Nano-Micro Biomolecular Sensors

Chih-Ting Lin

Gradate Institute of Biomedical Electronics and Bioinformatics

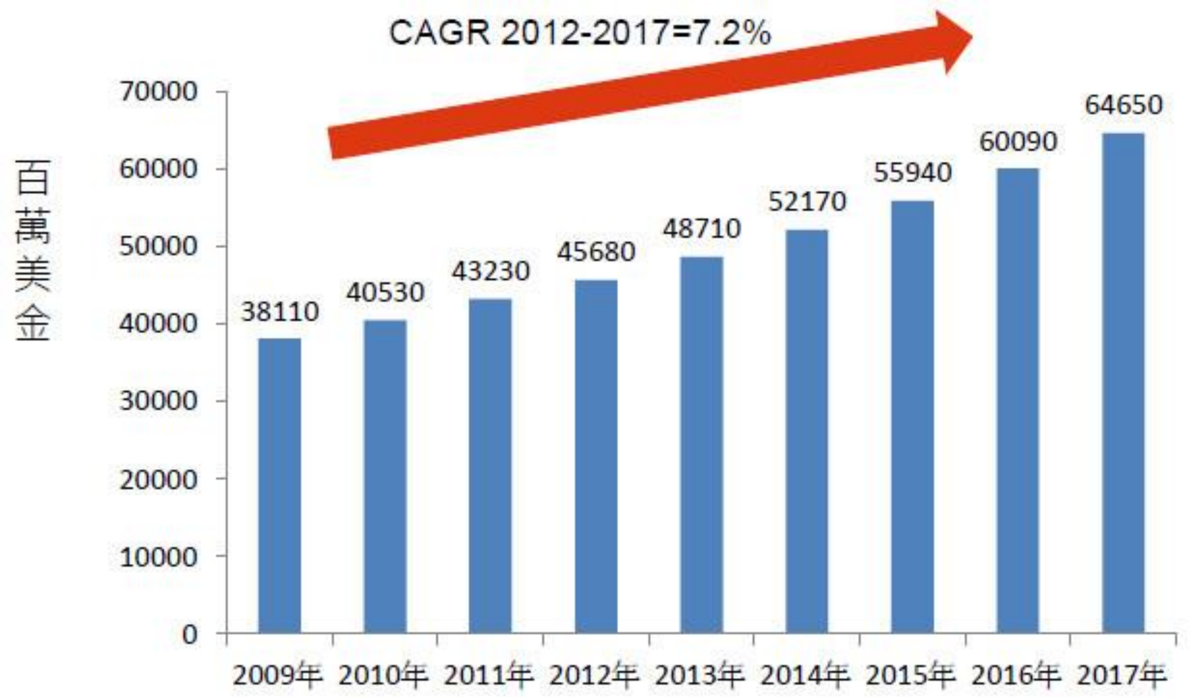
National Taiwan University

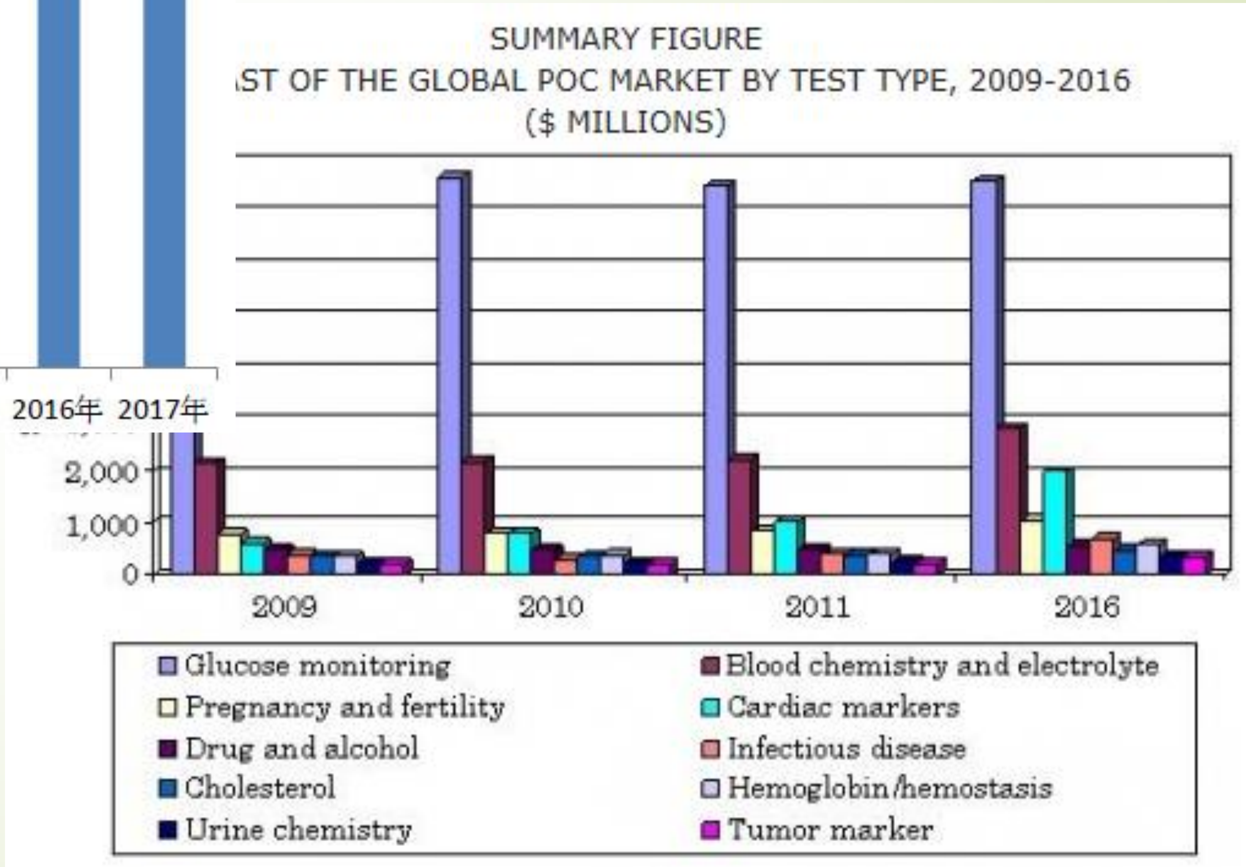
Electronic Devices for Diagnosis and Prognosis

- Traditional medical electronics
  - Signal processes: ECG, EMG, and EEG
  - Imaging systems: Ultrasonics, Magnetic Resonance Image (MRI), and Computerized Tomography (CT)

- Healthcare paradigm shift: personalized healthcare
- Emerging biotechnologies with nano/micro technologies
  - Biochip technologies

Growing Markets





Bio-Chip

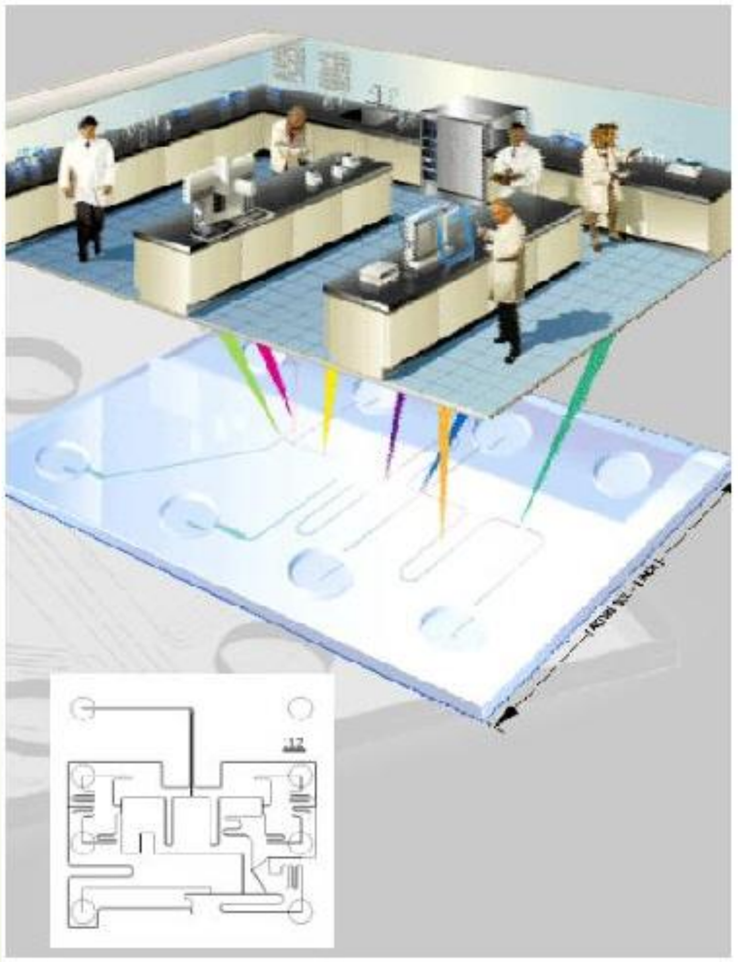
- Miniaturized bio-diagnosis devices or systems
  - Promoted by modern nano/micro technology
  - Used to detect or analyze the biomolecular composition
  - Utilizing different transduction methods to enhanced the sensitivity and reduce the cost
- It has become an emerging research field
  - To understand the life science
  - To identify diseases, drugs, and therapies

Necessity of Biochip

- Biomolecular sensing and diagnosis is the fundamental technique
  - Virus detection, protein analysis, cell reaction, and environmental monitoring
  - Life science and health care
  - The method must be simple, selective, and highly sensitive
- Traditional protein bio-diagnostics
  - Centrifugation, electrophoresis, chromatography, immunosorbent assay, and mass spectrometry
  - Time consuming, large amount of samples, High cost
- Miniaturized analysis systems
  - Bio-chip or Lab-on-a-chip
  - potential to reduce cost and waste of bio-diagnostics
  - Personalized health-care

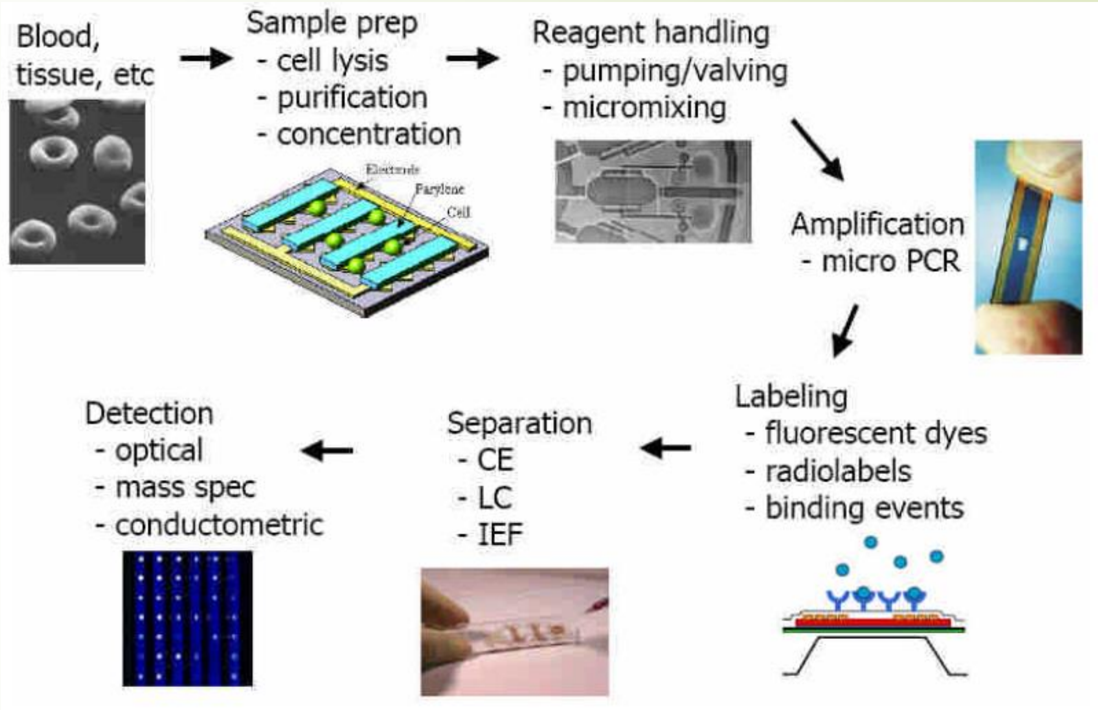
Lab-on-A-Chip I

- It can achieve miniaturization, automation, and full-integration
  - Parallel process
  - Save the reagent
  - High reproducibility
  - Anti false positive detection



Lab-on-A-Chip II

- It integrates
  - Sample loading
  - Fluidic transport
  - Sample preparation: concentration, separation, and reaction
  - Detection



