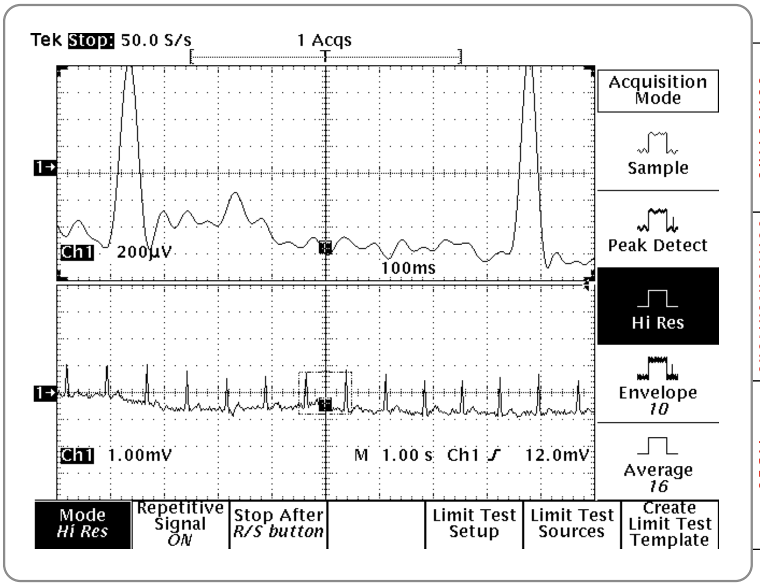
Biophysical Measurements Using the TDS400A Oscilloscope



Introduction

Measuring biophysical phenomena in biophysical experiments is a difficult challenge. The TDS400A oscilloscope, coupled with the ADA400A differential amplifier, provides a complete solution for bioscience measurements by delivering precise signal conditioning, outstanding acquisition confidence, comprehensive on-board signal processing and analysis, and accurate results-storage and report-generation capabilities.

How do you capture the tiny, microvolt-level electrical pulses that signal a firing neuron or a muscle response? Such events 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.

Making Biophysical Measurements

To acquire a biophysical signal, your system must meet certain conditions:

- You will need a system that can distinguish biophysical low-level signals and transients from background noise. You want something that can capture a wide dynamic range and then put that captured data through high quality signal conditioning to pinpoint the event in question.
- Certain measurements require long duration event capture, so you will need long record lengths, extensive storage capacity and single-shot acquisition capabilities.
- To view both the stimulus and response signals simultaneously, your measurement system must include multiple acquisition channels.

Once you acquire your signal, you must then analyze, document and report your results. You may need to store waveforms, waveform measurement results and test setups. 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, researchers constructed elaborate test equipment setups using expensive laboratory oscilloscopes, signal generators, chart recorders, pre-conditioning amplifiers, and filters to meet all of these requirements. Such systems were highly specialized and complicated and were often so bulky that they were difficult to move around the lab. In addition, data analysis was performed off-line with an external computer or controller – adding more expense to the research process.

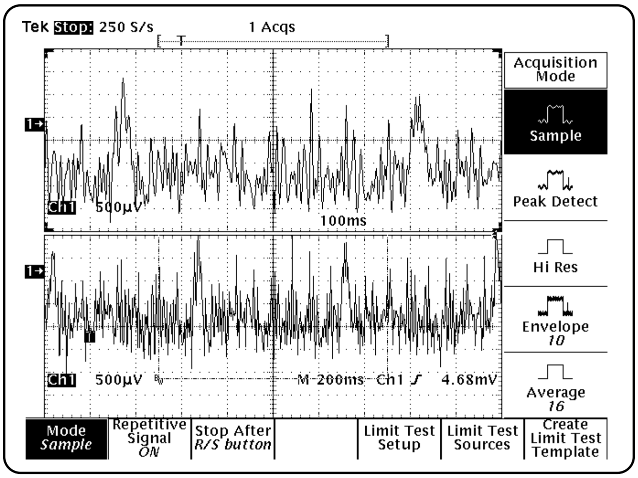


Figure 1.

