Lecture 5

- Biochemical sensors
- Biomolecular gain mechanism: Polymerase Chain Reaction (PCR, 聚合酶鏈反應)
- Micro Arrays
- Micro Total Analysis System

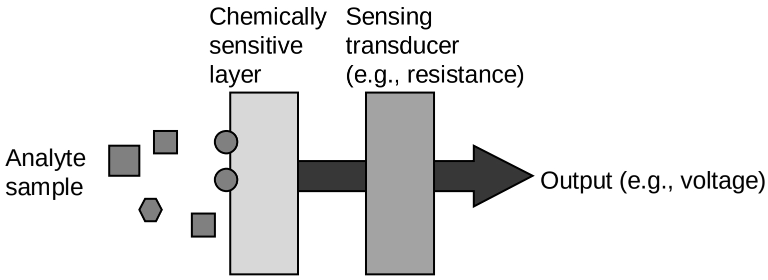
Biochemical Sensors

Biologix's PH sensor

Porton Diagnostic's Potassium sensor

Source: M. Madou
Fundamentals of microfabrication

Biochemical Microsensors

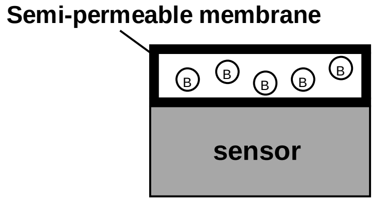


- A biochemical sensor is a device which is capable of converting a chemical (or biological) quantity into an electrical signal
- Basic components of the sensor include analyte molecule, chemically sensitive layer, and transducer

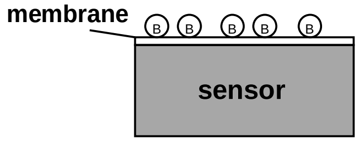
Principles

Principle	Measurand	Typical sensors
Conductimetric	Resistance	Tin oxide gas sensor
Potentiometric	Voltage	pH sensor
Amperometric	Current	Glucose sensor
Calorimetric	Heat/temperature	Pellistor gas sensor
Gravimetric	Mass	Surface acoustic wave (SAW) sensors
Coulometric	Charge/capacitance	Polymer humidity sensor

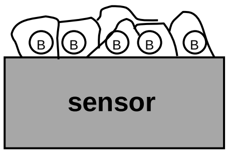
Immobilization of Biological Elements



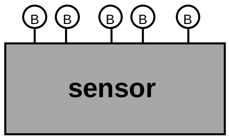
Membrane entrapment



Physical adsorption by a combination of van der Waals forces, hydrophobic forces, hydrogen bonds, and ionic forces



Matrix entrapment using a porous encapsulation matrix

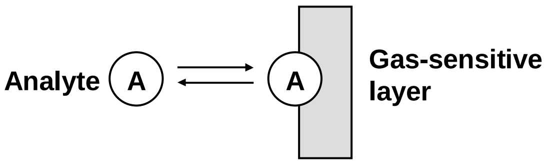


Covalent bonding using a processed sensor surface that has reactive group for binding

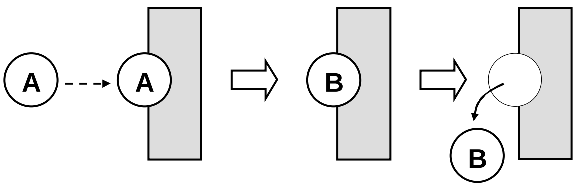
* After Dewa and Ko

Basic Mechanisms

- Reversible process (binding)

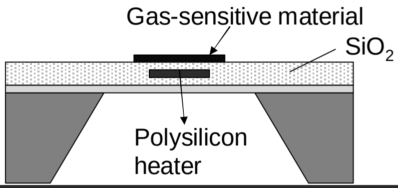


- Irreversible process (catalysis): most reactions are this type



Conductimetric Gas Sensor (Chemoresistor)

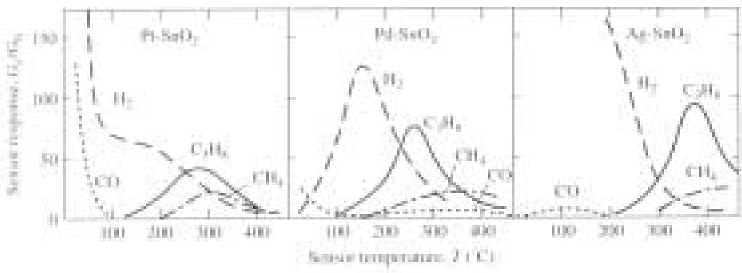
- **Semiconducting metal oxides (SnO_2, ZnO, Fe_2O_3) are commonly used (e.g., SnO_2 for CO, alcohol, H_2, H_2S, …etc)**
  - **Figaro Engineering in Japan is the famous manufacturer of the "Taguchi" tin oxide gas sensor**
- **The increase and decrease of resistance affected by:**
  - **Adsorption of O_2 on surface: $O_2 + 2e^-$ (due to vacant sites in SiO_2) $\rightarrow 2O^-$ (then R increases)**
  - **Reaction with combustible gases H_2: $H_2 + O^- \rightarrow H_2O + e^-$ (then R decreases)**
- **The devices are operated at high temperatures (typ. 300 – 400 °C)**
  - **Speed up the chemical reaction**
  - **Ameliorate the humidity effect**
  - **Yet consume a few hundred mW**



- **Image courtesy: Figaro Engineering, Inc., (http://www.figarosensor.com)**

Selectivity Enhancement by Doping

- Yamazoe et al., "Effects of additives on semiconductor gas sensors," *Sensors and Actuators B*, vol. 4, pp. 283-289, 1983



