MICROARRAYS

1. Introduction

Microarrays are analytical devices that utilize a platform technology for performing hundreds to thousands of individual reactions or assays simultaneously. These include nucleic acid probe assays, in which specific deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequences are detected and quantified; immunoassays using capture antibodies to detect multiple analytes; and enzyme assays, for metabolic assessment of multiple substrates and/or compounds acting on the enzymes. While microarrays were invented to meet the need for analyzing thousands of different sequences in DNA and RNA samples, they are also being applied to the detection and analysis of proteins, polysaccharides, and lipids; and to study the interactions of biomolecules with drugs and other bioactive agents.

2. Technology

Microarrays represent the evolution of biosensors, which measure a single analyte, to a platform able to measure multiple analytes simultaneously (1). As

Table 1. **Comparison of Biosensors and Microarrays**

Component–function	Biosensors	Microarrays
substrates	glass, membrane, polymer	same
receptors	antibodies and other proteins, oligonucleotides, DNA, RNA	same
transducers	optical, electrochemical, piezoelectric, surface acoustic wave, thermal, etc	none
detection	transducer output	radioactivity; fluorescence, chemiluminescence scanning
data processing	integrated software	scanner software

shown in Table 1, the basic components of a microarray and a biosensor are the same (1,2). Both utilize a biospecific surface for analyte capture (affinity or indirect) with a transducer or detector for qualitative or quantitative detection. They differ in that microarrays can have up to 1 million detection sites/cm^2, can perform simultaneous, multianalyte detection, and have required the development of highly sophisticated scanners for detecting and reporting capture events. Biosensor detection and data processing is built into the unit.

The basic components of a mircoarray include the following: the substrate to which the detection molecules are immobilized, usually made of silica, glass, or a polymer; an active layer containing receptors (oligonucleotides, DNA, RNA, antibodies, and other biomolecules) for the target analyte(s) immobilized to the substrate that capture the analytes being detected and quantified; and, in some cases, an optical or electrochemical transducer to detect the binding events or reactions occurring between the active layer and analytes.

In those cases, where an integrated transducer is not present, detection of the receptor–analyte complexes is utilized radioisotope films, or fluorometric or chemiluminescent scanning instruments.

Microarrays are also being developed as components in microfluidic lab-on-a-chip and MEMS (microelectromechanical systems) devices that are able to extract, amplify or concerntrate, and identify multiple analytes. For example, an integrated silicone chip has been developed containing all the components necessary to analyze nanoloter-size DNA samples (3). The device integrates electrophoretic separation, DNA amplification, and fluorescence detection of concentrations of DNA as low as 10 ng/mL in 1–2 min.

3. Microarray History

The first successful microarrays were fabricated in the late 1980s using photolithography to make peptide and dinucleotide arrays on glass slides (4). In this approach, amino acids and nucleotides are added to a growing peptide or oligonucleotide chain by step-wise synthesis using linkers containing photochemically removable protecting groups. At each addition step, a mask is used to direct

deprotection of specific chain end groups, and a specific monomer (also containing a protecting group on one of its two linker sites) is linked to the deprotected end of the growing chain. In this manner, high density arrays are produced with specific, known nucleotide or amino acid sequences. This technology became the basis for the first microarray company, Affymetrix, founded in 1991.

Other arrays and fabrication methods followed, concentrating on oligonucleotide arrays for gene expression analysis (5–7). In this approach, oligonucleotides of known sequence or DNA fragments are applied to discrete chip locations using contact or non-contact printing methods (see below).

Protein- and antibody-based microarrays developed from the need to identify the actual protein products from gene expression, a discipline commonly referred to as *proteomics*. Using many of the techniques developed for oligonucleotide and DNA arrays, together with classical protein immobilization methods, protein arrays were first developed using recombinant DNA expression libraries (8,9). Protein and antibody arrays have since been extended to other applications, such as immunoassay; detection or profiling of proteins and complexed proteins, such as proteoglycans and proteolipids; and biological warfare agent detection (10–12). For example, in studies to determine the binding of the secondary messenger phosphatidylinositol (PI) to specific cellular proteins, the entire yeast proteome of 5800 proteins was immobilized onto an array and used to identify specific proteins binding PI (13).

