Over a decade of rapid advances in biology has swept an avalanche of genetic information into scientists’ laps. But analysis of so vast an input, whether to deduce the inner workings of cells or to diagnose diseases, would be impractical without high-throughput technologies. Of these, DNA microarrays are in the lead. These gene chips or biochips, to use their popular name, allow scientists to look for the presence, productivity, or sequence of thousands of genes at a time. Just five years ago, no practical method could do that.

Analysts predict the US $300 million market in DNA microarrays will leap ahead by about 50 percent per year through 2005. Currently, one company, Affymetrix Inc., Santa Clara, Calif., dominates the market. But as microarrays have caught on among others, Affymetrix has found itself embroiled in patent disputes with a host of other life science firms. Competition from other technologies may also challenge the company’s lead. The potential of various devices for genetics research and drug discovery caught the eye of established high-tech firms like Motorola, Hitachi, Corning, and Agilent Technologies. In often novel ways, each of these firms is adapting existing tools—semiconductors, inkjet printers, flat panel displays—to the manufacture of microarrays, and are developing high-volume manufacturing techniques using fine printing pins and inkjet nozzles to challenge the leading technology, photolithography. Motorola and a few of its rivals are also

### Using Arrays to Figure Out which Genes Are Turned On

DNA microarrays can be used to study gene activity, or expression. In toxicology, for instance, the expression of genes in normal tissue might be compared to those expressed in cells treated with a drug. When a cell is making protein, it translates the genes (made of DNA) it needs for the job into ribonucleic acid (RNA). To perform a gene expression experiment, the RNA is extracted from the cells, and, in a series of chemical reactions, DNA copies of the RNA are constructed and tagged with fluorescent dyes.
putting active electronic elements into their microarrays to manipulate and sense DNA.

Few of these companies are typically identified with the life sciences, and some, like Motorola, are outright newcomers. All are hoping, though, that their technology will compete hard with Affymetrix and smaller competitors’ approaches.

Even with novel technologies, rivals of Affymetrix will need to target its weaknesses or markets it does not serve. “Affymetrix is in a solid position,” said Ken Rubenstein, chief executive officer of the Lion Consulting Group in Emeryville, Calif., and author of a recent microarray technology analysis. He expects the company to hold onto most of the research market for the foreseeable future, because of the manufacturing and market infrastructure it has already built up. But there are parts of the market where others might compete. “Agilent is going to be cutting in on the market for customized arrays, and Motorola hopes to make inroads into [low-density arrays], what is now being largely done on a home-brew basis,” he said.

The potential profits are huge, because pharmaceutical firms are in a rush to translate the human genome results into new products. First, though, they must figure out what the genes do, how they interact, and how they relate to disease. This is too tall an order for experiments that focus on one gene at a time, but microarrays can perform experiments with thousands of genes simultaneously. Arrays can take snapshots of which subset of genes in a cell is actively making proteins—the term biologists use is “which genes are expressed.” Other types of arrays can indicate where mutations lie that might be linked to a disease. Still others could be used to determine if a person’s genetic profile would make him or her more or less susceptible to drug side effects.

In 1999, a group of scientists at the Massachusetts Institute of Technology (MIT), Cambridge, performed an experiment that helped establish the importance of microarrays to the research community, particularly to those studying cancer. The MIT group took Affymetrix chips containing 6800 human genes and used them to analyze the expression of genes in cancer
cells from two types of blood cancer, namely, acute myeloid leukemia and lymphoblastic leukemia. A standard pathology examination finds it difficult to tell them apart. But the arrays showed a set of 50 genes that have different activity levels in the two cancers, and those genes can now be used to distinguish accurately between the two diseases in patients.

Microarray basics
A microarray starts with a piece of glass, or sometimes a silicon chip, the size of a microscope slide or smaller. Onto this substrate are fixed thousands of patches of single-stranded DNA, called probes, each patch measuring just tens of micrometers across. The location and sequence of each patch of DNA are known ahead of time.

