November 1986

AXOPROBE-1A MICROELECTRODE AMPLIFIER

OPERATOR'S MANUAL

Written for Axon Instruments, Inc.
by Alan Finkel, Ph.D.

QUESTIONS? Call (415) 340-9988

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VERIFICATION

THIS INSTRUMENT IS EXTENSIVELY TESTED AND THOROUGHLY CALIBRATED BEFORE LEAVING THE FACTORY. NEVERTHELESS, RESEARCHERS SHOULD INDEPENDENTLY VERIFY THE BASIC ACCURACY OF THE CONTROLS USING RESISTOR/CAPACITOR MODELS OF THEIR ELECTRODES AND CELL MEMBRANES.

DISCLAIMER

THIS EQUIPMENT IS NOT INTENDED TO BE USED AND SHOULD NOT BE USED IN HUMAN EXPERIMENTATION OR APPLIED TO HUMANS IN ANY WAY.

CONTENTS

INTRODUCTION

The AXOPROBE-1A multipurpose microelectrode amplifier contains two microelectrode amplifiers (ME1 and ME2). These amplifiers may be independently used for intracellular and extracellular voltage recording with simultaneous current passing, or differentially for recording with ion-sensitive electrodes. Many built-in features make the AXOPROBE-1A extremely convenient to use.

To learn how to make the most of these features, we advise first-time users of the AXOPROBE-1A to read this manual thoroughly and to familiarize themselves with the instrument using resistor/capacitor models of their electrodes and cells.

We will be pleased to answer any questions regarding the theory and use of the AXOPROBE-1A. Any comments and suggestions on the use and design of the AXOPROBE-1A will be much appreciated.

We would be most grateful for reprints of papers describing work performed with the AXOPROBE-1A. Keeping abreast of research performed helps us to design our instruments to be of maximum usefulness to you who use them.

Axon Instruments, Inc.

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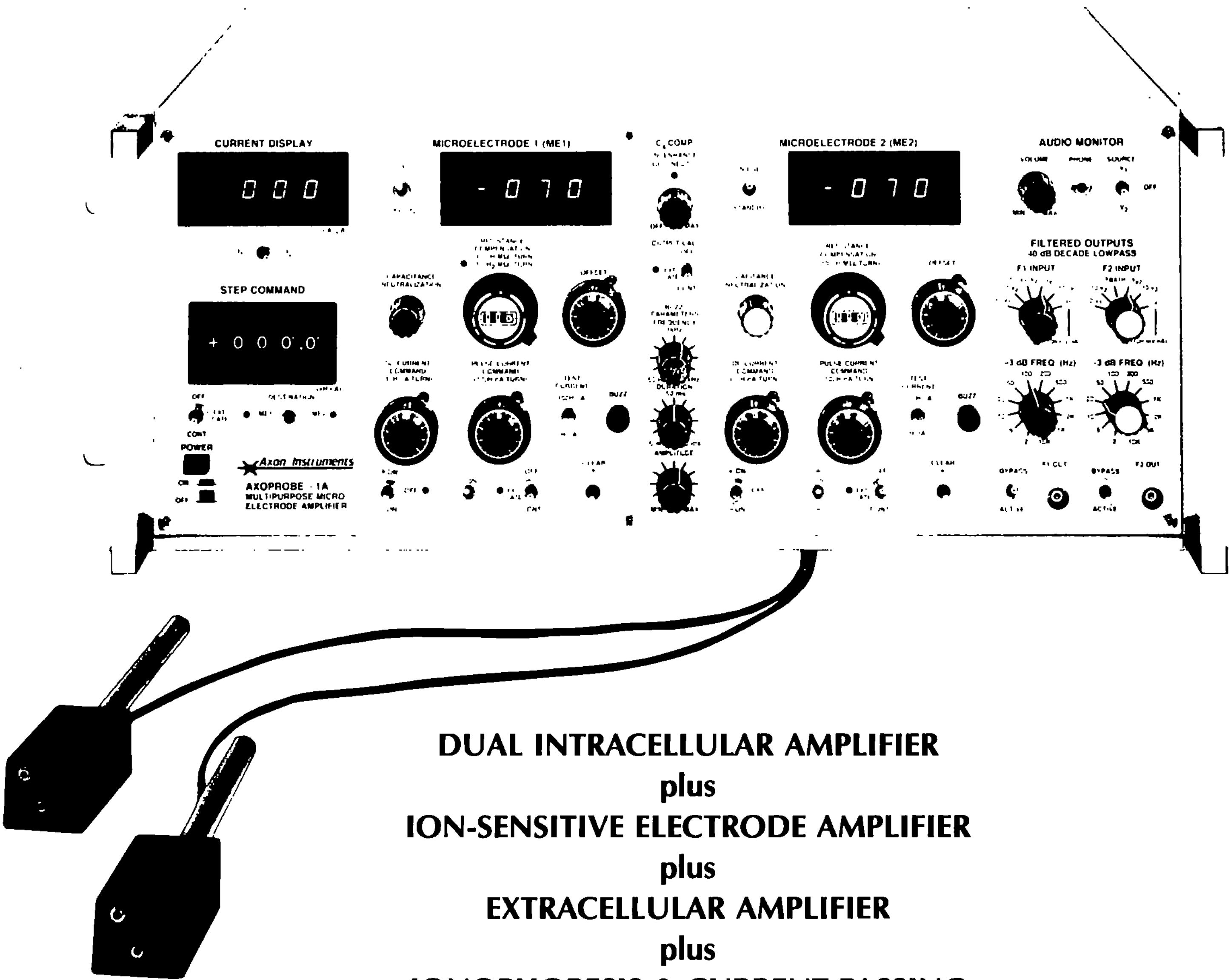
AXOPROBE–1A
Multipurpose Microelectrode Amplifier



DUAL INTRACELLULAR AMPLIFIER
plus
ION-SENSITIVE ELECTRODE AMPLIFIER
plus
EXTRACELLULAR AMPLIFIER
plus
IONOPHORESIS & CURRENT PASSING

The AXOPROBE–1A is a dual-channel microelectrode amplifier designed for a variety of experiments. High input resistances and differential recording make it ideal for ion-sensitive electrode measurements. Thirty-volt output compliances enable current passing through high-resistance electrodes for dye injection and ionophoresis. The AXOPROBE-1A is fast, low-noise, reliable and easy to operate. Command generators, digital voltmeters, lowpass filters and many other features are built in to enhance flexibility. Programmable "Buzz" and an audio monitor simplify cell impalement.

HEADSTAGES

The AXOPROBE-1A is designed to be used with the full range of microelectrode resistances, from **hundreds of kΩ to tens of GΩ.** To accomodate this range, a variety of interchangeable headstages are available. These all record at unity voltage gain but have different current-passing gains (H).

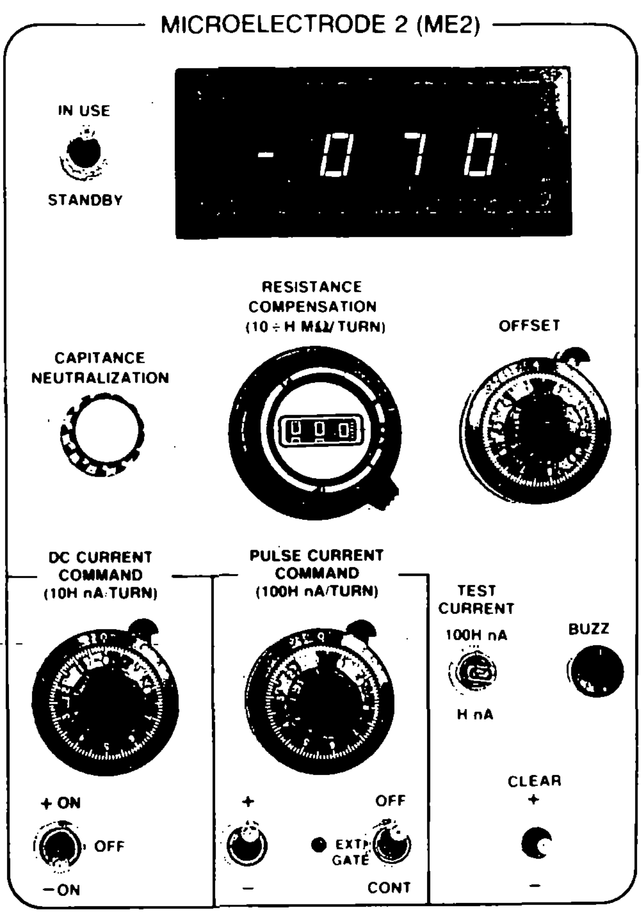
Headstages also come with a **choice of capacitance neutralization ranges.** The range depends on the size of the capacitor used for neutralization. The high-frequency noise is also proportional to this capacitor. Usually the headstages are supplied with the smallest capacitor (L version) but other choices are available.

The **actual current** in each microelectrode is measured. That is, the measurement falls to zero if the microelectrode blocks even though a current command is set up. During current passing, up to **±30 V** can be applied across the microelectrode to enable control of current in high-resistance electrodes.

For **ion-selective electrode** measurements either **one or both** headstages can be ultrahigh input impedance types.

VOLTAGE RECORDING and CURRENT PASSING

Voltage recording and current passing are always performed simultaneously. Passive voltage recording is simply achieved by switching off the current commands.



During current passing the voltage drop across the microelectrode can be eliminated from the electrode voltage by setting the **Resistance Compensation** control (also known as Bridge Balance control). At this setting the microelectrode resistance can be read from the dial. Alternatively, the microelectrode resistance can be read on the digital panel meter by using the **Test** switch.

Capacitance Neutralization uses a 10-turn control for maximum sensitivity.

To clear blocked tips, a **Clear** switch forces large hyperpolarizing and depolarizing currents through the electrode. In some preparations, flicking the Clear switch helps the microelectrode impale the cell.

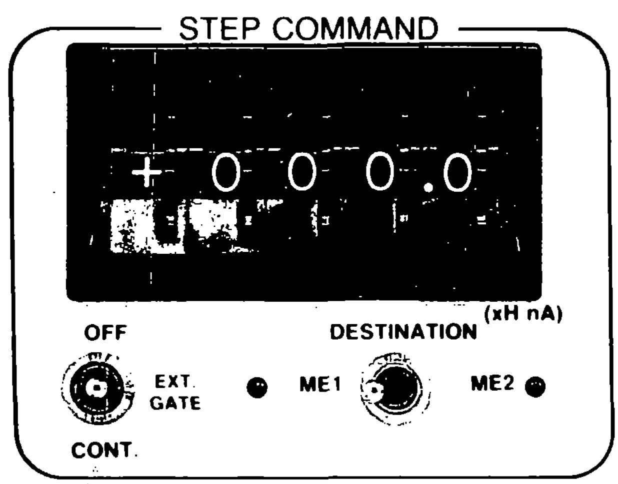
When using ultrahigh-impedance electrodes (e.g. ion-selective electrodes), there can potentially be a DC error introduced by leakage through the capacitor used for capacitance neutralization. This is totally eliminated in the AXOPROBE-1A by an original circuit that removes the DC voltage from across this capacitor, thus preventing any leakage.

A common problem when using stimulating electrodes is that some of the stimulus is directly coupled into the recording microelectrode. The AXOPROBE-1A has special circuits to **blank** the stimulus artifact.

COMMAND GENERATORS

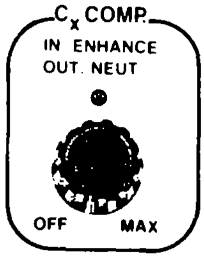
Several current commands can be generated internally. Each amplifier has a **DC Current Command** and a **Pulse Current Command.** In addition, a **Step Command** can be directed to either amplifier. The Step Command generator uses a thumbwheel switch to set the output of a digital-to-analog converter. Thus a high degree of precision and repeatability is achieved. Timing is set externally. **Indicator LEDs** light whenever an internal command is activated.

All commands add linearly. **External** command sources can be used simultaneously with the internal command sources.



