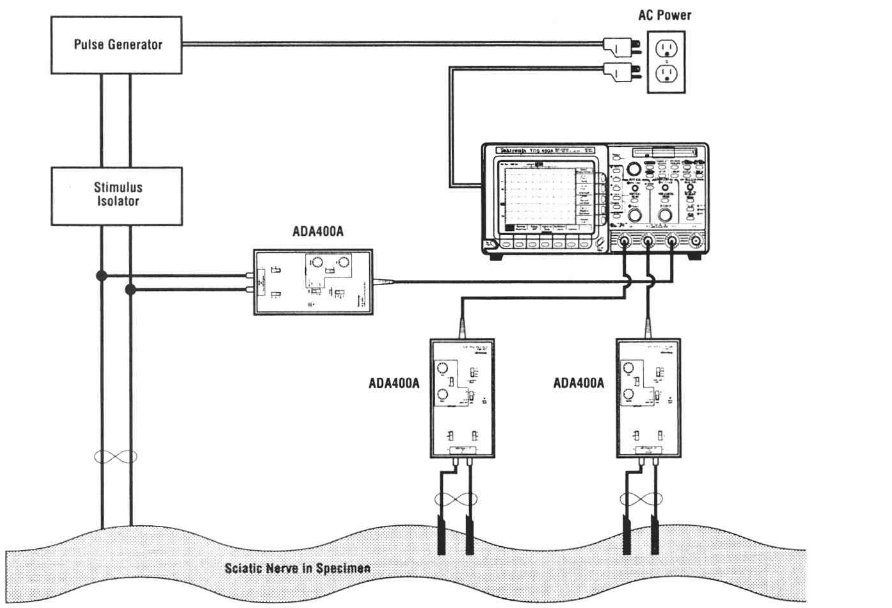
Application Note

Biophysical Measurements Using the TDS 400A Personal Lab Scope



Introduction

Measuring biophysical phenomena in biophysical experiments presents a number of unique challenges to signal measurement systems. The tiny, microvolt-level, electrical pulses that signal a firing neuron or a muscle response are typically shrouded 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 application note will discuss the basic techniques used to make low level stimulus-response measurements in biophysical research environments with a Tektronix TDS 400A Personal Lab Scope and an ADA400A Differential Amplifier. Part 1 of the note discusses the Personal Lab Scope capabilities and Part 2 describes the typical lab setup.

Making Biophysical Measurements

To distinguish biophysical low-level signals and transients from the surrounding noise, biophysical 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.

Furthermore, since bioscience researchers generally need to view both the stimulus and response signals simultaneously, the measurement system must include multiple acquisition channels.

Finally, proper documentation and reporting of results is a critical step in the bioscience research process. Bioscience researchers need the ability to store not only 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 retrieval, analysis and documentation capabilities.

In the past, elaborate test equipment setups using expensive laboratory oscilloscopes, signal generators, chart recorders, preconditioning amplifiers and filters were constructed to meet all of these requirements. Such systems were not only highly specialized and complicated, but were often so bulky they were difficult to move around the lab. In addition, data analysis usually had to be performed off-line with an external computer or controller – adding more expense to the research process.

Part 1

The Personal Lab Scope

A simpler, more flexible solution can be achieved by using a modern digitizing storage oscilloscope (DSO) equipped with a low noise differential amplifier. A Tektronix TDS 400A Series Personal Lab Scope coupled with the Tektronix ADA400A Differential Amplifier is a powerful, portable, and affordable measurement system for capturing and analyzing low-amplitude biophysical phenomena in the presence of noise.

Long, 120K-point record lengths on each of its four channels, gives researchers the ability to capture and analyze complex, slowly-varying events and view cause-and-effect relationships. And a unique **Roll Mode** Display allows users to observe changes as they happen, instead of waiting until the entire acquisition is completed. The Roll Mode is a comfortable way for scientists, who may already be familiar with the chart recording format, to examine the information.

Coupled with the ADA400A, the TDS 400A delivers 100,000:1 CMRR (common-mode rejection ratio) and microvolt level sensitivity for precise signal conditioning. It allows 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-proprietary Hi-Res™ acquisition mode effectively removes noise from single shot and repetitive events with real-time digital filtering. The Hi-Res mode and ADA400A combination increases the dynamic range and vertical resolution of the scope, allowing researchers to capture the fine nuances of microvolt-level electrophysiological signals – even single-shot signals, in the presence of much larger, common-mode noise signals.

