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Current focus

Biosensor technologies for detecting microbiological foodborne hazards

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Abstract

The convergence of molecular biology and miniaturized instrumentation has accelerated development of biosensors with the specifications necessary to support pathogen reduction and quality programs in the food supply. Advances in optoelectronics, thin layer deposition, and microfabrication have provided many options for achieving microbiological detection goals. Some promising technologies are reviewed.

Keywords: Biosensing techniques; Pathogen; Biotechnology; Food microbiology

1. Introduction

1.1. Problems in pathogen detection

Microbiological hazards can enter foods at any point during production, processing, transport, retailing, domestic storage or meal preparation. All of these areas undergo continual change, creating dynamic and highly complex environments in which our microbiological adversaries can elude detection and inactivation. Despite greater biological understanding and technological ingenuity, challenges continually arise in the form of familiar pathogens in new foods and as emerging pathogens in traditional foods. Efficient international distribution systems and rapid changes in consumer preferences can facilitate the swift penetration of pathogens through large populations, greatly shortening the reaction time available to public health agencies. Opportunities for intervention strategies diminish further with increasing consumer demands for raw and minimally processed foods, leading to additional needs for environmental surveillance and biosecurity.

1.2. Deployment of detection systems

Prevention of foodborne illness is increasingly dependent on the conscious application of hygienic practices throughout the food supply [1]. Systematic programs such as good manufacturing practices, hazard analysis, critical control point, good agricultural practices, and the food code represent approaches that significantly reduce pathogens in food animal production, aquaculture, arable agriculture, transportation, food-processing, effluent management, and retail settings. Activities indirectly contacting foods have also received scrutiny, as foodborne disease is increasingly traced to lax or unenforced environmental standards [2]. Traditional techniques of microbiological culture, isolation, and identification in epidemiological traceback, research, and clinical studies have played essential roles when major errors or process breakdowns have resulted in foodborne disease outbreaks. Although culture methods can provide great sensitivity and specificity, the efficacy of detection based on batch analysis is greatly constrained by the statistical limitations of sampling. In addition, the complexity and variety of food matrices, and the immense scale of the global food trade limit the deployment options for expensive batch-based systems which require any level of expertise [3].

1.3. Role for new technology in pathogen detection

Well-characterized processes which maintain consistently high levels of pathogen inactivation yield safe, microbiologically defined products. However, current trends toward minimal processing of foods minimize or eliminate pathogen reduction points and thereby impose additional performance requirements on pathogen detection methods. A vast number of rapid methods have been developed for clinical applications, and in many cases protocols have been adapted for foodborne hazards. Improvements continue in

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the rapidity and precision of enzyme-based amplification systems, and several protocols can resolve very low numbers of pathogens in well under 30 min. Minimizing the statistical limitations of sampling by developing continuous surveillance systems represents a more powerful and practical approach to ensuring pathogen inactivation than the alternative of end-product testing. However, in addition to exquisite sensitivity, specificity and continuous operation, efficient surveillance requires performance criteria including the prospect of remote, real-time pathogen detection at an acceptable unit cost [4–6]. Consumer demands, improving technology, and concerns over emerging microbial hazards will provide the drivers for improved pathogen detection [7].

2. Biosensors in food safety

Biosensors are defined as analytical devices which combine biospecific recognition systems with physical or electrochemical signaling. Biosensors have been used for many years to provide process control data in the pharmaceutical, fermentation, and food-processing industries [8]. The architecture of a generic system can be viewed as comprising three components: the biospecific interaction, the signal emitted when the target is bound, and the platform, which transduces the binding reaction into a machine-readable output signal. Significant progress has been made in this last component by developing platforms which exploit recent technological advances in microfabrication, optoelectronics and electromechanical nanotechnology. The interfaces between the biological and transducer modules are now routinely deposited on the nanometer scale, and dramatic improvements in device designs facilitated by new tools and instrumentation have increased biosensor sensitivities by orders of magnitude. New platforms can be arrayed in panels for the detection of numerous hazards, greatly simplifying method validation and reducing costs. Recently, these new concepts have begun to yield biosensors aimed at addressing pathogen detection issues in the modern global food industry, e.g. in poultry [9] and dairy [10]. Several approaches to biosensor development are described in the following, though the rapid pace of change precludes a comprehensive inventory. It remains to be seen, however, which, if any, technology can demonstrate its promised performance in independently corroborated trials.