DIFFERENTIAL MEASUREMENT

For **ion-sensitive** measurements and some current determinations in cylindrical cells it is necessary to measure V_1-V_2. This signal is provided as an output and can also be displayed on one of the digital panel meters. A **C_x Compensation** control enhances the cross capacitance (C_x) between the two electrodes thereby enabling their responses to common-mode potentials to be exactly matched.

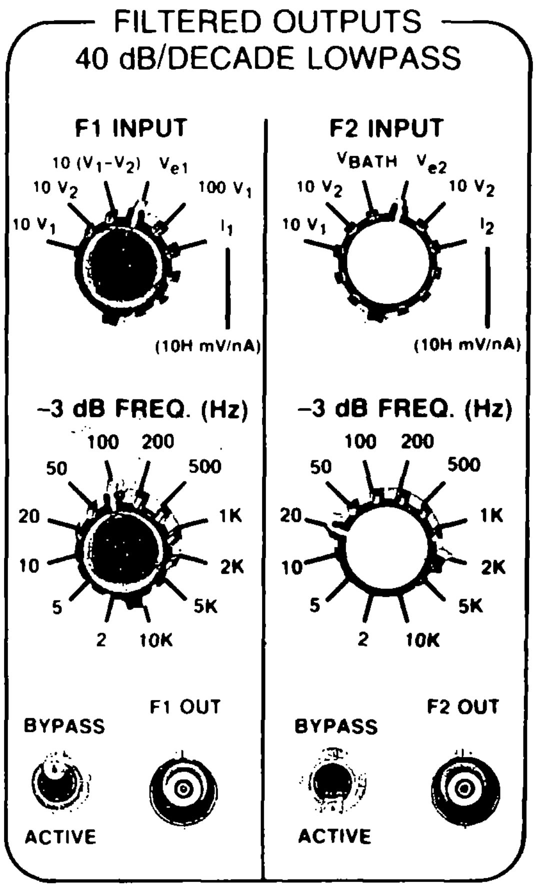


For **double-barrel** current-passing experiments the C_x Compensation control can be used to neutralize the cross capacitance. The coupling resistance can also be neutralized.

DISPLAYS, OUTPUTS and LOWPASS FILTERS

Two dedicated **digital panel meters** continuously display the microelectrode voltages and a third displays the current in the selected microelectrode. The decimal point of the current meter automatically shifts to comply with the various headstage current gains (H).

The **V_e** outputs are the raw electrode voltages. The **x10** outputs contain the Offset voltage and Resistance Compensation. The **x100** outputs are ultralow-noise AC-coupled outputs useful for extracellular measurements.

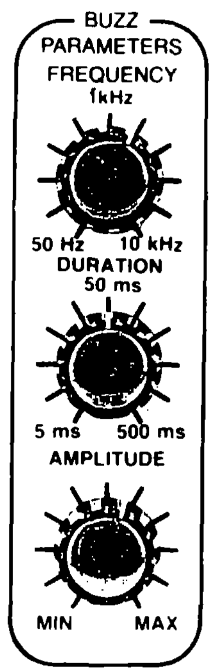


Internally or externally generated **calibration voltages** can be added to all of the outputs except V_e.

Two second-order **lowpass filters** have twelve selectable -3 dB frequencies. The filters have been designed for low noise and zero overshoot. The input to each filter can be switched to one of six signals. Thus all signals can be monitored on the two front-panel connectors without shuffling cables or cluttering up the equipment rack. The unfiltered and filtered signals are separately available at output connectors on the rear panel.

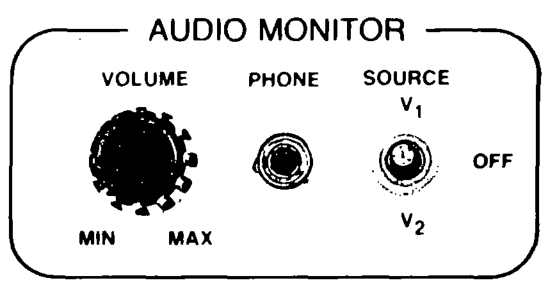
BUZZ

The most crucial stage of an intracellular experiment is obtaining a good impalement. A commonly used method is to press the microelectrode tip against the membrane and then oscillate (BUZZ) the microelectrode voltage. For unknown reasons (vibration of the tip and electrostatic attraction to bound charges on the inside membrane surface have been postulated) this causes the tip to penetrate. Until now, deliberately over utilized capacitance neutralization has been used to establish the oscillation. This produces widely variable results because the oscillation parameters are uncontrolled. In the AXOPROBE-1A a revolutionary approach is used. The experimenter is given complete control of the three essential Buzz parameters — **frequency, duration** and **amplitude** — so that requirements for easy impalement can be optimized. Buzz can be activated by a front-panel switch or by the footswitches provided.



AUDIO MONITOR

So the experimenter can watch the preparation without interruption, an audio monitor indicates the voltage change following a successful cell impalement. The moment that the electrode tip first touches the solution can also be clearly heard. The selected input (V_1 or V_2) determines the pitch of the monitor.



GENERAL

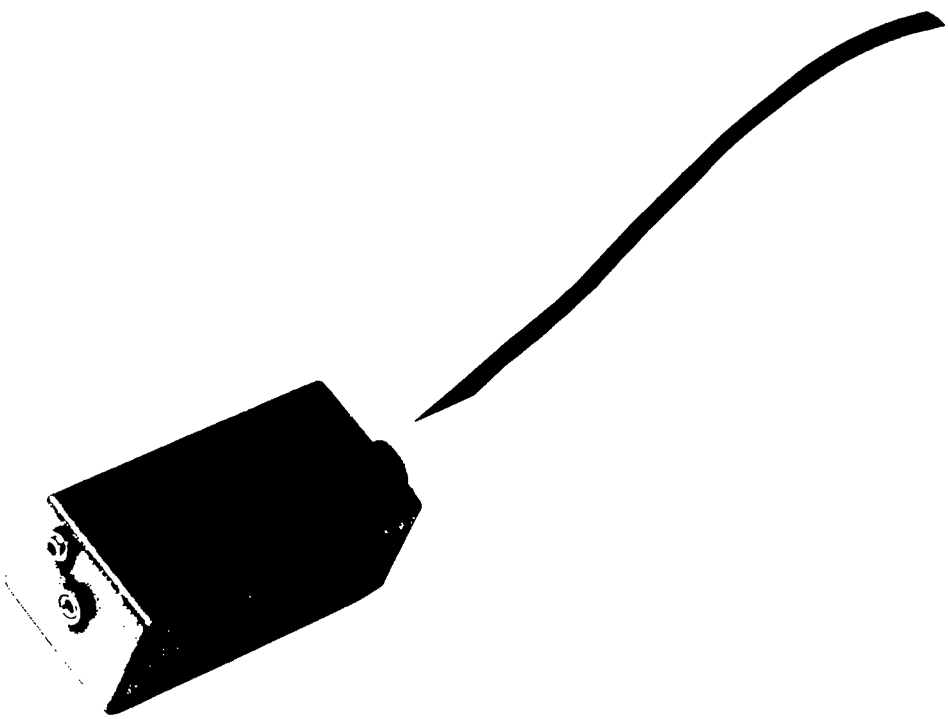
A third electrode can be used extracellularly to record the **bath potential.** To compensate for potential shifts caused by changing the bath solution or temperature, the bath potential is subtracted from the potentials recorded by the two main electrodes.

A specially constructed **low-radiation transformer** eliminates the source of line-frequency noise (hum). The incoming line voltage is filtered to remove radio-frequency interference (RFI).

Strong emphasis has been placed on **quality.** Precision ten-turn potentiometers, reliable switches and gold-plated connectors are used throughout. Ultralow-drift operational amplifiers are used in all critical positions and ICs are socketted for easy maintenance. Detailed operator's and service manuals are provided.

FURTHER INFORMATION and ORDERING

The Specifications Sheet contains complete technical details and ordering information. Please call the factory for answers to any questions you may have.



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November 1986

AXOPROBE–1A Multipurpose Microelectrode Amplifier

SPECIFICATIONS

Note: Numbered items are detailed in the Table.

HEADSTAGES

Types: HS–2 series headstages are standard.

Voltage Gain: All headstages record voltage at a unity gain.

Headstage Current Gain (H): Select on basis of cell input resistance (R_{in}) and maximum current capacity (I_{max}).

H = x 0.0001 for ion-sensitive electrodes with current passing.

H = x 0.01 for R_{in} greater than approx. 300 MΩ.

H = x 0.1 for R_{in} approx. 30–300 MΩ.

H = x 1 for R_{in} approx. 3–30 MΩ.

H = x 10 for R_{in} approx. 0.3–3 MΩ.

These ranges suggested for optimum performance. Considerable overlap is allowable.

Extracellular Electrodes: Operate with any H value (but check I_{max} and Input Leakage Current).

Ionophoresis: Typically uses H = x 1.

(1) **Current Setting and Measuring Resistance (R_o):** Located inside headstage. R_o determines H. R_o is *not* the input resistance.

(2) **Input Resistance:** Inversely proportional to H.

(3) **I_{max}:** Maximum current that can be passed with input grounded via indicated sum of cell resistance (R_{in}) and electrode resistance (R_e).

Compliance Voltage: ±30 V. Max voltage that can be applied across high-resistance electrodes.

(4) **Noise:** Values measured at V_e output with input grounded via R_e. Single-pole lowpass filter used to set measurement circuit −3 dB bandwidth. Capacitance Neutralization adjusted so step response of V_e is non-overshooting and so −3 dB bandwidth of v_e is equal to measurement circuit bandwidth.

Hum (Power Line Noise): Less than 20 µV peak-to-peak, grounded input, input-referred.

(5) **10–90% Settling Time (t_{10-90}):** Two values shown; $t_{10-90,V}$ for voltage step applied to input via R_e; $t_{10-90,I}$ for current step into same R_e. Capacitance Neutralization adjusted for fastest non-overshooting response.

Input Capacitance: Largely eliminated from step response considerations by bootstrapped power supplies and Capacitance Neutralization. See Settling Time specifications.

(6) **Capacitance Neutralization Range:** Ten-turn potentiometer. L version headstages have smallest range and lowest noise. M version headstages have larger range but more noise.

Capacitance Neutralization Leakage Current: Prevented by removal of DC potentials from neutralization capacitor and shield. Removal has 1 s or 10 s time constant.

(7) **Case/Shield:** Case and shield connector connected to Capacitance Neutralization or to unity-gain buffered electrode potential (V_e).

(8) **Input Leakage Current (I_{leak}) vs. Temperature.** Temperature dependance measured near 25°C. At fixed temperature input leakage current can be adjusted to zero.

PREAMPS (Two Channels)

Offset Range: ±500 mV. Ten-turn potentiometers.

Electrode Resistance Compensation Range: 10÷H MΩ/turn. Ten-turn potentiometers. Coupling resistance range, on ME1 control only, is 1÷H_2 MΩ/turn.

Test: For electrode resistance measurement. 100H mV/MΩ or H mV/MΩ. Res. Comp. must be off.

Clear: Forces ±100 nA through electrode.

In Use/Standby: Microelectrode 2 amplifier only. In Standby position disables panel meter and Capacitance Neutralization circuit.

