

big future for **MICROARRAYS**

The expectation that microarray technology will play a large role in shaping the future of pharmaceutical development and diagnostics has greatly increased due to new products and applications. Microarrays for gene expression have made a profound impact in the pharmaceutical and biomedical worlds. Information from newer microarray technologies such as CGH, ChIP-on-chip, splice variants and microRNAs, combined with gene expression data, can be applied to the drug discovery process enabling many exciting applications. We discuss the impact of these new microarray applications on the healthcare and pharmaceutical industries.

To date, DNA microarrays have enabled biologists to conduct large-scale experiments that have produced mass quantities of genetic information. These experiments have helped identify the mechanisms of disease and identify disease subphenotypes, predict disease progression, assign function to previously unannotated genes, group genes into functional pathways, and predict activities of new compounds. Directed at the genome sequence itself, microarrays have been used to identify novel genes, binding sites of transcription factors, changes in DNA copy number and variations from a baseline sequence, such as in emerging strains of pathogens or complex mutations in disease-causing human genes¹.

So why are researchers looking for new applications for genomic microarrays? Because gene expression only tells part of the picture. By combining gene expression data with information from newer microarray technologies such as CGH, ChIP-on-chip, splice variants and microRNAs, scientists can begin to apply themselves to many exciting applications, such as finding cures for infectious diseases, discovering the mechanisms involved in cancer and driving the developments of new therapeutics.

From the clinical perspective, microarrays are helping to diagnose many genetic disorders, such

as cystic fibrosis and autism and will hopefully aid in finding therapeutic interventions. The field of oncology has benefited greatly with the advent of expression and SNP microarrays. Genetic modifications such as insertions and deletions and signal transduction pathway analysis are providing researchers with valuable information into the cause and effect of many different types of cancers, leading to appropriate diagnoses and treatment.

In the future, microarray technology has the potential to help medical professionals select the most effective drugs, or those with the fewest side-effects, for individual patients. Since it can also help identify individuals with similar biological patterns, microarray analysis can assist drug companies in choosing the most appropriate candidates for participating in clinical trials of new drugs.

The importance of microarrays

Whether inherited or resulting from the body's response to environmental stresses like viruses or toxins, all diseases have a genetic component. Many human diseases are not caused by one genetic variation within a single gene, but are determined by complex interactions among multiple genes, environmental and lifestyle factors. A person's resistance or susceptibility to a disease and the severity and progression of the disease are all

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dependent on genetic factors. Since all of the factors involved in the intricate pathways of disease are not yet known, it has been difficult for researchers to develop screening tests for most diseases and disorders, such as diabetes, cardiovascular diseases, Alzheimer's disease and arthritis. By using microarray technology, researchers may begin to reveal relevant genes associated with a disease.

Microarrays have made a significant contribution to science both because they can survey a large number of genes quickly or study a small sample size. Microarrays have been used to assay gene expression within a single sample or to compare gene expression in two different cell types or tissue samples, such as in healthy and diseased tissue. However, this technology is branching out into many different applications, including genotyping, sequence analysis and many different types of microarrays, including carbohydrates, peptides, RNAi, microRNA, proteins and antibodies.

The ultimate goal is to use this genetic information to develop new ways to treat, cure, or even prevent the thousands of diseases that afflict humankind. But the road from gene identification

to effective treatments is long and fraught with challenges. In the meantime, biotechnology companies are racing ahead with commercialisation by designing diagnostic tests to detect errant genes in people suspected of having particular diseases or of being at risk for developing them².

Today's microarray experiments

There are a number of microarray types currently on the market and are used for a variety of applications. Table 1 details the current types of microarrays and applications.

Expression analysis

Microarray expression analysis determines the level at which a certain gene is expressed, and the arrays used in this kind of analysis are called expression chips.

Expression chips may be used to examine the expression of a gene under certain circumstances (expression patterns) as well as changes in gene expression over a given period of time, such as within the cell cycle. Analysis of gene expression data from a microarray experiment can reveal details of

