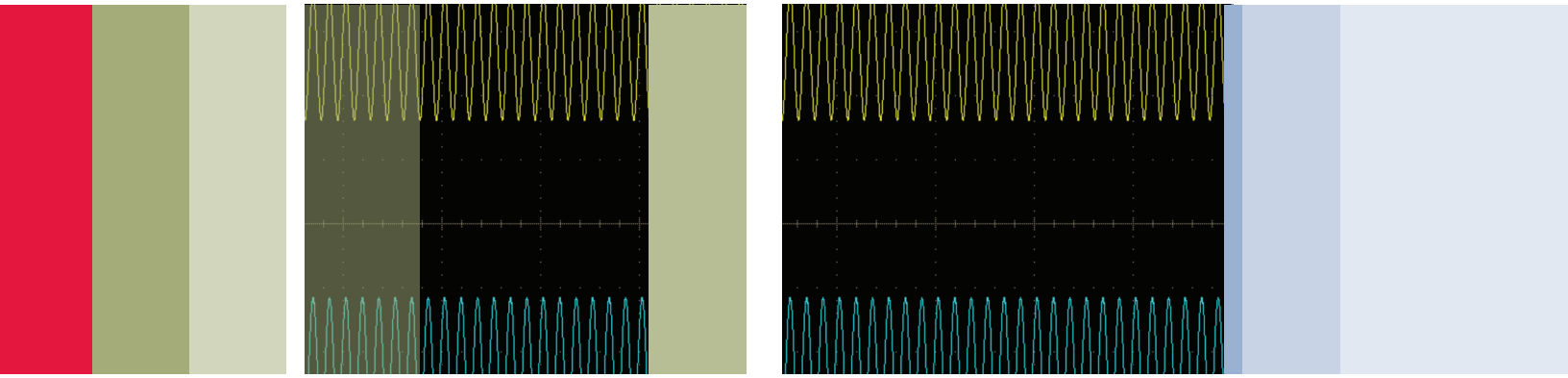

# Making MicroVolt Biomedical Measurements

## Technical Brief

### Introduction

Measuring electrical phenomena presents a number of unique challenges to biomedical signal measurement systems. The tiny, microVolt-level electrical pulses that signal a firing neuron or a muscle response are often obscured by high-amplitude noise and/or accompanied by significant DC potentials. Quite often, the signal of interest is a small transient pulse that occurs intermittently or only once. In some applications, minute chemical and catalytic changes occur over a matter of several minutes or even hours, making it critical that important experimental events are captured in a single acquisition.

This technical brief will discuss the basic techniques used to make low-level stimulus/response measurements in biomedical research environments with a Tektronix Digital Phosphor Oscilloscope and an ADA400A Differential Preamplifier.

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## Making Biomedical Measurements

To distinguish low-level signals and transients from the surrounding noise, biomedical measurement systems must provide not only wide dynamic range and high-quality signal conditioning, but also long record lengths for long duration event capture, extensive storage capacity, and single-shot acquisition capabilities with adequate time resolution. Finally, proper documentation and reporting of results is a critical step in the bioscience research process. Researchers and biomedical engineers need the ability to store not only acquired waveforms, but also waveform measurement results and test setups. Therefore, to allow for accurate reporting of the acquired data, the measurement system has to provide comprehensive data storage and retrieval, analysis, and documentation capabilities.

A Tektronix Digital Phosphor Oscilloscope (DPO), a TPA-BNC adapter, and an ADA400A low-noise differential amplifier provide a powerful, portable, and affordable measurement system for capturing and analyzing low-amplitude biomedical phenomena in the presence of noise. Coupled with the ADA400A, a DPO delivers 100,000:1 CMRR (common-mode rejection ratio) from DC to 10 kHz and microVolt-level sensitivity for precise signal conditioning. It allows engineers and researchers to pick up extremely small amplitude signals, such as neurons firing during an experiment or a muscle fiber response to external stimulus. The Tektronix HiRes™ acquisition mode effectively removes noise from single shot and repetitive events with real-time digital filtering. The combination of HiRes mode and the ADA400A increases the dynamic range and vertical resolution of the oscilloscope, allowing users to capture the fine nuances of microVolt-level electrophysiological signals - even single-shot signals, in the presence of much larger, common-mode noise signals.

