BIOSENOSRS

BIO 580

Optical Biosensors

WEEK-6

Fall Semester

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Topics that will be covered in the course

- ❑ History of biosensor development, applications and requirements of biosensors and classification
- ❑ Principles of molecular recognition and transduction signal acquisition
  - ✓Sources of Biological Recognition elements – enzymes/proteins, ssDNAs, antibody and Others
  - ✓Design considerations for use of recognition elements in biosensors
  - ✓Modeling of reactions for various biosensor applications- electrochemical, optical, piezoelectric, colorimetric, fluorometric and others.
- ❑ Modification of sensor surfaces and immobilization techniques
  - ✓Covalent modification of surfaces using surface chemistry
  - ✓Self Assembled Monolayers (SAM) and adsorptions
  - ✓Other ways to immobilize biological macromolecules on various solid surfaces
- ❑ Detection methods and Physical Sensors
  - ✓Electrodes/transducers – electrochemical (amperometric, potentiometric, and conductimetric transductions)
  - ✓Other sensors - for e.g., optical sensors (colorimetric/fluorimetric/luminometric sensors), Surface Plasmon Resonance (SPR) sensors, and piezoelectric resonators.
- ❑ Fabrication of biosensors
  - ✓Miniaturization-application of nano-materials, nanoparticles, carbon nanotubes (CNTs) and others
  - ✓Biocompatibility – stability, reproducibility and repeatability of biomolecules on transducer surfaces
- ❑ Data acquisition, statistical and error analysis
  - ✓Inter and Intra-assays and Coefficient of variation (CV)
  - ✓Signal to noise ratio
  - ✓Normalization/optimization and signal retrieval
- ❑ Examples of commercial biosensors

Electromagnetic Field

- ➢ The electromagnetic field is a physical field produced by electrically charged objects.

- ➢ It affects the behavior of charged objects in the vicinity of the field.

- ➢ Light is the electromagnetic field in a certain frequency range.

- ➢ At lower frequencies the electromagnetic field may be radio waves or infrared light, while at higher frequencies it may be UV light or x-rays, among others.

- ✓ The electromagnetic field extends indefinitely throughout space and describes the electromagnetic interaction. It is one of the four fundamental forces of nature (the others are gravitation, the weak interaction, and the strong interaction).

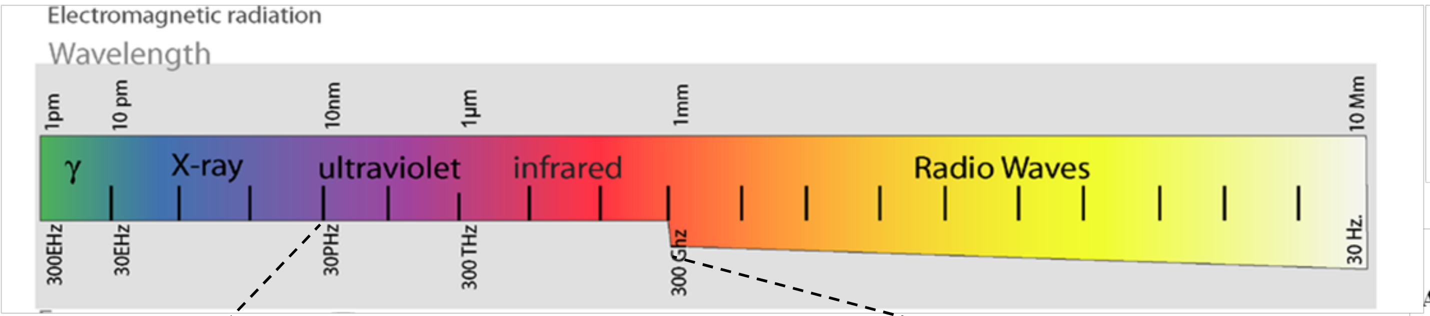
- ✓ The field propagates by electromagnetic radiation; in order of increasing energy (decreasing wavelength) electromagnetic radiation comprises: radio waves, microwaves, infrared, visible light, ultraviolet, X-rays, and gamma rays.

