



Diagnostic and analytical applications of protein microarrays

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DNA microarrays have changed the field of biomedical sciences over the past 10 years. For several reasons, antibody and other protein microarrays have not developed at the same rate. However, protein and antibody arrays have emerged as a powerful tool to complement DNA microarrays during the past 5 years. A genome-scale protein microarray has been demonstrated for identifying protein-protein interactions as well as for rapid identification of protein binding to a particular drug. Furthermore, protein microarrays have been shown as an efficient tool in cancer profiling, detection of bacteria and toxins, identification of allergen reactivity and autoantibodies. They have also demonstrated the ability to measure the absolute concentration of small molecules. Besides their capacity for parallel diagnostics, microarrays can be more sensitive than traditional methods such as enzyme-linked immunosorbent assay, mass spectrometry or high-performance liquid chromatography-based assays. However, for protein and antibody arrays to be successfully introduced into diagnostics, the biochemistry of immunomicroarrays must be better characterized and simplified, they must be validated in a clinical setting and be amenable to automation or integrated into easy-to-use systems, such as micrototal analysis systems or point-of-care devices.

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The concept of microarray technology emerged some 15 years ago. In its most simple form, a microarray experiment is a downsizing of many different biochemical solid-phase reactions. The surface-bound component serves as a sensor by capturing specific molecules in the sample (FIGURE 1). The surface-bound molecule is referred to as the probe and the captured molecules are referred to as the target. Many types of these solid-phase reactions exist that have been reformatted to the microarray platform. One of the most widely used applications today is perhaps the measurement of global gene expression in cells using DNA molecules as probes, which bind to specific nucleic acid targets in solution [1–4]. The ability to screen for up to 10^6 hybridization reactions using a sample volume below 100 μ l illustrates the parallel nature of microarray technology. This high-throughput analysis is enabled by miniaturization of the assay. Miniaturization itself increases the sensitivity

of the assay, provided that the spots in the microarray are sufficiently small [5]. However, many microarrays produced today have overly large spot sizes, indicating that the effect of miniaturization on sensitivity has not yet been fully achieved. Interestingly, miniaturization of capture area had little or no impact on sensitivity in a flow system in which the target availability was not limited by diffusion [6].

Comparison between DNA & protein microarrays

In comparison with DNA microarrays, microarray assays with a protein component are much more difficult to produce (TABLE 1), and they lack possibilities for target amplification. A DNA array is fabricated by first identifying specific probes using homology searches in databases. The probes are then synthesized either on the chip directly, enabling a high number of probes per chip [7], or off-chip and then spotted using a robot. If the sample is

limited or the molecular species is rare, nucleic acids can be amplified using, for example, PCR or T7-mediated amplification prior to detection, allowing differential gene expression measurements of a few cells [8]. To obtain a genome-wide proteome protein array containing nearly all 6300 proteins encoded by *Saccharomyces cerevisiae*, as described by Zhu and coworkers [9], is far more labor intensive than synthesizing 6300 DNA probes and immobilizing these onto a microarray. The proteome microarray was produced by cloning 6300 complementary DNAs in-frame with a glutathione S-transferase–His tag, expressing these clones in yeast and purifying the proteins prior to spotting. For immunoarrays (using antibodies as probes), the cumbersome process of producing specific antibodies towards the protein antigens precedes the fabrication of the array. Detection of analyte binding to protein microarrays is usually achieved by fluorescence. Immunoarrays have demonstrated a detection limit in the picomolar range [10], if the target is directly labeled, or in the subpicomolar range, if the target is detected using a sandwich assay [11]. These detection limits are sufficiently low for many clinical applications, but may be insufficient for analyzing small samples in research settings. Alternatively, immunoreactions can be measured using scanning electron microscopy with a limit of detection (LOD) as low as 5 pM [12], colorimetric detection of nanogold particles

with a LOD of 250 pM [13], or using bio-bar-codes in which the immunoreaction is detected by PCR with a LOD of 3 aM of protein [14]. A clear advantage of using protein or immunoarrays is that it can be a very simple assay for the end user compared with nucleic acid detection and characterization using DNA microarrays. Protein and small molecule detection can be as simple as mixing a sample with a buffer containing detection antibodies and incubating the mixture with the microarray (FIGURES 2B & C). By contrast, target preparation of nucleic acids is in most cases a lengthy process, involving a nucleic acid preparation step and an enzymatic reaction for labeling and/or amplification prior to hybridization. The washing, detection and quantification procedures are very similar for protein and nucleic acid detection.