CAUTION: Only certified test equipment can be used when making direct connections to human subjects.

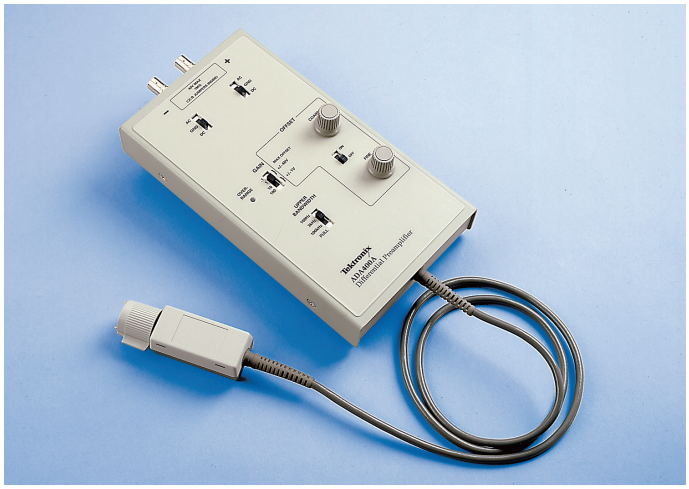

**Figure 1.** ADA400A Differential Preamplifier.

## Recording Low-level Signals in the Presence of Noise

A major challenge in measuring low-level signals is dealing with unwanted noise. When measuring signals in the microVolt range, noise can often be thousands of times greater in amplitude than the signal of interest. Noise in the biomedical environment can be divided into two categories: noise inherent in the signal and noise caused by the external environment. Inherently noisy response signals are usually caused by a noisy stimulus signal, or some other source of noise within the test and measurement apparatus itself. External noise is generated outside the test and measurement equipment by sources such as florescent lights, stray electric or magnetic fields, and poor shielding or grounding.

**Inherent Noise.** If the desired signal is inherently noisy, the noise will be amplified along with the signal of interest. Selective filtering can be employed to eliminate the noise. The ADA400A offers selectable low-pass filters that can be used to eliminate high frequency noise from the signal. In most cases, this noise filtering technique will not alter the essential character of the signal of interest. In cases of extreme noise, sharp cut-off notch filters or signal averaging may be required to extract the desired signal.

HiRes acquisition mode applies real-time digital filtering to the oscilloscope's digitizer output prior to writing the acquired signal to memory, allowing the oscilloscope to significantly reduce high-frequency noise on lower-frequency signals - even single shot signals. The HiRes mode does not rely on the presence of a stable trigger and, therefore, can be used on single-shot or non-repetitive events. It can provide a significant improvement in vertical resolution.

**Common-Mode Noise.** Noise that enters the measurement from the external laboratory environment and affects both the signal and the reference equally is called common-mode noise. If you touch your finger to an oscilloscope probe, for example, a large 50 or 60 Hz signal will likely be displayed on the oscilloscope's display. This is common-mode noise that your body, acting as an antenna, picks up from the environment. Biological specimens can pick up these same undesirable signals. Some of these common-mode signals can be eliminated by removing noise generating devices, such as fluorescent lights, from the laboratory. Surrounding the lab setup with a grounded electrical mesh will also help to eliminate common-mode noise. Even with these precautions, however, some common mode noise may still be present. This remaining common-mode noise is chiefly due to the inability to ground the biological specimen adequately. The solution to this problem is to use a high performance differential amplifier.

**Differential Amplifier.** A properly balanced differential amplifier has the unique ability to amplify very small signal differences, while attenuating common-mode noise. The ability of a differential amplifier to reject noise is called its common-mode rejection ratio (CMRR). The ADA400A Differential Preamplifier offers a CMRR of 100,000:1, allowing capture of small signals in the microvolt range (5-10 µV) when high-amplitude common-mode noise is present.

