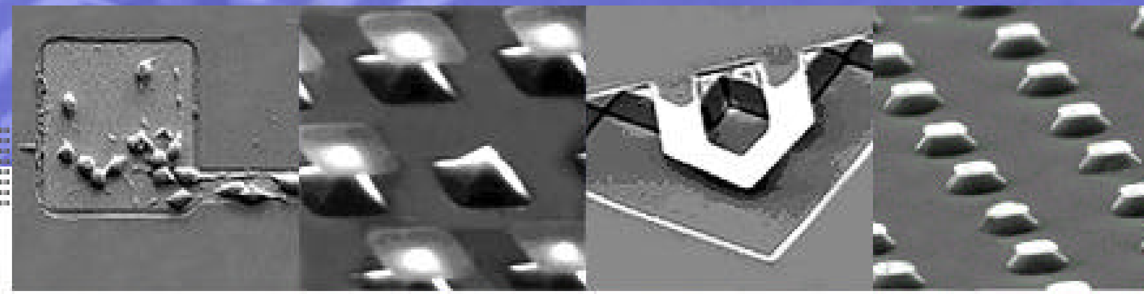
LIBNA is focused on research in BioMEMS & Bionanotechnology, in the areas of interface between micro, nanoengineering & life sciences



Introduction to BioMEMS & Bionanotechnology
Lecture 3

R. Bashir

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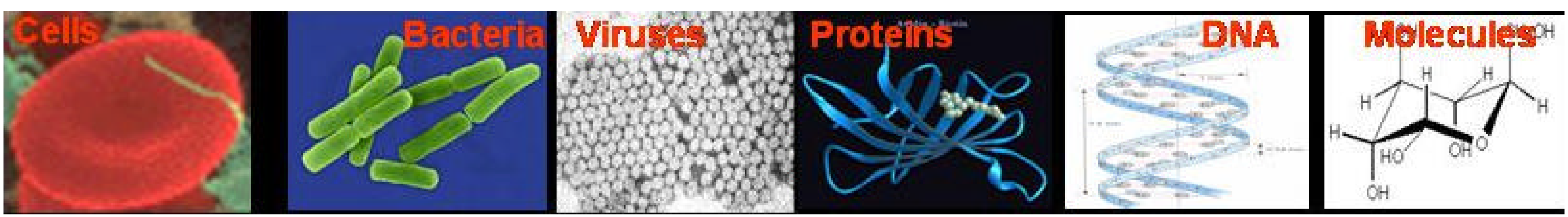
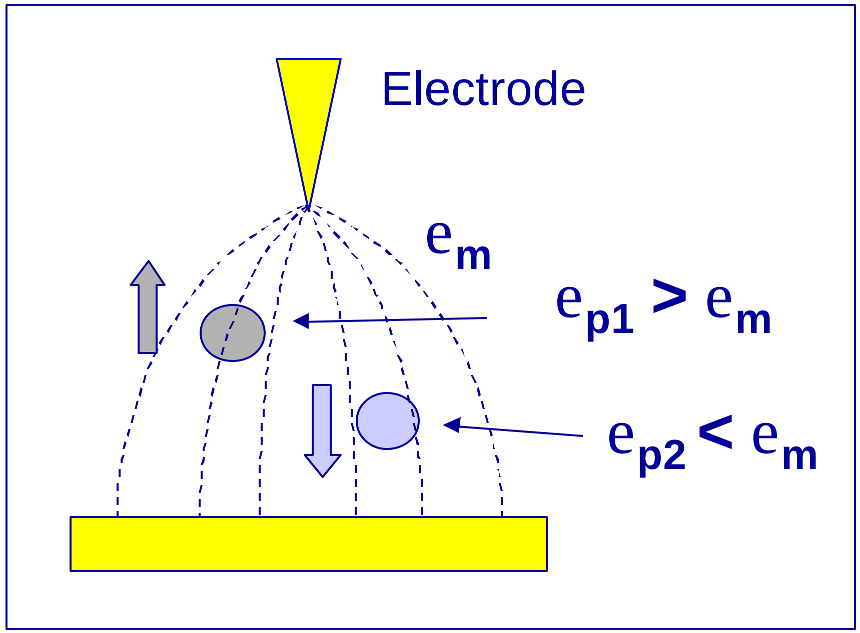
http://engineering.purdue.edu/LIBNA

Key Topics

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



Dielectrophoresis



Simplest approximation:

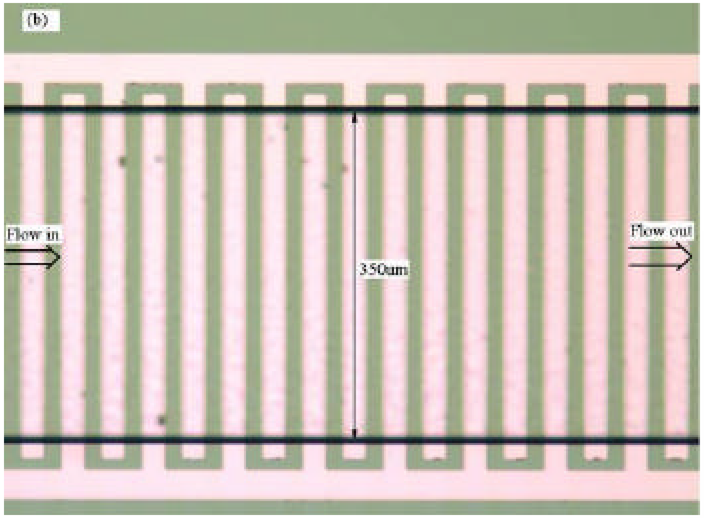
$$F = 2pe_0 e_m r^3 \,\mathrm{Re}\left[f_{CM} \right] \nabla \left| E_{RMS} \right|^2$$

$$\mathrm{f_{CM}}\left(\mathrm{e_p}, \mathrm{e_m}\right) = \frac{\mathrm{e_p} - \mathrm{e_m}}{\mathrm{e_p} + 2\,\mathrm{e_m}} \qquad \varepsilon_p = \varepsilon(\omega)$$

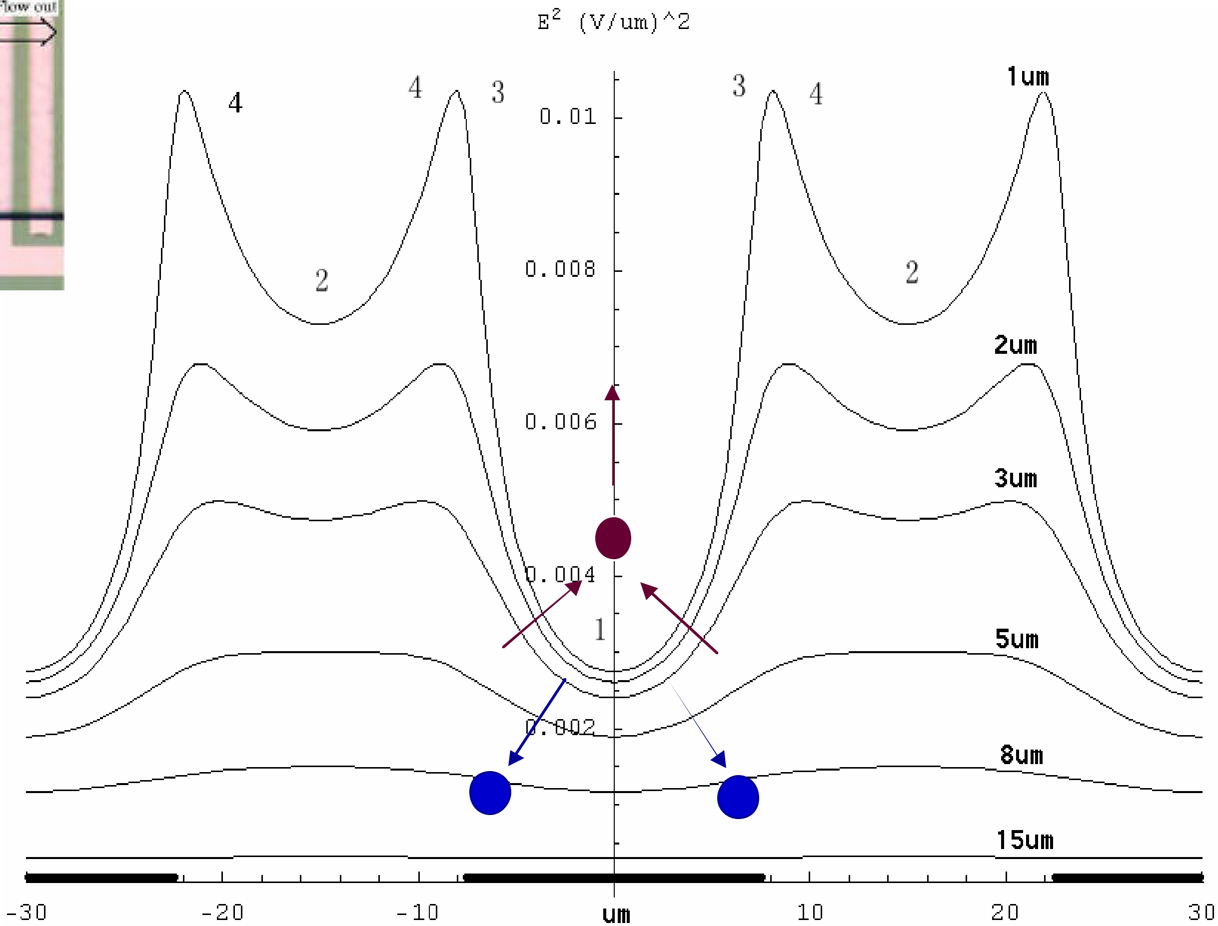
Dielectrophoresis on Interdigitated Electrodes

Polystyrene beads : $e_p < e_m \rightarrow$ negative DEP

Cells : $e_p < e_m \rightarrow$ Negative DEP
Cells : $e_p > e_m \rightarrow$ Positive DEP

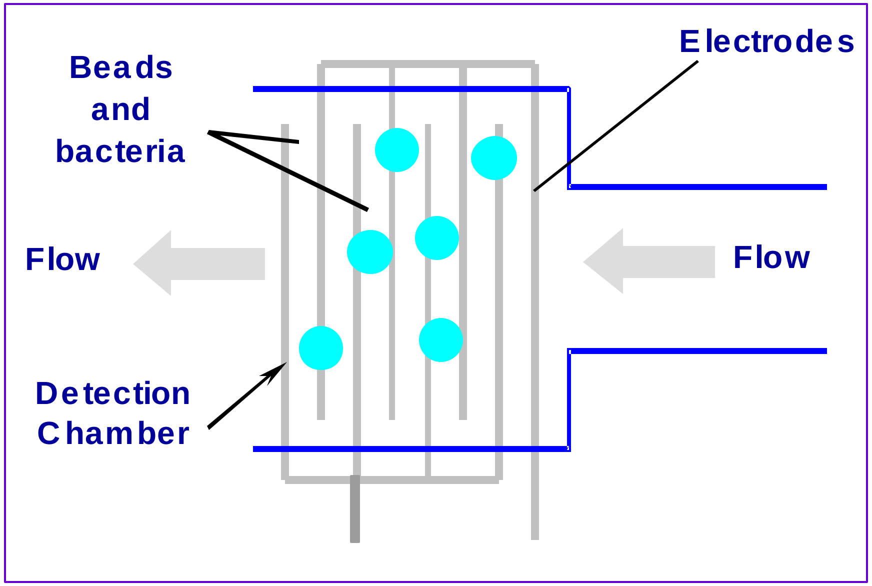


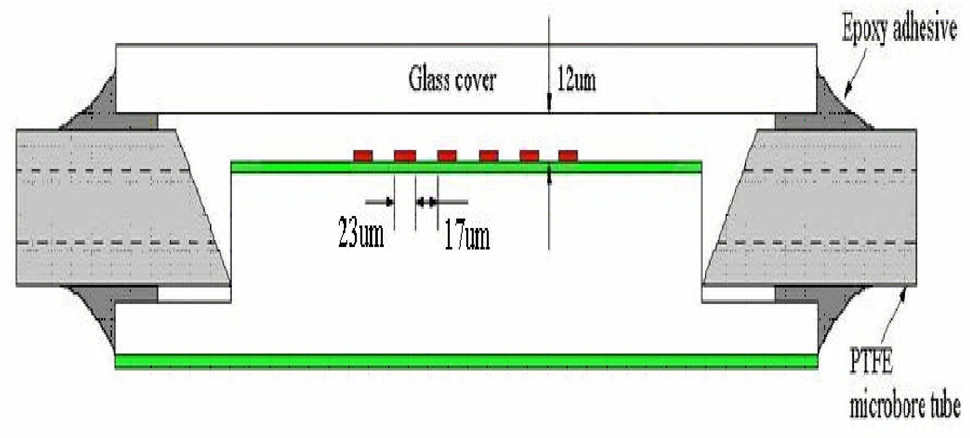
Interdigitated electrodes on a chip



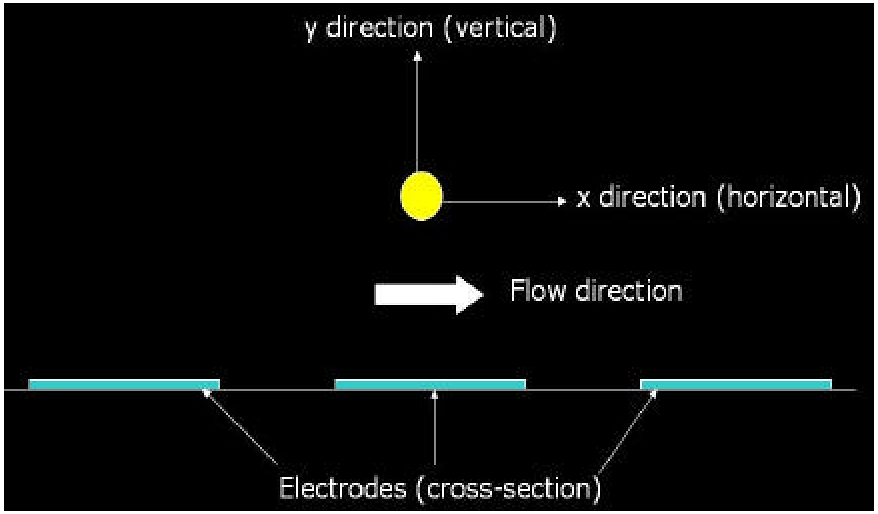
H. Li and R. Bashir, Sensors and Actuators, 2002, JMEMS, 2004

A Dielectrophoretic Filter





Schematic of the device cross-section



Forces on a particle in a micro-fluidic flow

- **1. DEP Force**
- **2. Sedimentation Force**

$$F_{sedi} = \frac{4}{3} p R^3 (r_p - r_m) g$$

- **3. Hydrodynamic Drag Force:**

$$F_{HD-drag} \approx 6pkRh(u_m - u_p)$$

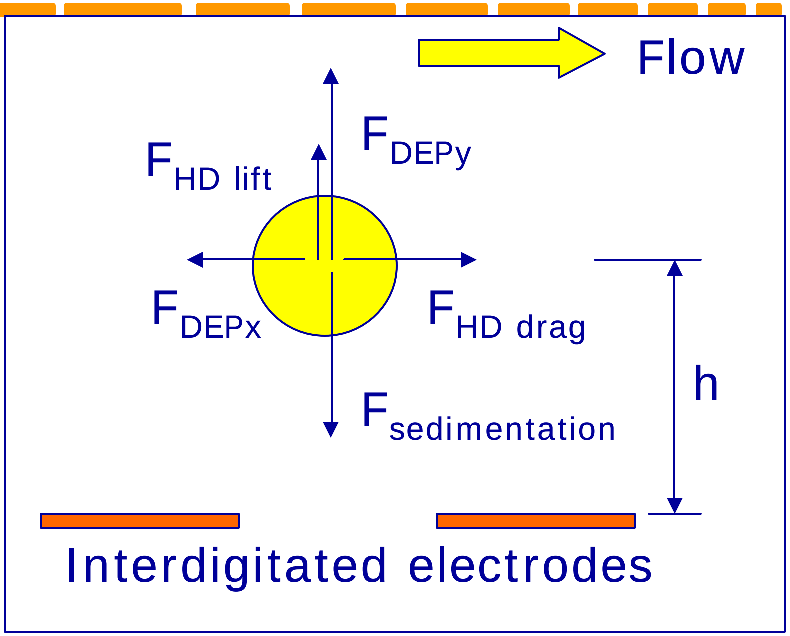
- **Assume a parabolic laminar flow profile:**

$$u = 6\langle u \rangle \frac{x}{h}\left(1 - \frac{x}{h}\right) \qquad \langle u \rangle = \frac{U}{wh}$$

