生物感測器　BIOSENSORS
精微機電與生物醫學的結合

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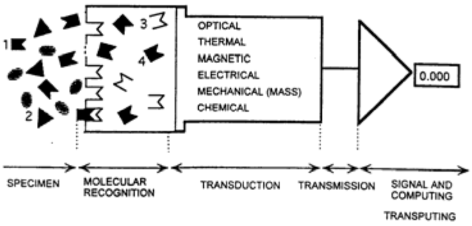
生物感測器

- 什麼是生物感測器?
- 需求點
- 歷史發展
- 現況
- 分類
- 生物感測器的特殊問題
- 應用
- 成功的挑戰
- 未來的發展

什麼是生物感測器?

- 使用生物要素作爲量測與分析的感測器 – 包括--antibodies, enzymes, cells;
  上述要素與傳感器的耦合電子裝置
- Biological molecule with the power of modern electronics



什麼是生物感測器?

- "A chemical sensor is a device that transforms chemcial information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal" – IUPAC
- "Biosensor - a subgroup of chemical sensors where biological host molecules, such as natural or artificial antibodies, enzymes or receptors or their hybrids, are equivalent to synthetic ligands and are integrated into the chemical recognition process.
  - High Specificity and selectivity
  - Restricted stabilities and life time

Biosensor – selective molecular recognition

Selective elements	Transducers
synthetic ionophores	electrochemical:
synthetic carriers	– potentiometric
supramolecular structures, clusters	– amperometric
solid layers: metals	– conductimetric
– metal oxides, crystals	– voltammetric, polarographic
– polymers, conducting polymers	– impedimetric, capacitive
organisms	– piezoelectric
microorganisms	optical:
plant and animal tissues	– transmission / absorbance / reflection
cells	– dispersion, interferometric
organelles	– polarimetric
membranes, bilayers and monolayers	– circular dichroism, ellipsometry
enzymes	– scattering
receptors	– emission intensity, photon counting
antibodies	(luminescence) decay time
nucleic acids	calorimetric
natural organic and inorganic molecules	acoustic / gravimetric:
micelles, reversed micelles	– surface photo-acoustic wave
	– quartz microbalance

Ref: Spichiger-Keller U.E., “Chemical Sensors and Biosensors for Medical and Biological Applications, Wiley-VCH, 1998

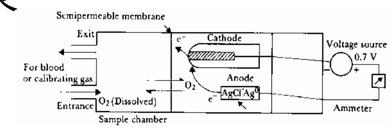
Biosensors - Features

Features	Benefits
targeted specificity, selectivity	versatility, dedicated systems
selective assay in complex samples	ease of use, front-line analysis, reagent-free or reagent-poor operation
short response time	fast measurements and high sample throughput
electronic processing and electronic control of calibration	consumer friendliness, ensuring safety of the assay
reversibility	continuous measurements, low waste, no consumption of the analyte
enzymatic steady-state	enzymatic turn-over of the analyte/substrate
availability, low cost	disposable or exchangeable elements

需求點

方法	空間解析度	時間解析度	監測能力	新陳代謝參數
X 光	0.1 mm	0.1 sec	有限	無
斷層掃瞄	< 1 mm	數秒	有限	無
正子掃瞄	3-5 mm	數分	無	微量元素
超音波影像	> 0.1 mm	即時	有	無
磁核共振影像	< 1 mm	數十分鐘	有限	H^+, P, C

歷史發展

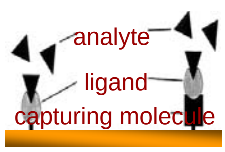


1930	pH glass electrode	MacInnes
1950	Ames test for urinary glucose	
1962	Blood pO2 sensor	Clark
1964	Quartz crystal sesnor Valinomycin for ion-selective effect In vivo Fiber optics Oximeter	King Moore Karpany
1966	Glucose sensor	Updike
1968	Fluorescent oxygen sensor	Bergman
1970	ISFET	Bergveld

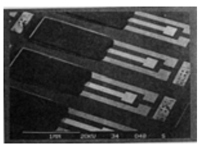
歷史發展

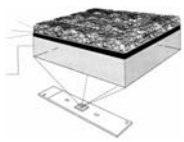
1975	First microelectrode with 1 um	Thomas
1977	Enzyme FET	Janata
1980	Fiber optic pH probe	Peterson
1983	First international meeting on Chemcial sensors	
1990	First world congress on Biosensors, Singapore	
1998	中華台灣化學感測器科技協會成立	

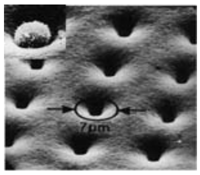
現況



- Most common Biosensors are enzyme or antibody based
- Miniaturization
- Many companies developing DNA-based biochip arrays
- Researchers working to affix entire living cells to a chip







分類 – 感測原理

Electrical transducers	Electrode
modes:	
voltage	potentiometric sensor
current	amperometric sensor
current–voltage	polarographic, voltammetric sensor
"work function"	FETs (field effect transistors)
	MOS (metal oxide semiconductor gas sensor)
charge transfer, resistance	coulometric sensor, chemiresistors, ion mobility, mass spectrometry
dielectricity	capacity sensor
Radiant or optical transducers	Optode, IOS (integrated optical sensor)
modes:	
absorption, intensity	transmission or absorbance in the UV-, VIS- or IR-region of the spectrum
	ATR (attenuated reflection) or evanescent field sensor
	SPR (surface plasmon resonance)
emission intensity	luminescence, photoemission, photon counting
emission and absorption	FOCS (fiber optic chemical sensor)
scattering:	e.g., Raman scattering
phase changes	refractive index sensor
	MIOS (miniaturized integrated optical sensor)
polarisation and absorption	OR (optical rotation), ellipsometry
	ORD (optical rotation dispersion)
	SPR (surface plasmon resonance)
opto–thermal effect	photo–acoustic effect
Thermal transducers	calorimetric sensor, pellistor
Magnetic transducers	NMR (nuclear magnetic resonance)
	permeability sensor
	mass spectrometry
Mechanical, frequency transducers	SAW (surface acoustic wave)
	piezoelectric oscillators, quartz balance

分類 – 依分子辨識機制

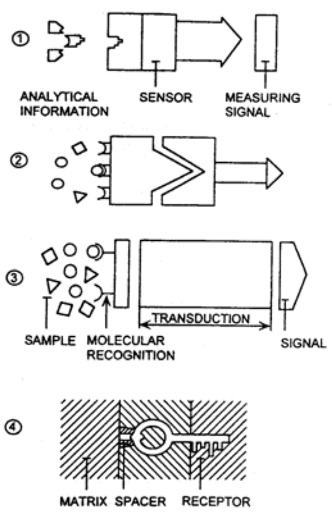
Sensor type	Reacting pairs	Chemical reaction recognition process
chemical sensors in the strict sense	host–guest; ligand–analyte; carrier–ion; ion, neutral species, and gas sensors	complexation association, addition, typical equilibrium reactions
oxide semiconductor sensors	inorganic metal oxide layer–reactive gases	absorption, reduction, oxidation
enzymatic sensors	active site–substrate–cosubstrate mediated sensing reactions	metabolic turnover, typical steady state,
	active site–mediator-electrode	kinetic reactions
immunochemical sensors	antibody (catalytic antibody)–antigen	affinity, association,
	antibody–antigenic protein–hapten	equilibrium reactions
receptrodes, living organs, bilayers hybrides as abzymes etc.	receptor–substrate	association, affinity, metabolic turnover

分類 – 依感測層種類

Surface active sensors:	surface layer furnished with immobilized dyes (indicators), enzymes, antibodies
Bulk-phase sensors:	
solid-state membranes and sensors	homogeneous: glass, single crystal, pressed cristalline body, organic and metal oxide semiconductors, metal oxide semiconducting gas sensors (MOS), heterogeneous: crystals implanted in an inert matrix (e.g., in silicone polymer)
solid-state sensors	liquid membranes with a solid-state internal reference system: e.g.: semiconducting elements, gate field effect transistors (GFETs), ISFETs, CHEMFETs, EN(ZYME)FETs
solvent polymeric membrane electrodes	synthetic carriers sensitive for anions and cations or for ions generated at a boundary by a selective chemical reaction or by hydration. carriers: charged, neutral or classical ion exchangers
optodes	containing synthetic carriers sensitive for ions or neutral species, gases. carriers: charged, neutral or classical ion exchangers
multilayer membranes	enzyme electrodes, optodes, EN(ZYME)FETs enzyme embodied electrode (EEE)
semipermeable coatings protecting layers	biocompatible membranes, interference-preventing layers ion-diffusion barriers, coatings, gas-permeable, gas-sensitive membranes
polymerized membranes	photo- and electropolymerized selective layers, selectrodes
tissues and microbial sensing adlayers	

作用模型

- The recognition process is in a separate layer of the sensor. (1)
- Surface reaction type and bulk membrane type with shape-fit relation. (2,3)
- The key-keyhole model (4)



生物感測器的特殊問題

- Testing Context
- Operator gives direct patient care
- Monitoring, rather than diagnosis
- Stability may be more important than accuracy
- Testing over limited time frame
- Testing does not involve separable specimen
- Method validation/verification difficult or impossible
- Calibration verification difficult or impossible

生物感測器的特殊問題(cont...)

- QC usually limited to comparison with specimens from the same patient tested in laboratory
- Proficiency testing usually impossible
- Inflexibility
- Calibration is frequently factory-set or performed only once before testing is begun
- Adverse testing conditions
- Bio-compatibility issues: thrombus formation or immune response

應用 - Invasive & Minimally-Invasive Testing

- Extra-corporeal sensor
  - – In-line testing
  - – Shunted outside
    - either returned to circulation or discarded
- Invasive sampling/external sensor

--Ex vivo sensors

--Arterial or venous specimen is shunted outside, tested and returned

- In vivo sensors

--continuous monitoring with indwelling sensors

--intravenous, intra-arterial or intra-peritoneal

Extra-Corporeal Circuits

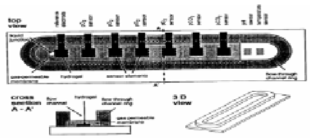
- Analytes: SpO_2, hematocrit, and change in blood volume
- Methodology: During dialysis, light is passed through the chamber to detect change in O_2 saturation. Hematocrit is derived from light scatter and absorption
- Calibration: Factory calibration
- Quality Control: "Verification filter" with specified tolerance
- Lifetime: Disposable after dialysis

Extra-Corporeal Circuits

- Analytes: pH, pC02, p02,K+, temperature, S02, Hct, Hgb
- Methodology: Continuous Intra-arterial testing. On demand, blood is shunted into contact with 4 optodes.
- Calibration: Tonometered calibration at the bedside.
- Quality Control: Simultaneous lab test
- Lifetime: Disposable after surgery

Ex Vivo

- Analytes: pH, pC02, p02,. Bicarb, BE, Sa02, and TCO2
- Methodology: Specimen is aspirated into sensor, tested & returned to circulation.
- Calibration: 2-level calibration using standards introduced by an independent Luer lock stopcock.
- Quality Control: Amid-level calibration using standards introduced after the device is attached to the patient
- Lifetime: 144 hours or 200 measurements



Ex Vivo

- Analytes: pH, pC02, p02, Hct, Na+, K+, bicarb, BE, TCO2
- Methodology: Specimen is withdrawn, tested & returned to circulation.
- Calibration: Initially a 2-level calibration; one-point calibrations can be done before each test
- Quality Control: Simultaneous test
- Lifetime 72 hours
- iSTAT

In Vivo

- Analytes: pH, pC02, and temperature
- Methodology: Indwelling arterial sensors that provides continuous monitoring using 3 separate optodes for fluorescent detection
- Calibration: 3 levels of Tonometered solution are used to calibrate just before insertion.
- Quality Control: None besides simultaneous lab test

比較

中央處理實驗室之自動分析儀(Autoanalyzer)

- 微量樣本（稀釋） （＜50 ul）
- 大量快速量測 （40-180 samples/hr）
- 高準確度 （2-3% C.V.）
- 正常工作之穩定度 （＞7天或＞200samples）

即時診斷用之分析儀(Point-of-Care)

- 微量樣本（不稀釋）
- 快速量測報告 （＜30 second）
- 價格低廉
- 準確度 （4-5% C.V.）

連續線上量測用之分析儀(On-Line)

- 高生物相容性
- 高穩定度

Challenges to Success

- Obstacles to reliable in vivo testing have not yet been overcome
- Biomolecules used for recognition are not stable or robust
- The trend toward miniaturization and full automation demand more advances

The Future

- Single molecule detection
- Nano-size sensors, telemetering capabilities
- Injectable
- Organ or tissue specific
- Nano-size HPLC, GC capable of self operation with telemetering capability
- multi-analyte arrays
- Non-inavsive for home care
- Auditory, olfactory, and visiual sensor
- Molecular architecture
- Phototransduction
- Interface between neurons and external world

