

# OPTICAL BIOSENSORS

The main types of photometric behavior which are exploited in biosensors applications:

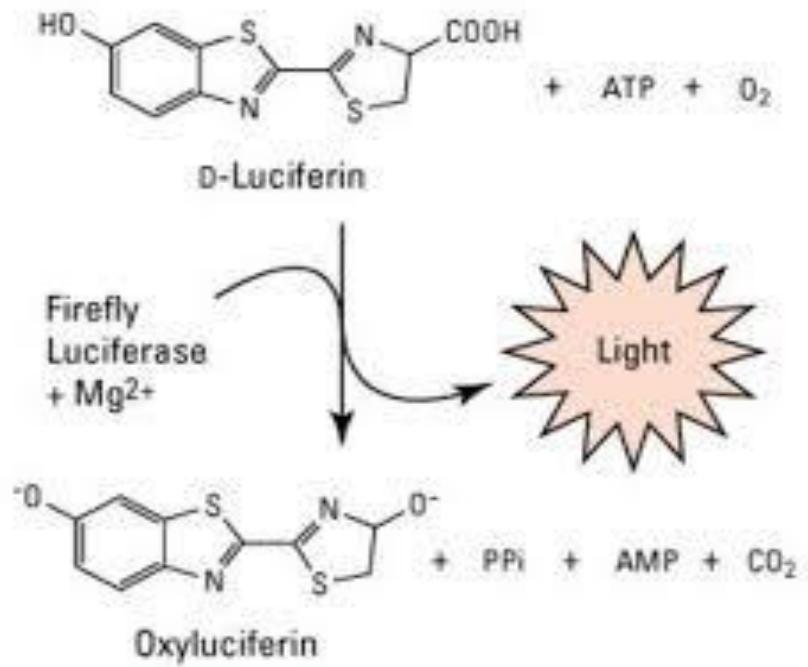
- UV-Visible absorption and reflection
- Fluorescence
- Phosphorescence
- Luminescence
- Internal reflection spectroscopy
- Laser light scattering methods

Typical photon detector characteristics<sup>a</sup>

Type	$D^*$ (cm Hz <sup>1/2</sup> W <sup>-1</sup> )	$R(\lambda)^b$	Linear range (decades)	Spectral range (nm) <sup>c</sup>	Rise time (ns)	Output
Photomultiplier tube	$10^{12}$ – $10^{17}$	$10$ – $10^5$ A W <sup>-1</sup>	6	110–1000	1–10	Current, charge
Vacuum phototube	$10^8$ – $10^{10}$	$10^{-3}$ – $10^{-1}$ A W <sup>-1</sup>	5	200–1000	1–10	Current
Si photodiode	$10^{10}$ – $10^{12}$	$0.05$ – $0.5$ A W <sup>-1</sup>	5–7	250–1100	1–10	Current
Photoconductive cell	$10^9$ – $10^{12}$	$10^4$ – $10^6$ V W <sup>-1</sup>	5	750–6000	50– $10^6$	Resistance change
Photovoltaic cell	$10^8$ – $10^{11}$	$100$ – $10^6$ V W <sup>-1</sup>	3	400–5000	1000	Current or voltage

**Example:**

Photometric detection of bacteria in food or clinical sample: Bacteria are specifically lysed and the ATP released reacted with D-luciferin and oxygen in a reaction which produces **yellow light** (562 nm) in high quantum yield.



Sensitivity of the photomultiplier-containing systems is  $< 10^4$  cells ml<sup>-1</sup>,  $< 10^{-12}$  M ATP. Firefly luciferase obtainable from the tails of wild fireflies is immobilized to reduce the cost of these analyses.