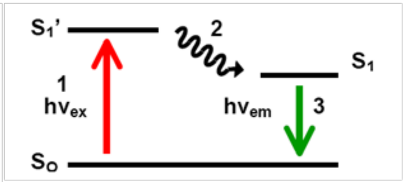
- ✓ The field can be viewed as the combination of an electric field and a magnetic field.

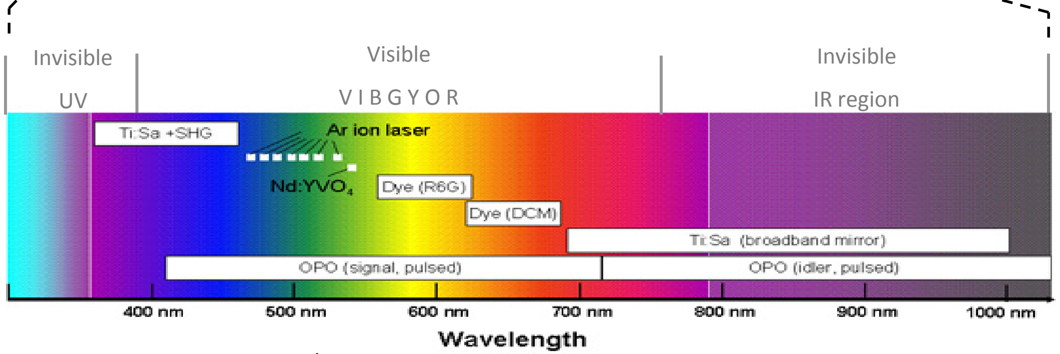
- ✓ The electric field is produced by stationary charges, and the magnetic field by moving charges (currents); these two are often described as the sources of the field.

- ✓ The way in which charges and currents interact with the electromagnetic field is described by Maxwell's equations and the Lorentz force law.

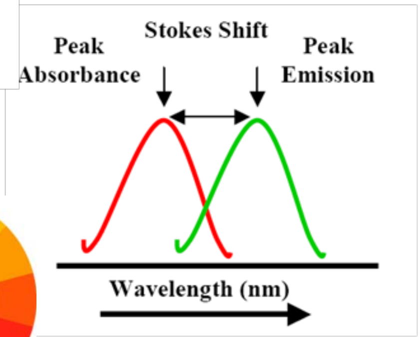
Spectrum, absorption and emission

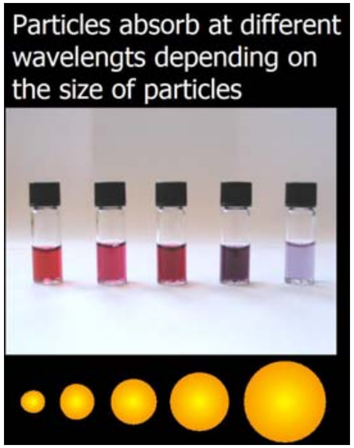


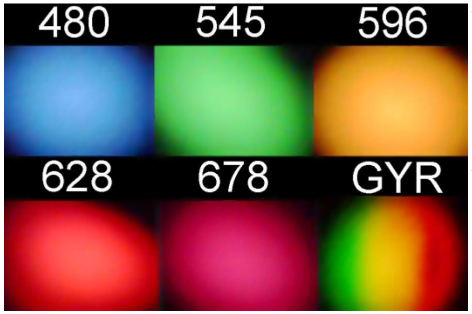




UV/VIS Spectrophotometer works in this region







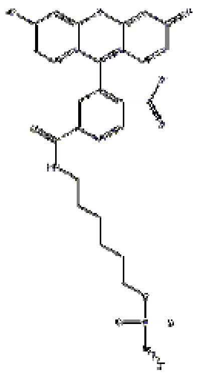
Wavelength (nm)

Color

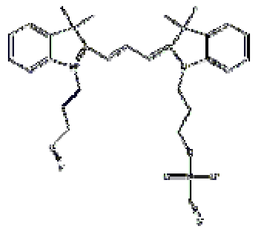
Wavelength (nm)

Color

Examples



Fluorescein (492/520)



Cy3 (552/570)

OPTICAL BIOSENSORS- an introduction

All molecules have the capability to interact with electromagnetic fields that pass through them because they contain atomic nuclei and a variety of electrons in various orbital states

Most fundamentally, electrons within molecules experience a force when they are exposed to the oscillating electromagnetic fields associated with the propagation of light. (Light is the electromagnetic field in a certain frequency range)

Molecules with an abundance of free electrons will become polarized by exposure to the light's electromagnetic field (one side of the molecule will be temporarily more negatively charged than the opposite side – resulting in the formation of an electric dipole)

The extent of the polarization may be different for any particular molecule depending on its size, shape, and orientation with respect to the electric field.