➢ Changing Boundaries and Definitions
➢ Evolving Standards and Regulations

Medical Challenges

Level of Difficulty	Challenge	Biotechnology approaches	Nanotechnology approaches
I	Minor symptoms & Minor physical trauma	Symptomatic suppression pharmacology	Pharmacytes, zippocytes, Respirocyte, etc.
II	Infectious agents	Antibiotics & vaccines	Nanobiotics
III	Mutation and cell diseases	Molecular diagnostics & Treatments; gene therapy	Nanobiotics, Cell repair machines
IV	Health maintenance & Aging	Gene therapy to enhance self-repair and immune function	Whole-Body cyto-assay; Cell repair machine
V	Major organ replacement or repair	Stem cell & tissue engineering; embryonic gene reactivation	Cell mills, tissue mills, organ mills, nanosurgery
VI	Morph-engineering	Control of natural morphogenetic systems	Nanosurgery, chromatin edition
VII	Emergency care; restoration of non homeostatic tissue	Biological repair nets; Bioengineered immune cells	Traumapods, Warm biotasis
VIII	Augmentation of natural structure or function	Cross-species & artificial morphogenetic supplementation	Cell engineering devices, autogenous control

Source: R. A. Freitas, Nanomedicine Vol.1

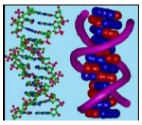
Part II

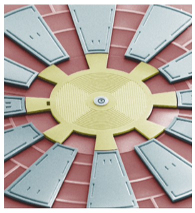
MEMS and Nano History

There's Plenty of Room at the Bottom

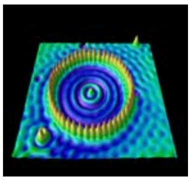
by Dr. Richard P. Feynman at Cal Tech, Pasadena, CA on Dec. 26, 1959

- Miniaturizing devices (information storage, computation, motor)
- Evaporation and lithograph
- Parallel microfabrication by a hundred tiny hands
  - **MEMS technology**
- Rearranging the atoms
- The marvelous biological system (DNA, RNA, Protein, Amino Acid, etc. for information processing, computation)
  - **Nanotechnology, Bio-Nano**





By TI, M Mehregany



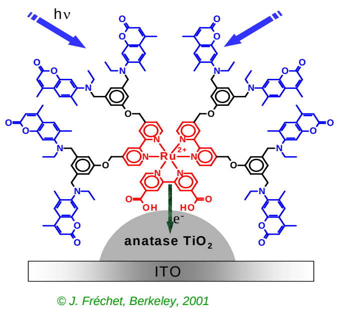
by IBM 1993

MEMS and Nano History

Infinitesimal Machinery

by Dr. Richard P. Feynman at JPL, Pasadena, CA on Feb. 23, 1983

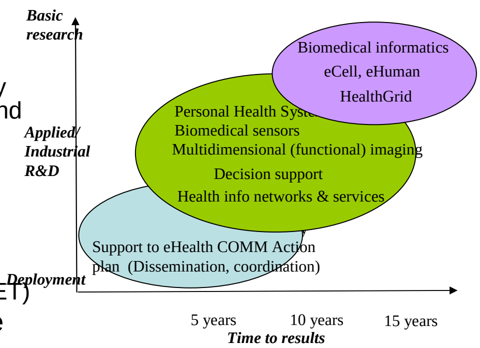
- Make, Use, and Power "Swallowable surgeon"
  - Electrostatic actuation
  - Electromagnetic field
  - Mobile microrobots powered by ATP or Optics (Autonomous machine for cellular operations or
- Friction, Sticking and Shaking of atoms
- Microfabrication by casting or by imprecise tools
- Quantum computation



- Biology is a guide (but not a perfect guide)
  - Various form changes by applying electrical field, which affect viscosity of fluid.

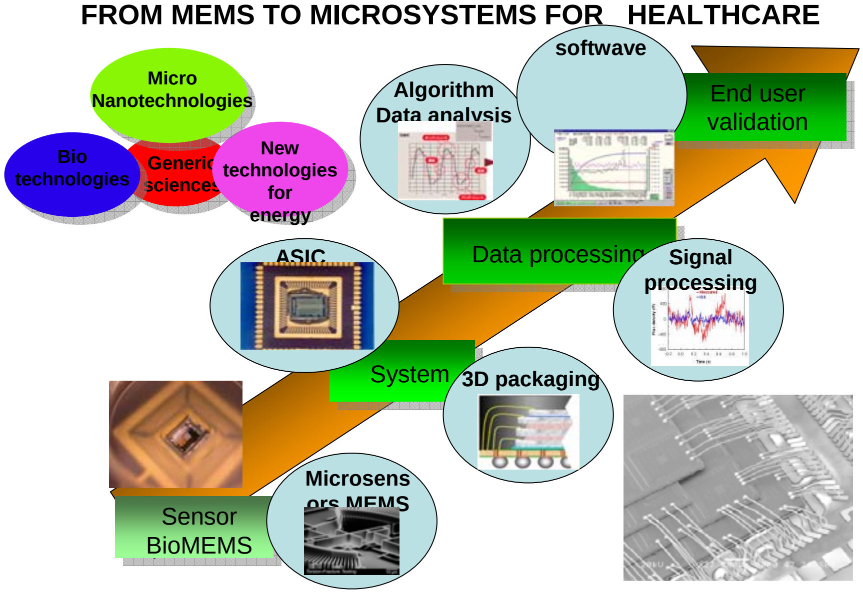
Technology Trends

- Mastering complexity by pioneering new approaches to cope with the infinitely small as well as the very large.
  - ScC in nanoelectronics, complexity in software development, boardband communications and Grids.
- Exploring multidisciplinary fields combining ICT with other science and technology fields.
  - Work programme in nanosystems, ICT for Health, cognitive systems, Future Emerging Technologies (FET)
- Promoting innovation from ICT use by bringing services and developments closer together.
  - Communications, nano and micro systems



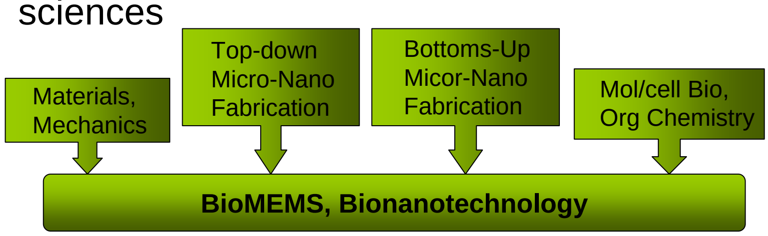
–From EU IST 2004 Work Programme 2005-2006

FROM MEMS TO MICROSYSTEMS FOR HEALTHCARE



Trends in Silicon-Based Biosensors

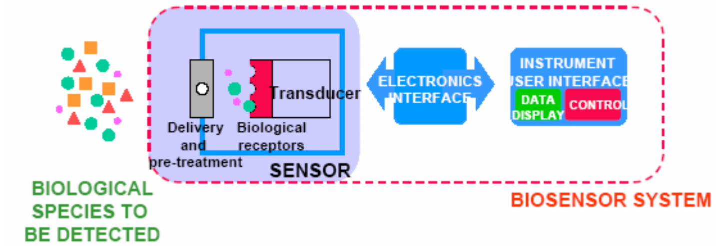
- Merger of Biotechnology and Micro/nanotechnology brings about unique and exciting possibilities.
  1. CMOS-based Integrated BioMEMS
  2. Optoelectronics & Photonics
  3. Wireless sensor network
- Engineers need to learn more about life-sciences



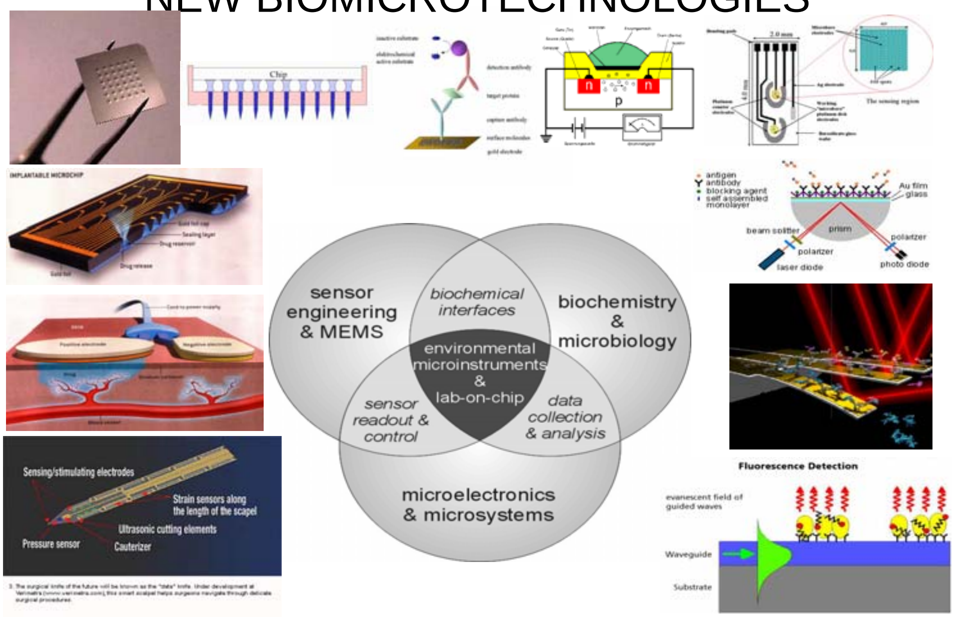
Health and comfort monitoring

Convergence of nano and biology

- Information capture
  - Affinity microarrays for DNA, proteins
  - Biosensors
  - Biological lab on chip, electropheresis,
  - DNA sequencers, mass spectrometry
- Drug delivery
- Bionics



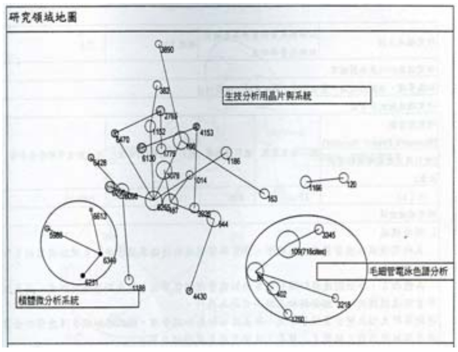
NEW BIOMICROTECHNOLOGIES

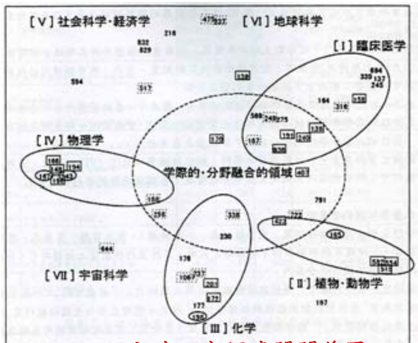


Global View in Bio Analysis Device

- 實際應用領域中，生物應用領域之生技分析裝置尚在各項技術研究階段
- 根據日本之科技中長期發展全瞻性預測調查，生技分析儀器爲**51**個急速發展之研究領域之一
- 主要內容：微體積分析系統 、多管道/微流體分析系統、微晶片電泳儀、毛細管電泳色譜分析儀、三次元微管道、**DNA**電泳機制

生物分析用裝置研究領域地圖





全球研究領域間關係圖

Wearable biosensors & Systems

Digital Finger Pulse Oximeter

http://www.nonin.com/products/9500.html

Actiwatch Actigraphs

http://www.minimitter.com/products/Actiwatch/

GPS Locator Watch For Alzheimer's Patients

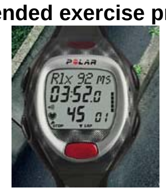
http://www.wirelesshomesecurityalarmsystems.com/

Telemonitoring Services

Bracelet Vivago

http://www.vivago.org/home.htm

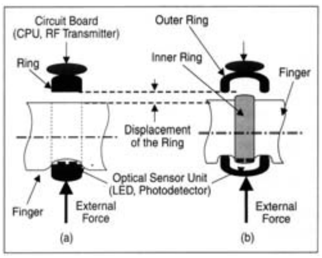
Exercise Set (extended exercise profiles)

