

Phenomena Behind Optical Biosensors: A Review

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Abstract:

The paper provides fundamentals of a sensor and its parameters. It explains the basic phenomena behind optical biosensors qualitatively. It also includes recent advancements in the field of optical biosensors giving emphasis on the plasmon enhanced electric field phenomenon. It creates interest to develop new sensing schemes based on fluorescence and SERS devices.

Keywords: Plasmon, florescence, Transducer, sensor chip, LSPR, SERS

1. Introduction:

A sensor is a device which measures a change in one physical parameter in terms of magnitude of a second different parameter which can be measured more conveniently and perhaps more accurately. For example, thermometer measures temperature in terms of the length of mercury column. Similarly, a conventional glucometer which is commercially available in the market, actually, measures current and it translates the change in current in terms of glucose concentration in blood. From the above examples one understands that sensors are of two groups: one group is a sensor which is quantitative, while the other sensor is purely qualitative.

2. Biosensor:

A biosensor [1] is any device that uses a specific biochemical reaction to detect chemical compounds in biological samples. In global market there is a huge potential and requirement for biosensors. That is why it is very important to study the concept of biosensors.

2.1. Components of a Biosensor:

In general, any type of biosensor will have the following components.

- A. Sensor surface: It will be a surface which interacts with biological molecule to be examined.
- B. Analytes: Analytes are chemical or biological or environmental elements or molecules that need to be sensed.
- C. (c). Bio-recognition element or bio-receptor (BRE): It leads to specific attachment with particular analyte and does not bind to anything else.
- D. Transducer: Transducers convert one form of energy to another form of energy. But, in a sensor it basically transforms the binding phenomenon or interaction phenomenon between the analyte and the BRE into a measurable output signal which can be an optical signal or electrical signal or acoustic or maybe change in dimensions or change in length as in thermometer.

3. Fabrication of a biosensor chip:

Various steps involved in the fabrication of the chip are shown in Fig.1. A cross linker is placed over a transducer surface. The job of the cross linker is to attach the bio-recognition element on top. If BRE is not directly get attached to the transducer surface then one need to put a cross linker. So a cross linker or a kind of adhesive is required to put this BRE(molecule) on the sensor surface. Now it can catch the analyte, but then there are lots of spaces on the surface which are not filled. So, it is very important to block all these sites so that nothing can go and bind on here. So anti-fouling agent is used. The job of this agent is to block all the nonspecific binding sites. Now the surface has only BREs open for binding, everything else is closed.

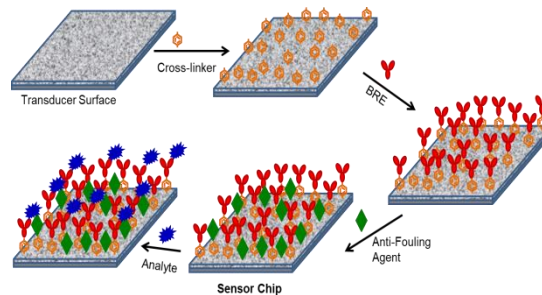


Fig.1 Biosensor chip

And now an analyte goes and binds on these sites. That is how a very good sensor surface is formed[1]. Among all types of biosensors optical biosensors are very small in size. They are flexible, they are very fast. However, the disadvantage is that the optical signal may not be strong enough many times.

4. Optical biosensors:

Optical biosensors are generally based on measurements of changes in any of the optical properties viz., absorbance in chemical reaction, reflectance and transmittance, refractive index, phase shift, polarization or light energy. So, it can either be a fluorescence-based sensor or Raman based sensors. Let us view the phenomena behind optical sensor qualitatively.

4.1. Surface plasmon resonance (SPR)[2,3]: Plasmons are basically quanta of longitudinal oscillations of free electrons in metal and we call it plasmon which is synonymous to plasma of charges. That means a metal has solid positive background with free electrons moving randomly and the oscillation is called plasmon oscillation. The free conduction electrons of a metal are influenced by a time dependent force opposite to that of the changing electromagnetic field of the incident light. The resulting motion of the electrons will be oscillatory, but 180° out of phase due to the charge of the electron, and with dampening caused by Ohmic losses. Like all oscillators, the conduction electrons have a characteristic frequency known as the plasma frequency. The plasma frequency depends on the density of electrons (n) and the effective mass (m_e). It reflects how easily the electrons move with respect to incident light. The free electrons in a bulk do not oscillate against the restoring force. If the light has a frequency above the plasma frequency i.e., in the ultraviolet (UV) range for metals, the electrons will not oscillate and the light will simply be transmitted or absorbed due to inter band transitions. If the light has a frequency smaller than the UV range, the electrons will oscillate 180° out of phase with the incident light, which causes a strong reflection. The combination of plasma frequency and inter band transitions gives metals their characteristic colour. Theoretically it can be shown that when the frequency of light is greater than plasma frequency the dielectric constant is positive and the light is transmitted. When the

frequency of light is less than the plasma frequency, the real part of the dielectric constant is negative, and the majority of the light is reflected. Thus the dielectric constant decides whether metal electrons can oscillate or not at the given frequency of light