Polarizability of molecules

A constant known as the *electric susceptibility, χ_e , quantifies the extent* of a molecule's "polarizability," and molecules with greater χ_e *are more easily* polarized.

When a polarizable molecule is placed in an electric field, the induced electrical dipole produces a secondary electric field such that the resulting electric field (i.e., the sum of the originally applied field and the secondary field) is of lower magnitude than the applied field.

Because an electromagnetic field associated with light is time-varying, the electrons within the molecule will experience a time-varying force so that electrons will oscillate within the molecule.

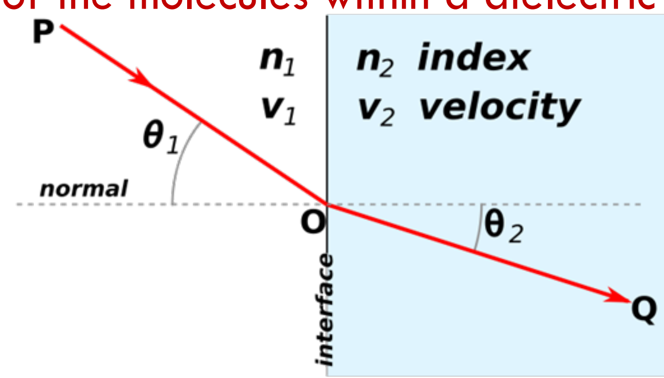
Moving electrons, by definition, produce an electrical current, so the molecule actually experiences a "polarization current" as a result of this electron motion.

The result of the polarization current is that light travels more slowly through the molecule than it would through free space.

The refractive index

Though free space has a permittivity defined by the constant ε_0, *(where $\varepsilon_0 = 8.85 \times 10^{-12}\,F/m$), the permittivity of a dielectric* material containing molecules is given by $\varepsilon = \varepsilon_r\,\varepsilon_0$, *where εr is known as the relative permittivity of the dielectric constant of the molecule.* ε_r *is directly related to* the polarizability of the molecule because it is mathematically defined as $\varepsilon_r = 1 + \chi_e$.

Many people are more familiar with the term *refractive index (n) to describe a* dielectric material. In an ordinary dielectric material at optical wavelengths, *n is* defined as $n = \sqrt{\varepsilon_r}$, *so the refractive index is directly related to the polarizability* of the molecules within a dielectric material.



[Refraction]() of light at the interface between two m
refractive indices, with $n_2 > n_1$. Since the phase vel
second medium ($v_2 < v_1$), the angle of refraction θ_2
angle of incidence θ_1; that is, the ray in the higher-
closer to the normal. (refractive index -Measure of
refraction of a beam of light on entering a denser r