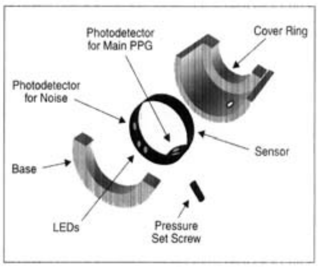


http://www.polar.fi/polar/channels/eng/segments/Running/S810i/allfeatures.html

HEART RHYTHM (ECG) MEASUREMENT DEVICE

Wearable Ring Sensor

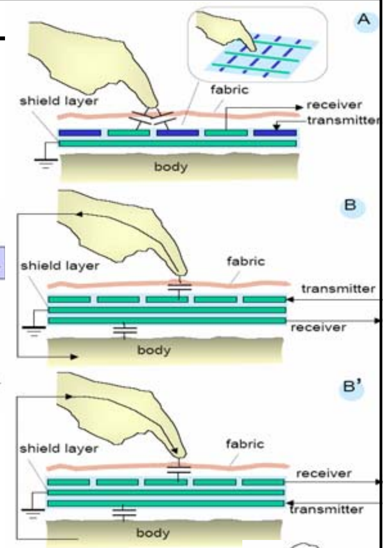


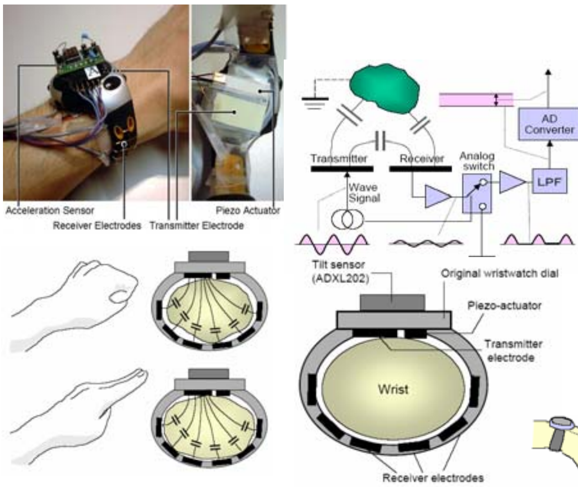


- Use and comfortable to wear for long periods of time
- This sensor is equipped with optoelectric components that allow forlong-term monitoring of a patient's arterial blood volume waveforms and blood oxygen saturation non-invasively and continuously
- The digital signals are transmitted by an RF wave through the standard RS-232 protocol.
- The whole process is scheduled and controlled by a single microprocessor on the ring.
- The inner ring is made up of an elastic material to maintain necessary pressure on the skin, while the outer ring is composed of a solid material such as aluminum to sustain the mass of the circuit boards and battery.

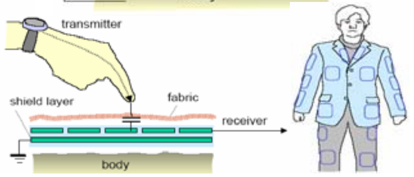
Sokwoo Rhee "Design and Optimization of an Artifact-Resistive Wearable Photoplethysmographic Device : The Ring Sensor",1999.8

wearable by using gesture-based commands

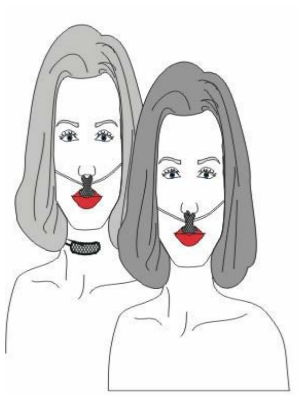


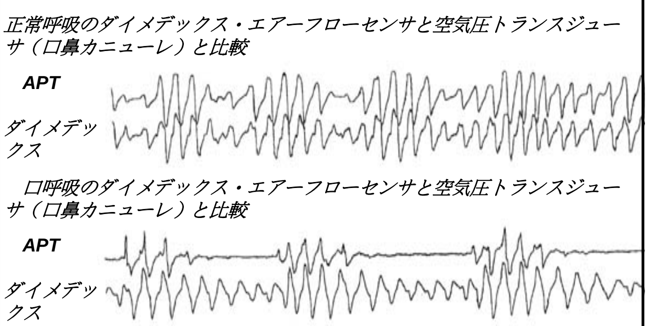


http://www.csl.sony.co.jp/person/rekimoto.html



Wearable breath sensor



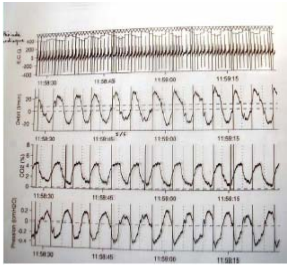
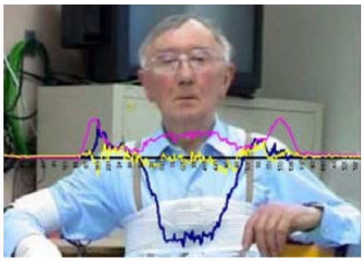



The breath sensor which uses the PVDF film it meaning accuracy of the ripple mark, and that sensitivity is superior gives the appraisal which is good to diagnosis.

http://www.miyuki-net.co.jp/hpage/respsensor.htm PVDF (Poly-vinylla-dene-Fluoride)

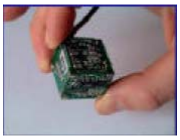
Wearable devices

- **Objectif : Wearable devices**
  Arythmie sinusale respiratoire

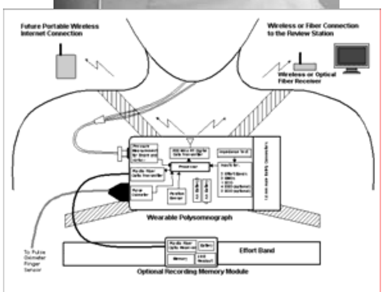
TIMC-PRETA & Sté RBI, G. BenChetrit, R. Baghi

- **Technological opportunities**
  – MEMS, motion capture device
  – Low power electronic design (ASICs)
  – Remote power supply / data transmission
  – System approach & information processing
  – Micro energy supply (or auto energy supply)




Polysomnograph

Advanced Medical Electronics Corporation

A full-featured 16-channel wearable polysomnograph that records locally, or transfers data off-body using a plastic optical fiber connection or wireless digital radio.

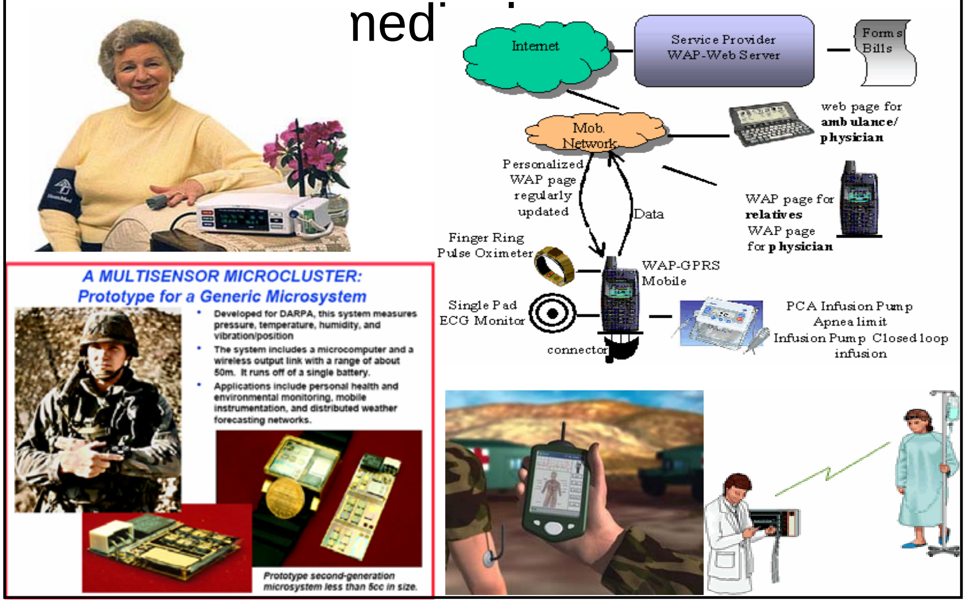
FEATURES:

· 16 data channels, including:
· Pulse Oximetry and Heart Rate,
· EEG (4 channels),
· EOG (2 channels),
· EMG (2 channels),
· EKG,
· Chest Effort (2 channels),
· Airflow (thermoresistive or optional pressure based),
· Body Position,
· Snore Sensor.

· Small Package: 0.85 x 3 x 6.5 inches.
· Lightweight: 8 ounces.
· Low Power: 2 AA batteries last 12 hours.
· Impedance Testing.
· Optical fiber connection (option).
· Wireless digital radio connection (option).
· Solid-state recording memory module (option).

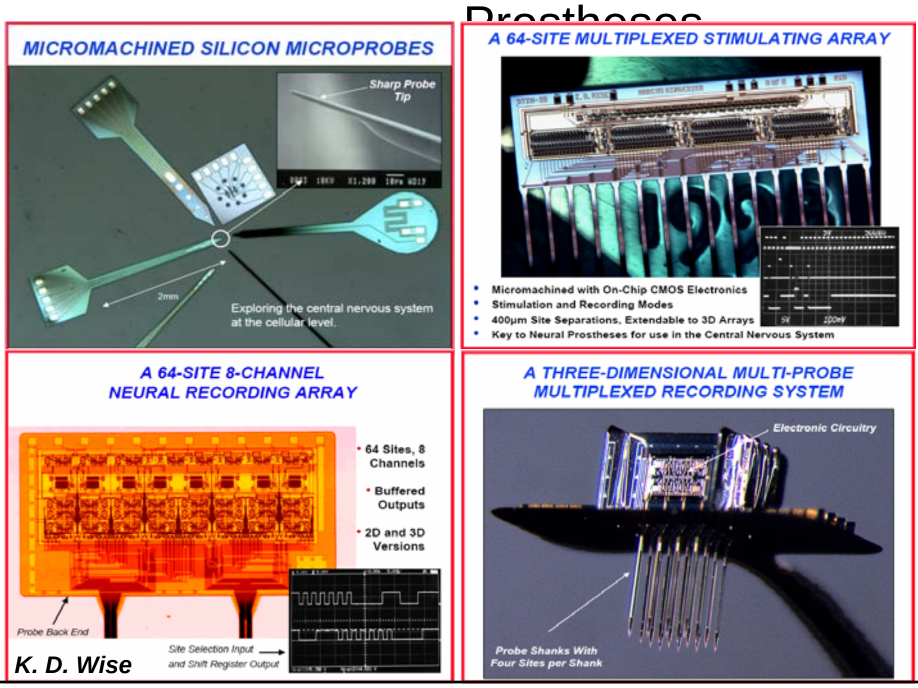
http://www.ame-corp.com/Wearable_Polysomnograph.htm

Wireless Systems for Wearable
ned



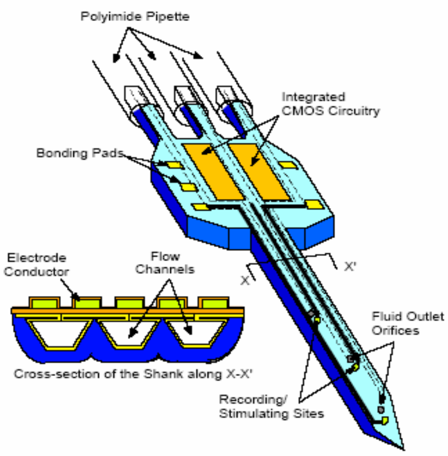
Minimum Invasive Silicon Microrobes: Recording Array for Neural Prostheses

3D Recording Array for Neural Prostheses



Integrated CMOS Circuitry with Microfluidic

TOWARDS MULTIPLEXED DRUG DELIVERY AT THE CELLULAR LEVEL



- Using only one additional mask, microchannels for drug delivery can be added to the probe substrate.
- Thus, electrical recording and stimulation can be done while controlling the chemical environment.
- Using no additional masks, shutters and in-line flowmeters can also be incorporated in the microchannels, allowing doses to be monitored to within a few picoLiters.
- Tentative designs for on-chip micropumps and microvalves for drug delivery have been identified.
- Such devices offer important new capabilities for research in neuropharmacology and for the treatment of important neurological disorders.