2.1. Biosensor architecture: providing specificity

Biosensors obtain their specificity from a biological binding reaction which may theoretically be derived from any of a wide range of interactions, specificity, and affinities [11], including antigen/antibody (Ag/Ab), enzyme/substrate/cofactor, receptor/ligand, energy transducer systems (e.g. photosystem reaction centers, redox components, and ion channels), synthetic chemical interactions, and nucleic acid hybridization.

2.1.1. Ag/Ab recognition

The Ag/Ab reaction has been used for almost a century and a half to identify specific interactions through either conformational or mass changes [12]. Advantages of Ag/Ab recognition systems include chemical stability, variable affinity from low (optimizing regeneration) to high (optimizing sensitivity), variable specificity from multiple (e.g. mixed antibodies responding to a group of hazards) to unispecific (e.g. monoclonal antibodies targeting specific epitopes), well-understood dynamics and low cost. Ag/Ab reactions can be engineered, and are highly compatible with well-understood conjugation chemistries. A panoply of labels and conjugation schemes developed over decades of work with enzyme-linked immuno-sorbent assay (ELISA) and western blots has provided many effective techniques to provide additional enzymatic amplification and enhance sensitivity, or emit a signal compatible with a convenient transducer. Examples include conjugates exploiting biotin/streptavidin, enzymes with chromogenic substrates, fluorescence (emission, quenching, polarization or energy transfer), H+ generation (detectable through a redox electrode) or luminescence [13]. In all these cases there are highly compatible optoelectronic, electrochemical, or mass-based signal transduction interfaces which can operate within flow-cells to signal antigen binding in near real-time. Increasing the antibody affinity generally necessitates increasing the harshness of binding-site regeneration, resulting in compromises between the sensitivity and the longevity of the probe. Recycling receptors while maintaining device performance remains a problem for most antibody–antigen-mediated systems; however, in defense, hazard analysis, critical control point, and similar surveillance systems it may be economic to optimize sensitivity, and replace sensors on breakthrough [14].

2.1.2. Biospecific enzyme/substrate sensors

Enzyme–substrate reactions currently lack the spectrum of Ag/Ab systems, yet they possess an inherent advantage in that generally they rapidly eliminate the target, release the product, and auto-regenerate the binding (active) site with minimal loss of affinity and specificity over large numbers of cycles. There is therefore no requirement for washing steps, and no trade-off between sensitivity and regeneration. Additional advantages include a wide range of enzymes available, resistance to fouling, compatibility with flow-cells (and thus continuous, real-time read-out), and amenability to advances in enzyme bioengineering which can provide high degrees of stability under either physiological or extreme conditions: for example, changes can be made in the performance characteristics of enzymes with regard to their optimum reaction conditions (temperature, pH optima, allosteric effects, etc.) and substrate specificity, by introducing site-specific mutations to the encoding sequences, and

