Biosensor/transducer qualification: a critical step for homeland security

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Mounting a defense against bioterroism here at home, and protecting soldiers on the battlefield from chemical and biological warfare agents requires fast, reliable environmental sensors that provide early detection of these threats. Accurate characterization of new sensor designs can speed up development, and instrument manufacturers can provide valuable insight on how it's done.

MANY sensors, transducers, and detection systems are being investigated for defensive applications. They vary greatly in their complexity and the amount of time it takes to get results. Biosensor/transducer technologies that generate electrical outputs offer numerous advantages in the design of detection systems to meet speed and ease of use criteria. With proper testing and calibration, electronic biosensors can meet these criteria while providing reliable results that minimize false positive and negative indications.

Current Biosensor Designs

The focus of this article is on electronic biosensor/transducer units that can be used in bioterrorism defensive systems, and the electrical characterization of these sensors. Hereafter, biosensor/transducer units are referred to simply as biosensors, and they are defined as devices that do one or more of the following:

1. Detect, record, convert, process, and transmit information regarding a physiological change or process
2. Utilize biological materials to monitor the presence of various chemicals in a substance (analyte)
3. Combine an electrical interface (transducer) with the biologically sensitive and selective element

More specifically, a biosensor contains a bioreceptor, which is a biomolecule that recognizes the target analyte. The transducer portion of the biosensor converts the recognition event into a measurable signal that correlates with the quantity or presence of the chemical or biological target that can cause physiological damage. A generalized biosensor model is illustrated in ***Figure 1***.

Performance criteria for a biosensor system include:

1. Speed and ease of use (testing/calibration/maintenance) by non-technical personnel
2. Selectivity (to target analyte)
3. Sensitivity/resolution
4. Accuracy/repeatability
5. Dynamic range (high analyte concentrations will not degrade sensor usability)
6. Environmentally robust (relatively insensitive to temperature, electrical noise, physical shock, vibration, etc.)
7. Usable lifetime/adaptability
8. Safety/integrity (for personnel, equipment, and analytes)

Current biosensor systems can accurately recognize organic chemicals and microbes

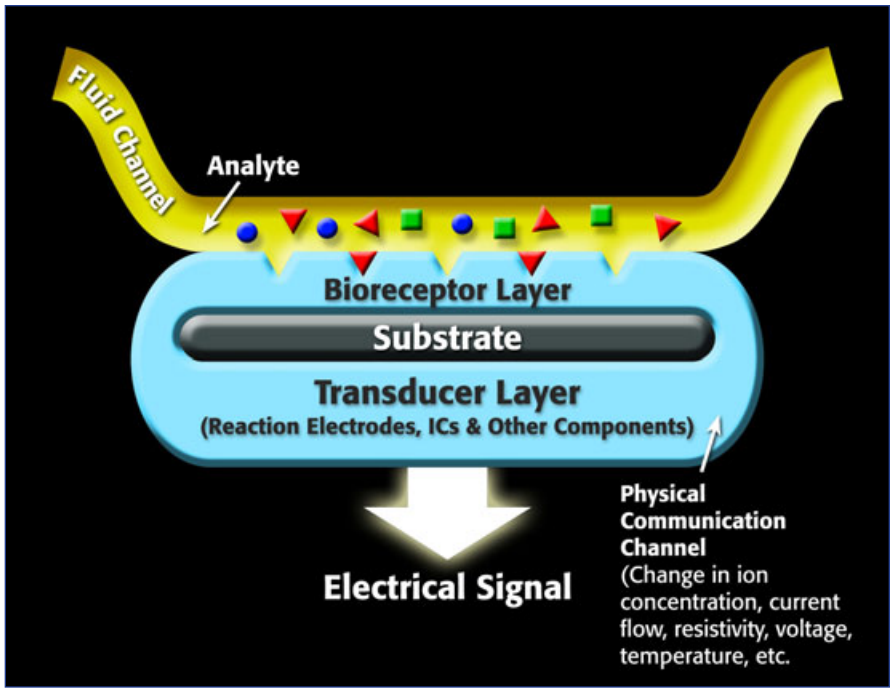

Figure 1. Representation of a generic biosensor.

utilizing various receptor technologies. For toxic airborne gases, inorganic sensor materials such as tin oxide, gold, platinum, and semiconductors are used. For microbes in liquid analytes (blood samples, etc.) bioreceptors such as enzymes, antibodies and nucleic acids are used. Chemical sensors for toxic gases can react quickly to their selected targets and provide a convenient electrical output. However, few commercial biosensor designs that utilize enzyme receptors, antigen-antibody reactions, ligand (DNA/RNA) binding, or whole cell metabolism can rapidly and automatically provide a high-level signal.