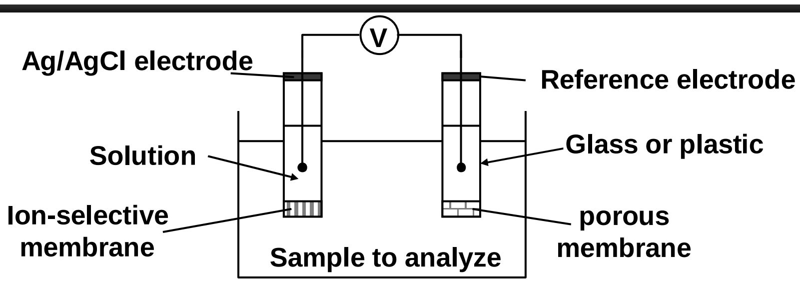
Potentiometric Electrochemical Sensing

- An electric potential is generated in response to a concentration change in a chemical sample
- Oxidation (forward reaction) and Reduction (reverse actuon):
  - D $\Leftrightarrow$ A + e^-
- The electric potential is governed by the Nernst equation:

$$E = \frac{RT}{nF} \ln \frac{a_{O_i}}{a_{R_i}}$$

  - R = 8.31441 J/(mol·K), T is the temperature in Kelvins, n is the valence for the ion, F = 9.64846×10^4 C/mol (Faraday's constant), and a is the ion activity (concentration and the degree of dilution)

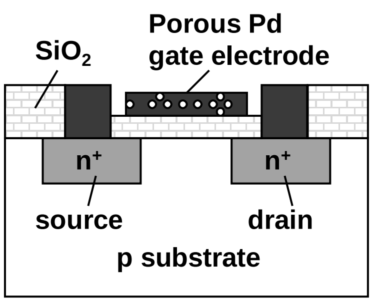
Measurement Arrangement using an ISE



- **Ion-selective electrode includes the membrane that is selective to the ion of interest, and the reference electrode has a non-selective membrane**
- **At 25 °C, the sensitivity is *59.12 mV* for each decade of variation in the change of activity for a *monovalent* cation**

Potentiometric MOSFET Gas Sensor

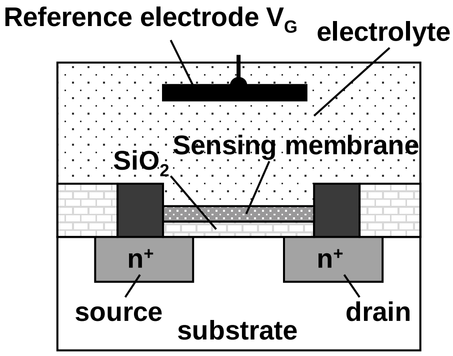
- **The gate electrode of MOSFET is made of gas sensitive**
  - **The nickname "CHEMFET"**
- **Hydrogen would dissociate and diffuse through to the interface, causing a shift in the threshold voltage V_{th}**
- **By using a constant current source to drive the transistor ⇒ $\Delta V_g = \Delta V_{th}$**
- **Other gate materials:**
  - **Platinum, iridium: typically operated at high temperature**
  - **Polymer (PolFET): room-temperature operation; susceptible to humidity**



I. Lundstrom, "Hydrogen sensitive MOS structures," Sensors and Actuators, vol. 1, pp. 403-426, 1981

Ion-Selective Field Effect Transistor (ISFET)

- Detect ions with a deposited ion-selective membrane on top of the gate oxide
  - The simplest form is the H^+ (pH) sensor in which the bare SiO_2 is the ion-selective layer (The use of Al_2O_3 gives more linear output though)
  - Na^+, Ca^{2+}, H^+, and K^+ for clinical applications
- The real gate voltage is associated with the potentials of the membrane and solution
- Can either sense V_G or i_D by fixing the other



P. Bergveld, *IEEE Trans. Biomed. Eng.*, vol. 19, pp. 70-71, 1970

Amperometric Sensing

- A voltage can be applied to an electrode in solution to measure the current as different species are oxidized or reduced
  - This is also called "Voltammetry"
- The voltages at which these reaction occur are indicative of the nature of each reaction an thus of particular species in solution
- Assume that the reactant species at the electrode surface is immediately depleted, the resulting current is ***diffusion limited***:

$$i(t) = nFAC\sqrt{\frac{D}{\pi t}} + \frac{nFACD}{r}$$

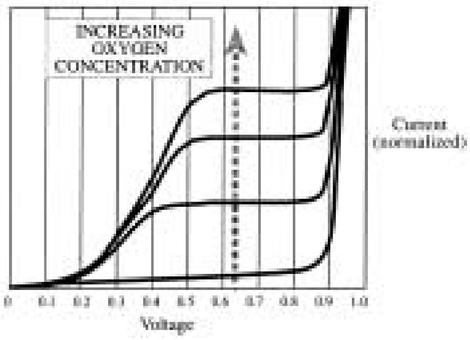
  - The first time-dependent term is known as the ***Cottrell equation***
  - n: number of electrons transferred, A: electrode area, C: concentration of the species of interest, D: diffusion coefficient of the species of interest, r: radius of the electrode, F: Faraday's constant

Amperometric Oxygen Sensing

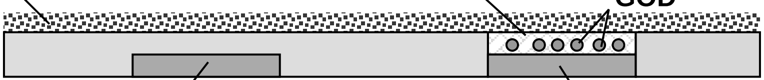
- Use a noble metal (e.g., gold, platinum, iridium) cathode coated with an oxygen-permeable membrane (e.g., Teflon™) in solution, and apply a voltage between the measuring electrode and the counter-electrode
  - The oxygen sensing electrodes also known as *Clark electrodes*
  - The reaction of dissolved oxygen at cathode:

$$O_2 + 2H_2O + 2e^- \rightarrow H_2O_2 + 2OH^-$$

$$H_2O_2 + 2e^- \rightarrow 2OH^-$$



Amperometric Glucose Sensor



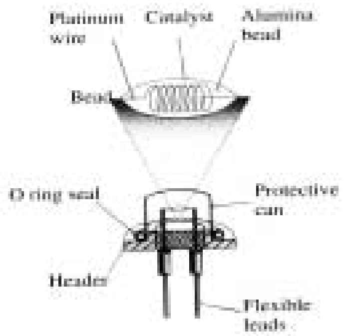
H. Hinkers et al., "Microdialysis system for continuous glucose monitoring," *Transducers 95*', pp. 470-473

