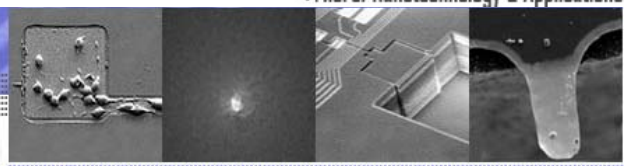
LIBNA

Laboratory of Integrated Bio Medical Micro/Nanotechnology & Applications

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



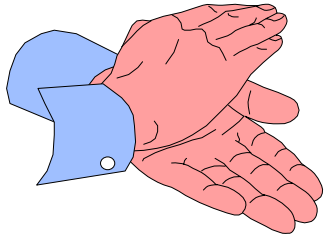
Interfacing Biology and Engineering at the Micro and Nano Scale

March 8th, 2008
Institute of Biological Engineering
2008 Annual Conference

Rashid Bashir

Micro and Nanotechnology Laboratory
Electrical and Computer Engineering & BioEngineering
University of Illinois, Urbana-Champaign
http://libna.micro.uiuc.edu/

Acknowledgements



Researchers:

- Prof. Demir Akin
- Dr. JeongMi Moon
- Piyush Bajaj
- Vincent Chan
- Brian Dorvell
- Oguz Elibol
- Yi-Shao Liu
- Kidong Park
- Bobby Reddy
- Shuaib Salamat
- Murali Venkatesan
- Nick Watkins

Recent Alums

- Prof. J. Jang, Prof. S. Iqbal, Prof. S. W. Lee, Prof. L. Yang,

BioVitesse, Inc. Co-Founder

Funding Agencies

- National Institute of Health, NIBIB
- NASA Institute on Nano-electronics and Computing (INAC)
- US Department of Agriculture (Center for Food Safety Engineering at Purdue)
- NSF NSEC at OSU
- NIH Nanomedicine NDC

Faculty Collaborators

- Prof. A. Alam (ECE, Purdue)
- Prof. D. Bergstrom (Med Chem, Purdue)
- Prof. A. Bhunia (Food Science, Purdue)
- Prof. S. Claire (IU-SOM)
- Prof. P. Guo (Vet Medicine, BME, Univ. of Cincinnati)
- Prof. M. Ladisch (Ag& Bio Engr, Purdue)
- Prof. M. Toner (Harvard Med School)
- Prof. J. P. Robinson (BMS, BME, Purdue)
- Prof. W. Rodriguez (Harvard Med School)

On Size and Scale !

Top-Down Fabrication

Feature Size

100mm — Organs

10mm — Tissue

1mm — Ants

100µm — Plant and Animal Cells

10µm — Most Bacteria

1µm

100nm — Virus

10nm — Proteins, Helical Turn of DNA

1nm — C-C Bond

0.1nm

Bottoms-Up Biological

CNT, QD, NS, NWs, AAO

Bottoms-Up Chemical

System on A board

System on a chip

MEMS

35nm Feat. of MOS-T (in 2008)

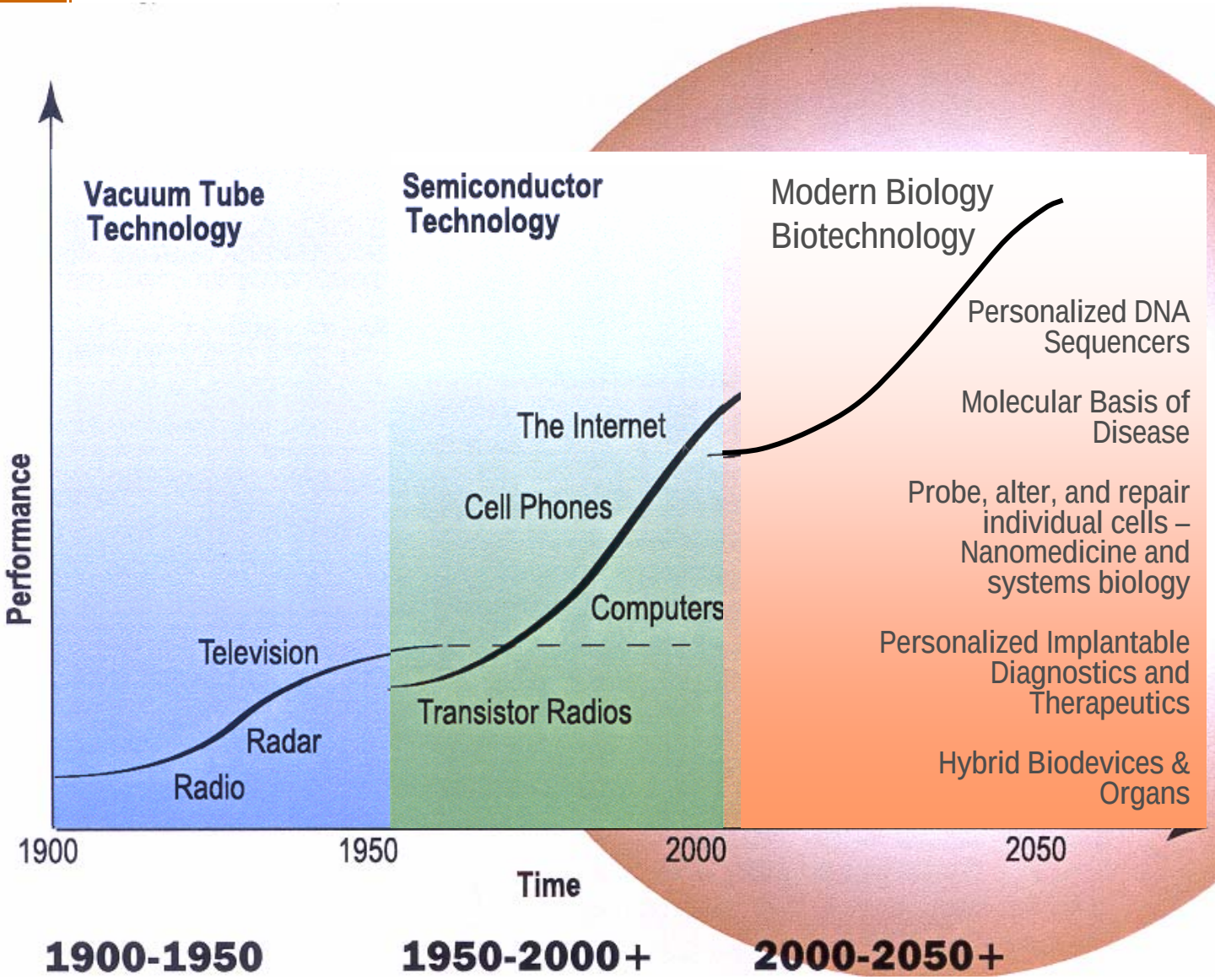
Nano pores

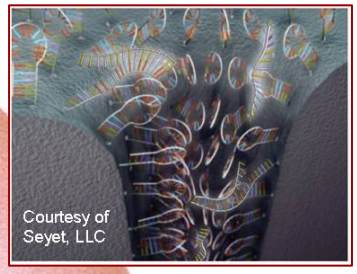
Gate Insulator

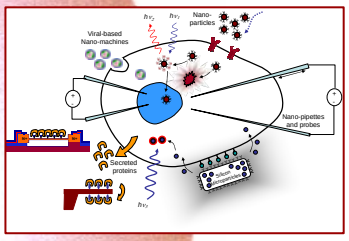
Microelectronic and MEMS

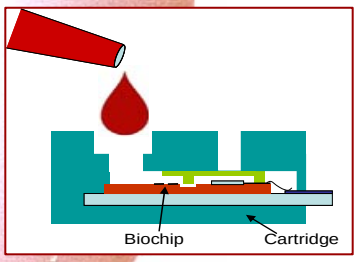
Nanoelectronics and Nanoscale Sensors

Evolution of Technologies



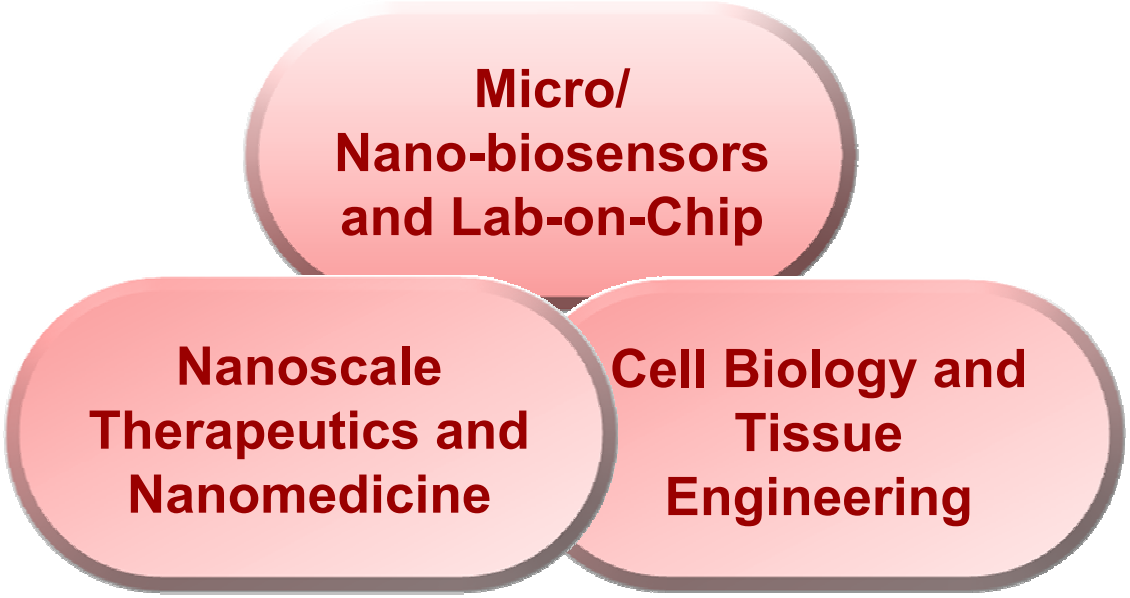






Research Projects

- Petri dish on a chip with electrical detection
- Cantilevers for virus and bacterial detection
- Detection of CD4+ cells from blood
- Nanopores for single DNA molecule characterization
- Label free field effect silicon sensors for DNA and proteins

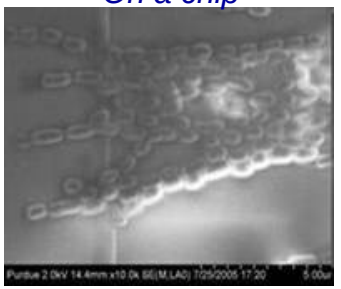


- Bacterial Mediated Delivery of Nanoparticles
- Macrophage Stealth Delivery of Nanovectors
- Phi-29 Nanomotor for Nanomedicine

- 3-D Tissue Engineering with Stereo-Lithography
- Nanomechanics for Cell Biology

Microfluidic-based technology platforms for bacterial detection

L. Monocytogenes
On a chip



R. Bashir, ECE, BME
A. Bhunia, Food Science
M. Ladisch, BME, ABE
J. P. Robinson, BMS, BME

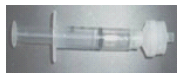
- **Rapid detection of live bacteria has wide applications**
  - **Industrial Microbiology (Pharmaceutical facility monitoring, Food Safety)**
  - **Blood transfusion**
  - **Human Diagnostics**
  - **Global Health**
- **Rapid time to result limited by:**
  - **Amplification, enrichment, culture**
  - **48-72 hours for growth and identification**
- **Center for Food Safety Engineering at Purdue (www.cfse.purdue.edu) through cooperative agreement with USDA-ARS**

USDA UNITED STATES DEPARTMENT OF AGRICULTURE

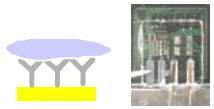
Overall Approach

Starting Sample

Sample Prep Concentration

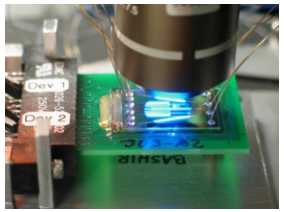


Detection/ ID



Data Analysis/ Results

Sample 100 – 250 ml



Automated Off-Chip Cell Concentration And Recovery - 1000X

On-Chip Concentration 1000X

Electrical Detection of cell Growth

Electronic Biomolecular identification

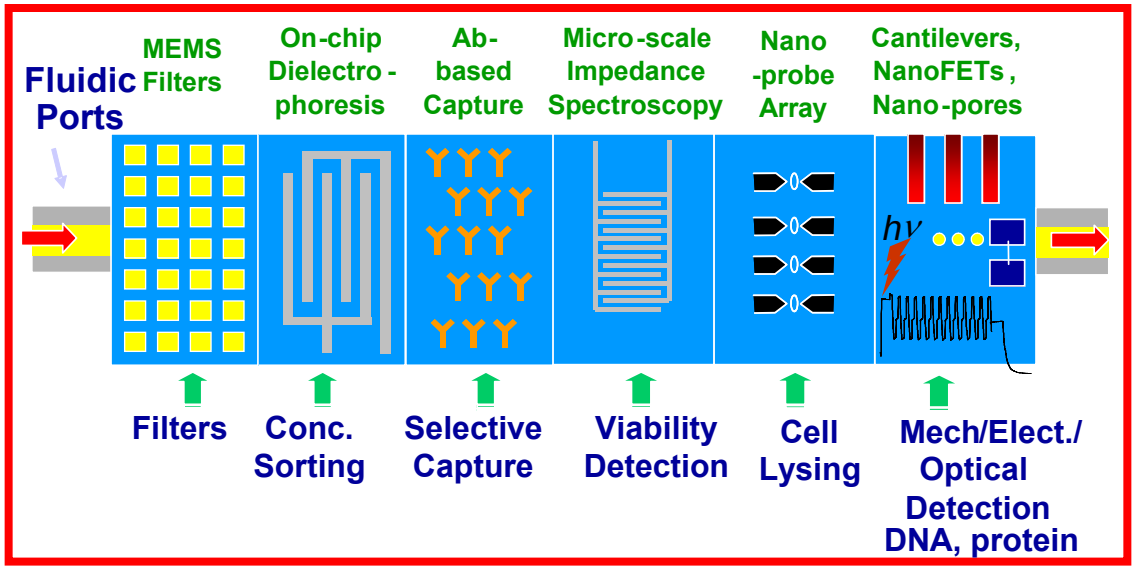
~ 15 - 30 min

~ 30 min

~ 1- 3 hr

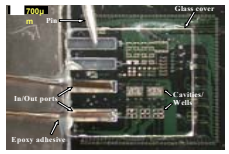
~ 1- 3 hr

Integrated Chips for Study of Microorganisms and Cells



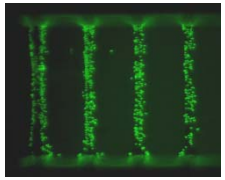
Lab-on-a-chip for Detection of Live Bacteria

Liu, Park, Li, Huang, Geng, Bhunia, Ladisch, Bashir



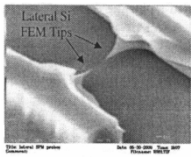
Dielectrophoresis Filters an Traps for Biological Entities

Li, Akin, Bhunia, Bashir



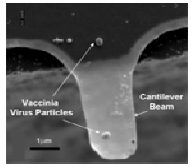
Trapping/Lysing of Bacteria/Viruses In Microfluidic Devices

Park, Akin, Bashir



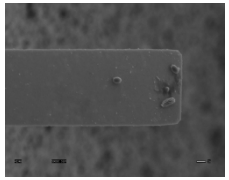
Nano-Mechanical Cantilever Sensors for Detection of Viruses

Gupta, Akin, Broyles, Ladisch, Bashir



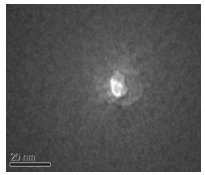
Micro-Mechanical Cantilevers for Detection of Spores

Davila, Walter, Aronson, Bashir



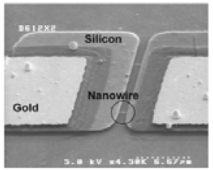
Nanopore Sensors for DNA Detection

Chang, Andreadakis, Kosari, Vasmatzis, Bashir



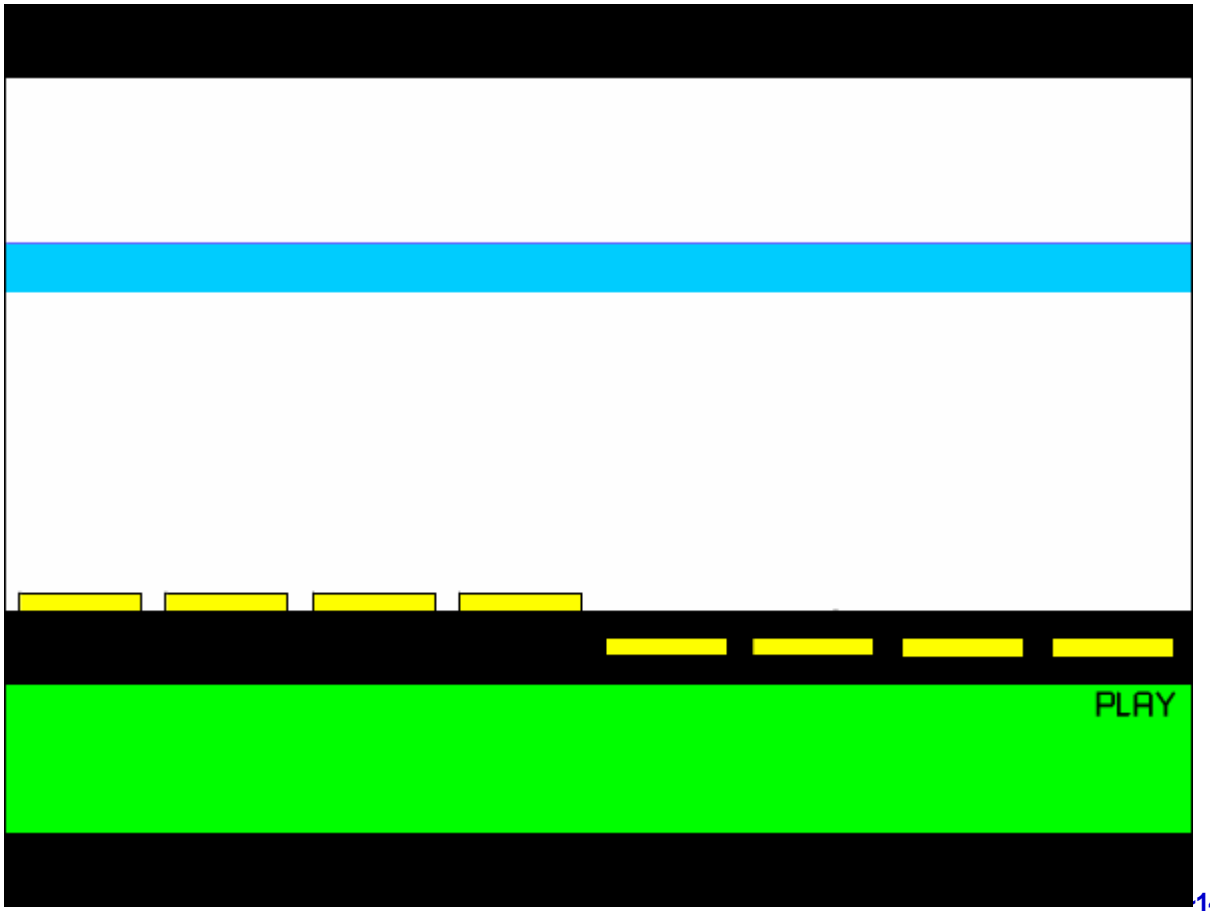
Silicon Nanowires and Nanoplates for DNA and Protein Detection

