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Biosensor Arrays for Environmental Monitoring

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1. Introduction

Environmental monitoring involves several steps such as sampling, sample handling and sample transportation to specialized laboratories, sample preparation and analysis. Traditional environmental monitoring approaches are based on discrete sampling methods followed by laboratory analysis. These approaches do not improve our understanding of the natural processes governing chemical species behavior, their transport and bioavailability, or the relationship between anthropogenic releases and their long-term impact on aquatic systems[1]. The challenge of environmental monitoring in situ requires new and improved analytical devices featuring precision, sensitivity, specificity, rapidity, and ease of operation to detect decreasing concentrations of an ever growing array of pollutants. Such devices must be comparable to or better than traditional analytical systems, and must be simple to handle, small, cheap, able to provide reliable information in real-time, and must be sensitive and selective for the analyte of interest, and suitable for in situ monitoring[2]. Biosensors not only fulfill all these requirements but also have applications in many areas such as clinical diagnostics, forensic chemistry, pharmaceutical studies, food quality control and environmental monitoring.

A biosensor is an analytical device for the detection of an analyte that combines a biological component with a physicochemical detector component. It consists of 3 parts: (1) the sensitive biological element (biological material such as tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc.), a biologically derived material or biomimic; (2) the transducer or the detector element (works in a physicochemical way; optical, piezoelectric, electrochemical, etc.) that transforms the signal resulting from the interaction of the analyte with the biological element into another signal that can be more easily measured and quantified; (3) associated electronics or signal processors that are primarily responsible for the display of the results in a user-friendly way[3]. Depending on the type of transduction mechanism applied and the bio-recognition element employed, the potential for these devices for detection can be enormous. The technological development and the success in single analyte detection propelled advances in the miniaturization of sensors along with multi-analyte detection with sensitivities ranging in the nano-mole to

atto-mole range. With advances in techniques for biosensor construction, it has been possible to miniaturize the whole biosensor system on a chip to fabricate biosensor arrays.
The Biosensor arrays developed at the Naval Research Laboratory (NRL) has successfully been used in the detection of a variety of protein toxins, organic molecules, physiological health markers, a virus and a number of bacteria, initially in buffer but increasingly in food, biological and environmental matrices [4]. These developed biosensors are rapid, simple to perform and require little-to-no sample pretreatment prior to analysis, even for more complex sample matrices. In addition, the two-dimensional nature of the slide sensing surface facilitates simultaneous analysis of multiple samples for multiple analytes. Research on biosensor arrays as multi-analyte bio-systems has generated increased interest in the last decade. The main feature of the micro-array technology is the ability to simultaneously detect multiple analytes in one sample by an affinity-binding event at a surface interface. Fifteen years ago, the gene expression analysis of *c*DNA on micro-arrays was one of the first applications that successfully detected thousands of labeled target DNA molecules in parallel. Also the first immuno-analytical biosensor array was described at the same time. In the meantime, a great variety of target analytes capable of interacting selectively with a bio-molecular receptor has been adapted to arrays[5]. The biosensor arrays have been envisioned as a tool for rapid, on-site screening of pollutants in whatever location they might be found. The goals of automation, weight reduction, minimal size, ease of use, and reliability have remained paramount as the system has been developed[6].
The challenge of continuous in situ monitoring of environmental pollution requires instruments that are robust and with sufficient sensitivity and long lifetime. Commonly used conventional methods are time-consuming, expensive, require skilled operators, and lack the required selectivity. Biosensor arrays have the advantage of being simple, uniform whole structures featuring direct transduction, high bio-selectivity, high sensitivity, miniaturization, electrical/optoelectronic readout, continuous monitoring, ease of use, and cost effectiveness. User advantages include low price, reliability, no sample preparation, disposability, and clean technology. Hence, biosensor arrays show the potential to complement both laboratory-based and field analytical methods for environmental monitoring. Biosensor arrays are based on one general principle—certain bio-molecular recognition elements are defined on a heterogeneous matrix. Each element is dedicated to an analyte and contains quantitative information. The matrix is a patterned surface where the recognition molecules are immobilized by micro-printing such as screen printed technique, micro fluidic or other micro-structuring processes[5]. The type of biosensor arrays involves DNA-based biosensor array, antibody-based biosensor array, aptamer-based biosensor array, enzyme-based biosensor array, and microorganism-based biosensor. Recent progress in the development of analytical detection methods for antibody arrays, enzyme arrays and aptamer arrays as well as microbial arrays are summarized in this review, and their applications in the environment monitoring are also discussed. Detection approach is focused on electrochemical and optical measurements including various electrochemical or florescent probes as well as label-free approach. The numerous fabrication methods of DNA capture probes, antibodies and aptamer for multiplexed biological targets are also discussed.

2. DNA-based biosensor arrays

Deoxyribonucleic acids (DNA) are arguably the most important of all bio-molecules. The unique complementary structure of DNA between the base pairs adenine/thymine and

cytosine/guanine has been the basis for genetic analysis over the last few decades. The ability of a single stranded DNA (*ss*DNA) molecule to 'seek out', or hybridize to, its complementary strand in a sample is the foundation of DNA-based detection systems. There is a great potential market for simple, cheap, rapid, and quantitative detection of specific genes. Areas of application include clinical, veterinary, medico-legal, environmental, and the food industry[7]. Development of DNA biosensors and DNA biosensor arrays has increased tremendously over the past few years as demonstrated by the large number of scientific publications. Numerous DNA detection systems based on the hybridization between a DNA target and its complementary probe, which is present either in solution or on a solid support, have been described[8]. Homogeneous assays allowing the determination of DNA sequences have been developed. These systems can be based on optical[9] or electrochemical[10] detection. However, they do not allow easy continuous monitoring and miniaturization. Heterogeneous DNA biosensors and DNA biosensor arrays offer promising alternatives to these methods. They allow continuous, fast, sensitive, and selective detection of DNA hybridization, and they also can be reused. DNA biosensors arrays (commonly called gene chips, DNA chips, or biochips) exploit the preferential binding of complementary single-stranded nucleic acid sequences. This system usually relies on the immobilization of a single-stranded DNA (*ss*DNA) probe onto a surface to recognize its complementary DNA target sequence by hybridization. Transduction of hybridization of DNA can be measured optically, electrochemically, or using other devices. The detection process is schematized in Figure 1[8].

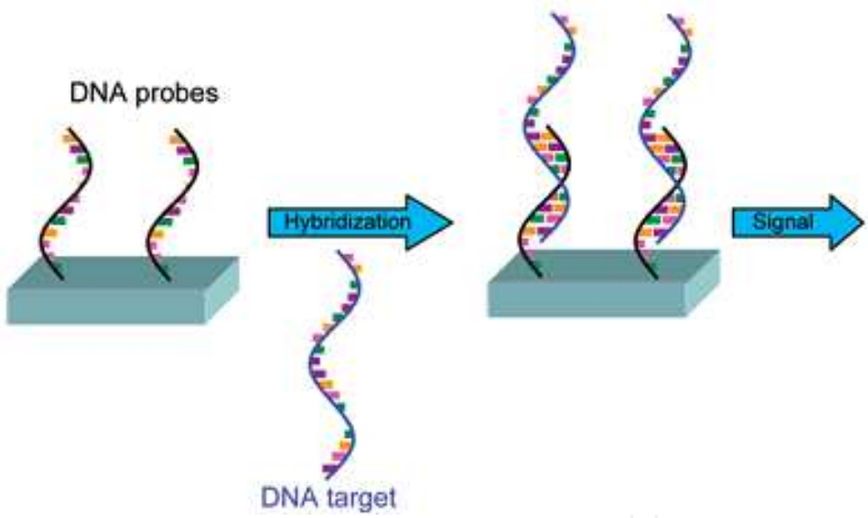

Fig. 1. Steps involved in the detection of a DNA sequence. Reprinted from ref. 8 with permission by the American Chemical Society.

In the case of DNA biosensors arrays, the immobilization of a DNA probe is achieved directly onto a transducer surface. DNA biosensor arrays are made from glass, plastic, or silicon supports and are constituted of tens to thousands of 10 – 100 µm reaction zones onto which individual oligonucleotide sequences have been immobilized. The exact number of DNA probes varies in accordance with the application. DNA biosensor arrays allow multiple parallel detection and analysis of the patterns of expression of thousands of genes in a single experiment.

We have presented an ultra-sensitive and direct electrochemical DNA biosensor array based on Ag aggregate tag and differential pulse voltammtery[11]. The scheme of detection is shown in Figure 2. The silver tags consist of Conjugate 1 (functionalized with capture probes and oligo A and Conjugate 2 (modified with oligo T). Hybridization between complementary oligo (d) A and oligo (d) T anchored on the silver nanoparticles produced aggregate tags. The hybridization-induced tags are successfully applied to bind with the DNA target via sandwich hybridization format and offer direct and amplified readout by differential pulse voltammetric method. We have found that the detection sensitivity by use of the aggregate tags can be improved by 3 orders of magnitude as compared to the single silver nanoparticle labels and a detection limit of 5 amol/L could be obtained.

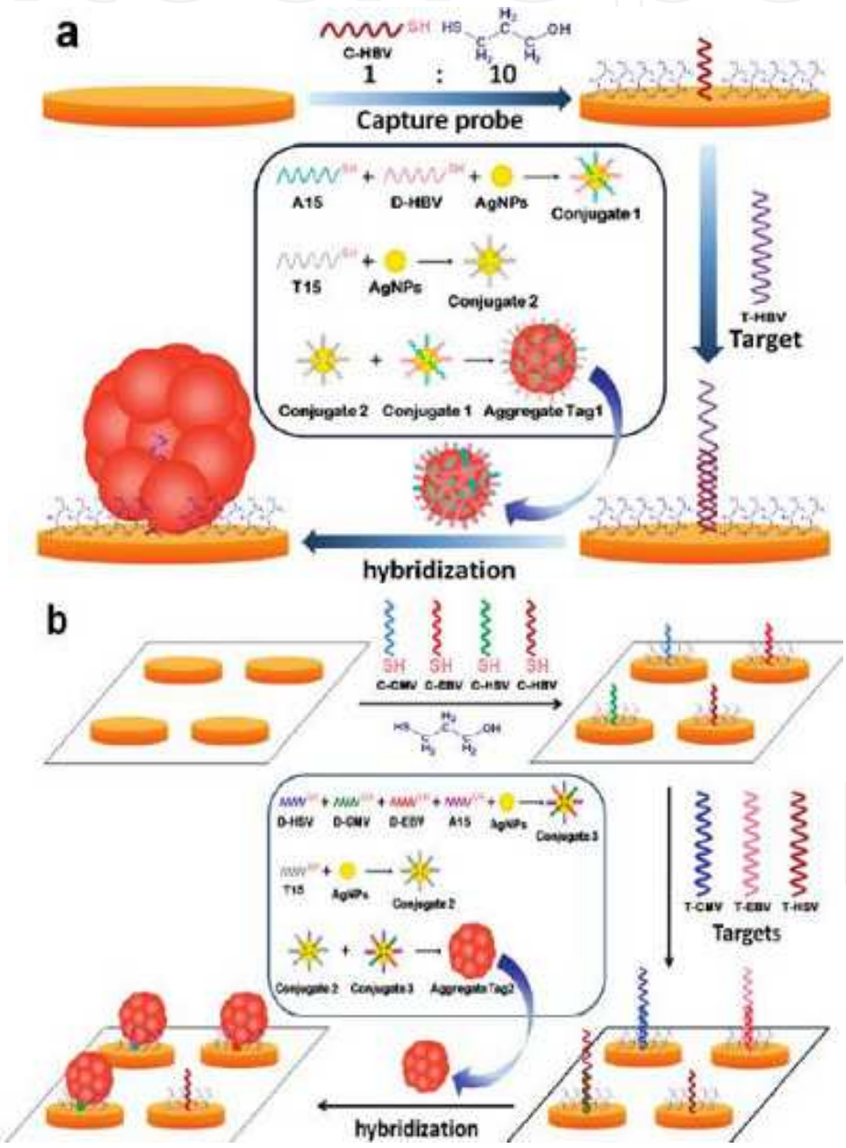

Fig. 2. Schematic illustration of the electrochemical Assay (a) and Multiplexed Assay (b) with silver nanoparticle Conjugates; Preparation of the aggregates is shown as well. Reprinted from ref. 11 with permission by the American Chemical Society.

