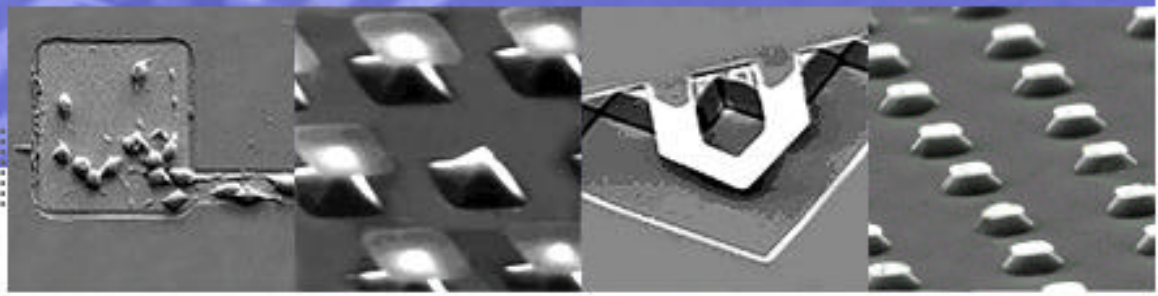
PURDUE UNIVERSITY

LIBNA

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



Introduction to BioMEMS & Bionanotechnology
Lecture 4

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School of Electrical and Computer Engineering,

Weldon School of Biomedical Engineering,

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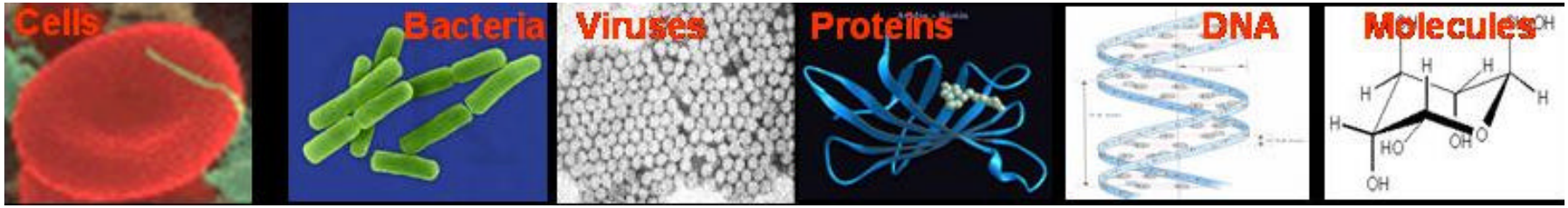
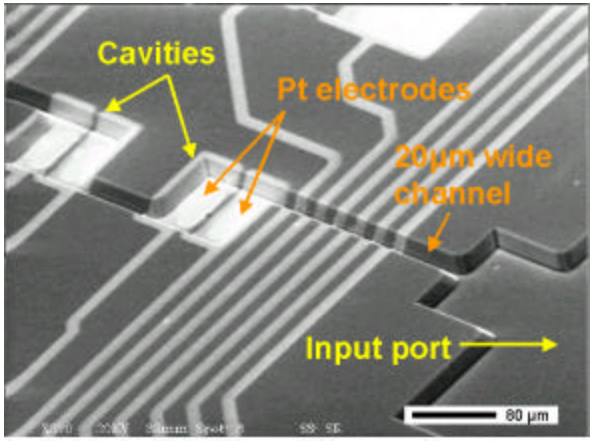
Key Topics

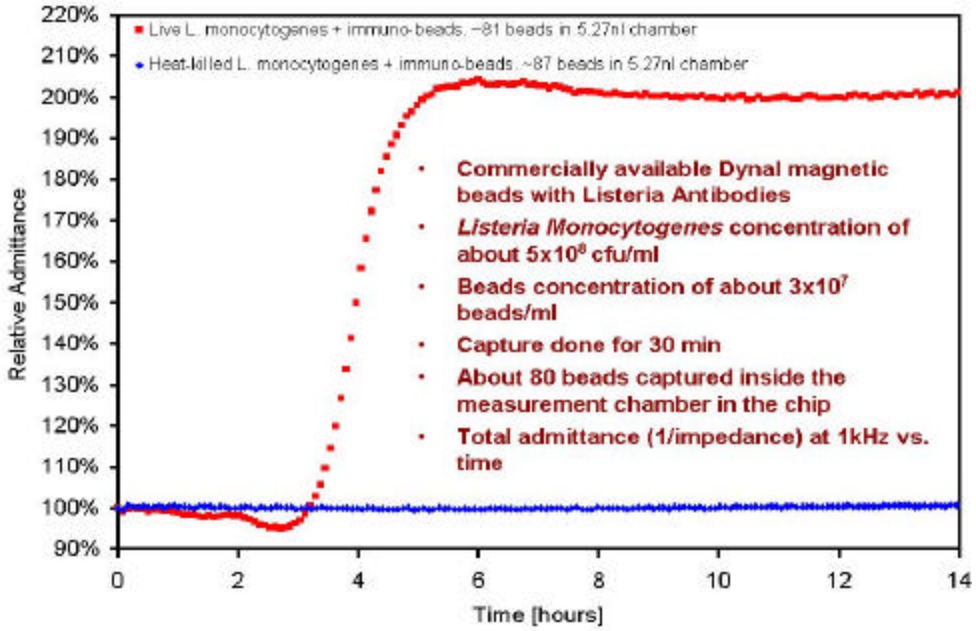
- **Biochips/Biosensors and Device Fabrication**
- **Cells, DNA, Proteins**
- **Micro-fluidics**
- **Biochip Sensors & Detection Methods**
- **Micro-arrays**
- **Lab-on-a-chip Devices**

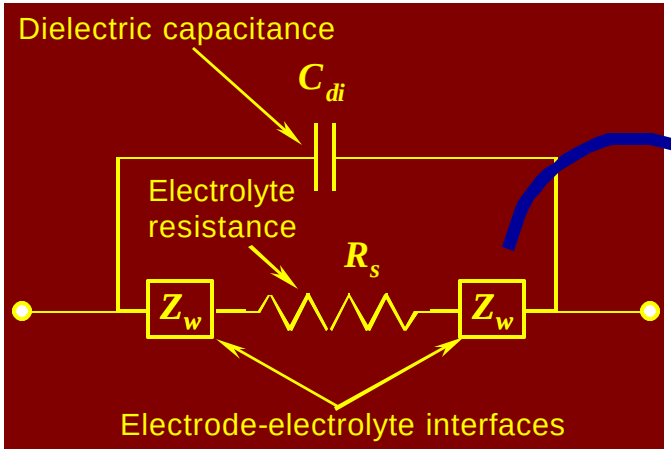


Micro-fluidic Devices for Conductance Detection of Bacterial Metabolism

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







Electrode-Electrolyte Interface Model:

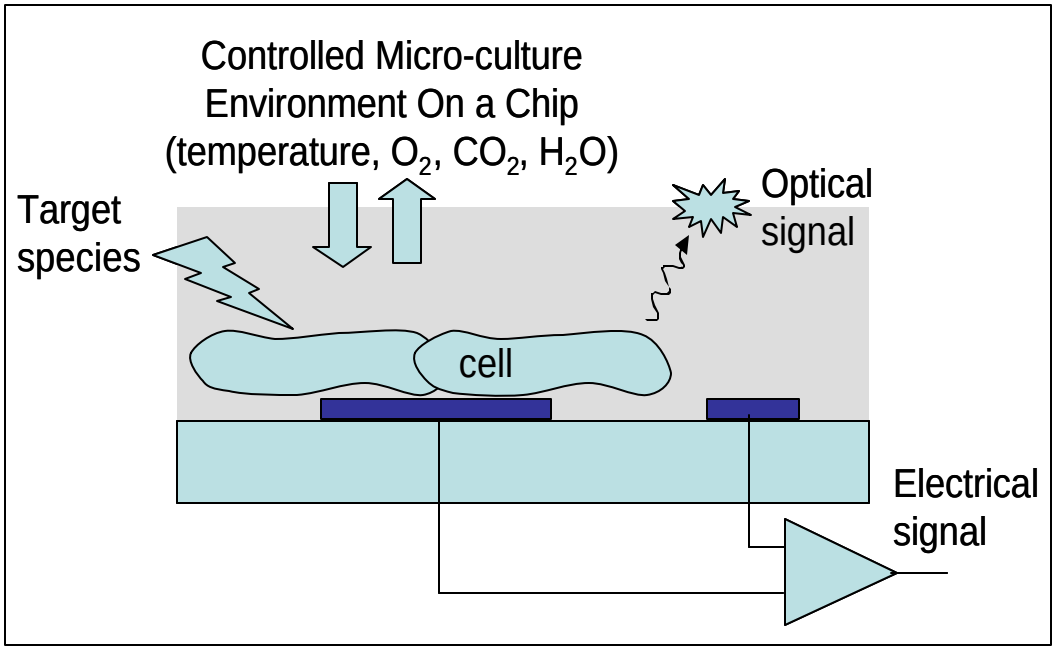
$$Z_w = \frac{1}{(jw)^n B}$$

Constant-angle impedance

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

4. Cell-Based Sensors/Biochips

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

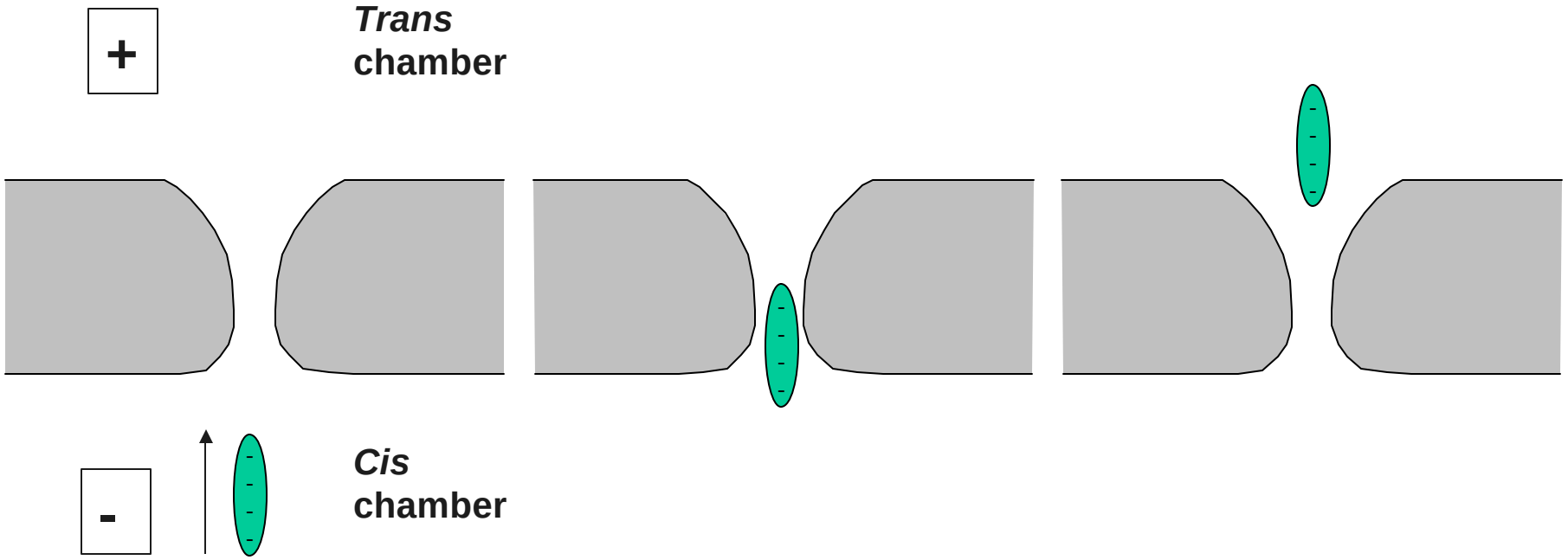


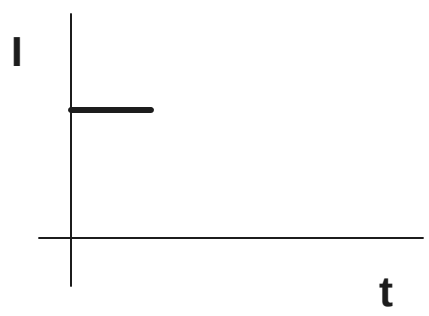
L. Bousse, Whole cell biosensors, Sensors and Actuators B (Chemical), Vol. B34, No. 1-3, August 1996, pp. 270-5.

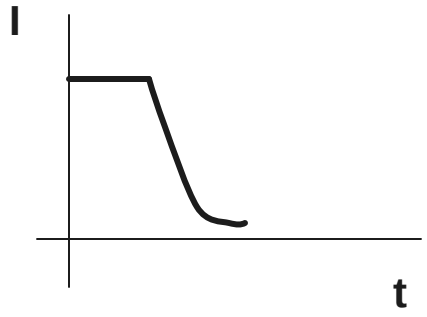
J.J. Pancrazio, J.P. Whelan, D.A. Borkholder, W. Ma, D.A. Stenger, Development and application of cell-based biosensors, Annals of Biomedical Engineering, Vol. 27, No. 6, November 1999, pp. 697-711.

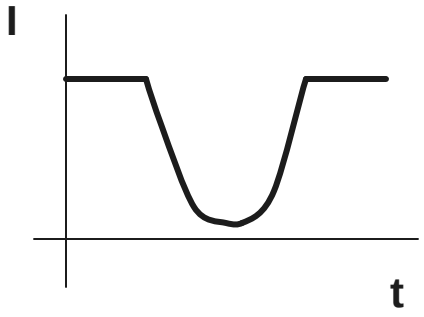
D.A. Stenger, G.W. Gross, E.W. Keefer, K.M. Shaffer, J.D, Andreadis, W. Ma, J.J. Pancrazio, Detection of physiologically active compounds using cell-based biosensors, Trends in Biotechnology, Vol. 19, No. 8, August 1, 2001, pp. 304-309.