Definition of immuno- & protein microarrays

In principle, any solid-phase immunoassay can be transferred to the microarray platform provided that no crossreactivity exists. In this review, protein and immunoarrays will be classified with regard to what is immobilized on the surface of the microarray. Examples will be given when a protein is immobilized on the surface to capture an antibody or other proteins (FIGURE 2A), and when immobilized antibodies are used to capture proteins such as in a classical sandwich enzyme-linked immunosorbent assay

(ELISA) (FIGURE 2B). The last group of microarrays is those that measure small molecules using competitive immunoassays (FIGURE 2C). Apart from these types of microarrays, it is expected that other types of probes will be available for future analytical development.

Antibody (immuno-) microarrays

Antibody arrays

A rather specialized application of antibody arrays was demonstrated by Wildt, who printed 18,000 recombinant antibody clones as an array on a filter support and then simultaneously screened all these clones for reactivity to a particular antigen [15]. However, in most cases, an antibody array is used to measure targets in solution. Using 146 arrayed antibodies as probes, Sreekumar and coworkers were able to identify specific proteins in a cancer cell line that were regulated by ionic irradiation [16]. Knezevic and coworkers studied protein expression in cancer tissues by producing protein extracts from cellular subpopulations within the tissue using laser capture microdissection. The protein extracts were subsequently analyzed on an antibody array and specific differences in protein expression were identified

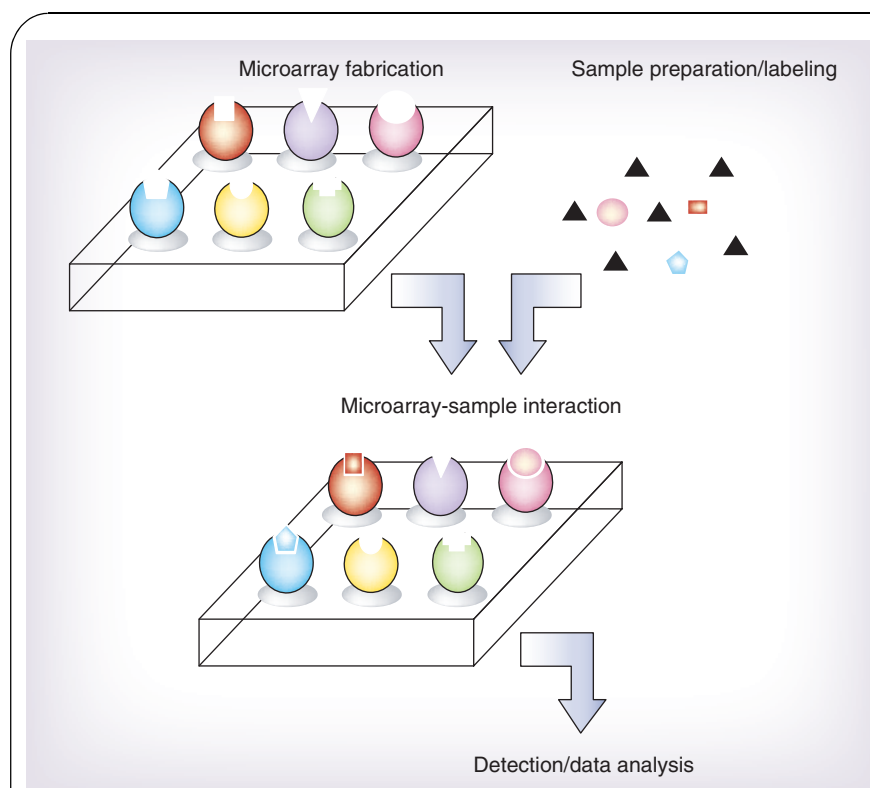


Figure 1. Principle of a microarray assay. The microarrays are fabricated by attaching the probe molecules in an ordered fashion, hence a certain probe has a determined coordinate in the microarray. The labeled target is incubated together with the microarray and after removal of unbound sample the microarray is scanned to detect interactions. The identity of the molecules in the sample is given by the location of the fluorescent signal in the microarray.

Table 1. Comparison between protein and DNA microarrays.

Protein arrays	DNA arrays
Target recognition by receptor binding (H-bonds, ionic bonds, van der Waals, hydrophobic/hydrophilic)	Target recognition by hybridization (H-bonds)
No correlation between the probe's linear amino acid sequence and the probe's specificity	Robust linear target-probe sequence hybridization correlation
No target amplification possible (signal only)	Target and signal amplification methods available
Difficult production or synthesis of antibody, proteins or receptors	Inexpensive and established DNA probe synthesis
Function is dependent on orientation of the probes	Simple immobilization
Biochemically unstable arrays	Biochemically stable arrays

between different cellular subpopulations [17]. This work demonstrates the advantage of microarrays in terms of their parallel nature and miniaturization, since a corresponding analysis would be difficult to perform with a standard ELISA. The protein variant of a cancer classifier was demonstrated by Miller and coworkers. This group could identify proteins showing a different expression pattern in prostate cancer patients as compared with healthy control subjects [18]. These results indicate that antibody arrays can be used in diagnostics, although further studies on larger patient groups must be performed to verify the applicability of microarrays in the clinic. A critical feature of microarray-based diagnostics is whether they are sensitive enough to detect early signs of illness (e.g., in cancer). A direct comparison of an antibody array that detected 16 different cytokines in a sandwich assay with a corresponding sandwich ELISA assay demonstrated that the microarray-based assay had very little cross reactivity, equal sensitivity (4–12 pg/ml), dynamic range and variance (1–23%) to ELISA [11]. However, careful selection of surface chemistry can increase the sensitivity of these ELISA-like microarray assays down to 1–10 fg/ml of target protein [19].

