Neuronal Signals - NBDS 5161
Session 5: Data Acquisition & Analysis

Abdallah HAYAR

**Lectures can be downloaded from
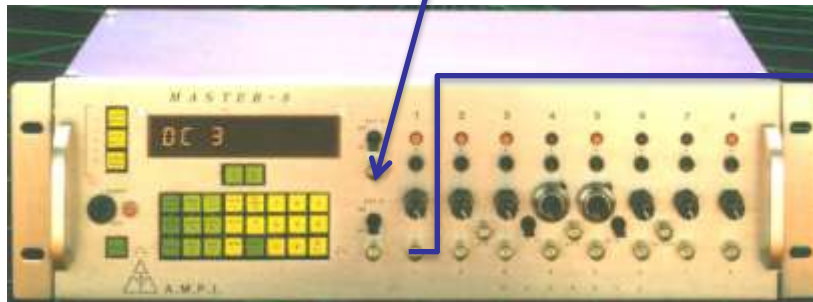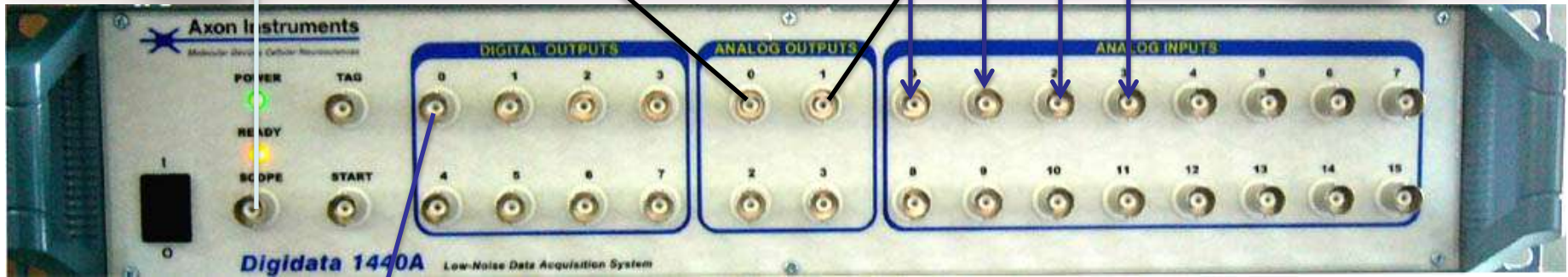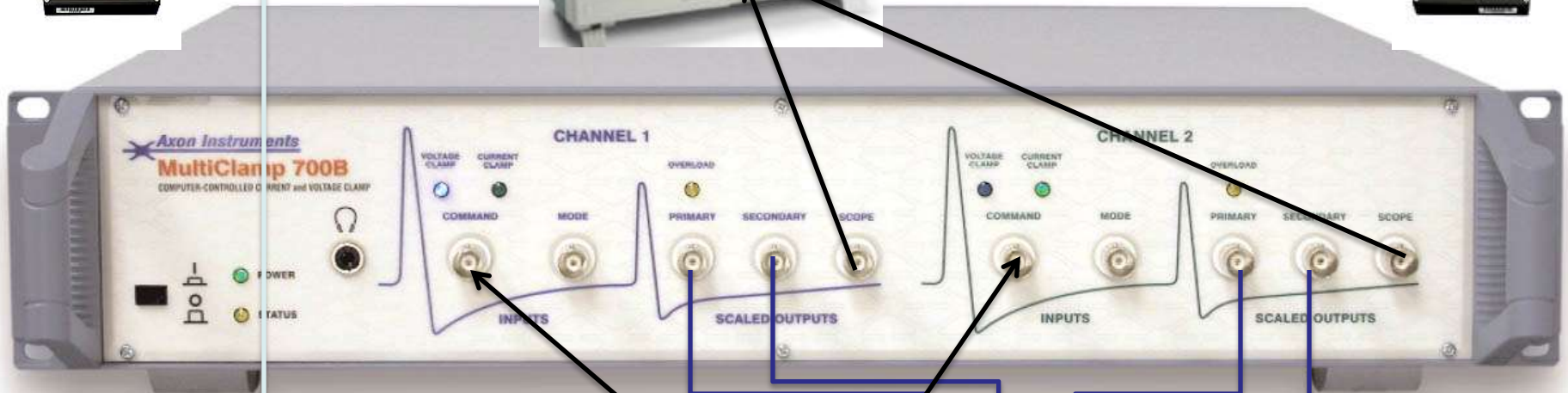http://hayar.net/NBDS5161**

Updated Tentative Schedule for Neuronal Signals (NBDS 5161)
One Credit–Hour, Summer 2010
Location: Biomedical Research Building II, 6th floor, conference room,
Time: 9:00 -10:20 am

Session	Day	Date	Topic	Instructor
1	Tue	6/1	Design of an electrophysiology setup	Hayar
2	Thu	6/3	Neural population recordings	Hayar
3	Thu	6/10	Single cell recordings	Hayar
4	Fri	6/11	Analyzing synaptic activity	Hayar
5	Mon	6/14	Data acquisition and analysis	Hayar
6	Wed	6/16	Analyzing and plotting data using OriginLab	Hayar
7	Fri	6/18	Detecting electrophysiological events	Hayar
8	Mon	6/21	Writing algorithms in OriginLab®	Hayar
9	Wed	6/23	Imaging neuronal activity	Hayar
10	Fri	6/25	Laboratory demonstration of an electrophysiology and imaging experiment	Hayar
11	Fri	7/9	Article presentation I: Electrophysiology	Hayar
12	Mon	7/12	Article presentation II: Imaging	Hayar
13	Wed	7/14	Exam and students' survey about the course	Hayar

Student List

|	Name	E-mail	Regular/Auditor	Department	Position
1	Simon, Christen	CSimon@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
2	Kezunovic, Nebojsa	NKezunovic@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
3	Hyde, James R	JRHyde@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
4	Yadlapalli, Krishnapraveen	KYadlapalli@uams.edu	Regular (form signed)	Pediatrics	Research Technologist – Mentor: Dr. Alchaer
5	Pathan, Asif	APATHAN@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Pharmacology – Mentor: Dr. Rusch
6	Kharade, Sujay	SKHARADE@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Pharmacology – 4th year - Mentor: Dr. Rusch
7	Howell, Matthew	MHOWELL2@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Interdisciplinary Toxicology - 3rd year - Mentor: Dr. Gottschall
8	Beck, Paige B	PBBeck@uams.edu	Regular (form signed)	College of Medicine	Medical Student – 2nd Year - Mentor: Dr. Garcia-Rill
9	Atcherson, Samuel R	SRAtcherson@uams.edu	Auditor (form signed)	Audiology & Speech Pathology	Assistant Professor
10	Detweiler, Neil D	NDDETWEILER@uams.edu	Auditor (form not signed)	Pharmacology & Toxicology	Graduate Pharmacology – 1st year
11	Thakali, Keshari M	KMThakali@uams.edu	Unofficial auditor	Pharmacology & Toxicology	Postdoctoral Fellow – Mentor: Dr. Rusch
12	Boursoulian, Feras	FBoursoulian@uams.edu	Unofficial auditor	Neurobiology & Developmental Sciences	Postdoctoral Fellow – Mentor: Dr. Hayar
13	Steele, James S	JSSTEELE@uams.edu	Unofficial auditor	College of Medicine	Medical Student – 1st Year – Mentor: Dr. Hayar
14	Smith, Kristen M	KMSmith2@uams.edu	Unofficial auditor	Neurobiology & Developmental Sciences	Research Technologist – Mentor: Dr. Garcia-Rill
15	Gruenwald, Konstantin	kjoachimg@gmail.com	Unofficial auditor	Neurobiology & Developmental Sciences	High school Student – Mentor: Dr. Hayar
16	Rhee, Sung	RheeSung@uams.edu	Unofficial auditor	Pharmacology & Toxicology	Assistant Professor
17	Light, Kim E	LightKimE@uams.edu	Unofficial auditor	Pharmaceutical Sciences	Professor

Basic Connections

The binary system

The binary numeral system, or base-2 number system, represents numeric values using two symbols, 0 and 1.

