Lecture 5 Biosensors

Outlines:

1. Introduction
2. Biological elements
   - Enzymes
   - Antibodies
   - Nucleic Acids
   - Receptors
3. Immobilization of biological component
   - Adsorption
   - Microencapsulation
   - Entrapment
   - Cross linking
   - Covalent bonding
4. biosensors
   - a. Optical detections
     - Spectrum related detections:
       - i. UV-visible absorption spectroscopy
       - ii. Fluorescent
       - iii. Chemiluminescence
       - iv. Bioluminescence
     - Reflectance Methods:
       - i. ATR (Attenuated total reflectance)
       - ii. TIRF (Total internal refection fluorescence)
       - iii. SPR (Surface Plasmon Resonance)
     - Light-scattering techniques:
       - i. QELS (Quasi-elastic Light-scattering Spectroscopy)
   - b. Electrical detections
     - Conductance
     - Impedance
   - c. Electrochemical detections
     - Basics:
     - Voltammetry:
     - Amperometry:
     - Field Effect Transistors:
   - d. Mechiancal/Physical detections

Ultrasound:
Cantilever:

e. Thermal detection
f. Hybrid biosensors

5. Molecular gain
   a. PCR
   b. Polypeptide
   c. Fluorescence chain
   d. Nano particles (lecture 7)

6. System examples

1. Introduction of Biosensors

Biosensor: a device incorporating a biological sensing element connected to a transducer
Transducer: converts an observed change (physical or chemical) into a measurable signal, usually a electronic signal whose magnitude is proportional to the concentration of a specific chemical or set of chemicals.

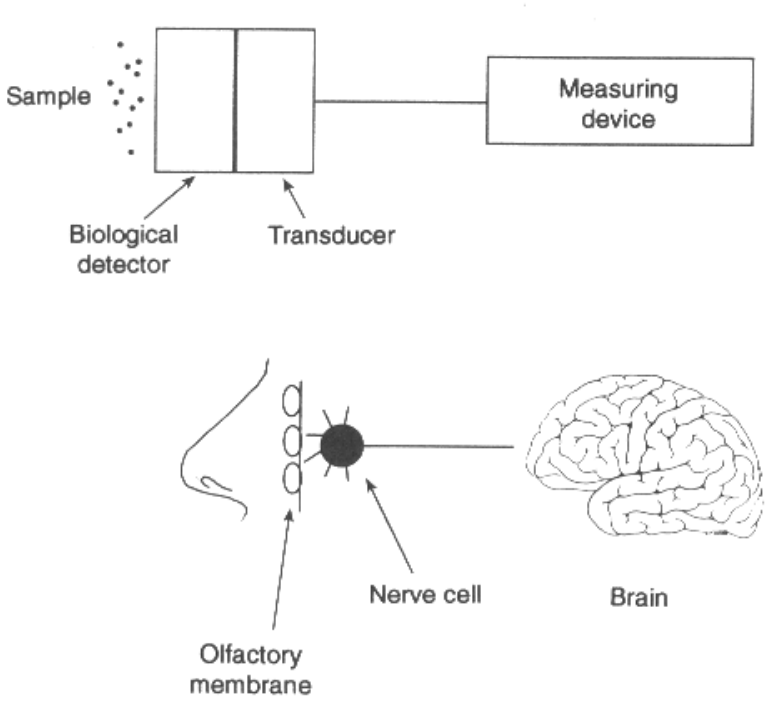

Figure 1.2 The nose as a biosensor

2. Biological Elements

Enzymes

i. pure enzymes

A large complex macromolecule, consisting largely of protein, usually containing a *prosthetic* group (義肢), which often includes one or more metal atoms. The mode of action involves oxidation or reduction.

Ex:

$$glucose + O_2 \xrightarrow{GOD} gluconic \quad acid + H_2O_2$$

Advantages:
(a). bind to substrate

(b) highly selective
(c) catalytic activity, improve sensitivity
(d) fast acting
(e) most commonly used biological component.

Disvantages:
(a) expansive
(b) often loss activity when immobilized on a transducer
(c) deactivation after a relatively short of time

ii. tissues

advantages:
(a) enzyme(s) is maintained in its natural environment
(b) enzyme activity is stabilized
(c) sometimes work when purified enzyme fail
(d) much less expansive
(e) loger life time

Disadvantages:
(a) loss of selectivity owning to containing multiple enzymes
(b) response may be slower

example:

Banana electrode:

Banana can be used to make biosensors for dopamine (an important brain chemical) detection. Dopamine is a catechol derivative. The enzyme polyphenol oxidase in banana catalyses the oxidation of the dihydroxy form of dopamine to the quinone form using ambient oxygen. The electrochemical reduction of the quinone back to the dihydroxy form causes a current to flow, which is directly proportional to the concentration of dopamine.

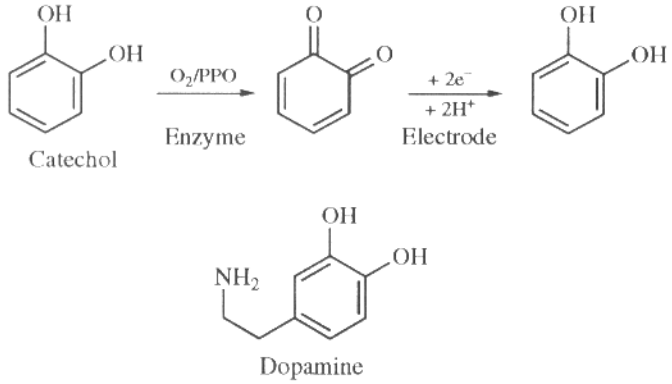

Figure 1.6 Reaction of catechol in a biosensor

iii. mitochondria (線粒體)

Sub-cellular multi-enzyme particles can be effective biocatalytic components. They are sometimes useful in improving sensor response and selectivity when the entire tissue lacks the necessary properties.

Table 2.3 Response characteristics of glutamine biosensors

Parameter	Enzyme	Mitochondria	Bacteria	Tissue
Slope/mV per decade	33–41	53	49	50
Detection limit/M	6.0×10^{-5}	2.2×10^{-5}	5.6×10^{-5}	2.0×10^{-5}
Linear range/mM	0.15–3.3	0.11–5.5	0.1–10	0.064–5.2
Response time/min	4–5	6–7	5	5–7
Lifetime/days	1	10	20	30

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Antibodies(Ab)

Proteins that can bind with and invading antigen(Ag) and remove it from harm. Widely used in immunoassays with labeled form.

$$Ab + Ag \Leftrightarrow Ab \cdot Ag$$

K=[Ab·Ag]/[Ab][Ag], K usually $>10^6$

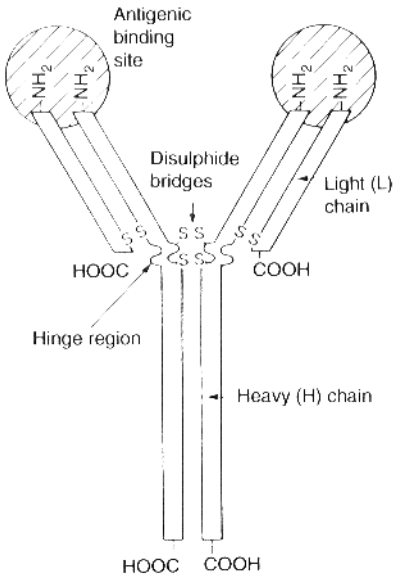


Figure 2.4 Scheme for a typical antibody composed of two heavy chains and two light chains. Reproduced by permission of the Open University Press from Hall (1990)

Advantages:
(a) very selective
(b) ultra-sensitive
(c) bind very powerfully

disadvantages:
(a)no catalytic effect

Nucleic Acids

The specific base pairing between strands of nucleic acid gives rise to the genetic code which determines the replicating characteristics of all parts of living cells and thus the inherited characteristics of individual members of a species. DNA probes can be used to detect generic diseases, cancers and viral infections.

Receptors

Much of the research into receptors has been with neuroreceptors and their recognition of neurotransmitters, neurotransmitter antagonists (對抗) and

neurotoxins. Binding a ligand (an agonist) to the receptor triggers an amplified physiological response: (a) io channel opening (b) second messenger systems (c) activation of enzymes. These biological receptors tend to have an affinity for a range of structurally related compounds rather than one specific analyte, which is often an attractive feature for biosensors.

3. Immobilization of Biological Component

a. Microencapsulation (membrane entrapment)

- Used in early biosensors. The biomaterial is held in place behind a membrane, giving close contact between the biomaterial and the transducer.
- Does not interfere with reliability of the enzyme, and limit contamination and biodegredation.
- Stable towards changes in temperature, pH, ionic strength, and chemical composition.
- Permeable to some materials: small molecules, gas molecules, and electrons.
- Main types:
  (a) cellulose acetate (dialysis membrane):excludes proteins and slows the transportation for interfering species such as ascorbate.
  (b) polycarbonate (Nuclepore), a synthetic material which is non-permselective
  (c) collagen: a natural protein
  (d) PTFE (Teflon), a synthetic polymer, selectively permeable to gases such as oxygen
  (e) Nafion
  (f) Polyurethane.

b. Entrapment

- The biomaterial is mixed with monomer solution, which is them polymerized to a gel, trapping the biomaterial.
- Most commonly used gel is polyacrylamide, others like starch gels, nylon, and silastic gels. Conduction polymers such as polypyrroles are particularly useful with electrodes.

- Issues: (a) large barriers, inhibiting the diffusion of the substrate for reaction. (b) Loss of enzyme activity.

c. Cross linking

- Using bifunctional agents to bind the biomaterial to solid supports. A useful method to stabilize adsorbed enzymes
- Distanvages:
  (a) causes damage to the enzyme
  (b) limits diffusion of the substrate
  (c) poor rigidity (mechanical strength)

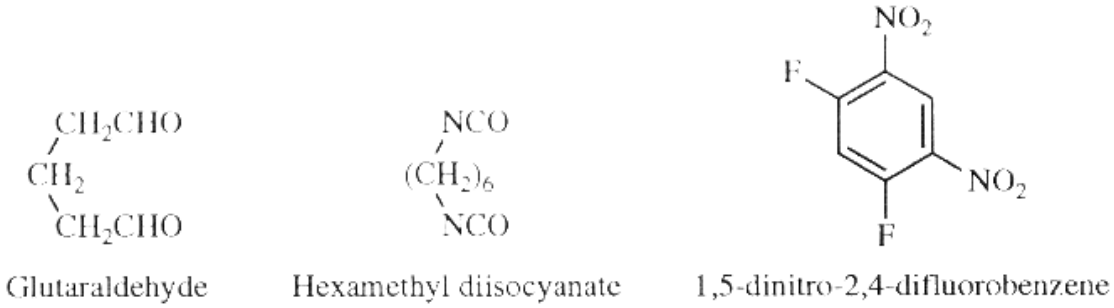


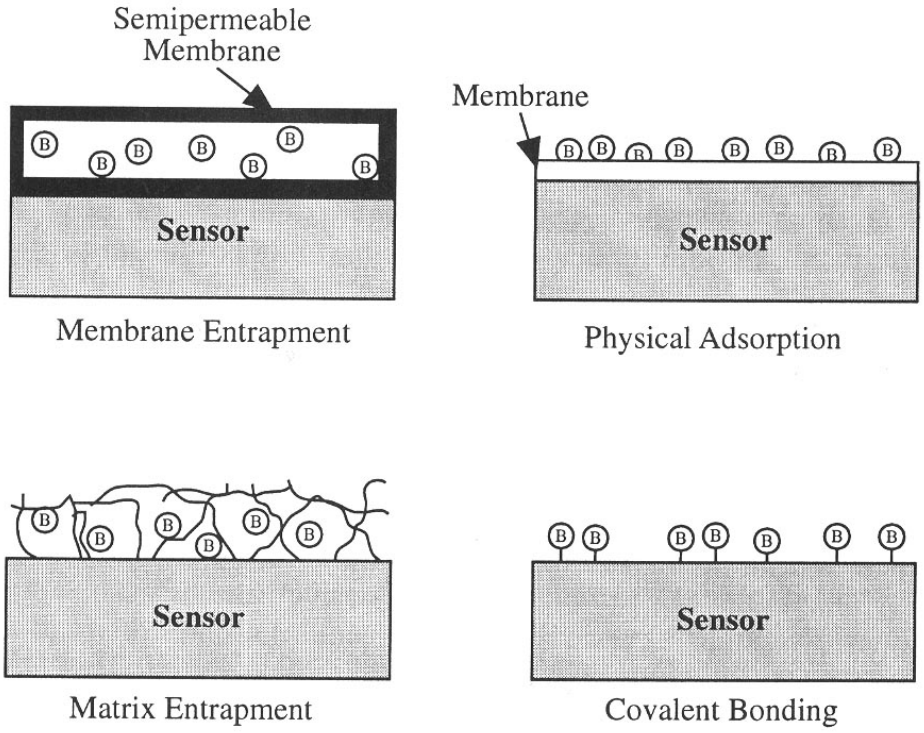
Figure 3.2 Some molecules used for cross-linking

d. Adsorption

- The simplest and involves minimal preparation. However, the bind is weak and this method is only suitable for exploratory work over a short time-span.
- Enzymes can be adsorbed on surfaces of: Alumina, charcoal, clay, cellulose, kaolin, silica gel, glass and collagen.
- Two classes:
  (a) physical adsorption (physisorption), very weak via the van deer Waals bonds, hydrogen bonds or charge-transfer forces.
  (b) chemical adsorption (cheminsorption), much stronger, involves the formation of covalent bonds.
- Adsorbed biomaterial is very susceptible to changes in pH, temperature, ionic strength and the substrate.