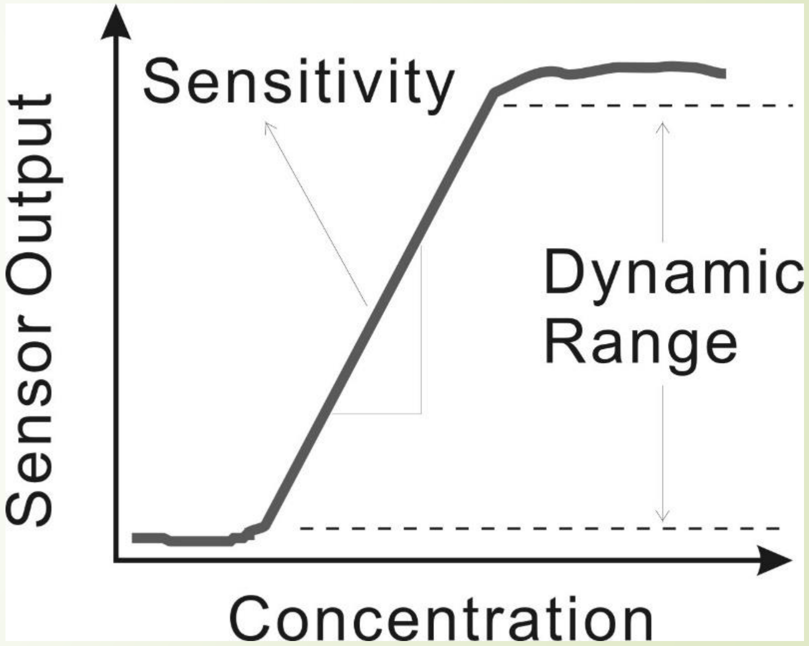
http://www.glud.umd.edu

Lab-on-A-Chip III

- Advantages
  - Low sample volume consumption
  - Higher analysis throughput and better efficiency
  - Better bio-process control because of faster response
  - Compact system
  - Lower device costs
- Disadvantages
  - Device/system performance highly depends on microfluidic condition
  - Relative low signal to noise ratio because of small sample volume
  - Not fully developed
- Implemented systems
  - PCR chip
  - Electrophoresis chip
  - Single-cell analysis chip

Bio-Sensor Characteristics I

- Sensitivity
  - The ability to describe the targeted biomolecular concentration
  - It is typically defined as the ratio of the signal response and the targeted biomolecular concentration
- Dynamic Range
  - The effective detection range of the device
  - Typically, it means the linear range



Bio-Sensor Characteristics II

- Selectivity
  - The ability to detect the targeted molecules among different kinds of molecules
  - It is defined as the sensitivity of targeted molecules divided by the sensitivity of non-targeted molecules
  - Typically it is based on the biomolecular binding affinity
  - However, it will be affected by the design of different sensing tranconduction methods
- Limit of Detection
  - The lowest molecular concentration can be identified by the sensor
  - Typical range is around nano-molar(10^{-9} M), some specific method can achieve atto-molar (10^{-17} M)

Bio-Sensor Characteristics III

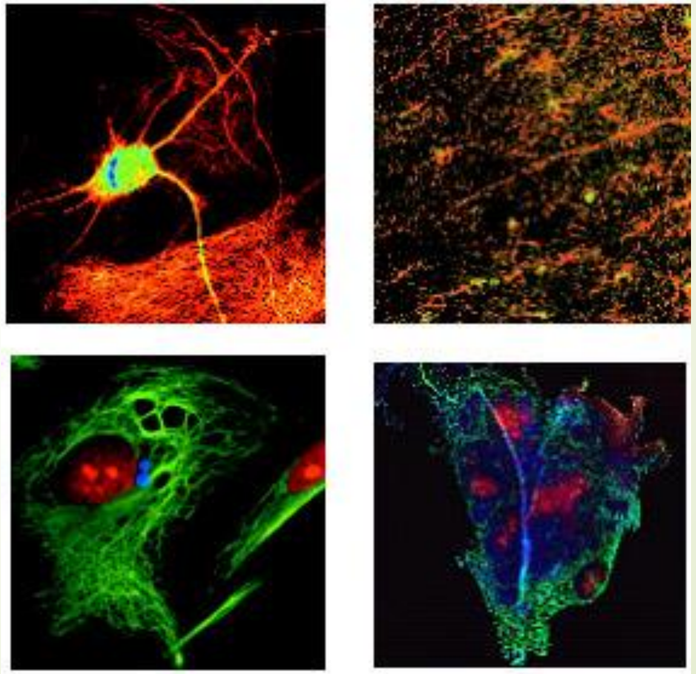
- Response time
  - It indicates how fast the sensor can response to the targeted molecular attachments
  - It is typically defined as the time required to obtain 95% signal strength
- Recovery time
  - It indicates how fast the sensor can be back to normal status after the targeted molecule removed
- Signal-to-noise ratio
  - This represents how clearly sensor signal can be obtained
  - In general, the noise source come optically or electronically
  - It is defined as the ratio of signal level divided by noise level

Biomolecular Detection Technologies

- Standard Diagnosis Technologies
  - Fluorescence detection
  - Amperometric detection
- Diagnosis Technology on Microchip
  - Surface plasma resonance
  - Microcantilever
  - Ion-sensitive field effect transistor
  - Nanowire

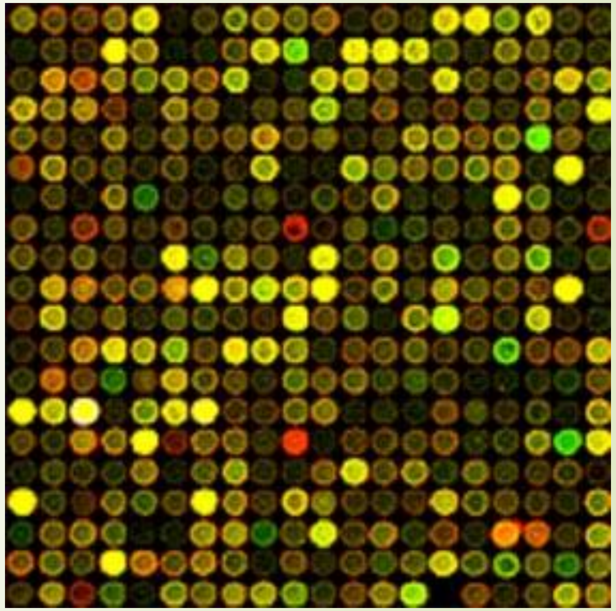
Fluorescence Techniques

- To see is to believe…
  - Fluorescence techniques have been widely used in most of biological diagnosis
  - Enzyme-linked immunosorbent assay (ELISA)
  - Polymerase chain reaction (PCR)
  - Fluorescent microscopy
  - DNA sequencing
  - Gel electrophoresis
  - etc…



Basics of Fluorescence Techniques

- Fluorescence combined with image systems is a sensitive and quantitative method for biological applications
- Advantages
  - Good sensitivity: with a good image system, single molecule image can be achieved
  - Multicolor detection: multiple targets using different fluorescent labels can be spectrally resolved
  - Good stability: compared with radio-labelled molecules, it has longer shelf-life
  - Low hazard and low cost



Fluorescence Imaging Systems

- Key elements
  - Excitation source
  - Light delivery optics
  - Light collection optics
  - Filtration of the emitted light
  - Detection, amplification and digitization

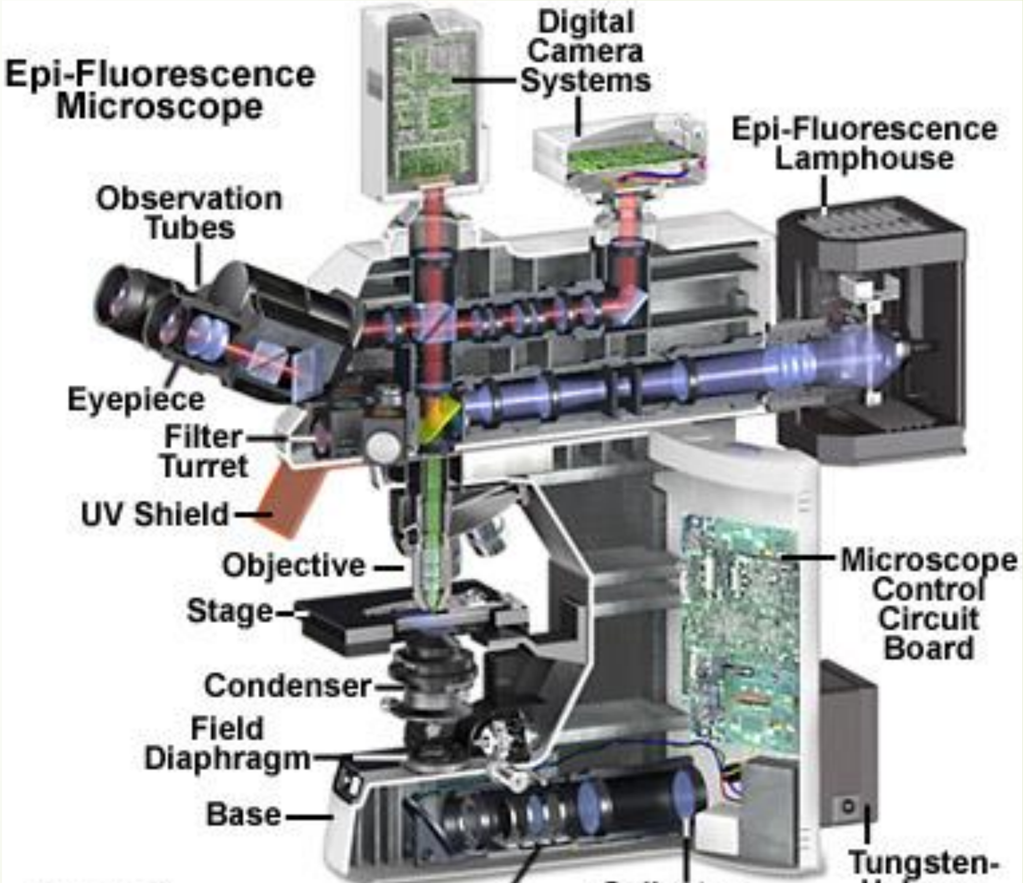
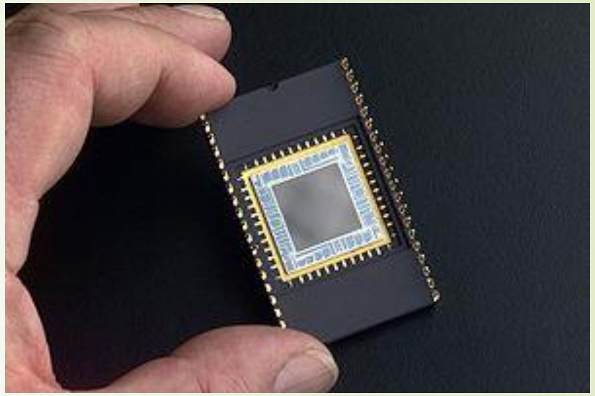
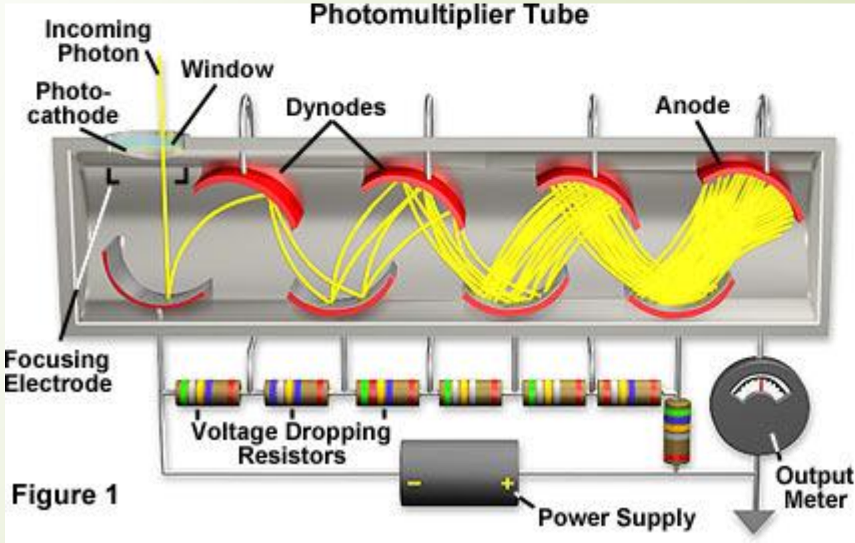


Figure 1

Detection

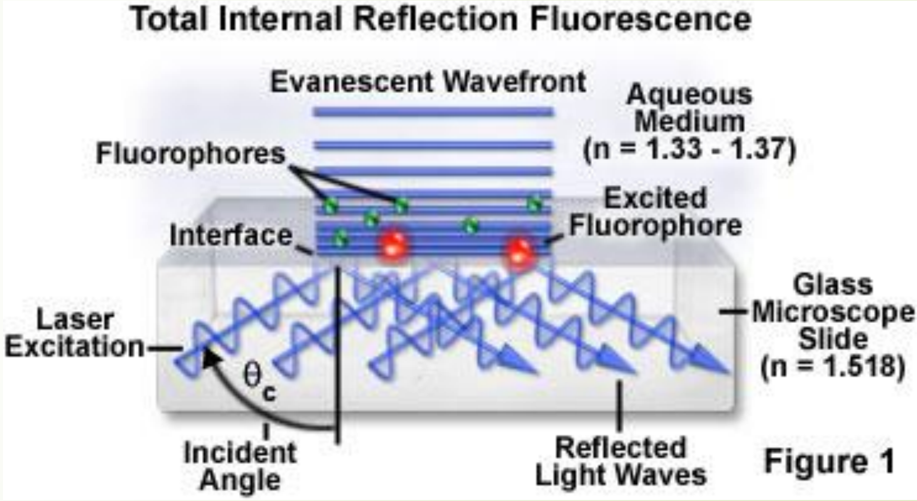
- For detection and quantitation of emitted light
  - Photomultiplier tube (PMT)
  - Charge-coupled device (CCD)
- PMT
  - An extremely sensitive detectors of light
  - Incident photons strike the photocathode material with electrons being produced as a consequence of the photoelectric effect
  - These electrons are directed by the focusing electrode toward the electron multiplier, where electrons are multiplied by the process of secondary emission