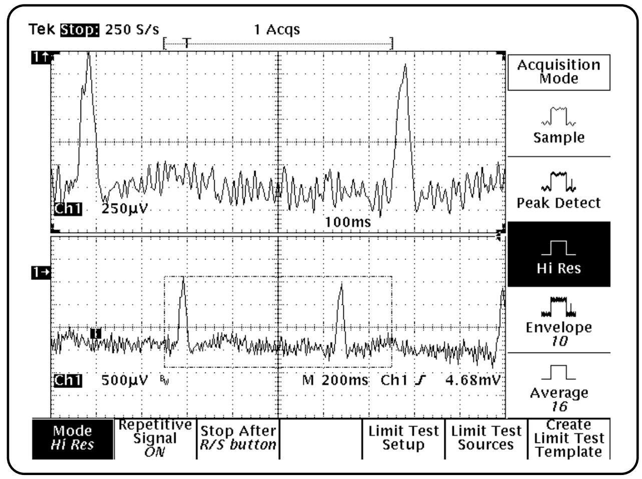


Figure 2.

Part 1

The TDS400A Oscilloscope

A simpler, more flexible solution can be achieved by using a modern digital storage oscilloscope (DSO) equipped with a low noise differential amplifier. A TDS400A Series oscilloscope coupled with the 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, 120 K-point record lengths on each of its four channels gives you the ability to capture and analyze complex, slowly-varying events, and to view cause-and-effect relationships. And a unique **Roll Mode** Display allows you to observe changes as they happen, instead of waiting until the entire acquisition is completed. Once you are familiar with the chart-recording format, the roll mode is a comfortable way for you to examine the information.

Coupled with the ADA400A, the TDS400A delivers 100,000:1 CMRR (Common-Mode Rejection Ratio – the ability of a differential amplifier to reject noise) and microvolt-level sensitivity for precise signal conditioning. It allows you to pick up extremely small amplitude signals, such as neurons firing during an experiment or a muscle fiber response to external stimulus. 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 oscilloscope, allowing you 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 oscilloscope 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, you can easily perform most analysis in real-time.

For the results generation and reporting phase of the research process, the oscilloscope includes a high-performance 3.5-inch DOS floppy drive. Using this capability, you can store waveforms in 14 different industry-standard waveform formats. You can save the 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 BMP – for use in Windows and Macintosh word-processing programs.

You can also access external storage facilities via the GPIB cable connector. With this robust access to additional storage, you can save data and test results for later retrieval, reporting, and analysis.

With bandwidths from 200 to 400 MHz, sample rates up to 100 MS/s per channel, and an advanced Peak Detect mode that captures significant yet rare events, the TDS400A oscilloscope assures you confidence in the accuracy of your waveform acquisition.

Part 2

Note: 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 fluorescent 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 TDS400A oscilloscope's proprietary Hi-Res mode applies real-time digital filtering to the oscilloscope's digitizer output prior to writing the acquisition to memory. This allows the oscilloscope 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, so you can use it on single-shot or non-repetitive events. It provides vertical resolution improvement – up to greater than 12 effective bits – for single-shot applications without the customary trade-off in the oscilloscope bandwidth.

The effectiveness of the Hi-Res mode is illustrated in Figures 1 and 2. Figure 1 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 2 allows you 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.

Note: For more information about the Hi-Res mode, please contact your local Tektronix sales representative.

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 oscilloscope'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 your 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 may be inescapable. Due to the nature of the experiment or the organism under test, you may not be able to fully ground your specimen. 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 ADA400A differential amplifier for the TDS400A offers 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 3). 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.

Table 1

CMRR - 100,000:1				
Source Voltage	Source Interface Impedence	Load	Displayed Voltage	CT divisions @50 µV/div
Differential 100 µV	Equal and Low	2 MΩ	100 µV	2 divs
Common mode 0.5 V		0.5 MΩ	5 µV	0.1 div

For example, the TDS400A/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 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 3). 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.