Owing to its straightforward implementation in digital electronic circuitry using logic gates, the binary system is used internally by all modern computers.

$2^0 = 1$, Remember $X^0 = 1$, ex: $10^0 = 1$

$2^1 = 2$, Remember $X^1 = X$

$2^2 = 2\text{x}2 = 4$

$2^3 = 2\text{x}2\text{x}2 = 8$

$2^4 = 2\text{x}2\text{x}2\text{x}2 = 16$

$2^5 = 2\text{x}2\text{x}2\text{x}2\text{x}2 = 32$

$2^6 = 2\text{x}2\text{x}2\text{x}2\text{x}2\text{x}2 = 64$

$2^7 = 2\text{x}2\text{x}2\text{x}2\text{x}2\text{x}2\text{x}2 = 128$

$2^8 = 2\text{x}2\text{x}2\text{x}2\text{x}2\text{x}2\text{x}2\text{x}2 = 256 = 1$ byte

$2^{10} = 2\text{x}2\text{x}2\text{x}2\text{x}2\text{x}2\text{x}2\text{x}2\text{x}2\text{x}2 = 1024 = 1$ Kbyte

$2^{16} = 65536$

Decimal	Hexa-decimal	Binary
0	0	0000
1	1	0001
2	2	0010
3	3	0011
4	4	0100
5	5	0101
6	6	0110
7	7	0111
8	8	1000
9	9	1001
10	A	1010
11	B	1011
12	C	1100
13	D	1101
14	E	1110
15	F	1111

Binary	1	0	0	1	0	1	0	1	1	0	1	
Decimal	$1\times2^{10} +$	$0\times2^9 +$	$0\times2^8 +$	$1\times2^7 +$	$0\times2^6 +$	$1\times2^5 +$	$0\times2^4 +$	$1\times2^3 +$	$1\times2^2 +$	$0\times2^1 +$	$1\times2^0 =$	1197

Decimal	1	1	9	7

Decimal	$1\times10^3 +$	$1\times10^2 +$	$9\times10^1 +$	$7\times10^0 +$	1197

Fundamental of data conversion
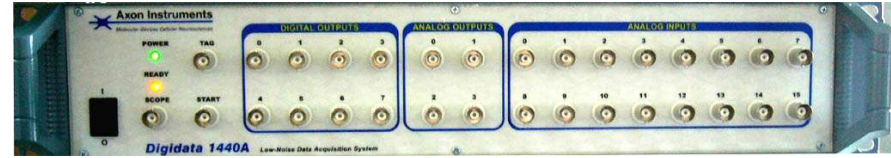
An analog-to-digital converter (abbreviated ADC, A/D or A to D) is a device which converts continuous signals to discrete digital numbers. The reverse operation is performed by a digital-to-analog converter (DAC).

In electrophysiological experiments, the data are most often in the form of voltage waveforms whose magnitudes vary with time.
Analog-to-digital conversion must be undertaken to convert the analog data into a compatible format for the computer.

Analog Temperature Graph

Digital Temperature Graph
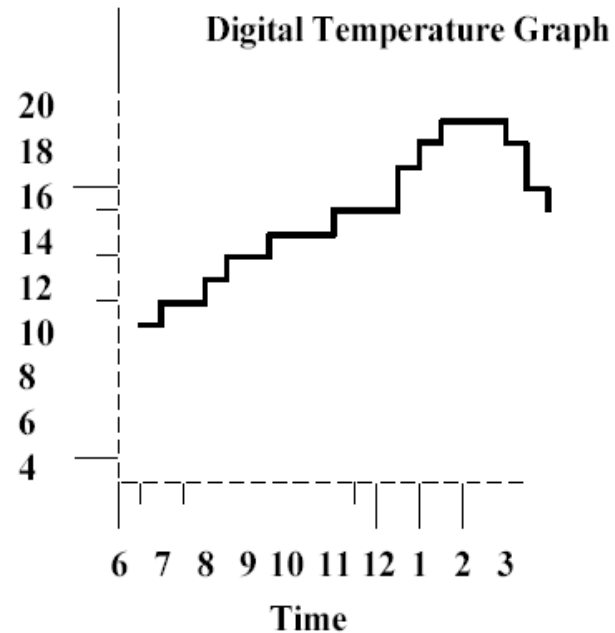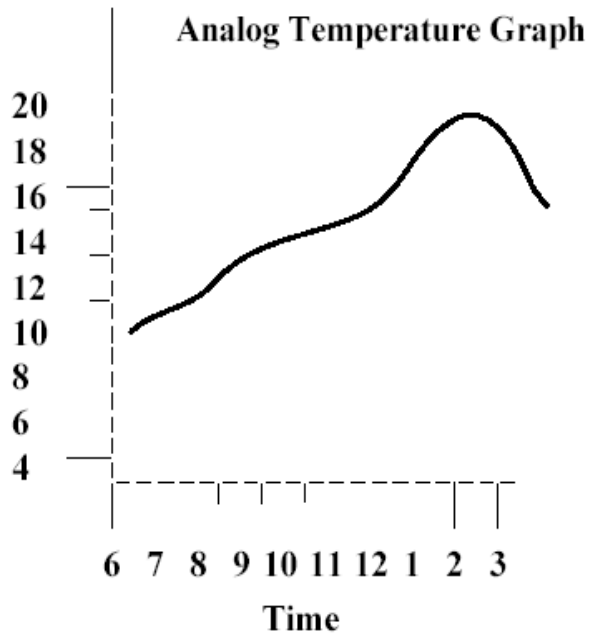
Time	T °C
6:00 AM	10
6:30 AM	11
7:00 AM	11
7:30 AM	12
8:00 AM	13
8:30 AM	13
9:00 AM	14
9:30 AM	14
10:00 AM	14
10:30 AM	15
11:00 AM	15
11:30 AM	15
12:00 PM	17
12:30 PM	18
1:00 PM	19
1:30 PM	19

Acquisition resolution

In an ADC, the total measurement range (e.g., 0–100 °C) is divided into a fixed number of possible values. The number of values is a power of two, often referred to as the number of "bits." Commonly, these values are:

8 bit = 2^8 = 256 values
12 bit = 2^{12} = 4,096 values
16 bit = 2^{16} = 65,536 values

To illustrate the impact on the resolution of using an 8-bit, 12-bit or 16-bit ADC, consider the temperature-measurement example where the electronic thermometer circuit generates an analog output from -10 V to +10 V for temperatures in the range -100 °C to +100 °C. In this case, the resolutions are:

8 bit → 78.4 mV = 0.784 °C
12 bit → 4.88 mV = 0.0488 °C
16 bit → 20 volts/ 2^{16} = 20/65536 = 0.305 mV = 0.00305 °C

Temporal Resolution or Sampling Rate

The sampling (Nyquist) theorem states that data should be sampled at a frequency at least equal to twice the highest frequency component in the signal in order to prevent an artifactual increase in the noise, due to a phenomenon known as aliasing.

Sampling is typically performed at 2.5 times the filter bandwidth. For example, if the data are filtered at 2 kHz they should be sampled at about 5 kHz. Slower sampling rates are unacceptable. Faster sampling rates are acceptable, but offer little advantage and increase the storage and analysis requirements.

If the resolution is too low, the reconstructed signal or image will differ from the original one, and an alias is seen.

