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Review

Evanescent wave fluorescence biosensors

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Abstract

Since discovery and first use in the mid-1970s, evanescent wave fluorescence biosensors have developed into a diverse range of instruments, each designed to meet a particular detection need. In this review, we provide a brief synopsis of what evanescent wave fluorescence biosensors are, how they work, and how they are used. In addition, we have summarized the important patents that have impacted the evolution from laboratory curiosities to fully automated commercial products. Finally, we address the critical issues that evanescent wave fluorescence biosensors will face in the coming years.

Keywords: Fluorescence; Biosensors; Evanescent; Planar waveguide; Fiber optic

Contents

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1. Introduction

Biosensors can be defined as opto/electronic detection devices that use biological molecules for detection and quantification of targets of interest. Distinct from single-use devices such as standard pregnancy tests or microtiter plate assays, biosensors have the additional requirement that they can be reused or regenerated for subsequent analyses.

The heart of the biosensor is the biological recognition element, which is chosen for its specificity and affinity, and can be an enzyme, receptor, antibody, chelator, nucleic acid, or antibiotic. For use in any optical sensor, the end result must be a change in an optical property induced by interaction of the recognition element with the target; these changes may be due to the formation of a fluorescent or luminescent product, association of molecules to fluoresce or to quench fluorescence, or modification of refractive index or absorption spectrum. For purposes of this article, we will discuss only those systems utilizing fluorescence, fluorescence quenching, or fluorescence resonance energy transfer (FRET) for signal generation upon evanescent wave excitation.

Well known now, evanescent wave excitation was first described in 1965 (Hirschfeld, 1965). Kronick and Little (1975) were the first to make use of evanescent wave excitation for a fluorescence immunoassay. Based on the principle of total internal reflectance, light launched into a waveguide placed into a dielectric medium of lower refractive index ($n_{\text{waveguide}} > n_{\text{medium}}$) will reflect all the light within the waveguide when the angle of incidence of light entering the waveguide, θ, is greater than the critical angle, θ_c, as defined by:

$$\theta_c = \sin^{-1}\left(\frac{n_{\text{medium}}}{n_{\text{waveguide}}}\right).$$

Under conditions of total internal reflectance, the Fresnel transmission coefficients for the transverse electric wave and the transverse magnetic wave are non-zero. This means that, although the light energy is totally reflected, an electromagnetic field extends out from the interface into the lower index medium. This field, the evanescent wave, decays exponentially with distance from the surface, generally over the distance of 100 nm to approximately a wavelength. For multimode waveguides, the penetration depth d_p, the distance from the surface at which the strength of this field is $1/e$ of its value at the surface, is a function of the two refractive indices, the angle of incidence of the light, and the wavelength:

$$d_p = \frac{1}{4\pi}[(n_{\text{waveguide}})^2 \sin\theta - (n_{\text{medium}})^2]^{1/2}.$$

The exponential decay of field strength essentially confines transducible optical signals to within a discrete distance from the waveguide's surface, minimizing optical interference or contribution from components in the lower index medium.

The surface-selectivity of the evanescent wave has been exploited by a number of biosensor types including: resonant mirrors, interferometers, surface plasmon resonance sensors, and fiber optic and planar array fluorescence sensors. All these sensors can measure surface-specific binding events in real time. Waveguides can be made of materials that both have suitable optical properties and are easily modified for attachment of recognition molecules. Sensor design is adaptable owing to the wide variety of visible and near IR light sources and detectors. Additionally, the systems described in detail here, fiber optic and planar array fluorescence sensors that utilize the evanescent wave for excitation of fluorescently tagged reporters, gain improved discrimination of specific binding from non-specific adsorption of sample components. Surface-selectivity of evanescent wave sensors, however, can prove a double-edged sword. Since the evanescent field interrogates only surface events, interactions occurring outside of the evanescent field are not significantly detectable. This can make analyzing large targets such as intact cells problematic. Furthermore, below a critical flow rate, mass transport may limit binding of target to immobilized recognition molecules.

The remainder of this article describes the means, methods, and materials by which this physical principle has been exploited for sensor development. Additionally, many of the critical inventions that have been made along this ongoing journey are recounted as well.

2. Materials

Materials for use in fiber optic and planar array evanescent wave sensors must satisfy several criteria: first, they must be transparent to the wavelengths used; second, they must be free from impurities that affect refractive indices or cause scattering; and, for fiber optic sensors, the physical characteristics must be such that they can be pulled into fibers. Glasses are the most widely used, due to their low cost and wide range of optical properties available through use of dopants. While fluoride and chalcogenide glasses are useful for applications in the infrared range, silica is the most commonly used. Fused silica waveguides are optically transparent over a wide range of wavelengths, from the ultraviolet range to the near-infrared range, and are chemically resistant to most biological buffers. Silica has a refractive index of approximately 1.457 ($\lambda = 633$ nm), but the refractive index can be modified by addition of dopants, such as GeO_2, P_2O_5, TiO_2, and Al_2O_3, which increase the refractive index, and B_2O_3 and F, which decrease the refractive index. Although fused silica media may exhibit fluorescence at lower wavelengths (Ligler et al., 1995; Zubia and Lomer, 2002), careful choice of light sources and fluorophores can minimize problems due to autofluorescence.

Typically, evanescent wave sensors using planar waveguides collect fluorescence emission perpendicular to the waveguide whereas most, but not all, fiber optic biosensors of this type collect the emitted light out the end of the fiber. The waveguide parameter of the optical substrate, or *V*-number, is an important factor in both evanescent excitation and coupling of the fluorescence emission back into the substrate if