Environmental applications of DNA biosensor arrays are in the field of species identification. For instance, DNA biosensor arrays are extensively exploited in the detection of pathogenic microorganisms relevant to food, bio-defense and environmental contamination applications. Mainly, DNA biosensor arrays have been coupled to PCR, as a specific detection method of the amplified base sequence. Zhang et al.[12] have developed a label-free electrochemical DNA biosensor array as a model system for simultaneous detection of multiplexed DNAs using micro-liters of sample. A novel multi-electrode array was comprised of six gold working electrodes and a gold auxiliary electrode, which were fabricated by gold sputtering technology, and a printed Ag/AgCl reference electrode was fabricated by screen-printing technology. The DNA biosensor array for simultaneous detection of the human immunodeficiency virus (HIV) oligonucleotide sequences, HIV-1 and HIV-2, was fabricated in sequence by self-assembling each of two kinds of thiolated hairpin-DNA probes onto the surfaces of the corresponding three working electrodes, respectively. The hybridization events were monitored by square wave voltammetry using methylene blue (MB) as a hybridization redox indicator. The oxidation currents of MB accumulated on the array decreased with increasing the concentration of HIVs due to higher affinity of MB for single strand rather than double strands of DNA. Under the optimized conditions, the peak currents were linear over ranges from 20 to 100 nmol/L for HIV-1 and HIV-2, with the same detection limits of 0.1 nmol/L (S/N= 3), respectively. The detection process is illustrated in Figure 3. The biosensor array showed a good specificity without the obvious cross-interference. Furthermore, single-base mutation oligonucleotides and random oligonucleotides can be easily discriminated from complementary target DNAs. Their work demonstrates that different hairpin-DNA probes can be used to design the label-free electrochemical biosensor array for simultaneous detection of multiplexed DNA sequences for various applications.

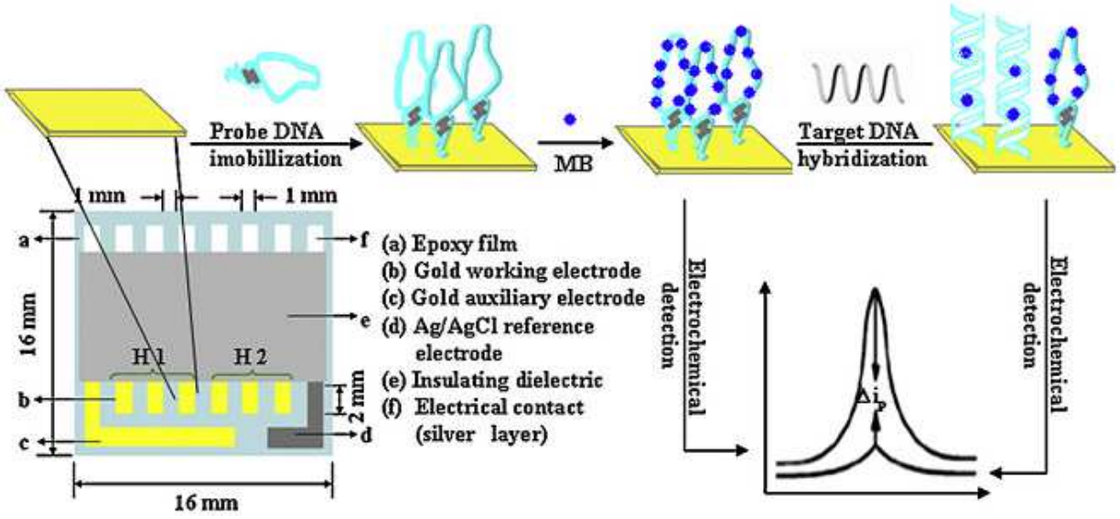

Fig. 3. Schematic diagrams of multi-electrode array and representation of biosensor array with fabrication steps and performance. Reprinted from ref. 12 with permission by the Elsevier.

Using electrochemical impedance spectroscopy (EIS) for biosensing applications typically requires repetitive experiments. To address this need, Bogomolova et al.[13] have designed a multi-specific electrochemical array with eight individually addressable 2 mm-diameter gold working electrodes for rapid biosensing data accumulation by EIS in the presence of redox agent. The array allows to incorporate multiple negative controls in the course of a single binding experiment, as well as to perform parallel identical experiments to improve reliability of detection. The array is fitted with attached electrochemical cell with Ag/AgCl mini reference electrode and can be used to process macro samples of 0.5–1 ml (Figure 4). The reported array is disposable, economical and is easy to use. Examples of array use for label-free genetic sensing of 2.7 kb-long target *Yersinia pestis* DNA and for protein sensing of Ricin Toxin Chain A (RTA) are presented. The authors suggest the reported array design as a tool for researchers in the area of EIS sensing.

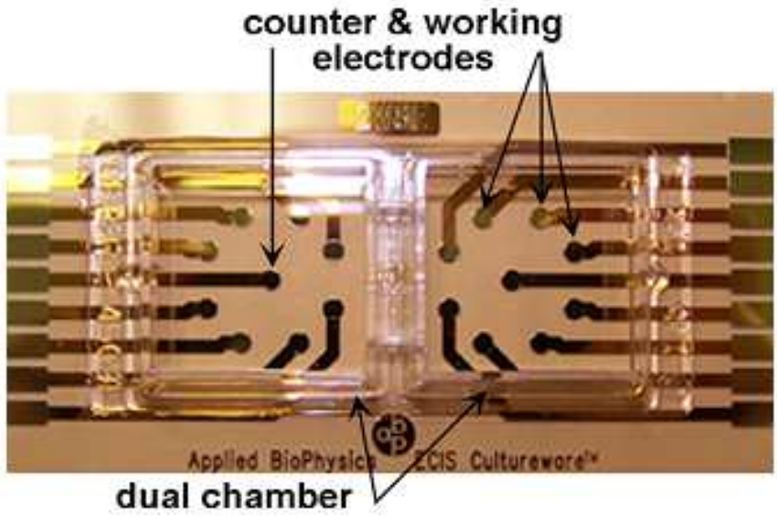

Fig. 4. Custom gold-sputtered dual array electrodes with attached chambers. Reprinted from ref. 13 with permission by the Elsevier.

Fu et al.[14] developed a piezoelectric quartz crystal microbalance (QCM) nucleic acid biosensor array using Au nanoparticle signal amplification to rapidly detect *S. epidermidis* in clinical samples. The synthesized thiolated probes specific targeting *S. epidermidis* 16S *r*RNA gene was immobilized on the surface of QCM nucleic acid biosensor arrays. Hybridization was induced by exposing the immobilized probes to the PCR amplified fragments of *S. epidermidis*, resulting in a mass change and a consequent frequency shift of the QCM biosensor. The results showed that the lowest detection limit of current QCM system was 1.3×10^3 CFU/mL. A linear correlation was found when the concentration of *S. epidermidis* varied from 1.3×10^3 to 1.3×10^7 CFU/mL. In addition, 55 clinical samples were detected with both current QCM biosensor system and conventional clinical microbiological method, and the sensitivity and specificity of current QCM biosensor system were 97.14% and 100%, respectively.

Doong et al.[15] fabricated a sol–gel-derived array DNA biosensors coupled with a fluorescence detection system and a robotic pin-printing platform to detect polycyclic aromatic hydrocarbons (PAHs) in water and serum samples (Figure 5). Parameters

including sol percentage, doped-amount of glycerol, dye probes, the surface coating, and DNA concentration were optimized. In their work, two fluorescent dyes, fluorescein isothiocyanate (FITC) and ethidium bromide (EDB) were selected and compared. Results showed that EDB was more sensitive to compete intercalators (PAHs) than FITC, and was selected as fluorescent dye for array-based DNA biosensors. The optimized procedure with *ds*DNA concentration of 23.5 g/mL allowed the fabrication of the DNA biosensor up to 50 spots within 10 min via the developed pin-printing system. For PAH detection, the developed array DNA biosensor effectively detected naphthalene and phenanthrene in the concentration range of 0-10 mg/L in aqueous solution, but was not sensitive to fluoranthene and benzo[a]pyrene. In the serum samples, the apparent water solubility of high-molecular-weight PAHs was greatly enhanced by the dissolved organic compounds in serum, and an obvious DNA toxicity was exhibited in the presence of three-to-five-ring PAHs. Benzo[a]pyrene showed high toxic effect at low concentration in serum samples, clearly showing that the sol-gel-derived array DNA biosensor with EDB as sensing probe can effectively detect PAHs in water and biological samples.

