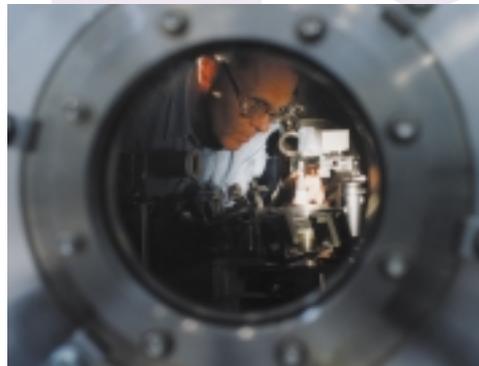


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THE NAVY'S CORPORATE LABORATORY

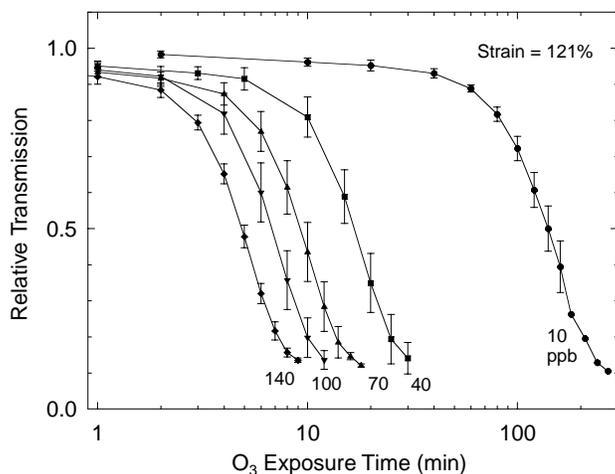


FIGURE 7
Change in optical transparency of polybutadiene stretched 121% while exposed to the indicated levels of ozone.

tion of the method, enhancing both the sensitivity and dynamic range.

Since ozone will not necessarily be the only air pollutant present, it is important to demonstrate that the test method is free from interference. We have determined that exposure to specific pollutants (sulfur dioxide, carbon monoxide, methane, nitrogen dioxide, nitrogen monoxide) has no influence on the response of the stretched elastomer to ozone. This is not surprising, since ozone is much more reactive than these gases.

Summary: Transparent rubber stretched in an ozone-laden environment initially develops micron-sized surface cracks due to ozone-induced chain scission. The consequent reduction in the transparency of the rubber provides a facile method for measuring ambient ozone levels. The loss of light transmission is linearly dependent on the ozone concentration, and is an increasing function of the strain. This method of detecting atmospheric ozone has high sensitivity (1 ppb) and a broad dynamic range. Envirionics, Inc. of Tolland, Connecticut, has obtained a license from the Navy to market the technology.⁴

Acknowledgments: We thank Envirionics, Inc. for the loan of their ozone generator and analyzer.
[Sponsored by ONR]

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The BARC Biosensor

L.J. Whitman,¹ P.E. Sheehan,¹ R.J. Colton¹, M.M. Miller,² R.L. Edelstein,³ and C.R. Tamanaha³
¹*Chemistry Division*
²*Materials Science and Technology Division*
³*Geo-Centers, Inc.*

Recent events in Yemen have made disastrously clear that acts of terrorism are one of the greatest threats to our Armed Forces. One of the most insidious threats is exposure to biological warfare agents. Unfortunately, treatments for infection by likely pathogens are currently limited, and may require treatment before any symptoms appear—the case for anthrax, for example. The invention of highly sensitive sensors for early detection of biological warfare agents is crucial. The Bead ARray Counter (BARC) system is a revolutionary tabletop biosensor we are developing at NRL to help solve this urgent national problem.