4. Microarray Fabrication

4.1. Characterization. Microarrays can be characterized using a number of parameters:

Density: Density defines the number of target spots per unit area of the microarray substrate. For example, 1000 complementary DNA (cDNA) spots on a 0.9×0.9-cm area (0.81 cm^2) results in 1235 spots/cm^2. However, because of the small area of each spot, which range from 10 to 300 μm^2, higher densities can be realized. For example, if a center-to-center distance between spots is 140 μm, then a density of 5102 reaction centers/cm^2 can be realized. Decreasing center-to-center distance to 100 μm increases reaction center density to 9172/cm^2.

There are limits, however, on microarray density that depend on the fabrication method used (see below). Printed arrays, eg, have a practical upper limit of ~10,000 reaction centers/cm^2 due to limitations on spot size achievable (75–300-μm diameter). Array fabrication using photolithography can achieve much higher densities: >250,000 reaction centers/cm^2 with spot sizes 10–40 μm in diameter.

Regularity: As important as density, regularity is a measure of evenness and reproducibility of spot location on an array. Today's microarrays can achieve a regularity of 90+%, ie, there is >10% variation in the spot size, and the center- to-center spacing of the spots. Differences and deviations are due to (a) irregularities in the substrate surface–coating (pits, uneven coatings, uneven functional group spacing, etc); (b) imprecision in

the spotting method, such as poor position of the applicator or microfluidic problems in sample delivery; and (c) environmental disturbances occurring during array fabrication, such as air currents, humidity, temperature extremes, and static electricity.

Receptor Purity and Reactivity: Microarray performance is dependent not only on how the receptor molecules are applied, but also on the purity of the receptors and retention of binding activity and specificity after immobilization. Purity can be compromised by incomplete receptor preparation, nozzle–pin carry-over between applications in printing methods, or incomplete washing and/or removal of protecting groups during photolithography fabrication methods. The activity and specificity of receptors can be changed and/or lost through incorrect choice of immobilization method, poor manufacturing methods and conditions, and method of storage before use. While oligonucleotides, DNA, and antibodies are relatively stable and resistant to microarray fabrication methods, it is usually accepted that up to 50% of the starting activity will be lost on the finished array.

4.2. Microarray Fabrication Methods. Generic steps in the construction and use of a microarray are as follows:

1. substrate—(surface treat/activate) → activated substrate (A*S)
2. A*S + receptor molecule → array
3. array + samples + controls → binding/hybridization (array*)
4. array* + label → scan
5. data reduction and interpretation.

There are variations in these steps depending on the methods used. For example, arrays are being developed that do not need the addition of labels and use of scanners for detection. All arrays, however, require a reactive surface (substrate) platform and a means of immobilizing receptor molecules onto the surface.

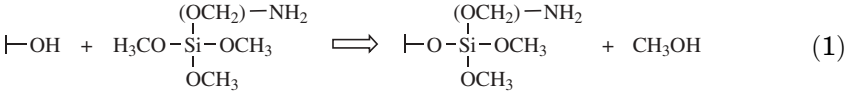
Substrates. The most commonly used microarray substrate is glass, including fused silica, borosilicates, aluminosilicates, and zinc titania. Glass slides and plates used for microarrays must have a high degree of purity and surface smoothness with low background fluorescence, low coefficients of thermal expansion, low reflectivity, and high light transmission properties. In addition to standard slide or plate geometry, glass arrays have also been developed as flow-through devices (14).

Other substrates for microarrays include porous materials, such as nitrocellulose and nylon membranes, polyacrylamide gels, and block copolymers (15,16). Such materials have a number of advantages over glass including larger available surface area, rapid uptake and adsorption of the receptor, higher uniformity of applied receptor spots, and less steric crowding of receptor–target pairs. The latter property has been optimized in polyacrylamide gel and polymeric microarrays which provide a three-dimensional (3D) matrix for receptor–target interactions (12,17).

Substrate Activation. Receptors can be attached to substrates with any of the common surface activation methods first developed for immunoassays and biosensors (18,19). The simplest of these is direct adsorption of the receptor to the surface. For silica substrates, such as glass, the net negative surface can be used to adsorb most peptides, proteins, and other receptors with neutral or net positive charge. However, adsorption to any substrate can also result is inactivation and/or blocking of active binding sites.

Since most microarrays to date have been developed for DNA analysis, surface activation chemistry has concentrated on either changing the surface of the substrate to have a net positive charge, or introducing groups that can link either to available groups in oligonucleotides (primarily the 5′- and 3′-hydroxy), or to modified oligos containing an amino group at its end(s). In most cases, however, the same activated surfaces can be used to link antibodies and other proteins.

