Field Guide to

Optical Biosensing

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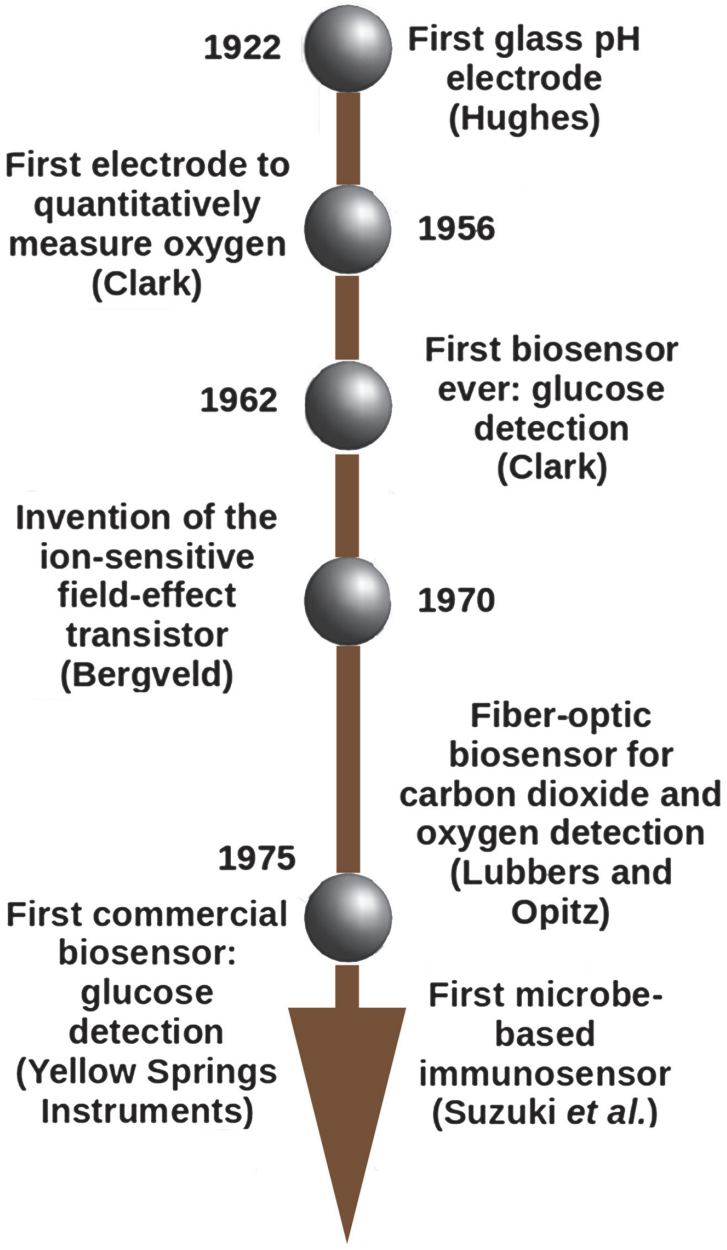
What Is a Biosensor?

A **biosensor**[1,2] is a self-contained measuring device that can detect an analyte of biochemical or biological origin and generate a quantifiable signal in response.

More specifically, a biosensor detects the analyte selectively and quantitatively using a **biorecognition** element (or **bioreceptor**), which provides specificity. A signal transduction element (or **transducer**) produces a physical signal that is generally processed by an electronic system.