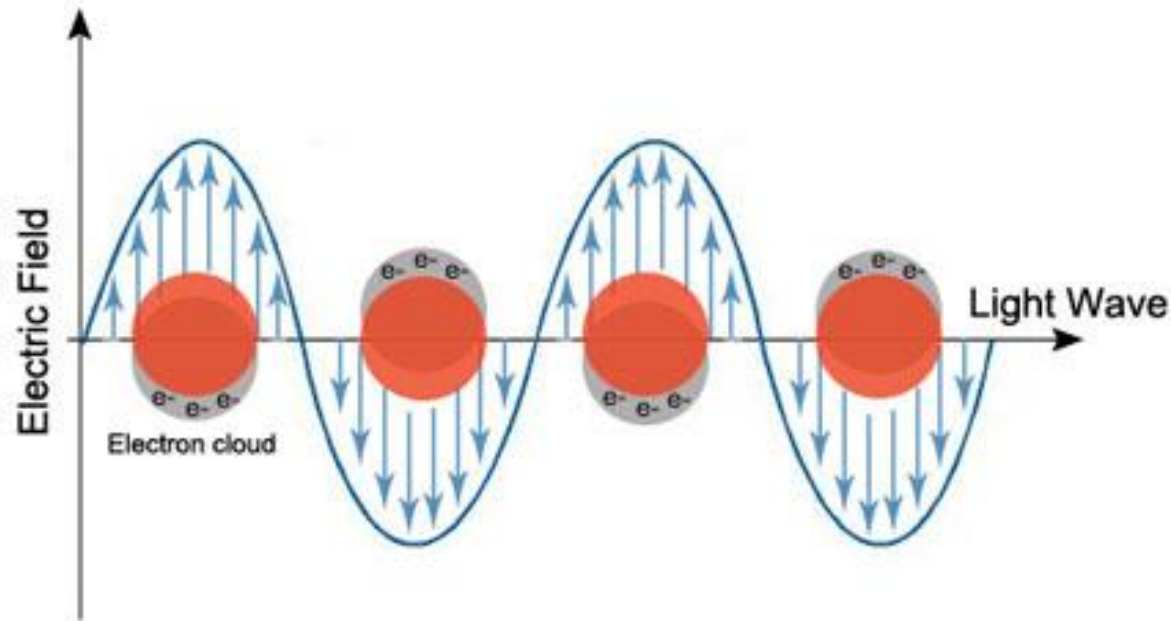
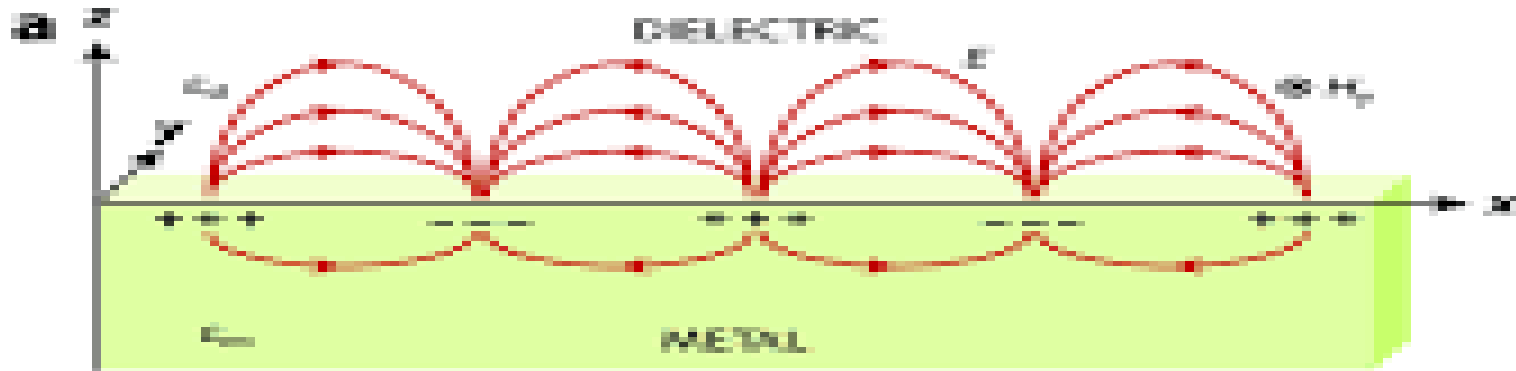
# Surface Plasmon Resonance (SPR)

SPR occurs on the surface of highly conducting metals, typically Au, Ag, Pt.

