biosensors—analytical devices based on molecules that recognize specific biological substances—have been the subject of extravagant claims and predictions over the last 30 years, leading some people to doubt their usefulness. Now, some of those predictions may be coming true, thanks to the development of new biological recognition systems, along with the use of mass-production techniques developed for microelectronics, printing, and photography that can improve durability and ease manufacturing. Biosensor technology may well find substantial new global markets in such varied fields as genomics, drug discovery, security, and process monitoring. With the addition of technology to feed the output signals from the sensors onto the Internet, it may even be possible to realize the long-cherished concept of e-medicine.

Biosensor technology has led to a solution addressing the need for biomedical diagnostics that allow reliable measurements to be made at the bedside, physician’s office, home, roadside, or workplace. By measuring levels of blood gases, electrolytes, and metabolites, doctors can monitor aberrant physiologies associated with pulmonary emphysema, respiratory disorders, heart failure, substance abuse, diarrhea, dehydration, diabetes, and kidney failure.
Our laboratory employs a simple reflection hologram as the interactive element in a sensor. The holographic element provides not only the analyte-selective polymer matrix but also the optical interrogation and reporting mechanism. Our system consists of a hydrogel (a smart polymer that swells or contracts when in contact with specific chemical or biological reagents) and a hologram that reports changes in the volume of the polymer. Hydrogels have the ability to respond to specific stimuli in an aqueous environment, although methods previously used to measure the volume changes in these polymers often lacked sensitivity and reliability. Our method involves coating a glass slide with a 10-µm-thick layer of unsensitized polymer that contains the appropriate receptor group. Next, we immerse the slide in solutions first of a silver salt (nitrate or perchlorate) and then of bromide ion containing a photosensitizing dye. This protocol, which we call the diffusion process, causes ultrafine rounded grains of photosensitive silver bromide (< 20-nm in diameter) to be precipitated within the volume of the smart polymer layer.

To create the holograms, we use a single 350-mJ pulse from a frequency-doubled (532 nm) neodymium-doped yttrium aluminum garnet (Nd:YAG) laser reflected from a plane mirror to create a classical standing wave pattern of interference fringes spaced half a wavelength apart. A polymer matrix coated onto an appropriate substrate records the standing wave pattern. After a conventional photographic development step, the fringe pattern appears as a distribution of ultra-fine grains of silver lying in layers within the thickness of the polymer film.

We construct the holographic sensor by cutting the substrate and placing a strip with its polymer side facing into a cuvette partially filled with a buffer solution. Since we use a reflection format, the sample and/or buffer can be opaque without impairing the optical properties of the hologram. Under white-light illumination, the developed grating acts as a reflector for a specific narrow band of wavelengths and holographically recreates the monochromatic image of the original mirror used in its construction. The constructive interference between partial reflections from each fringe plane gives a characteristic spectral peak with a wavelength \( \lambda_{\text{max}} \) described by the Bragg equation

\[
\lambda_{\text{max}} = 2nD \cos\theta
\]

where \( D \) is the fringe separation distance, \( n \) is the average refractive index, and \( \theta \) is the angle of illumination (see figure 2). A fiber-optic spectrometer measures the spectral peaks.

The peak reflectivity is dependent on the number of fringe planes and the modulation depth of the refractive index. Any physical, chemical, or biological mechanism that changes the fringe spacing or the average refractive index will generate observable changes in the wavelength or intensity of the reflection.
hologram. We can functionalize the sensor by incorporating receptor molecules in the supporting matrix of the hologram. When complementary ligands bind to these receptors, they will change the spacing of the fringes. The changes in fringe spacing appear as wavelength changes in the diffracted light.

**multiple chemical sensitivity**

We’ve used such smart polymers to fabricate chemically sensitive holograms responsive to water, alcohol, pH, and positively charged ions of alkali metal. For example, by co-polymerizing 2-(methyl methacryloyl)-18-crown-6 with hydroxyethyl methacrylate and ethylene dimethacrylate in a mole ratio of 47:50:3, we were able to produce durable polymers on glass slides that readily underwent the silver halide diffusion process and produced bright holograms. Using strips of sister holograms from one slide, we made a range of measurements of various mono- and bivalent ions. Rinsing the used hologram in de-ionized water completely reversed the swelling reaction.

The response of the hologram to the ions is predictable from the known stability constants for these ions binding in the cavity of 18-crown-6, and thus their ionic radii and charge densities. The selectivity for potassium ions over other ions likely to be found in blood, and the large response over the concentration range relevant for physiological measurements, suggested that 18-crown-6 holograms might be suitable for measuring potassium ions in blood. To test whether this would work, we repeated potassium measurements in a phosphate buffer solution containing sodium ions at physiological concentrations of about 150 mM. The response to potassium dropped significantly in the presence of high levels of sodium, but the sensor still responded to potassium. Crucially, the difference caused by varying the sodium background between the high (0.15 M) and low (0.13 M) extremes of the physiological range had relatively little effect on the potassium determination. Therefore, it seems likely that the sensor can be developed to give accurate blood potassium determinations. We also observed that holograms fabricated with polymers containing 15-crown-5 displayed preferential selectivity for sodium ions.