Table 1: Microarrays and applications

MICROARRAY PLATFORM	PURPOSE	APPLICATIONS
Genotyping	Used to identify genetic variation in individuals and across populations by identifying SNPs	Pharmacogenomics Pathogen subtyping Gene discovery/functional analysis Gene/disease association studies Bioterrorism/biowarfare Forensic applications Drug discovery
ChIP arrays	Used for isolation and identification of the DNA sequences occupied by specific DNA binding proteins in cells	Gene discovery/functional analysis Drug development Identify, assess, and monitor biomarkers Uncover and profile off-target events
CGH	Used to identify genomic insertions and deletions and copy number changes	Identify, assess, and monitor biomarkers Gene discovery/functional analysis Evolutional discoveries
Splice variants	To identify genes whose splice variants produce abnormal proteins which may trigger or contribute to the development of disease	Drug discovery Identify, assess, and monitor biomarkers Pharmacogenomics
Resequencing	To identify genes which allows geneticists to align and compare genetic codes	Gene discovery/functional analysis Disease association studies Drug development Biomarker development/discovery

Source: Beachhead Consulting

the cell cycle, providing valuable data on the points at which gene mutation leads to cancerous growth, as well as opportunities of therapeutic intervention.

Data produced from expression arrays can be an integral part of target discovery. For instance, if a certain gene is over-expressed in a particular form of cancer, researchers can use expression chips to see if the compound of interest will reduce over-expression and force the cancer into remission. Expression chips could also be used to diagnose diseases, such as the identification of new genes involved in environmentally triggered diseases which affect systems such as the immune, nervous, and pulmonary or respiratory systems.

Comparative Genomic Hybridisation (CGH)

Researchers use a microarray technique called Comparative Genomic Hybridisation (CGH) to look for genomic gains and losses or for a change in the number of copies of a particular gene involved in a disease state.

If the number of copies of a particular target gene has increased, a large amount of sample DNA will hybridise to those spots on the microarray that represent the gene involved in that disease, whereas comparatively small amounts of control DNA will hybridise to those same spots. As a result, those spots containing the disease gene will fluoresce red with greater intensity than they will fluoresce green, indicating that the number of copies of the gene involved in the disease has increased.

Single Nucleotide Polymorphism (SNP) chips

To detect mutations or polymorphisms in a gene sequence, the target, or immobilised DNA, of the microarray is usually that of a single gene. With this microarray technique, the target sequence placed on any given spot within the array will differ from that of other spots in the same microarray, sometimes by only one or a few specific nucleotides. One type of sequence commonly used in this type of analysis is called a Single Nucleotide Polymorphism or SNP. A SNP is a small genetic change or variation that can occur within a person's DNA sequence.

Once researchers have established that a SNP pattern is associated with a particular disease, they can use SNP microarray technology to test an individual for that disease expression pattern to determine whether he or she is at risk for developing that disease.

ChIP-on-chip – transcription factor analysis

Genome-wide location analysis, or ChIP-on-chip, is a technique for isolation and identification of the DNA sequences occupied by specific DNA binding

proteins in cells. These binding sites may indicate functions of various transcriptional regulators and help identify their target genes. They may also be used as a basis for annotating functional elements in genomes. The types of functional elements that one can identify using ChIP-on-chip include promoters, enhancers, repressor and silencing elements, insulators, boundary elements and sequences that control DNA replication³.

Overview of current application areas

Microarrays have been applied to the areas of environmental sciences, agriculture research, biodefence, diagnostics, forensics, pharmacogenomics and toxicogenomics.

Biodefence: Scientists at the Naval Medical Research Center (NMRC) have uncovered a way to quickly identify genetically engineered strains of anthrax bacteria and track their origins using resequencing microarrays. Results revealed that naturally occurring anthrax bacteria have undergone virtually no recombination and have a large excess of rare SNP variants; genetic characteristics that would make engineered strains easily distinguishable and traceable⁴.

Toxicology: Compound toxicity can be difficult to predict or find at early stages of drug discovery with current testing methods⁵. Identification of toxicity early in the discovery process can not only save the pharmaceutical companies money, but will help prevent harmful pharmaceuticals from ever going to market.

Gene discovery: Researchers at the National Alliance for Autism Research (NAAR) are conducting the largest study of the disease to date, the NAAR Autism Genome Project. Autism cases have increased over the past 20 years, and with the help of 170 of the world's top scientists and 50 academic and research institutions, the NAAR hopes to find genes linked with inherited risk of autism.

Cancer treatment: Current cancer treatments, although effective in curbing tumour growth, can be difficult to tolerate because of the damage they do to normal body cells. Although there are a few examples of targeted cancer therapy, there is an urgent need for more. The complexity of designing specific cancer therapies emerges from the different combinations of mutations that can give rise to cancer. Therapies may prove more efficacious if they are tailored to the type of cancer in a particular patient⁶.

