TECHVIEW: MOLECULAR BIOLOGY

Bead-based Fiber-Optic Arrays

David R. Walt

DNA microarrays have revolutionized the collection and analysis of genetic information. The monitoring of RNA expression and DNA variations has contributed dramatically to our understanding of basic biology and is having a direct impact in the clinic. Most DNA microarrays are prepared with one of three now-standard approaches (*1*). The Affymetrix GeneChip probe arrays are prepared using patterned, light-directed combinatorial chemical synthesis (*2*). Such arrays can contain hundreds to hundreds of thousands of probe sequences on a glass surface. To prepare spotted arrays, pins distribute preformed nucleic acid solutions to precise positions on various substrates (*3*–*6*). Arrays can also be created with ink-jet techniques in which oligonucleotides are synthesized base by base through sequential solution-based reactions on an appropriate substrate (*7*). A relative newcomer to the array field is the self-assembled bead array. This format is a departure from these three approaches and offers the molecular biologist an entirely new platform on which to study gene expression and DNA variation.

The bead arrays are assembled on an optical fiber substrate. Before describing the arrays, it is important to briefly review the basic principles of optical fibers and to describe how they can be converted into sensors. Optical fibers are made of two types of glass or plastic: the inner ring, called the core, has a slightly higher refractive index than the outer ring, known as the cladding (Fig. 1). Because of the mismatch in refractive indices, light is transmitted through the core over long distances by a process known as total internal reflection. This low-attenuation phenomenon is used routinely to carry light signals that encode most of our high-speed communications systems including telephone, Internet, and video signals.

Individual optical fibers can be converted into DNA sensors by attaching a DNA probe to the distal tip (*8*, *9*) or by removing the cladding and attaching the DNA probe to the outside of the core (*10*–*13*). Upon hybridization to its fluorescent target, labeled double-stranded DNA is formed that can be analyzed. When light at an excitation wavelength is focused onto the proximal end of the fiber, the fluorescent label on the distal end or on the core becomes excited. Isotropically emitted light from the fluorophore is captured by the same fiber and sent back to the proximal end where a detection system separates the excitation light signal from the emitted signal. Simple DNA arrays can be made from such optical fibers by physically bundling multiple fibers together (*14*). Advantages of optical fiber sensors are their small size and flexibility. Such features enable the sensors to be placed directly into sample solutions of DNA rather than bringing the samples to the sensor's surface.

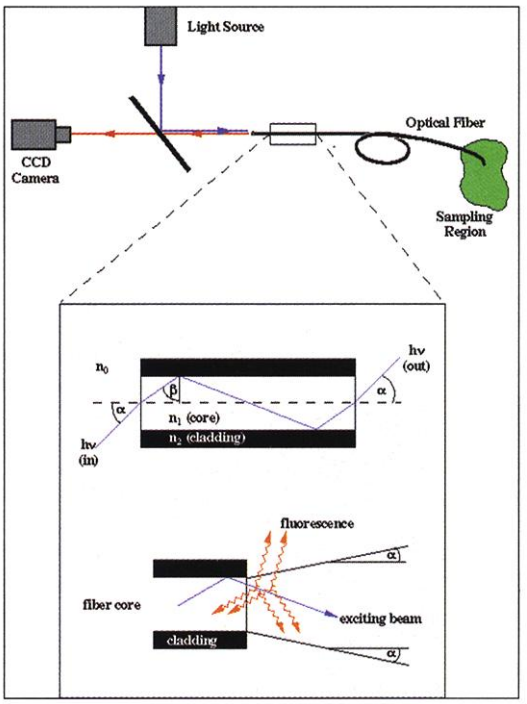

Fig. 1. Optical instrumentation used with an optical fiber array. Excitation light is launched into the fiber. Isotropically emitted light from fluorescent indicators on the fiber's distal tip is carried back along the fiber and filtered before image capture on a CCD camera.

Images cannot be carried over conventional optical fibers because the light signals become mixed and spatial resolution is not preserved. Imaging optical fibers have been created that contain an array of thousands of densely packed individual optical fibers fused into a coherent unitary bundle (*15*). These fibers are prepared by bundling larger optical fibers into a preform that is melted and pulled by a rotating drum to form the resulting fiber "thread", which has an identical structure and aspect ratio to the initial preform but is reduced in diameter. Typical imaging arrays contain between 5000 and 50,000 individual fibers, each 3 to 7 μm in diameter, creating a total array diameter of 300 to 1000 μm. Each fiber carries its own light signal; consequently, such arrays can be used to build up images with a pixel-by-pixel image reconstruction similar to that of an insect's compound eye. In one type of imaging fiber array, different DNA probes were attached to polymer spots distributed over the fiber's distal surface (*16*).

