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Biosensor/Transducer Qualification Using the Model 2450 Interactive SourceMeter® SMU Instrument

Introduction

Through much technological advancement in biology/medicine, semiconductor electronics, and nanoscience, bioelectronics has the potential to change personal healthcare, strengthen security systems, help protect the environment, food, and water supplies, and improve overall our way of life. Through semiconductor and nanoscale technologies, advancements in noninvasive physical biosensors, lab-on-a-chip tools, prosthetics/implants, and medical telematics systems are possible.

Significant research is taking place in the area of biosensors. A biosensor is an analytical device, typically used for the detection of an analyte, the substance or chemical constituent that is of interest. They are used to gain an understanding of bio-composition, structure, and function by converting a biological response into an electrical signal. In many cases, a device as simple as a transistor is the detector. But whether the design is using a semiconductor, electrochemical, or optical architecture approach, proper testing of the electrical portions of these sensors is needed to qualify the designs for further development.

Many 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.

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 is of interest. A generalized biosensor model is shown in **Figure 1**.

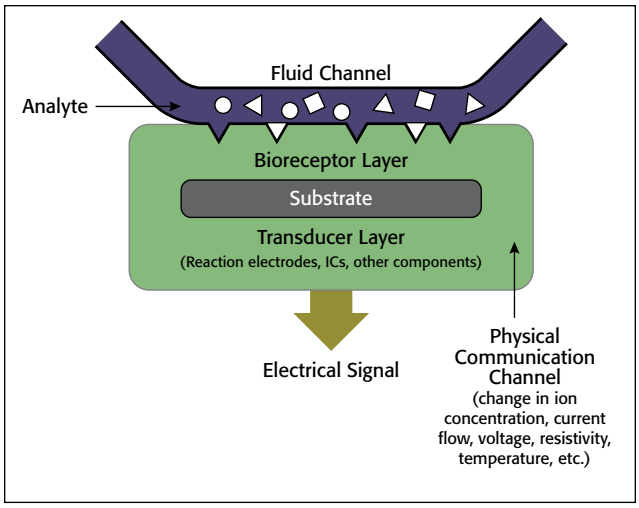

Figure 1. Representation of a generic biosensor.

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. Interference of chemicals must be minimized for obtaining the correct result.
3. Sensitivity/resolution.
4. Linearity. (Maximum linear value of the sensor calibration curve. Linearity of the sensor must be high for the detection of high substrate concentration.)
5. Accuracy/repeatability.
6. Dynamic range. High analyte concentrations will not degrade sensor usability.
7. Environmentally robust (relatively insensitive to temperature, electrical noise, physical shock, vibration, etc.)

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8. Usable lifetime/adaptability.

9. Safety/integrity (for personnel, equipment, and analytes.)

For faster detection with a readable electrical output, researchers are developing biosensors that combine bioreceptor functions with semiconductor devices for use in portable units in the field, such as in home medical monitoring systems. Similarly, biosensors are being researched for use with implantable medical systems.

Sensor Designs

There are various types of biosensor design approaches. One approach is to use 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. An SPR-based sensor can provide label-free studies of molecular interactions in real time using 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.

Electrochemical biosensors are also being applied for many applications. Electrochemical biosensors are normally based on enzymatic catalysis of a reaction that produces or consumes electrons. (Such enzymes are rightly called redox enzymes.) The sensor substrate may contain three electrodes: a reference electrode, a working electrode, and a counter electrode. The target analyte is involved in the reaction that takes place on the active electrode surface, and the reaction may cause either electron transfer across the double layer (producing a current) or can contribute to the double layer potential (producing a voltage.) One can either measure the current (rate of flow of electrons is now proportional to the analyte concentration) at a fixed potential or the potential can be measured at zero current (this gives a logarithmic response[1].)

Another example, the electrochemical potentiometric biosensor (potential produced at zero current), gives a logarithmic response with a high dynamic range. Such biosensors are often made by screen printing the electrode patterns on a plastic substrate that is coated with a conducting polymer, and then some protein (enzyme or antibody) is attached. They have only two electrodes and are extremely sensitive and robust.