U: flow rate in μl/min

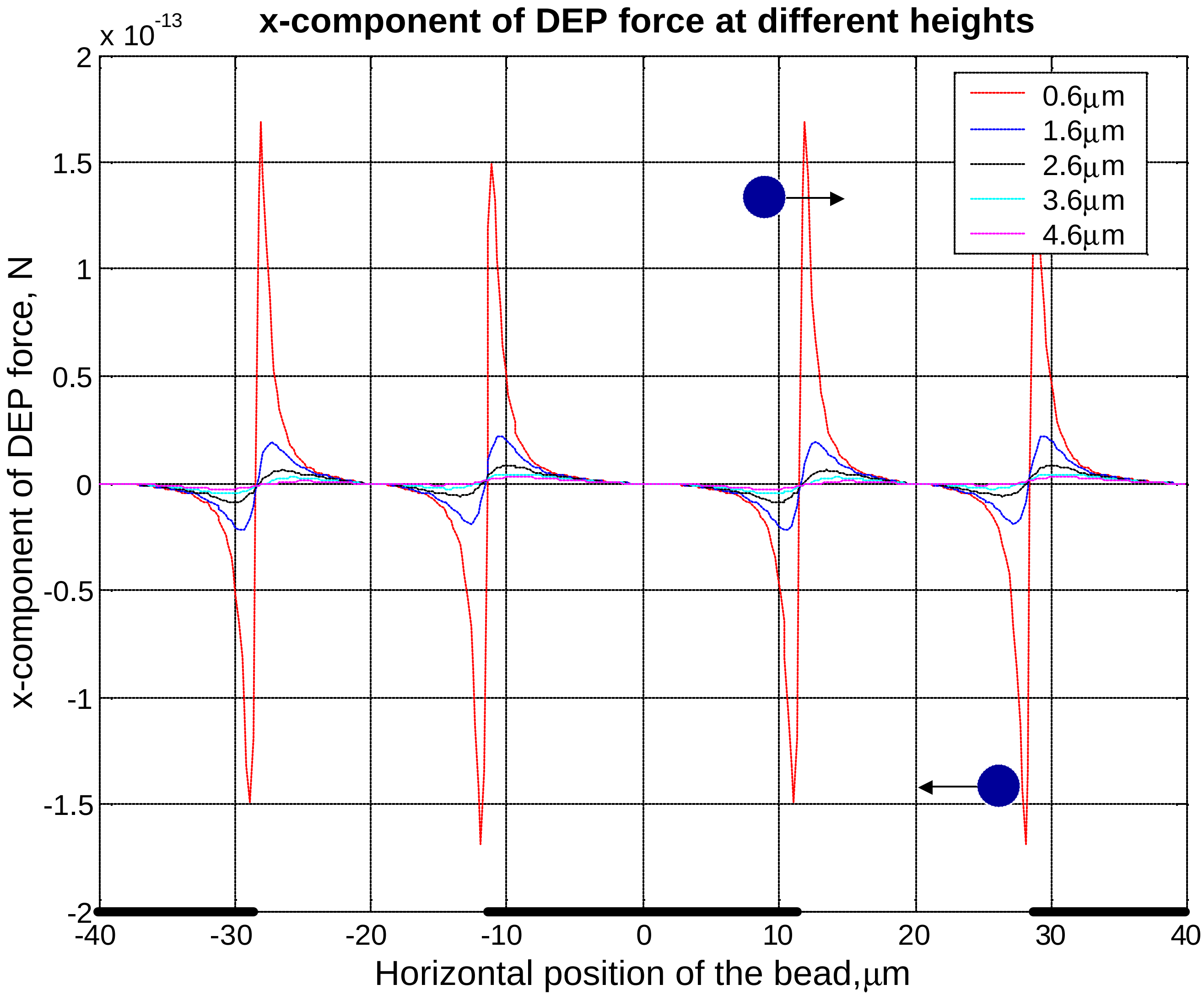
- **4. Hydrodynamic lifting force**

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



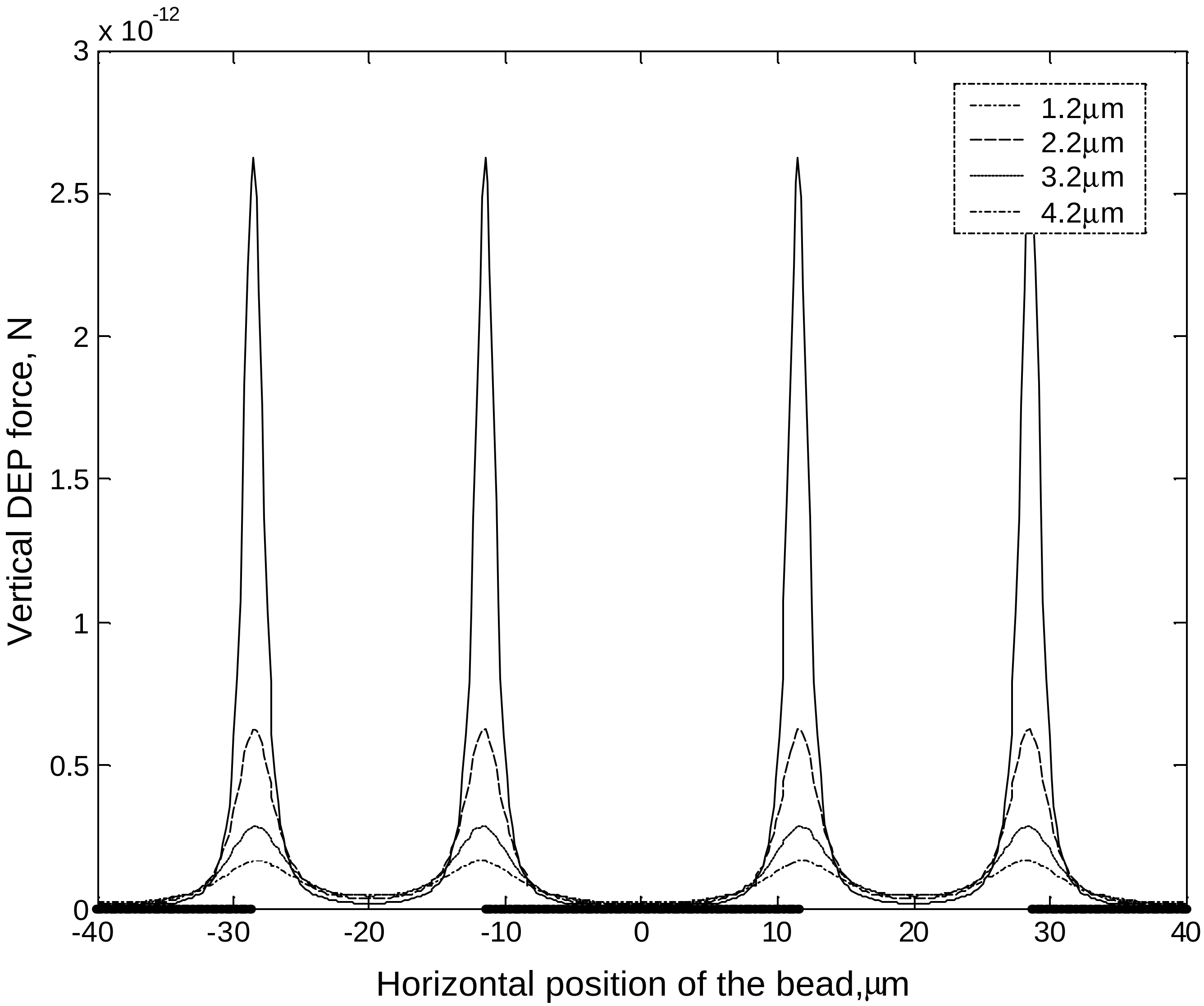
- **Two orders of magnitude smaller than typical DEP lifting force**
- **Neglected here**

X-component of DEP force at different heights



- Bead diameter: 0.7mm
- 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

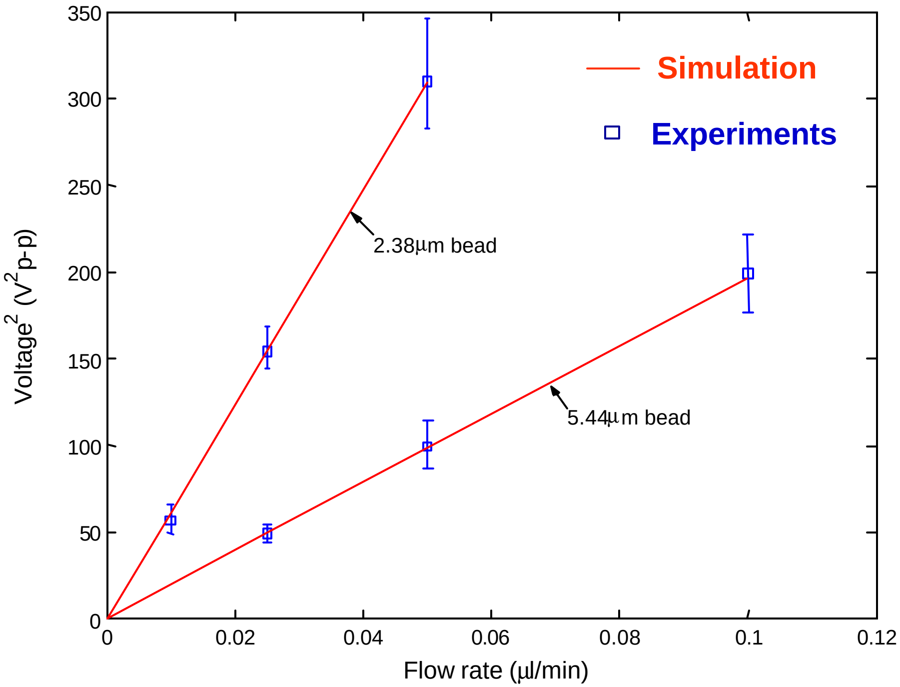
Y-component of DEP force at different heights

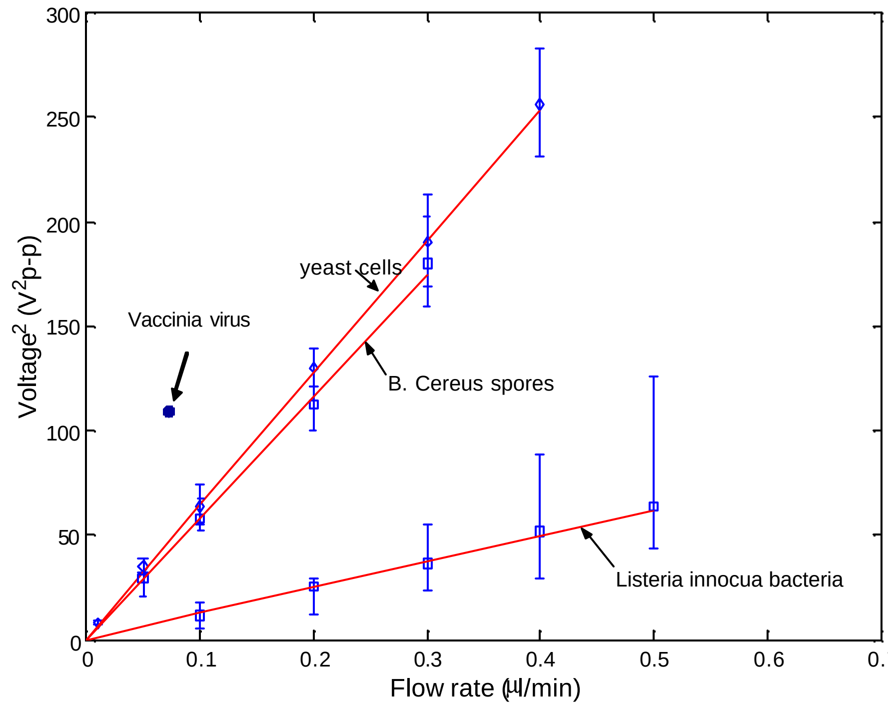


Purdue University

Trapping of beads (- DEP) and microorganisms (+ DEP)

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

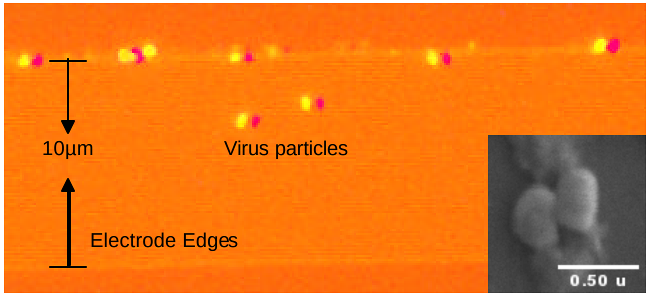




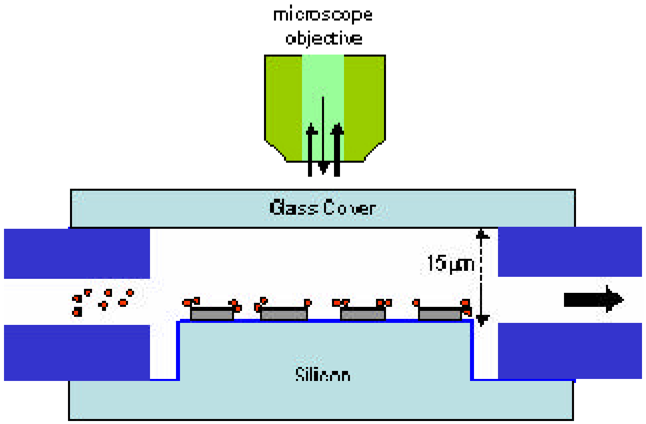
H. Li, Y. Zheng, D. Akin, R. Bashir, IEEE/ASME JMEMS, 2005

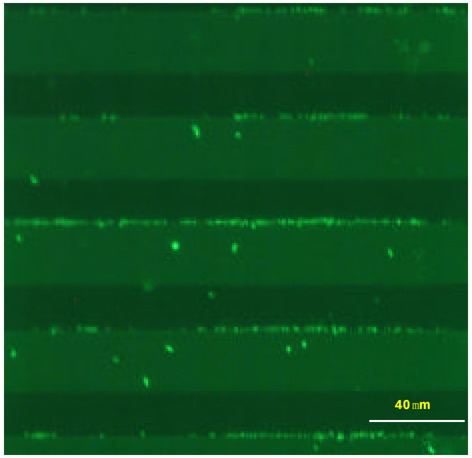
Dielectrophoretic Trapping of *Vaccinia* virus (positive DEP)