Adequately Sampled Signal

Aliased Signal Due to Undersampling
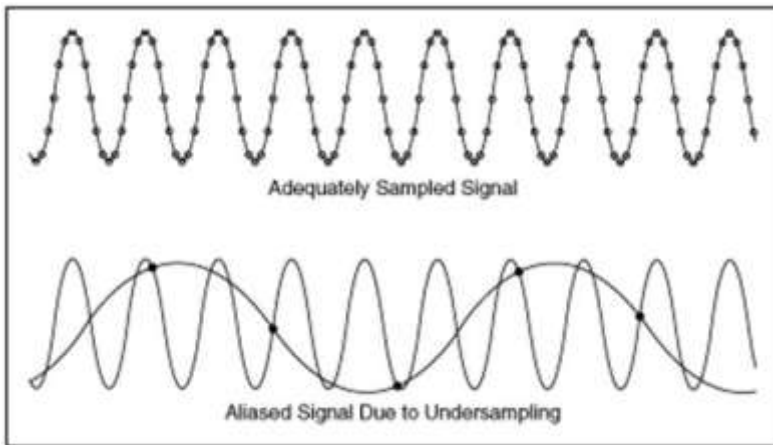
* The Nyquist sampling theorem requires that for accurate signal reconstruction, a signal must be sampled at a rate greater than 2 times the bandwidth of the signal. Sampling at 1x the sine wave frequency yields no information;
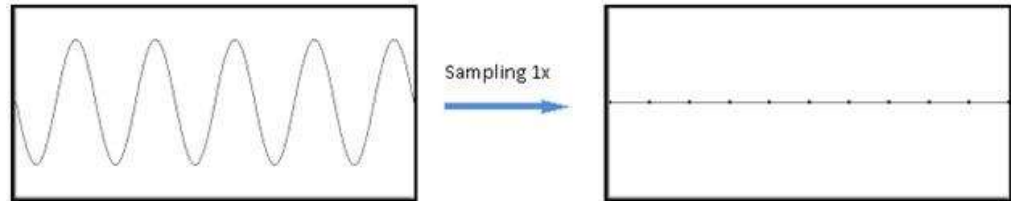
* Sampling at twice the signal bandwidth only preserves frequency information – amplitude and shape will not be preserved.
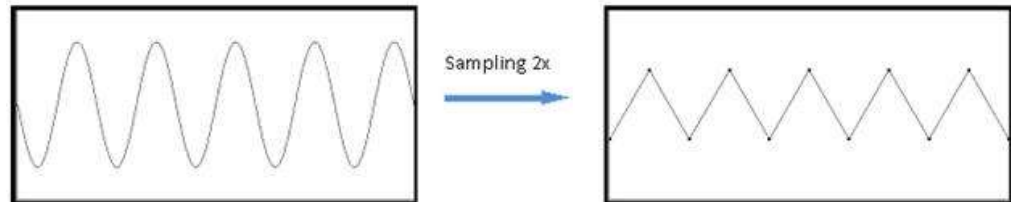
* For time-domain measurements, sampling at a rate at least 10x faster than the signal bandwidth accurately captures the frequency, amplitude and shape of the signal.

Sampling 1x

Sampling 2x

Sampling 10x
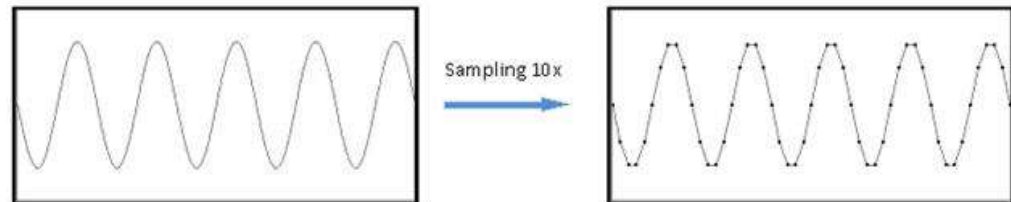
Data File Format

Axon Binary File (*.abf)
Data files are stored in Axon Binary File format. There are two types of ABF files – integer and floating point – which are used for acquired (raw) data and analyzed data respectively.

Axon Binary File (integer): The data in recorded ABF files are stored as 16-bit integers with a proprietary header to accommodate all the features of Axon data acquisition programs.
(00000000 00000000 to 11111111 11111111) = 0 to 65535 = 2^{16} values
16 bit $\rightarrow$ ± 10 volts = 20 volts/ 2^{16} = 20/65536 = 0.305 mV
00000000 00000001 = 0.305 mV
00000000 00000010 = 2 x 0.305 mV
00000000 00000011 = 3 x 0.305 mV

Axon Binary File (floating point): The data in analyzed ABF files are stored as 32-bit floating point values, so as to not lose data resolution if you have processed the data.

In computing, floating point describes a system for representing numbers that would be too large or too small to be represented as integers. Numbers are in general represented approximately to a fixed number of significant digits and scaled using an exponent. The base for the scaling is normally 2, 10 or 16. The typical number that can be represented exactly is of the form:

   significant digits × base exponent

The term floating point refers to the fact that the radix point (decimal point, or, more commonly in computers, binary point) can "float"; that is, it can be placed anywhere relative to the significant digits of the number. Example 0.000012345678 is represented as12345678 E-12

Axon Text File (ATF format)

ATF is a tab-delimited ASCII text format that can be read by typical spreadsheet programs such as Microsoft Excel. Thus, ATF files are easily imported into spreadsheet, scientific analysis, and graphics programs, and can also be edited by word processor and text editor programs.

An ATF text file consists of **records**. The group of records at the beginning of the file is called the file header. Each line in the text file is a record. Each record may consist of several **fields**, separated by a field separator (column delimiter). The tab and comma characters are field separators. Space characters around a tab or comma are ignored and considered part of the field separator.

The file header describes the file structure and includes column titles, units, and comments. Text strings are enclosed in quotation marks to ensure that any embedded spaces, commas and tabs are not mistaken for field separators.

Note: Data stored in a text format occupies much more disk space than data stored in a binary format

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.