K. D. Wise

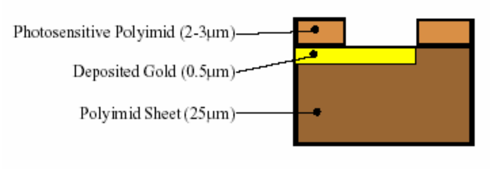
Flexible Surface Multipoint Microelectrode Array

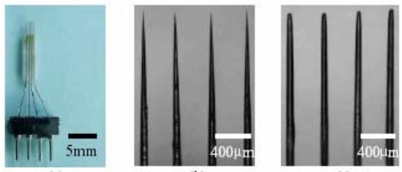
for Direct Recording of Auditory Evoked Potentials on the Auditory Cortex of a Rat

1.Microelectrode array

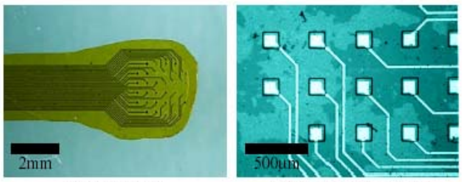
- 32 sites (100 μm2) in 2mm2
- Polyimide for biocopability and flexibiity

2. Spike microelectrode array





(a) (b) (c)

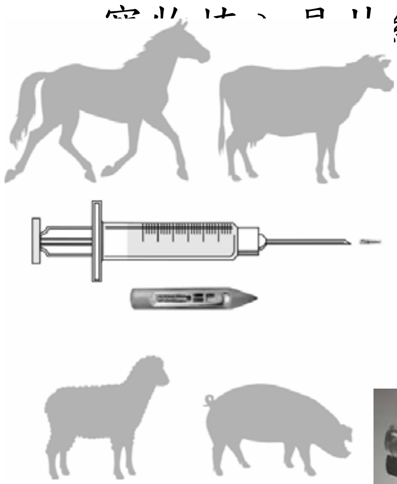


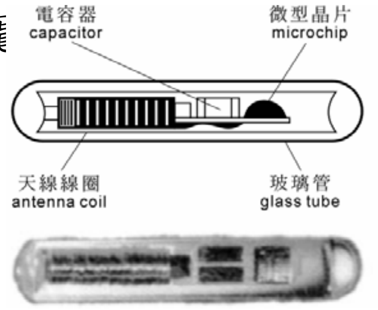
(a) Whole view (b) Magnification of the tip

Hirokazu Takahashi, Takayuki Ejiri, Masayuki Nakao, Kiyosi Matsumoto, Fumio Mase, Yotaro Hatamura, Thierry Hervé, Member, IEEE, Naoya Nakamura ,and Kimitaka Kaga:"Surface Multipoint Microelectrode for Direct Recording of Auditory Evoked Potentials on the Auditory Cortex of a Rat,"Proceedings of 1st Annual International IEEE-EMBS Special Topic Conference on Microtechnologies in Medicine & Biology, October, 2000, pp.512-517

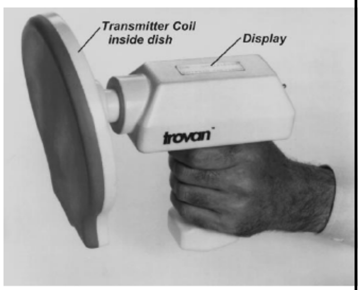
Implantable Microsystems

給寵





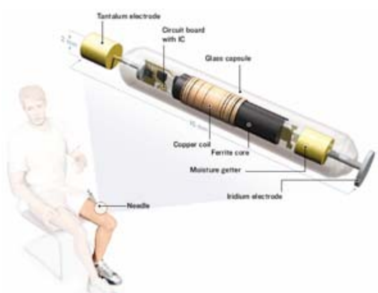
ISBN 7-01-002069-8



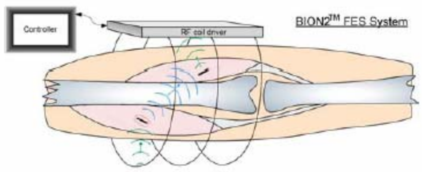
Microminiature implants

provides a long-term, wireless interface between an electronic controller and a neural function in the body

Microstimulator. At each end, a stimulating metal electrode exits the glass capsule. (Photograph courtesy of A. E. Mann Foundation.)



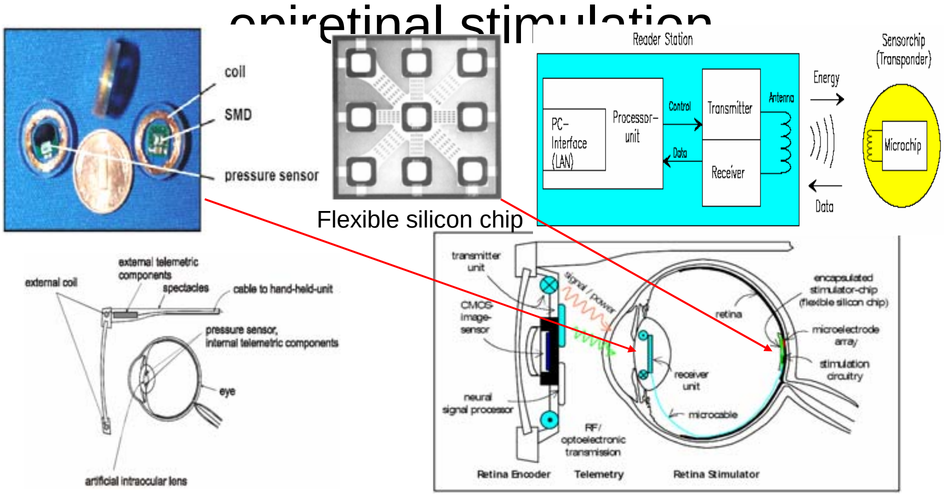
Injectable microstimulator for stimulation of paralyzed muscle. The single helical transmitter coil can power and command many individual microstimulators within the limb.



BIONic muscle spindle based on measuring mutual inductive coupling between implants.

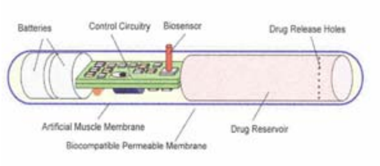
Philip R. Troyk. DEVELOPMENT OF BION TECHNOLOGY FOR FUNCTIONAL ELECTRICAL STIMULATION: BIDIRECTIONAL TELEMETRY.

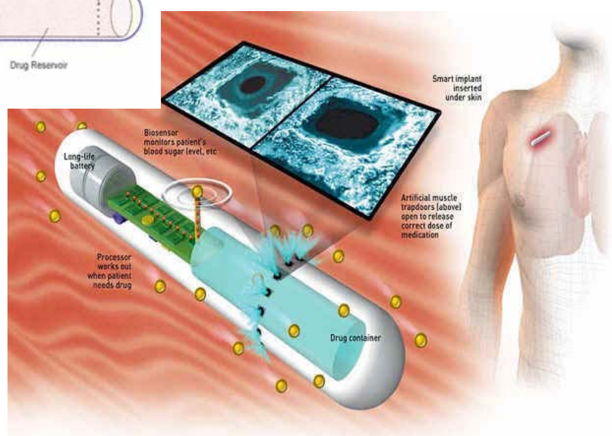
System concept of the visual for epiretinal stimulation



W. Mokwa, U. Schnakenberg: On-chip microsystems for medical applications Proc. Microsystem Symposium 1998, Delft, September 10-11,1998

The Smart Pill Demo

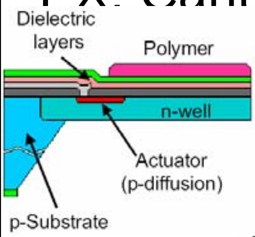


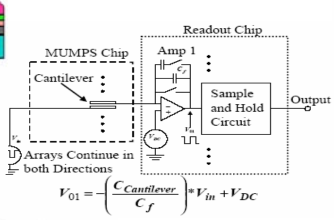


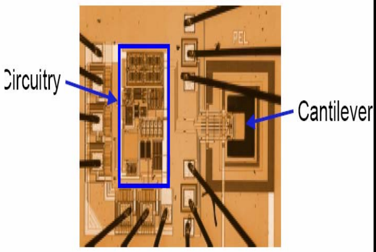
http://mmadou.eng.uci.edu/images/smartpill.swf

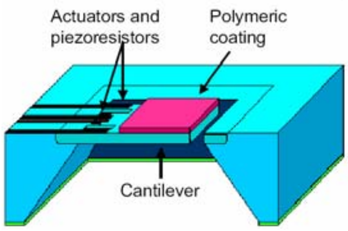
Research devices

EX: Cantilever and On-Chip Amplifier









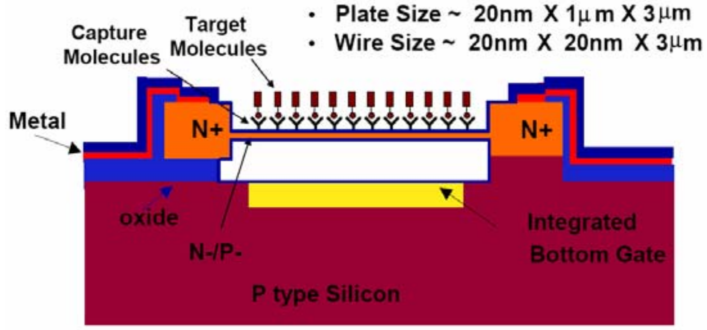
- ◆Chip size:1.2x1.8mm^2
- ◆Resonance frequency:380kHz and higher
- ◆Heating power~6mW
- ◆Quality factor:950 in air
- ◆On-chip pre-amplifier

D.Lange, C. Hagleitner, A. Hierlemann, O. Brand, Analytical Chemistry 74(2002) 3084-3095.

Integrated Nano-electromechanical Biomolecular Sensors

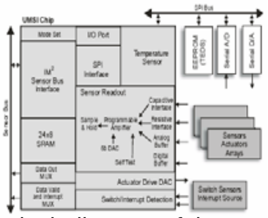
- Bio-sensors with electronic output
- Capability of dense arrays integrated with ULSI silicon
- Detection of DNA and Proteins
- Electrical and Mechanical sensing

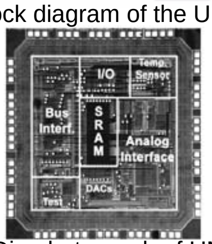
Bashir: Fabrication and testing
Bergstrom: DNA/PNA & Protein Chemistry
Rundell: Antibody/Antigen Interactions
Vasmatzis (Mayo): Bio Markers



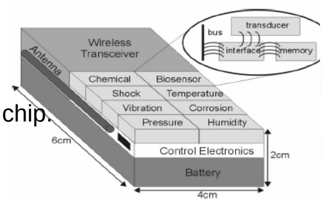
EX:A Modular Sensor Microsystem Utilizing a Universal Interface Circuit

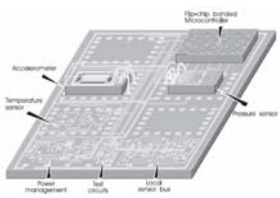
Multi Chip Module (MCM) Microsystems. J. H. Correia


Block diagram of the UMSI chip.



Die photograph of UMSI chip.



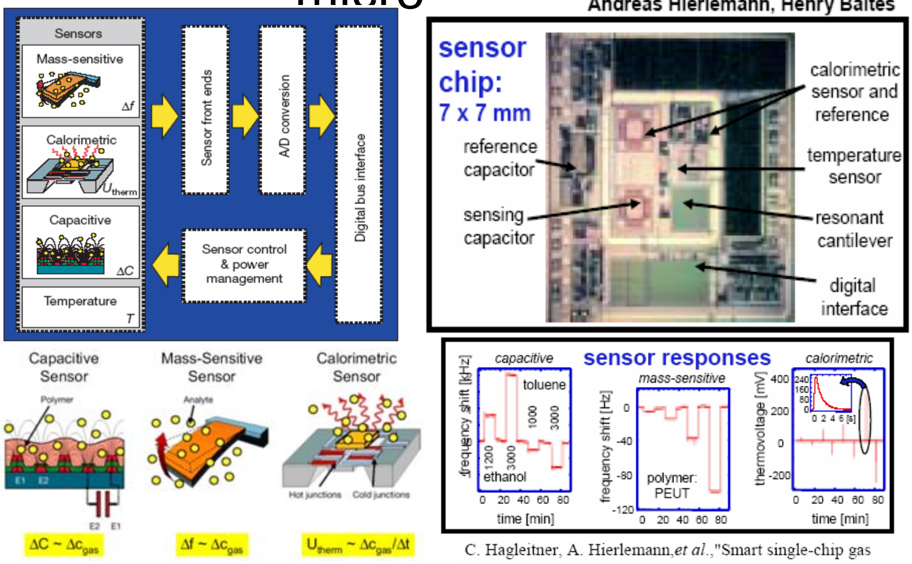


A compact modular microsystem packaging scheme showing sensor module components and microsystem module arrangement.

IEEE Int. Symposium on Circ. and Systems (ISCAS), Bangkok Thailand, May 2003.
A Low-Power Low-Voltage Digital Bus Interface for MCM-Based Microsystems.