- **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**

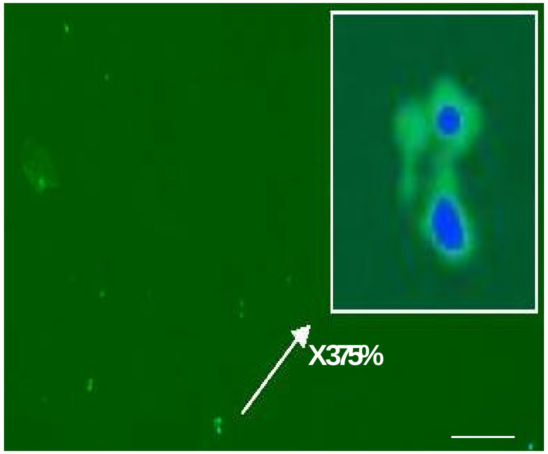


The dual (DiOC63, green and DiL, red) labelled viral particles





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



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

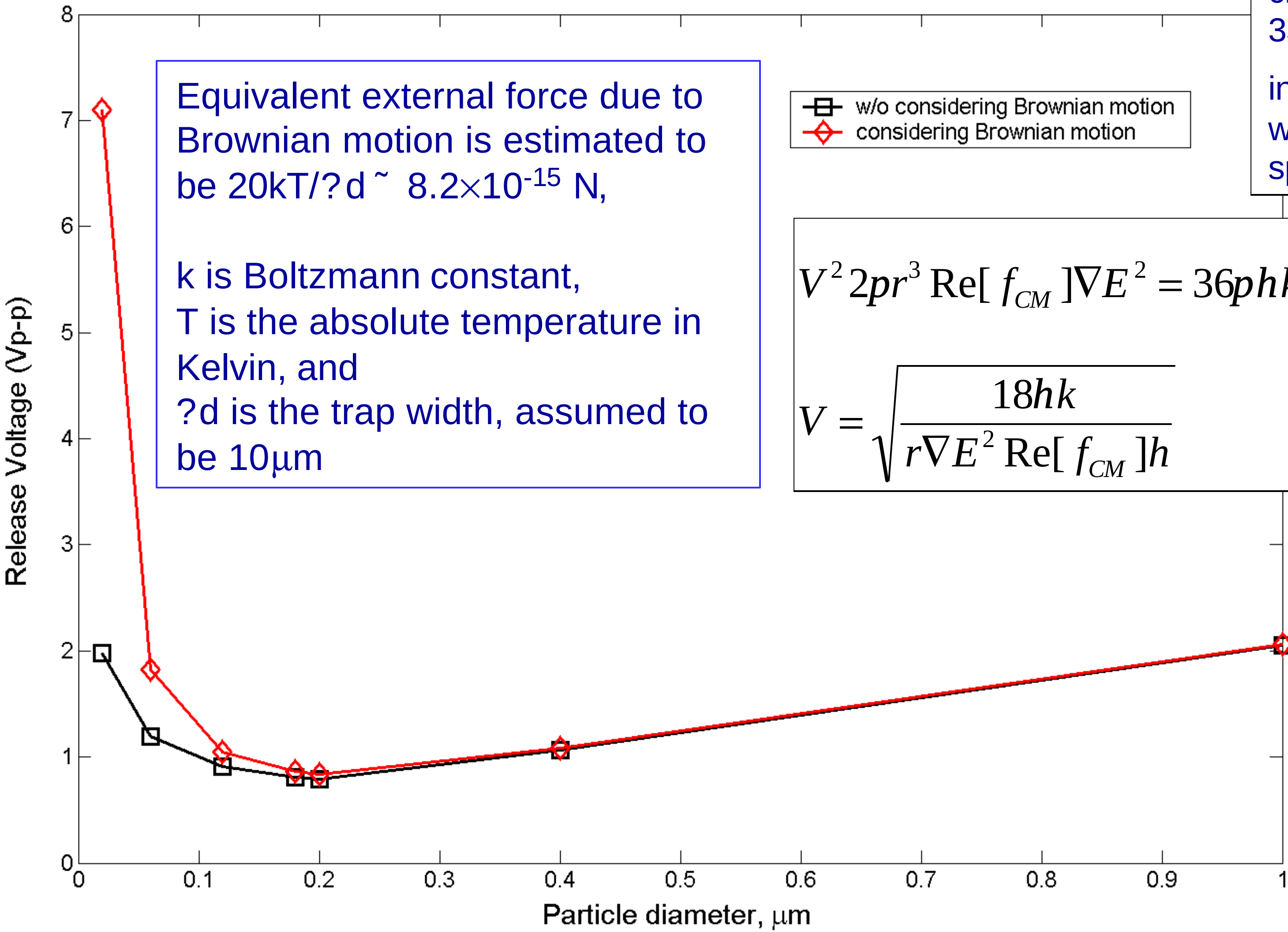
D. Akin, H. Li, R. Bashir, *Nano Letters*, 4, pp. 257 -259, 2004

Release voltage vs. diameter for particle collecting the electrode edge, considering the Brownian motion

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/?d ˜ 8.2×10^-15 N,

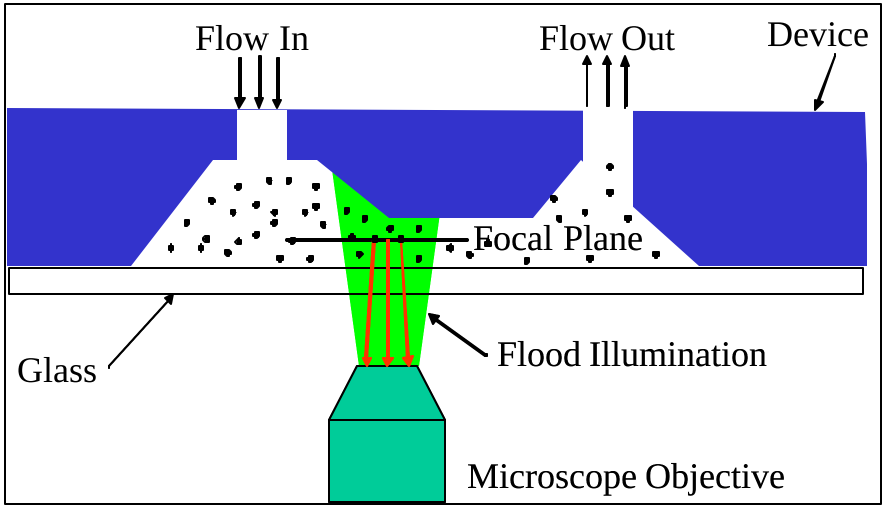
k is Boltzmann constant,
T is the absolute temperature in Kelvin, and
?d is the trap width, assumed to be 10µm

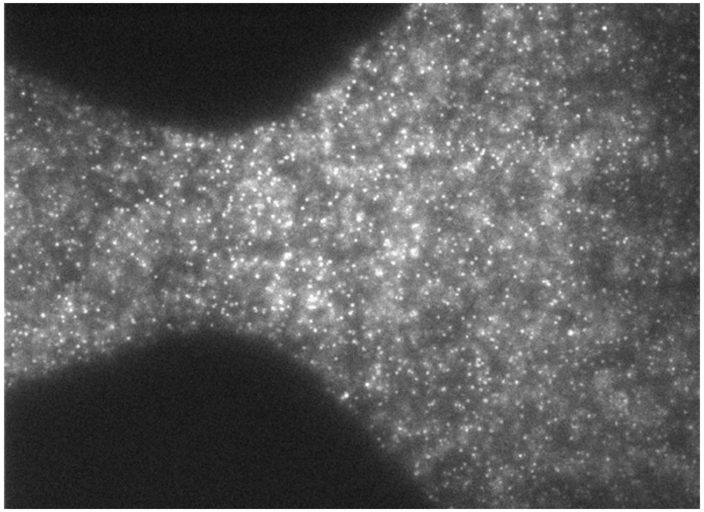
$$V^2 2pr^3 \,\mathrm{Re}[\,f_{CM}\,]\nabla E^2 = 36phkr\left(\frac{r}{h} - \frac{r^2}{h^2}\right) \approx 36phk\frac{r^2}{h}$$

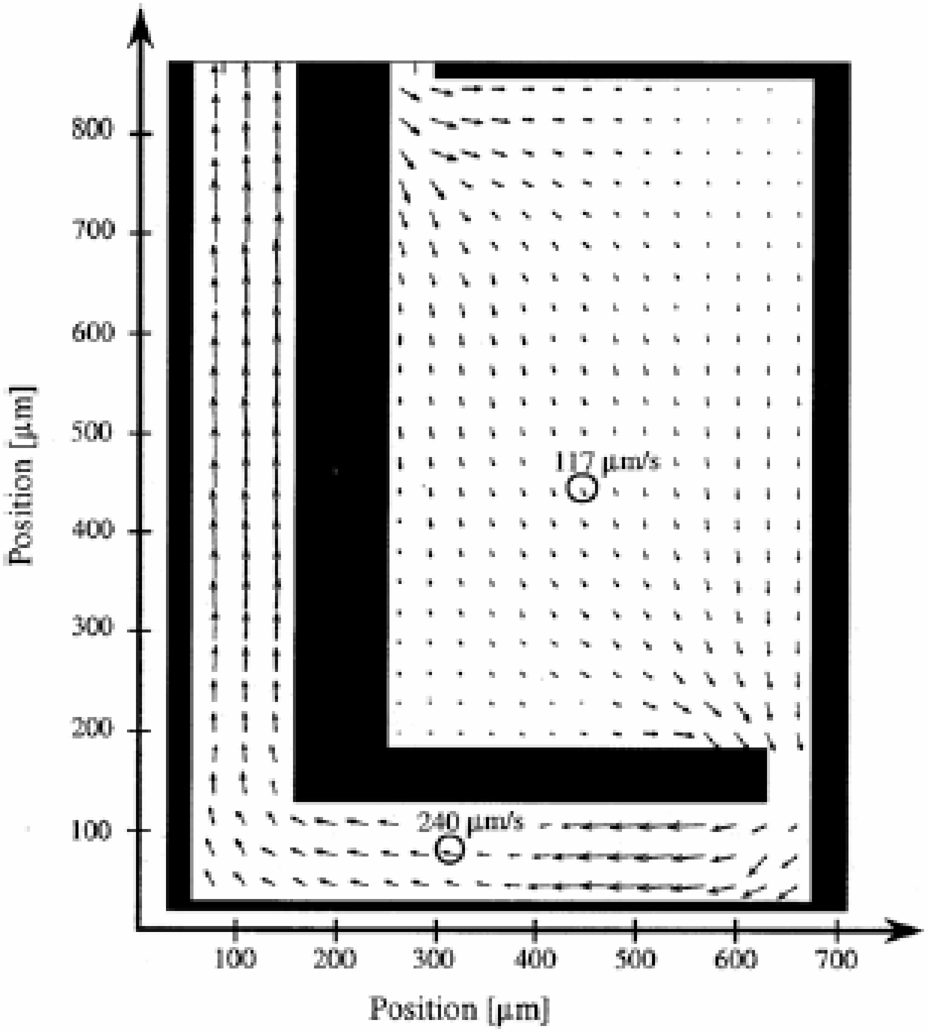
$$V = \sqrt{\frac{18hk}{r\nabla E^2 \,\mathrm{Re}[\,f_{CM}\,]h}}$$

Micro-fluidic Characterization

- Micro-Particle Imaging Velocimetry (μPIV)







Wereley, et al. Purdue

Gomez, et al. 2001

Key Topics

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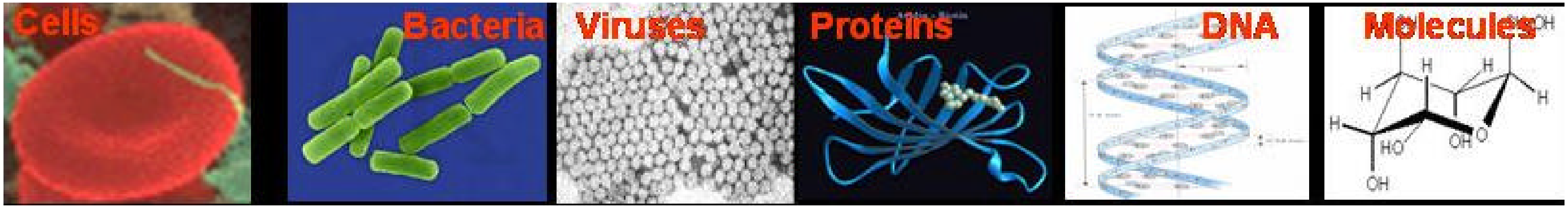

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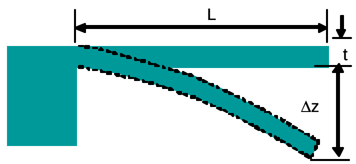
Biochip Sensors

- 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

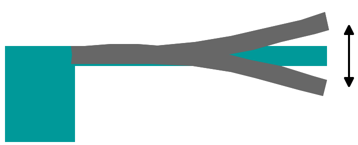
Surface Stress Change Detection



$$\Delta z = 4\left(\frac{l}{t}\right)^2 \frac{(1-n)}{E}(\Delta s_1 - \Delta s_2)$$

- Δz = deflection of the free end of the cantilever
- L = cantilever length
- t = cantilever thickness
- E = Young's modulus
- ν = poison's ratio
- Ds_1 change in surface stress on top surface
- Ds_2 change in surface stress on bottom surface

Mass Change Detection



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

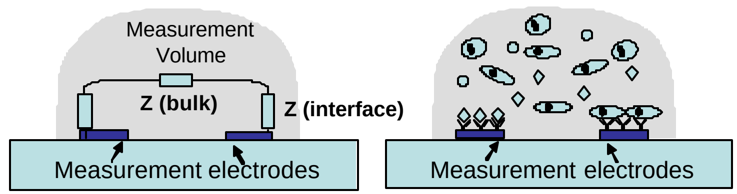
$$\Delta m = \frac{k}{4p^2}\left(\frac{1}{f_1^2} - \frac{1}{f_o^2}\right)$$

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

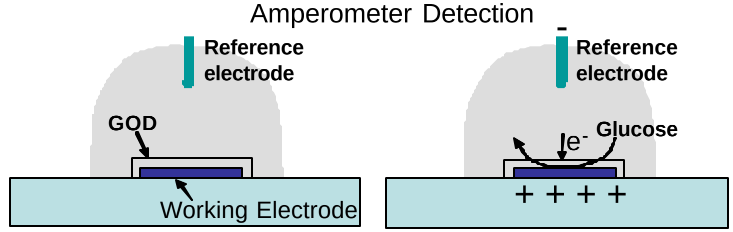
(a)

Electrical Detection

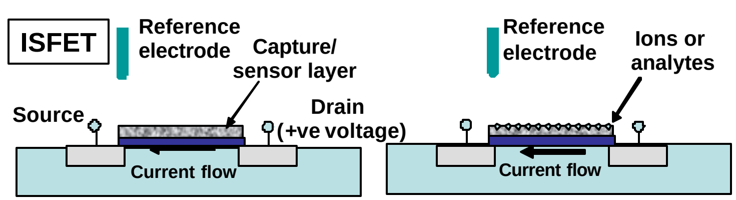
Conductometric Detection



Amperometer Detection

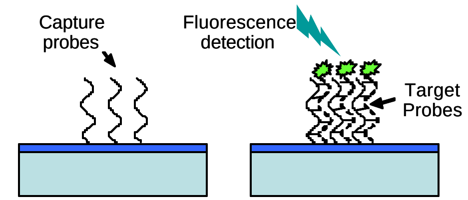


Potentiometric Detection

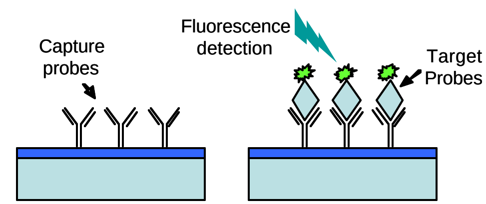


(b)

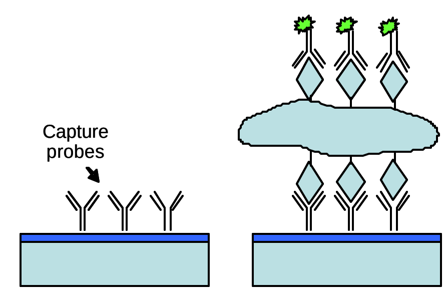
Optical Detection



DNA detection on chip surfaces



Protein detection on chip surfaces



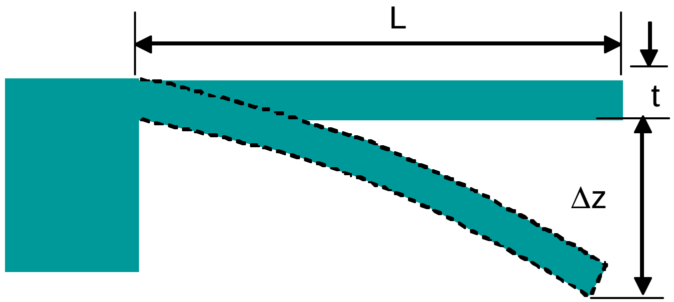
Cell detection on chip surfaces

(c)

1. Microcantilever Stress Sensors

Mechanical Detection

Surface Stress Change Detection



$$\Delta z = 4\left(\frac{l}{t}\right)^2 \frac{(1-n)}{E}(\Delta s_1 - \Delta s_2)$$