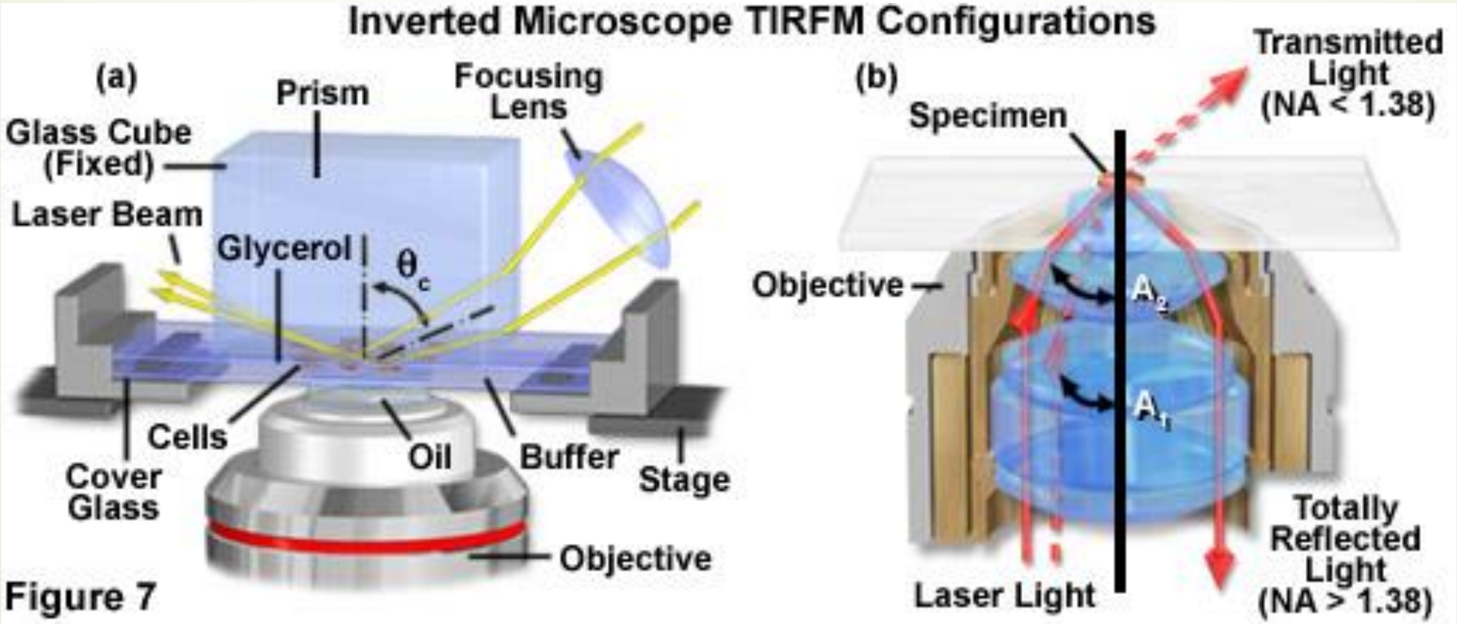
Total Internal Reflection Fluorescence Microscopy (TIRFM)

- The sensitivity of fluorescence can be enhanced by eliminating background fluorescence
  - Improve the signal-to-noise ratio and the spatial resolution of the features or events of interest
  - Refraction (or bending) of light as it encounters the interface between two media having different refractive indices (**n**) results in confinement of a portion or all of the light to the higher-index medium
  - Although light no longer passes into the second medium, the reflected light generates a highly restricted electromagnetic field adjacent to the interface

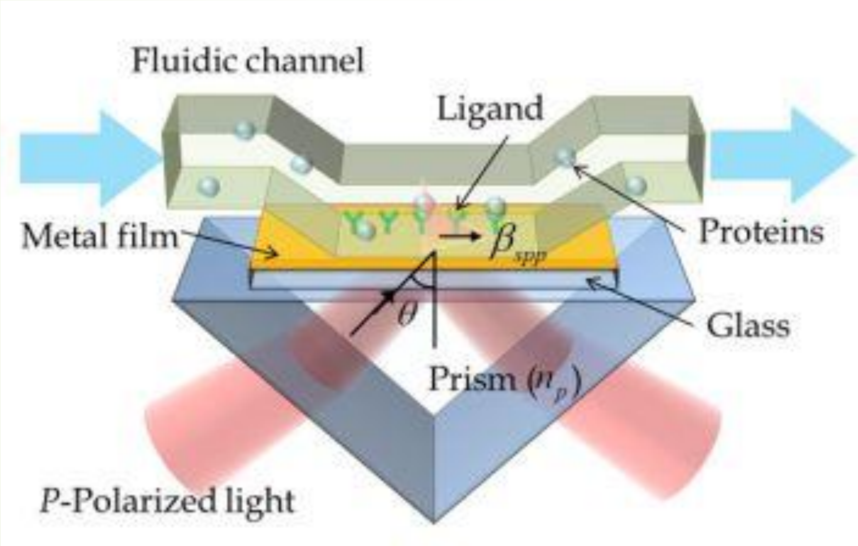


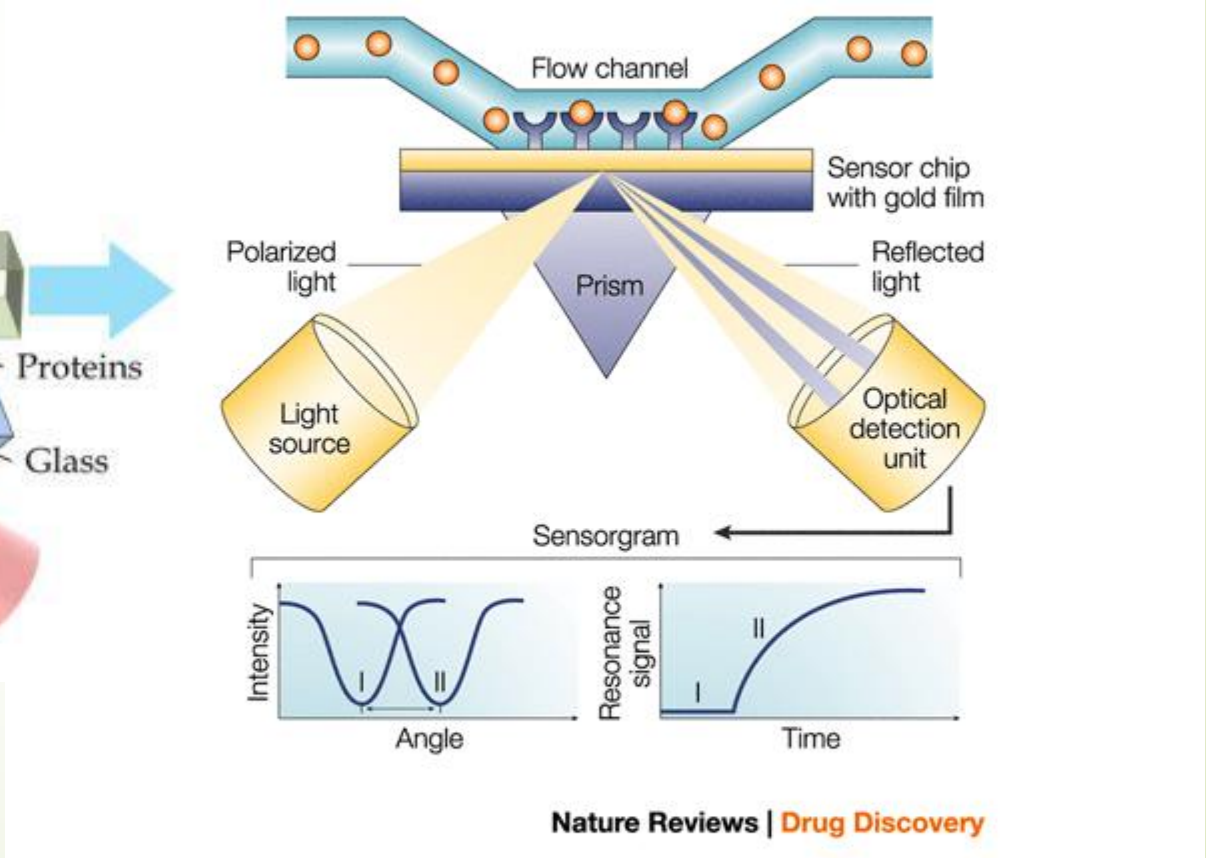
Total Internal Reflection Fluorescence Microscopy (TIRFM)

- An evanescent field is generated in the specimen medium immediately adjacent to the interface
  - The fluorophores nearest the glass surface are selectively excited
- Two basic approaches
  - Prism method
  - Objective lens method



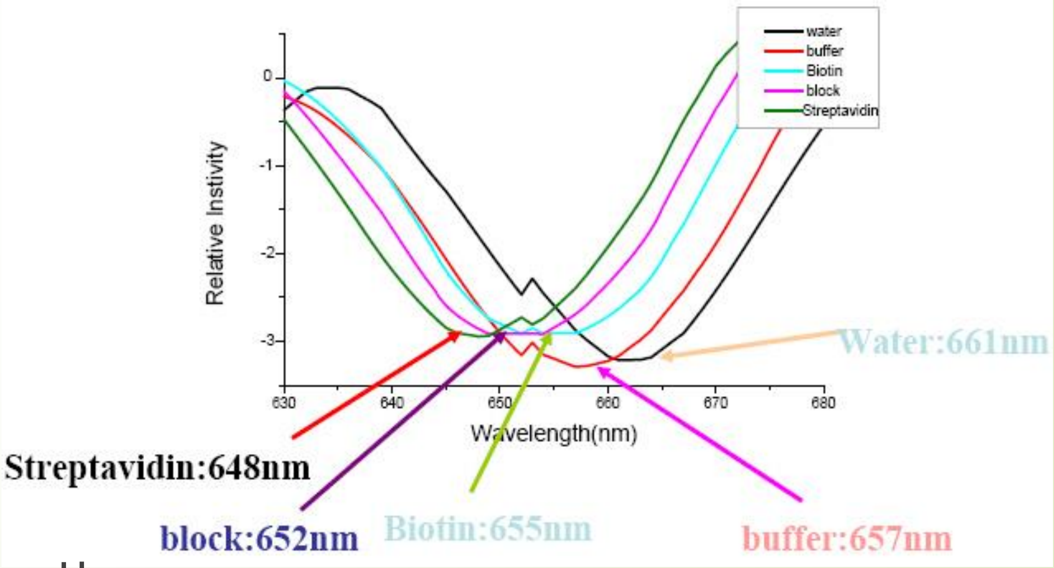
Surface Plasma Resonance Biosensor





Surface Plasma Resonance Biosensor

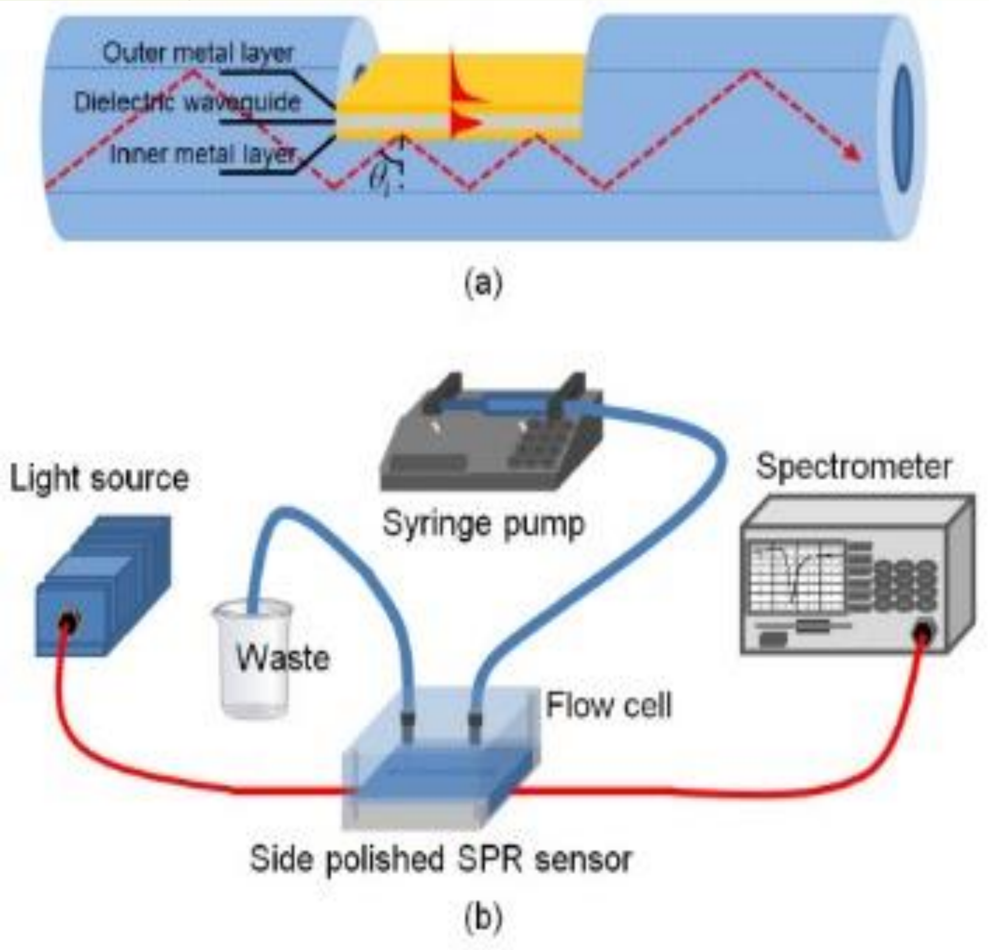
- The four basic elements for SPR
  - Light source: polarization, wavelength, angle, intensity, and phas modulation
  - Prism: couple photons to plasmons
  - Metal thin film: Au, Ag, Cu
  - Light dector
- Measurement methods
  - Angle modulation, wavelength modulation, intensity modulation, and phase modulation