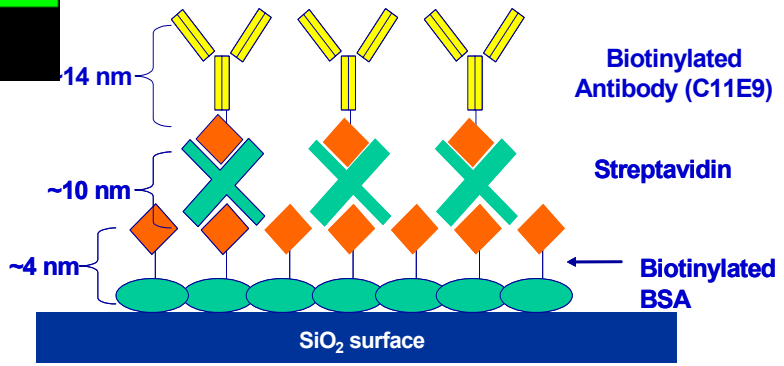
Elibol, Reddy, Nair, Bergstrom, Alam, Bsahir



"Lab on a Chip" with microfluidics and micro/nanosensors

Overall Detection Scheme

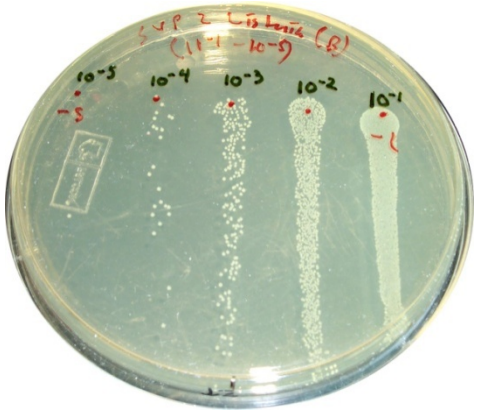




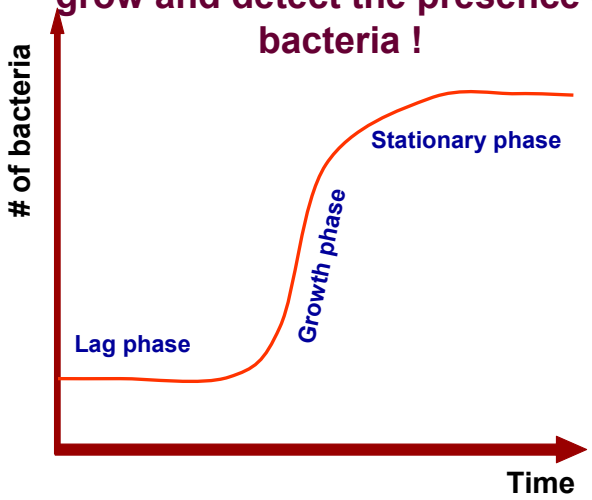
Gomez, Morisette, Bashir, IEEE/ASME JMEMS, 2005
Yang, Li, Akin, Huang, Bhunia, Ladisch, Bashir, Lab Chip, 2006

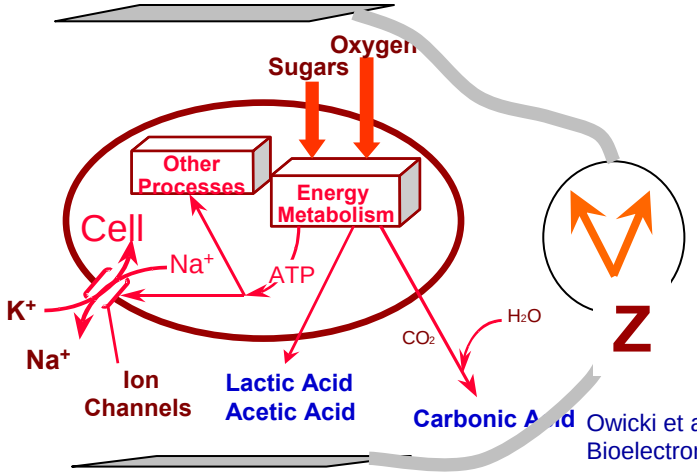
* Size information are obtained from Biochemistry 2nd edition, R. H. Garrett and C. M. Grisham, 1995.

Bacterial Growth & Impedance Microbiology !



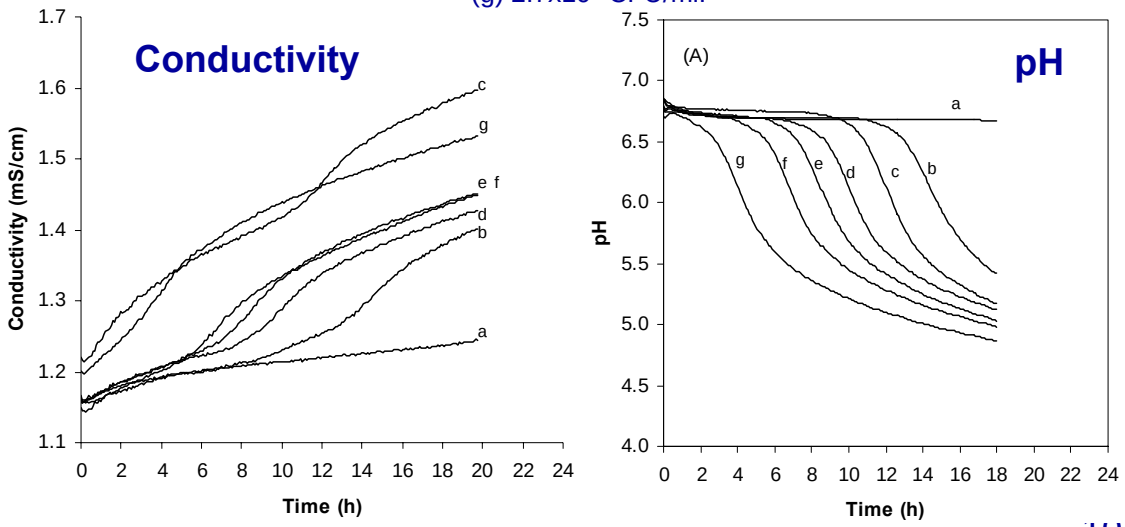
Invented in late 1800s (Petri and Koch)
Still the most widespread means to grow and detect the presence of bacteria !





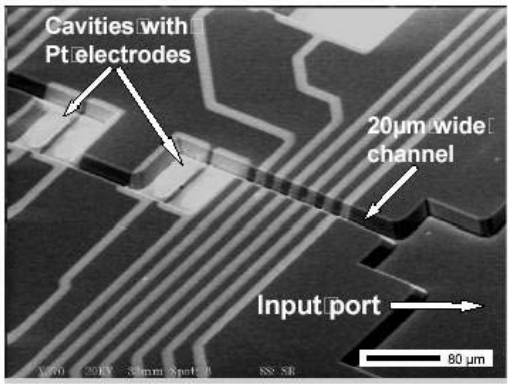
Owicki et al., Biosens. Bioelectron. (1992)

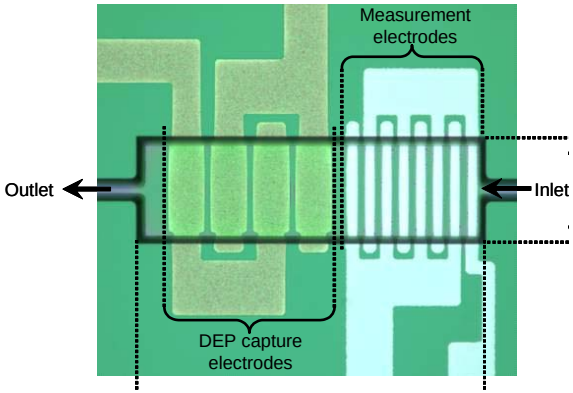
(a) no cell, (b) 2.5 x10^2, (c) 2.39x10^3, (d) 2.64x10^4, (e) 2.66x10^5, (f) 2.65x10^6, (g) 2.7x10^7 CFU/ml.

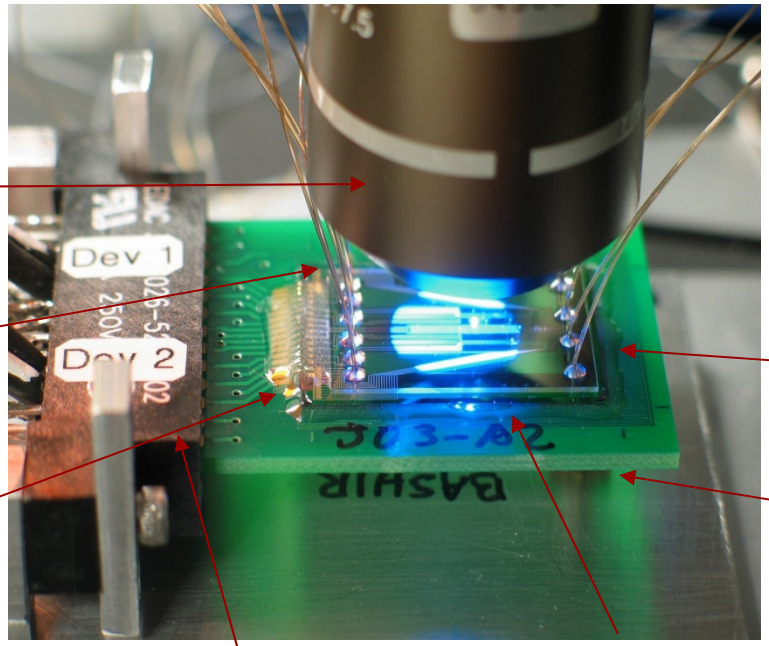


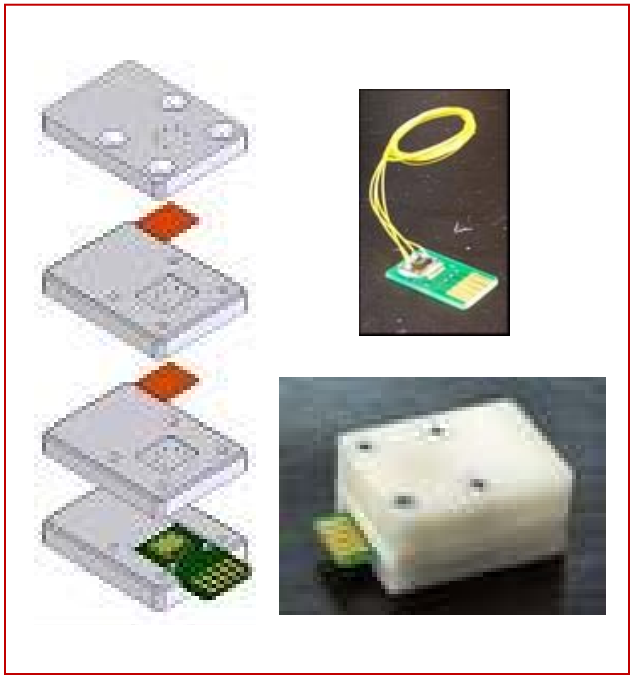
Yang, L, Banada, P., Bhunia, A. Bashir, R., Biotech Bioengr, 2005

Petri Dish-on-a-Chip





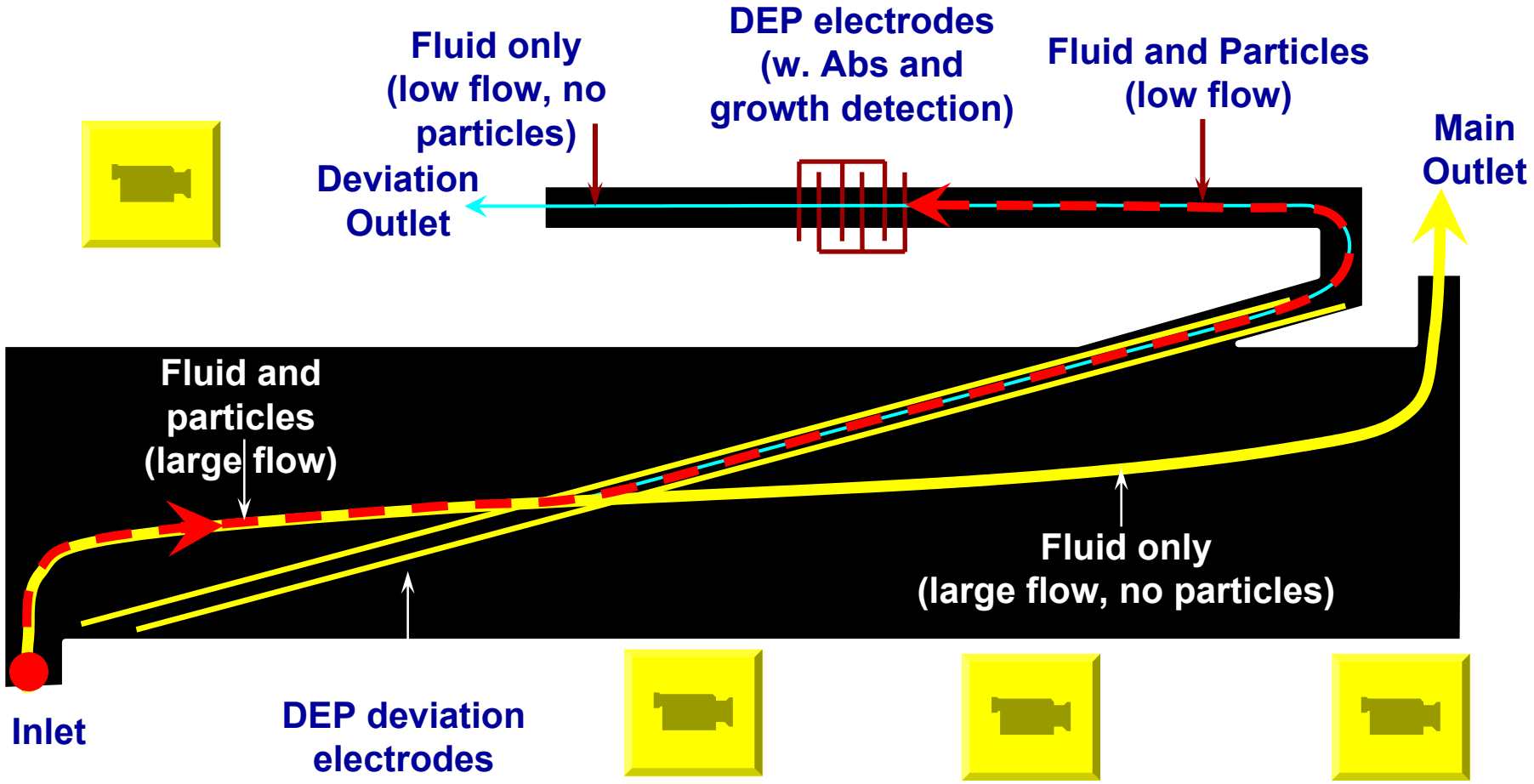




R. Gomez, D. Morisette, R. Bashir, IEEE/ASME JMEMS, 2005

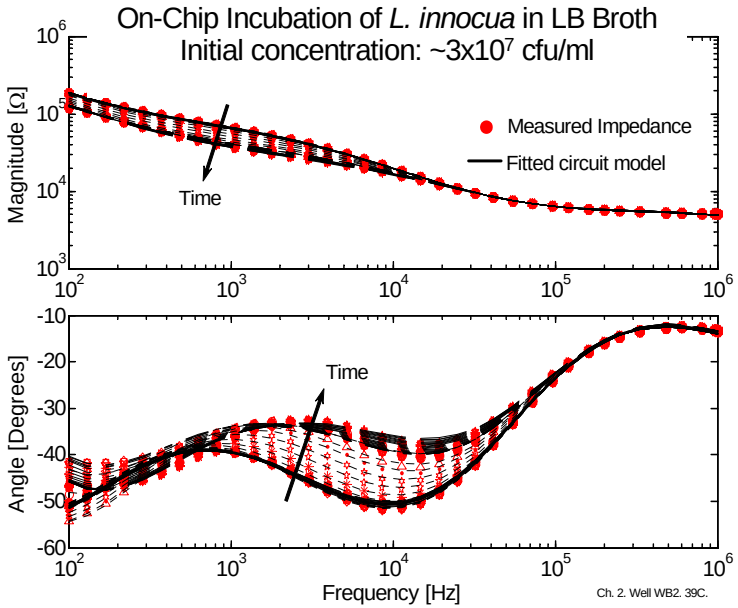
Bacterial Cell Concentration on-Chip

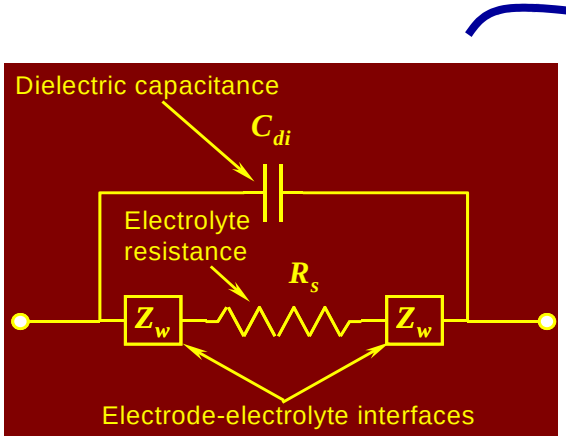
- DEP-based concentration system collects particles from a large flow stream and diverts them to a smaller stream



