The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

2268-4

Conference on Nanotechnology for Biological and Biomedical Applications (Nano-Bio-Med)

10 - 14 October 2011

Biomimicking Cell Microenvironment for Cancer Detection

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Biomimicking Cell Microenvironment for Cancer Detection

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ICTP-KFAS Nano-Bio-Med 2011
13 October 2011

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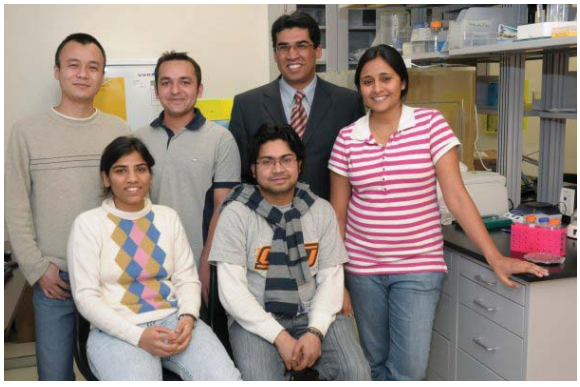
- Andrew Ellington (UT-Austin)
- Ed Kolesar (Late)

Wan **Asghar**



Goyal **Noor** **Ramachandaran**

Funding

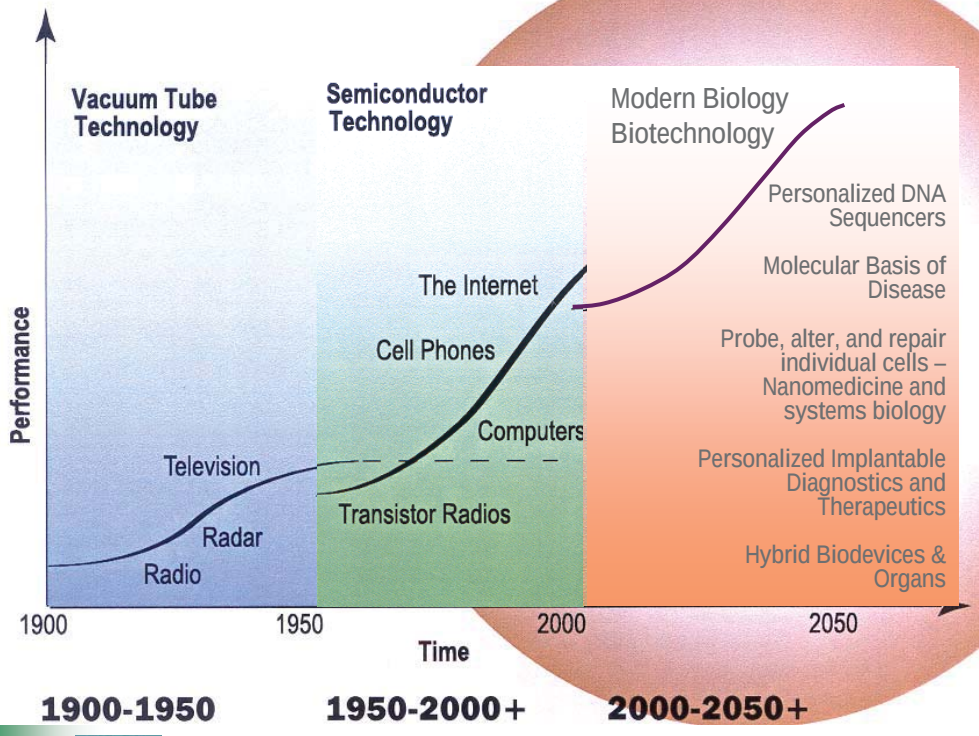
NSF

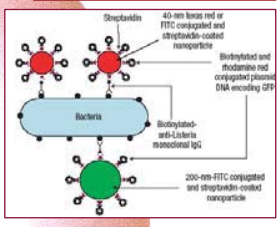
NATIONAL INSTITUTES OF HEALTH

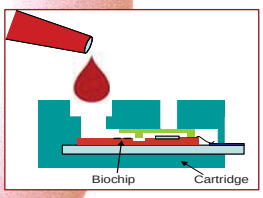
MRC EDM

Metroplex Research Consortium For Electronic Devices And Materials

Evolution of Technologies







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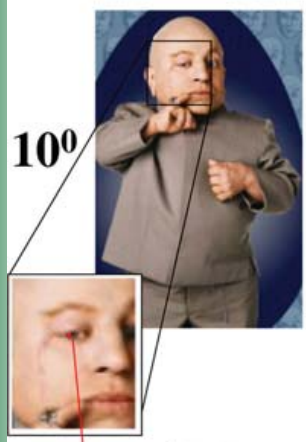
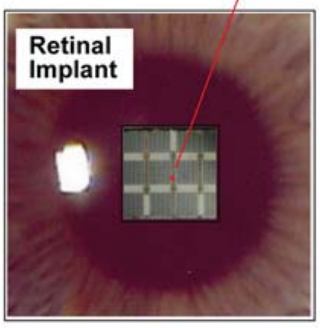
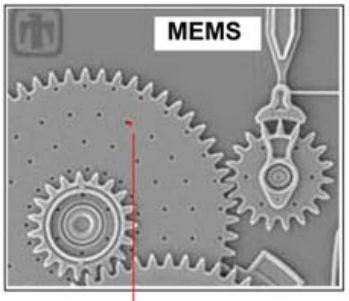
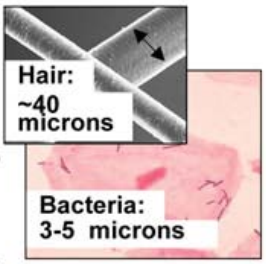
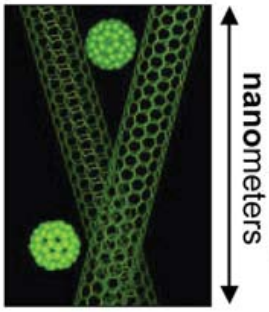
Size and Scale: Factors of 1000

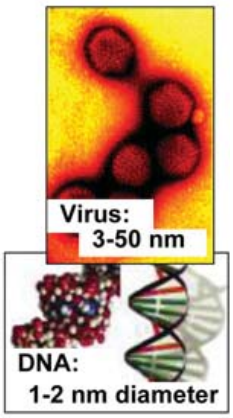












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http://www.mrsec.wisc.edu/Edetc/nanoscale/index.html

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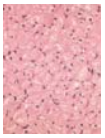
Biological Information Superhighway !

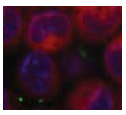
Body

Organ

Tissue



Cell-Cell/ECM



Cells

Protein Interactions, Metabolites

Proteins

mRNA

DNA

- Understand smaller parts to reverse engineer
- Cells ultimately determine behavior
- Biomolecules regulate cells through pathways
- Hardware and software

Polymers and hydrogels, Ink-jet Printing, 3-D Stereo-lithography,

Micro-fluidics, Soft-Lithography,

Nano-pores, Cantilevers, NWs, NTs, Quantum Dots, Nano-particles,

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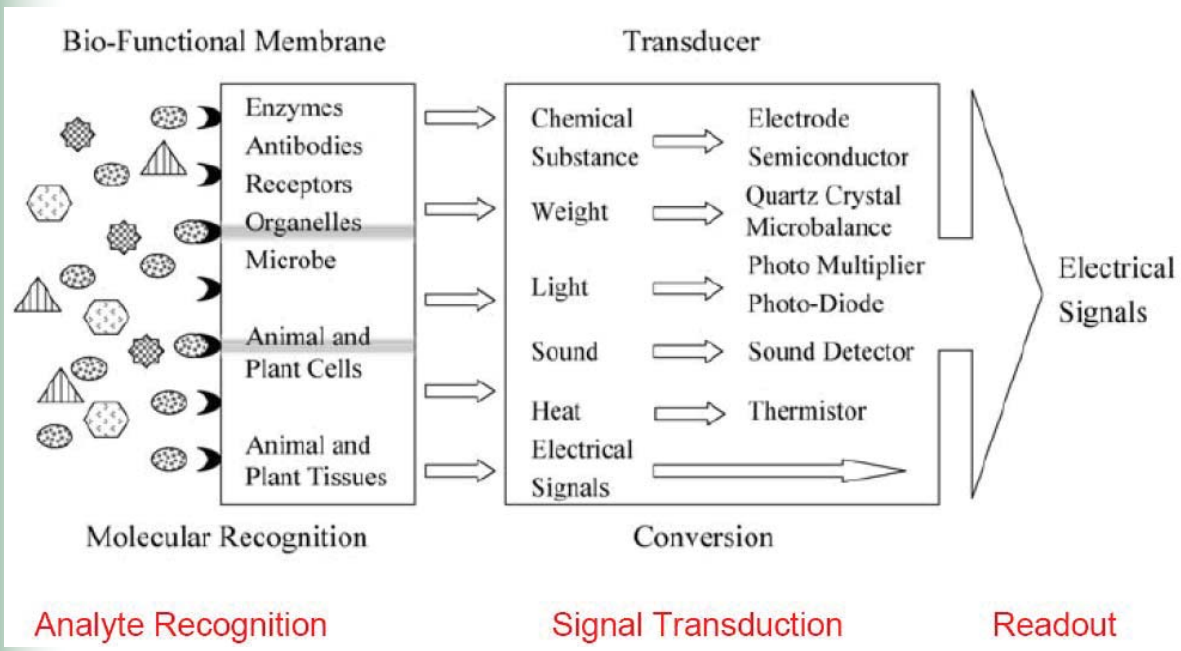
Principles of Sensing



[Nakamura et al., *Anal Bioanal Chem*, 2003]

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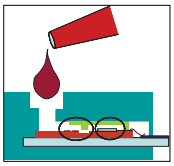
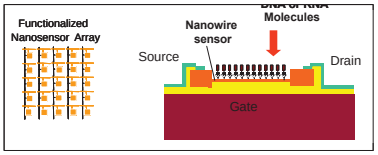
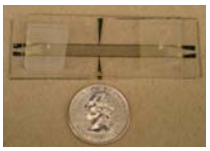
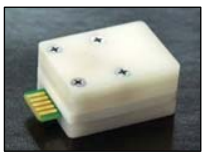
POC Biosensors!

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









- **Focus on translation: Top-down integrated nano-structures/sensors**
- **POC sensors for biomolecules**
- **System level**
  - **From 'Molecules to Systems'**
  - **Fluidics, Transport, Packaging, Interfaces, etc.**

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POC Biosensors!

Disposable Cartridge

- A single credit card sized cartridge can yield quantitative results for multiple markers simultaneously.
- Requires only a finger-stick of whole blood.
- Capability for quantitative analysis of blood, serum, urine, and other bodily fluids.