The distal end of a fiber's core can be selectively etched relative to the cladding when exposed to various chemical etching agents such as hydrofluoric acid. Wells of different depths are created depending on the strength of the etching agent and the exposure time (*17*). Figure 2 (left) shows a scanning force micrograph image of the etched surface of an optical imaging fiber. In this image, a section of an array containing wells about 2 μm deep is shown. At the bottom of the wells are the distal ends of the fiber that compose the array. Thus, each well is optically wired so that it can be tested individually. Latex or silica beads can be loaded into the wells either by dipping the etched fiber into a bead-containing solution or by applying a small aliquot of bead solution directly to the fiber tip. Upon drying, the beads are held firmly in the wells (*18*) (Fig. 2, right).

Fiber-optic oligonucleotide arrays can be

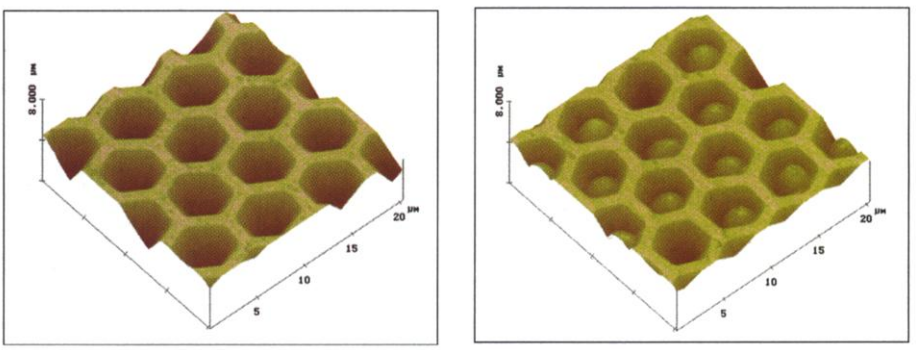

Fig. 2. Scanning force micrograph showing contours before (left) and after (right) microspheres were distributed into the array of etched wells on the optical fiber's surface.

The author is in the Department of Chemistry, Tufts University, 62 Talbot Avenue, Medford, MA 02155, USA. E-mail: david.walt@tufts.edu

prepared by attaching DNA probes to microspheres and then filling each well with a microsphere carrying a different DNA probe. Microspheres can be inserted directly into a conventional DNA synthesizer and the desired sequence can be built up base by base using conventional phosphoramidite chemistry. Alternatively, preformed oligonucleotides may be added directly to surface-activated microspheres. Individual populations of microspheres, each containing a different probe sequence, are then mixed to form a stock solution. This library of microspheres is distributed randomly over the fiber array surface. The microspheres are all the same size and are matched to the size of the etched wells so that only one microsphere occupies each well. Because the resulting array is randomly distributed, any of the sequences can be positioned in any given well and so a strategy must be devised for registering each array.

Several methods are available to accomplish this task. Each different type of microsphere is tagged with a unique combination of fluorescent dyes either before or after probe attachment. This "optical bar code" is simply a combination of fluorescent dyes with different excitation and emission wavelengths and intensities that allow each bead to be independently identified. This type of encoding is similar to that used by Luminex in its multiplexed flow cytometry assays (*19*); however, additional degrees of freedom are available in the fiber-optic format in terms of the number of excitation and emission wavelengths that can be used. The optically bar-coded arrays can be decoded in a matter of seconds with conventional image processing software by collecting a series of fluorescence images at different excitation and emission wavelengths and analyzing the relative intensities of each bead.

An alternative approach to decoding the array involves hybridizing labeled DNA molecules to the array (*20*). There are sequential hybridization-dehybridization cycles with different decoding solutions. Image processing techniques are also used to evaluate the images and register the positions of each bead type. Beaded optical fiber arrays differ markedly from other microarray formats in that the position of each probe in the array is not registered by deliberate positioning during array fabrication but is spectrally registered subsequent to its random distribution in the wells. Thus, each array is unique with different microspheres positioned in a different pattern from array to array. Although these arrays are easy to prepare, there are several disadvantages. As every array has the sensing probes arranged randomly, every array must be decoded. Each oligonucleotide probe also must be synthesized individually as opposed to the combinatorial synthesis approaches available with light-directed or ink-jet techniques.

Bead assays are becoming increasingly popular, being used in, for example, the Luminex system for flow cytometry (*19*), surface plasmon resonance–binding assays (*21*), DNA colorimetric nanoparticle assays (*22*), and solution-based DNA hybridization (*23*). A salient feature of microsphere-based analyses is the sheer scale of bead preparation. With a composition of 20% solids, 1 ml of a 3 μm bead suspension contains 10^{10} beads.

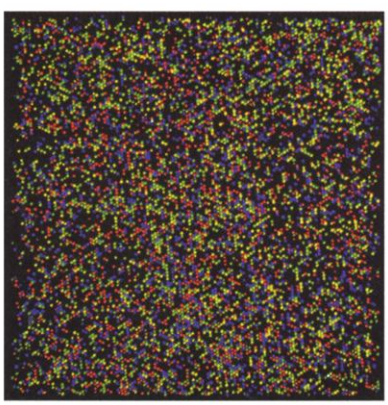

Fig. 3. An image of a 13,000-well square fiber bundle microarray, with a diameter of ~1.2 mm. This entire array is about the size of a single spot on a traditional spotted array.