5. Micro/Nano-scale Coulter Counter

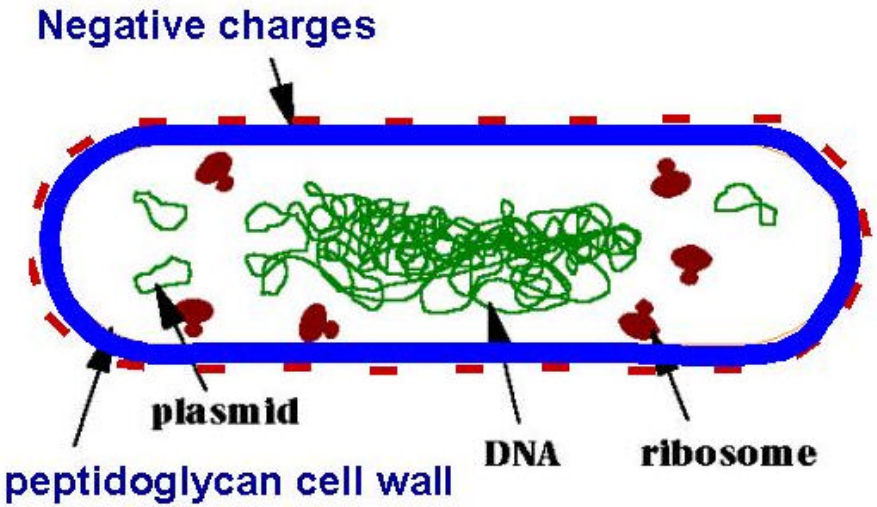


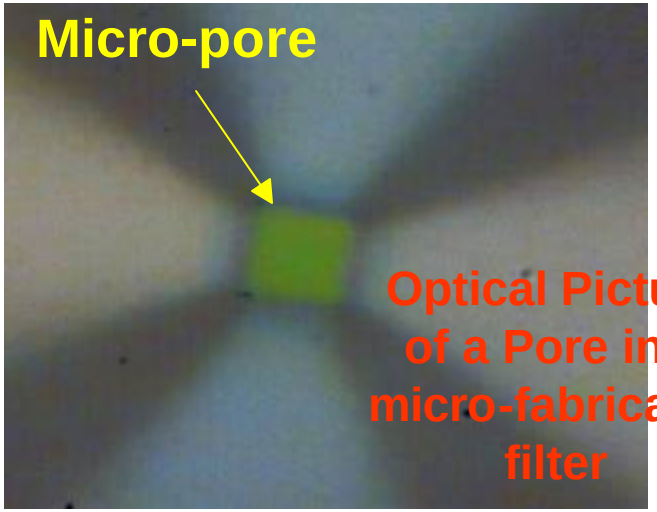




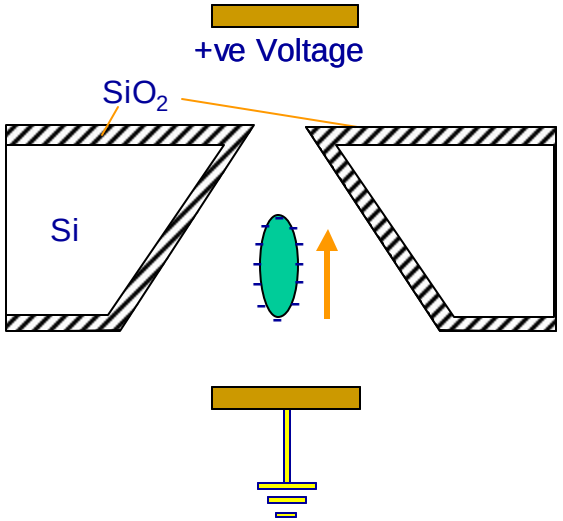


Micro-pore for cellular studies



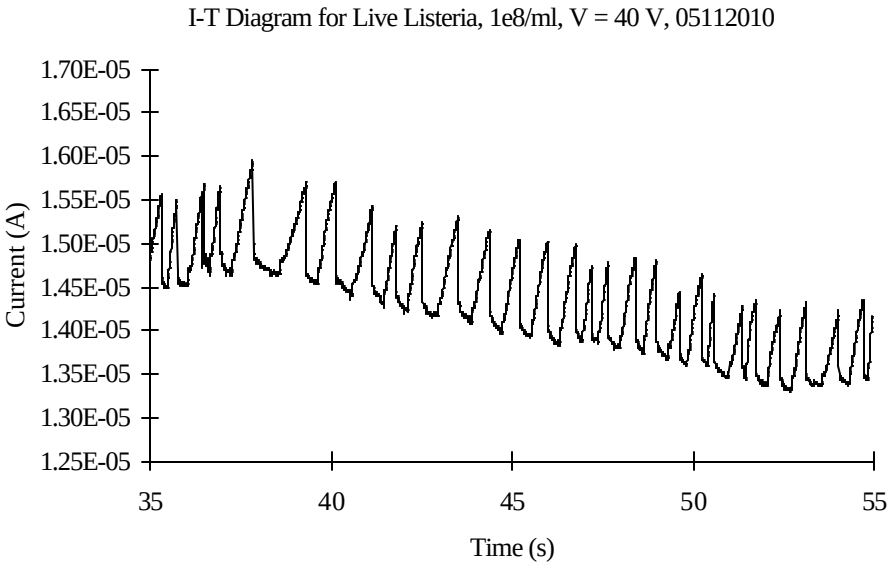


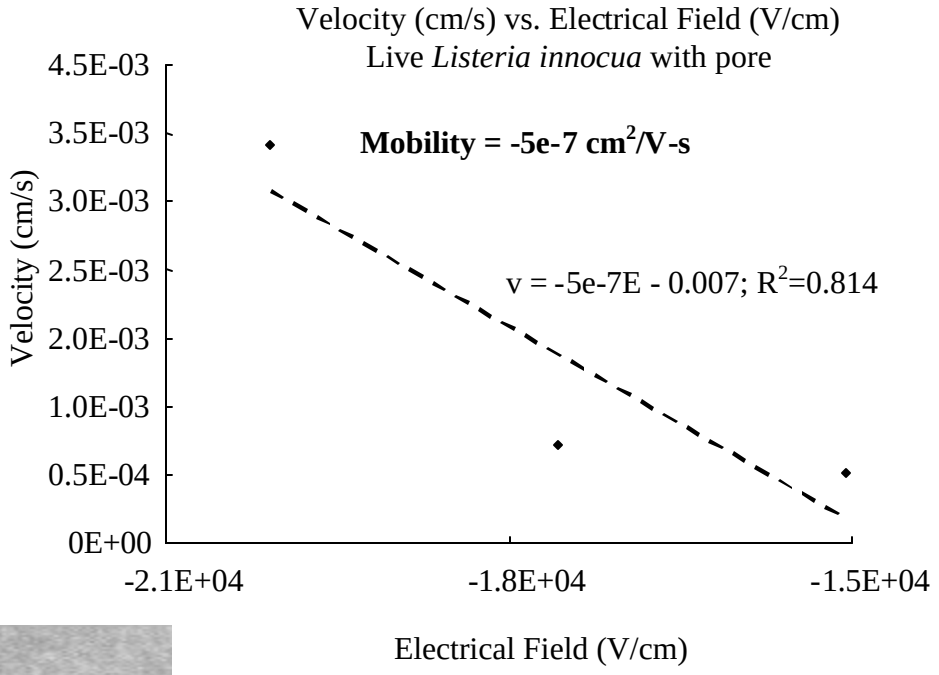
- **Micro-devices for single cell characterization – utilize the charge properties**
- **Micro-fabricate a pore where single entity can pass**



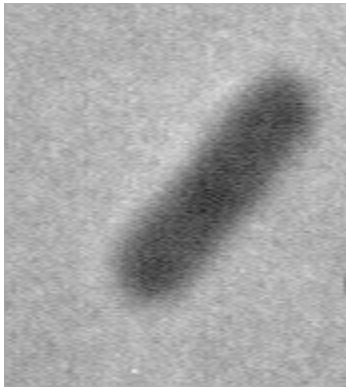
Cross section of micro-fabricated pore

Microscale Coulter Counter





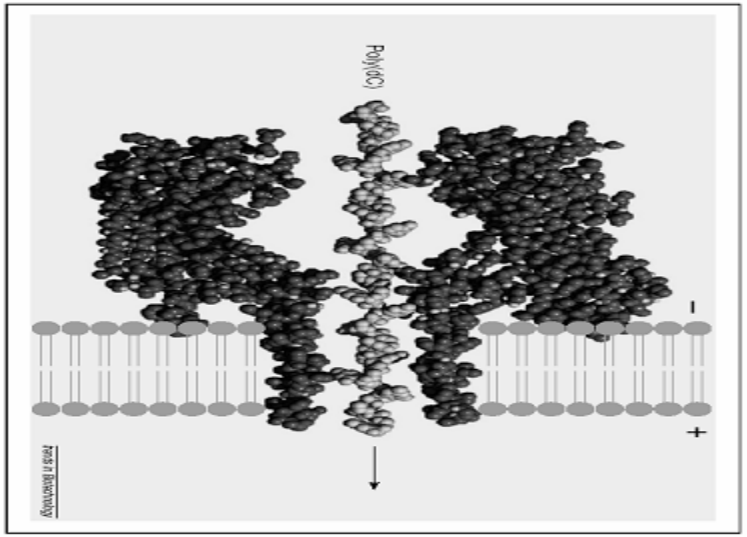
Live *Listeria innocua* with a well-defined cell wall



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

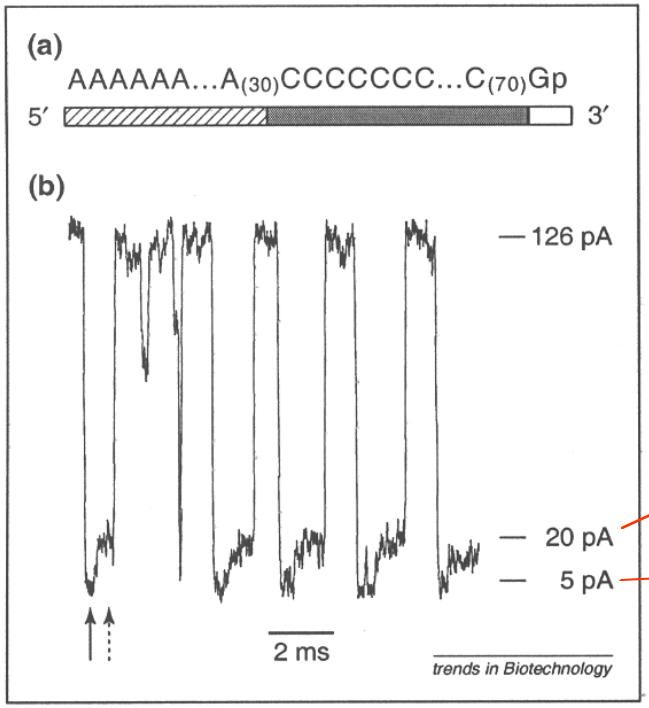
Nanoscale DNA Coulter Counter

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



a-hemolysin nanochannel

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



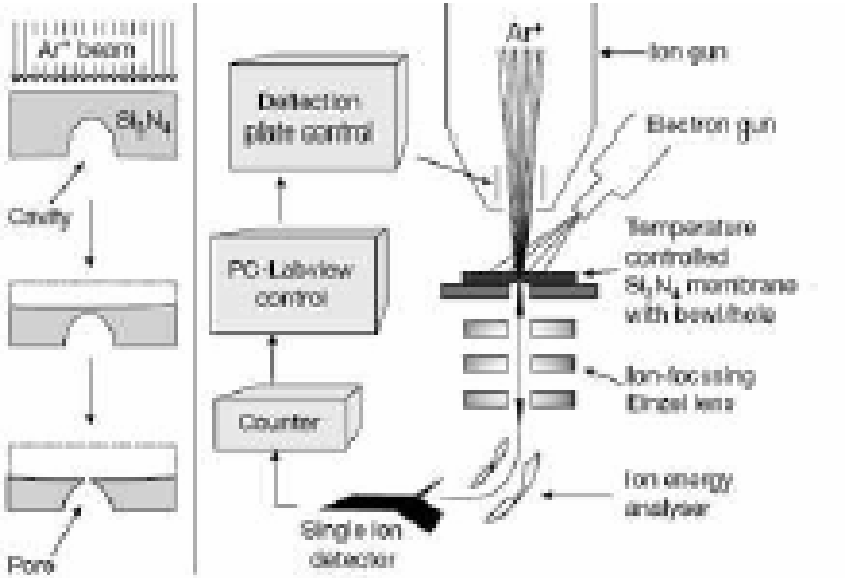
Poly-A

Poly-C

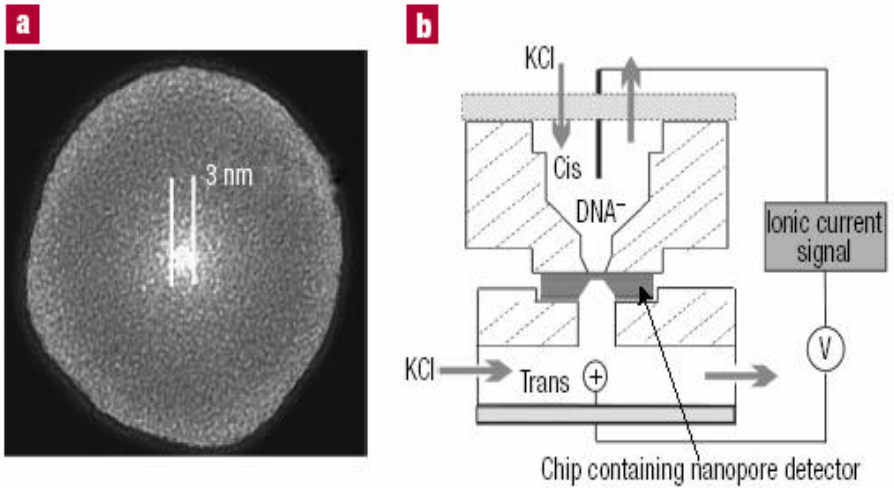
Kasianowicz et al., 1996, Meller, et. al. 2000.