New Sensor Designs

For faster detection with a readable electrical output, researchers are developing biosensors that combine these or similar functions with semiconductor devices in compact portable units for field use. One approach uses an oligonucleotide sensor and nucleic acid reaction to indicate the presence of a pathogen. Another design employs surface plasmon resonance (SPR) to detect biological molecules such as protein and DNA. One SPR-based sensor reportedly provides label-free studies of molecular interactions in real time. It has a sensor chip interface that facilitates attachment of specific ligands to the transducer surface, and provides a sensitive measurement of surface concentrations.

Tissue-based sensors are also being developed. They utilize living cells on chips that can react functionally to the presence of both biological and chemical threat agents. Because they are designed to mimic the function of multi-cellular human tissue, these sensors should respond to both known and previously uncharacterized agents. The transducer senses small changes in electrical charges on the surface of the living cells.

A developmental liquid crystal detection system has been demonstrated that can selectively identify pathogens in only minutes. The liquid crystals amplify antibody structural changes that are induced by antigen-antibody binding. A change in the heavy molecular chain deforms the liquid crystal array alignment. Through the use of polarizing filters, light transmission through the liquid crystal cells is altered. This change can be converted to an electrical signal that alerts personnel when pathogenic agent antigens bind to the receptor housed in the liquid crystal matrix.

Multiple technologies can be combined to create biosensors with different performance characteristics. These include nucleic acid probes, PCR-amplified nucleic acid reactions, various enzyme-linked immunosorbant techniques, and many others. Some sensors designed around biologically engineered molecules may surpass the limits of binding measurable by other methods. Still, most of these devices trade off speed for sensitivity or lower cost. Typically, they require upwards of 10,000 organisms for detection, and may also require highly skilled operators.

Depending on the analyte and bioreceptor, the transducer portion of a biosensor could utilize one of the following mechanisms:

Amperometric: Amperometric devices detect changes in current. They measure currents generated when electrons are exchanged between a biological system and an electrode.

Potentiometric: Some reactions cause a change in voltage (potential at constant current) between electrodes that can be detected or measured.

Conductive: Conductimetric devices detect changes in conductivity between two electrodes.

Resistive: Resistivity is the inverse of conductivity, and can be measured with similar methods.

Capacitive: When the biorecognition reaction causes a change in the dielectric constant of the medium in the vicinity of the bioreceptor, capacitance measurement method can be used as a transducer.

Piezoelectric: In a piezoelectric material there is a coupling between its mechanical and electrical properties. It can be used to create an electrical oscillator whose frequency can be varied and measured by varying a mass applied to its surface. In the case of a biosensor, that mass can change due to the reaction taking place on the surface.

Thermal: These devices measure changes in temperature.

Optical: Optical biosensors correlate changes in concentration, mass, or number of molecules to direct changes in the characteristics of light. For this method to work, one of the reactants or products of the biorecognition reaction has to be linked to colorimetric, fluorescent or luminescent indicators. Sometimes, an optical fiber is used for guiding light signals from the source to the detector.

Sensor Characterization: First Step in Validation Process

Development programs are aimed at overcoming design limitations in current biosensor systems. For example, one of the problems in biosensor design is achieving a stable, reproducible interface between the biological affinity elements and an inorganic transducer element. The desire to miniaturize biosensors for handheld portability, and still achieve adequate sensitivity, imposes significant technical challenges in the coupling of biomolecules to transducer surfaces. Therefore, fast and accurate electrical characterization of biosensors in the development lab and in production is essential for qualifying the sensor/transducer interface, and the ultimate operation of a biodetection system.

