Development of an Automated Handheld Immunoaffinity Fluorometric Biosensor

Micah A. Carlson, C. Brent Bargeron, Richard C. Benson, Allan B. Fraser, John D. Groopman, Harvey W. Ko, Terry E. Phillips, Paul T. Strickland, and James T. Velky

A new immunoaffinity fluorometric biosensor has been developed for detecting and quantifying aflatoxins, a family of potent fungi-produced carcinogens that are commonly found in a variety of agricultural products. They have also been cited as biological agents under weapons development. The handheld, self-contained biosensor is fully automatic, highly sensitive, quick, quantitative, and requires no special storage. Concentrations from 0.1 to 50 parts per billion can be determined in less than 2 min with a 1-mL sample volume. The device operates on the principles of immunoaffinity for specificity and fluorescence for a quantitative assay. The analytic procedure is flexible so that other chemical and biological analytes can be detected with minor modifications to the current device. Advances in electro-optical components, electronics, and miniaturized fluidics were combined to produce this reliable, small, and versatile instrument. (Keywords: Aflatoxin, Automated, Biosensor, Fluorescence, Handheld, Immunoaffinity.)

INTRODUCTION

Aflatoxins are a group of chemically related mycotoxins formed by common fungi (*Aspergillus flavus*, *A. parasiticus*, and *A. nomius*) found in corn, cottonseed, peanuts, and other nuts, grains, and spices.[1] Fungal infection and aflatoxin production can occur at any stage of plant growth, harvesting, drying, processing, and storage. Both the infection process and aflatoxin accumulation are strongly affected by environmental conditions such as insect damage, temperature, and humidity. The molecular structures of the four most frequently occurring aflatoxins, B_1, B_2, G_1, and G_2, are shown in Fig. 1, along with two common metabolic by-products, M_1 and M_2, which are secreted in the milk of lactating animals that have consumed feed contaminated with aflatoxin.

Exposure by ingestion or inhalation of aflatoxins may lead to the development of serious medical conditions that vary considerably depending on the animal species, dose, diet, age, and gender. Acute effects are primarily observed in structural and functional liver damage, including liver cell necrosis, hemorrhage, lesions, fibrosis, and cirrhosis. Additionally, hepatic encephalopathy, immunosuppression, lower respiratory infections, gastrointestinal hemorrhage, anorexia,

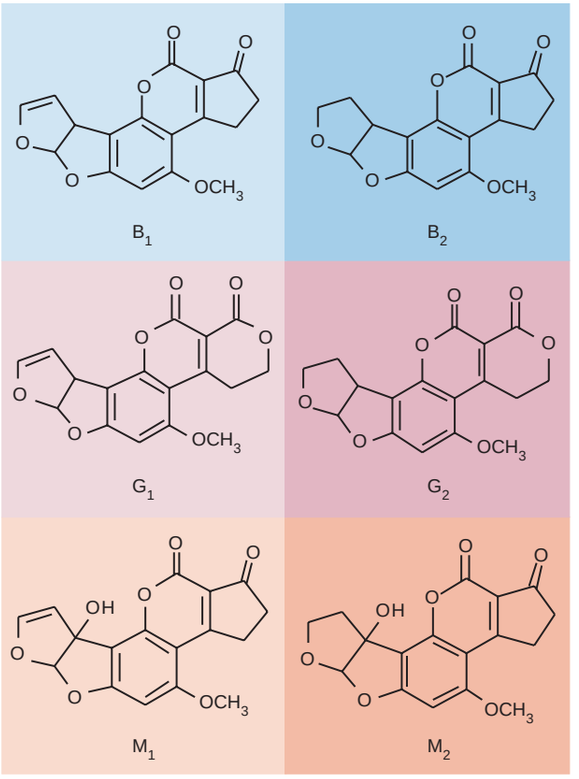


Figure 1. Chemical structure of the most frequently occurring forms of aflatoxin. The most toxic is the B_1 form, which is classified as "extremely carcinogenic." The others are somewhat less dangerous, being classified as "highly carcinogenic," "carcinogenic," and/or "extremely poisonous." The two type M aflatoxins are found in the milk of lactating animals that have ingested type B aflatoxins.

malaise, and fever have been observed. Chronic exposure to aflatoxins often leads to liver cancer as well as carcinomas of other organs (kidney, lung, colon, and the nervous system).[1]