- Δz = deflection of the free end of the cantilever
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- Ds_1 change in surface stress on top surface
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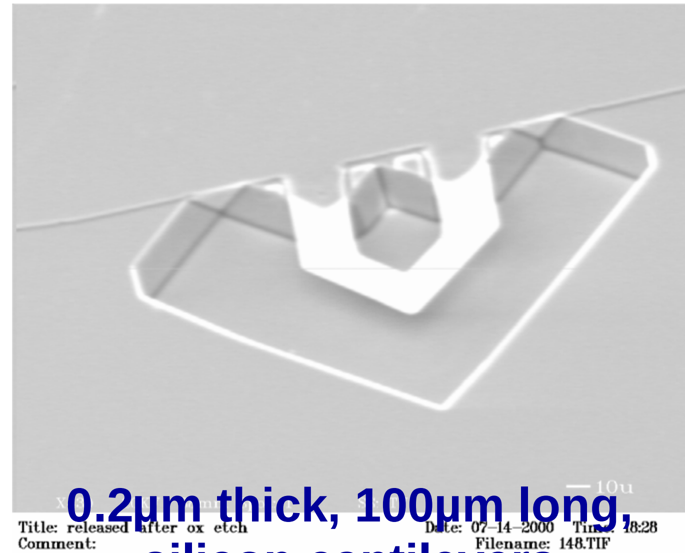
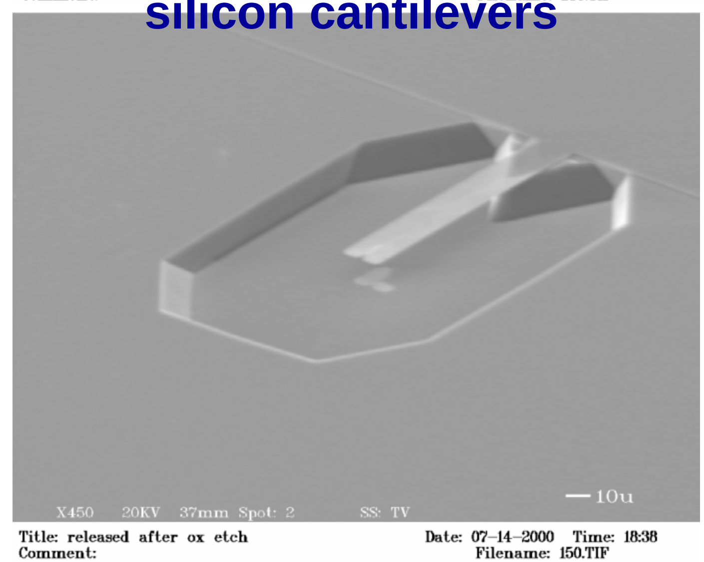
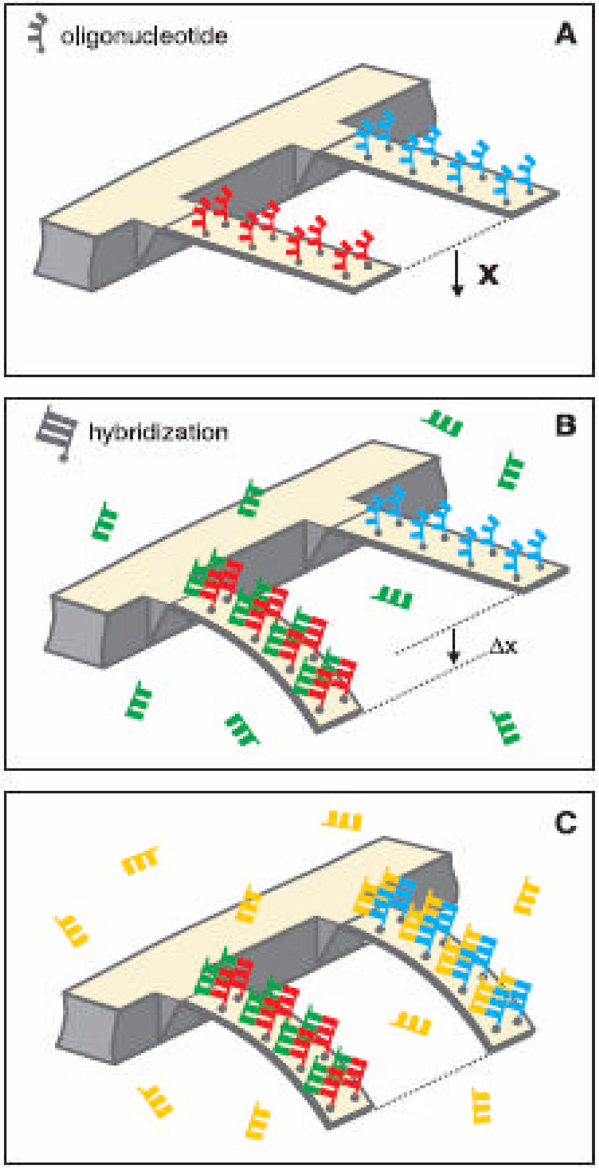


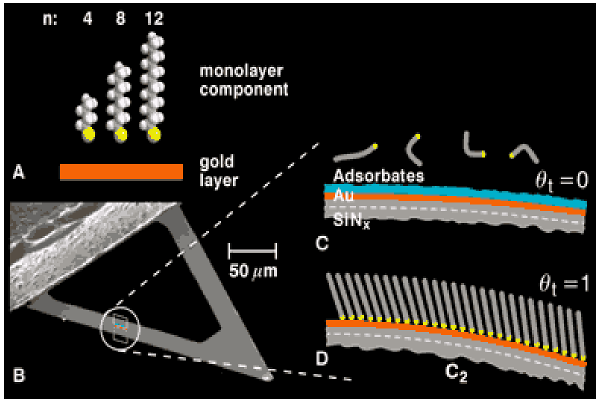
0.2μm thick, 100μm long, silicon cantilevers

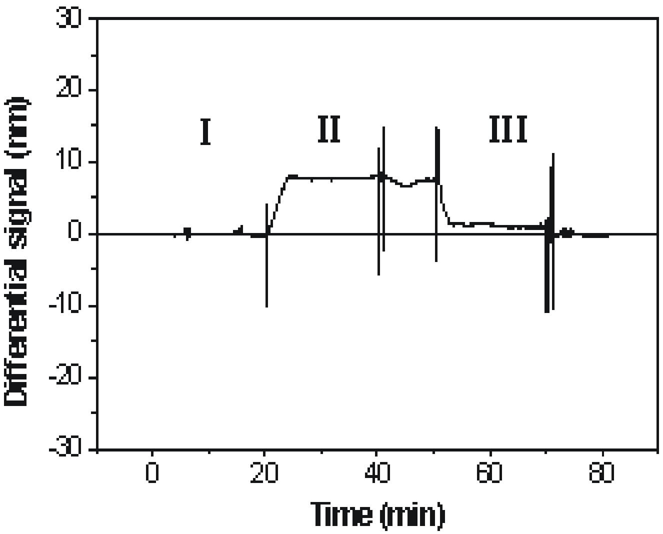


Microcantilever Stress Sensors



IBM Zurich Research: DNA Detection

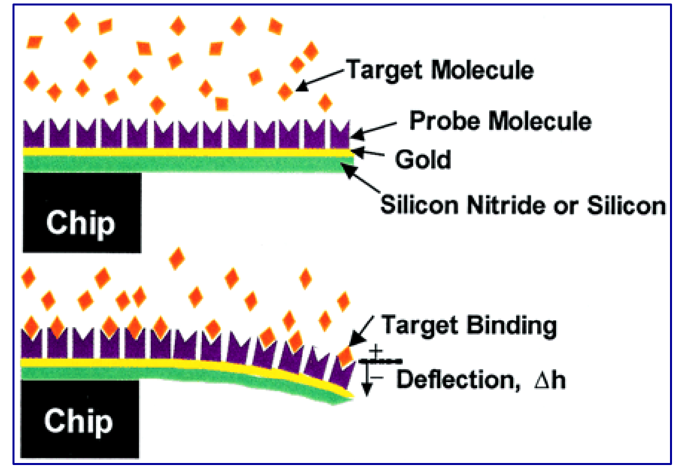


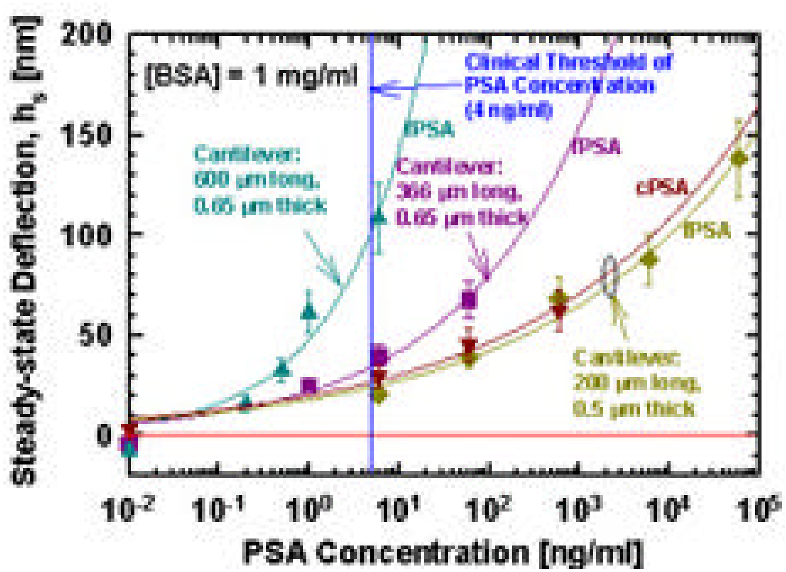


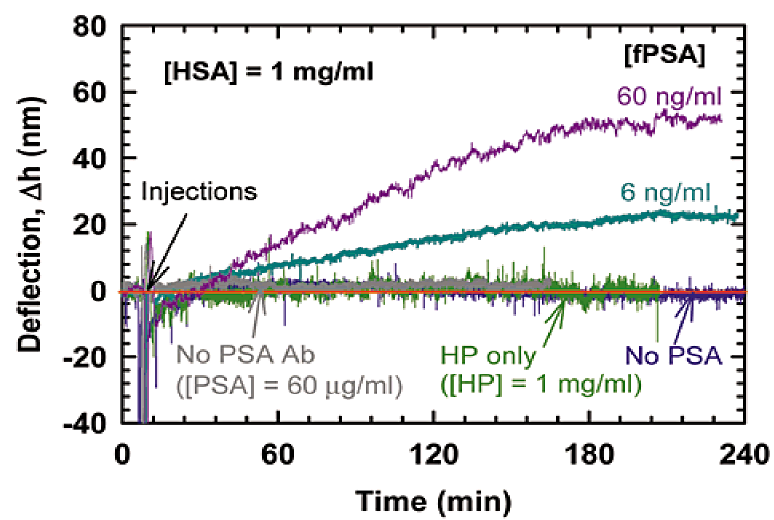
Fritz et al, *Science*, **288,** April 2000

Microcantilever Stress Sensors

Detection of PSA,
Prostate Specific Antigen
(cancer marker protein in blood)







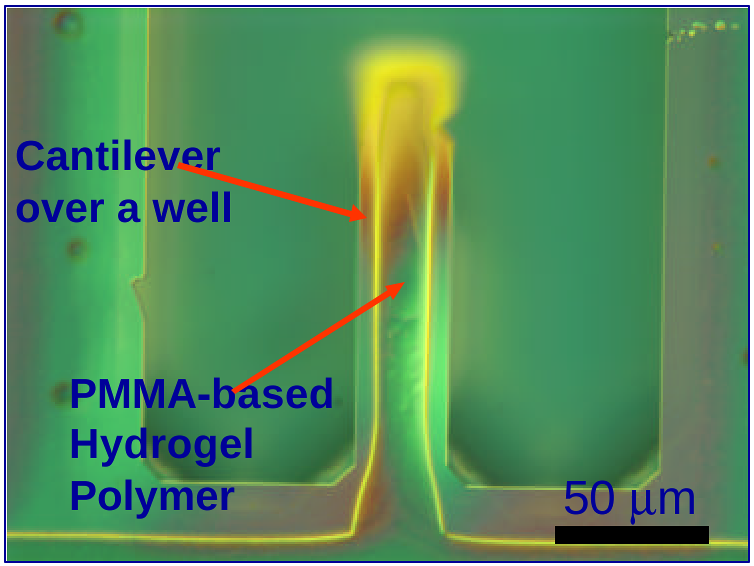
- PSA ~ 30kDa ~ 30 x 1e3 x 1.66e-24gm
- In 1ng/ml ~ 2e10 molecules/ml
- Area of 20um x 60um, each protein 10nm x 10nm → ~1e8 proteins

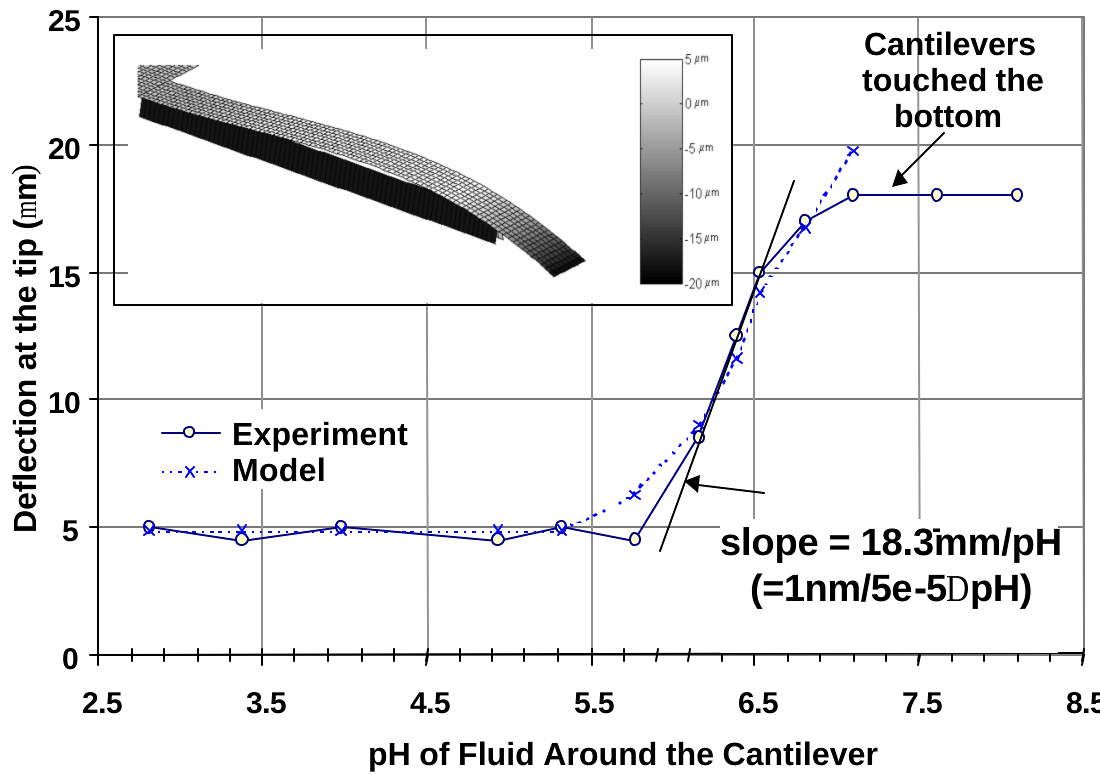
Wu et al, Nature Biotechnology, **19,** September 2001

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**





- DpH = 1-10e-5

- pH = 6.5 → ~ 1.9e5 H^+ in 1000mm^3

- DpH = 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

PURDUE UNIVERSITY

2. Microcantilever Mass Sensors

Unloaded Resonant Frequency :

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

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

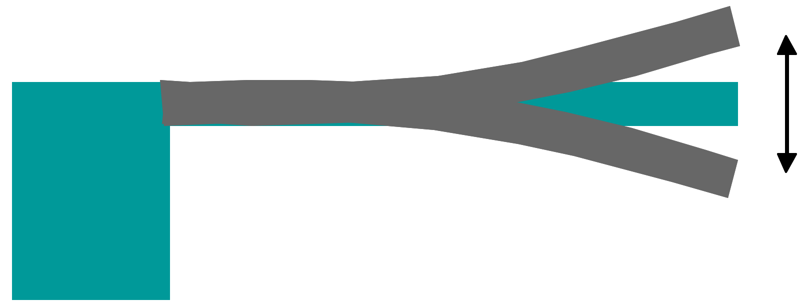
Loaded Resonant frequency : $f_1 = \frac{1}{2p}\sqrt{\frac{k}{m*+dm}}$

