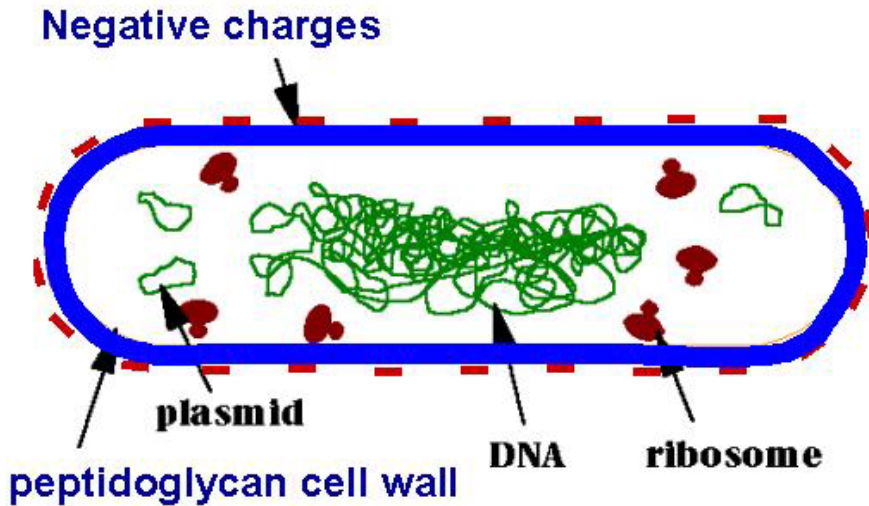
- **Micro-devices for single cell characterization – utilize the charge properties**
- **Micro-fabricate a pore where single entity can pass**