Genes, the instructions for making the proteins that do a cell’s work, are encoded in the sequence of chemicals that make up DNA. These chemicals, called nucleotides, each contain a sugar and phosphate backbone plus one of four molecules called bases—adenine, guanine, cytosine, or thymine (A, T, G, C).

In the DNA molecule, the nucleotides are stacked atop each other in two strands forming a twisted ladder. The rungs of the ladder are the bases, with adenine always across from cytosine and guanine always across from thymine; however, under certain conditions the DNA helix can unzip along the rungs of the ladder to form two single strands.

Microarrays draw on the so-called hybridization reaction. Two lengths of single-stranded DNA will bind together, or hybridize, only if the bases on one strand find complementary bases on the other strand. In practice, every adenine base must match up with a thymine, and each guanine with a cytosine.

A leading use of DNA microarrays is in determining which subset of a cell’s genes are expressed, or are actively making proteins, under certain conditions, scientists extract the RNA and then build single-stranded DNA copies of it. To aid in detecting the DNA on the microarray, they build fluorescent molecules, or tags, into the new DNA.

When the tagged single-stranded DNA is washed over the array, it sticks fast only to any single-stranded probe DNA that has a complementary gene sequence to its own. A scan of the array with a laser or other excitation source causes any DNA that has found a tagged match to fluoresce, and that glow is picked up by a detector. The detector can consist of a charge-coupled device or a photomultiplier tube.

The image from the detector is then fed to a computer, which analyzes the location, color, and brightness of each patch of DNA. Because the sequence of the array’s DNA in each spot is known, the sequence of any DNA captured on that spot is also known. Comparing the colors found at those points on the array reveals the difference in gene expression between the two cells.

Genetic variations called single nucleotide polymorphisms (SNPs) can also be uncovered by microarrays. SNPs are variations or mutations at a single spot in a gene’s sequence. Since single-stranded DNA prefers to hybridize only with its perfect complement, arrays can determine the presence of such a mutation. SNPs are thought to be key to why people vary in their susceptibility to diseases.

Experimental arrays containing partial genomes of organisms such as yeast and humans are the bread-and-butter of most firms; but many companies also custom-build arrays from gene sequences that customers upload to them. Most of these sequences for building arrays are available in public databases or from private genomics firms like Incyte Pharmaceuticals Inc., Palo Alto, Calif.

Straight from the wafer fab
There are a number of ways to make arrays, but Affymetrix, the current market leader in microarrays and owner of the now commonly used term “gene chips,” may have the one to beat. It makes its high-density arrays with a method familiar to anyone in the semiconductor industry, namely, photolithography. This technology, known in the microarray industry as light-directed in situ synthesis, builds DNA probes one base at a time right on the chip [see “Making Microarrays,” opposite page].

Construction begins with a glass slide that has been chemically primed with sites ready to bind nucleotides. The sites are capped by a photosensitive chemical that detaches under illumination. Light is shone through a patterned mask onto the chip, causing the capping chemical to break away from the areas it strikes, thus exposing the primed spots. A solution containing one of the four types of nucleotides (each molecule of which is itself attached to a capping molecule) is then washed over the chip. The nucleotides bond only to the areas that have been exposed, and add a capping layer themselves. As the process can be repeated with another mask and a different nucleotide, a variety of DNA sequences can be built on the chip.

Photolithography offers the highest density of probes per unit area of any technique in use. Production-scale chips can pack 400 000 probes in 20-µm patches [see “Is There a Moore’s Law for Gene Chips?,” p. 59]. The single-stranded DNA reaches only 25 nucleotides in length, so that it takes several such patches to positively identify a single gene, but Affymetrix claims the redundancy improves detec-
Microarrays can be constructed in a variety of ways, but most fall into one of two categories. Using either printing pins or inkjets, droplets containing many copies of a sequence of DNA can be stuck to a substrate. Contact printing involves wetting a printing pin with the DNA solution and tapping it to the microarray surface. Inkjetting ejects uniform droplets of solution onto the substrate.

