SIGNAL CONDITIONING AND SIGNAL CONDITIONERS

It is rare for biological, physiological, chemical, electrical or physical signals to be measured in the appropriate format for recording and interpretation. Usually, a signal must be "massaged" to optimize it for both of these functions. For example, storage of recorded data is more accurate if the data are amplified before digitization so that they occupy the whole dynamic range of the A/D converter, and interpretation is enhanced if extraneous noise and signals above the bandwidth of interest are eliminated by a low-pass filter.

Why Should Signals Be Filtered?

A filter is a circuit that removes selected frequencies from the signal. Filtering is most often performed to remove unwanted signals and noise from the data. The most common form of filtering is *low-pass filtering*, which limits the bandwidth of the data by eliminating signals and noise above the corner frequency of the filter (Figure 6-1). The importance of low-pass filtering is apparent when measuring currents from single-ion channels. For example, channel openings that are obscured by noise in a 10 kHz bandwidth may become easily distinguishable if the bandwidth is limited to 1 kHz.

High-pass filtering is required when the main source of noise is below the frequency range of the signals of interest. This is most commonly encountered when making intracellular recordings from nerve cells in the central nervous system. There are low-frequency fluctuations in the membrane potential due to a variety of mechanisms, including the summation of synaptic inputs. The small, excitatory synaptic potentials that the user might be interested in are often smaller than the low-frequency fluctuations. Since excitatory synaptic potentials are often quite brief, the low-frequency fluctuations can be safely eliminated by using a high-pass filter. High-pass filtering is also referred to as AC coupling.

Another type of filter that is often used in biological recording is the *notch filter*. This is a special filter designed to eliminate one fundamental frequency and very little else. Notch filters are most commonly used at 50 or 60 Hz to eliminate line-frequency pickup.

Fundamentals of Filtering

Filters are distinguished by a number of important features. These are:

-3 dB Frequency

The -3 dB frequency (f_{-3}) is the frequency at which the signal *voltage* at the output of the filter falls to √1/2, *i.e.*, 0.7071, of the amplitude of the input signal. Equivalently, f_{-3} is the frequency at which the signal *power* at the output of the filter falls to half of the power of the input signal. (See definition of decibels later in this chapter.)

Type: High-pass, Low-pass, Band-pass or Band-reject (notch)

A low-pass filter rejects signals in high frequencies and passes signals in frequencies below the -3 dB frequency. A high-pass filter rejects signals in low frequencies and passes signals in frequencies above the -3 dB frequency. A band-pass filter rejects signals outside a certain frequency range (bandwidth) and passes signals inside the bandwidth defined by the high and the low -3 dB frequencies. A band-pass filter can simply be thought of as a series cascade of high-pass and low-pass filters. A band-reject filter, often referred to as a notch filter, rejects signals inside a certain range and passes signals outside the bandwidth defined by the high and the low -3 dB frequencies. A band-reject filter can simply be thought of as a parallel combination of a high-pass and a low-pass filter.

Order

A simple filter made from one resistor and one capacitor is known as a *first-order filter*. Electrical engineers call it a single-pole filter. Each capacitor in an active filter is usually associated with one pole. The higher the order of a filter, the more completely out-of-band signals are rejected. In a first-order filter, the attenuation of signals above f_{-3} increases at 6 dB/octave, which is equal to 20 dB/decade. In linear terminology, this attenuation rate can be re-stated as a voltage attenuation increasing by 2 for each doubling of frequency, or by 10 for each ten-fold increase in frequency.

Implementation: Active, Passive or Digital

Active filters are usually made from resistors, capacitors and operational amplifiers. Passive filters use resistors, capacitors and inductors. Passive filters are more difficult to make and design because inductors are relatively expensive, bulky and available in fewer varieties and values than capacitors. Active filters have the further virtue of not presenting a significant load to the source. Digital filters are implemented in software. They consist of a series of mathematical calculations that process digitized data.

Filter Function

There are many possible transfer functions that can be implemented by active filters. The most common filters are: Elliptic, Cauer, Chebyshev, Bessel and Butterworth. The frequency responses of the latter two are illustrated in Figure 6-1. Any of these filter transfer functions can be adapted to implement a high-pass, low-pass, band-pass or band-reject filter. All of these filter transfer functions and more can be implemented by digital filter algorithms. Digital filters can even do the seemingly impossible: since all of the data may be present when the filtering begins, some digital filters use data that arrive after the current data. This is clearly impossible in an analog filter because the future cannot be predicted. Typical digital filters are the box-car smoothing filter and the Gaussian filter.

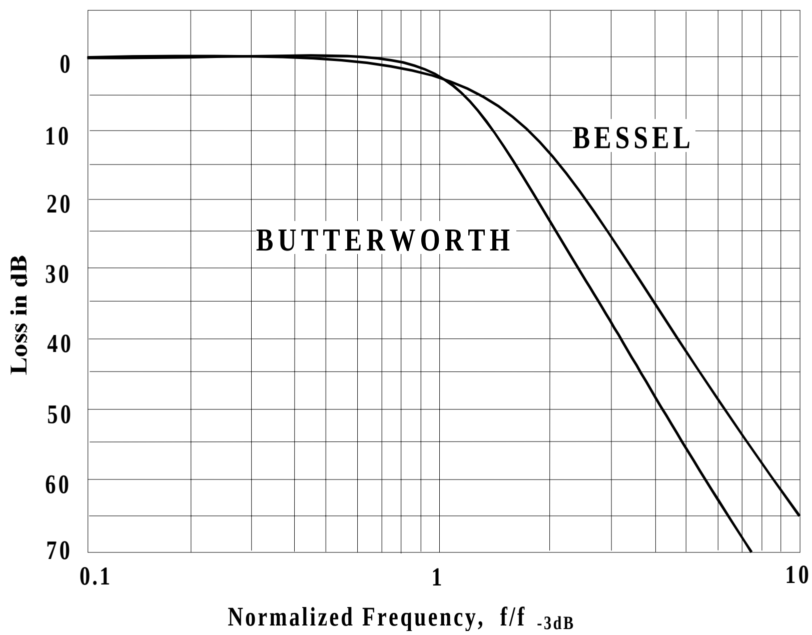

Figure 6-1. Frequency Response of 4th-Order Bessel and Butterworth Filters
The spectra have been normalized so that the signal magnitude in the pass band is 0 dB. The -3 dB frequency has been normalized to unity.

Filter Terminology

The terminology in this section is defined and illustrated in terms of a low-pass filter. The definitions can easily be modified to describe high-pass and band-pass filters. Many of these terms are illustrated in Figure 6-2.