The plasmon is a group of excited electrons surrounding the atomic lattice sites of a metal behave like a single electrical entity

Surface plasmon resonance (SPR) is **the collective oscillation of conduction band electrons that are in resonance with the oscillating electric field of incident light**, which will produce energetic plasmonic electrons through non-radiative excitation.

Surface plasmons (or surface plasmon polaritons) are **surface electromagnetic waves** that propagate parallel along a metal-dielectric interface (e.g. metal-air).



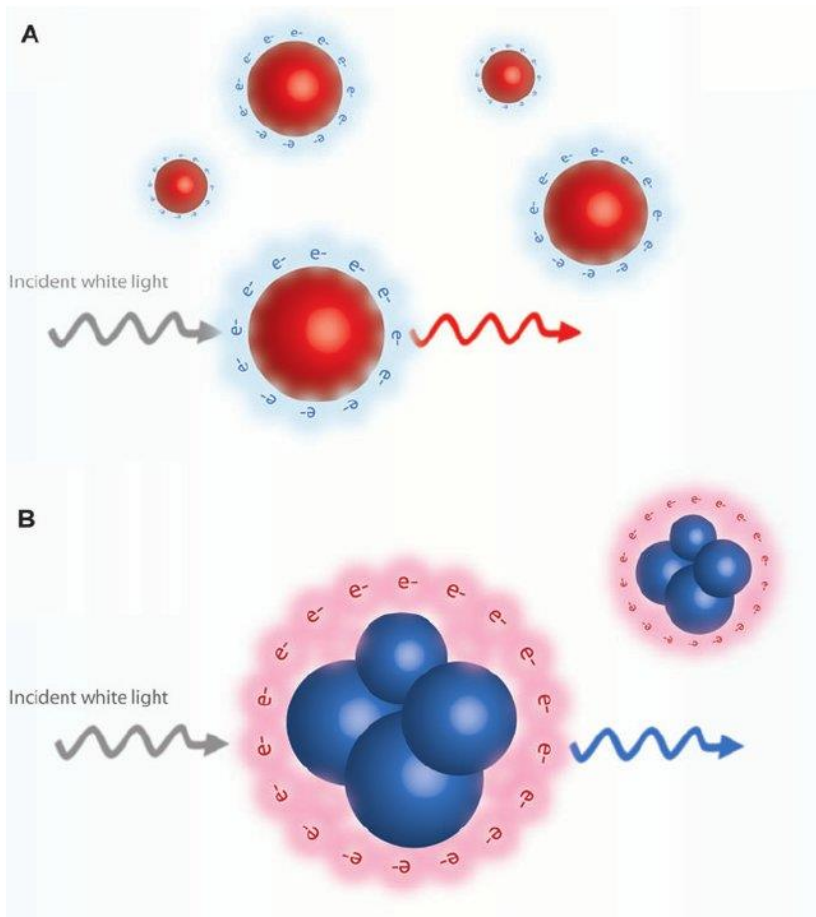
***LSPR*** : *localized surface plasmon resonance* for nanometer-sized metallic structures.

A localized surface plasmon (LSP) is the result of the confinement of a surface plasmon in a nanoparticle of size comparable to or smaller than the wavelength of light used to excite the plasmon.

When the electron cloud is displaced relative to its original position, a restoring force arises from Coulombic attraction between electrons and nuclei. This force causes the electron cloud to oscillate.

The oscillation frequency is determined by the density of electrons, the effective electron mass, and the size and shape of the charge distribution. Therefore, LSPR is highly sensitive to size, size distribution, and shape of the metal nanostructures, as well as the environment that surrounds them.

