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Review Articles

# FUTURE OF SCIENCE IN ONE CHIP: THE MICROARRAY

**NAZMA I, V. B. KOTWAL AND R. T. THUBE**

*Allana College of Pharmacy, K.B. Hidayatullah Road, Azam Campus, Pune − 411001*
*E-mail: nazma13@yahoo.co.in*

***ABSTRACT***

*The sequencing of the human genome and other ongoing sequencing projects have accelerated the pace of gene discovery and caused the identification of thousands of new genes. Microarrays provide the ability to measure the expression of thousands of genes in parallel. In particular, microarrays have applications in genome annotation, they contribute to the improvement in disease understanding and can be used in the drug development pipeline to improve selection of biological targets and lead compounds. This review explains in brief the principles underlying DNA microarrays, traditional microarray techniques and highlights the uses to which they are being put to investigate the molecular basis of different diseases and disorders.*

**Keywords:** Microarray, Formats, Types, Applications, Limitations.

## INTRODUCTION

A microarray can fit in the palm of our hands, its miniature size belies the extraordinary power and this technology is having a powerful impact on many domains of science[1]. One of the most powerful new technologies to emerge from the age of genome sequencing comes from this tiny microarray slide, which carry the capacity to comparatively scan genome and wide patterns of gene expression for any organism with a sequenced genome. It was first developed in research laboratories during examining the model organisms like yeast and mustard. Microarrays are being used worldwide and encompass fields like cancer biology, drug development and evolutionary biology of microbes too. Already the basics of array technology are being adapted to include the diagnosis of disease predisposition in humans, rapid identification of specific viruses in infected humans and protein analysis through the burgeoning field of proteomics[2].

Although the concept of using microarrays can be traced back 25 years to the introduction of southern blot[3], modern microarray analysis was introduced in 1995 by a Stanford university research team led by Pat Brown and Ron Davis. Their seminal publication was titled, 'quantitative monitoring of gene expression patterns with a complementary DNA microarray'[4]. Although the principle of nucleic acid hybridization is not new, microarrays have opened the way for the parallel detection and analysis of the patterns of expression of thousands of genes (currently about 20, 000−40, 000) in a single experiment.

## WHAT IS A MICROARRAY?

Microarrays are typically glass slides, on to which DNA molecules are attached at fixed locations. There are several synonyms for this technology - microarrays, DNA chips, gene chips, and DNA arrays - and there are no standard definitions for which type of microarray technology should be called by which name[5].

A higher throughput tool is needed to assess global gene activity (expression) simultaneously and this need led to the development of DNA microarrays in the early 1990s[6, 7]. A DNA microarray consists of a flat, solid substrate (typically glass) with an organic coating, typically an organo-functional group alkoxysilane. The coated glass is then grafted (by printing or in situ synthesis) with various known DNA probes at predefined locations, as shown in fig. 1. The DNA microarray can be considered as a glass-based, biological sensor that can contain over 30, 000 distinct, known probes at prespecified locations. This powerful, glass-based, biological sensor provides researchers with the ability to characterize the human genomic state fully with a single, miniature experiment. The array elements bind specifically to labeled

molecules that are 'targets', present in complex molecule mixture or sample generating signals that reveal the identity and the concentration of the interacting labeled species. The 'probe' (protein /cell/ tissue / small molecule/carbohydrate/cDNA) is immobilized directly or via an affinity tag (optional) on an organic layer that forms a thin film on the array substrate. Each array is comprised of a precise micrometer scale and a 2-d addressable grid of biological molecules immobilized on an organic thin film coated on a substrate[8].

The two most commercially successful DNA microarray companies, affymetrix and agilent use in-situ microarray fabrication processes. During in-situ synthesis, techniques such as combinatorial photochemistry (Affymetrix) or phosphoramidite coating supported by ink jet printing (Agilent) are used to build up unique DNA probe sequences at specific locations on a coated glass substrate surface[9]. Such in-situ processes can lead to more probe versatility, as the probes are synthesized directly on the coated surface, thus eliminating the need to work with large probe libraries, which have to be stored in microtiter plates.

