Types of Biosensors II

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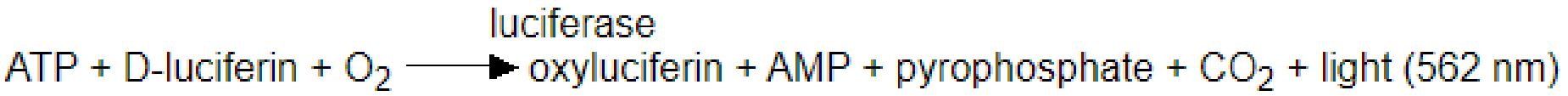
3. Optical Biosensor

There are two main areas of development in optical biosensors. These involve determining changes in light absorption between the reactants and products of a reaction, or measuring the light output by a luminescent process. The former usually involve the widely established, if rather low technology, use of colorimetric test strips. These are disposable single-use cellulose pads impregnated with enzyme and reagents. The most common use of this technology is for whole-blood monitoring in diabetes control. In this case, the strips include glucose oxidase, horseradish peroxidase (EC 1.11.1.7) and a chromogen (e.g., *o*-toluidine or 3,3',5,5'-tetramethylbenzidine). The hydrogen peroxide, produced by the aerobic oxidation of glucose, oxidising the weakly coloured chromogen to a highly coloured dye.

$$\text{chromogen(2H)} + H_2O_2 \xrightarrow{\text{peroxidase}} \text{dye} + 2H_2O$$

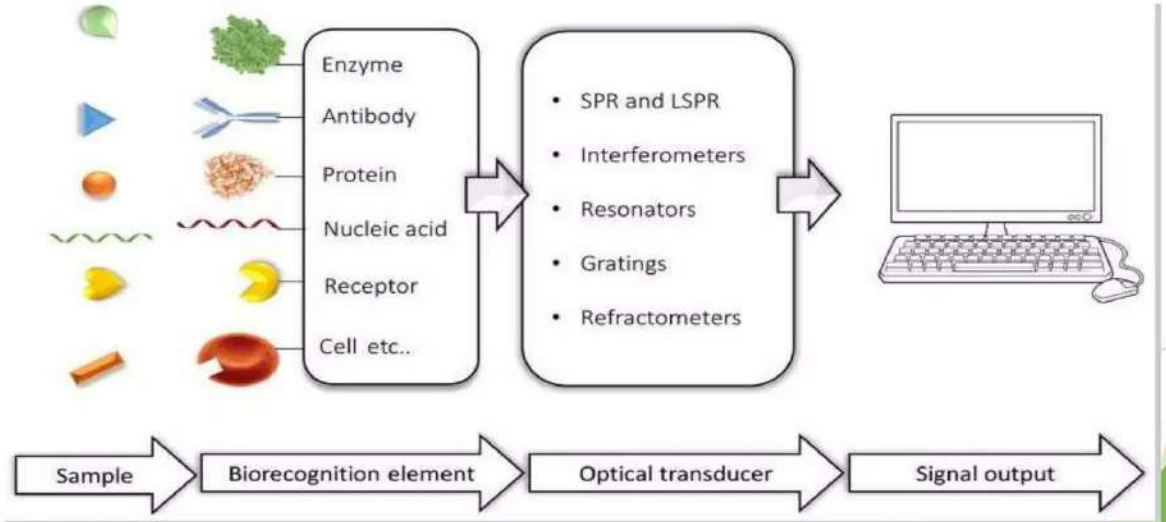
The evaluation of the dyed strips is best achieved by the use of portable reflectance meters, although direct visual comparison with a coloured chart is often used. A wide variety of test strips involving other enzymes are commercially available at the present time.A most promising biosensor involving luminescence uses firefly luciferase (*Photinus*-luciferin 4-monooxygenase (ATP-hydrolysing), EC 1.13.12.7) to detect the presence of bacteria in food or clinical samples. Bacteria are specifically lysed and the ATP released (roughly proportional to the number of bacteria present) reacted with D-luciferin and oxygen in a reaction which produces yellow light in high quantum yield



The light produced may be detected photometrically by use of high-voltage, and expensive, photomultiplier tubes or low-voltage cheap photodiode systems. The sensitivity of the photomultiplier-containing systems is, at present, somewhat greater (< 10^4 cells ml^{-1}, < 10^{-12} M ATP) than the simpler photon detectors which use photodiodes. Firefly luciferase is a very expensive enzyme, only obtainable from the tails of wild fireflies. Use of immobilised luciferase greatly reduces the cost of these analyses.