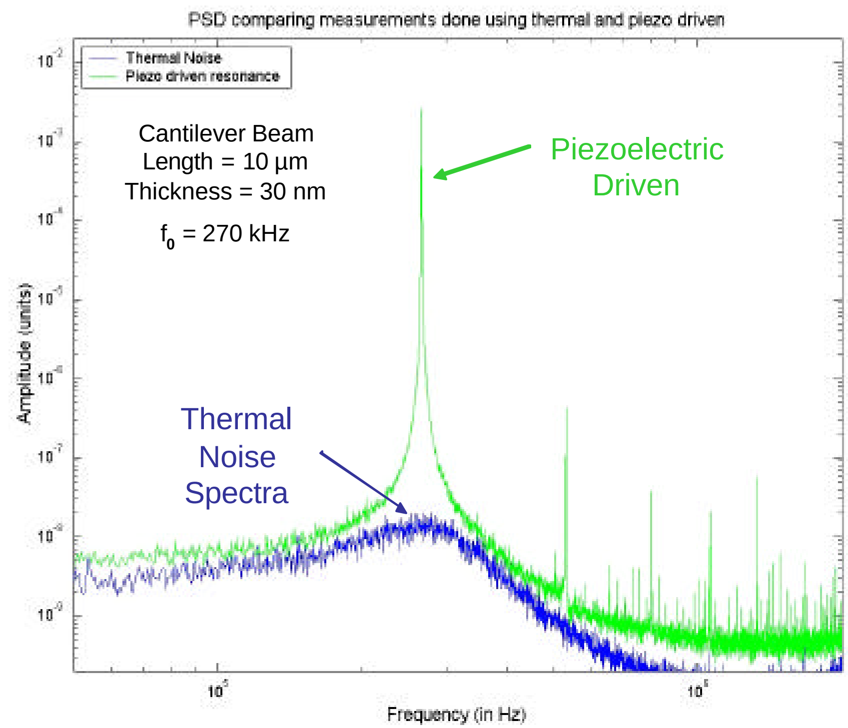
δm is the added mass

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

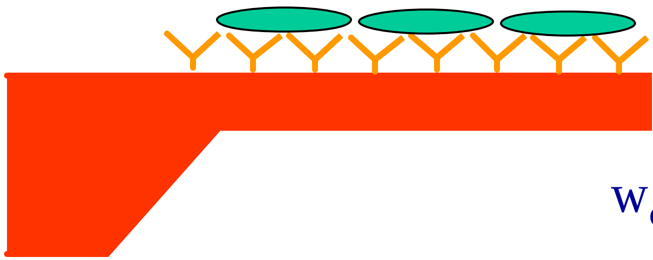
- k = spring constant
- m = mass of cantilever
- *f_0 = unloaded resonant frequency*
- *f_1 = loaded resonant frequency*