R. Gomez, D. Morisette, R. Bashir, IEEE/ASME JMEMS, 2005

On-Chip Incubation of *L. Monocytogenes*

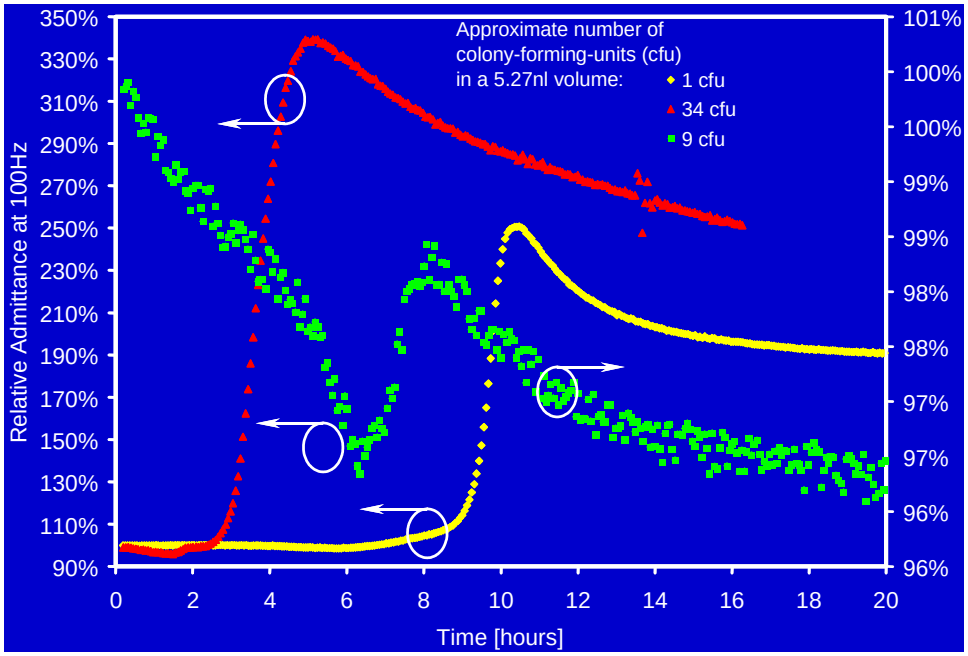


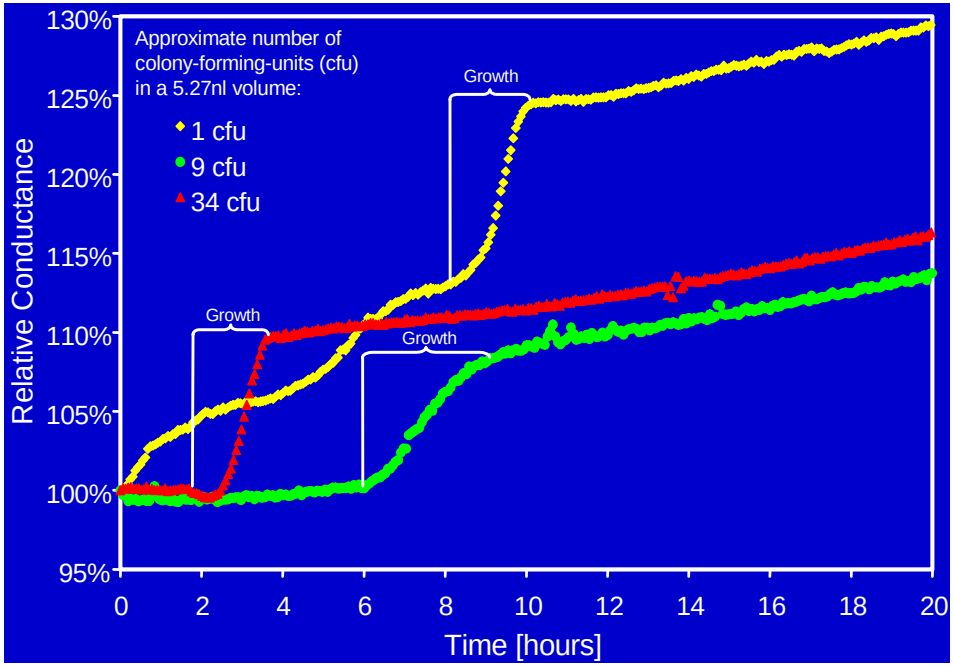


Electrode-Electrolyte Interface Model:

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

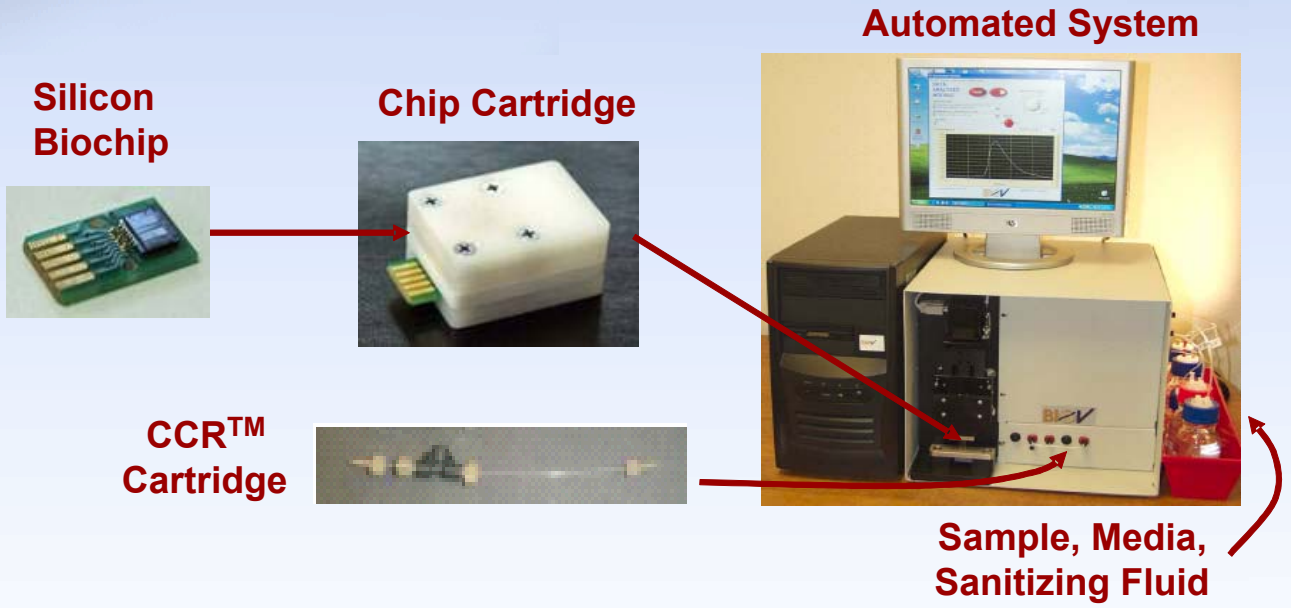
Constant-angle impedance



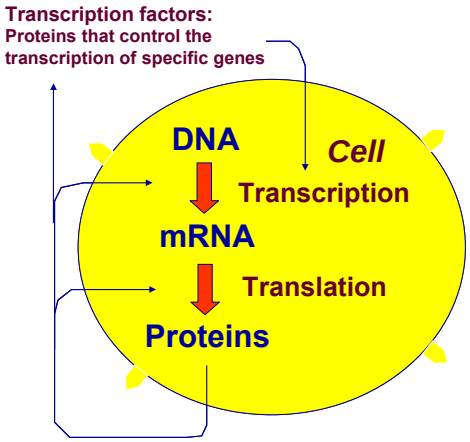


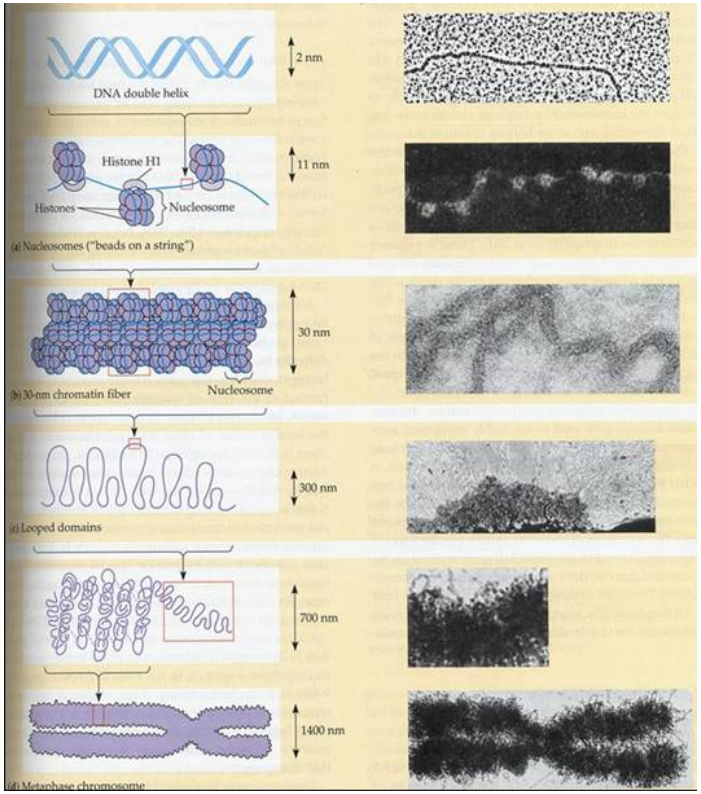
BioVitesse, Inc.

- ‘Petri dish on a chip’ to miniaturize impedance microbiology
- To quickly and reliably detect and identify *live bacteria* in 2 to 4 hours, instead of 2 to 10 days
- Provides in-process quality control monitoring systems
- To the industrial microbiological market (Bio/Pharma and Food Safety)

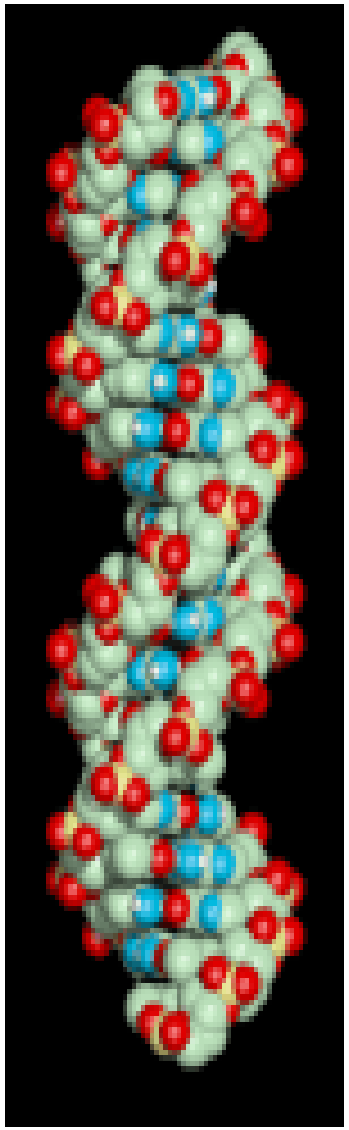


DNA – the code of life in all Cells!

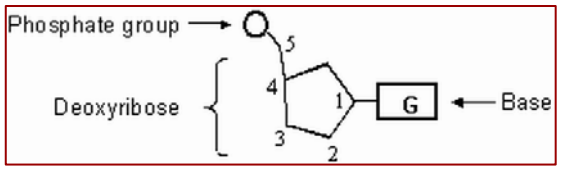


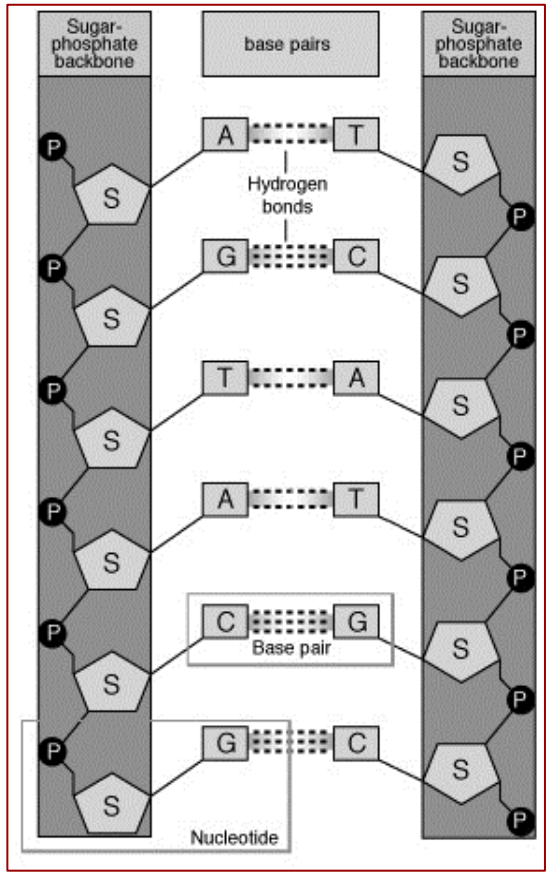


Molecular Biology of the Cell, Alberts, Bray, et al.



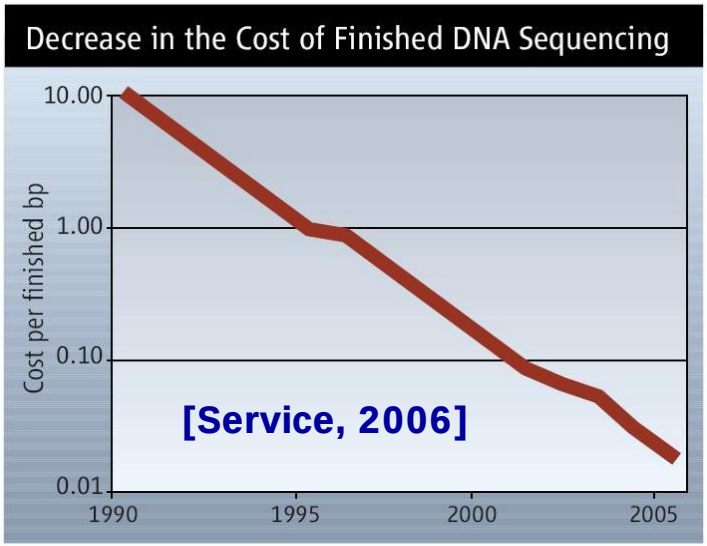
http://www.chromosome.com/dna.html

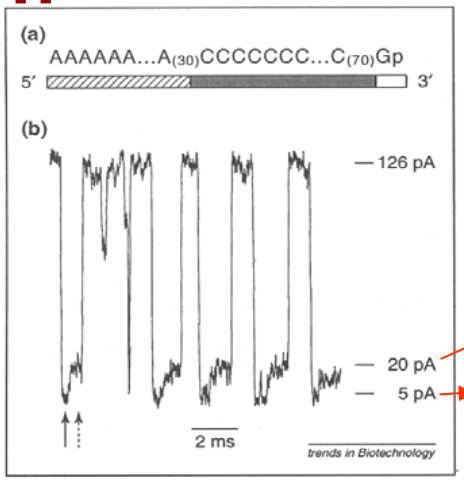




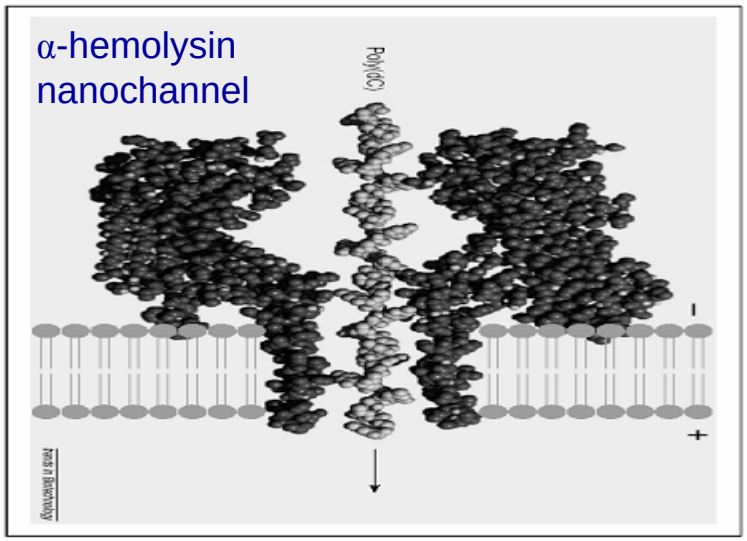
Nanopores for single DNA molecule characterization

2c/bp x 3e9bp = $60M !





- **α-hemolysin channel, a biological protein based-pore**
- **Diameter of 2.6 nm.**
- **Translocation of ssDNA using electrophoresis**
- **Nanoscale coulter counter**



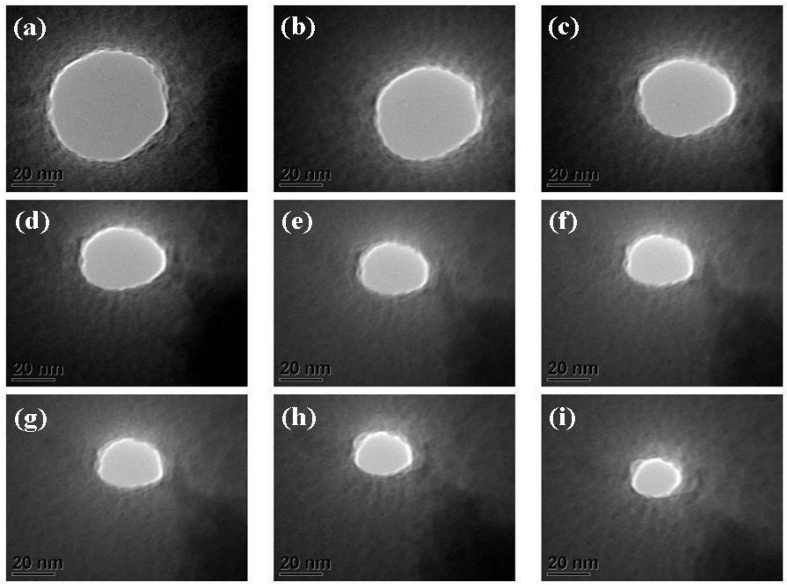
The model of DNA passing through an α-hemolysin channel.