TABLE

PARAMETER	UNITS	HS-2 x 10M	HS-2 x 1M	HS-2 x 1L	HS-2 x 0.1L	HS-2 x 0.01M	HS-2M x 0.0001
(1) R_o	MΩ	10^6	10^7	10^7	10^8	10^9	10^{11}
(2) Input Resistance	Ω	10^{10}	10^{11}	10^{11}	10^{12}	10^{13}	10^{15}
(3) I_{max} (R_{in} + R_e)	nA	10,000 (2MΩ)	1,000 (20MΩ)	1,000 (20MΩ)	100 (200MΩ)	10 (2GΩ)	0.1 (200GΩ)
(4) Noise (R_e, Bandwidth)	µVrms	24 (1MΩ) (10kHz)	70 (10MΩ) (10kHz)	54 (10MΩ) (10kHz)	53 (100MΩ) (1kHz)	40 (1GΩ) (100Hz)	30 (10GΩ) (10Hz)
(5) $t_{10-90,V}$(R_e)	µs	4	12	11	34	40	2,000
$t_{10-90,I}$(R_e)	µs	4 (1MΩ)	13 (10MΩ)	12 (10MΩ)	36 (100MΩ)	300 (1GΩ)	3,000 (100GΩ)
(6) Capacitance Neutralization Range	pF	0 to 22	0 to 22	0 to 8	0 to 8	0 to 22	0 to 22
(7) Case/Shield Connected To	—	Cap Neut	Cap Neut	Cap Neut	Cap Neut	V_e	V_e
(8) I_{leak} vs. Temp	pA/°C	30	3	3	0.3	0.1	0.005
(9) I Output Sensitivity	mV/nA	1	10	10	100	1,000	0.1V
Maximum Meter Reading		1.999 µA	199.9 nA	199.9 nA	19.99 nA	1.999 nA	19.99 pA
(10) C_x Enhancement/Neutralization Range	pF	40	40	15	15	15	40

BUZZ
Frequency: Approx 50 Hz–10 kHz. Logarithmic potentiometer.
Duration: Approx. 5–500 ms. Logarithmic potentiometer.
Amplitude: 0 to ±30 V. Attenuated by input capacitance. Linear potentiometer.
Activation: Front-panel debounced switch, footswitches, or logic HIGH level on rear-panel connector.

OUTPUTS
[9] **I_1 and I_2:** *Actual* electrode currents. 10÷H mV/nA.
[9] **Current Meter:** Recognizes H of headstage in use and sets decimal point accordingly. Maximum meter reading is less than headstage current limit (I_{max}). Currents exceeding current meter range can be measured on I_1 and I_2 outputs. Display selections are I_1 and I_2.
Voltage Meters: Range ±1999 mV. Separate meters for V_1 and V_2. V_1 meter also displays V_1–V_2.
V_e: Raw headstage voltage. 0.1%
10V: x10 output with Resistance Compensation and Offset. 0.1%
10(V_1–V_2): Difference of 10V_1 and 10V_2. Matched to 0.01%
100V: x100 output. AC coupled (1 Hz). 2%.
V_{BATH}: x1 bath potential. 1%
Output Impedances: 500Ω.
[10] **C_x Compensation:** Used to compensate for the coupling capacitance between electrodes. ENHANCE position adds ME2 voltage via a capacitor into ME2 headstage input, thus matching electrode responses to common-mode signals. Used for ion-sensitive electrode recording. NEUTRALIZE position subtracts ME2 voltage from ME2 headstage input. Used for double-barrel current passing.

LOWPASS FILETERS
Two independent filters: F1, F2. Second-order.
–3 dB frequencies: Twelve. 2, 5, 10, 20, 50, 100, 200, 500, 1K, 2K, 5K, 10K Hertz. Continuous rotation.
F1 Inputs: Switch selected. 10V_1, 10V_2, 10(V_1–V_2), V_{e1}, 100V_1, I_1.
F2 Inputs: Switch selected. 10V_1, 10V_2, V_{BATH}, V_{e2}, 100V_2, I_2.
Bypass Switch: In ACTIVE position signals are filtered. In BYPASS position signals are wideband.

INTERNAL COMMANDS
Note: Commands from all sources sum linearly.
DC Current Command: One for each preamp. ±100H nA max. Ten-turn potentiometers.
Pulse Current Command: One for each preamp. ±1000H nA max. Ten-turn potentiometers. Activated by HIGH control signal on PULSE GATE input or by front-panel switch.
Step Command: Shared by preamps. Destination switch determines which preamp command goes to. ±199.9H nA max. Set on thumbwheel switch. Activated by HIGH control signal on STEP GATE input or by front-panel switch.

EXTERNAL COMMANDS
Sensitivities: 20H nA/V
Input Impedance: 100 kΩ
Max. Input Voltage: ±30 V

CALIBRATION SIGNAL
Internal: Activated by HIGH control signal on CAL. GATE input or by front-panel switch. Input-referred values: 10 mV on x 10 outputs, 1 mV on x 100 outputs, 10H nA on I outputs.
External: Proportional to applied voltage. Input-referred values: 2 mV/V on x10 outputs, 0.2 mV/V on x100 outputs, 2H nA/V on I outputs. 100 kΩ input impedance.
Accuracy: 1% typical.
Audio Monitor: Pitch proportional to V_1 or V_2. Internal speaker bypassed when earphone plugged in.

BATH POTENTIAL SUBTRACTION
Signal recorded by bath headstage or by external amplifier is subtracted from x10 outputs. Subtraction band-limited to 10 kHz. If bath potential not measured system automatically reverts to using 0 V as reference potential. Standard headstages work as bath headstages if plugged into bath headstage connector.

GROUNDING
Signal ground is isolated from chassis and power ground.

CONTROL INPUTS
Above 3 V accepted as logic HIGH. Below 2 V accepted as logic LOW. Inputs protected to ±15 V.

PAIRING BRACKET (BR–1)
BR–1 bracket (optional extra) for mounting two headstages as a pair.

HEADSTAGE DIMENSIONS
Case is 2.25 x 1.14 x 0.87" (57.2 x 29.0 x 22.1 mm). Mounting rod is 4 (102) long. Available mounting rod diameters (D) are ½, 5/16 or ⅜ (6.3, 7.9 or 9.5). Specify required mounting rod diameter with order. Cable length is 10 feet (3 m).

HEADSTAGE CONNECTORS
Sockets for microelectrode input, shield drive and ground output are 0.08" (2 mm) diameter. Input socket is Teflon insulated.

CABINET DIMENSIONS
7 (177) high, 19 (483) wide, 12.5 (317) deep. Mounts in standard 19" rack. Handles included.

SUPPLY REQUIREMENTS
Line Voltage: 100–125 VAC or 200–250 VAC. User selectable by an internal switch.
Line Frequency: 50–60 Hz.
Power: 20 W.
Fuse: 0.5 A slow. 5 x 20 mm.
Line Filter: RFI filter is included.
Line cord: Shielded line cord is provided.

ACCESSORIES PROVIDED
Operator's Manual
Service Manual
2 mm plugs for use with headstage
Low-capacitance test resistor for each headstage
Spare fuse
Footswitches to operate Buzz of both electrodes.

ORDERING INFORMATION
When ordering please specify:
1. Current gain (H) and type of two headstages provided.
2. Current gain (H) and type of any extra headstages.
3. Diameter (D) of headstage mounting rods.

Unless you specify otherwise, the AXOPROBE–1A will be supplied with one HS-2 H=x0.1L and one HS-2 H=x1L headstage, each with D=5/16 (7.9 mm). Domestic and international sales are direct from the factory.

10% discount applies to simultaneous purchase of two or more AXOPROBE–1As by a single group. For non-simultaneous purchases, 10% discount applies to second and subsequent AXOPROBE–1As purchased by a single group within 12 months. Discount must be requested when placing order.

WARRANTY
12 months parts and labor from date of receipt.

SERVICE
Service is available at the factory. A detailed service manual is supplied with each AXOPROBE–1A.

For further information call us. A factory expert will be pleased to answer your technical and ordering inquiries.

AXON INSTRUMENTS, INC.
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GLOSSARY OF FRONT PANEL ABBREVIATIONS

CAL.	Calibration.
CONT.	Continuous.
C_x COMP	Cross capacitance compensation.
EXT.	External.
F1/F2	Lowpass filter.
H	Headstage current gain.
I_1/I_2	Current in microelectrode 1/2.
ME1/2	Microelectrode 1/2.
V_{BATH}	Bath electrode potential. Unity gain.
V_{e1}/V_{e2}	Raw electrode potential. Unity gain.
V_1/V_2	Unity-gain electrode potential. Derived by dividing $10V_1/10V_2$ by ten.
$10V_1/10V_2$	x10 electrode potential. Includes Resistance Compensation, Offset, bath potential subtraction, Calibration.
$10(V_1-V_2)$	Difference between $10V_1$ and $10V_2$.
$100V_1/100V_2$	x100 electrode potential. Includes Calibration. AC coupled.

QUICK GUIDE TO OPERATIONS

The controls and operation of the AXOPROBE-1A are very briefly described in this section. Detailed explanations are given in the alphabetically organized section E of this manual.

D1. REST POSITIONS OF CONTROLS

Current meter display: I_1

Step Command: 000.0

OFF

ME1

Panel meters: V_1, IN USE

Capacitance Neutralization: Counterclockwise

Resistance Compensation: Counterclockwise (zero)

Offset: Mid position (approx. 5.0)

DC Current Command: OFF

Counterclockwise (zero)

Pulse Current Command: OFF

Counterclockwise (zero)

C_x Compensation: Counterclockwise, IN

Output Calibration: OFF

Buzz frequency: Mid position

Buzz duration: Mid position

Buzz amplitude: Mid position

Audio Monitor: OFF

Counterclockwise

Filters: $10V_1$, $10V_2$

10 kHz

Active

D2. HEADSTAGES

HS-2 series headstages are standard. Two supplied with AXOPROBE-1A.

All HS-2 headstages record voltage at unity gain.

Available in several headstage current gains (H). Front-panel controls read directly in indicated units when H = x1. All H values are powers of 10. Small H values for high-resistance cells and electrodes. Large H values for passing large currents.

H = x10, x1, x0.1, x0.01 for general purpose.
H = x0.0001 for ion-sensitive electrodes.

Headstages normally supplied in L version (low-noise, low capacitance-neutralization range).
M version can be supplied to compensate large capacitances.
Red connector: Microelectrode input
Gold connector: Driven shield; case
Yellow connector: Ground output

D3. MICROELECTRODE 1 (ME1)

Complete intracellular/extracellular electrometer.

Capacitance Neutralization:

Neutralizes electrode input capacitance. Clockwise rotation reduces effective input capacitance and speeds response. Overutilization oscillates headstage.

Resistance Compensation:

Compensates electrode voltage drop during current passing. Resistance (scaled by H) read on ten-turn dial. If cross-capacitance compensation (C_x COMP) is "on" and in the NEUT (neutralize) position, then this control compensates electrode coupling resistance, scaled by H_2 (H of ME2).

Test Current:

Applies constant current to electrode. Electrode response in mV corresponds to resistance. Resistance Compensation setting must be zero.

Offset:

Compensates ±500 mV electrode tip potential. Works an x10 output only. Use to zero electrode voltage while extracellular.

DC Current Command:

For injection of constant current. Magnitude set on ten-turn dial. Polarity set on switch. LED indicates when current injection activated.

Pulse Current Command:

For injection of pulsed or constant current. Magnitude set on ten-turn dial. Polarity set on switch. CONT. position for continuous activation. EXT. GATE position for gating by logic HIGH level applied to rear-panel PULSE GATE input. LED indicates activation.

Clear:

Passes large hyperpolarizing and depolarizing current to clear blocked electrodes or to impale cell.

Display:

Digital panel meter displays either V_1 or $V_1 - V_2$.

Buzz:

Use for cell impalement. Connects oscillating voltage to microelectrode. Parameters of oscillation set in Buzz Parameters section. Activate by front-panel pushbutton switch, or by external switch or logic device to connect 5 V TO BUZZ input on rear panel.

D4. MICROELECTRODE 2 (ME2)

Independent intracellular/extracellular electrometer similar to ME1. One difference is Standby switch replacing Display switch. Standby position used when ME2 not required (disables digital panel meter and capacitance neutralization). Second difference is that when cross compensation is used it is applied from ME2 to ME1, not vice versa.

D5. BUZZ PARAMETERS:

For optimization of headstage oscillation used to assist impalement. Frequency, Duration and Amplitude of oscillation independently set.

D6. FILTERED OUTPUTS

Two independent two-pole filters. One of six output signals can be selected for each filter. Twelve —3 dB cutoff frequencies can be selected. Bypass/Active switch determines whether signal is filtered (active position) or wideband (filter bypassed).