Mass Change Detection

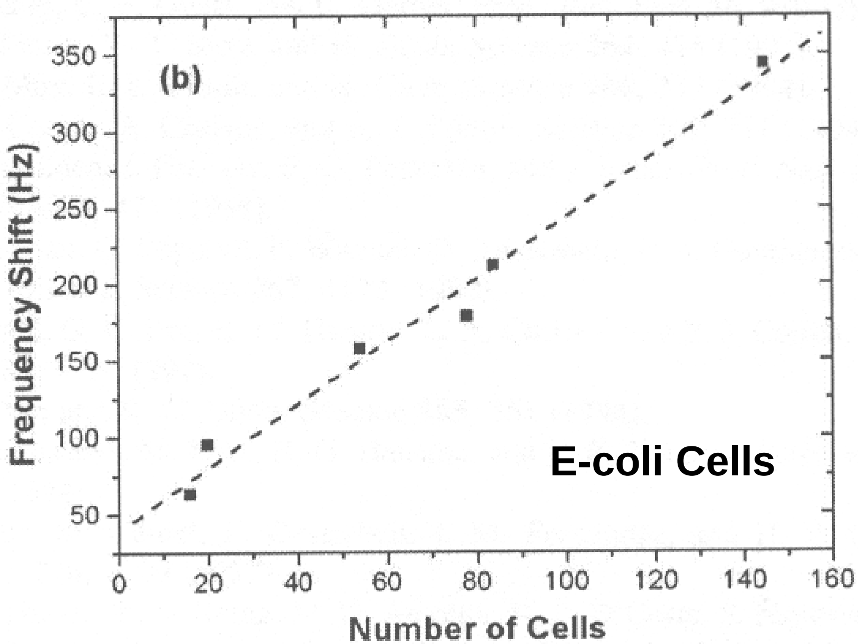


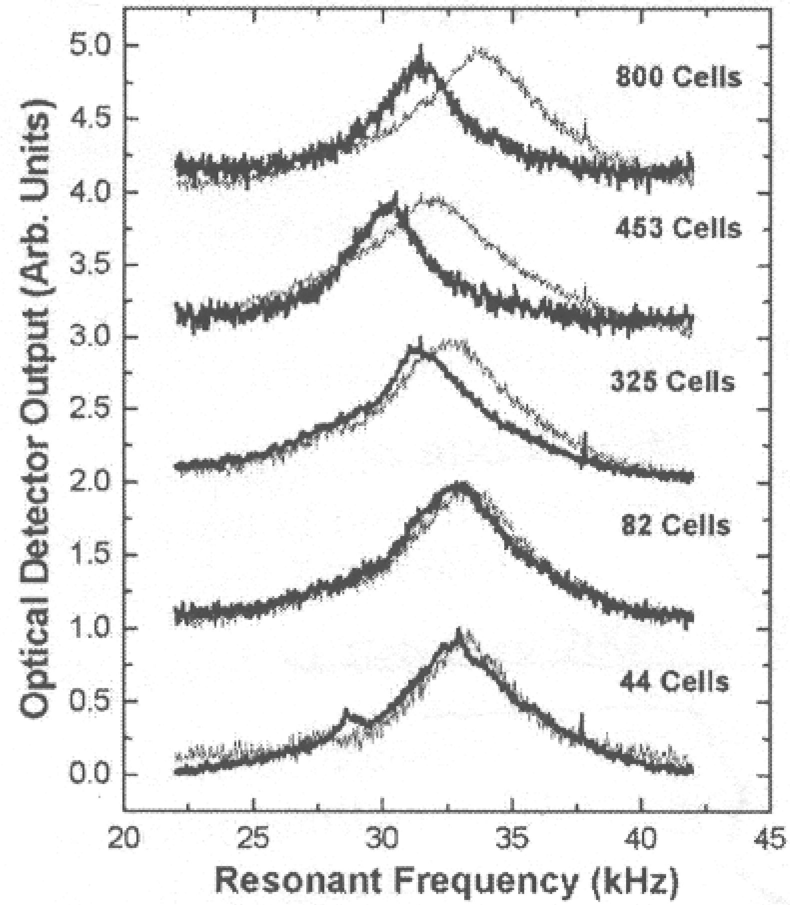


Detection of Bacterial Mass



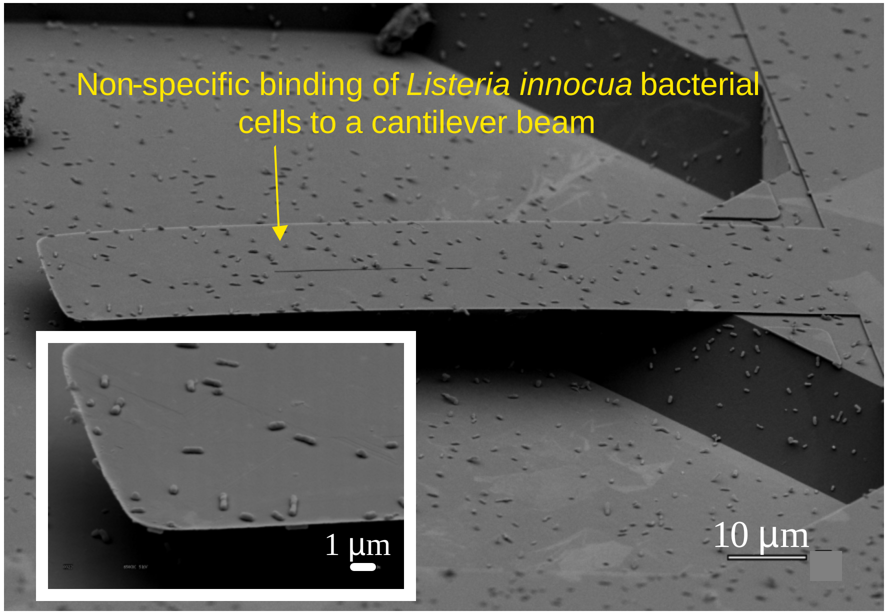
$$w_o^2 = \frac{k}{m}$$

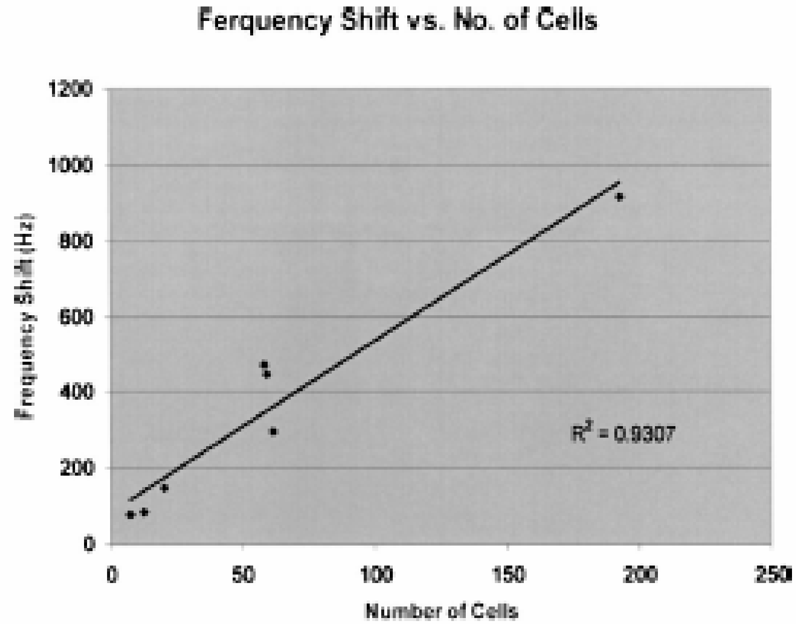


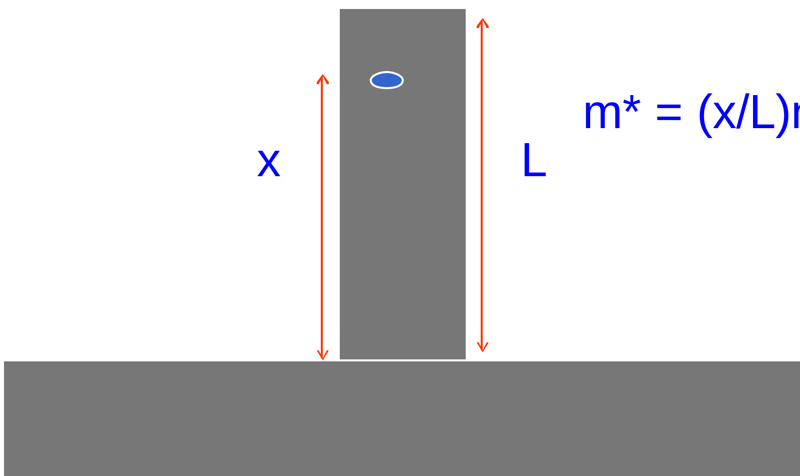


Craighead, et al. APL, 77, 3, 17th July 2001, 450-452

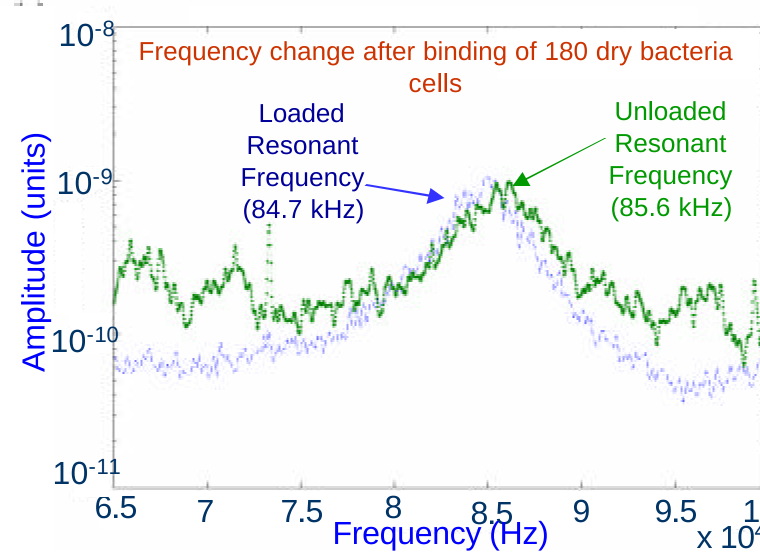
Detection of Listeria Cell Mass







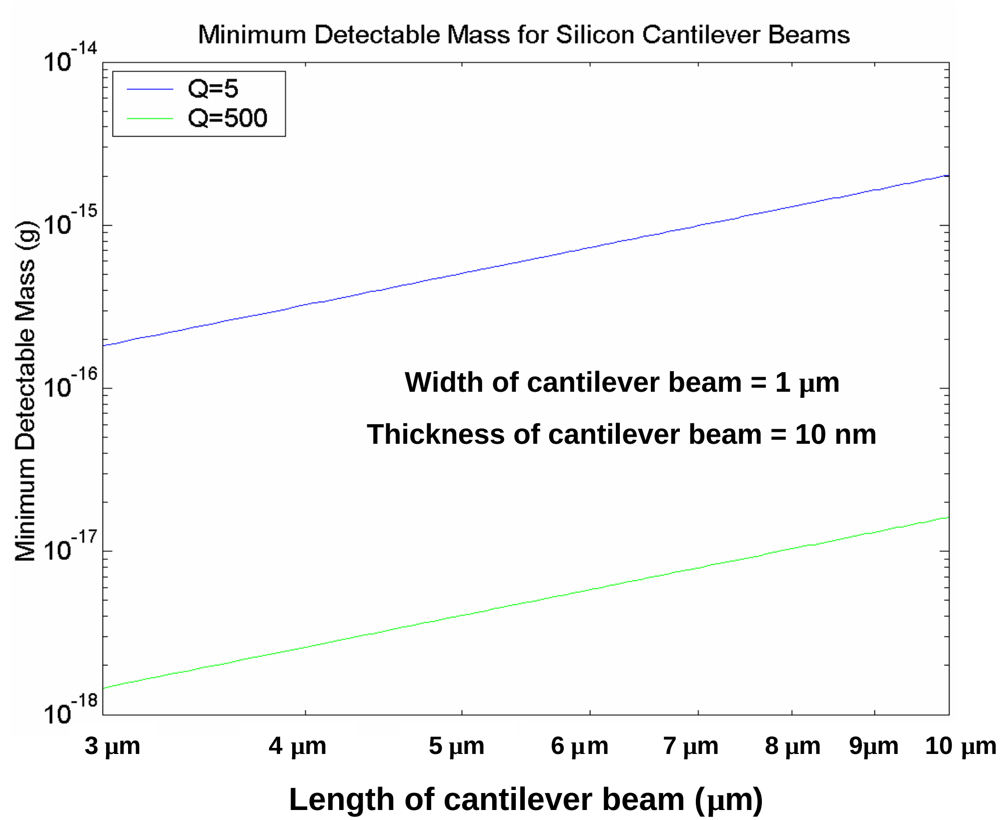
$$m^* = (x/L)m$$



A. Gupta, D. Akin, R. Bashir, JVST-B, 2004.

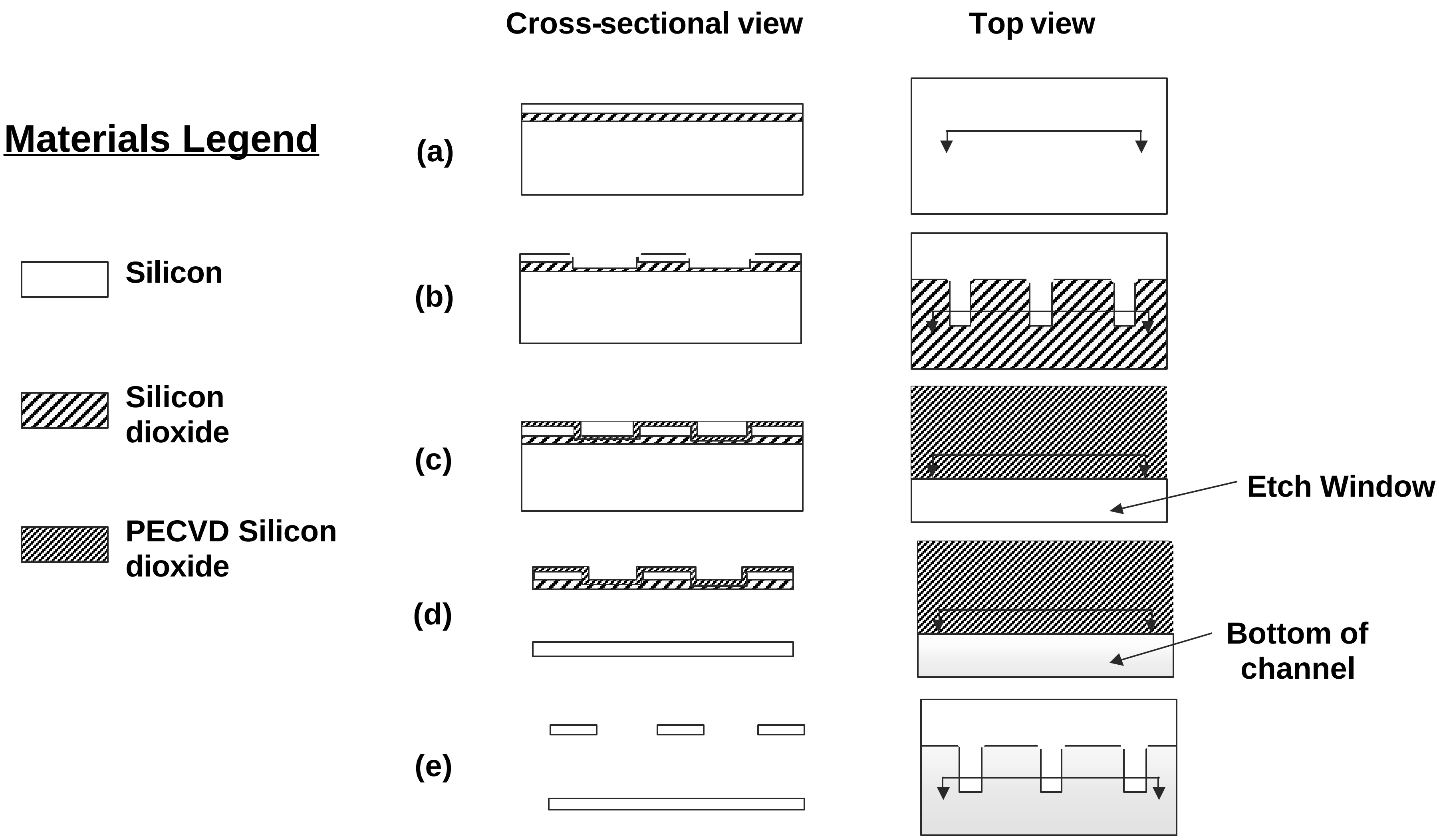
Minimum Detectable Mass

- The frequency measurement is limited by thermo-mechanical noise on the cantilever beam.
- Minimum Detectable Frequency, ?f,min = $$\frac{1}{A}\sqrt{\frac{f_0 k_B TB}{2p\,kQ}}$$
- Minimum Detectable Mass, ?m,min = $$\frac{1}{A}\sqrt{\frac{4k_B TB}{Q}}\,\frac{m_{eff}^{5/4}}{k^{3/4}}$$
- k_B = Boltzmann constant
- T = Temperature in Kelvin
- B = Bandwidth measurement, (~ 1 kHz)
- Q can increase by 100X by driving the cantilevers

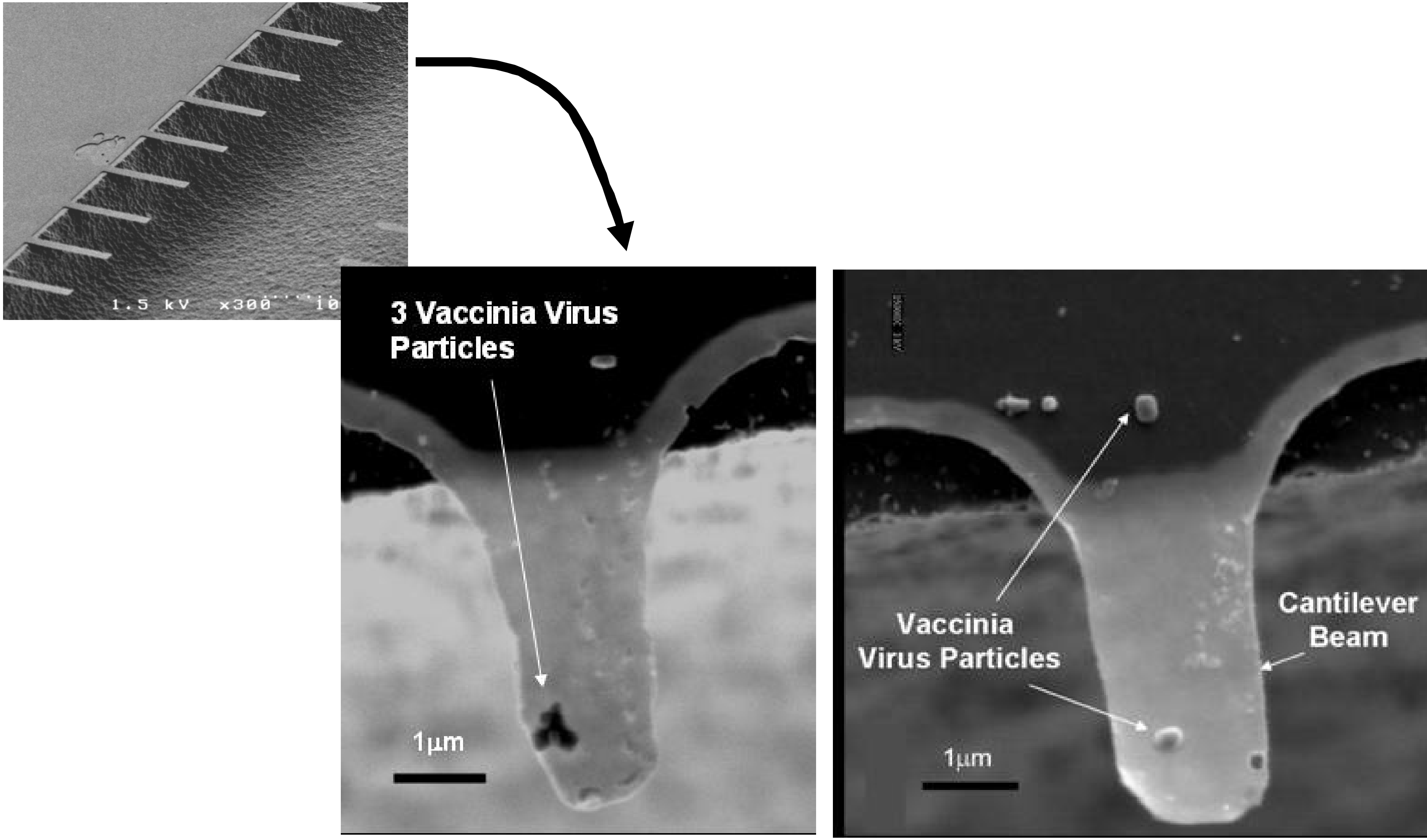


Roukes, et al.

Fabrication Process Flow

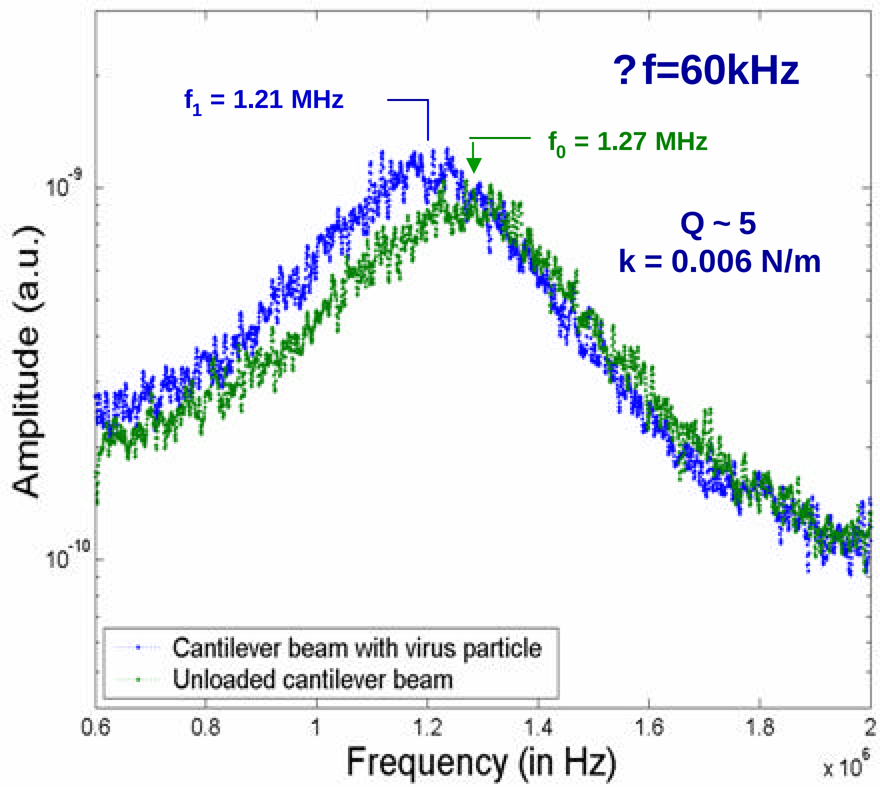


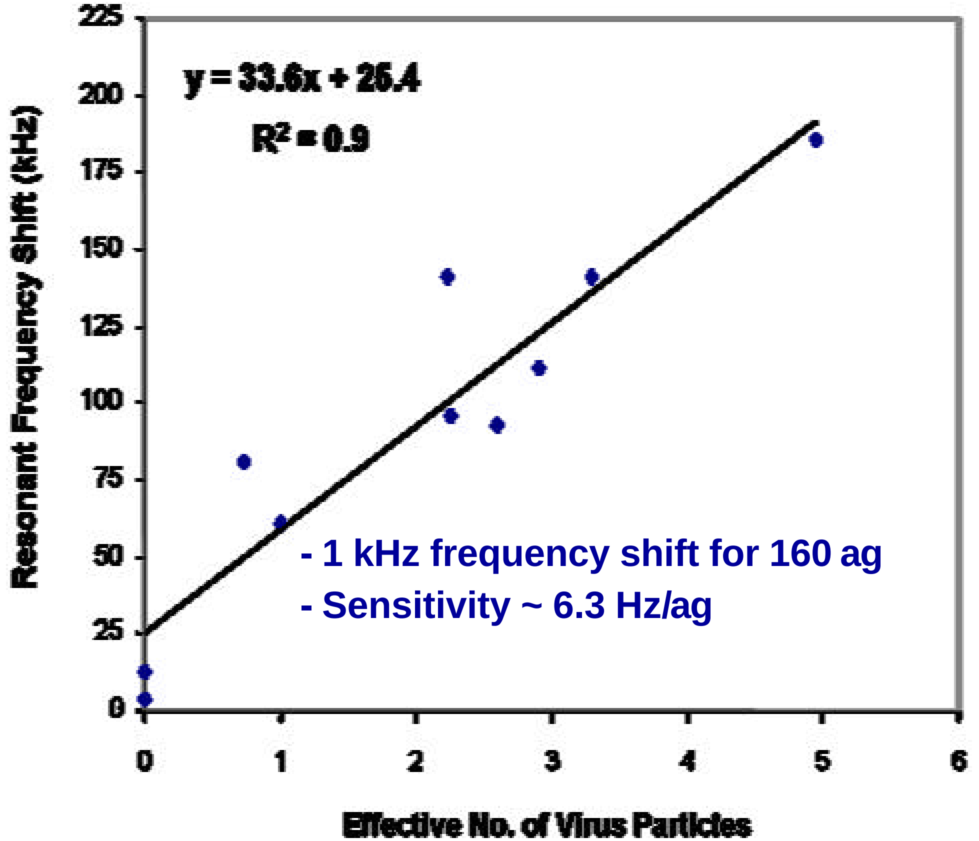
SEM Pictures of Cantilevers



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

Frequency Shift vs. No. of Particles

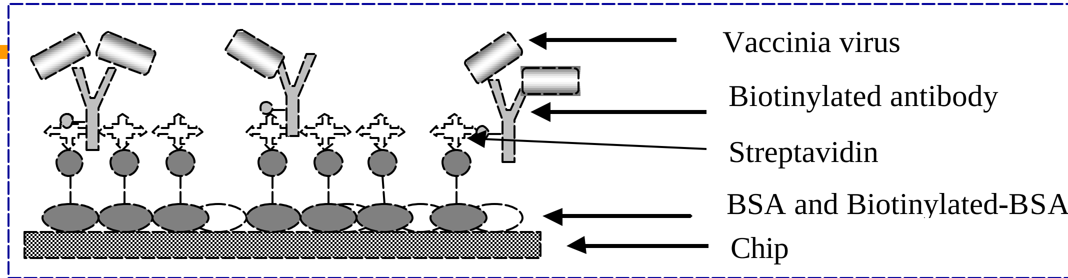


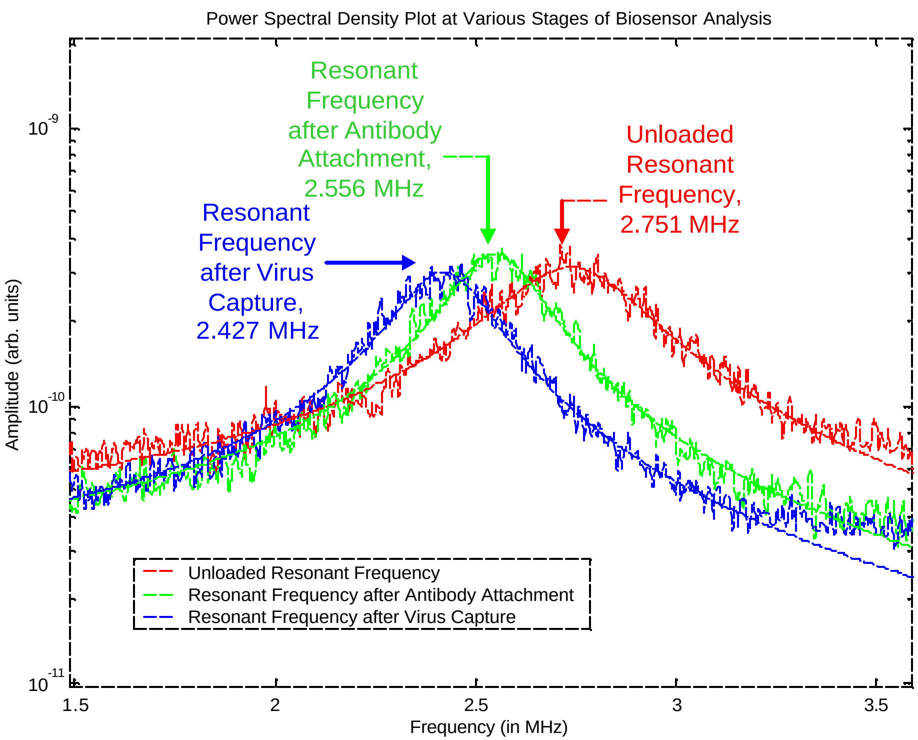


- Average mass of Vaccinia Virus ~ 9.5fg
- Work on going to integrated concentration elements
- Integrated Abs on cantilevers

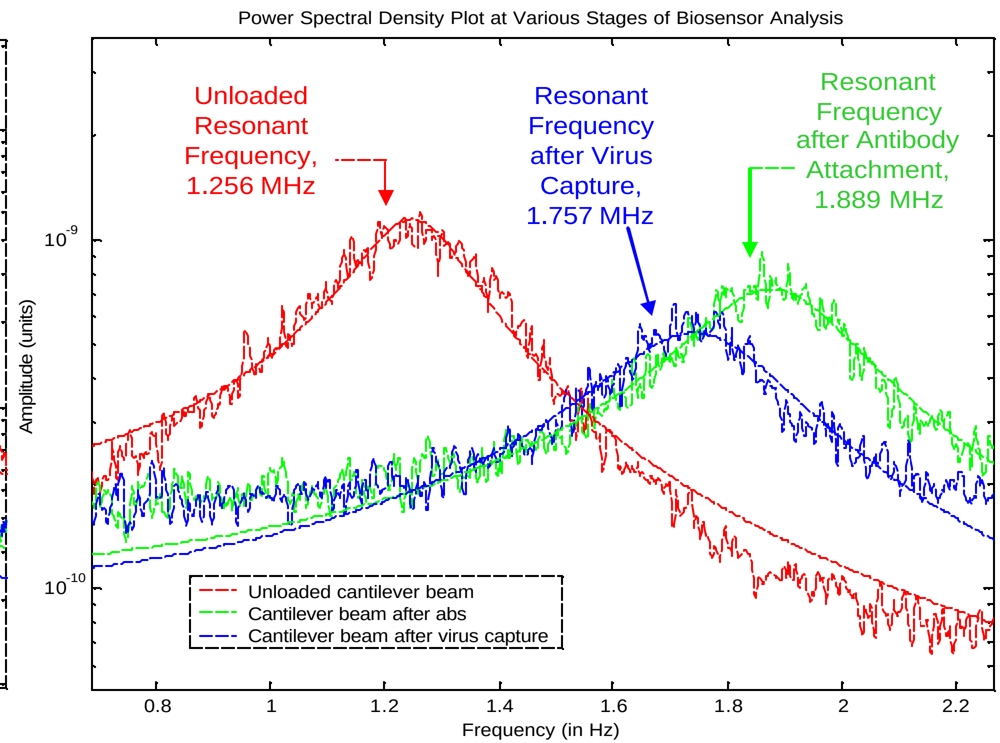
A. Gupta, D. Akin, R. Bashir, *Applied Physics Letters*, March 15, 2004.