e. Covalent bonding

- Some functional groups which are not essential for the catalytic activity for an enzyme can be convalently bonded to the support matrix (transducer or membrane). This method uses nucleophilic groups for coupling such as NH_2, CO_2H, OH, C_6H4OH, SH and imidazole.
- Reactions need to be performed under mild conditions-low temperature, low ionic strength and pH in the physiological range.



4. Biosensors

a. Optical detections

- Most bioassays were originally of the photometric type. They express changes in a species that involve a strong change in photometric properties.

For example:

$$pyruvate + NADH + H^+ \xrightarrow{LDH} L-lactate + NAD^+$$

- NADH has a strong absorbance at 340 nm and gives fluorescence at 400 nm, but NAD^+ has no absorbance at this wavelength. (NAD^+ and NADH are coenzyme: for electron or proton accepter or donor in enzyme catalysis process)

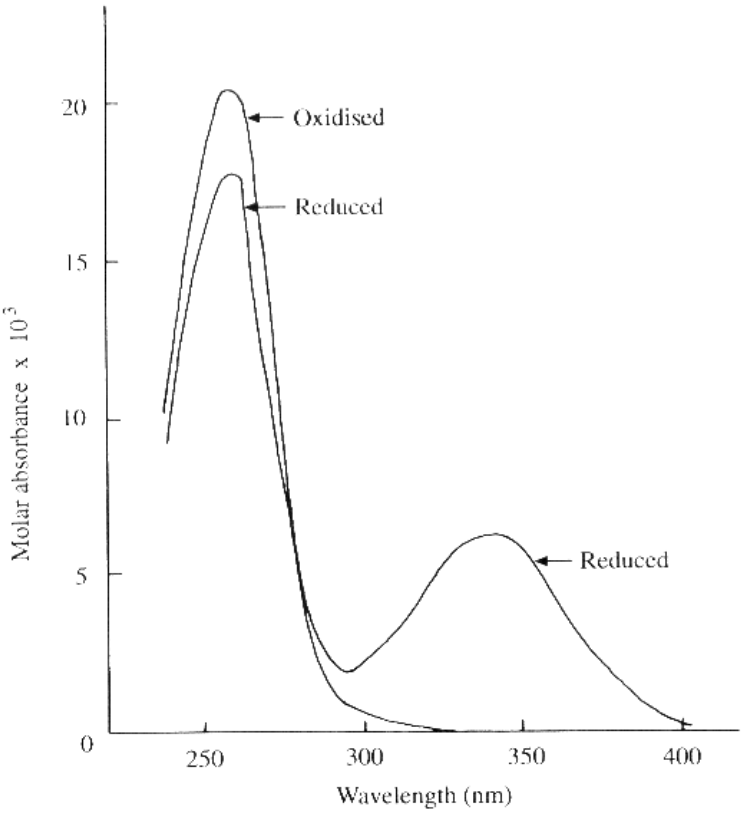


Figure 5.1 Absorption spectra of NAD in oxidised and reduced forms. Reproduced by permission of the Open University Press from Hall (1990)

- The basic optical response is based on the beer-Lambert Law:

Beer's law

$$\log(I / I_0) = A = \varepsilon Cl$$

where:

I_0=intensity of incident light
I=intensity of transmitted light
A=absorbance (usually measured directly by instrument)
ε=extinction coefficient
C=concentration of analyte
l=path length of light through solution

Advantages:

i. No electrical interference, No reference electrode need
ii. Sample contact is no necessary required
iii. Highly stable measurement
iv. Multi-wavelengths can be involved for different reagents

Disadvantages:

i. need appropriate reagent phase
ii. background ambient light interference
iii. limited dynamic range typically 10^2, compared to 10^6-10^{12} for ion selective electrodes
iv. large in size, hard to be miniaturized
v. Response may be slow due to the mass transfer of analytes to the reagent phase.

- Waveguides for different wavelengths:

λ>450 nm (plastic)
λ>350 nm (glass)
λ<350 nm (fused silica)
λ>1000 nm (germanium crystal guedes)

- Basic device construction:

i. direct method

An analyte, A, can complex with the reagent, R, as follows

$$A + R \leftrightarrow AR$$

$$K = \frac{[AR]}{[A][R]}$$

if {R} is the total reagent concentration:

$$\{R\}=[R]+[AR]$$

$$[AR] = \frac{K\{R\}[A]}{1 + K[A]}$$

if K[A]>>1, [AR]~{R} (response[AR] is propotional to {R})
if K[A]<<1, [AR]~K{R}[A]~K[R][A], {R}~[R], [AR] does not change with [R]=>saturation.

A better approach is to use two wavelengths, one for AR and one for R (reference), and there will be a linear relationship between the ratio of the absorbances at λ_{AR} and λ_R and the concentration of A. This relationship is independent of {R}:

$$\frac{[AR]}{[R]} = K[A]$$

ii. indirect method

If neither the analyte or the reagent show a spectral change on binding, a competitive binding method by employing *analyte analogue* will be used.

Ex: In the determination of glucose, a bioreceptor concanvalin A (ConA), and a suitable agalogue dextran labeled with fluorescein siothiocyanate (FITC-dextran), will be employed for compete reaction.

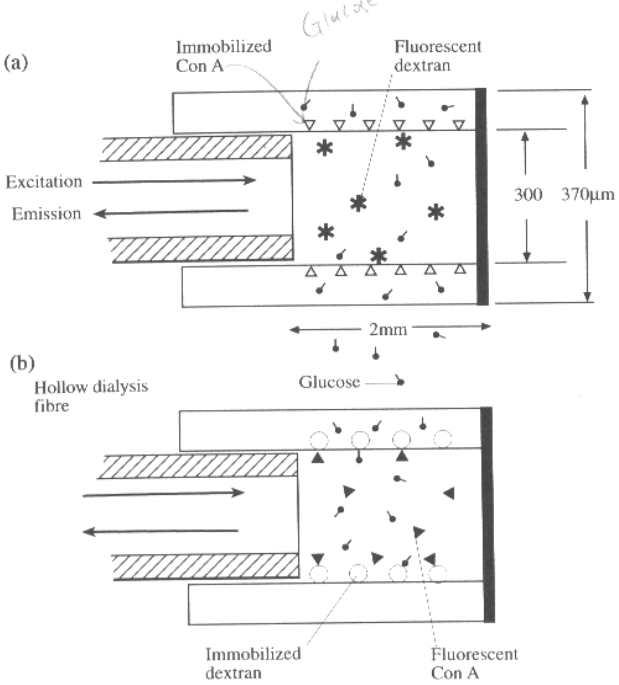


Figure 5.6 Two modes of biosensor for glucose using fluorescein isothiocyanate (FITC)-labelled dextran and Con A. Reproduced by permission of Oxford University Press from Schultz (1987)

$$A+R <=> AR$$
$$A^*+R <=> A^*R$$

$$K=[AR]/[A][R]$$
$$K^*=[A^*R]/[A^*][R]$$

$$\{R\}=[R]+[AR]+[A^*R]$$
$$\{A^*\}=[A^*]+[A^*R]$$

Solve the above four equations, one can find the ratio of the bound receptor to the total receptor concentrations:

$$([A^*]/\{A^*\})^2 + \{A^*\}/[A^*](((\{R\}/\{A^*\} - 1) + \frac{K[A]+1}{\{A^*\}K^*}) - \frac{K[A]+1}{[A^*]K^*} = 0$$

[A*]/{A*}: normalized response between 0 and 1. The sensitivity is related to {A*}

The normalized response is a function of two terms {R}/{A*} and (K[A]+1)/{A*}K*.

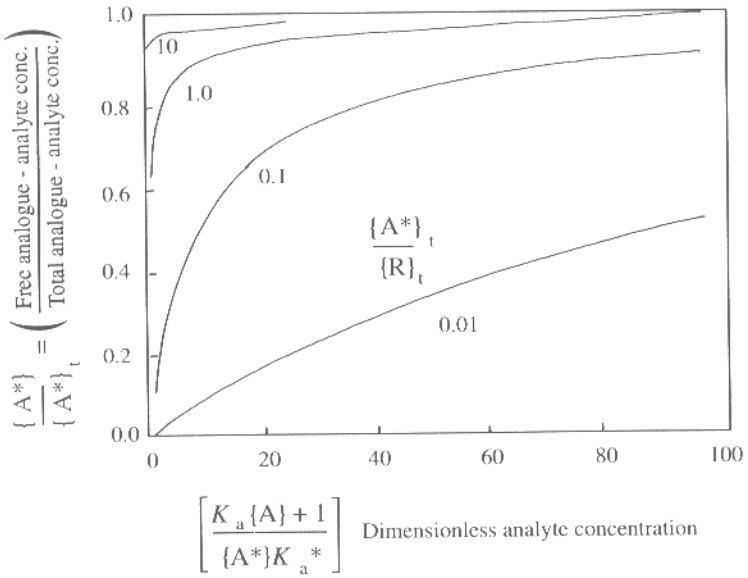


Figure 5.8 Parametric plot of equation 5.1. Changes in {A*}/{R} show the effect of relative levels of analyte-analogue concentration within the detection chamber. Reproduced by permission of Oxford University Press from Schultz (1987)

iii. solid-phase absorption label sensors

For a portable device, low power components are desirable, such as LED sources and photodiode detectors.