+ve Voltage

SiO_2
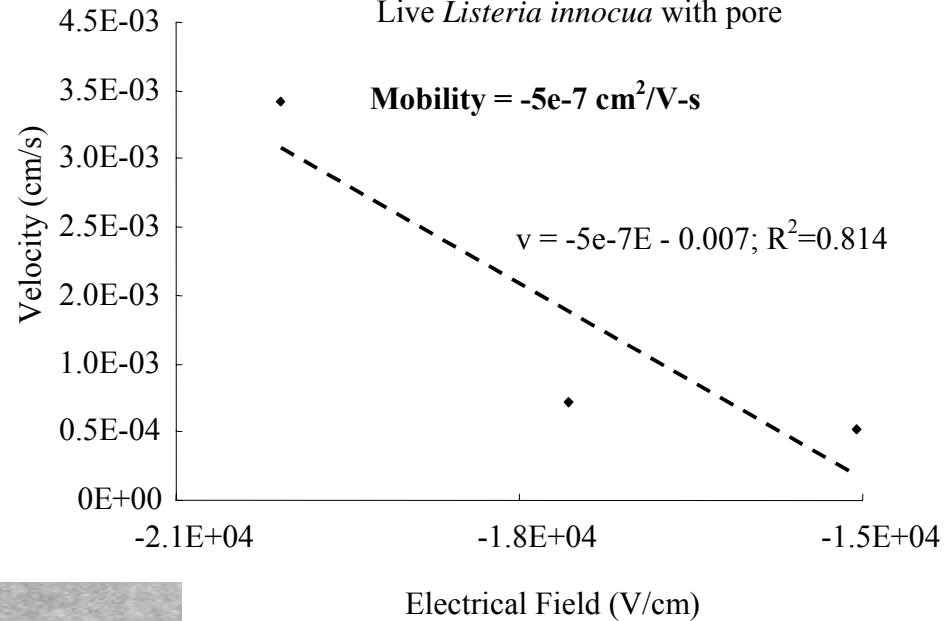
Si

Cross section of micro-fabricated pore

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Microscale Coulter Counter

I-T Diagram for Live Listeria, 1e8/ml, V = 40 V, 05112010

Velocity (cm/s) vs. Electrical Field (V/cm)
Live *Listeria innocua* with pore

Mobility = -5e-7 cm^2/V-s

$v = -5e-7E - 0.007;$ $R^2 = 0.814$

Live L*isteria innocua*
with a well-defined
cell wall

H. Chang, A. Ikram, T. Geng, F. Kosari, G. Vasmatzis, A. Bhunia, and R. Bashir, "Electrical characterization of microorganisms using microfabricated devices", Journal of Vacuum Society and Technology B, 20, 2058 (2002).
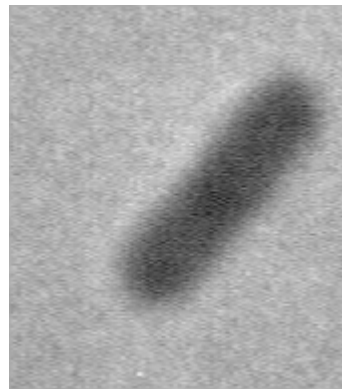
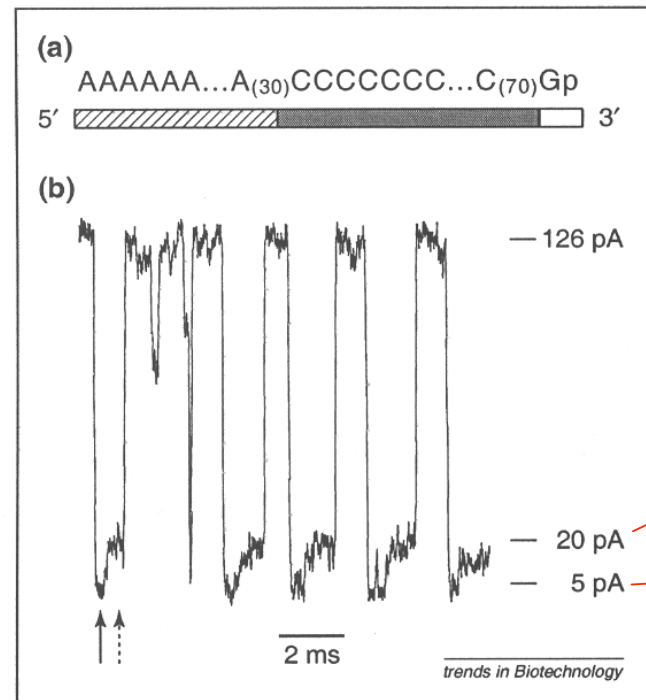# Nanoscale DNA Coulter Counter

- α-hemolysin channel, a biological protein based-pore, was utilized.
- Pore size is 2.6 nm.
- Both RNA and DNA molecules were observed traversing the nanochannel.
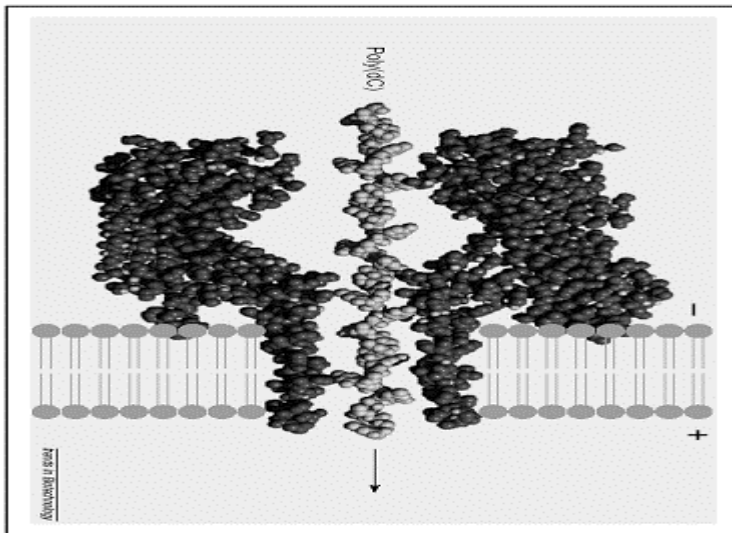
α-hemolysin nanochannel

The model of DNA passing through an α-hemolysin channel.