Analyzer

- Can be packaged as a bench-top instrument or battery-operated hand-held unit.
- No sample preparation or user intervention.
- Intelligent QC (monitor each assay step to guarantee result accuracy).
- Results are available in less than 15 minutes.
- Wireless, wired, or printer output capable.

Claros Diagnostics: www.clarosdx.com

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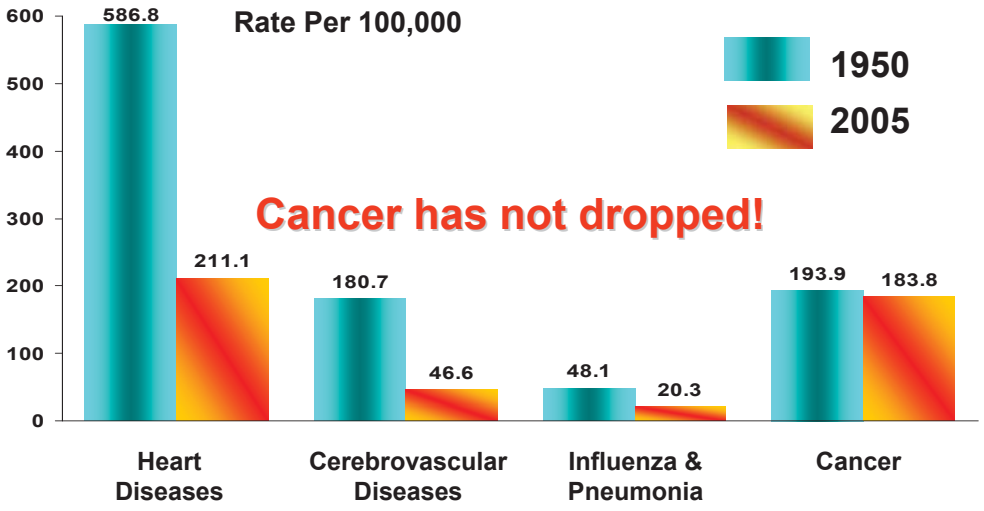
Challenges!

Change in Death Rates by Cause (USA)



Cancer has not dropped!

Sources: 1950 Mortality Data - CDC/NCHS, NVSS, Mortality Revised.
2005 Mortality Data: US Mortality Data 2005, NCHS, Centers for Disease Control and Prevention, 2008.

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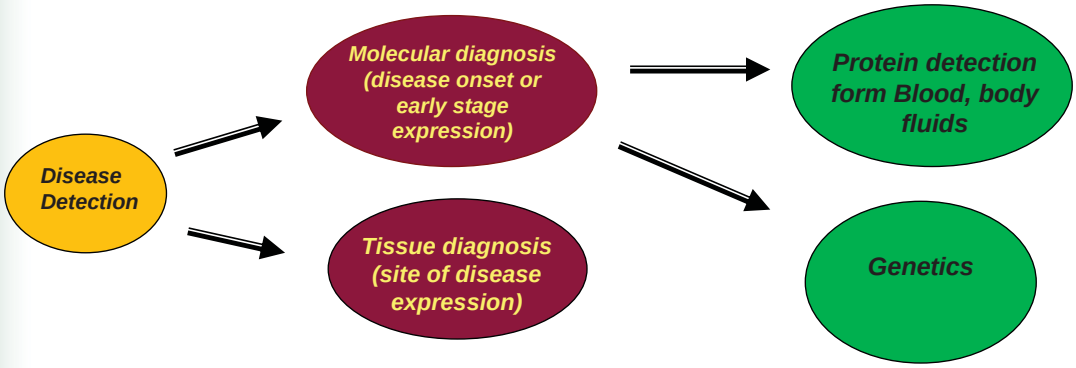
Disease Diagnostics – Genes and Proteins



Clinically Relevant Biomarkers		
Biomarker	**Type and Source**	**Disease**
Carcinoembryonic Antigen	Glycoprotein in Serum	Colorectal, Pancreatic, Breast cancer
Epidermal Growth Factor	Protein	Glioblastoma, Lung cancer
Prostate Specific Antigen	Protein in serum	Prostate cancer
Nuclear Matrix Protein	Protein in urine	Bladder cancer
Beta Amyloidal peptide	Body Fluid	Alzheimer's disease

Disease Diagnostics – Genes and Proteins

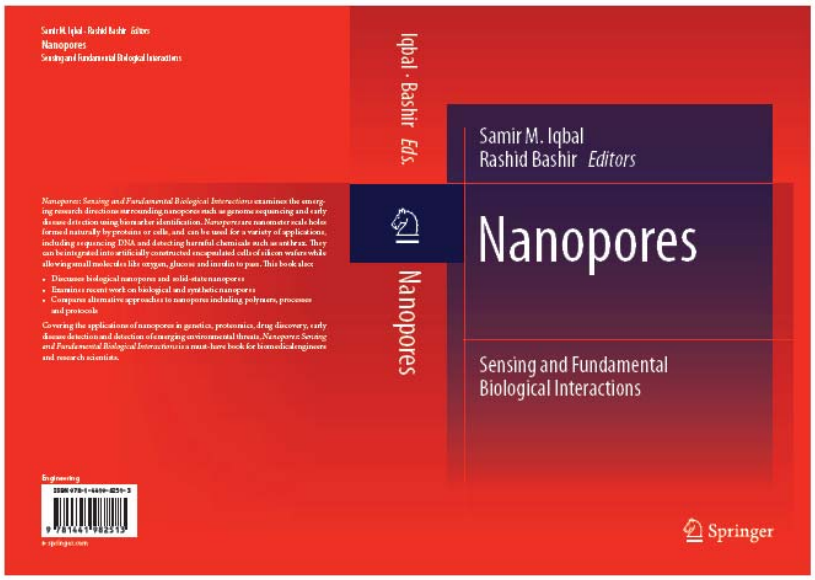
Disease	Biomarker
Breast Cancer	CEA, HER-2, EGFR
Cervical Cancer	Human Pappiloma Virus, EGFR
Lung Cancer	EGFR, KRAS, BRAF
Bladder Cancer	EGFR, fatty acid binding protein, HSP 27, Annexin
Ovarian Cancer	EGFR, haptaglobin α, CA-125
Esophageal Cancer	EGFR, periplakin

[Klijn JG, et al., Endocr. Rev., 1992], [Yim EK, et al., Expert. Rev. Proteomic., 2006], [Hirsch FR, et al., J. Clin. Oncol., 2006], [Sheng KH, et al., Proteomics, 2006], [Ahmed N, et al., Proteomics, 2005], [Nishimori T, et al., Proteomics, 2006]

Tumor Type	% of Tumors Overexpressing EGFR
Head & Neck	80-100
Kidney	50-90
Lung	40-80
Glioma	40-50
Ovarian	35-70
Bladder	31-48
Pancreatic	30-50
Colon	25-77
Breast	14-91

[Hong W and Ullrich A, Oncol. Biotherapeut., 2000]

Nanopores

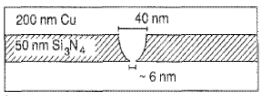


Biological and Solid-state Nanopores

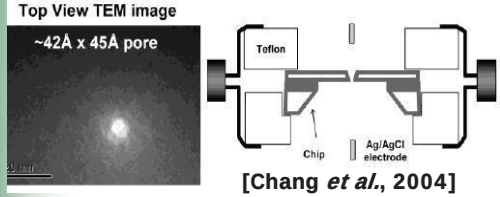
- 2.6 nm α-hemolysin channel
- Patterns to discriminate targets
- DNA–nanopore

- Nanopores in membranes
- DNA Characterization

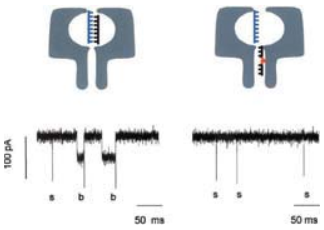
DNA through an α-HL channel [Kasianowicz *et al.*, 1996]



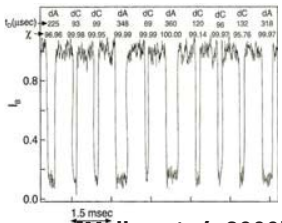
[Ralls *et al.*, 1989]



[Chang *et al.*, 2004]



[Howorka *et al.*, 2001]

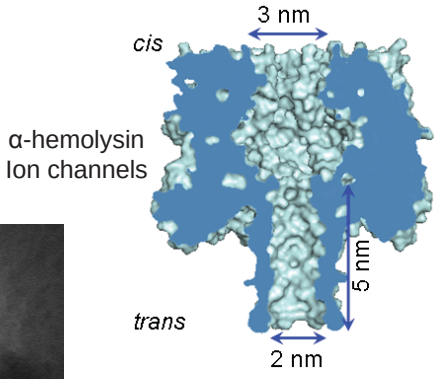


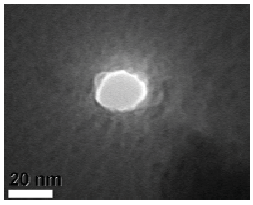
[Meller *et al.*, 2000]

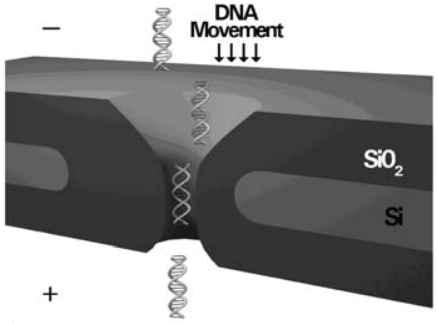
Solid-state Nanopores

- Why solid-state nanopores?
- DNA Characterization

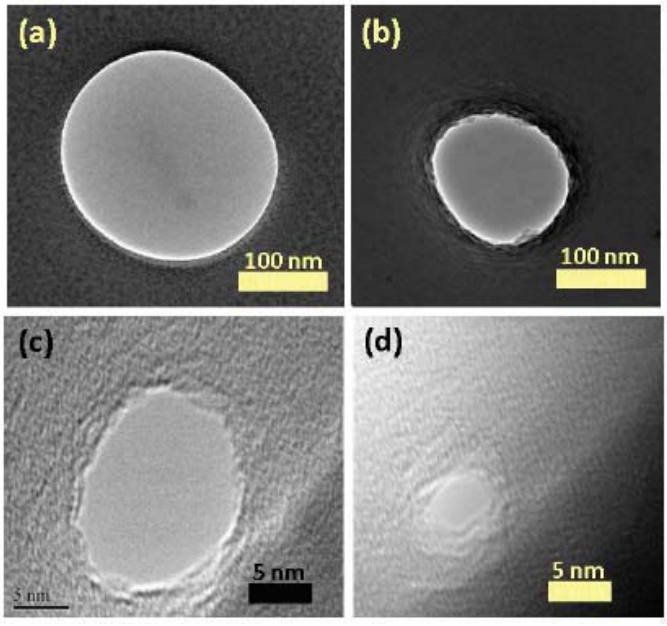
- DNA analyzer
  - Molecule events: Nanoscale
  - Interface: Macroscale
- Nanopores for interactions of:
  - DNA-DNA
  - DNA-protein
  - Biomarkers-Drug
  - Aptamers-Cells → Micropores