Early Milestones in the Development of Biosensors

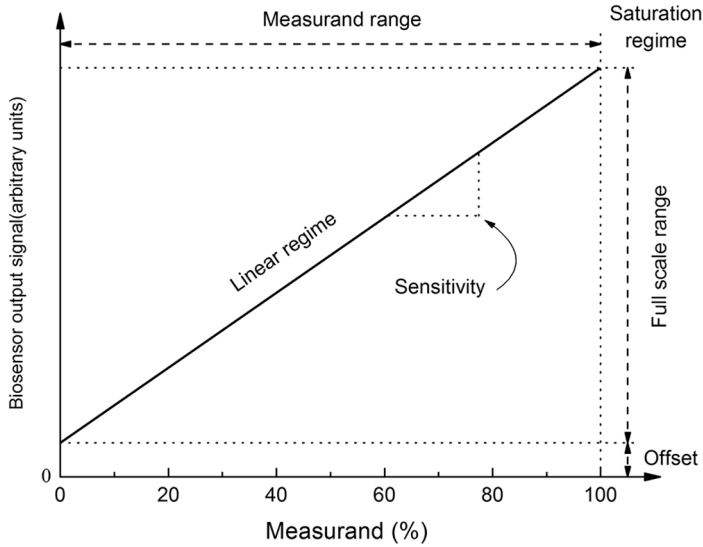


Sensitivity

The **sensitivity** of any detection device is defined as the ratio of the change in sensor output to the change in the value of the measurand. Its value is given by the slope of the calibration curve, i.e., the marginal increase in output for a marginal stimulus increase.

For a sensor in which the output y is related to the measurand x by the equation $y = f(x)$, the sensitivity $S(x_a)$ at point x_a is given by

$$S(x_a) = \left.\frac{dy}{dx}\right|_{x=x_a}$$



The sensitivity of a biosensor should be sufficiently high to allow convenient measurement of the transducer output signal with electronic instrumentation used for signal processing. Also, it is often desirable to have constant sensitivity, as in the case of the calibration curve shown here. Ideally, the sensitivity of a given biosensor should remain constant during its lifetime, although in practice this parameter experiences variation.

Transmission, Absorption, and Scattering

Transmission is the passage of electromagnetic radiation through a given material. As in the case of reflection, there is **direct transmission**, also called regular transmission, when the process follows the laws of geometrical optics, and **diffuse transmission**, which leads to scattered radiation. The overall transmission process is characterized by its **transmittance** T, which is the ratio of the energy flux reflected to the total amount of electromagnetic flux incident on the surface.

Scattering is defined as the process of deflecting a unidirectional beam into many directions. As such, it will comprise diffuse reflection, diffuse transmission, and other volume light diffusion processes. It is characterized by S, the ratio between scattered and total incident radiation.

Absorption is the transformation of radiant power to another type of energy by interaction with matter. This is usually heat, although the absorption of electromagnetic radiation can trigger the emission of radiation by the absorbent. This process is characterized by its **absorptance** A, defined as the ratio of the radiant energy absorbed by a material to that incident upon it.

Adding the contribution of the four basic optical processes gives

$$R + T + A + S = 1$$

This equation can be considered an expression of the principle of the conservation of energy. These magnitudes (T, R, A, and S) show a spectral behavior, i.e., a dependence on wavelength. As such, the previous equation is valid for every single wavelength.

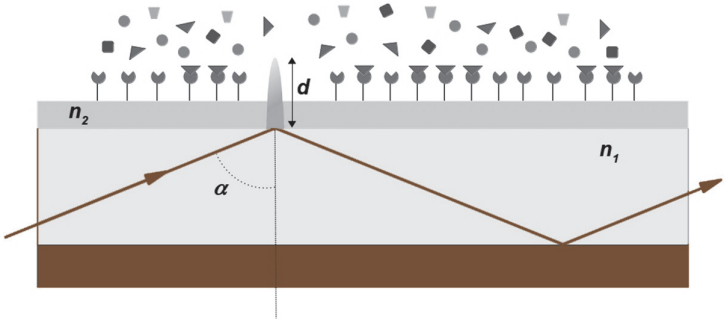
Note that reflection, transmission, and scattering are optical processes that leave the frequency of the incident radiation unchanged.

Evanescent Wave Biosensing

When light traveling through a medium is reflected at the interface with an optically-less-dense medium at an angle exceeding the critical angle, **total internal reflection (TIR)** occurs. An electromagnetic wave called an **evanescent wave (EW)** is generated close to the interface in the less dense medium. The EW travels parallel to the interface of the two media but decays exponentially in amplitude with respect to distance into the medium of lower index of refraction. The expression of the EW penetration depth d at a given wavelength λ is given by