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

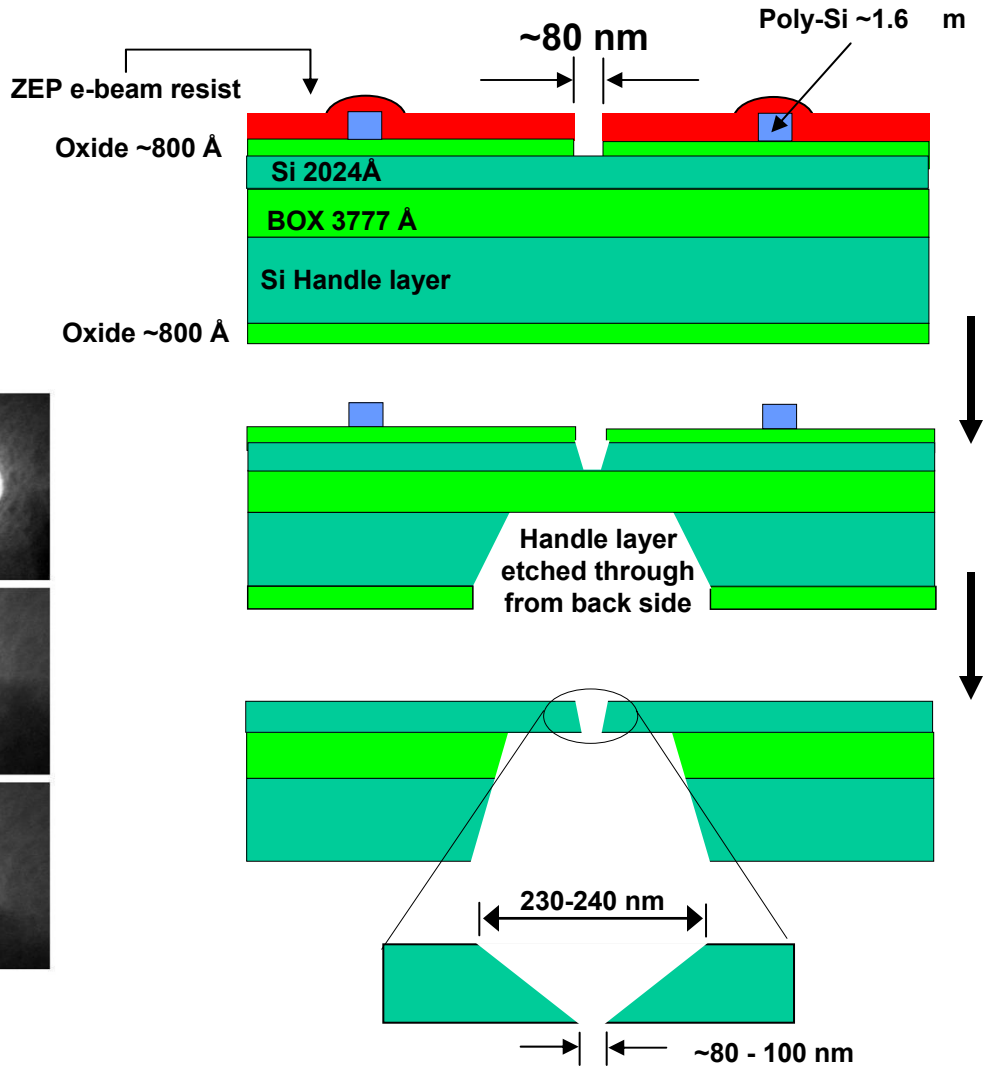
Nanopore Fabrication

Key steps

- EBL to write dots
- TMAH etch
- Pore in SOI
- Oxidation
- Shrinking in TEM

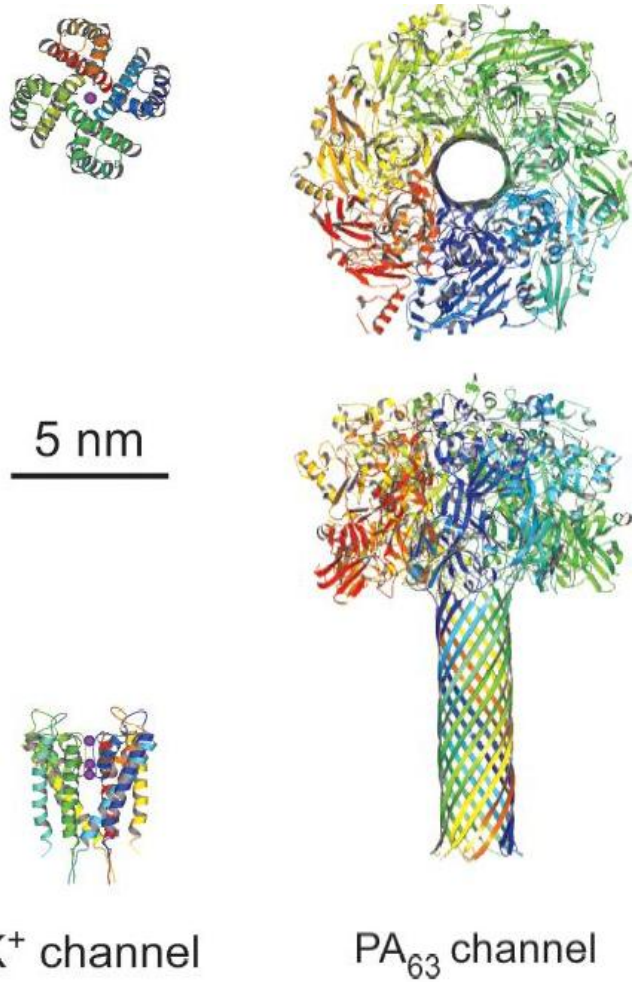


Pore shrinking temporal profile of a nanopore channel
All Images at 1,000,000X. (NPC-2)



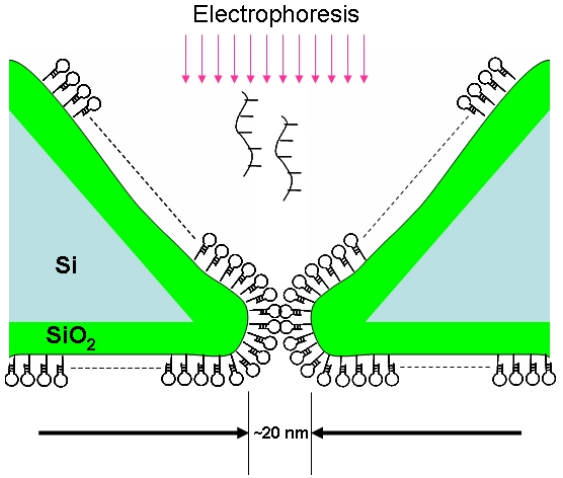
Adding DNA Selectivity to Solid State Nanopores

Protein Ion Channels



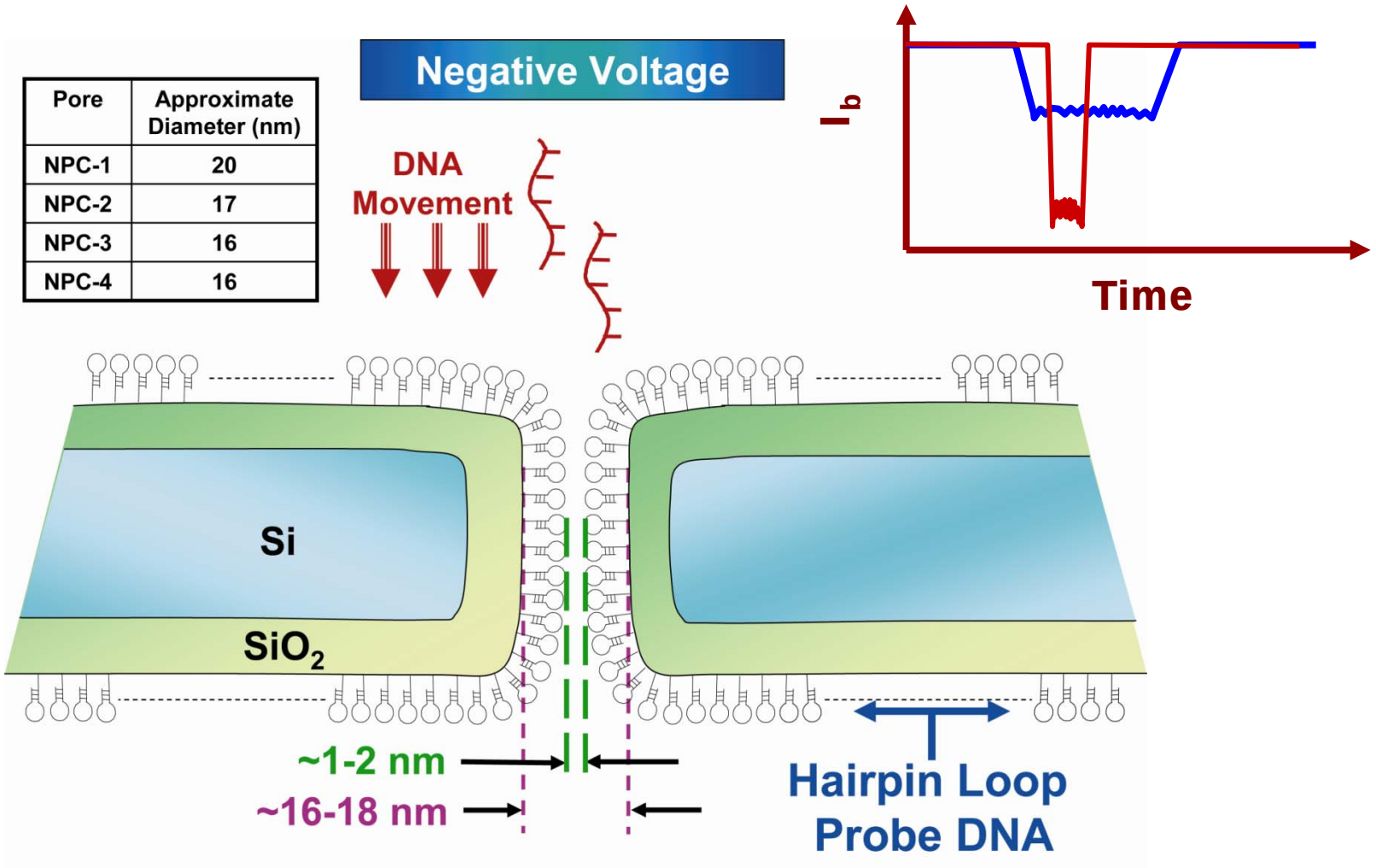
[Kasianowicz *et al.*, 2006]

Nanopores with selectivity towards specific targets



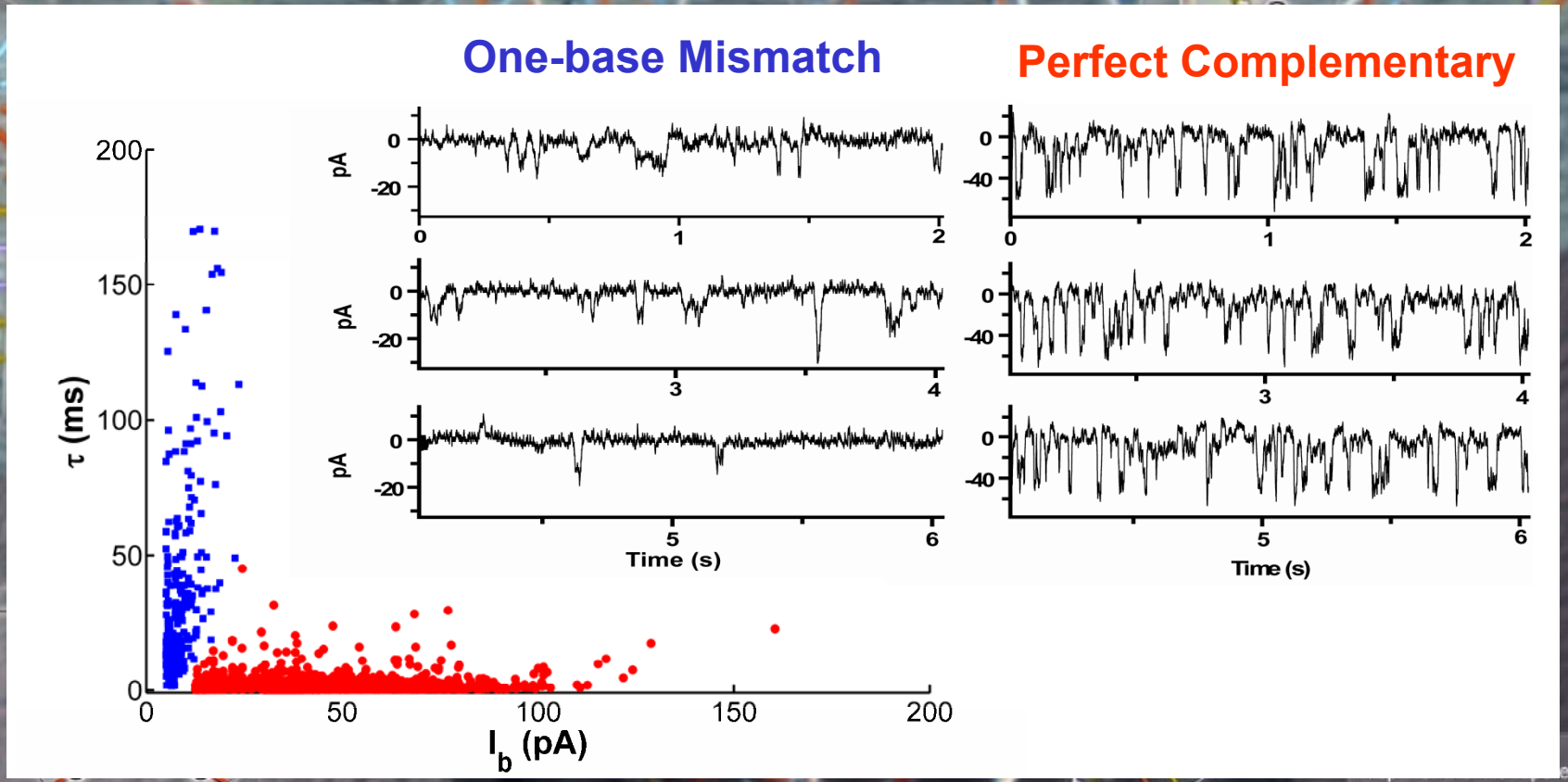
Selective Nanopores Measurements Overview

Pore	Approximate Diameter (nm)
NPC-1	20
NPC-2	17
NPC-3	16
NPC-4	16



S. M. Iqbal, D. Akin, R. Bashir, Nature Nanotechnology, April 2007

Courtesy of
Seyet, LLC



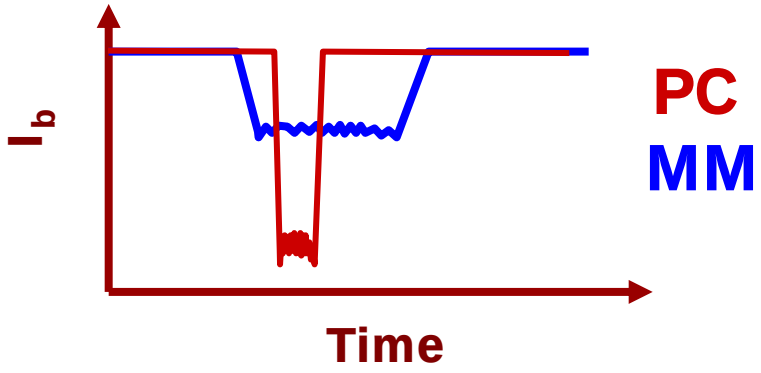
NPC-1	1MM-DNA (in 120 min)		PC-DNA (in 120 min)		1MM-DNA after PC-DNA (in 120 min)	
Signature of pulses	τ (ms)	I_b (pA)	τ (ms)	I_b (pA)	τ (ms)	I_b (pA)
Mean	178.8	28.9	10.2	31.2	92.0	29.1
Sigma	260.3	31.7	30.4	27.8	78.2	23.0
Number of pulses	3,353		96,876		2,896	

Si

SELECTIVE TRANSPORT
ECULES THROUGH
SOLIDSTATE NANOPORE CHANNELS

Single-Base Mismatch Selectivity

- Channel-Molecular Interaction
  - **MM-DNA vs. PC-DNA**
- PC-DNA
  - **Interactions with Binding Sites**
  - **Faster and More than MM-DNA**
- MM-DNA
  - **Electrostatic Friction**
  - **Mechanical Resistance**
  - **Inability to open HPL**



$$J = \frac{n}{\tau}(c_1 - c_2)$$

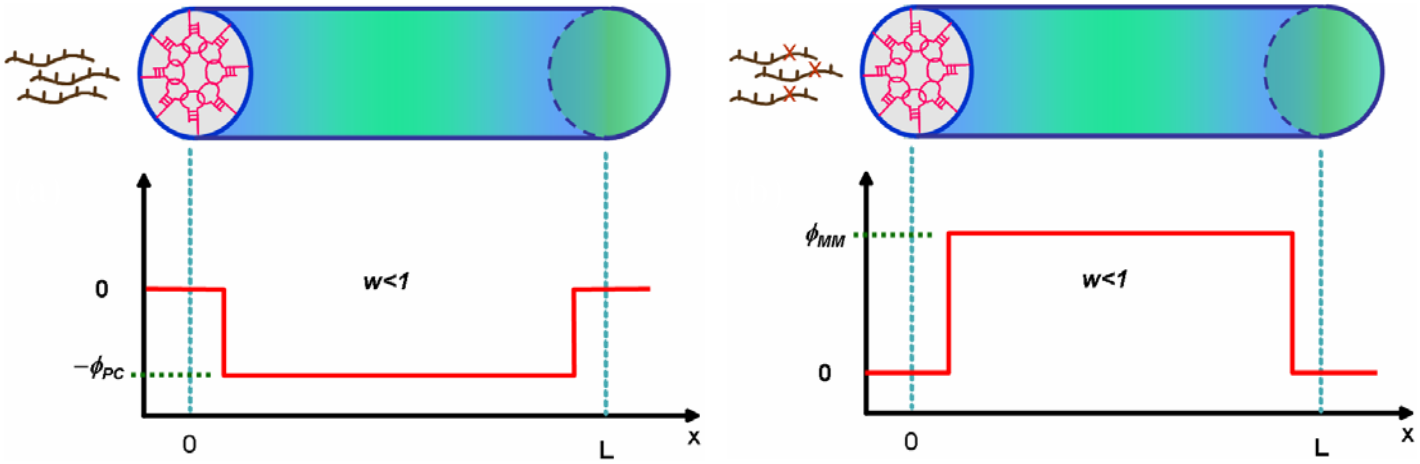
S. M. Iqbal, D. Akin, R. Bashir, Nature Nanotechnology, April 2007

Channel Interaction
Attractive vs. Repulsive Potential

Key Assumptions

- PC/HPL: Attractive Potential
- MM/HPL: Repulsive Potential
- Magnitudes of Potentials
- ϕ span part of the channel

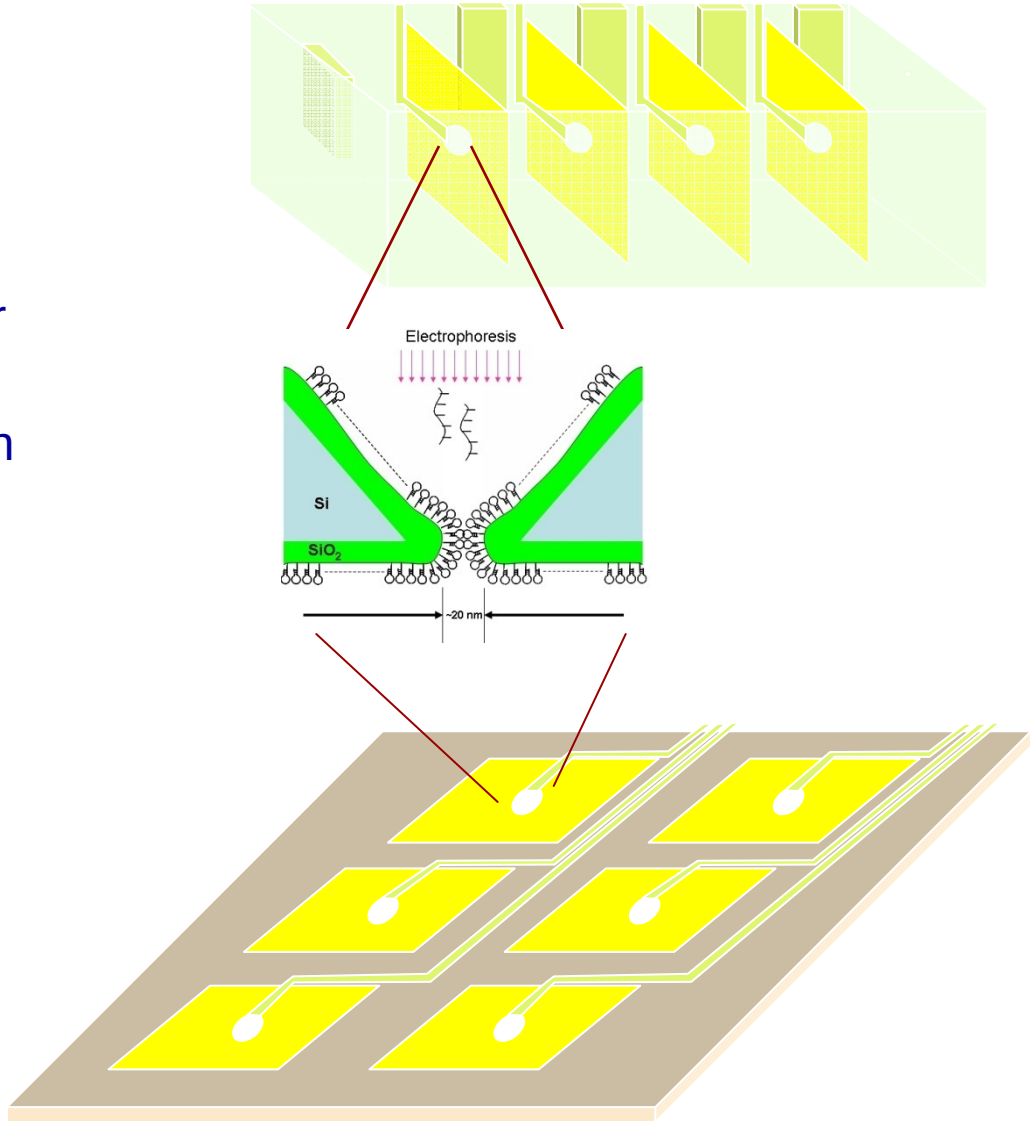
$$\frac{\tau_{MM}}{\tau_{PC}} = e^{|\phi_{MM}|-|\phi_{PC}|}$$