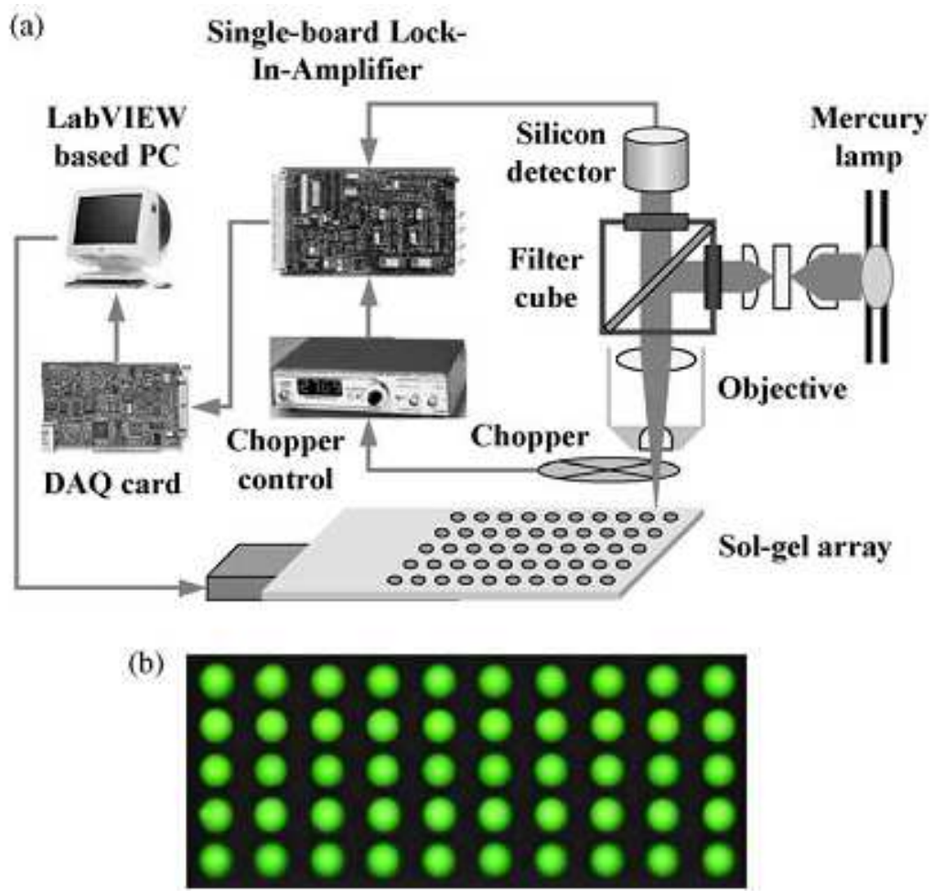

Fig. 5. (a) the schematic diagram of the developed fluorescence detection system and (b) images of the array DNA biosensor using the sol-gel processes. Reprinted from ref. 15 with permission by the Elsevier.

3. Antibody based biosensor array

Immunoassays gained popularity for biomedical applications in the 1970s because of the impressively low detection limits and high selectivity for analyzing complex samples that could be achieved with relatively simple procedures and instrumentation. The availability of highly selective antibodies for an increasingly wide variety of important analytes was also an important factor in the growth of the method over the following decades. The development of more sensitive labels and detection devices also improved the sensitivity of the assays even further. Once immunoassays became more common, the development of more convenient immuno-sensors that are easier and faster to use gained momentum[16].

Antibody-based biosensors are inherently more versatile than enzyme-based biosensors in that antibodies have been generated which specifically bind to individual compounds or groups of structurally related compounds with a wide range of affinities. There are, however, several limitations in the use of antibody-based biosensors for environmental monitoring applications. These limitations include the complexity of assay formats and the number of specialized reagents (e.g., antibodies, antigens, tracers, etc.) that must be developed and characterized for each compound and the limited number of compounds typically determined in an individual assay as compared to the multiple compounds that contaminate environmental samples[17].

Antibody-based biosensor arrays are a powerful tool for analytical purposes. Immuno-analytical micro-arrays are a quantitative analytical technique using antibodies as highly specific biological recognition elements[5]. They can be designed for a variety of analytical applications producing rapid results with low limits of detection (LOD). The detection antibodies are in direct contact with the sample, without prior sample cleanup. Immobilized haptens in combination with an indirect competitive immunoassay (IA) are the common format for the detection of multiple small molecules (e.g. pesticides, pharmaceuticals, small toxin targets). Haptens provoke an immune response if coupled to a protein by use of their functional groups. Hapten micro-arrays use analyte derivatives as immobilized recognition molecules. Antibody micro-arrays quantify proteins, bacteria, or viruses by using a sandwich immunoassay format. Recent advances reported for antibody-based biosensor arrays for environmental applications have primarily been focused toward these. For example, Jin et al.[18] have developed a fluorescent NP-mediated Ab micro-array system for the detection of bioterrorism agents exemplified by ricin, CT, and SEB toxins (results are displayed in Figure 6). High sensitivity, specificity, and reproducibility were achieved by using their antibody biosensor array. They found that substituting monoclonal antibodies (mAb) with highly purified polyclone antibodies (pAb), even though having similar titer by ELISA, could dramatically improve the micro-array performance. A likely explanation is that pAb enhances the Ag capture by binding multiple sites on the analyte. The micro-array format allows a multiplexed, high-throughput, and high-parallel assay. Furthermore, the miniature feature permits low consumption of both sample and reagents, reducing the amounts of biohazard waste as well as the costs to conduct the assay.

Seidel et al.[19] fabricated an automated chemiluminescence (CL) read-out system for analytical flow-through micro-arrays based on multiplexed immunoassays. The micro-array chip reader (MCR 3) is designed as a stand-alone platform, with the goal to quantify multiple analytes in complex matrices of food and liquid samples for field analysis or for routine analytical laboratories. The CL micro-array platform is a self-contained system for

the fully automated multiplexed immuno-analysis comprising the micro-array chip, the fluidic system and the software module that enable automated calibration and determination of analyte concentrations during a whole working day. The detection of antibiotics in milk was demonstrated to validate this device. Therefore, an automated multi-analyte detection instrument is needed for the simultaneous and rapid quantification of antibiotics. Also regeneration is required to avoid replacing the assay surface. The European Union, for example, has defined maximum residue levels (MRLs) for a number of antibacterial compounds. However, despite the obvious demand for quantitative multi-residue detection methods that can be carried out on a routine basis, there is currently a lack in the development of such systems. In particular, an automated multi-analyte detection instrument is needed that is capable of quantifying several antibiotics simultaneously within minutes. Seidel's group[20] developed a new hapten based micro-arrays for the parallel analysis of 13 different antibiotics in milk within six minutes by applying an indirect competitive chemiluminescence micro-array immunoassay (CL-MIA). To allow multiple analyses, a regenerable micro-array chip was developed based on epoxy-activated PEG chip surfaces, onto which micro-spotted antibiotic derivatives like sulfonamides, b-lactams, aminoglycosides, fluorquinolones and polyketides are coupled directly without further use of linking agents. Using the chip reader platform MCR 3 (Figure 7), this antigen solid phase is stable for at least 50 consecutive analyses.

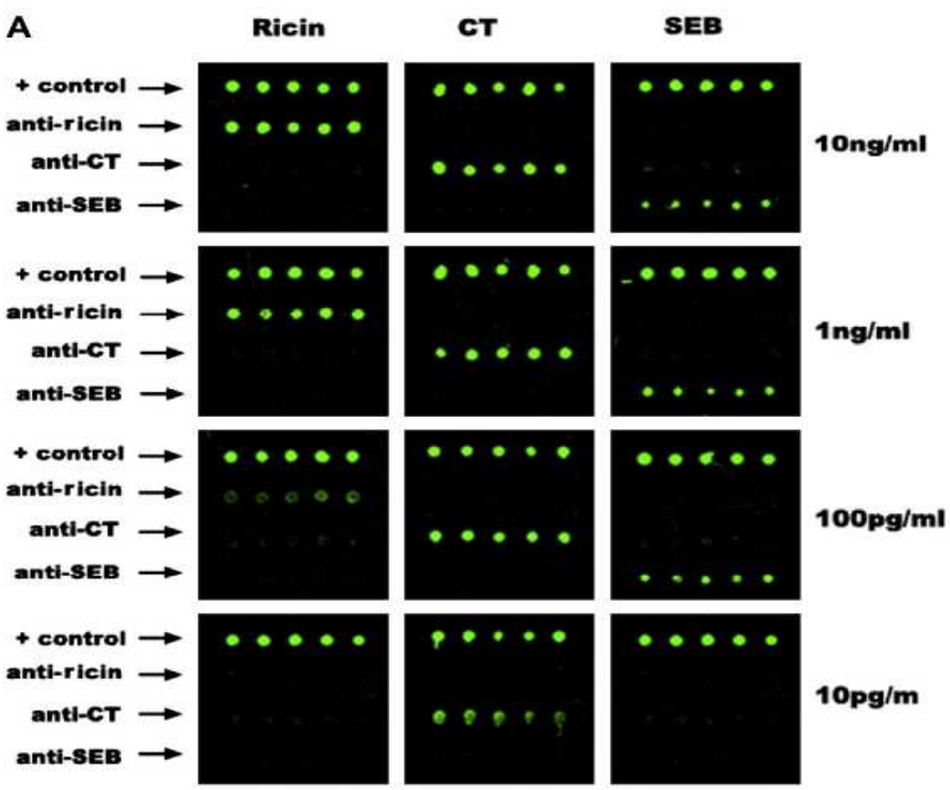

Fig. 6. The detection results of the different toxins by using antibody arrays. Reprinted from ref. 18 with permission by the Elsevier.

An impedance biosensor based on interdigitated array microelectrode (IDAM) coupled with magnetic nanoparticle–antibody conjugates (MNAC) was developed and evaluated for rapid and specific detection of *E. coli* O157:H7 in ground beef samples by Li et al.[21]. MNAC were prepared by immobilizing biotin-labeled polyclonal goat anti-*E. coli* antibodies onto streptavidin-coated magnetic nanoparticles, which were used to separate and concentrate *E. coli* O157:H7 from ground beef samples. Magnitude of impedance and phase angle were measured in a frequency range of 10 Hz to 1 MHz in the presence of 0.1mol/L mannitol solution. The lowest detection limits of this biosensor for detection of *E. coli* O157:H7 in pure culture and ground beef samples were 7.4×10^4 and 8.0×10^5 CFU/mL, respectively. The regression equation for the normalized impedance change (NIC) versus *E. coli* O157:H7 concentration (N) in ground beef samples was NIC=15.55N−71.04 with R^2= 0.95. Sensitivity of the impedance biosensor was improved by 35% by concentrating bacterial cells attached to MNAC in the active layer of IDAM above the surface of electrodes with the help of a magnetic field. Based on equivalent circuit analysis, it was observed that bulk resistance and double layer capacitance were responsible for the impedance change caused by the presence of *E. coli* O157:H7 on the surface of IDAM. Surface immobilization techniques, redox probes, or sample incubation were not used in this impedance biosensor. The total detection time from sampling to measurement was 35 min.