Aflatoxins and Public Health

By the early 1960s, it became evident that aflatoxins represent a significant public health concern. Although contamination from ubiquitous fungi such as *Aspergillus* cannot be eliminated, exposure to the toxins can be minimized. Consequently, aflatoxin levels in animal feed and various human food products are now monitored and tightly regulated by various U.S. government agencies. Action levels above which products are removed from commerce are typically 20 parts per billion (ppb) for human foodstuffs, with the exception of milk, which has a more stringent action level of 0.5 ppb for one of the aflatoxin metabolites. Somewhat higher action levels (100–200 ppb) are allowed for some livestock feeds, depending on the species' sensitivity to aflatoxins and the ultimate use of the animals.[1]

It seems to be generally accepted that current regulatory mechanisms adequately control the level of aflatoxin in our domestic food supplies. However, in remote areas of several foreign countries, the lack of effective regulation and adequate control of animal feed and human food production frequently results in many people consuming foods with high aflatoxin levels. The fact that aflatoxins have been developed by foreign powers as weapons of mass destruction[2] and have caused serious worldwide public health concerns prompted initial investigations into the design and construction of a field-portable sensor for aflatoxin as well as other toxins, agents, and analytes of interest.

The APL sensor design was a proof of concept for a miniature, sensitive, rapid aflatoxin sensor. It demonstrated that the essential components of a portable sensor could be produced quickly and inexpensively in a small, handheld package. The sensor could be used to sample solid or liquid food products, various bodily fluids and materials (saliva, blood, urine, or stool samples), or possibly air, water, and soil in civilian or military environments. All of these very different scenarios require significantly different approaches to collecting and preparing samples. For this prototype instrument, the sample requirement is simply a small volume (1 mL) of nominally clean aqueous solution, which may be prepared by one of many sampling procedures.

Measurement Techniques

There are several different accepted measurement techniques available for determining aflatoxin levels. In general, most of these techniques require three steps: extraction of the aflatoxin from complex mixtures of materials in which it is found, purification to remove interferents, and finally, detection and quantification. The more traditional techniques used to purify aflatoxin before presenting it to a detector for quantification use some form of chromatography, a mainstay of analytical chemistry. Examples include thin-layer, gas, and high-pressure liquid chromatography. These methods are well proven and widely accepted, however, they are often viewed as laborious and time intensive, requiring a significant investment in equipment, materials, and maintenance.

Some more modern methods of sample extraction and purification rely on immunochemical techniques. These techniques use aflatoxin-specific antibodies to efficiently extract and purify aflatoxin from complex mixtures. Antibodies are fundamental components of immune systems, which protect against various disease-causing organisms and foreign molecules. They are proteinaceous biomolecules that identify and tightly bind a specific antigen or target molecule, such as a coat protein on a flu-causing virus. Once bound, the antibody–antigen complex is marked for destruction by other elements of the immune system.

Outside the body, this same antibody specificity has been exploited by microbiologists to enable faster sample preparation and to provide for very sensitive antigen identification schemes. Whereas the target antigen is typically some foreign protein on the surface of a bacterium or virus, it is also relatively easy to grow antibodies that are specific for many other chemical compounds, including aflatoxin. There are a number of immunochemical-based assays that are used for detecting aflatoxin[3]: radioimmunoassay, enzyme-linked immunosorbent assay, and immunoaffinity column assay (ICA), which is used in the APL aflatoxin sensor and will be described in the following section.

Some immunochemical techniques require relatively little equipment, materials, and maintenance, but they are still labor intensive. Our intent in designing and building the prototype instrument was to use the best immunochemical techniques while minimizing the required manual manipulations. The result was a handheld, self-contained, automated sensor that can replace the "bench chemistry" methods along with their large, cumbersome, chemical laboratory analysis systems. The sensor also provides a quick, cost-effective means for determining the presence of aflatoxin in remote locations. We also intended to incorporate a modular design that would permit the rapid substitution of other reagents to detect various toxins within the same sensor platform. The sensor could be used to measure other toxins of agricultural, public health, and defense interests as well.