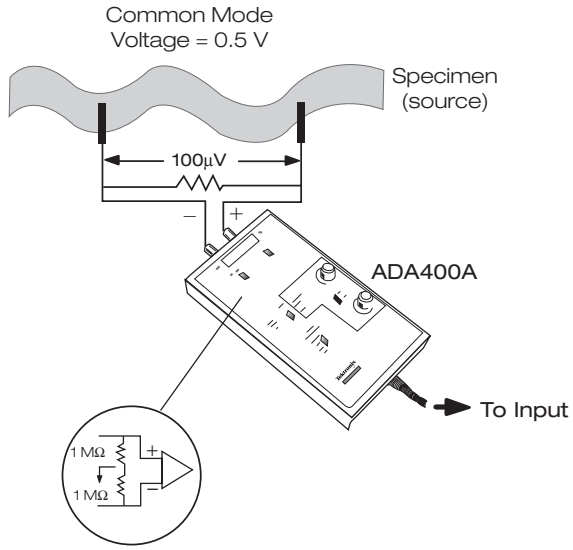


**Figure 2.** Differential Amplifier Connection with Balanced Source Impedances.

Differential amplifiers have two inputs, both of which designed to be connected to the specimen (see Figure 2). Neither of these inputs are grounded: in other words, the amplifier floats above ground potential. A ground electrode is sometimes connected to the specimen to reference it to the measurement system. When the two differential inputs are connected to the specimen and the impedance at the two connections are reasonably well matched to the amplifier's impedance, the amplifier "sees" only the true difference signal.

The standard oscilloscope differential amplifier input impedance at the frequencies encountered in biophysical research is 1 MΩ, each side to ground, or 2 MΩ across the differential inputs. This impedance level occurs because the input impedance of the oscilloscope is typically set to 1 MΩ and "1X" passive voltage probes are used for minimum attenuation, resulting in a 1 MΩ input resistance of each input to ground. These values produce a net 500 kΩ impedance to ground for common-mode signals.

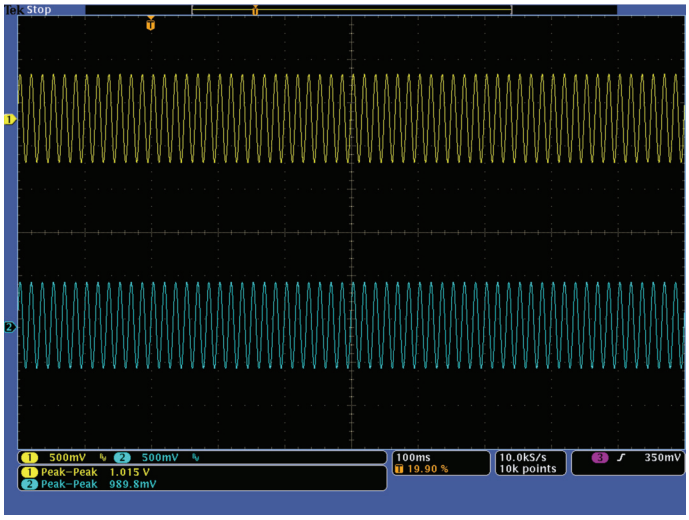


**Figure 3a.** Large scale 60 Hz sinusoidal noise with (yellow Channel 1) and without (blue Channel 2) small scale cardiac output signal.

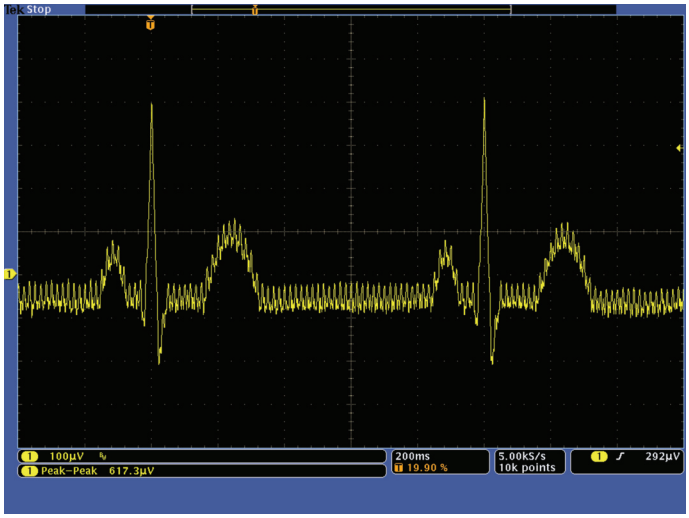


**Figure 3b.** Small scale cardiac signal after ADA400A preamplifier set to 100x gain. Note, 617.3 µV pk-pk vs. composite signal of sinusoidal noise and cardiac output in Figure 2a with pk-pk voltage of 1.015 V.