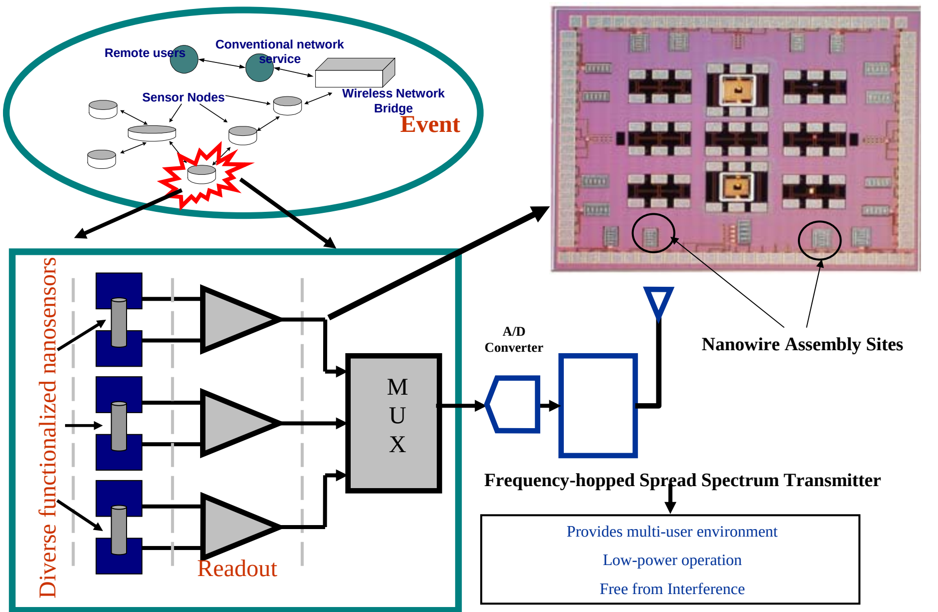
EX: Smart single-chip gas sensor microsystem

Andreas Hierlemann, Henry Baltes



C. Hagleitner, A. Hierlemann, *et al.*, "Smart single-chip gas sensor microsystem", *Nature 414*, 293-296 (2001).

Architecture of Wireless Sensor Networks



Prof. Stephane Evoy

Domestic developments

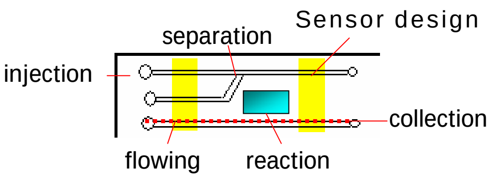
- RFID –
  - 工研院　電子所, 生醫中心
  - 台灣大學 呂學士 教授
- FES –
  - 成功大學 陳家進　教授
  - 台灣大學　賴金鑫　教授
- Artificial Retina –
  - 交通大學 吳重雨　教授
  - 陽明大學 張寅 教授
- Swallowable camera
  - 成功大學　羅錦興　教授
  - 中山科學院
- CMOS Thermal Actuator
  - 清華大學 方維倫 教授
- ChemFET
  - 中原大學　熊慎幹　校長
  - 雲科大　張榮泉　教授
- NanoSensor
  - 成功大學　張憲彰　教授
  - 台灣大學　陳炳輝　教授
  - 台灣大學　黃榮山　教授
- Sensory electrode array
  - 陽明大學　楊順聰　教授
- MEA
  - 中研院生醫所　許百川　教授
  - 交通大學 林志生 教授
- Lab-on-a-chip
  - 清華大學 曾繁根 教授
  - 成功大學 李國賓 教授

SPR Biochip

Motivation 1 – Biochip application

1. Accurate and minimized device with analytical or diagnostic function.
2. Substrates can be the glass, silica wafer, or plastic.
3. High sensitivity and specificity, high speed, small size, small sample requirement, multi-channel and parallel analyzing

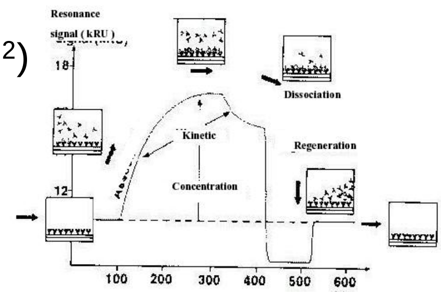
- Solid – liquid interface interaction
- Nonlabeling method



Bio-sensors in surface detection
- 1.QCM. (piezoelectricity)
- 2.Electrochemical measurement
- 3.**SPR**
- 4.TIRF/TIRS
- 5.Fluorescence
- 6.Ellipsometry
- 7.AFM

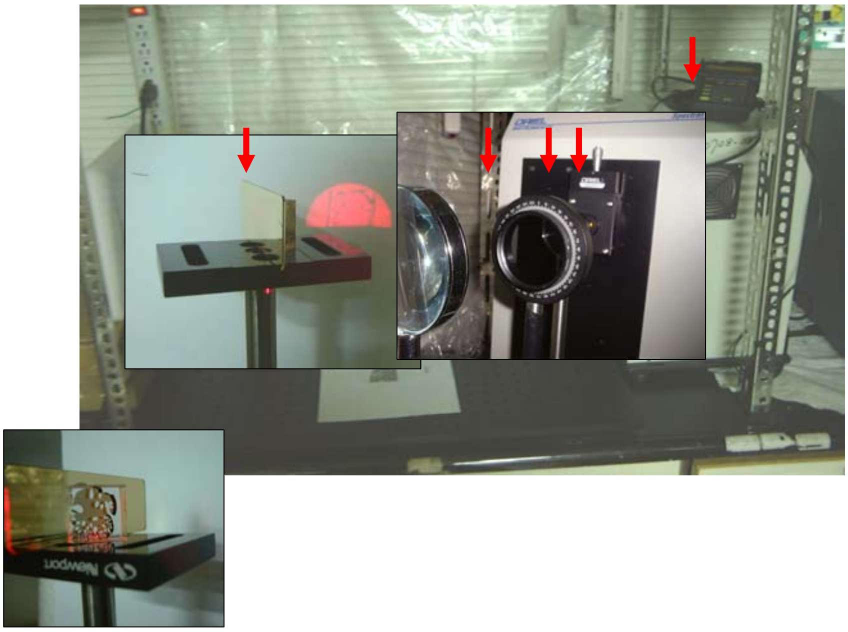
Advantage of SPR sensor

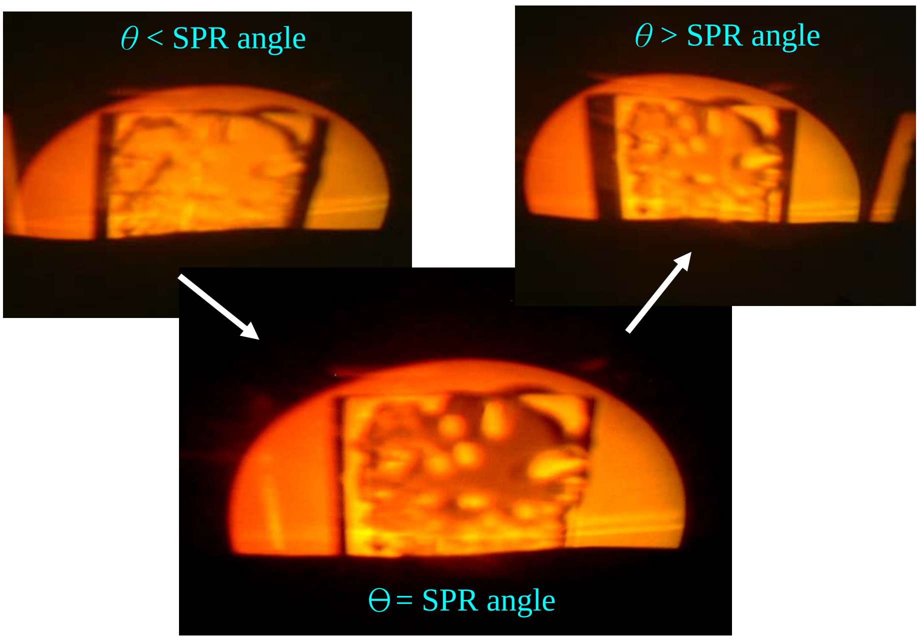
1. Utilizing in liquid-solid interface
2. Real time sensing
3. non-labeling (comparing: ELISA, fluorescence, autoradiograph)
4. high sensitivity (1 pg/mm^2)
5. multichannel parallel detection



Homola, Sinclair S. Yee, Surface plasmon resonance sensors: review , (1999)

SPR phenomena





k1

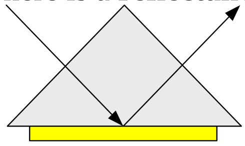
SPR sensor structure

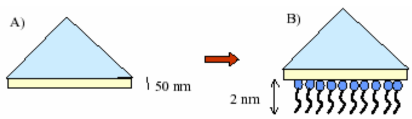
SPR (surface plasmon resonance)

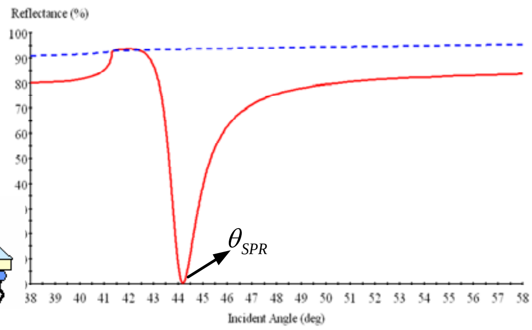
1.Excited by **TM-wave** in the surface between **metal and dielectric**.

2.Use the **prism coupling method** by total internal reflection to couple the light into the SP wave.

3.There is a **reflectance dip** called SPR resonance angle (θ_{SPR})







Motivation 2: OBmorph System

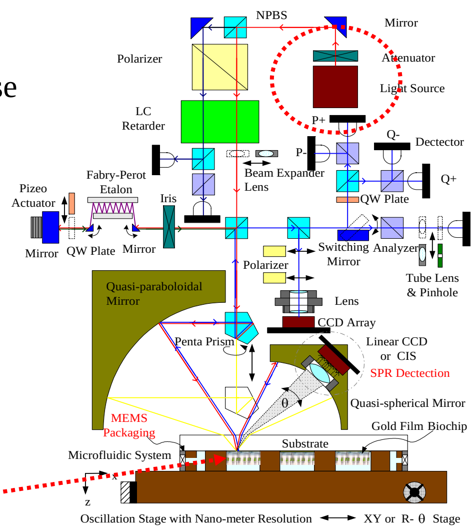
- Ellipsometer
- SPR for amplitude and phase detection

Problems:

- Incident angle > 74 degree for BK7/Au/Water

Possible solutions

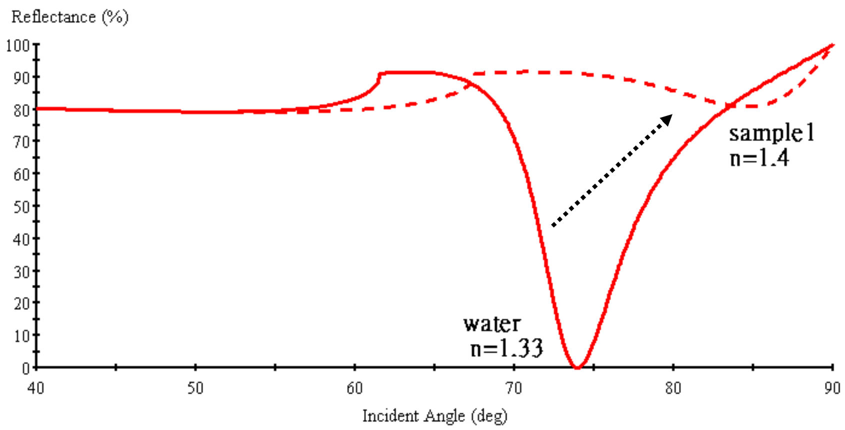
1. NIR excitation
2. Higher refractive index coupling prism
3. **SPR thinfilm device**



k1 20世紀初被發現,1970年代由otto & kretchsmenn用菱鏡偶合激發,1990年代開始商品化.
kobela, 2003/12/14

Motivation

• Too big SPR angle for the BK7 prism, 633 solid laser system in the liquid phase.