APPROACH

After many of the accepted test methods were evaluated, such as the ones mentioned previously, it was decided that the ICA was most suited to automation in a small, inexpensive, and fieldable instrument. A diagram of an ICA is shown on the far left-hand side of Fig. 2. To the right of this, the analysis steps are presented. In an ICA, the antibodies are attached to small (10- to 100-μm-dia.) beads of agarose, sepharose, other organic polymers, or membranes. The analysis begins by forcing a filtered, unknown liquid sample through a volume of these small antibody-coated beads (Fig. 2, Step A). Because of the large surface area and tortuous fluid path intrinsic to the packed beads, the aflatoxin can be captured in a much shorter time than that required by many other immunochemical techniques. The beads with the bound aflatoxin are subsequently rinsed (Fig. 2, Step B) to remove any unbound or nonspecifically bound impurities and interferents (labeled "junk" in the figure). After the rinse, an eluant solution, in this case consisting of about 50% phosphate-buffered saline and 50% methanol, is passed through the beads (Fig. 2, Step C), causing the antibodies to release the bound aflatoxin. The released aflatoxin is then collected and placed in a fluorometer, where its intrinsic fluorescence is measured and used to quantify the concentration.

To automate this intensive manual operation, the sample preparation and analysis procedures currently used in the ICA operation were refined and modified according to the size limitations and the complexity envisioned for the instrument. The techniques implemented in the automatic sensor replicate the steps that would normally be performed by a skilled laboratory technician. Thus, by following a standardized protocol, the sensor will determine toxin levels reliably and automatically by implementing well-tested and accepted analysis techniques. Since antibodies can be produced that recognize many different analytes, there are many potential applications for the technology to detect and measure a variety of substances such as proteins, toxins, viruses, bacteria, or other substances contained in a complex mixture.

SENSOR DESCRIPTION

In Fig. 3, the instrument is shown with its cover removed and its various components are identified. There are essentially two subsystems within this instrument: a fluidics subsystem, which performs mechanical sample-handling and processing, and an electro-optical system incorporating a miniature fluorometer that measures and reports the toxin level to the user. The

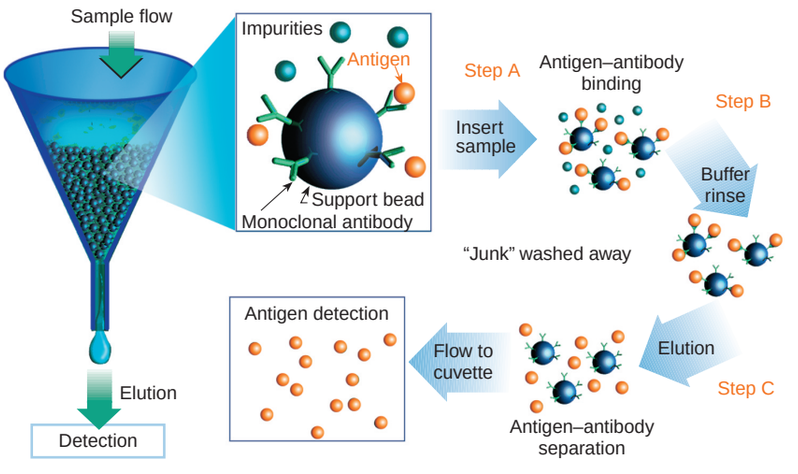


Figure 2. The immunochemical-based capture, purification, and detection process.

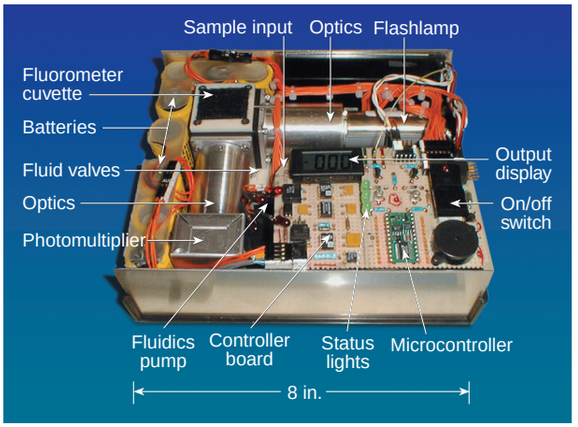


Figure 3. The interior of the sensor. Not visible are the liquid reagent bags and peristaltic pump located beneath the controller board at the bottom right of the instrument.

two systems are controlled by a microprocessor that directs the systems to perform the sequential steps in the analysis, which would normally be performed by a laboratory technician.