- Reactions at the Pt electrode:
  - Glucose oxidized in the presence of oxygen and GOD:
    - » Glucose + $O_2 \rightarrow$ gluconolactone + H_2O_2
  - Hydrogen peroxide is oxidized and cause the current flow:
    - » $H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$

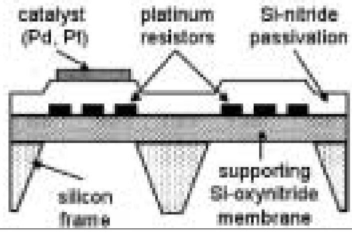
Microcalorimeters

- Measure the amount of energy (enthalpy) released or absorbed during the biochemical reaction through the associated change in *temperature*
  - A thermally isolated structure is desired for measurement
- Three types of calorimetric sensors:
  - Thermistor: $\Delta T = -\Delta E_h / c_p$, $R = R_o (1 + \alpha \Delta T)$
  - Pellistor
  - Thermopile

Pellistor



Conventional pellistor
(City Technology Ltd, UK)

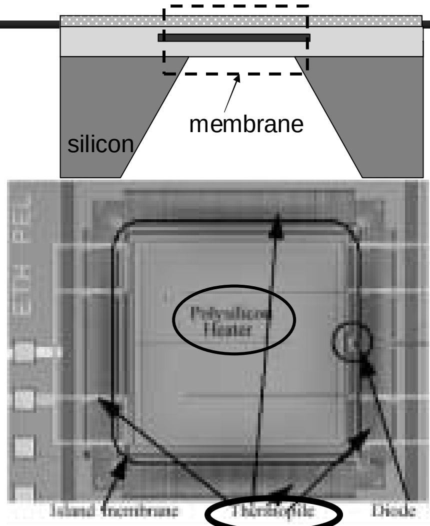


- Pellistor originally refers to a device consisting of a small platinum coil embedded in a ceramic bead, which is impregnated with a noble metal catalyst
- It is a catalytic thermal sensor that measures the heat evolved during the controlled combustion of flammable gaseous compounds (e.g., methane) in ambient air on surface of a hot catalyst by resistive sensing

M. Zanini et al, *Sensors and Actuators A* Vol. 48, pp. 187-192, 1995

A CMOS-MEMS Microcalorimeter

- A suspended n-well island is obtained by a back-side anisotropic silicon etch using KOH
- Use thermopile for sensing
- Use polysilicon heater to emulate the produced heat for calibration
- A sensing layer is sprayed-coated on top of the CMOS-MEMS membrane for ethamol, toluene, and humidity measurements



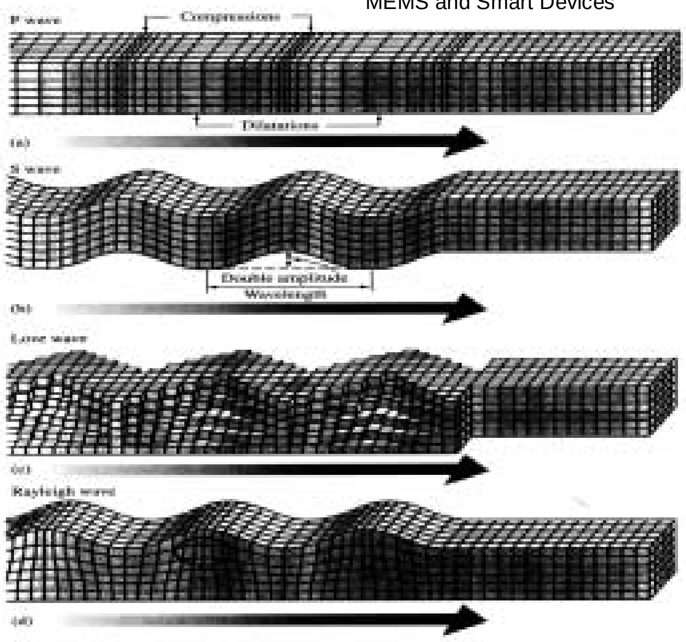
* N. Kerness et al., calorimetric chemical sensor, MEMS 2000

Mass-Sensitive Chemical Sensors

- The change of mass is often converted to a change of frequency
  - Immunoassay, electrochemical analysis, film deposition, etc
- Typically devices:
  - Piezoelectric actuation: can operate in solutions
    - » Quartz Crystal Microbalance (QCM)
    - » Surface Acoustic Wave (SAW) devices
  - Electrostatic actuation: usually for gas sensing
    - » Resonant beams

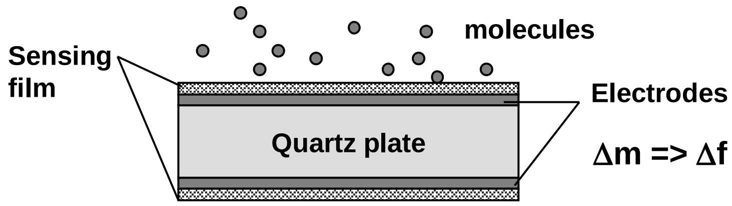
Different Acoustic Waves

Image from J.W. Gardner et al., Microsensors MEMS and Smart Devices



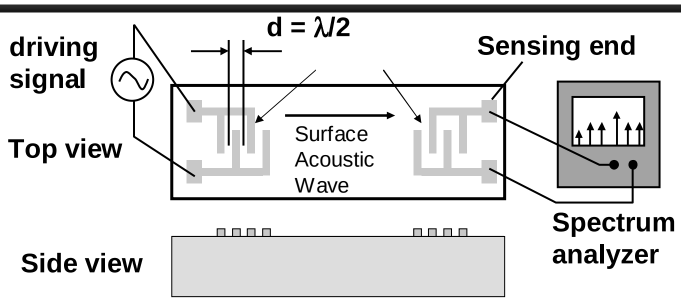
Surface Acoustic Wave →

QCM Sensor



- **Acoustic wave propagated through the bulk**
- **The change of frequency: $\Delta f = -2f_o^2\Delta m/[A(\rho\mu)^{1/2}]$; *Sauerbrey equation***
  - » **f_o is the fundamental resonant frequency, A is the electrode area, ρ is the density of the quartz (2648 kg/m^3), and μ is the shear modulus of quartz (2.95×10^{10} kg/m/s^2)**