Amino Derivatization. In this method, silylamines are used to introduce an amino functional group onto the array substrate. For example, 3-aminopropyltrimethoxysilane can be used to activate a glass surface through free hydroxyl groups:

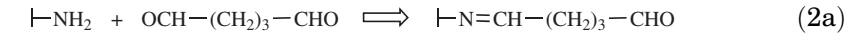


(1)

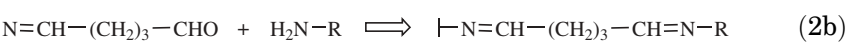
Electronegative oligonucleotides and nucleic acids may then be adsorbed to the now positively charged surface by electrostatic interactions; or a number of agents, such as carbodiimides, glutaraldehyde, isothiocyanate, hydrazine, or divinylsulfone can be used to covalently link the free amino groups on the substrate to amino-terminal ologinucleotides or to proteins.

In a variation of the adsorption approach, an amine, such as lysine or poly-L-lysine is covalently immobilized on the substrate via the silylamine groups. The resulting, net positive amine coatings then ionically bind the negative backbone of oligonucleotides, DNA, or RNA. The lysine layer also masks unreacted, negatively charged areas of the glass surface, providing a more uniform layer of positive charges for adsorption. The available amino groups in the layer can also be reacted with the amino and carboxyl groups of proteins and peptides for covalent immobilization to the substrate.

Active Aldehyde. The free amino groups on silylamine-treated surfaces can be reacted with glutaraldehyde to result in active aldehyde groups (2a):



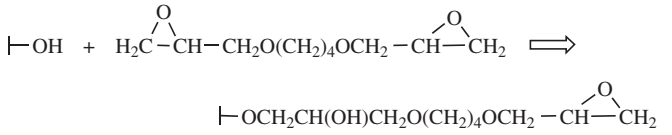
(2a)



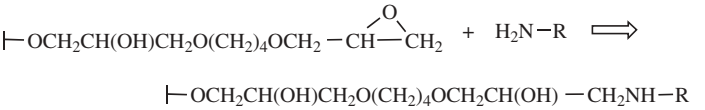
(2b)

The free aldehyde groups will react spontaneously with free amino groups in proteins or on amino-terminal oligonucleotides (2b). This method also allows the introduction of a spacer arm, five carbon atoms long in the case of glutaraldehyde, which aids in moving the receptor away from the substrate surface and facilitating better interaction with the target molecule.

Epoxy Activation. A bis(oxirane) can be used to activate any substrate with free hydroxy, amino, or sulfhydryl surface groups to result in an active epoxy surface (18). For example, 1,4-butanediol diglycidyl ether will react with glass surfaces at room temperature to produce an active epoxy layer (3a):



(3a)

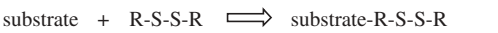


(3b)

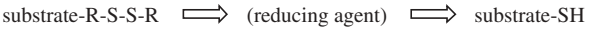
The active epoxy group will react spontaneously at basic pH with amino, hydroxyl, or sulfhydryl groups on proteins or peptides (3b); or with the 3′- or 5′-hydroxy groups of oligonucleotides, DNA, or RNA. Again, this approach also introduces a spacer arm between the substrate and the receptor.

Thiol Activation. A commercially available microarray substrate (20) pretreats the substrate to contain disulfide bonds (4a) for attachment of modified oligonucleotides. The oligonucleotide or nucleic acid to be attached to the substrate is treated with acrydite phosphoramidite to yield an acrylamide group on the 5′ end of the oligo or DNA (21).

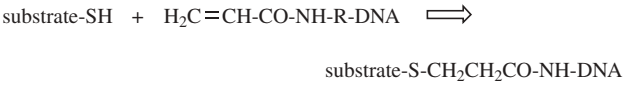
For immobilization, a reducing agent [tris(2-carboxymethyl)phosphine HCl] breaks the disulfide bonds (4b) and the free sulfhydral groups react with the modified oligonucleotide (4c):



(4a)



(4b)



(4c)

Biospecific Binding. Originally developed for immunoassays, the high avidity of biotin for the protein avidin can be used to immobilize biotin-containing oligonucleotides, peptides, or proteins to avidin or stepavidin adsorbed to or covalently bound to the substrate (22,23). The resulting biotin–avidin complex is essentially irreversible. In addition, the addition of biotin can be directed to a specific part of the receptor allowing for directional immobilization to the substrate.