isolating genetically modified enzymes from expression vehicles. In addition, enzyme-mediated reactions may yield intermediates which facilitate electrochemical signal transduction. When enzyme reactions are coupled to reduction of nicotinamide adenine dinucleotide (phosphate) [NAD(P)] to NAD(P)H, the oxidation of the reducing equivalent can be coupled to a fluorescent emission [12]. Amperometric biosensors, which harness cofactors (usually on the tips of finely drawn optic fibers) have found numerous food-processing and clinical applications for the detection of analytes and contaminants [15]. The high performance of these optoamperometric and other optical nanosensor constructs has allowed the analysis of subcellular processes [16]. Changes in pH are compatible with some of the highest performing, simplest and cheapest transducing systems, e.g. in clinical applications several robust urea biosensors have employed urease to liberate CO_2, generating pH changes at an electrode surface, which can be transduced by any of a range of highly sensitive electrochemical and fluorescent methods [16]. Dill et al. [17] applied this principle to detect *Salmonella* in poultry using biotin–fluorescein-conjugated anti-*Salmonella* antibodies with an anti-fluorescein urease conjugate to the immunocomplex. This step provided signal amplification and a reported sensitivity of 119 CFU. Amperometric circuits can also employ coupled enzymes to amplify the signal and minimize the accumulation of undesirable intermediates [18]. Che et al. [19] developed a *Salmonella* assay system using alkaline phosphatase-conjugated anti-*Salmonella* antibody to bind bacteria to latex beads containing ferritin. This immunomagnetic separation (IMS) technique was used to attract the target to the surface of an amperometric biosensor bearing a tyrosinase carbon paste electrode. Alkaline phosphatase converted phenylphosphate to phenol, which was catalyzed to quinone in the presence of oxygen. The reduction of quinone back to phenol at the electrode surface prevented fouling, and provided amplification of the electrical signal. A sensitivity of 5×10^3 ml^{-1} CFU of *Salmonella* was reported in chicken carcass washes. Several enzyme–substrate biosensors are currently in use in the food industry for process control, particularly in fermentations (e.g. for ethanol, glucose, and vitamins) [20]; however, examples of food safety applications, most notably, include substrate-based assays for the detection of alkaline phosphatase as a surrogate marker in pasteurization. Assay of metabolites can indicate product quality, and increases in intermediary metabolites can indicate fermentation imbalance or spoilage. Microbial contamination in commercial fermentations is monitored by measuring the presence of acetic acid production as an indicator for the contamination of ethanol production.

2.1.3. Biospecific thermal sensors

Thermal biosensors represent a subset of enzyme–substrate sensors which catalyze specific exogonic reactions to sensitive thymistors [21]. Almost all enzyme reactions release energy, and in some cases substantial heat is generated, for example glucose oxidase mediates an enthalpy change of 100 kJ mM^{-1}, which corresponds to a temperature change of 0.015 °C mM^{-1} substrate. The system comprises a specific catalytic enzyme providing specificity, and a semiconducting thermal resistor which transduces temperature changes into changes in conductivity in an electrical circuit. Temperature changes of 0.0001 °C relative to a reference channel can be reported. The conjugation chemistry is straightforward, with effective thermal sensors developed by immobilizing enzyme onto silanized glass beads with a cross-linker such as glutaraldehyde. The glass beads are packed into a column surrounding the thymistor probe. Several dozen analytes have been measured with these biosensors, and illustrative examples include ethanol (linear range of 0.6–45 mM), L-lactate (lactate oxidase: 0.3–30 mM) and penicillin V (either B-lactamase: 0.1–300 mM; or penicillin V acylase: 0.5–150 mM) [22]. Enzymes may be coupled into multienzyme processes which can expand the dynamic range, amplify signals generated from very low concentrations of starting material, or measure the concentrations of both the substrate and the product of fermentations. Genetic engineering offers the prospect of future progress in the development of new thermal biosensor enzymes with favorable characteristics (e.g. pH and temperature optima), specificity, ruggedness and cost.

2.1.4. Translocating biosensors

Energy transfer reactions using electron transfer cytochromes or chlorophylls may energize fluorescent reducing equivalents, which in turn may emit a signal through an optical fiber to a photoelectric reporter, or be coupled through artificial electron acceptors to an oxygen electrode. Reactions in photosystems, cytochromes and ion channels all represent protein-mediated energy-linked processes, although the product is not a new chemical structure, rather the concentrative translocation of an ion or electron in space, which may be measured electrochemically, for example, by the ion channel switch [23].