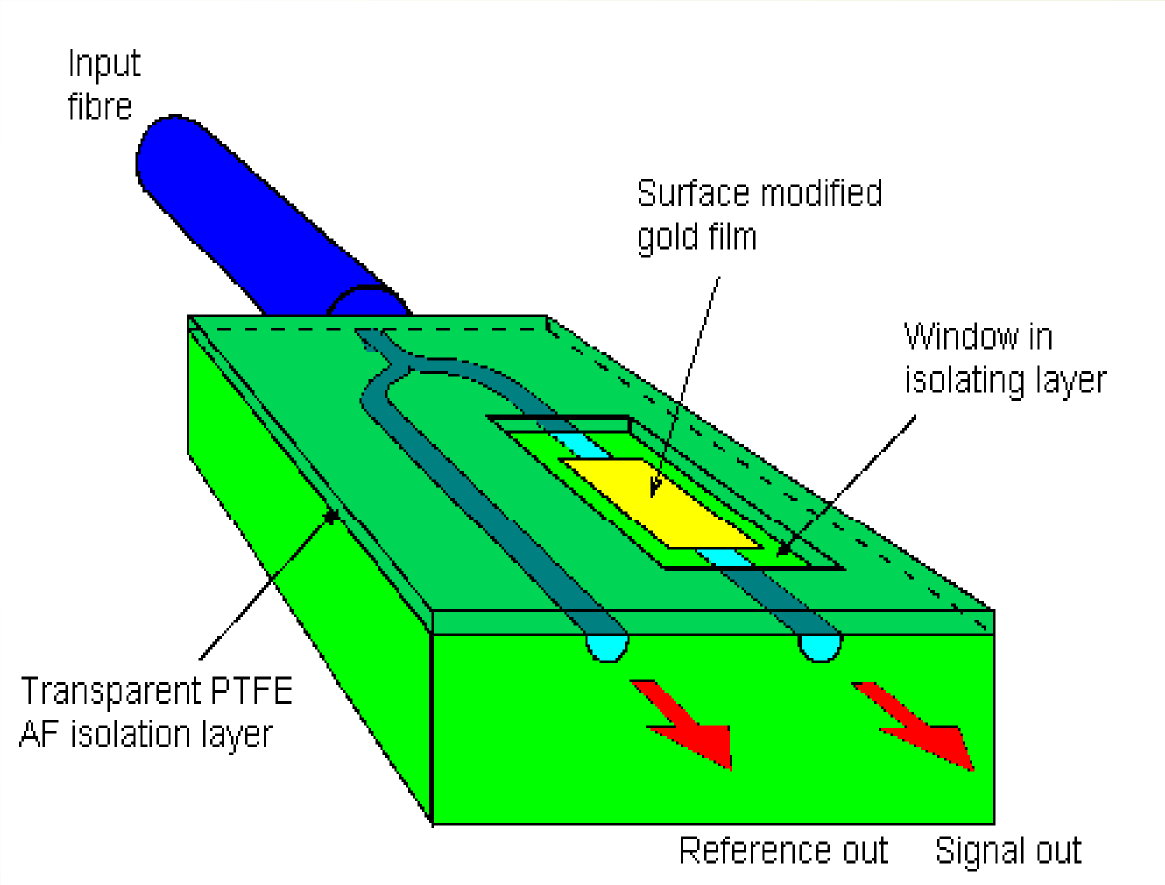


Optical Wave Guide

- The micro cavity structure used for optical filtering/coupling has been developed for years
  - It can be fabricated by micro-fabrication
  - The optical coupling effect is highly depended on the gap between the micro ring and wave guide
  - The optical coupling is also affected by the surface effective reflection index
  - The biomolecules binding will affect the surface effective reflection index
  - The optical spectrum shifting will be proportional to the biomolecular concentration and species

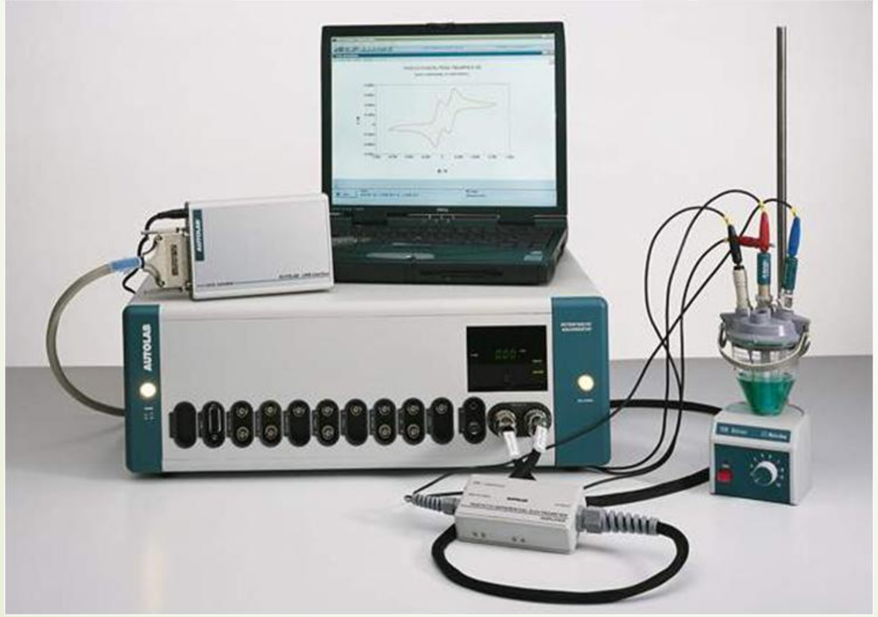
Fiber Biosensors

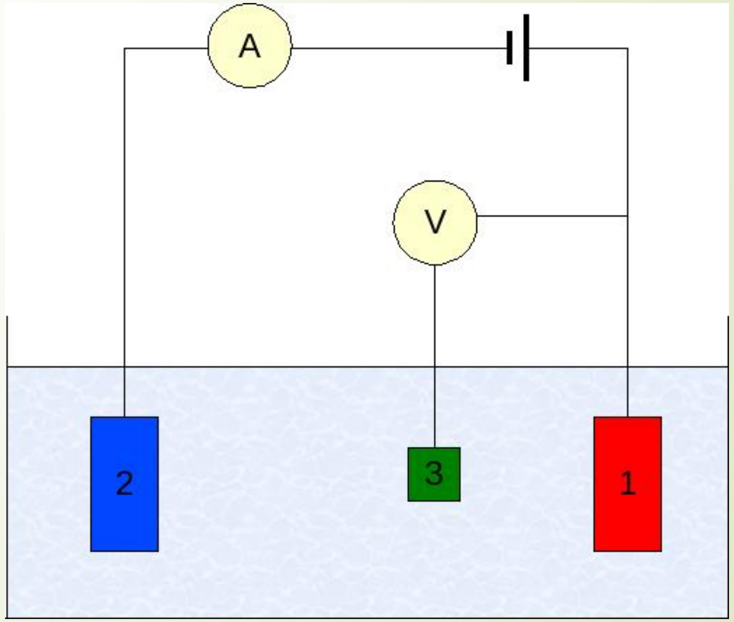




Amperometric detection

- Detection of ions in a solution based on electric current or changes in electric current
  - A potential is applied to a working electrode. Electro-active compounds are either oxidized or reduced at the working electrode, and the resulting current is monitored

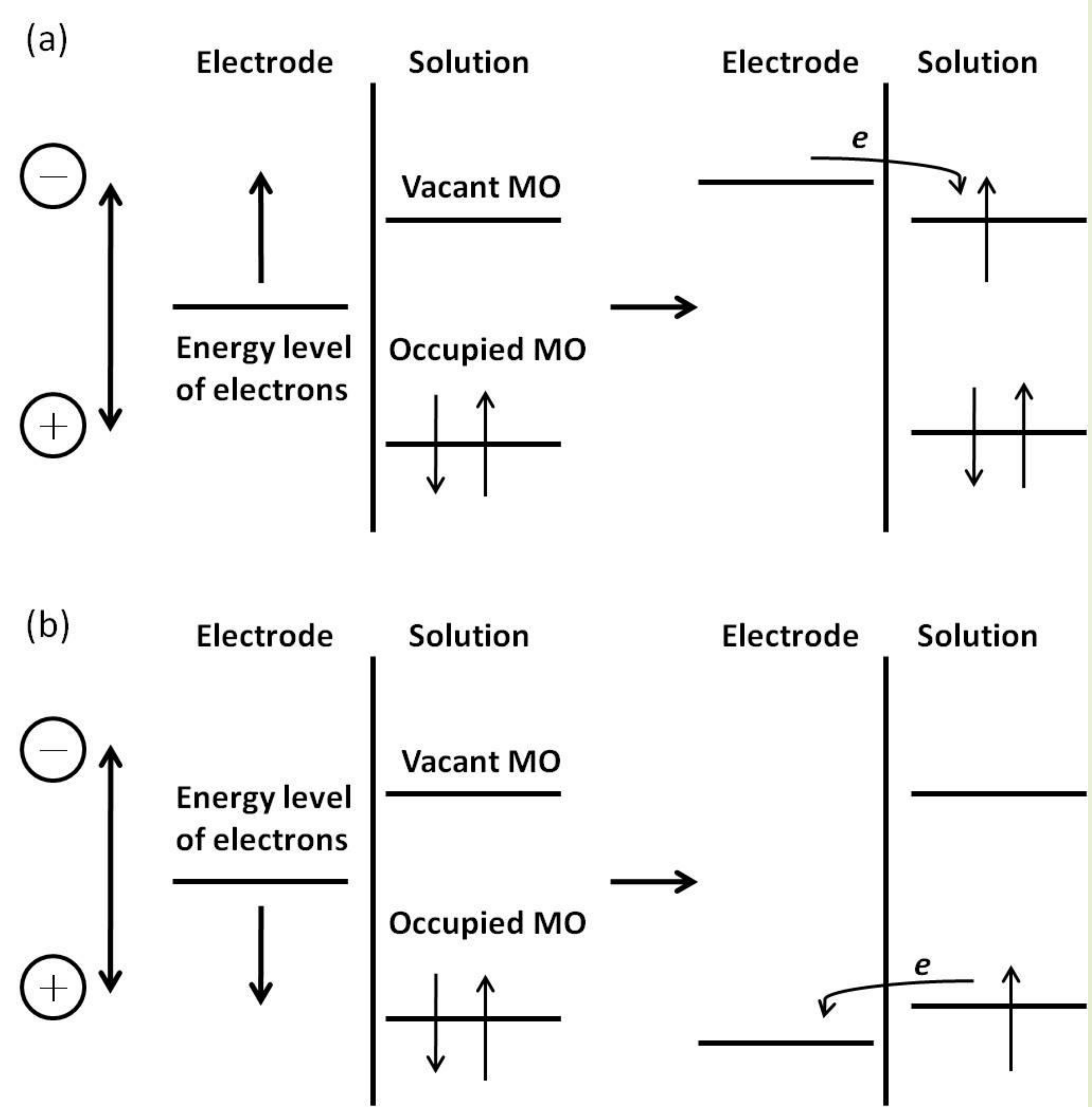




Amperometric detection

- Based on simple redox chemistry involving the transfer of electrons from target analyte to the working electrode
- When electroactive compound pass by, working electrode either receives or supplies an electron and the current be measured
- Reference electrode acts as a zeroing point
- If oxidization is occurring at the working electrode, reduction occurs at auxiliary electrode
- Sensitivity can be tuned by adjusting potential across working electrode and reference electrode

Amperometric detection

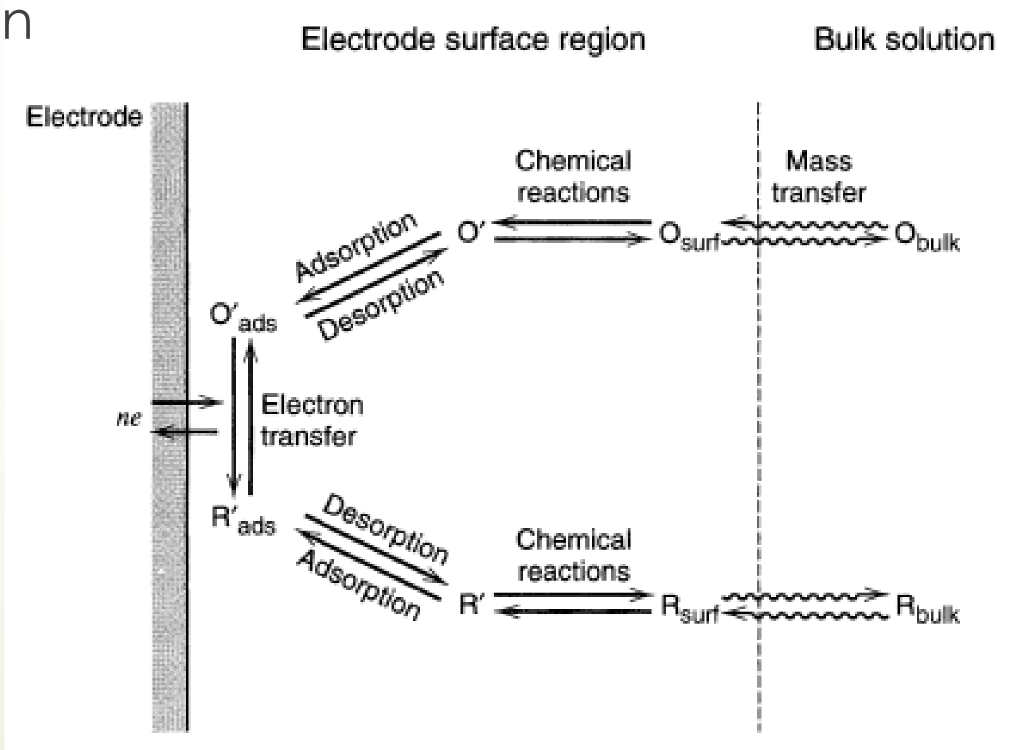


Faraday and Non-Faraday Current

- Faraday current
  - Electrons across the interface (electrolytes/electrodes) by reduction-oxidation processes
- Non-faraday current
  - The change of electrode potentials induces ions redistribution on the top of electrodes
- For amperometry, the major current is faraday current