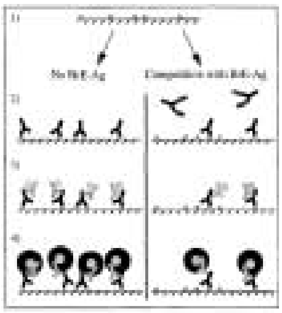
Interdigital Transducers in SAW Devices

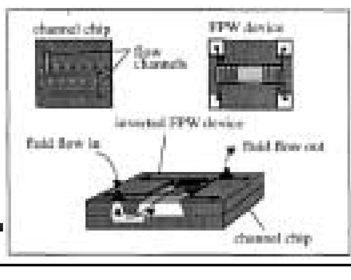


- Synchronous frequency $f_o = v/\lambda$, ranging from a few MHz to a few GHz
- Energy transfer: electrical input → piezoelectric actuation → piezoelectric sensing → electrical signal
- Change in the sensed physical nature (mass, density, viscosity, etc) gives a perturbed velocity, thus a ***phase shift***

Ultrasonic Immunoassay for Detection of Breast Cancer Antigens

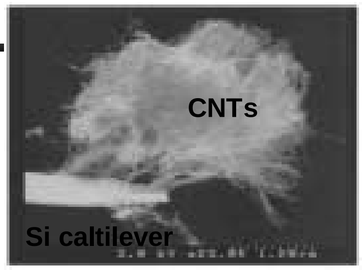
- A.W. Wang, *Int. Conf. on Solid-State Sensors and Actuators* (Transducers 97'), pp. 191-194 (UC Berkeley)
- Patient with breast cancer exhibit elevated blood serum levels of breast epithelial mucin antigen (BrE-Ag)
- Use of a gravimetric immunoassay with a mass-amplifying label to provide an alternative to radioimmunoassay
- Shift of original resonant frequency (f = 1.5 MHz) is less with more BrE-Ag

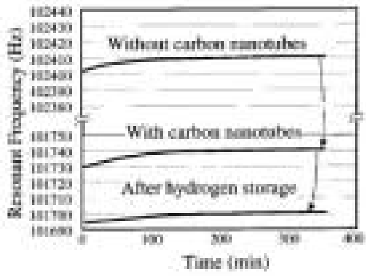




Mass Sensing using a Resonating Silicon Cantilever

- **The resonating silicon cantilever (170 nm thick) is annealed in ultrahigh vacuum to enhance the quality factor**
- **Electrostatic actuation**
- **Measured mass (hydrogen) resolution is 10^{-18} g, and the frequency stability is 5 Hz/day**

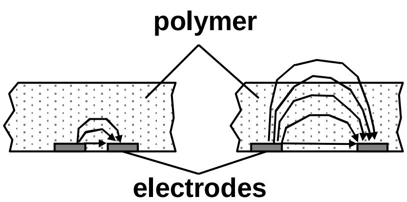




Esashi et al., *Rev. of Sci. Instr*., vol.74 No.3, pp. 1040-1043, 2003

Chemocapacitors

- **Polymer is often used as the dielectric material in the capacitor**
  - **Sense CO, CO_2, N_2, CH_4, humidity, etc**
- **The capacitance changes based on two mechanisms:**
  - **Dielectric constant changes as the analyte of interest dissolves into the non-conductive polymer**
  - **The polymer swells as it absorbs the analyte (not desired)**
- **Consume little power (no heating required)**

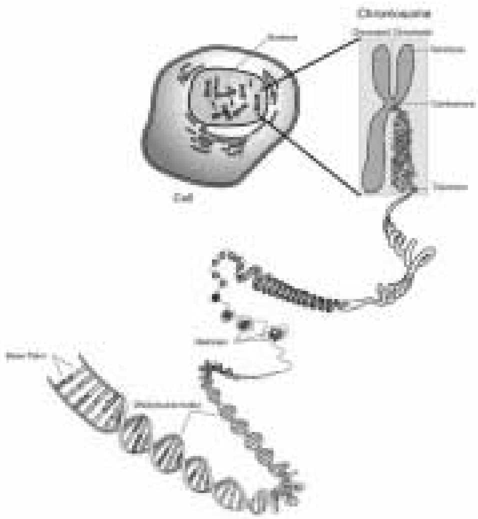


H. Baltes,"CMOS-based microsensors," *Proc. of Eurosensors XIV*, pp. 1-8, 2000
The electrodes with narrow gap won't measure the swelling effect as the E field is confined within the polymer

- Biomolecular Gain (PCR)
- Microarrays

Cell, Chromosome, and Deoxyribonucleic Acid (DNA)

