



## MICROARRAY APPROACHES IN PROTEOMICS

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**ABSTRACT:** Proteomics is one of the fastest growing researches. It analyzes proteins to yield more direct understanding of function and regulation of genes. The most important challenge in proteomics is determining the function of every isoform of each protein. Recently, numerous advances in high throughput protein production and microarray surface technologies have been developed. The microarray format provides a robust and convenient platform for the simultaneous analysis of thousands of individual protein samples under highly specific conditions. The microarrays have broad applications for both discovery and quantitative analysis. Currently, they have applications in drug discovery, drug screening, biomarker identification and molecular profiling of cellular and biological material.

**Keywords:** Microarray, Approaches, Proteomics.

### INTRODUCTION

Microarray technology is usually used for gene expression profiling. It provides a practical method for measuring the expression level of thousands of genes simultaneously. (1, 2) About 10 yr before microarray technology became a widely used tool, the concept of it was first published in the late 1980s and was termed a “multianalyte immunoassay,” for analyzing an array of antibody–antigen interactions (3) This technology is a powerful platform for biological exploration (4). Gene expression profiling of cancers represents the largest research category using microarrays and appears to be the most robust approach for molecular characterization of cancers (4, 5) Such gene expression data allow us to identify genes which are expressed in a given cell type under a particular condition and time, to identify key players or target genes in signaling pathways, to recognize new targets of drugs, to find molecular markers in disease diagnosis (6, 7)

Depending on the type of probes used, microarray systems are classified as oligonucleotide , cDNA, protein, peptide, glycan, lipid microarrays .(4)

This review will be focused on how microarray technology is aiding proteomics for diagnosing of diseases. At the first different types of microarrays will be presented and then discussed about benefits of this approach in biomarker discovery.

### MICROARRAY APPROCHES

#### Oligonucleotide Microarrays

A typical DNA microarray-based method plays a valuable role in high throughput sequence analysis and is less time-consuming (4, 8, 9). Oligonucleotide microarray is a method for rapid mutation analysis of selected gene sequences, and is effective in sequence analysis, diagnosing genetic diseases and gene polymorphism studies(4, 10). It can be used for gene expression, mutation, SNP (single nucleotide polymorphism) and genotyping analyses as well. Oligonucleotide microarrays consist of a hybridization slide spotted with oligonucleotides ranging in length from 16-70-mer. signals mutations or SNP can be detected by discriminating between

perfectly matched and mismatched. Oligonucleotide microarrays is a high sensitivity method and reliable genetic devices in terms of point mutation detection, which simplifies detecting mutations.(4)

### **cDNA Microarrays**

cDNA microarrays are usually used for analyzing gene expression at the one time. It consists of a slide spotted with cDNA probes ranging from a few hundred to 1,000 bp. (4, 11). cDNA spots are usually made by PCR-amplified sequences from bacterial libraries, onto glass slides (4, 12). cDNA microarrays comprise relatively long DNA molecules immobilized on a solid surface and are mostly used for large-scale screening and expression studies. Unlike Oligonucleotide microarrays which can be used both in mutation-genotyping and gene expression analysis, cDNA microarrays can only be used in gene expression analysis,(4, 5).

### **Glycan Microarrays**

Glycan modifications play crucial roles in the function of many proteins. With glycan arrays study in cell interaction and for characterizing glycan-binding proteins, including antibodies are becoming available.(13-17) Carbohydrate arrays have helped identify autoantibodies such as in Crohn's disease and Hodgkin's lymphoma.(18, 19) Glycosylation of proteins is important in tumor malignancy, so with better characterization of glycans, the development of tumor vaccine can be achieved (20, 21)

### **Lipid Microarrays**

Lipids are important in the pathogenesis of autoimmune diseases, as well as several microbial diseases. They can be immobilized on microarrays for the study of biology and disease such as multiple sclerosis. Lipid microarrays can help discover key factors which leading us to the development of better treatments or preventions for diseases.(22)

### **Peptide Microarrays**

Peptide arrays are much easier to produce and array onto microarrays than whole proteins. They can be synthesized chemically on the microarray "chip," without the need for live transcription and translation machinery. One significant benefit of peptides is that many peptides can be screened simultaneously for antibody interactions relatively easily using phage display(23). Peptide antigens can be spotted or synthesized on microarrays to evaluate their usefulness in identifying biomarkers and disease-specific antibody profiles. Several groups have recently taken advantage of phage display combined with microarray technology to identify autoantibody profiles of different cancers.(24-26)