| Source Voltage | Source Interface Impedance | Load | Displayed Voltage | Displayed Signal Height @ 50 µV/div |
|---|---|---|---|---|
| Differential 100µV | Equal and Low | 2 MΩ | 100 µV | 2 divisions |
| Common-mode 0.5 V | | 0.5 MΩ | 5 µV | 0.1 divisions |

**Table 1.** CMRR = 100,000:1.

The effectiveness of the ADA400A common mode rejection is illustrated in Figures 3a and 3b. Figure 3a shows unfiltered monitor signals. Note, the signal on channel one contains both a simulated cardiac signal, similar to what would be seen on an ordinary heart monitor, as well as a large 60 Hz sinusoidal noise trace. The signal on channel two contains the same 60 Hz sine wave but without the cardiac signal. Because the sine wave noise is significantly larger than the cardiac signal it's difficult to view the anomalies in the "at-rest" area following the main beat. After connecting the ADA400A to the composite signal the large "common-mode" signal is removed between the two inputs and the resulting "differential" is displayed as shown in Figure 3b.

An as another example the ADA400A is used to measure a 100 µV signal from a specimen that produces a 0.5 V common-mode signal. In this experiment, the specimen interface impedances were low and matched. Using a vertical scaling on the oscilloscope of 50 µV/div, the resulting display shows the amplitude of the signal of interest occupying 2 vertical divisions of the screen, while the common-mode noise takes up only 0.1 division (see Table 1). The 100,000:1 CMRR of the differential preamplifier causes the common-mode noise to be attenuated from 0.5 V to 0.5 µV, essentially eliminating it from the measurement.

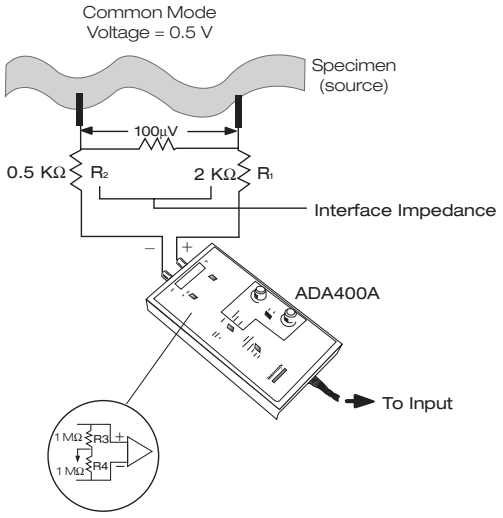


**Figure 4.** Differential Amplifier Connection with Unbalanced Source Impedances.

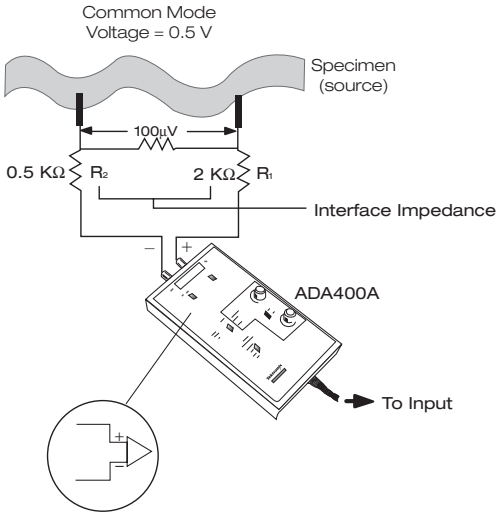


**Figure 5.** ADA400A with Increased Input Impedances.

| Source Voltage | Source Interface Impedance | Load | Displayed Voltage | Displayed Signal Height @ 50 µV/div |
|---|---|---|---|---|
| Differential 100µV | Mismatched and High | 2 MΩ | ~100 µV | 2 divisions |
| Common-mode 0.5 V | | 0.5 MΩ | ~750 µV | 15 divisions |

**Table 2.** CMRR = 666:1.

This example illustrates the usefulness of the ADA400A Differential Preamplifier for measurements when the source impedances are low and well matched (see Figure 2). In practice, however, you may not always have control over the source impedances. In such situations the CMRR of the differential amplifier will be degraded.