Kasianowicz et al., 1996, Meller, et. al. 2000.
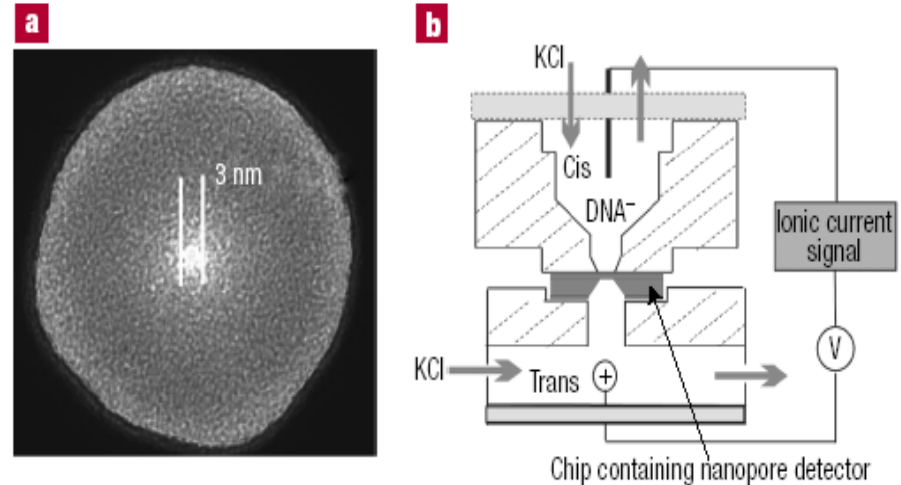
Fabrication Techniques

- **Solid-state based nanopore. Made in silicon nitride membrane.**
- **Pore size: 3 nm and 10 nm.**
- **The relation among DNA lengths and translocation times and applied biases were determined.**
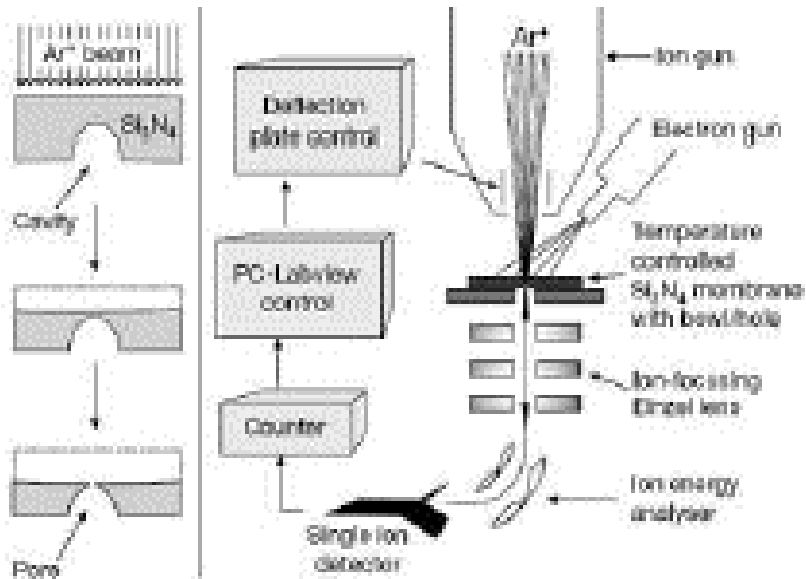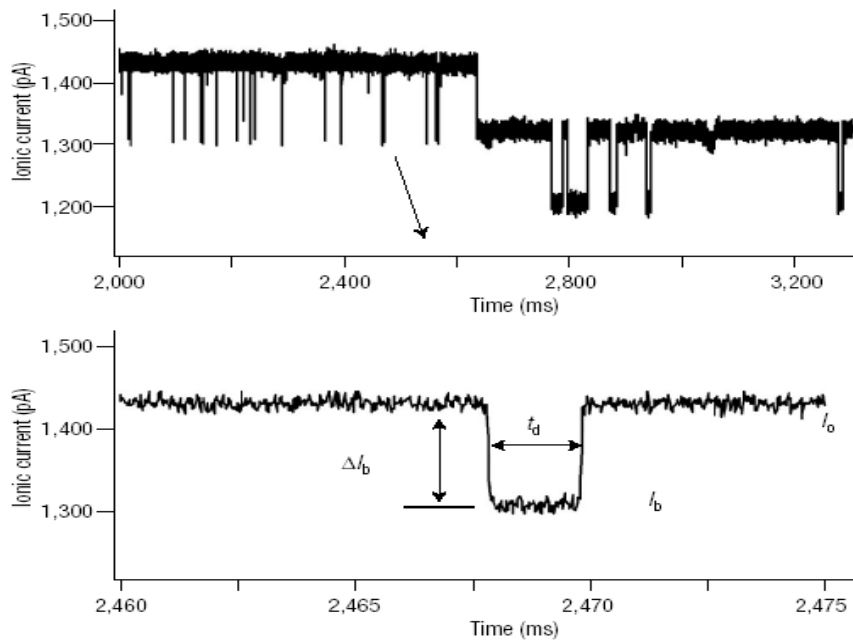