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 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 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:

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

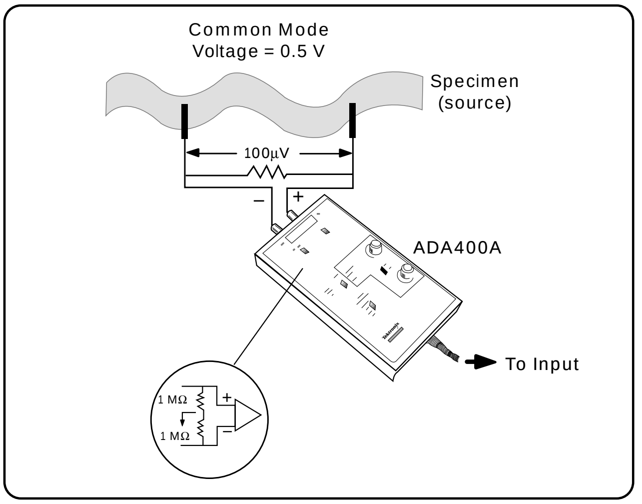


Figure 3.

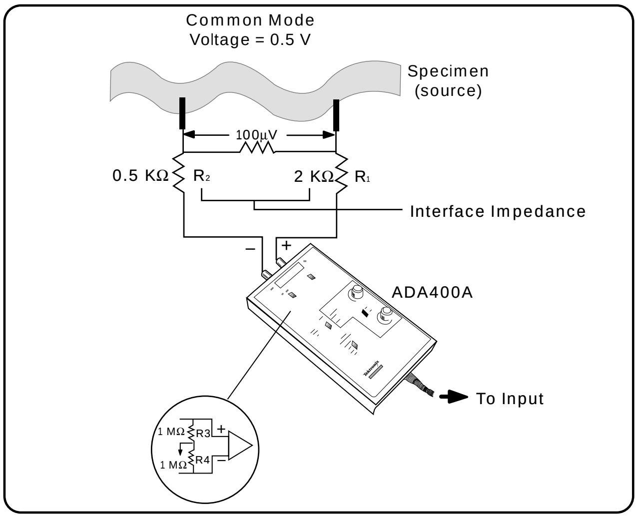


Figure 4.

Table 2

		CMRR - 666:1		
Source Voltage	Source Interface Impedence	Load	Displayed Voltage	CT divisions @50 µV/div
Differential 100 µV	Mismatched and High	2 MΩ	–100 µV	2 divs
Common mode 0.5 V		0.5 MΩ	–750 µV	15 divs

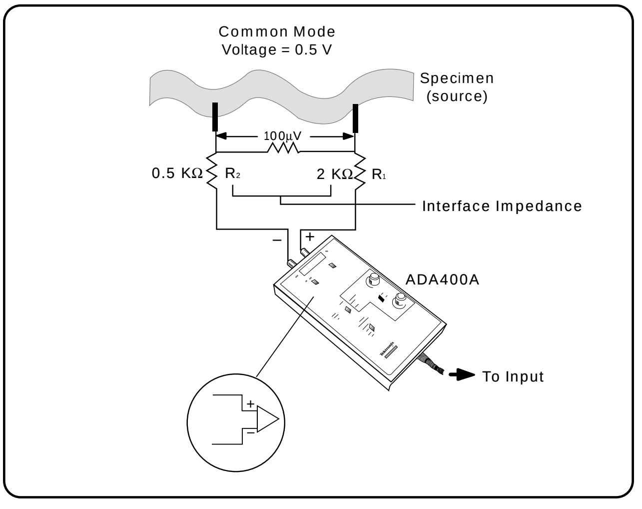


Figure 5.