- 3 dB Frequency

f_{-3}, defined previously, is sometimes called the *cutoff* frequency or the *corner* frequency. While most engineers and physiologists specify a filter's bandwidth in terms of the -3 dB frequency, for obscure reasons some manufacturers label the filter frequency on the front panel of their instruments based on a frequency calculated from the intersection frequency of the pass-band and the stop-band asymptotes. This is very confusing. To make sure that the filter is calibrated in terms of the -3 dB frequency, a sine wave generator can be used to find the -3 dB frequency.

Attenuation

Attenuation is the reciprocal of gain. For example, if the gain is 0.1 the attenuation is 10. The term *attenuation* is often preferred to *gain* when describing the amplitude response of filters since many filters have a maximum gain of unity. (For accurate measurements, note that even filters with a stated gain of unity can differ from 1.00 by a few percent.)

Pass Band

Pass band is the frequency region below f_{-3}. In the ideal low-pass filter there would be no attenuation in the pass band. In practice, the gain of the filter gradually reduces from unity to 0.7071 (*i.e.*, -3 dB) as the signal frequencies increase from DC to f_{-3}.

Stop Band

Stop band is the frequency region above f_{-3}. In the ideal low-pass filter, the attenuation of signals in the stop band would be infinite. In practice, the gain of the filter gradually reduces from 0.7071 to a filter-function and implementation-dependent minimum as the signal frequencies increase from f_{-3} to the maximum frequencies in the system.

Phase Shift

The phase of sinusoidal components of the input signal is shifted by the filter. If the phase shift in the pass band is linearly dependent on the frequency of the sinusoidal components, the distortion of the signal waveform is minimal.

Overshoot

When the phase shift in the pass band is not linearly dependent on the frequency of the sinusoidal component, the filtered signal generally exhibits overshoot. That is, the response to a step transiently exceeds the final value.

Octave

An octave is a range of frequencies where the largest frequency is double the lowest frequency.

Decade

A decade is a range of frequencies where the largest frequency is ten times the lowest frequency.

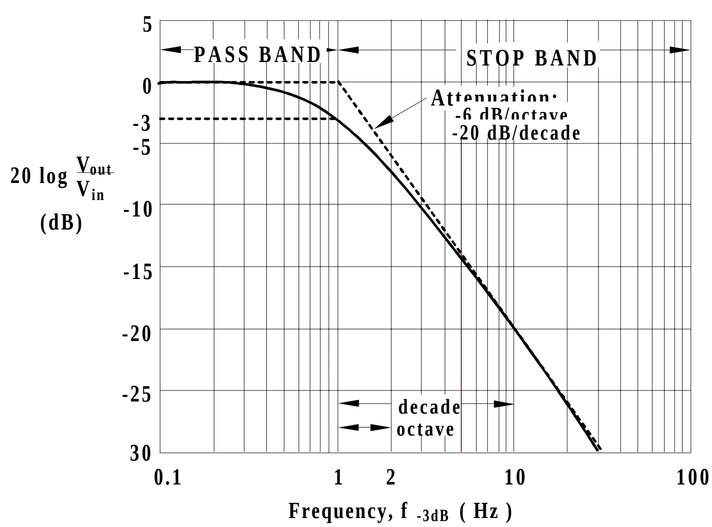

Figure 6-2. Illustration of Filter Terminology

A number of the terms used to describe a filter are illustrated in the context of a single-pole, low-pass filter.

Decibels (dB)

Since filters span many orders of magnitude of frequency and amplitude, it is common to describe filter characteristics using logarithmic terminology. Decibels provide the means of stating ratios of two voltages or two powers.

Voltage:

$$\mathrm{dB} = 20 \log \frac{V_{out}}{V_{in}} \tag{1}$$

Thus, 20 dB corresponds to a tenfold increase in the voltage.
-3 dB corresponds to a $\sqrt{2}$ decrease in the voltage.

Power:

$$\mathrm{dB} = 10 \log \frac{P_{out}}{P_{in}} \tag{2}$$

Thus, 10 dB corresponds to a tenfold increase of the power.
-3 dB corresponds to a halving of the power.

Some useful values to remember:

Decibels	Voltage Ratio	Power Ratio
3 dB	1.414:1	2:1
6 dB	2:1	4:1
20 dB	10:1	100:1
40 dB	100:1	10,000:1
60 dB	1,000:1	1,000,000:1
66 dB	2,000:1	4,000,000:1
72 dB	4,000:1	16,000,000:1
80 dB	10,000:1	10^8:1
100 dB	100,000:1	10^{10}:1
120 dB	1,000,000:1	10^{12}:1

Order

As mentioned above, the order of a filter describes the number of poles. The order is often described as the slope of the attenuation in the stop band, well above f_{-3}, so that the slope of the attenuation has approached its asymptotic value (see Figure 6-2). Each row in the following table contains different descriptions of the same order filter.

Pole	Order	Slopes	
1 pole	1st order	6 dB/octave	20 dB/decade
2 poles	2nd order	12 dB/octave	40 dB/decade
4 poles	4th order	24 dB/octave	80 dB/decade
8 poles	8th order	48 dB/octave	160 dB/decade

Typically, the higher the order of the filter, the less the attenuation in the pass band. That is, the slope of the filter in the pass band is flatter for higher order filters (Figure 6-3).

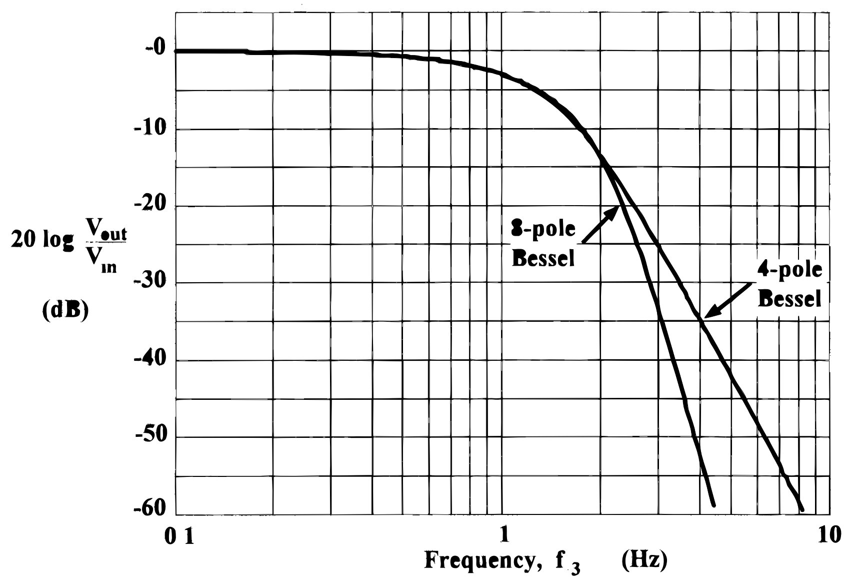

Figure 6-3. Difference Between a 4th- and 8th-Order Transfer Function

10-90% Rise Time

The 10-90% rise time ($t_{10\text{-}90}$) is the time it takes for a signal to rise from 10% of its final value to 90% of its final value. For a signal passing through a low-pass filter, $t_{10\text{-}90}$ increases as the -3 dB frequency of the filter is lowered. Generally, when a signal containing a step change passes through a high-order filter, the rise time of the emerging signal is given by:

$$t_{10\text{-}90} \approx 0.3/f_{-3} \qquad (3)$$

For example, if f_{-3} is 1 kHz, $t_{10\text{-}90}$ is approximately 300 µs.