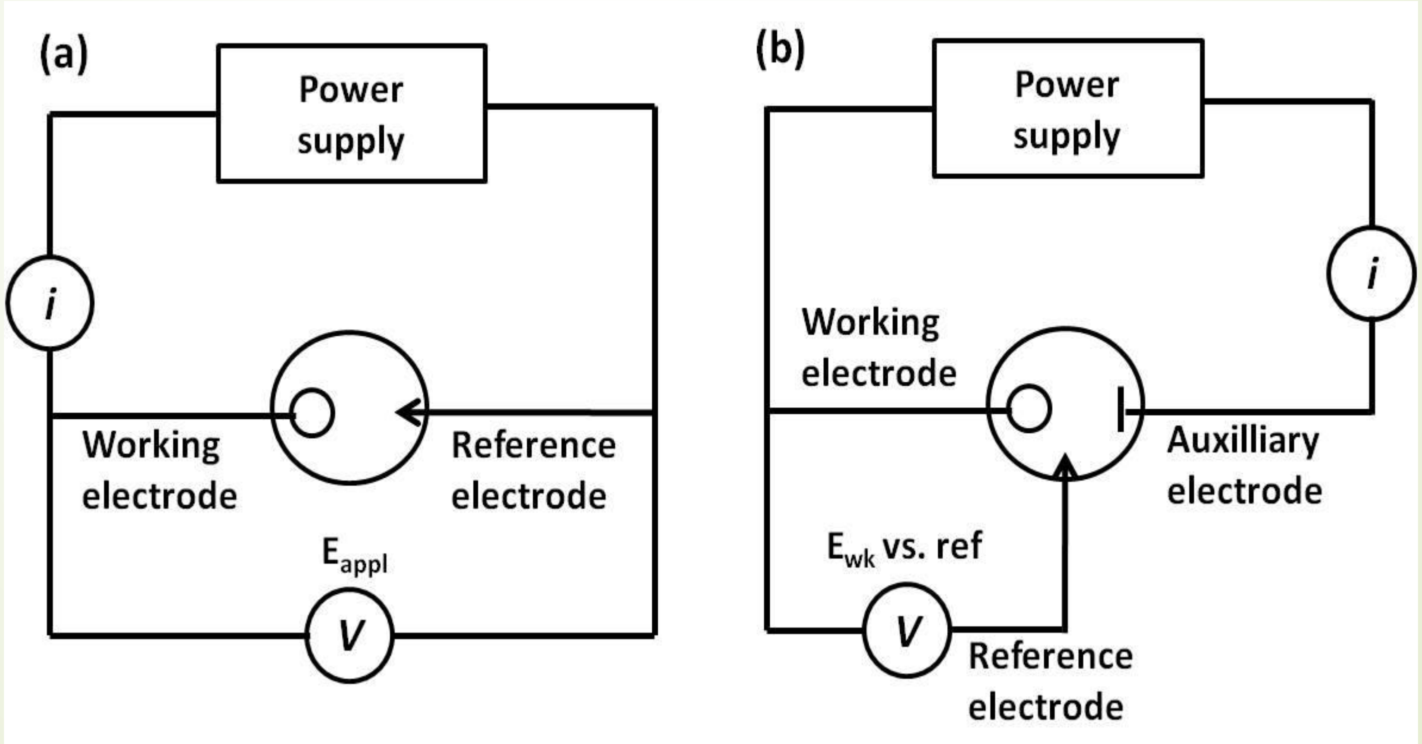
Processes of Electrochemical Reactions

- The electrochemical reactions can be characterized by
  - Electron transfer on the interface of electrodes
  - Reactants mass transportation
  - Surface adsorption and desorption
- Mass transportations
  - Migration
  - Diffusion
  - Convection

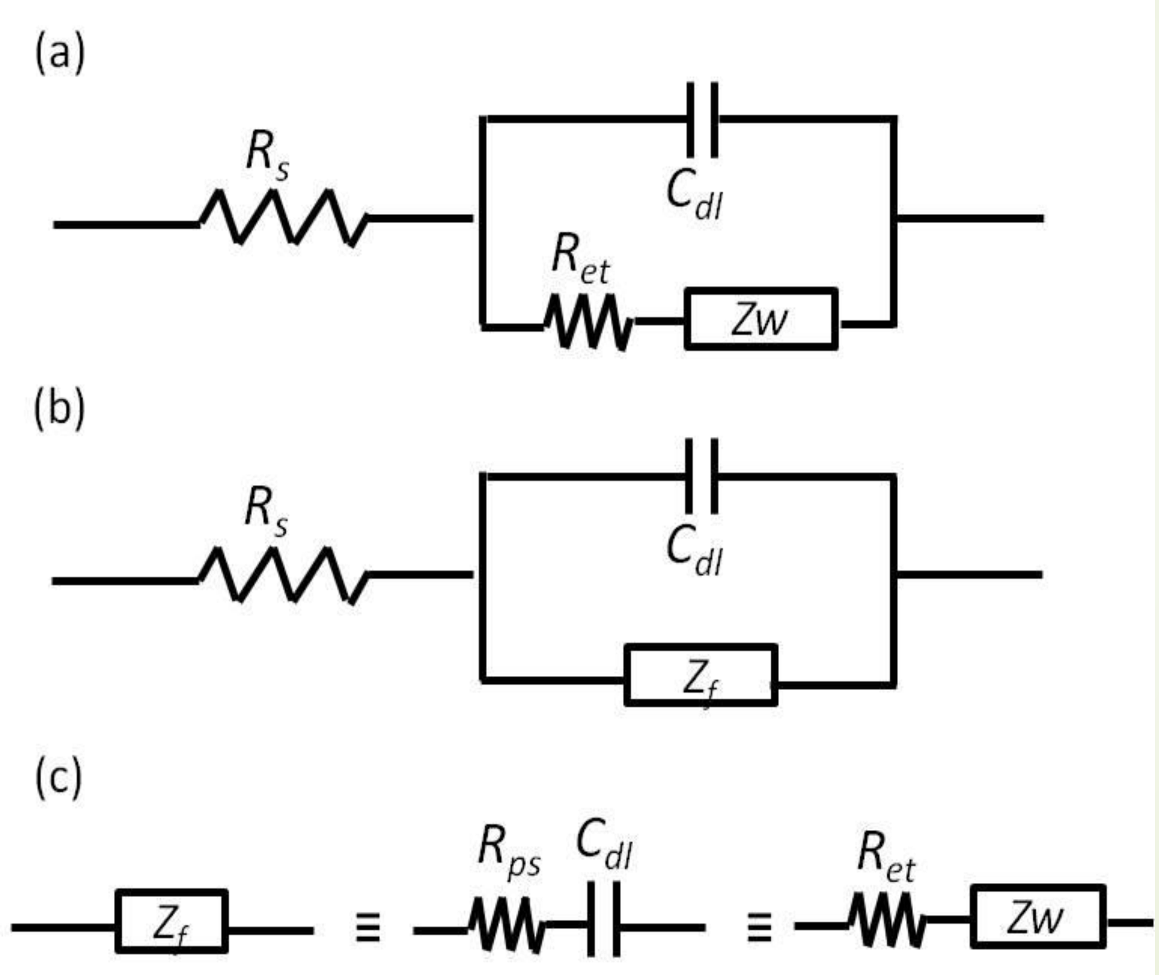


System of Amperometry

- Two-electrode system and Three-electrode system



Equivalent Model of Interfaces

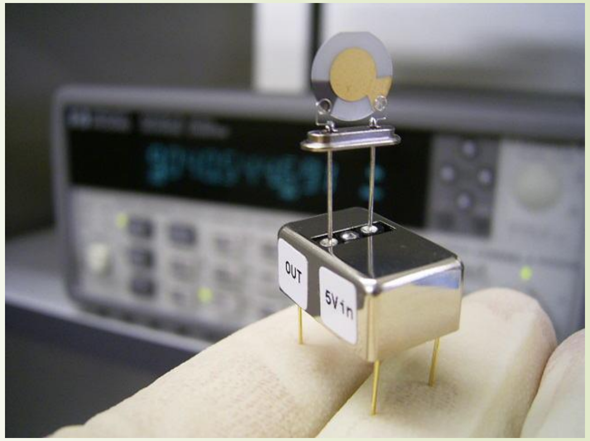


Mechanical Resonance

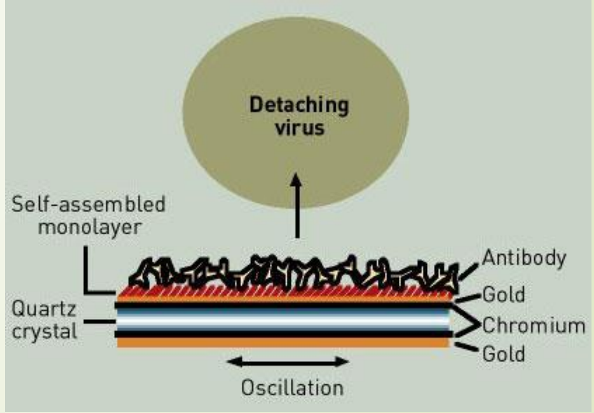
- Utilizing the fundamental mechanics
  - Resonance frequency is highly depending on the mass and boundary condition of the structure
  - As the biomolecular binding to the surface, it locally changes the condition of the mechanical prosperities
  - Utilizing the frequency analysis, the targeted biomolecular concentration is proportional to the frequency shifting

Mechanical Resonance

- Quartz crystal microbalance (QCM)
  - Based on the quartz resonator, the resonance frequency is very sensitive to the mass of the quartz thin film
  - There are two electrodes on each side of the quartz thin film
  - The bio-recognition molecules can be bounded on to the surface
  - The resonant frequency change is linearly related to the changes of mass from biomolecules