Provide frequency selectivity

Phase shaping or equalization
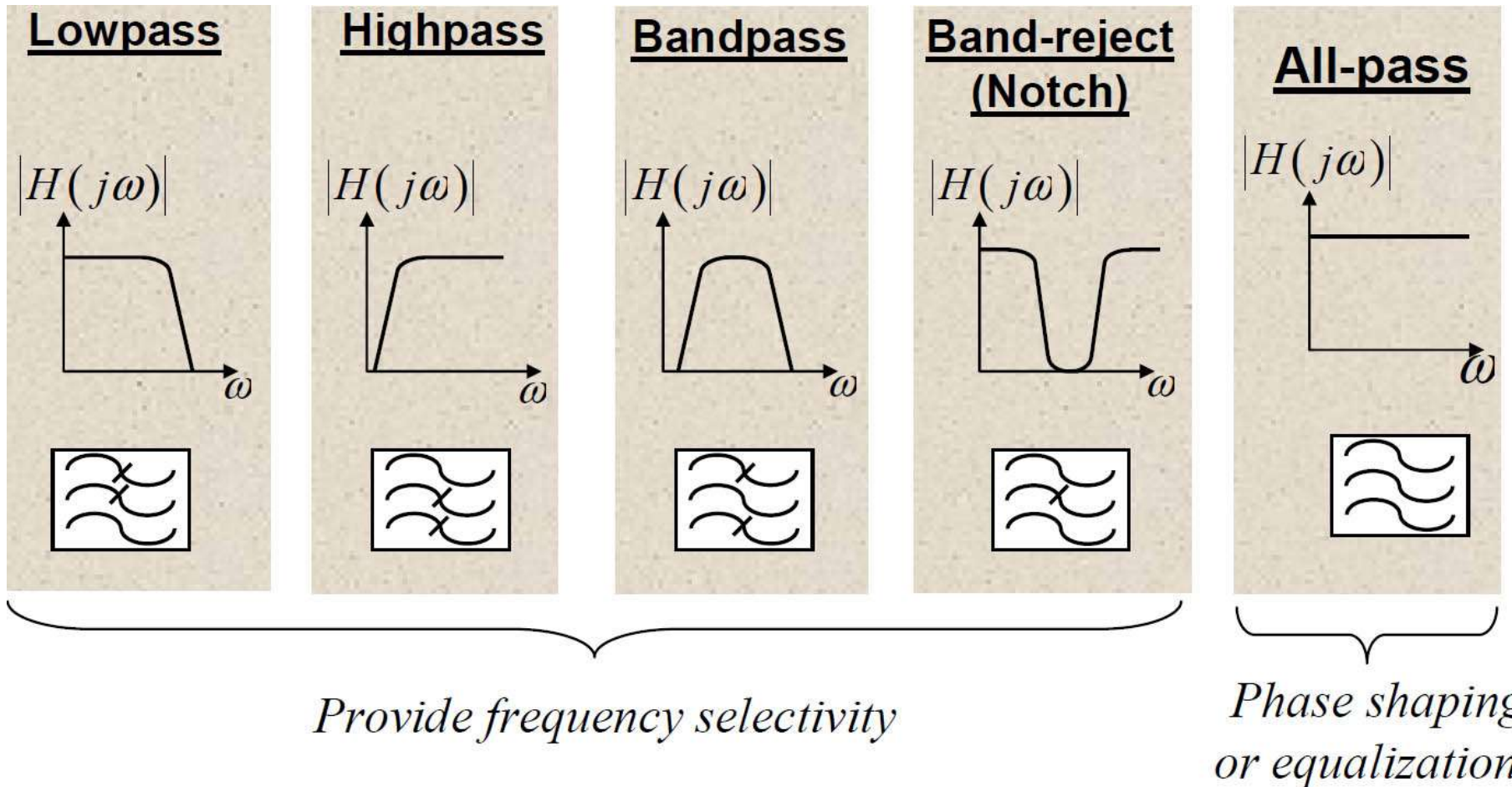
Filter terminology
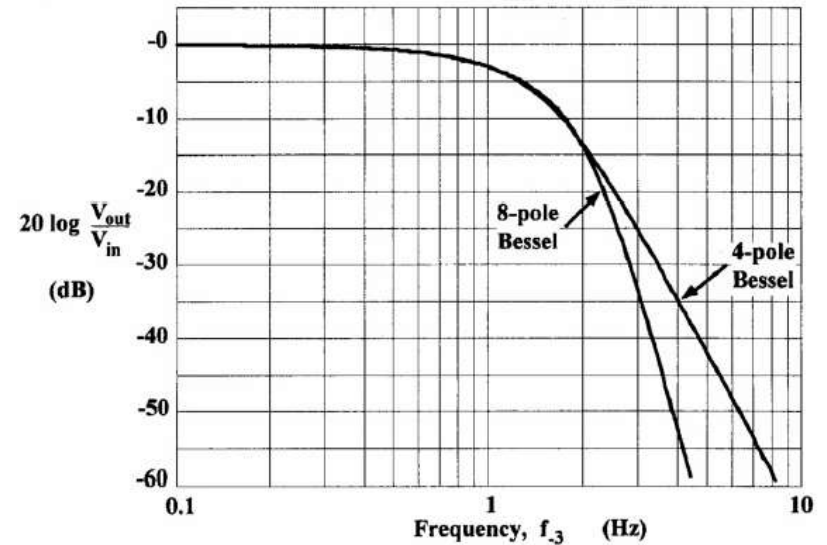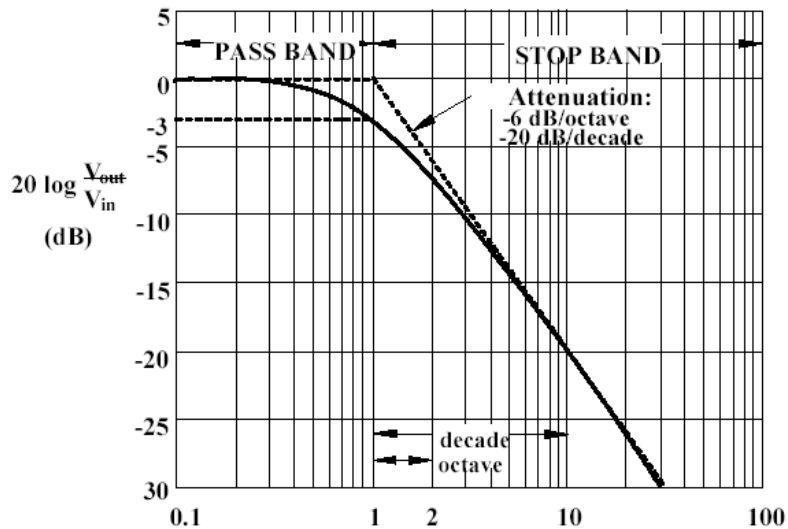
The -3 dB frequency (f-3, cutoff or the corner frequency) is the frequency at which the signal voltage at the output of the filter falls to .1/2, *i.e., 0.7071, of the amplitude of the input signal. Equivalently, f-3 is the* frequency at which the signal power at the output of the filter falls to half of the power of the input signal.

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

$$B = \log_{10} \frac{P_2}{P_1} \qquad dB = 10 \log_{10} \frac{P_2}{P_1} \qquad 10 \log_{10} \frac{1}{2} = -3.01\, dB \approx -3\, dB$$

The **decibel** is defined as:
1 bel = 10 decibels (dB)

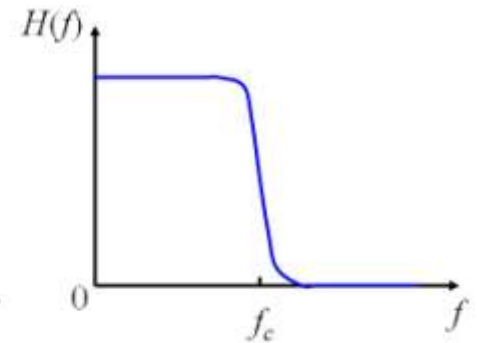A common dB term is the **half power point** which is the dB value when the P_2 is one-half P_1

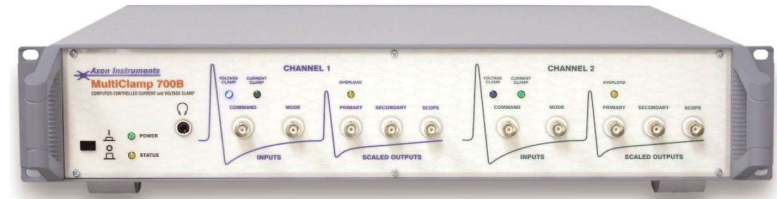# Comparison of Various Type Low-Pass Filter Magnitude Response

Ideal "brick wall" filter

Practical filter

Bessel

Butterworth

Chebyshev I

Chebyshev II

Elliptic

Bessel Filter

This is the analog filter used for most signals for which minimum distortion in the time domain is required. The Bessel filter does not provide as sharp a roll-off as the Butterworth filter, but it is well behaved at sharp transitions in the signal, such as might occur at capacitance transients or single-channel current steps.

Butterworth Filter

This is the filter of choice when analyzing signals in the frequency domain, *e.g. when making power spectra for noise analysis. The Butterworth filter has a sharp, smooth roll-off in the frequency domain, but introduces an overshoot and "ringing" appearance to step signals in the time domain.*

High-pass Filter

The Primary Output and Scope signals can be high-pass filtered by setting the AC value in the Output Gains and Filters section of the main MultiClamp 700B Commander panel. This is typically done in order to remove a DC component of the signal. When the filter cutoff is set to DC this high-pass filter is bypassed.