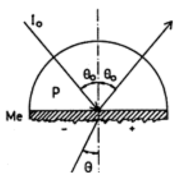
Novel concept development

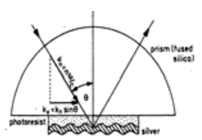
1.SPR on smooth, rough, periodic structure (grating) surface

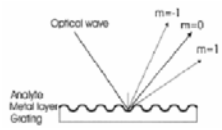
- Smooth – theory
- Rough – directional scattering
- Grating – enhanced SPR with h factor of S(x) = h sin (2 π /a)x. (smaller h for larger enhancement)

2.Coupled SPR & Long range SPR

Various thickness of dielectric layer for enhancement of SPR signal (coupling & wave guide effect)

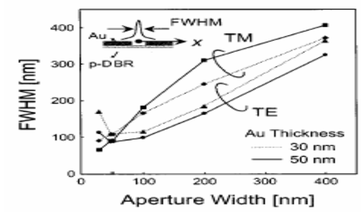


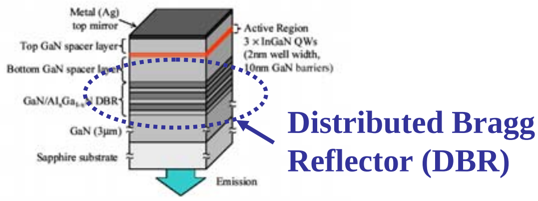


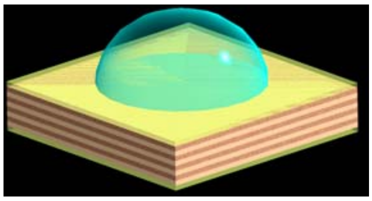


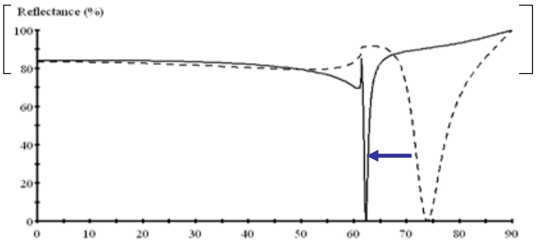
Active SPR Device

Combination of the traditional single metal layer and the VCSEL (vertical cavity surface-emitting laser)

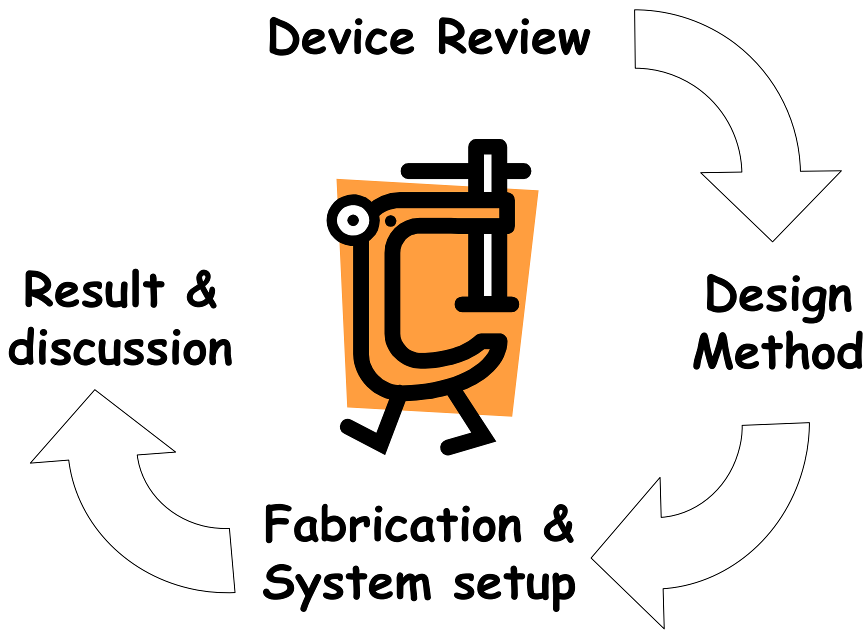




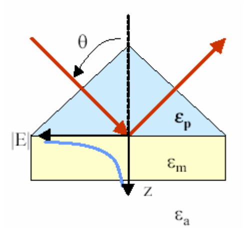


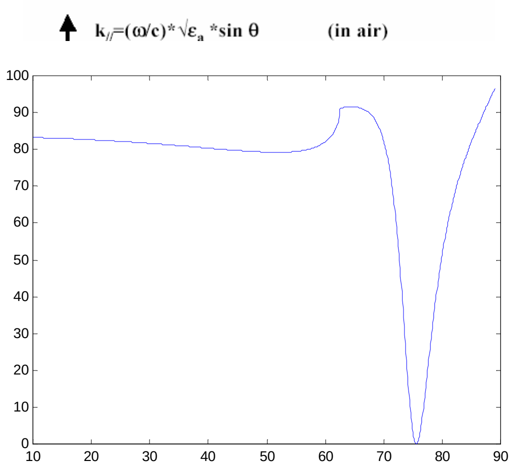


•We want to **enhance** the SPR signal and **modulate** the resonance angle



Single metal layer





$$K_x(\theta)=(\frac{\omega}{c})\cdot\sqrt{\varepsilon_p}\cdot\sin\theta_{SP}$$

While the wavelength is 633nm

$\varepsilon_p = (1.5)^2$

$\varepsilon_a = (1.33)^2$,

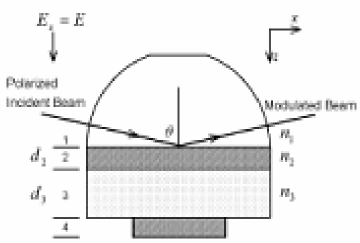
$\varepsilon'_m = -10.1055 = \mathrm{Re}[(0.15 - i*3.3)^2]$ Au

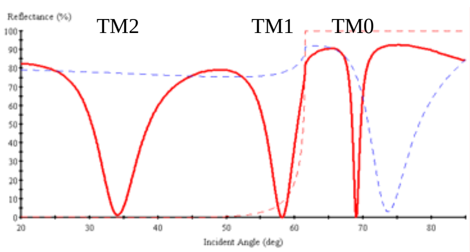
→ We can calculate the angle is 74 degree

CPWR (Coupled plasmon- waveguided resonators)

-By using a thick dielectric layer to cause the multi dips in the reflection curve

-only TM0 is SPR wave, the others are belonged to guided wave, and can't be used for sensing.





633nm, BK7-Au(25)-SiO2(700)-Au(25)-H2O

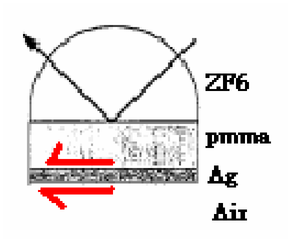
Low voltage electro -optic polymer light modulator using attenuated total internal reflection, *2001,* Y. Jiang, Z. Cao,

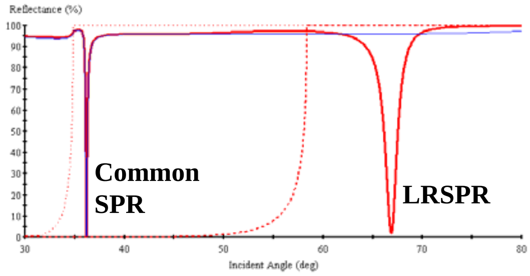
LRSPR (Long range surface plasmon resonance)

-In front of the metal layer, putting a space layer (dielectric).

-LRSP will cause Im (Ksp) smaller, so it will have longer propagation length.

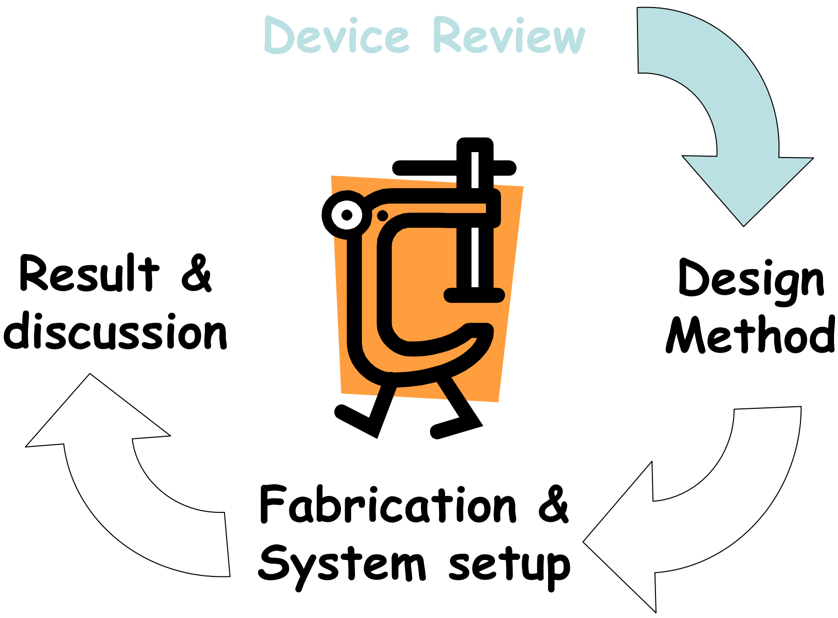
-LRSPR stimulate the SPR at the both sides of the metal.





633nm, ZF6-pmma(400nm)-Ag(55)-Air

Improved SPR Technique for Determination of the thickness and Optical Constants of thin Metal Films, 2002, Y.Ding, Z.Q. Cao, Q.S.Shen



Optical Thin-film calculation

Single layer

Yo

Ys

Fresnel's Eq

$$R = \left(\frac{Y_0 - Y_S}{Y_0 + Y_S}\right)^2$$

Multilayer

Yo
Y1
Y2
Y3
Y4
Y5
Ys

Characteristic matrix

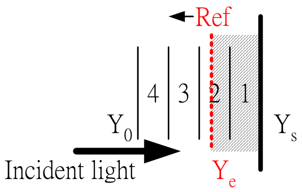
$$\begin{bmatrix} B \\ C \end{bmatrix} = \prod_{j=1}^{a} \begin{bmatrix} \cos\delta_j & i \cdot \sin\delta_j / \eta_j \\ i \cdot \eta_j \sin\delta_j & \cos\delta_j \end{bmatrix} \begin{bmatrix} 1 \\ \eta_{sub} \end{bmatrix}$$

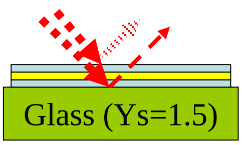
Yo

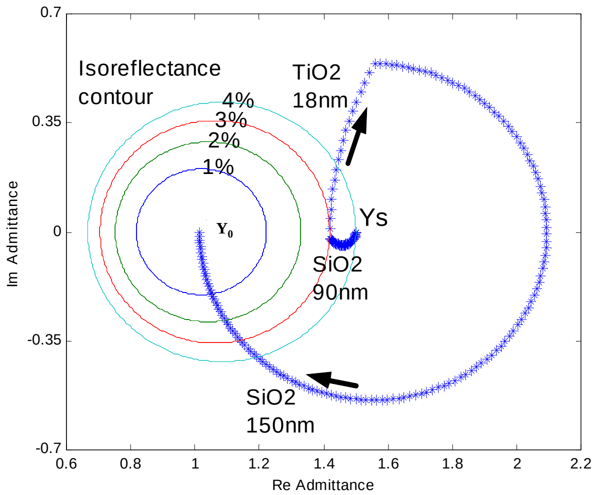
Ye

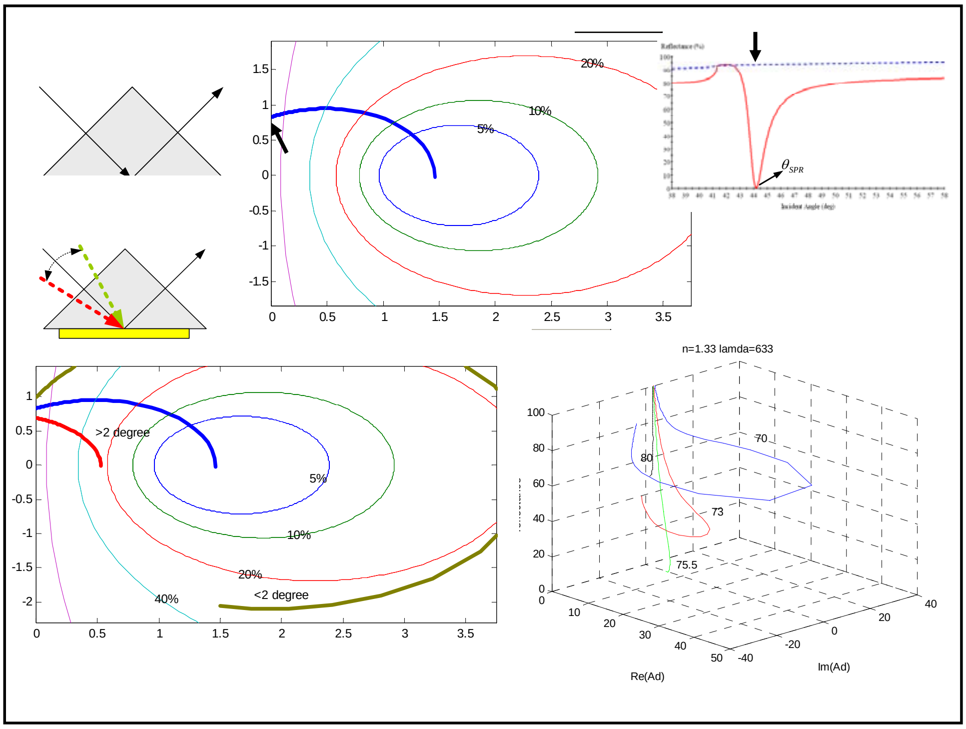
$$R = \left(\frac{Y_0 - Y_E}{Y_0 + Y_E}\right)^2$$

