Innovations

Chemistry on a pinhead
Illumina, Inc.

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The standard microtiter plate is undergoing a makeover, with any number of companies squeezing 384, 1536 or even 6144 wells into the space that once held just 96 wells. But for Illumina Inc. (San Diego, California) these increases in capacity are almost comically small. Illumina is crowding wells together at a density of twenty million per square centimeter, with each well individually addressable by light.

What makes it all possible is optical fibers. A one-step acid treatment of a commercial optical fiber turns the fiber into an array of microscopic wells. The cladding of the fibers resists acid treatment, even as the fiber cores are etched away to make the wells. Each well can then be viewed at the other end of the fiber using a microscope with a charge-coupled device (CCD).

Coded beads — synthesized in bulk in large pools and then randomly distributed into the wells — act as the sensors. “We’ve exchanged the very difficult problem of synthesizing things on a very small scale with the more tractable problem of decoding,” says Illumina founder and chief scientific officer Tony Czarnik. Now the company has to decide what, of all the things that could be detected by a bead-based sensor, should be the focus of the company.

Sensing with optical fibers

Illumina is based on the work of David Walt (Tufts University, Medford, Massachusetts). Initially Walt used single-core fibers to transmit the optical readout of a chemical event. Based on this technology, Walt founded Optical Sensors, Inc. (Minneapolis, Minnesota), which makes a group of three fibers to measure blood pH, carbon dioxide and oxygen levels without the need to draw blood. Ariano Technologies, Inc. (Cambridge, Massachusetts) uses the fibers to detect hydrocarbon vapors around leaking gas storage tanks, and YSOS Inc. (Yellow Springs, Ohio) does bioprocess monitoring during the production of drugs, vaccines, food and beverages.

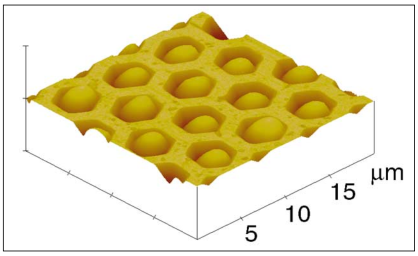
The next step for Walt was sensor arrays. By shining light through a single fiber in a bundle, he could use photoactivatable groups to attach sensors to a small area on the bundle tip. Up to 30 different sensors could be localized to different areas on a single bundle tip.

That density is still not high enough for what Illumina has in mind. The path to the Illumina arrays began with a modest paper in a 1996 issue of *Chemistry of Materials*. The paper reported the chemical etching of fiber tips to create optically wired wells. The next step was to place plastic microspheres (and the chemicals attached to them) in each well. Happily, approximately 96% of the wells could be filled by pipetting a suspension of the microspheres onto the end of the bundle and allowing the suspension to dry (Figure 1). “When my student Karri Michael showed me the micrographs [of the beads in the wells], our eyes were bugging out of our heads,” says Walt. “We were amazed that these things were working themselves into the wells.”

Before Walt contacted the venture capitalists there was one more problem to face. “We just had no idea what the things were good for,” says Walt. “We sat on the result for months and months.”

The simple regimen of pipette, settle, and dry was great for getting the beads into the wells, but it left the researchers with no clues about the location of different beads in the array.

Figure 1



Beads in femtowells. This false-color image is based on an atomic force micrograph of ~3.6-µm-diameter wells each containing a single 3.1-µm-diameter microsphere. Reprinted with permission from *Anal. Chem.*, 1998, 70, 1242–1248. Copyright 1998 American Chemical Society.

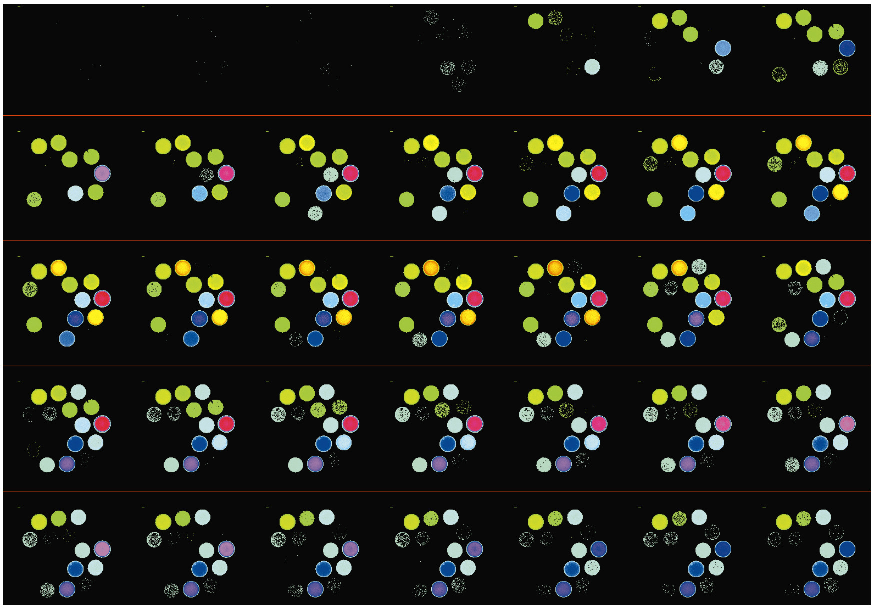
Walt’s collaboration with W. Clark Still (Columbia University, New York) inspired him with at least a temporary fix to the problem. Still’s chemical tags identify the beads used in combinatorial chemistry; a similar set of tags that generate an optical readout would allow Walt to locate every one of his microspheres. As described in a 1998 paper in *Analytical Chemistry*, Walt soaked each bead type, with its sensor attached, in a mixture of dyes of a particular identity and concentration. By correlating two optical readouts — one from the dye mixture and the other from the binding of molecules such as avidin and biotin to the various sensors — the researchers found that the system could differentiate six different types of beads with 92% accuracy.