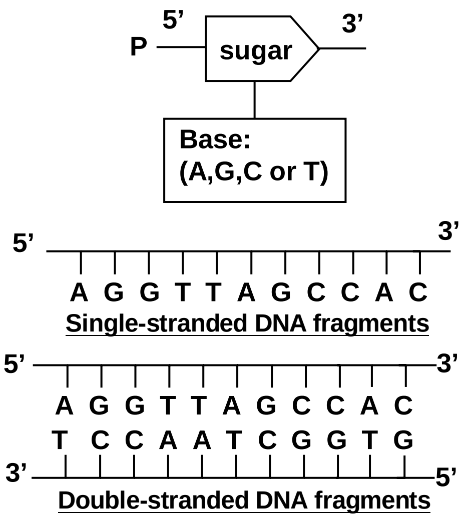
- **Genetic information in humans is stored in the cell chromosomes, which consist of long, compactly packed DNA strands**
  - **Each of 46 chromosome is 50 – 400 $\times$ 10^6 unit long**
- **The units of a single DNA strand are called nucleotides**



source: www.geneinformation.org

Nucleotides

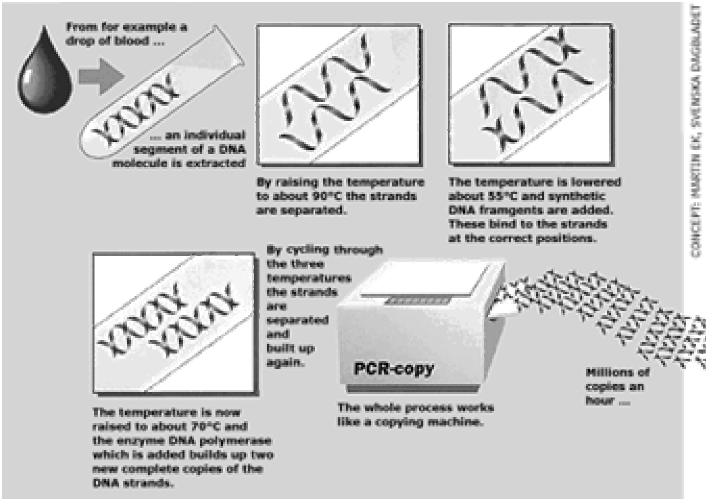
- Consist of a sugar linkage, a phosphate linking group at one end, and an amino acid group called a ***base*** attached to the sugar (A: adenine, C: cytosine, G: guanine, and T: Thymine)
- The label 5’ is attached to the 3’ end of the next nucleotide
- The double-helix molecule have complementary pairs of bases bonded to each other (G-C and A-T)



Biomolecular Gain: Polymerase Chain Reaction

- Selective amplification of signals (e.g., DNA) without noise is useful for biosensors
- Invented by Kary Mullis (Nobel prize in Chemistry 1993)
- DNA amplification and analysis is of great importance for sequencing the human genome, genetic profiling, forensics, and unknown sample identification and classification
- In PCR, the specific ***taq polymerase*** (聚合酶) is first obtained from a bacterium that thrives in the hot springs of Yellowstone National Park in 1969
- Micromachined PCR provides faster ***thermal cycles*** for DNA amplification than the traditional approach

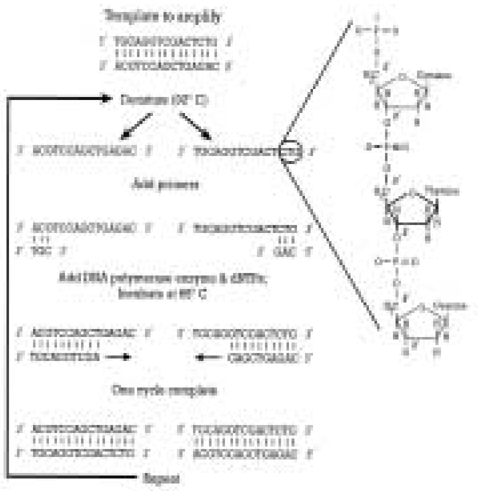
PCR



Courtesy: www.nobel.se

Cont'd

- PCR performs repeated thermal cycles:
  - Denaturing: raise temperature to ~95 °C; separate two bound strands
  - Annealing: ~65 °C; primer (a synthetically produced single-strand molecule) added to initiate attachment
  - Extension: ~72 °C; add nucleotides and polymerase enzyme to promote extension



Courtesy: N. Maluf, An introduction to Microelectromechanical Systems engineering

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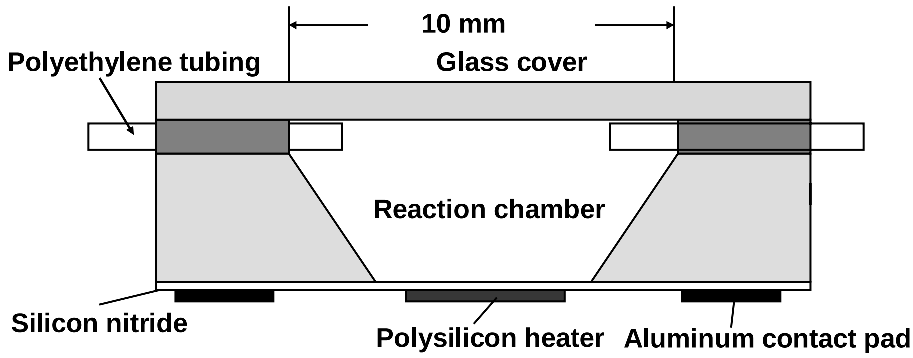


M.A. Northrup et al. "DNA amplification with a microfabricated reaction Chamber," *Transducers 93*', pp. 924-926 (Lawrence Livermore National Laboratories and Cepheid, Inc.)