(a) Attractive potential (b) Repulsive potential, spanning part of the channel

Nanopore Channel Sensor Array for Characterization of Single Molecules

- Miniature DNA analyzer
  - Transduction events at single molecule, nanoscale
  - Interface to the macroscale
  - Array of nanopores – serial or parallel ?
  - Site selective functionalization strategy
- Sequencing by digestion
  - Smaller molecules – bases !
  - Digest and detect



Microcantilever Mass Sensors

Unloaded Resonant Frequency :

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

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

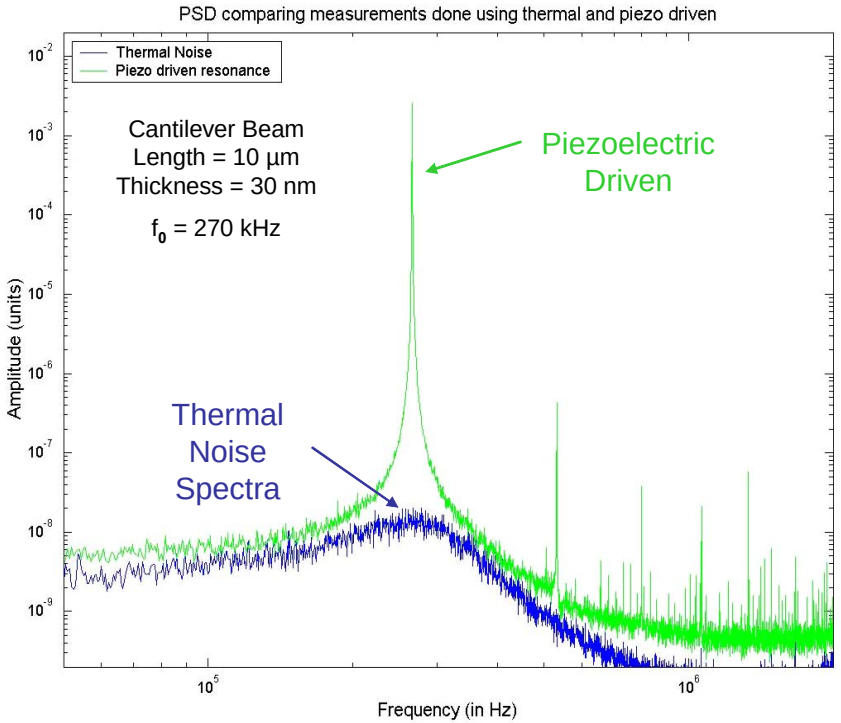
Loaded Resonant frequency : $f_1 = \frac{1}{2\pi}\sqrt{\frac{k}{m*+\delta m}}$

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

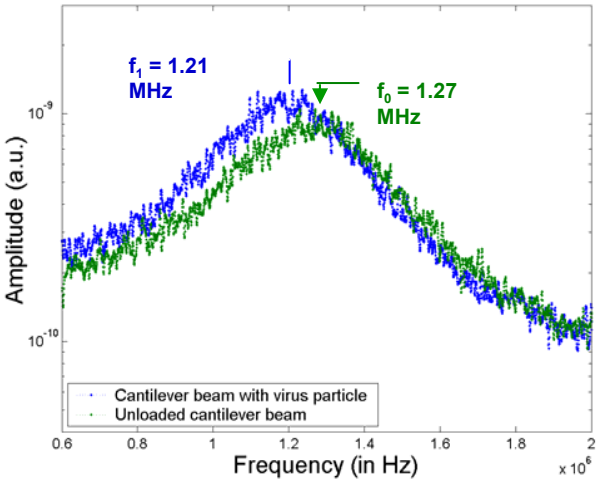


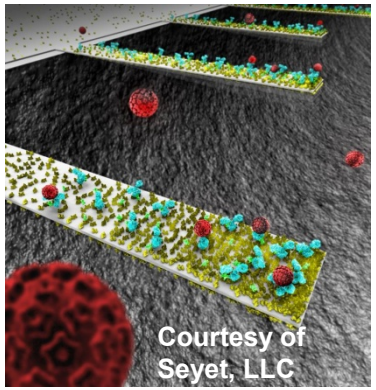
Nanomechanical Sensors for Viral Detection

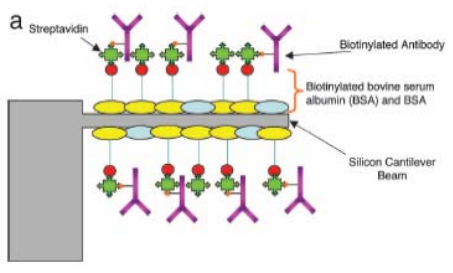
Objectives:

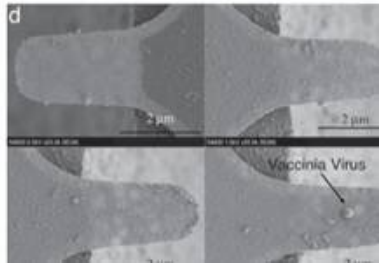
To develop technology for the rapid detection of virus particles in fluid and air using Nanomechanical Cantilever Sensors

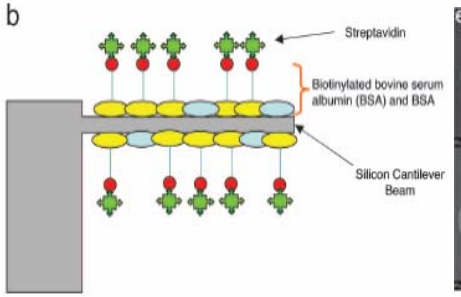
Frequency Shift, Δf = 60 kHz
⇒ Mass change, Δm = 9 fg
⇒ This corresponds 1 vaccinia virus.

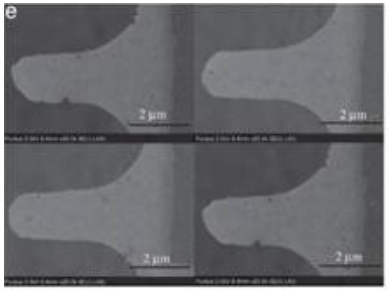


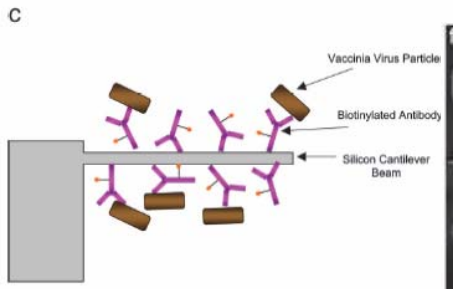


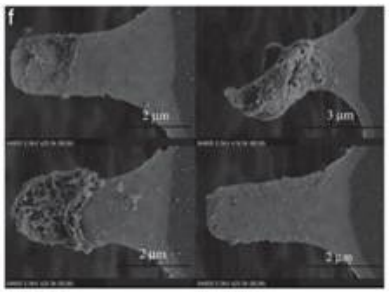


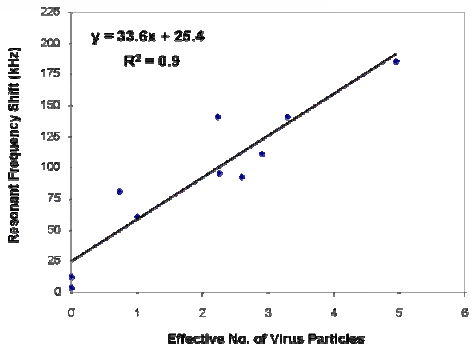










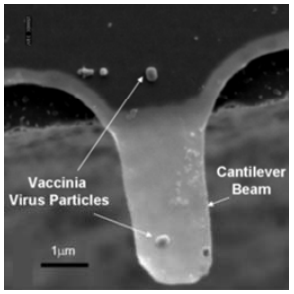


A. Gupta, et al. Proc. Nat. Acad. Sci. 2006

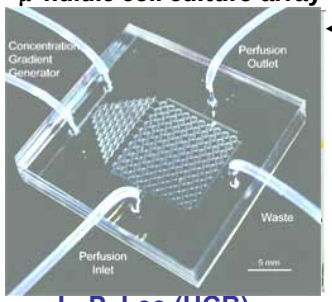
Living Cantilever Arrays

Measuring the growth-rate of a single cell, under various **combination of stimuli**.