Array Spotting and Synthesis. Capture receptors are applied to a microarray substrate by three major methods using nanoliter (nL) to picoliter (pL) amounts of DNA, RNA, or protein. Receptor application can be done by hand on the substrate, but now is routinely carried out using one of the many commercially available automated robotic array spotters marketed by

companies, such as Affymetrix, Agilent, Cartesian Technologies, GeneMachines, Genetix, and others.

Direct Spotting. In the most common method currently used in microarray fabrication, oligonucleotides, isolated and amplified DNA, antibodies, and other receptors are spotted directly onto preactivated substrate.

Typically, direct spotting is used for arrays containing between 100 and 10,000 reaction centers, although higher densities (up to 50,000 reaction centers) are possible. Currently available spotters can deposit from 10 pL to 10 nL of receptor solution per reaction center resulting in spot diameters from 50 to 1000 μm. While higher reaction center densities are possible with spotting methods such as photolithography (see below), direct spotting has shown to produce arrays with high fidelity with no real limitation of oligonucleotide, nucleic acid, or protein molecular size.

While direct spotting can be done by hand, automated robotic spotting instruments are used for routine and reproducible production of arrays. Such instruments generally use one of two methods.

(*1*) *Contact Printing:* Rigid pins carry the sample to the substrate and make contact with the substrate while depositing the receptor solution. The pin heads are usually hollow and engineered to hold a precise amount of liquid. For example, one approach draws up from 250 to 600 nL of receptor solution into each pin head and delivers as little as 0.6 nL to each array spot (24). The pins and associated instrument can take up samples from standard 96- and 384-well plates, producing up to 100 microarrays each containing up to 10,000 spots in 6 h (25). In another approach, a ring at the end of a solid pin captures receptor sample and the sample is deposited on the substrate by pushing the pin through the ring (26).

(*2*) *Noncontact Printing:* The primary method for noncontact application of receptor to substrate uses piezoelectric ink-jet technology to "print" the receptor reaction centers. Essentially, capillary tubes surrounded by a transducer pick up the samples that are then propelled onto the substrate using electric pulses (27,28). Droplets from the capillary can be as small as 1 nL. In addition, unlike hollow pin applicators, excess sample can be recovered from the piezoelectric print head.

In Situ Synthesis. Used to produce some of the first microarrays (4,5), *in situ* synthesis synthesizes oligonucleotide reaction centers on the chip itself, building each oligonucleotide base-by-base. By combining synthesis with photolithography and masking, specific areas of the chip can be chosen to contain specific oligonucleotide reaction centers. The method also allows variation in the length of oligonucleotides in different reaction centers.

On-substrate (or on-chip) synthesis using photolithography was first reported in 1991 (4) using nucleic acid bases containing photolabile groups. The process starts with a silane-treated, amino functional (usually glass) surface to which the first base in the oligonucleotide to be synthesized is attached through a phosphorylamidite functional group on the 3′ position of its ribose (or deoxyribose). The bases also have a methylnitropiperonyloxycarbonyl (CH_3NPOC) group attached to the 5′ ribose position. The CH_3NPOC is a

photolabile group that, while stable under normal reaction conditions, is lost when exposed to ultraviolet (uv) light for ~30 s. After light exposure, another specific base containing phosphoramidite and CH_3NPOC is added, reacts via the phosphoramidite group with the (now) available 5′ position of the previously immobilized base, and adds to the growing oligonucleotide chain.

The process can be repeated (cycled), with different bases to build oligonucleotides of 20–40 bases in a stepwise series of reactions. To control which spot receives a new base at each step, a chrome photomask is used to direct which spot is exposed to the uv light, activating the 5′ group at its growing end.

Photolithography and *in situ* synthesis have produced the highest density arrays possible, with hundreds of thousands of reaction centers on a single chip. The method is limited, however, to synthesis of oligonucleotides no larger than ~30 bases in length.