A typical test program task is developing or verifying performance metrics for the biosensor. Because of the complexity in extracting cell and tissue signatures of agent activity and response, it is often desirable to conduct direct current-voltage (I-V) characterization on key components of the biosensor. I-V characterization requires only a small fraction of the time needed for most types of functional testing, but is a powerful predictor of full fledged operation. For example, I-V data can be used to study anomalies, locate maximum or minimum curve slopes, and perform reliability analyses. Depending on design specifics, I-V characterization is often suitable for sensors based on amperometric, potentiometric, conductive, resistive, and thermal principles.

Usually, I-V testing applies a voltage or current to the device under test (DUT), and measures its response to that stimulus. Temperature measurements may also be taken. The test procedures may involve probing of integrated circuits to apply the stimulus to certain connections pads, and measure the DUT response on others.

Depending on the DUT, signal levels may be quite low. This calls for highly sensitive source and measurement instruments, and test techniques that minimize external sources of error. Where an optical mechanism is involved, I-V characterization may

also involve simultaneous measurements of the wavelength or intensity of a light output with a photodetector. This is called L-I-V testing.

Characterizing the Performance of Biosensors

In many cases, biosensors used by medical practitioners, military personnel, and public safety forces will be part of a portable system. This places restrictions on the sensors' operational power requirements, and may dictate the level of voltage or current output that can be provided to measurement circuitry. In battery operated systems, sensor output current can range from nanoamps to milliamps, and voltage from nanovolts to volts. Different measurement techniques and tools are required for signal levels at the opposite ends of such wide ranges.

Voltage instruments. Characterizing biosensors that have a voltage output greater than, say, tens of microvolts should be relatively easy. A sampling data acquisition system based on a PC plug-in board may provide adequate resolution, as would many programmable digital multimeters (DMMs) and self-contained data loggers. For example, most laboratory grade DMMs provide enough range and resolution to make voltage measurements from 1mV up to 1000V. For the PC-based data acquisition board solution, you can measure over a variety of voltage levels as shown in ***Table 1***, depending on the resolution of the A/D converter and its gain.

For measurements below 1mV, a nanovoltmeter should be considered instead of a data acquisition board or DMM solution. A nanovoltmeter is a very sensitive digital voltmeter with an A/D resolution in the 20-24 bit range. This type of instrument is optimized for accurate, low level voltage measurements, even when the signal is approaching the theoretical (lower) limit associated with sensors having a low output impedance. (Low output impedance requires a voltmeter with a high input impedance to avoid measurement errors.) Although the input impedance of a nanovoltmeter is similar to a DMM, it has much lower voltage noise and drift. This gives the nanovoltmeter much better voltage sensitivity – sometimes as good as 1nV.

Noise in voltage measurements. No matter which tool is used for voltage measurements, noise is often a problem. Significant errors can be generated by noise that originates in the sensor, the measuring instrument, and sources external to the test circuit. External sources include electromagnetic fields, measuring circuit ground loops, and thermal EMFs. Another form of thermal noise that occurs in every electrical component is called Johnson noise, which establishes the ultimate limitation on the signal level that can be measured. For accurate measurements of any sort, all noise sources should be minimized as much as possible. (A discussion of error sources and how to control them can be found in Ref. 2.)

Some types of noise are difficult to avoid. When that happens, measurement compensation techniques are needed. These are included in most benchtop DMMs and nanovoltmeters, which minimize electrical noise from AC lines, and from random noise sources. These techniques are less likely to be available in PC-based data acquisition systems, but knowledgeable users can program a system for signal averaging to help reduce external noise.

Current measurements. Amperometric sensors require a different characterization approach. Electrical currents can be measured with data acquisition systems, but the method selected will depend on the current level and number of required measurement channels. Otherwise, I-V characterization of a current loop sensor system is uncomplicated as long as the current source output voltage is high enough to overcome any test lead resistance. The corollary is that current loop sensors are ideal where there is appreciable distance between the signal source and the instrumentation.