D7. C_x COMP

IN: Enhances capacitance coupling of V_2 into V_1. Speeds ME1 response to common signals so that common signals are recorded identically by ME1 and ME2. Useful for ion-sensitive electrode recording.

OUT: Neutralizes resistance and capacitance coupling of V_2 into V_1. Useful for current passing through one barrel of a double-barrel electrode.

D8. OUTPUT CAL.

Adds a 100 mV signal to current outputs, x10 and x100 voltage outputs. Input-referred values are 10H nA, 10 mV and 1 mV respectively. CONT. position for continuous activation. EXT. GATE position for gating by logic HIGH level applied to rear-panel CAL. GATE input. Additional calibration signals separately generated by applying a signal to the EXT. CAL. SIGNAL input on the rear panel.

D9. CURRENT DISPLAY

Meter displays average current. Decimal point automatically placed to suit H. Display is in nA for H = x1, x0.1, x0.01; pA for H = x0.0001; µA for H = x10. Display I_1 or I_2.

D10. STEP COMMAND

D/A convertor generates precision command voltages. Destination switch selects either ME1 or ME2 to be target for command. Thumbwheel switch sets magnitude with 0.05% resolution. Magnitudes are scaled by H. CONT position for continuous activation. EXT. GATE position for activation by logic HIGH level applied to rear-panel STEP GATE input.

D11. AUDIO MONITOR

Pitch of audible tone depends on potential of selected input (V_1 or V_2). Potentiometer sets volume. Earphone can be plugged into phone jack. If so, speaker is disabled.

D12. BATH POTENTIAL

Use a low-resistance microelectrode with an HS-2 headstage to record potential of bath solution (V_{BATH}). This potential is subtracted from $10V_1$ and $10V_2$ outputs. If not required, do not plug headstage into rear-panel connector. V_{BATH} recorded by outside equipment can be subtracted by connecting to EXT. V_{BATH} connector.

D13. INPUTS AND OUTPUTS

Located on rear-panel, but F1 OUT and F2 OUT repeated on front-panel.

F1, F2 outputs:

Filtered outputs.

V_e output:

Raw electrode potential.

10V output:

Modified electrode potential. x10 gain. Includes Offset, Resistance Compensation, CAL and bath potential.

$10(V_1-V_2)$ output:

Difference signal.

100V output:

AC-coupled (1 Hz) electrode potential. x100 gain. Includes CAL.

V_{BATH} output:

Potential recorded by bath electrode.

I output:

Electrode current.

Logic Levels:

Over 3 V accepted as logic HIGH. Below 1 V accepted as logic LOW. ±15 V safe operating range.

Step Gate input:

Logic HIGH activates Step Command.

Pulse Gate input:

Logic HIGH activates Pulse Current.

100 mV CAL. Gate input:

Logic HIGH activates 100 mV OUTPUT CAL.

EXT. CAL. Signal input:

Voltage on this input converted into proportional calibration signal.

EXT. ME Command input:

Voltage on this input converted into proportional current.

EXT. V_{BATH} input:

Bath potential recorded by outside equipment subtracted from 10V outputs.

V_1 Blank Gate input:

Logic HIGH causes V_{e1} to be sampled and held. Used for stimulus artifact rejection.

Buzz ME input:

Logic HIGH on this input activates Buzz.

+5V output:

Used with footswitches to generate logic HIGH level for Buzz. Protected by 150 ohm series resistor.

DETAILED GUIDE TO OPERATION

The controls and operation of the AXOPROBE-1A are described in this section. The topics are arranged in alphabetical order.

AUDIO MONITOR

The audio monitor is a voltage controlled oscillator (VCO) that drives a small speaker. A switch is used to select V_1 or V_2 as the control voltage (V_C). As V_C varies, a so too does the pitch of the audio tone.

When $V_C = 0$ the frequency is about 2.25 kHz. This frequency drops by approximately 3 octaves as V_C decreases to -100 mV.

The Audio Monitor enables changes in the electrode potential to be recognized without having to look at the oscilloscope or panel meters. Thus one can detect a successful cell impalement while still looking through the microscope. There is also an abrupt change in tone when a new electrode first touches the solution. Thus the electrode can be lowered towards the preparation very rapidly and stopped as soon as the tone change indicates contact with the solution.

The volume control on minimum makes the tone inaudible. The volume control can be left in its usual position and the Audio Monitor switched off by using the center position of the input-selector switch.

An earphone can be plugged into the phone jack provided. This disables the speaker.

BATH PROBE

In certain experimental circumstances it is desirable to make all voltage measurements relative to a reference point in the bathing solution rather than relative to ground. (These conditions may include precision measurements during changes of temperature or ion content of the saline, or cases of restricted access from the extracellular space to the grounding point.)

All measurements are normally made relative to the system ground. However, if a unity-gain headstage is plugged into the rear-panel Bath Headstage connector, measurements by both ME1 and ME2 are made relative to the potential recorded by this headstage. The bandwidth of the bath potential is limited to 10 kHz before it is subtracted from the potentials recorded by ME1 and ME2. The bath microelectrode cannot be used for current passing.

If there is no unity-gain headstage plugged into the Bath Headstage connector, a reference potential from an external amplifier can be subtracted by connecting it to the EXT. V_{BATH} connector.

BLANKING

A common problem when using stimulating electrodes is that some of the stimulus is directly coupled into the recording microelectrode. The best way to minimize or even eliminate this artifact is at the source, by using small stimuli, isolated stimulators, placing an earthed shield between the stimulating electrodes and the microelectrodes, etc. Often, though, it is not possible to reduce the artifact to manageable levels. Artifact pickup is particularly apparent on the x100 output because the capacitor used for AC coupling acquires a net charge from the artifact. This charge may take several hundred milliseconds to decay.

The AXOPROBE-1A can circumvent the effects of the stimulus artifact by Blanking. At the moment the logic level of the V_1 BLANK GATE input goes HIGH the value of V_1 is sampled and saved.. For the duration of the HIGH signal, this saved value is used instead of the actual potential.

BUZZ

When the Buzz switch is pressed, an oscillating voltage is applied to the microelectrode via a capacitor in the headstage. (This capacitor is normally used for capacitance neutralization.)

If this is done while the tip of the microelectrode is pressing against the cell membrane, the oscillation will often help the microelectrode impale the cell. The mechanism is unknown, but it may involve attraction between the charge at the tip of the microelectrode and bound charges on the inside of the membrane.

To see the oscillating microelectrode voltage, look at V_e without filtering. The Buzz waveform consists of an alternating series of positive and negative spikes which decay substantially when the oscillation frequency is low.

The Duration control governs the overall period of the burst of oscillation. The pushbutton Buzz switches are debounced. That is, bursts cannot be accidentally triggered when the button is released.

The Frequency control governs the frequency of the oscillation.

The Amplitude control governs the amplitude of the oscillation. This control is linear. The amplitude is attenuated by the electrode capacitance when it is more that a few pF.

The Buzz Parameter controls are shared by the two electrodes. However, the Buzz oscillation only goes to the electrode whose Buzz button is pressed.

Buzz can be activated by the front-panel pushbutton switches. It can also be activated by applying a logic HIGH voltage to the Buzz jack on the rear panel. The logic HIGH voltage can arise from a switch (such as the footswitch provided) used to connect +5 V (red jack) to the Buzz input (violet jack), or by using an external logic device.

CALIBRATION SIGNAL

A calibration signal can be simultaneously superimposed on all of the voltage and current outputs except V_e.

A +100 mV internally generated calibration voltage can be activated by switching the front-panel switch to the CONT. position. With the switch in the EXT. GATE position the +100 mV calibration voltage is off unless a logic HIGH level is applied to the rear-panel 100 mV CAL. GATE input. The OFF position disables the external logic command.

The +100 mV calibration voltage is added to the outputs. The input-referred values depend on the gain of each output.

A calibration voltage proportional to an external signal can be added to the output by applying the external signal to the EXT. CAL. SIGNAL input. The voltage appearing on the outputs will be 20 mV per volt of external signal. Thus the input-referred calibration voltages will be 2 mV/V on the 10V outputs, 0.2 mV/V on the 100V outputs, and 2H nA/V on the I outputs.

CAPACITANCE NEUTRALIZATION AND INPUT CAPACITANCE

The Capacitance (C_{in}) at the input of the headstage amplifier is due to the capacitance of the amplifier input itself (C_{in1}) plus the capacitance to ground of the microelectrode and any connecting lead (C_{in2}). C_{in} combined with the microelectrode resistance (R_e) acts as a lowpass filter for signals recorded at the tip of the microelectrode. Two techniques may be used to increase the recording bandwidth.

(1) Primary

A special technique is used in the headstages to keep the contribution to C_{in} from the input amplifier as small as possible. This consists of adding the input signal voltage to the power supply voltages used by the input stages. This technique, known as bootstrapping, fixes the voltage drop across C_{in1} to a constant value thereby preventing current flow through C_{in1}. The effective value of C_{in1} is thus reduced to well below its real value.

(2) Secondary

A commonly used technique known as capacitance neutralization is used to negate C_{in2} and the effective remnant of C_{in1}. The capacitance neutralization circuit attempts to inject into the headstage input a current which it anticipates will be required to charge and discharge C_{in} during signal changes. To use the capacitance neutralization circuit the voltage response to a current step should be observed on an oscilloscope. Advance the capacitance neutralization control as far as possible without introducing overshoot in the step response. This setting is optimal for current passing and is also optimal for recording potentials at the tip of the microelectrode.

It is important to recognize that the capacitance neutralization circuit is not more than 90% effective even for ideal microelectrodes. This is because of the finite frequency responses of the headstage amplifiers and the capacitance neutralization circuit, and also because C_{in} does not behave ideally as a linear lumped capacitor. Consequently, the amount of C_{in} that the circuit must neutralize should be kept as small as possible. To this end, avoid using long lengths of shielded cable to connect the microelectrode to the input. If possible, plug the microelectrode holder directly into the input. Use shallow bathing solutions. Avoid having grounded objects near the electrode. Do not ground the headstage case.

If metal objects (such as the microscope) must be very near the electrode, they may be disconnected from ground and connected to the gold shield socket in the headstage. This technique may improve the microelectrode response speed.

See also the section titled Microelectrodes for Fast Settling.

CLEAR

There is one Clear switch for each microelectrode. It is used to pass up to ±1000H nA through the microelectrode. "+" and "-" correspond to depolarizing and hyperpolarizing currents respectively. The Clear switch is used for two purposes:

(1) When the microelectrode tip resistance goes high this condition can often be cleared by rapidly toggling the Clear switch from + to -. Because of the large current passed this should only be done extracellularly.

(2) Sometimes microelectrode tips press against the cell membrane but fail to penetrate. A quick flick of the Clear switch will often force the microelectrode to penetrate. Whether to use a hyperpolarizing or depolarizing current depends on the preparation and must be determined by trial and error. Like Buzz, the mechanism for impalement is unknown.

COMMAND GENERATORS

Current commands can be obtained from the internal Step Command generator, from the internal DC Command generators, from the internal Pulse Command generators, and from external sources.

All commands are scaled by the headstage current gain (H). Depolarizing commands are indicated by "+", hyperpolarizing commands by "-".

The durations of the internal commands are either continuous, or gated by external logic HIGH levels used to activate the commands.

Step Command Generator

The Step Command generator is shared by the two microelectrode amplifiers. Use the Destination switch to direct the command to ME1 or ME2.

The current indicated on the thumbwheel switch (scaled by H) is passed through the selected microelectrode when the front-panel switch is in the CONT. position. With the switch in the EXT. GATE position the command is off unless a logic HIGH level is applied to the rear-panel STEP GATE input. The OFF position disables this external gating.

The maximum command which can be set on the thumbwheel switch is 199.9H nA. An LED corresponding to the selected microelectrode lights up when the Step Command is activated. When rotating the thumbwheel switch, be decisive. If the switch is rotated slowly the output will momentarily fall to zero as the switching contacts pass through an open-circuit state.