Figure 4 shows an example of a situation where specimen interface impedances of 2 kΩ and 0.5 kΩ were created when the research procedure was unable to establish good control between the electrodes and the specimen.

If the specimen interface creates a high and possibly different impedance between the electrode pairs, as in Figure 4, the measured signal will not truly represent the signal at the specimen interface. Also, the voltage dividers thus created are different, causing the CMRR to degrade according to the following formula:

$$CMRR = |(R_3 \text{ or } R_4)/(R_1-R_2)|$$
$$= 1\ M\Omega\ /\ (2\ k\Omega - 0.5\ k\Omega) = 666{:}1$$

When the CMRR is degraded like this, the displayed common-mode noise is much greater. If, in the example given above, the CMRR of the differential amplifier is degraded to 666:1, the amplitude of the common-mode noise will occupy the equivalent of 15 vertical divisions on the oscilloscope's display (which extends beyond the top and bottom of the screen). Even with the high gain for the differential signal, the 15 division display of the common-mode noise will make the 2 division response signal unreadable (see Table 2).

## Raising the Input Impedance of the Differential Amplifier

The solution to the problem of degraded CMRR is to raise the input impedance of the differential amplifier. If the differential amplifier had an essentially infinite input impedance, the circuit in Figure 4 would look like the circuit in Figure 5. In this case there is essentially no voltage divider action due to the mismatched interface impedances, and the full CMRR can be very nearly attained.

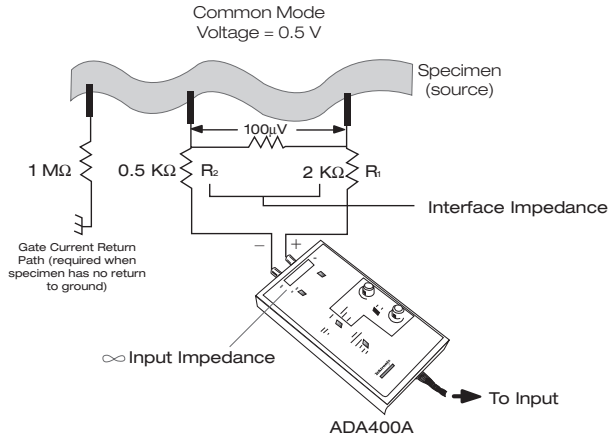


**Figure 6.** ADA400A with Increased Input Impedances and Gate Current Return Path.

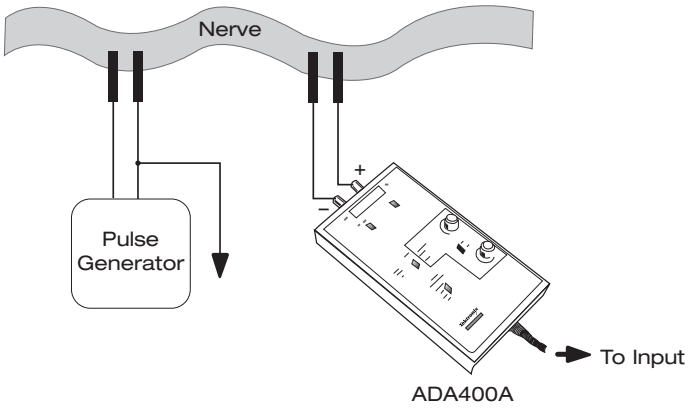


**Figure 7.** Differential Measurements with One Grounded Input Signal.

The Tektronix ADA400A incorporates removable jumpers which allow the internal 1 MΩ resistors to be disconnected, thus presenting an essentially infinite impedance to the source. (This mode is effective only for the 100X and 10X gain ranges.) Also, the gate current, generally less than 25 picoAmps of the Field Effect Transistor (FET) at the amplifier input, must have an external path to instrument ground. This path is usually provided by the specimen or the signal source itself. In the unlikely event that the source is purely capacitive, some conductance must be added, either in the amplifier itself or at the source, to instrument ground. (Because the gate current is very low, this path can be resistive.) Refer to Figure 6.

