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TREND

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Optical microarray biosensors

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Introduction

Most biosensors are dedicated to specific tasks, such as glucose measurement, and are therefore not transferable between applications. In addition, some of them lack the specificity to be useful without any confirmation analysis. This leads us to the current situation, where, with an increasing number of parameters and the need for quantification, other analytical techniques, such as chromatography, are more attractive. To overcome these limitations, multianalyte biosensors have been developed. Most of them are based on the parallelization of conventional biosensors. However, the increasing technical expenditure with increasing number of parameters limits this approach to only a few channels. Despite the tremendous success of microarray technology, which is based on pioneering work performed in the 1980s [1, 2, 3] and at the beginning of the 1990s, the application of microarrays in biosensing is a quite recent development.

Comparison of microarray biosensors

In 1999, one of the first biosensors based on microarray technology [4–6] was presented in the field of environmental analysis (Table 1). The system was designated a ''parallel affinity sensor array (PASA)'', a designation which emphasizes both the spatial array pattern and the fully parallel readout, which differentiated it from older systems which achieved multianalyte detection by sequential collection of data and subsequent evaluation. The PASA systems consist of a series of precision syringe pumps, a flow cell and a cooled (high-sensitivity) CCD camera for parallel measurement. The assays are analogous to immunoassays performed in microtitration plates; however, chemiluminescence detection is used instead of a chromogenic substrate. Peroxidase-catalyzed chemiluminescence (luminol/hydrogen peroxide) shows very good sensitivity, and, more relevant in this context, it leads to a very simple and efficient optical system, which needs neither wavelength-selective elements (like filters or spectrometers) nor light sources (like lasers) for excitation, in contrast to fluorescence. The PASA system has been extended in several steps and applied in different fields, including allergy diagnosis (specific and total IgE detection in human blood serum [8]) and the high-speed detection of antibiotics in milk [9]. In the latter instrument, an assay time of less than 5 min was achieved for ten analytes. Other systems [10, 11, 12, 13] were based on total internal reflection fluorescence (TIRF). Mobile systems designed for field deployment were applied in the field of biological warfare agents and explosives. Up to nine analytes could be determined simultaneously [12]. A second system based on TIRF was demonstrated for pesticide analysis in water [14]. Recently, the parallel detection of three analytes was presented [15]. In Table 1 some systems are compared with their key specifications. Multianalyte capillary systems often rely on several capillaries, which are coated with different reagents [16, 17, 18]. In 2002, however, a biosensor with four different reagent zones in one capillary was demonstrated [19].

Multivariate and univariate microarrays

Single analyte immunoassays always show some degree of cross-reactivity and so are not able to deliver analyte concentrations. Only analyte equivalents are available.

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Table 1 Comparison of microarray biosensors based on [7]

System	Principle	Analyte group	Number of analytes	Reagent spots/sample (max)	Cycle time (approx., min)	Sensitivity (μg/l)		Year/ references
PASA	CCD/CL	Environmental contaminants	5	800	30	0.2	(TNT)	1999 [4]
PASA	CCD/CL	Triazine herbicides	4	200	120	0.04	(Atrazine)	1999 [5]
PASA	CCD/CL	Environmental contaminants	5	396 (1600)	30	0.02	(Terbuthylazine)	1999 [6]
NRL	CCD/TIRF	Biological warfare simulants	3	6[a]	20	10	(SEB)	1999 [10]
NRL	CCD/TIRF	Biological warfare simulants	6	6[a]	20	100	(SEB)	2000 [11]
NRL	CCD/TIRF	Biological warfare simulants	9	36	15	100	(SEB)	2002 [12]
NRL	CCD/TIRF	TNT	1	6[a](36)	12	20	(TNT)	2002 [13]
RIANA	PD/TIRF	Pesticides	3	3[a]	15	0.03	(Atrazine)	2002 [14]
NCSR	PM/capillary	Pesticides	4	4[a]	35	0.04	(Mesotrione)	2002 [19]
PASA	CCD/CL	Allergy diagnosis/IgE	24	96	60	0.2	(PLA2)	2003 [8]
PASA	CCD/CL	Antibiotics	10	140	< 5	0.2	(Cloxacillin)	2004 [9]
RIANA	PD/TIRF	Water contaminants	3	3[a]	15	0.05	(Isoproturon)	2004 [15]