All biosensors usually involve minimal sample preparation as the biological sensing component is highly selective for the analyte concerned. The signal is produced by electrochemical and physical changes in the conducting polymer layer due to changes occurring at the surface of the sensor. Such changes can be attributed to ionic strength, pH, hydration and redox reactions, the latter due to the enzyme label turning over a substrate. Field effect transistors (FETS), in which the gate region has been modified with an enzyme or antibody, can also detect very low concentrations of various analytes as the binding of the analyte to the gate region of the FET causes a change in the drain-source current[1].

Recently, in the field of nanoscience, there have been many advances in biosensors through the use of graphene. Graphene was discovered in 2004 and is drawing significant attention due to its unique physiochemical, high sensitivity, and superior mechanical, thermal, and electrical properties. Graphene-based biosensors can potentially have a much higher sensitivity because graphene is a two-dimenstional single atomic layer of graphite that can maximize the interaction between the surface dopants and adsorbates. Graphene has much lower Johnson noise compared to carbon nanotubes that are functionalized for bio-detection applications. Johnson noise is the noise in a resistive material caused by thermal motion of charge carriers. Therefore, a very small variation of the carrier concentration in a graphene biosensor can cause a notable variation of electrical conductivity that can be measured.

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. An optical fiber is sometimes used for guiding light signals from the source to the detector.

Sensor Characterization: The First Step in the Validation Process

Development programs are aimed at overcoming design limitations in 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, while still achieving adequate sensitivity, imposes 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, as well as the ultimate operation of a biodetection system.

A typical test program task is to develop or verify 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, requiring highly sensitive source and measurement instruments and test techniques that minimize external sources of error.

Characterizing the Performance of Biosensors with a Source Measure Unit (SMU) Instrument

In many cases, biosensors will be used in portable systems by medical practitioners, military personnel, public safety forces, and even for home health care monitoring. This places restrictions on the sensors' operational power requirements and may dictate the level of voltage or current output that can be provided to the 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.

One of the best tools for performing I-V characterization is the source measure unit (SMU) or SourceMeter® SMU Instrument. In I-V characterization, the integration of a DC source and measuring instrument can be problematic because of intricate triggering issues. Stated in the simplest possible terms, an SMU instrument integrates the capabilities of a precision power supply (PPS) with those of a high-performance digital multimeter (DMM) in a single instrument. For example, SMU instruments can simultaneously source or sink voltage while measuring current, and source or sink current while measuring voltage. **Figure 2** illustrates an SMU instrument configuration as a constant current source and voltmeter to measure the response from the DUT.

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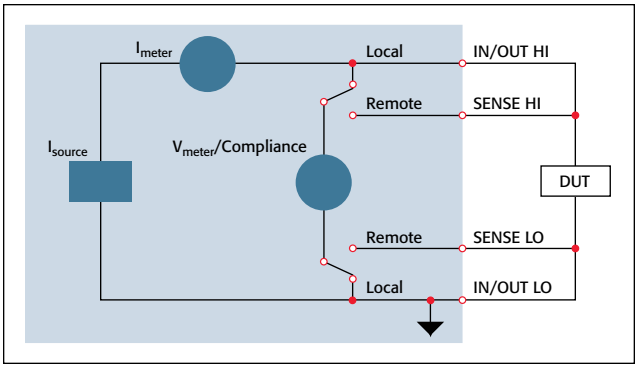

Figure 2. SMU instrument configured as a constant current source and voltmeter to measure the response from the DUT.

SMU instruments can also be used as stand-alone constant voltage or constant current sources, as stand-alone voltmeters, ammeters, and ohmmeters, and as precision electronic loads. Their high performance architecture also allows using them as pulse generators, as waveform generators, and as automated current-voltage (I-V) characterization systems.

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 an SMU 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.

With so many researchers from disciplines like biology, chemistry, materials, and electrochemistry working on biosensors and other bioelectronics technologies, ease of use and a low learning curve of the instrument is very important. Such researchers may not have been exposed to electrical characterization tools like the SMU instrument but need to perform I-V characterization of their devices in the lab.