Fluidics System

In the initial development of the fluidics system, a flow-through cuvette was selected as the best interface between the fluidics and electro-optical systems. This style of cuvette allows for easy cleaning of the fluid path in the sensor, thereby eliminating potential contamination between samples.

A pumping system drives various fluids at differing times through portions of the system. Because of its low power consumption, small size, cleanliness, accuracy, and flexibility, a small peristaltic pump (INSTEC Model P625) was selected to provide adequate fluid movement in a small, low-power package.

The valve design was developed to provide the numerous fluidic switching sequences required during an analysis. The need to perform multiple analyses before servicing was a major driver in this design. This requirement resulted in a setup that is small and uses minimal fluid. Presently, the instrument can hold about 500 mL (total) of reagents, which is enough for about 100 analyses before replacement is necessary. A second, more important driver was the need for cleanliness. It was essential that the fluidics system not allow any liquids remaining from a previous measurement to mix with the current sample. The design shown in Fig. 4a was selected to use a minimum number of valves while reducing the required fluid volumes for sample processing, and still maintain the required cleanliness.

The valves facilitate the fluid sequence that is required to prepare the sample for the fluorescence measurement in the sampling cuvette. After the sample is introduced into the chamber, approximately 0.05 mL of fluid in the syringe is drawn through the bubble release valve into the waste chamber to eliminate any bubbles that may adversely affect the final measurement. A volume of sample, ranging from 0.05 to 10 mL, is then drawn through the affinity column containing 100-μm sephorose beads to which the aflatoxin-specific antibodies are attached. As the liquid moves over the beads, any aflatoxin in the sample is tightly bound to the antibodies, removing it from the liquid flow. The volume of sample selected is determined by the range of aflatoxin concentrations in which the analyst is interested. High concentrations (ppm) require small sample volumes, and low concentrations (ppb) require larger sample volumes.

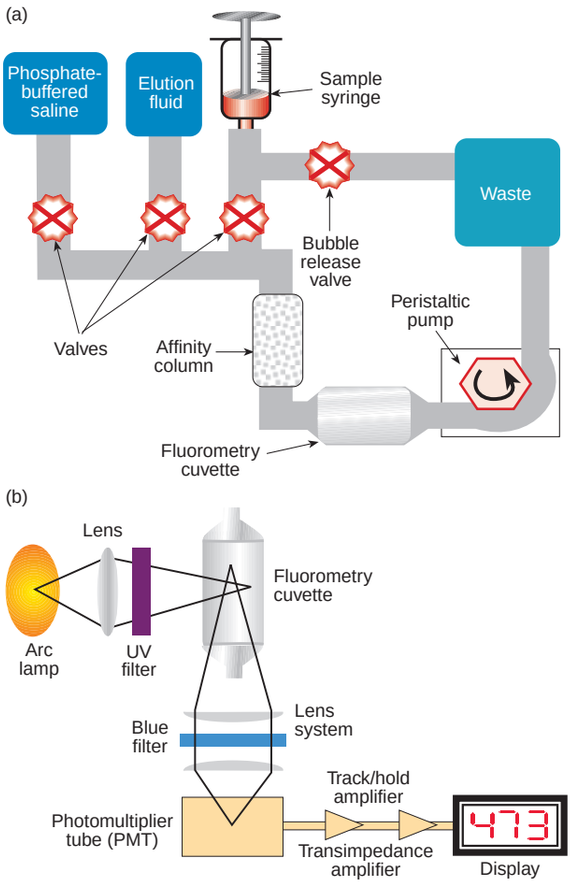


Figure 4. (a) Schematic diagram of the fluidics system. The primary fluid flow is counterclockwise from the valve manifold at the top left, through the affinity column, the fluorometry cuvette, the pump, and ending in the waste chamber. When the sample is first injected, a small amount (0.05 mL) of liquid is moved clockwise through the bubble release valve direction into the waste chamber to remove any inadvertently added air bubbles. (b) The electro-optics system contained within the instrument. A focused and filtered light pulse (365 nm) from the lamp is directed into the fluorometry cuvette, where the aflatoxin absorbs this energy and re-emits some of it in the blue region of the spectrum (445 nm). The PMT and associated electronics measure the amount of emitted blue light, which is proportional to the concentration of aflatoxin in the fluorometry cuvette.