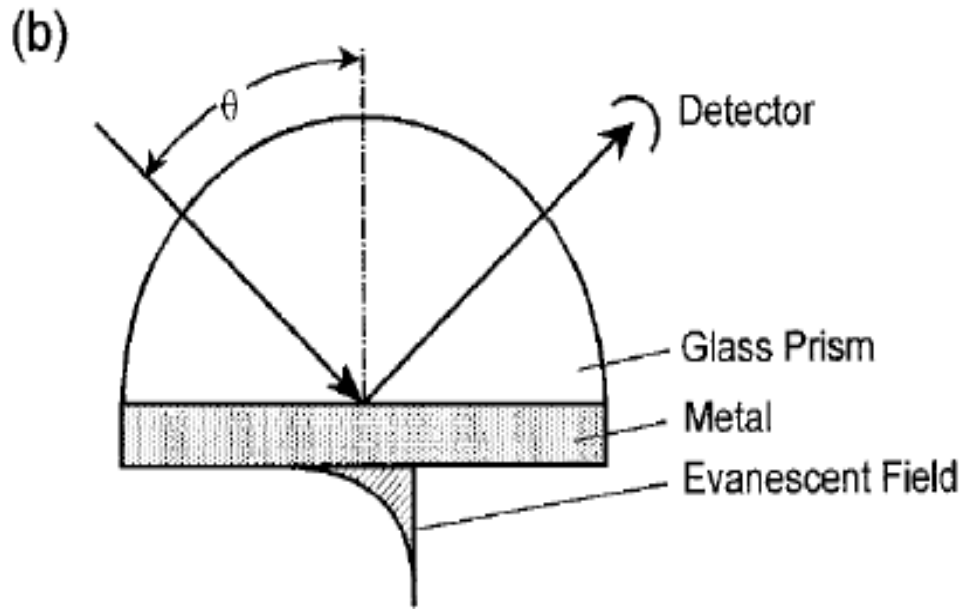
Hence, LSPR is the fundamental principle behind many color-based biosensor applications and different lab-on-a-chip sensors.



(A) Dispersed AuNPs absorb visible light at their LSPR absorbance maximum (~520 nm for AuNPs of approx. 15 nm) solution color appears red.

(B) When AuNPs undergo aggregation, their electronic clouds overlap and thus the aggregated particles behave as a larger particle with a SPR absorbance maximum at longer wavelength (red-shift). Thus, the solution color appears blue.

# Configuration of SPR



## Kretschmann ATR configuration

Most of the SPR sensors are based on **Kretschmann configuration** of the attenuated total internal reflection (ATR) method.

A light wave (usually plane polarized) passed through a high refractive index prism is reflected at the prism base covered with a thin Au film.

The light evanescently passes through the thin film and excited the plasmon at the outer boundary of the film *if the incident light wave and plasmon are closely phase-matched.*

Prism in plasmon resonance experiments given its larger refractive index equal to 1.5 compared to air equal to 1. Also the prism deviates the light.

To obtain the TIR at the glass/metal interface such that the evanescent field reaches the metal/air interface where the plasmon is excited (the thickness of the film is critical!), a prism is needed. In the prism configuration the incident ray has a relatively small angle (relative to the normal to the side of the prism) so bending is minimal and TIR can be achieved (and monitored on the opposite side) under well controlled angles.

p-polarized light is required to satisfy the boundary conditions necessary to excite SPR for sensing applications that can be achieved by using a prism. Also the prism deviates the light that helps to generate evanescent wave and detect reflected light.