Fabrication Techniques

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

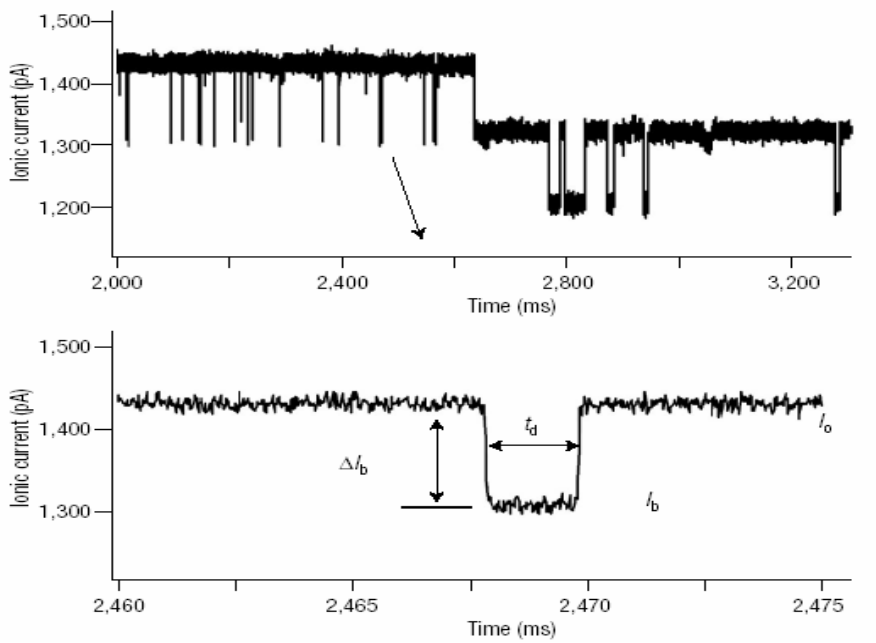


The fabrication of Li's nanopore. From *Li et. al. Nature, 2001.*

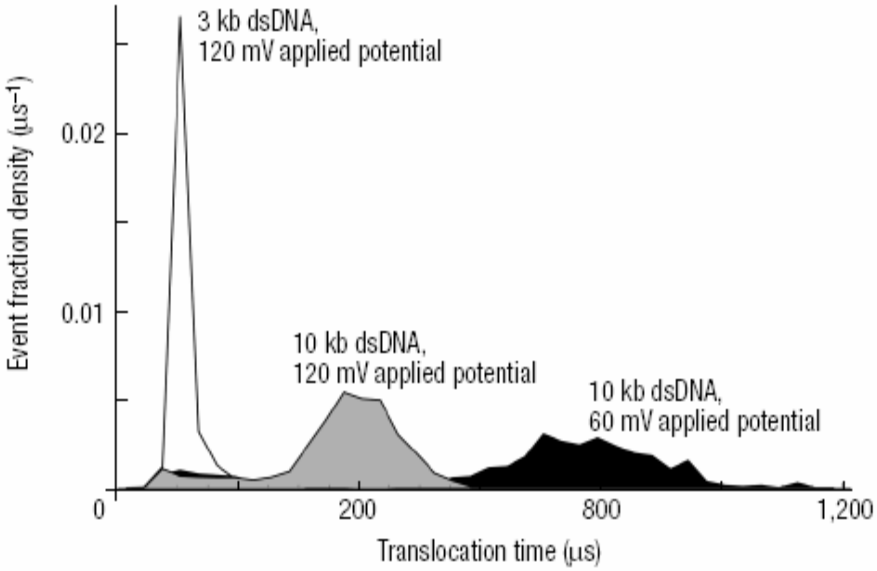


TEM of Li's nanopore. b. DNA measurement setup in Li's work. From *Li et. al. Nature Materials, 2003*

DNA Translocation



Current fluctuations when DNA was passing through the pore



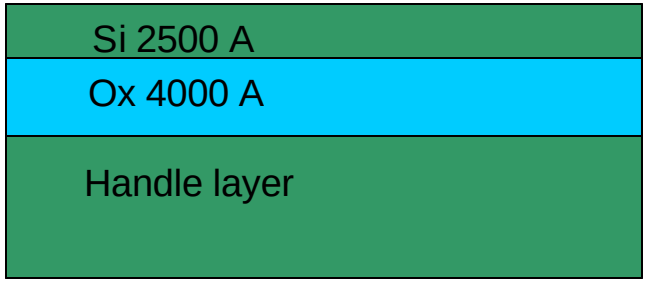
Histograms of relation among DNA lengths, translocation times and applied biases.

Li et. al. 2003

Silicon Based Nanopore

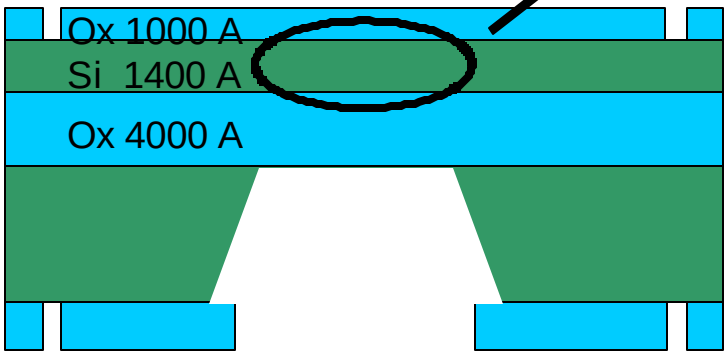
(Not to scale)

1.



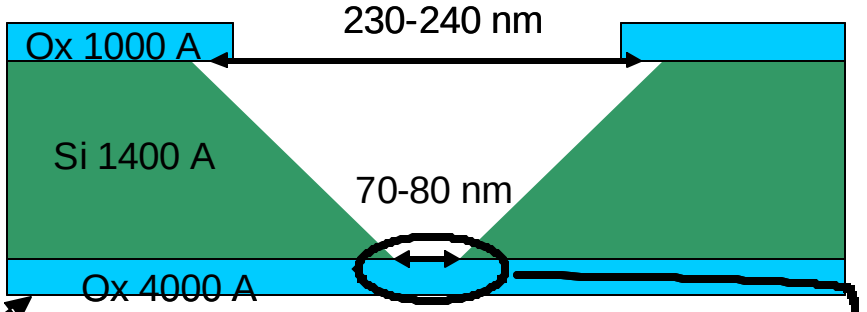
Start with a (100) 4 inch SOI wafer. Thickness : 525 um. SOI : 250 nm, Buried oxide layer: 400 nm.

2.



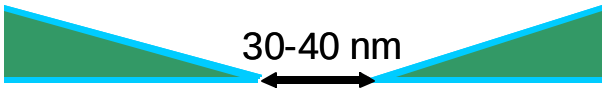
Grow thermal oxide on wafer surface and open etch window to etch through the handle layer. Etch stops on buried oxide layer.

3.



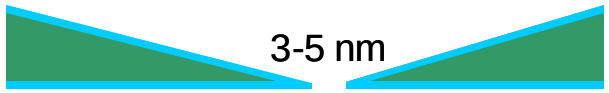
On SOI layer, open another etch window to etch through the SOI layer. Etch stops on buried oxide layer.

4.



Remove buried oxide layer and regrow 100 nm thermal oxide.

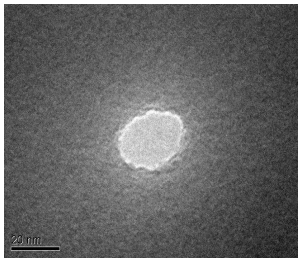
5.



Shrink the pore to 3 –5 nm by TEM

Pore shrinking and shape changing (After Thermal Oxidation, Oxide Thickness = 50 nm)

41 x 26 nm
(300,000 X)

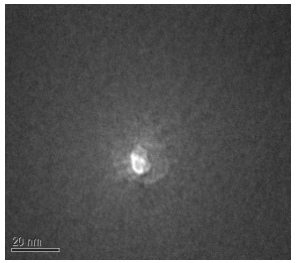


23 nm
(1,000,000 X)

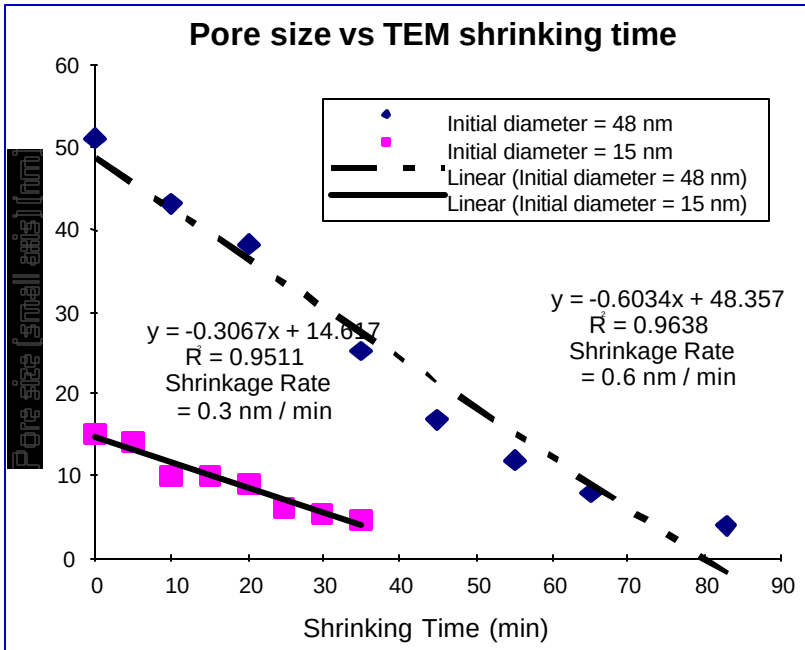
19 nm
(1,000,000 X)

11 nm
(1,000,000 X)

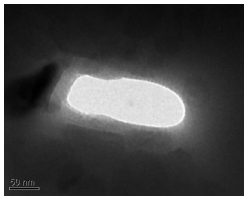
7 nm
(1,000,000 X)



4.2 nm x 4.6 nm
1,000,000 X)

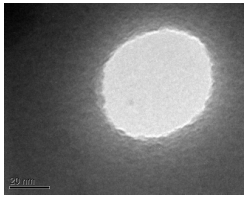


Slopes in the plot are the shrinkage rates. Different initial pore size had different shrinkage rates.