An alternative, known as *in situ* fabrication, builds the DNA sequence at each site one nucleotide at a time. This is done using either inkjets or photolithography. In inkjetting, solutions of nucleotides are ejected from the nozzle onto the substrate, then chemically fixed to the surface. The next set of nucleotides are jetted onto the first and chemically fixed to those. The process is repeated until the desired set of DNA is complete.

In photolithography, light at 365 nm is shone through a mask to illuminate a subset of regions on a substrate, which is coated with a photosensitive capping chemical. The light releases the capping chemical, exposing parts of the substrate. A solution containing a single type of nucleotide attached to a photosensitive chemical is then washed over the substrate. The nucleotides attach to the unprotected sites, adding their own capping layer. The process is repeated, building up sequences of DNA.
tion and quantification of the target gene. One weakness to the current photolithography method is that a new set of masks must be produced for every new type of array. Help may be in the offending for the latter problem, however. Scientists at the University of Wisconsin in Madison, the University of Texas Southwestern Medical Center in Dallas, and Xeotron Corp. in Houston have demonstrated a maskless technique that uses an array of micromirrors that reflect onto the appropriate spots on the chip.

**Gene spotting**

Perhaps the most straightforward array-making method is contact printing. A pin is first dipped into a solution containing pieces of DNA of uniform sequence that have been synthesized in the lab. The pin is pressed to the array surface, leaving behind a droplet of solution (again, see “Making Microarrays,” previous page). Researchers and companies have developed several variations on this basic technique. The most obvious is the replicator pin, whose point must be rewetted after each deposition. Alternatively, pins with a split tip or a hollow tip hold a reservoir of fluid. In a third method, utilized by

### Transatlantic Scuffle

**BY CHRISTOPHER MORRISON**

*Contributing Editor*

**On 2 November 2000,** the stock price of Affymetrix skyrocketed more than 30 percent. But there was no laboratory “Eureka!”—no cancer cure, or financial windfall behind this sprint to the top of the Nasdaq charts. In fact, the Santa Clara, California-based biotechnology company had yet to turn a profit.

Instead, investors were responding to a British Court of Appeals decision favoring the company over Oxford Gene Technology (OGT) Ltd., Oxford, in a lawsuit over the use of the technological gems known as the Southern Patents. Those patents describe technology for synthesizing short sequences of DNA and other nucleic acids on glass surfaces. Affymetrix and industry analysts touted the ruling as a huge victory, an announcement that cemented its leadership position in the commercialization of DNA microarray technology.

The OGT lawsuit had sought to take a big bite out of Affymetrix’ revenues. While OGT has a small, in-house business dedicated to microarray design and service agreements, the company’s primary focus is to license the Southern Patents, named for Edwin Southern, their inventor and an Oxford University biotechnology pioneer. Affymetrix began cross-licensing negotiations with the British company for use of the Southern Patents in 1997.

The talks did not go smoothly, however, and in July 1998, they broke down owing to Affymetrix’ “excessive demands” when it came to giving OGT access to its own microarray patent estate, said Chris Shelley, a lawyer with the Oxford- and London-based firm Manches, who represents the British company. Eventually, though, Affymetrix gained a license to the Southern Patents by purchasing the microarray business of Beckman Coulter Inc., in Fullerton, Calif., which had licensed the patents back in 1991.

Oxford Gene Technology then sued Affymetrix in June 1999, arguing that the firm could not transfer the license from Beckman Coulter and that it had infringed the Southern Patents. Affymetrix responded that not only did the Beckman buy give it a license, but the OGT patents were invalid as well—a common claim in patent disputes. In April 2000, a British High Court sided with the Oxford biotechnology firm. Affymetrix appealed the decision, leading to its 2 November victory when the three-judge Court of Appeals panel unanimously said the purchase, and hence the license, was legal. The validity issue was not decided.

**An open market?**

But OGT isn’t cashing the checks. It has bigger things in mind. “[It] would like Affymetrix to open up the market and license [Affymetrix’] competitors under its high-density array patents,” said Shelley. “Affymetrix is holding back a market that would be moving much faster.”