Refractive index (n): The ratio of the velocity of light in a vacuum (c) to its velocity in a specified medium (v).

$$n = \frac{c}{v}$$

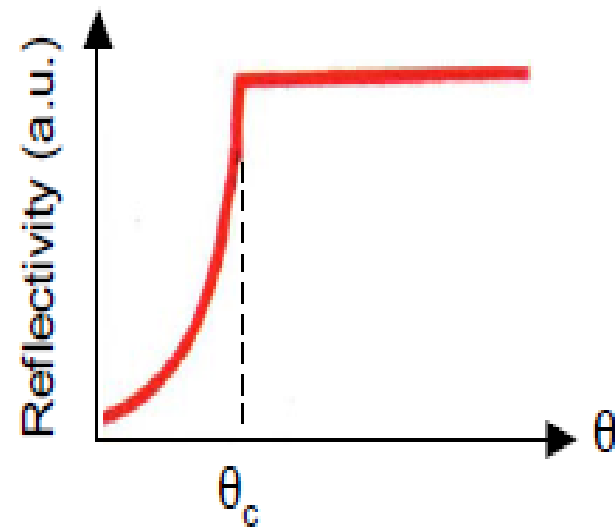
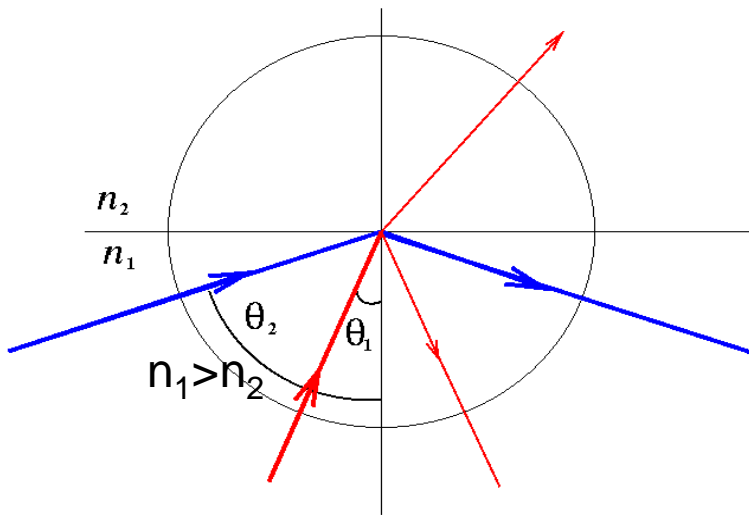