The Personal Lab Scope also allows on-board signal processing and analysis of waveform data. The intuitive graphical user interface delivers easy access to 25 automated measurements, including FFT and Template testing, plus a comprehensive suite of waveform math functions. With these on-line tools, researchers can easily perform most analyses in real-time.

For the all-important results-generation and reporting phase of the research process, the Personal Lab Scope provides a high-performance **3.5-inch DOS floppy drive**. Using this capability, researchers can store waveforms in fourteen different industry-standard waveform formats. They can save information in a spreadsheet format for use in report-generation programs such as EXCEL®, Lotus 1-2-3®, QuatroPro®, and MathCad®; or, in popular desktop publishing formats – PCX, TIFF, and BITMAP – for use in Windows and Macintosh wordprocessing programs.

Waveform data and test results can also be downloaded via GPIB to external storage facilities for later retrieval and reporting.

With bandwidths from 200 to 400 MHz, sample rates up to 100 MS/s per channel, and an advanced Peak Detect triggering mode that captures significant yet rare events, the TDS 400A Personal Lab Scope provides the acquisition confidence traditionally offered only by expensive lab oscilloscopes.

Part 2

[This article does not discuss the subjects of biological specimen preparation or interpretation of waveforms.]

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

Recording Low Level Signals in the Presence of Undesirable 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 bioscience environment can be divided into two categories: noise inherent to 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 Tektronix ADA400A Differential Amplifier offers selectable high cut-off filters that can be used to create a frequency window – eliminating high frequency noise from the measurement.

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.

The TDS 400A Personal Lab Scope's proprietary Hi-Res™ Mode[1] applies real-time digital filtering to the scope's digitizer output prior to writing the acquisition to memory. This allows the scope to eliminate high-frequency noise from lower frequency signals – even single shot signals. The Hi-Res mode does not rely on the presence of a stable trigger and, therefore, can be used on single-shot or non-repetitive events. It provides vertical resolution improvement – from 1 to 4 additional bits – for single-shot applications without the customary tradeoff in scope bandwidth.

The effectiveness of the Hi-Res mode is illustrated in Figures 1a and 1b. Figure 1a shows a simulated cardiac beat-like signal (1 Hz), similar to what would be seen on an ordinary monitor. The top waveform shows the main heart beat clearly, but noise prevents close examination of anomalies in the "at-rest" area following the main beat. The bottom waveform uses the Hi-Res mode to effectively remove the noise for a clear view of the entire signal.

The additional waveform detail provided by the Hi-Res™ mode in Figure 1b allows researchers to gain a clearer understanding of underlying phenomena. And unlike traditional averaging, the Hi-Res mode does not need to wait for repetitive acquisitions to remove the noise – noise removal takes place in a single acquisition.

[1]For more information about the Hi-Res mode, please contact your local Tektronix sales representative and ask for "*Increase Vertical Resolution Using the TDS Hi-Res Mode.*" – Tek Publication #3GW-8222-2

0.4 V. Differences between electrode-pair contact potentials produce an offset potential, which shows 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 most biophysical signals contain low frequency information. Also, AC coupling can not be used in the "high impedance" mode described earlier in this discussion.