The 2 November decision effectively saves Affymetrix from that scenario, at least for now. Had Affymetrix lost, explained analyst Darren Mac of New York City’s Gruntal & Co., the other side could have forced the company to cross-license its own patents, which would open the market further for OGT customers such as Agilent Technologies and Incyte Pharmaceuticals.

Despite the UK victory and the subsequent run-up in Affymetrix’ share price, the fight is not over. OGT is appealing the 2 November decision to the House of Lords. If an appeal is granted, it will probably be at least a year before a final decision. The British company is also aiming for a retrial of the willfulness issue decided on 10 November, arguing that the Delaware judge gave the jury bad instructions. Finally, the validity of all these patents will come before the UK courts in March.

**Gorilla in the chips**

The OGT feud isn’t the only patent spat involving Affymetrix. The company has been sued by Hyseq Inc., of Sunnyvale, Calif., which alleges that Affymetrix’ GeneChips infringed its chip technology. Affymetrix has countersued. Affymetrix has also sued Incyte for infringement, after Incyte purchased microarray developer Synteni in 1998. All three companies claimed a partial victory in a preliminary ruling at the end of January.

(To be sure, others are in microarray legal disputes. Nanogen and Motorola, for example, are suing each other over a patent on electronic hybridization. A trial date for the case won’t be set until next October.)

Should Affymetrix be worried? “When you’re the 800-pound gorilla, people will come at you from all different angles,” said analyst Scott Greenstone, of Thomas Weisel & Partners, San Francisco. He notes that despite competing technologies and various lawsuits, Affymetrix has too much of a head start on the competition to lose too much of its market share. “At the end of the day, all this litigation will be a speed bump,” he predicted. Better buck up anyway.
a recently acquired division of Affymetrix, a pin passes through a ring near its tip before contacting the array surface. The ring, once dipped into a solution of DNA, acts as a reservoir for the pin.

One technology company now moving into microarrays, Coming Inc., of Corning, N.Y., chose the simple replicator pin design but executed it in a novel manner. It prints a thousand spots of DNA simultaneously onto a glass microarray, far more than any other contact printing method.

To break into high-density microarrays, Corning had to develop a system that did not infringe on anybody else's intellectual property (IP). "There is a significant amount of IP in place and we took some time looking at it," said Tom Hinman, division vice president and general manager of Corning's microarray business [see "Transatlantic Scuffle," opposite]. What the company came up with was a printing system that culled technologies from far-flung corners of Corning's own IP storehouse.

For its array, the company cobbled together several technologies it had developed over the years for other purposes. For instance, a three-year-old technique intended for printing color filters onto LCDs was applied to building an array of DNA-printing pins.

That technology involved a type of photosensitive glass into which features on the order of 100 µm can be etched. A pattern is projected onto the glass, which is then doused with hydrofluoric acid. The process yields a print head with 1000 pins about 100 µm in diameter, each separated by 100–120 µm.

Figuring out how to wet each pin with a unique DNA sample was another challenge. Here the company borrowed an extrusion process it had invented in the early 1970s for making porous substrates for catalytic converters. It combined that procedure with one of its techniques for drawing optical fibers, and the result was a funnel-shaped reservoir of 1000 tightly packed conical cells.

To make an array, a high-precision robotic system dips the pin head into the tip of the reservoir and then places it onto a glass slide. Coming currently uses a series of ten 1000-pin heads and reservoirs to produce arrays with 10 000 features each. The company started low-volume production last September, but Hinman told IEEE Spectrum that it will be up to full-scale by the end of this spring.

From inks to nucleic acids

Experience making inkjet printers has been parlayed into a microarray business by Hewlett-Packard spinoff Agilent Technologies Inc., headquartered in Palo Alto, Calif. Inkjetting has two capabilities: it can print spots of DNA sequences synthesized in the lab and also, in a process called in situ fabrication, it can build up parts of genes on the array one base at a time [again, see "Making Microarrays," p. 57]. Agilent recently started up a new plant in Santa Clara, Calif., which does both processes.