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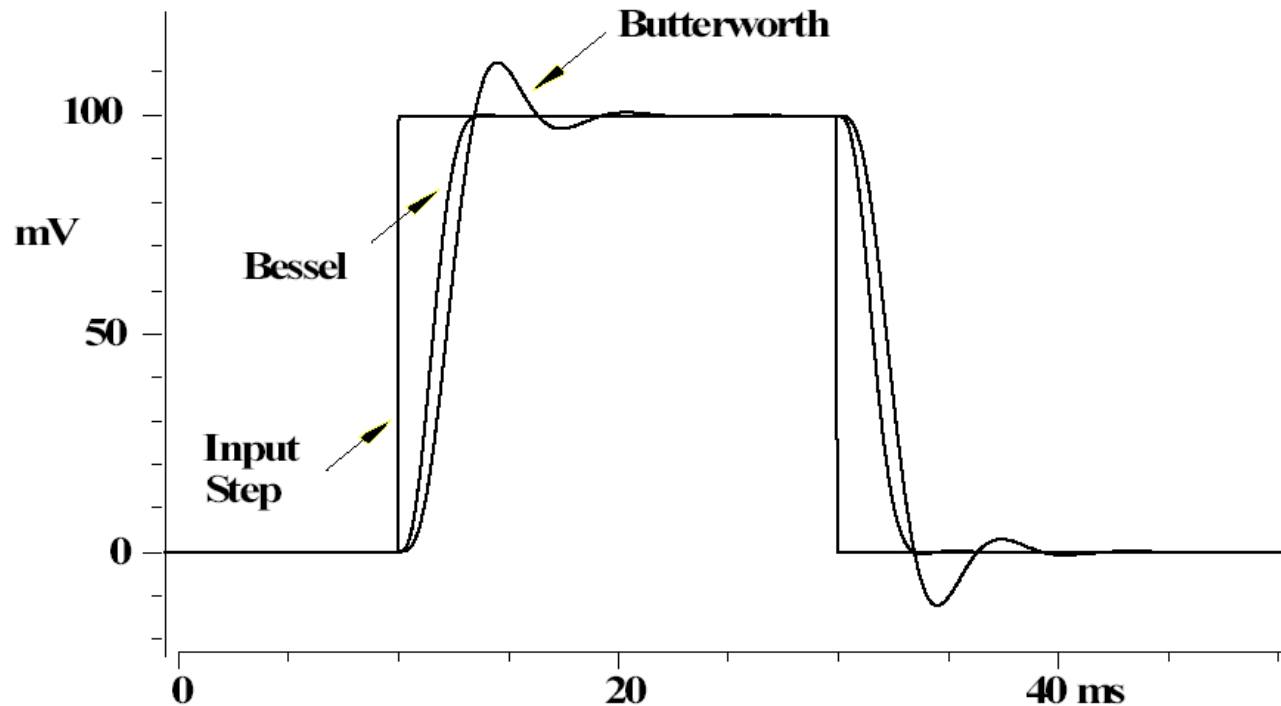
In general, the best filters to use for time-domain analysis are Bessel filters because they add less than 1% overshoot to pulses. The Bessel filter is sometimes called a "linear-phase" or "constant delay" filter. All filters alter the phase of sinusoidal components of the signal.
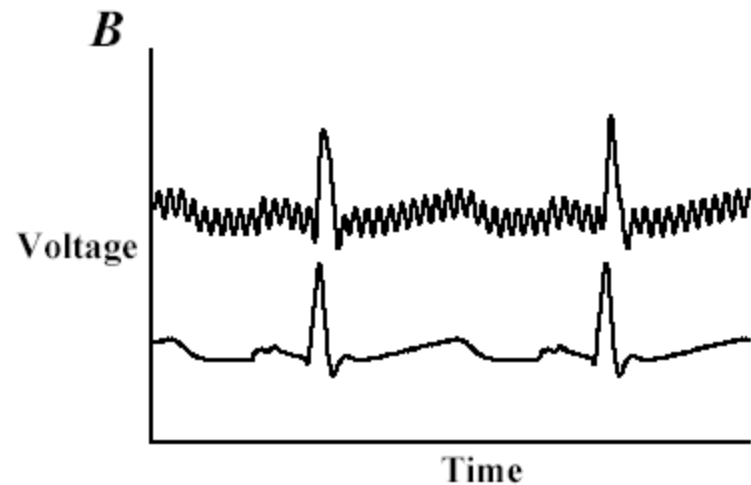In a Bessel filter, the change of phase with respect to frequency is linear. Put differently, the amount of signal delay is constant in the pass band. This means that pulses are minimally distorted. Butterworth filters add considerable overshoot: 10.8% for a fourth-order filter; 16.3% for an eighth-order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

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.
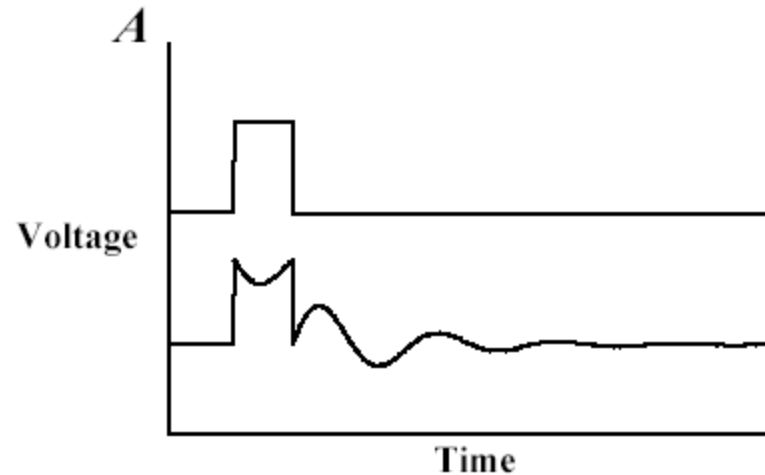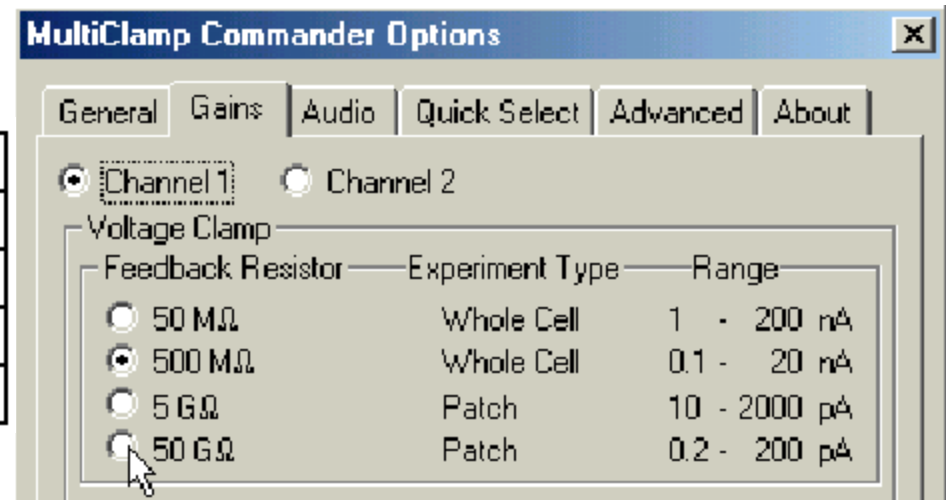
B. Shows an appropriate use of the notch filter. An EKG signal is corrupted by a large 60 Hz component that is completely eliminated by the notch filter.

Changing Headstage Gain

Feedback Resistor	Noise*
50 MΩ	2.0 pA rms
500 MΩ	0.8 pA rms
5 GΩ	0.5 pA rms
50 GΩ	0.15 pA rms

MultiClamp Commander Options

General | Gains | Audio | Quick Select | Advanced | About

⦿ Channel 1   ○ Channel 2

Voltage Clamp

| Feedback Resistor	Experiment Type	Range
○ 50 MΩ	Whole Cell	1 - 200 nA
⦿ 500 MΩ	Whole Cell	0.1 - 20 nA
○ 5 GΩ	Patch	10 - 2000 pA
○ 50 GΩ	Patch	0.2 - 200 pA

Increasing the size of the feedback resistor, which is located in the headstage, increases the gain of the headstage. As a rule of thumb, the larger the value of the feedback resistor, the smaller the noise of the headstage but the smaller the range of the output. For this reason, *larger feedback resistors are usually* selected for patch recording, where low noise is more important than range.

Current Clamp: Electrode Resistance Neutralization: Bridge Balance

The flow of current through the microelectrode produces a voltage drop across the electrode that depends on the product of I and the microelectrode resistance (Re). This unwanted IRe voltage drop adds to the recorded potential.