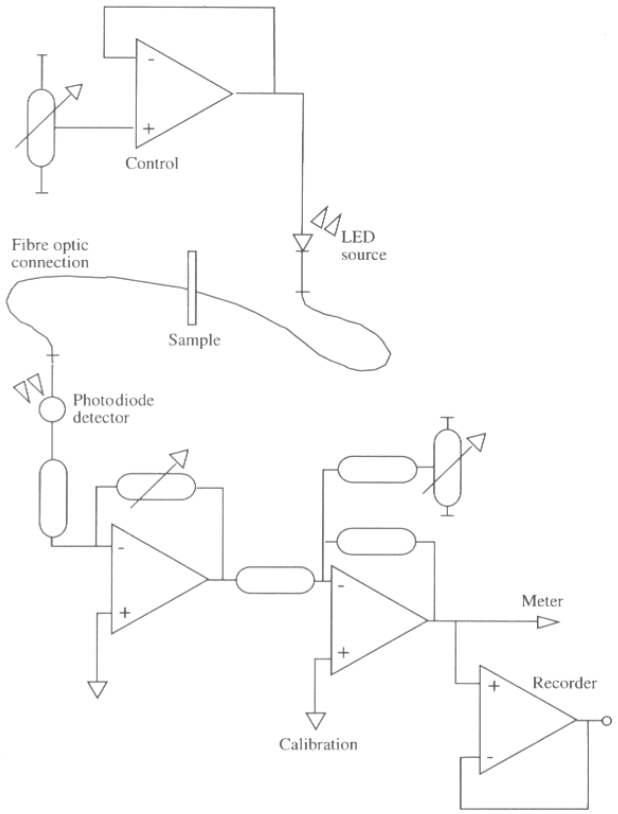


Figure 5.9 Basic control circuit for optical assay employing LED light source and photodiode detector. Reproduced by permission of the Open University Press from Hall (1990)

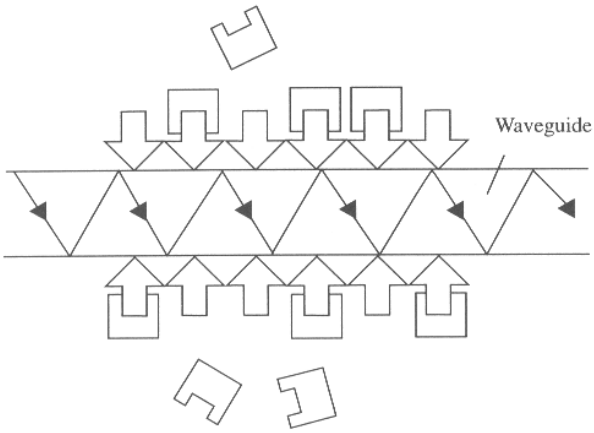


Figure 5.10 Optical sensor: evanescent field monitoring. Reproduced by permission of the Open University Press from Hall (1990)

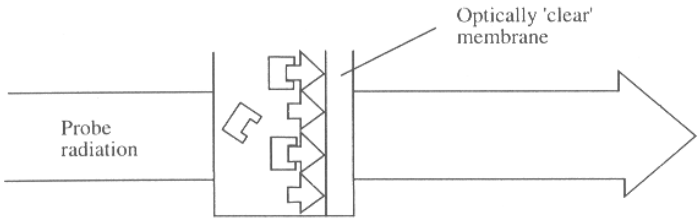


Figure 5.11 Solid-state optical sensor. Reproduced by permission of the Open University Press from Hall (1990)

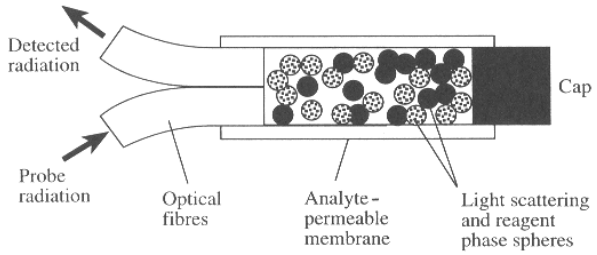


Figure 5.12 Optical sensor: light-scattering probe with common source and detection wavelength. Reproduced by permission of the Open University Press from Hall (1990)

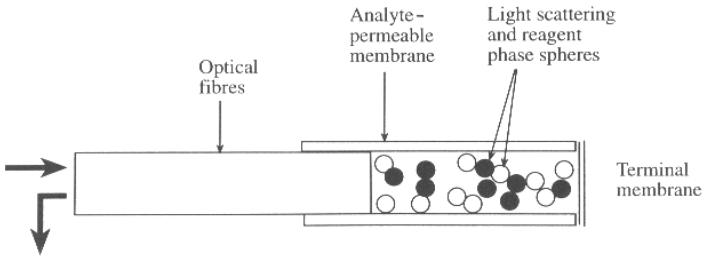


Figure 5.13 Optical sensor: light-scattering probe with different source and detection wavelengths. Reproduced by permission of the Open University Press from Hall (1990)

iv. Catalysis

The immobilized reagent can be a catalyst to convert the analyte into a substance with different optical properties.

- Spectrum related detections:

i. UV-visible absorption spectroscopy

PH variation:

(a) Many dyes act as pH indicators, however, the pH range is small—less than 2 pH units. This does not matter too much with biochemical systems as they generally operate within fairly limited pH ranges.

(b) Methyl red is a suitable dye which has a distinctive visible spectrum with well separated maxima for the acid and basic forms of the dye.

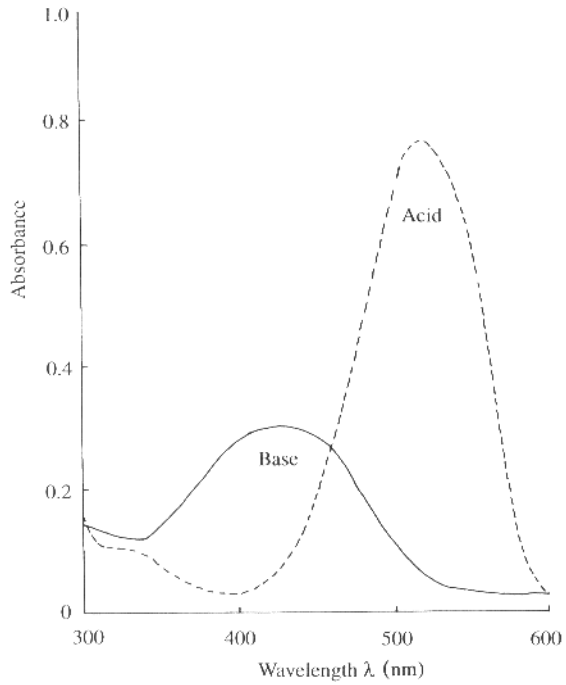


Figure 5.14 The ultraviolet–visible absorption spectrum of methyl red in its acid and base forms. Reproduced by permission of the Open University Press from Hall (1990)

ii. Fluorescent

Most developed area for photometric sensors and biosensors.

Ion selective sensors for pH detection:

The best fluorescent reagents for pH sensors: trisodium 8-hydroxy-1, 3, 6-trisulphonate. Absorption (excitation) bands occur at 405 nm (acid form) and 470 nm (basic form) with emission (fluorescence) at 520 nm. It operates over the pH range 6.4-7.5 (±0.01)

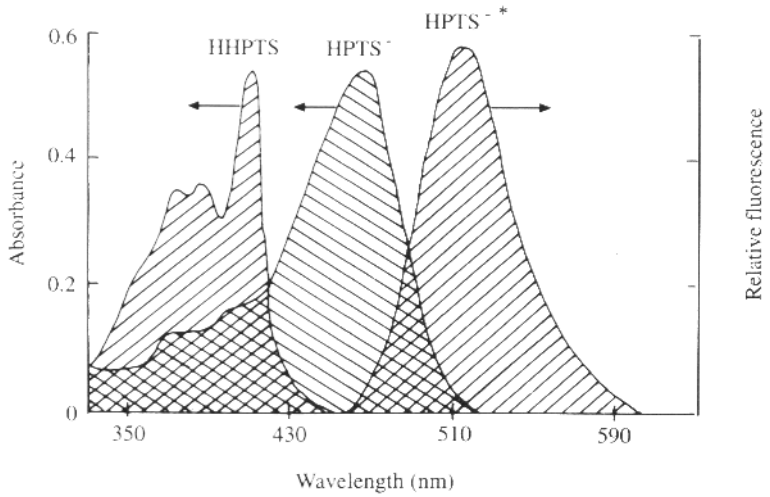


Figure 5.16 Absorption spectra of 8-hydroxy-1,3,6-trisulphonic acid (HPTS) in acid and base forms and fluorescent spectrum of base form. Reproduced by permission of the Open University Press from Hall (1990)

iii. Chemiluminescence

Chimiluminescence occurs by the oxidation of certain substances, usually with oxygen or hydrogen peroxide, to produce visible light in the cold and in the absence of any exciting illumination.
The best known of these is luminol, normally used as a label. It can be used in any assay involving oxygen, hydrogen peroxide or peroxidase, and very useful with imunoassays. However, the sensitivity is limited because the quantum yield is only 1%.

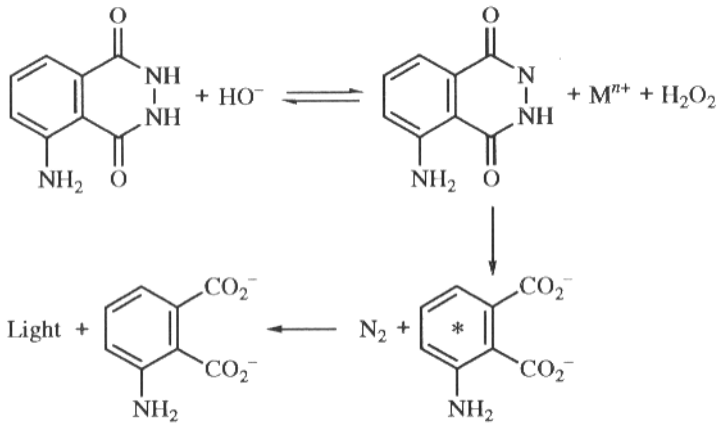


Figure 5.17 Reaction mode of luminol. Reproduced by permission of Oxford University Press from McCapra (1987)

iv. Combination of luminescence and fluorescence for competitive immunoassay. (fluorescent –labelled antibody and chemiluminescent-labled antigen)

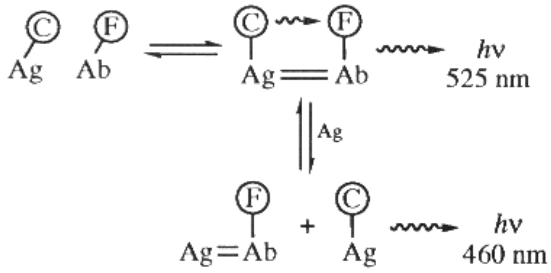


Figure 5.18 Competitive immunoassay employing a fluorescent-labelled antibody and chemiluminescent-labelled antigen. The fluorophore acts as the acceptor of the chemiluminescent energy in the doubly labelled complex. Reproduced by permission of Oxford University Press from McCapra (1987)

v. Bioluminescence

Certain biological species, principally the firefly, can emit luminescence by enzyme-catalyzed oxidation of luciferin:

$$luciferin \xrightarrow{luciferase, O_2} oxyluciferin + h\nu(562nm)$$

Some of the luciferase reactions couple with cofactors such as ATP, FMN, and FADH

$$ATP + luciferin + O_2 \xrightarrow{luciferase} AMP + pyrophosphate + oxyluciferin + CO_2 + H_2O + h\nu$$

The reaction is very sensitive down to femtomole concentrations.

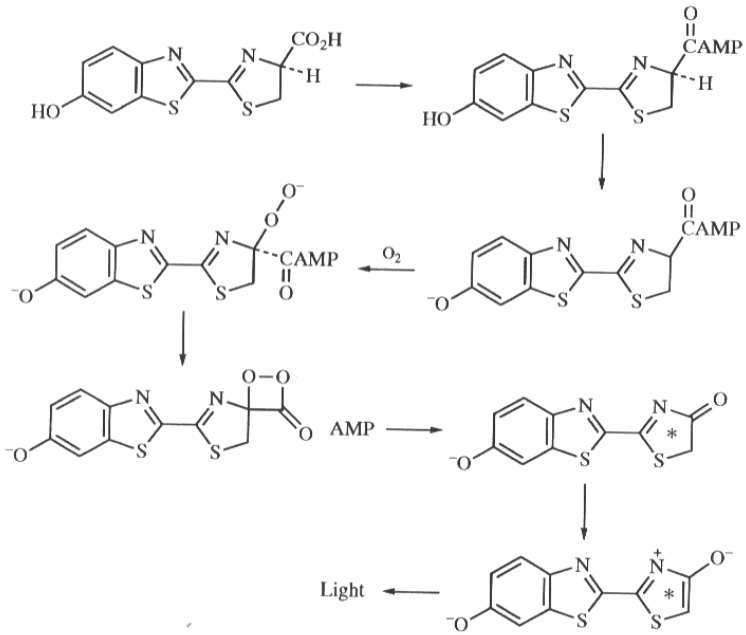


Figure 5.20 Examples of luciferins. Reproduced by permission of Oxford University Press from McCapra (1987)

vi. Optical diffraction: antibody-antigen binding area shows diffraction grating. Using latex or colloidal gold to increase the effective size of the bound analyte to amplify signal.