One thing to keep in mind is that it may be desirable to qualify a biosensor's output in terms of engineering units, such as concentration of the target analyte in mg/dl. In the case of an amperometric output, the current is an indicator of the phenomenon actually being measured. Therefore, it's desirable to have instrumentation that makes it easy to do the conversion. This means having internal calculation and scaling features that convert current readings to appropriate engineering units. For example, a biosensing transducer with a 4–20mA current loop output might be calibrated for a concentration of zero mg/dl at 4mA, and a full scale concentration of 100mg/dl at 20mA.

In the early stages of product development, it may not be necessary to do this conversion, because the researcher understands the significance of the biosensor's output current level. In this case, the current reading is the parameter of interest, and a voltage stimulus may serve as a proxy for the biological event during I-V characterization. Under these circumstances, the current signal can be relatively high, but may require care in selecting a dropping resistor for the measurement. The current passes though the dropping resistor at the input stage of a data acquisition system, and voltage is measured to determine the current level using Ohm's Law. (This type of scaling is a common feature of data acquisition systems.)

However, unlike voltage measurements,

Table 1. Voltage measurement resolution and maximum ranges for different A/D converter resolutions.

	Converter Bits (n)			
	8	10	12	16
Output States (2n)	256	1024	4096	65,536
Resolution, 0–10V input	39.06 mV	9.765 mV	2.441 mV	152.59 µV
Resolution, 0–5V input	19.53 mV	4.883 mV	1.221 mV	76.29 µV
Resolution, ±10V input	78.12 mV	19.53 mV	4.883 mV	305.2 mV
Resolution, ±5V input	39.06 mV	9.765 mV	2.441 mV	152.59 µV
Resolution, ±2.5V input	19.53 mV	4.883 mV	1.221 mV	76.29 µV
Resolution, ±1.25V input	9.76 mV	2.441 mV	610.35 µV	38.15 µV
Max. input, 0–10V (res x 2n–1)	9.960 V	9.990 V	9.9976 V	9.99985V

Source: Data Acquisition and Control Handbook, 1st Edition. Keithley Instruments, Inc. 2001 [1].

current measurements may be subject to "voltage burden" errors. Voltage burden is defined as the voltage drop across the input of an ammeter when it is inserted into a circuit. In ***Figure 2***, the dropping resistor (R) and A/D voltage input constitute an ammeter, and the current flow can be calculated from the voltage drop across the resistor. (See Ref. 2 for a more complete discussion of voltage burden.) Noise considerations similar to those for voltage measurements also come into play, and are exacerbated by voltage burden.

In ***Figure 2***, the resistor value will normally be selected to provide a voltage drop corresponding to the A/D board's full input range when the maximum anticipated current flows. For example, a 20mA current produces a 10V drop across a 500Ω resistor. Note that the sensor (i.e., current source) must be capable of developing a minimum output potential of around 10–11V in order to achieve the full voltage drop across the resistor. This may not be a problem in the lab, but if a portable biosensor circuit like the one in ***Figure 2*** is powered by only 6V, it cannot drive more than 12mA through the resistor (6V/500Ω). Furthermore, the resistor must have an adequate power rating (I^2R) for the current and resistance values.

Issues such as these can be avoided by performing current measurements with benchtop instruments that use either a small dropping resistance, or a feedback ammeter circuit. In the former, highly sensitive voltage measurements allow a small dropping resistance value. A feedback ammeter solves the problem by having an operational amplifier for its input circuitry, which has very low input resistance and a voltage burden that typically ranges from about 10μV to 1mV. This is the type of current measurement circuit typically used in a complete biosensor instrument.

Digital multimeters (DMMs) typically use dropping resistor ammeter circuits, whereas picoammeters and electrometers use feedback ammeter circuits. In either case, making connections to the signal source is straightforward, and they are suitable for measuring currents up to several amperes. At the other end of the scale, DMMs can measure currents down to about 10nA, and picoammeters can measure currents as low as 10fA. Another advantage of these