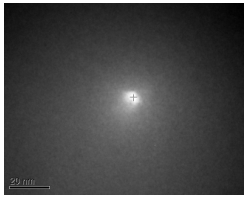


9nm x 198nm
Initial pore

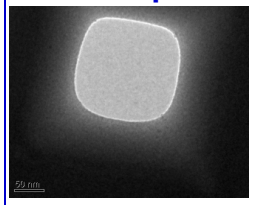
80nm x 156nm
90 min



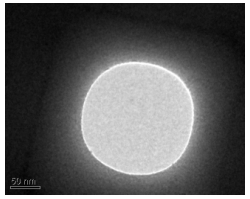
48nm x 57nm
250 min



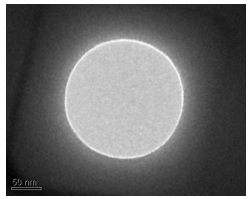
3.3nm x 3.5nm
334 min



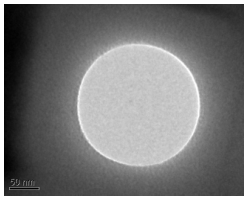
168nm x 172nm



187nm x 189nm
20 min



200nm x 201nm
45 min

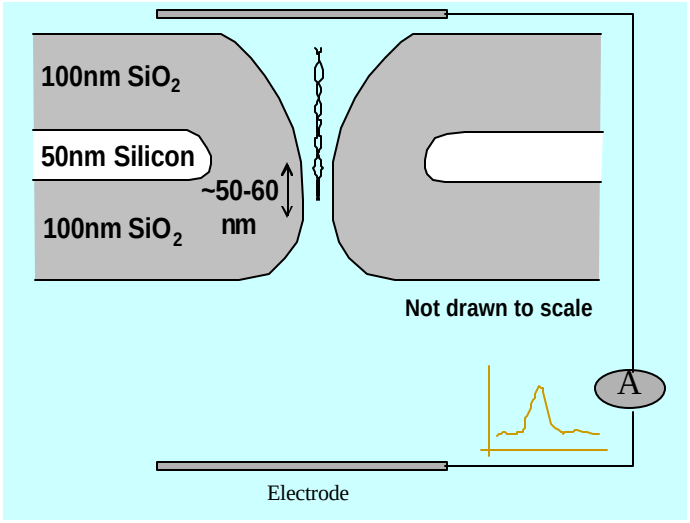


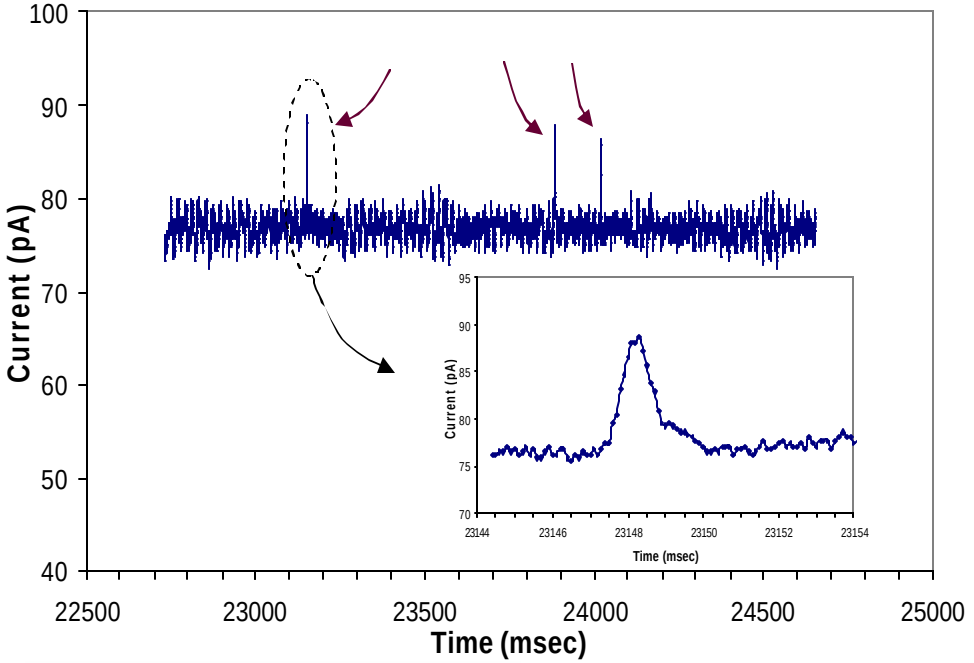
206 nm x 208 nm
58 min

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

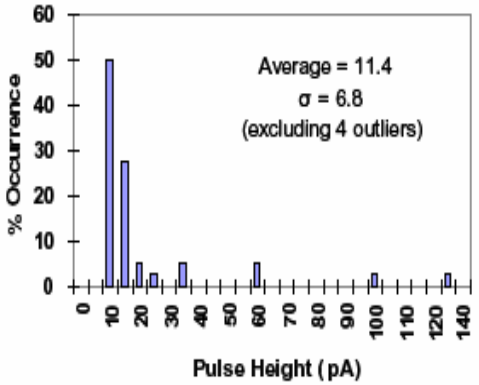
H. Chang, F. Kosari, G. Andreadakis, G. Vasmatzis, E. Basgall, A. H. King, and R. Bashir, "Towards Integrated Micro-Machined Silicon-Based Nanopores For Characterization Of DNA", Hilton Head MEMS conference, 2004, Hilton Head, South Carolina.

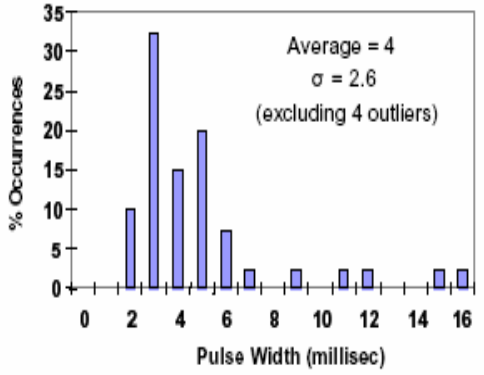
‘Nanopore Channel’ Sensors for Characterization of Single Molecule dsDNA



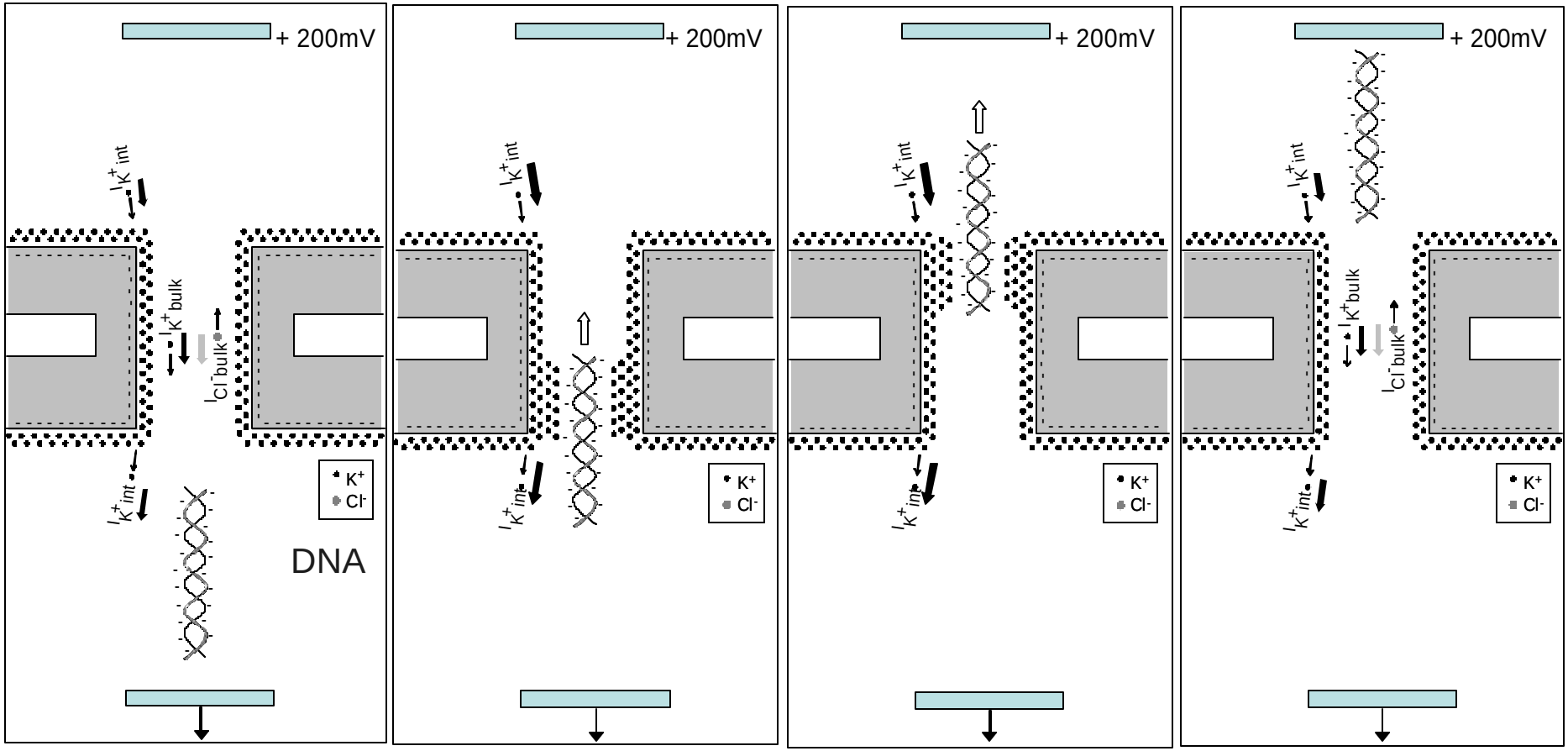


- 200bp DNA was used. Concentration of 0.3 mg/ml.
- Buffer solution : 0.1 M KCl, 2 mM Tris (pH 8.5)
- Ag/AgCl electrodes were utilized.
- Bias : 200 mV.
- Time sampling interval : 100 us



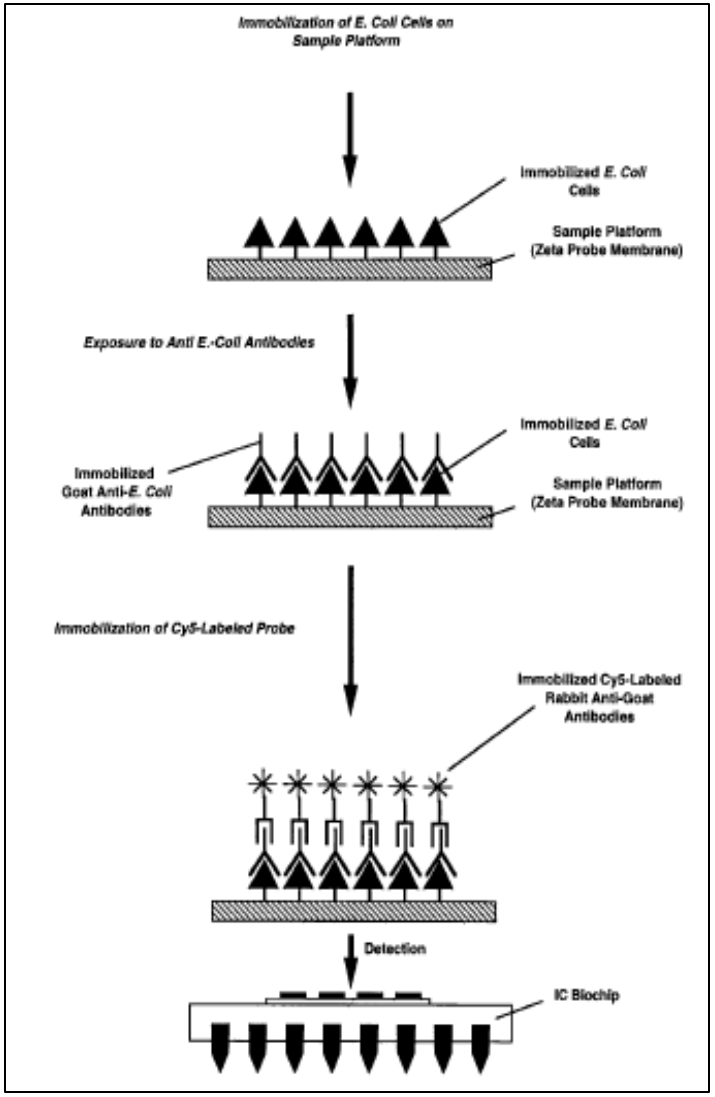


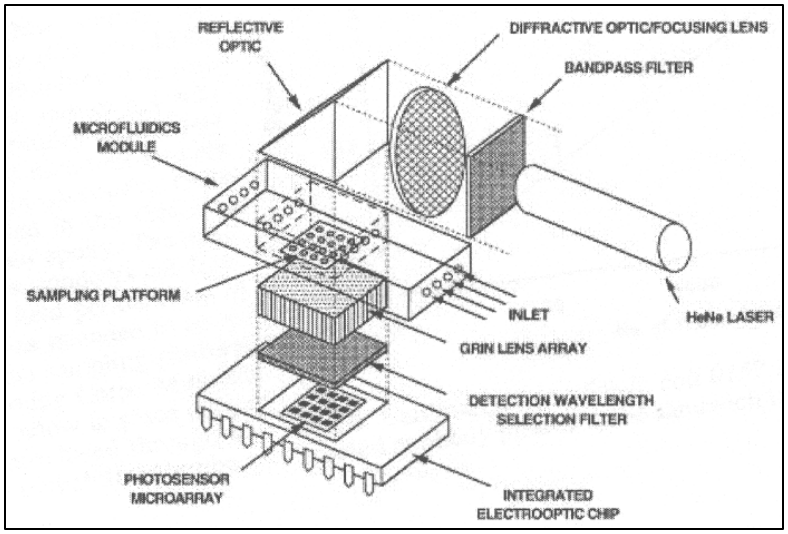
Explanation of Current Pulses

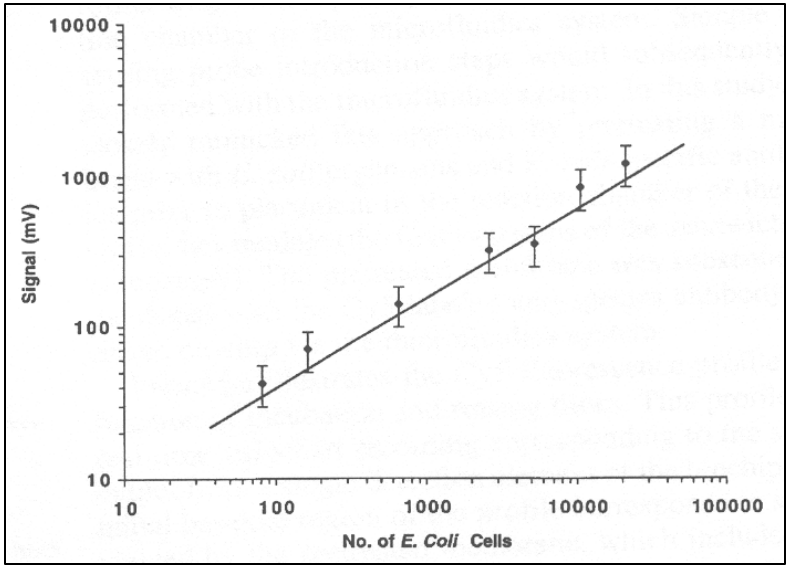


DNA induces extra potassium ions when passing through the nano-channel. The interface current of K ions thus increases. At the same time bulk currents decrease because of DNA blocking.