Specific Capture of Virus Particles



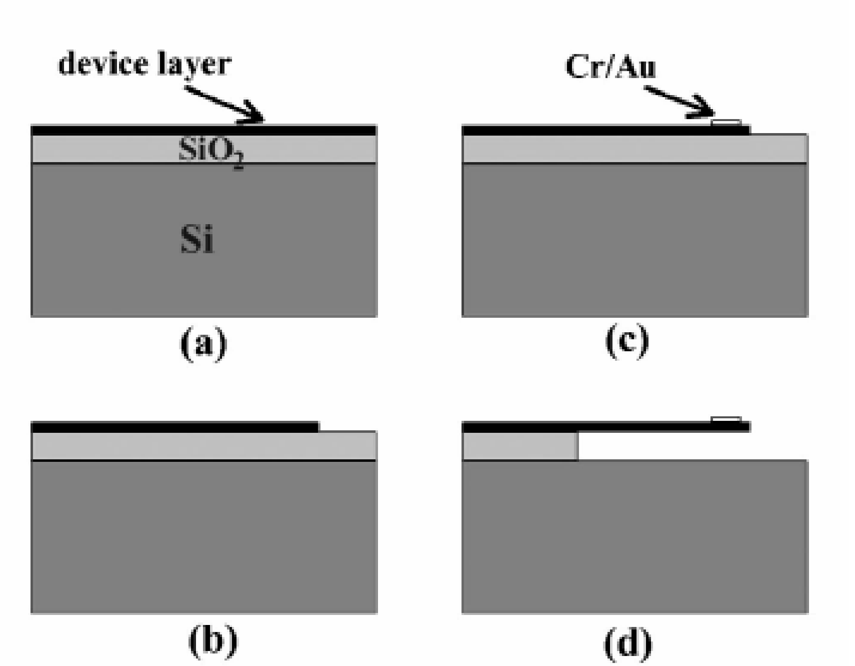


Virus capture experiment: w_o **decreases** with Ab attachment and with antigen capture

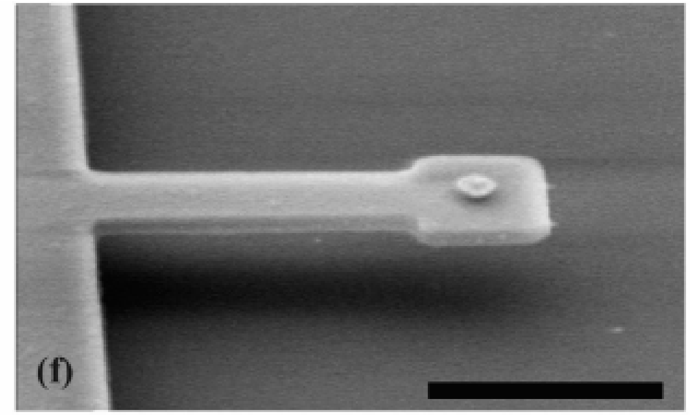


Virus capture experiment: w_o **increases** with Ab attachment and **decreases** with antigen capture

Mass of Molecules



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.



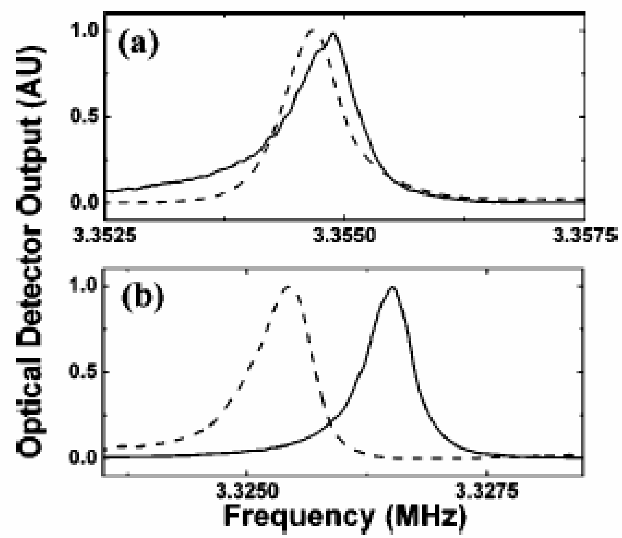


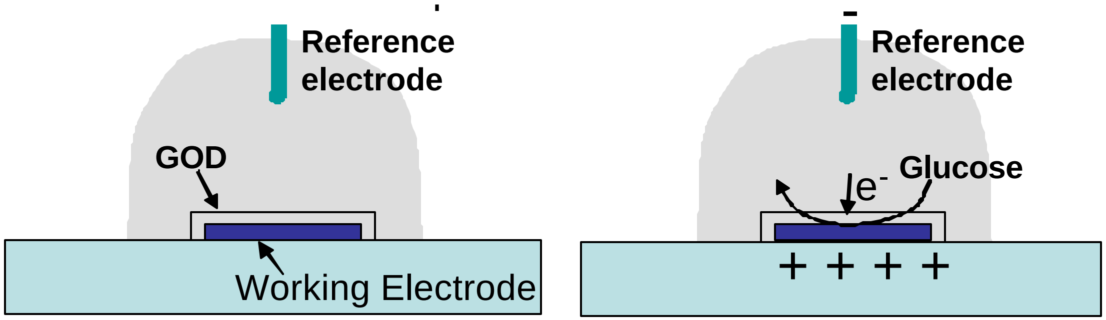
FIG. 14. Experimentally measured frequency spectra before (solid line) and after (dashed line) the adsorption of the thiolate on (a) 50- and (b) 400-nm-diam Au contact. Rectangular beam dimensions were $l = 10\ \mu m$, $w = 1\ \mu m$, and $t = 250$ nm.

Ilic, B., Craighead, H.G.; Krylov, S.; Senaratne, W.; Ober, C.; Neuzil, P.
Source: Journal of Applied Physics, v 95, n 7, 1 April 2004, p 3694-703

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

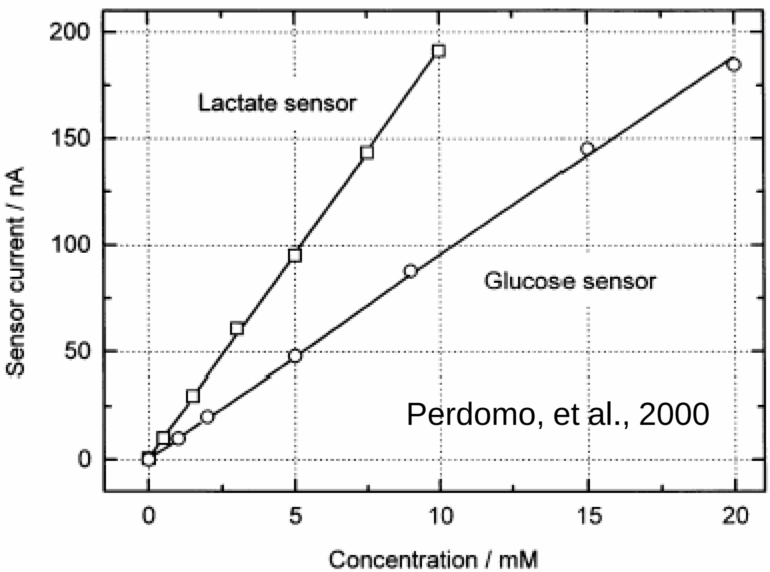


Glucose Oxidase

b**-D-Glucose + O_2 + H_2O → D-gluconic acid + H_2O_2**

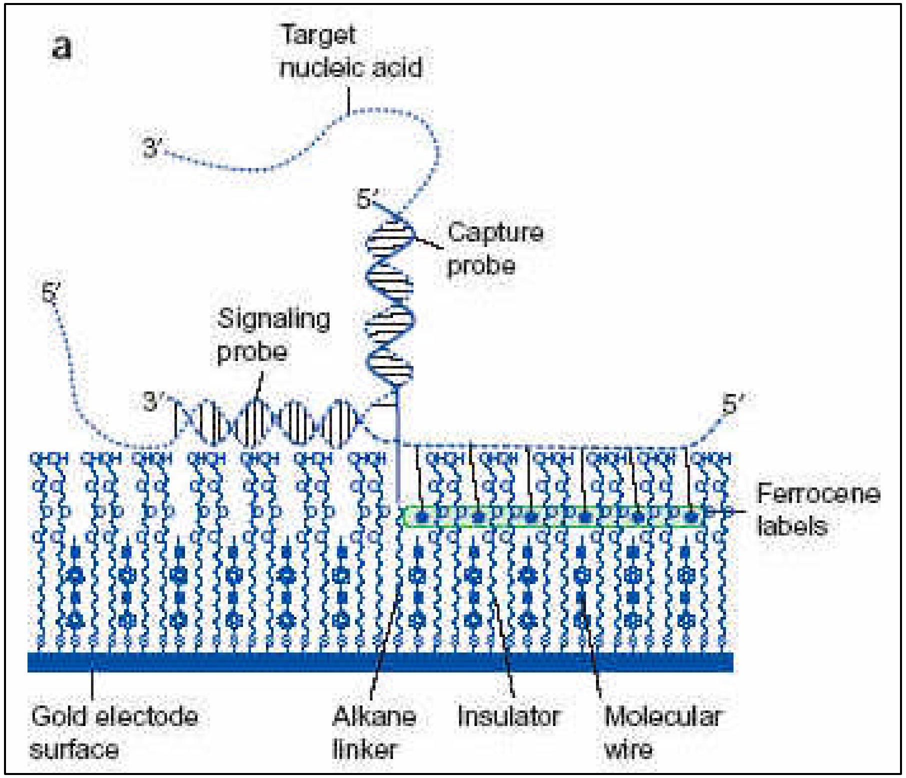
hydrogen peroxide is reduced at -600mV at Ag/AgCl anode reference electrode.

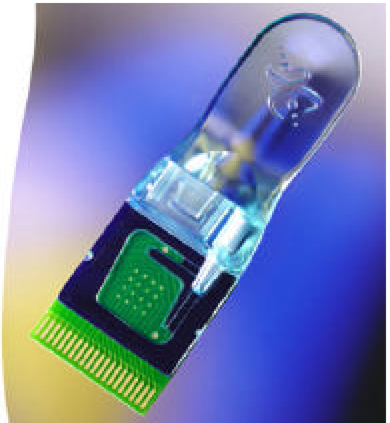
- Detection of Glucose, Lactate, Urea, etc.
- Enzyme entrapped in a gel
- Surface regeneration and sensor reusability



Detection of DNA Hybridization

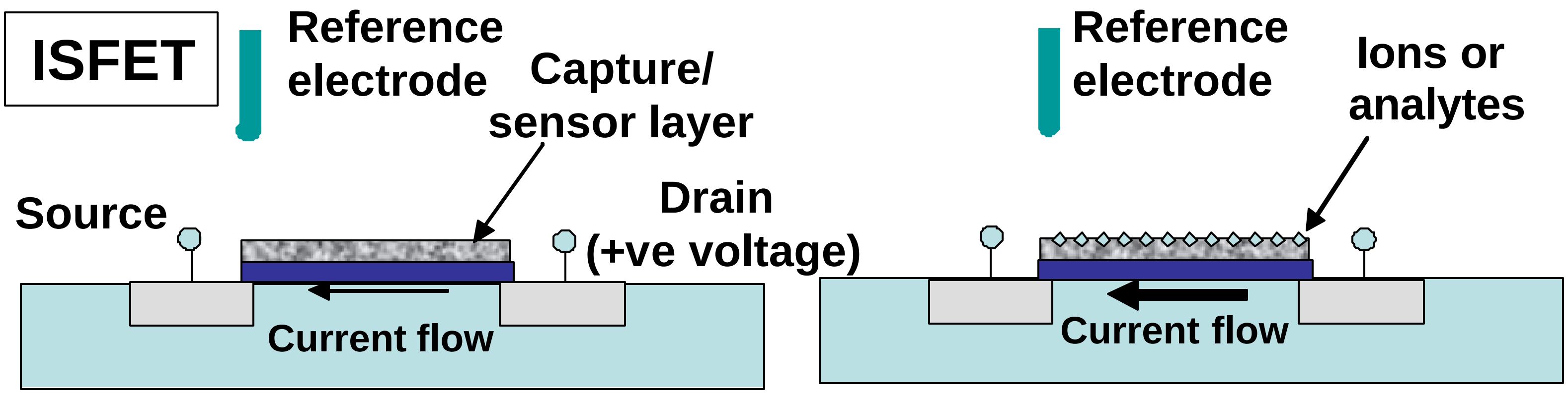
- Capture probes are attached to electrodes.
- Target DNA binds to complementary probes
- DNA sequences, called signaling probes, with electronic labels attach to them (ferrocene-modified DNA oligonucleotides, E1/2 of 0.120 V vs. Ag/AgCl, act as signaling probes).
- Binding of the target sequence to 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.





