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Evanescent wave fluorescence biosensors: Advances of the last decade

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ABSTRACT

Biosensor development has been a highly dynamic field of research and has progressed rapidly over the past two decades. The advances have accompanied the breakthroughs in molecular biology, nanomaterial sciences, and most importantly computers and electronics. The subfield of evanescent wave fluorescence biosensors has also matured dramatically during this time. Fundamentally, this review builds on our earlier 2005 review. While a brief mention of seminal early work will be included, this current review will focus on new technological developments as well as technology commercialized in just the last decade. Evanescent wave biosensors have found a wide array applications ranging from clinical diagnostics to biodefense to food testing; advances in those applications and more are described herein.

1. Introduction

Since the theory and practice of fluorescence-based biosensors that exploit the surface sensitive nature of the evanescent wave first appeared in the mid-1970's, the types of evanescent wave biosensors and the applications for which those biosensors have been utilized has exploded. In our first review (Taitt et al., 2005), we covered the history, theory and practice of evanescent wave biosensors up to that date. Now, some 10 years later, the time has come to provide an update on the field and describe the highlights of the last decade.

Biosensors encompass the entire range of instruments which utilize a biological component for the target recognition/capture step. For this review we are limiting ourselves to only those instruments where that biological recognition step and subsequent signal transduction of the binding event occurs within the confines of an evanescent wave. The evanescent wave is remarkable phenomena, the details of which are often poorly understood – even by those responsible for assay development on the biosensor systems.

The evanescent wave, first described by Hirschfeld (1966) arises from the manner in which light behaves when confined in an optical waveguide. Guided light is totally internally reflected when it meets the interface of the waveguide and a surrounding medium with a lower index of refraction. For 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 ($n_{waveguide}$ and, n_{medium}), the angle of incidence of the light, and the wavelength (λ). Looking at these parameters, one can appreciate the key to generating a strong evanescent field is the angle of incidence of the light at the interface. One can easily show that nearly all the power in the evanescent wave comes from light that contacts the interface at an angle just above that required to be become leaky, an important consideration during the instrument design phase (Anderson et al., 1994).

Since the evanescent wave is such a near-surface phenomena, biosensors which employ evanescent wave excitation to generate the fluorescent signal are by their nature surface-sensitive measurements, meaning that only fluorescent molecules near the surface are excited. This geometric limitation can help minimize unwanted background signal from a bulk sample while enhancing just the signal from fluorophores captured on the surface. What has been most remarkable is the profuse variety of biosensors that have been developed based on the same fundamental scientific principle.

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2. Fast forward to the present

The first biosensors to take advantage of technology evolving from the optical communications community relied on optical fibers and relatively large lasers. Since then, lasers have gotten much smaller, cheaper, and so easy to use that they are found in consumer goods. In many cases, they have been replaced by light emitting diodes (LEDs) and even polymer LEDs. Concurrent advances in waveguide materials have ushered in a transition from silica fibers to planar waveguides. The expansion from silicon and silica materials to polymers has not only decreased the cost and facilitated manufacture, but also opened the doors to a much wider variety of geometries for waveguiding and signal detection. This geometric flexibility is leading to integrated, automated biosensors where the sample processing, excitation and fluorescence signal collection can all occur on a single substrate – which could even be disposable.

The fiber optic biosensors of today have evolved significantly from the initial devices that relied on long, partially clad, tapered silica fibers (Thompson and Villarruel, 1991) or unclad rods of glass with diameters large enough for coupling light easily into the end (Hirschfeld and Block, 1985). The molecular recognition elements are still attached to the fiber surface and the fluorescence is excited with evanescent light – and the advantages of tapering the fiber to improve the delivery of light to the surface are well appreciated. However, fibers used by most groups have evolved into small, molded polystyrene disposable probes with the in-coupling lens integrated with the fiber. Research International in particular (www.resrchintl.com, Monroe, WA USA) currently sells two fully automated systems with the same fiber probes: the RAPTOR, which requires manual sample introduction, and the BioHawk, which is integrated with an air sampler in a back pack for use by first responders and the military (Anderson and McCrae, 2007; Saaski, 2009). The RAPTOR has four probes per cassette with an off-chip reservoir of fluorescent reagents (Jung et al., 2003), while the BioHawk has eight probes contained with reagents in a reusable cassette to test for 8 different targets simultaneously. Separating the reagents according to the specific assay on the probe has the benefit of eliminating the problem of crossreacting antibodies as cocktails of tracer reagents become more complex in more highly multiplexed assays. The other difference is that the signal in the RAPTOR is collected back up the fiber and through a ball lens into the detector while the BioHawk collects the signal normal to the fiber probe (Fig. 1). In a recent modification of the Research International probes, Ton et al. (2015) demonstrated a "reagentless" assay format, coating the probes with a fluorophore-containing molecularly imprinted polymer; the researchers detected a small molecule target in the low nM range without the addition of fluorescent reagents during the assay.