DC Current Command

The current indicated on the ten-turn dial is passed through the microelectrode when the switch is in the +ON or -ON position. The DC Current Command cannot be gated on and off by external logic signals. An LED illuminates when the command is on. The maximum DC Current Command is 100H nA.

Pulse Current Command

The current indicated on the ten-turn dial is passed through the microelectrode when the activation switch is in the CONT. position. With the switch in the EXT. GATE position the command is off unless a logic HIGH level is applied to the rear-panel PULSE GATE input. The OFF position disables this external activation. An LED illuminates when the command is on. Polarity is set on a separate switch.

The maximum Pulse Current command is 1000H nA. There may be a small residual current command whem the dial indicates zero. Switching to the OFF position will eliminate this residual command.

External Command Inputs

Two external command inputs are provided. These are for setting the current in ME1 (EXT. ME1 COMMAND) and the current in ME2 (EXT. ME2 COMMAND). These external commands are active simultaneously with the internal command generators. The sensitivity is 20H nA per volt of external signal.

The external command inputs are DC connected. Therefore, any deviation from zero volts in the external signal source while it is in its "off" state will cause a DC current to flow in the electrode.

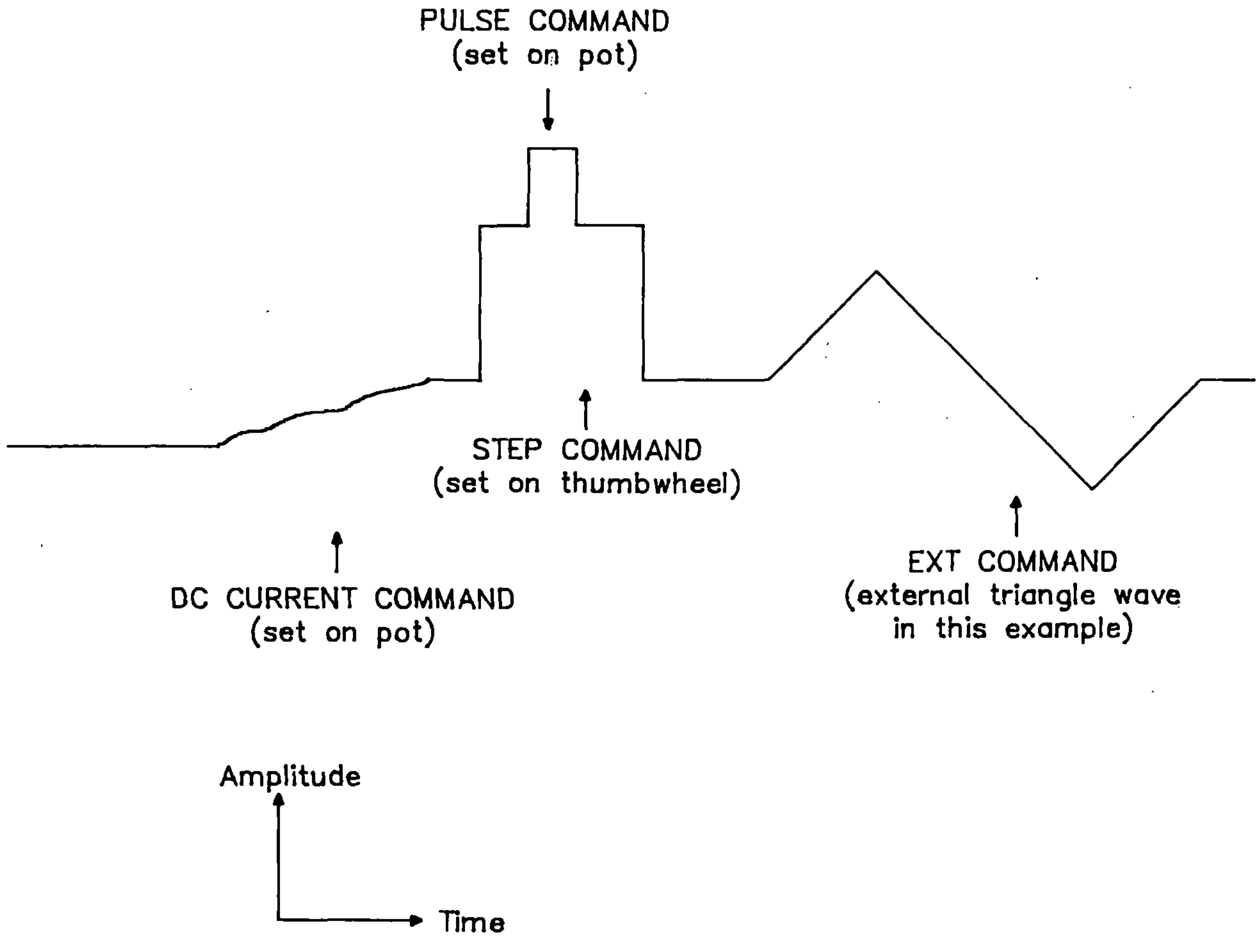
This can be avoided by using:

(1) A very high-quality external source which puts out a true zero voltage level in its off state or which can be trimmed to do so.

(2) An isolated external source.

Mixing Commands

Complex command waveforms can be generated by appropriately mixing the Step Command, the DC Command, the Pulse Command and the Ext. Command. For example, the command waveform in the following figure can be used to establish the current injected into ME1 by setting the Destination switch to the ME1 position and using the ME1 DC Command, the Step Command, the Pulse Command and the Ext. ME1 Command input.



SUMMATION OF COMMANDS

This figure shows the command potential that would result if all command sources were switched on one at a time and left on.

CURRENT MEASUREMENT

The actual current through each microelectrode is independently measured. If the electrode blocks, the measured current falls to zero even though a current command may exist.

The current output is proportional to the voltage drop across a resistor (R_O) inside the headstage. The value of R_O depends on the headstage current gain (H). Thus the current output scaling depends on H. It is $10 \div H$ mV/nA.

C_x COMP

The Cx COMP control is used to compensate microelectrode 1 for voltage transients occurring in microelectrode 2. There are two modes of operation:

1) ENHANCEMENT: for ion-sensitive electrode recording

When the differential signal $10(V_1-V_2)$ is recorded there is often a common-mode signal on both electrodes. For example, when ion concentrations are measured the signal of interest is the difference between the signals recorded by the ion-sensitive electrode (ME1) and the reference electrode (ME2). If the electrode pair is intracellular both electrodes will record changes in membrane potential.

Unless the response speeds of the two electrodes are identical there will be a transient in the differential signal due to imperfect cancellation of the common-mode signal (membrane potential).

The primary means of eliminating this transient is to try to match the electrode response speeds. Use the Capacitance Neutralization controls to speed up the slower electrode and to slow down the faster electrode. In addition, consider using a high-resistance electrode for the reference (e.g. a neutral-resin electrode).

When the two response speeds cannot be matched this way the C_x COMP control can help. This control enhances the effective coupling capacitance (C_x) from ME2 into ME1. This speeds up the ME1 response to common-mode signals so that common-mode signals are recorded identically by ME1 and ME2. Thus the transient in the differential signal is eliminated. Note that the speed for recording differential signals is not improved.

2) NEUTRALIZATION: for double-barrel current passing

In some experiments, one barrel (ME2) of a double-barrel microelectrode is used for current-passing while the other barrel (ME1) is used to record the voltage response. Neglecting complications, this technique is preferrable to the more usual one of using a single-barrel electrode for both tasks because fluctuations in the electrode resistance during current passing do not affect the recorded potential.

There are two complications which reduce the usefulness of the double-barrel technique. These are: 1) transient coupling through the coupling (or cross) capacitance (C_x), and 2) DC coupling through the coupling (or cross) resistance (R_x) (see Figure at the end of this section).

Transient Coupling

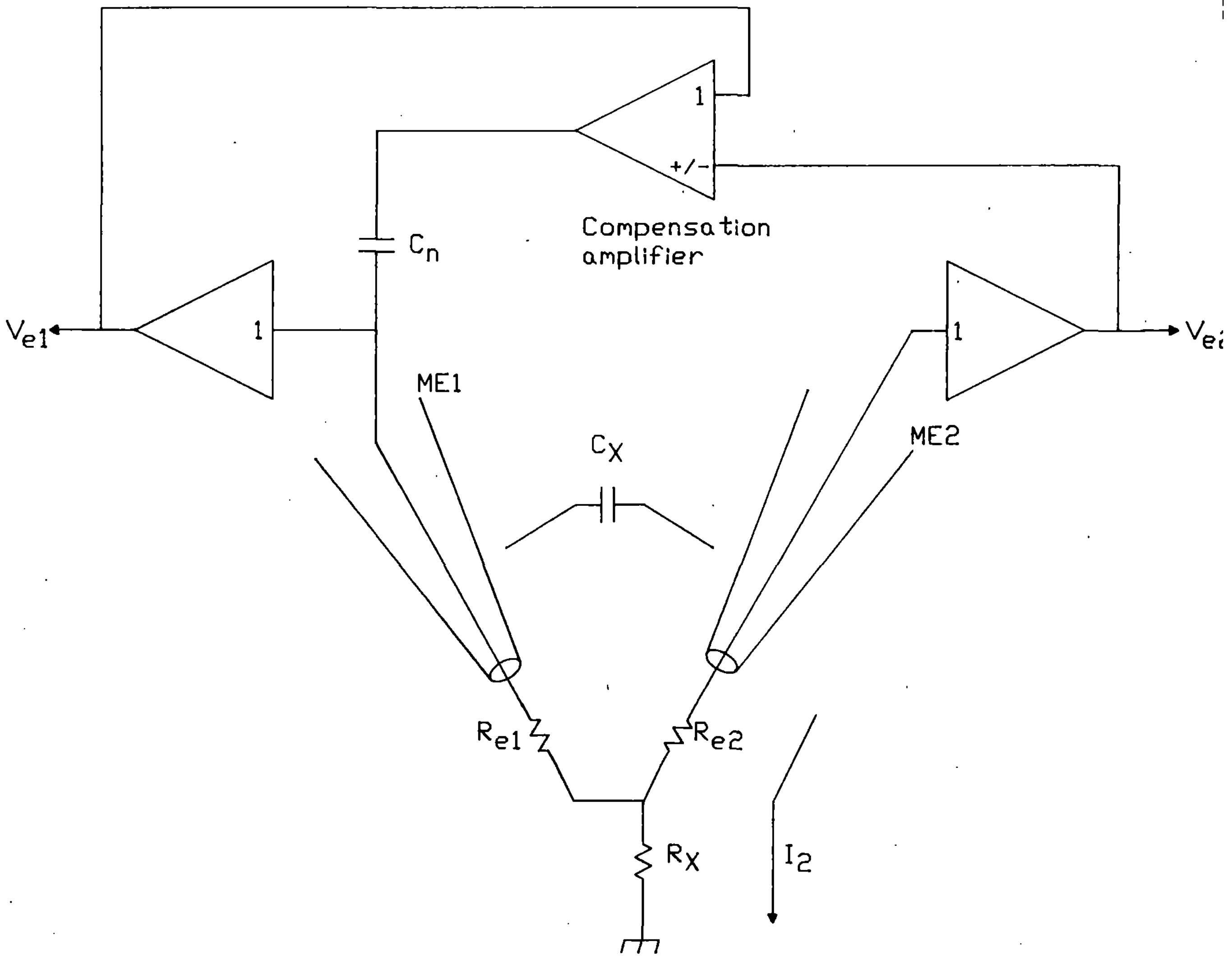
When a current step is applied to the ME2 barrel, a voltage transient couples through the capacitance (C_x) of the separating glass wall into the ME1 barrel. The C_x COMP potentiometer can be used to eliminate this transient. First, pull the knob to select NEUTRALIZE mode. Second, while a repetitive current step is being applied to ME2, rotate the C_x COMP potentiometer until the transient on $10V_1$ is eliminated.