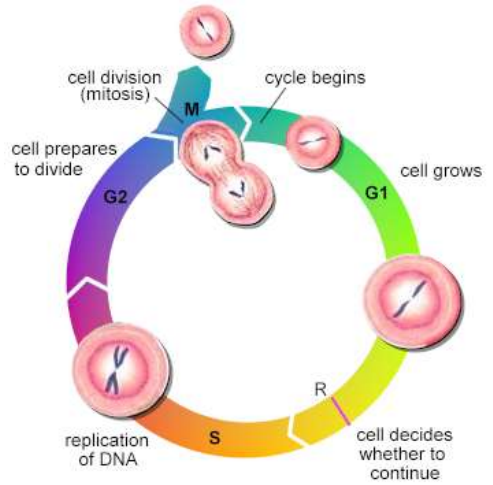
Cantilever mass sensing

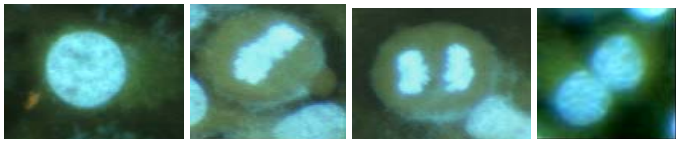


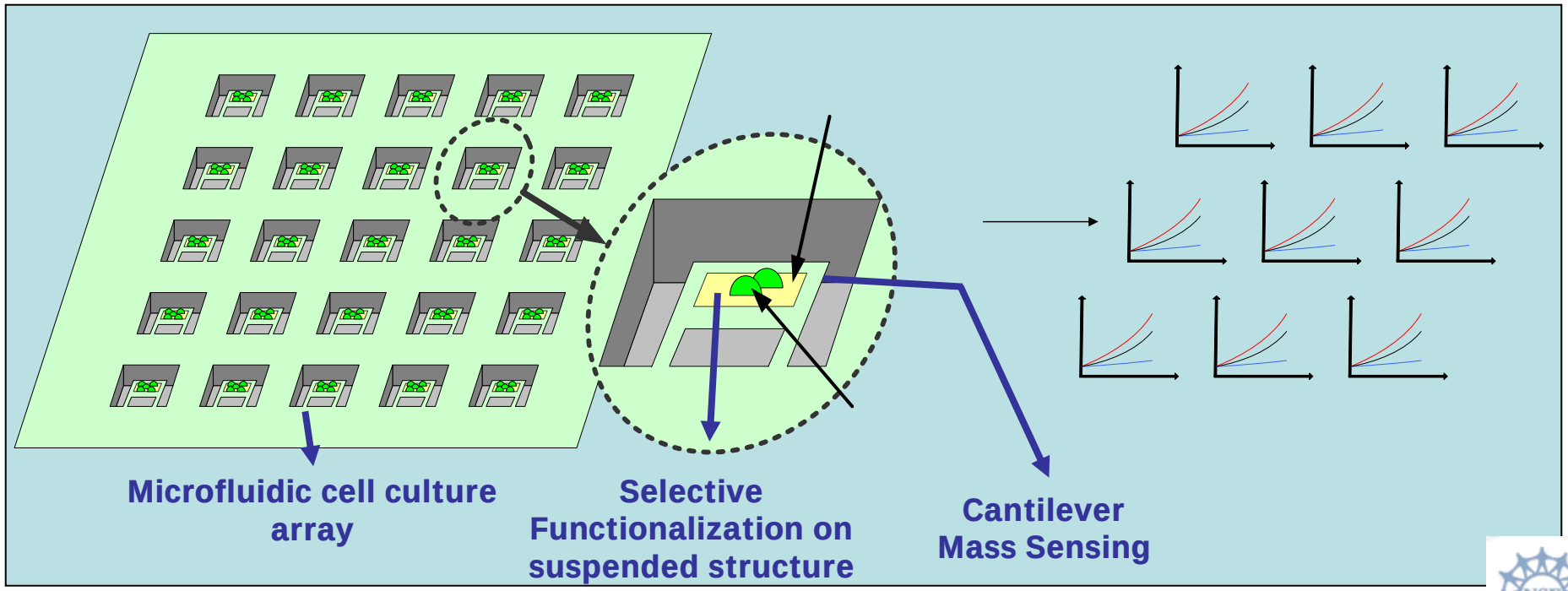
μ-fluidic cell culture array



L. P. Lee (UCB)

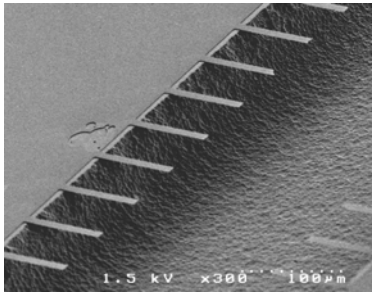


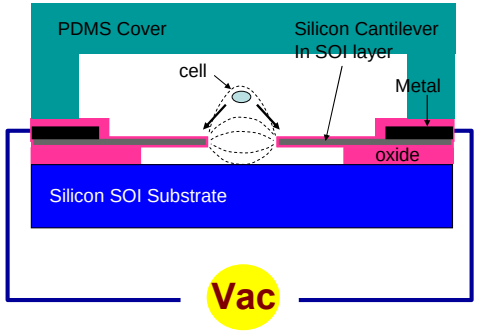


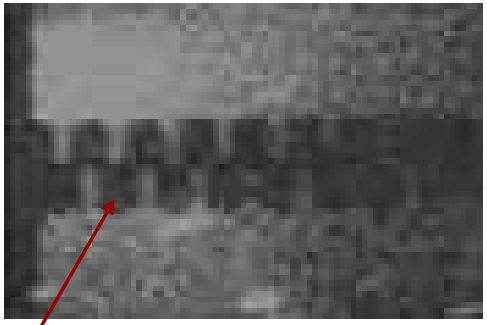


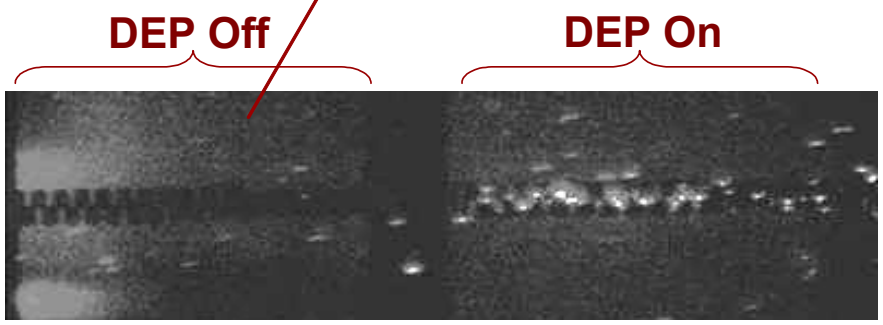
Cell Attachment on Cantilevers

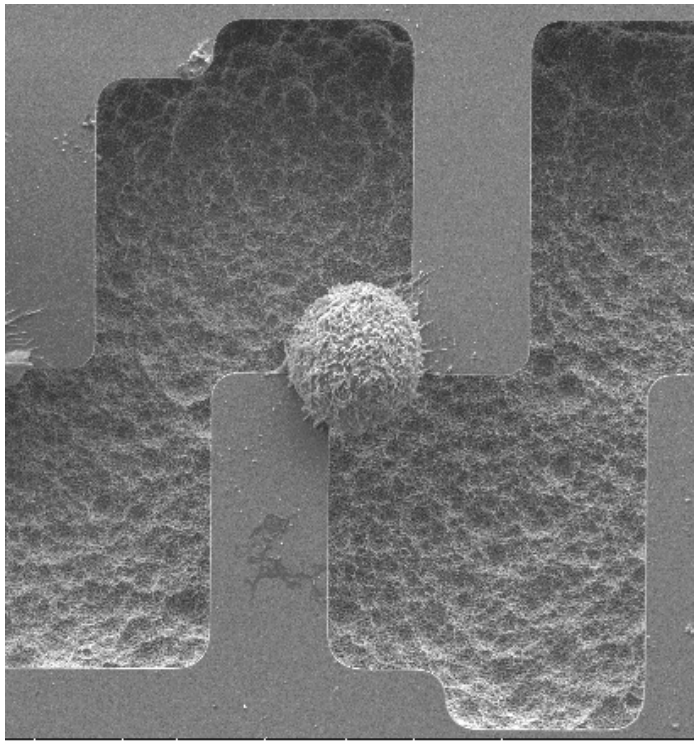
- DEP Assisted Capture





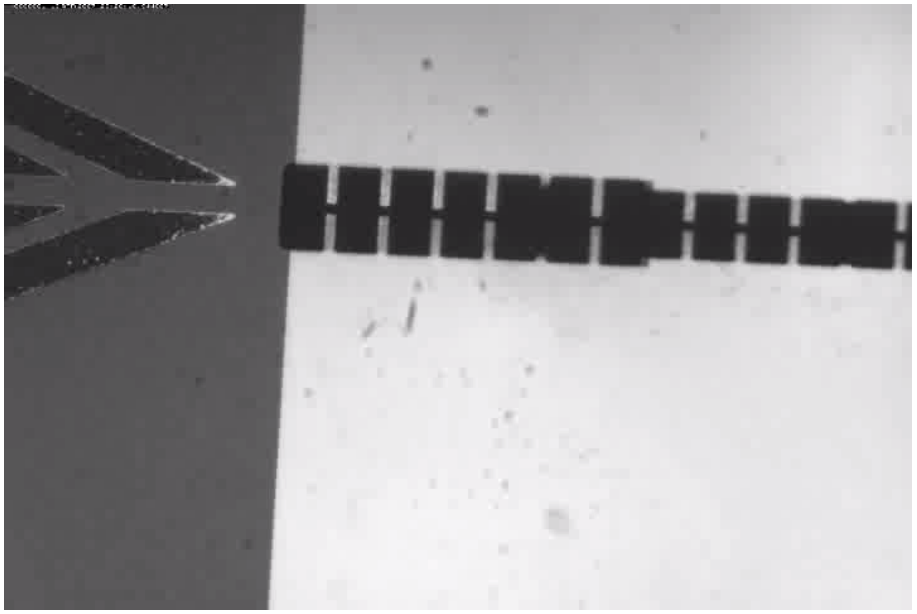


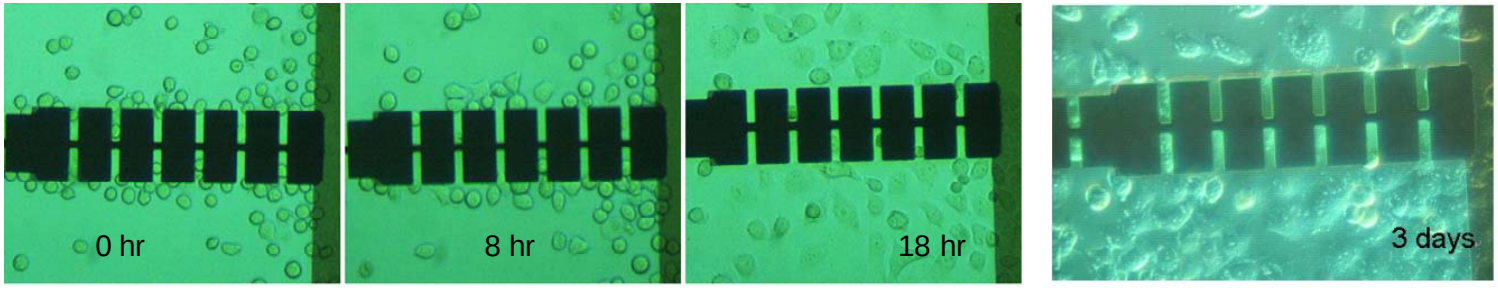




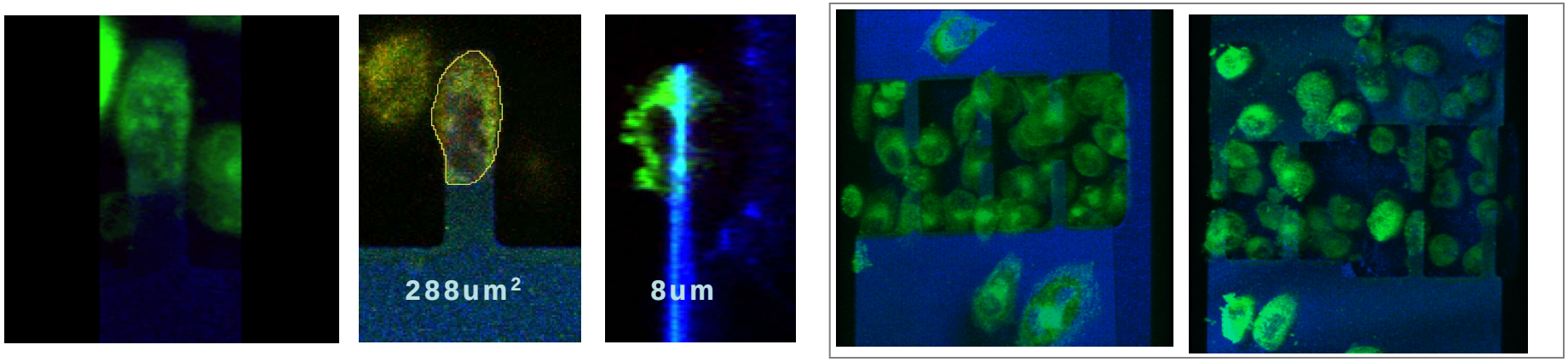
Hela cells, captured with DEP. Conductive silicon electrode generate DEP forces to capture Hela cells.

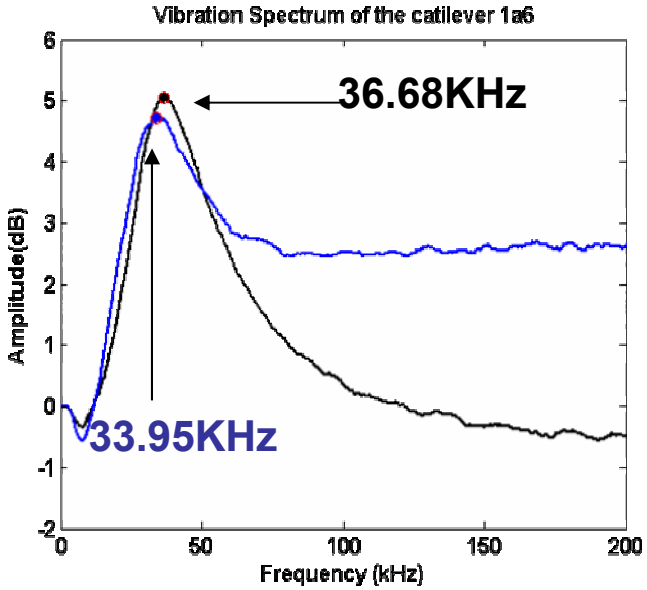
Living Cantilever Arrays





Living Cantilever Arrays





Calculated Mass: 2.06 ng

Volume: 2349 um³
Density[3]: 1.055g/cc
Estimated mass : 2.48 ng

Point of Test Biosensors

Abbott/iSTAT
(Glucose)

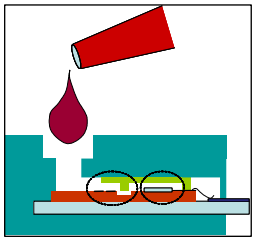
Accuteck
(LDH, Theophylline)

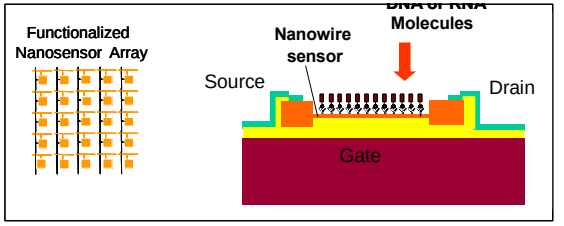
Abbott/iSTAT
(gases, ions, markers)

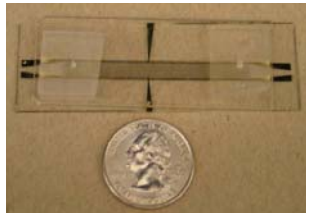
Pregnancy Tests

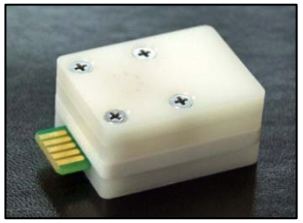
Silicon Based BioChips !

- Disposable, one-time-use devices
- Rapid, sensitive, integrated
- Detection and monitoring of disease and state of health





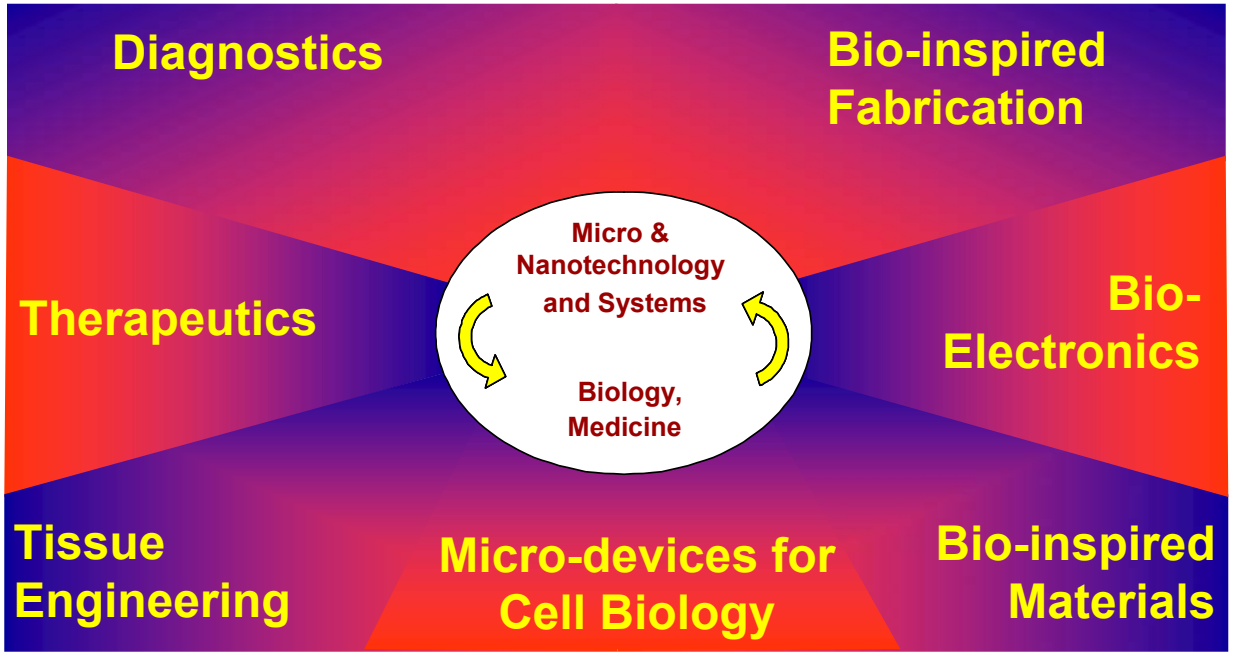




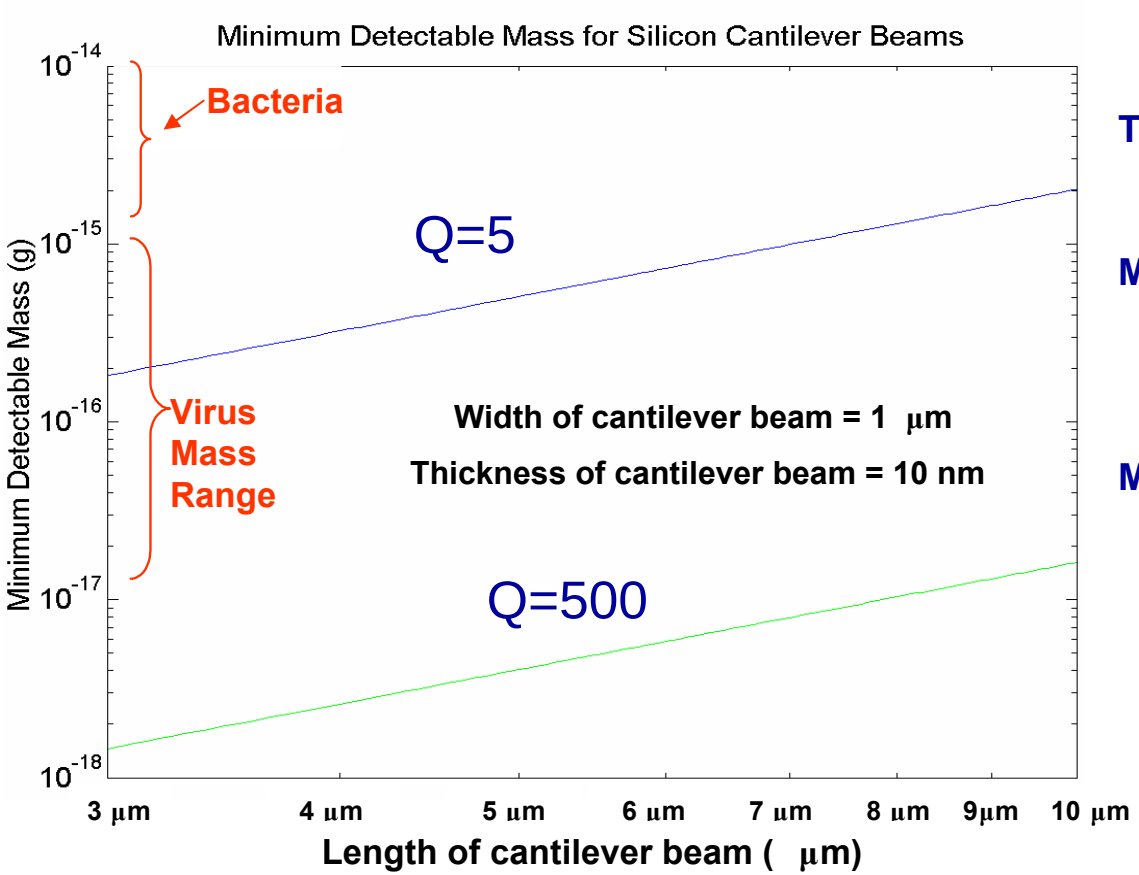
- **Focus on translation: Top-down integrated nano-structures and nano-sensors platforms – POC sensors for biomolecules**
- **Chip makers and diagnostic industry**
- **System level fabrication and modeling/simulations (from ‘molecules to systems’) with considerations of integrated systems, fluid and biomolecule transport, packaging and interfaces, etc.**

BioMEMS and Bionanotechnology

Apply micro-systems and nanotechnology to develop novel devices and systems that have a biomedical impact or are bio-inspired



Minimum Detectable Mass



100kDa Protein ~ 10^{-19} g

DNA bp ~ 10^{-21} g

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

Minimum Detectable Frequency, $\Delta f_{,min}$ =

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

Minimum Detectable Mass, $\Delta m_{,min}$ =

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

k_B = Boltzmann constant

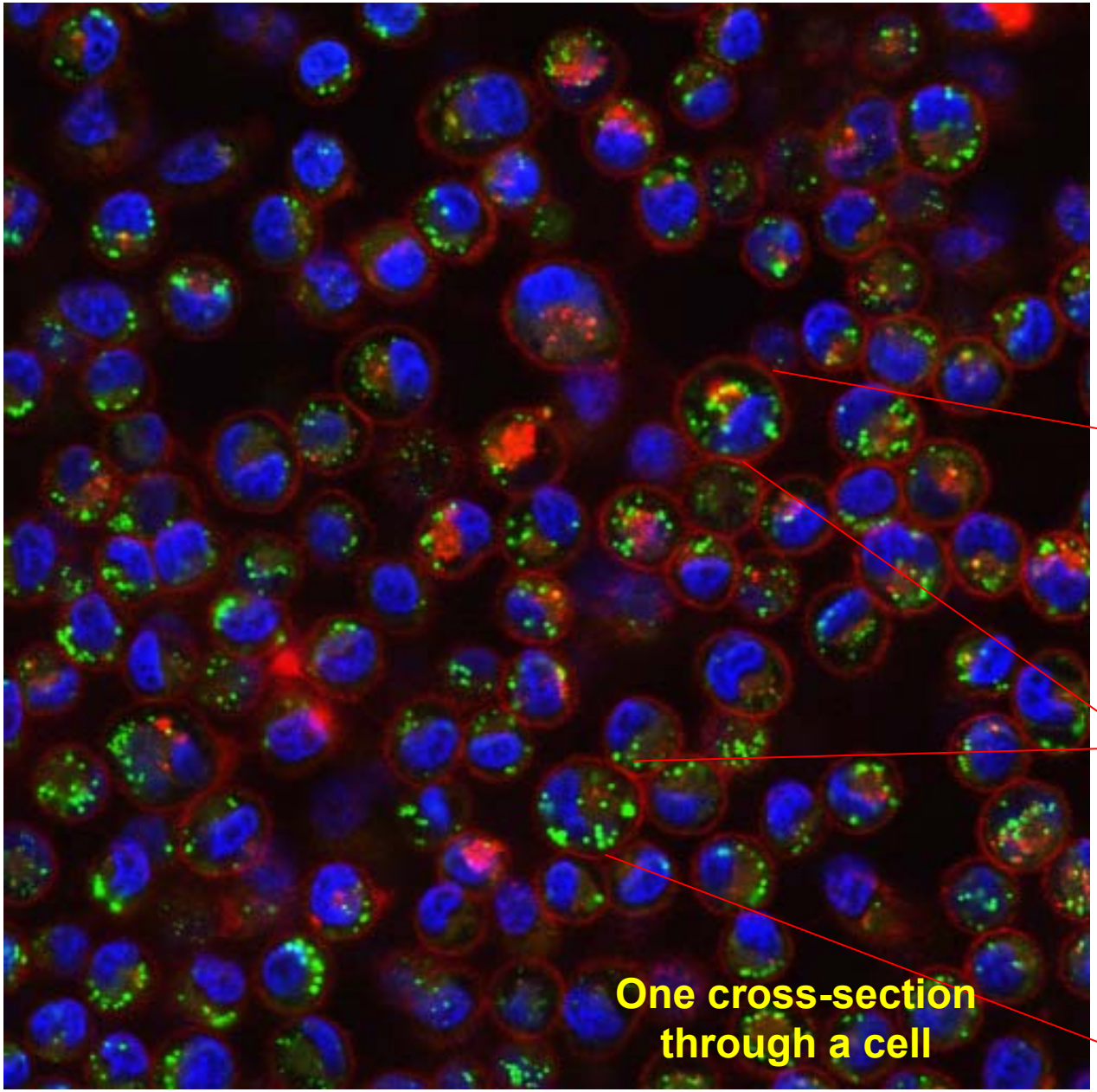
T = Temperature in Kelvin

B = Bandwidth measurement, (= $2\pi f_0/2Q$)

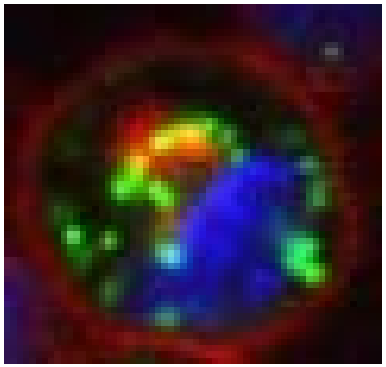
Q can increase by 100X by driving the cantilevers

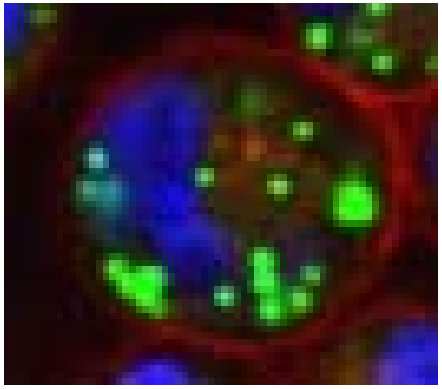
Roukes, et. al.