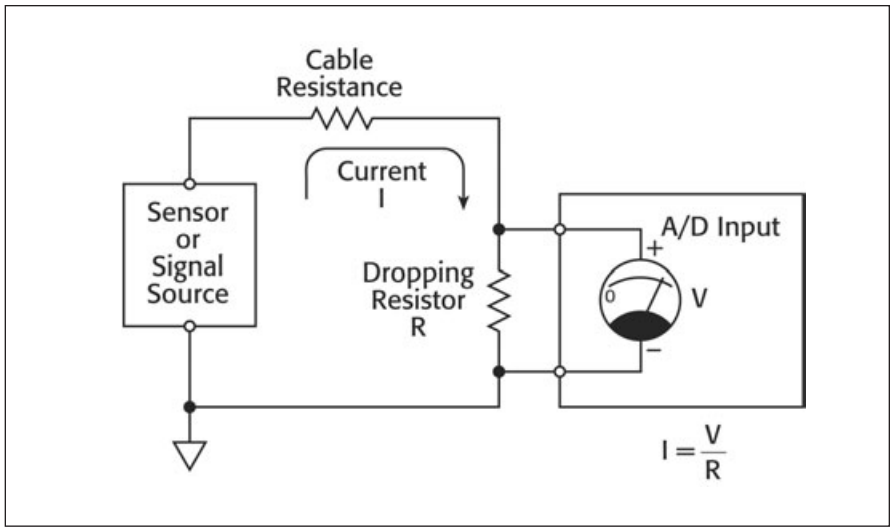

Figure 2. Current measurement using a dropping resistor and voltmeter.

instruments is built-in signal conditioning circuitry. DMMs are available that provide voltage, current, resistance, and temperature (thermocouple or thermistor) measurements on up to 200 channels.

Source-Measure Instruments. In I-V characterization, the integration of a DC source and measuring instrument can be problematic because of intricate triggering issues. Such issues can often be avoided by using a tightly integrated source-measure unit (also referred to as an SMU or SourceMeter® instrument). These high precision instruments can act as either a voltage or current source with sweep, pulse, and compliance limit capabilities, and simultaneously measure I and V parameters. See ***Figure 3***. Typical resolutions are in the range of microvolts and picoamperes.

The bipolar voltage and current sources of these instruments are controlled by a microprocessor, making I-V characterization much more efficient, and simplifying instrumentation set up. When a SourceMeter instrument is used, many different test sequences can be stored in its program memory and executed with a simple trigger signal. Test data can be stored in a buffer memory until an I-V sweep is completed, and then downloaded to a PC for processing and analysis.

3 C's of Accurate Testing: Cabling, Conductors, and Capacitance

Regardless of the instrumentation used,

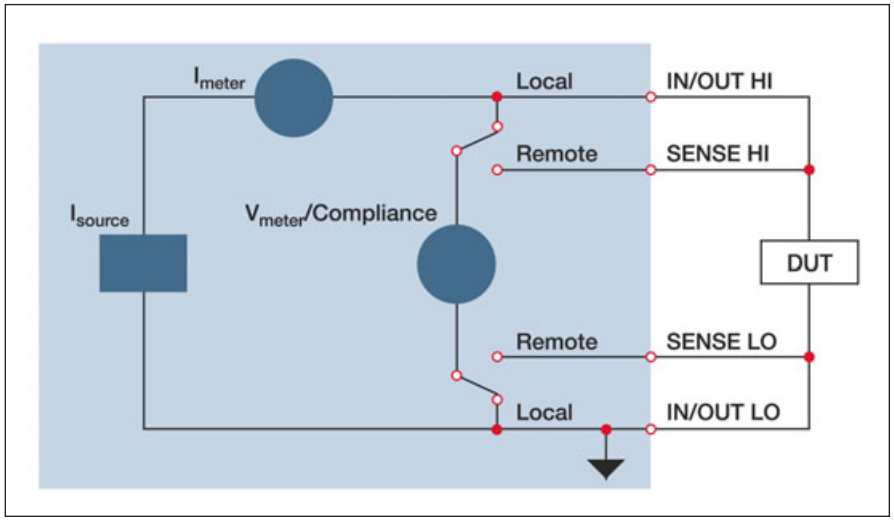

Figure 3. SourceMeter instrument configured as a constant current source and voltmeter that measures the DUT response. Its compliance function makes sure that voltage does not exceed a preset level.

connections between it and the DUT are important parts of a measurement system. Understanding and managing the limitations of these connections is crucial for accurate measurements. Noise sources, cable length, and cable capacitance can affect the quality of any measurement, but the lower the signal level, the more important these issues become. To minimize problems, the measurement circuit, its cables, and connectors should be matched to test signals. In addition, cables and test leads should be carefully routed and mounted.