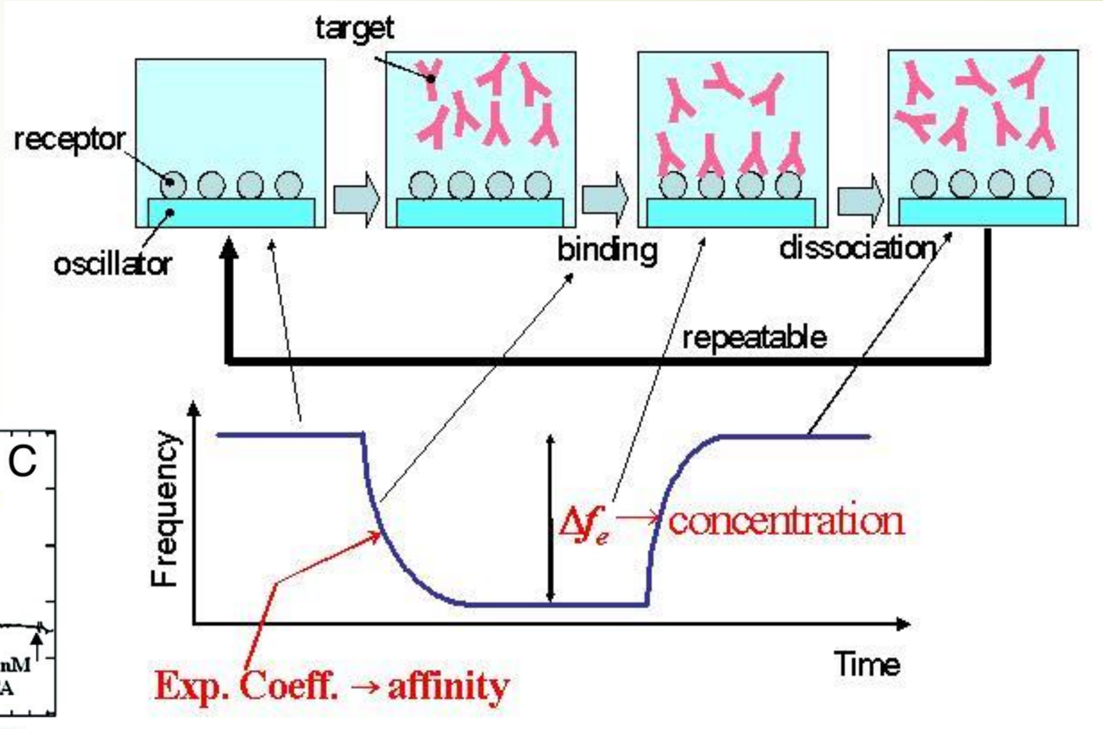


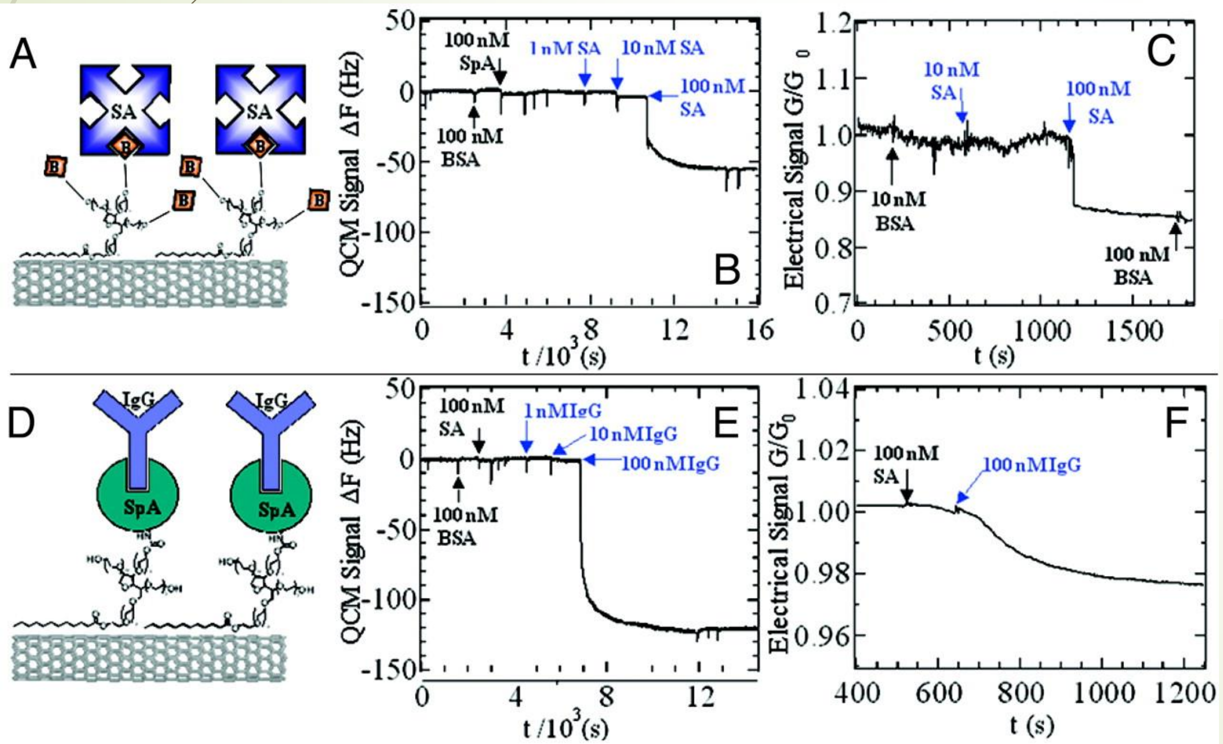
http://www.tamadevice.co.jp



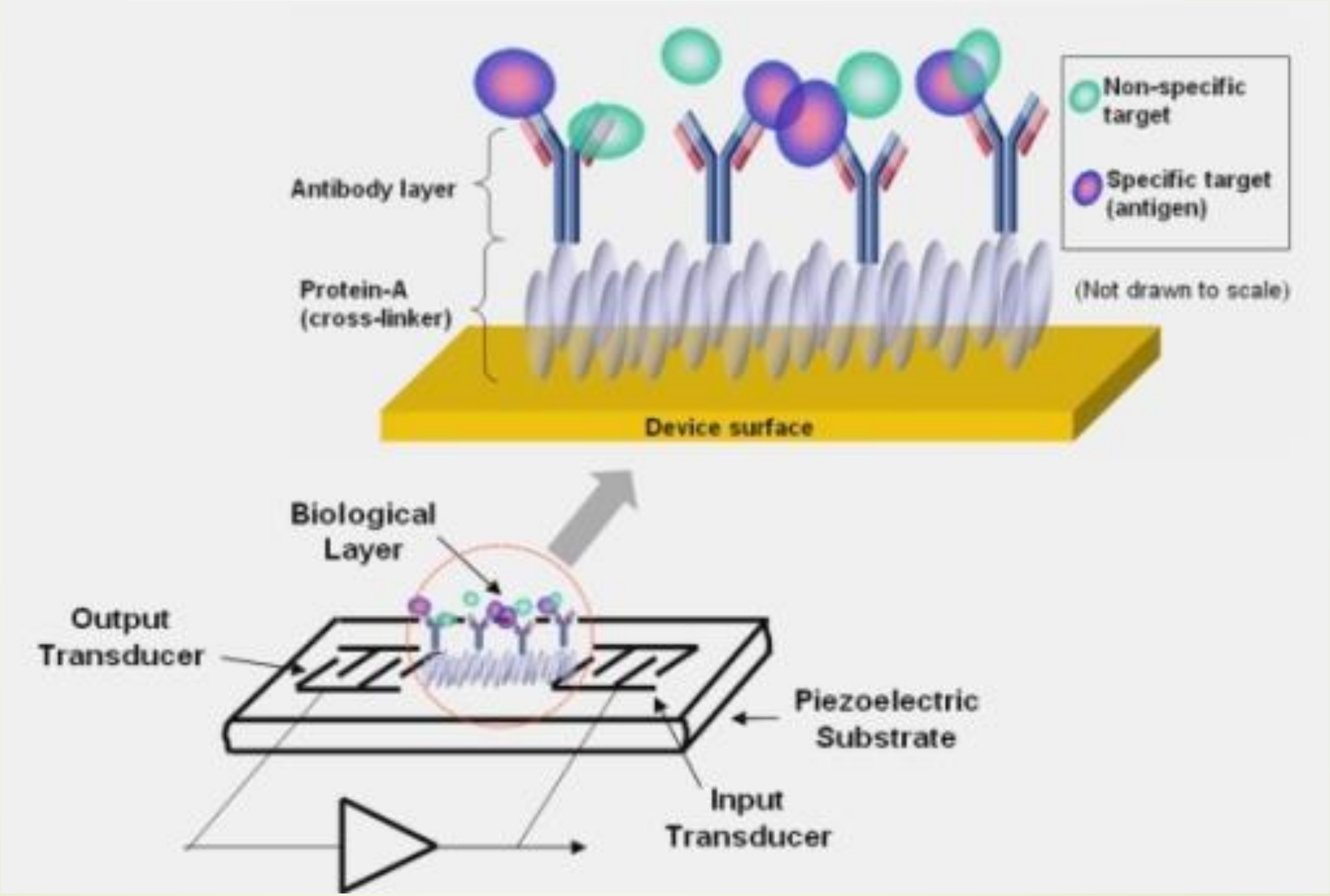
http://pubs.acs.org

Quartz Crystal Microbalance Biosensors

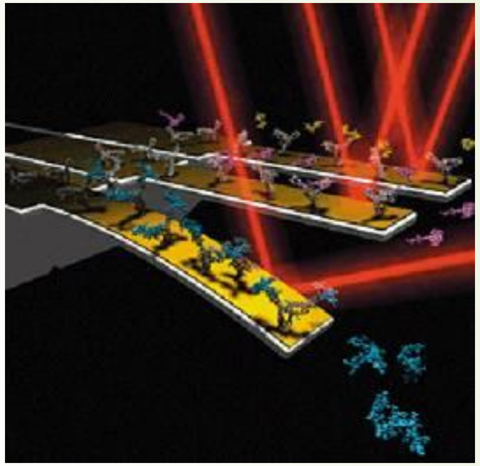


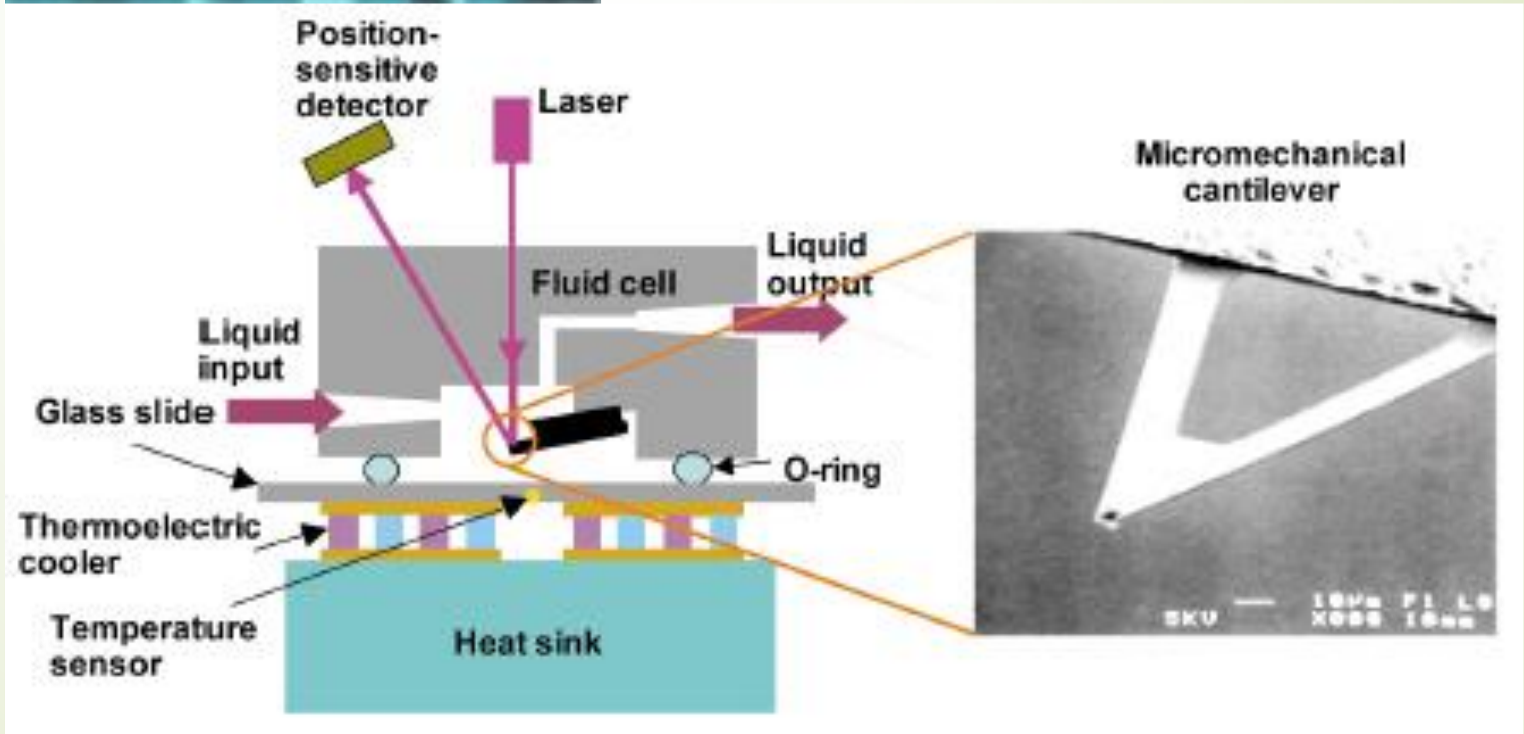


Surface-Acoustic-Wave Biosensor

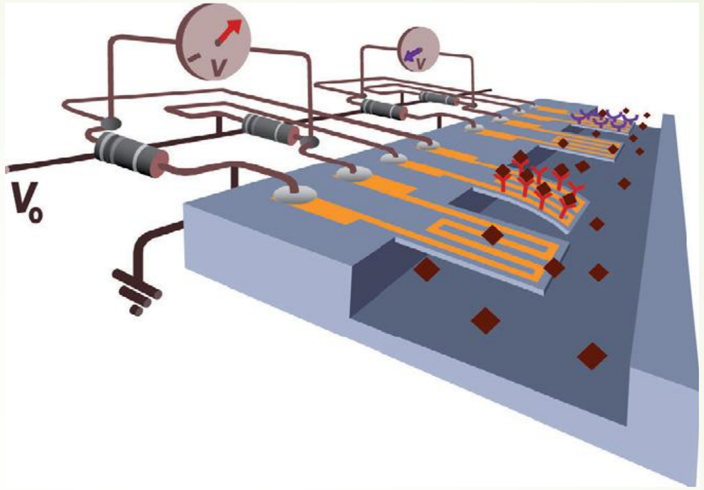


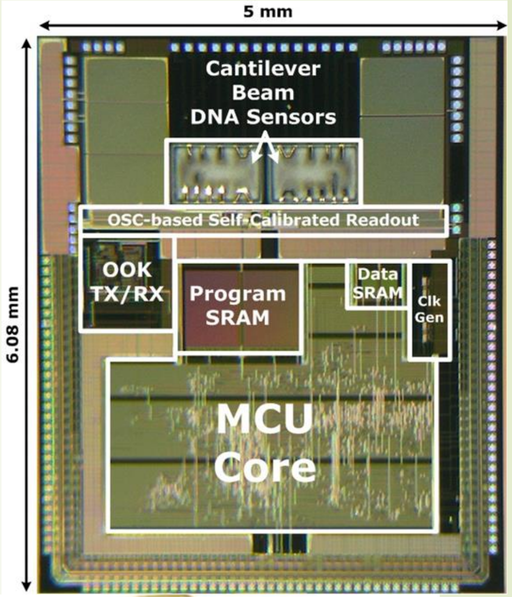
Micromechanical Biosensors

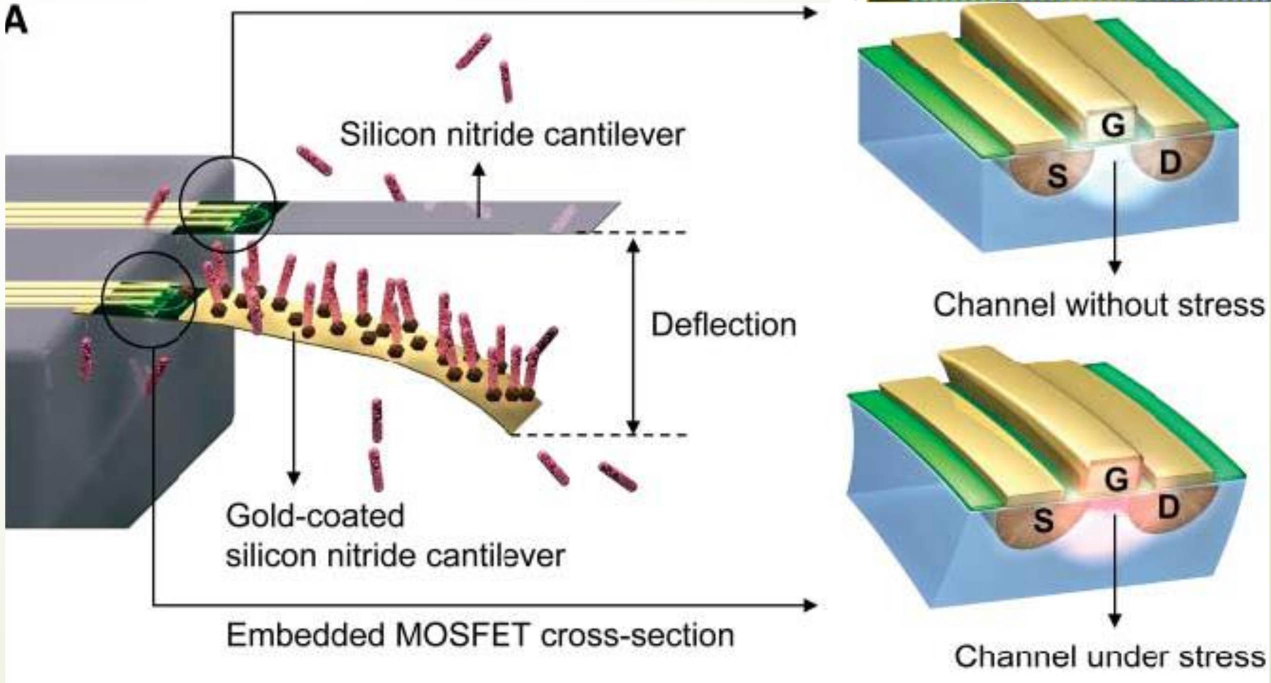




Micromechanical Biosensors

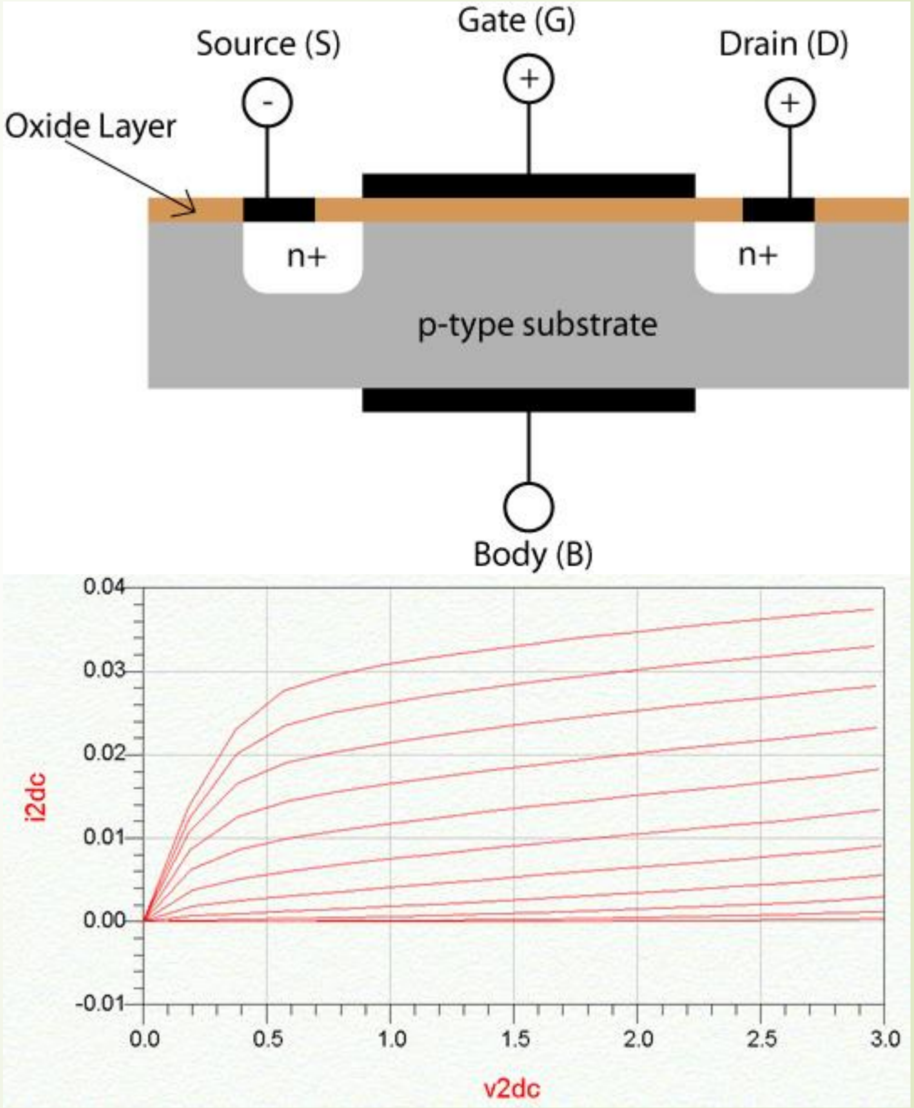






Field-Effect Transistor

- The characteristic I-V curve of a field effect transistor is modulated by gate voltage
  - By changing the gate potential, the current flows throught the conduction channel wil be changed



http://www.doitpoms.ac.uk

Ion-Sensitive Field-Effect Transistor (ISFET)

The ISFET is in fact nothing else than a MOSFET with the gate connection separated from the chip in the form of a reference electrode inserted in an aqueous solution which is in contact with the gate oxide.

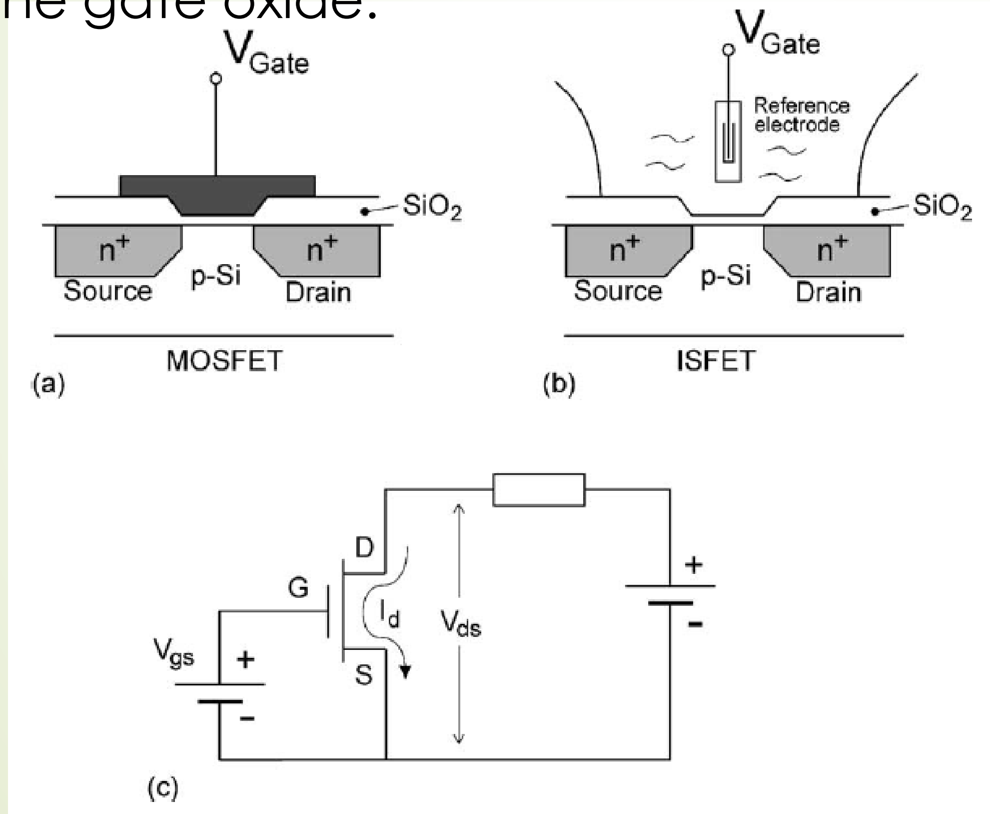