With advances in touchscreen technology and the proliferation of smart phones and tablets that drive intuitive operation, touchscreen graphical user interfaces (GUI) on bench instrumentation can significantly reduce the learning curve and overall ease of use. With a touchscreen approach, users feel comfortable that they cannot "do anything wrong;" they instinctively understand how to use the interface. Touchscreen systems can make everyone an "expert user" from the first touch, whether a new instrument user or the most experienced user. Compared to traditional training methods, using touchscreens can drastically reduce training time, increase operator accuracy, and improve overall operational efficiencies.

Figure 3. Advanced capacitive touchscreen GUI on Keithley's Model 2450 SourceMeter® SMU Instrument.

Keithley's Model 2450 Interactive SourceMeter® SMU Instrument eases the learning curve for non-traditional users and to significantly reduce the struggle to configure measurement functions using cumbersome, multi-layer menu structures and confusing multi-function buttons. The Model 2450 uses an icon-based flat menu system just like that used on smart technology consumer products, like the array of application icons displayed on a tablet or smartphone.

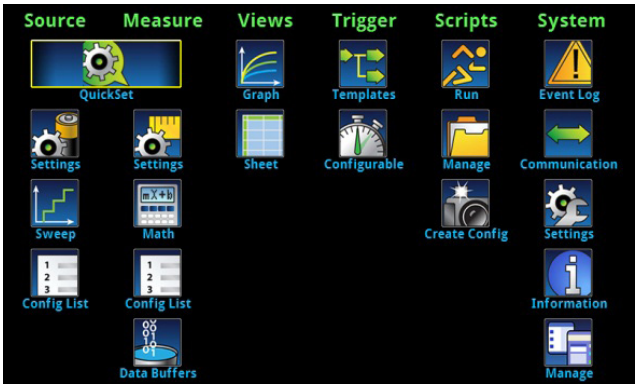

Figure 4. The Model 2450 on-screen menu.

Testing a BioFET Sensor

As previously mentioned, a transistor field effect transistor (FET) can be fabricated to work with bio-materials to become a biosensor. The FET is a transistor that uses an electric field to control the shape and hence the conductivity of a channel

of one type of charge carrier in a semiconductor material. A BioFET contains the following parts: a semiconductor transducer, a dielectric layer, a biofunctionalized surface, the analyte, and a reference electrode (the gate in FET terms) as shown in **Figure 5**.

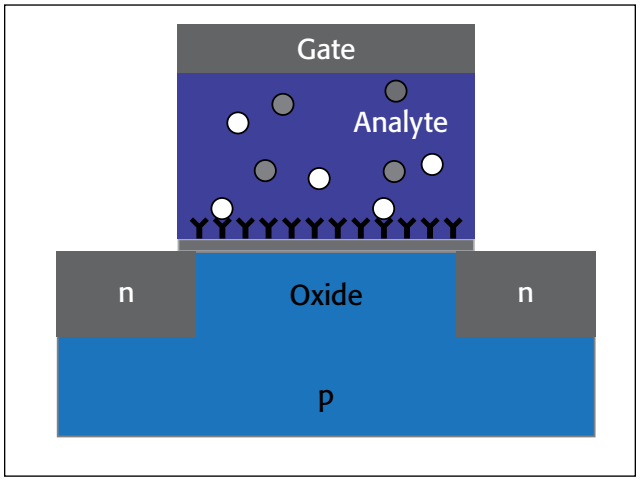

Figure 5. Conceptual drawing of a BioFET.

The semiconductor BioFET transducer is fabricated as follows. The dielectric layer is an oxide, such as silicon dioxide, and has two tasks. The first task is to isolate the channel of the FET from the liquid and the second is to electrostatically couple the surface layer charge into the channel. On top of the dielectric is a biofunctionalized layer that exhibits immobilized biomolecule receptors able to bind the desired molecule. The analyte is a solution that contains the dissolved sample molecules. The reference electrode allows adjusting the device so its sensitivity will be maximized. If the target molecules bind to the receptors, a change in the surface charge density occurs. This change alters the potential in the semiconductor and thus the conductivity in the channel of the FET[2].