40nm NPs in KB Cancer Cells

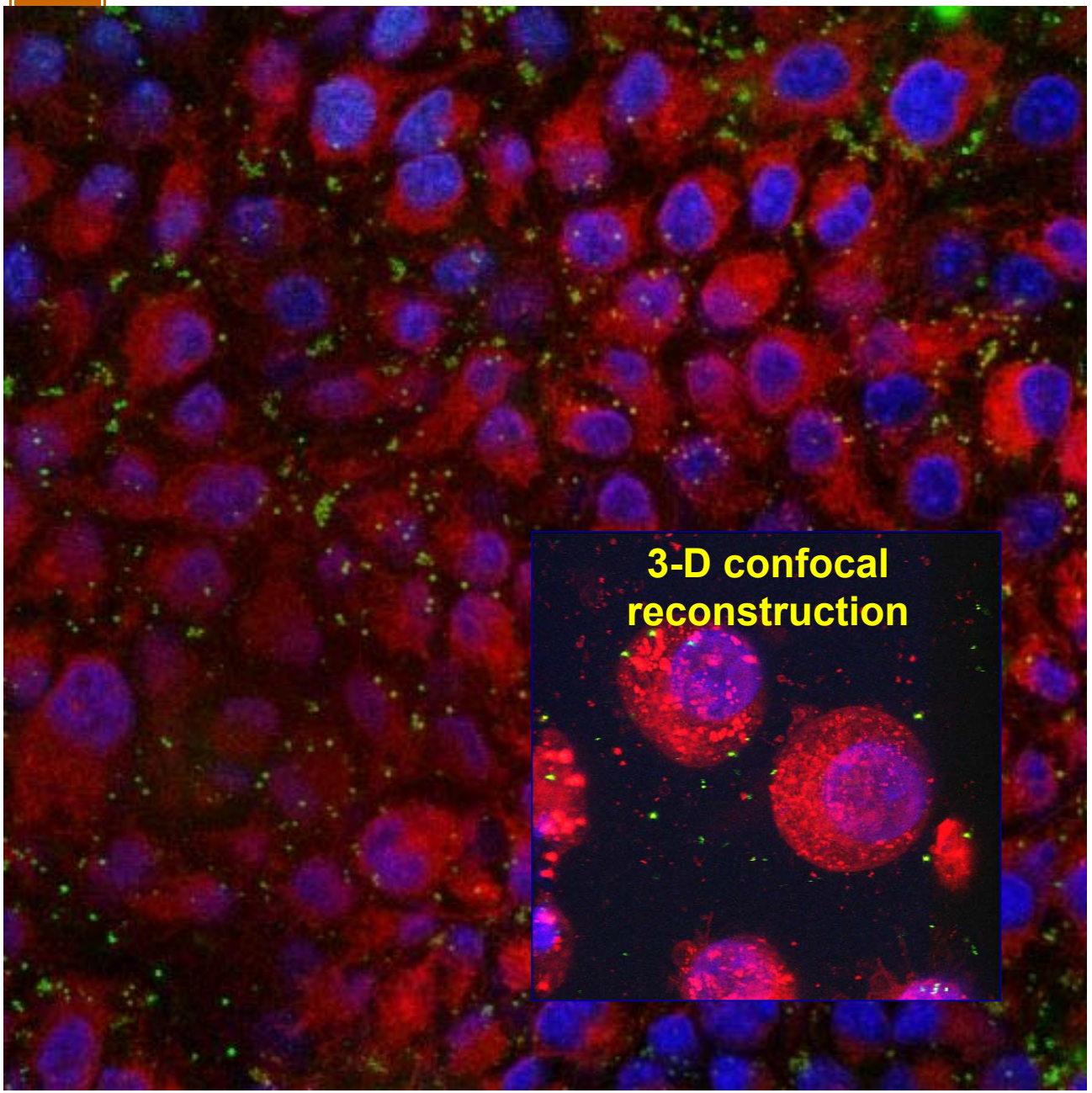


- Bead incubation for 1 hour
- Particles are internalized by cells
- External beads rinsed and quenched by trypan blue dye

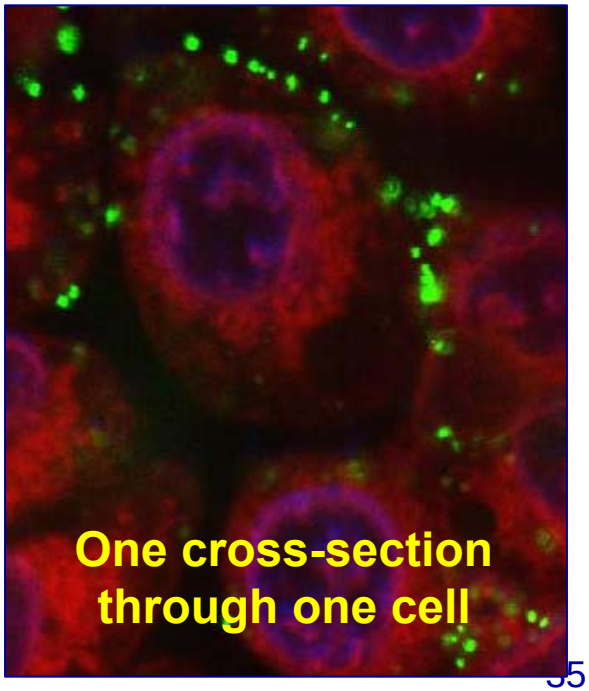




200nm Beads in KB Cells

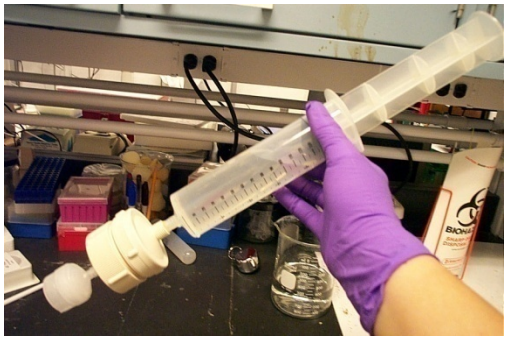


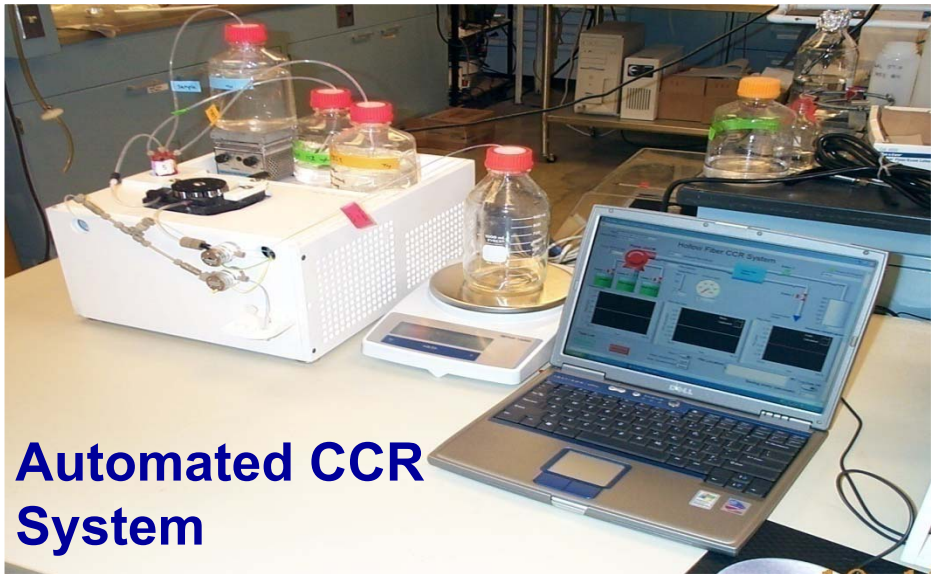
- Bead incubation for 1 hour
- Confocal images – NPs are NOT internalized
- Cross-sectional scans



Cell Concentration and Recovery

- **Proprietary technology for cell concentration and recovery in small volumes – CCR™ kit**
- **One-time use filter cartridge**
- **Recovery rates ~ 50%**





Chen, et al. Biotech Bioengr, 2005
Huang, et al. Submitted, 2007

Ladsich, et al. USDA Review, 2004

Recovery Results

E. coli

100 ml sample in PBST

Run#	Initial cell conc. (cells/ml)	Final pressure drop (psi)	Recovery collection 1 (viable cells)	Total cells in collection 1	Recovery collection 2 (viable cells)	Recovery collection 3 (viable cells)	Leakage through permeate side	Total recovery (3 collections)
1	52±12	46	72.4±11.6%	3767±603	4.9±0.9%	0.4±0.1%	0.0±0.0%	77.7%
2	52±12	40	62.2±15.5%	3233±808	2.9±1.6%	0.5±0.3%	0.0±0.0%	65.5%
3	52±12	38	62.8±14.7%	3267±1531	4.0±1.1%	0.2±0.1%	0.0±0.0%	67.0%

Listeria innocua

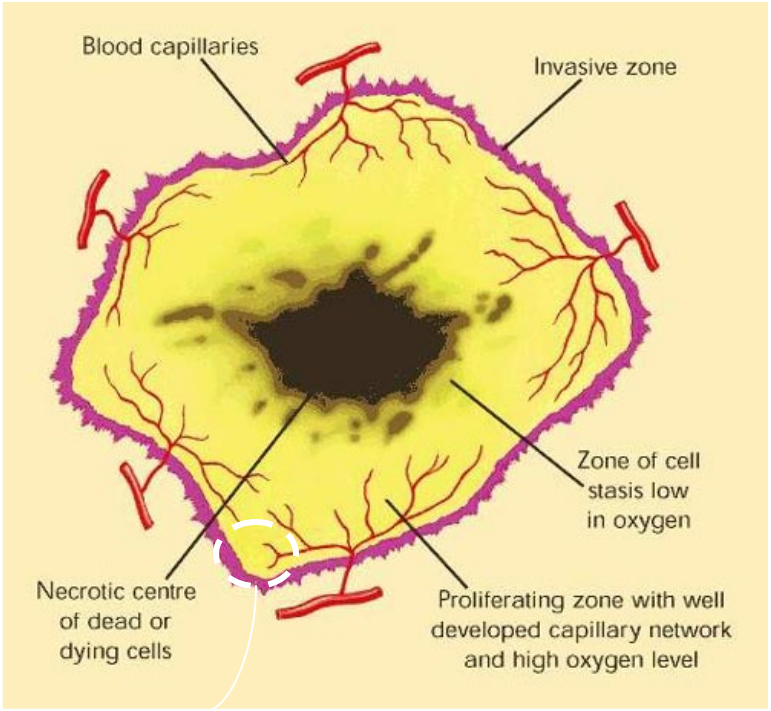
Run#	Initial cell conc. (cells/ml)	Final pressure drop (psi)	Recovery collection 1 (viable cells)	Total cells in collection 1	Recovery collection 2 (viable cells)	Recovery collection 3 (viable cells)	Leakage through permeate side	Total recovery (3 collections)
1	66±22	31	56.6±3.8%	3510±236	12.7±2.4%	0.9±0.4%	0.0±0.0%	70.2%
2	66±22	30	60.0±3.9%	3717±244	13.7±2.4%	2.3±0.7%	0.0±0.0%	75.9%
3	66±22	28	37.2±3.9%	2303±243	25.1±2.6%	6.0±0.6%	0.0±0.0%	68.2%

Streptococcus faecalis

Run#	Initial cell conc. (cells/ml)	Final pressure drop (psi)	Recovery collection 1 (viable cells)	Total cells in collection 1	Recovery collection 2 (viable cells)	Recovery collection 3 (viable cells)	Leakage through permeate side	Total recovery (3 collections)
1	109±24	40	53.7±1.9%	5867±208	5.6±2.4%	1.7±0.3%	0.0±0.0%	61.0%
2	109±24	40	37.3±8.3%	4067±907	17.3±0.9%	2.4±0.3%	0.0±0.0%	56.9%
3	109±24	40	56.2±16.6%	6133±1815	6.4±0.3%	2.9±0.5%	0.0±0.0%	65.5%

Huang, Chen, Ladisch, et al. 2007 (submitted)

Targeted Nano-therapeutics

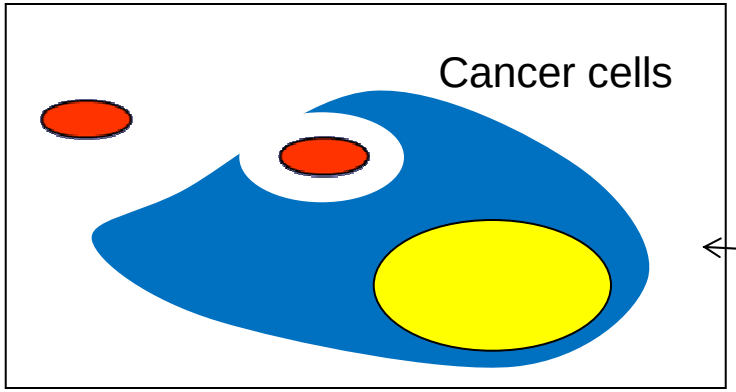


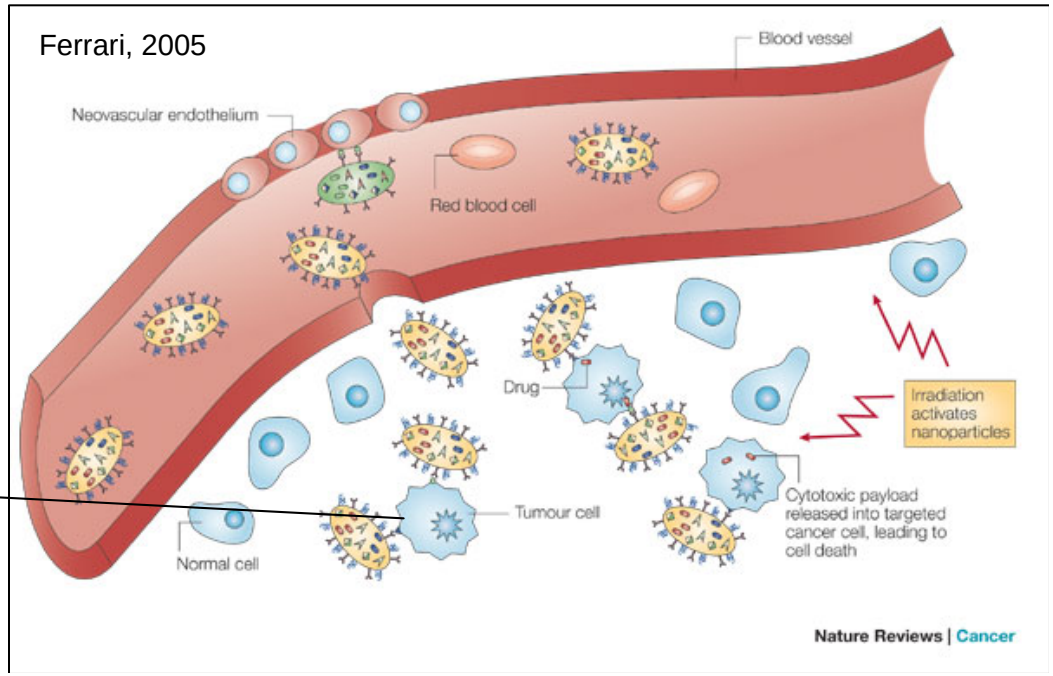
Objective:

To develop novel means to deliver nano-therapeutics to cancer cells and tumors

- Current drugs are ineffective at penetrating to the tumors due to inadequate vascularization at these tumor sites.
- Nanoparticles have the similar challenge of reaching tumor sites if adequate vascularization does not exist.
- Penetrate multiple barriers

- Need active transport to the cells and tumors





- Current drugs are ineffective at penetrating through the solid tumors due to inadequate vascularization at these tumor sites.
- Nanoparticles have the similar challenge of reaching tumor sites if adequate vascularization does not exist since they are carried by blood flow.

Bacterial Mediated Delivery of Nano-particles in Cells

D. Akin, J.P. Robinson, S. Muhammad, A. Bhunia, R. Bashir

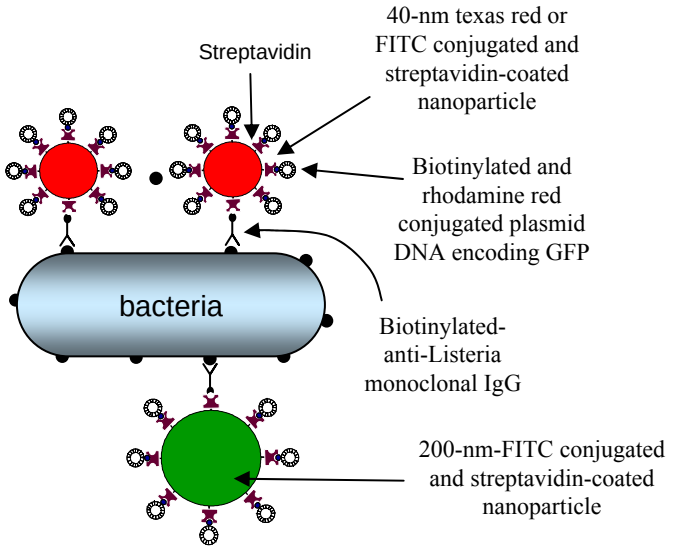
Objective:

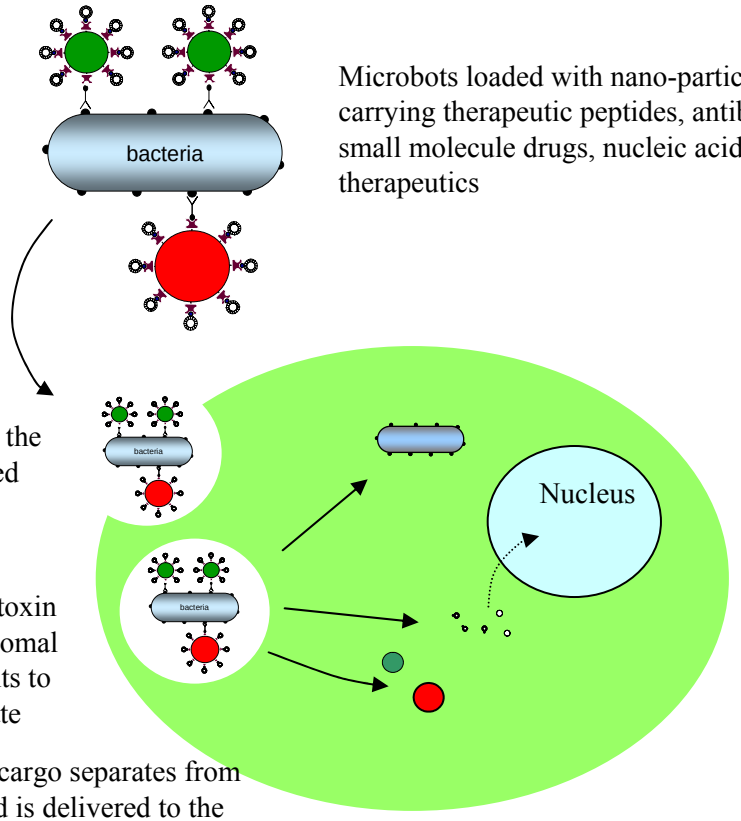
To develop biologically-inspired intelligent devices for intracellular monitoring and therapeutic intervention of signal transduction networks

Bacteria as a carrier !