Pathogen detection

Pathogen detection utilizing parallel antibody protein recognition has been described for different bacteria, such as *Bacillus globigii*, *Salmonella enterica*, Staphylococcal enterotoxin B antigen (SEB) or *Yersenia pestis* F1 antigen, using an optical waveguide system [20–22]. In this assay format, biotinylated capture antibodies were immobilized onto avidin-coated surfaces and the sample was subsequently added via a microchannel system. The

reported detection limit after 15 min incubation was approximately 8×10^4 colony-forming units, 1 ng/ml for the SEB antigen and 25 ng/ml for the F1 antigen. Both the SEB and F1 antigen were detectable at concentrations below their relevant physiologically encountered concentrations. In comparison with simultaneous ELISA testing, detection by protein array demonstrated an approximately tenfold higher detection level, expressed as a percentage of positive *B. globigii* or SEB assays.

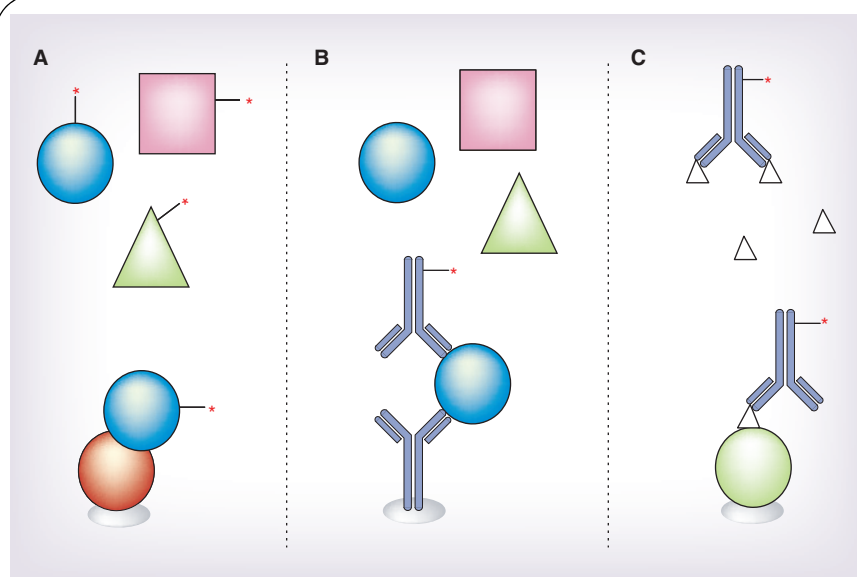


Figure 2. Different examples of protein-based microarrays. (A) Direct protein assays. In this example, the probes consist of proteins that specifically recognize the target protein in a mixture. In such assays, the target is, in most cases, labeled by fluorescence prior to the interaction with the microarray. **(B)** Sandwich immunoassay in which the target protein is immobilized to the surface via a specific antibody. The reaction is subsequently detected using a secondary antibody binding to the target protein. Alternatively, the target can be directly labeled as shown in **(A)**. This is particularly useful as many different kinds of antibody–antigen interactions are analyzed on the array. **(C)** Competitive immunoassay is often used to detect small molecules since small molecules can only bind one antibody, which makes a sandwich assay impossible. Instead, the target, which is identical to the target being analyzed, is immobilized on the surface and competes for antibody binding occurring in solution. Large amounts of target in solution will thus result in low signals and conversely low target concentration in solution results in high signals. Alternatively, the antibody can be immobilized and the sample is spiked with a labeled target molecule that competes with the unlabeled target from the sample for the surface-bound antibodies.

Cell surface antigen analysis using immunoarrays

Taking advantage of the fact that living cells express a variety of protein antigens on their surface, Belov and coworkers fabricated an immunoarray for differentiation of leukocytes [23,24]. The microarray consisted of more than 50 antibodies towards different cluster of differentiation (CD) antigen. Adding patient cells to the arrays, distinct phenotypes could be resolved, and the patients could be classified into distinct types of leukemia. In the future, this technology may complement standard flow cytometry for cancer cell classification.