Polymeric Embedding. A more recent method developed for the inexpensive mass production of microarrays utilizes entrapment of the receptor into a multilayered polymer matrix (12,29). The method applies the probe or cDNA directly to a membrane as a reagent line (Fig. 1). Many (10–100) membranes are then stacked, compressed, and polymerized by adding a polymerizing agent. The resulting blocks are then sliced to result in reproducible arrays. In one example, five lines containing different oligonucleotides from a preparation of λ phage DNA were laid down onto the membranes and arrays produced. The resulting array membranes were then interrogated with λ phage DNA ^{32}P probes and detected. Each arrayed DNA fragment retained absolute specificity for its probe, spot densities and placement were highly reproducible, and up to 5000 identical arrays can result from a 10-cm block of stacked arrays. This method holds promise for the rapid, inexpensive production of microarrays.

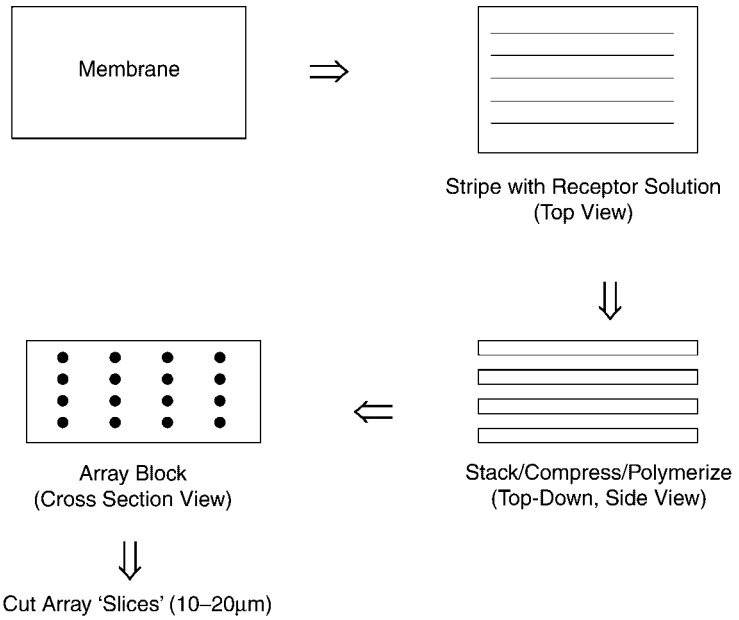


Fig. 1. Basic steps for mass production of a polymeric microarray (12,29).

Detection. Once the target analytes have been captured by the receptor on the microarray substrate, a means must be available to detect and quantify the receptor–analyte complexes. The current method of choice is to label the receptor complex for either genomic or proteomic arrays with a fluorescent or chemiluminescent label, and then to detect and quantify the complexes by fluorescence or light emission. These methods are derived directly from methods originally developed for immunoassays and nucleic acid probes (30, 31). Detection limits are possible down to attomole ($10^{-18}M$) concentrations of receptor–target complex.

Labels can be introduced into oligonucleotide probes by synthesis in the presence of labeled nucleotides or PCR (polymerase chain reaction) primers. Fluorescent labels emitting light at different wavelengths are commonly used to label sets of probes enabling comparitive analysis of probe-binding patterns to an array. The most common fluorescent probes for this approach are the cyanine (Cy2, Cy3, Cy5, etc) and Alexa (Alexa 488, 532, 546, 568, etc) fluorescent dyes. Other commonly used fluorescent dyes include fluorescein, rhodamine 6G, and phycoerythrin.

In another common approach, the probes are labeled with biotin. After hybridization on the array, fluorescently labeled strepavidin, such as strepavidin-phycoerythrin (32) is added to detect the hybridized probes. Biotinylation is also used for labeling proteins in a sample prior to analysis on a protein microarray. After binding to the microarray containing defined protein-binding sites, the proteins present are detected by adding strepavidin labeled with various fluorophores (33).

Receptor-target fluorescent complexes are detected on microarrays using a scanner (Table 2). Scanners commonly use a laser or white light source for

Table 2. **Examples of Microarray Scanners**

Scanner/Supplier website	Properties
Affymetrix GeneChip www.affymetrix.com	single laser, PMT scanner for use with Affymetrix GeneChips only
1700 Chemiluminescent Microarray www.appliedbio-sytems.com	CCD camera-based system limited to use with PE custom cartridges, 20-μ resolution, 10^3 dynamic range
DNA Microarray Scanner www.chem.agilent.com	Dual laser, PMT scanner for 1 × 3-in. slides, 10^3 dynamic range, 5–10-μm resolution
Applied Precision arrayWoRx www.api.com	CCD camera scanner for 96–1536 well microtiter plates, detects up to four fluorescent signals/well, 5-μm resolution, 10^4 dynamic range
Axon Instruments GenePix www.axon.com	dual laser, PMT scanner for 1 × 3-in. slides, 5-μm resolution, 10^4 dynamic range
GeneTac www.genomicsolutions.com	dual laser, PMT scanner for 1 × 3-in. slides, <5 μm resolution, 3 emission filters
ProScan Array www.perkinelmer.com	four laser, 11 emission filter, PMT scanner for 1 × 3-in. slides, 10^4 dynamic range
Tecan LS www.tecan.com	four laser, 28 emission filter, 2 PMT scanner for various substrates from slides to microtiter plates, <5 μm resolution, 10^5 dynamic range

