

# BIOSENOSRS

BIO 580

Optical Biosensors

WEEK-7

Fall Semester

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Faculty of Engineering & Natural Sciences

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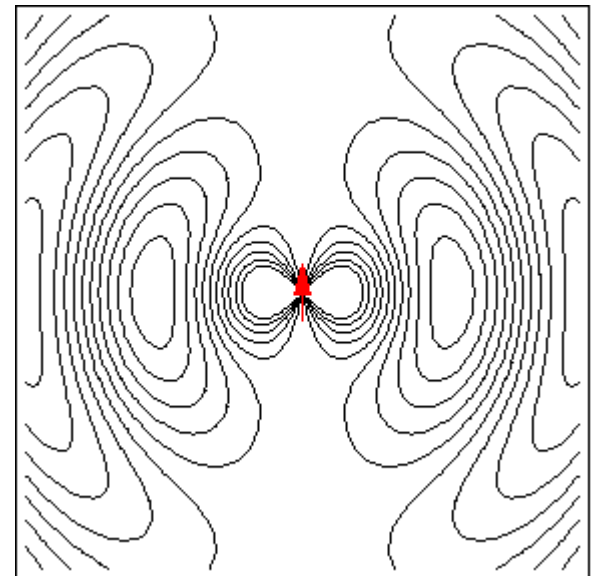
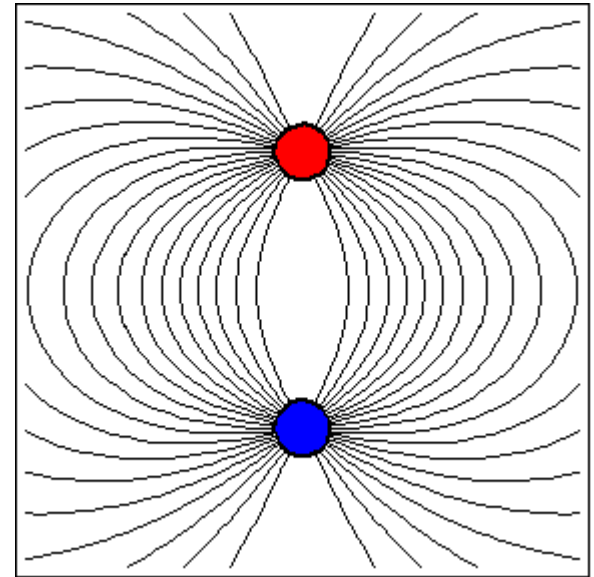


## Topics that will be covered in the course

- ❑ 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

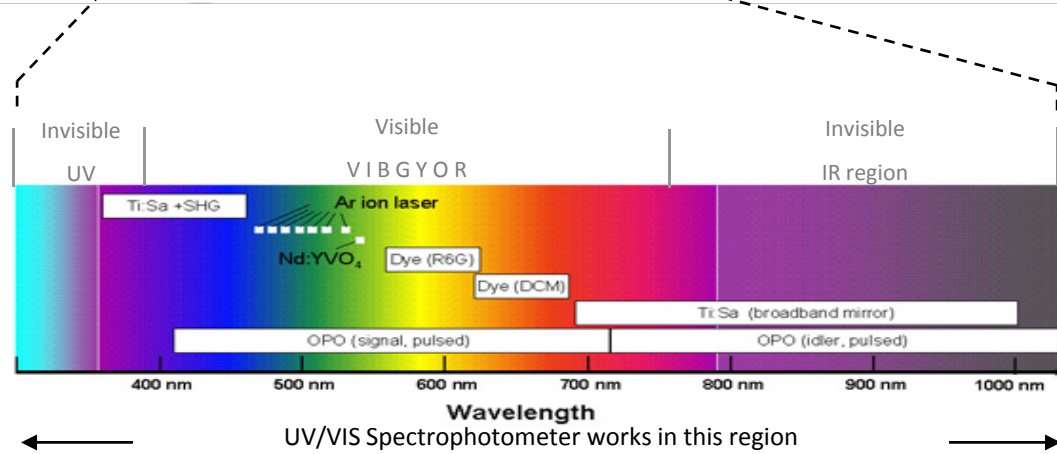
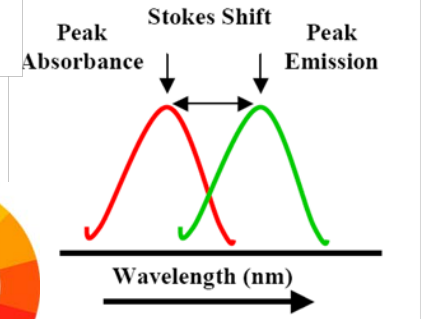
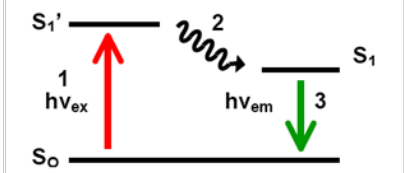
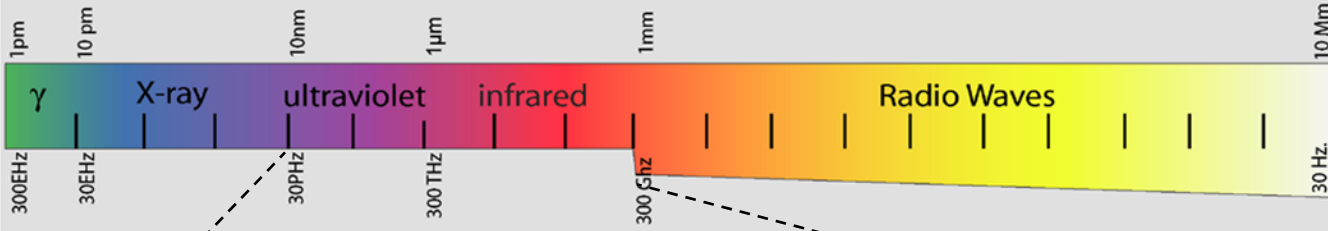
- The electromagnetic field is a physical field produced by electrically charged objects.
- Light is the electromagnetic field in a certain frequency range.
- At lower frequencies the electromagnetic field may be radio waves or infrared light, while at higher frequencies it may be UV light or x-rays, 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.