## TYPES OF MICROARRAY EXPERIMENT

Microarrays can be used in two main ways, genotyping and the study of gene expression. Genotyping (analyzing the genomic DNA content of the pathogen) has been used to identify and characterize bacteria, viruses, parasites and fungi[10].

## STEPS IN A TYPICAL MICROARRAY EXPERIMENT

**1. Microarray fabrication** The term 'micro fabrication' refers to all techniques used for fabricating devices and systems on the micrometer scale[11]. Various traditional microarray techniques[12] applied are shown in Fig. 2.

**2. Target preparation** The sample molecules are labeled with fluorescent dyes such as cyanine-3 (which fluoresces with green color), cyanine-5 (which fluoresces with red color). Depending on the availability of the sample size and type, pretreatments like purification, extraction and amplification can be done[13].

**3. Hybridization and analysis:** The sample is made to react with the probe on microarray, that means; target molecules are hybridized with probes (Fig.3). After hybridization under stringent conditions and excitation, a scanner records the intensity of the fluorescence emission signals those are proportional to transcript levels in the biological samples. The microarray data is analyzed using specific software's like Generic that enables clustering of genes with similar expression patterns, assuming that they share common biological functions[14]. The Generic software computes the intensities of the two fluorochromes at each location and provides readout of the data as a spreadsheet linked to the image. The next step in the analysis pipeline is the image girding that allows mapping the location of the pixels representing each spot. The image is then processed (normalization background substraction and signal to noise calculation) and the software calculates the intensity values of each probe as well as the quantitative ratio of the fluorescence intensity levels between the two samples being compared. These values provide an indication of the relative amounts of given molecular target species in the samples[15].

**4. Biostatistical analysis of Microarray data** Microarray experiments generate a tremendous amount of data that can be integrated and analysed. The central analytical task here is to reduce the complexity of the datasets so that trends and structure are revealed and to group together the genes that are biological samples based on the similarity of their expression profiles. The fundamental assumption in the comparative analysis of transcriptomers is that coregulated genes often share common functions. Possible roles of genes of unknown function can be suggested on basis of their association with genes of known function. Similarly samples of unknown physiological status can be classified based on their association with samples of known physiological state[16]. There are numerous mathematical and statistical methods that can be brought to perform the analysis. The two main methods used are Principal Component Analysis (PCA) and Cluster Analysis[17, 18].

## DIFFERENT MICROARRAY FORMATS

Today many different chip formats to study the proteome have been developed. These include macro- and microarrays, which were spotted on filters and glass slides, chips bearing microwells, microfluidic and liquid-chips[19], as well as alternative formats, such as Surface-Enhanced Laser/Desorption Ionisation Technology (SELDI)20, and Surface Plasmon Resonance (SPR) [21].

## TYPES OF MICROARRAYS

**DNA microarrays:** The two main formats of DNA microarrays are cDNA microarrays and oligonucleotide microarrays[22]. The cDNA microarrays are manufactured using mRNA isolated from a cell. This mRNA is then reverse transcribed into cDNA. Each spot on the cDNA microarray is composed of a particular sequence of cDNA isolated from the organism. The second type of DNA microarrays, oligonucleotide microarrays are made from DNA sequences directly synthesized on to a slide. With this method there is more control over what sequences go on to the slide and what the size of the sequences are. The equipment to automate such complex DNA synthesis is extremely expensive but more powerful.

**Antibody microarray technology** In this type the important issue is the source of antibodies to be used on microarrays. Antibody fragments can be selected and produced using inexpensive media and purification methods for E. coli[23, 24]. To further increase the properties of selected antibodies, new strategies completely working in vitro have been developed using ribosomal display[25, 26] or mRNA–protein fusions[27]. At the same time other binding scaffolds than immunoglobulin domains like antibodies[28], fibronectin[29,] lipocalin[30]or repeat domains[31] are explored to meet the requirements associated with antibody array technology[32].