Introduction

- Optical Biosensor consists of biological equipment, detector element and transducer associated with both components.
- The biological component is generally a protein target (bio receptors) which is immobilized on the surface of the transducer.
- The OBs are coupled with microfluidics which introduces an analyte as well as separates unbound molecules and is a powerful biophysical tool for characterization quantitatively.

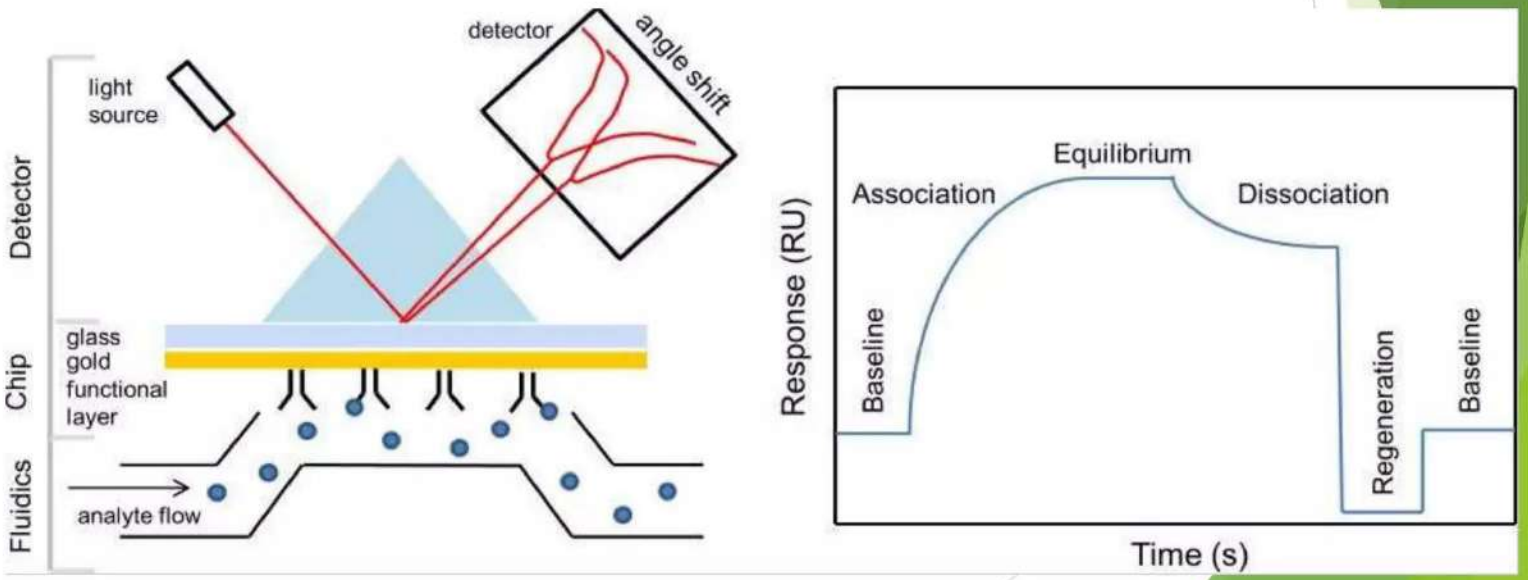


TYPES OF OB

Surface Plasmon Resonance

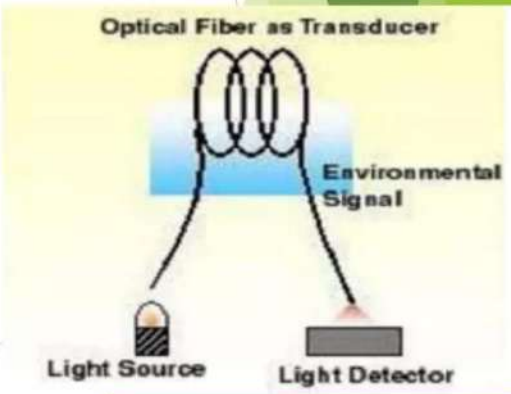
- Physical phenomenon for which the electrons in thin gold film can be excited into collective oscillation by bombarding it with photons (light) of particular frequency.
- These occurs on the surface of metal at the interface of two media (usually glass and liquid) when it is illuminated by polarized light at a specific angle.
- This generates surface Plasmon and consequently a reduction of the intensity of reflected light at a specific angle known as the resonance angle. This effect is proportionate to the mass on the surface.
- SPR sensing is an exceedingly powerful and quantitative probe of the interactions. These interactions of variety of biopolymers with various ligands, biopolymers and membranes including protein:ligand,protein:protein,protein:DNA and protein: membrane.
- The SPR phenomenon enables direct, label-free and real-time changes of refractive index at the sensor surface, which is proportionate to the biomolecule concentration.

