Broadband Biodetection: Holmes on a Chip

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Sherlock Holmes never knew where the next clue might lie, so he kept his sensory channels wide open:

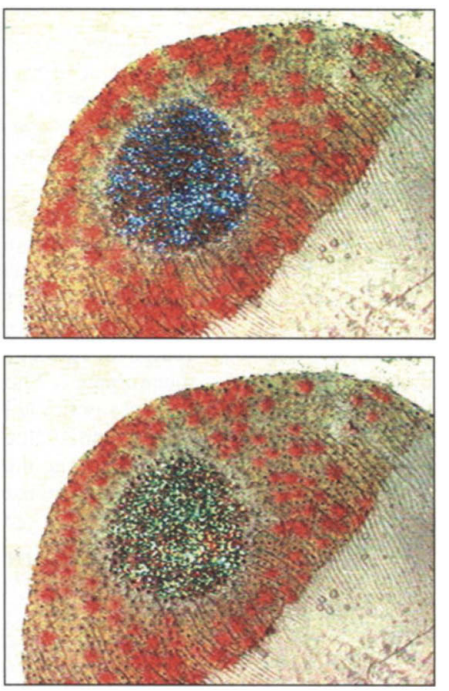
> "As he spoke, [Holmes] whipped a tape measure and a large round magnifying glass from his pocket. With these two implements he trotted noiselessly about the room...As I watched him I was irresistibly reminded of a pure-blooded, well-trained foxhound as it dashes backwards and forwards...until it comes across the lost scent." (*1*)

Biosensors and cytosensors are instruments that use biological elements to detect molecular clues with Holmesian breadth of sensitivity.

Biosensors

Biosensors use biological molecules—usually an enzyme, antibody, or nucleic acid—to recognize sample molecules of interest (*2*) via hydrogen bonding, charge-charge interactions, and other biochemical interactions. A secondary process, such as a colorimetric or fluorescent indicator reaction or an amplified bioelectric or biomagnetic signal, flags the primary molecular recognition event for the user. Some molecular recognition biosensors are used in familiar consumer products, including glucose monitors (enzyme-based), pregnancy test strips (antibody-based), and paternity test kits (nucleic acid–based). Others are used commonly in industry, such as the LAL (Limulus amebocyte lysate) biosensor, which uses blood-clotting proteins from the horseshoe crab to detect bacterial endotoxin contamination of pharmaceuticals. Of great recent interest are molecular recognition biosensors proposed for rapid fieldwork in response to bioterrorism. These include rugged and portable biosensors capable of rapid amplification of nucleic acid molecules to detect "fingerprints" of organisms known as bioterror agents.

The list of biological molecules that could potentially be used in biosensors is ever-growing, in part due to the newly described proteins and nucleic acid sequences emerging from the characterization of organismal genomes and proteomes. But a major obstacle to the use of many of these molecules is their delicacy. Biosensor molecules often are easily damaged and denatured by relatively mild experimental conditions, so the care and feeding of them is not a trivial matter. Therefore, much research is underway to develop stable biosensor molecules. Even so, many biosensors of the future will probably carry instruction labels such as "keep out of direct sunlight" and "avoid extremes in temperature."



An optically monitored cytosensor. The appearance of an isolated fish scale is shown before (top) and after (bottom) exposure to a chemical warfare agent. The colors change due to thousands of living chromatophores.

Though nature does not always provide the desired primary detection molecules, it is thought that a stitch here or a tuck there could refashion certain molecules into a useful synthetic form. For example, the nervous system enzyme acetylcholinesterase, a common target of organophosphate neurotoxic agents, may eventually be modified by genetic engineering or synthetic chemistry into a biosensor that can discriminate between relatively benign agricultural pesticides and far more hazardous chemical warfare agents such as sarin. One wholly synthetic biosensor, inspired by nature, uses nanocrystalline layers of silicon that change the distances between layers and thus their light-refracting properties when exposed to residues of explosive chemicals (*3*). The iridescent colors that result resemble those found in butterfly wings.

Many current biosensors involve miniature components that will enable them to one day be assembled within densely packed chips. A given single biosensor may serve as just one element in a large chip array with versatile detection capabilities. In a hunt for clues, the reliability and performance of each individual biosensor element could be fallible as long as the array as a whole is sensitive to many substances.