As a general rule, when a signal with $t_{10\text{-}90} = t_s$ is passed through a filter with $t_{10\text{-}90} = t_f$, the rise time of the filtered signal is approximately:

$$t_r = \sqrt{t_s^2 + t_f^2} \qquad (4)$$

Filtering for Time-Domain Analysis

Time-domain analysis refers to the analysis of signals that are represented the same way they would appear on a conventional oscilloscope. That is, steps appear as steps and sine waves appear as sine waves. For this type of analysis, it is important that the filter contributes minimal distortion to the time course of the signal. It would not be very helpful to have a filter that was very effective at eliminating high-frequency noise if it caused 15% overshoot in the pulses. Yet this is what many kinds of filters do.

In general, the best filters to use for time-domain analysis are Bessel filters because they add less than 1% overshoot to pulses. The Bessel filter is sometimes called a "linear-phase" or "constant delay" filter. All filters alter the phase of sinusoidal components of the signal. In a Bessel filter, the change of phase with respect to frequency is linear. Put differently, the amount of signal delay is constant in the pass band. This means that pulses are minimally distorted. Butterworth filters add considerable overshoot: 10.8% for a fourth-order filter; 16.3% for an eighth-order filter. The step response of the Bessel and Butterworth filters is compared in Figure 6-4.

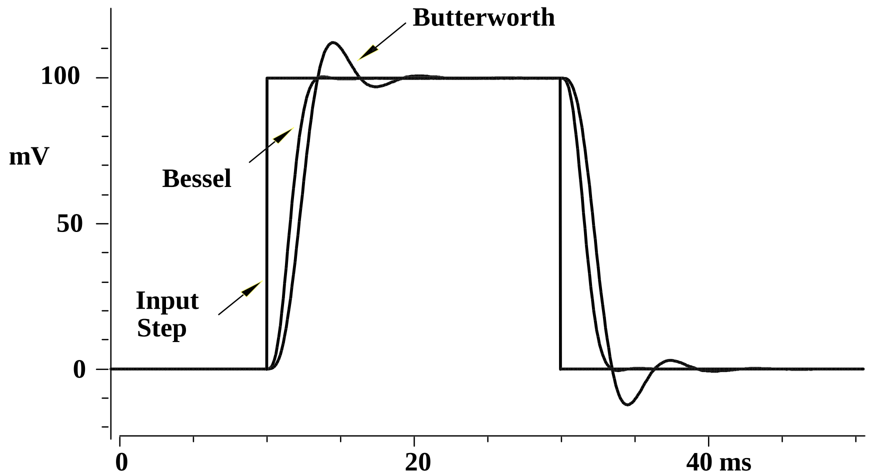

Figure 6-4. Step Response Comparison Between Bessel and Butterworth Filters
The overshoots of fourth-order Bessel and Butterworth filters are compared.

In many experiments in biology and physiology (*e.g.*, voltage- and patch-clamp experiments), the signal noise increases rapidly with bandwidth. Therefore, a single-pole filter is inadequate. A four-pole Bessel filter is usually sufficient, but eight-pole filters are not uncommon. In experiments where the noise power spectral density is constant with bandwidth (*e.g.*, recording from a strain gauge), a single-pole filter is sometimes considered to be adequate.

In the time domain, a notch filter must be used with caution. If the recording bandwidth encompasses the notch filter frequency, signals that include a sinusoidal component at the notch frequency will be grossly distorted, as shown in Figure 6-5. On the other hand, if the notch filter is in series with a low-pass or high-pass filter that excludes the notch frequency, distortions will be prevented. For example, notch filters are often used in electromyogram (EMG) recording in which the line-frequency pickup is sometimes much larger than the signal. The 50 or 60 Hz notch filter is typically followed by a 300 Hz high-pass filter. The notch filter is required because the high-pass filter does not adequately reject the 50 or 60 Hz hum (see below).

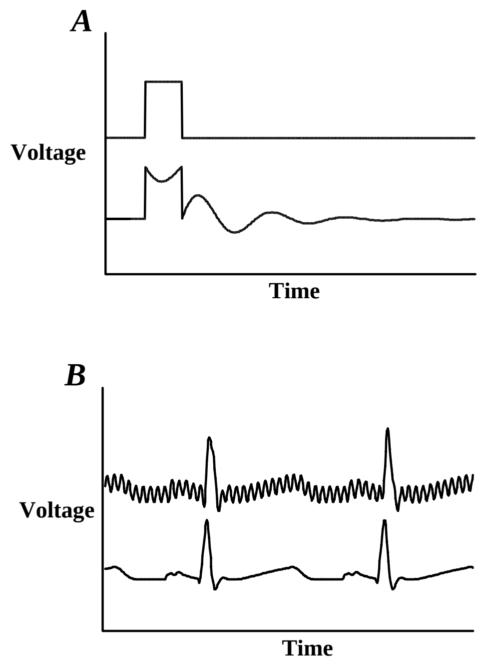

Figure 6-5. Use of a Notch Filter: Inappropriately and Appropriately

A shows an inappropriate use of the notch filter. The notch filter is tuned for 50 Hz. The input to the notch filter is a 10 ms wide pulse. This pulse has a strong component at 50 Hz that is almost eliminated by the notch filter. Thus, the output is grossly distorted. ***B*** shows an appropriate use of the notch filter. An EKG signal is corrupted by a large 60 Hz component that is completely eliminated by the notch filter.