If the bulk metal is reduced into the thin film, oscillation will exist only at the surface and the corresponding propagating charge waves called as surface plasmon polaritons (SPP) or evanescent waves. The interface between metal surface and surroundings limits frequencies of oscillating electrons. The wave vector or momentum of charge wave will be always greater than that of massless photons. So extra momentum is provided to photons by propagating them through prism or using grating and SPP are excited. At a specific angle i.e. at resonance a dip is observed in the reflection or transmission spectrum.

The problem with SPP sensors is that they require suitable glass prism or grating arrangement

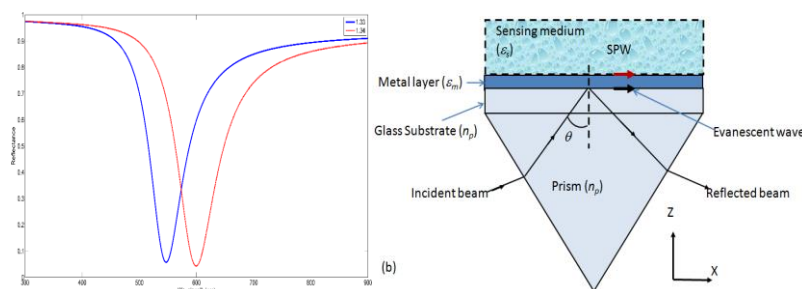


Fig.2(a).Red shift of wavelength and (b)SPR Biosensor

The SPR sensor in kretschmann configuration (where there is very thin metallic film is placed between prism and dielectric or sensing medium) is shown in fig.2(b). When light is incident there will be dip in reflection spectrum at a particular angle as shown by the blue curve in Fig.2(a). If the sensing medium contains analytes the dip shifts as shown by red curve because of change of dielectric constant or refractive index which is known as red shift. This shift is a measure of sensitivity of a biosensor.

4.2. Local surface plasmon Resonance (LSPR) [4-7]: The restrictions of SPP can be overcome changing a two-dimensional (2D) metal film into a zero-dimensional (0D) nano particle. Plasmon resonances of metal nano particles are basically quanta of charge density oscillations of metal nano particles. When metallic nano sphere whose size is smaller than wavelength as shown in the Fig.3 is subjected to a time varying electric field, if the electric field is pointing in upward direction, the electron cloud would slightly shift to the downward direction, leaving behind a dipole here with electrical field in the opposite direction as shown in Fig.3. If applied field is varying at a particular frequency then this dipole will also be oscillating at the same frequency.

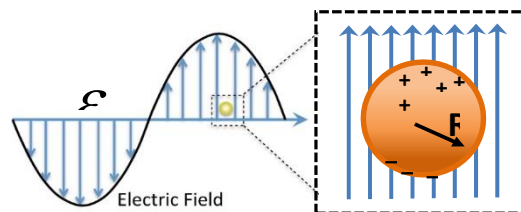


Fig.3. Effect of applied field on nano particle

This kind of oscillations, when the frequency of the incident light becomes equal to the oscillation frequency of conduction electrons is attributed as localized surface plasmon resonance. When there is

resonance, light will get absorbed by these plasmons. This absorbance as well as the resonance frequency is highly dependent on the size, shape and environment of the nanoparticles. As the change of refractive index of medium surrounding nano particle causes change of resonance frequency, LSPR can be used for sensing.