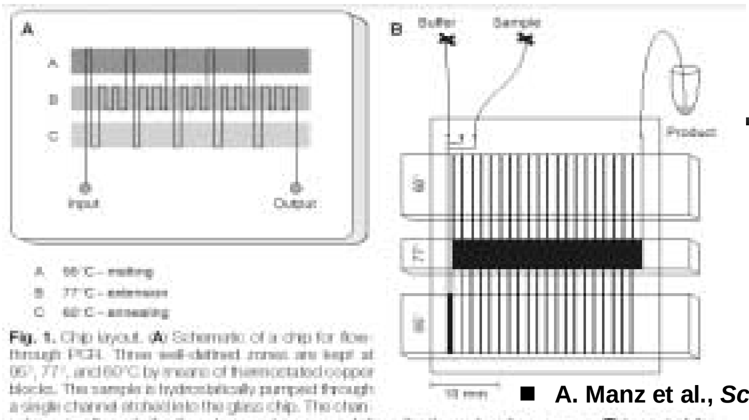


Fig. 1. Chip layout. (**A**) Schematic of a chip for flow-through PCR. Three well-defined zones are kept at 95°, 77°, and 60°C by means of thermostated copper blocks. The sample is hydrodynamically pumped through a single channel etched into the glass chip. The channel passing through the three temperature zones defines the thermal cycling process. (**B**) Layout of the device used in this study. The device has three inlets on the left side of the device and one outlet to the right. Only two inlets are used: one carrying the sample, the other bringing a constant buffer flow. The whole chip incorporates 20 identical cycles, except that the first one includes a threefold increase in DNA melting time. The chip was fabricated in Corning 0211 glass at the Alberta Microelectronic Centre, Canada. All channels are 40 μm deep and 90 μm wide; the etched glass chip and the cover plate are each 0.55 mm thick. Access to the channels is provided by holes (400 μm) drilled into the cover plate. Standard fused silica capillaries (outside diameter 375 μm, inside diameter 100 μm) are glued with epoxy into the holes of the chip. Virtually no dead volume is introduced by this connection. Two precision syringe pumps (Boehm 50300, 25 μl) deliver the PCR sample and the buffer solution onto the chip. The pumps are controlled by a program written in Labview running on a PC. Product is collected at the outlet capillary and then analyzed by slab-gel electrophoresis. The copper blocks are heated by 5-W heating cartridges, and the surface temperature is monitored by a Pt100 thin-film resistor mounted on the surface of the block near the chip contact area. Cooling fins passively cool the two blocks at 77° and 60°C. The temperature controllers are built with standard PID (proportional, integral, and derivative) digital temperature controllers (CAL 3200), power supplies, and switching electronics for the heating cartridges.

- **A. Manz et al., *Science,* 1998**

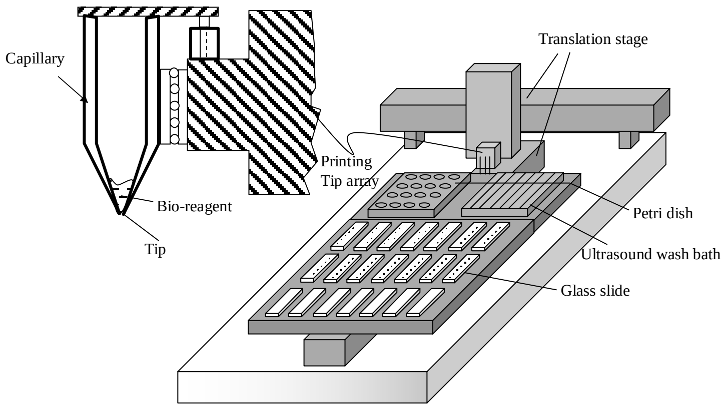
DNA Microarray Technology

- DNA arrays consist of large numbers of DNA molecules spotted in a systematic order on a solid substrate
- Advantages:
  - Faster
  - Parallelism: users can simultaneously screen hundreds to thousands of targets in a single experiment
  - Economic: less reagents and samples are needed
  - Automation: analysis can be automated through hardware and software

Types of DNA Microarrays

- Based on manufacturing:
  - Mechanical micro-spotting (delivery to chip)
  - Photolithography (synthesis on chip)
  - Ink jetting (delivery to chip)
  - Electrode addressing

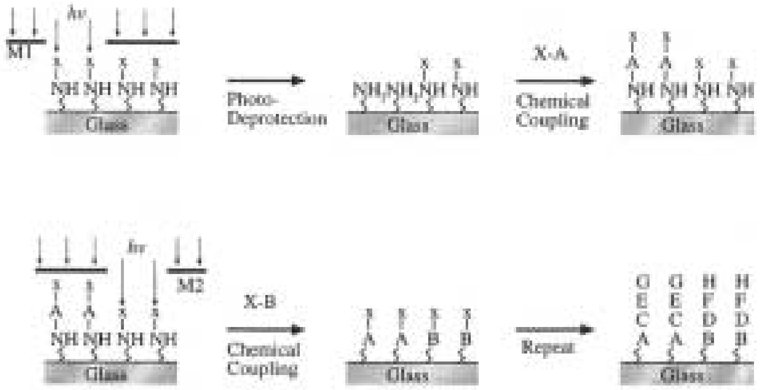
Mechanically-Spotted DNA Array



- **Image courtesy: Professor Patrick Brown, Stanford University**

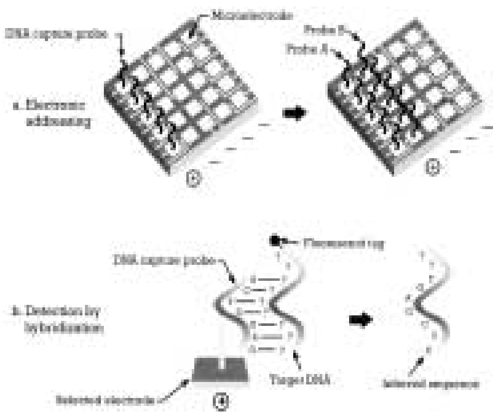
Synthesized Array by Affymetrix, Inc

- **Chemically synthesized oligonucleotides (低聚核苷酸) and peptides (肽) to detect complementary sequences for gene mapping, fingerprinting, and diagnostics**
- **40,000 different compounds in 1 cm^2**
- **4 lithography steps are required for DNA array (A, C, G, and T)**