Filtering for Frequency-Domain Analysis

Frequency-domain analysis refers to the analysis of signals that are viewed to make a power spectrum after they are transformed into the frequency domain. This is typically achieved using a fast Fourier transform (FFT). After transformation, sine waves appear as thin peaks in the spectrum and square waves consist of their component sine waves. For this type of analysis it does not matter if the filter distorts the time-domain signal. The most important requirement is to have a sharp filter cutoff so that noise above the -3 dB frequency does not get folded back into the frequency of interest by the aliasing phenomenon (see **Chapter 12**).

The simplest and most commonly used filter for frequency-domain analysis in biological applications is the Butterworth filter. This filter type has "maximally flat amplitude" characteristics in the pass band. All low-pass filters progressively attenuate sinusoidal components of the signal as the -3 dB frequency is approached from DC. In a Butterworth filter, the attenuation in the pass band is as flat as possible without having pass-band ripple. This means that the frequency spectrum is minimally distorted.

Usually, notch filters can be safely used in conjunction with frequency-domain analysis since they simply remove a narrow section of the power spectrum. Nevertheless, they are not universally used this way because many experimenters prefer to record the data "as is" and then remove the offending frequency component from the power spectrum digitally.

Sampling Rate

If one intends to keep the data in the time domain, sufficient samples must be taken so that transients and pulses are adequately sampled. The Nyquist Sampling Theorem states that a bare minimum sampling rate is twice the signal bandwidth; that is, the -3 dB frequency of the low-pass filter must be set at one half the sampling rate or lower. Therefore, if the filter -3 dB frequency is 1 kHz, the sampling theorem requires a minimum sampling rate of 2 kHz. In practice, a significantly higher sampling rate is often used because it is not practical to implement the reconstruction filters that would be required to reconstruct time-domain data acquired at the minimum sampling rate. A sampling rate of five or more times the -3 dB frequency of the filter is common.

If you wish to make peak-to-peak measurements of your data for high-frequency signals, you must consider the sampling rate closely. The largest errors occur when samples are equally spaced around the true peak in the record. This gives the worst estimate of the peak value. To illustrate the magnitude of this problem, assume that the signal is a sine wave and that the following number of samples are taken per cycle of the sine wave. The maximum errors that one could get are then as follows:

5 samples/cycle	19.0% error
10 samples/cycle	4.9% error
20 samples/cycle	1.2% error

The sampling of the peak values varies between no error and the maximum as stated above.

If one intends to transform the data into the frequency domain, Butterworth or Elliptic filters are more suitable than the Bessel filter. These filters have a sharper cutoff near the -3 dB frequency than the Bessel filter, and thus better prevent the phenomenon known as aliasing. With a fourth-order or higher Butterworth filter, it is usual to set f_{-3} to about 40% of the sampling rate. Frequently, researchers do not have a Butterworth filter handy. If a Bessel filter is available, it can be used instead, but f_{-3} would normally be set to about 25 - 30% of the sampling rate. This is because the Bessel filter attenuation is not as sharp near the -3 dB frequency as that of a Butterworth filter.

Filtering Patch-Clamp Data

The analog filters that are typically used with a patch clamp are the Bessel and Butterworth types, in either 4-pole or 8-pole versions. If simply regarded from the noise point of view, the Butterworth filter is the best. Empirical measurements carried out at Axon Instruments reveal that the rms noise passed by the filters (relative to a 4-pole Bessel filter) when used with an Axopatch 200 in single-channel patch mode is as follows:

Noise	**4-Pole Bessel**	**8-Pole Bessel**	**4-Pole Butterworth**	**8-Pole Butterworth**
1 kHz	1.0	0.97	0.95	0.93
5 kHz	1.0	0.97	0.85	0.82
10 kHz	1.0	0.94	0.83	0.80

The table shows that the main reduction in noise is gained by using a Butterworth filter instead of a Bessel filter. The improvement achieved by going from a 4-pole filter to an 8-pole filter of the same kind is small.

However, for the reasons discussed above, the Butterworth filter cannot be used for time-domain analysis. Since this is the most common kind of analysis performed on patch-clamp data, Bessel filters are almost invariably used.

Digital Filters

Some researchers prefer to record data at wide bandwidths to prevent the loss of potentially important information. An analog filter is used to provide anti-alias filtering, followed by a digital filter implemented at various lower -3 dB frequencies during the analysis. There are many types of digital filters. Two types are described here:

Nonrecursive Filter

The output of a nonrecursive filter depends only on the input data. There is no dependence on the history of previous outputs. An example is the smoothing-by-3's filter:

$$y_n = \frac{x_{n-1} + x_n + x_{n+1}}{3} \qquad (5)$$

where y_n and x_n are the output and input samples at sample interval n.

Nonrecursive filters are also known as "finite impulse response" filters (FIR) because their response to a single impulse endures only as long as the newest sample included in the formula (*i.e.*, x_{n+1} in the smoothing-by-3's filter).

Another example of a nonrecursive digital filter is the Gaussian filter. It has a similar form to the smoothing-by-3's filter described above, except that typically there are more terms and the magnitudes of the coefficients lie on the bell-shaped Gaussian curve.

These types of filters have the advantage of not altering the phase of the signal. That is, the mid-point for the rise time of a step occurs at the same time both before and after filtering. In contrast, analog filters always introduce a delay into the filtered signal.

A problem with digital filters is that values near the beginning and end of the data cannot be properly computed. For example, in the formula above, if the sample is the first point in the data, x_{n-1} does not exist. This may not be a problem for a long sequence of data points; however, the end effects can be serious for a short sequence. There is no good solution other than to use short filters (*i.e.*, few terms). Adding values outside the sequence of data is arbitrary and can lead to misleading results.

Recursive Filter

The output of a recursive filter depends not only on the inputs, but on the previous outputs as well. That is, the filter has some time-dependent "memory." Recursive filters are also known as "infinite impulse response" filters (IIR) because their response to a single impulse extends indefinitely into the future (subject to computer processing limitations).

Digital-filter implementations of analog filters such as the Bessel, Butterworth and RC filters are recursive.

Correcting for Filter Delay

The delay introduced by analog filters necessarily makes recorded events appear to occur later than they actually occurred. If it is not accounted for, this added delay can introduce an error in subsequent data analysis. The effect of the delay can be illustrated by considering two common questions: How long after a stimulus did an event occur (latency measurement)? And, what was the initial value of an exponentially decaying process (extrapolation back to the zero time of a fitted curve)? If the computer program was instructed to record 50 points of baseline and then apply a stimulus, it would be natural, but incorrect, to assume in the subsequent analysis that zero time begins somewhere between points 50 and 51 of the recorded data, since zero time may actually be at point 52 or later.