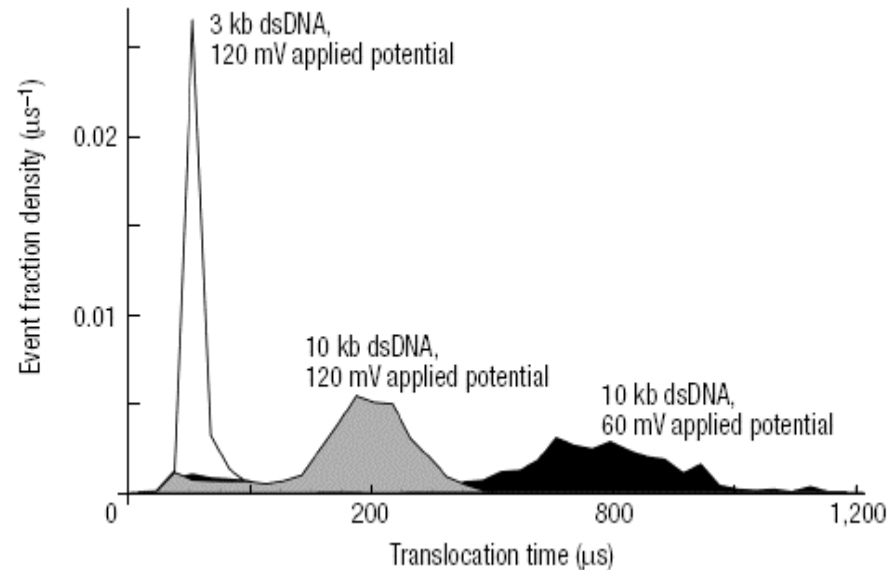The fabrication of Li's nanopore. From *Li et. al. Nature, 2001.*

TEM of Li's nanopore. b. DNA measurement setup in Li's work. From *Li et. al. Nature Materials, 2003*
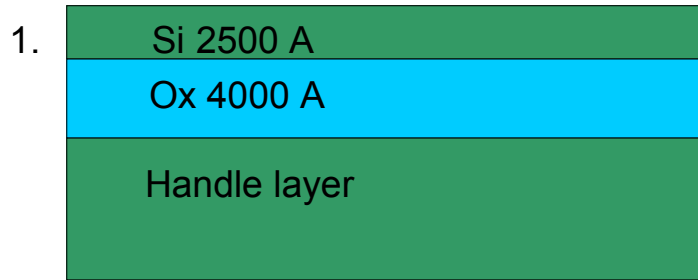
DNA Translocation

Current fluctuations when DNA was passing through the pore

Histograms of relation among DNA lengths, translocation times and applied biases.

Li et. al. 2003

(Not to scale)

1.