Next, the affinity column is washed with phosphate-buffered saline to remove any nonspecifically bound, dissolved, or suspended materials that may later interfere with the fluorometric assay. After the toxin has been concentrated and purified on the column, a small quantity of elution fluid is passed through the affinity column. The elution fluid causes the toxin to be released quickly from the antibodies and redissolve. The fluid with antibodies moves into the fluorometric cuvette for analysis. The total volume of reagents (buffer and elution fluid) required to get the purified sample to the fluorometric cuvette is on the order of 2 mL. After the electro-optical system measures the fluorescence of the aflatoxin, another 2 mL of reagents is required to flush away the old sample and prepare the instrument for the next sample.

Electro-optical System

The fluorometer is similar to standard crossed-beam laboratory instruments, but the size and power requirements were reduced significantly for its use in a hand-held sensor. The system shown in Fig. 4b uses a xenon arc lamp to generate a microsecond flash and a lens to focus the radiation onto the cuvette. A filter passes only the radiation in the near-ultraviolet band centered around 365 nm that optically excites the aflatoxin fluorescence. The optically excited aflatoxin fluoresces in proportion to its concentration by emitting blue light centered on 445 nm. A second lens captures some of this emitted light and focuses it through a second blue-transmitting filter onto the photomultiplier tube (PMT) detector. The PMT produces a microsecond-long electrical pulse having a total charge that is proportional to the light input and therefore to the aflatoxin concentration. A voltage proportional to this charge is captured by the transimpedance and track/hold amplifiers in a way that makes timing noncritical and reduces electrical noise. The voltage is then digitized and presented on the sensor's display.

Microprocessor

An onboard microprocessor controls all of the fluid movement and fluorometric analysis sequences. It acts as the technician in the operation of the system, but with more reproducibility. The sequence of events is the same as that performed by laboratory technicians for the commercial fluorometric analysis process. The various processes controlled by the processor are illustrated in Fig. 5. The steps indicated in the diagram include the sample injection, washing, eluting, injection into the cuvette, various power supplies being cycled on and off to conserve power from the system's lithium battery pack, ultraviolet lamp pulse initiation, amplifier control, PMT measurement, display of results, and cleaning sequences. Empirical testing of the system was used to determine the actual times required by each of the sequences.

SYSTEM TESTING

Limited testing was done on the instrument to measure sensitivity, column viability, and interference rejection, and to answer design questions. All plumbing materials were selected primarily for their leaching characteristics and manufacturability. The materials in contact with liquid were required to exhibit minimal leaching to minimize the background fluorescence. Structural materials investigated included aluminum, stainless steel, titanium, Scotch 3031 epoxy, buna-n, delrin, Teflon, isoplast, nylon, polyolefin, zellite, and PVC. Isoplast and platinum-cured silicone were used for their low leaching characteristics and for their manufacturability. The column (glass) and the chamber (quartz) do not leach appreciably. Background levels on the fluids were also minimal (all were within the 0.1-ppb equivalent aflatoxin concentration range).

An example of the sensitivity as well as the operation of the instrument is shown in Fig. 6. In a normal operating mode, the instrument measures the

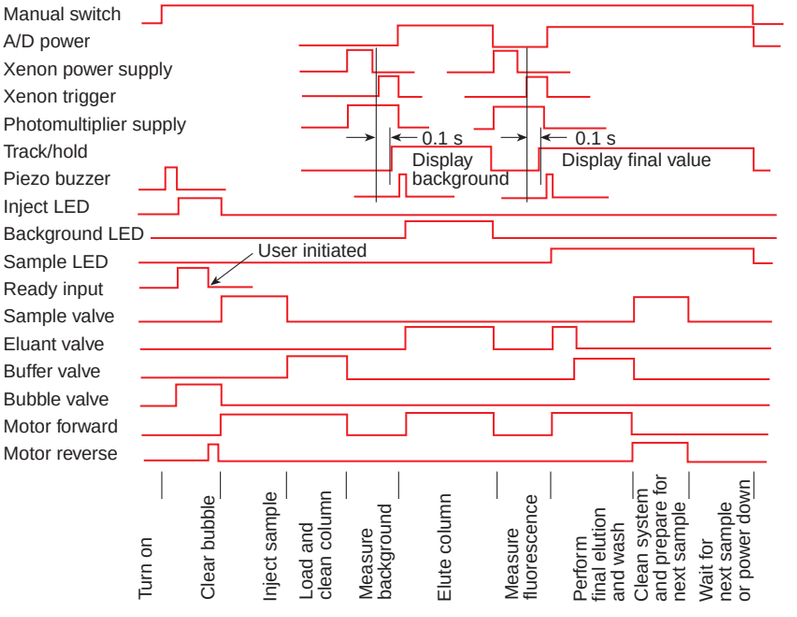


Figure 5. Sensor processes occurring during a measurement cycle.