Among the merits of biosensors is their specificity: the ability to pick out from innumerable molecules those few of specific interest (*4*). Broadband specificity may be the highest merit of all, meaning that a biosensor can even recognize molecules that do not exactly match known standards. Pregnancy test strips, for example, use antibodies imprecise enough to recognize the many variants of the signature pregnancy molecule, hCG (human chorionic gonadotropin). Though they may differ in molecular mass, charge, and other physical attributes, hCG molecules share the same fundamental shape across the human population and thus are recognized by a single biosensor. The biosensor recognizes the distinctive features of a substance just as our brains interpret the words of a message whether it's written in pencil or in red ink.

Cytosensors

Cytosensors are detection instruments that use living cells as sensor elements (*5*). Cytosensors can be used even if the structure of a desired target is not known in advance because they detect "activity," the effect a substance has on the workings of a living system. Activities are conventionally detected by tests with live animals, such as the canary test for suffocants and the rabbit eye test for irritants. Cytosensors are broadband replacement technologies for animal tests, with foreseeable applications in the monitoring of indoor and outdoor environments for toxic substances, the discovery of new drugs in libraries of natural and synthetic com-

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pounds, measurements of toxicity in foods and cosmetics, the gathering of forensics information, and the diagnosis of disease.

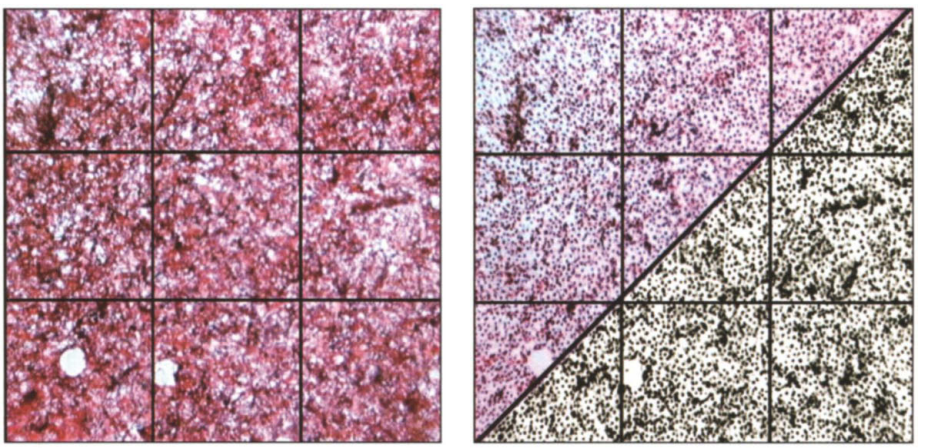
Perhaps the most influential cell-based detection system has been the Ames test, a remarkably prescient technology that uses bacterial cells to screen substances for their potential activity as DNA-mutating compounds. Such genotoxic compounds cause damage to the DNA blueprint in the bacteria, causing a change in microbial growth in culture. Bacterial cells can be engineered to give other measurable kinds of readouts, including flashes of light when a genotoxic substance interferes with the normal processes of gene expression such as RNA transcription and protein translation.

If a compound is not genotoxic, it may be cytotoxic, meaning that it interferes with the even higher processes of a cell, sometimes killing it. Of great use will be cytosensors that can serve as surrogates for specialized human sensitivities. Forseeably, a panel of several kinds of cells—liver, immune, cardiac, nerve cells, and others—could collectively detect many of the activities that affect humans. Plant cells, algal cells, protozoans, invertebrate cells, and other cell types could also be valuable in such a panel because they offer sensitivities to certain broad classes of active substances (e.g., herbicides).

The challenge of providing the living cells for cytosensors is being met by a nascent industry, involving firms such as Cambrex/BioWhittaker, Cellomics, and Rainwater Research, Inc., whose focus includes the production of primary cells, stem cells, and cell lines that are rugged, stable, and capable of detecting an abundance of clues about analytical samples. Innovative means are being developed for packaging cells into sealed chambers for use as consumable cartridges that are loaded into a reusable cell reader apparatus. A further challenge of detecting the often-weak signals from living cells is taking many interesting directions including the development of sensitive electronic and optical methods that can monitor in real time the multiple responses of large populations of single cells (*6*).