$$d = \frac{\lambda}{2\pi\sqrt{n_1^2 \sin^2\alpha - n_2^2}}$$

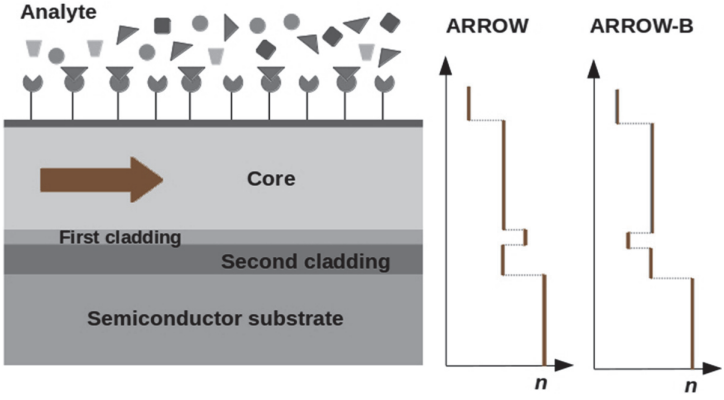
where n_1 and n_2 are the index of refraction of the two media, and α is the angle of incidence. The penetration depth increases with a low refractive index contrast, a long wavelength, and an incident angle near the critical angle.



Optical interaction of the EW with analytes close to or at the surface is used as a transduction technique. This interaction can be monitored as, for example, a change in the intensity of light emerging from the optically denser medium. This biosensing technique[39] allows the continuous monitoring of the presence of analytes with minimal interference from substances distant from the interface, i.e., at distances on the order of a light wavelength. EW biosensors allow real-time analysis.

Antiresonant Reflecting Optical Waveguides

Antiresonant reflecting optical waveguide (ARROW) architectures confine light using antiresonant Fabry–Pérot reflectors. In ARROWs, waveguiding takes place in a thick, low-refractive-index layer (such as silicon oxide) rather than in a thin, high-refractive-index layer. The indices of refraction and the thicknesses of the individual cladding layers must be properly adjusted to ensure high reflectivity for the working wavelength and, therefore, optimal guiding characteristics. In a conventional ARROW, the index of refraction of the cladding is higher than that of the core, while in ARROW-B structures, it is lower.

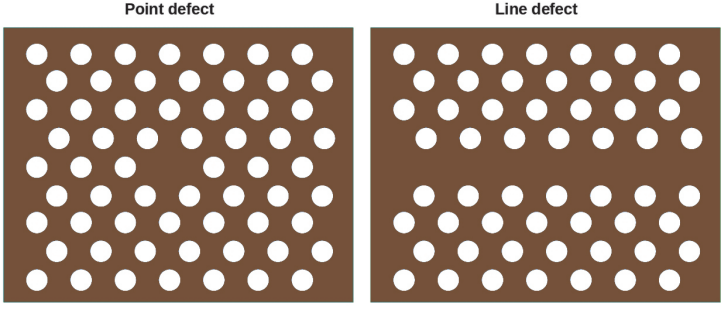


Although ARROW modes are leaky, low-loss propagation over relatively large distances (typically micrometers rather than nanometers) can be achieved. Besides, ARROWs exhibit good sensitivity.

ARROWs may be either symmetrical or asymmetrical. In the first case, light confinement at both boundaries is achieved by antiresonant reflectors, while in the latter case light is confined by an antiresonant reflector at one boundary and total internal reflection at the other. Asymmetrical ARROWs are preferred for biosensing since the TIR boundary can be placed between the bioreceptor and the analyte.

Photonic Crystal Cavity Biosensors

Introducing disturbances to the periodic structure of a **photonic crystal**, i.e., defects, can create specific modes within the band gap at well-defined wavelengths. As such, an incoming light beam with a wavelength corresponding to the defect mode will be confined to the defect area of the photonic crystal. Since the spectral position of the resonant defect mode is highly sensitive to any changes in the local environment close to the defect region, i.e., to variations of the effective index of refraction, these devices can be used as biosensors. Defects can be realized by introducing point or line defects in the structure of the photonic crystal, for instance, by eliminating a single hole or a row of holes, or by substituting a hole with another one with a different radius or index of refraction.