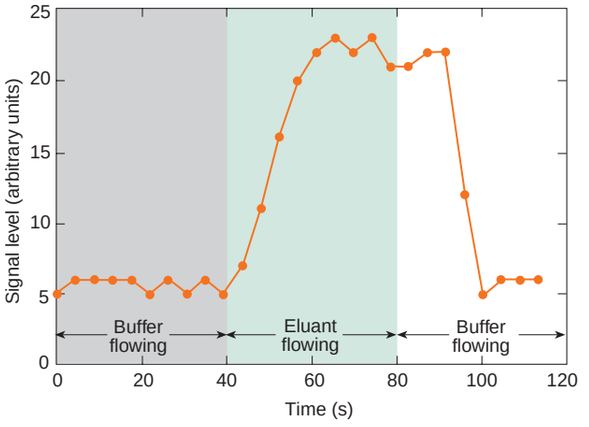


Figure 6. The operation of the sensor in the diagnostic mode in response to the introduction of 1.44 ng (equivalent to 1 mL of a 1.44-ppb solution) of aflatoxin B_2 at 0 s. The signal level on the abscissa is proportional to the amount of light striking the PMT at each measurement point. From 0 to 33 s, the buffer liquid is flowing, loading the sample onto the column and cleaning any impurities from the system. At 33 s the buffer flow is replaced by elution fluid, releasing the aflatoxin into the solution, as evidenced by the increased signal level. In the normal mode of operation, only these signal values occurring at the peak (around 60–70 s) would be reported to the user. At 80 s, the buffer flow is reestablished, purging the aflatoxin and preparing for the next sample.

fluorescent signal only twice during a single measurement cycle: once to get a background measurement and again when the maximum aflatoxin fluorescence signal is present. However, in Fig. 6, the data were obtained as the instrument was being operated in a diagnostic mode, where the fluorescent signal is monitored continuously every 4 to 5 s as the instrument proceeds through its normal sequences. The data are presented as "units," which are just the readings taken from the onboard 3.5-digit voltmeter. They represent the amount of charge generated by the PMT in response to a light pulse, and are therefore proportional to the aflatoxin concentration in the fluorometer chamber.

At 0 time in Fig. 6, 1 mL of a 1.44-ppb aflatoxin sample had already been loaded into the fluidics system. From 0 to 30 s, the buffer fluid was being pumped through the column and fluorometer chamber. This sequence completes the loading of the sample onto the column and the rinsing away of nonbound material. It also corresponds to the final stage of Steps A and B of Fig. 2. Note that the background signal was on the order of 5 to 6 units, indicating that very little fluorescent material is in the fluorometer chamber, and both optical and electrical leakage are low. At 30 s, the buffer fluid flow was switched off and replaced with an eluant flow, which is used to release the bound aflatoxin from the column (see Step C of Fig. 2). About 10 s into this elution phase, the fluorescent signal intensity begins to climb, reaching a maximum at around 60 s, as the aflatoxin is released from the antibodies in the column and flows into the fluorometer chamber. At this point in the normal operating mode of the instrument, the fluorometer would have been turned on, with the measured value being reported to the user. At 80 s, the buffer fluid flow is reestablished, purging the system of aflatoxin (as evidenced by the fluorescent signal level dropping back to the background levels), and thus preparing the instrument for another measurement cycle.

SUMMARY

The aflatoxin sensor provides portable multisample measurement and rapid sample throughput with minimal sample handling and consumables. It uses accepted testing procedures in a simple, one-button-operation unit. It provides very high sensitivity to aflatoxin, below 1 ppb, and can make over 100 measurements with one installation of fluids, batteries, and an immunoassay column.

The experience gained from integrating the various fluidics, optics, electronics, and biological subsystems into a successful sensor has revealed several further refinements and adaptations that may be applied to the sensor platform. First, the present instrument could be significantly reduced in size, possibly by 50 to 60%. Additionally, this basic platform design can be easily modified or extended to detect and measure many other chemical and biological substances of importance in the areas of chemical and biological warfare, environmental or agricultural science, law enforcement, and clinical medicine and health care. Because there is an obvious need for such measurement capabilities, APL is actively working on the modifications that will extend the application of this platform to a broader collection of substances. Some potential analytes being considered are as follows.