Si 2500 A

Ox 4000 A

Handle layer

Start with a (100) 4 inch SOI wafer.
Thickness : 525 um. SOI : 250 nm,
Buried oxide layer: 400 nm.

2.

Ox 1000 A
Si 1400 A
Ox 4000 A
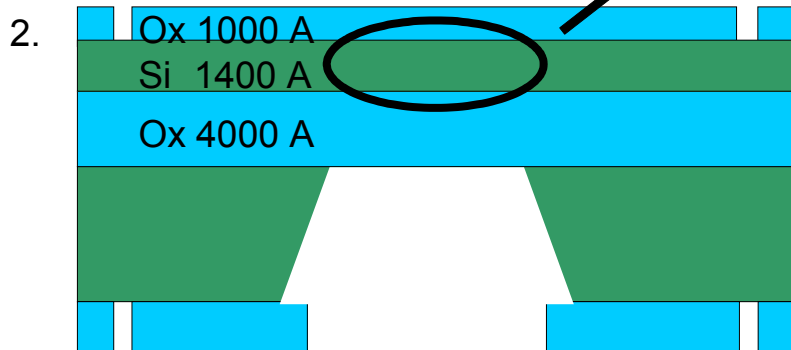
Grow thermal oxide on wafer surface and
open etch window to etch through the
handle layer. Etch stops on buried oxide
layer.

3.

Ox 1000 A

230-240 nm

Si 1400 A

70-80 nm

Ox 4000 A
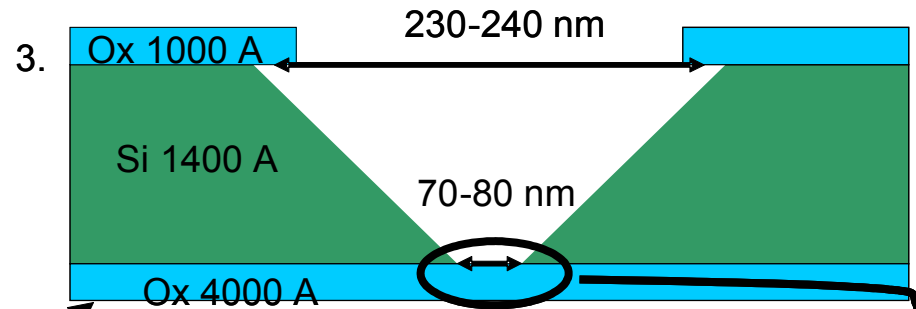
On SOI layer, open another etch window to
etch through the SOI layer. Etch stops on
buried oxide layer.

4.

30-40 nm
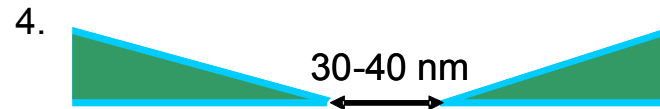
Remove buried oxide layer and
regrow 100 nm thermal oxide.

5.

3-5 nm

Shrink the pore to 3 –5 nm by
TEM

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41 x 26 nm (300,000 X)   **23 nm (1,000,000 X)**   **19 nm (1,000,000 X)**   **11 nm (1,000,000 X)**   **7 nm (1,000,000 X)**   **4.2 nm x 4.6 nm 1,000,000 X)**

Pore size vs TEM shrinking time

- Initial diameter = 48 nm
- Initial diameter = 15 nm
- Linear (Initial diameter = 48 nm)
- Linear (Initial diameter = 15 nm)