The Bridge Balance control can be used to balance out this voltage drop so that only the membrane potential is recorded. The term "Bridge" refers to the original Wheatstone Bridge circuit used to balance the IR voltage drop and is retained by tradition, even though operational amplifiers have replaced the original circuitry.

A differential amplifier is used to subtract a scaled fraction of the current I from the voltage recorded at the back of the microelectrode, Vp. The scaling factor is the microelectrode resistance (Re). The result of this subtraction is thus the true membrane potential, Vm.

$$(V_p = V_m + IR_e)$$
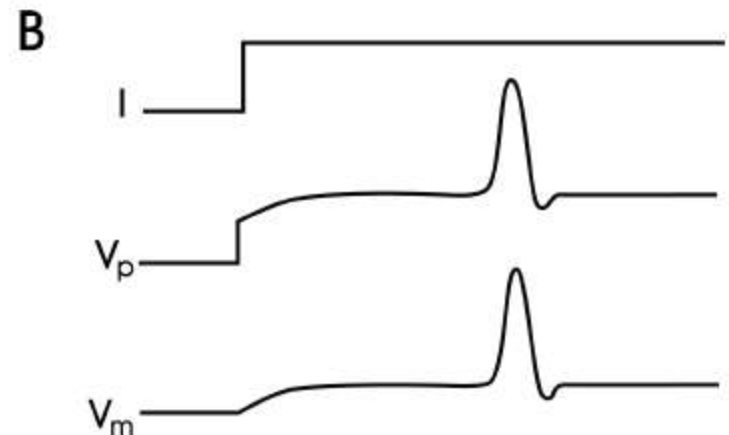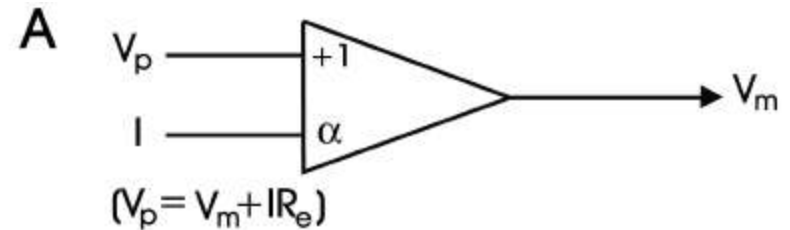
The Bridge Balance should be frequently checked when inside a cell, because the electrode resistance can drift.

Current Clamp: Electrode Capacitance Neutralization

☑ Pipette Capacitance Neutralization:

The capacitance (Cin) at the input of the headstage amplifier is due to the capacitance of the amplifier input itself (Cin1) plus the capacitance to ground of the microelectrode and any connecting lead (Cin2). Cin combined with the microelectrode resistance (Re) acts as a low-pass filter for signals recorded at the tip of the microelectrode. For optimal performance at high frequencies this RC time constant must be made as small as possible.

Check the Tuning checkbox and choose amplitude (nA) and frequency (Hz) parameters that result in a sawtooth pattern of about 10 mV amplitude on "Primary Output: Membrane Potential". Carefully increase the Pipette Capacitance Neutralization value until overshoot just starts to appear in the step response. This is easiest to see if you have already adjusted Bridge Balance. **If you go too far the overshoot may become a damped** oscillation, which may escalate into a continuous oscillation, killing the cell.

Sometimes the overshoot is difficult to see. In this case, you may prefer to look at the "Primary Output: Membrane Potential" trace at high gain on an oscilloscope, advancing the Pipette Capacitance Neutralization value until the trace becomes noisy and oscillations seem imminent. It is usually prudent to reduce the Pipette Capacitance Neutralization setting slightly from the optimal, in case the capacitance changes during the experiment.

Voltage clamp: Electrode capacitance compensation
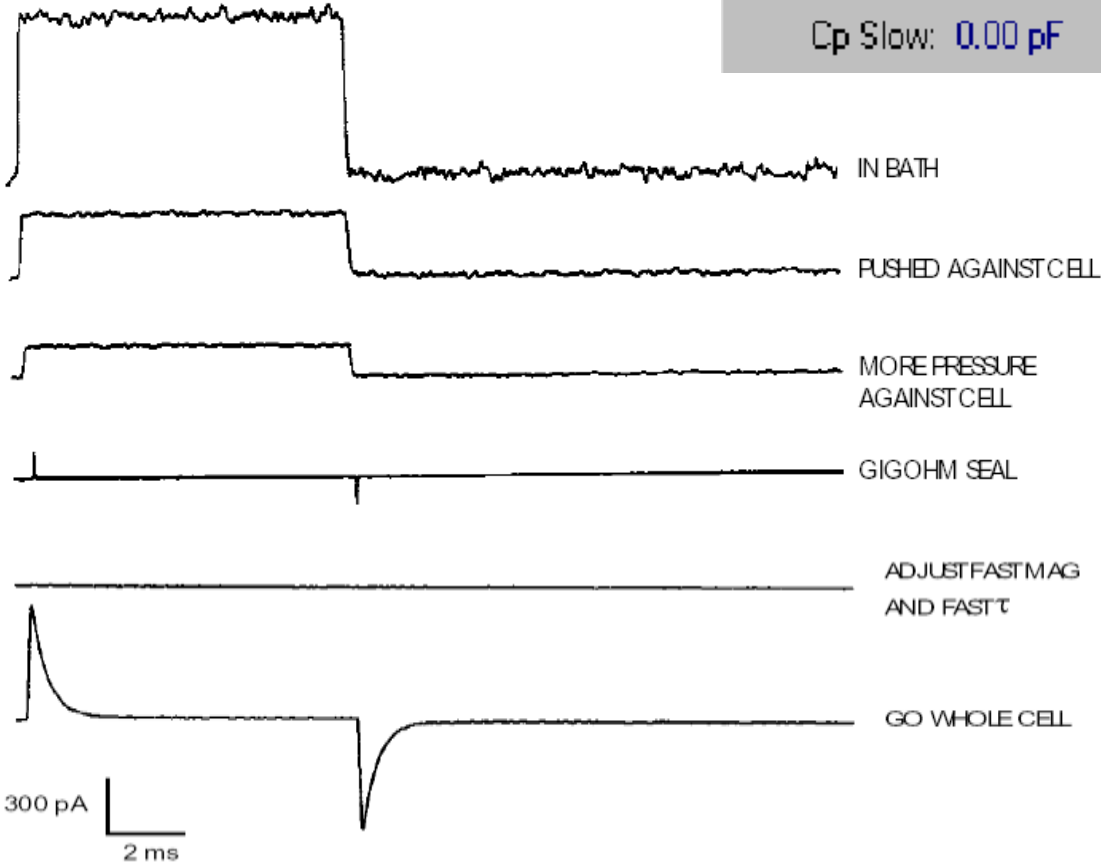
Voltage clamp: Whole-Cell Capacitance Compensation

☑ Whole Cell
32.94 pF ⊕ 10.01 MΩ
Auto
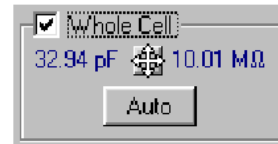
When the membrane potential is stepped, there is a significant current transient required to charge the membrane capacitance. The purpose of these adjustments is to offload the burden of this task from the feedback resistor, Rf.

In many cells, even a small command voltage of a few tens of millivolts can require such a large current to charge the membrane that it cannot be supplied by Rf.