Integrated Optical Detection







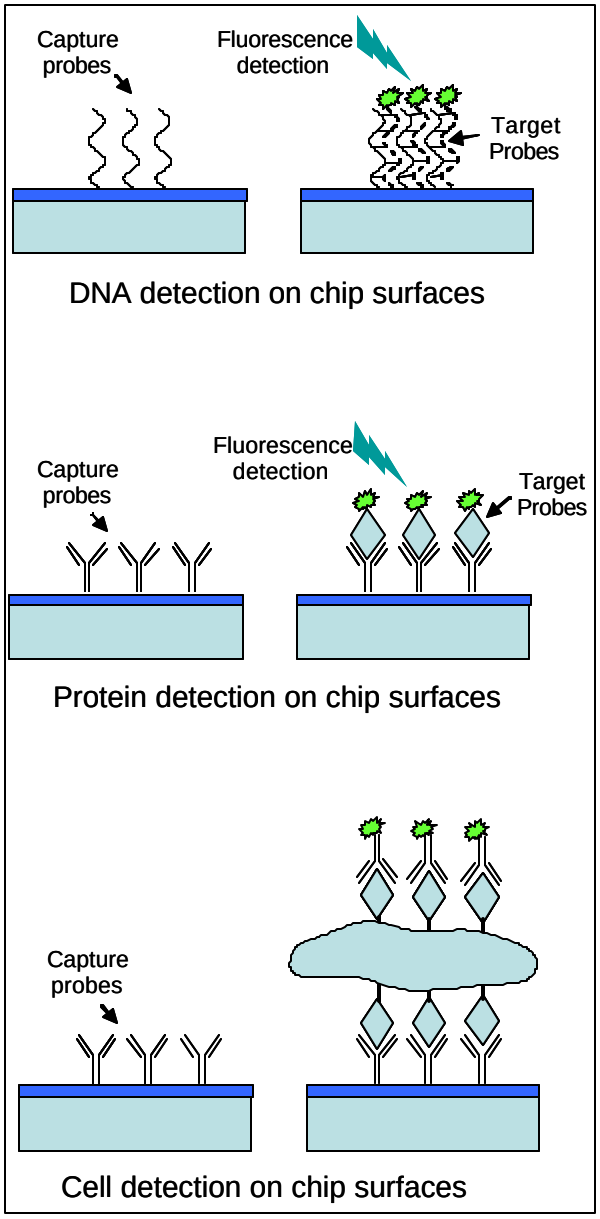
Stokes, Griffen, Vo-Dinh, Fresenius J Anal Chem, 369,:295-301, 2001

Optical Detection in Biochips

1. Fluorescence: Markers that emit light at specific wavelengths and enhancement, or reduction (as in Fluorescence Resonance Energy Transfer) in optical signal can indicate a binding reaction
2. Chemiluminescence: Generation of light by the release of energy as a result of a chemical reaction.

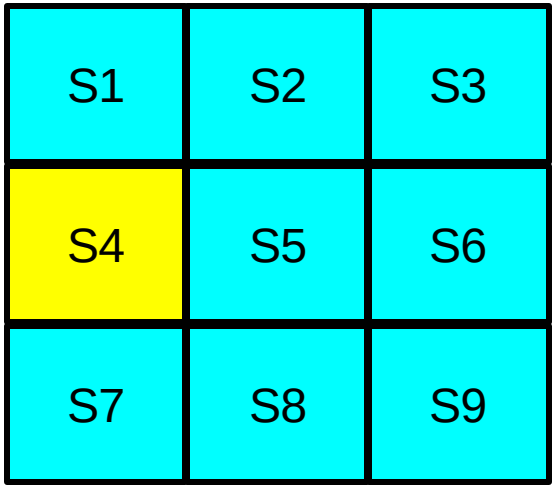
   - Light emission from a living organism is termed bioluminescence (sometimes called biological fluorescence),

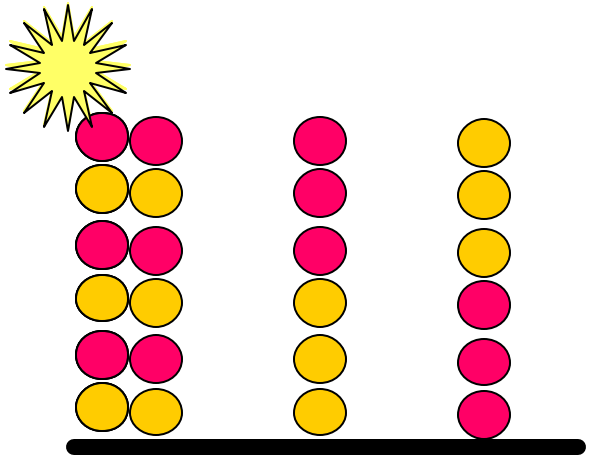
   - light emission which take place by passage of electrical current is designated electrochemiluminescence.



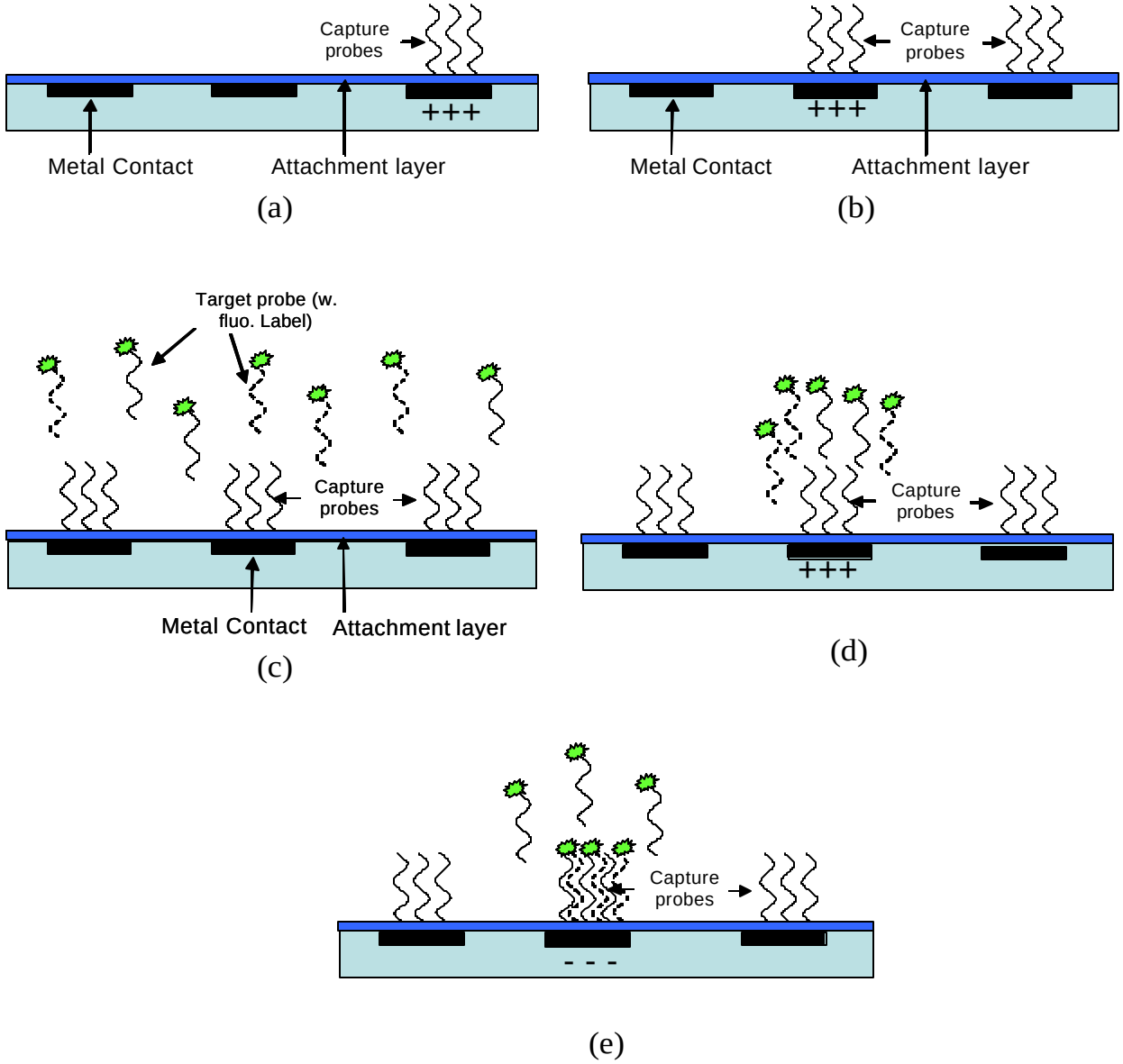
DNA Hybridization in Microarrays

- Basis for detection of unknown nucleotides
- Example: Bio-chips for identification of DNA
  - Hybridization of an unknown, flourescently tagged strand with a many known strands - reaction will determine the sequence of the unknown (or vice versa)
  - Strands can be lithographically (Affymetrix) or electronically (nanogen) defined at a specific location





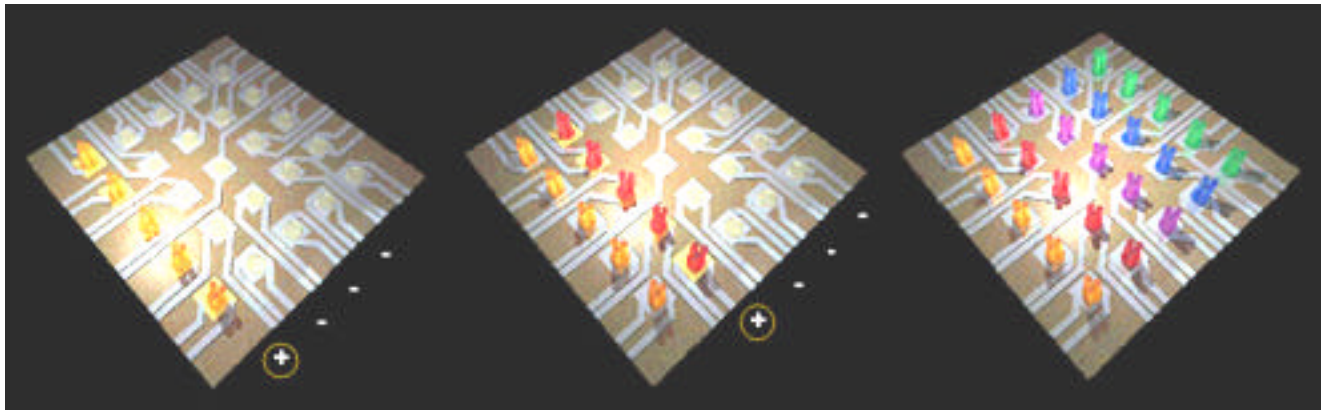
Electronic Placement of DNA Probes



DNA Biochips (Nanogen)

Technology Features:

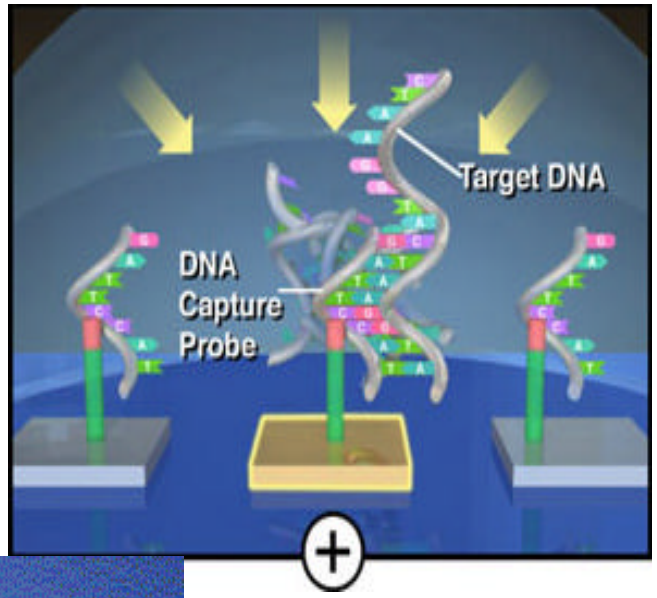
- Biochips for DNA detection, antigen-antibody, enzyme-substrate, cell-receptor and cell separation techniques.
- Takes advantage of charges on biological molecules.
- Small sequences of DNA capture probes to be electronically placed at, or "addressed" to, specific sites on the microchip.

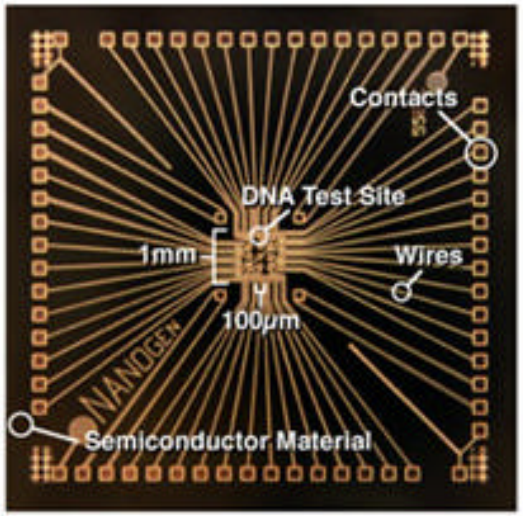


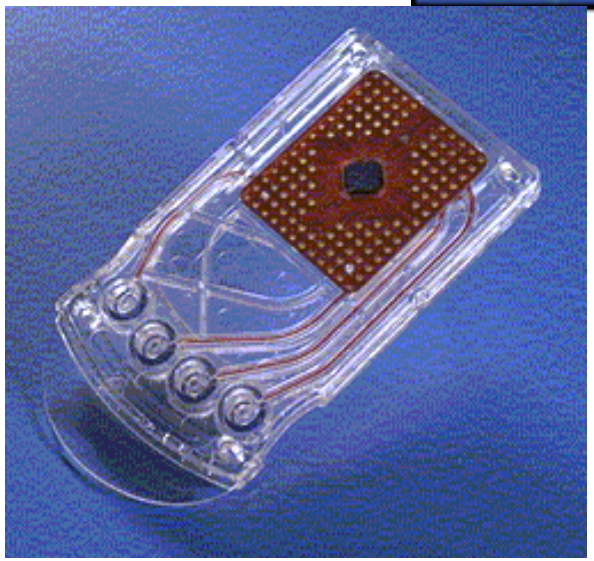
Technology Features

Hybridization.