- Reflectance Methods:

These methods are concerned with studying material adsorbed on an optical surface. They are particularly suitable for use with immunoassays. Three principles: ATR, TIRF, and SPR.

Basic arrangement:

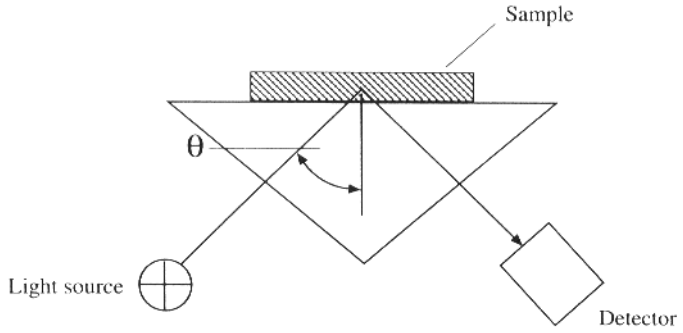


Figure 5.24 Attenuated total reflection. Reproduced by permission of Oxford University Press from Sutherland and Dähne (1987)

$$\sin\theta_c = n_2 / n_1 \quad and \quad n_1 > n_2$$

When $\theta > \theta_c$, there is an evanescent wave refracted through the interface in the Z direction with penetrate the n_2 medium a distance d_p, which is of the or of wavelength. The electrical field E decays exponentially with distance (Z):

$$E = E_0 \exp(Z / d_p)$$

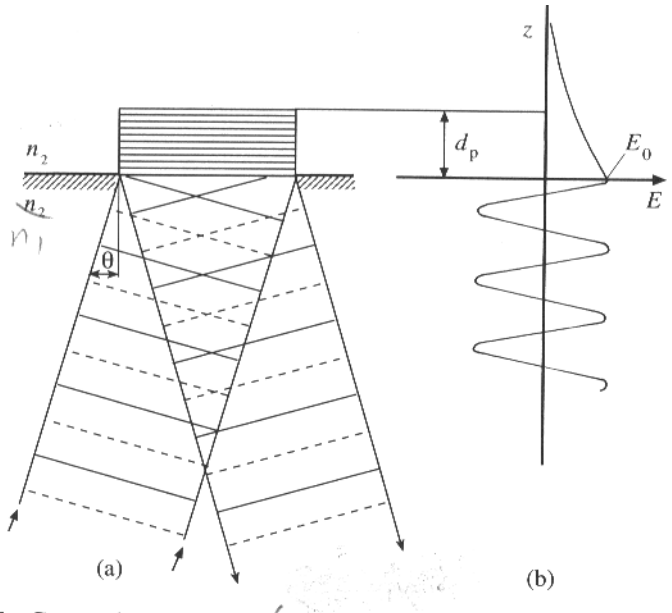


Figure 5.25 Generation of the evanescent wave at an interface between two optical media. Reproduced by permission of Oxford University Press from Sutherland and Dähne (1987)

The depth of the penetration, d_p, can be related to other factors by the equation:

$$d_p = \frac{\lambda / n_1}{2\pi[\sin^2\theta - (n_2/n_1)^2]^{1/2}}$$

d_p decreases with increasing θ and increases as n_2/n_1 tends to unity.

Factors for determining the attenuation of reflection:

i. d_p
ii. polarization-dependent electric field intensity at the reflecting surface.
iii. Sampling area
iv. Matching of the two refractive indeces n_1 and n_2

An effective thickness d_e takes account of all these factors.

In order to enhance the sensitivity, multiple reflections may be used:

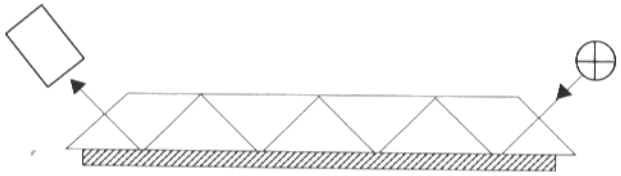


Figure 5.26 Total internal reflection showing multiple reflections. Reproduced by permission of Oxford University Press from Sutherland and Dähne (1987)

a. ATR (Attenuated total reflectance)

An absorbing material is placed in contact with the reflecting surface of an internal reflection element (IRE), causing attenuation of the internally reflected light. ATR has been used for immunoassays in the IR, visible, and UV regions.

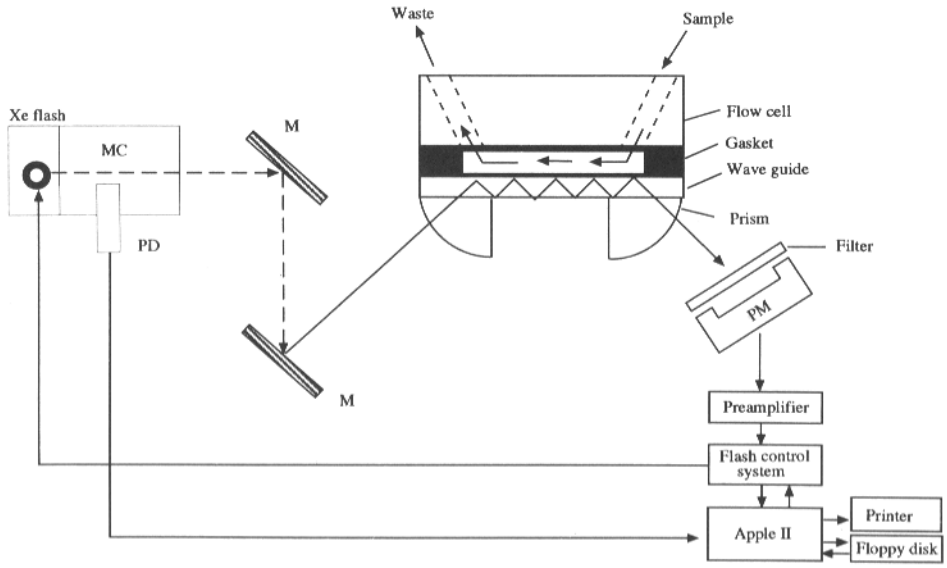


Figure 5.27 Diagram of instrumental layout used for measuring immunoassays with a multiple-internal reflection plate. PM, photomultiplier tube; PD, photodiode; MC, monochromator; M, mirrors. Reproduced by permission of Oxford University Press from Sutherland and Dähne (1987)

b. TIRF (Total internal refection fluorescence)

The emitted fluorescence be detected either by a detector at right-angles to the interface or in line with the primary beam. The later one results in an enhancement of up to 50-fold. By avoiding measurement of the fluorescence through the bulk of the sample solution round the surface, interference is minimized.

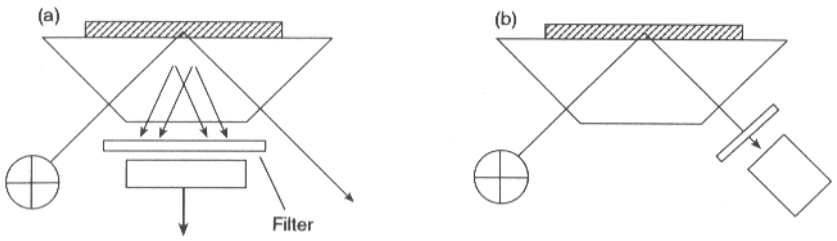
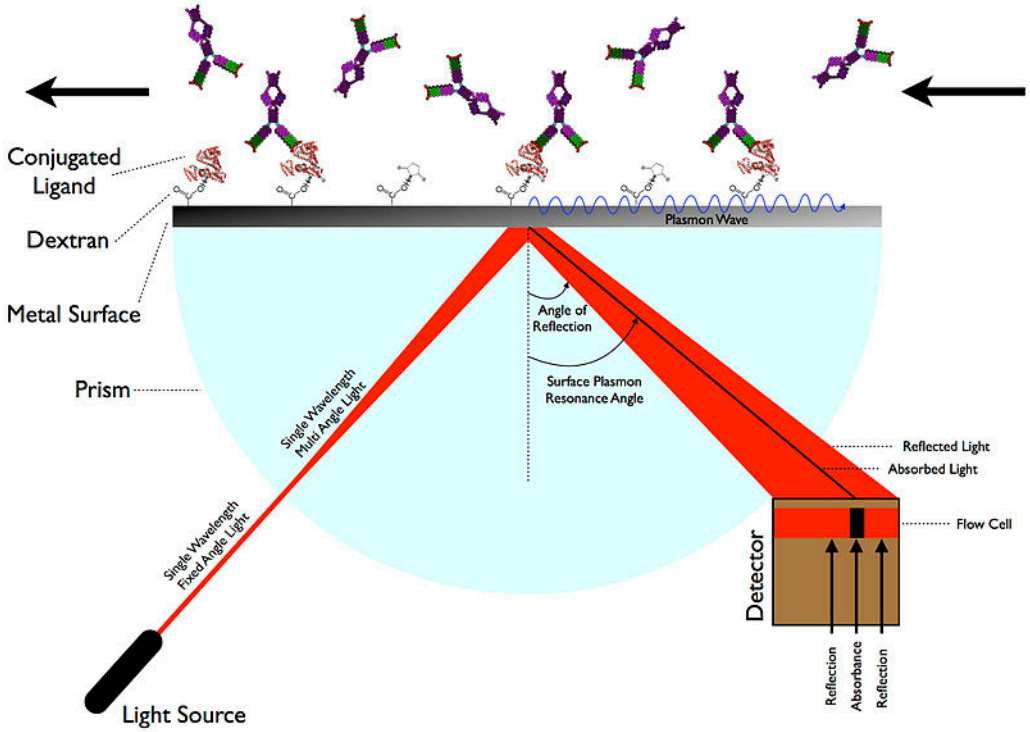


Figure 5.29 Detection of internal reflection by (a) right-angled fluorescence and (b) in-line fluorescence. Reproduced by permission of Oxford University Press from Sutherland and Dähne (1987)

c. SPR (Surface Plasmon Resonance)



i. These are formed in the boundary of a solid (metal or semiconductor). The electrons behave like a quasi-free electro gas. Quanta oscillations of surface charges are produced by exterior electrical fields in the boundary. These charge oscillations couple with high-frequency electromagnetic fields extending into space.

ii. They can be excited by electron beams or by light. The most useful are non-radiative plasmons excited by evanescent light waves. The plasmon is characterized by an exponential decrease in the electric filed with increasing distance from the boundary.

iii. The p-polarized incident field, which has an angle θ such that the photon momentum along the surface, matches the plasmon frequency, so that the light can couple to the electron plasma in the metal.— surface plasmon resonance.

n_2:metal, such as 60 nm siliver, $n_1 > n_3$

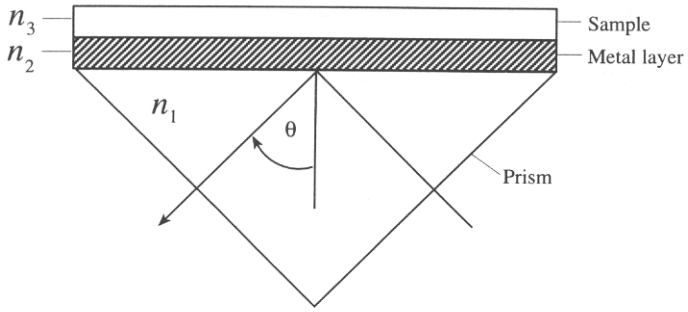


Figure 5.30 Attenuated total reflection method of exciting non-radiative plasmons using the Kretschmann arrangement. n_1, n_2 and n_3 are the refractive indices of the glass prism, metal layer and sample, respectively. Reproduced by permission of Oxford University Press from Sutherland and Dähne (1987)

- The intensity of the totally reflected light shows a sharp drop with increase in θ, depending on the depth and width and the characteristic absorbance and thickness of the metal. $H(x)^2$ means the energy density across the metal layer for different angles of incidence.