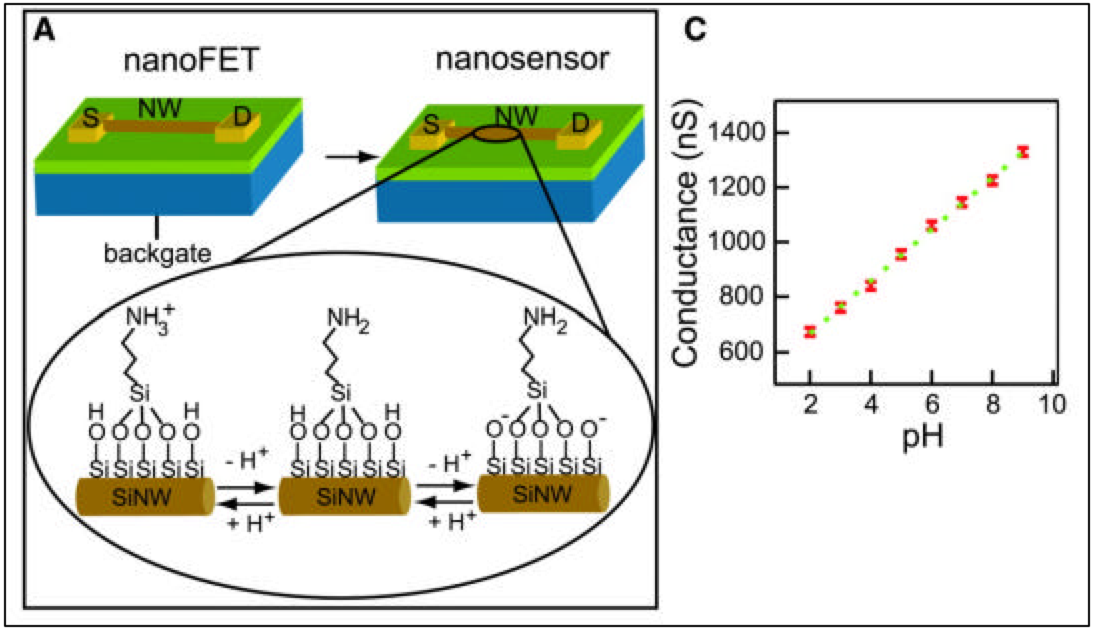
Umek, et al. J. Molecular Diagnostics, 3, 74-84, 2001
Drummond, Hill, Barton, Nature Biotech, v21, n10, Oct 2003, p1192
http://www.motorola.com/lifesciences/esensor/tech_bioelectronics.html

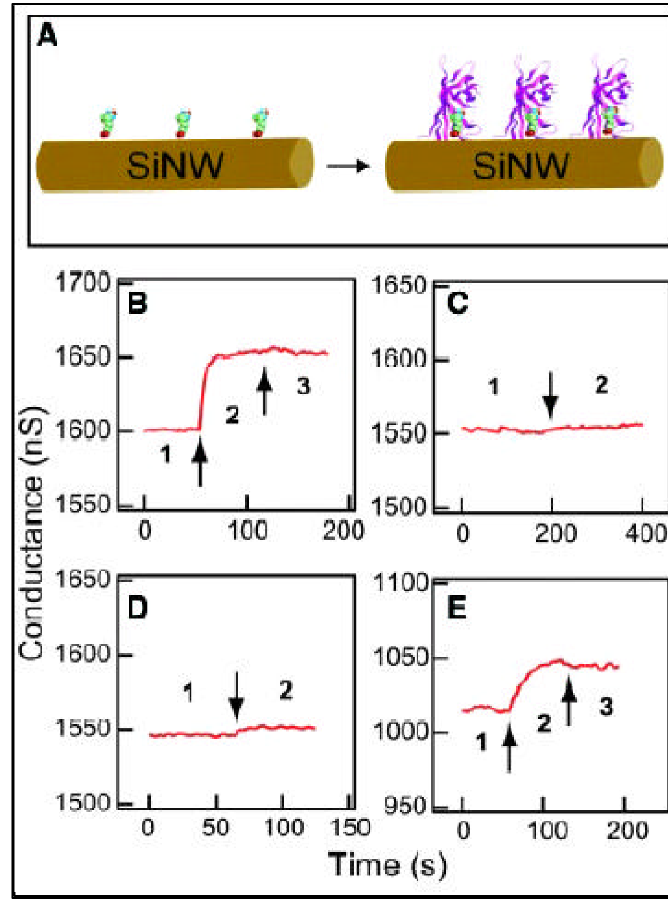
2. Potentiometric Sensors



- 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.

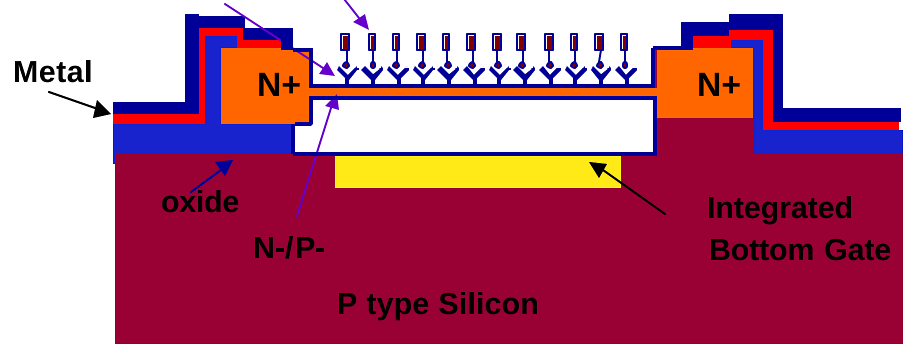
Y. Cui, Q. Wei, H. Park, and C.M. Lieber. Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. *Science*, 293:1289–1292, 2001.

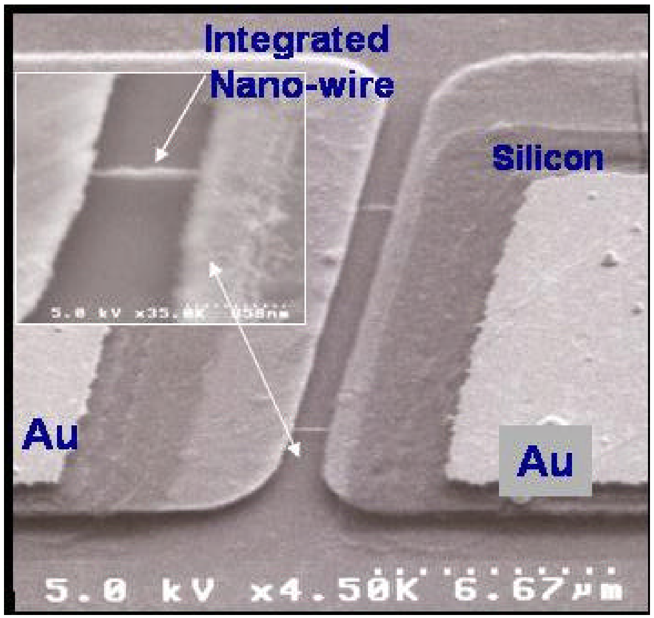
Integrated Silicon Nanowire Sensors

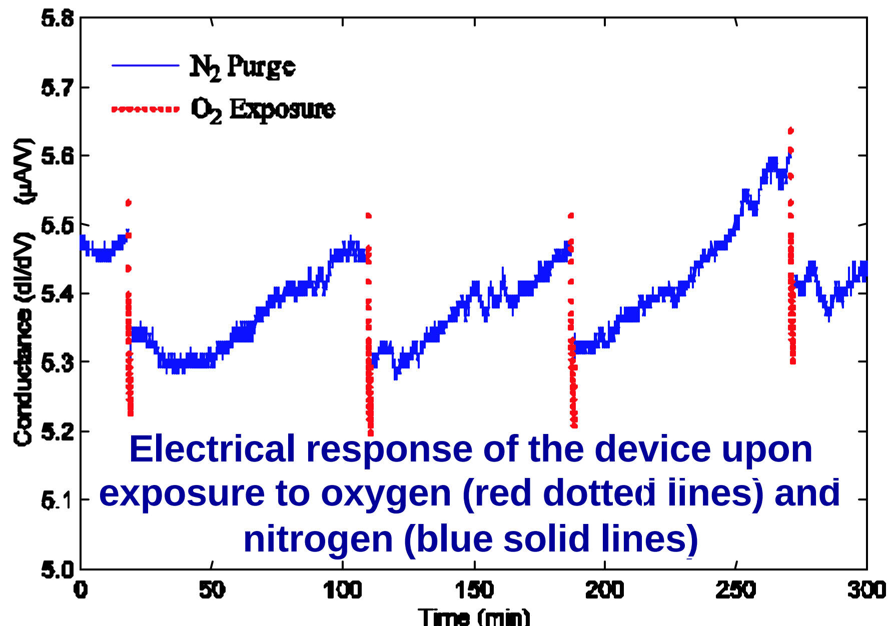
Objectives:

- 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 1mm X 3mm
- Wire Size ~ 20nm X 20nm X 3mm



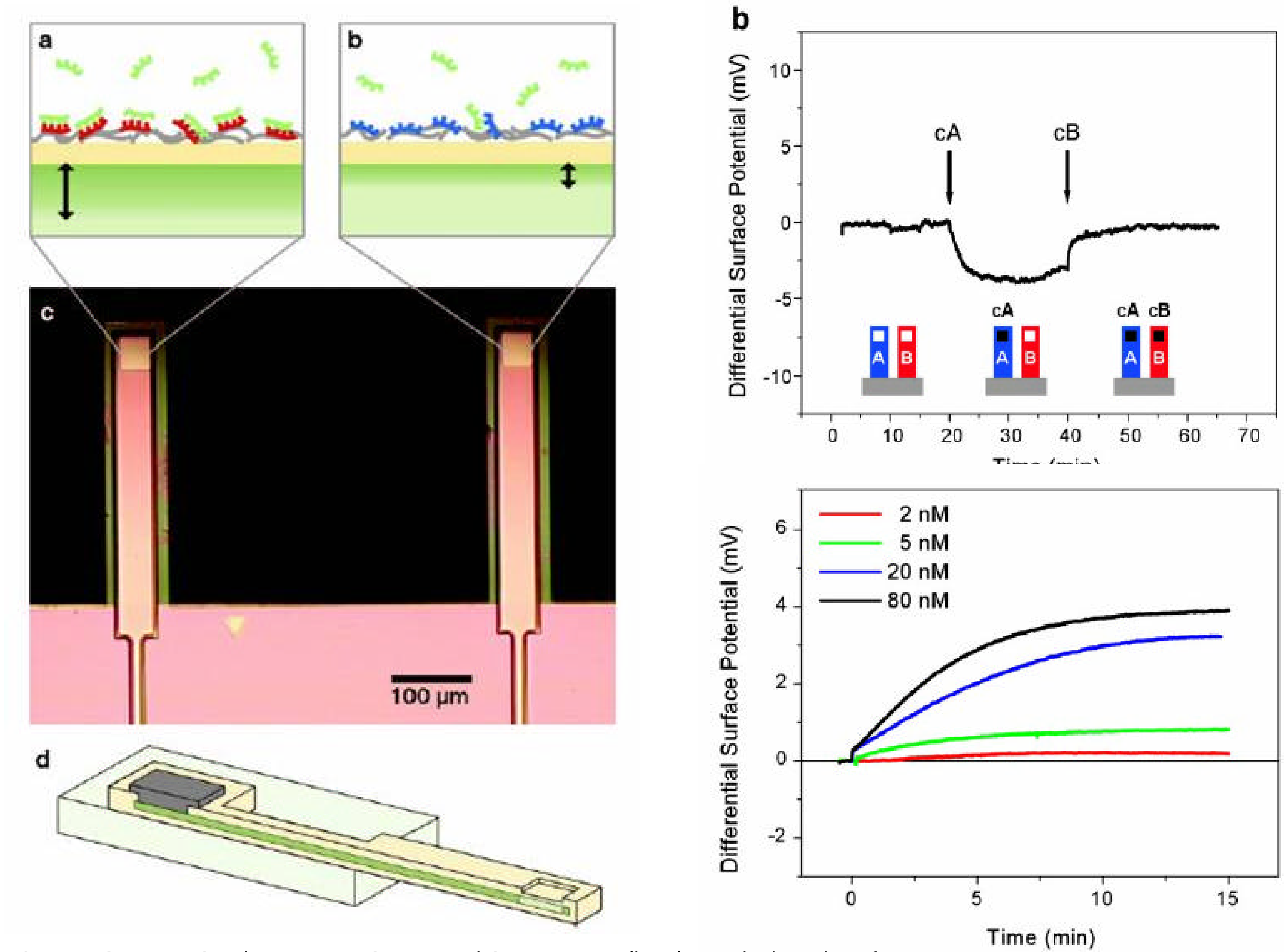




Electrical response of the device upon exposure to oxygen (red dotted lines) and nitrogen (blue solid lines)

O. H. Elibol, D. Morisette, D. Akin, R. Bashir, Applied Physics Letters. Volume 83, Issue 22, pp. 4613-4615, December 1, 2003

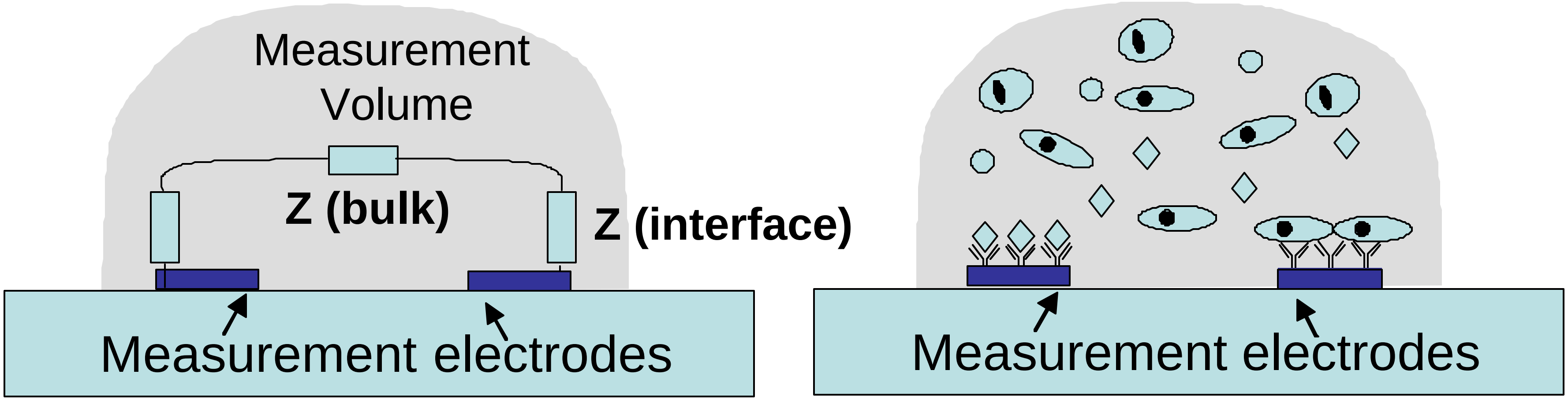
Field Effect Sensing of DNA



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.

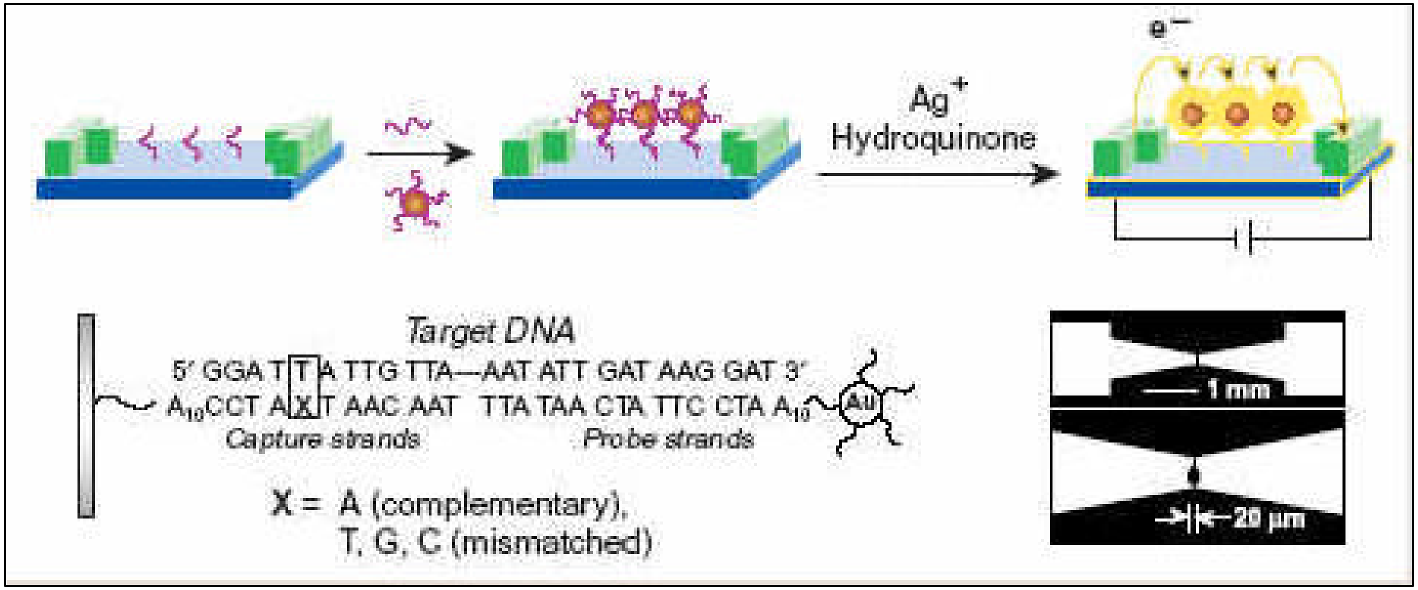
3. Conductometric Biochips

- 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.



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.