**Tissue Microarrays (TMAs)** Hundreds of cylindrical samples measuring up to 4 mm (usually 0.6 mm) in diameter are removed from formalin fixed or frozen tissues and are deposited into one recipient block[33.] Sections from such TMA blocks containing hundreds of different tissues are then placed on a glass slide. TMAs allow a high-throughput analysis of large numbers of well-characterized tissues by in situ methods. As such, TMAs allow a new dimension of molecular tissue analysis. Thousands of normal and diseased tissues can be analyzed in one project. With the number of genes implicated in cancer biology increasing rapidly, tissue microarrays offer an in vivo system to validate the results of microarray data[34.] For example breast tumors[35] and bladder cancer[36] can be analyzed for the over expression of a particular protein in hundreds of patients through the use of a single tissue microarray slide. TMAs are also used for toxicity and biological evaluation[37, 38].

**Protein microarrays** In protein microarray, instead of many copies of a particular segment of a gene on a spot, many copies of protein antibody or membrane protein occupy one spot. Such protein microarrays allow scientists to screen protein-protein interaction or identify protein targets of small molecules. Proteins are also very efficiently handled and produced by cDNA expression libraries using E. coli. To obtain proteins, that are toxic to their expression hosts, strategies involving in vitro transcription and translation systems have been developed[39]. Another generation of samples for protein microarrays works by the chromatographic separation of a crude mixture like cell culture extracts[40]. While investigators are still finetuning protein microarray production, there is enormous promise in protein microarrays as future diagnostic tools[41.]

**Small molecule microarrays** Nowadays drugs are often discovered based on their ability to bind to a particular protein. In order to accelerate this process, microarrays are now fabricated with hundreds of different small molecules or possible drugs adhered to a slide[42]. A protein target of interest is applied to the microarray and analyzed for attachment to any particular small molecule. If there is attachment, this small molecule can then be further analyzed for its effects and possible efficacy. In addition to possibility of facilitating drug discovery, the small molecules identified may also have effects that are helpful for basic biochemical research[43.]

**Bacterial artificial chromosomes (BAC's) microarray** An important aspect to BAC microarray is the issue of gene specificity[44]. Each BAC insert should contain only one human gene to maintain binding specificity at level of single genes. BACs have been used successfully for a technique called 'Comparative Genomic Hybridization' (CGH). CGH is a molecular cytogenetic method of screening for genetic changes. The alterations are classified as DNA gains and losses and reveal a characteristic pattern that includes mutations at chromosomal and sub chromosomal levels. This array format can be used to develop in vitro diagnostic devices[45].

**Carbohydrate microarray[46]** Carbohydrates cover the surface of practically all-living cells in the form of diverse glycoconjugates. There is a need to develop innovative approaches in order to identify the proteins in proteomes that interact with carbohydrates and to characterize the oligosaccharide sequence they bind to. The carbohydrate protein interaction participates in folding of nascent proteins in the endoplasmic reticu-

lum, targeting of newly synthesized lysosomal enzymes to lysosomes, activation and trafficking of leukocytes in mechanisms of inflammation and adhesion of microbes during infection[47].

## SOME UNIQUE APPLICATIONS OF MICROARRAY TECHNOLOGY

**Toxicology** Microarrays can help in determining the therapeutic index of a compound by helping to determine its potential toxic liabilities[48]. Microarrays are being applied to this area despite of considerable technical challenges[49], and they are a key component within the new field of research known as toxicogenomics. They also help in classifying toxicants[50].

**Disease characterisation** Microarray analysis has been used to provide an insight into schizophrenia51 and host–pathogen interactions are being investigated using this technology[52]. It is useful in classifying cancers based on their gene expression profiles; for example, acute leukaemias[53], breast cancers[54] and melanomas[55]. A good example was the classification of B-cell lymphomas[56].

**In infectious diseases** Gene expression can be analysed using microarrays in either pathogen or host, thus allowing investigation of both sides of the host-pathogen interaction. This technology is further explored for discovery of novel pathogens in epidemiological investigations, to differentiate between strains[57], to determine pathogenicity and virulence[58] for diagnosis and for organism identification[59]. Microarrays have also been used to advance our understanding of the genetic processes involved in immune cell development[60, 61] and how B and T cell immunity develops[62, 63]. It is also helpful in differentiating between infectious agents[64, 65], differentiate between the host response to extracellular and intracellular parasites and also between different intracellular parasites[66, 67,68].