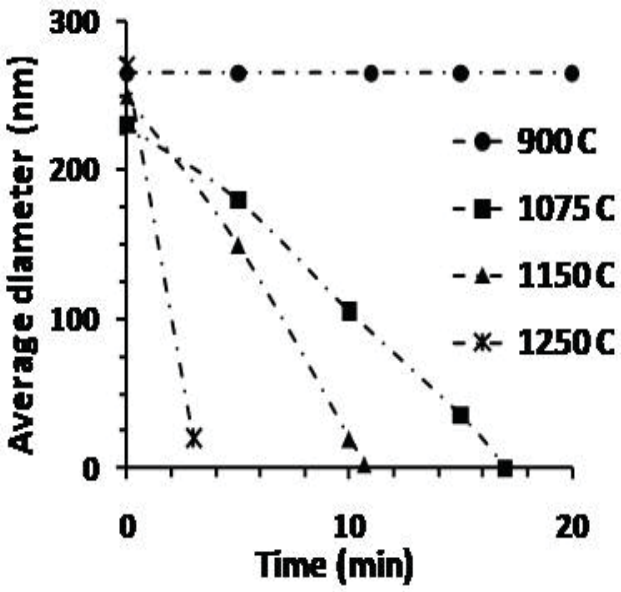
Nanopore Shrinking



[Asghar et al. *Nanoscale Research Letters*, 6:372, 2011]

Nanopore Shrinking

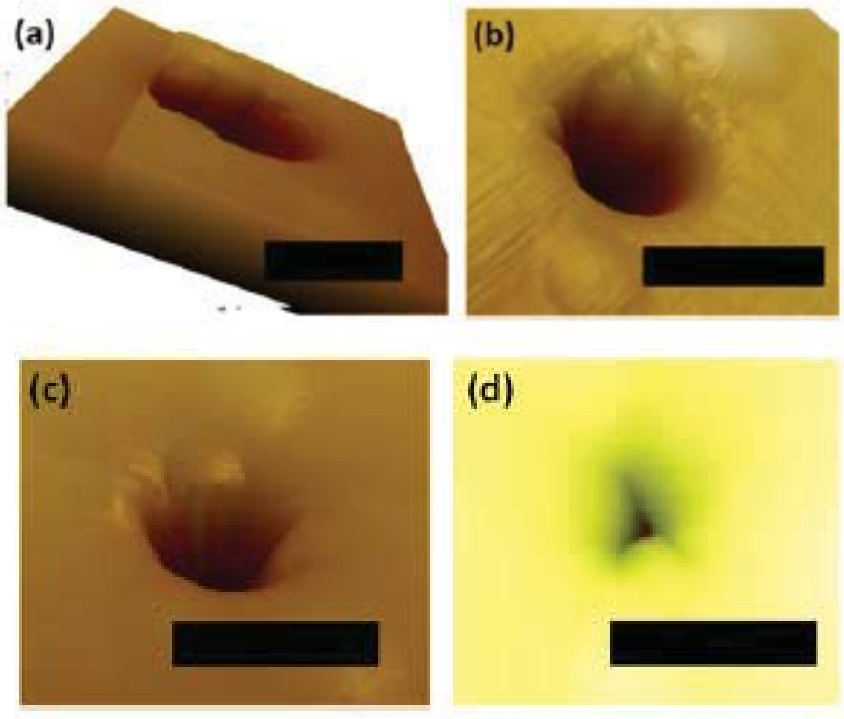
- At 1150 °C:
  - 22 nm/s
- At 1250 °C:
  - 80 nm/s
  - Thermal stresses can crack membrane
- <900 °C
  - No effect



[Asghar et al. *Nanoscale Research Letters*, 6:372, 2011]

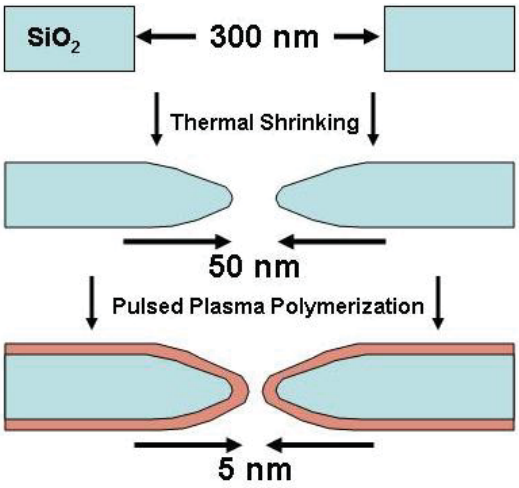
Nanopore Control of Surface

scale bars: 200 nm

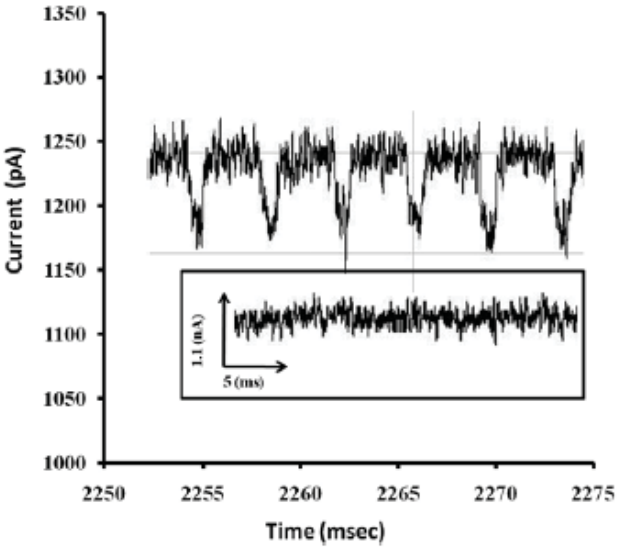


[Asghar et al. *Nanotechnology*, 22, 2011]

Nanopore Control of Surface



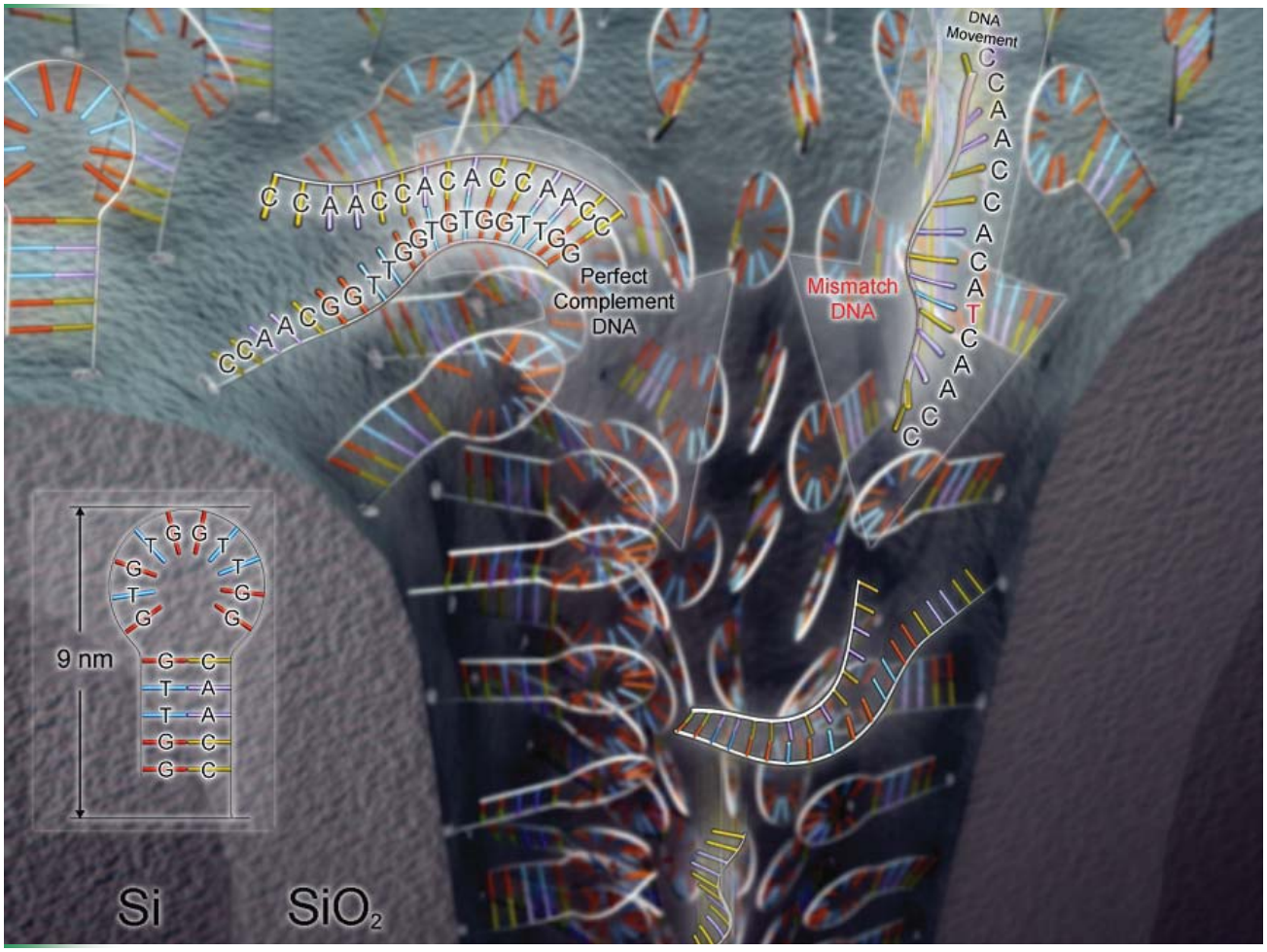
Nanopore Control of Surface



λ-DNA translocation

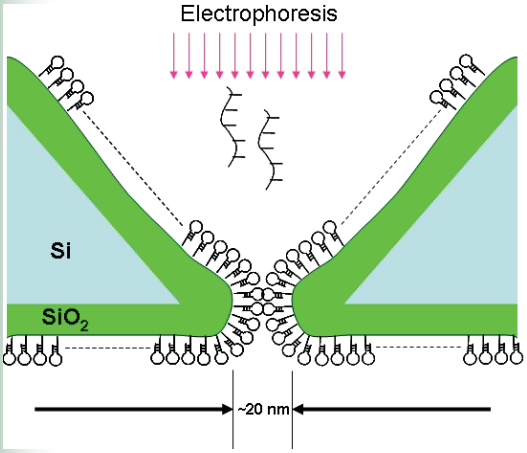
[Asghar et al. *Nanotechnology*, 22, 2011]