The use of short glass rods as waveguides took a very different evolutionary turn. Ligler, Feldstein et al. (2000) realized that a glass capillary could serve as a waveguide while the sensing molecules were attached to the inner wall and the lumen used to deliver sample and reagents. This system had three other advantages: 1. The excitation could be provided easily from the side, either at a critical angle or normal to the capillary. 2. The emission signal could be integrated as it guided down the capillary/waveguide into a simple photodiode. The geometric integration of the signal without concomitant increase in stray excitation light enhanced the signal-to-noise ratio significantly. 3. The capillaries could be bundled and easily used in parallel with a single light source. This unique geometry has been used by two other groups to develop biosensor products, Creatv Microtech (www.creatvmicrotec.com, Potomac, MD, USA) and an Italian consortium funded by the European Union CAREMAN project. Creatv's Signalyte™-II uses a single fused-silica capillary waveguide to harvest target from relatively large volumes and achieve sensitivities 1000 times that of a fluorescent plate reader. Samples tested include clinical fluids, food, water and environmental samples. In concert with immunomagnetic separation and antibody-coated capillaries, the Signalyte™-II system achieved a detection level of 10 *Escherichia coli* cells per mL in water and beef homogenate (Zhu et al., 2011). The Italian consortium led by Francesco Baldini has produced a polymethylmethacrylate (PMMA) chip with parallel channels and multiple waveguides in the roof (Fig. 2). Flood illumination from an LED provides the excitation light normal to the waveguides and

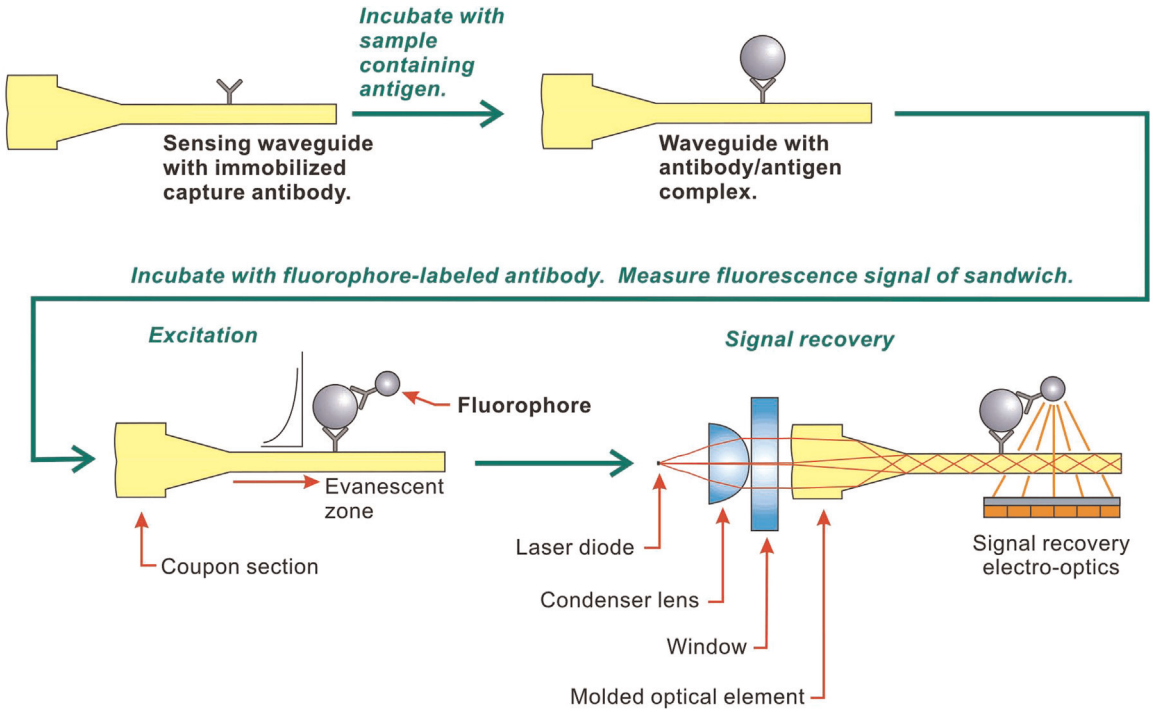

Fig. 1. Fiber probe waveguide and biosensing scheme used in the BioHawk (Saaski, 2009). Reprinted with permission from SPIE and the author.

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