## Electrode Contact Potential

Electrode potentials exist whenever metallic electrodes interface with the specimen via an electrolyte. Differences between electrode-pair contact potentials produce an offset potential, typically in the range of hundreds of milliVolts, which appear as a DC voltage source in series with the desired signal. The nominal DC-coupled amplifier load of 2 MΩ will tend to discharge these “batteries”, but residual offset may displace the desired signal off-screen, especially at high sensitivities. There are several ways of cancelling the effects of this offset potential:

1) Some differential amplifiers include a DC offset adjustment. The ADA400A, for example, has a DC OFFSET control that can be used to compensate for electrode offset potentials, while preserving DC coupling and differential operation.
2) If the displayed offset is small, the differential amplifier display position control can be used to position the display screen.
3) AC coupling will also remove the DC component from the waveform. However, AC coupling attenuates frequencies below 2 Hz and many biophysical signals contain low frequency information in this frequency range. Also, AC coupling cannot be used in the “high impedance” mode described earlier in this discussion.

## Eliminating Noise at the Source

Clearly, it is desirable not to have noise signals to contend with in the first place. Eliminating noise sources such as fluorescent lighting or constructing a grounded mesh around the test setup are good first steps. But other steps can be taken.

**Signal Sources.** Connecting the stimulus pulse generators through stimulus isolators presents the stimulus pulse across a discrete area. Leakage currents to ground through the specimen are thus avoided. Stimulators with one lead grounded could produce large ground currents through the specimen. If these currents flow through the response pick off point, the resulting potential drop will show as an unwanted signal. If a grounded stimulator is used, the grounded electrode should always be placed between the signal electrode and the measurement electrodes, as shown in Figure 7.

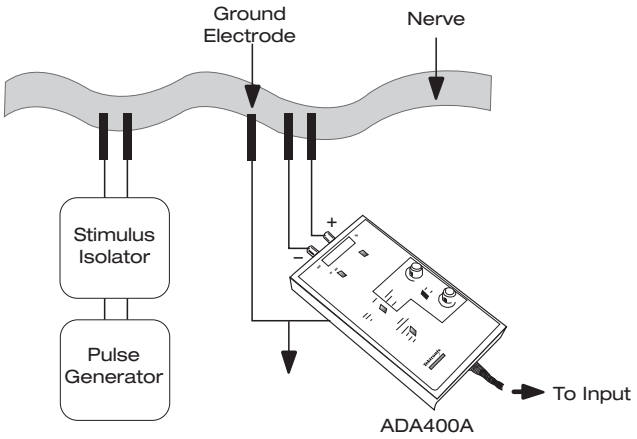


**Figure 8.** Differential Measurements with Floating Input Signals.

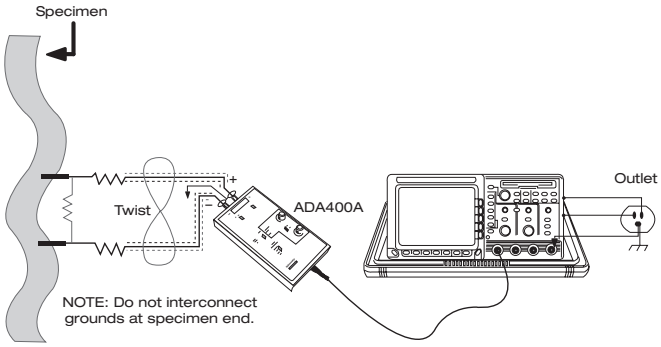


**Figure 9.** Proper Grounding Technique for Differential Measurements.

An extension of this principle can be applied when making stimulus-response measurements on an excised nerve of a biological specimen (see Figure 8). A grounded electrode could be placed across the nerve between the stimulus isolator and the recording electrodes to effectively bypass surface currents to ground. The recording electrodes will then see the conducted action potential with very little stimulus artifact.

NOTE: Ground in this discussion refers to circuit ground, preferably located at the differential amplifier. The triangular ground circuit symbol is used to signify circuit ground. Safety ground or earth ground, discussed below, is denoted by the rake ground symbol.