A rugged and sensitive cytosensor system has been developed at Oregon State University, based on the optical signals produced by living chromatophores. Chromatophores are the brilliantly colorful cells in the skin of fish, frogs, and other cold-blooded animals (*7*). These cells can change their appearance within a short time by powering movements of their internal colorants to camouflage the animal or help it attract a member of the opposite sex. By monitoring the optical changes in chromatophores as a function of exposure to a substance, one can use chromatophore cytosensors to detect many kinds of active agents (see figure, previous page). Some chromatophores, such as the black melanophores and red erythrophores, respond to active agents by moving their pigmented organelles to become darker or paler in appearance within seconds. Other chromatophores change their spectral hue in response to active agents, shifting between iridescent blue, green, yellow, orange, and red. Some chromatophores may show no initial color changes with exposure to an agent, but effects of the agent are revealed later when the cells are found incapable of undergoing normal optical changes when exposed to a natural nervous system substance like norepinephrine.

For this kind of cytosensor, the inclusion of additional cell types together with chromatophores can further widen the breadth of sensitivity of a test. For example, neuronal cells added to the cell chambers react to neuroactive substances by releasing natural neurotransmitters that, in turn, cause optically detectable changes in the chromatophores (*8*). All told, several dozen families of substances, potentially representing thousands of active agents, produce distinct changes in chromatophores. The living colors presented by 1000 isolated chromatophores in a 1 mm^2 cell chamber can take on a staggering number of combinatorial possibilities (see figure, above). Digital image analysis can dissect these many varieties of optical change and categorize the identity of an unknown agent (*9*).



Cell chambers. Chromatophores appear different before (left) and after (right) exposure to a blood pressure–regulating drug. The chamber is divided into a 3 by 3 matrix (1 mm^2) for statistical cross-checking of its performance. The triangular section (lower right) shows an example of a step in digital image analysis, which in this case pinpoints the chromatophores whose pigment has aggregated to the center of the cell as a consequence of exposure to the active substance.

Cytosensor research has been catalyzing mergers between cell science, bioengineering, and information technologies. It takes a multidisciplinary team effort to build a cytosensor, especially one ready for use in the field. As the technology develops, living cells will find their way into rugged, portable cytosensors for applications in the home, microelectronic gear, robotic control systems, and so on.

Detection instruments could ultimately combine the use of cytosensors and biosensors to gather the initial clues and set the course for further analysis by additional instruments such as mass spectrometers. Sherlock Holmes himself often did not connect all the clues until the last pages of the novel. But his broadband gathering of initial clues—the color of a cigarette ash or the depth of a footprint in the mud—was the key to breaking a case and identifying the guilty party.

References and Notes

1. A. C. Doyle, *A Study in Scarlet.* (Oxford Univ. Press, Oxford, UK, reprint. ed., 1993), p. 32.
2. Special Issue: Selected Papers for the Sixth World Congress on Biosensors, *Biosens. Bioelectron.*, **16**, 609–1142 (December 2001).
3. H. Sohn, R. M. Calhoun, M. J. Sailor, W. C. Trogler, *Angew. Chem. Int. Ed. Engl.* **40**, 2104 (2001).
4. A. P. F. Turner, "Biosensors: Past, present and future" [online] Cranfield Univ., Cranfield, Befordshire, UK, 1996 [cited September 2002]. Available from Cranfield University at www.cranfield.ac.uk/biotech/chinap.htm.
5. D. A. Stenger, *et al.*, *Trends Biotechnol.* **19**, 304 (2001).
6. I. Biran, D. R. Walt, *Anal. Chem.* **74**, 3046 (2002).
7. A. M. Karlsson *et al.*, *Biosens. Bioelectron.* **17**, 331 (2002).
8. R. R. Preston, P. N. McFadden, *Biosens. Bioelectron.* **16** 447 (2001).
9. T. R. Danosky, P. N. McFadden, *Biosens. Bioelectron.* **12**, 925 (1997).

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