- **A test sample can be analyzed for the presence of target DNA molecules by determining which of the DNA capture probes on the array bind, or hybridize, with complementary DNA in the test sample.**
- **Fluorescence output**

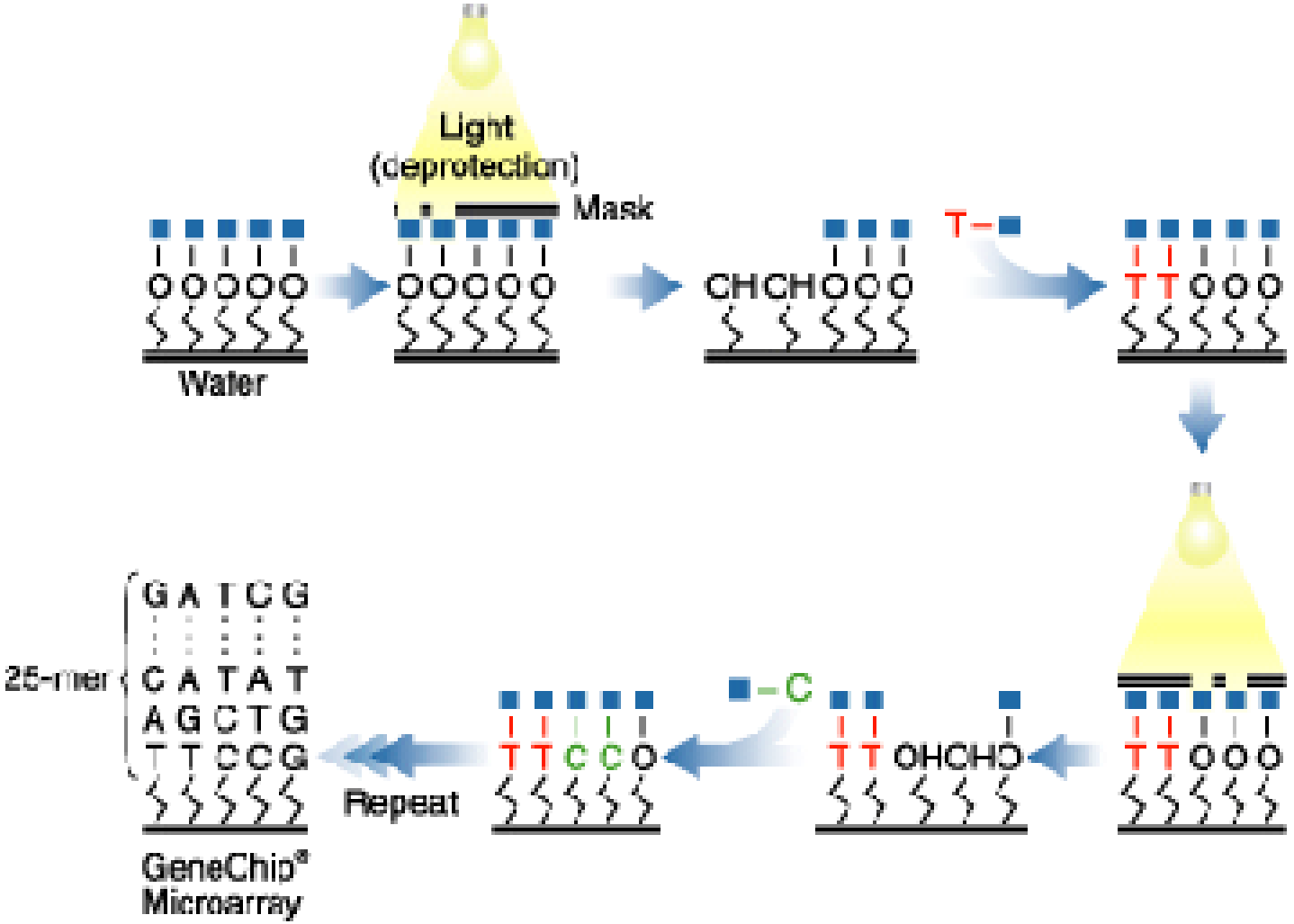






www.nanogen.com

Light Directed DNA Synthesis on a chip (Affymetrix)



Fodor, et al. 1991, www.affymetrix.com

Light Directed DNA Synthesis on a chip (Affymetrix)

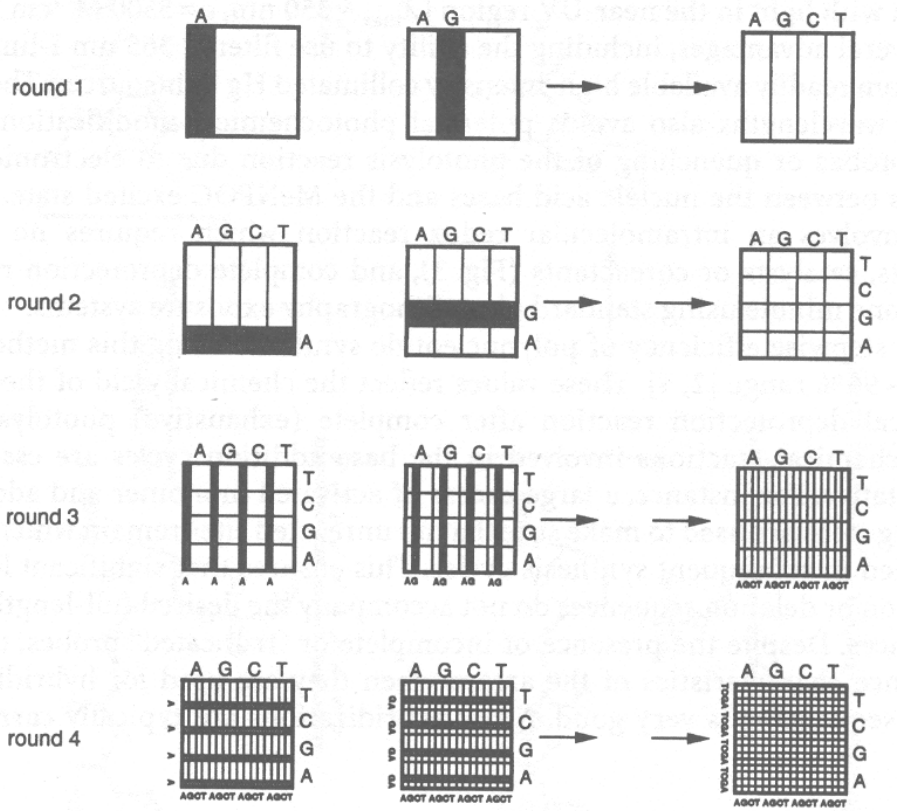
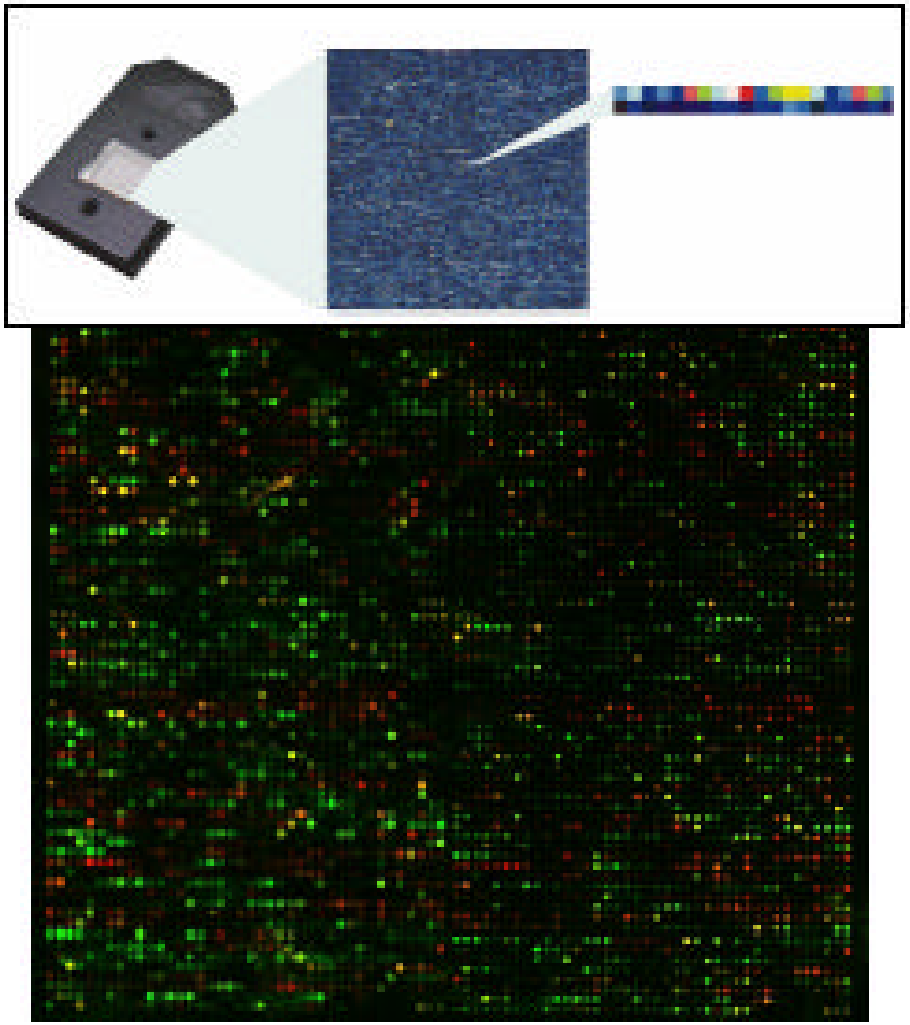


Table 1. Combinatorial synthesis of polynucleotide probe arrays

Probe Length	Chemical Steps	Number of Possible Probes
4	16	256
8	32	65 536
12	48	16 777 216
16	64	$\sim 4.3 \times 10^9$
20	80	$\sim 1.1 \times 10^{12}$

Fodor, et al. 1991,
www.affymetrix.com

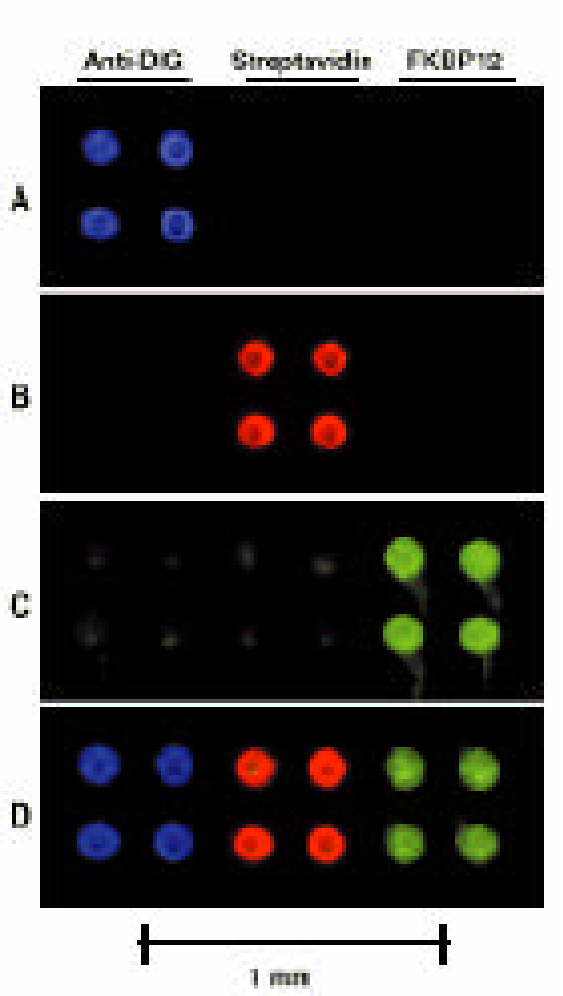
Light Directed DNA Synthesis on a chip (Affymetrix)

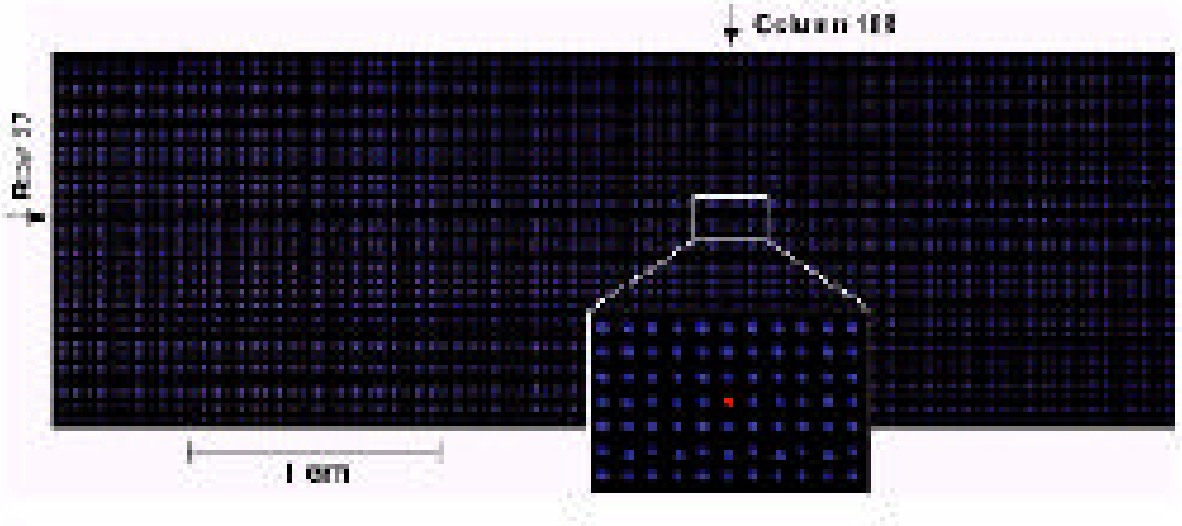


- Fluorescence detection
- Ultimately will limit size of pixel in array

Applications:
Polynucleotide array
HIV resequencing
mRNA expression monitoring

Protein Arrays





- Protein-Protein Interactions
- Protein small molecule interactions
- Derivatized substrates – glass, plastics
- High Throughput screening of chemical compounds

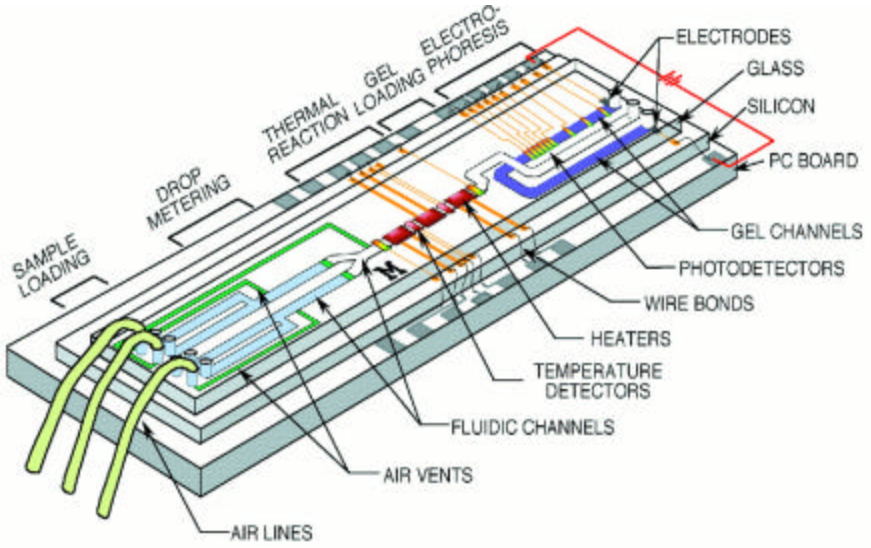
G. MacBeath, and S.L. Schreiber, Printing proteins as microarrays for high-throughput function determination, Science, 289, 1760, 2000.