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Table 2: Profile of new genomic microarray applications in pharmaceutical R&D

	CURRENT ADOPTION BY PHARMA R&D	POTENTIAL APPLICATIONS IN BIOMARKER DISCOVERY	POTENTIAL APPLICATION IN CLINICAL SCREENING	NEED FOR HIGH DENSITY ARRAYS	OVERALL POTENTIAL IN PHARMACEUTICAL R&D	POTENTIAL FOR DIAGNOSTIC APPLICATIONS
SNP arrays	●	●	●	●	●	●
Array CGH	◐	◐	◐	◐	◐	◐
Resequencing	●	◐	◐	●	◐	◐
ChIP/Chip	○	○	○	●	◐	○
Splice variants	◐	◐	◐	●	◐	◐
MicroRNA	◐	◐	◐	◐	◐	○
RNAi arrays	◐	◐	○	◐	◐	○
Mitochondrial DNA arrays	○	○	○	○	○	○
Methylation	◐	◐	○	●	◐	○

Source: Beachhead Consulting

Current microarray technology in research

Genotyping microarrays

Technology and resources generated by the Human Genome Project and other genomics research are already having a major impact on research across the life sciences. With this technology, researchers can identify the genetic sources of many diseases, provide valuable information during clinical trials regarding metabolism of potential new drugs, help predict the outcomes of transplants, and identify possible diseases *in utero* or in newborn babies. Other applications of this genetic research include molecular medicine, energy sources and environmental applications and DNA forensics.

SNPs

Single nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide (A, T, C or G) in the genome sequence is altered. A variation must occur in at least 1% of the population for it to be considered a SNP. Although more than 99% of human DNA sequences are the same across the population, variations in DNA sequence can have a major impact on how humans respond to disease. SNPs do not cause disease, but they can help determine the like-

lihood that someone will develop a particular disease. This makes SNPs of great value for biomedical research and for developing pharmaceutical products or medical diagnostics. SNPs are also evolutionarily stable, meaning they do not change much from generation to generation, making them easy to follow in population studies.

Scientists believe SNP maps will help them identify the multiple genes associated with complex diseases such as cancer, diabetes, vascular disease and mental illness.

Understanding the role of genetic polymorphisms in drug response will facilitate drug efficacy and decrease adverse effects.

New microarray technologies

Bead-based microarrays

Bead based arrays are likely to become the future of microarrays, due to their ease of use, conservation of size and scalability, however this technology is currently in developmental stages. Bead-based arrays contain hundreds of thousands to millions of individual probes in which reactions take place on the surface of microbeads. They are thought to be one of the most promising technologies for the vast number of tests that will be needed for applications

such as drug discovery and for diagnosis and treatment of patients. Three companies involved in bead-based microarrays for genotyping include Illumina, Inc, BioArray Solutions and Tm Bioscience.

Microarray-based Comparative Genomic Hybridisation (a-CGH)

The first efficient approach to scanning the entire genome for variations in DNA copy number was Comparative Genomic Hybridisation (CGH). Array-based CGH is a powerful technology capable of identifying chromosomal imbalance at a high resolution. While it began as an effective tool for genome scanning, array-CGH is becoming one of the most powerful diagnostic and prognostic tests in clinical pathology. Congenital abnormalities, haematopoietic and solid tumours and infertility are just a few of the areas where CGH microarrays are being applied.

The application of comparative genomic hybridisation microarrays has been focused in the field of oncology. Application of array CGH to tumour specimens makes genetic diagnosis of cancers possible and may help to differentiate tumour subtypes allowing for more accurate diagnosis and treatment decisions.

ChIP-on-chip microarrays

ChIP-on-chip is a microarray-based technique for understanding gene regulation in disease. It uses chromatin immunoprecipitation (ChIP) to discover how regulatory proteins interact with the genome of living cells. Regulatory proteins bind to genomic DNA to control chromosome replication and gene activity, thereby functioning as switches in the regulatory circuitry of cells.

Dr Richard Young and several colleagues at the Whitehead Institute for Biomedical Research developed their own system for decoding gene regulators, the ChIP-on-chip technology. Dr Young, along with Dr David Gifford, an MIT professor, and an expert in computational biology, founded Computational Biology Corporation (Cambridge, MA). Prior to its first anniversary, Computational Biology Corporation was acquired by Agilent Technologies and gained exclusive access to a critical patent and intellectual property for ChIP-on-chip analysis, enabling the company to offer a unique microarray solution for disease research, drug discovery and development.

ChIP-on-chip technology has been used to discover the mechanism by which human embryonic stem cells retain the ability to become any type cell, called pluripotency, and how this ability is lost once the cells begin to differentiate⁷.