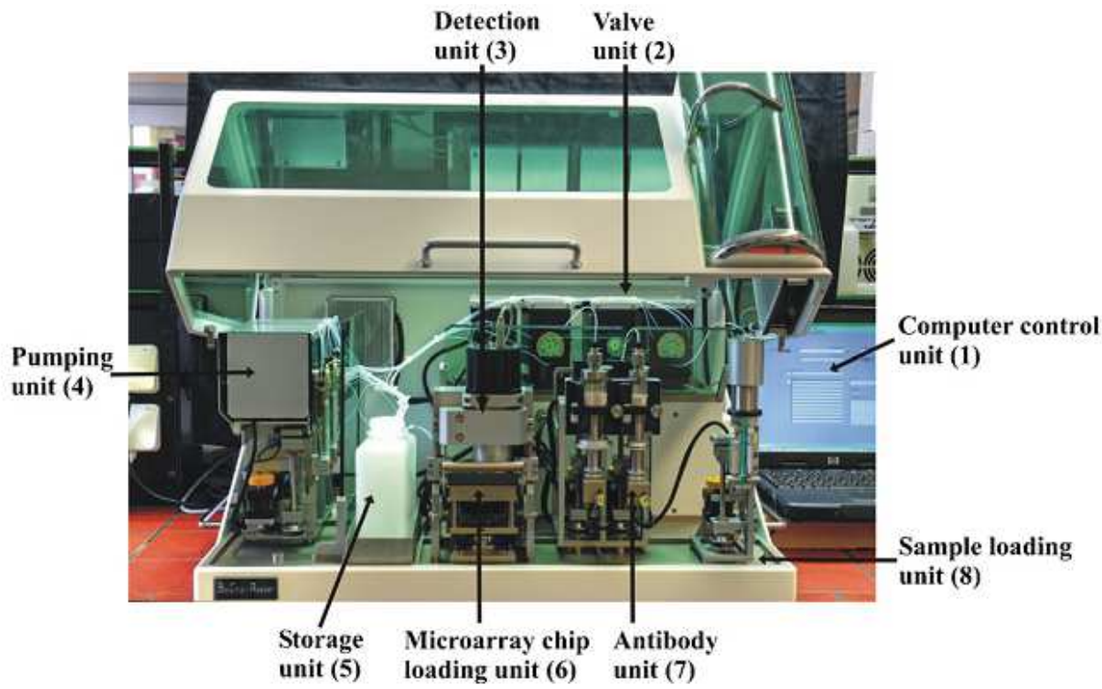

Fig. 7. Image of the MCR 3 system. Reprinted from ref. 20 with permission by the Royal Society of Chemistry.

Because of the potential health risks of aflatoxin B1 (AFB1), it is essential to monitor the level of this mycotoxin in a variety of foods. An indirect competitive immunoassay has been developed using the NRL array biosensor (Figure 8) by Shriver-Lake et al.[22], offering rapid, sensitive detection, and quantification of AFB1 in buffer, corn and nut products. AFB1-

spiked foods were extracted with methanol and Cy5-anti-AFB1 was added to the resulting sample. The extracted sample/antibody mix was passed over a waveguide surface patterned with immobilized AFB1. The resulting fluorescence signal decreased as the concentration of AFB1 in the sample increased. The limit of detection for AFB1 in buffer, 0.3 ng/mL, was found to increase to between 1.5 and 5.1 ng/g and 0.6 and 1.4 ng/g when measured in various corn and nut products, respectively.

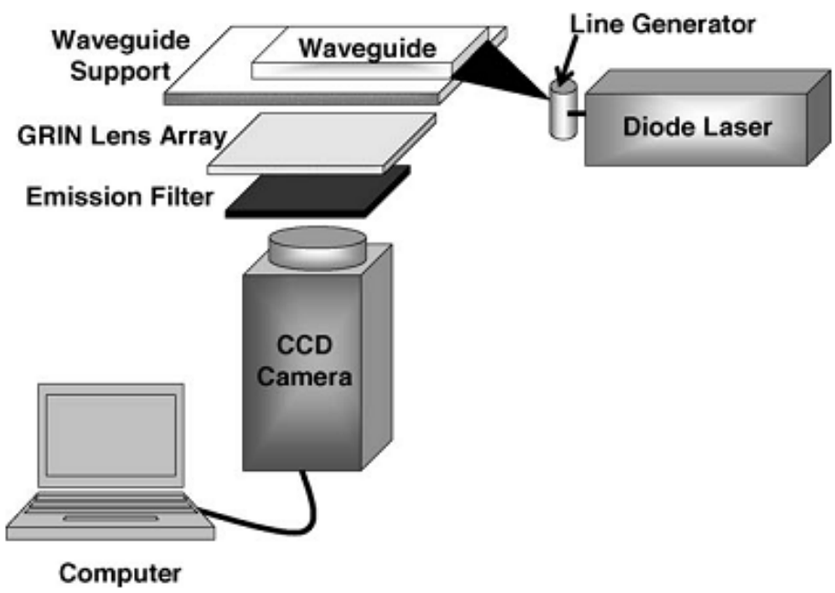

Fig. 8. Schematic of the NRL array biosensor. Reprinted from ref. 22 with permission by the Elsevier.

Ligler et al.[4] has clearly demonstrated the versatility of the biosensor arrays for the detection of both large and small food contaminants either individually or simultaneously. The bacterial pathogen *C. jejuni* was measured using a sandwich immunoassay both in buffer and additional complex food matrices with LODs ranging from 500 to 3780 CFU/ml. The mycotoxins OTA, DON, AFB1 and FB were detected simultaneously on a signal substrate using a competitive-based immunoassay format taking only 15min. The combination of sandwich and competitive immunoassay formats on a single substrate was demonstrated, allowing the simultaneous detection of both large (*C. jejuni*) and small (AFB1) food pathogens with LODs in buffer of 500 CFU/ml and 0.3 ng/mL, respectively.

Deoxynivalenol (DON), a mycotoxin produced by several *Fusaruim* species, is a worldwide contaminant of foods and feeds. Because of the potential dangers due to accidental or intentional contamination of foods with DON, there is a need to develop a rapid and highly sensitive method for easy identification and quantification of DON. In Taitt's study[23], they have developed and utilized a competitive immunoassay technique to detect DON in various food matrixes and indoor air samples using a biosensor array. A DON biotin conjugate, immobilized on a NeutrAvidin-coated optical waveguide, competed with the DON in the sample for binding to fluorescently labeled DON monoclonal antibodies. To demonstrate a simple procedure amenable for on-site use, DON-spiked cornmeal, cornflakes, wheat, barley, and oats were extracted with methanol water (3:1) and assayed

without cleanup or pre-concentration. The limits of detection ranged from 0.2 ng/mL in buffer to 50 ng/g in oats. The detection limit of DON spiked into an aqueous effluent from an air sampler was 4 ng/mL.

Parro et al.[24] have developed antibodies and a multi-array competitive immunoassay (MACIA) for the detection of a wide range of molecular size compounds, from single aromatic ring derivatives or polycyclic aromatic hydrocarbons (PAHs), through small peptides, proteins or whole cells (spores). Multiple biosensor arrays containing target molecules are used simultaneously to run several competitive immunoassays. The sensitivity of the MACIA for small organic compounds like naphthalene, 4-phenylphenol or 4-terbutylphenol is in the range of 100–500 ppb, for others like the insecticide terbutryn it is at the ppt level, while for small peptides, as well as for more complex molecules like the protein thioredoxin, the sensitivity is approximately 1-2 ppb, or 104-105 spores of Bacillus subtilis per milliliter. For organic compounds, a water–methanol solution was used in order to achieve a better dissolution of the organics without compromising the antibody–antigen interaction. The above-mentioned compounds were detected by MACIA in water–(10%) methanol extracts from spiked pyrite and hematite-containing rock powder samples, as well as from a spiked-sand sample subjected to organic extraction with dichloromethane–methanol (1/1).

Sandwich immunoassays have also been conducted by running the samples through a multiple channel (one per flow channel) over the array of capture antibodies for a period of time sufficient for the antibody to bind any target agent in the sample. In most of the assays described by Ligler et al.[25], they use 8–10 min for binding in order to balance assay sensitivity with keeping the total assay time short (under 15 min). The sensitivity is clearly greater if longer periods of time are used, particularly if the samples are viscous and the rate of diffusion of the target within the sample is slow. In this array, biotinylated capture antibodies were exposed to the avidin-coated waveguide using flow channels molded out of polydimethylsiloxane as described previously. TNT (trinitrotoluene) antibody spots bound a Cy5-labeled TNB (trinitrobenzene) tracer molecule in the tracer cocktail to provide a positive control. Assays were conducted using staphylococcal enterotoxin B (SEB), ricin toxin, cholera toxin, mouse IgG and Bacillus globigii (*B. globigii* is an anthrax spore simulant) as targets. The Cy5-TNB bound to the appropriate antibodies in all lanes. Samples containing SEB, cholera toxin, mouse IgG and *B. globigii* bound cleanly to the appropriate spots without showing any cross-reactivity against other capture antibodies.

Contamination of food by mycotoxins occurs in minute quantities, and therefore, there is a need for a highly sensitive and selective device that can detect and quantify these organic toxins. Taitt et al.[26] reported the development of a rapid and highly sensitive array biosensor for the detection and quantitation of ochratoxin A (OTA). The array biosensor utilizes a competitive immunoassay format. Immobilized OTA derivatives compete with toxin in solution for binding to fluorescent anti-OTA antibody spiked into the sample. This competition is quantified by measuring the formation of the fluorescent immuno-complex on the waveguide surface. The fluorescent signal is inversely proportional to the concentration of OTA in the sample. Analyses for OTA in buffer and a variety of food and beverage samples were performed. Samples were extracted with methanol, without any sample cleanup or pre-concentration step prior to analysis. The limit of detection for OTA in several cereals ranged from 3.8 to 100 ng/g, while in coffee and wine, detection limits were 7 and 38 ng/g, respectively.

Golden et al.[27] have developed a "do-it-yourself" biosensor array for the simultaneous detection of multiple targets in multiple samples within 15-30 min. The biosensor is based on a planar waveguide, a modiWed microscope slide, with a pattern of small (mm^2) sensing

regions. The waveguide is illuminated by launching the emission of a 635 nm diode laser into the proximal end of the slide via a line generator. The evanescent Weld excites Xuorophores bound in the sensing region and the emitted Xuorescence is measured using a Peltier-cooled CCD camera. Assays can be performed on the waveguide in multichannel Xow chambers and then interrogated using the detection system described in their paper. This biosensor can detect many different targets, including proteins, toxins, cells, virus, and explosives with detection limit rivaling those of the ELISA detection system.

Kramer et al.[28] presents a new, versatile, portable miniaturized flow-injection biosensor array which is designed for field analysis. The temperature-controlled field prototype can run for 6 h without external power supply. The bio-recognition element is an analyte-specific antibody immobilized on a gold surface of pyramidal structures inside an exchangeable single-use chip, which hosts also the enzyme-tracer and the sample reservoirs. The competition between the enzyme-tracer and the analyte for the antigen-binding sites of the antibodies yields in the final step a chemiluminescence signal that is inversely proportional to the concentration of analyte in the given range of detection. A proof of principle is shown for nitroaromatics and pesticides. The detection limits reached with the field prototype in the laboratory was below 0.1 g/L for 2,4,6-trinitrotoluene (TNT), and about 0.2 g/L for diuron and atrazine, respectively. Important aspects in this development were the design of the competition between analyte and enzyme-tracer, the unspecific signal due to unspecific binding and/or luminescence background signal, and the flow pattern inside the chip.