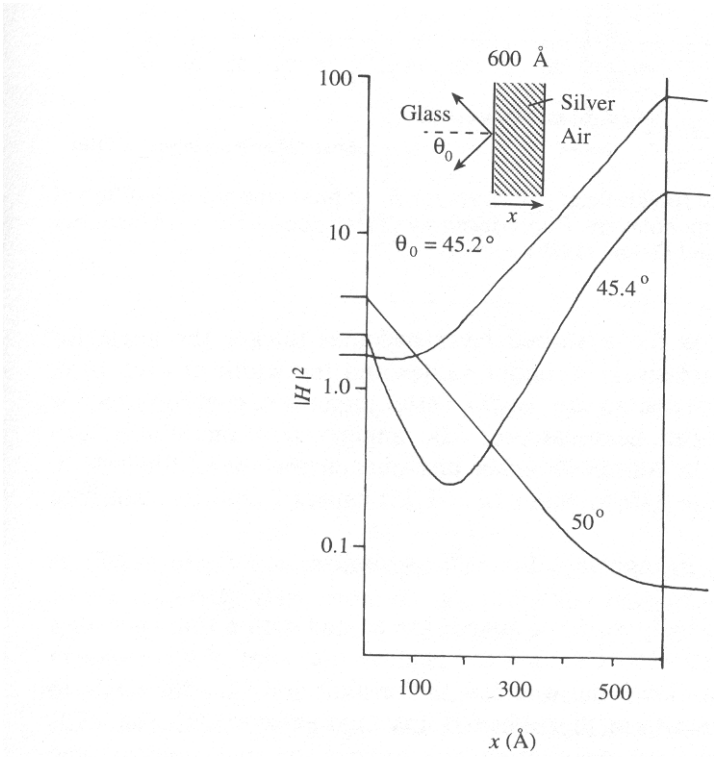
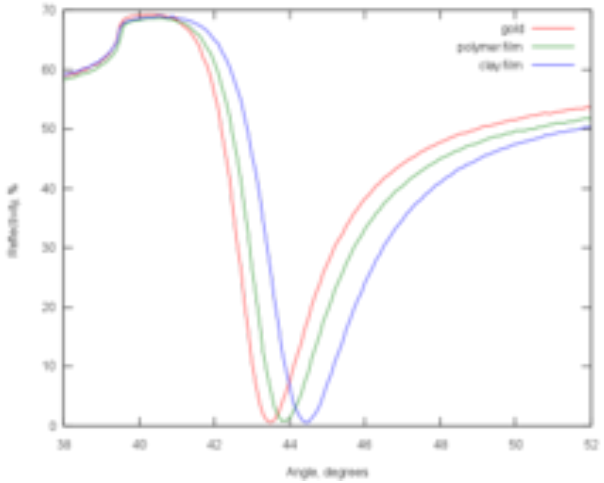
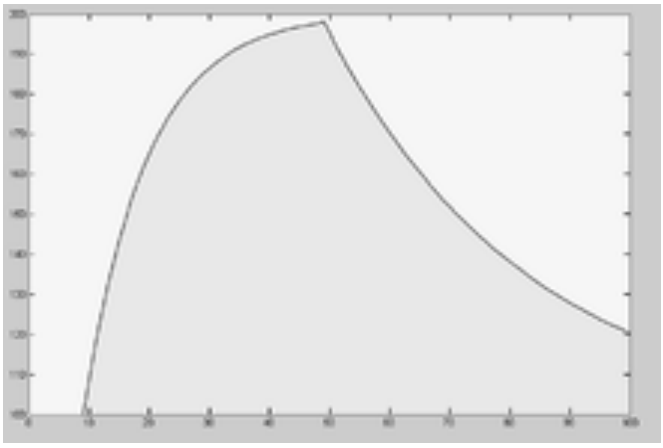


Figure 5.31 The calculated electromagnetic density $|H(x)|^2$ in a 600 Å silver film with the angle of incidence as the parameter. Reproduced by permission of Oxford University Press from Sutherland and Dähne (1987)

- Angular position is very sensitive to changes in refractive index just outside the metal film. Changing from air (n=1.0) to water (n=1.33) causes a shift in resonance angle of 25°. IRE prisms, usually made of quartz, coated with Au, Ag, Al or Cu. Several ways to achieve the point of resonance: (a). keep the wavelength constant and vary the angle to obtain maximum intensity the reflection wave. Angular shifts as small as 0.0005° have been detected. (b). keep the angle constant and vary the wavelength to obtain the same effect.



- Dynamic measurement of immunoassays.



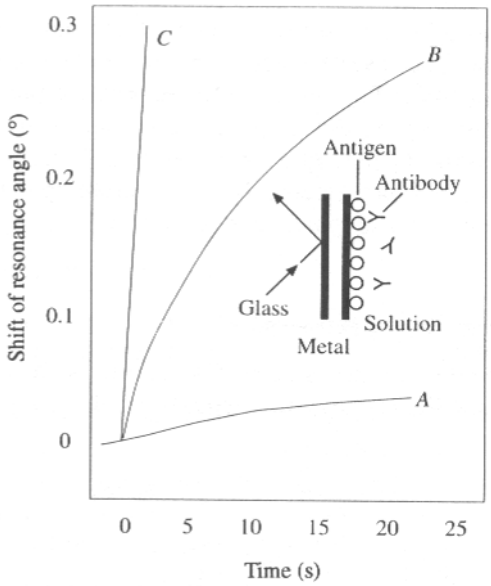


Figure 5.33 Shift in resonance angle versus time for three anti-IgG concentrations: (A)2, (B)20 and (C)200 μg ml^{-1}. The inset illustrates the antibody-binding event. Reproduced by permission of Oxford University Press from Sutherland and Dähne (1987)

Detection limit: 0.02-0.05 μg/cm^{-2} is possible

- **Light-scattering techniques:**

Dynamic Light Scattering Technology

Particle size can be determined by measuring the random changes in the intensity of light scattered from a suspension or solution. This technique is commonly known as dynamic light scattering (DLS), but is also called photon correlation spectroscopy (PCS) and quasi-elastic light scattering (QELS). The latter terms are more common in older literature.

Applications for Dynamic Light Scattering

DLS is most commonly used to analyze nanoparticles. Examples include determining nanogold size, protein size, latex size, and colloid size. In general, the technique is best used for submicron particles and can be used to measure particle with sizes less than a nanometer. In this size regime (microns to nanometers) and for the purposes of size measurement (but not thermodynamics!) the distinction between a molecule (such as a protein or macromolecule) and a particle (such as nanogold) and even a second liquid phase (such as in an emulsion) becomes blurred. Dynamic light scattering can also be used as a probe of complex fluids such as concentrated solutions. However, this application is much less common than particle sizing.

Stokes Einstein: Relating Particle Size to Particle Motion

Small particles in suspension undergo random thermal motion known as Brownian motion. This random motion is modeled by the Stokes-Einstein equation. Below the equation is given in the form most often used for particle size analysis.

$$D_h = \frac{k_B T}{3\pi\eta D_t}$$

The Stokes-Einstein relation that connects diffusion coefficient measured by dynamic light scattering to particle size.

where

D_h is the hydrodynamic diameter (this is the goal: particle size!)

D_t is the translational diffusion coefficient (we find this by dynamic light scattering)

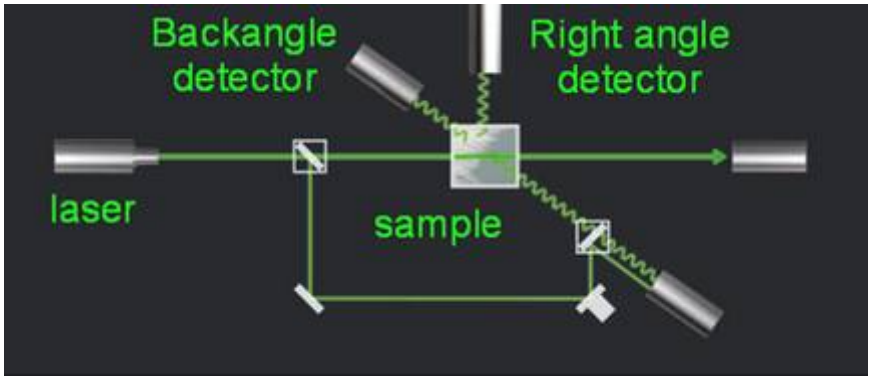
k_B is Boltzmann's constant (we know this)

T is thermodynamic temperature (we control this)

η is dynamic viscosity (we know this)

How to Measure Particle Motion I: Dynamic Light Scattering Optical Setup

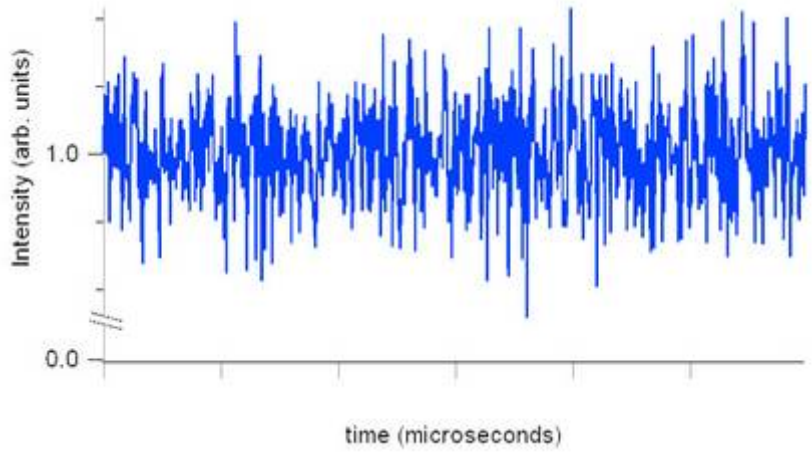
A top view of the optical setup for DLS is shown below.



Optical setup for dynamic light scattering (DLS) nanoparticle size analyzer

Light from the laser light source illuminates the sample in the cell. The scattered light signal is collected with one of two detectors, either at a 90 degree (right angle) or 173 degree (back angle) scattering angle. The provision of both detectors allows more flexibility in choosing measurement conditions. Particles can be dispersed in a variety of liquids. Only liquid refractive index and viscosity needs to be known for interpreting the measurement results.

The obtained optical signal shows random changes due to the randomly changing relative position of the particles. This is shown schematically in the graph below.

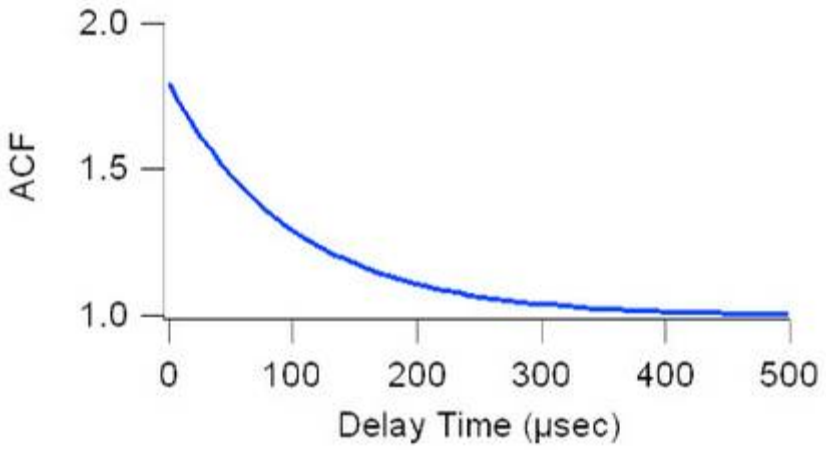


Optical signal from a nanoparticle sample on a microsecond timescale.