# The phase-matching condition:

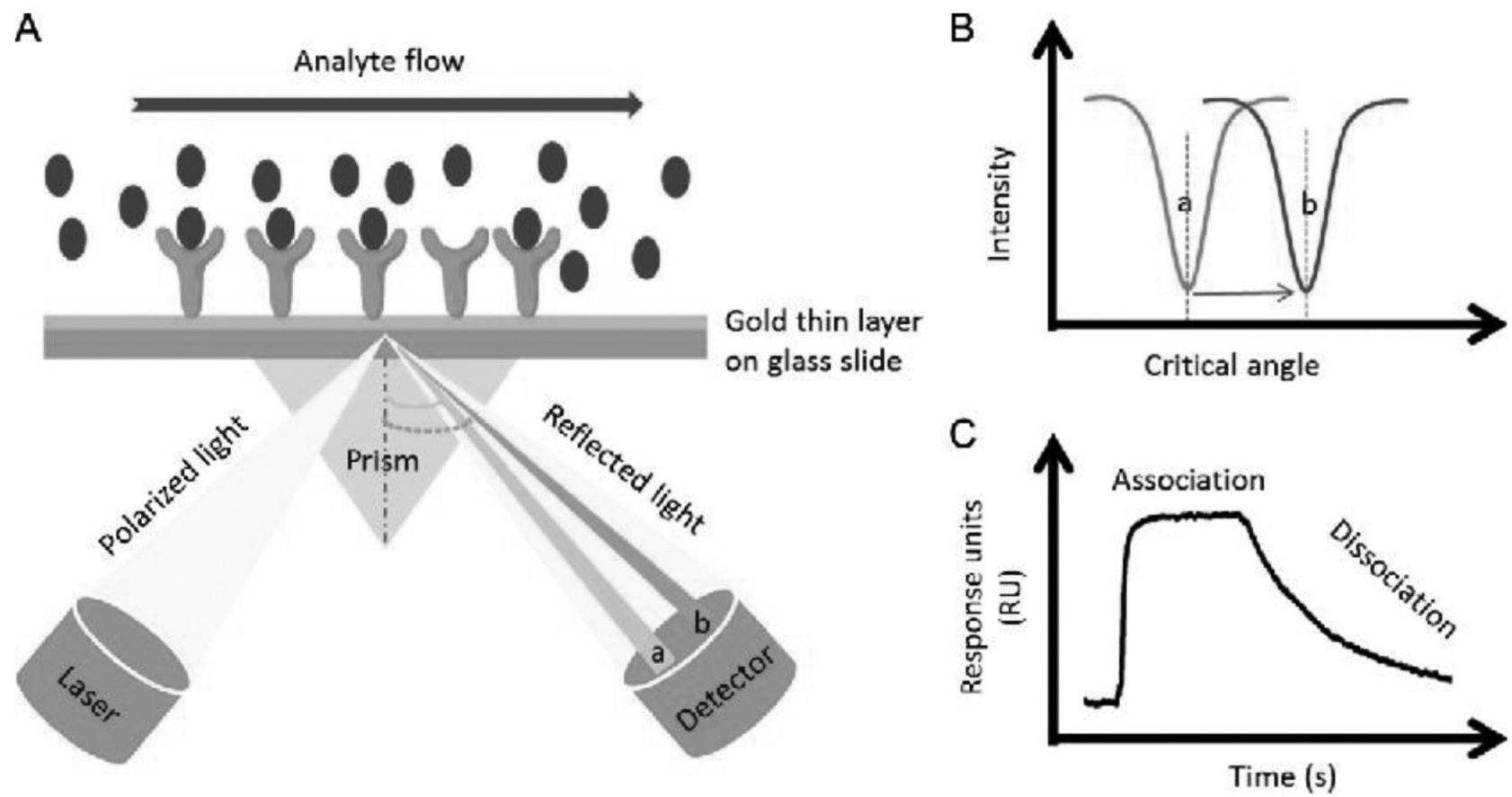
$$n_p \sin \theta = n_{ef}$$

$n_p$  is the refractive index of the coupling prism and  $\theta$  is the angle of incidence on the metal film (in the prism),  $n_{ef}$  is the effective refractive index of the dielectric medium.

If  $\theta > \theta_c$ , the entire ray reflects from the boundary, this is called **total internal reflection (TIR)**.



# SPR sensing platform



- (A) The ligand is immobilized on the sensor chip, which is composed of a gold thin layer on a glass slide.
- (B) Reflected light intensity shifts upon a critical angle change from “a” to “b,” resulting from a binding interaction event (change the chemical environment).
- (C) Light intensity shifts are transformed into sensorgrams, a plot of response units (RU) versus time

- ❖ The fully reflected beam leaks an electrical field intensity (i.e. evanescent field wave) into the low refractive index medium.
- ❖ No photons exit the reflecting surface but their electric field decreases exponentially with distance from the interface, decaying over a distance of  $\sim 1/4$  wavelength beyond the surface.
- ❖ If the lower refractive index media has a non-zero absorption coefficient, the evanescent field wave may transfer the matching photon energy to the medium.