$y = -0.6034x + 48.357$
$R^2 = 0.9638$
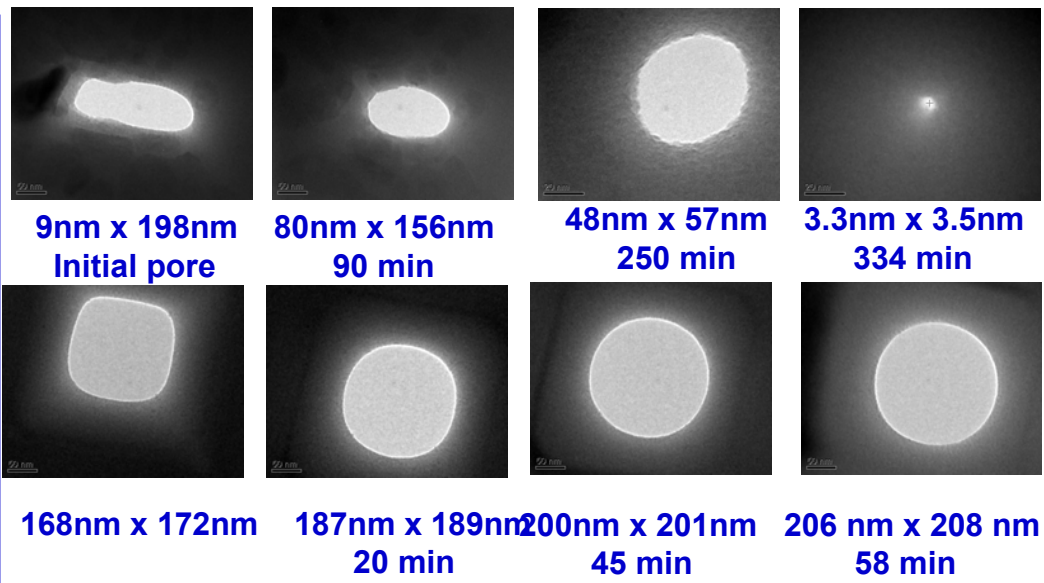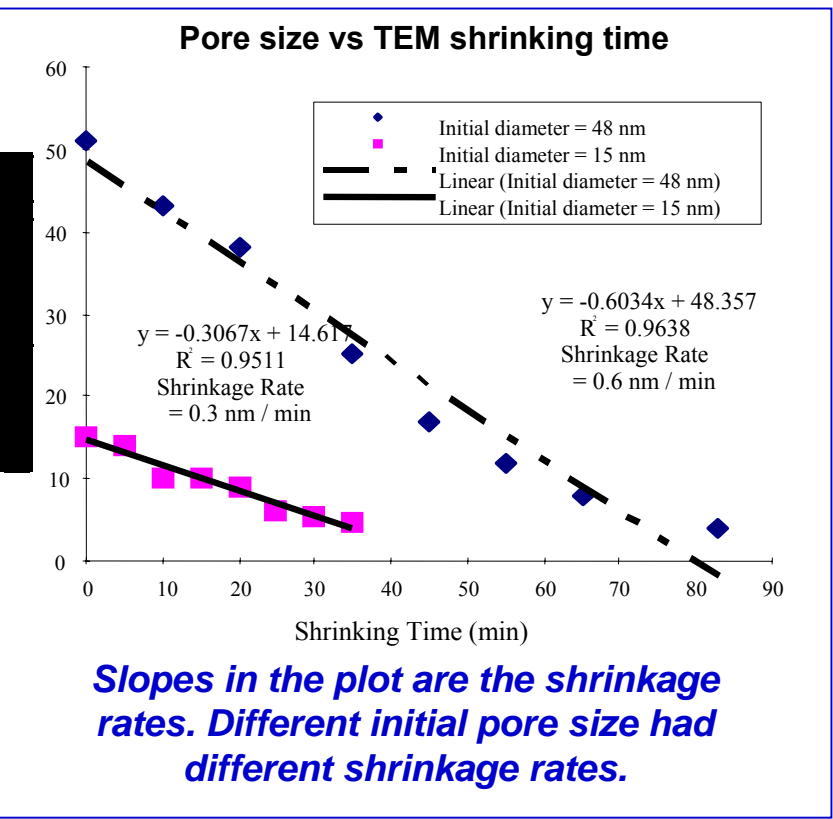Shrinkage Rate = 0.6 nm / min

$y = -0.3067x + 14.617$
$R^2 = 0.9511$
Shrinkage Rate = 0.3 nm / min

Shrinking Time (min)

Slopes in the plot are the shrinkage rates. Different initial pore size had different shrinkage rates.