**Establishing a Common Earth Ground.** Very often a multitude of line operated equipment is used to perform biomedical experiments. The way this equipment is connected together can greatly affect the level of noise generated in the measurement system. The voltage of third wire ground connection, for example, at various wall outlets may not be at exactly the same ground potential, or at the same level between outlets. If two or more pieces of equipment are connected together via coaxial cables (as they should be), it is possible for circulating line currents to flow in the outer braid. This “ground loop” can inject line ripple into the inputs of susceptible devices such as amplifiers. To avoid these problems, safety grounds should be solid and all equipment to be used in the measurement should be connected to the same ground bus.

**Electromagnetic Induction.** Any cable, shielded or otherwise, can pick up induced currents if they pass close to power transformers, line cords, or other AC current carrying leads. Care has to be taken to route “single-ended” signal leads away from such sources, and paired differential leads are often twisted together to cancel out induced currents. The ADA400A Differential Preamplifier, however, places the differential amplifier circuitry at the probe end, where it is as close as possible to the specimen being tested (see Figure 8). This virtually eliminates problems from induced currents. The signal of interest is amplified before it can be degraded by electromagnetic induction.

Probes that interface with the animal or specimen should be shielded and grounded at the equipment end. Never ground both ends of signal leads as this immediately sets up a ground loop. Figure 9 shows the correct grounding technique. Using this test setup, the differential amplifier eliminates the effects of ground loops while keeping the oscilloscope safely grounded.

CAUTION: In the United States the Occupational Health and Safety Administration (OSHA) warns that floating test equipment above ground can be very hazardous and increase chances of electric shock. To be safe, Tektronix recommends that you NEVER “float” the instruments by disabling the safety ground connection.

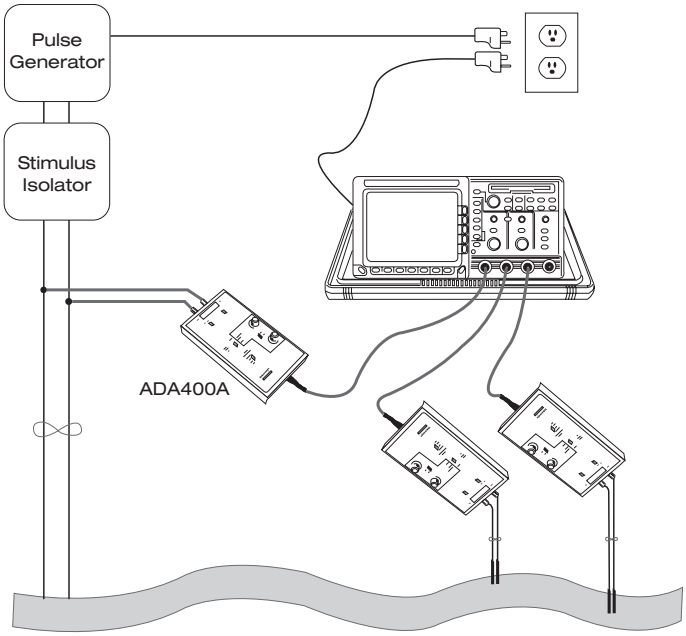


**Figure 10.** Differential Measurements on Grounds within Biomedical Equipment.

**Determining When Ground Isn't Ground.** A multitude of line-powered equipment is often used to perform biomedical experiments. The way this equipment is connected together can greatly affect the level of noise generated in the measurement system. The voltage of third wire ground connection, for example, at various wall outlets may not be at exactly ground potential, or at the same level between outlets. If two or more pieces of equipment are connected together via coaxial cables (as they should be), it is possible for circulating line currents to flow in the outer braid. This "ground loop" can inject line ripple into the inputs of susceptible devices such as amplifiers. To avoid these problems, safety grounds should be solid and all equipment to be used in the measurement should be connected to the same ground bus.

## Summary

In this application note we've demonstrated that paying careful attention to the grounding of the equipment, isolation of the signal generators, and shielding of the probes and leads, can produce very refined biomedical measurements without complicated and expensive test equipment setups, preconditioning equipment, and external filters. Using a Tektronix DPO and the ADA400A Differential Preamplifier, engineers and researchers can obtain complete solutions to their biomedical measurements. This advanced test system delivers precise signal conditioning, outstanding acquisition confidence, comprehensive on-board signal processing end analysis, and accurate results storage and report generation capabilities making it versatile enough to solve a variety of complex measurement problems in the areas of manufacturing test, bioscience research, power electronics/power supply design, and electronic product service end repair.

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