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The incoming beam has to match its momentum to that of the plasmon

- The refractive index change ( $\Delta n_b$ ) caused by the binding of specific analyte molecules at the sensor surface can be expressed as (often referred to as the
- **de Feijter formula):**

$$\Delta n_b = \left( \frac{dn}{dc} \right)_{vol} \Delta c_b = \left( \frac{dn}{dc} \right)_{vol} \frac{\Delta \Gamma}{h}$$

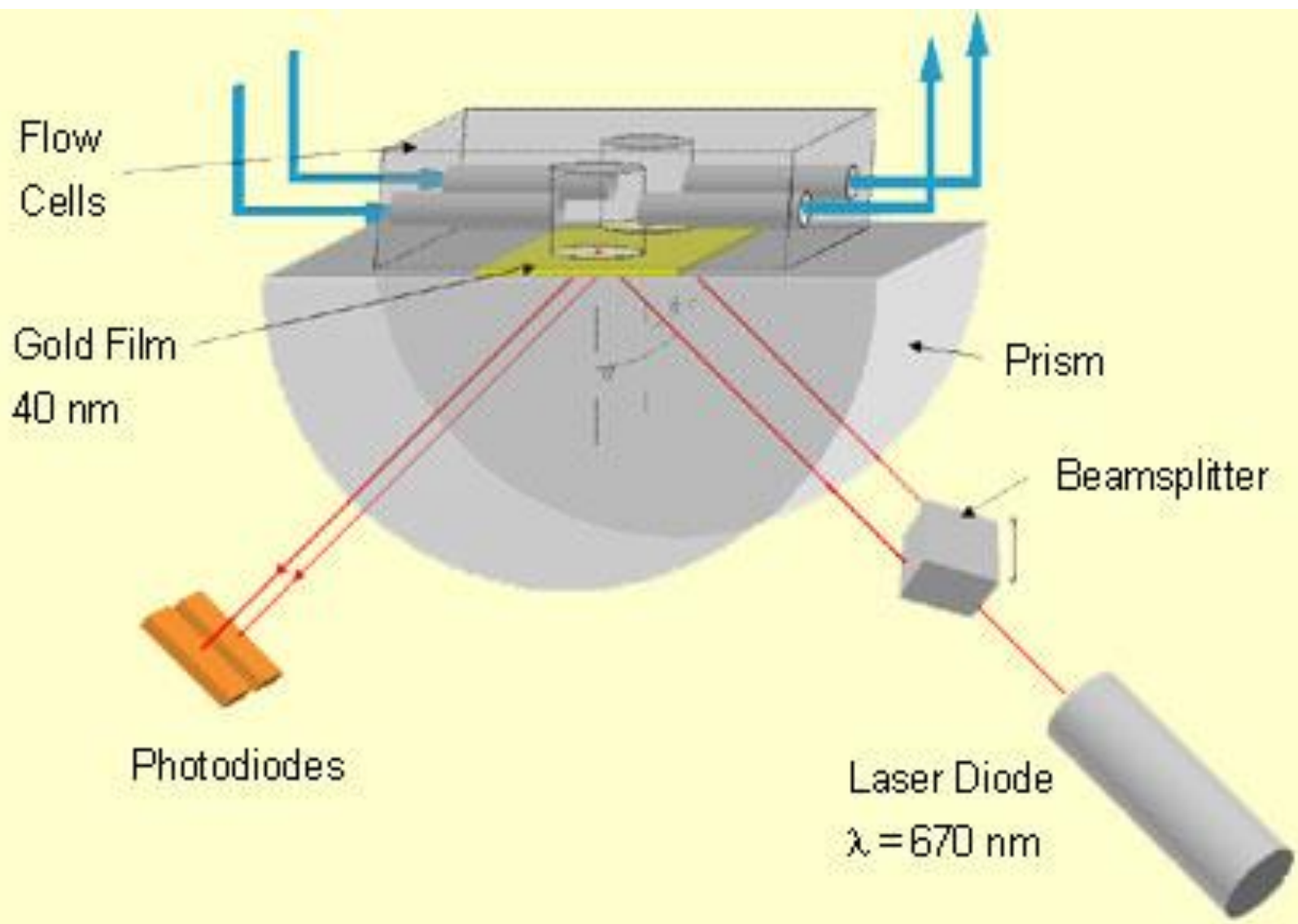
where  $(dn/dc)_{vol}$  is the refractive index increment,  $\Delta c_b$  is the wt/vol concentration of bound molecules within the sensitive layer with the thickness  $h$ , and  **$\Delta \Gamma$  is the corresponding surface concentration** (mass per surface area).