DNA Addressing with Microelectrodes

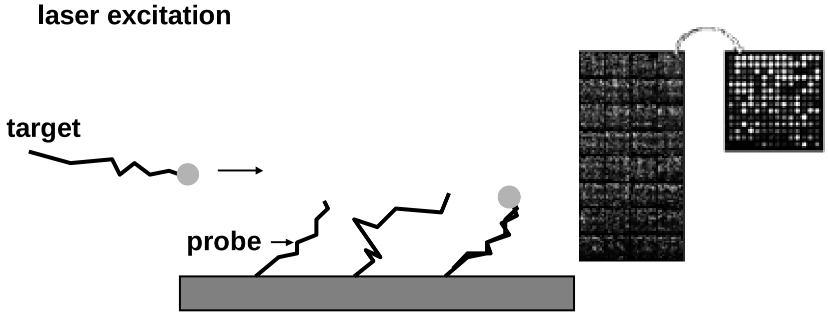
- ***Electronic addressing***: Use a positive voltage to attract DNA capture probes, repeatedly in different solution
- ***Detection by hybridization***: unknown DNA fragment attaches to the complementary sequence, as revealed by the fluorescence microscopy



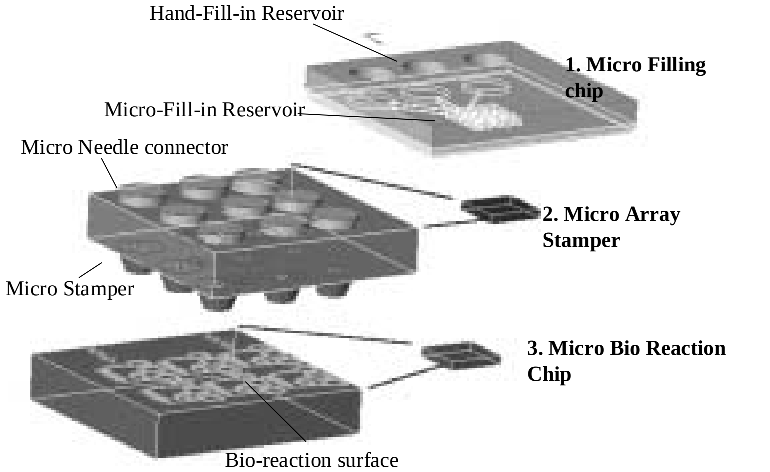
Nanogen, Inc. (image courtesy: Nadim Maluf)

DNA Microarray Assay Format

(1) Spotting DNA on solid substrate
(2) Sample isolation and labeling
(3) Probe/target hybridization
(4) Presence of bound DNA is detected by fluorescence following laser excitation

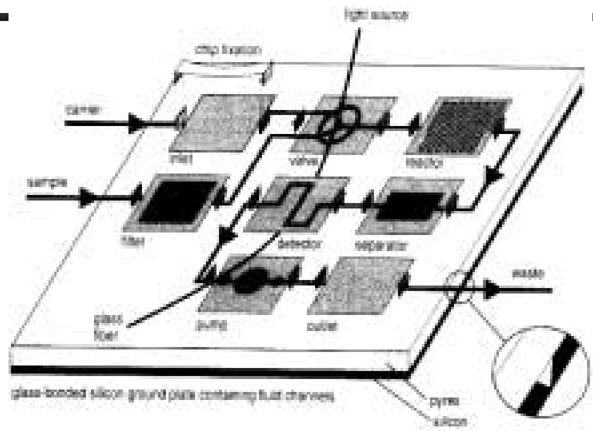


Micro Protein Array



- **Image courtesy: Professor Tseng, National Tsing Hua University**

Micro Total Analysis System (μ-TAS)



Source: G.K. Lebbink, Mikroniek, 3, pp. 70-73, 1994

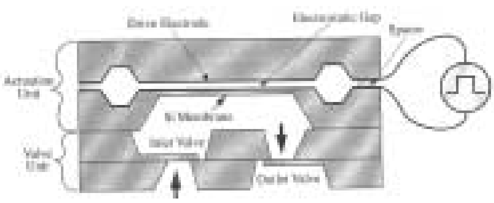
- **Includes moving samples, calibration of fluid through tiny conduits, control of fluid temperature of fluids, and detection, etc**

Microfluidic Devices

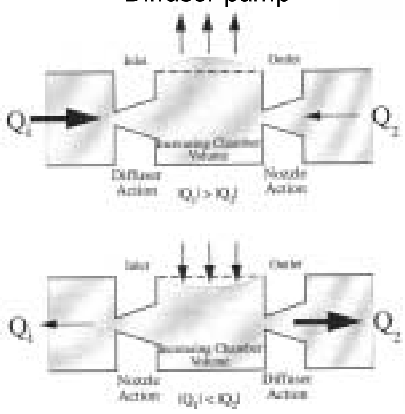
- For moving fluid:
  - Micropump
  - Acoustic streaming
    - » Use an oscillating sound field at the solid-fluid boundary
  - Electrophoresis and Electro-osmosis
  - Etc
- Mixers
- Flow channels
- Valves

Pumps

Electrostatic pump



Diffuser pump



- The required pumping pressure has to consider flow resistance

Fluidic Resistance

- Fluidic resistance is defined as the ratio of pressure drop over flow rate:

$$R = \frac{\Delta P}{Q} \quad in\ N \cdot s / m^5$$

- For a pipe (length L) with a circular cross section (radius r), assuming laminar flow (μ: viscosity):

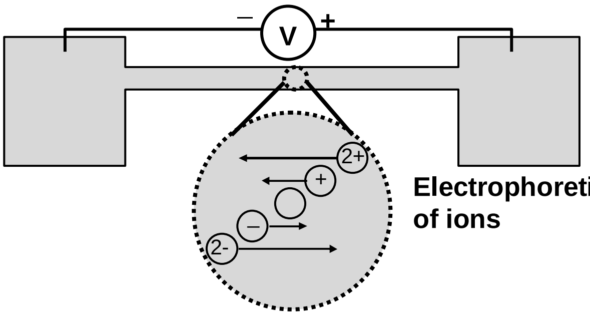
$$R = \frac{8\mu L}{\pi r^4}$$

- For rectangular channels (*w* >> *h*):

$$R = \frac{12\mu L}{wh^3}$$

- Reduction of channel size increases the fluidic resistance

Electrophoretic and Electroosmotic



Electrophoretic movement of ions

- The two popular mechanisms used in microfluidic chips for moving/pumping liquids
- Electrophoresis is essentially the movement (drift) of ions relative to solvent molecules under an externally generated electric field, and it is the ions themselves that are "pumped"