**enzyme watch**

We used two different approaches to monitor enzyme reactions with holographic gratings. First, we used changes in pH, generated by the release or uptake of hydrogen ions that occur in some enzymatic reactions, to alter the state of protonation, and hence hydration, of the holographic matrix. Second, we used enzymes to degrade the structure of the grating. A grating made from a smart polymer synthesized with pendant acidic functions could readily act as a pH sensor over a range approximately equal to the pKₐ of the functional group. If the pH of the solution around the grating increased, the acid groups in the grating became ionized, thus attracting more water molecules. The resultant increase in osmotic pressure caused swelling that the system registered as a red shift in the peak of the diffracted wavelength. Conversely, when the active functional groups of the polymer were predominantly basic, an increase in pH neutralized positive charges. The accompanying decrease in attraction for water triggered a contraction that caused a blue shift (see figure 3).

Changes in pH induced enzymatically by the action of biological substances urease, glucose oxidase, or penicillinase on their respective substrates allow us to construct metabolite-sensitive holograms. An alternative approach to monitoring an enzyme reaction uses the enzyme to lyse (dissolve) the polymer supporting the hologram. We demonstrated this concept by using trypsin to lyse the gelatin matrix of a proprietary brand of holographic recording material. Subsequently, we demonstrated hydrolysis of poly-(L-lysine) but not poly-(D-lysine)-based holograms. With the same technique, we monitored α-amylase levels using a hologram fabricated in a starch matrix.

The ability to make rapid measurements of high levels of α-amylase activity is important for the detection of acute pancreatitis, which causes raised levels of α-amylase in blood and urine. We fabricated holographic gratings in an interpenetrating network of starch and polyacrylamide. Treatment of these gratings with α-amylase resulted in partial lysis of the starch, causing changes in the spatial regularity of the holographic fringes throughout the volume of the hologram. This caused a blue shift in the λ_max of the diffracted light from approximately 680 nm to 620 nm over a period of about 10 minutes. It also caused the reflectivity of the hologram to weaken at an initial rate dependent on the concentration of enzyme.

As the reaction progressed, the regularity of the fringe structure recovered but at a shorter wavelength due to contraction. Amylase activity could be determined by the rate of change of either reflectivity or peak wavelength. Interestingly, we saw no apparent effect in further experiments using relatively high levels of maltase or β-amylase. Starch holograms have extended shelf lives and can respond

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**Figure 3** Poly-hydroxyethyl-methacrylate (HEMA) holograms constructed with 6 mol% of either DMAEM (squares) or MAA (circles) were incubated with buffers of varying pH (10 mM, I = 200 mM, adjusted with NaCl) at 30°C and the resultant diffraction wavelength monitored. The data plots show response over a pH range of 2.5 to 9.5.
Afer getting a PhD in biochemistry, Christopher Lowe discovered that, in the end, physics was all that mattered. Born in Barkshire, England, Lowe discovered early that he loved the sciences. “When I was young, I was always interested in chemistry, biology, and physics,” says Lowe, now director and a professor at the University of Cambridge Institute of Biotechnology (Cambridge, UK). “As a young scientist, I pursued applied chemistry, even though I remained interested in biology and physics.”

Lowe received a BS and PhD in biochemistry from the University of Birmingham in 1967 and 1970, respectively. He continued his postdoctoral research in Liverpool and Sweden before moving on to the University of Southampton. While residing as senior lecturer at Southampton, Lowe says he realized that his nagging love for physics and the need for scientists with multidisciplinary backgrounds were pushing him in new directions. “I wanted something real to come out of what I was doing,” he says. Making the difficult possible brought acclaim and awards, including the Pierce Award, the David Curnow Prize, the Schlumberger Stichting Prize, the Jubilee Medal, and the Queen’s Award for Technological Achievement.

During the next 20 years, Lowe started seven companies, the most recent of which, Smart Holograms Inc. (Cambridge, UK), is the culmination of his life’s work and the scientific disciplines that motivated him. “We have a healthy patent portfolio,” he says. “I have a strong feeling about biosensors, and I think [this company] is going to be a winner.”

In addition to Smart Holograms Inc., Lowe sits on the boards of five companies and a score of research councils and government scientific committees, all while supervising PhD students at one of the world’s oldest universities. Oh, and did we mention his 250 publications, seven monographs, and 40 patents? —Winn Hardin

Christopher Lowe is a professor and the director; Jeff Blyth, Satyamoorthy Kabilan, Blanca Madrigal-Gonzalez, Xiao-Ping Yang, and Colin Davidson are research associates; and Alex Marshall, Anthony James, Felicity Sartain, and Mei-Ching Lee are PhD students at the Institute of Biotechnology, University of Cambridge, Cambridge, UK. For questions, contact Lowe at +44 1223 334160 (phone); +44 1223 334162 (fax); or orcrl1@biotech.cam.ac.uk (e-mail).

References