4.3 Surface enhanced Raman spectroscopy (SERS)[8-11]. If a molecule is shined with a laser, most of the light gets scattered from this is at the same wavelength or frequency of that of the laser. This is called Rayleigh's scattering. But, very small fraction of this light, which is getting scattered from this molecule will have frequencies which are either smaller or larger than the frequency of laser incident and that small shift is very important which is known as Raman shift. This small change in the frequency is a fingerprint of molecular bonds and crystalline structure of that molecule. Measuring the Raman spectra of different materials, one can say which one is what material. But the problem is that out of 10 million photons only one gets Raman scattered. But if the molecule is brought close to the metallic nanostructure, it will experience enhanced electromagnetic field and can enhance its optical signals - its spectroscopic signals. This is called SERS. It is not just the case for enhancing the Raman signal, it can enhance the fluorescence signal, can enhance absorption - all these things. Thus SERS continues to be attractive technique for chemical sensing, biomedical applications and has advantages including unique spectral signatures of analytes, easy operation without complicated sample preparation. Raman spectroscopy can be used to stop fraud. For example comparing Raman spectra for calcium carbonate - natural pearl, faux pearl one can say whether pearl is pure or not[12]. The components of drug tablet can be determined by identifying colors of Raman spectra images. The two constraints of SERS are the molecule under investigation has to be very close to metallic structure and it has to be small. However SERS is also useful to detect bacteria of size of few micron and not very close to nano particle by a method known as non-direct sensing.

5. Various sensors and their mechanisms: Based on the above phenomena various sensors can be fabricated.

5.1 Localized surface Plasmon enhanced electric field(LSPR) biosensor: LSPR is like a dipole sitting at the center of the nanoparticle and this was oscillating. The oscillating dipole there causes the enhancement of the electric field in the vicinity of the nanoparticle on light incidence. The enhanced electromagnetic fields can be used for enhancing optical signals. During sensing the light which is scattered from the nanoparticles is captured by the camera. So we can see different colors, which are pertaining to different shapes and sizes of the nanoparticles and here it can be seen that they can correspond to different resonances for different dimensions

We can have single silver particle biosensors. Fig.4 shows one single nanoparticle (i) before and (ii) after exposure of 10 nanomolar streptavidin that a shift of about 13 nanometers occurs by adding streptavidin. These measurements were collected in a nitrogen environment. So, that is how nanoparticles are used for localized surface plasmon resonance space sensing[13].

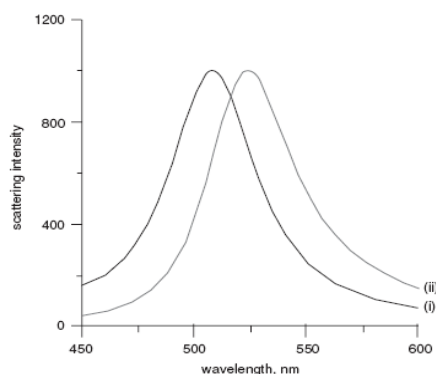


Fig.4. Single Ag nanoparticle biosensor

5.2 Surface enhanced fluorescence(SEF) biosensor: ‘Fluorescence’ was named by George Gabriel Stokes when he saw mineral fluorite which lights up when illuminated with ultraviolet and that is how he named it fluorescence. Actually if a molecule is shined light of certain wave length, it gets excited to higher state and after certain time, it comes back to the ground state emitting light. That is called fluorescence. The fluorescence time is much smaller than phosphorescence time. Common Fluorophores are Flourosences, BOZ 7, RHODAMINE 6G.

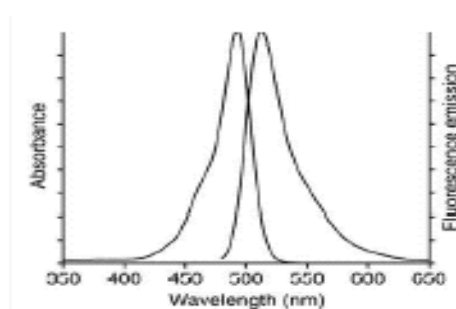


Fig. 5. Fluorescein molecule red shift

For example if Fluorescein molecule is shined with certain light, peak absorption takes place at about 575 nm and gets adsorbed there. After this kind of vibrational relaxation, it comes down emitting light of wavelength 525 nm which is larger than the absorption wavelength as depicted in Fig.5. This is called fluorescence. In case of a fluorescence biosensor we attach this fluorophore to the receptor via some linker. The linker provides the means for triggering the change in the fluorescence of the attached fluorophore. If we bring this kind of fluorophore near a metallic nano structured surface - plasmonic surface, then its fluorescence can be enhanced and that is why it is called surface enhanced fluorescence[14,15].