The founding of a company

With the *Analytical Chemistry* paper came the birth of Illumina, which opened for business in June 1998. Czarnik arrived from IRORI (see *Chem. Biol.* **5**, R39–R40) and Mark Chee from Affymetrix (see *Chem. Biol.* **4**, 157–158).

“When I joined the company, we identified, I think correctly, that the method for refining the array was largely developmental,” says Czarnik. “We didn’t have a lot of discovery — we were assembling from known components. Where the invention part of the company lies is in the encoding.”

Figure 2



Detection of benzene by an optical nose. The figure shows 35 sequential images of the bundle of optical fibers that make up the optical nose. Each fiber has a different sensing chemical attached; these chemicals differ in the kinetics of their interaction with benzene and the effect of that interaction on their emission spectrum. The sum of all the changes in the different images is a molecular signature for benzene, which allows it to be differentiated from many other chemicals. In this experiment, benzene is applied between frames 4 and 19, with the entire set of images covering less than nine seconds. Image courtesy of Todd Dickinson, John Kauer, and David Walt. Reprinted with permission from Elsevier Science.

Walt's encoding solution works for a few different sensors, but it soon runs into problems with the overlap of multiple similar spectra. "This," says Czarnik, "is the intellectual nucleus of the company — how you can reliably encode thousands of beads in a robust way." Illumina is hoping that the answer, for both the thousands of beads in an oligonucleotide array and the millions of beads in a combinatorial chemistry array, is buried in paperwork now at the US patent office. "We've got solutions and they are part of patent applications," says Czarnik. "The proof-of-concept experiments for our first scheme have been completed successfully."

What is it good for?

Illumina's strength is the simplicity of array construction. "We are able to create something with very fine structure with only bulk processes," says Czarnik. "And the technology is very scalable. The method for creating 250,000 wells on a fiber is the same as that for creating one."

But who will buy the arrays, and what will they use them for? Illumina is initially focusing on single nucleotide polymorphism genotyping, and may move on to expression analysis. "I think the genomics application absolutely needs to be pursued in the short term," says Walt. "The number of companies interested in functional genomics or expression patterns is way up, and people are fixing on a platform. So if we are not out there now showing what we have, there won't be a market in a couple of years. It has to be a quick process."

One of Illumina's primary competitors will be Affymetrix, who have an issued patent covering expression analysis using any type of high density array. "Affymetrix is the leader in the DNA array synthesis world," admits Czarnik. But, he says, "we wouldn't even have begun our company if we weren't confident of our ability to practice." Illumina has the exclusive license to Walt's technology, which Walt claims will prove superior. "The small size of the array and the amount of information you can get from the array is tremendous relative to the other platforms," he says. "[Illumina's customers] will be able to capture any lost time just because of the high-throughput capabilities."

The next priority will be drug discovery: testing small molecules attached to the beads against protein targets. Antibodies on the beads could be used for proteomics or diagnostics.

The science-fiction side of the company is the optical-nose project (Figure 2). In collaboration with Tufts neuroscience professor John Kauer, Walt has been interested for several years in using optical fibers and fancy pattern recognition software to mimic the way that smells are detected by the nose and decoded by the brain. As in the nose, the fiber system recognizes patterns of binding interactions, rather than relying on specific binding events between one analyte and one sensor. Walt has used the system to detect the residue of explosives left on the outside of landmines (what he calls "the needle in a haystack problem"), and Illumina may develop it for quality control in industrial settings. But eventually Walt envisions a consumer product that could act as a smoke, gas and carbon monoxide detector, then tell its user if last week's chicken is off. Preventing a *Salmonella* infection, and finding the drugs to treat it, may both come down to the same bundle of optical fibers.

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