Selectivity in Nanopores

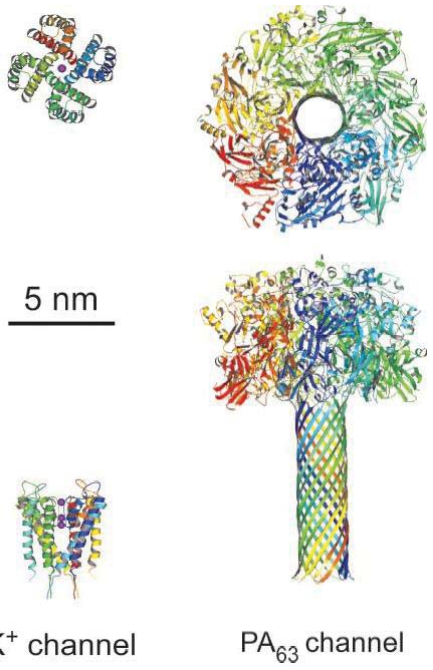


Selective Nanopore Channels

- Nanopores
- Selectivity towards specific Targets



- Protein Ion Channels

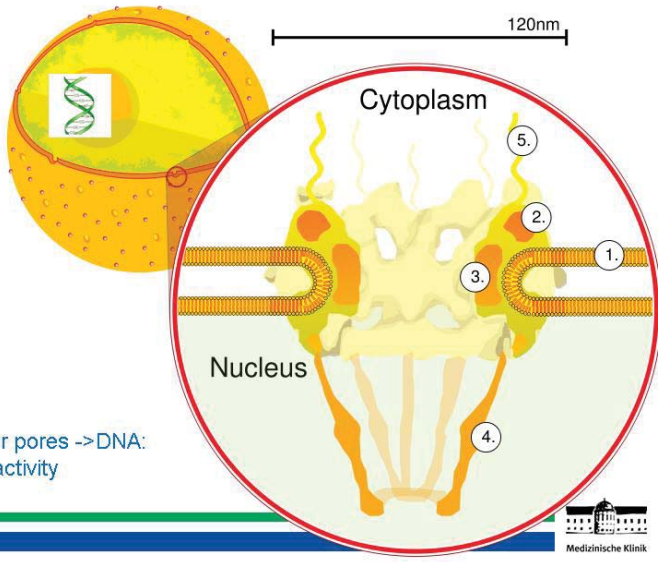


K^+ channel

PA_{63} channel

[Kasianowicz *et al.*, 2006]

Nuclear pores: Transport between nucleus and cytoplasm



Transport via nuclear pores ->DNA:
Regulation of gene activity

Medizinische Klinik

Dr. Wolfgang R. Bauer, from 2 days ago

Selective Nanopores

Type	Sequence
Immobilized Hairpin Probe	5′-Amine-C6-CCAACGGTTGGTGTGGTTGG-3′ *
Perfect Complement (PC-DNA)	3′-CCAACCACACCAACC-5′
Single-base Mismatch (1MM-DNA)	3′-CCAACCACACTAACC-5′
Two-base Mismatch (2MM-DNA)	3′-CCAACCACATTAACC-5′
Three-base Mismatch (3MM-DNA)	3′-CCAACCACATTTACC-5′

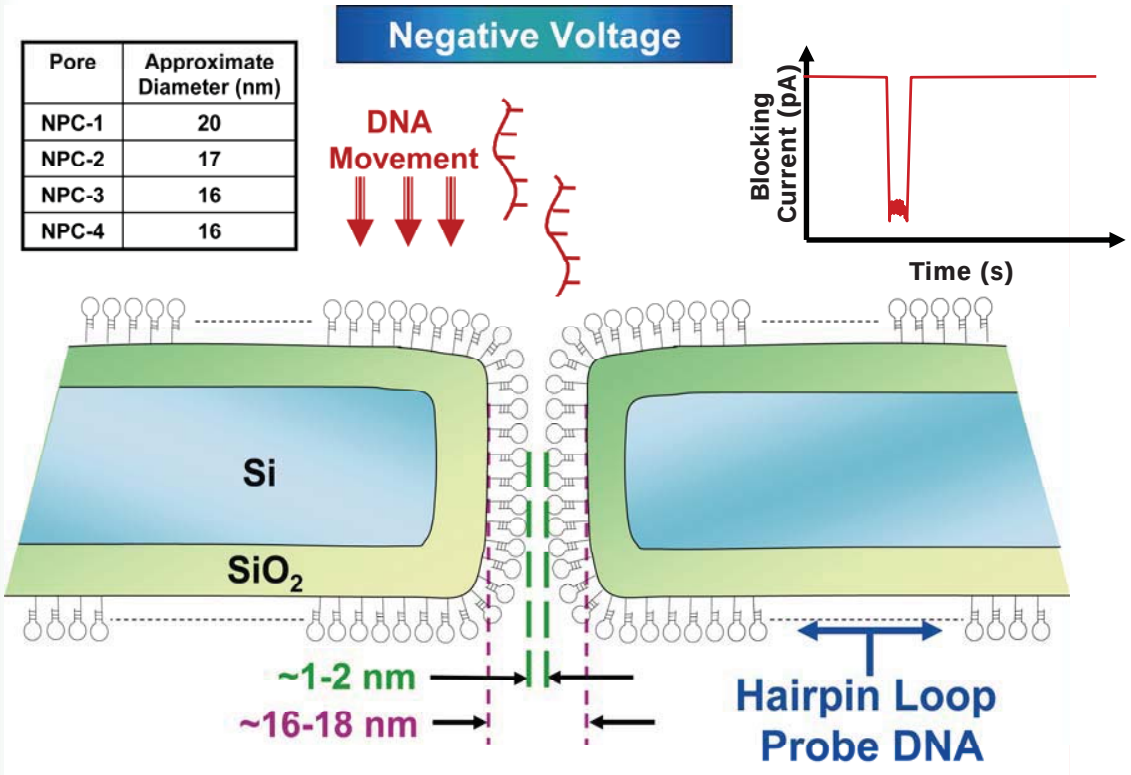
- Underlined bases: Stem of the HPL
- Red bases: Mismatched nucleotides

* [Hamaguchi *et al.*, 2001]

[Iqbal *et al.*, *Nature Nanotechnology*, 2, 243-248 (2007)]

Selective Nanopores

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



[Iqbal *et al.*, *Nature Nanotechnology*, 2, 243-248 (2007)]

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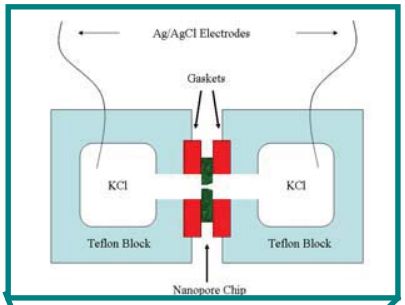
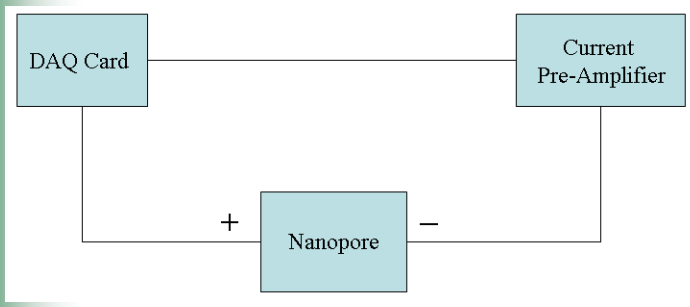
Selective Nanopores

- LabView
- Data Acquisition Card
- Current PreAmp
- Ag/AgCl Electrodes

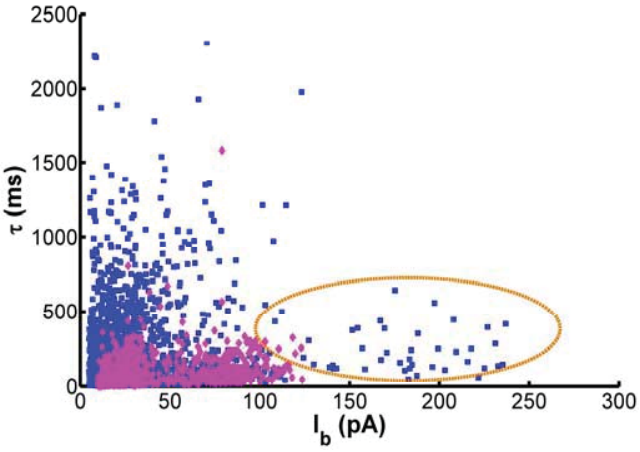




[Iqbal *et al.*, *Nature Nanotechnology*, 2, 243-248 (2007)]

Selective Nanopores

- 20 nm dia nanopore
- MM-DNA target vs. Subsequent PC-DNA
  - Faster Translocation
  - Smaller Mean Passage Time
- 1MM-DNA target
  - before and
  - after PC-DNA