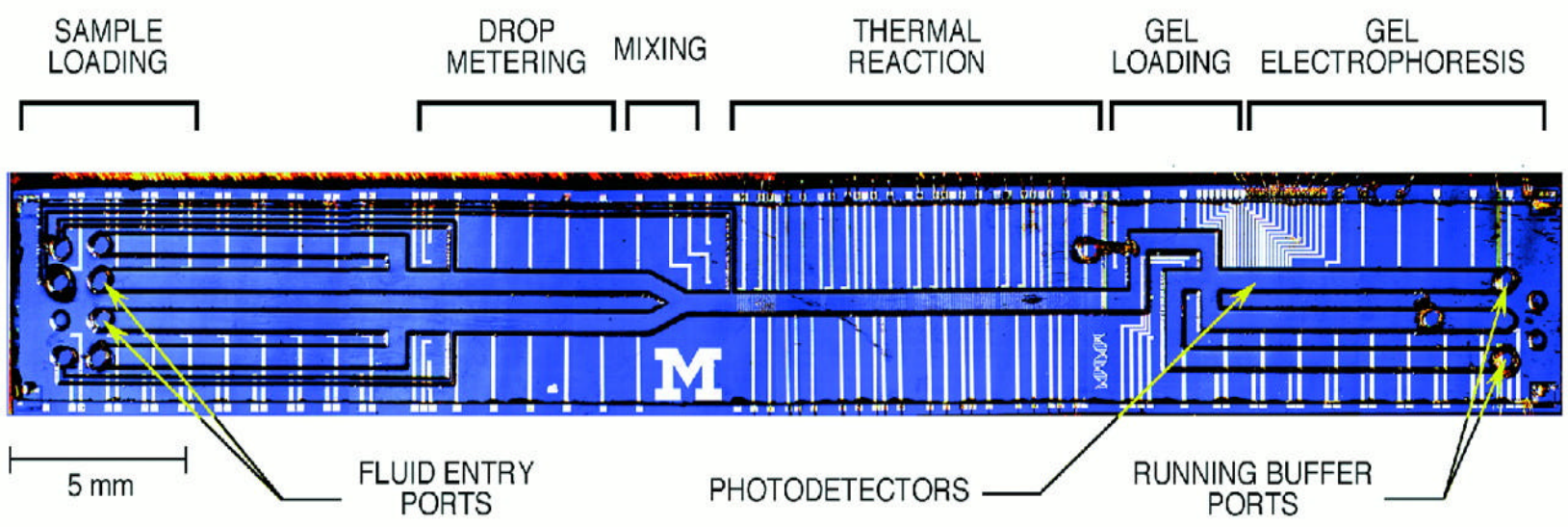
Note: Sensor Arrays

- Any of the individual sensors described earlier can be used in an array format to make micro/nano sensor arrays.
- The sensors in the array need addressing
- Each sensor can be functionalized with different bio-receptor molecule to detect different entities
- Examples, cantilever array, electrochemical detection in electrode arrays, cellular arrays for chemical detection, etc.

Lab-on-a-Chip/Integrated Devices

- Single chip device for DNA electrophoresis
- Sample loading and metering
- PCR on a chip (faster temperature cycling due to reduced thermal mass)
- Gel electrophoresis on chip

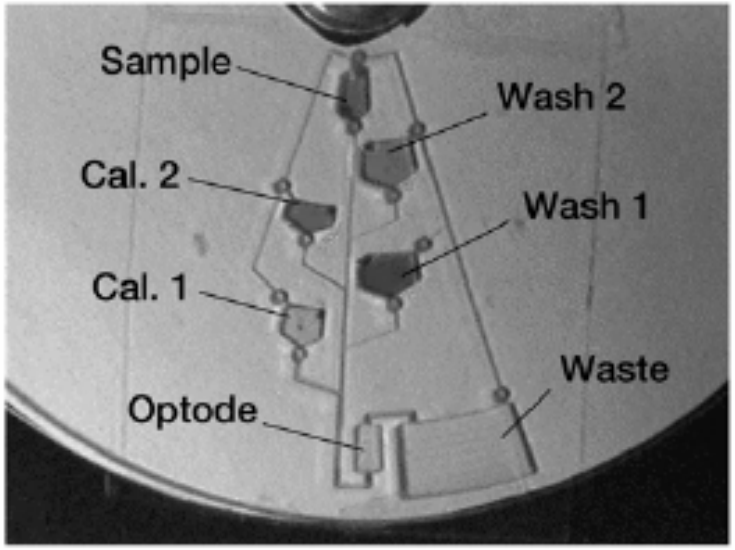




Burns, et al. 1998, Science, v 282, n 5388, Oct 16, 1998, p 484-487

CD Format Biochips

- Micro-fluidic devices on a CD type platform using centrifugal and capillary forces for liquid transport
- Cheap plastic CDs
- Optical detection systems

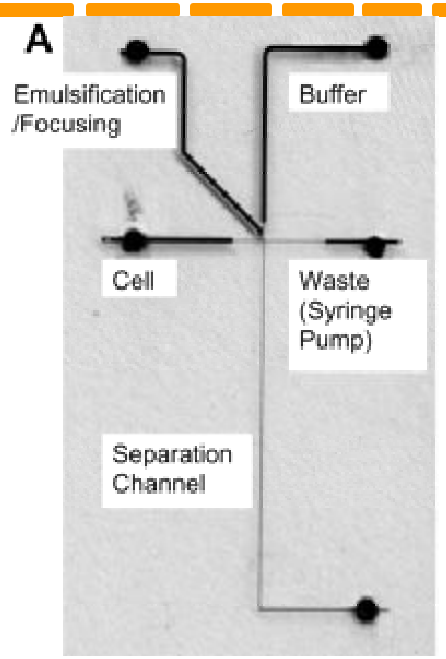


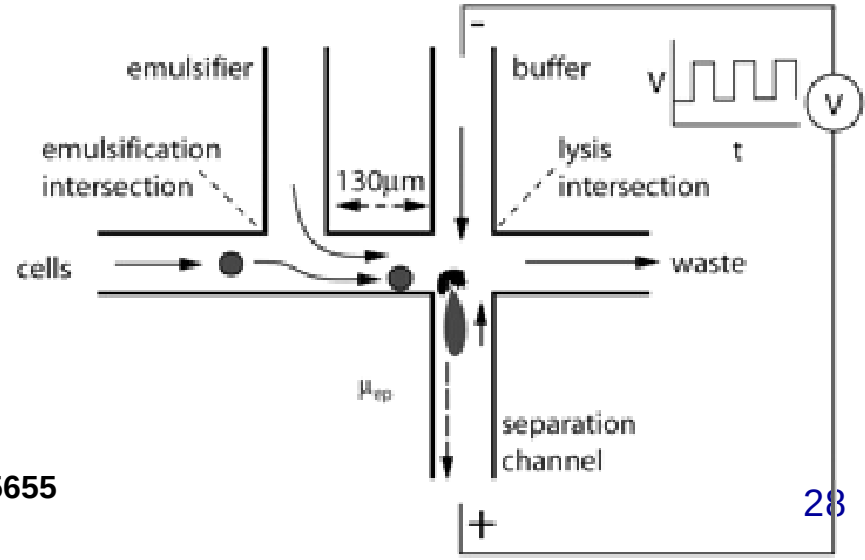
Flow order: Cal. 1 → Wash 1 → Cal. 2 → Wash 2 → Sample

Madou et al., 2001, Biomedical MicroDevices, v 3, n 3, 2001, p 245-54

Cellular Analysis on Chip

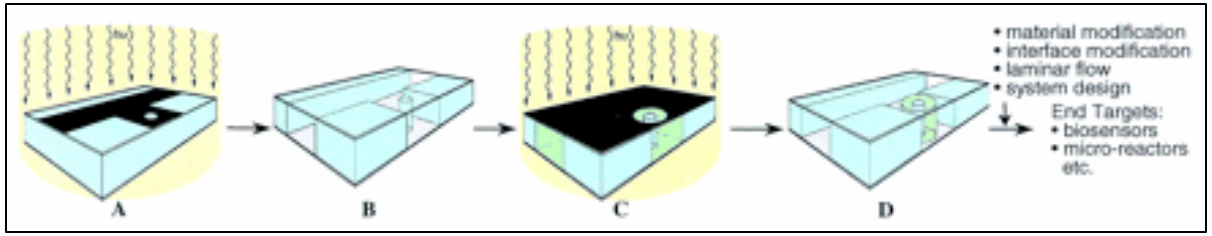
- Plastic biochips using hydrodynamic transport of cells
- Electric field mediated lysing
- Fluorescence detection (off-chip detectors)
- Analysis time of about 10 cells/minute



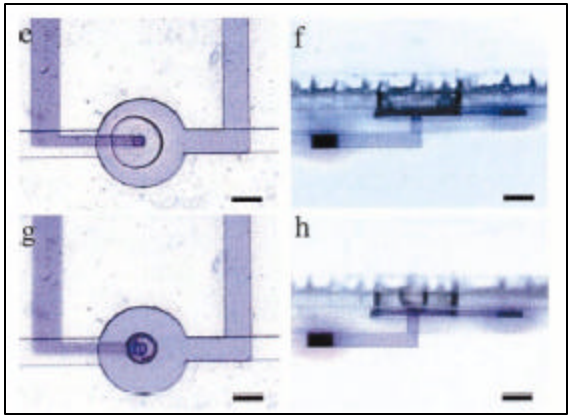


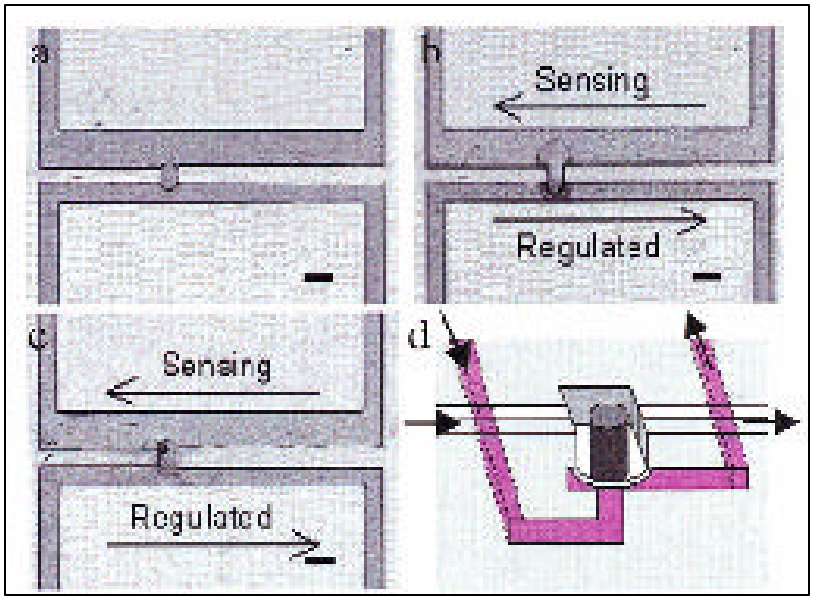
McClain, et al. Analytical Chemistry, v 75, n 21, Nov 1, 2003, p 5646-5655

Polymer μSensor and Actuator

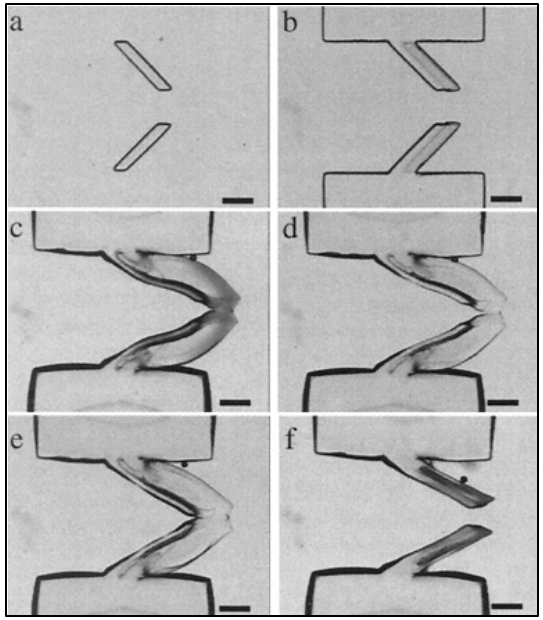


Process flow for the preparation of a hydrogel valve.