9nm x 198nm Initial pore   **80nm x 156nm 90 min**   **48nm x 57nm 250 min**   **3.3nm x 3.5nm 334 min**

168nm x 172nm   **187nm x 189nm 20 min**   **200nm x 201nm 45 min**   **206 nm x 208 nm 58 min**

A. J. Storm, J.H. Chen, X.S. Ling, H.W. Zanderbergen and C. Dekker, "Fabrication of solid-state nanopores with single-nanometre precision", Nature Materials, 2, 537 (2003).

H. Chang, F. Kosari, G. Andreadakis, G. Vasmatzis, E. Basgall, A. H. King, and R. Bashir, "Towards Integrated Micro-Machined Silicon-Based Nanopores For Characterization Of DNA", Hilton Head MEMS conference, 2004, Hilton Head, South Carolina.

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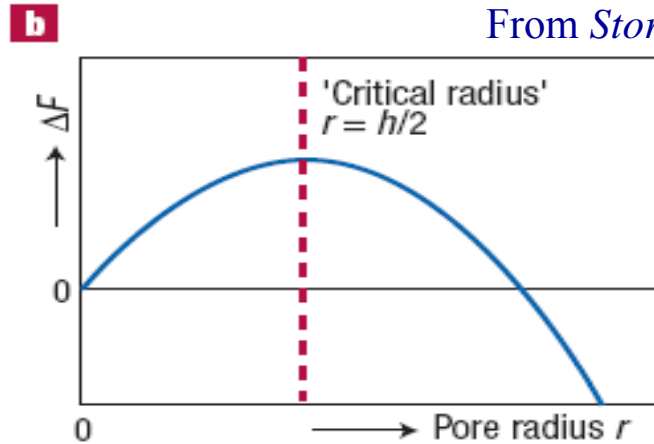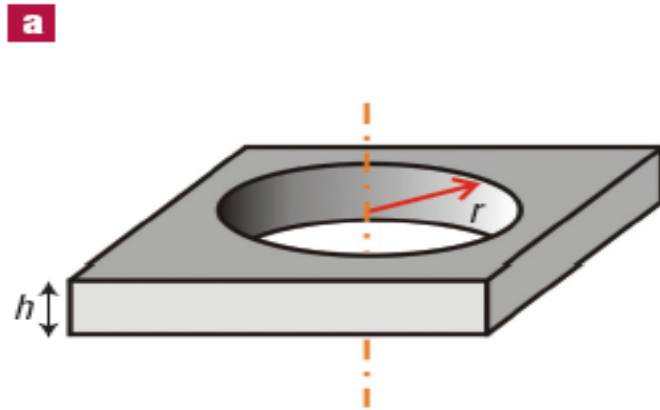
From *Storm et. al. 2003*

Surface free energy:

$$\Delta F = \gamma \, \Delta A = 2\pi\gamma(rh - r^2)$$

where γ is the surface tension of the fluid,

ΔA is the change in surface area.

r is the radius of the pore right after the final oxidation,

h is oxide thickness.

The shrinkage/expanding of pores with different radii and oxide thicknesses.

Sample name Shrinkage/Expansion (S/E)	Average radius of the pore r (nm)	Grown oxide thickness d (nm)	The ratio of r/d
103003_4 S	9	79	0.11
102503_4 S	38	100	0.38
110603_2 S	51.5	130	0.40
091603_6m E	82	90	0.91
082703_5m E	138	120	1.15
091603_7m E	110	54	2.04

H. Chang, F. Kosari, G. Andreadakis, G. Vasmatzis, E. Basgall, A. H. King, and R. Bashir, "Towards Integrated Micro-Machined Silicon-Based Nanopores For Characterization Of DNA", Hilton Head MEMS conference, 2004, Hilton Head, South Carolina.

Integrated Optical Detection

Stokes, Griffen, Vo-Dinh, Fresenius J Anal Chem, 369,:295-301, 2001