Note that this control works in one direction only. It operates by injecting a compensating current into ME1 derived from the voltage in ME2.

DC Coupling

The coupling resistance (R_x) is poorly named. It is actually a fraction of the ME2 resistance whose voltage drop is measured by ME1. This voltage drop is proportional to R_x and I_2.

The ME1 Resistance Compensation potentiometer can be used to eliminate this transient. When the C_x COMP control is in the NEUTRALIZE position, an LED lights to indicate that the ME1 Resistance Compensation is reconfigured to compensate the ME1 voltage for the ME2 current. While a repetitive current is being applied to ME2, rotate the ME1 Resistance Compensation potentiometer until the steady-state step on $10V_1$ is eliminated.



RESISTANCE COMPENSATION PROCEDURE

Direct coupling between two microelectrodes.

R_x; coupling resistance. C_x; coupling capacitance.

GROUNDING AND HUM

A perennial bane of electrophysiology is line-frequency pickup (noise), often referred to as hum. Hum can occur not only at the line frequency but also at multiples of it.

The AXOPROBE-1A has inherently low hum levels (less than 20 µV peak-to-peak). To take advantage of these low levels great care must be taken when integrating the AXOPROBE-1A into a complete recording system. The following procedures should be followed.

(1) **Only ground the preparation bath by directly connecting it to the yellow ground connector on the ME1 headstage.**

(2) Place the AXOPROBE-1A in a position in the rack where transformers in adjacent equipment are unlikely to radiate into its electronics. The most sensitive part of the AXOPROBE-1A electronics is the right-hand half of the bottom side looking from the front.

(3) Initially make only one connection to the AXOPROBE-1A. This should be to the oscilloscope from the F1, V_{e1} or $10V_1$ outputs. Ground the ME1 headstage input to the yellow ME1 ground connector. After verifying that the hum levels are low, start increasing the complexity of the connections one lead at a time. Leads should not be draped near transformers located inside other equipment. In desperate circumstances the continuity of the shield on an offending coaxial cable can be broken.

(4) Try grounding auxilliary equipment from a ground distribution bus. This bus should be connected to the AXOPROBE-1A via the yellow banana (4 mm) socket on the rear panel. This socket is connected to the AXOPROBE-1A's signal ground (i.e. the outer conductors of all the BNC connectors). The signal ground in the AXOPROBE-1A is isolated from the chassis and power ground.

(5) If more than one headstage is used, all the headstage cables should run from the AXOPROBE-1A to the preparation in a bundle. The bundle can be formed either by gently twisting the cables together or by loosely tying them together.

(6) Experiment. While hum can be explained in theory (e.g. direct pickup, earth loops), in practice the ultimate theory is the end result. Following the rules above is the best start. The final hum level can often be kept to less than 100 µV peak-to-peak referred to V_e. One technique that should **not** be used to reduce the hum is the delicate placement of cables so that a number of competing hum sources cancel out. Such a procedure is too prone to accidental alteration.

HEADSTAGES

The headstage buffers the high impedance of the microelectrode, making the potential recorded by the microelectrode available to the rest of the circuitry. It also provides the means for injecting current into the microelectrode and for neutralizing the input capacitance.

The standard headstages for the AXOPROBE-1A are the HS-2 series.

The Meaning of H

A precision resistor (R_o) inside the headstage sets the headstage curent gain (H). Larger H values correspond to smaller R_o values. The particular value of H used affects the Resistance Compensation range, the sensitivity to current commands, the sensitivity of the current monitors, the input resistance and the input leakage current. The effects on the control ranges (see Table 1) are clearly marked on the front and rear panels, and since they always appear in multiples of 10 they are easy to calculate. The effects on headstage performance are listed in the table in the specifications.

Which Headstage to Use

The H value required depends on the typical input resistances (R_{in}) of your cells. The recommended H values are in Table 1.

Table 1

H - dependent values

H = x0.0001 for ion-sensitive electrodes.
H = x0.01 for R_{in} greater than approx. 300 MΩ.
H = x0.1 for R_{in} approx. 30-300 MΩ.
H = x1 for R_{in} approx. 3-30 MΩ.
H = x10 for R_{in} approx. 0.3-3 MΩ.
Electrode Resistance Compensation: $100 \div H$ MΩ max.
DC Current Command: 100H nA max.
Pulse Current Command: 1000H nA max.
Step Command: 199.9H nA max.
Current Output: $10 \div H$ mV/nA.
$R_o = 10 \div H$ MΩ.

Some overlap in these recommendations is allowable. The guiding principles are these:

(1) For fastest responses use the **largest** feasible H value.

(2) A limitation on using large H values is that as R_o becomes smaller the input leakage current of the headstage becomes more prone to increase with time and temperature (see Input Leakage Current later in this section).

(3) A further limitation on using large H values is that if R_o is less than the microelectrode resistance (R_e) the high-frequency noise increases.

(4) The H value sets the current-passing sensitivity. Hence it should be chosen for sensitivities suitable for your cells.

(5) If $R_e \gg R_{in}$ a smaller H value should be favored.

Capacitance Neutralization Range

HS-2 Series headstages are available with L or M suffixes permitting a maximum of 8 or 22 pF of Capacitance Neutralization respectively. The increased Capacitance Neutralization range is a trade-off against microelectrode noise. The HS-2L has the lowest noise, close to the theoretically predicted thermal noise of the electrode. The HS-2M has about 20% extra noise.

Headstage Connectors

There are three teflon-insulated 2 mm (0.08 inch) sockets in the headstage (see Figure at end of this section). These are standard-diameter sockets.

1. Microelectrode Input Connector

The **red** socket is the microelectrode input. The connection between the microelectrode and this socket should be kept as short as possible. Two excellent methods are to:

(i) Solder a silver/silver-chloride wire directly to one of the 2 mm plugs supplied. Use the wire to connect to the microelectrode which can be supported on a separate mounting.

(ii) For greater mechanical stability, use an HL-2 series microelectrode holder form Axon Instruments.

(iii) Plug a standard microelectrode holder (2 mm plug) directly into the input socket. The teflon input socket should allow enough clearance for most standard holders.

(iv) Use a BNC-type microelectrode holder. This requires an HLB-2 adaptor from Axon Instruments.

2. Shield Drive Connector

The Shield drive (**gold** socket) is connected to the gold-plated socket and to the case. The drive is protected against continuous short circuits, however for best frequency response **the case must not be grounded.** In general, this necessitates using an insulated mounting for the headstage (such as the rod provided).

The shield connection is provided primarily for driving the shield of microelectrodes prepared for deep immersion (see notes in Microelectrodes for Fast Settling Section). It may also be used for driving metal objects near the input, or even the hutch in which the preparation is housed. It can be used for driving the shield of a coaxial cable used to connect the microelectrode to the input, although it is not recommended that the microelectrode be connected in this way (see below). If not used, the shield socket is simply left unconnected.

There are two reasons why we do not recommend using shielded cable to connect the microelectrode to the headstage:

1) Shielded cables add significant input capacitance. The shield drive circuit mostly removes the effect of this capacitance on electrode response speed. However, from a noise point of view the capacitance remains and causes an increase in high-frequency electrode noise.

2) The leakage resistance of shielded cable can degrade the input resistance when used with ion-sensitive and other high-impedance electrodes. If shielded cable is used it should have teflon as the insulating material between the shield and the inner conductor.

To optimize the response speed of low and medium impedance electrodes (up to approx. 300 MΩ) when a driven shield is used, the shield of headstages with H = 0.1 and larger is driven from the capacitance neutralization circuit. To optimize the headstage input resistance when a driven shield is used, the shield of headstages with H = 0.01 and smaller is driven from the output of the unity-gain buffer inside the headstage.

If a shielded cable is being used and unusual electrode responses are observed, try disconnecting the shield.

3. Ground Output Connector

The **yellow** ground socket of the ME1 headstage is used for earthing the preparation. Using this connection as the preparation ground minimizes hum.

Tip Potentials - Detection

During the passage of current the tip potentials of many electrodes change. Changes in tip potential are indistinguishable from the membrane potential and can therefore represent a serious source of error. To prevent this error the following checks should be made.

(1) While the microelectrode is outside the cell, set the offset to zero. Pass a constant current into the bath for about 10 seconds. The current magnitude should be the same as the maximum sustained current likely to be passed during the experiment. When the current is switched off the recorded

potential should return to zero within a few milliseconds at most. Some electrodes either return very slowly to zero potential, or not at all. These electrodes should be discarded.

(2) Once the experiment is in progress occasionally check the resistance of the microelectrode. Changes in tip potential are usually accompanied by changes in electrode resistance.

Tip Potentials - Prevention

Not much can be done to prevent tip potentials from changing but the following may be helpful.

(1) Sometimes the slow changes in tip potentials are worse when standard microelectrode holders with an embedded AgCl pellet are used instead of an Ag/AgCl wire. Some holders are alright while other ostensibly identical holders are not. Therefore holders should be tested and selected.

The variability of the tip potentials may in some way be related to pressure developed when the microelectrode is pressed into the holder. A narrow hole drilled into the side of the holder to relieve pressure might help.

(2) Using filling solutions with low pH, or adding small concentrations of polyvalent cations like Th^{4+}, may reduce the size of the tip potential (Purves, 1981) and therefore the magnitude of any changes.

Interchangeability

Any unity-gain headstage in the HS-2 series can be used for ME1 or ME2. The equipment will not be damaged if headstages are exchanged while the AXOPROBE-1A is switched on.

Cleaning

To clean salt spills from the input connectors wipe with a damp cloth. Avoid spilling liquids on the headstage.

Input Leakage Current and How to Trim It to Zero

All DC-connected systems suffer from the problem of drift. With changes in temperature and the passage of time, the DC offsets of all semiconductor devices can drift by many millivolts away from their initial values. The major worry in a microelectrode system is that the cumulative effects of drift in various parts of the circuit may lead to the development of a DC offset across the resistor (R_o) used to set H. As a result, an undesirable DC leakage current is injected into the microelectrode.

Careful consideration to this problem has been applied throughout the design of the AXOPROBE-1A, and the overall DC offset has been made as insensitive as possible to drift in the integrated circuits. As well, special low-drift integrated circuits have been used in all critical positions. The magnitude of the DC leakage current increases with H. This normally introduces no greater error in the DC offset voltage developed across the microelectrode or the cell membrane because larger H values are usually used with lower-resistance cells and microelectrodes.

Before leaving the factory, the DC offset voltage of each HS-2 headstage is trimmed so that the input leakage current is no more than

100	pA	for	H = x10
10	pA	for	H = x1
1	pA	for	H = x0.1
1	pA	for	H = x0.01
10	fA	for	H = x0.0001

These input current levels are very low and cause negligible shifts in the cell membrane potential (V_m) when the headstages are used with the recommended ranges of cell input resistances (see Table 1).

If you ever suspect that the input current has grown to a level where V_m is significantly affected, it can be re-adjusted by the following procedure.

(1) Switch off all current commands and disconnect any external current commands. Set the Resistance Compensation potentiometer to zero.

(2) Remove the plastic cap from the access hole in the headstage cover.

(3) Ground the headstage input via a resistor equal to $R_o \div 10$ (where R_o is given in Table 1). On an oscilloscope at 2 mV/div observe the 10V output through the filter set to a 10 Hz cutoff frequency. Use the Offset control to center the trace on the screen.

(4) Now ground the headstage input via a resistor equal to R_o (2) in Table 1. Observe the shift of the oscilloscope trace.

(5) Repetitively swap from grounding via $R_o \div 10$ to grounding via R_o. Adjust the trim pot inside the headstage until there is no shift.

Note 1. For values of 1 GΩ or more it is important to clean the surface of the resistor thoroughly to remove leakage pathways.

Depending on the reason for a trim being necessary, the trim procedure may have to be repeated if the headstage is changed.