Drawbacks of antibody arrays for expression profiling

A serious drawback of protein and antibody microarrays (and ELISA) is the potential immobilization of not only the desired target from a complex sample but also any protein that is binding to that target in solution [25]. Such complexes can yield signals that can be falsely higher in an assay. Mass spectrometry analysis of the microarray spot could rapidly identify such impurities. Furthermore, mass spectrometry analysis could also identify minute changes in the proteins, such as phosphorylation, which can be difficult to discriminate using antibodies only, in a microarray assay. Such systems exist, such as Ciphergen's surface-enhanced laser desorption/ionization (SELDI) instruments, which couple an affinity array to a time-of-flight mass spectrometer. However, the sensitivity of these instruments is in the femtomole range compared with fluorescence, which is easily detected in the subattomole range using a standard fluorescence microarray scanner. The coupling of affinity arrays to SELDI avoids some of the detection limit problems since analytes can be enriched to certain spots.

Protein arrays

Protein arrays for antibody diagnostics

Printing protein solutions on a microarray surface was initially performed for protein expression microarrays identifying bacterial clones expressing a recombinant protein, which was then detected by antibody binding [26]. By printing autoantigens, Joos and coworkers demonstrated that the protein microarray technique could be used for screening of patients for circulating autoantibodies, enabling fast and highly sensitive autoimmunity diagnosis [27]. Similarly, allergens were microarrayed for the detection of specific immunoglobulin (Ig)G and E antibodies to diagnose and profile an allergy [28]. The microarray-based analysis of autoantibodies and allergen reactivity were both found to correlate well with ELISA and clinical data results [27–29].

Proteome analysis

The impressive work carried out by Zhu and coworkers to immobilize nearly all proteins encoded by *S. cerevisiae* in a microarray demonstrated that a complete proteome microarray could be fabricated. This proteome array can be probed with specific proteins, DNA or small molecules to search for protein–protein, protein–DNA and protein–small molecule interactions [9,30]. These types of arrays are a fast and efficient tool that can replace one- and two-hybrid screenings in yeast, which are much more

time consuming. However, a drawback of these arrays is that the probes are proteins produced under certain culture conditions and the proteins can thus be subjected to certain secondary modifications that may influence the results on target binding.

Small molecule arrays

Pesticides

Quantitative measurements of small molecules was achieved by Belleville and coworkers [31,32] and Han and coworkers [13], utilizing direct detection of small molecules with monoclonal antibodies in a competitive microarray (FIGURE 2). The detection limit of the microarray assay was down to 1 pg/ml (5 pM) for fluorescent detection, which is far below the European Union detection limit of 100 pg/ml. Similar low detection limits (0.6 pg/ml) on competitive immunoassay of pesticides has been obtained by Tschmelak and coworkers [33]. This group employed a portable fluorescence detection system (river analyser) rather than large microarray scanners for detection. This demonstrated a practical setup for distributed use. Furthermore, the assay takes only minutes to perform but requires a 900 µl sample, in contrast with microarray-based pesticide analysis, which only uses 10 µl samples. The low detection limit obtained in these assays is extremely relevant for monitoring physiologic concentration of drugs and their metabolites in samples such as blood or urine. Including more than one analyte, Belleville and coworkers demonstrated the ability to measure several, even closely related, analytes in parallel without loss of resolution or increase in detection limit [31].

Antibiotics

Circumventing the need for fluorescently labeled antibodies for microarray detection, Knecht and coworkers utilized the enzymatic generation of light using secondary horse radish peroxidase-labeled antibodies [34]. Using ten different specific monoclonal antibodies directed against ten different antibiotics, this group were able to detect and quantify the analytes simultaneously. The detection limits of the assay ranged from 120 ng/ml (cephapirin) to 32 µg/ml (neomycin). Also improving the process time was the operation of the microarray in a flow system, enabling very short incubation times (70 s) resulting in a total assay time of less than 5 min. The demonstration and use of chemiluminescence for online signal generation and detection is a significant step forward in microarray chip technology, demonstrating a new method for systems development.

It is without doubt that microarrays will be used in the future not only as a powerful research tool but also as a diagnostic tool, as illustrated by the examples above. The reasons for this include that microarrays:

- Are highly sensitive
- Require little starting material due to miniaturization
- Can be implemented into micrototal analysis system (µTAS) devices
- Enable rapid multiplex analysis of a wide variety of analytes ranging from small molecules to cells

However, many protein microarrays are not likely to be regarded as straightforward as DNA microarrays, and a likely development is outlined in TABLE 2. DNA arrays are much simpler to produce and have been available for 10 years. They offer expression profiles at the transcript level and high-throughput microarray-based mutation detection. Compared with DNA microarrays, the development of similar diverse protein arrays is hampered by the lack of adequate parallel methods for probe design/synthesis, target amplification and binding conditions. Despite the immediate meager outlook, some protein array applications are more relevant than their DNA counterparts since, for example, the protein expression profile or detection of protein variants more closely reflects the *in vivo* situation, suggesting that protein microarrays in diagnostics may be more widely used in the future than DNA microarrays.