fluorescence excitation, and either a PMT (photomultiplier tube) or a CCD camera for detection of emitted light. The PMTs both amplify and measure low levels of fluorescent emissions while CCD scanners–imagers utilize emission filters to focus the fluorescent light onto the CCD camera. Since CCDs tend to be less sensitive than PMTs, most CCD detectors integrate emission signals over time, leading to longer scan times.

Most commercially available scanners have two lasers (usually at 532 and 635 nm), use a PMT detector, can scan 1×3-in. glass slides, and have a pixel resolution in the 1–10-μm range. More advanced models (Tecan, Perkin Elmer) have four lasers. The Tecan LS scanner also has two PMTs, and can scan a range of array substrates, from slides to microtiterplates. All scanners have integrated software programs for signal interpretation and report output.

Chemiluminescence has primarily been applied to protein microarrays and immunoassay arrays. The most common approach is to label the detection probe (usually an antibody) with an enzyme such as peroxidase that can oxidize luminol producing luminescence. The signal is then detected using either a CCD camera-based imager or X-ray film (34).

Labeling probe molecules with radioactive isotopes is still used in some instances in spite of the issues surrounding safe use and disposal of radioactive waste. Labeling a protein with ^{32}P is preferred in some cases for use with protein arrays to avoid problems with undirected, multisite labeling when an indiscriminant label, such as biotinylation is used. For example, ^{32}P-phosphorylated fusion protein probes for protein microarrays have been produced using protein kinase A (35).

Another, more novel approach has been developed for detection, analysis, and identification of target analytes on microarrays and chips utilizing mass spectroscopy (36,37). The capture array or chip contains spots with one of several available surface chemistries including hydrophobic, hydrophilic, ion exchange, and immobilized metals. The sample containing the target analyte(s) is applied to the chip and binds. After washing the chip to remove unbound material, the chip is analyzed using TOF (time-of-flight) mass spectrometry. The target analyte is desorbed from the chip prior to analysis using laser desorption. This approach had been applied to the analysis of many analytes including tumor markers, glycoproteins, hormones, cardiac distress markers, and cell signaling protein; and is especially powerful for analyzing proteins and peptides in biological samples. It is also being applied for proteomic expression and difference mapping (38).

5. Microarray Applications

The total global market for microarrays was ~$596 million in 2003, including both instruments and arrays, and is expected to reach nearly $1 billion by 2010 (39). The primary products in the market to date have been for genomic analysis and drug discovery. However, microarray technology is now being extended into other areas. Currently, there are four major areas of microarray development and application:

(*1*) *Genomic Analysis*: The analysis of whole and targeted segments of the genomes of humans, other animals, plants, and microbes to define the genetic compliment of each, and to search for differences that can be exploited in the development of new drugs, diagnostics, plant species, and other products.

(*2*) *Expression Analysis*: The analysis of the messenger RNA (mRNA) in human or other cells to define changes in the genome and to direct the development of new drugs, diagnostics, and directed therapy for disease states (personalized or individualized medicine).

(*3*) *Proteomics*: The analysis of the proteins present in a cell or tissue to detect and define any protein content differences in disease and abnormal states to direct development of new drug therapies and diagnostic tests.

(*4*) *Diagnostics*: The extension of current clinical, environmental, food and chemical testing to multiplex arrays able to detect and identify multiple targets on a single array.

These applications are leading to intensive R&D to discover and develop new microarrays not only for genome analysis, but also for clinical diagnostics (infectious diseases, blood proteins, cancer, and cardiac markers), environmental analysis (pollutants; chemical waste), food analysis (microbes, toxic contaminants), and, chemical and biological warfare agents.