The inkjet technology used in the Santa Clara plant is essentially the same as that found in a desktop printer. Jets of fluid are pressed through nozzles and broken into uniform droplets by the print head. For in situ synthesis, the four colors of ink—cyan, magenta, yellow, and black—are replaced with nucleotides of DNA having the four types of bases—adenine, guanine, cytosine, and thymine.

This system can build lengths of DNA up to 60 nucleotides long, according to Bill Buffington, vice president and
Agilent Technologies applied its experience with inkjet printers to depositing DNA onto microarrays

100 jet nozzles, each spitting out a unique sequence of DNA. For both methods, the spot size ends up being 70–120 µm in diameter, allowing for arrays with about 25,000 features.

Among the other companies using inkjet spotting to deposit laboratory-synthesized DNA sequences is Motorola Inc. The key difference between its arrays and others is a third dimension. In July 2000 the company inked a $25 million joint development deal with SurModics Inc., Minneapolis, Minn., for making microarrays with a coating of acrylimide, a gel commonly used in genetics experiments. Coating a glass substrate creates a three-dimensional jungle of polymer to which DNA can be fixed, as it would be on a flat inkjet array. Motorola claims the gel allows certain enzymatic reactions to occur that might be important to future lab-on-a-chip applications.

In silico
Motorola is also looking at the lower-density-array markets. But many firms have already established a foothold there, and the array by first washing it in single-stranded probe DNA, biasing the desired spot on the chip, and then chemically fixing the DNA to that spot.

The electronics are also useful during the hybridization reaction, where single strands of DNA find their matches. Pooling DNA onto electrically charged sites speeds the reaction by a factor of as much as 1000, claimed Bob Martinsons, vice president of systems engineering at Nanogen. Conversely, a reverse voltage shaves loose imperfectly matched DNA, leading to more accurate results, he said.

Electric fields might find other uses in microarrays. Motorola is working on a method of detecting hybridized DNA using electrical signals rather than optical ones, Spectrum learned from Nancy Schmelkin, director of marketing for Motorola BioChip Systems. The technology is incompatible with the glass arrays the company is developing because it requires addressable electrodes and other embedded circuitry. So Motorola has begun designing a silicon-based technology to take advantage of its semiconductor manufacturing experience. It plans to combine that experience with technology from the recently acquired Clinical Micro Sensors, Pasadena, Calif., which has developed a process that detects hybridization through a change in conductance.

The future in medicine and research
Nanogen is hoping the improved hybridization speed of its chip will give it a leg up in the medical diagnostics market. That sector is practically nonexistent right now as far as DNA microarrays are concerned, but many in the industry see it as one day becoming the largest market for arrays. For instance, companies envision microarrays that can detect the presence of genetic variations that make one drug therapy more efficacious than another.

Some chips are already being tested in clinical laboratories, but estimates of when microarray-aided diagnostics will take off vary. For them to become an important market, the technology will have to improve. In particular, sample preparation time and complexity will have to decrease, so firms expect their current arrays to evolve into more complete on-chip laboratories capable of performing all the necessary procedures to extract genetic material from tissue or blood samples and then analyze it as well.

The research market is shifting as well. As more and more DNA sequences are completed, biologists are looking downstream of DNA for clues to how the body works. And so their attention is turning to understanding proteins and their interactions.

Microarray makers already have their eye on this market. Some start-ups such as Ciphergen Biosystems Inc., of Fremont, Calif., are building their business around arrays of chemicals such as antibodies that will bind and identify proteins much as the DNA devices do. Several firms, including Agilent and Corning, believe their basic microarray platform will be compatible with producing such arrays. And one firm, Packard Instrument Co., of Meriden, Conn., has given up its DNA array business in favor of protein chips. Only time will tell whether protein chips eclipse DNA arrays in importance and whether the big technology firms will succeed in either field.