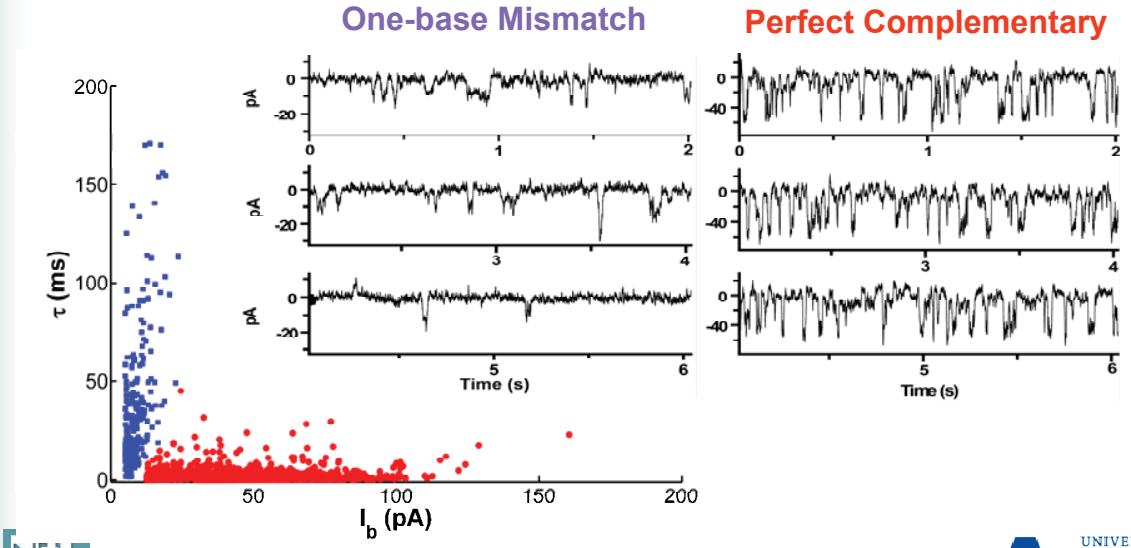


NPC-1	1MM-DNA (in 120 min)		PC-DNA (in 120 min)		1MM-DNA after PC-DNA (in 120 min)	
Signature of pulses	τ (ms)	I_b (pA)	τ (ms)	I_b (pA)	τ (ms)	I_b (pA)
Mean	178.8	28.9	10.2	31.2	92.0	29.1
Sigma	260.3	31.7	30.4	27.8	78.2	23.0
Number of pulses	3,353		96,876		2,896	

[Iqbal *et al.*, *Nature Nanotechnology*, 2, 243-248 (2007)]

Selective Nanopores

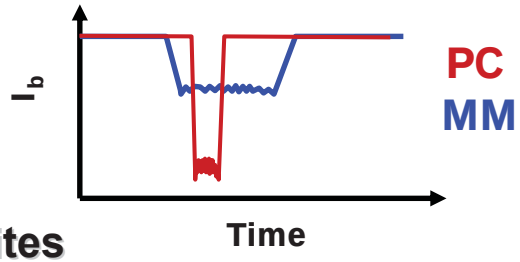
- Smaller Pore
- More Selective
- More I_b → Higher % of the nanopore blocked



[Iqbal *et al.*, *Nature Nanotechnology*, 2, 243-248 (2007)]

Selective Nanopores

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



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

[Iqbal *et al.*, *Nature Nanotechnology*, 2, 243-248 (2007)]

Selective Nanopores – Channel Interactions

- Protein channels provide selective pathways
- Binding sites affect transport
- PC-DNA transport with interactions
  - Shorter Mean First Passage Time (τ)
  - Higher Flux (J)
- The flux of particles interacting with channel

$$n = \frac{L}{2}\left\langle e^{-\phi} \right\rangle \qquad \tau = \frac{L^2}{2D}\left\langle e^{\phi} \right\rangle \left\langle e^{-\phi} \right\rangle \Longrightarrow J = \frac{D}{L}\frac{1}{\left\langle e^{\phi} \right\rangle}(c_1 - c_2)$$

[Bauer and Nadler, 2005 and 2006]

Selective Nanopores – Channel Interactions

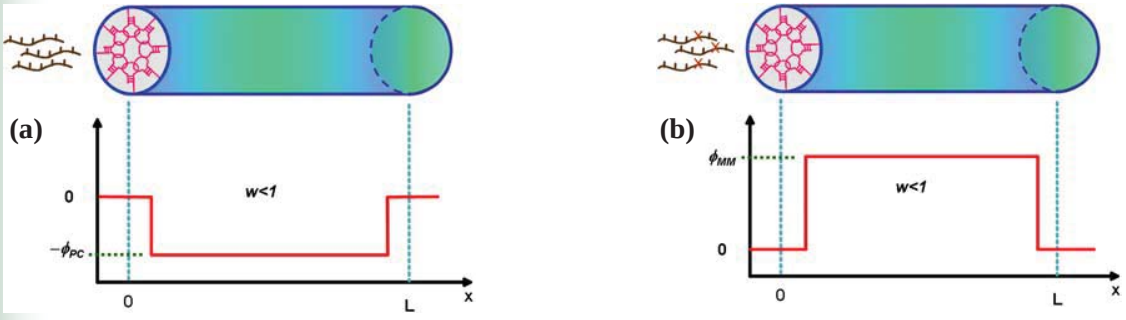
Attractive vs. Repulsive Potential

Key Assumptions

- PC/HPL: Attractive Potential

MM/HPL: Repulsive Potential

- Magnitudes of Potentials
- ϕ span part of the channel



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

Selective Nanopores – Channel Interactions

W = 1

$$J_{PC} = \frac{D}{L}\frac{1}{e^{-\phi_{PC}}}c_1 \quad \& \quad J_{MM} = \frac{D}{L}\frac{1}{e^{\phi_{MM}}}c_1 \rightarrow \frac{J_{PC}}{J_{MM}} = e^{\phi_{MM}}e^{\phi_{PC}}$$

$$J_{PC} > J_o > J_{MM}$$

$$\tau_{MM} = \tau_{PC} = \frac{L^2}{2D}$$

W < 1

$$\tau = \left(\frac{L^2}{2D}\right)w(1-w)e^{|\phi|} \rightarrow \frac{\tau_{MM}}{\tau_{PC}} = e^{|\phi_{MM}|-|\phi_{PC}|}$$

$$J_{PC} = J_o\frac{1}{1-w} \quad J_{MM} = J_o we^{-\phi_{MM}} \rightarrow \frac{J_{PC}}{J_{MM}} = \frac{w}{1-w}e^{\phi_{MM}}$$

$$J_{PC} > J_o > J_{MM}$$

$$\tau_{MM} > \tau_{PC} > \tau_o$$

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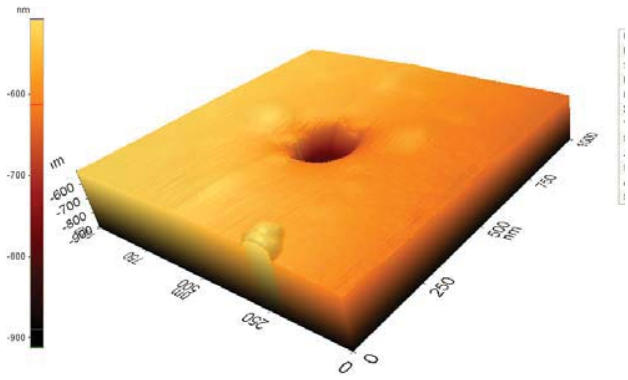
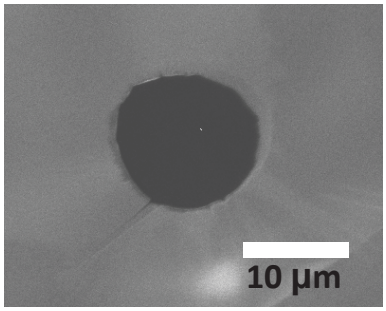
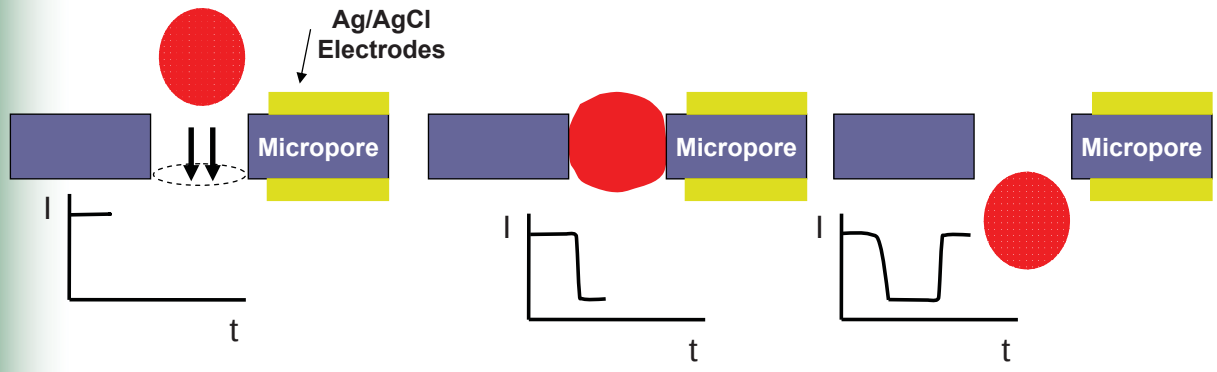
Micropores