5.1. Genomics and Expression Analysis. Microarrays were first developed for analyzing the overwhelming amount of information available from genomic analysis carried out in sequencing the human genome. The primary use for these arrays was *genomic* and *expression analysis*, to determine both the base sequence of DNA and to define gene activity and function in a sample. Refinement of DNA and expression analysis led to analysis of individual genes and gene fragments and determination of differences, such as identification of SNPs (single nucleotide polymorphisms) and their linkage to disease states.

Table 3 lists a number of commercially available microarrays for expression analysis and whole genome characterization and comparison studies. While many can be used with any scanner, others like those from Affymetrix can only be used with the company's equipment.

Affymetrix (Santa Clara, Calif.) was the first company to develop large arrays, packing upward of 100,000 oligonucleotides onto a $1\,cm^2$ chip. As described earlier, Affymatrix utilizes *in situ* synthesis of oligonucleotides, directing the synthesis of oligonucleotides on specific parts of the chip using photolithography, resulting in chips for whole genome and SNP analysis, and for diagnosing diseases, such as acquired immune deficiency syndrome (AIDS). The latter chip, GeneChip HIV PRT detects known mutations in two enzymes from human immunodeficiency virus-1 (protease and reverse transcriptase). Knowing whether these mutations are present can direct therapy in a patient.

An example of a slide-based array for human genome analysis is shown in Fig. 2. The array contains 44,000, 60-mer oligonucleotides for whole-genome profiling. The array is produced on a conventional 1×3-in. slide to be compatible with most commercial microarray scanners.

Table 3. **Examples of Commercially Available Microarrays**

Company/website	Examples of Arrays Available
Affymetrix www.affymetrix.com	quartz wafer-based human, mouse, and rat DNA or oligonucleotide whole genome; arabidopsis, rice, barley and other plant; yeast, bacterial, viral and other microbes
Applied Biosystems www.appliedbiosystems.com	nylon-coated glass slides with human, mouse or rat genome expression arrays
BD Biosciences Clontech www.bdbiosciences.com/clontech	nylon, glass, and plastic substrate arrays and kits for human, mouse, and rat expression profiling
EMD Biosciences/Novagen www.emdbiosciences.com	glass slide-based protein arrays for human cytokines
Invitrogen www.invitrogen.com	nitrocellulose coated slide-based human and yeast protein and kinase arrays
Panomics www.panomics.com	membrane-based arrays for gene expression (human, mouse), signal transduction (human, mouse cytokines), and human proteins
Protein One www.proteinone.com	nitrocellulose-based arrays for transcription factors, nuclear receptors, tumor suppressors, and onco-proteins
Quansys BioSciences www.quansysbio.com	microtiter plate-based antibody arrays for human and mouse cytokines; human autoantibodies, hormones, and cancer markers
RayBiotech, Inc. www.raybiotech.com	membrane-based antibody arrays for human and mouse cytokines, inflammation, angiogenesis, and artherosclerosis
TeleChem International, Inc. www.arrayit.com	polymer coated glass slide-based arrays for the human genome (25,000 elements) and human proteins
Whatman/Schleicher & Schuell www.schleicher-schull.com	nitrocellulose coated slide-based antibody and capture protein arrays for mouse and human cytokines; human cancer serum proteins, chemokines, and angiogenesis

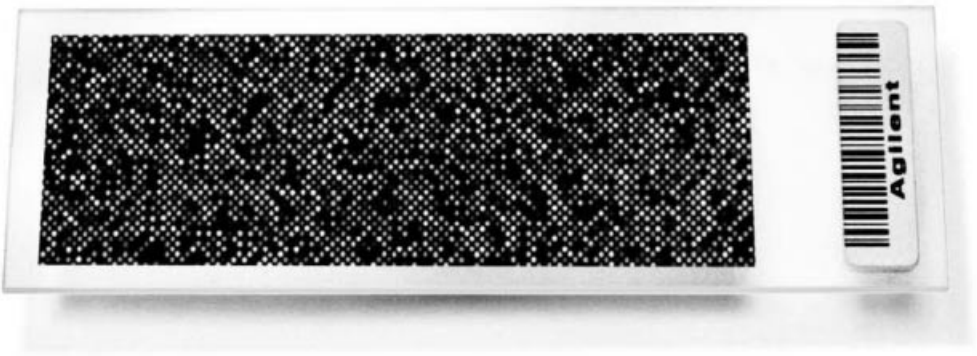


Fig. 2. Agilent whole human genome microarray. (Courtesy of Agilent Technologies.)