## LIMITATIONS FOR USE OF MICROARRAYS

There are various significant bottlenecks for the extensive use of microarrays in clinical care[69], these are mentioned below.

**Cost** A DNA microarray has yet to become accessible to many research teams and hospitals due to their high cost and difficulty to use. A typical array can cost over 1000 dollars. Combined with the cost of RNA isolation and amplification, a single sample could cost up to 2000 dollars. Also the cost of obtaining a clean sample of RNA is high. The procedure of micro dissecting specimens is very expensive at the moment and limits a large number of studies. Also, when tissue is obtained, it is difficult to select just the diseased cells that one wants to study, as normal and mutant cells can be very close together and even overlap.

**Poor reproducibility of microarray results** There are potential difficulties due to technical reasons such as slide heterogeneity, printer-pin variation and spot size differences resulting from variability in the quantity of target DNA synthesized or spotted. These difficulties are generally overcome by the use of control target features included at different sites on the array. In addition, variation between slides is normalized using replicate data and bio-informatics software[70, 71]. The key is then to extract biological knowledge from these data.

**Lack of data / significant data** Lack of data is the result of high cost of microarray experiments and the need for a database that researchers may quickly access for past experimental results. A database would eliminate the necessity of obtaining expression profiles already researched by other teams.

**Intrinsic microarray properties** While the gene expression profile allows a whole genome approach to study the cell, there are other factors effecting cell phenotype. More over, some expressed genes do not lead to proteins, as downstream regulation can occur within the cell. That is why the study of proteomics is important.

**Surfaces and hardware** Since most of the hardware equipments were adopted from DNA microarray science, such as the microscope slide format and its surface chemistry, fluorescent detection, the spotting devices and scanners, many of these will have to be optimized to meet the requirements of different microarrays.

**New technology bugs** Many researchers still do not trust the data obtained from microarrays because of the high variability. Repeated studies seem to yield significant differences in the scale factor and present call percentages. These difficulties currently faced in the use of microarrays may be solved in near future with advances in techniques like laser capture microdissection, improved RNA extraction, amplification and data analysis.

**TABLE 1. A BRIEF SUMMARY OF TYPES OF MICROARRAYS AND THEIR APPLICATIONS**

| Sr. no. | Sample type | Interaction | Application |
|---|---|---|---|
| 1 | Antibodies | Antibody-antigenAntibody-RNA Antibody-DNA Antibody-cell surface Antibody-organelle | Epitope mapping, evolution, gene expression, genotyping - post-translational analysis and proteomics |
| 2 | Bacterial artificial Chromosomes (BAC) | DNA-protein, DNA- DNA, DNA-RNA | Evolution, genotyping, mapping, systemic analysis and comparative genomic hybridization. |
| 3 | Carbohydrates | Sugar-protein, sugar-antibody, sugar-receptor | Docking and Signaling |
| 4 | Enzymes | Enzyme-substrate, enzyme-effector, enzyme-inhibitor | Kinetics, substrate specificity and inhibitor in analysis |
| 5 | Oligonulcleotides | DNA-DNA, DNA-protein | Evolution, gene expression, genotyping, mapping, SNP mapping, SNP detection and two-hybrid analysis. |
| 6 | Proteins and Peptides | Protein-protein, Protein-RNA Antigen-antibody. Protein-DNA, protein-small molecule, protein-receptor | Epitope mapping, gene expression, genotyping, post translational analysis, proteomics and drug discovery. |
| 7 | Small molecules | Small molecule-protein | Binding kinetics and drug discovery. |
| 8 | tRNA, rRNA, mRNA | RNA-DNA, RNA-RNA | Genotyping, mapping, SNP detection, structure function analysis. |
| 9 | Whole cells | Receptor-hormone, receptor-antibody, sugar-antibody | Cell typing, secretion studies and clonal analsis. |