Warning

If an external source is connected to the Ext. ME1 or ME2 Command input, any time this source is nonzero a proportional current will flow in the microelectrode. Many external sources do not put out a true zero voltage when in the "off" state, thus there may be an unwanted electrode current due to the fact that an external source is connected. To avoid this, use an external source in which you can adjust the off-state voltage, or use an isolated external source.

DC Removal

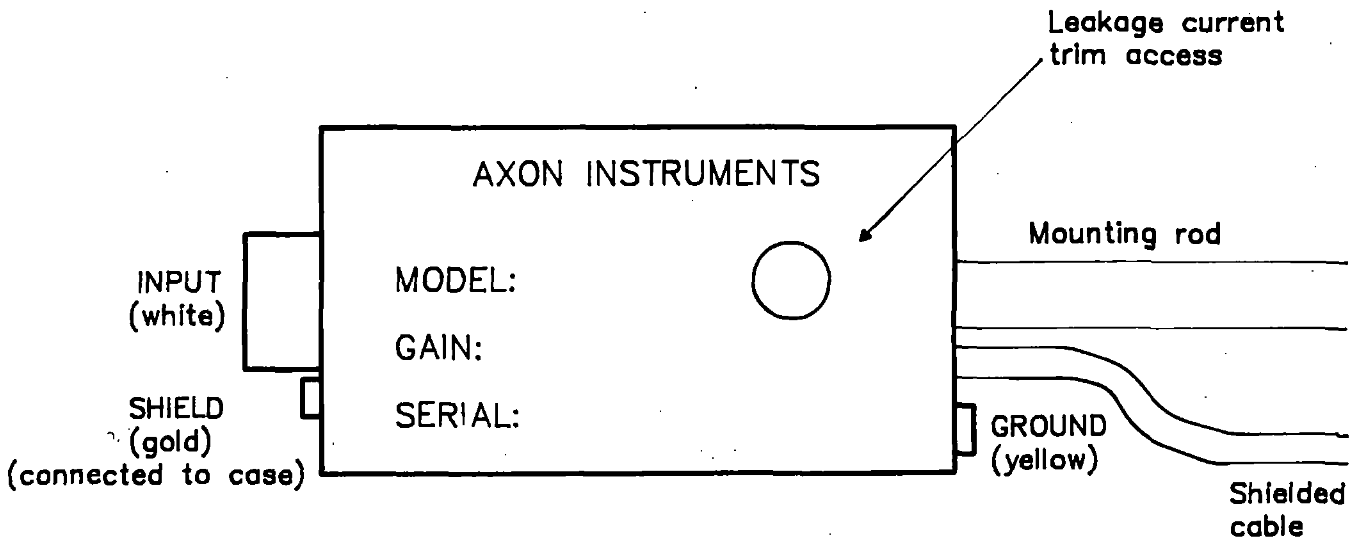
One potential source of a small but variable input leakage current is due to DC current flow through the dielectric of the capacitor (C_n) used for capacitance neutralization. For example, the electrode potential might be 200 mV (though the experimenter does not see this potential because of the output offset compensation). To compensate several pF of input capacitance the gain of the capacitance neutralization circuit might be 2. Thus 400 mV would be fed back to C_n resulting in 200 mV across it. If the dielectric resistance of C_n were 10^{11} Ω (the guaranteed minimum of high-quailty capacitors) there would be 2 pA flowing through the capacitor.

To eliminate this source of leakage current, a DC removal circuit in all HS-2 series headstages removes the DC voltage from across C_n. The DC removal circuit operates with a 1 s or 10 s time constant. There may be a transient shift in the electrode voltage while the Capacitance Neutralization control is being adjusted, but no net charge is injected into the electrode. The DC voltage is also removed from the shield drive.

Input Resistance

The input resistance of the headstages is predommantly related to R_o. A circuit inside the AXOPROBE-1A called a constant current source (CCS) controls the voltage across R_o. Ideally, the voltage across R_o is independent of the electrode voltage. The accuracy of the CCS in controlling the voltage across R_o is preset at the factory. Extremely stable components are used in the CCS so that the accuracy will not deteriorate with time. In general, the CCS is effective to one part in 10^4 so that the input resistance is $10^4 R_o$.

Other possible factors which would decrease the input resistance are minimized. For example, the field effect transistor (FET) input of the headstage amplifier is referenced to the input voltage rather than to ground. This technique is known as bootstrapping. Thus the effective resistance of the input is much greater than the already high resistance of the FET. Leakage current and resistive loading through the insulation of the input socket are minimized by using Teflon insulation and by driving the case with the DC input voltage.



Notes: "Model" may be HS-2L or HS-2M

"Gain" refers to headstage current gain (H)

HS-2 HEADSTAGE CONNECTION DIAGRAM

HOLDERS

Features

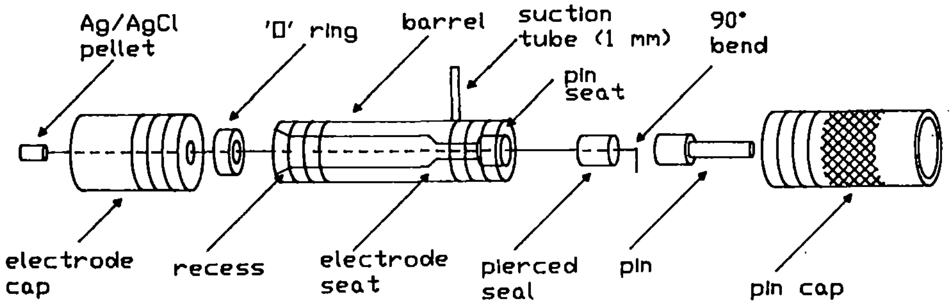
The HL-2 series holders have been designed for low-noise mechanically stable microelectrode recordings with or without suction. The body of the holders are made out of polycarbonate for lowest noise and easy cleaning. Maintenance is simple because the holder can be fully disassembled for cleaning and parts replacement.

Mechanical stability of the electrode is assured several ways. For example, as the electrode cap is closed, the 'O' ring is forced into a special recess and pulls the electrode firmly back into the holder so that its end presses tightly against the electrode seat. The holder mates firmly with the special teflon connectors on the HS-2, HS-4 and VG-2 series headstages. A 2 mm diameter pin is used for the electrical connection.

The holders are designed to emerge along the long axis of the headstage. A right-angle adapter can be purchased if it is necessary for the holder to emerge at 90° from the headstage. A BNC-to-Axon adaptor (HLB-2) can be purchased if you wish to use third-pary BNC-style holders.

Parts

The various parts of the holders are shown in the exploded view:



Five spare 'O' rings and one spare pierced seal are provided with each holder. Additional 'O' rings, pierced seals, pins and Ag/AgCl pellet assemblies can be purchased from Axon Instruments.

HL-2-12 holders use a plain Ag wire and 'O' rings with a 1.2 mm hole. HL-2-17 holders use a Ag/AgCl pellet assembly and 'O' rings with a 1.7 mm hole.

To replace the silver wire, insert the nonchlorided end through the hole of the pierced seal and bend the last 1 mm over to an angle of 90°. Press the pierced seal and the wire into the pin seat. Push the large end of the pin down onto the bent-over wire and into the pin seat. This assures good electrical contact. Screw the pin cap down firmly but without excessive force.

USE

Insertion of electrode

Make sure the electrode cap is loosened so that pressure on the 'O' ring is relieved, but do not remove the electrode cap. Push the back end of the electrode through the electrode cap and 'O' ring until it presses against the electrode seat. Gently tighten the electrode cap so that the electrode is gripped firmly.

To minimize cutting of the 'O' ring by the sharp back end of the electrode, you can smooth the electrode edges by rotating the back end of the electrode in a bunsen burner flame.

Cleaning

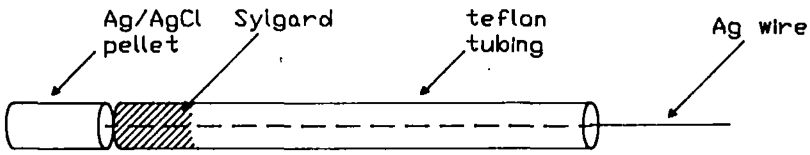
For lowest noise, keep the holder clean. Frequently rinse the holder with distilled water. If heavier cleaning is required, briefly wash in ethanol or mild soapy water. Never use methanol or strong solvents.

Filling electrodes

Only the taper and a few millimeters of the shaft of the electrode should be filled with solution. The chlorided tip of the wire should be inserted into this solution. Avoid wetting the holder since this will increase the noise.

Silver chloriding

The HL-2-17 holders are supplied with a Ag/AgCl pellet that should give you many months of DC-stable recordings. The silver wire is surrounded by a Sylgard-sealed teflon tube. This ensures that the electrode solution only contacts the Ag/AgCl pellet.



It is not practical to make a pellet small enough to fit inside the shaft of the narrow glass electrodes used in the and HL-2-12 holders, therefore these holders are supplied with a piece of 0.25 mm silver wire. It is up to you to chloride the end of this wire as required. Chloriding procedures are contained in many electrophysiology texts (e.g. Purves, 1981). Typically the chlorided wire will need to be replaced every few weeks.

Heat smoothing the back end of the electrode extends the life of the chloride coating by minimizing the amount of scratch damage. Another way to protect the AgCl coating is to slip a perforated teflon tube over the chlorided region.

The chlorided region should be long enough so that the electrode solution does not come in contact with the bare silver wire.

Glass Dimensions

Use the HL-2-12 holders for glass from 1.0 to 1.2 mm outside diameter (OD). The optimal dimensions are 1.15 mm OD and >0.5 mm ID.

Use the HL-2-17 holders for glass from 1.5 to 1.7 mm outside diameter (OD). The optimal dimensions are 1.65 mm OD and >1.1 mm ID.

For other glass dimensions you can drill out the bore of the HL-2-12 holder.

IN USE/STANDBY

The In Use/Standby switch is used to put the microelectrode 2 amplifier into a standby state when it is not being used. During Standby: 1) the digital panel meter is turned off to indicate that ME2 is not in use and 2) the Capacitance Neutralization of ME2 is set to its minimum value. This ensures that the open-circuited headstage will not oscillate and interfere with the ME1 recording.

IONOPHORESIS

Either of the electrodes can be used for ionophoresis.

Set the retaining current on the DC Current Command control.

Set the ejection current on the Pulse Current Command control. Remember that commands add. Therefore the ejection current will be smaller than the commanded Pulse current by the amount of the retaining current. The panel meter and the current outputs will indicate the true currents.

e.g. For retaining current = -5nA
ejection current= 80nA

Set DC Current Command = -5nA
Pulse Current Command = 85nA

Not all of the 30 volt output of the AXOPROBE-1A is applied to the electrode. Some of it is lost as a voltage drop across the current-setting and measuring resistor (R_o) inside the headstage. Therefore choose a headstage in which $R_o < R_e$ (the electrode resistance). The H = 1 headstage (with R_o = 10 MΩ) is the best general choice for ionophoresis. In this case, with a 100 MΩ electrode, passage of 250 nA results in 25 V being applied to the electrode and 2.5 V being dropped across R_o.

ION SENSITIVE ELECTRODES - SPECIAL CONSIDERATIONS

Buzz

If the Buzz amplitude and duration are above a certain level some resins will charge up during Buzz and take several minutes to decay to their normal potential. The maximum allowable amplitudes and durations must be determined by experiment for each type of resin and electrode.

Capacitance Neutralization

When adjusting the capacitance neutralization a transient change in electrode potential may be seen. It is due to displacement current through the capacitance neutralization capacitor. There is no DC change because the DC removal circuit (see Headstages section) inside the headstage removes the DC voltage from across the headstage. Even the minute charge which flowed during the transient is ultimately removed from the electrode.

MICROELECTRODES FOR FAST SETTLING

MICROELECTRODE CAPACITANCE

To get extremely fast settling it is essential to minimize the transmural capacitance (C_t) from the inside of the microelectrode to the external solution. C_t is usually 1-2 pF per mm of immersion. Two applications requiring different approaches are discussed here.

(1) Target cell near surface of solution.