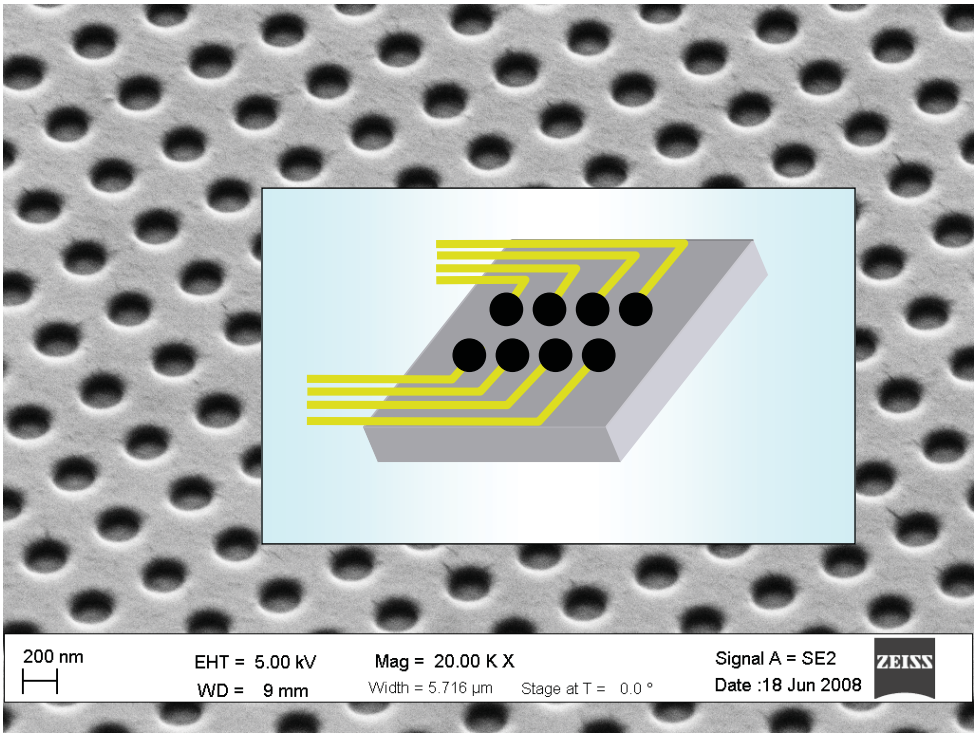


Micropores for Cancer Detection

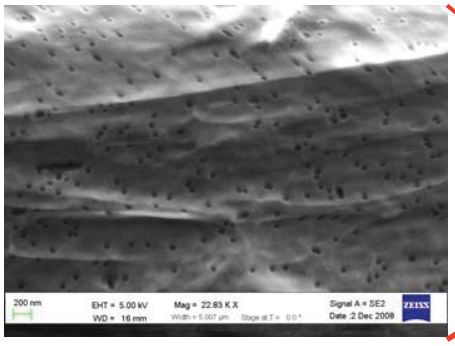


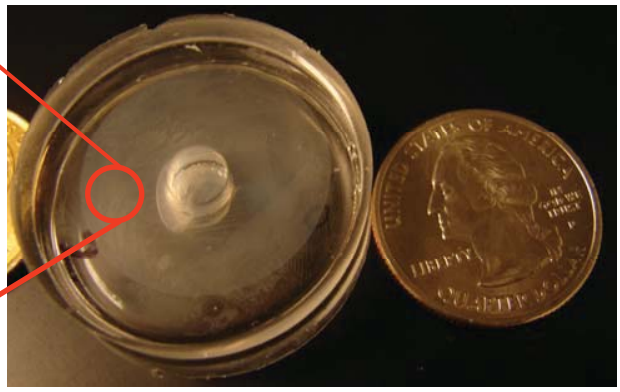


Micropore Array for Cancer Detection



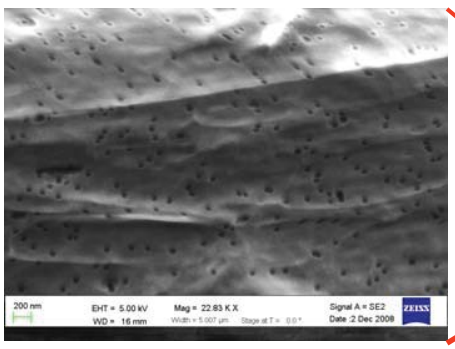
Active Membranes for the Enrichment of Biomarkers

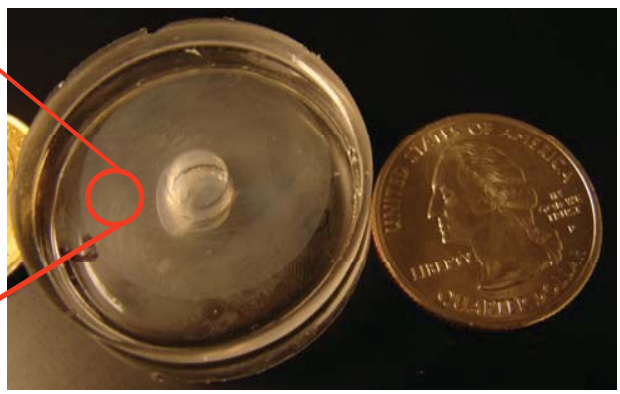




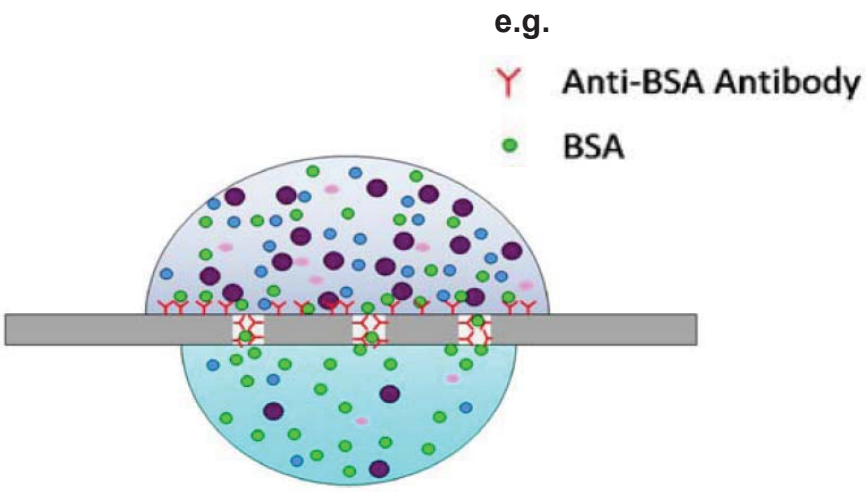
aka

Facilitated Transport through Functionalized Membranes



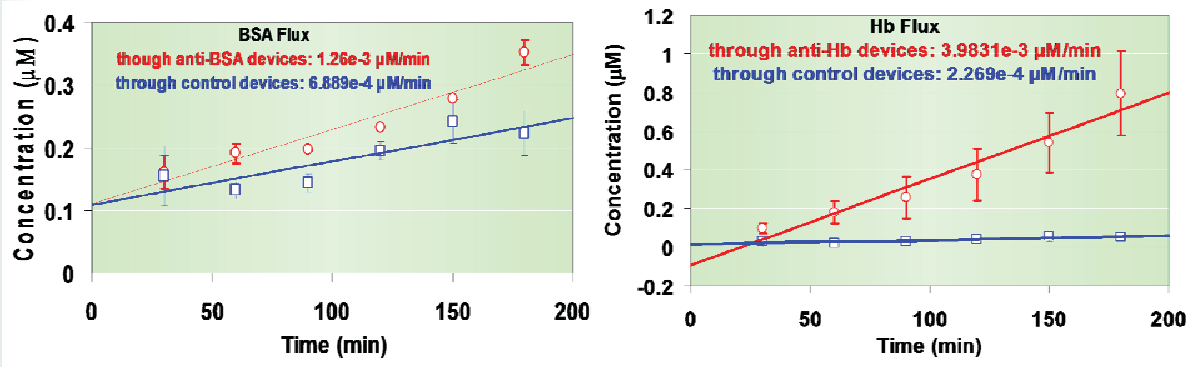


Active Protein Separation



[Goyal, et al., "Active and biomimetic nanofilters for selective protein separation," *Biomedical Microdevices*, vol. 12, pp. 317-324, 2010.]

Active Protein Separation



Functionalization	Transport rate of molecules (μM/min)	
	BSA	Hb
Control	6.8871e-4	2.2693e-4
Anti-BSA antibody	1.2608e-3	2.2941e-4
Anti-Hb antibody	3.0281e-3	3.9831e-3

[Goyal, et al., "Active and biomimetic nanofilters for selective protein separation," *Biomedical Microdevices*, vol. 12, pp. 317-324, 2010.]

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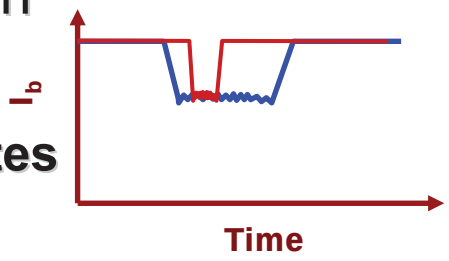
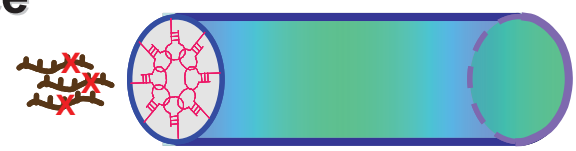
Active Protein Separation – Facilitated Transport

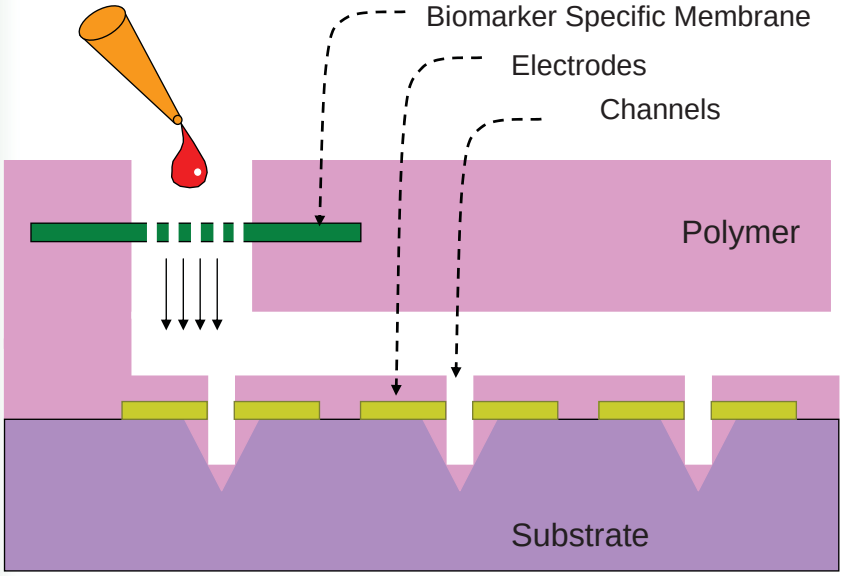
- Channel-Molecular Interaction
- Interacting Molecules
  - **Interactions with Binding Sites**
  - **Faster and More**
- Non-Interacting Molecules
  - **Electrostatic Friction**
  - **Mechanical Resistance**
  - **Inability to bind**

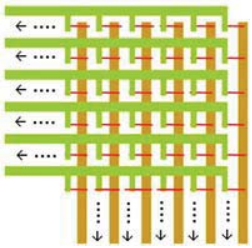


$$J = \frac{n}{\tau}(c_1 - c_2)$$

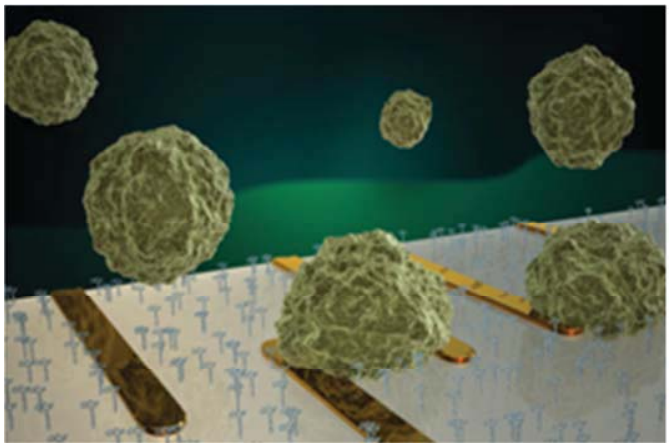


Diagnostics!