Admittance loci design method

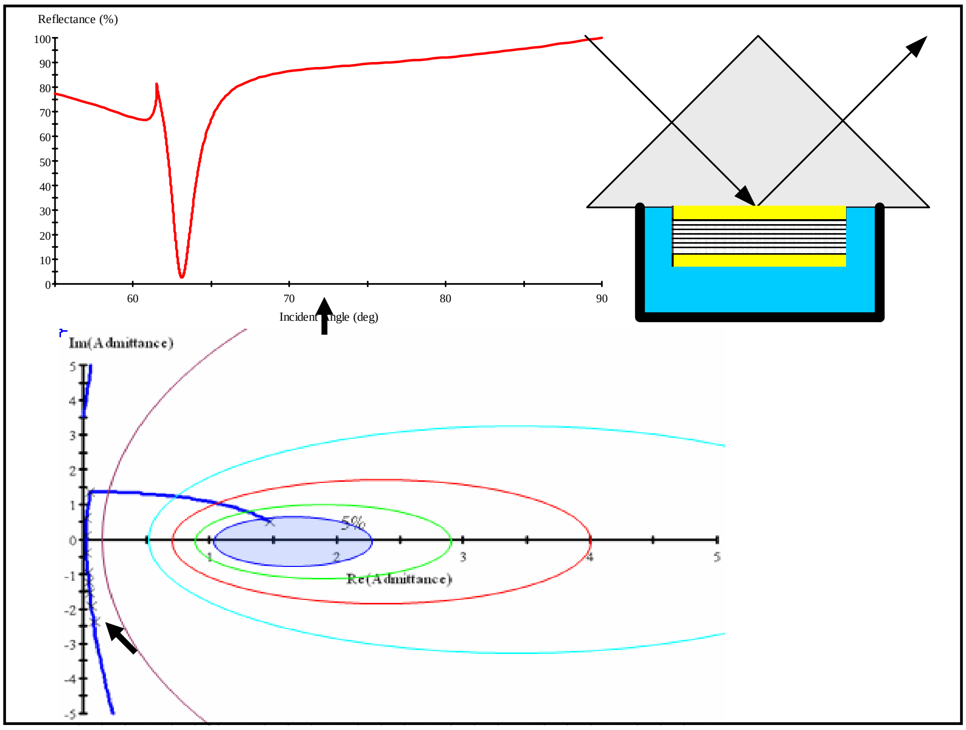








n=1.33 lamda=633
>2 degree
<2 degree
5%
10%
20%
40%
70
80
73
75.5
Re(Ad)
Im(Ad)

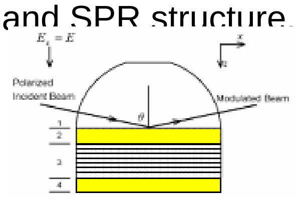


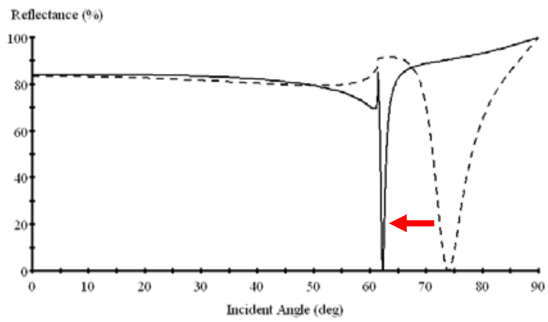
Reflectance (%)
Incident Angle (deg)
Im(Admittance)
Re(Admittance)
5%

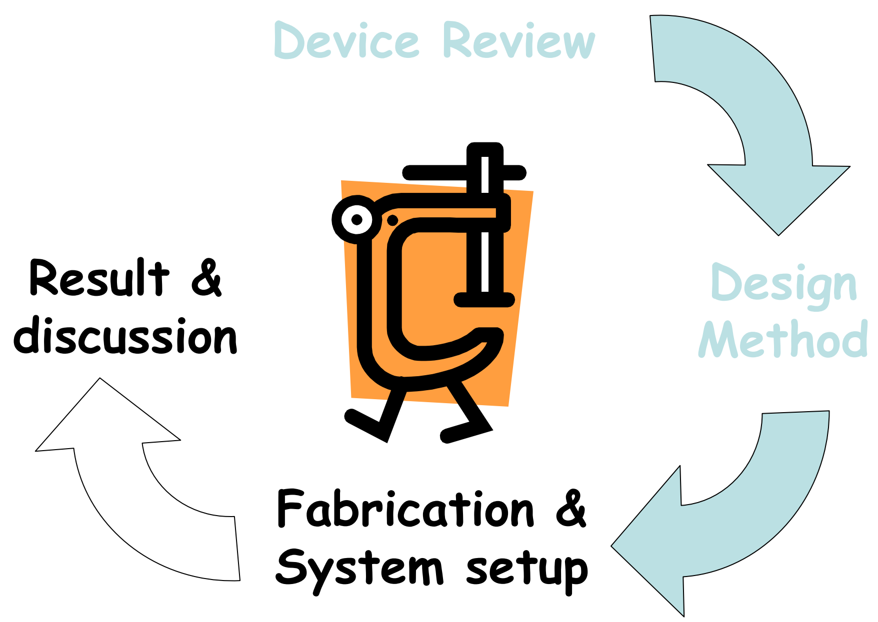
Multilayer design

- Combine the VCSEL and SPR structure, enhance the signal.

Au	30nm
SiO2	20nm
TiO2	20nm
SiO2	20nm
TiO2	20nm
SiO2	20nm
TiO2	20nm
SiO2	20nm
TiO2	20nm
Au	40nm
Glass slide	







Material

1.material:

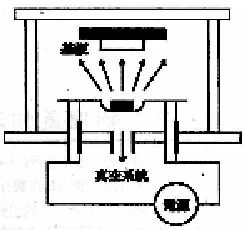
- substrate: glass slide (n~1.515)
  - SuperFrost – 100 Lames porte-Objet Microscopy slides, MENZEL-GLASER ,German, 76 * 26 mm.
- Target :Au, TiO_2, SiO_2, Cr (>99.99%)
- Coupling prism: BK7 right angle prism (2.5cm*2.5cm)
- Sample medium: water (n=1.33)
- Matching oil (n=1.52)

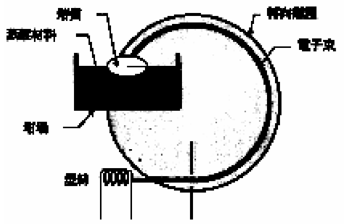
Target	n	k	Melt	Density	Z-ratio
Au	0.162	3.212	1063	19.3	0.381
SiO2	1.457	0	1610	2.202	1.07
TiO2	2.279	0	1825	4.26	0.4

n, k value @ λ=643nm

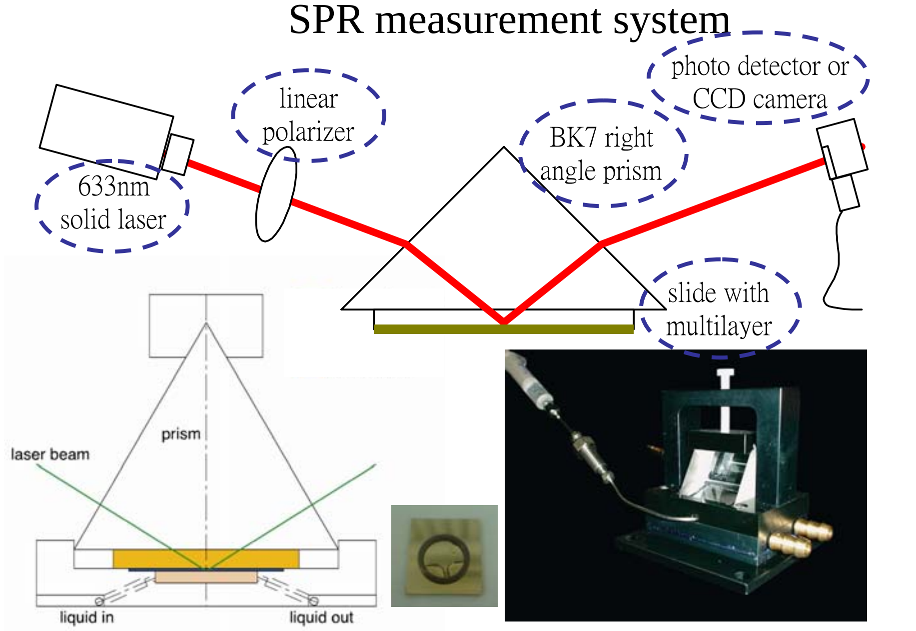
Process

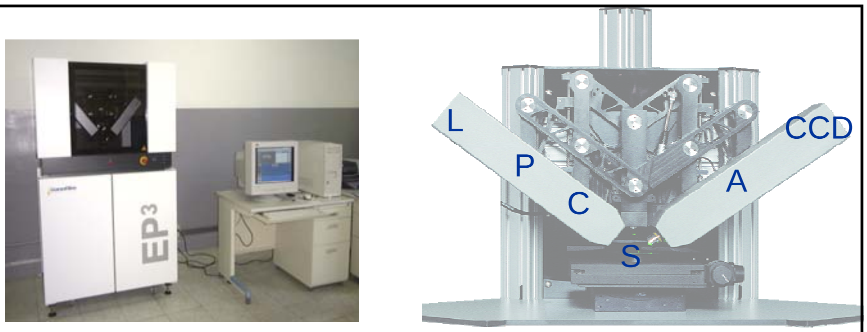
1. Clean substrate, piranha solution -> detergent -> ultrasonic -> remove water stain
2. Put the sample and target in to E-beam evaporator, pumping the chamber < $1.6*10^{-5}$ torr, and heating substrate to 60℃.
3. E-beam power: 9000V, 3-20mA, shooting the traget, let the evaporating rate~2Å/s.
4. Input the material parameter (density, and Z-ratio), and use QCM to control the thickness



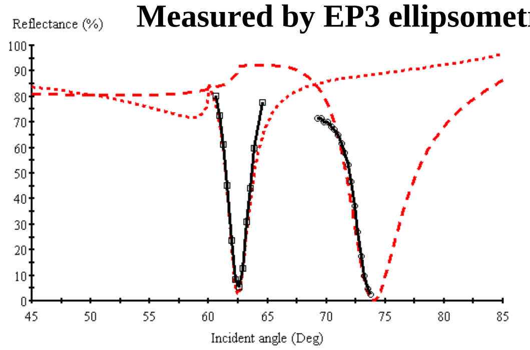


SPR measurement system



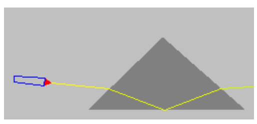


Measured by EP3 ellipsometry



643nm solid laser

Angular resolution: 0.001 degree

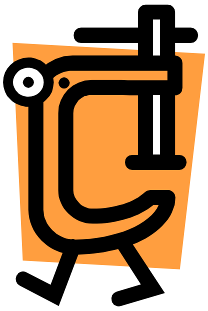


Device Review

Design Method

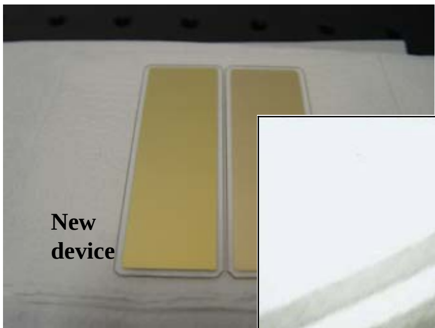
Fabrication & System setup

Result & discussion

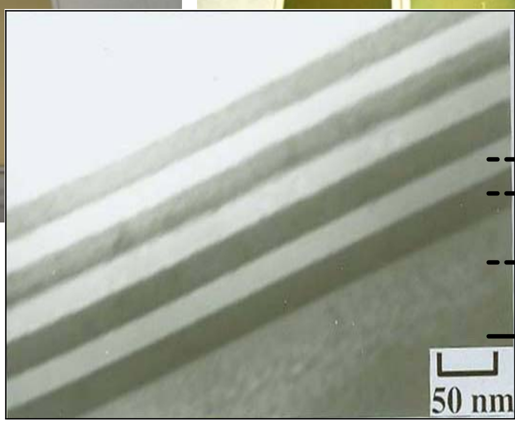


Result

Outlook comparing with 50nm Au



TEM scanning of the multilayer structure



Material test (n, k, d) by ellipsometry

TiO_2

QCM	d	n	k	MSE
20	24.84±0.08	1.4477	0.0063	8.3E-03
50	56.69±0.54	1.4562	0.003	8.16E-03

SiO_2

QCM	d	n	k	MSE
20	33.24±0.33	2.0537	0.0026	16.8E-03
50	63.79±0.45	2.1948	0.0011	12E-03

Au

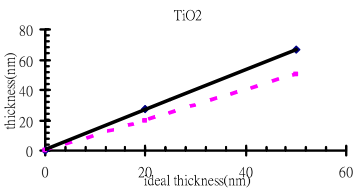
QCM	d	n	k	MSE
30	29.18±0.13	0.1636	3.4858	6.04E-03
50	46.17±1.2	0.1621	3.5387	4.01E-03

1. The thickness is different with the QCM monitor.
2. n, k values change with the different thickness.

Ts= 60℃

$1.5*10^{-5}$ Torr

For dielectric, the fitting thickness is thicker than QCM, and the fitting n value is smaller than bulk material.