The multianalyte array biosensor (MAAB) is a rapid analysis instrument capable of detecting multiple analytes simultaneously. Rapid (15 min), single-analyte sandwich immunoassays were developed for the detection of Salmonella enterica serovar Typhimurium by Taitt et al.[29], with a detection limit of 8×10^4 CFU/mL; the limit of detection was improved 10 fold by lengthening the assay protocol to 1 h. S. enterica serovar Typhimurium was also detected in the following spiked foodstuffs, with minimal sample preparation: sausage, cantaloupe, whole liquid egg, alfalfa sprouts, and chicken carcass rinse. Cross-reactivity tests were performed with *Escherichia coli* and *Campylobacter jejuni*. To determine whether the MAAB has potential as a screening tool for the diagnosis of asymptomatic Salmonella infection of poultry, chicken excretal samples from a private, noncommercial farm and from university poultry facilities were tested. While the private farm excreta gave rise to signals significantly above the buffer blanks, none of the university samples tested positive for S. enterica serovar Typhimurium without spiking; dose-response curves of spiked excretal samples from university-raised poultry gave limits of detection of 8×10^3 CFU/g.

Campylobacter and Shigella bacteria are common causes of food- and water-borne illness worldwide. There is a current need in food, medical, environmental, and military markets for a rapid and user-friendly method of detecting such pathogens. The array biosensor developed at the NRL encompasses these qualities. Ligler et al.[30] reported on a sandwich immunoassay-based biosensor array that was developed for the detection of Campylobacter and Shigella species in both buffer and a variety of food and beverage samples. The limit of detection for Shigella dysenteriae in buffer and chicken carcass wash was 4.9×10^4 CFU/mL, whereas Campylobacter jejuni could be measured at concentrations as low as 9.7×10^2 CFU/mL. The limits of detection and dynamic range were found to vary depending on the sample matrix, but could be improved by running the sample over the waveguide surface for longer periods of time. Samples were analyzed with no pre-concentration or enrichment steps and little-to-no sample pretreatment prior to analysis, and the total analysis run time was 25 min.

Biosensor array that is capable of detecting multiple targets rapidly and simultaneously on the surface of a single waveguide has also been studied. Ligler et al.[31] developed a

sandwich and competitive fluoroimmunoassays to detect high and low molecular weight toxins, respectively, in complex samples. Antibodies were first immobilized in specific locations on the waveguide and the resultant patterned array was used to interrogate up to 12 different samples for the presence of multiple different analytes. Upon binding of a fluorescent analyte or fluorescent immunocomplex, the pattern of fluorescent spots was detected using a CCD camera. Automated image analysis was used to determine a mean fluorescence value for each assay spot and to subtract the local background signal. The location of the spot and its mean fluorescence value were used to determine the toxin identity and concentration. Toxins were measured in clinical fluids, environmental samples and foods, with minimal sample preparation. Results were reported for rapid analyses of staphylococcal enterotoxin B, ricin, cholera toxin, botulinum toxoids, trinitrotoluene, and the mycotoxin fumonisin. Toxins were detected at levels as low as 0.5 ng/mL.

4. Aptamer based biosensor array

Aptamers are single-stranded (ss) DNA or RNA molecules, typically <100 monomer units, which have the ability to bind to other molecules with high affinity and specificity. They are selected from random oligonucleotide pools by a process called Systematic Evolution of Ligands by Exponential enrichment (SELEX). Conceptually, the SELEX process is based on the ability of these small oligonucleotides to fold into unique three-dimensional (3-D) structures which can interact with a specific target with high specificity and affinity through such interactions as van der Waals surface contacts, hydrogen bonding and base stacking interactions. Aptamers can offer a strong and reliable role as biological recognition elements in most analytical applications. The specificity and high affinity of aptamers to a wide variety of targets, coupled with the ease of design and molecular engineering, as outlined earlier, make aptamers highly suitable for development as molecular biosensors. By moving in this direction, investigators have made appreciable efforts in recent years to utilize these unique features of aptamers to devise appropriate strategies with which to effectively and efficiently apply aptamers for biological agent recognition, identification, characterization and quantification[32].

Analogous to immunoassays based on the antigen-antibody interaction, aptamer-based biosensors can adopt different assay configurations to transduce bio-recognition events. Since aptamers have been selected to bind very different targets, ranging from small molecules to macromolecules, such as proteins, various assay configurations have been designed and reported. Nevertheless, the majority of these designs fall into two categories of configuration (Figure 9): single-site binding and dual-site binding[33]. The use of aptamers as new biological receptors in biosensor arrays can accelerate the development of biosensors of practical relevance. Because of their exceptionally high stability, selectivity and sensitivity, aptamer-based biosensor arrays have the potential to overcome the lacking functional and storage stability of most biosensors[34]. For example, an electrochemical impedance spectroscopy method of detection for aptamer-based electrochemical biosensor array (Figure 10) is reported in which the binding of aptamers immobilized on gold electrodes leads to impedance changes associated with target protein binding events by Xu et al.[35]. Human IgE was used as a model target protein and incubated with the aptamer-based array consisting of single-stranded DNA containing a hairpin loop. To increase the binding efficiency for proteins, a hybrid modified layer containing aptamers and cysteamine was fabricated on the photolithographic gold surface through molecular self-assembly. Compared to immunosensing methods using anti-human IgE antibody as the recognition element, impedance spectroscopy detection could

provide higher sensitivity and better selectivity for aptamer-modified electrodes. The results of this method show good correlation for human IgE in the range of 2.5-100 nmol/L. A detection limit of 0.1 nmol/L was obtained, and an average of the relative standard deviation was 10%. The method describes the first label-free detection for arrayed electrodes utilizing electrochemical impedance spectroscopy.

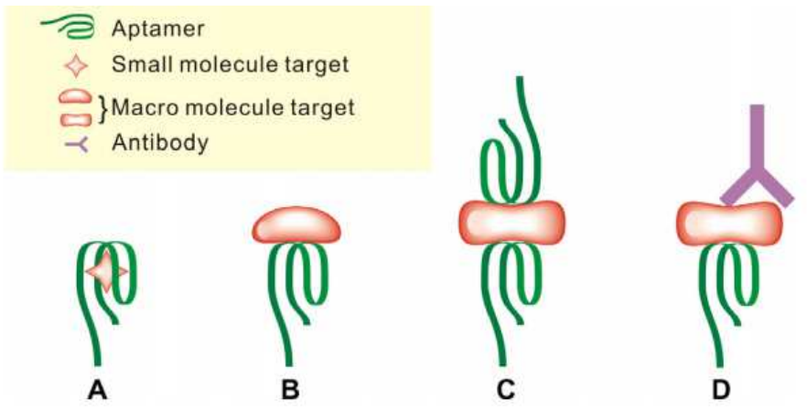

Fig. 9. Aptamer-based assay formats. (A) Small-molecule target buried within the binding pockets of aptamer structures; (B) single-site format;(C) dual-site (sandwich) binding format with two aptamers; and, (D) ''sandwich'' binding format with an aptamer. Reprinted from ref. 33 with permission by the Elsevier.

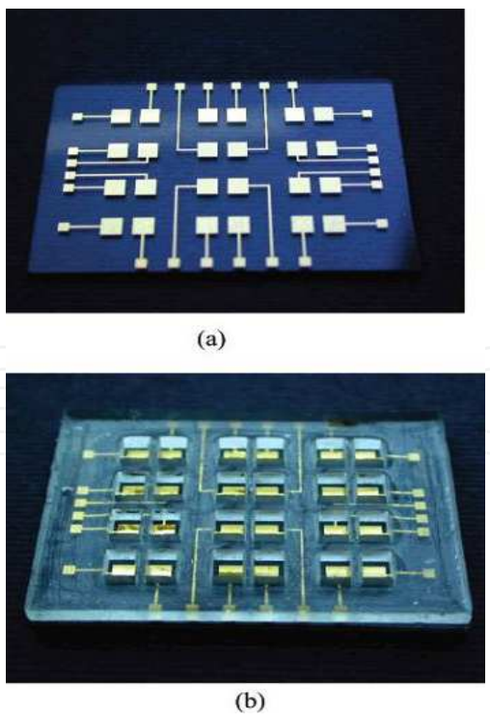

Fig. 10. Construction of the gold array electrode chip: (a) a photolithographic gold film array electrode; (b) a home-constructed PDMS frame containing 24-microwells for the immobilization. Reprinted from ref. 35 with permission by the Royal Society of Chemistry.

Quandt et al.[36] designed a Love-wave biosensor array by coupling aptamers to the surface of a Love-wave sensor chip. The sensor chip consists of five single sensor elements and allows label-free, real-time, and quantitative measurements of protein and nucleic acid binding events in concentration-dependent fashion. The biosensor was calibrated for human-thrombin and HIV-1 Rev peptide by binding fluorescently labeled molecules and correlating the mass of the bound molecules to fluorescence intensity. Detection limits of approximately 75 pg/cm^2 were obtained, and analyte recognition was specific. The sensor can easily be regenerated by simple washing steps. They further demonstrated the versatile applicability of the sensor by immobilizing single-stranded DNA (*ss*DNA) for the detection of the corresponding counter-strand.

The large quantity of aptamers which have been selected to bind complex molecules of low molecular weight leads to the possible use of these aptamers not only in diagnostic assays, but also in a wider range of applications, such as environmental analytical chemistry[37]. Selection of DNA ligands to the chloroaromatics, 4-chloroaniline (4-CA), 2,4,6-trichloroaniline (TCA) and pentachlorophenol (PCP), was performed by a novel method utilizing magnetic beads (MBs) having a linker arm for immobilization[38]. Moreover, Labuda et al.[39] reported for the first time the selection of RNA aptamers for the recognition of hydrophobic aromatic carcinogens. In particular, RNA aptamers with a K_d in the low micro-molar range have been selected for aromatic amines residues using as a model methylendianiline, which is a common industrial chemical employed to manufacture plastics, glues and foams.