Gene Chips: The BARC biosensor is based on a so-called "gene chip." The function of a gene chip is to simultaneously look for a large number of distinct segments of deoxyribonucleic acid (DNA), the unique genetic material of life. Identification is accomplished by taking advantage of the unusual structure of DNA: two complementary strands that recognize one another, even in a complex mixture. An array of DNA spots (the "probes") is deposited on a surface (the "chip"), with each spot containing *single* strands of one particular segment of interest. When matching segments are present in a sample, they combine (hybridize) with their complements on the chip. The resulting double-stranded DNA molecules are typically labeled with fluorescent molecules. Then

a bright light is focused on the chip, and a special optical system “reads” which spots fluoresce.

Although the first commercial gene chips are proving useful for medical research, they fall short for biological warfare defense. Because very little light is emitted by the labeled DNA, a large, powerful, and complex optical system is needed to read a chip. Furthermore, to achieve the desired sensitivity—the detection of a few thousand DNA molecules or less per milliliter in the original sample—fluorescence-based systems require extensive sample preparation that may take hours to complete.

The BARC Approach: In the BARC biosensor, the fluorescent labels are replaced by magnetic labels that can be detected *individually* using an array of magnetic field microsensors embedded in the chip (Fig. 8).^{1,2} The magnetic labels are commercial beads, 1 to 2 microns in diameter, containing some magnetic material. The magnetic field sensors are wire-like structures on the chip, a few microns wide, made of a special metal alloy that displays giant magnetoresistance (GMR). When a magnetic bead is present above a sensor wire, the wire’s resistance decreases by a small, but detectable, amount. The more beads present, the larger the change in resistance. Hence, the BARC approach eliminates the optical system required for fluorescence-based detection, replacing it with simpler, more sensitive, and less expensive microelectronics.

The biochemistry underlying the BARC biosensor is illustrated in Fig. 8. The chip containing the GMR sensors is coated with thin layers of silicon nitride (~1 μm) and gold (~50 nm) to protect the electronic circuitry from the saline solution containing the DNA. A custom-designed arraying system then deposits 0.3 mm-diameter spots of single-stranded probe DNA over the GMR sensors, with one end of

each DNA molecule specially modified with a sulfur atom to bond to the gold surface. The DNA segments are from genes of pathogens likely to be used for biological warfare, such as *Botulinum*, *Francisella tularensis*, and *Yersinia pestis* (responsible for botulism, tularemia, and plague, respectively). The genetic information was provided by the Naval Medical Research Center.

After depositing the probes, the remaining surface is coated with polyethylene glycol, a polymer that inhibits the sticking of DNA or microbeads in the areas outside of the spots. The sample is then introduced and allowed to hybridize with the probes. At this point in our system development, the sample DNA must first be prepared so that it is single-stranded and tagged with biotin, a small ligand molecule that binds very selectively (and strongly) with a special receptor molecule, streptavidin. Magnetic microbeads precoated with streptavidin are then allowed to settle on the chip, bonding strongly with any biotin present and thereby labeling the captured sample DNA. The beads that are not bonded in this way are easily rinsed off, and the remaining beads are counted by the GMR sensors, indicating the identity and concentration of any pathogens present.

The Sensor System: The BARC assay is done in a small, glass flow cell mounted on the sensor chip (Fig. 9). The chip carrier board is housed in a disposable plastic cartridge that contains the reagents for the assay. The cartridge plugs into the electrical control system and interfaces with a miniature pump (not shown) that regulates the fluid flow. The overall system is operated with a laptop computer. A demonstration of the BARC biosensor is illustrated in Fig. 10. The eight sensor regions on the chip were each coated with gold. Two of each were arrayed with four different probes (one a positive control), and an assay

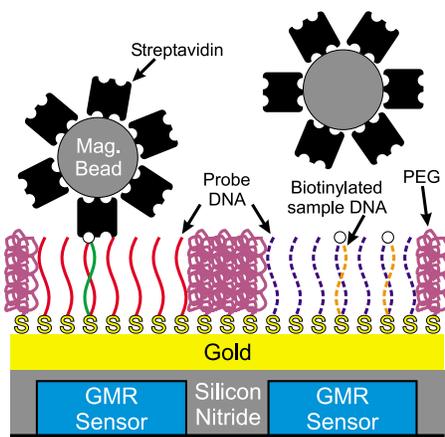


FIGURE 8
The BARC biosensor approach. Note that the elements are not to scale; in particular, the beads and sensors are much larger in proportion to the molecular components.