### **Protein Microarrays**

In their basic form, a protein microarray, also known as a protein chip, is a solid support (typically glass) on which thousands of different proteins (e.g., antigens, antibodies, enzymes, substrates, etc) are immobilized in discrete spatial locations, forming a high density protein dot matrix.(27-30) The immobilized material may be heterogeneous or homogeneous in nature, and may consist of cell or phage lysates, body fluids, an antibody, body fluid, or recombinant/expressed proteins (27-29, 31). These molecules are then queried by probing with an analytic specific Molecule that is coupled to a second signal-generating molecule. The signal can be from a chemiluminescent, colorimetric, fluorescent, radiometric or electrochemical read-out. In theory, the resulting signal intensity of each spot is a proportional to the quantity of applied tagged molecules bound to the bait molecule. Then the spot pattern of image is captured, analyzed and correlated with biological information.

In the literature three classes of protein microarrays are made: a) antibody arrays also known as forward phase arrays (FPPA), b) sandwich arrays, and c) reverse phase protein arrays (RPPA) . (32, 33).

In the FPPA formats, the analytes are captured by a capture molecule . Each spot on an array contains one type of immobilized antibody or bait protein. Each array is incubated with one test sample which represents a specific treatment condition, and multiple analytes from that sample are measured simultaneously. Cellular lysates or serum samples are common probes and the analyte of interest is directly labeled, typically with a fluorescent molecule .The forward phase array permits the simultaneous analysis of multiple analytes present in one sample. (34, 35).

The sandwich array employs a two-antibody system for binding and detecting the protein of interest. One antibody is required to bind the analyte of interest to the substratum and a second antibody binds a different epitope on the same molecule, which functions as a detection molecule (31, 35). Unlike forward phase arrays, Reverse phase protein microarrays (RPPA) are comprised of an immobilized cellular lysate that is probed with a primary antibody as is shown in figure 1. The term “reverse phase” refers to the fact that the analyte (antigen) is immobilized as a capture molecule, rather than immobilizing an antibody as the capture molecule (27, 35-38). The RPPA technology is designed for quantitative, multiplexed analysis of specific protein modifications (phosphorylation, glycosylation, processing or some combination thereof), or total forms of cellular proteins from a limited amount of samples. (33, 35, 39-41)

## PROTEOMICS AND MICROARRAY

Proteomic offers powerful technologies for protein separation and identification.(29, 42, 43) In order to find key defects in a diseased tissue and approach the ultimate goal, patient tailored therapies, proteomics emphasizes on the analysis of biological samples like biopsy specimens and body fluids .(29, 39, 44) Most proteomic technologies have technological limitations because of analytical sensitivity. when the analysis is of the very small tissue samples, these limitations are dramatically revealed. Most tissue biopsy specimens contain only a few thousand cells. Widely used proteomic platforms require relatively large numbers of cells for any significant results – many orders of magnitude greater than the quantity procured during a clinical biopsy (29, 45, 46). New technologies are needed that can utilize microscopic amounts of cellular material. The RPPA has demonstrated the critical ability to measure the level of phosphorylation of signaling pathways using small numbers of human tissue cells from biopsy specimens. (27, 29, 32, 47-50) because the RPPA employs denatured lysates, antigen retrieval, a significant limitation for tissue arrays, antibody arrays, and immunohistochemistry technologies, is not problematic, so the RPA offer unique advantages over other array based platforms such as tissue arrays (29, 51) or antibody (forward phase) arrays(29, 52, 53). The ability to generate quantitative data from minute quantities of cellular input without a two-site assay also enables a marked improvement in reproducibility, sensitivity and robustness of the assay over other techniques (27, 29).

## MICROARRAY AND BIOMARKER

Proteomic technology greatly facilitates the comprehensive analysis of protein expression and post-translational modifications. Most of the cancers are characterized by particular alterations in certain signaling pathways, and the identification these alterations could facilitate the selection of an effective therapy targeted at that specific pathway.(33) Profiles of protein expression and post-translational modifications are powerful tools for identifying specific therapeutic targets in cancers.(33) PTMs ranging from the simple conjugation of a phosphate group to the complex addition of ubiquitin can drastically alter the function of a protein and they are essential for the proper function of many proteins. (54, 55). Despite the importance of these modifications in human diseases, identifying which proteins are modified in cells on a proteome-wide scale has proven technically difficult. (55-57) To overcome these technical limitations, protein microarrays as a platform for profiling PTM activities are used.(55)

The human proteome consists of 20 500 non-redundant proteins . Antibody-based proteomics plays a basic role in the cancer biomarker discovery. The use of antibodies for protein profiling on a global scale facilitate the system examination of the cancer proteome. Antibody-based approaches can be used in conjunction with a wide range of high-throughput assays such as immunohistochemistry (IHC) on tissue microarrays (TMAs) and protein microarrays(33, 58-60).