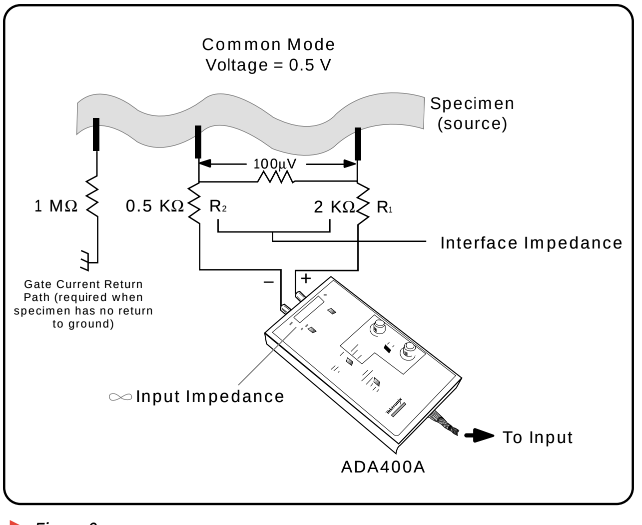


Figure 6.

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 oscilloscope's CRT (see Table 2 above). 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 the degraded CMRR is to raise the input impedance of the differential amplifier. If the differential amplifier had 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.

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 6.

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 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 the 2 MΩ will tend to discharge these "batteries," but residual offset may displace the desired signal off-screen, especially at high sensitivities.

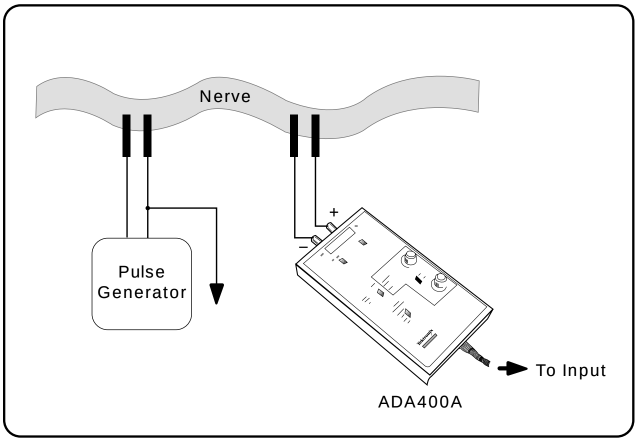


Figure 7.

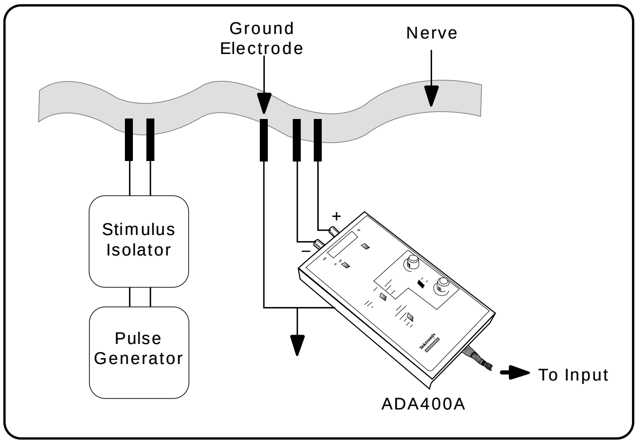


Figure 8.

These are several ways of canceling the effects of this offset potential:

- Some differential amplifiers include 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.
- If the display offset is small, the differential amplifier display position control can be used to position the display screen.
- 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 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.

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 circuit symbol ⏚ is used to signify circuit ground. Safety ground or earth ground (see next page) is denoted by the symbol ⏚ .