Commercialization

The commercialization of DNA microarrays has shown that only very few companies can bring forward a solution that will gain acceptance. Most notably is the implementation of

Affymetrix arrays and equipment. However, making a similar proteome-wide system for protein expression profiling is unlikely to happen any time soon, at least not for protein arrays based on antibodies, due to prohibitive high development costs. Closest today is a product from BD Biosciences, containing a set of 500 specific antibodies towards human antigens.

Interestingly, the global yeast proteome array, which was developed by Zhu and coworkers, is now available in a commercial variant from Invitrogen [9]. However, one array costs approximately US\$1200, making its use in routine diagnostics less interesting. Producing a human proteome variant will require addressing the host organism ultimately influencing the protein modifications, and thereby the target binding properties. Antibody arrays do not have the same problem, since an antibody can be directed to both the modified and unmodified version of a protein, which may indicate that antibody arrays are more suitable for diagnostics than protein arrays. However, the proteome array is a very efficient research tool for high-throughput screening of protein–protein, protein–DNA and protein–drug interactions and cannot be replaced by antibody arrays.

Table 2. Summary: 5-year view.

Technology	Current status	5 years
Binder selection	Difficult antibody/proteins synthesis	Antibodies/protein and other synthetic binders such as aptamers, single chain Fab fragments, phages
Binder specificity	Medium to high	High (more alternatives)
Immobilization chemistries	Limited knowledge	Well characterized
Absolute quantification	Possible	Standard
Sensitivity	Equal to ELISA	Better than ELISA
Detection/readout	Expensive	Less expensive, more suppliers
Micrototal analysis systems solutions	Possible	Yes
Proteome arrays		
Commercial proteome arrays	One (yeast)	Maybe for human and some other organisms
Price	High	High
Complexity of assay protocol/readout	High	High
Immunodiagnostic arrays		
Commercial immunoarrays	Very few with limited sets of probes	Larger sets of probes, possibly more companies offering tests
Noncommercial immunoarrays (locally developed)	Few	Many
Price	High	Lower
Complexity of assay protocol/readout	High	Low
High-throughput diagnostics	None/limited	Few
Distributed diagnostics	No	Doubtful
ELISA: Enzyme-linked immunosorbent assay.		

These products for screening a sample for a large number of targets at the same time may not meet the requirements of diagnostic arrays since the described microarrays are expensive and provide much more information than is really necessary. It is thus likely that for diagnostics we will see an increasing number of small focused microarrays (<50 antibodies). This will keep the fabrication costs down and also ensure the physical dimensions of the microarrays are small enough to integrate into microtiter plate wells. These small focused arrays will contain antibodies towards known and accepted biomarkers that have been found using a variety of proteomic methods. There are several companies that offer similar microarray products, for example, Zyomyx, which have a human and a murine immunoarray towards cytokines. Multiplexed immunoassays are also provided by Luminex and BD Biosciences. However, these systems are based on the immobilization of probes to a bead with a particular fluorescent characteristic. An array of probe beads can be mixed together with the sample and, after target binding, the array of beads is resolved using flow cytometry. Interestingly, some of the multiplexed bead-based immunoassays have been approved by the US Food and Drug Administration (FDA), strongly indicating that other multiplexed immunoassays such as microarray-based assays can obtain FDA approval.

Expert opinion & five-year view: future requirements for protein microarrays in diagnostics

Biochemistry & analytical capabilities of protein microarrays

The understanding of immobilizing proteins to surfaces by microarraying is still rudimentary and will require more effort in the coming years. This is essential for building diagnostic devices as the orientation of the protein/antibody, the substrate itself and spotting conditions, heavily determines the performance of the assay [9,19,35–37]. Performance is not only determined by LOD, it should also be highly reproducible, preferably with a variation of less than 10%. For this to be achieved, large efforts must be made to find suitable microarray assay conditions. Parameters such as surface immobilization chemistries, spotting procedures (contact vs. noncontact printing), probe concentration on the surface and incubation conditions (e.g., temperature, stirring and buffers) could affect both sensitivity and reproducibility. Proteins are expressed at widely different concentrations and a large, all-purpose diagnostic array should be able to handle a large dynamic range. Interestingly, Lee and coworkers have demonstrated a protein microarray substrate that has a dose response range between 1 fg/ml and 100 ng/ml (ten orders of magnitude) of target molecules captured by an immobilized antibody, suggesting that different proteins expressed at highly different levels could be analyzed on the same chip [19].