- Chemical/biological warfare threats (botulism toxin, nerve gases, sarin, sabun, ricin toxin, saxotoxin)
- Bacterial spore agents (anthrax, botulism, *Campylobacter*, *F. tularensis*, *Heliobactor pylori*, hemolytic *E. coli*, *Listeria, Pfiesteria, V. cholera, Y. pestus*)
- Drugs of abuse (amphetamines, cocaine, heroin/morphine, LSD, PCP, THC)
- Environmental/agricultural substances (aflatoxins, ochratoxins, fumonisins, heterocyclic amines, pesticides, polycyclic aromatic hydrocarbons)
- Health/medical interests (metabolites of agents, toxins, threats, metabolites of therapeutic drugs to set dose, pathogens)

REFERENCES

[1]Eaton, D. L., and Groopman, J. D. (eds.), *The Toxicology of Aflatoxins*, Academic Press, Inc., New York, pp. 521–523 (1994).
[2]*Presidential Advisory Committee on Gulf War Veterans' Illnesses: Final Report*, U.S. Government Printing Office, Washington, DC (Dec 1996).
[3]Chu, F. S., "Immunoassays for Mycotoxins: Current State of Art, Commercial and Epidemiological Applications," *Vet. Hum. Toxicol.* (Suppl.) **32**, 42–50 (1990).

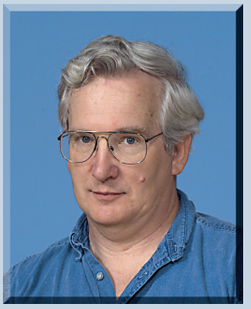
ACKNOWLEDGMENTS: The authors would like to acknowledge David R. Kohler and Charles W. Anderson for their valued involvement with initial aspects of the electro-optics and fluidics systems, A. E. Skip Colvin and R. William Lynn for providing valuable guidance as we worked to formulate the initial concept of this sensor design, and Mildred Donlon for her program support. This work was funded by DARPA and the Nonproliferation Center under contract MDA 972-96-D-002.

THE AUTHORS

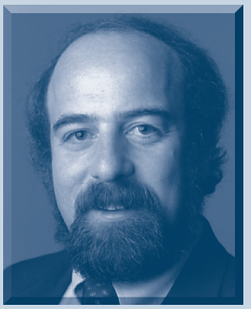
MICAH A. CARLSON is a mechanical engineer in the APL Submarine Technology Department's Engineering and Test Group. He received a B.S. in mechanical engineering and a B.A. in music from the University of Colorado in 1996. Mr. Carlson's specialties include structural design and analysis, materials engineering, system integration, and the development and application of miniaturized fluidics handling systems. His current work areas include the development of coastal monitoring buoy systems and an aerosol collection system for use in a field-portable miniature mass spectrometer. Mr. Carlson is currently pursuing his M.S. in mechanical engineering from The Johns Hopkins University and is also a member of ASME. His e-mail address is micah.carlson@jhuapl.edu.

C. BRENT BARGERON earned a Ph.D. degree in physics at the University of Illinois in 1971 and joined APL that year as a member of the Milton S. Eisenhower Research Center, now the Research and Technology Development Center. He is currently a member of the Sensor Science Group. Since joining APL, Dr. Bargeron has focused on solid-state physics, light scattering, chemical lasers, arterial geometry, corneal damage from infrared radiation, quality control and failure analysis of microelectronic components, electron physics, surface science, and sensor science. His e-mail address is brent.bargeron@jhuapl.edu.

RICHARD C. BENSON was educated at Michigan State University (B.S. in physical chemistry, 1966) and the University of Illinois (Ph.D. in physical chemistry, 1972). Since joining APL in 1972, he has been a member of the Milton S. Eisenhower Research and Technology Development Center and is currently supervisor of the Sensor Science Group. Dr. Benson's technical background is in physical chemistry, sensors, materials science, and lasers. He is currently involved in research and development on miniature sensors, counterfeit deterrence features for U.S. currency, the properties of materials used in microelectronics and spacecraft, and the application of optical techniques to surface science. He is a member of the IEEE, American Physical Society, American Vacuum Society, and the Materials Research Society. His e-mail address is richard.benson@jhuapl.edu.