Cabling. When evaluating a cable for the measurement application, consider these issues:

- How much electrical noise exists in the test environment? Noise can be defined as any undesirable signal that is impressed upon a signal of interest. Sources of electromagnetic noise include AC power lines, motors and generators, transformers, fluorescent lights, CRT displays, computers, radio transmitters, etc. Depending on the nature of the signal and the noise, it may not be possible to separate them once the signal has been acquired at the instrumentation input terminals. To the extent possible, route cables and test leads so their exposure to noise sources is minimized. Then mount them rigidly in place so they cannot move and cause the generation of spurious EMFs in the presence of electromagnetic fields.
- What is the distance between the signal source and measurement system terminals? Wire has electrical resistance, which depends on its composition, length, and diameter. Resistance increases with increasing length and decreasing wire diameter. This resistance is a component of the total cable effects that become part of a measurement circuit's analog input (***Figure 2***). High cable resistance in conjunction with low A/D input resistance can result in a significant voltage drop through the interconnect wiring, resulting in measurement errors.
- Is the data acquisition channel a single-ended or differential input type? Single-ended signals, i.e., those referenced to ground, can be transmitted with two wires or with a shielded cable where the shield is tied to ground. For differential signals, at least two wires are needed to transmit the signal, which consists of a signal high and a signal low, neither of which is referenced to ground. Two individual conductors will work, but a twisted pair or shielded twisted pair provides greater noise immunity.

Conductors. The conductors used in shielded or unshielded cable can be solid or stranded wire. Solid wire results in minimum signal attenuation, but stranded conductors provide more flexibility and may be easier to route and mount. Conductors may be bare copper, plated with silver, or tinned with solder. Connector and conductor materials should match to minimize resistance and thermally generated EMFs.

For the highest signal integrity, use cables with shielded conductors. Shielding reduces electromagnetic noise picked up by signal leads. It is also helpful in reducing electromagnetic radiation from conductors carrying high frequency signals. Shielding is constructed with different types of wire braid, or a combination of wire braid and foil. Multilayer or multi-braid shields are more effective than a single layer in attenuating signal pickup and radiation. However, this tends to make cables stiffer and more difficult to route and mount.

Consider these points when selecting shielded cable:

- Higher frequency noise is more difficult to attenuate, and requires more elaborate shielding.
- Simple spiral wire wrap foil is the least effective type of shielding.
- Tight braiding, double braiding, or braiding plus foil offer more effective shielding.
- Caustic atmospheres, moisture, etc. can reduce the effectiveness of shielding. In some cases, these contaminants can leach into a cable and degrade the shielding far beneath the outer insulating jacket. If possible, avoid testing in such environments.

Capacitance. For many biosensors, the output signal can be modeled as a voltage source in series with a resistance. Similarly, an analog instrument input can be modeled as a meter in parallel with an input resistance. (Refer to ***Figure 2***.) During a measurement, the instrument input absorbs a small bias current that the source must be able to supply. The interconnect cabling is an essential part of this circuit, and can introduce resistance, capacitance, and inductive effects that depend on length, gauge, composition, routing, and the physical environment.

For high speed, rapidly changing signals, circuit inductance and capacitance can be serious obstacles to measurement speed, even if signal source and instrument impedances are properly matched. Often, spurious capacitance is more of a problem than inductance. Signals originating from a high impedance source take longer to stabilize at the instrument input, because the signal's limited current level requires more time to charge the cable capacitance. In that case, taking a measurement before the signal has settled leads to erroneous readings.

Conclusions

Qualifying sensors for biodetection systems and analytical instruments can be simplified in the early stages of development by using I-V characterization techniques. In many cases, these same techniques can be carried over to production testing of the sensors. Instrument manufacturers are a valuable source of information in applying these techniques to a wide range of sensor types, and in the selection of the best measuring instruments for both R&D and production testing. KEITHLEY

References:

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