The delay can be seen in Figure 6-4. The times to the 50% rise or fall point of a step signal (delay) are approximately $0.33/f_{-3}$ for a fourth-order low-pass Bessel filter, and $0.51/f_{-3}$ for an eighth-order Bessel filter. In practice, when using a fourth-order Bessel filter with f_{-3} = 1 kHz and sampling the trace at 6 kHz, the filter delay is 330 µs. So, the whole record will have to be shifted by 2.0 points with respect to the stimulus events that were programmed.

Preparing Signals for A/D Conversion

Analog-to-Digital (A/D) converters have a fixed resolution and measure signals in a grainy manner. This means that all signals lying between certain levels are converted as if they are the same value. To minimize the impact of this undesirable "quantization" effect, it is important to amplify the signal prior to presenting it to the A/D converter. Ideally, the gain should be chosen so that the biggest signals of interest occupy the full range of the A/D converter but do not exceed it. In most laboratory systems, the full range of the A/D converter is ±10 V, but other ranges such as ±5 V and ±1.25 V are often used in industrial applications.

Where to Amplify

Amplification is possible at a number of different points along the signal pathway. Since the amplification, filtering and offset circuits can themselves introduce noise, the location of the amplification circuitry must be carefully considered. There are several options:

Inside the Recording Instrument

The ideal place to amplify the signal is inside the instrument that records the signal. For example, the Axopatch patch clamp from Axon Instruments contains a variable gain control in the output section that can be used to provide low-noise amplification of the pipette current or membrane potential. The advantage of placing the amplification inside the recording instrument is that the amount of circuitry between the low-level signal and the amplifying circuitry can be minimized, thereby reducing extraneous noise contributions.

Between the Recording Instrument and the A/D Converter

A good place to amplify the signal is after it emerges from the recording instrument, before it is sent to the A/D converter. The CyberAmp signal conditioners from Axon Instruments can be used for this purpose. For the best noise performance, a small amount of initial amplification should be provided in the recording instrument if the signal levels are low. The main advantage of using a multi-channel amplifier such as the CyberAmp is that the gain of each recording pathway can be independently set by the computer. An instrument such as the CyberAmp has more gain choices than are usually available in a recording instrument.

In either of the examples discussed so far, anti-aliasing filtering is conveniently provided on a per-channel basis after the gain amplification.

After the Channel Multiplexor on the A/D Converter Board

A common place to provide amplification is in a programmable gain amplifier (PGA) located after the channel multiplexor on the A/D converter board. Briefly, in the A/D converter board, many channels are typically digitized by a single A/D converter. The signals are sequentially presented to the A/D converter by a multiplexor circuit. A PGA located after the multiplexor is very economical, since only one PGA is required regardless of the number of channels.

The main advantage of locating a PGA after the multiplexor on the A/D board is that it is inexpensive. However, there are significant disadvantages:

(1) The PGA has to have extremely wide bandwidth since it must be able to settle within the resolution of the A/D converter at the multiplexing rate. Depending on the number of channels being sampled, this could mean that the bandwidth of the PGA has to be ten to several hundred times greater than the bandwidth of the analog signals. Such fast amplifiers are difficult to design, but in many cases the required speed can be achieved. The less obvious problem is that every amplifier introduces intrinsic noise, and the amount of noise observed on the amplifier output increases with bandwidth. Since the PGA may have several hundred times more bandwidth than the analog signal, it is likely to contribute more noise to the recording than is inherent in the signal. This problem cannot be eliminated or even reduced by filtering because filtering would lengthen the settling time of the PGA. This serious problem limits the usefulness of a shared PGA. This problem does not exist in the previously discussed systems in which there is an amplifier for each channel.

(2) If the PGA is located on the A/D board, the low-level signals must be brought by cables to the A/D board. Typically, these cables are a couple of meters or more in length and provide ample opportunity for pick up of hum, radio-frequency interference and cross-talk between signals. With careful attention to shielding and grounding, these undesirable effects can be minimized. In the alternative approaches, in which the signals are amplified before they are sent to the A/D converter, the relative impact of these undesirable interferences are reduced in proportion to the amount of early amplification.

Some of Axon Instruments' A/D systems include a PGA on the A/D board. This is useful when the signals require only a small amount of amplification (ten fold or less) or when the user cannot afford or has not invested in external amplifiers.

Pre-Filter vs. *Post-Filter Gain*

When low-level signals are recorded, it is essential that the first-stage amplification be sufficient to make the noise contributed by succeeding stages irrelevant. For example, in a microphone amplifier the tiny output from the microphone is first coupled into an extremely low-noise pre-amplifier. After this first-stage amplification, circuits with more modest noise characteristics are used for further amplification and to introduce treble and bass filtering.

If the only rule was to maximize the early gain, all of the gain would be implemented before any low-pass filter, notch filter or offset stages. However, with some signals, too much gain in front of the low-pass filter can introduce a different problem. This occurs when the signal is much smaller than the out-of-band noise.

This problem is illustrated by the signal in Figure 6-6. Panel ***A*** shows an input signal consisting of a pair of 1 pA current pulses significantly corrupted by instrumentation noise. When the noisy signal is first amplified by x100 and then low-pass filtered, the amplified noisy signal saturates the electronics and the noise is clipped. (Note that for clarity, these traces are not drawn to scale.) After low-pass filtering, the signal looks clean, but its amplitude is less than x100 the original signal because the low-pass filter extracts a signal that is the average of the non-symmetrical, clipped noise. When the noisy signal is first amplified by x10, then low-pass filtered before a further amplification by x10, saturation is avoided and the amplitude of the filtered signal is x100 the original input. Panel ***B*** shows the two outputs superimposed to illustrate the loss of magnitude in the signal that was amplified too much before it was low-pass filtered.