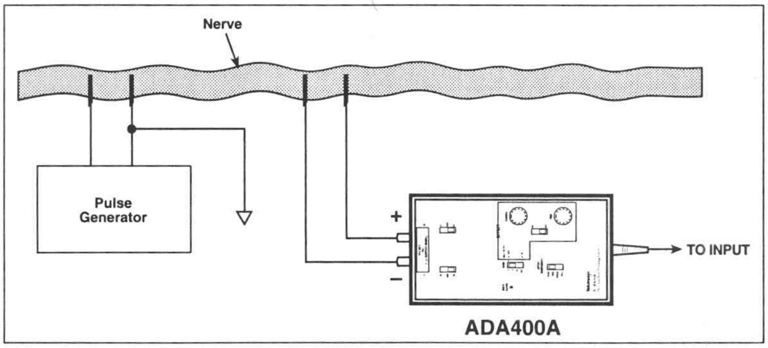


Figure 6

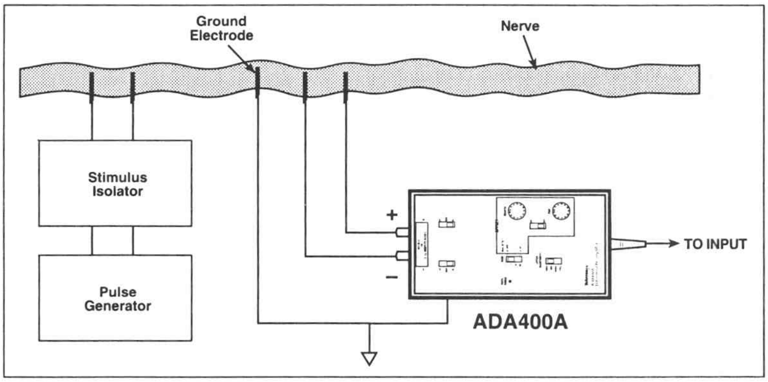


Figure 7

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 6.

An extension of this principle can be applied when making stimulus-response measurements on an excised nerve of a biological specimen (see Figure 7). 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 circuit symbol ⏚ is used to signify circuit ground. Safety ground or earth ground, discussed below, is denoted by the symbol ⊥.

Establishing a Common Earth Ground. Very often a multitude of line operated equipment is used to perform biophysical experiments. The way this equipment is hooked together can greatly affect the level of noise generated in the measurement system. The third wire ground levels, 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. In the past, care had to be taken to route "single-ended" signal leads away from such sources, and paired differential leads were twisted together to cancel out induced currents.

Figure 3 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.

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. These values produce a 500 kΩ impedance to ground for common-mode signals.

If the specimen interface creates a high, and possibly different impedance between the electrode pairs, as in Figure 3, 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:

$$\text{CMRR} = \left|\frac{R_3 or R_4}{R_1 - R_2}\right| = \frac{1M\Omega}{2k\Omega - 0.5k\Omega} = 666{:}1$$

When the CMRR is degraded like this, the common-mode noise displayed 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 15 divisions on the scope's CRT (see Table 2 below). Even with the high gain for the differential signal, the 15 divisions display of the common-mode noise will make the 2 division response signal unreadable.

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 3 would look like the circuit in Figure 4.