Fig. 3. Schematic representation of MOSFET (a), ISFET (b), and electronic diagram (c).

- ✓ The general expression for the drain current of the MOSFET

$$I_d = C_{ox}\mu \frac{W}{L}\left[(V_{gs} - V_t)V_{ds} - \frac{1}{2}V_{ds}^2\right]$$

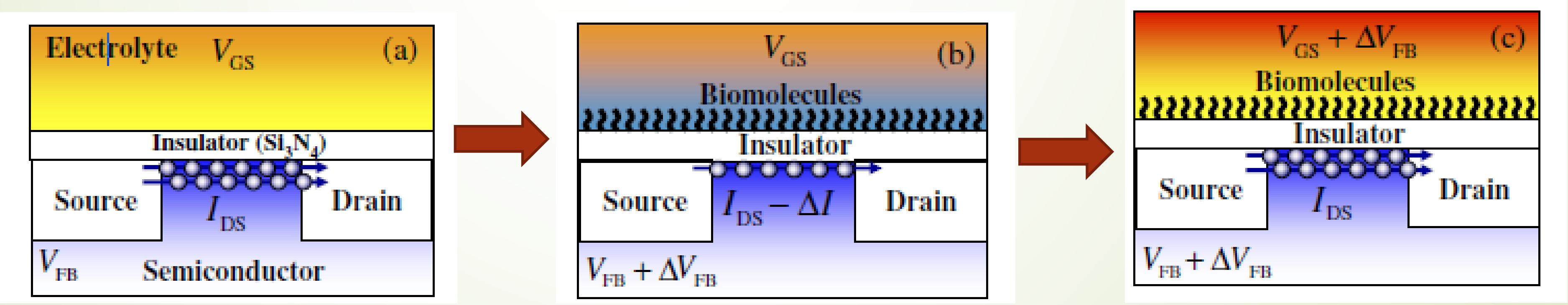
Non-saturated mode, "C_{ox}" is the oxide capacity per unit area, "W and L " the width and the length of the channel, respectively, and "μ" is the electron mobility in the channel.

P. Bergveld, "Thirty years of ISFETOLOGY What happened in the past 30 years and what may happen in the next 30 years," *Sensors and Actuators B*, 88 (2003) 1–20

ISFET

$$V_t = E_{ref} - \Psi + \chi^{sol} - \frac{\Phi_{Si}}{q} - \frac{Q_{ox} + Q_{ss} + Q_B}{C_{ox}} + 2\phi_f$$

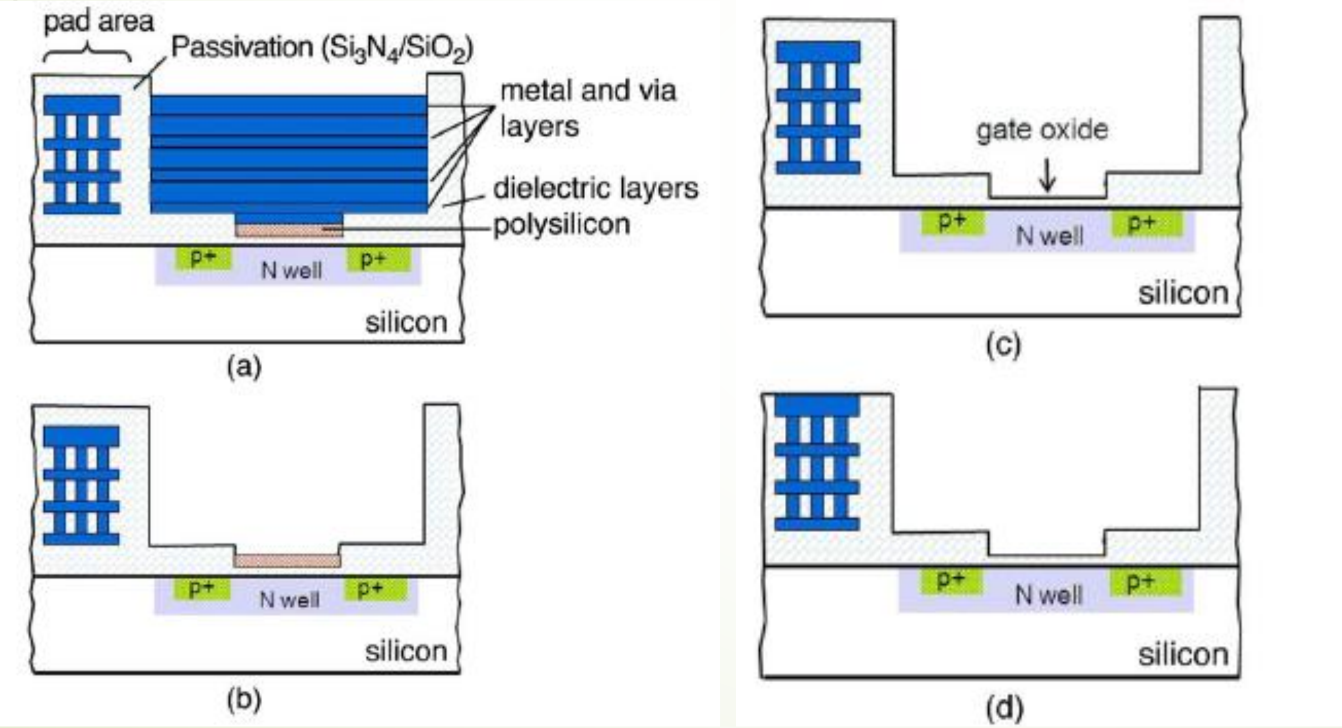
The constant potential of the reference electrode, E_{ref}, and the interfacial potential $\Psi + \chi^{sol}$ at the solution/oxide interface of which Ψ is the chemical input parameter, shown to be a function of the solution pH and χ^{sol} is the surface dipole potential of the solvent and thus having a constant value. Note that the parameter ϕ_M is "buried" by definition in the term E_{ref}.