2.1.5. Artificial olfaction

The human nose has long played a key role in the microbiology of food safety. Volatile organic metabolites indicating contaminated cultures, spoiled food, degraded environments, and bacterial infection can be detected and analyzed by olfaction systems and sophisticated data presentation techniques. Olfactory recognition components are generally sensitive, specific, stable, compatible with environmental matrices, and easily regenerated (with several notable exceptions). Artificial olfaction benefits from an easily handled matrix (air) which diffuses to the sensor rapidly and predictably from its point source. Although many compounds and mixtures remain undetectable, a wide range of alcohols, amines, ethers, phosphines, phosphites, thioethers, thiols, and to a lesser extent arenes, halocarbons,

and ketones will bind metalloporphyrin dyes and induce large spectral shifts based upon the polarizability of the ligand. A major problem with artificial olfaction systems has been devising data handling methods which can identify closely related members of a chemical family, and distinguishing the components in a mixture. Rakow and Suslick proposed an approach to integrating data from numerous metalated tetraphenylporphyrins [24]. Various dye solutions were spotted onto silica plates which were housed as sensor arrays in an N_2 flow-cell. To provide a transduction system capable of accommodating the quantity of data generated, the sensor array was simply installed above a flatbed scanner. When ligating vapors were passaged, distinctive colorimetric patterns were recorded. The color patterns offered powerful, readable, and highly expandable presentation tools to the human eye and to increasingly competent pattern recognition software. Although this system does not currently engage a biological recognition component beyond the use of the human eye in pattern recognition. It is notable that the reported drawbacks of this system, namely, poor reversibility, poor sensitivity in sub-saturating conditions, and poor performance in humid conditions are particular strengths of biological olfactory systems. Gas-phase analysis combining synthetic dyes and purified or cloned and expressed olfactory receptor proteins (ORPs) may become valuable in food quality analyses, detecting spoilage, evaluating waste management, and identifying hazards in many food-processing, environmental, and defense settings.

2.2. Biosensor architecture: signaling specific binding

Advances in interfacing biological recognition to signal transduction components have been critical to improving the scope and sensitivity of biosensors. New materials, improved micromachining, and advances in methods to modify, derivatize and coat surfaces have brought biosensor design to the nanometer scale [25]. Converging technologies have opened doors to numerous innovative biosensor platforms based on the analysis of refractive index changes (e.g. surface plasmon resonance (SPR) and optical interferometry), measurement of attached mass (e.g. electromechanical microdevices and piezoelectric (PZ) systems), measurement of fluorescent or luminescent emissions, and a diverse range of colorimetry, electrochemical, charge, mass, and spectral effects.

Binding or enzymatic interactions which yield measurable changes at the sensor surface include most of the interactions listed above, e.g. Ag/Ab reactions, nucleic acid hybridization, and numerous colorimetric assays involving the generation and precipitation of salts, as in western blots. The products of these reactions can be measured through a wide variety of techniques and combinations of techniques, and several spectacular technological advances are accelerating progress in this area. Generally, binding or precipitation reactions can be analyzed using electromechanical devices or optical systems, although within each category there is a range of approaches.

2.2.1. Electromechanical devices

For many years it has been known that the concentration of mass can yield measurable changes in several parameters. Acoustic and optical analysis can identify increased density or refractive index changes in specific molecular traps. Advances in instrumentation continue to improve the sensitivity of these methods. Several mechanical and electromechanical microgravimetric devices utilize mobile or resonant platforms to measure mass specifically attached to a modifiable surface. Rapid improvements in microfabrication and optoelectronics technology have brought these devices to the cellular and sub-cellular scale, where measurements on the femtogram scale of individual bacteria become feasible with devices which are compatible with mass production.