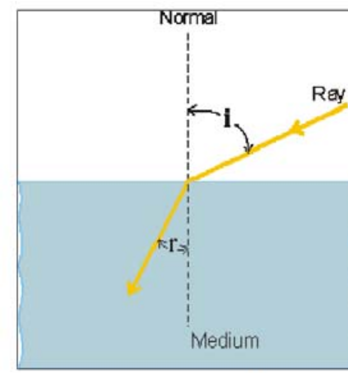
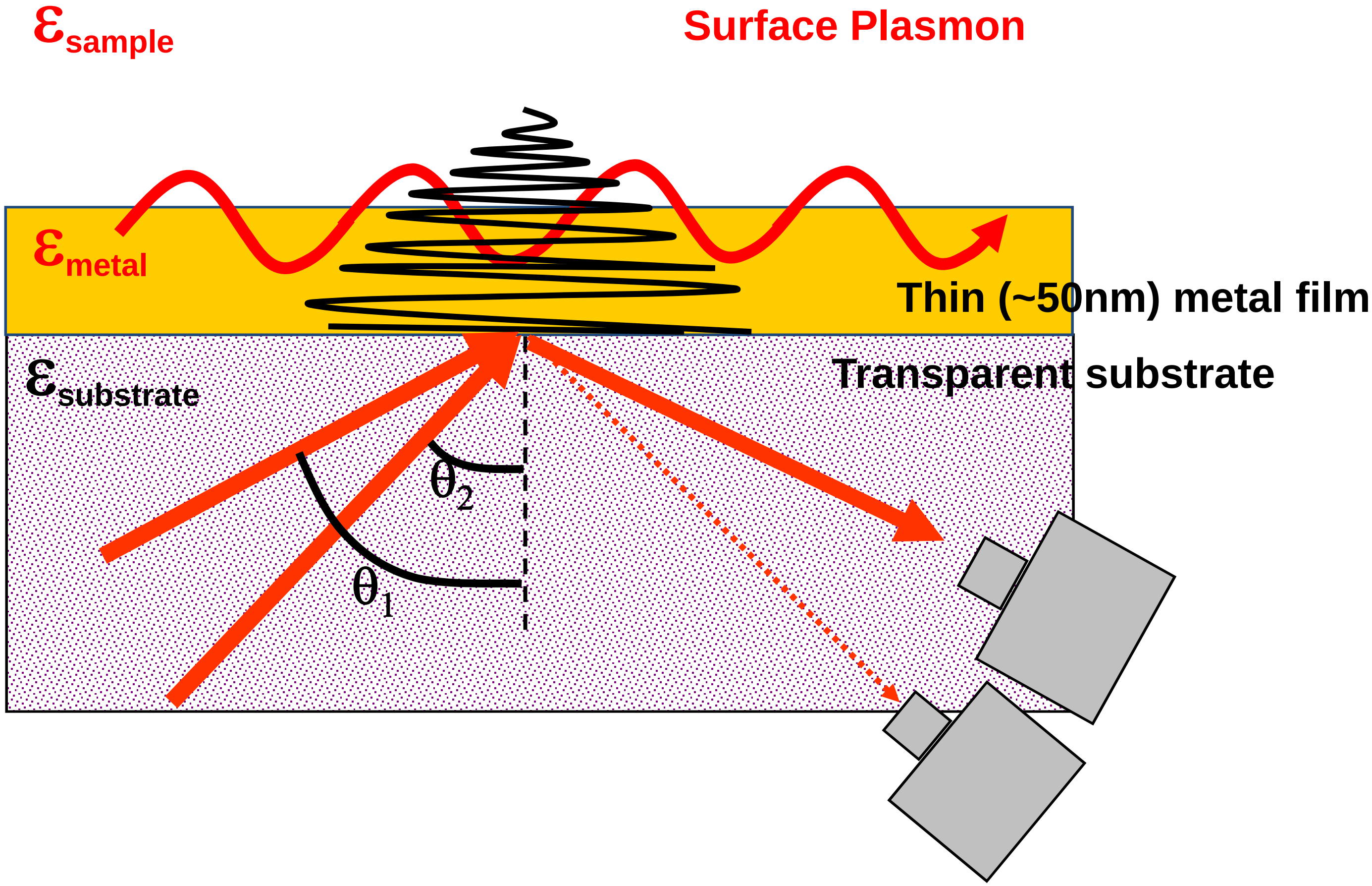


Figure 1: Refraction of light.