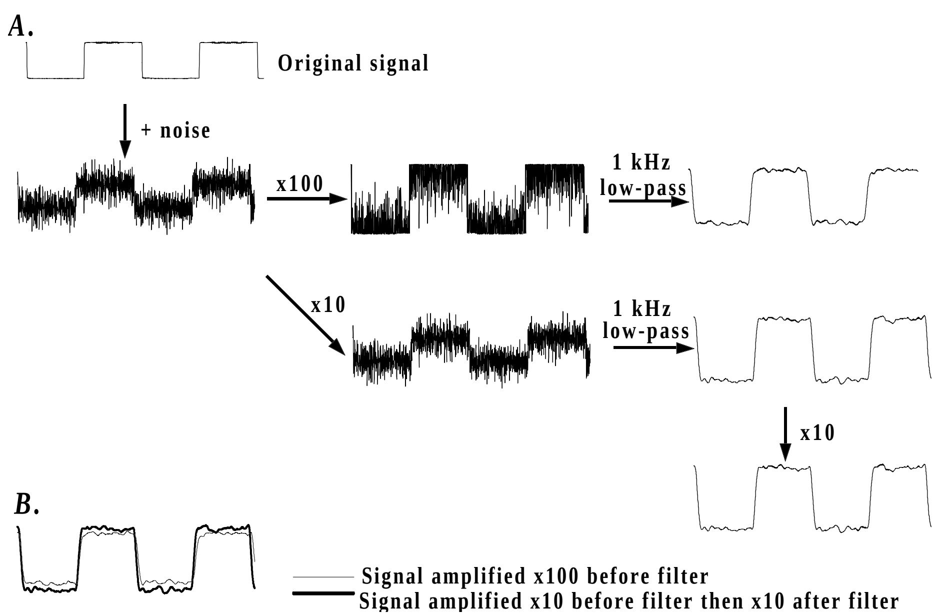

Figure 6-6. Distortion of Signal Caused by High Amplification Prior to Filtering

Offset Control

In some cases, it is necessary to add an offset to the signal before it is amplified. This is necessary if the gain required to amplify the signal of interest would amplify the DC offset of the signal to the point that it would cause the gain amplifier to saturate.

An example is provided by an electronic thermometer. A typical sensitivity of an electronic thermometer is 10 mV/°C, with zero volts corresponding to 0°C. A 12-bit A/D converter can measure with an approximate resolution of 5 mV, corresponding to 0.5 C in this example. If the data are to be analyzed at 0.01°C resolution, amplification by a factor of at least x50, and probably x100, would be necessary. If the temperatures of interest are between 30°C and 40°C and are amplified by x100, these temperatures will correspond to voltages between 30 V and 40 V — values well beyond the range of the A/D converter. The solution is to introduce an offset of -350 mV before any amplification, so that zero volts will correspond to 35°C. Now when the signal is amplified by x100, the 30 - 40°C temperature range will correspond to voltages between -5 V and +5 V.

AC Coupling and Autozeroing

AC coupling is used to continuously remove DC offsets from the input signal. Signals below the -3 dB frequency of the AC coupling circuit are rejected. Signals above this frequency are passed. For this reason, AC-coupling circuits are more formally known as high-pass filters. In most instruments with AC coupling, the AC-coupling circuit is a first-order filter. That is, the attenuation below the -3 dB frequency increases at 20 dB/decade.

When a signal is AC-coupled, the DC component of the signal is eliminated and the low-frequency content is filtered out. This causes significant distortion of the signal, as shown in Figure 6-7.

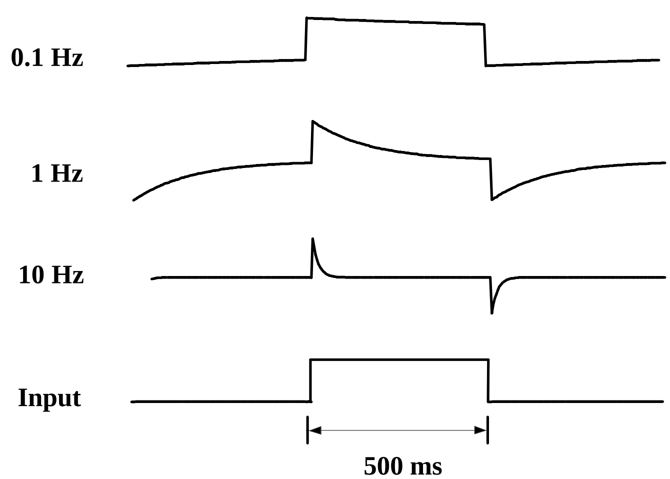

Figure 6-7. Distortion of Signal Caused by AC Coupling at High Frequencies

A 1 Hz square wave is AC-coupled at three different frequencies. The distortion is progressively reduced as the AC-coupling frequency is reduced from 10 Hz to 0.1 Hz. In all cases, the DC content of the signal is removed. The problem when using the lowest AC-coupling frequencies is that slow shifts in the baseline may not be rejected and transient shifts in the baseline might take a long time to recover.

Since there is less distortion when the AC-coupling frequency is lower, it is tempting to suggest that the AC coupling should always be set to very low frequencies, such as 0.1 Hz. However, this is often unacceptable because some shifts in the baseline are relatively rapid and need to be eliminated quickly.

A significant difference between using an AC-coupling circuit and setting a fixed DC offset is the way the two circuits handle ongoing drift in the signal. With fixed DC offset removal, the ongoing drift in the signal is recorded. With AC coupling, the drift is removed continuously. Whether this is an advantage or a disadvantage depends on whether the drift has meaning.

For very slow signals, even the lowest AC-coupling frequency causes significant distortion of the signal. For these signals, an alternative technique known as *autozeroing* can be used. This technique is available in some signal conditioners, such as the CyberAmp, as well as in some recording instruments, such as the Axopatch-1. In this technique, the signal is DC-coupled and a sample of the signal is taken during a baseline period. This DC sample value is stored and continuously subtracted by the instrument from the signal until the next sample is taken. In early instruments, the sample was taken using an analog sample-and-hold circuit. These circuits exhibit the problem known as "droop." That is, the sampled value drifts with time. Later systems, such as the ones used in the CyberAmp and the Axopatch-1D, use droop-free, digital sample-and-hold circuits.

The efficacy of the technique is illustrated in Figure 6-8.

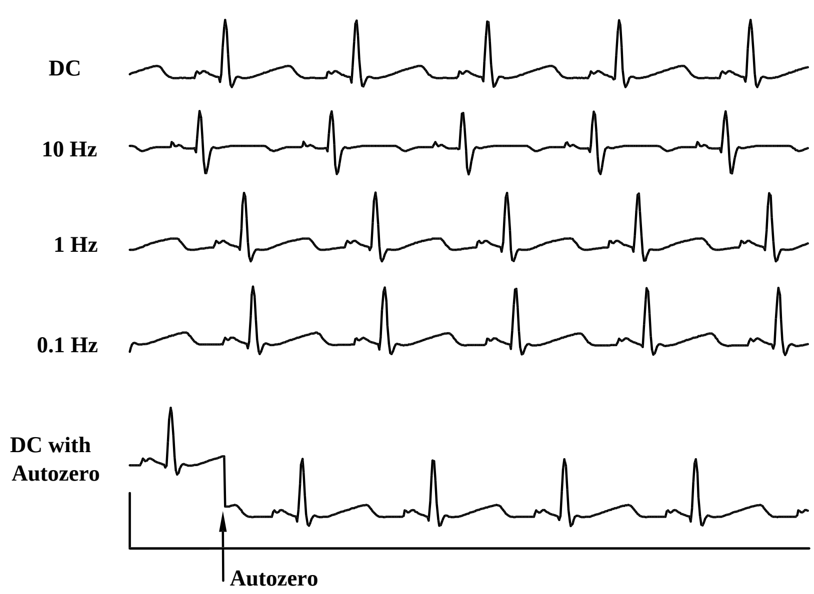

Figure 6-8. Comparison of Autozeroing to AC Coupling

The top trace shows an EKG signal that is sitting on a 5 mV offset resulting from electrode junction potentials. At AC-coupling frequencies of 10 Hz and 1 Hz, the signal is distorted. In the bottom trace, an Autozero command is issued to the signal conditioner (the CyberAmp 380) at the time indicated by the arrow. The DC component is immediately removed, but the transients are unaffected.