2.2.2. Piezoelectric devices

The most extensively evaluated method involves the PZ materials popularized in the form of quartz crystal microbalance (QCM) devices. When mechanically stressed, PZ materials create an electrical charge [26]. The change in oscillating frequency of a PZ crystal is proportionate to the mass variation on the metallic electrodes, which can be derivitized to provide a biospecific surface [27]. Numerous PZ devices have been described, the majority of which were immunosensors targeting intact bacteria because antibody is relatively simple to immobilize, and entrapped bacterial cells can accumulate into a significantly large, detectable mass [28]. Applications have been proposed for many PZ devices in clinical and food pathogen identifications, for example QCM devices coated with antibody, protein A, or other specific receptor molecules have been described for recognition of a wide range of antibodies, pathogenic organisms (including *Vibrio*, *Salmonella*, *Campylobacter*, *Escherichia coli*, *Shigella*, *Yersinia*, viruses, and protozoa) and PCR amplicons [29]. The rapid expansion of PZ systems into a range of important foodborne and clinical applications attests to the simplicity and flexibility of the QCM format. Although the sensitivity of these sensors is only suitable for very dense bacterial cultures, food safety applications have been proposed for situations where bacterial enrichment and growth is required to demonstrate bacterial viability. The QCM device can be coated with specific antibody and integrated into the culture enrichment tube, which can be sealed after inoculation. This format resolves several problems in food safety testing, albeit at the expense of rapidity and cost. Several investigators have used antibody-coated latex beads containing ferritin to provide an IMS technique to extract the target from the matrix and concentrate it onto the biosensor surface. Additionally, sentinel IMS beads can be entrapped within a size-selective flow-cell to restrict large interferents from abrading the biosensor surface, and massive beads can

provide additional signal amplification of the bound mass, and thus go some way towards overcoming the serious sensitivity limitations of PZ mass sensors [26]. There are numerous biological components which can be coupled to mass aggregation and deposition chemistries, largely because the signal amplification required in most ELISA, western, hybridization and dot blot detection systems entail accumulation of precipitated mass in the process of generating a detectable signal. Among the ELISA systems that have been modified for mass sensors are Ag/Ab binding and DNA–DNA hybridization, and indirect, amplified systems such as DAAP, avidin/streptavidin enzyme-conjugated secondary antibodies.

Wu described a novel PZ sensor coated with isolated ORPs from bullfrogs (*Rana* spp.), yielding a sensitive detector for several volatile organic compounds [30]. ORPs generally have favorable characteristics of sensitivity, stability and regeneration, and can be used to develop 'taste' or 'smell' sensors, for example in artificial olefaction systems. Devices such as these are valuable for food research and development, and in the future could potentially play an important role in detecting spoilage in a variety of settings.

2.2.3. Nanoelectromechanical devices

Among the various proposed designs for micro- and nanomechanical devices, the cantilever has emerged as a particularly interesting tool [31]. These devices are machined from silicon wafers using microfabrication techniques developed in the electronics industry. Cantilevers on the micrometer and nanometer scales naturally oscillate in ambient conditions under thermal energy, and the resonant frequency can be determined using a high-resolution HeNe laser-based optoelectronics package. The silicon surface of the device is readily modified to covalently attach antibodies. Upon binding the target, the resonant frequency of the device shifts in proportion to the change in attached mass. A sensitivity to 44 *E. coli* organisms was recently obtained with an antibody-coated device [32]; however, with improvements in nanomachining technology and more specific receptors, further miniaturized devices appear practical in the near future. Advances in the field are already developing analytical techniques for the measurement of mass and molecular forces on the sub-cellular scale [31,33]. These devices are representative of the nascent nanobiotechnology field, in which nanofabricated probes constructed on the same or smaller scale than their targets offer significant theoretical advantages. Detailed descriptions of intramolecular forces have already been reported using cantilever devices constructed on a biomolecular scale [31,34,35]. Key points of particular significance are the outstanding early results of resonant cantilever devices for the resolution of mass on the scale of individual bacteria, the simplicity of the 'dip, shake and measure' assay [32], the suitability of cantilever arrays for additional capabilities, and the possibility of mass-production techniques to dramatically reduce costs. Research exploring the extremities of small scale also raised the possibility of integrating the entrapment and measurement of subcellular targets, for example specific nucleotide sequences and proteins [34].