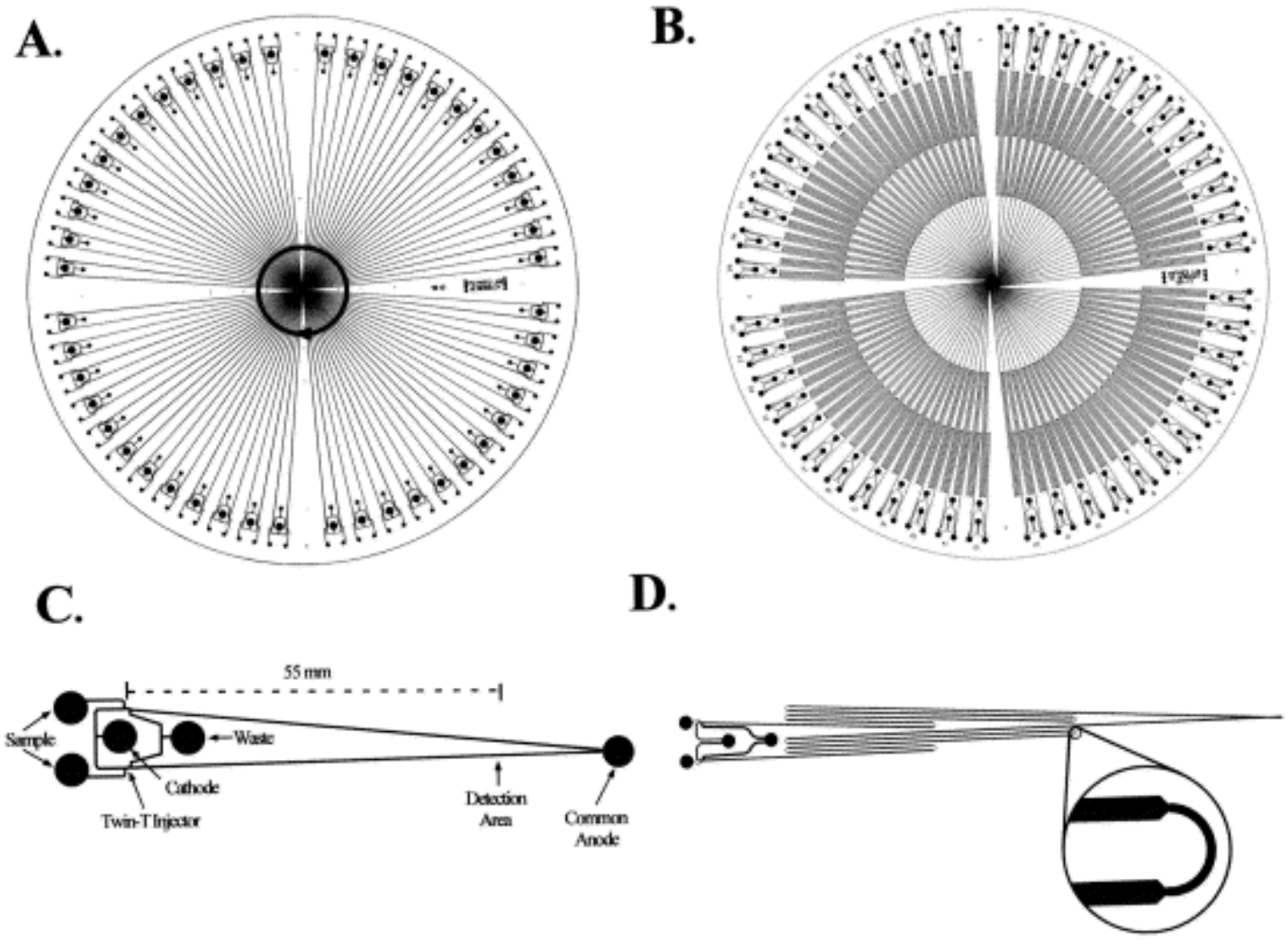
Hydrogel valve designs (2D and 3D)



A biomimetic valve based on bistrip hydrogel.

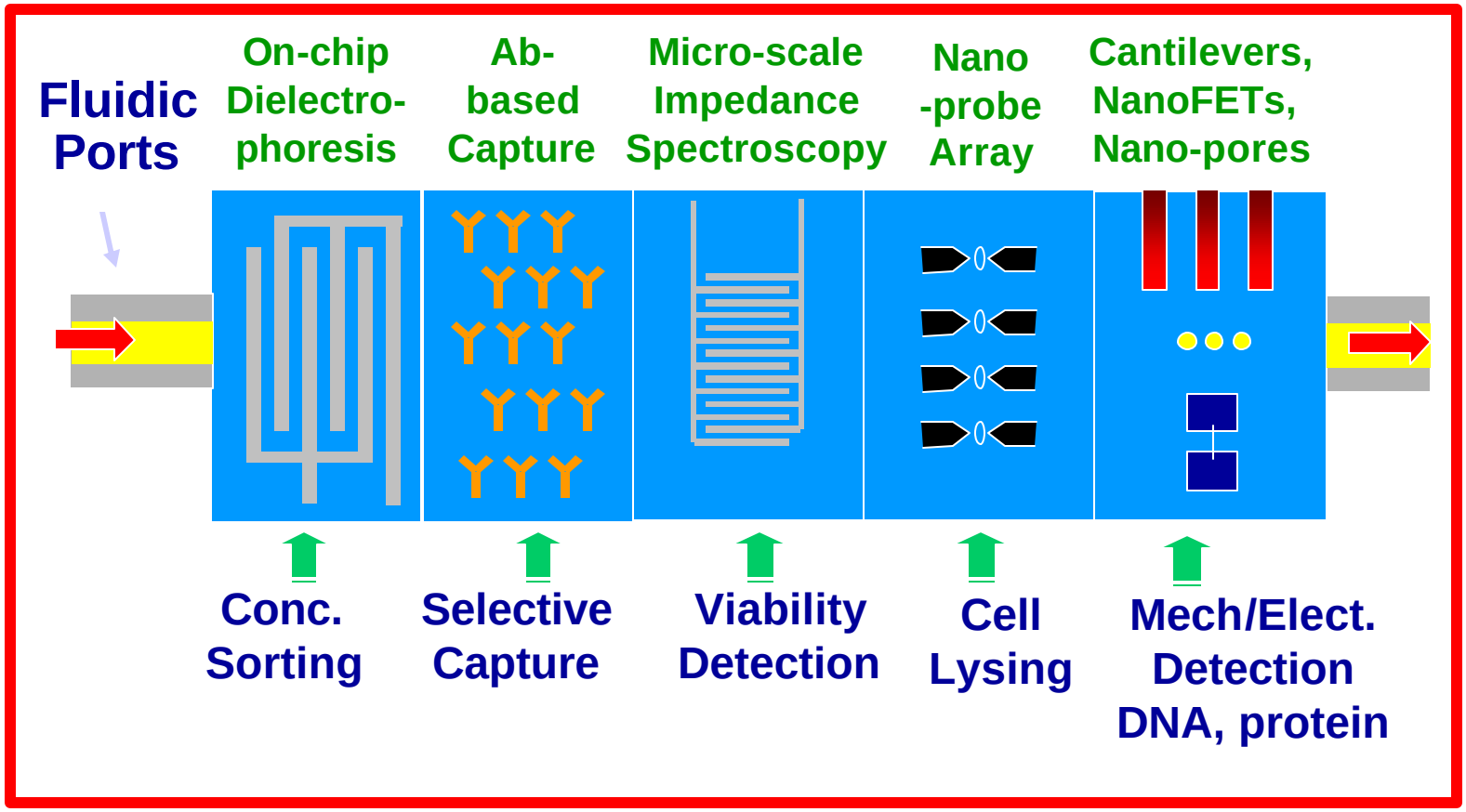
D.J. Beebe et al., Proc. Natl. Acad. Sci. U.S.A. **97**, 13488 (2000).

DNA Capillary Electrophoresis



Design of the 96-channel CAE microplate and radial scanner. Mask pattern used to form the 96 straight channel radial microplate on a 150-mm diameter wafer.

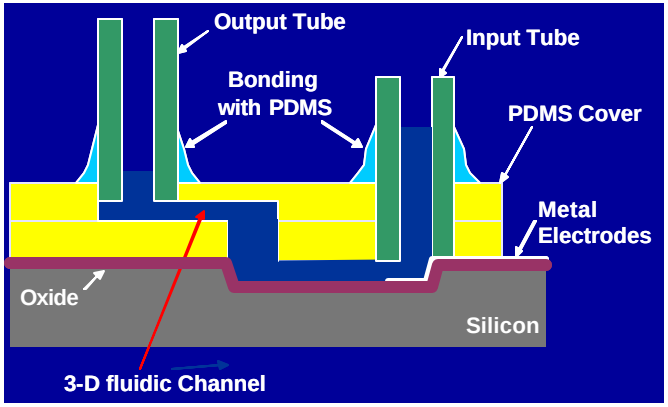
Integrated Systems for Study of Microorganisms and Cells

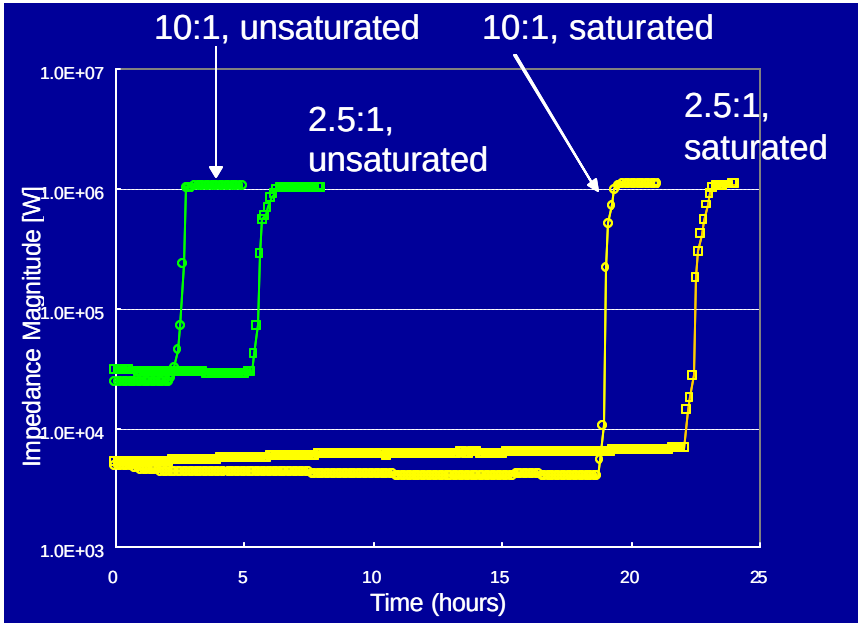


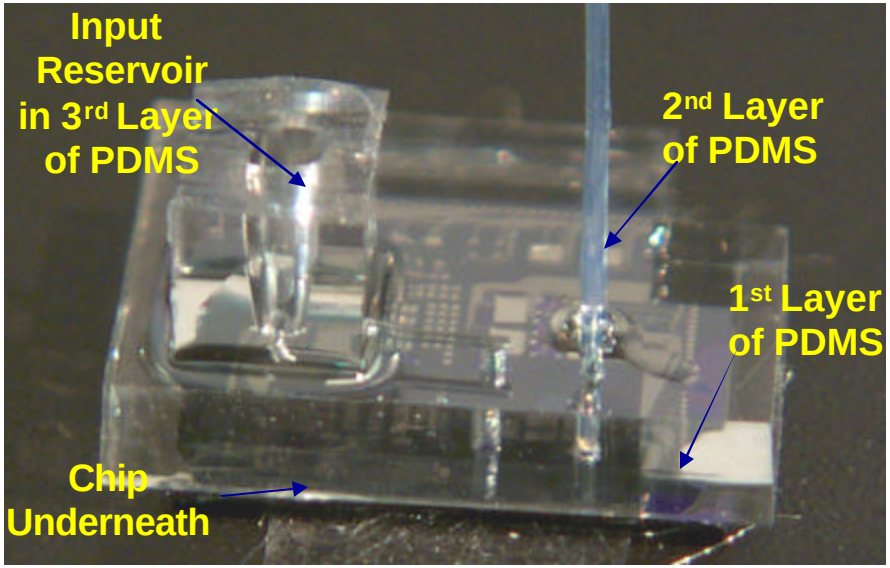
"Lab on a Chip" for
Enabled by BioMEMS and Bionanotechnology

Micro-fluidic Polymer Devices for Culture Bacteria and Spores

- Growth of bacteria inside a micro-fluidic polymer chip
- Rapid detection and reduced time to result





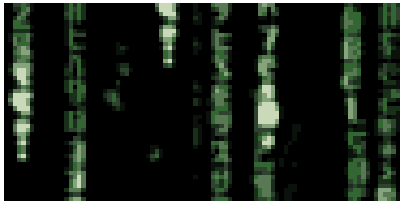


Silicon Base, 3 PDMS layers, Top I/O port

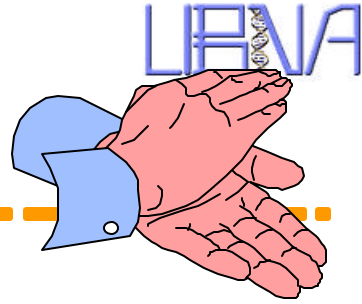
Woo-Jin Chang, Demir Akin, Miroslav Sedlek, Michael Ladisch, Rashid Bashir, , "Hybrid Poly(dimethylsiloxane) (PDMS)/Silicon Biochips For Bacterial Culture Applications", Biomedical Microdevices 5:4, 281-290, 2003,

Future Directions

- Integrated device for analysis of single cells – applications and fundamental science
- Building cell by cell/tissue engineering using micro and nano fabrication techniques
- Integrated diagnostics and therapeutics (drug delivery)
- Tools for genetic manipulation of microorganisms and viruses – synthetic biology



Acknowledgements



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- Samir Iqbal
- Oguz Elibol
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- Kidong Park

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- National Institute of Health
- DARPA Nanotechnology Research
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- Prof. A. Bhunia (Food Science)
- Prof. M. Ladisch (Ag& Bio Engr)

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