Expression arrays are also being applied to *pahramacogenomics*, the prediction of whether a patient will respond to a specific drug based on the mRNA and proteins produced by the patient. Pharmacogenomics can also look at the potential toxicity of a drug. For example, one of the primary and first sites of drug toxicity is the liver. Microarrays are available that contain immobilized single-stranded DNA that binds to DNA coding for most liver proteins. The function of each protein is known and many can be linked to liver damage. Essentially, two samples of liver cells are obtained from the patient and are cultured. One of the cultured samples is exposed to a new drug. After a specific time, the mRNA is collected form both cell cultures and transcribed into cDNA using reverse transcriptase and fluorescent markers. The cDNA is then placed on the chip and allowed to hybridize. After hybridization and washing, the chip is scanned and the amount of each DNA in the sample quantified. The results from this analysis will indicate which mRNAs were expressed more or less in the presence of drug. If it turns out the mRNA coding for proteins that can lead to liver damage are expressed at a higher level with the drug present, then liver toxicity by the drug is indicated.

5.2. Proteomics. Proteomics is the analysis of the proteome or total expressed protein in a cell. While such analysis has traditionally relied on complex and challenging methods, such as electrophoresis and analytical chemistry, protein microarrays are evolving to answer the challenges of identifying and quantifying the expressed protein in a normal or diseased cell, or a cell treated with a specific drug. Proteomics can also be used to study basic mechanisms in cell function and cell structure.

For example, one of the first eucaryotic protein microarrays has been constructed from the cloned, 5800 protein proteome of yeast (13). Each protein was produced, purified, and bound to glutaraldehyde-activated slides, immobilizing the entire compliment onto a 1 cm^2 chip, in duplicate. In one series of experiments, the chip was used to assess the specific binding of PI, a second messenger in transmembrane cellular communication, to the immobilized proteins. After binding and washing, the bound PI was detected by labeling with an avidinfluorophone and scanning. A differential binding of the PI by the yeast membrane proteins was found, supporting the interaction of PI with specific membrane proteins and further clarifying transmembrane signaling mechanisms. Studies such as these can be used to direct new drug therapies in dysfunctional cells.

5.3. Diagnostics. Current human and veterinary diagnostics depend primarily on analytical chemistry or molecular diagnostic methods, such as immunoassay and nucleic acid probe technology. Microarrays are being developed that utilize these established technologies by multiplexing assays on a chip, increasing the number of analyses that can be performed at one time, and decreasing cost and labor. For example, a number of commercially available immobilized antibody-based microarrays are listed in Table 3. While simply extensions of immunoassay technology, they represent the first generation of arrayed diagnostic assays.

The first U.S. Food and Drug Administration (FDA) approved microarray for human diagnostic use is based on the Affymetrix gene chip. The AmpliChip CYP450 Test (40) detects genetic variations in the genes for cytochrome P450 isoenzymes 2D6 and 2C19. Variations in these genes and the expressed enzymes

can change the metabolism, and resulting toxicity and efficacy of specific drugs in individuals. By determining the nature of such variations, a physician can better prescribe specific drugs.

Further developments in microarray-based diagnostics using technologies, such as gene and antibody chips, and the polymeric embedding microarray technology already described (12,29), promise to revolutionize diagnostic assays including those for clinical diagnostics, infectious diseases, protein disease markers, food pathogens, and harmful environmental agents.

6. Conclusion

Microarrays represent a whole new technology for analysis, detection, and diagnostics of analytes, from whole cells and DNA to environmental pollutants. Currently, microarrays are complex to use and costly to manufacture. This will change as microarrays based on proteins as well as nucleic acids find increasing applications.

Within the next 10 years, microarrays will be routinely applied not only to the study of genes and genomes (*genomics*), but in nearly every area requiring analysis and detection. These will include new applications, such as *pharmacogenomics* (therapy based on the genome of an individual), *toxicogenomics* (definition of the toxic effect of substances on the genome), and proteomics (definition of expressed protein in a cell); as well as traditional areas, such as clinical diagnostics, drug discovery, food and environmental analysis, and chemical and biological warfare defense. Microarrays are also the basis for a new generation of analytical devices, MEMS and lab-on-a-chip, which integrate functions, such as DNA amplification, separation, analysis, and detection with microfluidics on a single platform.

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RICHARD F. TAYLOR
TC Associates Inc.