The BioFET can be characterized using two Model 2450 SourceMeter SMU Instruments to perform I-V characterization of the sensor. Determining the I-V parameters of a BioFET helps ensure that they function properly in their intended applications and that they meet specifications. There are many I-V tests that can be performed with the Model 2450, including gate leakage, breakdown voltage, threshold voltage, transfer characteristics, and drain current. The number of Model 2450 SMU instruments required for testing depends on the number of FET terminals that must be biased and measured.

This example application shows how to perform a drain family of curves (Vds-Id) on a three-terminal FET. The techniques could be applied to BioFET devices.

Equipment required

- Two interactive Model 2450 SourceMeter® SMU Instruments
- Four triaxial cables (Keithley part number 7078-TRX-10)
- A metal-shielded test fixture or probe station with female triaxial connectors
- A triaxial tee connector (Keithley part number 237-TRX-T)
- Cabling for external hardware triggers is different depending on the command set being used:
  - For SCPI commands: one DB-9 male-to-male 9-pin cable to connect the digital I/O ports on the back of the Model 2450 instruments to each other.
  - For TSP® commands: one TSP-Link® crossover cable (one Keithley Model CA-180-3A is included with the Model 2450) to connect the TSP-Link ports on the rear panel of the Model 2450 instruments to each other.
- Cabling from the computer to the Model 2450 instruments is different depending on the command set being used:
  - For SCPI commands: two GPIB cables, two USB cables, or two Ethernet cables
  - For TSP commands: one GPIB cable, one USB cable, or one Ethernet cable

Set up remote communications

This application can be run from any of the supported communication interfaces for the instrument (GPIB, USB, or Ethernet).

The rear-panel connection locations for the remote communication interfaces are shown in the following figure.

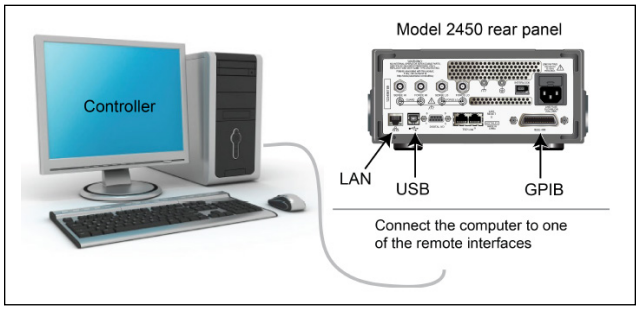

Figure 6: Model 2450 remote interface connections.

Set up external hardware triggers

To enable synchronization between the two Model 2450 SMU instruments for stepping and sweeping voltages, connect the

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external triggers of each instrument to the other. The cabling used depends on the Model 2450 programming command set chosen to control the test.

Connections for the SCPI command set

If you are using the SCPI command set, connect a DB-9 male-to-male cable between the digital I/O connectors on the back of each of the instruments, as shown in the figure below.

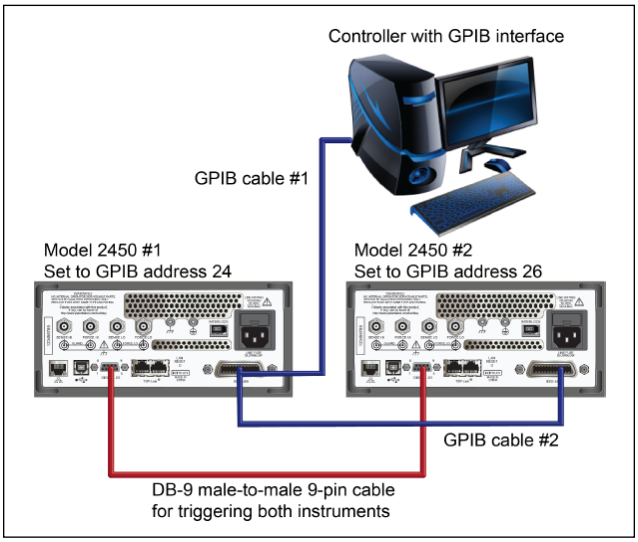

Figure 7: GPIB and DB-9 cable connections for the SCPI programming example.