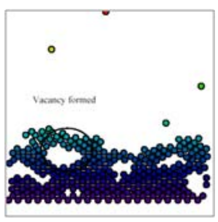
Regression	TiO2	SiO2	Au
	y=1.2556x+3.0509	Y=1.1283x+0.8549	Y=0.9274x+0.3882
R^2	0.9807	0.9981	0.9987

	TiO2	SiO2	Au
n_B	2.276	1.4567	0.1501
k_B	0.0002	0	3.30

Packing density (p):

$$p = \frac{\text{volume of solid part of film}}{\text{total volume of film (including pores)}}$$

$$p = \frac{n_f - n_0}{n_B - n_0}$$



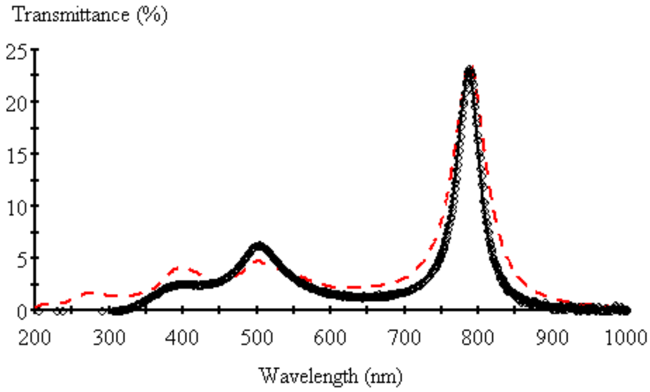
	20nm TiO2	50nm TiO2	20nm SiO2	50nm SiO2	30nm Au	50nm Au
p	82.16%	93.49%	97.96%	99.82%	98.4%	98.58%

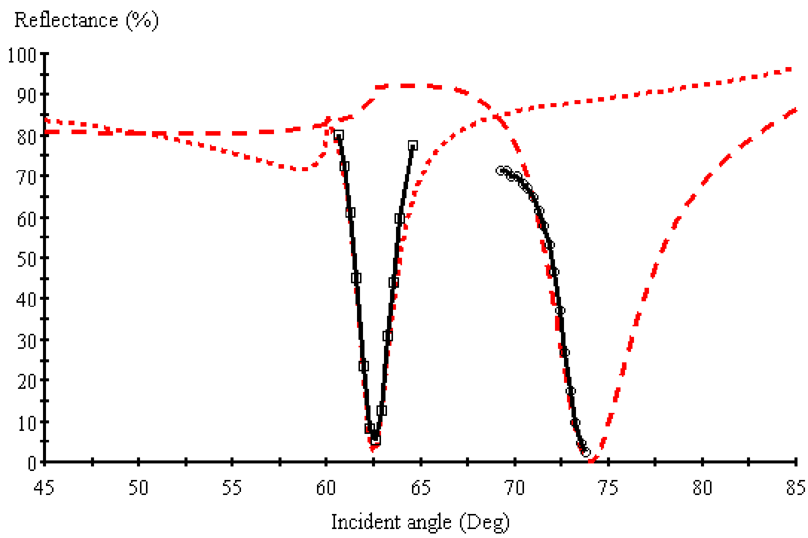
H.A. Macleod, "Thin film optical filter (1986)

Discussion

In transmittance curve, the error of dielectric layer thickness will cause the peak shift, and metal thickness error will change the peak value.

Au	30nm
SiO2	20nm
TiO2	20nm
SiO2	20nm
TiO2	20nm
SiO2	20nm
TiO2	20nm
SiO2	20nm
TiO2	20nm
Au	40nm
Glass slide	

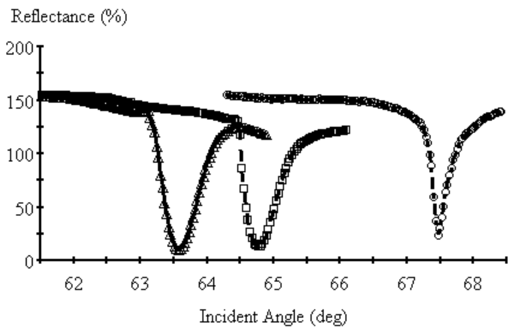


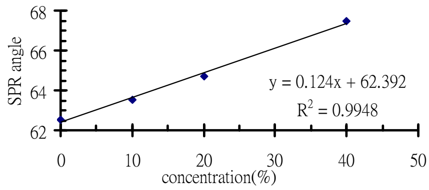


Application Experiment

1. Input 10%, 20%, 40% glucose.

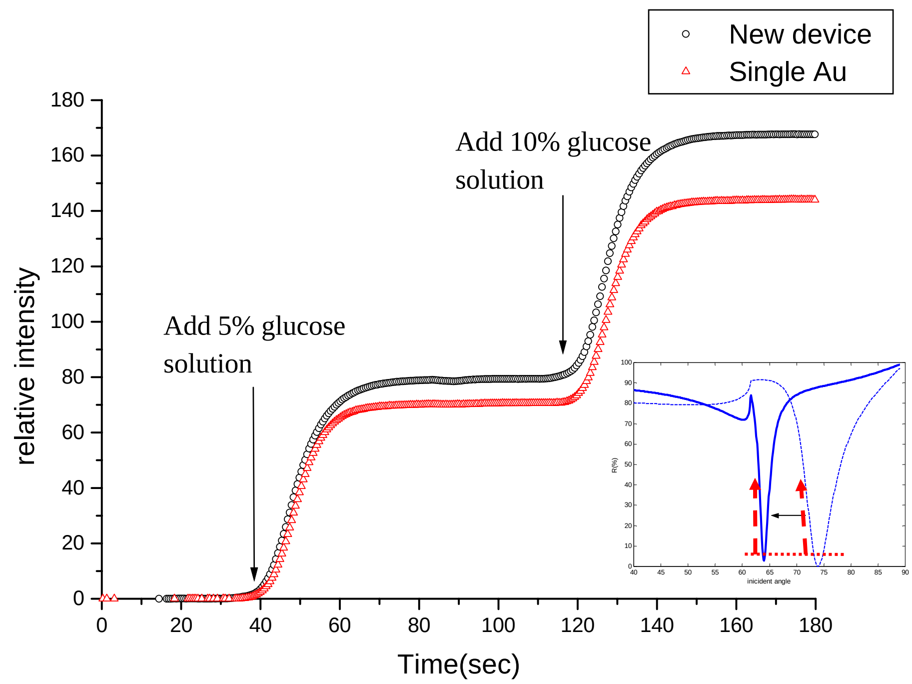
concentration	SPR angle
dH2O	62.54
10%	63.55
20%	64.7
40%	67.46





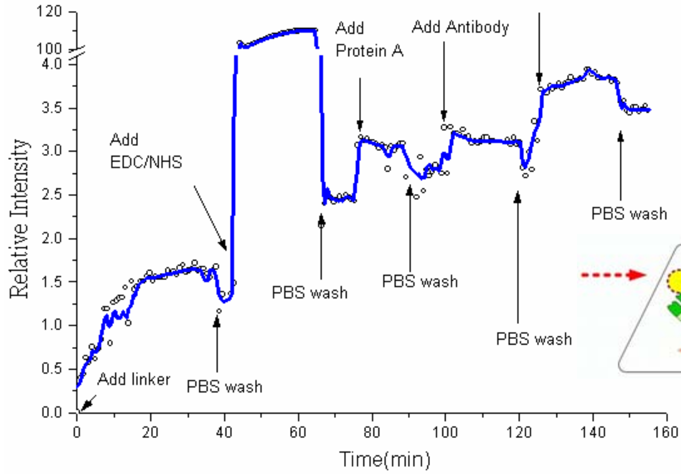
Sensitivity of Glucose:

16 pg/mm²



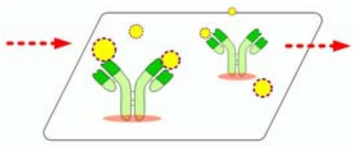
Protein-protein interaction

	PBS	Linker	EDC/NHS	protein A	Ab (IgG)	Ag (ALV)
Concentration	10x	20uM	400mM/100mM	0.1mg/ml	0.3ug/ml	~30ng/ml

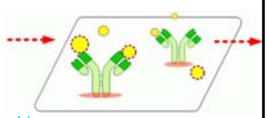


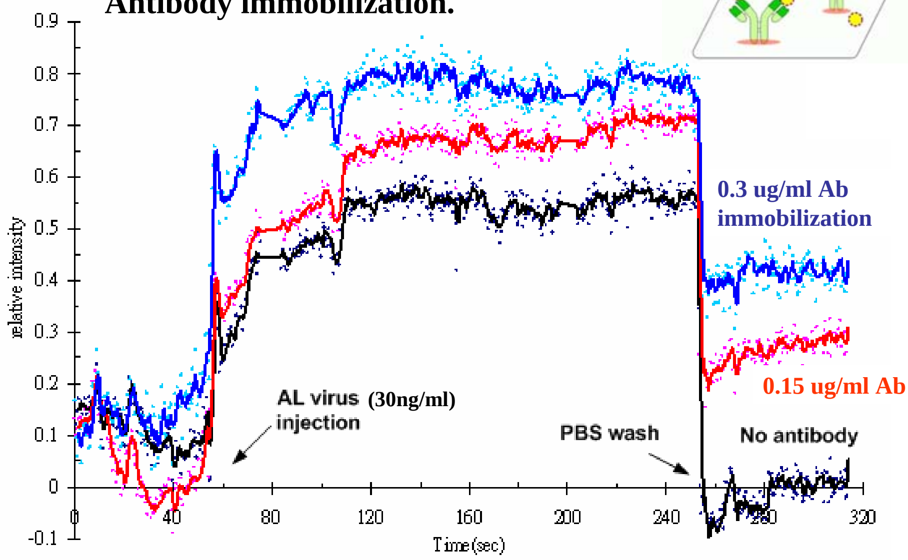
IgG: MW= 150 kDa, size: ~10nm

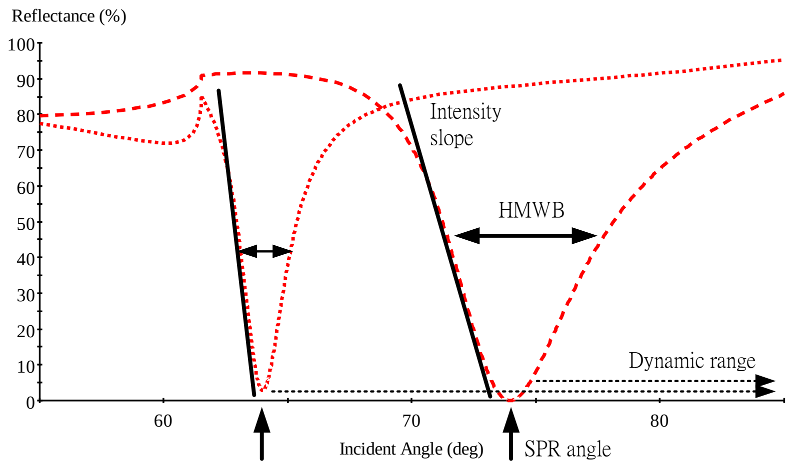
ALV:Avian Leukosis Virus, size: 50~100nm



Different concentration Antibody immobilization.







	SPR angle	HMBW	Dynamic range	resolution (RIU)	Intensity slope	Intensity sensitivity
Single Au	73.9	6.5	1.331-1.38	$8.33*10^{-6}$	58.65	6900
multilayer	62.54	2	1.331-1.50	$12.5*10^{-6}$	127.5	10000

Home work

- Read the references to write an assay about how to use biosensor (SPR) for drug screening based on molecular interactions.

1. Biosensor profiling of molecular interactions in pharmacology, Current Opinion in Pharmacology 2003, 3:557–562, www.current-opinion.com
2. SPR analysis of dynamic biological interactions with biomaterials, Biomaterials 21 (2000) 1823-1835
3. Assay and screening methods for bioactive substances based on cellular signaling pathways, **Reviews in Molecular Biotechnology** Volume 82, Issue 4, February 2002, Pages 357-370

Due on 11/03, sent to cwlinx@ntu.edu.tw