Photonic crystal cavities[53] exhibit strong spatial and temporal light confinement, in addition to a long photon lifetime, i.e., high quality factor Q. Accordingly, these devices greatly enhance the interaction strength between the optical field and the defect region. These characteristics make photonic crystal cavities suitable for multiplexing and for very localized sensing. From a practical point of view, a high Q translates into a low limit of detection, while the strong spatial confinement translates into very small sensing areas and thus the need for less analyte and the possibility for very high miniaturization degrees.

Infections Caused by Pathogens[60]

Type of Pathogen	Disease
Virus	Influenza (the flu) Common cold Coronavirus disease (COVID-19) Measles Chickenpox (varicella) Shingles (herpes zoster) Viral gastroenteritis Meningitis HIV and AIDS Hepatitis A, B, C, D, and E Yellow fever Warts, including genital warts Dengue fever Oral and genital herpes
Bacteria	Bacterial gastroenteritis (e.g., salmonella food poisoning or *E. coli* infection) Tuberculosis Strep throat Bacterial meningitis Urinary tract infection Cellulitis Lyme disease Gonorrhea
Fungus	Oral candidiasis (oral thrush) Dermatophytosis (ringworm) Vaginal yeast infection *Tinea pedis* (athlete's foot) Onychomycosis (fungal infection of the nail) *Tinea cruris* (jock itch)
Parasites	Malaria Head lice infestation Giardiasis Toxoplasmosis Trichomoniasis Intestinal worms

Important Biological Warfare Agents[70]

Biological Agent	Type	Disease
Variola major	Virus	Smallpox
Marburg	Virus	Marburg hemorrhagic fever
Lassa	Virus	Lassa hemorrhagic fever
Machupo	Virus	Bolivian hemorrhagic fever
Bacillus anthracis	Bacterium	Anthrax
Francisella tularensis	Bacterium	Tularemia
Clostridium botulinum, including its toxins	Bacterium producing botulinum toxin	Poisoning by toxin
Ricin	Toxin from the plant *Ricinus communis*	Poisoning by ricin
Burkholderia mallei	Bacterium	Glanders
Burkholderia pseudomallei	Bacterium	Melioidosis
Brucella melitensis	Bacterium	Brucellosis
Chlamydia psittaci	Bacterium	Chlamydiosis
Escherichia coli O157:H7, including its shiga toxins	Bacterium	Foodborne illness, poisoning by shiga toxin
Rickettsia prowazekii	Bacterium	Typhus
Vibrio cholerae	Bacterium	Cholera
Staphylococcus aureus, including its toxins	Bacterium producing a group of staphylococcal enterotoxins	Staphylococcal infections, poisoning by staphylococcal enterotoxins

Wearable and Implantable Biosensors

Wearable biosensors[75,76] are minimally or non-invasive sensing devices that are integrated into various wearables, such as clothes, watches, glasses, bandages, contact lenses, rings, and tattoos, or even into the skin. These devices are receiving growing attention given their enormous potential to measure and track a broad variety of physiological parameters, including blood pressure, heart rate, body temperature, and respiration rate.

Wearable biosensors can perform real-time measurements of biochemical markers in biological fluids, such as sweat, saliva, tears, and interstitial fluid. Tracking these biochemical compounds (e.g., antigens, antibodies, abnormal enzymes, or hormones) will provide early warning of many abnormal health conditions.

These devices provide improved comfort, in a great deal due to the tremendous advance in the micro- and nanofabrication techniques. In fact, aiming at improving wearability and ease of operation, multiplexed biosensing, microfluidic sampling and transport systems have been integrated, miniaturized and combined with flexible materials. Besides, with the universalization of smartphones and other mobile microelectronic devices,[77] wearable and implantable biosensors are receiving renewed attention owing to the possibility of developing apps to monitor the data measured by the sensor. These devices have become widely accepted by the public. It is expected that wearable and implantable optical biosensor technologies will have a broad impact on our daily lives in the next years.

An implantable fluorescence-based sensor that allows the continuous response to blood glucose concentration changes for up to 140 days has been demonstrated. The implanted fiber transmits fluorescent signals transdermally, according to blood glucose concentration. A system has been developed for the direct, *in vivo* observation of glucose Raman peaks to determine the glucose concentration in blood.