2.2.4. Flexural-plate-wave sensor

The ultrasonic flexural-plate-wave (FPW) sensor is one of a family of gravimetric sensor devices that can estimate the mass of a vibrating element. FPW sensors possess a thin membrane which can propagate an acoustic wave along its surface. When the FPW sensor is in contact with a liquid, a thin film of the liquid is forced to vibrate with the membrane. Any changes therefore in the mass of the membrane or the density of the vibrating thin liquid film measurably modifies the acoustic wave and can be detected with high resolution. The FPW thin film can accommodate biosensing components such as antibodies, antigens, or degradable polymers for the measurement of enzyme activity. FPW sensors can perform in gels, permitting size exclusion or other forms of selectivity which can be used to restrict access or protect the sensing surface. Bacterial cells can settle onto the sensor under the influence of gravity [36] or specificity can be applied by coating the transducer surface with an immunoaffinity layer or any of a number of possible ligands. The reported mass-detection limit is at the nanogram level. Pyun et al. [37] targeted *E. coli* surface antigens to detect a suspension of approximately 10^5-10^7 cells per ml. Although several settings such as industrial fermenters, and wastewater treatment facilities could accommodate these limitations, improved sensitivity may be obtained by the inclusion of IMS beads. The advantages of FPW sensors are their on-line, real-time performance, compatibility with aqueous samples, and variable surface chemistry. A particularly interesting feature of FPW (which also applies to resonating cantilevers) is their potential for the sentinel activities in remote or inaccessible locations. Specific FPW and QCM biosensors may also play a useful role as a simple, inexpensive, and low cost read-out when combined with microbiological enrichment cultures.

2.3. Optical systems

Recent developments in instrumentation have permitted the development of powerful analytical techniques based on the behavior of light at the interface of changes in refractive index. Particularly, powerful approaches currently receiving attention for food safety and microbiological applications include SPR and optical interferometry; both of which measure changes in the refractive index of an aqueous layer when certain molecular interactions occur.

2.3.1. Surface plasmon resonance

Surface plasmons refer to the collective oscillations of free electrons at the surface of a metal film, usually gold. The plasmons can be set to resonate when illuminated under appropriate conditions, and this resonance absorbs light. The resonance, and thus the absorption of light, is exquis

itely sensitive to the refractive index of the milieu of the metal surface. The surface is illuminated with polarized laser light from a light-emitting diode, the incident light is reflected in the gold film, and exits to a light-sensitive diode-array at an angle related to the refractive index, and thus can be related to the mass in the surface milieu. Although the refractive index is not necessarily proportional to mass or density, for certain analyses conducted under certain conditions, changes in the refractive index of the medium above the gold film have been reported to be proportional to the mass associated with the surface [38]. This surface can be modified to bind receptors, ligands, proteins, nucleic acids, or other molecules, and estimates can be made of the concentration of specific ligands, enzymes, antigens, or homologous sequences. SPR is thus an optical technique which makes quantitative measurements of changes in refractive index close to a modifiable surface.

The instrumentation to conduct these analyses comprises a sensor chip, an SPR detector, software for control and data analysis and a microfluidics system to introduce reagents and analytes to the sensor chip surface. The sensor chip comprises a prism to which a thin gold film is attached. To the gold surface is immobilized a bioactivated matrix, e.g. a 100-nm thick layer of hydrophilic 2–3% flexible dextran. The dextran surface can be derivatized with a wide range of ligands to provide specificity for any of a number of analytes.

Research applications for SPR extend into many areas of biochemical analysis [39,40] because the analysis module does not interfere with the interaction being measured, so measurements can be made under changing experimental conditions. In addition, because labeling is not required, native activities can be measured. Association and dissociation rate constants and kinetic measurements of impure analytes can therefore be calculated as analytes are adsorbed or desorbed from the specific derivatized surface, and analysis can proceed as reagents are added or removed [40].

Progress is rapid in the application of SPR to microbiology, food safety and associated problems including detection of a range of microbial components, including amplicons generated by PCR amplification of virulence genes (reviewed in [39,40]). The flexibility of SPR was shown to include the detection of enzymatic activity. Sumner et al. [41] attached a polymer film of the alpha-chymotrypsin substrate poly-(esteramide) to the gold surface, and measured the digestion of the substrate over time in the presence of alpha-chymotrypsin. An additional advantage is the capacity for SPR to conduct analyses while preserving biocontainment, and its compatibility with flow-through or valved sample chambers. SPR has been used in conjunction with other high-resolution techniques, e.g. matrix-assisted, laser-desorbed ionization time-of-flight (MALDI-TOF) has been used in conjunction with SPR to demonstrate a method for the capture and elution of staphylococcal toxins by antibody immobilized on an SPR surface. A sensitivity of 1 ng ml^{-1} was obtained for staphylococcal enterotoxin B in milk and mushroom samples [42].