TMA is a method of assembling multiple tissue samples from an individual paraffin block to simultaneously evaluate multiple biomarkers using IHC. TMA can potentially become an accelerated molecular method for using a large-scale library of antibodies to examine the association between molecular biomarkers and clinical outcomes (33, 61). When proteins on a protein microarray are viewed as potential antigens, it becomes a powerful tool in biomarker identification. The principle is straightforward: when an auto-antibody presented in human sera associated with a human disease (e.g., auto-immune diseases) recognizes a human protein spotted on the array, it can be readily detected with fluorescently labeled anti-human immunoglobulin antibodies (e.g., anti-IgG) and a profile of auto-antibodies associated with a disease thus created, providing a rapid approach to identifying potential disease biomarkers.(30)

## CONCLUSION

Genomic profiling using gene expression arrays has shown considerable potential for the classification of patient populations (35, 47). Nevertheless, transcript profiling, by itself, doesn't provide a complete picture of the dynamic molecular network for a number of clinically important reasons (35, 62). RNA transcripts also provide little information about protein-protein interactions and the state of the cellular signaling pathways (35, 63). The applications described above specially protein microarrays are most useful in basic research during recent years (30, 60).

RPPA technology is specifically developed for clinical proteomics and clinical applications. (27, 29, 39) RPPA may be used to monitor changes in protein phosphorylation over time, before and after treatment, between disease and non-disease states and responders versus non-responders, allowing one to infer the activity levels of the proteins in a particular pathway in each patients. (29)

Although microarray technology is still at a relatively early stage of development, it has become obvious that the protein microarray platform is a suitable tool for the large-scale, high-throughput biology, especially in the areas of profiling PTMs and in analysis of signal transduction networks and pathways (30, 64, 65).

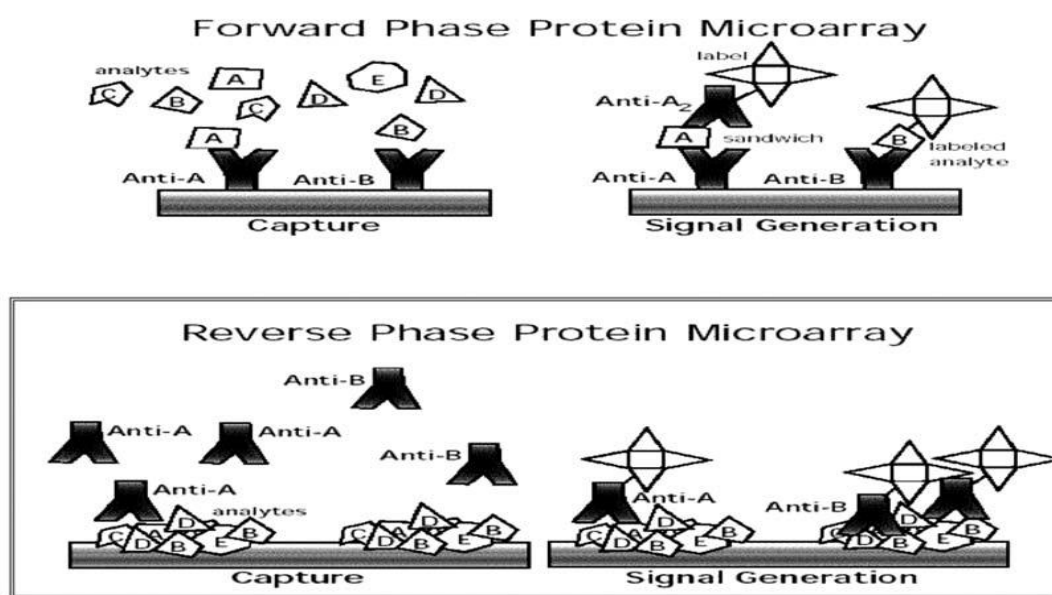


Figure 1. Example format for forward (top) and reverse (bottom) phase protein microarrays. (29)

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