[a]One-dimensional "array" (line)
CL: Chemiluminescence; *CCD*: Charge-coupled Device; *NCSR*: National Centre for Scientific Research "Demokritos"; *NRL*: Naval Research Laboratory; *PASA*: Parallel Affinity Sensor Array; *PD*: Photodiodes; *PLA2*: Phospholipase A2; *PM*: Photomultiplier; *RIANA*: River Analyzer; *SEB*: *Staphylococcus aureus* Enterotoxin B; *TIRF*: Total Internal Reflection Fluorescence (waveguide); *TNT*: Trinitrotoluene

Without any further information, equivalents cannot be converted into concentrations. That is one of the main reasons why many immunoassays are used for screening purposes only, and need some confirmation analysis (for instance, by HPLC). Even if "generic antibodies" (group-selective antibodies) are used, only semi-quantitative results can be expected, because most cross-reactivity patterns are not "flat" enough. Microarrays and other highly parallel techniques offer a powerful approach for overcoming this problem. By using overlapping cross-reactivities, even complex mixtures may be resolved and quantified. This chemometric approach could be termed a "multivariate microarray" [20]. For this purpose a larger set of antibodies (or other reagents) directed against one analyte group is needed. Today the production of antibodies is still the limiting step and so this approach is rarely used. It is much easier to set up a "univariate microarray" with many antibodies with essentially non-overlapping cross-reactivities. The design of such a system is relatively straightforward; only some unexpected cross-reactivities have to be dealt with, for example by the exchange of an antibody. In "univariate microarrays" all of the assays can be evaluated independently. There is, in fact, no difference to simple immunoassays. Unfortunately, the advantages of "multivariate microarrays", such as substance identification and quantification, are lost in "univariate" systems. However, they can be used for screening purposes, similar to their microtitration plate counterparts. Their main benefit is their ability to screen against many compounds in one run, and so they are perfectly suited for economic non-target analysis.

High-speed microarrays

Microarrays are acknowledged to be powerful research tools. Unfortunately, many microarray experiments are very slow and typically need several hours or sometimes even days to be completed. Consequently, the application of microarrays to fast sensing applications was mostly disregarded. However, taking into account that many immunoassays are performed in a kinetic mode anyway, it is obvious that microarrays can be favorably used for fast applications, too. Currently, assay durations of less than 5 min can be achieved [9]. Even shorter assay times may be conceivable. Alternatively, encoded microsphere assays based on flow-cytometer technology might be used for the development of fast multianalyte biosensors [21]. However, their on-line potential is still largely unexplored.

Outlook

Recent work in the field of microarray biosensors shows that there is much potential for novel multianalyte applications. However, techniques for the fast and economic production of high-quality antibodies are still needed to fulfill the needs of the growing field of protein microarrays. Unfortunately, recombinant techniques have not lead to the expected breakthrough, as yet. In addition, problems with intellectual property may have delayed the widespread and economic use of existing antibodies in microarrays. Furthermore, fierce patent battles have surrounded microarray technology, some of which are not completely settled, and these may have deterred many companies from entering the market. Another problem to be solved is the complexity involved in manufacturing protein microarrays.

However, the additional dimensions in the designs of such arrays in relation to standard immunoassays provide almost infinite opportunities for researchers to solve their analytical problems. The ability to overcome cross-reactivity problems, hence the potential for quantitative

results, the excellent scalability, and the high speed of such microarray biosensors makes them a powerful analytical technique.

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