Isolating Circulating Tumor Cells with Aptamer Biochip



Antibody vs. Aptamer

Property	EGFR Antibody	Anti-EGFR Aptamer
Specificity	Low (≈ 60%)	High (≈ 90%)
Binding Conditions	Physical	pH, Tm, Salt Conc.
Shelf Time	Short	Long
Kd	≈1 nM	2.4 nM

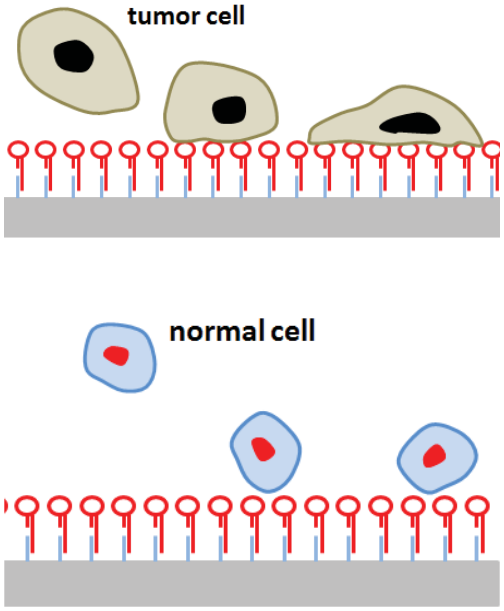
[Dadparvar S, et al., Cancer, 1994], [Nagrath S, et al., Nature, 2007], [Vona G, et al., Am. J. Pathol, 2000], [Sullenger BA, et al., Nature, 2002], [Cho EJ, et al., Ann. Rev. Anal. Chem, 2009]

Aptamers… growing list

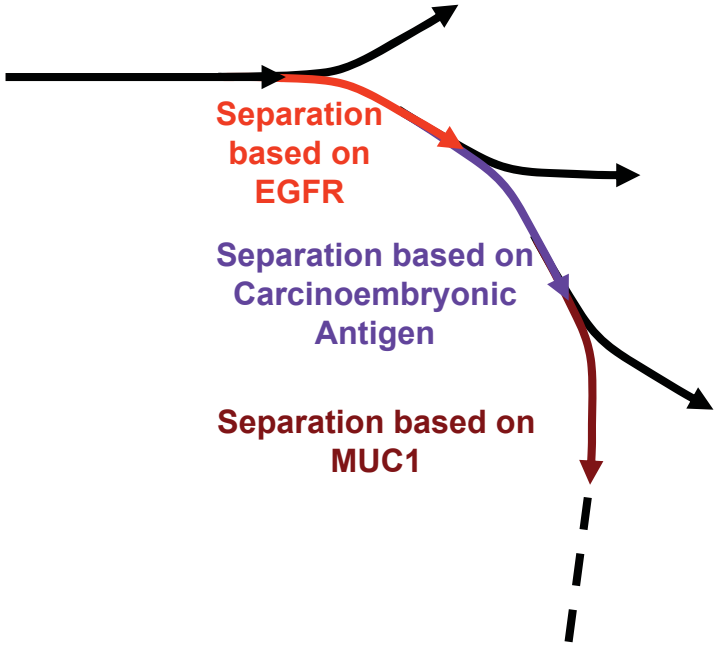
- Anti-EGFR aptamer to detect over-expression of EGFR (which occurs in cancer cell)
- RT-26: a high-affinity DNA aptamer than can be used for the detection of the reverse transcriptase of the HIV type-1 as the target protein
- Anti-thrombin aptamer: identified to interact with thrombin protein (important for blood clot formation),
- Aptamer for rapid detection of immunoglobulin E (IgE) (high level of IgE is seen in patients with allergic asthma & immune deficiency-related diseases, such as AIDS)
- Aptamers for the detection of β-amyloid peptide (Alzheimer's disease is characterized by the deposition of this peptide in the brain)
- Aptamers for bio-warfare agent detection (Anthrax, Ricin, Tularemia, Influenza virus)

Aptamers for Tumor Cell Capture

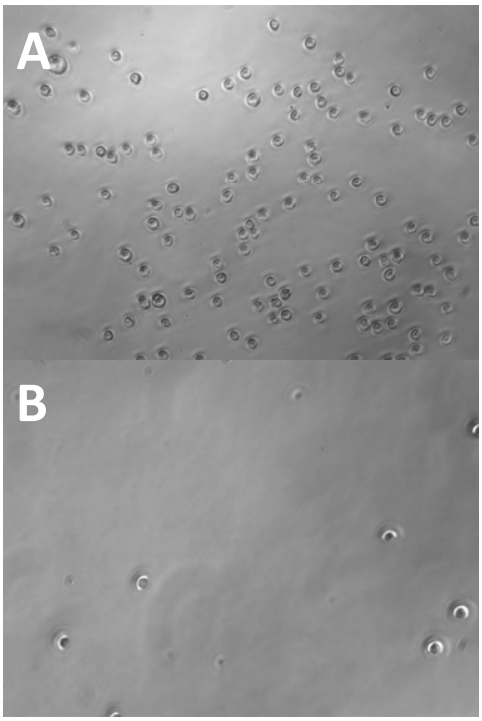
- **High specificity and affinity**
- **Chemical synthesis**
- **Stable at various conditions**
- **Labeled with fluorescent dye**
- **Reversible denaturation**

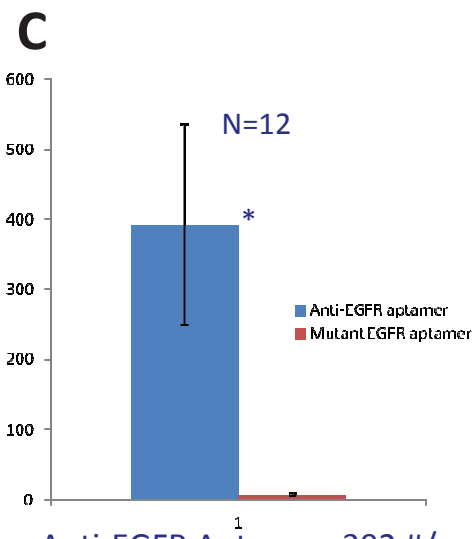


Biomarker-based Tumor Cell Capture



Tumor Cell Binding to Anti-EGFR Aptamer vs. Mutant Aptamer



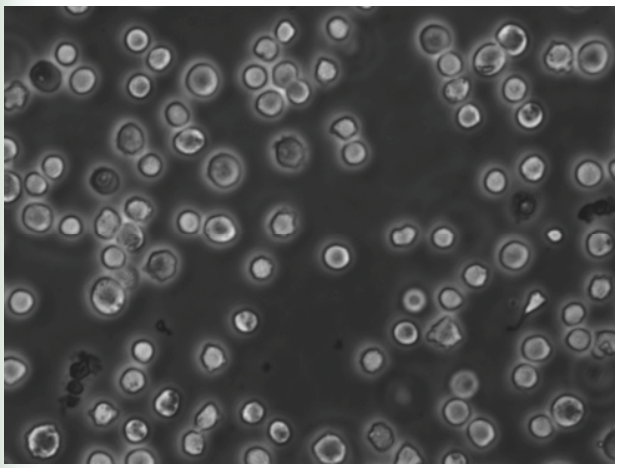


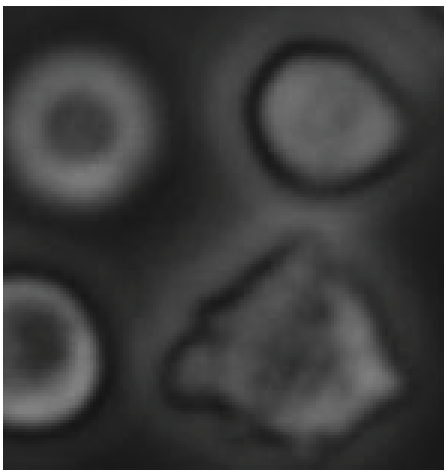
Anti-EGFR Aptamer: 392 #/mm^2

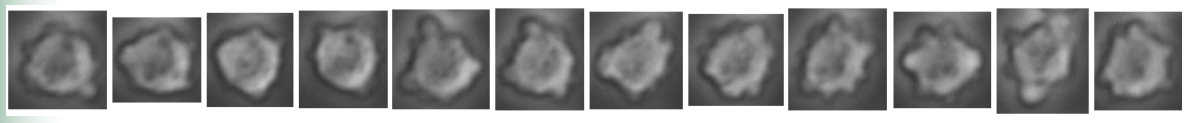
Mutant Aptamer: 7 #/mm^2

Wan, et al., "Surface Immobilized Aptamers for Cancer Cell Isolation and Microscopic Cytology," *Cancer Research*, 70(22), pp. 9371-9380, (2010)

CTC Chip – A New Cytology Tool







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Wan, et al., "Surface Immobilized Aptamers for Cancer Cell Isolation and Microscopic Cytology," *Cancer Research*, 70(22), pp. 9371-9380, (2010)

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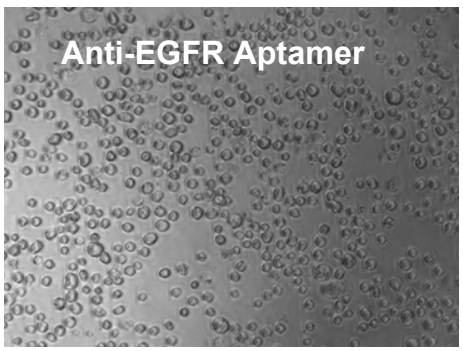
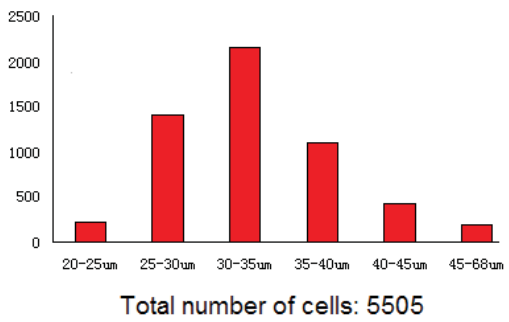
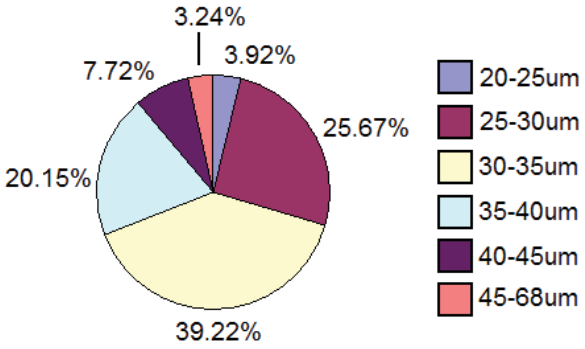
CTC Chip





Total number of cells: 5505



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Wan, et al., "Surface Immobilized Aptamers for Cancer Cell Isolation and Microscopic Cytology," *Cancer Research*, 70(22), pp. 9371-9380, (2010)