A toxin-related work based on aptamers arrays have been published by Ellington et al.[40] . The authors reported the adaptation of a chip-based micro-sphere array (the ‘‘electronic taste chip’’) to aptamer receptors. Their detection system is illustrated in Figure 11. Unlike most protein-based arrays, the aptamer chips could be stripped and reused multiple times. The aptamer chips proved to be useful for screening aptamers from in vitro selection experiments and for sensitively quantitating the bio-threat agent ricin. The system composed of a flow cell connected to a fast performance liquid chromatography pump and a fluorescence microscope for observation. The flow cells contained silicon chips with multiple wells in which beads modified with the sensor elements were deposited. Commercially available streptavidin agarose beads were modified with biotinylated aptamers; RNA anti-ricin aptamers were used to demonstrate the possibility of quantifying the labeled protein. A sandwich assay format was also optimized using anti-ricin antibodies, to directly detect the unlabelled protein. In the first type of assay, the aptamer was bio-tinylated, immobilized and put in contact with the solution containing fluorescently labeled ricin, once introduced into the chip wells. The fluorescence intensities of the captures proteins were used to construct a calibration plot for ricin and a detection limit of 8 mg/ml was obtained. In the sandwich assay, the anti-ricin aptamer acted as a capture reagent and unlabelled ricin bound to the aptamer could interact with fluorophore-labeled fabricated an aptamer-based biosensor array for protein detection.

Environmental allergenic disease is a major cause of illness and disability, and there is broad consensus that the prevalence of type I allergy is increasing worldwide. Recent advances in biotechnology have yielded potentially useful functional binding aptamers that can enable low cost, high affinity allergen measurement. Aptamers are selected in vitro from combinatorial oligonucleotide libraries and therefore have several advantages over the traditionally used antibodies for detection of allergens. Aptamer-based methods could be used for measuring environmental allergens. Integrating the resulting aptamer-based

allergen measurements to enhance quantization in an ongoing and complementary environmental childhood asthma epidemiological study forms the basis for the third and final aim. Successful use of aptamers for measuring environmental allergens should lead to a more cost effective, flexible, and health relevant method and thereby provides the potential for a more fundamental understanding of the role of environmental allergens in respiratory health.

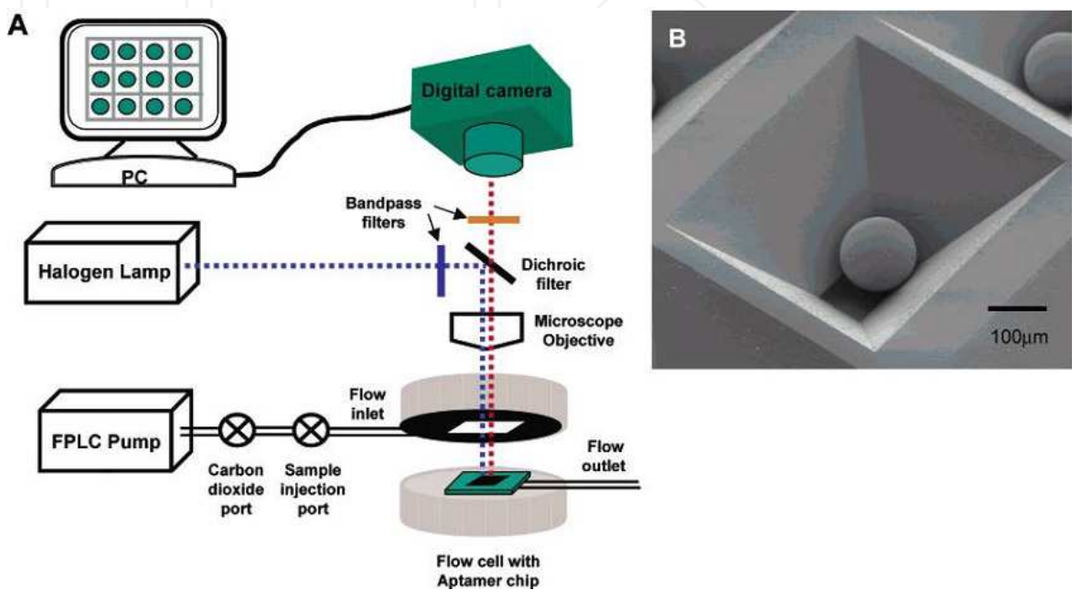

Fig. 11. Detection systems. (A) The electronic tongue setup contains a fluid delivery system, fluorescence microscope, digital camera, flow cell in which the aptamer chip will be loaded, and computer for data analysis. (B) Close-up look at a bead in a rectangular-shaped micro-machined well of the aptamer chip. Reprinted from ref. 40 with permission by the Royal Society of Chemistry.

5. Enzyme based biosensor array

Enzyme-based technology relies upon the natural specificity of given enzymatic protein to react biochemically with a target substrate or substrates. Like ion channels, there are many enzymes that participate in cellular signaling and, in some cases, are targeted by compounds associated with environmental toxicity. In general, enzyme-based biosensors employ semi-permeable membranes through which target analytes diffuse toward a solid-phase immobilized enzyme compartment. Ion selective, amperometric, or pH electrodes measure reaction components such as hydrogen peroxide (from oxidation of glucose by glucose oxidase) or ammonium ions (from urease metabolism of urea)[41]. Enzymes were historically the first molecular recognition elements included in biosensors and continue to be the basis for a significant number of publications reported for biosensors in general as well as biosensors for environmental applications. There are several advantages for enzyme biosensors. These include a stable source of material (primarily through bio-renewable sources), the ability to modify the catalytic properties or substrate specificity by means of genetic engineering, and catalytic amplification of the biosensor response by modulation of the enzyme activity with respect to the target analyte[17].

Recent progress with respect to enzyme biosensors for environmental applications has been reported in several areas[42]. These areas include the following: genetic modification of enzymes to increase assay sensitivity, stability and shelf life; improved electrochemical interfaces and mediators for more efficient operation; and introduction of sampling schemes consistent with potential environmental applications. More recently, enzyme-based biosensor arrays also have been used in the application of environmental monitoring. For example, Kukla et al.[43] developed a multi-enzyme electrochemical biosensor array. Their sensor array is based on capacitance pH-sensitive electrolyte–insulator–semiconductor (EIS) sensors with silicon nitride ion-sensitive layers and different forms of cholinesterase, urease and glucose oxidase as sensitive elements. With this sensor array, the authors used a multi-enzyme analysis to recognize the heavy metal ions in solutions containing a mixture of different metal ions, as well as for determination of the metal ion content in the analyzed samples. The content of toxic elements was determined by estimation of the residual activity of enzymatic membranes after the injection of analyzed samples. The conditions for enzyme sensors operation, such as buffer capacity, substrate concentration, time of incubation and time of response signal measurement, were optimized to reach the maximal sensitivity of multi-sensor for analysis of heavy metal ions in the investigated solutions. The results show that multi-enzyme analysis followed by mathematical processing is an efficient approach to develop biosensor arrays for toxic substrates detection.

Organophosphate pesticides (OPs) used to be widely used in agriculture due to their high efficiency as insecticides. OPs have been shown to result in high levels of acute neuron-toxicity and carcinogenicity, with the majority being hazardous to both human health and to the wider environment. A rapid, reliable, economical and portable analytical system will be of great benefit in the detection and prevention of OPs contamination. A biosensor array based on six acetylcholinesterase enzymes coupled with a novel automated instrument incorporating a neural network program has been reported by Hart et al.[44]. The biosensor array and the instrument is illustrated in Figure 12. Electrochemical analysis was carried out using chronoamperometry and the measurement was taken 10 s after applying a potential of 0 V vs. Ag/AgCl. The total analysis time for the complete assay was less than 6 min. The array was used to produce calibration data with six organophosphate pesticides (OPs) in the concentration range of 10^{-5} mol/L to 10^{-9} mol/L to train a neural network. The output of the neural network was subsequently evaluated using different sample matrices. There was no detrimental matrix effect observed from water, phosphate buffer, food or vegetable extracts. Furthermore, the sensor system was not detrimentally affected by the contents of water samples taken from each stage of the water treatment process. Their biosensor array system successfully identified and quantified all samples where an OP was present in water, food and vegetable extracts containing different OPs. There were no false positives or false negatives observed during the evaluation of the analytical system. Their biosensor arrays and automated instrument were evaluated in situ in field experiments where the instrument was successfully applied to the analysis of a range of environmental samples.

Recently, many studies have focused on the development of biochemical sensors, which are well suited for the rapid, simple and selective analysis of pesticides. Specially, they combine the selectivity of the enzymatic reactions with operational simplicity and simple detection schemes. Valle et al.[45] developed an electronic tongue, employing an array of inhibition biosensors and Artificial Neural Networks (ANNs). The array of biosensors was made up of three amperometric pesticide biosensors that used different acetylcholinesterase (AChE) enzymes: a wild type from electric eel (EE) and two different genetically modified enzymes

(B1 and B394). In order to model the response to dichlorvos and carbofuran mixtures, a total amount of 22 solutions were prepared, with random concentrations. Chronoamperometric responses of the biosensor array were used in order to obtain the inhibition bioelectronic tongue. Mean values of concentration of pesticides evaluated were 0.79 nmol/L for dichlorvos and 4.1 nmol/L for carbofuran. Good prediction ability was obtained with correlation coefficients better than 0.918 when the obtained values were compared with those expected for a set of 6 external test samples not used for training.

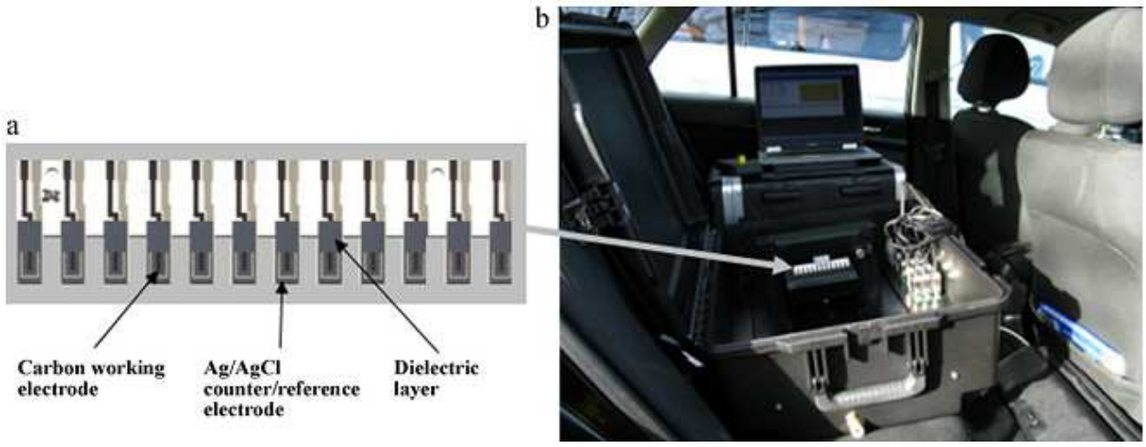

Fig. 12. (a) electrode array comprising 12 screen-printed carbon electrodes and an Ag/AgCl counter/reference electrode printed on an alumina substrate; (b) array in the prototype biosensor system operating in the field powered from a car battery via the lighter socket. Reprinted from ref. 44 with permission by the Elsevier.