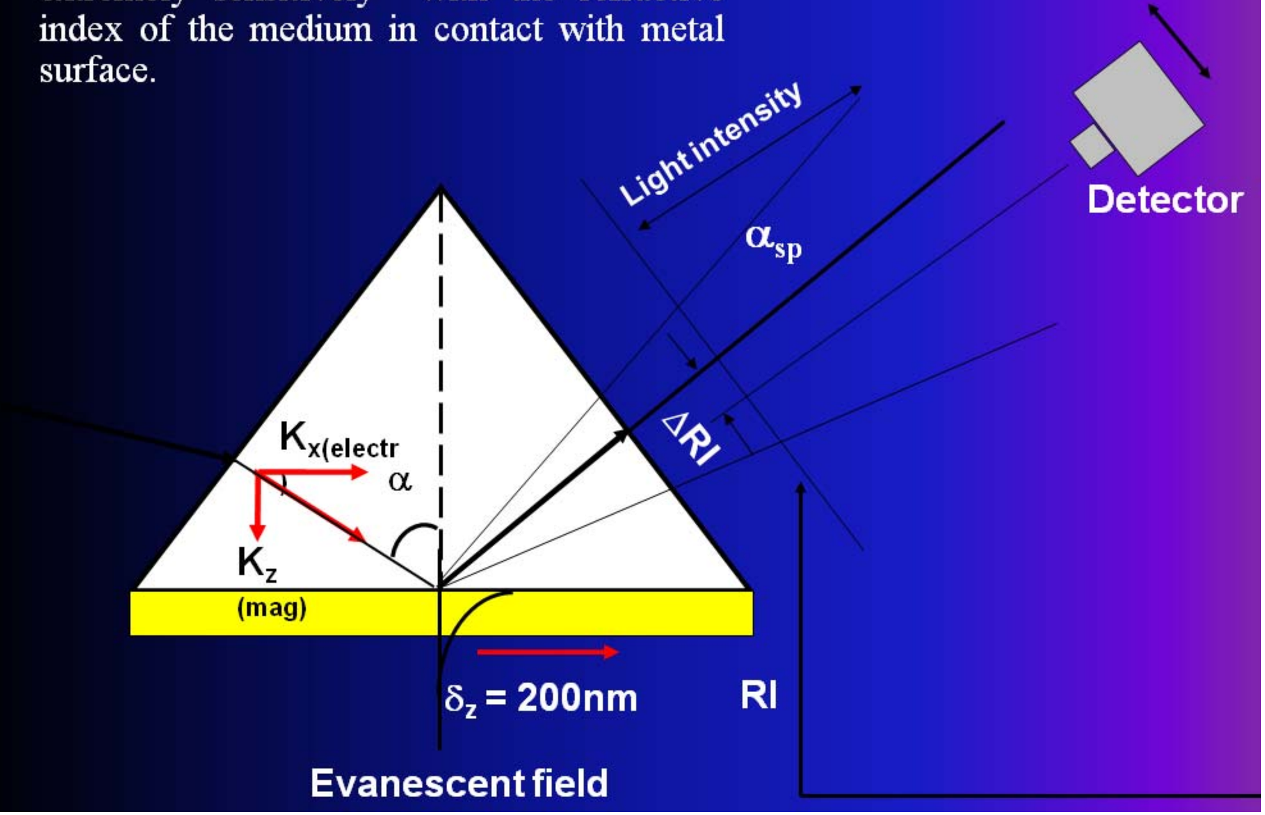
Generally, the refractive index is a quantity defined for a bulk material, while electric susceptibility and dielectric permittivity can apply to individual molecules such as those adsorbed to optical biosensor surfaces.

Principle of SPR system



Surface Plasmon Resonance

The angles at which this occurs vary extremely sensitively with the refractive index of the medium in contact with metal surface.



Optical Biosensor

The key behind optical biosensors' ability to detect biological analytes is that biological molecules, including proteins, cells, and DNA, all have dielectric permittivity greater than that of air and water.

Therefore, these materials all possess the intrinsic ability to reduce the propagation velocity of electromagnetic fields that pass through them.

Optical biosensors are designed to translate changes in the propagation speed of light through a medium that contains biological material into a quantifiable signal proportional to the amount of biological material present on the sensor surface.

In the design of optical biosensors, the detected biological material is often modeled as a thin film with a finite refractive index, although this is a simplification.

Several studies have been performed to characterize the dielectric properties of representative molecular monolayer films.

Therefore, if a biosensor transducer surface is covered with water, and if biological molecules can adsorb to the transducer surface, a small quantity of water molecules is displaced and replaced with a molecule that is more easily polarized by electromagnetic fields associated with light.

Therefore, the design goal for all optical biosensors is to provide a transducer with some externally measurable characteristic that is modified by changes in dielectric permittivity on its surface.

In this way, optical biosensors do not measure the mass of adsorbed material (as sometimes stated), although often the mass of deposited material is often related to the change in dielectric permittivity.