The “noise” is actually due to particle motion and will be used to extract the particle size. In contrast to laser diffraction, DLS measurements are typically made at a single angle, although data obtained at several angles can be useful. In addition, the technique is completely noninvasive; the particle motion continues whether or not it is being probed by DLS.

How to Extract Particle Diffusion Coefficient: Dynamic Light Scattering Data Interpretation

The signal can be interpreted in terms of an autocorrelation function. Incoming data is processed in real time with a digital signal processing device known as a correlator and the autocorrelation function as a function of delay time, τ, is extracted.



Autocorrelation Function from dynamic light scattering. The decay of this function is used to extract particle size. Faster decays correspond to smaller particles.

For a sample where all of the particles are the same size, the baseline subtracted autocorrelation function, C, is simply an exponential decay of the following form:

$$C = \exp(-2\Gamma\tau)$$

Exponential decay of autocorrelation function. The decay constant is proportional to the diffusion coefficient.

Γ is readily derived from experimental data by a curve fit. The diffusion coefficient is obtained from the relation $\Gamma=D_t q^2$ where q is the scattering vector, given by $q=(4\pi n/\lambda)\sin(\theta/2)$. The refractive index of the liquid is n. The wavelength of the laser light is λ, and scattering angle, θ. Inserting D_t into the Stokes-Einstein equation above and solving for particle size is the final step.

Analyzing Real Particle Size Distributions I: The Method of Cumulants and Z-average

The discussion above can be extended to real nanoparticle samples that contain a distribution of particle sizes. The exponential decay is rewritten as a power series:

$$C = \exp(-2\bar{\Gamma}\tau + \mu_2\tau^2 - \cdots)$$

Exponential decay of autocorrelation function. The linear decay constant is proportional to the average diffusion coefficient and is used to extract average particle size.

Once again, a decay constant is extracted and interpreted to obtain particle size. However, in this case, the obtained particle size, known as the z-average size, is a weighted mean size. Unfortunately, the weighting is somewhat convoluted. Recall that the decay constant is proportional to the diffusion coefficient. So, by dynamic light scattering one has determined the intensity weighted diffusion coefficient. The diffusion coefficient is inversely proportional to size. So, in truth, the “z-average size” is the intensity weighted harmonic mean size. This definition differs substantially from that of the z-average radius of gyration encountered in the light scattering study of polymers.

Despite the convoluted meaning, the z-average size increases as the particle size increases. And, it is extremely easy to measure reliably. For these reasons, the z-average size has become the accepted norm for particle sizing by dynamic light scattering.

Analyzing Real Particle Size Distributions II: Size Distribution Data

While a detailed discussion is beyond the scope of this work, it is possible to extract size distribution data from DLS data. One can convert the measured autocorrelation function into what is known as an electric field autocorrelation function, $g_1(\tau)$. Then use the following relationship between $g_1(\tau)$ and the scattered intensity, S, for each possible decay constant, Γ. The overall electric field autocorrelation function is the intensity weighted sum of the decays due to every particle in the system.

$$g_1(\tau) = \int S(\Gamma) \exp(-\Gamma \tau) d\Gamma$$

Electric field autocorrelation function as a sum of exponential decays. The decay constants are inversely proportional to the particle size.

Inversion of this equation, that is using experimentally determined values of $g_1(\tau)$ to find values of S(Γ), will lead to information about the size distribution. Unlike the cumulants analysis discussed above, this is an ill-posed mathematical problem. Even so, the technique remains useful for interpreting DLS data.

b. Electrical detections

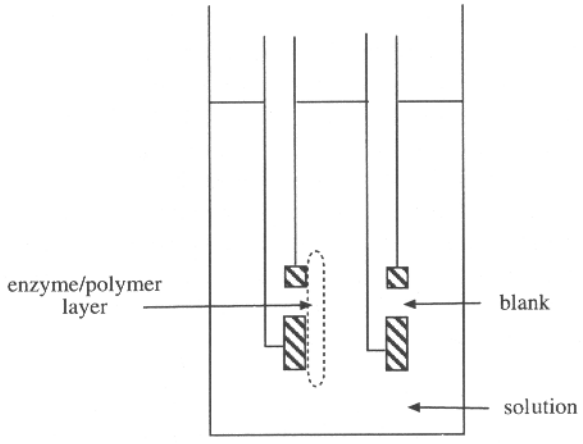
Conductance

Conductance is the inverse of resistance.
Ohm's law:

E=IR

Conductance L=1/R

Conductivity varies with (a) charge on the ion (b) the mobility of ion (c) the degree of dissociation of the ion. L is measured by alternative current. The technique itself has no selectivity.



Example:

$$urea + 2H_2O \xrightarrow{urease} 2NH_4^+ + HCO_3^-$$

this reaction involve change in ions. Conductance measurements are unaffected by colour or turbidity.

Impedance sensing

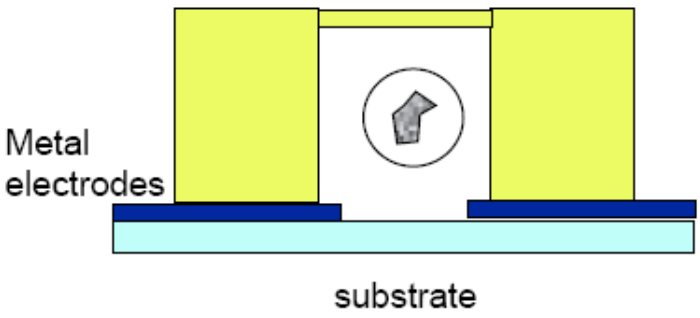
(Fuller et al. microTAS 2000)

- Coulter counter: utilizes DC impedance to measure small particles in suspension
- A system that can perform broad-band impedance characterizations of particles to enable particle differentiation via features in their impedance spectra is desired
- This paper was able to demonstrate AC, multifrequency measurements on cells and other particles
- The system measured the volume, cytoplasmic conductivity, and membrane capacitance of human peripheral blood granulocytes

Capacitance Cytometry

(Sohn et al. PNAS 2000, vol.97, no.20, p.10687)

- Utilize AC capacitance allows one to probe the polarization response of a wide range of materials, both organic and inorganic, to an external electrical field
- Can be used to determine cell size and cellular membrane capacitance for cell-cycle progression and to differentiate normal and malignant WBCs
- This paper detects and quantifies the polarization response of DNA within the nucleus of single eukaryotic cells at 1kHz



c. Electrochemical detections

Basics:

Three basic electrochemical processes: (a) ***Potentiometry***, the measurement of a cell potential at zero current (b) ***voltammetry (amperometry)***, in which an oxidizing (or reducing) potential is applied between the cell electrodes and the cell current is measured. (c) ***conductimetry***, where the conductance of the cell is measured by an alternating current bridge method.(introduced previously)

Potentiometry

i. If a piece of metal is placed in an electrolyte solution, there is charge separation between the metal (electrode) and the solution, and a potential will be set up which can not be measured directly. It requires a combination of two half-cells to become an electrochemical cell.

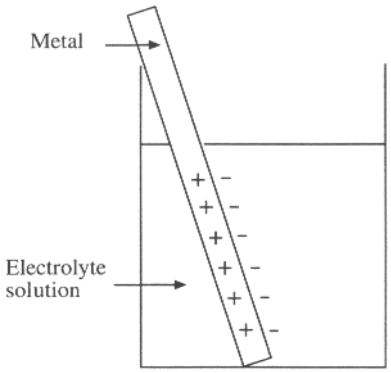


Figure 4.1 A metal electrode dipped in electrolyte solution. One-half cell

i. The two half cells must be connected internally by means of an electrically conducting bridge or membrane. Then the two electrodes are connected externally by a potential measuring device. There is a very high internal impedance (~10^{12} Ω), such that very little current will flow through it.

ii. The e.m.f. of the cell is the difference between the electrode potentials of the two half-cells. This potential depending on (a). the nature of the

electrodes M_1 and M_2 (b) the nature and concentrations of the solutions S_1 and S_2 and (c) the liquid junction potential at the membrane (Salt bridge)

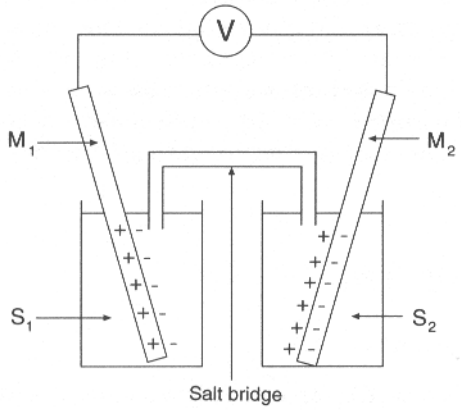


Figure 4.2 Two half-cell electrodes combined, making a complete cell

iii. Gibbs free energy is simply related to the e.m.f. of the cell:

$\Delta G = -nFE$ (for one concentration, usually 1M)
n: electrons transferred
F: Faraday constant=96487 $Cmol^{-1}$
E: e.m.f. of the cell

=> find ΔG_1 and ΔG_2=> find E_1 and E_2

iv. Define a reference reaction of ***hydrogen oxidation***: ΔG=0 (NHE: normal hydrogen electrode)

$$H^+ + e^- = 1/2H_2$$
$$E^{\theta}_{H^+/H_2} = 0$$

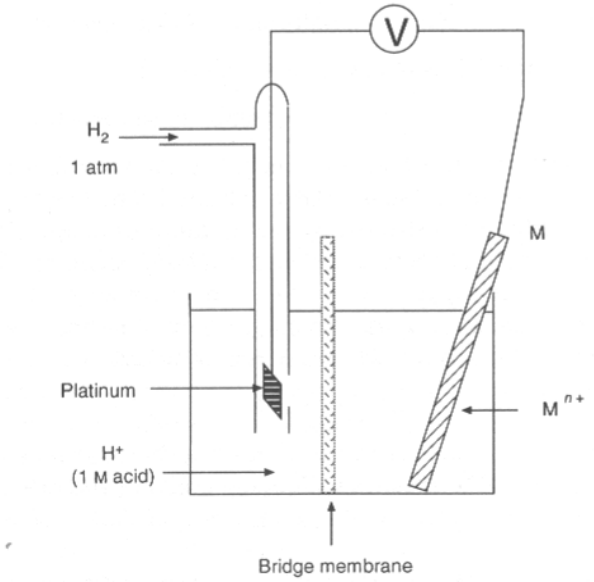


Figure 4.4 A hydrogen electrode connected with another half-cell

Silver-Silver Chloride electrode:

$$AgCl + e^- = Ag + Cl^- \quad E^\theta = 0.22V$$

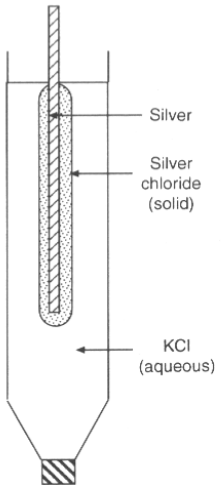


Figure 4.5 A silver/silver chloride reference electrode

Saturated calomel electrode (SCE)

$$Hg_2Cl_2 + 2e^- = 2Hg + 2Cl^- \quad E^\theta = 0.24V$$

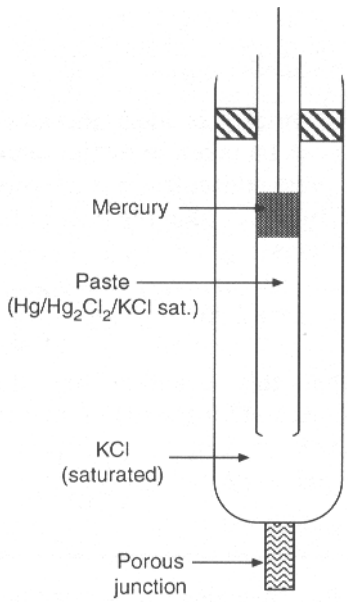


Figure 4.6 A saturated calomel electrode (SCE)

v. The Nernst Equation

Consider the effect of different concentrations on the electrode potential, from thermaldynamic

$$\Delta G = -RT \ln K$$

So for the half-cell reaction, $Ox+ne^-=R$, the Nernst equation is

$$E = E^{\theta} + \frac{RT}{nF} \ln(\frac{a_{ox}}{a_R})$$

a_{ox} or a_R are activities, i.e. ideal thermodynamic concentrations.