The figure above also shows the communication cabling for using the GPIB remote communication interface. GPIB cable #1 connects the GPIB port on the computer (controller) to the IEEE-488 connector on the rear panel of Model 2450 #1. GPIB cable #2 is connected between the IEEE-488 connectors of the two Model 2450s.

When connecting the computer and Model 2450 SMU instruments using USB cables, each instrument must be connected to the computer with a separate USB cable.

When connecting the computer and Model 2450 SMU instruments using an Ethernet connection, the instruments and computer must be connected using an Ethernet switch or hub.

Connections for the TSP command set

When using the Test Script Processor (TSP®) command set for remote programming, a Model CA-180-3A crossover cable (one is included with the Model 2450) connects the TSP-Link ports on the rear panels of the Model 2450 instruments to each other (see figure below).

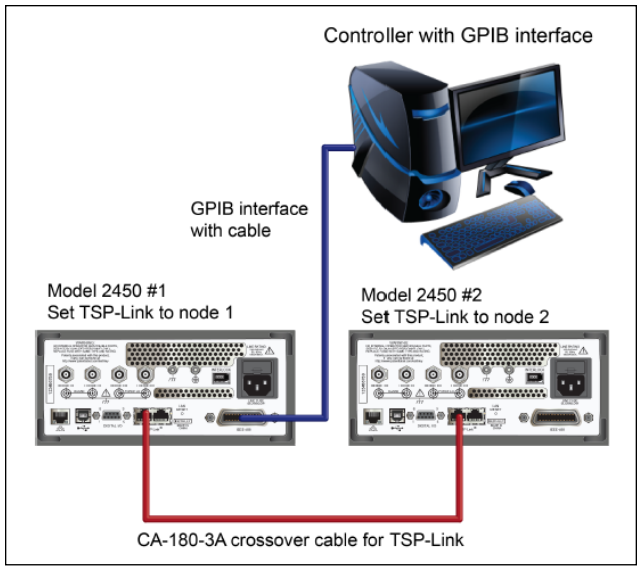

Figure 8: Connections for the TSP command set.

For GPIB communication from the computer to the Model 2450 instruments, only one cable is necessary from the GPIB interface to one of the Model 2450 IEEE-488 interfaces (Model 2450 #1 in the figure above). Set the TSP-Link node of Model 2450 #1 to node 1 and the TSP-Link node of Model 2450 #2 to node 2.

To change the Model 2450 TSP-Link nodes from the front panel:

1. Press the communication status indicator on the upper left corner of the home screen, and then select Change Settings. The SYSTEM COMMUNICATION window opens.
2. On the TSP-Link tab, select the button next to Node and enter the node number you want.
3. Select Initialize.
4. Press the MENU key to return to the home screen.

Repeat this instruction for all Model 2450 SMU instruments in the TSP-Link network.

Device connections

To perform a drain family of curves, configure both Model 2450 SMU instruments to source voltage and measure current. In this circuit, the Force HI terminal of Model 2450 #2 is connected to the gate of the BioFET, and the Force HI terminal of Model 2450 #1 is connected to the drain. The source terminal of the BioFET is connected to the Force LO terminals of both Model 2450 SMU instruments. A third Model

2450 is required when sourcing and measuring from all three terminals. An I-V test configuration for a BioFET using two Model 2450 SMU instruments is shown in the following figure.

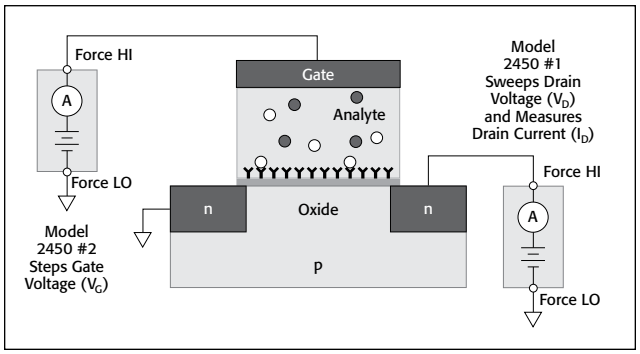

Figure 9: Three-terminal I-V test configuration for a BioFET.