5.3 ESP-LSP coupling based ultra high enhancement biosensor: If there are surface plasmons then enhancement of the electromagnetic field on the surface will be up to order of 10^2 . If there are LSP, the enhancement of field will be about 10^3 . The ESP-LSP coupling can enhance field by 10^5 . Supposing this kind of structure, these are extended plasmons coupled to localized plasmons and slightly displaced, say about 1 or 2 nanometres, we find a high enhanced electromagnetic field here which is called plasmonic hotspot. And if we bring a molecule here, basically you can enhance its electromagnetic signal. So, the molecule placed experiences an electromagnetic field of about 10^5 in the hotspot.

If a fluorescent molecule is placed at the hot spot, it experiences enhanced fluorescence intensity and do not see any enhancement when it go off ESP with fluorescence intensity going down[16,17]. Thus there

is better control over fluorescence by controlling the ESP resonance angle and hence better sensitivity. The diagram of ESP-LSP coupled biosensor is shown in Fig.6.

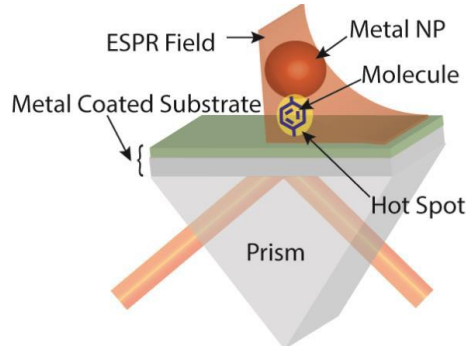


Fig.6. ESP-LSP coupled biosensor

5.4 Colorimetric biosensors; In this kind of biosensors, it is the change of colour when the interaction occurs. So, it is a kind of sensor where just by seeing - using eye, one can say that this is the particular analyte that is making the change here. For example, the pH strip is dip into the solution, it changes the colour and then we know how much pH it occurred. So, mostly it is qualitative[18].

5.5 Fibre optic Fabry-Perot Interferometer(FPI) based biosensors: Fabry-Pérot interferometer, basically is a thin film which has two mirrors facing each other. When a ray of light enters to it, it gets partially reflected from one boundary and then transmitted through the medium and then move back and forth between mirrors. Because of multiple reflections, an interference pattern of more contrast and visibility than that of Michelson Interferometer, is formed as it has a greater number of reflections. In case of fibre optic Fabry-Pérot biosensor two small pieces of this optical fibre are separated a gap called Fabry-Perot cavity. The ends of fibre pieces have metallic coatings which act as mirrors as shown in fig.7. This can be used for detection of ammonia.

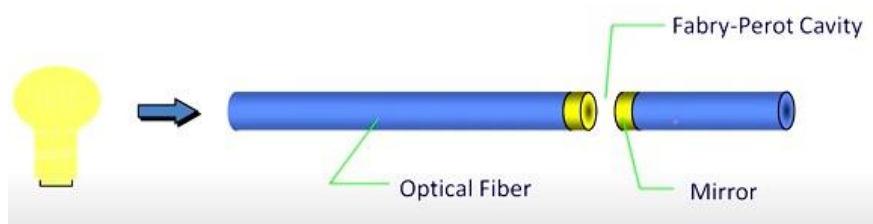


Fig.7 Fibre optic FPI biosensor

6. Recent Trends in the area of biosensors:

The recent advances are in COVID-19, including the insights in the virus, the responses of the host cells, the cytokine release syndrome, and the therapeutic approaches to inhibit the virus and alleviate the cytokine storm. Detection of biomarkers has raised much interest recently due to the need for disease diagnosis and personalized medicine in future point-of-care systems. Among various biomarkers, antibodies are an important type of detection target due to their potential for indicating disease progression stage and the efficiency of therapeutic antibody drug treatment[19-24].

7. Conclusion:

Colorimetric biosensors are simple and can be constructed easily but they have low sensitivity. The ESP-LSP coupling can enhance electric field but not that much as high enhancement as in case of SERS. On the other hand Fluorescence enhanced biosensors and SERS are widely used in various fields because of their high sensitivity, low cost and abundant availability. Among available biosensors, SERS biosensors are relatively new and have outstanding capability of performing and imaging at larger penetration depths when compared other optical biosensors. But SERS instruments are large in size and expensive so they have limited applications. Nowadays with the availability of commercial bench top or palm sized Raman readers, plasmon enhanced SERS technology is developing towards compact SERS biosensors. Recent research focus is on SERS substrates because of their flexibility, conformability and easy uptake of analytes. In the future, plasmonic are developed for not only enhancing the sensing signal but also to develop new sensing schemes based on fluorescence and SERS devices.

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