V_{cmd} Vcmd is the patch-clamp voltage command

V_m Vm is the voltage across the cell membrane

I_{Rf} IRf is the current across the patch-clamp feedback resistor;

I_{C2} IC2 is the current injected across the patch-clamp compensation capacitor

Before
Whole-cell Compensation

After
Whole-cell Compensation
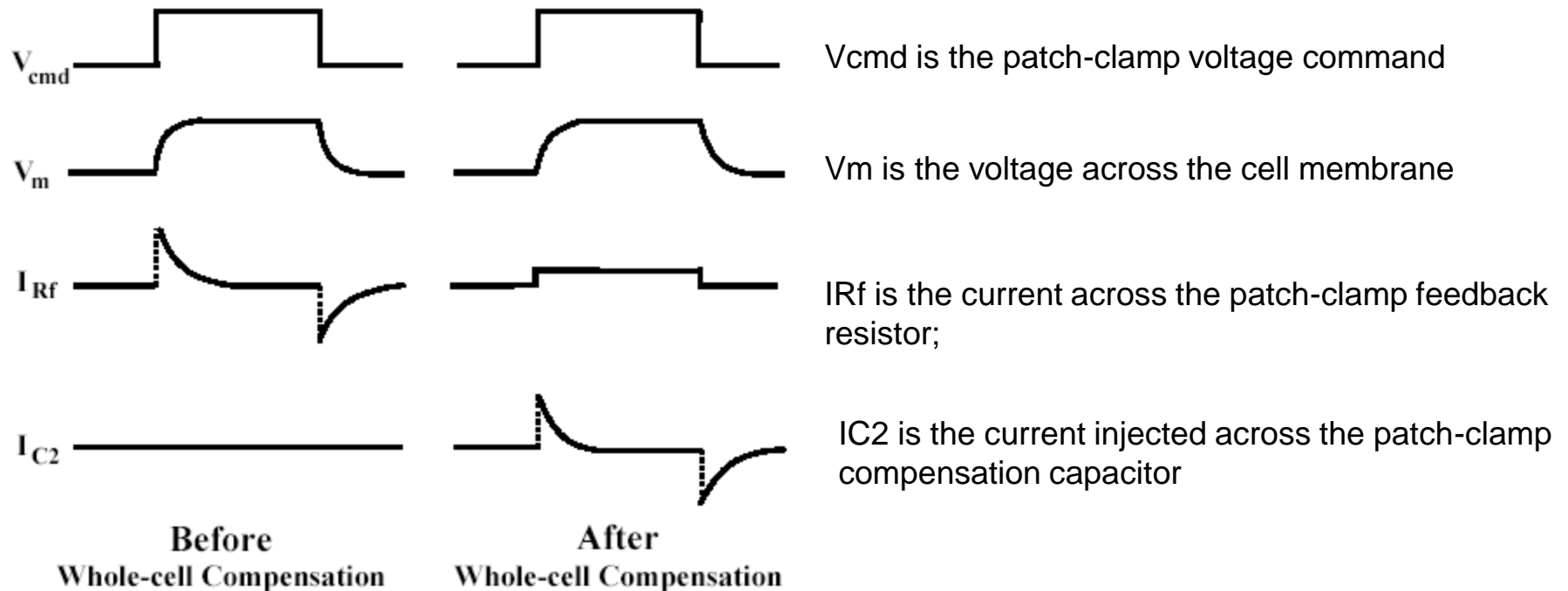
The absolute value of the membrane capacitance is displayed on the whole-cell capacitance dial after the whole-cell current transient has been eliminated. This value may be used to estimate the surface area of the cell assuming that the membrane capacitance per unit area is 1 µF/cm2.

Voltage Clamp: Series Resistance Compensation

Series resistance (Rs) is defined as the total resistance that is interposed between the circuitry of the headstage and the membrane of the cell. Contributors to Rs include:

• The resistance of the solution inside the electrode, dominated by that at the narrow tip. (Re or Rp)
• The resistance caused by intracellular organelles that partially clog the electrode tip. (Raccess)
• The resistance due to glial cells or connective tissue that cover the cell membrane (usually minor).
• The resistance of the bath solution and the bath electrode (usually minor).
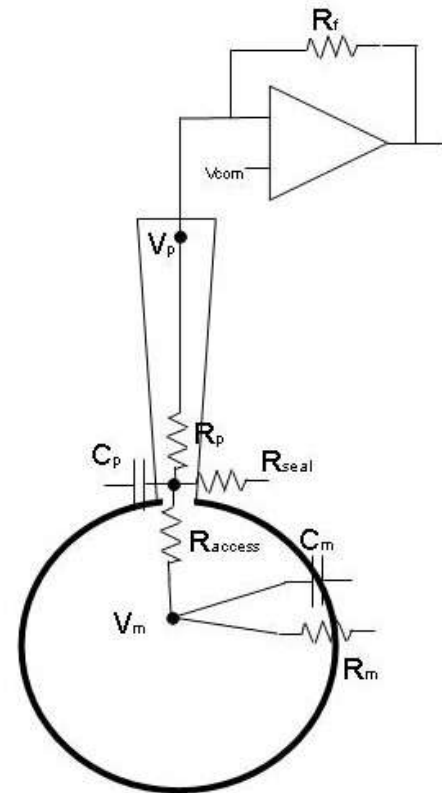
The size of Rs can be estimated by selecting the Whole Cell checkbox in the MultiClamp 700B Commander and pressing the Auto button to compensate the whole-cell capacitance.
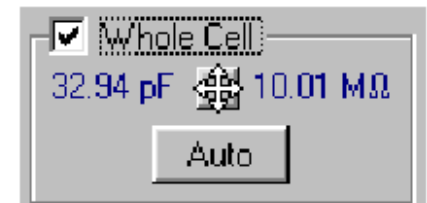
Is Rs Compensation Necessary?

•If Rs = 10 MOhm and the maximum membrane current you anticipate is 100 pA, the steady-state voltage error will be at most 10 M. x 100 pA = 1 mV which is probably insignificant.

•Application of Rs compensation can greatly improve the fidelity of the voltage clamp.

•In whole-cell recordings, it is best to try Rs compensation to see if it makes a difference.

•In order to see the improvement brought about by Rs compensation, check and uncheck the Rs Compensation checkbox. A dramatic speeding-up of the Membrane Current should be apparent with the compensation correctly adjusted.

•Compensation is rarely useful with isolated membrane patches, which typically have small capacitance and membrane currents.

•If Rs compensation is found not to be necessary, it is best to turn it off. This is because Rs compensation increases noise.

Voltage Clamp: Series Resistance Errors

1- Steady-state voltage errors. Suppose you are measuring a 1 nA membrane current under V-Clamp. If Rs = 10 MOhm, there will be a voltage drop of IRs = 1 nA x 10 MOhm = 10 mV across the series resistance. Since Rs is interposed between the headstage and the cell membrane, the actual cell membrane potential will be 10 mV different from the command potential at the headstage.

2. **Dynamic voltage errors.** Following a step change in command potential, the actual cell membrane potential will respond with an exponential time course with a time constant given by tau = RsCm, where Cm is the cell membrane capacitance. This time constant is 330 µs for the model cell provided with the MultiClamp 700B (Rs = 10 M., Cm = 33 pF). This means that the actual membrane potential response to a step voltage command will have a 10-90% risetime of more than 0.7 ms and will not settle to within 1% of its final value until about 1.5 ms after the start of a step command. If you are interested in fast membrane currents, like sodium currents, this slow relaxation of the voltage clamp is unacceptable.

3- Bandwidth errors. The Rs appears in parallel with the membrane capacitance, Cm, of the cell. Together they form a one-pole RC filter with a –3 dB cutoff frequency given by 1/2TauRsCm. This filter will distort currents regardless of their amplitude. For the parameters of the model cell, this filter restricts true measurement bandwidth to 480 Hz without Rs compensation.