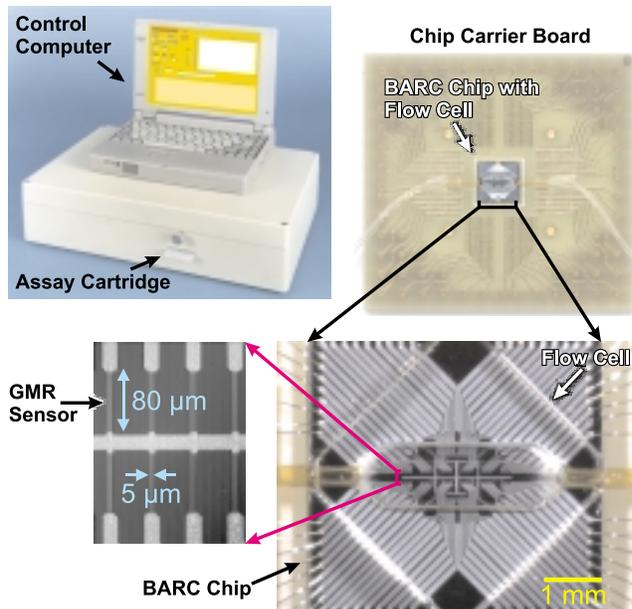
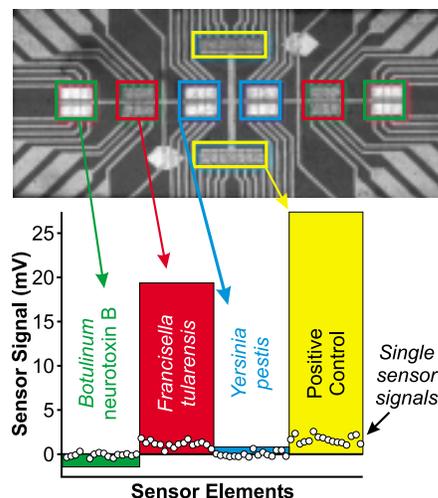


FIGURE 9
The BARC system and some of the internal components. The 5-mm-wide BARC chip (with the flow cell on top) is mounted on the chip carrier board, which is housed within the assay cartridge. The prototype chip shown has 64 GMR sensor strips, $5 \times 80 \mu\text{m}$ each, in groups of eight.

FIGURE 10
The results of a three-analyte assay. A view of the chip surface after the assay is shown, with the eight sensing regions outlined. The dark regions are almost completely covered with microbeads. For each analyte, the graph shows the measured signals from the individual sensing elements (symbols) and the integrated signal for all 16 elements (colored bar). The assay for *Francisella tularensis* was performed with 10 nM DNA hybridized for 30 min. The total assay required about 60 min.



was performed for *Francisella tularensis*. Afterward, the control and *tularensis* regions are densely covered with beads, while the other four sensor regions are nearly bead-free. The amount of DNA in the sample is measured by summing the signals from the sensors addressing each probe type (16 each).

Prospects for Further Development: The GMR sensor used in the BARC system is similar to technology being developed for a new type of computer memory, magnetic RAM. It should be possible to adapt this technology to make inexpensive BARC chips with millions of sensors. Ultimately, we hope to develop a small cartridge that can be plugged into

a hand-held computer for rapid, sensitive detection of all known biological warfare agents. Such a system would also have broad commercial applications in the fields of biomedical research, diagnostics, drug discovery, forensics, agriculture, and environmental testing.

[Sponsored by DARPA and ONR]

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