

New frontiers in microarray technology development

Editorial overview

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Within the past decade, microarray-based assays have moved from being technology-driven to application-oriented high-output assay systems. The basic principles of microarray technology were already described in the early 1980s by Ekins' Ambient Analyte Theory (Ekins RP: **Multi-analyte immunoassay**. *J Pharm Biomed Anal* 1989, 7(2):155–168). The driving force behind this theory was the quest for increased sensitivity in the determination of low concentrations of diagnostically important substances, such as hormones. However, developments within the field of microarray technology have been driven by the urgent demand within the field of genomics to provide global analytical tools to process large amounts of information. This could only be accomplished by testing for all possible analytes simultaneously ("Massive parallel testing", 2002, "Chipping Forecast II." *Nat Genet*, 32(supplement):461–552). The initial format developed by Schena and colleagues was simply spotted sets of cDNAs immobilized in a microarray format that were able to hybridize to fluorescently labeled RNA (Schena *et al.*: *Science* 1995, 270(5235):467–470). They could analyze the expression level of approximately a 1000 genes at the RNA level, a significant accomplishment at the time. Fast-forward about a dozen years and the term 'microarray' can be found in every area of molecular biology and microarray-based technologies are entering routine applications (see recent review by Hoheisel J: *Nat Rev Genet* 2006, 7:200–210). Currently, a scientist can take advantage of a wide variety of array platforms containing probes for whole transcriptome analysis, millions of single nucleotide polymorphisms, fragments of genomic DNA, antibodies, cell lysates, purified proteins, tissue sections, and embedded cells. As important as the development of the basic platforms have been, the growth of bioinformatic and statistical approaches that provide the ability to understand and mine the vast amounts of information generated have been equally important and have matured in parallel (for an introduction see review by Quackenbush J: *N Engl J Med* 2006, 354(23):2463–2472).

Although it may not be possible for those unfamiliar with the historical aspects of microarrays to appreciate, at this juncture it is not possible to review all microarray technologies and applications. We have placed our emphasis on several up and coming uses of microarrays. Innovative protein array applications and technologies are discussed in three papers in order to give the reader an appreciation for the breadth and technological diversity of the area. Microarray analysis of genomic integrity, with an emphasis on cancer, is reviewed, including the development of biomarkers. Once potential biomarkers are elaborated, they need to be further validated and two reviews are focused on application of tissue and cell microarrays. As

microarrays are no longer just a research tool, we have included a review on the assessment of data quality and technical performance, which is particularly important as regulatory decisions, and use in diagnostics becomes the standard. Finally, in two papers we take a brief look at the informatics approaches and statistical best practices that are absolutely required to permit sufficient confidence and bring meaning to these very large data sets. In total, these reviews should give the reader a sense of the larger microarray field and the technological advances, particularly with protein arrays, as well as some best practices to keep in mind.

Michael Taussig and colleagues have reviewed several technologies that are applicable to the preprogrammed production of protein microarrays. Overcoming the limitations of printing proteins directly, which requires the upfront cloning, expression, and purification steps, is a major objective of the field. The described approaches (e.g. PISA, NAPPa) permit 'on demand' protein array production as they utilize printed nucleic acids to produce proteins directly on the arrays via coupled transcription and translation reactions. Localized attachment of the resulting proteins to the surface of the arrays is accomplished in a variety of ways that are described. The authors point out several advantages, one of which is the fast development time from cloning of a new gene to expression on the array, for example, for interaction analysis.

The paper by **Leming Shi and colleagues** reviews the development of the Microarray Quality Control (MAQC) project, in which they have been integral contributors and organizers. The authors explain the rationale behind the studies and some of the conclusions till date, which will have direct effects on the use of microarray data by the FDA and in the development of microarray-based diagnostics. Many disparate procedures are available to normalize and standardize RNA microarray data and **Shi** provides some guidance on current best practices and how the MAQC project will contribute to the further development of microarray analysis methods.

Mark Basik and colleagues review tissue microarrays (TMAs), a microarray application in which hundreds of tissue cores from diseased and normal samples are arrayed and interrogated with antibodies or other probes specific for particular proteins. **Basik** poises the question, 'with so many putative biomarkers being developed from other array technologies, how are they going to be validated?' TMA technology is presented as a viable way to accomplish this goal and the authors review additional uses of TMAs including correlation with clinical pathology, disease prognosis, and characterization of target distribution. The reader will also find comments on the limitations of TMAs and discussion of TMA scoring and quantitation issues as well as related applications, for example, cell arrays.

Microarray data presents a wealth of opportunities to ask new questions as well as a myriad of new statistical issues that one needs to be aware of in order to reach appropriate conclusions. **Richard Simon's** review focuses on several key areas in which particular attention is needed: study design, how to address false positive issues inherent in data sets with tens of thousands of gene measurements across hundreds of samples and class prediction. In each section, the author encourages best practices and provides practical examples of what to do and not to do in order to increase the success of microarray experiments.

Sophia Hober and colleagues describe the generation of protein expression atlases to complement the many RNA-based expression databases that are available. The authors review the cloning and selection of antigens and high-throughput antibody production efforts. They also demonstrate how these antibodies are being further qualified with protein arrays and then used to characterize the expression pattern of human proteins in a wide variety of normal and diseased tissues. The use of both TMAs and cell arrays are illustrated. There is a particular focus on their own initiative, the 'Human Protein Atlas', for which quantitated results for thousands of proteins are available on the web.

Anne Kallioniemi reviews the power of array-based comparative genomic hybridization (aCGH) to analyze genetic alterations, which are commonly observed in cancer cells. These genetic alterations are associated with tumor progression, therapy response, or patient outcome. aCGH enables a global analysis of copy number aberrations and identification of putative target genes. Using oligonucleotide arrays increases the resolution of a CGH and permits a precise definition of aberration boundaries and breakpoints. The current potential of aCGH is presented, as well as the demand for further functional analyses of the actual contribution of putative target genes to cancer status and progression.

Within the past few years protein microarray-based research has moved from technology development into application-oriented research. Protein microarrays have been successfully used in a variety of applications like the identification, quantification, and functional analysis of proteins in basic and applied proteome research. **Satoshi Nishizuka and Brett Spurrier** review reverse-phase lysate microarrays for systems biology applications. Reverse-phase arrays allow the quantitative measurement of complex cellular reactions within the protein signaling networks using well-defined cell culture assays. The authors provide an overview of the current approaches used to generate relevant data sets for the elucidation of protein networks.

Thomas Werner provides an overview of current bioinformatics applications for pathway analysis of microarray data.

For the past several years bioinformatic analyses of microarray data have gone beyond simple clustering and statistical analysis. The power of microarrays allows one to analyze changes within whole transcriptomes in the context of a single experiment. Sophisticated bioinformatics tools reveal immediately the list of upregulated and down-regulated genes. However, one has to be aware that most genes serve multiple context-dependent functions and that some changes in mRNA level are secondary to the initial treatment effects. [Werner](#) gives an overview of the different bioinformatics tools available, which enable examination of relevant pathways and signaling networks.

[Carl Borrebaeck and Christer Wingren](#) review the direct labeling approach of antibody microarrays. Starting with proof-of-concept designs, protein microarrays have become established high-performing technology platforms for the analysis of nonfractionated complex proteomes. Robust protocols have been designed for direct labeling of whole proteomes compatible with a sensitive fluorescent-based detection. Successful approaches implemented in the field of DNA microarray technology like general standard operating procedures and bioinformatic standards are discussed in the context of antibody microarray data analysis.