Autozeroing should be restricted to cases where the time of occurrence of signals is known, so that the Autozero command can be issued during the baseline recording period preceding the signal.

Time Constant

The AC-coupling frequency is related to the time constant of decay, τ (see Figure 6-7):

$$\tau = \frac{1}{2\pi f_{-3}} \tag{6}$$

The time constants for some common AC-coupling frequencies are:

f_{-3} (Hz)	τ (ms)
100.0	1.6
30.0	5.3
10.0	16.0
3.0	53.0
1.0	160.0
0.1	1,600.0

In one time constant, the signal decays to approximately 37% of its initial value. It takes approximately 2.3 time constants for the signal to decay to 10% of the initial value.

Saturation

The AC-coupling circuit is the first circuit in most signal conditioning pathways. If a large step is applied to the AC-coupled inputs, the AC coupling rejects the step voltage with a time constant determined by the AC-coupling frequency. If the amplifiers are set for high gain, the output might be saturated for a considerable time. For example, if the gain is x100, the AC coupling is 1 Hz and the step amplitude is 1 V, the output will be saturated until the voltage at the output of the AC-coupling circuit falls to 100 mV from its initial peak of 1 V. This will take about 2.3 time constants. Since the time constant is 160 ms, the output will be saturated for at least 370 ms. For the next several time constants, the output will settle towards zero.

Overload Detection

An amplified signal may exceed the ±10 V acceptable operating range inside the instrument in two places: at the input of the various filters and at the output of the final amplifier stage. An example of an overload condition at the input of an internal low-pass filter is shown in Figure 6-6.

It is common practice to place overload-detection circuitry inside the instrument at these points. The overload-detection circuitry is activated whenever the signal exceeds an upper or lower threshold such as ±11 V. The limit of ±11 V exceeds the usual ±10 V recommended operating range in order to provide some headroom. There is no difficulty in providing this headroom since the internal amplifiers in most instruments operate linearly for signal levels up to about ±12 V.

Normally, the overload circuitry simply indicates the overload condition by flashing a light on the front panel. In more sophisticated instruments such as the CyberAmp, the host computer can interrogate the CyberAmp to determine if an overload has occurred.

Averaging

Averaging is a way to increase the signal-to-noise ratio in those cases where the frequency spectrum of the noise and the signal overlap. In these cases, conventional filtering does not help because if the -3 dB frequency of the filter is set to reject the noise, it also rejects the signal.

Averaging is applicable only to repetitive signals, when many sweeps of data are collected along with precise timing information to keep track of the exact moment that the signal commences or crosses a threshold. All of these sweeps are summed, then divided by the total number of sweeps (N) to form the average. Before the final division, the amplitude of the signal in the accumulated total will have increased by N. Because the noise in each sweep is uncorrelated with the noise in any of the other sweeps, the amplitude of the noise in the accumulated signal will only have increased by $\sqrt{N}$. After the division, the signal will have a magnitude of unity, whereas the noise will have a magnitude of $1/\sqrt{N}$. Thus, the signal-to-noise ratio increases by $\sqrt{N}$.

Line-Frequency Pick-Up (Hum)

An important consideration when measuring small biological signals is to minimize the amount of line-frequency pickup, often referred to as hum. Procedures to achieve this goal by minimizing the hum at its source are discussed in **Chapter 2**.

Hum can be further minimized by using a notch filter, as discussed above, or by differential amplification. To implement the latter technique, the input amplifier of the data acquisition system is configured as a differential amplifier. The signal from the measurement instrument is connected to the positive input of the differential amplifier, while the ground from the measurement instrument is connected to the negative input. If, as is often the case, the hum signal has corrupted the ground and the signal equally, the hum signal will be eliminated by the differential measurement.

Peak-to-Peak and rms Noise Measurements

Noise is a crucially important parameter in instruments designed for measuring low-level signals.

Invariably, engineers quote noise specifications as root-mean-square (rms) values, whereas users measure noise as peak-to-peak (p-p) values. Users' preference for peak-to-peak values arises from the fact that this corresponds directly to what they see on the oscilloscope screen or data acquisition monitor.

Engineers prefer to quote rms values because these can be measured consistently. The rms is a parameter that can be evaluated easily. In statistical terms, it is the standard deviation of the noise. True rms meters and measurement software are commonly available and the values measured are completely consistent.

On the other hand, peak-to-peak measurements are poorly defined and no instruments or measurement software are available for their determination. Depending on the interpreter, estimates of the peak-to-peak value of Gaussian noise range from four to eight times the rms value. This is because some observers focus on the "extremes" of the noise excursions (hence, the x8 factor), while others focus on the "reasonable" excursions (x6 factor) or the "bulk" of the noise (x4 factor).

Axon Instruments has developed software to measure the rms and the peak-to-peak noise simultaneously. The peak-to-peak noise is calculated as the threshold level that would encompass a certain percentage of all of the acquired data. For white noise, the corresponding values are:

Percentage of Data Encompassed	Peak-To-Peak Thresholds
95.0%	3.5 - 4 times rms value
99.0%	5 - 6 times rms value
99.9%	7 - 8 times rms value

These empirical measurements can be confirmed by analysis of the Gaussian probability distribution function.

In this *Guide* and various Axon Instruments product specifications, noise measurements are usually quoted in both rms and peak-to-peak values. Since there is no commonly accepted definition of peak-to-peak values, Axon Instruments usually uses a factor of about 6 to calculate the peak-to-peak values from the measured rms values.

In an article from Bell Telephone Laboratories (1970), the authors define the peak factor as "the ratio of the value exceeded by the noise a certain percentage of the time to the rms noise value. This percentage of time is commonly chosen to be 0.01 per cent." This ratio, from tables listing the area under the Gaussian distribution, turns out to be 7.78 times the rms value. Thus, according to this article, the appropriate factor to calculate the peak-to-peak values from the measured rms values is closer to x8.