Another approach is by using Organophosphorus hydrolase (OPH). OPH is a 72 kDa homodimeric, metalloenzyme, containing two zinc ions in the active site involved in catalytic and/or structural functions. OPH catalyzes the hydrolysis of Organophosphates (OPs) resulting in its detoxification. Some of the biosensors that were developed exploiting OPH as the bio-recognition element on different detection platforms have been reported. Though highly sensitive and selective towards different OPs, their inability to provide simultaneous measurements of different analytes was a major shortcoming. Simonian et al.[46] developed a biosensor array (Figure 13) with the potential for direct detection of organophosphates using OPH, conjugated with a pH-sensitive fluorophore, carboxynaphthofluorescein (CNF). The presence of reference spots allows the discrimination of the enzymatic and non-enzymatic based pH changes; bovine serum albumin (BSA) was used as a non-enzymatic scaffold protein for CNF attachment at the reference spots. An array biosensor unit developed at the Naval Research Laboratories (NRL) was adopted as the detection platform and appropriately modified for enzyme-based measurements. A planar multi-mode waveguide was covered with an optically transparent TiO_2 layer to increase the surface area available for immobilization. The biosensor enabled the detection of 2.5 μmol/L paraoxon, and 10 μmol/L parathion respectively. Very short response time of 30 s can be achieved with a total analysis time of less than 2 min. When operated at room temperature and stored at 4 ℃, the waveguide retained reasonable activity for greater than 45 days.

An array-based optical biosensor for the simultaneous analysis of multiple samples in the presence of unrelated multi-analytes was fabricated by Doong et al.[47]. The authors used

Urease and acetylcholinesterase (AChE) as model enzymes, which were co-entrapped with the sensing probe, FITC-dextran, in the sol-gel matrix to measure pH, urea, acetylcholine (ACh) and heavy metals (enzyme inhibitors). Environmental and biological samples spiked with metal ions were also used to evaluate the application of the array biosensor to real samples. The biosensor exhibited high specificity in identifying multiple analytes. No obvious cross-interference was observed when a 50-spot array biosensor was used for simultaneous analysis of multiple samples in the presence of multiple analytes. The sensing system can determine pH over a dynamic range from 4 to 8.5. The limits of detection of 2.5-50 µmol/L with a dynamic range of 2-3 orders of magnitude for urea and ACh measurements were obtained. Moreover, the urease-encapsulated array biosensor was used to detect heavy metals. The analytical ranges of Cd(II), Cu(II), and Hg(II) were between 10 nmol/L and 100 mmol/L. When real samples were spiked with heavy metals, the array biosensor also exhibited potential effectiveness in screening enzyme inhibitors.`

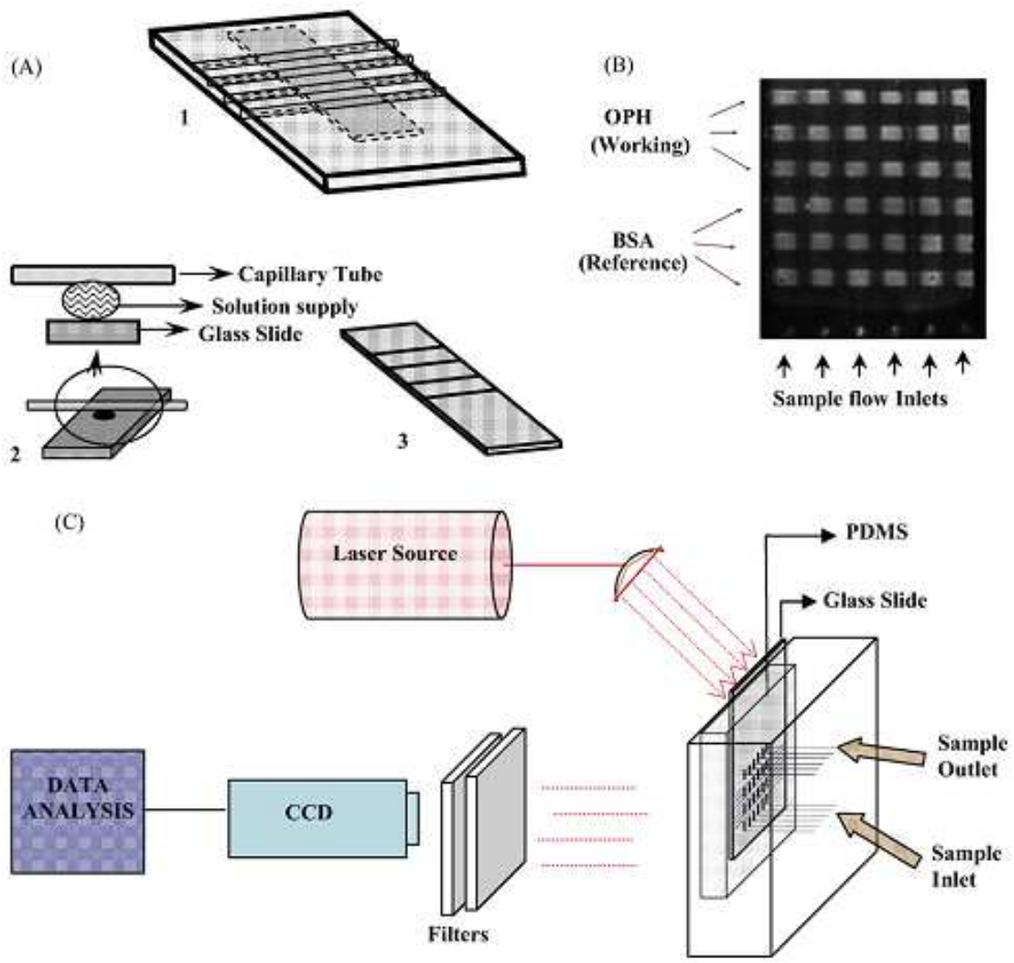

Fig. 13. (A) Schematic of modified process for incubation using thin glass tubes. (B) Schematic of the glass slide with immobilized proteins and fluorophores. (C) Schematic of the array biosensor. Reprinted from ref. 46 with permission by the Elsevier.

Solna et al.[48] use screen-printed four-electrode system as the amperometric transducer for determination of phenols and pesticides using immobilized tyrosinase, peroxidase, acetylcholinesterase and butyrylcholinesterase. Acetylthiocholine chloride was chosen as substrate for cholinesterases to measure inhibition by pesticides, hydrogen peroxide served as co-substrate for peroxidase to measure phenols. In their work, the compatibility of hydrolases and oxidoreductases working in the same array was studied. The detection of p-cresol, catechol and phenol as well as of pesticides including carbaryl, heptenophos and fenitrothion was carried out in flow-through and steady state arrangements. It was demonstrated that electrodes modified with hydrolases and oxidoreductases can function in the same array. The limit of detection for catechol using tyrosinase was equal to 0.35 and 1.7 μmol/L in the flow and steady systems. Lower limits of detection for pesticides were achieved in the steady state system: carbaryl 26 nmol/L, heptenophos 14 nmol/L and fenitrothion 0.58 nmol/L. Similar multi-enzyme-based electrochemical biosensor arrays for the determination of pesticides[49-52] and phenols[53] have been reported by other workers.

6. Microorganism-based biosensor array

A microbial biosensor is an analytical device which integrates microorganism(s) with a physical transducer to generate a measurable signal proportional to the concentration of analytes. In recent years, a large number of microbial biosensors have been developed for environmental, food, and biomedical applications[54].

Enzymes are the most widely used biological sensing element in the fabrication of biosensors. Although purified enzymes have very high specificity for their substrates or inhibitors, their application in biosensors construction may be limited by the tedious, time-consuming and costly enzyme purification, requirement of multiple enzymes to generate the measurable product or need of cofactor/coenzyme. Microorganisms provide an ideal alternative to these bottle-necks. The many enzymes and co-factors that co-exist in the cells give the cells the ability to consume and hence detect large number of chemicals; however, this can compromise the selectivity. They can be easily manipulated and adapted to consume and degrade new substrate under certain cultivating condition. Additionally, the progress in molecular biology/recombinant DNA technologies has opened endless possibilities of tailoring the microorganisms to improve the activity of an existing enzyme or express foreign enzyme/protein in host cell. All of these make microbes excellent biosensing elements[55].

Microorganism-based biosensor arrays classically used for environmental biosensing are mainly bacteria and yeasts, and to a lesser extent algae. Various strains have been exploited, from commercial and well-characterized cells harboring a broad range of substrates to genetically engineered organisms specially constructed to detect specific molecules or groups of molecules, passing through environmental cells isolated from polluted sites offering greater robustness and more specific enzymatic properties[56].

Rapid identification of *Escherichia coli* strains is an important diagnostic goal in applied medicine as well as the environmental and food sciences. Mikkelsen et al.[57] reported an electrochemical, screen-printed biosensor array, where selective recognition is accomplished using lectins that recognize and bind to cell-surface lipopolysaccharides and coulometric transduction exploits non-native external oxidants to monitor respiratory cycle activity in lectin-bound cells. Ten different lectins were separately immobilized onto porous

membranes that feature activated surfaces. Modified membranes were exposed to untreated *E. coli* cultures for 30 min, rinsed, and layered over the individual screen-printed carbon electrodes of the sensor array. The membranes were incubated 5 min in a reagent solution that contained the oxidants menadione and ferricyanide as well as the respiratory substrates succinate and formate. Electrochemical oxidation of ferrocyanide for 2 min provided chronocoulometric data related to the quantities of bound cells. These screen-printed sensor arrays were used in conjunction with factor analysis for the rapid identification of four *E. coli* subspecies (*E. coli* B, *E. coli* Neotype, *E. coli* JM105 and *E. coli* HB101). Systematic examination of lectin-binding patterns showed that these four *E. coli* subspecies are readily distinguished using only five essential lectins.

The last decade has witnessed a significant increase in interest in whole-cell biosensors for diverse applications, as well as a rapid and continuous expansion of array technologies. The combination of these two disciplines has yielded the notion of whole-cell array biosensors. Belkin et al.[58] presented a potential manifestation of this idea by describing the printing of a whole-cell bacterial bioreporters array (Figure 14). Exploiting natural bacterial tendency to adhere to positively charged abiotic surfaces, they describe immobilization and patterning of bacterial ''spots'' in the nanoliter volume range by a non-contact robotic printer. They show that the printed Escherichia coli-based sensor bacteria are immobilized on the surface, and retain their viability and biosensing activity for at least 2 months when kept at 4℃. Immobilization efficiency was improved by manipulating the bacterial genetics, the growth and the printing media and by a chemical modification of the inanimate surface. The result suggests that the methodology presented by them may be applicable to the manufacturing of whole-cell sensor arrays for diverse high throughput applications. In the course of the study, they have also described a novel specific reporter for the detection of respiratory inhibitors. Sodium azide, a chemical with a constantly increasing world distribution, served as the model toxicant. The sensor's response was rapid (20 minutes after exposure) and dose-dependent, and could be maintained for at least 2 months at 4 ℃.