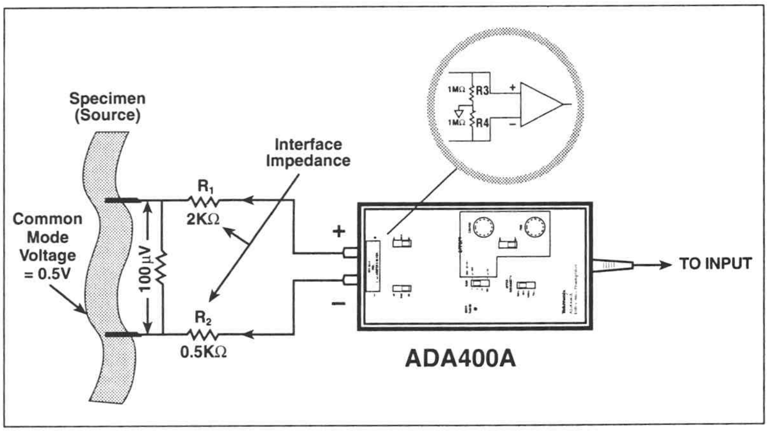


Figure 3

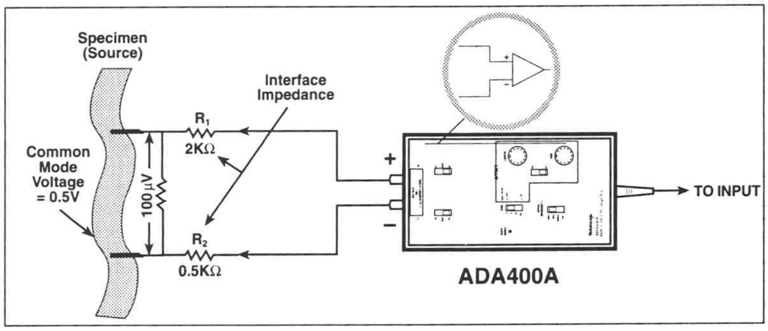


Figure 4

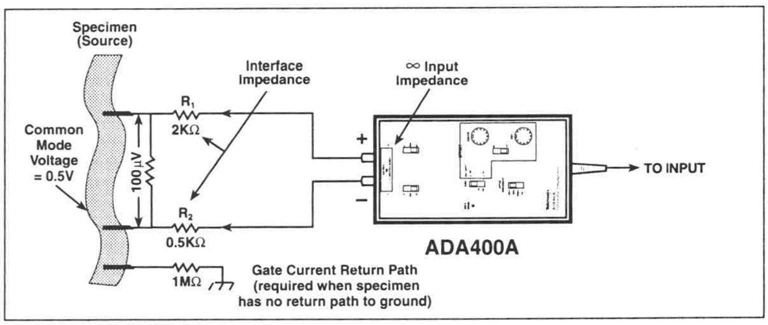


Figure 5

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.

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 pA (10^{-12} amps) 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 5.

Electrode Contact Potential. Electrode potentials exist whenever metallic electrodes interface with the specimen via an electrolyte. Typical half-cell chlorided electrode potentials (Ag-AgCl) are in the region of

Table 2

CMRR ∼ 666:1

Source Voltage	Source Interface Impedance	Load	Displayed Voltage	CRT divs @ 50 µV/div
Differential 100µV	Mismatched	2 MΩ	∼ 100 µV	2 divs
Common mode 0.5 V	and High	0.5 MΩ	∼ 750 µV	15 divs

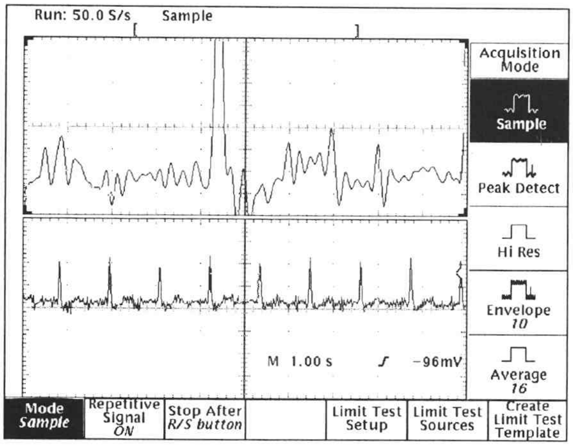


Figure 1a

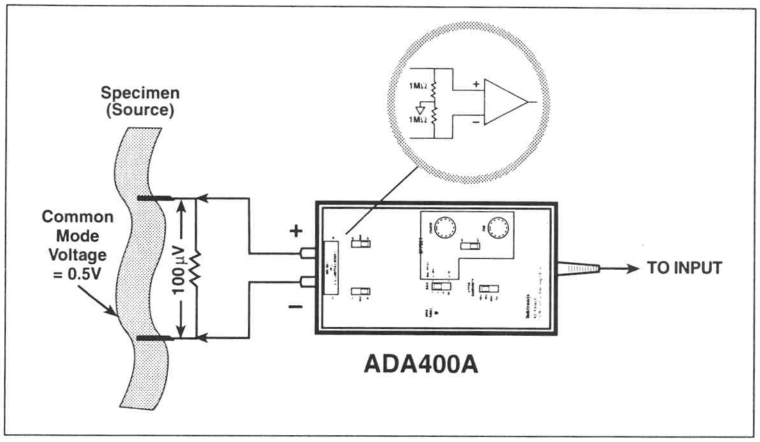


Figure 2

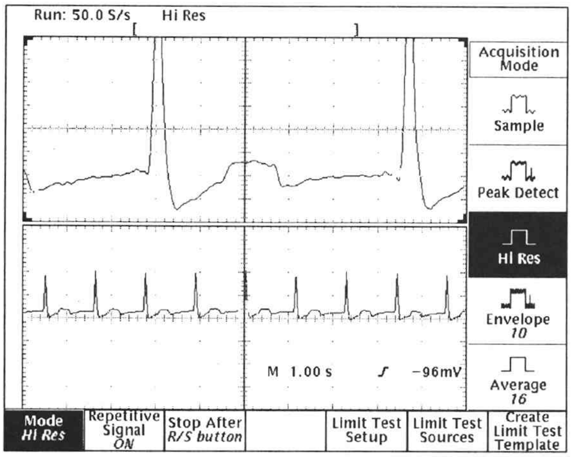


Figure 1b

Common-Mode Noise. Noise that enters the measurement from the external laboratory environment is called common-mode noise. If you touch your finger to an oscilloscope probe, for example, a large 60 Hz signal will be displayed on the scope's CRT. 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 properly. 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 signals, while at the same time attenuating common-mode noise. The ability of a differential amplifier to reject noise is called its common-mode rejection ratio (CMRR). The ADA400A Differential Amplifier for the TDS 400A 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.