Transfer to natural log form:

$$E = E^{\theta} + 2.303 \frac{RT}{nF} \log(\frac{[Ox]}{[R]})$$

R is often a metal, in which case it has a constant concentration (activity)=1, so the equation can be simplified to :

$$E = E^{\theta} + 0.06 \log([Ox])$$

vi. Concentration Cells

If we incorporate a similar half-cell with the same redox couple but with a different concentration of Ox

$$\Delta E = E_1 - E_2 = S \log(\frac{[Ox]_1}{[Ox]_2})$$

for $[Ox]_2$ keep a constants, $\Delta E \sim \log[Ox]_1$

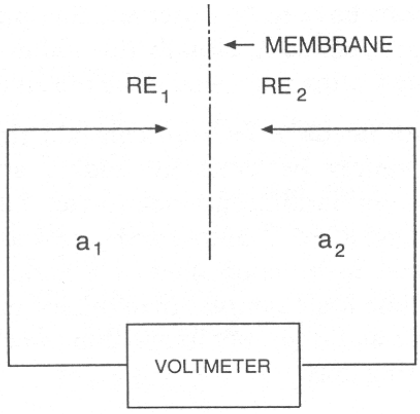


Figure 4.8 A concentration cell. RE_1 and RE_2 = reference electrodes

Voltammetry/Cyclic voltametry:

i. Linear Sweep Voltammetry

The application of linearly varying potential between a working electrode and a reference electrode in a electrochemical cell to monitor the current through the cell.

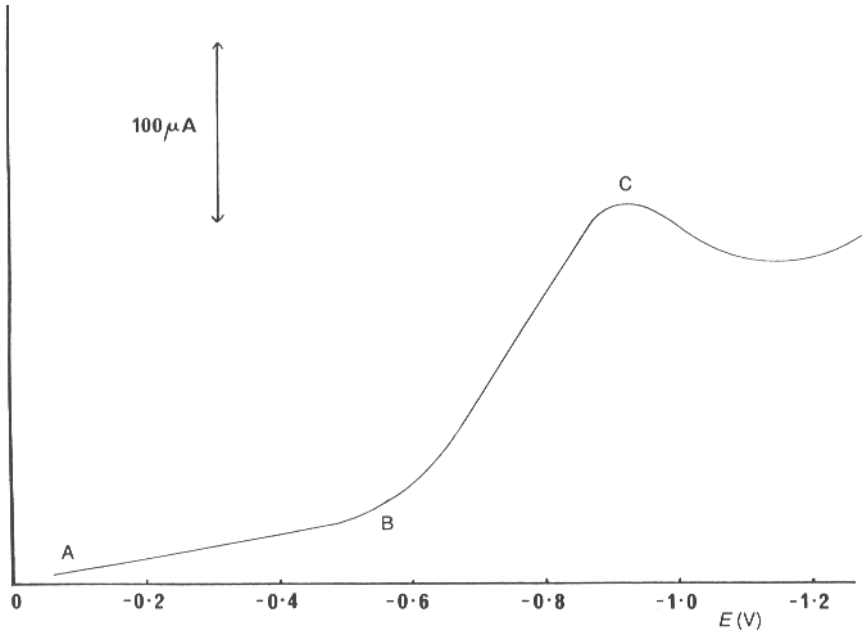


Figure 4.14 A linear sweep voltammogram

A=>B: rising slowly owing to the residual current (from impurities) and double layer charging

At point B: the potential approaches the reduction potential of the Ox. The increasing potential causes electrons to transfer from electrode to Ox at an increasing rate.

The rise does not continue infinitely, and will be limited by the

depletion of Ox, and the current is limited by the decreasing rate of diffusion of fresh Ox from the bulk of the solution.

The peak can be expressed by Randels-Sevcik equation:

$$i_p = 2.68 \times 10^5 n^{3/2} A D^{1/2} C_{Ox} \nu^{1/2}$$

The peak current is directly proportional to the concentration of Ox (C_{Ox})

ii. Cyclic Voltammery

While the amount of Ox at the electrode surface becomes depleted by the reduction, it is of course replaced by R which diffuses away into the solution. If we reverse the potential sweep from the positive side of the peak we shall observe the reverse effect => R species start to be reoxidized to Ox. The current will now increase in the negative (oxidizing) direction until an oxidation peak is reached.

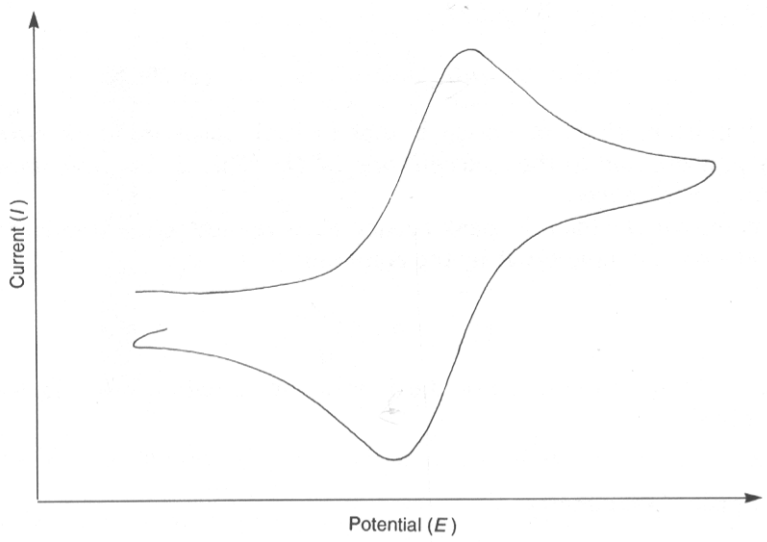


Figure 4.17 A reversible cyclic voltammogram

The peak potentials are shifted by 0.056/n V relate to each other. The average of the two peak potentials is equal to the standard redox potential regardless of the concentration of substrate or its diffusion coefficients or rates of electron transfer.

iii. Chronoamperometry

Apply a square wave to a potential just past where the peak would be in linear sweep voltammetry. (diffusion control)

The current decays because of the spreading out of the diffusion layer, and is proportional the reciprocal of the square root of time. Cottrell equation:

$$i_d = \frac{nFAD^{1/2}C_{Ox}}{\pi^{1/2}t^{1/2}}$$

this can be used to determine n, A, D, or C.

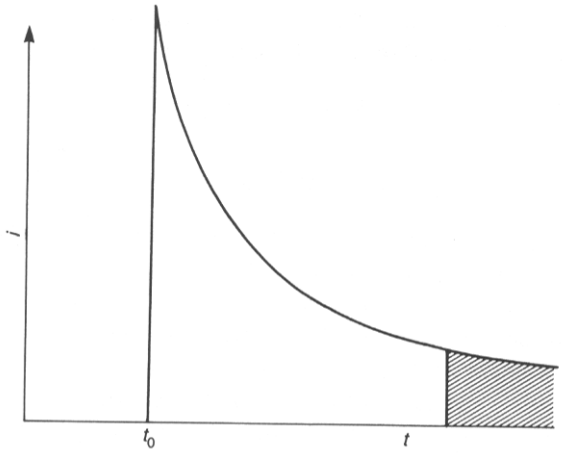


Figure 4.20 The current–time profile associated with a potential step redox process

iv. Amperometry

With certain cell and electrode configurations, the decaying current reaches an approximately steady state after a certain time. (as the shadow area of the above figure) The current has become effectively independent of time:

$$i_d = \frac{nFADC_{Ox}}{\delta}$$

δ: a constant related to the diffusion layer thickness

Field Effect Transistors:

i. The metal of the gate is replaced by a chemically sensing surface. The chemically sensitive membrane is in contact with the analyte solution. A reference electrode completes the circuit via the V_G bias. V_G is corrected by the membrane potential minus the solution potential:

$$I_D = K(V_G - V_T - E_{ref} - (\frac{RT}{n_i F})\ln a_i - V_D/2)V_D$$

The current can be measured directly at constant V_G (fig. 4-28), or keep I_D constant by changing V_G and measuring V_G (Fig. 4-29):

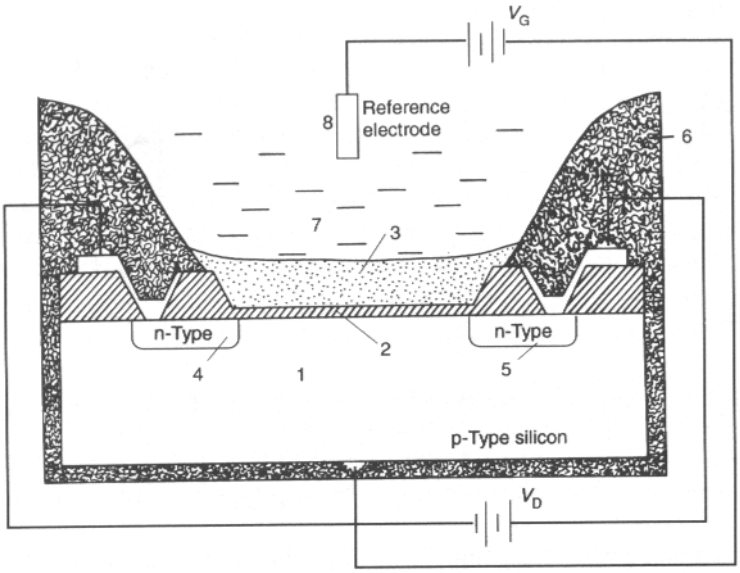


Figure 4.27 Diagram of the CHEMFET. 1, Silicon substrate; 2, insulator; 3, chemically sensitive membrane; 4, source; 5, drain; 6, insulating encapsulant; 7, analyte solution; 8, reference electrode

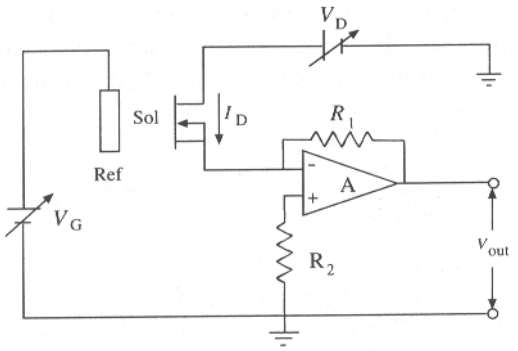


Figure 4.28 Schematic diagram of circuit for measuring I_D at constant gate voltage. A, Operational amplifier