Li et al.[59] developed a double interdigitated array microelectrodes (IAM)-based flow cell for an impedance biosensor to detect viable *Escherichia coli* O157:H7 cells after enrichment in a growth medium. Their study was aimed at the design of a simple flow cell with embedded IAM which does not require complex microfabrication techniques and can be used repeatedly with a simple assembly/disassembly step. The flow cell was also unique in having two IAM chips on both top and bottom surfaces of the flow cell, which enhances the sensitivity of the impedance measurement. *E. coli* O157:H7 cells were grown in a low conductivity yeast–peptone–lactose–TMAO (YPLT) medium outside the flow cell. After bacterial growth, impedance was measured inside the flow cell. Equivalent circuit analysis indicated that the impedance change caused by bacterial growth was due to double layer capacitance and bulk medium resistance. Both parameters were a function of ionic concentration in the medium, which increased during bacterial growth due to the conversion of weakly charged substances present in the medium into highly charged ions. The impedance biosensor successfully detected *E. coli* O157:H7 in a range from 8.0 to 8.2×10^8 CFU/mL after an enrichment growth of 14.7 and 0.8 h, respectively. A logarithmic linear relationship between detection time (T_D) in h and initial cell concentration (N_0) in CFU/mL was $T_D = -1.73 \log N_0 + 14.62$, with $R^2 = 0.93$. Double IAM-based flow cell was more sensitive than single IAM-based flow cell in the detection of *E. coli* O157:H7 with 37–61% more impedance change for the frequency range from 10 Hz to 1 MHz. The double IAM-

based flow cell could be used to design a simple impedance biosensor for the sensitive detection of bacterial growth and their metabolites.

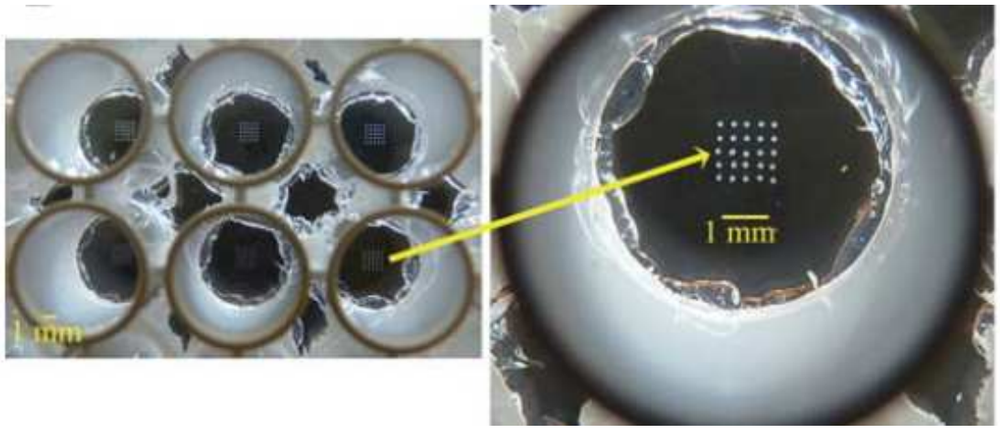

Fig. 14. Twenty five spots, 1 nl each, of strain SM118 in ectoine, printed onto the wells of 96-well plate with an APTES coated glass bottom. Reprinted from ref. 58 with permission by the Royal Society of Chemistry.

Worldwide herbicide discharge into the aquatic environment is also a growing concern. Adverse effects induced by herbicide contamination are impacting a great variety of organisms and ecosystems, ranging from the primary producers to animals and humans. Biosensors for the rapid detection of herbicides in the environment have also been explored. A multiple-strain algal biosensor was constructed for the detection of herbicides inhibiting photosynthesis by Podola et al.[60]. Nine different microalgal strains were immobilized on an array biochip using permeable membranes. The biosensor allowed on-line measurements of aqueous solutions passing through a flow cell using chlorophyll fluorescence as the biosensor response signal. The herbicides atrazine, simazine, diuron, isoproturon and paraquat were detectable within minutes at minimal LOEC (Lowest Observed Effect Concentration) ranging from 0.5 to 100 µg/L, depending on the herbicide and algal strain. The most sensitive strains in terms of EC50 values were Tetraselmis cordiformis and Scherffelia dubia. Less sensitive species were Chlorella vulgaris, Chlamydomonas sp. and Pseudokirchneriella subcapitata, but for most of the strains no general sensitivity or resistance was found. The different responses of algal strains to the five herbicides constituted a complex response pattern (RP), which was analyzed for herbicide specificity within the linear dose-response relationship.

Recombinant bioluminescent bacterial strains are increasingly receiving attention as environmental biosensors due to their advantages, such as high sensitivity and selectivity, low costs, ease of use and short measurement times. Gu et al.[61] use a cell-based array technology that uses recombinant bioluminescent bacteria to detect and classify environmental toxicity followed by developing two biosensor arrays, i.e., a chip and a plate array. Twenty recombinant bioluminescent bacteria, having different promoters fused with the bacterial lux genes, were immobilized within LB-agar. About 2 µl of the cell-agar mixture was deposited into the wells of either a cell chip or a 384-well plate. The bioluminescence (BL) from the cell arrays was measured with the use of highly sensitive cooled CCD camera that measured the bioluminescent signal from the immobilized cells and then quantified the pixel density using image analysis software. The responses from the

cell arrays were characterized using three chemicals that cause either superoxide damage (paraquat), DNA damage (mitomycin C) or protein/membrane damage (salicylic acid). The responses were found to be dependent upon the promoter fused upstream of the lux operon within each strain. Therefore, a sample's toxicity can be analyzed and classified through the changes in the BL expression from each well. Moreover, a time of only 2 h was needed for analysis, making either of these arrays a fast, portable and economical high-throughput biosensor system for detecting environmental toxicities.

Because of their ability to perform functional sensing, living cell-based biosensors are drawing increased attention. The work reported by Walt et al.[62] demonstrates the ability to fabricate an optical imaging fiber-based living bacterial cell array for genotoxin detection. A biosensor composed of a high-density living bacterial cell array was fabricated by inserting bacteria into a micro-well array formed on one end of an imaging fiber bundle. The size of each micro-well allows only one cell to occupy each well. In this biosensor, *E. coli* cells carrying a *recA::gfp* fusion were used as sensing components for genotoxin detection. Each fiber in the array has its own light pathway, enabling thousands of individual cell responses to be monitored simultaneously with both spatial and temporal resolution. The biosensor was capable of performing cell-based functional sensing of a genotoxin with high sensitivity and short incubation times (1 ng/mL mitomycin C after 90 min). The biosensors demonstrated an active sensing lifetime of more than 6 h and a shelf lifetime of two weeks. Their group reported another live cell biosensor array[63], which was fabricated by immobilizing bacterial cells on the face of an optical imaging fiber containing a high density array of micro-wells. Each microwell accommodates a single bacterium that was genetically engineered to respond to a specific analyte. A genetically modified *Escherichia coli* strain, containing the *lacZ* reporter gene fused to the heavy metal-responsive gene promoter *zntA*, was used to fabricate a mercury biosensor. A plasmid carrying the gene coding for the enhanced cyan fluorescent protein (ECFP) was also introduced into this sensing strain to identify the cell locations in the array. Single cell *lacZ* expression was measured when the array was exposed to mercury and a response to 100 nmol/L Hg^{2+} could be detected after a 1-h incubation time. The optical imaging fiber-based single bacterial cell array is a flexible and sensitive biosensor platform that can be used to monitor the expression of different reporter genes and accommodate a variety of sensing strains.

7. Conclusion and future direction

In recent years, there have been dramatic advances in a new analytical format, the biosensor array, a tool that has revolutionized our ability to characterize and quantify biologically and envitonmetally relevant molecules. The biosensor arrays address the need for rapid, sensitive, and specific screening for multiple pollutants at the site of sample collection. The biosensor arrays have several very significant advantages for such applications: (1) The number of analyte which can be detected simultaneously can be expanded as need dictates and specific analyte become available. (2) The biosensor arrays and tracer reagents are reusable if no target agent binds to the array surface. This feature significantly decreases the cost and operational burden for the user and simplifies automation for extended monitoring applications. (3) The biosensor array is simple to use. It is easily portable for first responder applications. The insertion of the sensor array, tracer reagents and samples is very simple with no requirement for alignment operations by the user. (4) The biosensor array is a low-cost system which can be made even more cost effective with mass production. (5) The

biosensor array can be easily adapted for continuous monitoring operations by integration with a computer-controlled sampler to format automatic analytical system. Because of these advantages, more and more biosensor arrays are applied in varied areas including environmental monitoring. An overview of the applications for environment by using biosensor arrays, which are not mentioned in this review, are listed in Table 1.

Target	Biosensor array type	LOD	Reference
Herbicide Subclasses	Array of photosystem II mutants	$3x10^{-9}$ mol/L	[64]
Metal ions	All-solid-state potentiometric biosensor array	10^{-6} mol/L	[65]
Microbial species	Electrochemical biosensor array	Not given	[66]
Escherichia coli	Quantum dot-based array	10 CFU/mL	[67]
Bio-hazardous agents	Planar waveguide biosensor array	5×10^{5} CFU/mL	[68]
aflatoxin B_1	NRL biosensor array	0.6 ng/g	[69]
Ochratoxin A	Antibody-based biosensor array	3.8 ng/g	[70]
Odour	Colorimetric biosensor array	Not given	[71]
Escherichia coli	Antimicrobial Peptides based biosensor array	10^{7} CFU/mL	[72]
Yersinia pestis F1	Antibody-based biosensor array	25 ng/mL	[73]
Bacillus globigii	Antibody-based biosensor array	10^{5} CFU/mL	[74]
Shigella dysenteriae	Antibody-based biosensor array	5×10^{4} CFU/mL	[75]

Table 1. Applications of biosensor arrays for environmental monitoring

Despite the high number of biosensor arrays under development and the amount of research literature on this area, few practical systems are currently enjoying market acceptance for environmental applications. The Naval Research Laboratory (NRL) biosensor arrays are the most successful type of biosensor arrays that have found commercial application not only in environmental monitoring but also in the monitoring of bio-molecular interaction events in general. Biosensor arrays still need more research and development in order to achieve the stability, sensitivity, specificity, and versatility that will attract confidence of potential users, especially for biotechnology and environmental applications.

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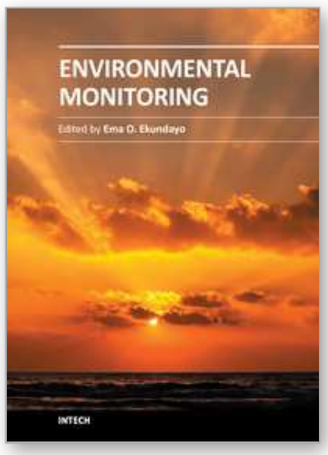

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