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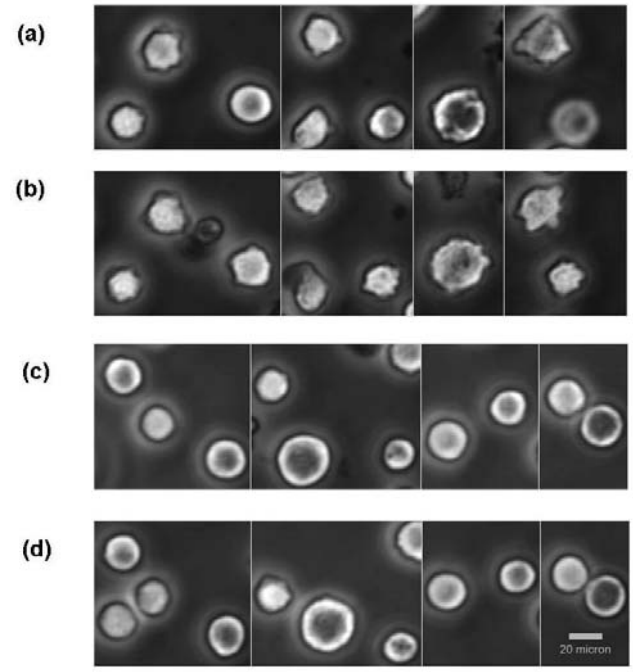
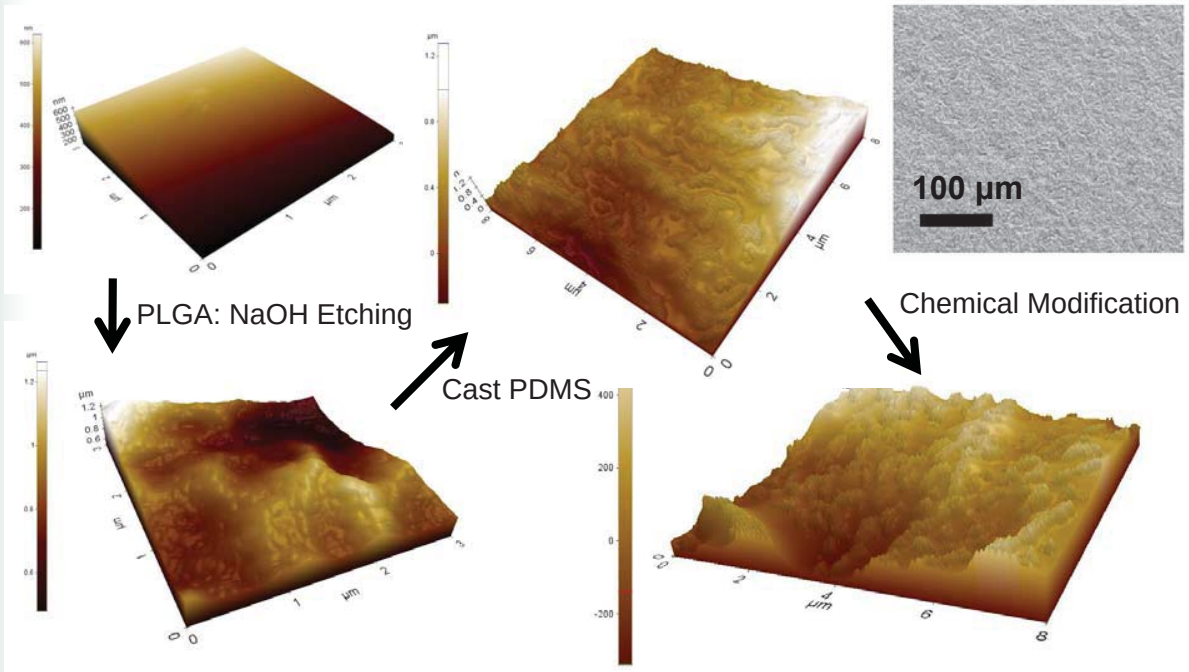
CTC Chip

- Tumor cells grow
- Shapes change from smooth circular to irregular
- Antennae formed after capture
- Rate of deformation and shape change



Wan, et al., "Surface Immobilized Aptamers for Cancer Cell Isolation and Microscopic Cytology," *Cancer Research*, 70(22), pp. 9371-9380, (2010)

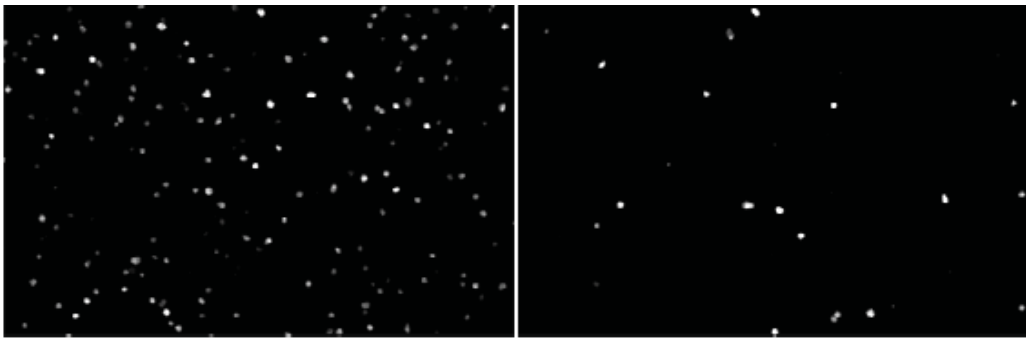
Nano-texturing Chip Surface



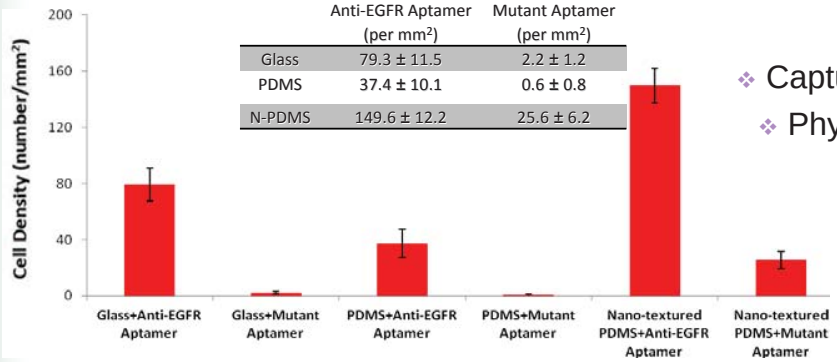
[Wan, et al., *CANCER*, 2011]

Nano-texturing Chip Surface

N-PDMS



	Anti-EGFR Aptamer (per mm²)	Mutant Aptamer (per mm²)
Glass	79.3 ± 11.5	2.2 ± 1.2
PDMS	37.4 ± 10.1	0.6 ± 0.8
N-PDMS	149.6 ± 12.2	25.6 ± 6.2



- Captured Cancer Cells ↑↑
- Physical Absorption ↑↑

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[Wan, et al., *CANCER*, 2011]

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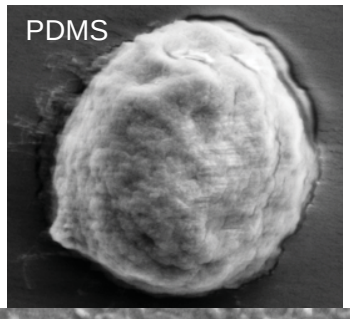
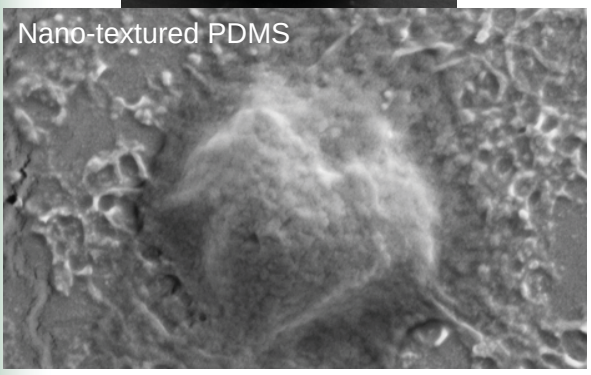
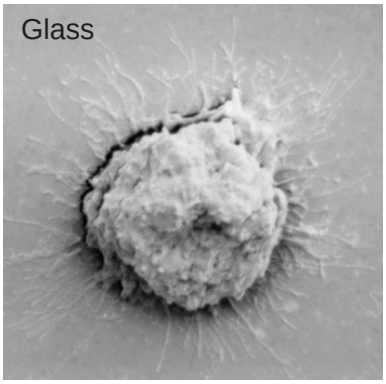
Nano-texturing Chip Surface





Captured tumor cell on PDMS, nano-textured PDMS, and glass substrate



- Contact Area
- Morphology

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[Wan, et al., *CANCER*, 2011]

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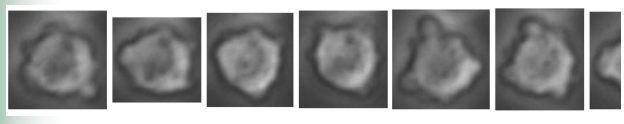
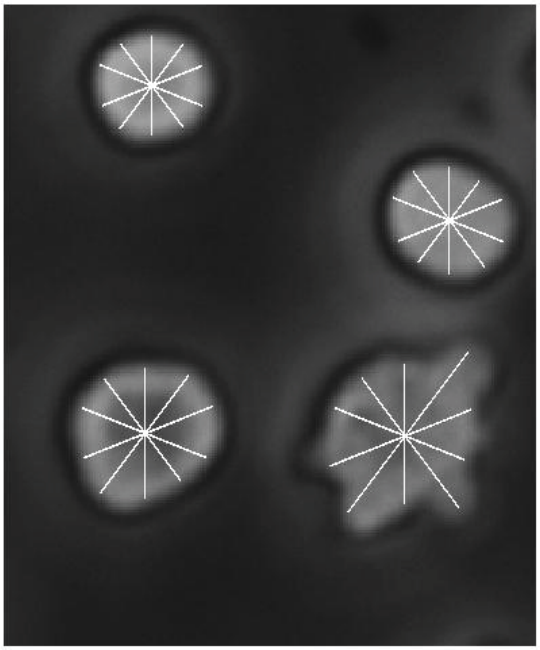
CTC Chip





CTC: Electrical Sensing

- Cancerous tissues are known to have notably higher water content by weight than healthy tissues
- Known different dielectric properties
- AC measurements can measure different changes in the electrical impedance on the electrodes
- Impedance data (Amplitude, phase, permittivity, conductivity) of the two types of cells is expected to be significantly different!!

Opportunities

Cells, Biomarkers, Genes

Energy, Environment, Natural Resources

Questions

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SMIQBAL@uta.edu