ALLAN B. FRASER was an engineering science student at the Pennsylvania State University and completed his Ph.D. in biophysics at JHU in 1970. After joining APL in 1975, he worked in the Milton S. Eisenhower Research Center and in biomedical programs. Most of his career at APL involved nonacoustic submarine detection as the Chief Engineer of the Submarine Technology Department. Mr. Fraser is now retired, consults at APL and elsewhere, and is a member of the Society of Vertebrate Paleontology. His e-mail address is alflaurf@abs.net.



JOHN D. GROOPMAN is the Anna M. Baetjer Professor and Chair of the Department of Environmental Health Sciences at the JHU School of Hygiene and Public Health. He is also Professor of Oncology at the JHU School of Medicine and is the Associate Director of the Oncology Center for Cancer Prevention and Control. Dr. Groopman received his Ph.D. from the Massachusetts Institute of Technology in 1979. He joined the School of Public Health at Boston University in 1981, and in 1984 was promoted to Associate Professor. He became the Associate Dean at the school in 1987. In 1989, Dr. Groopman joined the faculty at the JHU School of Hygiene and Public Health. His main research interests have been in the development of biomarkers reflective of exposure and risk from environmental carcinogens. Dr. Groopman's e-mail address is jgroopma@jhsph.edu.

HARVEY W. KO is the Chief Scientist in APL's Submarine Technology Department. He obtained a B.S. in electrical engineering in 1967 and a Ph.D. in electrophysics in 1973, both from Drexel University. Since joining APL in 1973, he has been active in nonacoustic antisubmarine warfare, ocean electromagnetics, radar propagation, and biomedical engineering. He holds several patents in biomedical engineering for technology methods in brain edema, osteoporosis, and magnetoencephalography. His current research interests include bio-impedance, low-frequency electromagnetic holography, and chemical and biological detection. As manager of the counterproliferation efforts at APL, he is involved in the development of mass spectrometers and miniature electronic biosensor systems. He is a member of the IEEE and the Association for the Advancement of Medical Instrumentation. His e-mail address is harvey.ko@jhuapl.edu.

TERRY E. PHILLIPS is a chemist in the Sensor Science Group of the Milton S. Eisenhower Research and Technology Development Center at APL. He received a B.A. from Susquehanna University in 1970 and a Ph.D. in organic chemistry from JHU in 1976. After completing postgraduate studies at Northwestern University in low-dimensional organic conductors, he joined APL in 1979. He has studied photoelectrochemical energy conversion; inorganic optical and electrical phase transition compounds; high-temperature superconductors; and material characterization with X rays, nuclear magnetic resonance, mass spectroscopy, and optical spectroscopic techniques. His e-mail address is terry.phillips@jhuapl.edu.

PAUL T. STRICKLAND received a Ph.D. in environmental health sciences from New York University (1978) and received postdoctoral training at the Paterson Laboratories of the Christie Hospital and Holt Radium Institute in Manchester, England. He is currently Professor of Environmental Health Sciences in the JHU School of Hygiene and Public Health, and Associate Professor of Oncology in the JHU School of Medicine, Comprehensive Cancer Center. He was awarded National Institutes of Health and IARC Research Fellowships. His research interests include mechanisms of human carcinogenesis, monitoring exposure and risk in human populations, biomarkers of environmental exposure and individual susceptibility, and development of rapid biosensors for environmental toxins. He has published over 90 research papers, reviews, and chapters on these and related topics. Dr. Strickland's e-mail address is pstrickl@jhsph.edu.

JAMES T. VELKY is an associate engineer in the Engineering and Test Group of APL's Submarine Technology Department (STD). He received an associate's degree in electronics from Catonsville Community College in 1974. He joined APL in 1975 as a group leader of the Technical Support Department. In 1979, he transferred to the Submarine Test Team where he assisted with the development of sensors for towed oceanographic systems. Since joining the STD Engineering and Test Group (STE), his primary interests have included fluorometry, power management, and engineering of grounding and shielding techniques. He is currently involved with the development of the Tiny-TOF Mass Spectrometer. Mr. Velky is a member of the Design Review Committee for STE Group projects. His e-mail address is james.velky@jhuapl.edu.