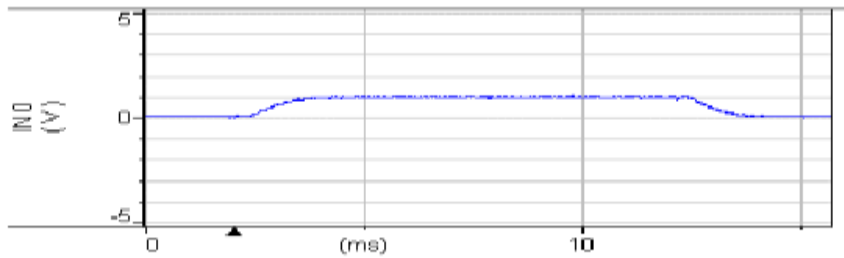# Voltage Clamp: How to Adjust Series Resistance Compensation
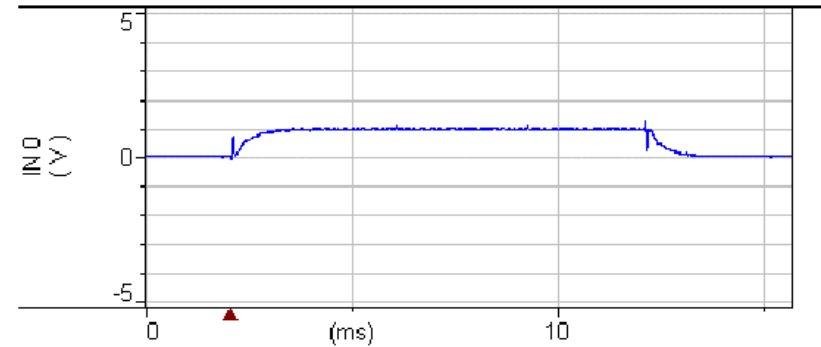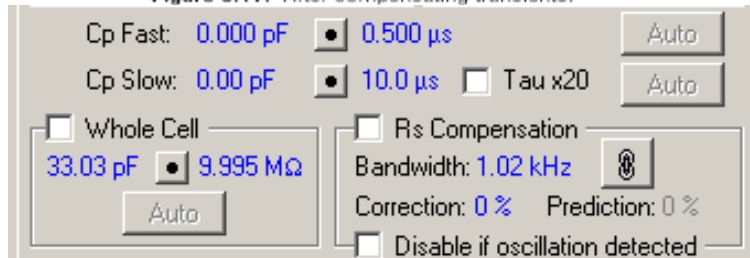

Figure 5.17. After compensating transients.

Cp Fast: 0.000 pF ● 0.500 µs [Auto]
Cp Slow: 0.00 pF ● 10.0 µs ☐ Tau x20 [Auto]

☐ Whole Cell
33.03 pF ● 9.995 MΩ [Auto]

☐ Rs Compensation
Bandwidth: 1.02 kHz
Correction: 0 %   Prediction: 0 %
☐ Disable if oscillation detected

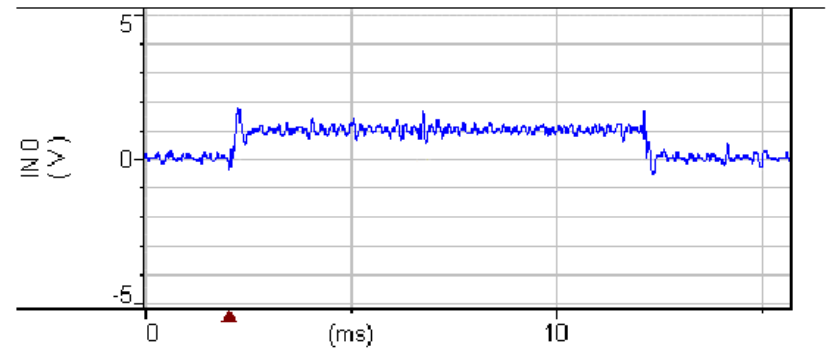**Figure 5.18.** After setting Prediction = 90%, Correction = 0%.

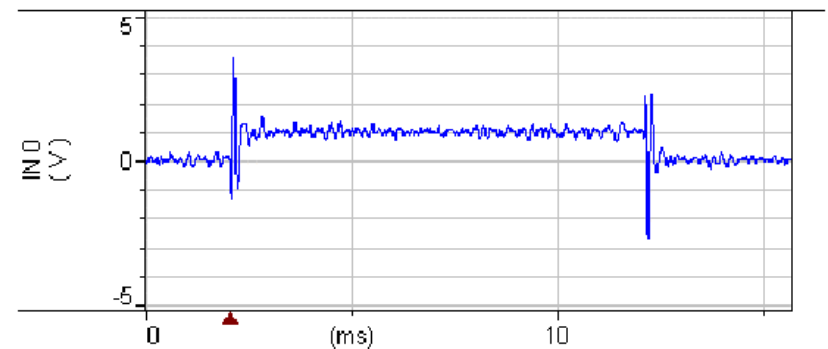**Figure 5.19.** After setting Prediction = 90%, Correction = 90%.

Figure 5.20. After optimizing C_f, R_s and C_m to minimize transients.

Carefully increase the Correction value to equal that under Prediction. A rather large transient should appear in the current at the beginning and end of the command step.

Its peak-to-peak amplitude should be 2-4 nA and it should undergo several distinct "rings" requiring 1 ms to disappear into the noise (Figure 5.19). To eliminate this transient, begin by reducing by a few percent the value of Rs (M.) displayed under Whole Cell. As you reduce this setting, the amplitude of the transient first decreases and then begins to increase. A distinct minimum exists and the desired value of Rs is at this minimum.

Next, slightly adjust the Cp Fast settings, trying to further minimize any fast leading-edge transients. When this has been done, small adjustments in the Whole Cell capacitance (pF) value should completely eliminate any remaining transients (Figure 5.20). If this is not possible in the real experiment, iterative fine adjustments of Cp Fast and Whole Cell Rs may achieve the desired cancellation. If all of this fails and the oscillations are too severe, you may need to go back to the beginning and set the Prediction and Correction controls to lower values.
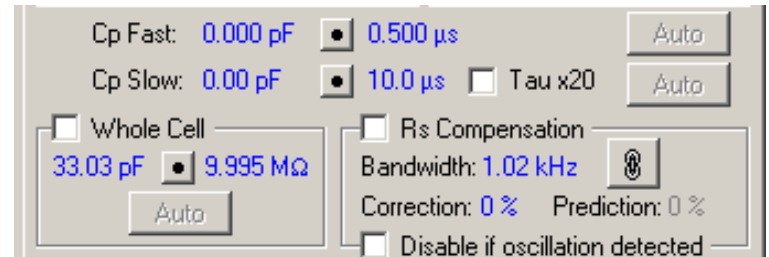
Voltage Clamp: Series Resistance Compensation Bandwidth

The relationship of Bandwidth (BW) to Lag is defined as:
BW = 1 / (2 * π * Lag)
The default MultiClamp Rs Correction Bandwidth value
is 1 kHz, which equates to a Lag value of 160 µs.
(2 kHz BW = 80 µs Lag, 10 kHz BW = 16 µs Lag, etc.)

By reducing the Bandwidth control under Rs Compensation you can usually increase the percent compensation without instability. However, this is likely to be a false improvement if it is pushed too far.

Reducing the Bandwidth slows down the feedback circuit used in Rs compensation, reducing its dynamic response. For example, if you limit the Bandwidth to 1 kHz, the Rs Compensation will be reduced substantially for conductance changes faster than 160 us.

Bottom line: if you increase the Bandwidth value, you can measure faster conductance changes, but you sacrifice Rs compensation stability.

Voltage Clamp: Leak Subtraction

Leak Subtraction provides a quick method of subtracting linear leak currents from the Membrane Current in V-Clamp mode.

Leak Subtraction is typically used when you are trying to measure single-channel currents that are sitting on top of a relatively large leak current. Imagine, for example, a channel that opens during a 100 mV voltage step that is applied to a patch with a 1 G. seal resistance. The seal (leak) current during the step will be 100 pA. Because of this relatively large leak current, the gain of the MultiClamp 700B cannot be turned up very far without saturating the amplifier, but at a low gain setting the single-channel openings may not be resolved very well.

Leak Subtraction solves this problem by subtracting from the membrane current, in this case, a 100 pA step of current before the Output Gain is applied. The Primary Output signal will now be a flat line on which the single-channel activity is superimposed.

When it is correctly adjusted, voltage steps that are known to elicit no active currents (*e.g. small hyperpolarizing steps) will produce a flat line in the* Membrane Current signal.

For subtracting leak currents in whole-cell recordings, it is safer to use a computer program like pCLAMP, which allows off-line leak correction.