MicroRNA (miRNAs) arrays

Although the first published description of a miRNA appeared 10 years ago⁸, only in the last two to three years has their importance been better understood. The function of miRNAs appears to be in gene regulation. Most of the genome sequences encoding miRNAs occur in areas of the genome that are not associated with known genes; many are found in fragile sites in human chromosomes and appear to be independently transcribed. Experiments involving miRNA demonstrate similar profiles of miRNA between related organs, such as heart and skeletal muscle, while the brain has a very different profile. This expression data suggest that miRNAs are important factors in differentiating tissues in adult organisms.

Splice variant microarrays

Alternative splicing plays a significant role in physiology and disease. Approximately 30-60% of genes undergo alternative splicing. Splice variants are variable sequences of RNA produced from the same gene in DNA, resulting in the creation of different proteins potentially affecting cellular regulation. Scientists developing therapeutics are increasingly interested in this emerging field as the expression of splice variants can provide novel targets, may indicate disease states and can be altered by exposure to drugs and toxins.

All stages of drug development, predictive toxicology, molecular diagnostics and clinical biomarkers in oncology and haematology can benefit from splice variant microarray technology.

Other microarrays

RNA interference (RNAi) microarrays

RNA interference, or RNAi, is a powerful mechanism for inhibiting gene expression. RNAi appears to be a highly potent and specific process which is actively carried out by the RNA interference machinery. Once RNAi finds a double-stranded RNA molecule, cuts it up with an enzyme known as Dicer, separates the two strands and then destroys other single-stranded RNA molecules that are complementary to one of those segments. dsRNAs direct the creation of small interfering RNAs (siRNAs) which target RNA-degrading enzymes (RNAses) to destroy transcripts complementary to the siRNAs.

Mitochondrial resequencing array

During the past 10 years, it has become increasingly clear that mutations of mitochondrial DNA contribute to a variety of human diseases, including cancer, diabetes, obesity, neurodegeneration and ageing.

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The mitochondrial array provides complete sequence information, captures genetic variation and enables the detection of both known and novel mutations in the mitochondrial genome associated with complex diseases.

Affymetrix (Santa Clara, CA) is the only company currently producing mitochondrial microarrays (for research purposes only) which can interrogate the entire 16kb mitochondrial genome on one array.

These arrays are relatively new and have limited adoption into drug discovery and development.

Market penetration and potential growth

In 2005, revenue for the total US DNA microarrays market totalled approximately \$450 million and is expected to rise to \$530 million by the end of 2012. The compound annual growth rate (CAGR) for 2005 to 2012 is estimated at 10.9%⁹.

Most of this growth will come from SNP revenues along with continued growth of traditional array applications and new technologies and applications.

Our market model shows that the percentage of the array market attributed to SNP arrays in 2004 was approximately 17%. We expect this percentage to grow rapidly to approximately 45% in 2009 due to quality improvements with clinical samples and high density arrays and because many cancer biomarker programmes centre on genotyping, the main driver being the effort to find SNP markers for specific tumour types to correlate with treatment. Price erosion and the establishment of more stringent FDA regulations could be a tempering factor.

Many applications have their place in basic target or disease research but will not be run on thousands of samples, therefore reducing their overall market potential.

Conclusion and potential for adoption

The importance of DNA microarrays in research for disease diagnosis, understanding drug interactions and determining drug candidates is expected to keep the market growing substantially. Currently, however, these technologies have not substantially impacted the delivery of therapeutics, or the bottom line of the pharmaceutical industry. Fundamentally, we have only just begun to fully characterise the specific variations in genomic data that are relevant and beneficial to the development of diagnostics, therapeutics and the dream of 'personalised medicine'.

While microarrays are already part of many drug development projects; there is room for improved

utility such as better solutions for cost, automation, high-throughput and data management.

Companies involved in development of new applications must work closely with their target customers, namely pharmaceutical, biotechnology and diagnostic research and development groups to understand the most efficient productisation path. Market share growth can occur rapidly for companies that take the time to understand both the application and the workflow involved with the target customer. **DDW**

The impact of these new microarray applications on the healthcare and pharmaceutical industries is examined in a newly released report, 'Beyond Expression Arrays, Emerging Applications for Genomic Microarray Technology' from Beachhead, LLC (www.beachhead.com). Based on in-depth interviews of leading scientific researchers and biotechnology executives, the report presents the current applications and products contributing to the development of tools for clinical and pharmacological applications. The report addresses the commercial adoption and market potential for these technologies, given pharmaceutical objectives and trends.

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