2.3.2. Resonant mirror

A variation of SPR is the resonant mirror system which utilizes a series of polarizing filters to block internally reflected light. At one incident angle (the resonant angle), light is diverted through a low refractive index spacer layer to a high refractive index guide, and appears as a peak of intensity on a dark background. SPR with resonant mirror was used to characterize the interaction of *Helicobacter pylori* with sialoglyco-conjugates [43].

2.3.3. Colorimetric interference

Colorimetric interference is a specific nucleic acid detection system which yields a visual color signal in response to nucleic acid hybridization [44]. Capturing oligonucleotides are coated on an optical surface where they bind target DNA, which in turn binds a biotin-labeled probe sequence, which initiates the formation of an organic thin film on the optical surface. Anti-biotin conjugated to horseradish peroxidase is then added with tetramethylbenzidrine substrate, which deposits a layer of 10–20 Å. Incident light is reflected from the optical surface and the deposited layer, yielding destructive interference and a color change in the reflected light. This is seen as a change in color from gold to purple in the presence of target. This system required no instrumentation, and yielded single-base specificity and a sensitivity of 150 attomoles of DNA in under 3 min [44].

2.3.4. Optical interferometry

Several biosensor formats exploit the refractive index changes which occur when specific receptor/ligand interactions occur. When light passes along a thin film of < 1 μm, extending into the outer region by the order of 1 wavelength is what is called the ‘evanescent region’. When analytes displace water (or vice versa) within the evanescent region, there may be a change in the refractive index of the surface layer, which affects the phase of the lightwave propagating in the thin film. Optical interferometry is based on the detection of an interference pattern when a polarized light source is split into a reference beam and an analysis beam. The analysis beam is directed along a waveguide juxtaposed to a selectively binding thin film, and then recombined with the reference beam. Differences in phase due to differences in refractive index between sample and reference thin films are identified as an interference pattern in a diode-array. Optical interferometry shares with other evanescent wave techniques advantages such as real-time assay, liquid phase, flow-through, modifiable specificity, and minimal handling. Although these devices perform in the absence of labels, improved performance can be attained with the use of sandwiching conjugates such as nanoparticles which can amplify and control the refractive index changes when the specific analyte is bound to the thin film. Antibody-based optical interferometry has been applied to *Salmonella* detec

tion [45] with a sensitivity of 5×10^5 CFU ml^{-1}, and is used in the man-portable analyte identification system (MANTIS) biological weapon detector [46]. A direct DNA–DNA hybridization assay was developed based on an immobilized single-stranded 37-bp probe and four synthetic oligonucleotides to determine a detection capability estimated at ~4 ng ml^{-1} (or 10^{11} copies per ml).

3. Conclusion

All areas of biological detection, including food hazard identification and microbiology are undergoing a period of unusually rapid progress, as technological advances allow bridging among individually powerful disciplines. Impressive performance specifications have already been reported, despite the newness of many approaches. Several methods currently being proposed as platforms for microbiological detection possess such resolving power that they have yet even to be fully exploited as research tools; indicating that the potential of these methods is currently very unclear. Furthermore, traditional microbiology and enzyme-based rapid methods continue to accomplish impressive progress, especially in clinical applications, and biosensor systems should be viewed as complementary. Amid the excitement and raised expectations of accelerating bioanalytical capabilities, two inescapable challenges remain: pre-empting the extraordinary ability of microbiological agents to identify and exploit weaknesses, and developing a responsive and systematic approach to the independent evaluation of methods. Although the list of proposed tests and their advertised applications expands daily, only a minority of food pathogen detection methods have, however, been independently evaluated in collaborative studies, such as those approved by the Association of Official Analytical Chemists.

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