- To measure a ligand-analyte interaction, one interacting molecule must be immobilized on the sensor surface.
- The SPR chip contains a functional layer which enables the immobilization of interacting molecules.
- The first commercial SPR-based biosensor instrument was launched by Pharmacia Biosensor AB, which was later renamed Biacore.

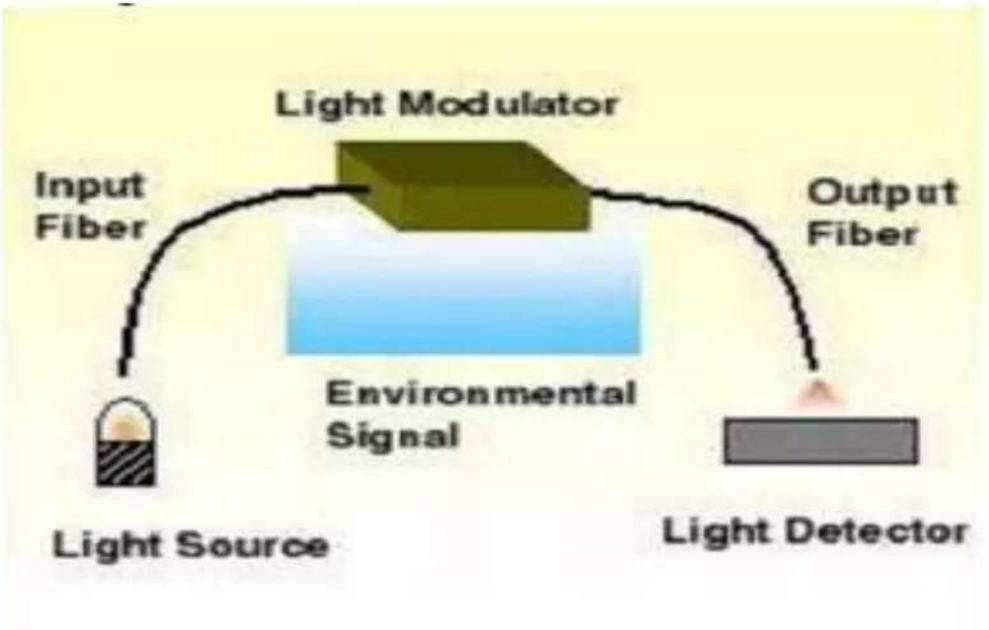


Fibre Optic Biosensor

- Analytical devices in which fibre optic device serves as transduction element and it is to produce a signal that is proportional to the concentration of chemical or biochemical to which the biological element reacts.
- Transmits light on the basis of the principle of Total Internal Reflection(TIR).
- These are based on the transmission of light along with silica glass fibre or plastic fibre optic to the site of analysis.
- Classified into 2 different categories:
  - Intrinsic sensors: the internal property of optical fiber itself converts the environmental changes into a modulation of light signal. This modulation of light signal may be in form of intensity, phase and frequency or may be polarization.



- Extrinsic sensors: In contrast to intrinsic sensing, in extrinsic sensing; the fiber may be used as information carriers that lead to a black box and that will generate a light signal depending on the information arrived at black box. This black box may be made of mirrors, gas, liquid cell, or many other mechanisms that will proceed to generate an optical signal.



ABSORBANCE BASED

- Low technology
- Colorimetric test strips
- Cellulose pads embedded with enzymes and reagents
- Used in diabetes regulation:

$$\text{1 glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$\text{2 chromogen}(2H) + H_2O_2 \xrightarrow{\text{peroxidase}} \text{dye} + 2H_2O$$

- Strip includes: glucose oxidase, horseradish peroxidase, and a chromogen When oxidized, the chromogen will be colored

LUMINESCENCE BASED

- Luminescence-light that is not primarily generated from heat.
- Light output from a biochemical reaction.
- Can be used in detection of bacteria

  --Uses Firefly luciferase(from the tails of wild fireflies).

  --Bacteria is lysed and yellow light is given off.

$$ATP + Luciferin + O_2 \longrightarrow AMP + PPi + Oxyluciferin + CO_2 + h\gamma$$

ADVANTAGES

- Small
- Flexible
- Fast
- Safe, no electrical device interacts with the body
- Good biocompatibility (fibers are glass)

DISADVANTAGES

- High cost of some instrumentation
- May be Invasive
- Fluorescent signal may not be strong enough