Blanking

In certain experiments, relatively huge transients are superimposed on the signal and corrupt the recording. This problem commonly occurs during extracellular recording of nerve impulses evoked by a high-voltage stimulator. If the isolation of the stimulator is not perfect (it never is), there is some coupling of the stimulus into the micropipette input. This relatively large artifact can sometimes cause the coupling capacitors in subsequent AC amplifiers to saturate. There might be some time lost while these capacitors recover from saturation, and thus valuable data might be wasted.

If it is not possible to prevent the stimulus coupling, the next best thing to do is to suppress the artifact before it feeds into the AC-coupled amplifiers. This is made possible in the Axoclamp, Axoprobe and Axopatch 200 amplifiers by providing sample-and-hold amplifiers early in the signal pathway. The user provides a logic-level pulse that encompasses the period of the transient. This logic-level pulse forces the sample-and-hold amplifiers into the "hold" mode. In this mode, signals at the input of the sample-and-hold amplifier are ignored. Instead, the output of the sample-and-hold amplifier is kept equal to the signal that existed at the moment the logic-level pulse was applied.

Audio Monitor — Friend or Foe?

When monitoring data, experimenters need not be limited to their sense of sight. The data may also be monitored with great sensitivity using the sense of hearing. In such cases, the data are input to an audio monitor and fed to a loudspeaker or a set of headphones.

Two types of audio monitor are used. The first is a power amplifier that applies the signal directly to the speaker, thereby allowing signals in the audio bandwidth to be heard. This type of audio monitor is frequently used in EMG monitoring or in central nervous system recording. Each spike is heard as an audible click, and the rate and volume of clicking is a good indicator of the muscle or nerve activity. This type of audio monitor is either called an AM (amplitude modulated) audio monitor, or it is said to be operating in "click" mode.

The second type of audio monitor is a tone generator whose frequency depends on the amplitude of the input signal. Usually, the oscillator frequency increases with increasingly positive signals. This type of audio monitor is often used for intracellular recording. It provides an extremely sensitive measure of the DC level in the cell. This type of audio monitor is either called an FM (frequency modulated) audio monitor, or it is said to be operating in "tone" mode.

Most researchers have a very strong opinion about audio monitors; they either love them or hate them. Those who love audio monitors appreciate the supplementary "view" of the data. Those who hate audio monitors have probably suffered the annoyance of having to listen for interminable lengths of time to the audio output from another rig in the same room. A constant tone can be irritating to listen to, especially if it is high pitched, unless it has an important message for you, such as "my cell is maintaining its potential admirably."

Audio monitors are standard features in most of Axon Instruments' equipment. To minimize the potential for aggravation, we include a headphone jack so that users can listen to the audio output without testing the patience of their colleagues.

Electrode Test

It is useful to be able to measure the electrode resistance for two reasons. The first reason is to establish the basic continuity of the electrode circuit. Sometimes, electrode leads can break, leaving an open-circuit input and, consequently, no incoming data. The second reason is to verify that the electrode is acceptably attached. For example, it may be that to achieve the low-noise recording levels needed in an EMG recording, the electrode resistance must be less than 5 kΩ.

Some transducer amplifiers allow the electrode impedance to be easily measured. For example, the CyberAmp amplifiers have an Electrode Test facility. When this is activated, an approximate 1 $\mu A_{p\text{-}p}$, 10 Hz square wave is connected to every input via individual 1 MΩ resistors. The electrode resistance can be directly determined from the amplitude of the voltage response.

Common-Mode Rejection Ratio

In general, the information that the researcher wants to record is the difference between two signals connected to the positive and the negative amplifier inputs. Often, these signals contain an additional component that is common to both, but that does not contain relevant information. For example, both outputs of a strain gauge might include a DC potential of 2.5 V that arises from the excitation voltage. However, the strain on the gauge generates a microvolt-size *difference* between the two outputs, but does not affect the 2.5 V "common-mode" voltage. Another example is often seen when recording EMG signals from an animal. Both the positive and the negative electrodes pick up line-frequency hum that has coupled into the animal. The hum picked up by the electrodes may be as large as 10 mV, but it is identical on both electrodes. The EMG signal of interest is the small difference between the potentials on the two electrodes; it may be as small as a few tens of microvolts.

To prevent the common-mode signal from swamping the much smaller differential signal, the positive and negative gains of the amplifier must be nearly identical. In the above EMG example, if the amplifier inputs are exactly unity, the 10 mV of hum that appears equally on both electrodes does not show up at all on the amplifier output. The only signal to appear on the output is the small signal proportional to the EMG potential generated between the two electrodes.

In practice, the positive and negative inputs of the amplifier are never exactly equal. The quality of their matching is measured by the common-mode rejection ratio (CMRR). This is normally quoted in dB, where 20 dB corresponds to a factor of ten. Returning to the EMG example, if the amplifier operates at unity gain with a CMRR of 60 dB (*i.e.*, one part in a thousand), the 10 mV of common-mode hum results in 10 µV of hum appearing on the amplifier output. This is small, but still significant compared with the smallest EMG signals, so an amplifier with higher CMRR, *e.g.*, 80 dB, may be desirable.

The CMRR of an amplifier varies with frequency. It is best at very low frequencies, while above a certain frequency it diminishes steadily as the frequency of the common-mode signal increases. It is therefore important to verify the CMRR of the amplifier at a frequency that exceeds the expected frequency of the common-mode signal.

The CMRR of the recording system is adversely affected by imbalances in the source resistances of the recording electrodes. This is because the source resistance of each electrode forms a voltage divider with the input resistance of the amplifier. If the source resistances of the two electrodes are not identical, the voltage dividers at the positive and negative inputs of the amplifier are not equal. Returning to the EMG example, if the resistance of one electrode is 9 kΩ, the resistance of the other is 10 kΩ, and the amplifier input resistances total 1 MΩ, then the gain for one electrode is 0.9901 instead of unity, while the gain for the other electrode is 0.9911. The difference is 0.001. Thus, even though the amplifier may have a CMRR of 80 dB or more, the system CMRR is only 60 dB. In some cases, 60 dB is acceptable, but in others it is not. The solution to this problem is to use an amplifier that has very high input resistances of 100 MΩ or more.

If there is a large common-mode signal and a source imbalance of more than a few kilohms, a high input resistance amplifier probe should be used. Several AI 400 series probes are available from Axon Instruments that have input resistances of 10 gigohms (10^{10} Ω) or more. These probes are distinguished on the basis of noise, cost and size.

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Further Reading

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