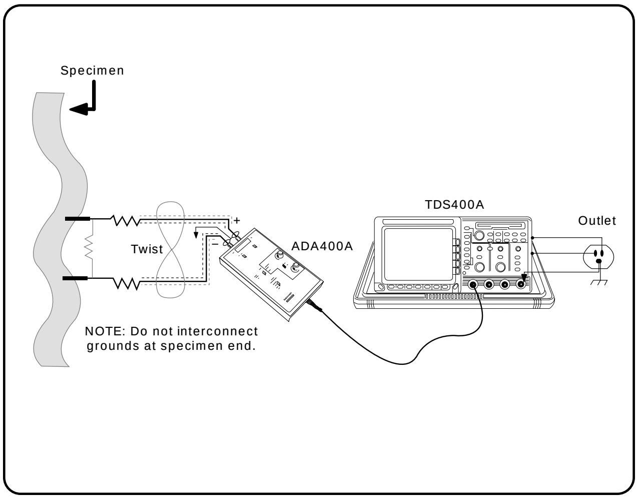


Figure 9.

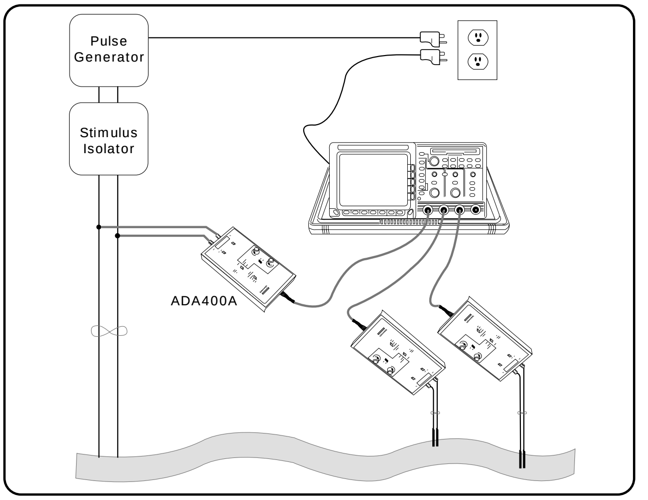


Figure 10.

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.

The ADA400A differential amplifier, 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.

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.

The Test Setup

Figure 10 shows the test setup 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 the 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

Paying careful attention to the grounding of equipment, isolation of signal generators, and shielding of probes and leads, can produce very refined biophysical measurements without complicated and expensive test equipment setups, precondition equipment, or external filters. Using the TDS400A oscilloscope and the ADA400A differential amplifier, you can obtain complete solutions to your 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.

For Further Information

Tektronix maintains a comprehensive, constantly expanding collection of application notes, technical briefs and other resources to help engineers working on the cutting edge of technology.

Please visit “Resources For You” on our Web site at **www.tektronix.com**

Contact Tektronix

ASEAN Countries (65) 356-3900

Australia & New Zealand 61 (2) 9888-0100

Austria, Central Eastern Europe, Greece, Turkey, Malta & Cyprus +43 2236 8092 0

Belgium +32 (2) 715 89 70

Brazil and South America 55 (11) 3741-8360

Canada 1 (800) 661-5625

Denmark +45 (44) 850 700

Finland +358 (9) 4783 400

France & North Africa +33 1 69 86 81 81

Germany +49 (221) 94 77 400

Hong Kong (852) 2585-6688

India (91) 80-2275577

Italy +39 (2) 25086 501

Japan (Sony/Tektronix Corporation) 81 (3) 3448-3111

Mexico, Central America & Caribbean 52 (5) 666-6333

The Netherlands +31 23 56 95555

Norway +47 22 07 07 00

People’s Republic of China 86 (10) 6235 1230

Poland (48) 22 251 5340

Republic of Korea 82 (2) 528-5299

South Africa (27 11) 254-8360

Spain & Portugal +34 91 372 6000

Sweden +46 8 477 65 00

Switzerland +41 (41) 729 36 40

Taiwan 886 (2) 2722-9622

United Kingdom & Eire +44 (0)1344 392000

USA 1 (800) 426-2200

For other areas, contact: Tektronix, Inc. at 1 (503) 627-1924

08/01 HB/XBS 3GW-10379-1

Tektronix
Enabling Innovation