The connections from the rear panel terminals of both Model 2450 SMU instruments to the BioFET are shown in the following figure.

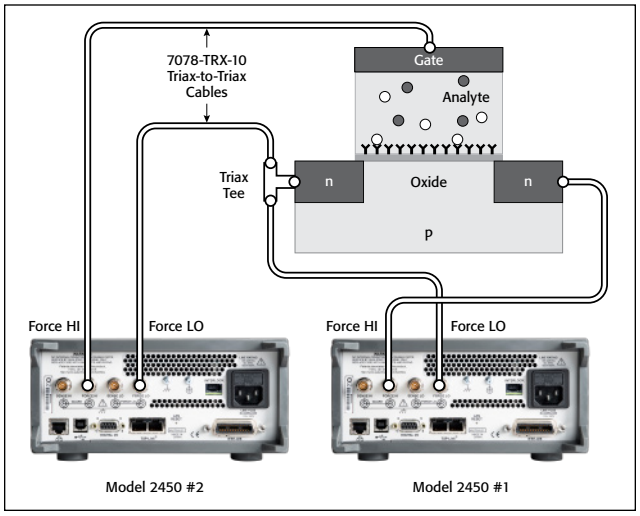

Figure 10: Two Model 2450s configured to test a three-terminal BioFET.

For this example, four triaxial cables (Model 7078-TRX-10) are used to connect from the Model 2450 rear-panel female triaxial connectors to the BioFET device, which is mounted in a metal-shielded test fixture with female triaxial connectors. The Force LO terminals of the both Model 2450 instruments are connected to the Source terminal of the BioFET using a triaxial tee connector (Model 237-TRX-T).

For the SCPI or TSP programming sequences to test the FET, please refer to the Model 2450 User's Manual, Section 7, Measuring I-V Characteristics of FETs.

The following figure shows a graph of a typical FET family of curves. The results may vary for a BioFET and the type of bio-functionalized materials used.

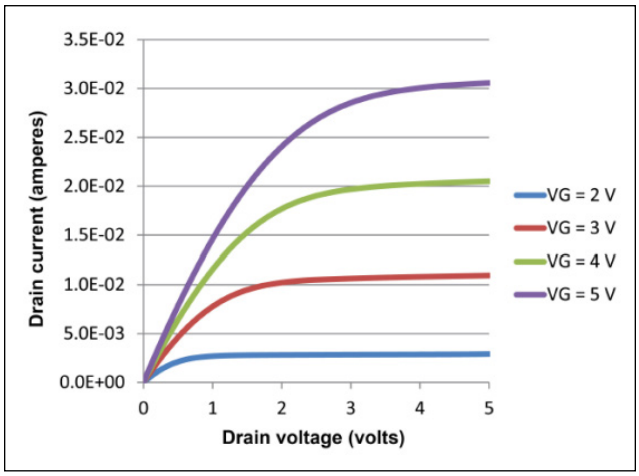

Figure 11: Typical FET drain family of curves generated with two Model 2450s.

3 Cs of Accurate Testing: Cabling, Conductors, and Capacitance

Regardless of the instrumentation used, 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 and 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. High cable resistance in conjunction with low analog to digital input resistance can result in a significant voltage drop through the interconnect wiring, resulting in measurement errors.

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. Multi-layer 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. 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 bio detection 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.

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[1] Wikipedia, http://en.wikipedia.org/wiki/Biosensor.

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[3] 2450 Interactive SourceMeter® Instrument Users Manual, 2450-900-01 Rev. B.

[4] "Biosensor/transducer qualification: a critical step for homeland security," Jonathan Tucker, Keithley Instruments, March 2004.

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