Detection systems

The detection systems based on fluorescence are sufficiently sensitive for most applications and it is likely that fluorescence, although expensive in terms of equipment, will remain the most used detection method. Alternative methods such as scanning electron microscope [12] and bio-bar-codes [14] will not be

easily implemented into diagnostics since they are either laboratory intensive or require expensive equipment. However, integrating an optical detection of chemiluminescence could be within reach of a 5-year perspective [34].

Alternative probes

The perfect probe should be specific, stable and function efficiently on a surface. Furthermore, it should be easy to select and produce. In essence, a probe can be anything provided that it fulfils these requirements. The perfect probe does not currently exist. Antibodies are difficult to isolate but are highly characterized. It is likely that other types of probes will emerge in the future, such as DNA or RNA aptamers. These types of probes are isolated very fast and are inexpensive when compared with antibodies and still have high specificity with binding constants in the micromolar range for small molecules and in the picomolar range for proteins [38–40]. Finally, such nucleic acid-based binders are stable reagents (at least DNA) and there exist a number of well-characterized immobilization chemistries to attach DNA to glass and plastics. DNA aptamer microarrays are thus likely to become more robust compared with protein- or antibody-based microarrays.

Automation & μ TAS

Many of the microarrays used today, as well as DNA microarrays, are solely focused on screening purposes in a research setting. Fundamentally, these microarrays are too difficult to use today since they require a large number of laboratory and analysis steps. For the microarray to be used as a diagnostic tool in wider perspective, every step needed for an analysis must be automated. Microarrays have been spotted into microtiter plate wells allowing hundreds of analytes to be analyzed at the same time [41]. The use of microtiter plates also enables easy integration of microarray technology with standard laboratory automation equipment for high-throughput analysis. Beckman Coulter's A2 platform fulfils most of the requirements of a high-throughput immunoarray device, using microarrays printed in microtiter plate wells with charge-coupled device-based fluorescent reader and automated analyses of the data. Although no clinical assay is commercially available for this platform as yet, it is expected that within the next 5 years it should be possible to build clinical applications around such a solution. Still, this platform and similar solutions must be operated by highly skilled personnel in centralized laboratories, in contrast to the envisioned μ TAS devices, which will also be intended for distributed analysis. Such devices may fully automate analysis, including sample preparation, target binding, washing and detection. For example, if a pesticide analysis could be transferred to a μ TAS platform, a do-it-yourself test could determine the water quality at home for a very low cost. Similarly, rapid pathogen detection and identification at a general practitioners office could make the treatment of bacterial infection more efficient. A μ TAS device for immuno- and protein arrays is thus desired. Such devices could also include more

advanced functions such as automatic generation of a dilution series for standards. As demonstrated by Haab and coworkers, only a fraction of 115 antibodies tested could bind a dilution series of antigens that corresponds to the theoretical expectations, indicating that standard curves are highly critical for quantification [10]. Furthermore, unlike many ELISA assays, microarray-based analyses do not have any mixing during incubation and movements of target molecules to the microarray spots is only dependent on diffusion. Mixing on microarrays has shown that the binding kinetic is increased up to fivefold and that more homogenous reaction conditions are obtained over the microarray [42,43]. The authors expect that such μ TAS devices will exist, at least at

research stage, in 5 years' time. Protein arrays are exceptionally suitable for integration into μ TAS systems since they require no heating and the analyte is often in the pico- to micromolar range, which can be detected using fluorescence, chemiluminescence or colorimetric methods [13,21,33,34,44].

Concluding remark

Ultimately, the success of microarrays in diagnostics will be determined by economics and not by the current limitation of the technology, as the numerous examples above demonstrate. However, it will take several years for protein and antibody arrays to establish themselves in both clinical and distributed settings.

Key issues

- Protein/antibody microarrays can be highly parallel and sensitive detection systems.
- Protein arrays can be absolutely quantitative.
- Antibodies/proteins can detect a wide range of analytes ranging from small molecules to whole cells.
- Before adaption of protein arrays, several technology issues must be addressed such as micrototal analysis system solutions, biochemistry, probe specificity and alternative type of binders that are less expensive to develop and to produce.
- In a 5-year view, small focused protein and antibody microarrays (20–50 immobilized antibodies or proteins) will become increasingly used at specialized laboratories for research, quality control and clinical laboratories.
- A few immunodiagnostic tests with the complexity and cost of a pregnancy test will be commercially available.
- A yeast proteome array is commercially available today and a human counterpart may be commercially available within 5 years. No immunoarray for global human proteome analysis is likely to be available.
- Microarrays must be validated as a diagnostic tool in large-scale clinical studies.