One consequence of the fabrication technique is that replicates of each bead type are present in every array because of the necessity to ensure that the array contains at least one bead of each oligonucleotide probe. It is possible to calculate precisely the probability of preparing a random array that contains every bead type by applying Poisson statistics; a redundancy of 15 provides a high degree of confidence. With larger arrays there is a price to pay for redundancy because the area coverage for each probe sequence increases. The small sizes of individual beads make this aspect of bead-based fiber optic arrays acceptable. In addition, redundancy in bead arrays provides two advantages. First, a voting scheme in which replicates must agree can be used to virtually eliminate false positives and false negatives. Second, redundancy enhances sensitivity: Signal-to-noise ratios scale as the square root of the number of identical sensing elements so sensitivity can be enhanced by looking at all the identical probe microspheres in the array.

The ability to bring the fiber-optic arrays directly to the DNA sample facilitates the use of small sample sizes. Sample sizes of about 1 μl enable DNA to be detected after a limited number of amplification cycles. Typically, fibers are dipped directly into microtiter plates containing the target solution. Because the sensors are small, diffusion limitations typical of large-area planar surfaces are reduced. In addition, a simple high-temperature denaturation or organic solvent treatment can accomplish dehybridization. Fiber-optic microarrays have been used for over 100 hybridization-dehybridization cycles with less than 2% degradation (*24*). Furthermore, by adjusting the stringency with either temperature or solvent, single-base mismatches between the probe and target DNA can be detected.

When a DNA target sequence binds to its probe on a particular microsphere, the small size of individual beads ensures a high local concentration when only a few DNA target copies are bound. For example, a 3-μm bead occupies a volume of only a few femtoliters. When only 1000 labeled target molecules hybridize to the probe microsphere, a local target concentration of 1 μM is achieved—a relatively easy concentration of fluorescent dye to detect. This high local concentration of fluorescent probe combined with the signal-to-noise enhancements from redundant array elements, enables detection limits of femtomolar concentrations and absolute detection limits of zeptomoles (10^{-21} moles) of DNA.

Optical sensor arrays also provide a high degree of flexibility. As new sequences of interest are identified, new probe microspheres are simply added to the probe library. Thus, the incremental cost in both materials and time is negligible. This flexibility offers a particular advantage to the individual researcher. Finally, all the techniques for enhancing the flexibility of arrays, such as zip codes for universal fabrication (*25*) and molecular beacons (*24*, *26*) for label-less detection, can be used with optical sensor–beaded arrays.

References

1. S. C. Case-Green *et al., Curr. Opin. Chem. Biol.* **2**, 404 (1998).
2. S. A. Fodor, *Science* **277**, 393 (1997).
3. D. J. Duggan *et al., Nature Genet.* **21 (Suppl.)**, 10 (1999).
4. M. Schena *et al., Science* **270**, 467 (1995).
5. P. O. Brown and D. Botstein, *Nature Genet.* **21 (Suppl.)**, 33 (1999).
6. L. McAllister *et al., Am. J. Hum. Genet.* **61 (Suppl.)**, 1387 (1997).
7. A. P. Blanchard *et al., Biosens. and Bioelectron.* **11**, 687 (1996).
8. G. J. Zhang *et al., Anal. Lett.* **32**, 2725 (1999).
9. F. Kleinjung *et al., Anal. Chim. Acta* **350**, 51 (1997).
10. A. H. Uddin *et al, Nucleic Acids Res.* **25**, 4139 (1997).
11. P. A. E. Piunno *et al., Anal. Chem.* **67**, 2635 (1995).
12. F. F. Bier *et al., Sens. Actuators B Chem.* **38**, 78 (1997).
13. A. P. Abel *et al., Anal. Chem.* **68**, 2905 (1996).
14. J. A. Ferguson *et al., Nature Biotechnol.* **14**, 1681 (1996).
15. D. R. Walt, *Acc. Chem. Res.* **31**, 267 (1998).
16. B. G. Healey *et al., Anal. Biochem.* **251**, 270 (1997).
17. P. Pantano and D. R. Walt, *Chem. Mater.* **8**, 2832 (1996).
18. K. L. Michael *et al., Anal. Chem.* **70**, 1242 (1998).
19. R. J. Fulton *et al., Clin. Chem.* **443**, 1749 (1997).
20. M. Chee and D. R. Walt, unpublished results.
21. L. A. Lyon *et al., Anal. Chem.* **70**, 5177 (1998).
22. J. J. Storhoff *et al., J. Am. Chem Soc.* **120**, 1959 (1998).
23. R. Elghanian *et al., Science* **277**, 1078 (1997).
24. F. J. Steemers *et al., Nature Biotechnol.* **18**, 91 (2000).
25. N. P. Gerry *et al., J. Mol. Biol.* **292**, 251 (1999).
26. J. Liu and W. H. Tan, *Anal. Chem.* **71**, 5054 (1999).

CREDIT: KEVIN GUNDERSON AND MARK CHEE OF ILLUMINA, INC.