Differential amplifiers have two inputs, both of which are 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 and low with respect to the amplifier's impedance, the amplifier "sees" only the true difference signal.

For example the TDS 400A/ 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 scope 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.

This example illustrates the usefulness of the ADA400A Differential Amplifier 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.

Table 1

CMRR - 100,000:1

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

The ADA400A Differential Amp, however, places the differential amplifier circuitry at the probe end, where it is as close as possible to the specimen being tested (see Figure 7). 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 8 shows the correct grounding technique.

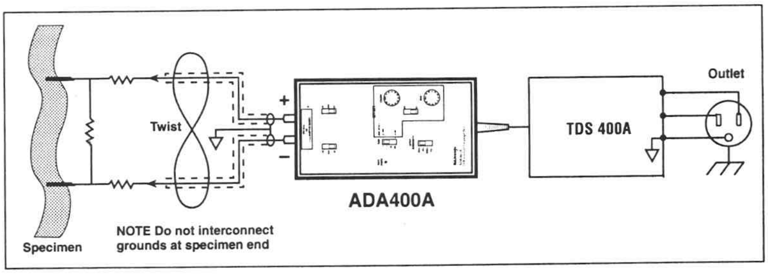


Figure 8

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.

Using this test setup, the differential amplifier eliminates the effects of ground loops while keeping the oscilloscope safely grounded.

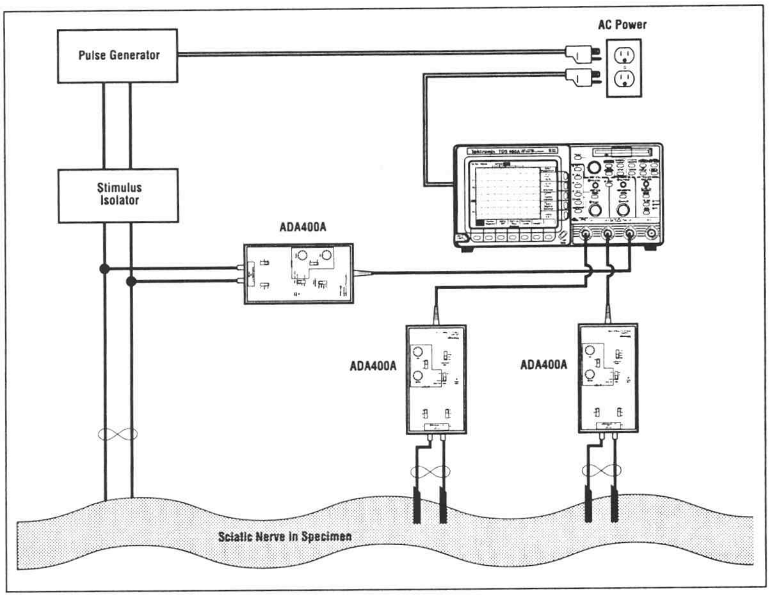


Figure 9

The Test Set-Up

Figure 9 shows the test set-up for examining compound action potentials in the sciatic nerve of a specimen. By using differential inputs on two channels, and acquiring the stimulus signal on the third, you can not only compare response to stimulus, but also make direct measurements of the conduction time (propagation delay) over specific lengths of the nerve.

Note that both the stimulus pulse generator and the oscilloscope are connected to the same earth ground bus. The pulse generator is also isolated from the specimen through stimulus isolators. The specimen can be referenced to the oscilloscope input ground either by means of a ground plane (shield) under the specimen, or an actual ground reference point on the specimen itself. All cabling from the specimen to the oscilloscope and pulse generator is through shielded coaxial cables. In addition, only incandescent lights are used in the laboratory where the experiment is being conducted.

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 biophysical measurements without complicated and expensive test equipment setups, precondition equipment, or external filters. Using the TDS 400A Personal Lab Scope and the ADA 400A Differential Amplifier, researchers can obtain complete solutions to their bioscience measurements. This advanced test system delivers precise signal conditioning, outstanding acquisition confidence, comprehensive on-board signal processing and 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 and repair.

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