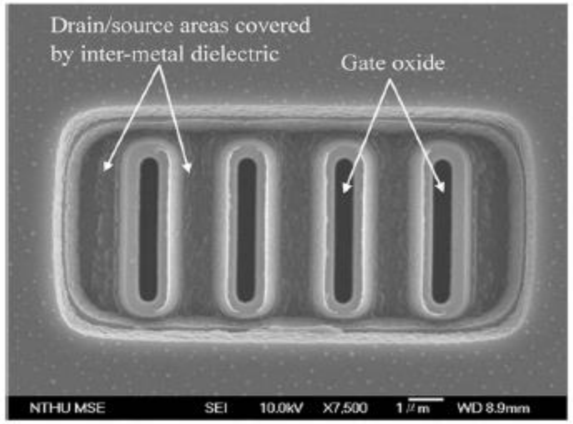


P. Bergveld, "Thirty years of ISFETOLOGY What happened in the past 30 years and what may happen in the next 30 years," *Sensors and Actuators B*, 88 (2003) 1–20

Shigeyasu Uno, et. al., "Full Three-Dimensional Simulation of Ion-Sensitive Field-effect Transistor Flatband Voltage shift Due to DNA immobilization and Hybridization," Japanese Journal of Applied Physics, 49, 2010

Ion-Sensitive Field-Effect Transistor Biosensor





(e)

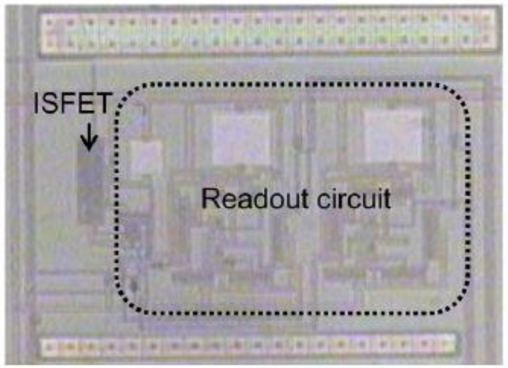
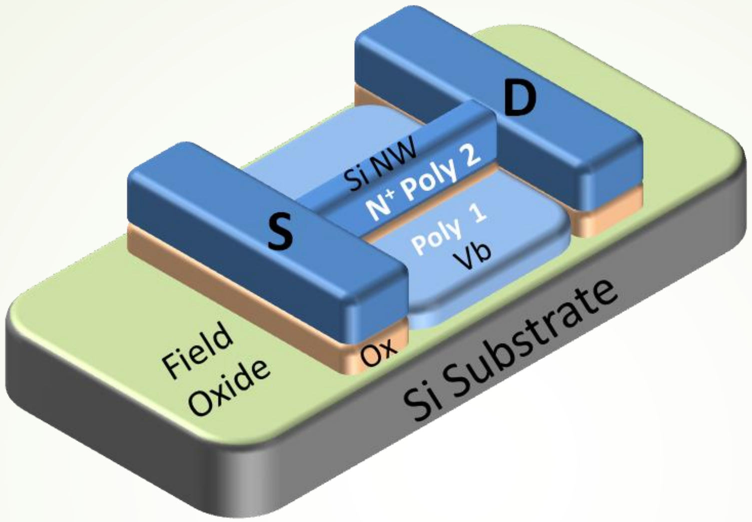
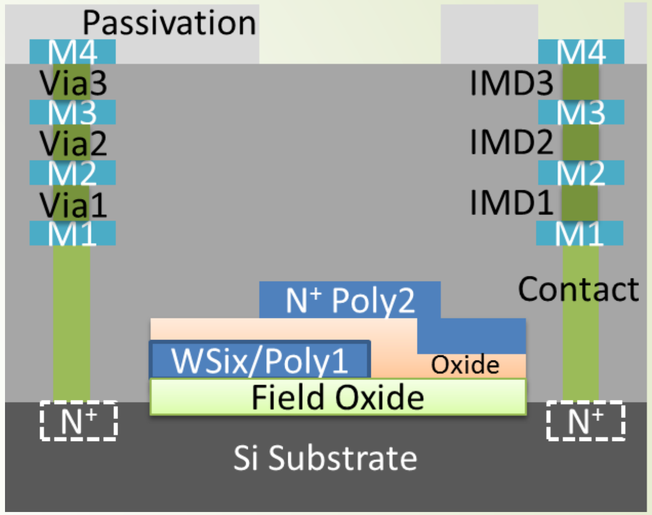
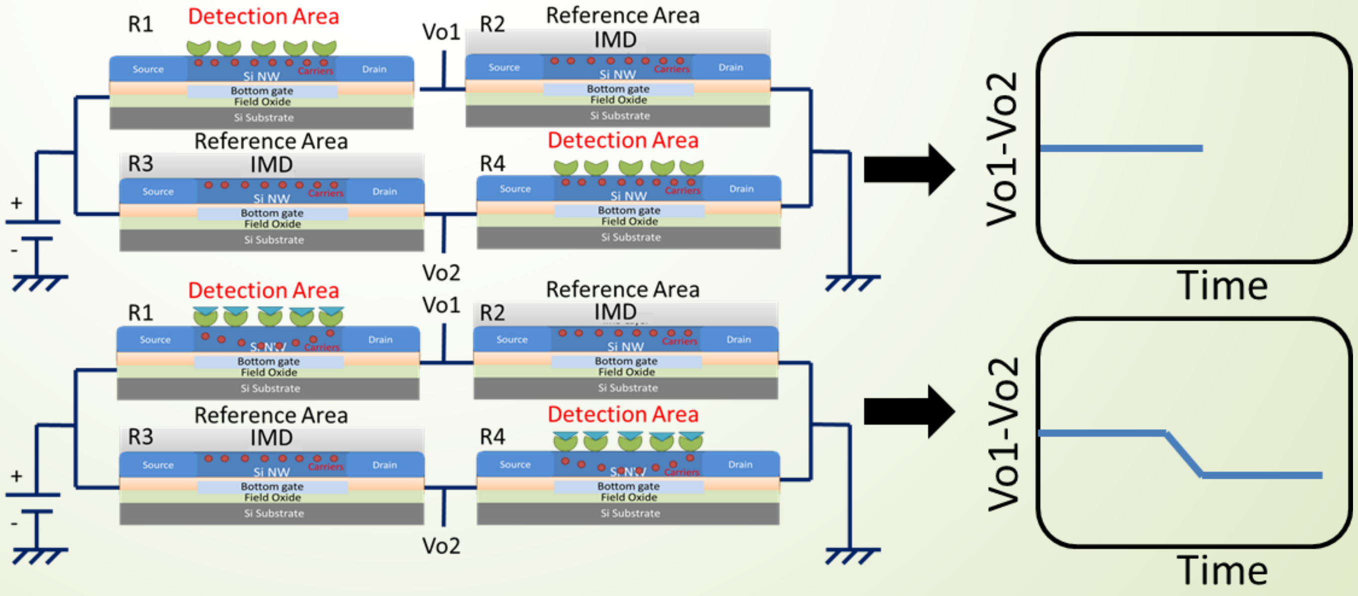


Fig. 7. Photograph of the ISFET device with CMOS readout circuit.

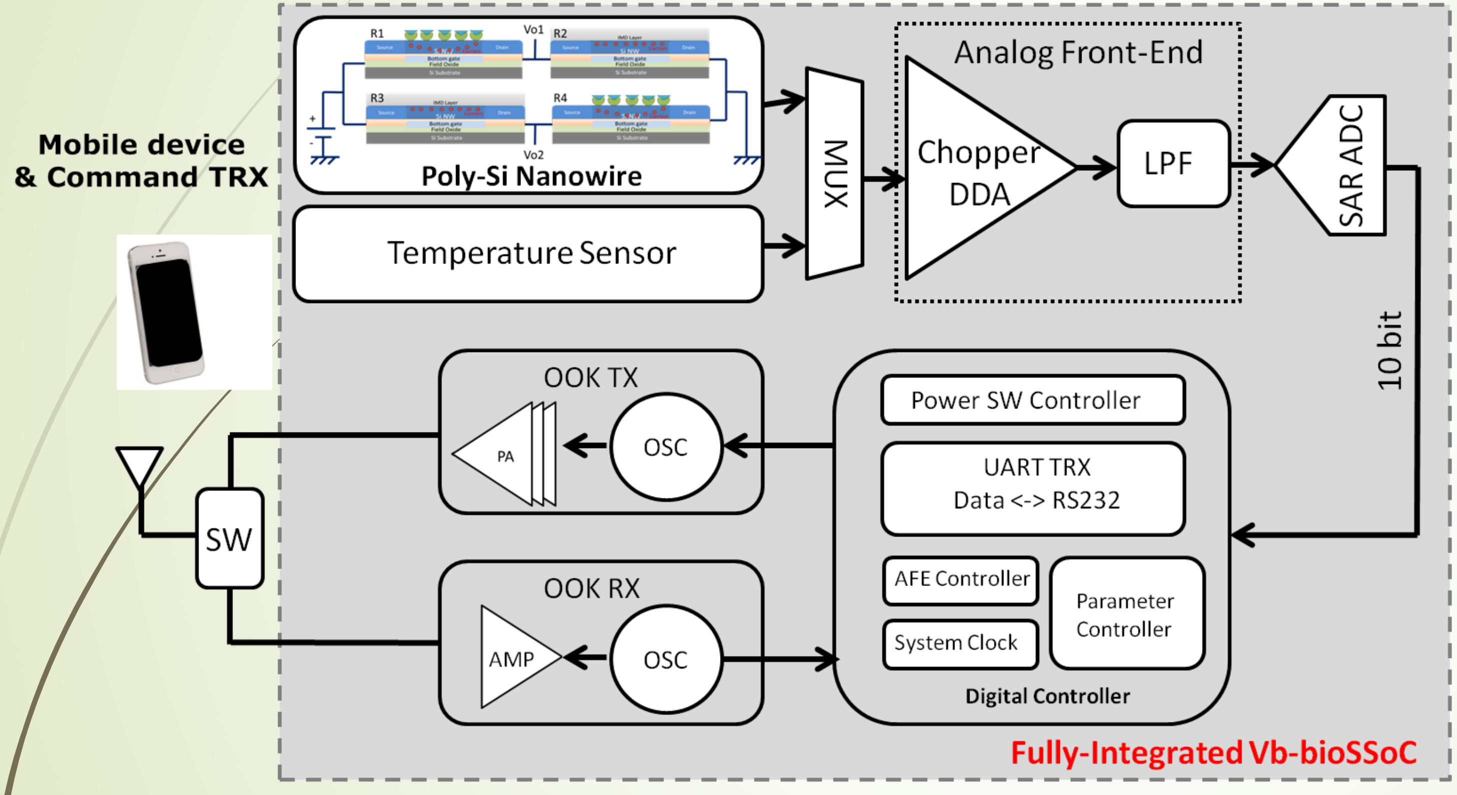
Poly-Si Nanowire Biosensor

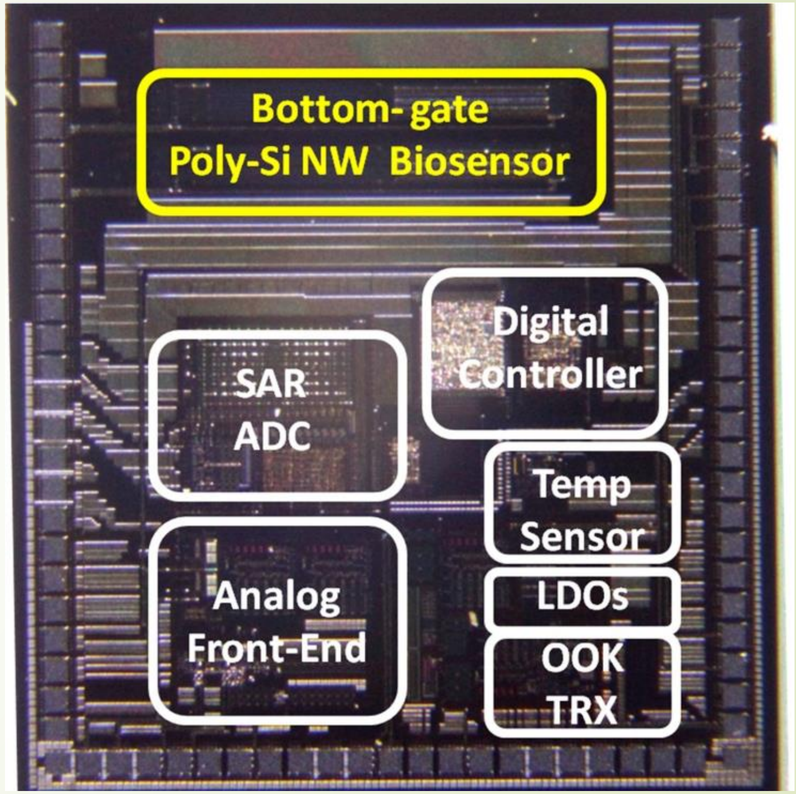






Poly-Si Nanowire Biosensor





Conclusion

- Biomoelcular diagnosis technologies have become an emerging biomedical engineering applications
- Microfluidics and microsensors are integrated to perform wearable healthcare devices
- Integrated with diagnosis tools and data analysis to realize personalized healthcare system