- **Intracellular bacteria may provide means to penetrate and deliver therapeutic compounds to solid tumors that are otherwise inaccessible to drugs delivered by oral and parenteral routes.**
- **Bacteria can be engineered to have affinity for certain tumors.**
- **Bacteria can be engineered to express proteins on their surface for therapeutic or diagnostic intervention**

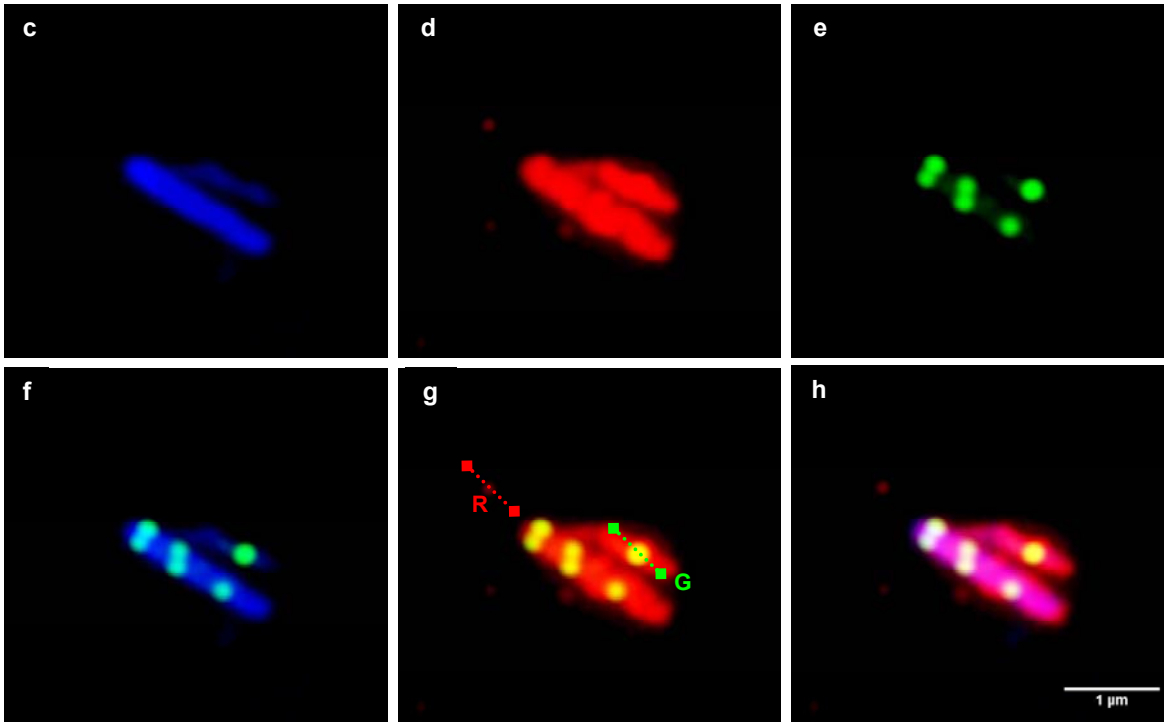
Functionalization Strategy

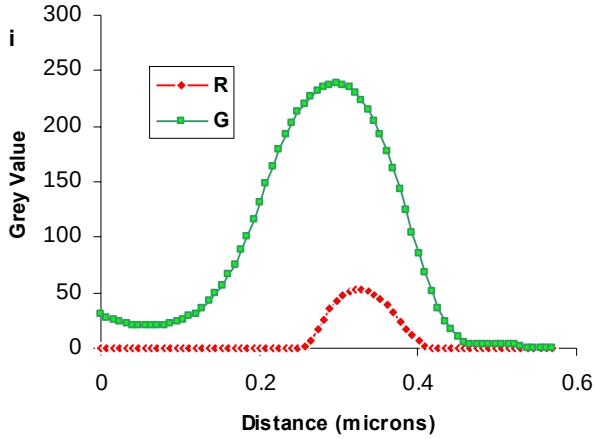


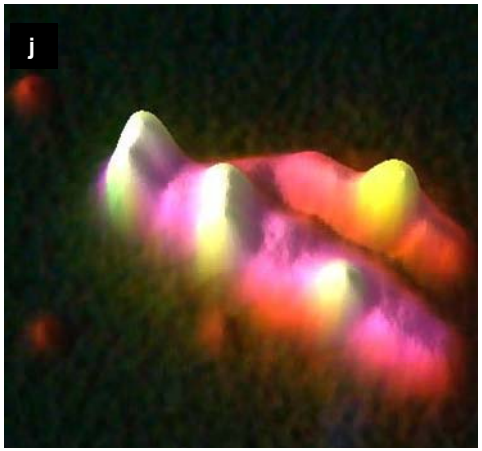


D. Akin, J. Sturgis, K. Reghab, K. Burkholder, S. Muhammad, A. Bhunia, J. Robinson, R. Bashir, Nature Nanotechnology, 2007

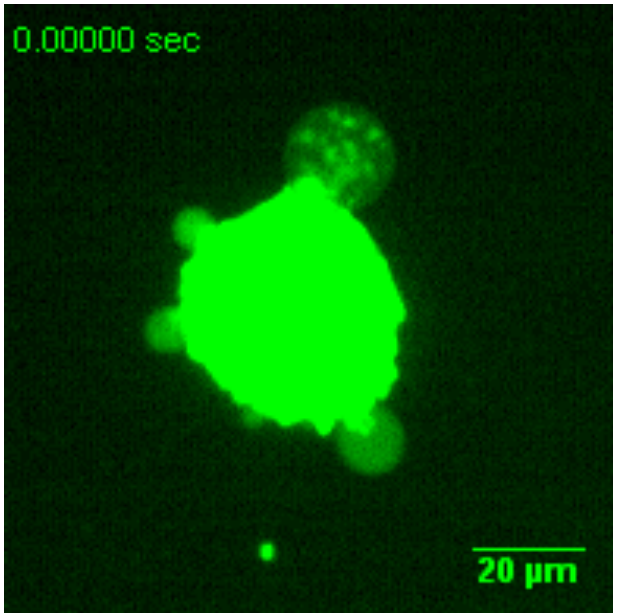
Nanoparticles on Bacteria

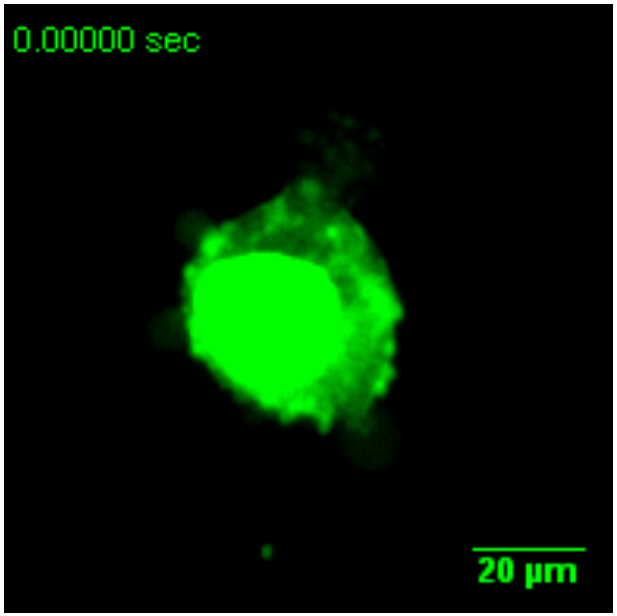




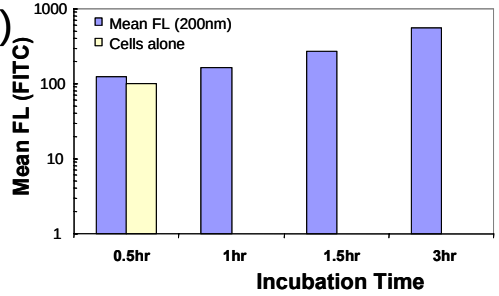


Bacterial Mediated Delivery of NP

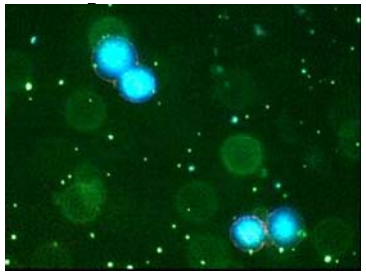




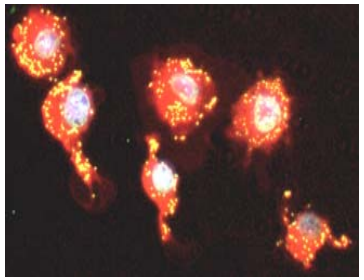
(a)



(b)



(c)



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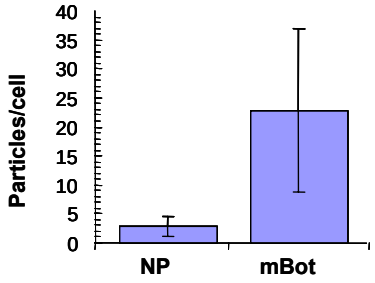
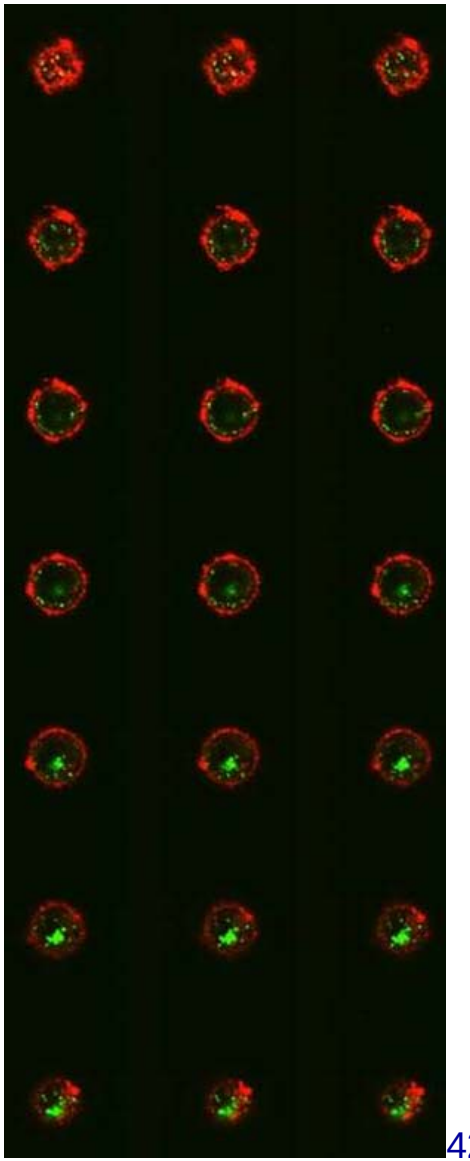
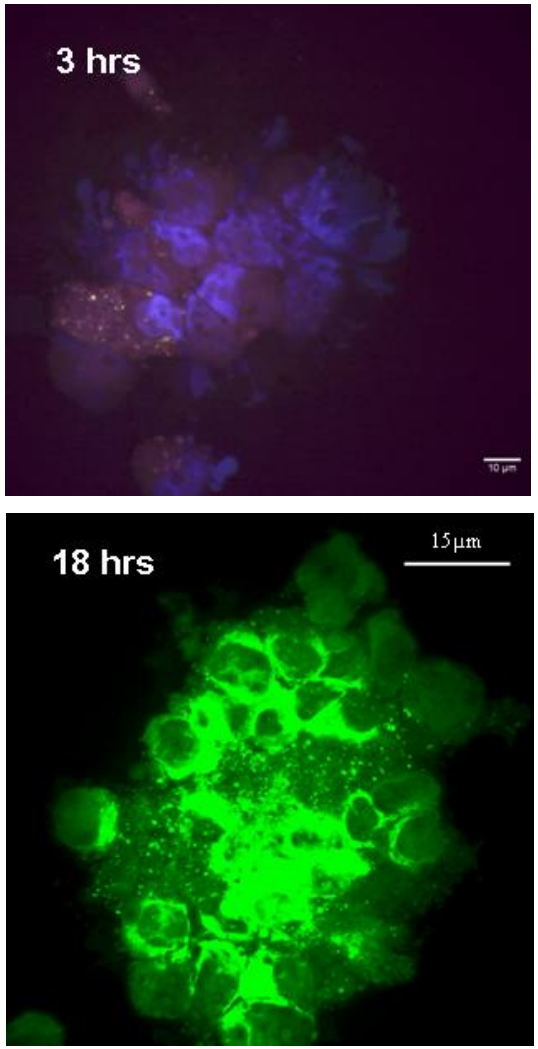


(d)

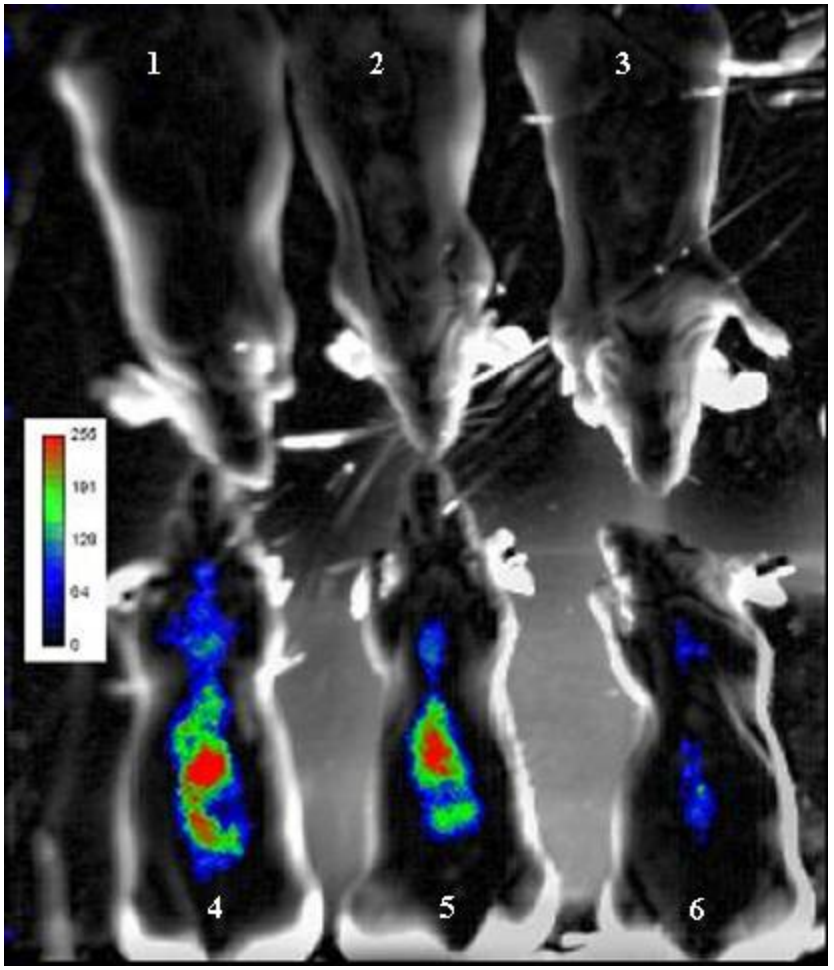


Expression of GFP in KB Cells

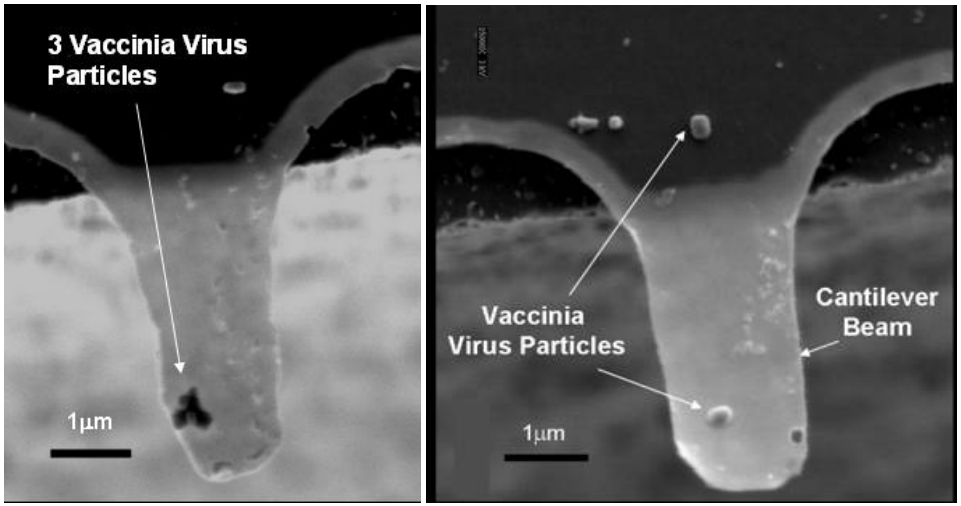
Expression in-vitro in cells

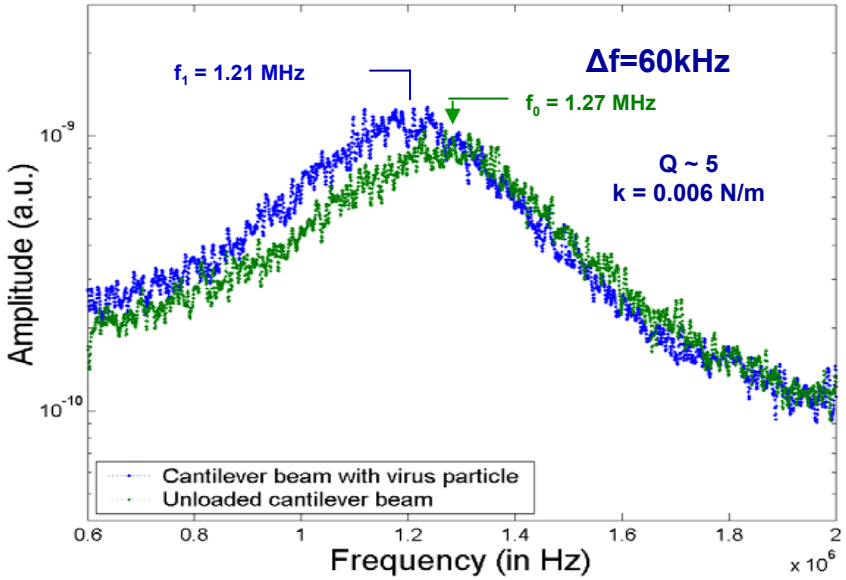


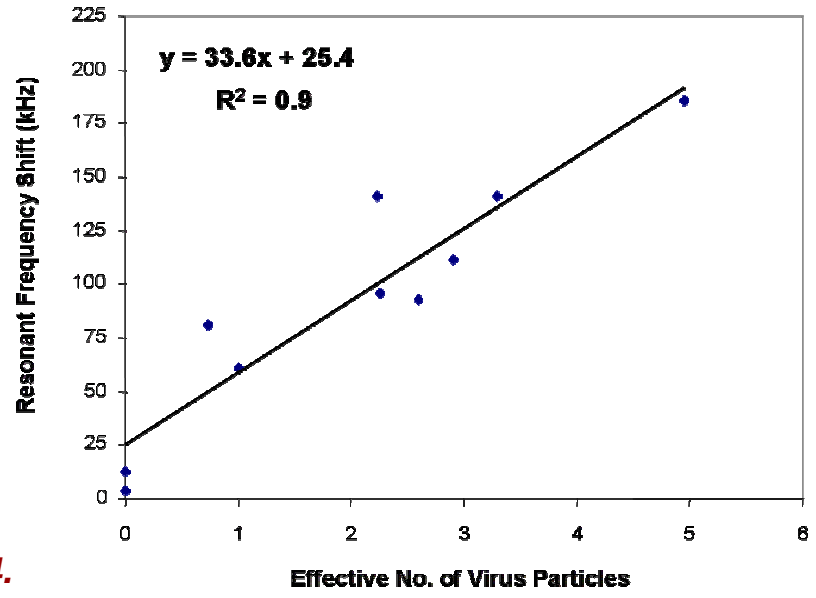
Expression in-vitro in mice



D. Akin, J. Sturgis, K. Reghab, K. Burkholder, S. Muhammad, A. Bhunia, J. Robinson, R. Bashir, Nature Nanotechnology, 2007







Work featured in Popular Science, EE Times, Nature News, and CNBC TV Show

A. Gupta, D. Akin, R. Bashir, Applied Physics Letters, 2004.
A. Gupta, et al. Proc. Nat. Acad. Sci. 2006