Principles of Electrophoresis

- The velocity v of an ion with charge n is proportional to the applied electric field:

$$v = \mu E$$

- Ion mobility μ ($m^2/(V \cdot s)$) is related to the total charge and the friction coefficient f :

$$\mu = \frac{ne}{f}$$

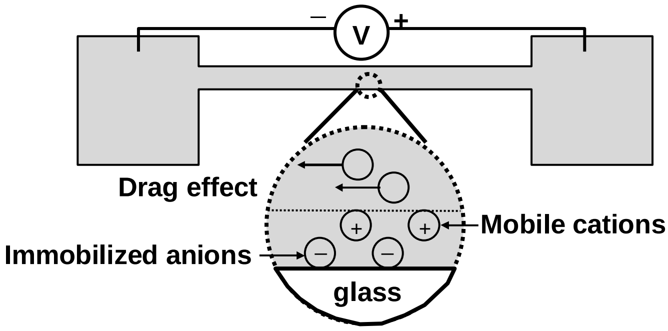
- f is related to ion radius r, liquid viscosity μ_l by:

$$f = 6\pi r \mu_l$$

- Therefore:

$$v = \frac{ne}{6\pi r \mu_l} E$$

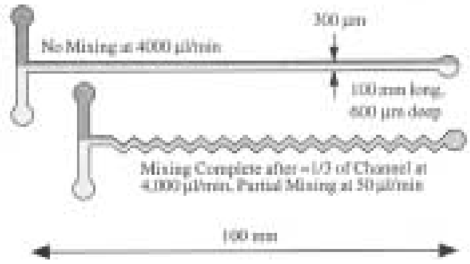
Principles of Electroosmosis



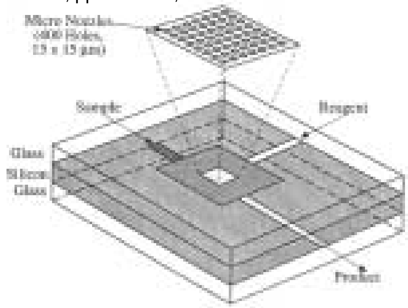
- It is more of a bulk phenomenon than electrophoresis, and results in pumping of electrically neutral fluid, if there are immobilized charges on the walls of the flow channel
- The ***double layer***, where most charges exist, in ionic solutions can be moved under external E field to drag the fluid

Mixers

- The mixing of two fluids requires that their contact area is maximized
- Turbulent flow can be used in macro- and some meso-scopic fluidic devices, but is difficult to be realized in micromachined devices

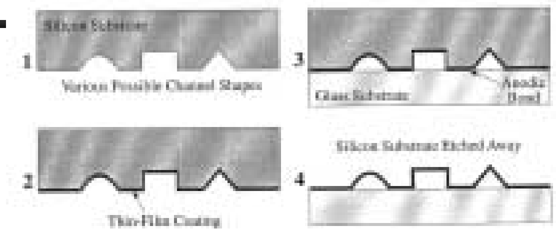


Branebjerg et al., *Proc. of Micro Total Analysis Systems Conference*, pp. 141-151, 1994



Miyaki et al., pp. 265-270, MEMS 1993

Channels



Bulk micromachining

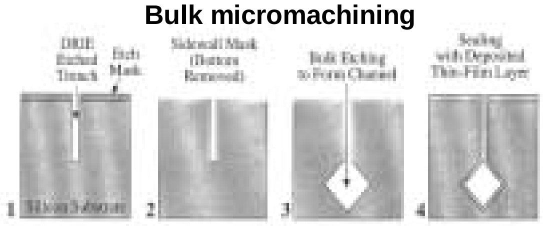


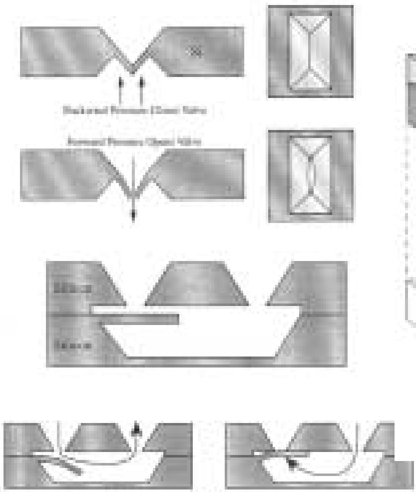
Illustration of a process for forming buried, bulk-etched fluidic channels in a silicon substrate, and subsequently sealing them with a deposited thin-film layer. Adapted from Tjerkstra, et al. (1997).

Source: G. Kovacs

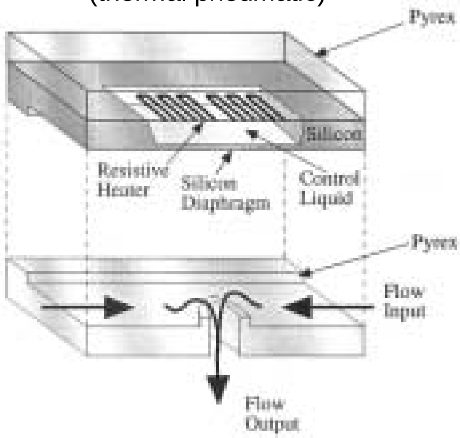
Buried micro-channel

Valves

Check valves (passive)



Active valves
(thermal pneumatic)



Source: G. Kovacs