References

Papers of special note have been highlighted as:

- of interest
- of considerable interest

- 1 DeRisi JL, Penland L, Brown PO *et al.* Use of a cDNA microarray to analyze gene expression patterns in human cancer. *Nature Genet.* 14(4), 457–460 (1996).
- 2 DeRisi JL, Iyer VR, Brown PO. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278(5338), 680–686 (1997).
- 3 Lockhart DL, Dong H, Byrne MC *et al.* Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nature Biotechnol.* 14(13), 1675–1680 (1996).
- 4 Schena M, Shalon D, Heller R *et al.* Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. *Proc. Natl Acad. Sci. USA* 93(20), 10614–10619 (1996).
- 5 Ekins R, Chu F, Biggart E. Multispot, multianalyte, immunoassay. *Ann. Biol. Clin. (Paris)* 48(9), 655–666 (1990).
- 6 Sapsford KE, Liron Z, Shubin YS, Ligler FS. Kinetics of antigen binding to arrays of antibodies in different sized spots. *Anal. Chem.* 73(22), 5518–5524 (2001).
- 7 Pease AC, Solas D, Sullivan EJ *et al.* Light-generated oligonucleotide arrays for rapid DNA sequence analysis. *Proc. Natl Acad. Sci. USA* 91(11), 5022–5026 (1994).
- 8 Luo L, Salunga RC, Guo H *et al.* Gene expression profiles of laser-captured adjacent neuronal subtypes. *Nature Med.* 5(1), 117–122 (1999).
- 9 Zhu H, Bilgin M, Bangham R *et al.* Global analysis of protein activities using proteome chips. *Science*. 293(5537), 2101–2105 (2001).
- **First description of a proteome microarray. Almost all open reading frames of yeast were expressed, purified and printed onto a microarray and functionally tested by protein and phospholipid interactions.**
- 10 Haab BB, Dunham MJ, Brown PO. Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. *Genome Biol.* 2(2), (2001).
- **Describes the sensitivity and linearity of protein and antibody microarrays probed with directly labeled antibodies or proteins.**
- 11 Knight PR, Sreekumar A, Siddiqui J *et al.* Development of a sensitive microarray immunoassay and comparison with standard enzyme-linked immunoassay for cytokine analysis. *Shock* 21(1), 26–30 (2004).
- **Shows that microarray-based sandwich assays have similar sensitivity and reproducibility to standard enzyme-linked immunosorbent assays.**
- 12 Levit-Binnun N, Lindner A, Zik O, Eshhar Z, Moses E. Quantitative detection of protein arrays. *Anal. Chem.* 75, 1436–1441 (2003).
- 13 Han A, Dufva M, Belleville E, Christensen CB. Detection of analyte binding to microarrays using gold nanoparticle labels and a desktop scanner. *Lab. Chip.* 3(4), 329–332 (2003).
- 14 Nam JM, Thaxton CS, Mirkin CA. Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. *Science* 301(5641), 1884–1886 (2003).
- 15 de Wildt DM, Mundy CR, Gorick BD, Tomlinson IM. Antibody arrays for high-throughput screening of antibody-antigen interactions. *Nature Biotechnol.* 18(9), 989–994 (2000).