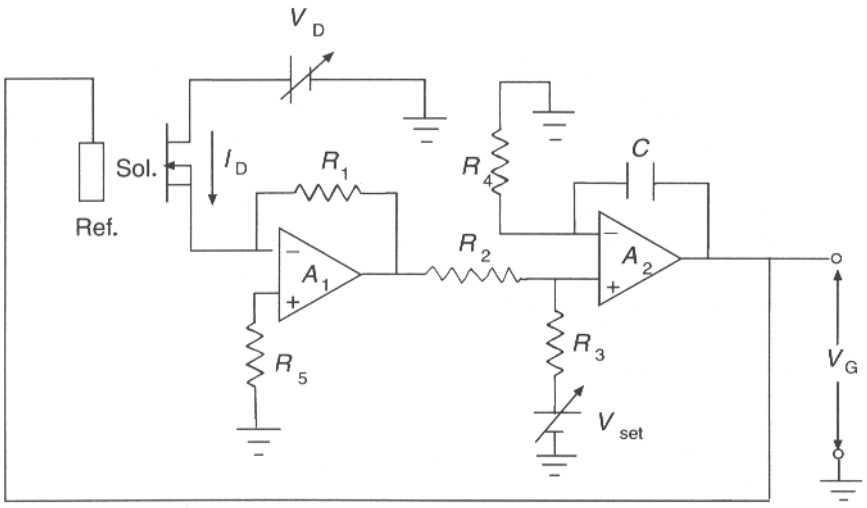


Figure 4.29 Schematic diagram of circuit for measuring changes in V_G at constant drain current. A_1, A_2 = Operational amplifiers

d. Mechiancal/Physical Detections

i. Ultrasound:

$$\frac{\Delta f}{f_0} = S_M \Delta M$$

S_M: Mass sensitivity of device
ΔM: added mass per unit area

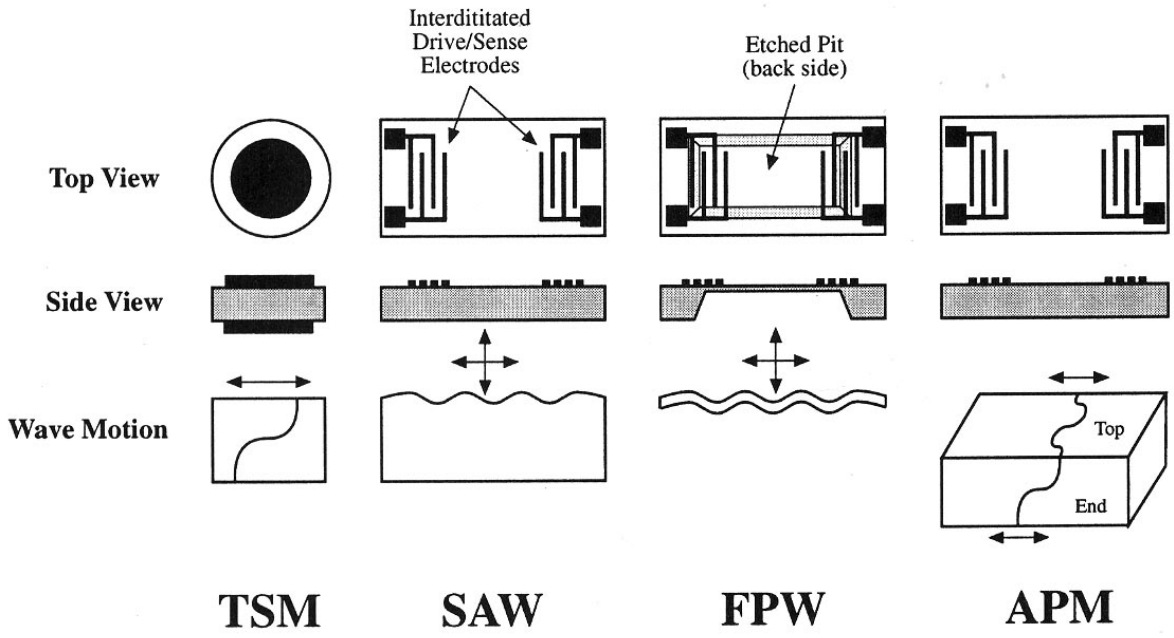


Illustration of the four major classes of acoustic wave transducers. After Grate, et al. (1993).

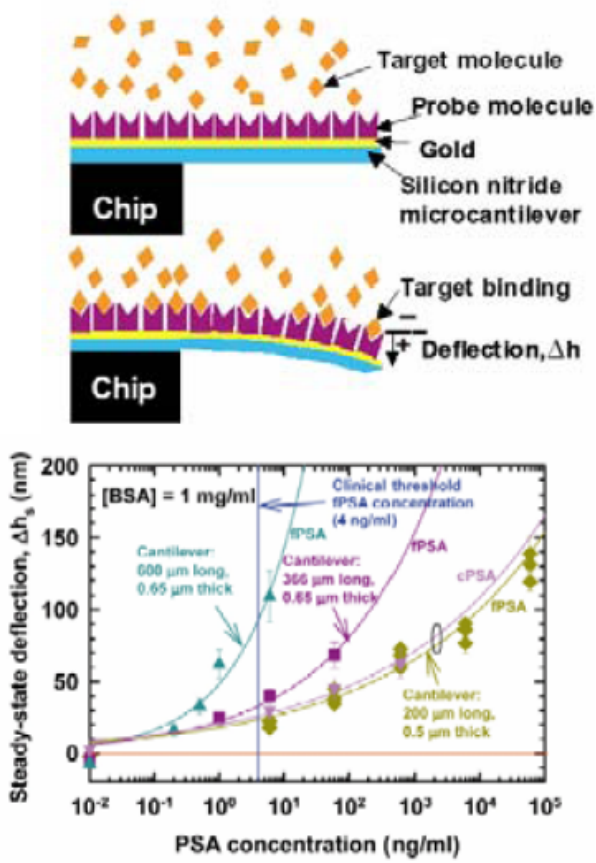
Detection limit~ 1pg.

ii. Cantilever:

MEMS Bioassays for Cancer Diagnostics

- Bioassay of prostate-specific antigen (PSA) using microcantilevers
- cantilever motion originates from the free-energy change induced by specific biomolecular binding (protein-protein) that can be optically detected
- may offer a common platform for high-throughput label-free analysis of protein–protein binding, DNA hybridization, and DNA–protein

interactions, as well as drug discovery.

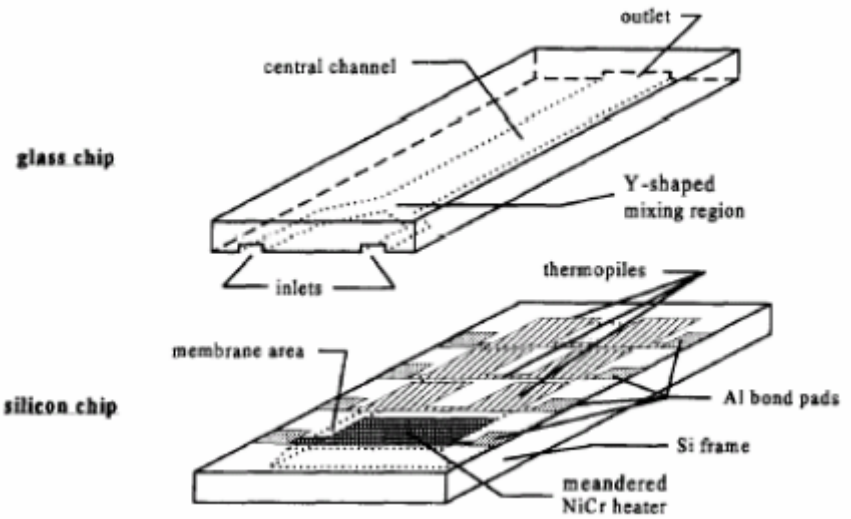


Wu et al., Nature Biotechnology, vol.19, Sep 2001

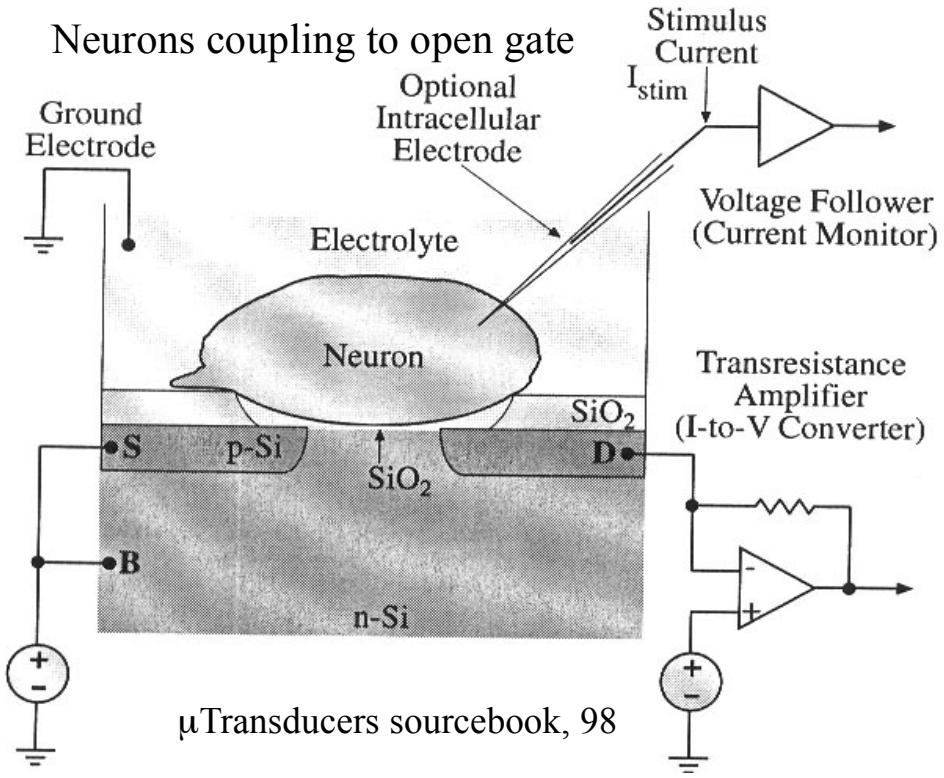
e. Thermal Detection

Microcalorimetry
(Kohler and Zieren, Thermochimica Acta 310 (1998), p.25)

- Thermal transduction principles can characterize chemical substances and processes since all chemical reactions, phase transitions, and even mixing and wetting processes are accompanied with exchange of heat
- Chemical compounds can be distinguished by different specific heats
- Thin film thermopiles or thermoresistors are used to sense the temperature changes



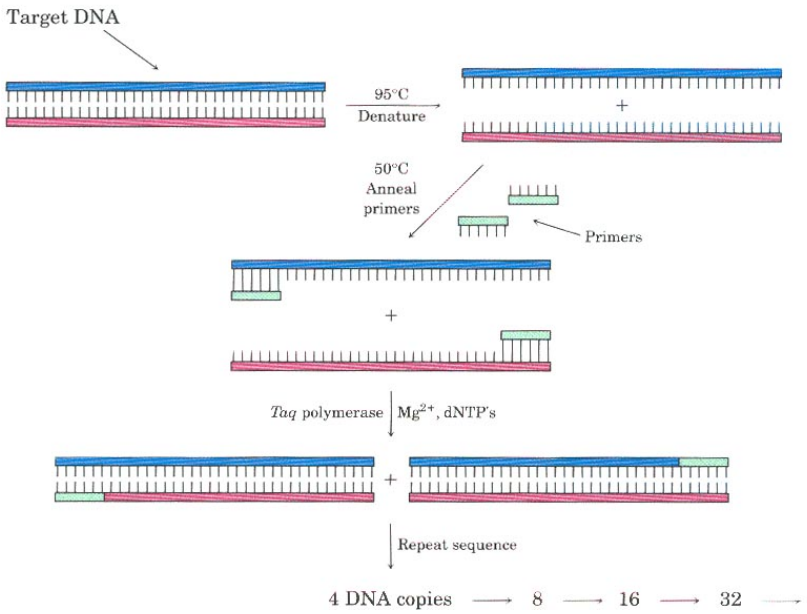
f. **Hybrid biosensors**



μTransducers sourcebook, 98

5. Molecular gain

a. PCR (Polymerase Chain Reaction)



b. Polypeptide
c. Fluorescence chain
d. Nano particles (lecture 7)

6. **Detection System**

Sample Detection in microTAS

- Integration
- Sensitivity
- SNR (Signal to Noise Ratio)
- Bandwidth
- Sample readout

Reference:

1. Egins, Brian R., “Biosensors, an introduction”, Wiley and Teuner, New York, USA1996.
2. Micromachined Transducers source book, Gregory T.A. Kovacs, McGraw Hill, 1998.