The  $(dn/dc)_{vol}$  is a well-characterized property for most of the biochemical species and ranges typically from 0.1 to 0.3 cm<sup>3</sup>g<sup>-1</sup>. A change in the effective refractive index of the surface plasmon due to the capture of analyte can be expressed as:

$$n_{ef} = K \Delta \Gamma \quad \text{where } K \text{ is a constant } (n_{ef}, \text{ change in effective refractive index})$$

SPR optical reader measures changes in a characteristic of a plasmon-coupled light wave resulting from changes in the effective refractive index

- The velocity of the surface plasmons (and hence the light energy for resonance) changes with the change in the refractive index near the metal surface.
- The refractive index in turn is greatly dependent on the chemical environment of the metal-dielectric medium.
- The binding of the analyte to the biorecognition layer over the sensor surface gives rise to a refractive index change close to the sensor surface, which can be measured by the optical reader.
- To measure the SPR angle, the **change in the intensity of the reflected light** with the angle of incidence is monitored.



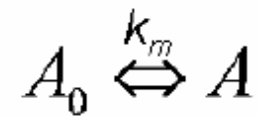
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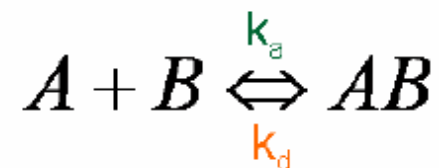


## Analyte Mass Transport



The concentration of analyte at the surface (**A**) is related to the concentration of injected analyte (**A<sub>0</sub>**) by  $k_m$ , the mass transport coefficient.

## Complex (AB) Formation



Where the forward (on) and reverse (off) rates are  $k_a$  and  $k_d$ , respectively.

Figure 4: Interpretation of Binding Response Curves.



# APPLICATIONS of SPR

**Equilibrium measurements** : The time it takes to reach equilibrium is determined primarily by the dissociation rate constant  $K_D$  or  $k_{off}$ . A useful rule of thumb is that an interaction should reach 99% of the equilibrium level within  $4.6/k_{off}$  seconds.

High affinity interactions ( $K_D < 10$  nM) usually have very slow  $k_{off}$  values and are therefore unsuitable for equilibrium analysis. Conversely, very weak interactions ( $K_D > 100$   $\mu$ M) are easily studied.

Binding constant determination: It is the equilibrium value for the product quotient and is the association rate divided by the dissociation rate.

$$A + B \rightleftharpoons C$$
$$K_{ass} = \frac{k_{on}}{k_{off}} = \frac{[C]}{[A] \times [B]} \quad K_{diss} = \frac{k_{off}}{k_{on}} = \frac{[A] \times [B]}{[C]}$$

## Evaluation of macromolecules and analysis of mutant protein

Chauhan, M., & Kumar Singh, V. (2021). Review on recent experimental SPR/LSPR based fiber optic analytical sensors. Optical Fiber Technology, 64. <https://doi.org/10.1016/j.yofte.2021.102580>

## Advantages of SPR:

- Label-free detection technique
- Distinguishes surface-bound material from bulk material
- Monitor molecular interactions in real-time (kinetics)
- Highly-sensitive (  $d_{\text{film}}$  of  $\sim 1\text{-}2\text{ \AA}$  or nanograms of adsorbed mass)
- Works in turbid or opaque samples

## Problems of SPR:

- Limited to choice of metal which results in SPR
- Sample preparation (attaching probe to metal surface) tedious.
- Non-specific interactions also results SPR signal.
- Refractive index is temperature dependent.
- Not suitable for High throughput assays, concentration assay, small analytes ( $M_r < 1000$  give very small responses).

END