- 16 Sreekumar A, Nyati MK, Varambally S *et al.* Profiling of cancer cells using protein microarrays: discovery of novel radiation-regulated proteins. *Cancer Res.* 61(20), 7585–7593 (2001).
- **Demonstrates that antibody microarrays can be used as a proteomic tool.**
- 17 Knezevic V, Leethanakul C, Bichsel VE *et al.* Proteomic profiling of the cancer microenvironment by antibody arrays. *Proteomics* 1(10), 1271–1278 (2001).
- 18 Miller JC, Zhou H, Kwekel J *et al.* Antibody microarray profiling of human prostate cancer sera: antibody screening and identification of potential biomarkers. *Proteomics* 3(1), 56–63 (2003).
- **Demonstrates serum protein profiling using antibody arrays and the identification of novel marker for prostate cancer.**
- 19 Lee Y, Lee EK, Cho YW *et al.* ProteoChip: a highly sensitive protein microarray prepared by a novel method of protein immobilization for application of protein–protein interaction studies. *Proteomics* 3(12), 2289–2304 (2003).
- 20 Rowe CA, Scruggs SB, Feldstein MJ, Golden JP, Ligler FS. An array immunosensor for simultaneous detection of clinical analytes. *Anal. Chem.* 71(2), 433–439 (1999).
- 21 Rowe CA, Tender LM, Feldstein MJ *et al.* Array biosensor for simultaneous identification of bacterial, viral, and protein analytes. *Anal. Chem.* 71(17), 3846–3852 (1999).
- 22 Taitt CR, Shubin YS, Angel R, Ligler FS. Detection of *Salmonella enterica* serovar typhimurium by using a rapid, array-based immunosensor. *Appl. Environ. Microbiol.* 70(1), 152–158 (2004).
- 23 Belov L, de la Vega O, dos Remedios CG, Mulligan SP, Christopherson RI. Immunophenotyping of leukemias using a cluster of differentiation antibody microarray. *Cancer Res.* 61(11), 4483–4489 (2001).
- **A microarray of antibodies towards cluster of differentiation antigens is used to phenotype leukemia cells. These arrays can complement flow cytometry analysis.**
- 24 Belov L, Huang P, Barber N, Mulligan SP, Christopherson RI. Identification of repertoires of surface antigens on leukemias using an antibody microarray. *Proteomics* 3(11), 2147–2154 (2003).
- 25 MacBeath G. Protein microarrays and proteomics. *Nature Genet.* 32(Suppl), 526–532 (2002).
- 26 Lucking A, Horn M, Eickhoff H *et al.* Protein microarrays for gene expression and antibody screening. *Anal. Biochem.* 270(1), 103–111 (1999).
- 27 Joos TO, Schrenk M, Hopfl P *et al.* A microarray enzyme-linked immunosorbent assay for autoimmune diagnostics. *Electrophoresis* 21(13), 2641–2650 (2000).
- **Demonstrates that antigens can be arrayed and used to diagnose patients for autoimmune diseases.**
- 28 Hiller R, Laffer S, Harwanegg C *et al.* Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J.* 16(3), 414–416 (2002).
- 29 Jahn-Schmid B, Harwanegg C, Hiller R *et al.* Allergen microarray: comparison of microarray using recombinant allergens with conventional diagnostic methods to detect allergen-specific serum immunoglobulin E. *Clin. Exp. Allergy* 33(10), 1443–1449 (2003).
- 30 Hall HA, Zhu H, Zhu X *et al.* Regulation of gene expression by a metabolic enzyme. *Science* 306(5695), 482–484 (2004).
- 31 Belleville E, Dufva M, Aamand J *et al.* Quantitative microarray pesticide analysis. *J. Immunol. Methods* 286(1–2), 219–229 (2004).
- **Demonstrates a microarray-based small molecule quantification method with sensitivity down to 5 pM.**
- 32 Belleville E, Dufva M, Aamand J, Bruun L, Christensen C. Quantitative assessment of factors affecting the sensitivity of a competitive immuno-microarray for pesticide detection. *BioTechniques* 35, 1044–1051 (2003).
- 33 Tschmelak J, Proll G, Gauglitz G. Ultra-sensitive fully automated immunoassay for detection of propanil in aqueous samples: steps of progress toward sub-nanogram per liter detection. *Anal. Bioanal. Chem.* 379(7–8), 1004–1012 (2004).
- **A portable detection system for sensitive detection of small molecules.**
- 34 Knecht BG, Strasser A, Dietrich R *et al.* Automated microarray system for the simultaneous detection of antibiotics in milk. *Anal. Chem.* 76(3), 646–654 (2004).
- 35 Lee C, Kim S, Kim BG. Improvement of protein stability in protein microarrays. *Biotechnol. Lett.* 24(10), 839–844 (2002).
- 36 Angenendt P, Glokler J, Sobek J, Lehrach H, Cahill DJ. Next generation of protein microarray support materials: evaluation for protein and antibody microarray applications. *J. Chromatogr. A* 1009(1–2), 97–104 (2003).
- 37 Peluso P, Wilson DS, Do D *et al.* Optimizing antibody immobilization strategies for the construction of protein microarrays. *Anal. Biochem.* 312(2), 113–124 (2003).
- 38 Yang Q, Goldstein IJ, Mei HY, Engelke DR. DNA ligands that bind tightly and selectively to cellobiose. *Proc. Natl Acad. Sci. USA* 95(10), 5462–5467 (1998).
- 39 Hermann T, Patel DJ. Adaptive recognition by nucleic acid aptamers. *Science* 287(5454), 820–825 (2000).
- 40 Famulok M. Oligonucleotide aptamers that recognize small molecules. *Curr. Opin. Struct. Biol.* 9(3), 324–329 (1999).
- 41 Mendoza LG, McQuary P, Mongan A *et al.* High-throughput microarray-based enzyme-linked immunosorbent assay (ELISA). *BioTechniques* 27(4), 778–780, 782–776, 788 (1999).
- 42 Liu R, Lenigk R, Druyor-Sanchez R, Yang J, Grodzinski P. Hybridization enhancement using cavitation microstreaming. *Anal. Chem.* 75(8), 1911–1917 (2003).
- 43 Yuen P, Li G, Bao Y, Muller U. Microfluidic devices for fluidic circulation and mixing improve hybridization signal intensity on DNA arrays. *Lab. Chip.* 3(1), 46–50 (2003).
- 44 Delehanty JB, Ligler FS. A microarray immunoassay for simultaneous detection of proteins and bacteria. *Anal. Chem.* 74(21), 5681–5687 (2002).

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