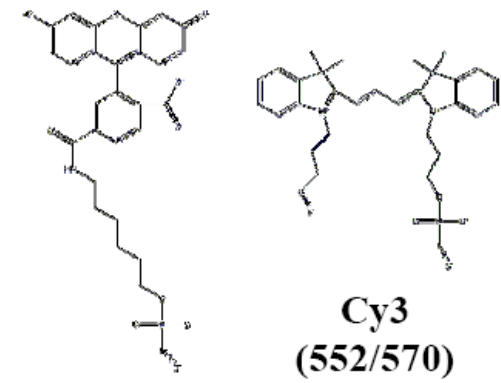
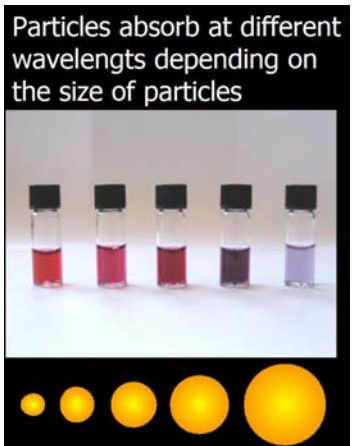
# Spectrum, absorption and emission

Electromagnetic radiation

Wavelength



## 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*,  $\chi_e$ , quantifies the extent of a molecule's "polarizability," and molecules with greater  $\chi_e$  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.

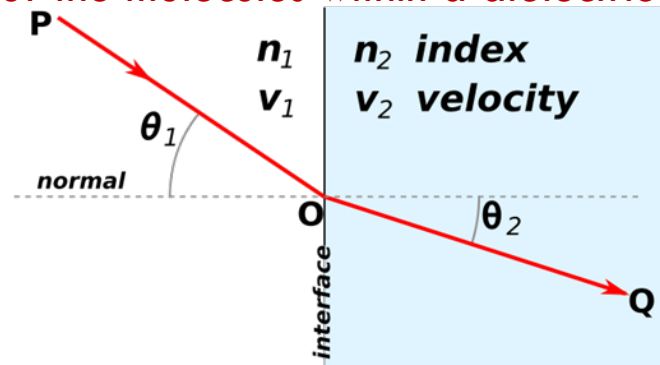
Moving electrons, by definition, produce an electrical current, so the molecule actually experiences a "polarization current" as a result of this electron motion.

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  $\epsilon_0$ , (where  $\epsilon_0 = 8.85 \times 10^{-12} \text{ F/m}$ ), the permittivity of a dielectric material containing molecules is given by  $\epsilon = \epsilon_r \epsilon_0$ , where  $\epsilon_r$  is known as the relative permittivity of the dielectric constant of the molecule.  $\epsilon_r$  is directly related to the polarizability of the molecule because it is mathematically defined as  $\epsilon_r = 1 + \chi_e$ .

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  $n = \sqrt{\epsilon_r}$ , 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 media with different refractive indices, with  $n_2 > n_1$ . Since the phase velocity in the second medium ( $v_2 < v_1$ ), the angle of refraction  $\theta_2$  is smaller than the angle of incidence  $\theta_1$ ; that is, the ray in the higher-index medium is closer to the normal. (refractive index - Measure of refraction of a beam of light on entering a denser medium)

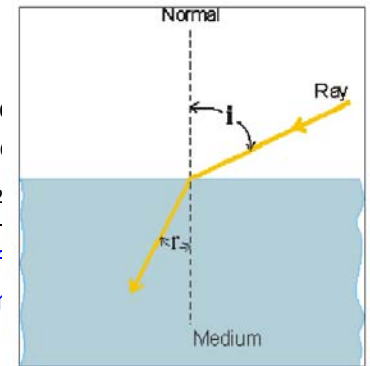


Figure 1: Refraction of light.

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.

## 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.

### **EVANESCENT-WAVE PHENOMENON**

Total internal reflection of light at a surface–solution interface produces an electromagnetic field, or evanescent wave, that extends a short distance (100–200 nm) into the solution.

SPR is an evanescent- wave phenomenon that occurs at certain metallic surfaces.