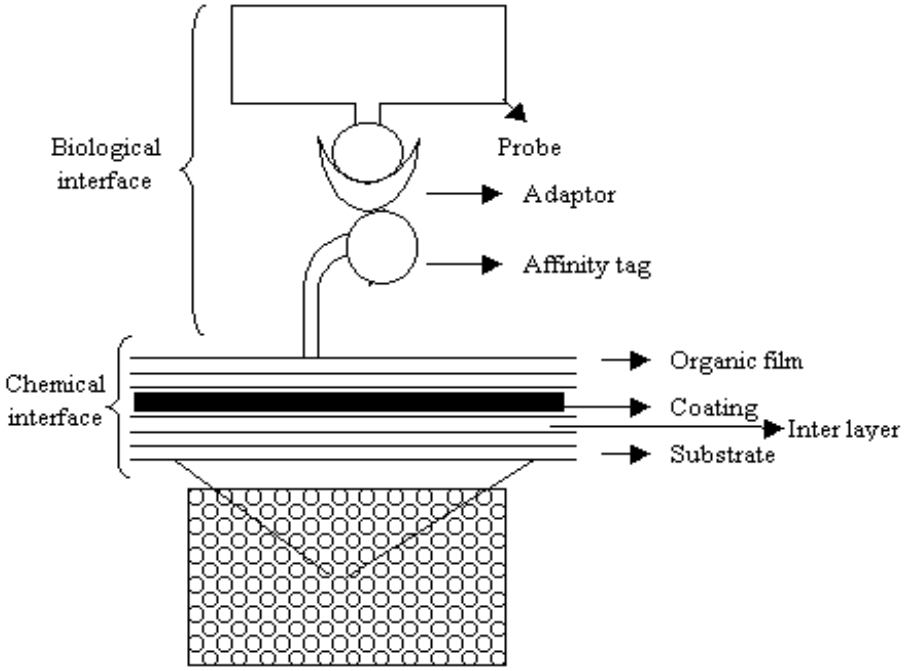


**Figure 1: Schematic representation of microarray architecture.**

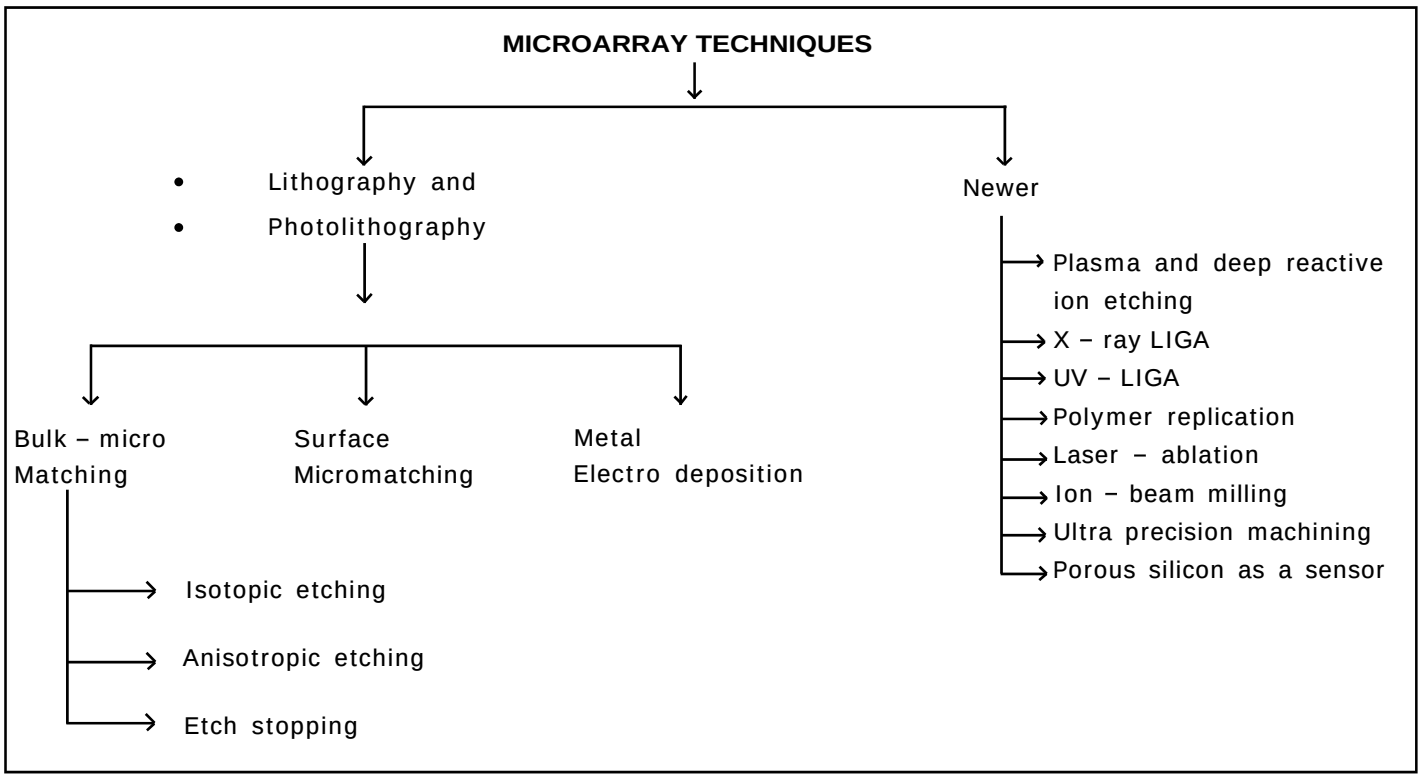


**Figure 2: Schematic representation of traditional microarray techniques.**

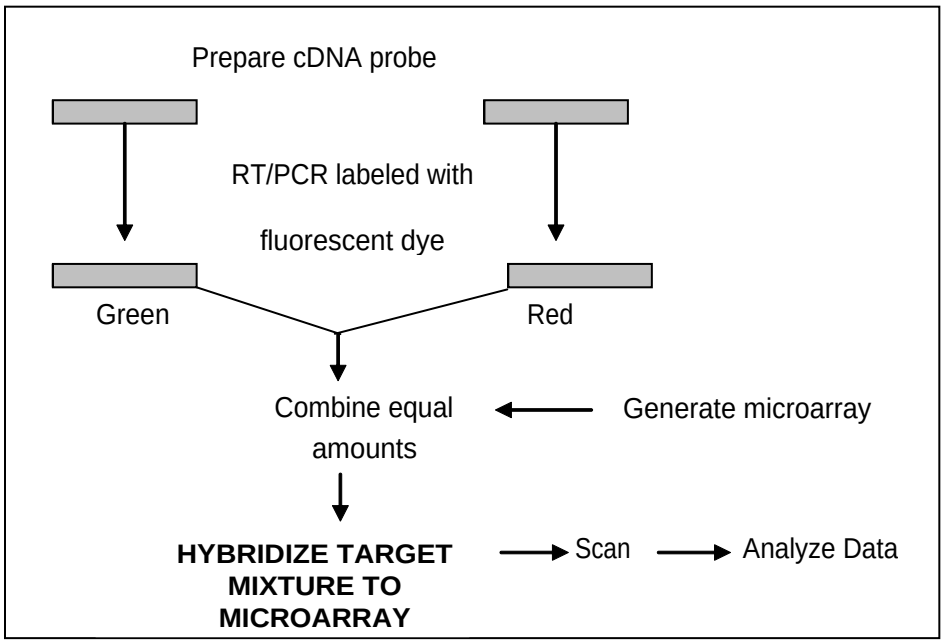


**Figure 3: Schematic representation of hybridization and analysis of Microarray data.**

## CONCLUSION

The use of microarrays to monitor gene expression is a rapidly evolving technology that continues to gain momentum. The contribution of cDNA microarrays, tissue microarrays, protein microarrays and small molecule microarrays to the understanding of the role of genetics in disease has been and will be incredible and immeasurable. In contrast to the traditional reductionist scientific approach, genome-wide surveys of gene expression are leading the biologist to a more holistic or 'systems' view of biology. This technology has significant impact on the direction of biological and medical research.

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