In an isolated preparation, C_t can be reduced by lowering the surface of the solution as far as possible. Precautions must be taken to prevent surface tension effects from drawing a thin layer of solution up the outer wall of the microelectrode. If this film of saline is allowed to develop, C_t will be much worse that otherwise. Because the film of saline has axial resistance the contribution to C_t will be very nonlinear, and the capacitance neutralization circuit will not be able to cope with it. To prevent the saline film from developing, the electrode should be coated with a hydrophobic material. This can be done just before use by dipping the **filled** microelectrode into silicone oil or mineral oil. Another method is to coat the electrode with Sylgard (Hammill et al., 1981).

(2) Target cell deep in solution.

In some preparations, e.g., in vivo CNS, the target cell is several millimeters below the surface of the solution. In this case the more difficult procedure of guarding the electrodes may have to be used. This involves coating the outside of the microelectrode with a metal layer and connecting this layer to the case socket of the headstage. This procedure does not reduce C_t. Instead, it reduces the **effect** of C_t by controlling the voltage across it. The metal guard layer must be insulated from the preparation solution. For different approaches to this method see Schwartz & House (1970), Suzuki, Rohlicek & Fromter (1978), Sachs & McGarrigle (1980) and Finkel & Redman (1983).

Because of the distributed nature of the axial resistance of the microelectrode, of the axial resistance of the metal layer, and of C_t, the shielding technique is not perfect. In practice, the effect of these nonidealities is to cause the step response of the microelectrode to overshoot. To overcome this tendency, the Capacitance Neutralization circuit has a minimum less than unity.

MICROELECTRODE RESISTANCE

Another important aspect of the microelectrode is the tip resistance (R_e). This should be as low as possible consistent with good impalements of the cell. There are two advantages associated with low values of R_e:

(1) Settling time.

The decay time constant for the microelectrode voltage after a current pulse depends strongly on R_e. Hence, lower R_e values produce faster settling times. As well, high R_e values are sometimes associated with a slow final decay even after C_t has been eliminated.

(2) Stability

R_e of most microelectrodes changes with time and with current passing. R_e is affected not only by the magnitude of the current but also by its polarity. In general, microelectrodes of low resistance are more stable during current passing than microelectrodes of high resistance.

FILLING SOLUTIONS

The best filling solution to use depends on the preparation under investigation and the experience of the investigator. Although KCl gives one of the lowest tip resistances for a given tip diameter it is not necessarily the fastest to settle after a current pulse. K-citrate is sometimes faster.

It is important to be aware that during current passing, large amounts of ions from inside the microelectrode can be ionophoresed into the cell. For example, if current is passed by the flow of ion species A from the microelectrode into the cell, then after 50 seconds of current at 1 nA (or 1 second of current at 50 nA) the change in concentration of A inside a cell 100 µm in diameter is 1 mM. If A is an impermeant ion, the cell may swell due to the inflow of water to balance the osmotic pressure.

RECOMMENDED READING

A small book by Purves (1981) serves as an excellent general reference on microelectrode techniques.

MODELS

We recommend that you practice using the AXOPROBE-1A on an RC cell model. The resistor provided with each headstage can be conveniently used to simulate the microelectrode and the RC cell model can be soldered directly to the free end.

OFFSET

The Offset controls compensate for the junction potentials in the experimental setup.

The offset compensation works by adding a DC voltage to the 10V output. The electrode voltage and current are not affected.

The compensation range is ±500 mV. The no-compensation point is in the middle of the range of the multiturn dials. Each turn of the dials is approximately 100 mV. The dials can be locked after setting.

The normal procedure for using the Offset controls is to zero the output when the microelectrode is outside the cell. All subsequent readings are then with respect to the potential of the extracellular solution.

OUTPUT FILTERS

Two filters are provided. These are second-order lowpass filters. Twelve -3 dB frequencies (f_L) can be selected.

Order

The "order" of a filter refers to the number of poles (RC sections). Each pole attenuates the high-frequency noise by 20 dB/decade (= 6 dB/octave).

Type

An ideal lowpass second-order filter passes all signals at frequencies below f_L with no attenuation, and attenuates all signals at frequencies above f_L by 40 dB/decade. Practical lowpass filters only approximate this characteristic. There is a gradual attenuation within the pass band, reaching -3 dB at f_L.

Filters can be designed to closely approximate the ideal amplitude vs. frequency response (e.g. Butterworth or Chebyshev) but in this case the step response has considerable overshoot. By allowing a poorer approximation, the overshoot in the step response can be made smaller (e.g. < 1% in a Bessel filter) or eliminated altogether (e.g. multiple coincident pole). The AXOPROBE-1A filters are of the multiple coincident pole type. For aficionados this means that Q = 0.5; zeta = 1.

Rise Time

Lowpass filters also slow the rise time of the signal. The rise time depends on f_L. The 10-90% rise time of each filter in the AXOPROBE-1A is:

$$t_r \approx 0.35(f_L)^{-1}$$

where f_L is in Hz.

If an external filter is used and it has < 10% overshoot then its t_r will be within a few percent of the t_r value just given. Some manufactures specify the -3 dB frequency based on the phase response of the filter instead of its amplitude response. Before using an external filter it is advisable to check t_r for a step signal applied to the input.

When a signal with 10-90% rise time t_1 is passed through a filter with 10-90% rise time t_2 the rise time of the output signal is approximately

$$t_r = \sqrt{(t_1^2 + t_2^2)}$$

OUTPUT IMPEDANCE AND PROTECTION

All outputs (except V_{BATH}) are protected by 500 Ω output resistors. When loaded by the 1 MΩ input resistance of typical laboratory recording instruments this output resistance causes 0.05% error.

All protected outputs can withstand a continuous short circuit to ground or any voltage in the ±15 V range. However, in keeping with normal practice, such short circuits should be avoided.

The V_{BATH} output has < 1 Ω output impedance so that it can drive the relatively low external bath input impedance of another AXOPROBE. This output can withstand a continuous short circuit to ground but not to ±15 V.

PANEL METERS

Voltage

One meter can display V_1 or V_1-V_2. The second displays V_2. The displays are in mV. These unity-gain voltages are derived from the x10 outputs. Thus they include the correction potentials (Resistance Compensation, Offset, Bath Potential, Calibration).

Since the x10 outputs saturate at about $\pm$13 V, the digital displays saturate at about $\pm$1300 mV.

Current

The third meter displays I_1 or I_2. The required current is selected on the Display switch.

The decimal point is automatically placed to correspond to the current gain (H) of the headstage in use. For H = x1, x0.1, x0.01 the display is in nA. For H = x0.0001 the display is in pA. For H = x10 or more the display is in µA.

The current meter is placed directly above the thumbwheel switch of the Step Command generator. When setting this switch one can avoid the mental effort of scaling by H by taking the decimal point position directly from the current meter.

Round-Off Error and Zero Error

Digital meters are often out by one unit in the least significant (right-most) digit. This is because the reading is rounded off to the closest value.

The current meter may indicate 001 even when no current is commanded. It is most likely that this is a zero error in the current measurement circuit and meter, and not due to input leakage current.

POWER-SUPPLY GLITCHES

The AXOPROBE-1A has been designed to minimize the effects of power-supply transients (glitches). This is achieved by 1) taking the incoming power through a radio frequency interference (RFI) filter, and 2) capacitively isolating the transformer primaries and secondaries.

Nevertheless, some power-supply glitches do get through. These can cause transients to appear on the voltage and current outputs which may corrupt high-gain recordings (for example, during noise analysis).

The only completely effective way to gain immunity from mains glitches is to eliminate them at their source. Most glitches are due to the switching on and off of other equipment and lights on the same power-supply circuit. Precautions to be taken include:

(1) Avoid switching equipment and lights on or off while recordings are being made.

(2) Water baths, heaters, coolers etc. should operate from zero-crossing relays.

(3) RFI filters should be installed in glitch-producing equipment.

In most circumstances occasional transients on the outputs are inconsequential and therefore no precautions will have to be taken.

POWER SUPPLY VOLTAGE SELECTION & FUSE CHANGING

Supply Voltage

The AXOPROBE-1A can work from all international supply voltages. The two input ranges are:

(1) 115 V : For 100 Vac to 125 Vac operation.

(2) 230 V : For 200 Vac to 250 Vac operation.

To change the supply voltage setting:

(1) **Disconnect the power cord**

(2) Remove the top cover

(3) Locate the slide switch labeled "S2" at the back of the power supply board. The power supply board is the small horizontal board in the left side of the instrument.

(4) For 115 V operation slide S2 to the left towards the label "115".

For 230 V operation slide S2 to the right towards the label "230".

(5) Replace the top cover.

(6) Re-connect the power cord.

(7) Mark the new operating voltage on the identification plate on the rear of the instrument.

Changing the Fuse

The AXOPROBE-1A uses a 0.5 A 250 V slow-acting 5 x 20 mm fuse on both voltage ranges. Before changing the fuse investigate the reason for its failure.

To change the fuse:

(1) **Disconnect the power cord.**

(2) Use a screwdriver or something similar to lever out the fuse holder.

(3) Discard the fuse from the active slot, i.e. the slot which places the fuse closest to the inside of the instrument.

(4) Shift the spare fuse from the spare slot (i.e. the slot which places the fuse towards the outside of the instrument) to the active slot.

(5) Re-connect the power cord.

RESISTANCE COMPENSATION

Description

Associated with the current flow (I) in a microelectrode is a voltage drop across the microelectrode which depends on the microelectrode resistance (R_e). This unwanted IR voltage drop adds to the recorded potential. The Resistance Compensation control can be used to balance out this voltage drop so that only membrane potential is recorded. The particular setting required to achieve balance is a measure of the microelectrode resistance. On earlier amplifiers the Resistance Compensation control was named "Bridge".

Suggested Use

Set the Destination switch to ME1/2 and externally trigger the Step Command generator so that pulses of current are repetitively injected into ME1/2. Start with the Resistance Compensation control set to zero. Advance the dial until the fast voltage steps seen at the start and finish of the current step are just eliminated. The residual transient at the start and finish of the current step is due to the finite response time of the microelectrode. No attempt is made to remove this transient because 1) it only covers a very brief period, 2) it is a useful indication of the response time of the microelectrode, and 3) no valid information can be recorded in this period even if the transient is artificially removed. The transient can be minimized by correctly setting the Capacitance Neutralization.

The balancing procedure is illustrated in the following figure. The trace in A was recorded in a model cell when the Resistance Compensation control was correctly set. In response to a positive current pulse the membrane potential began to charge up. Before the membrane potential reached its final value the current pulse was terminated and the membrane potential exponentially decayed to its final value.

The traces in B were recorded at a sweep speed which was fast compared with the membrane time constant, hence the membrane responses look like straight lines. The top trace shows the voltage recorded when no Resistance Compensation was used. The response was dominated by the IR voltage drop across the electrode. In the middle trace the Resistance Compensation was optimum and in the bottom trace it was slightly overused.

When the Resistance Compensation is optimum the resistance of the microelectrode can be read directly from the dial. The sensitivity is 10 ÷ H MΩ per turn.

The Resistance Compensation controls operate on the 10V outputs. The controls saturate when the IR voltage drop exceeds ±1200 mV referred to the input.

Intracellular Balancing

The traces in the figure were all recorded with the electrode inside the cell. Since the electrode response and the oscilloscope sweep speed were fast compared with the membrane time constant (as in Figure part B), the correct Resistance Compensation setting was easy to see, even through the electrode was inside the cell.

It is sometimes useful to inject a brief small current pulse at the start of each oscilloscope sweep in order to continually check the Resistance Compensation setting during the course of an experiment.

FIGURE:

Illustration of Resistance Compensation techniques. All traces were recorded from the $10V_1$ output. The model cell was 10 MΩ//1 nF. R_e was 10 MΩ. Recording bandwidth: 30 kHz. Vertical calibration: 20 mV referred to V_1.

A. Response to +5 nA 10 ms current pulse. Compensation optimum. Trace is membrane response only. Cal. bar: 20 ms.

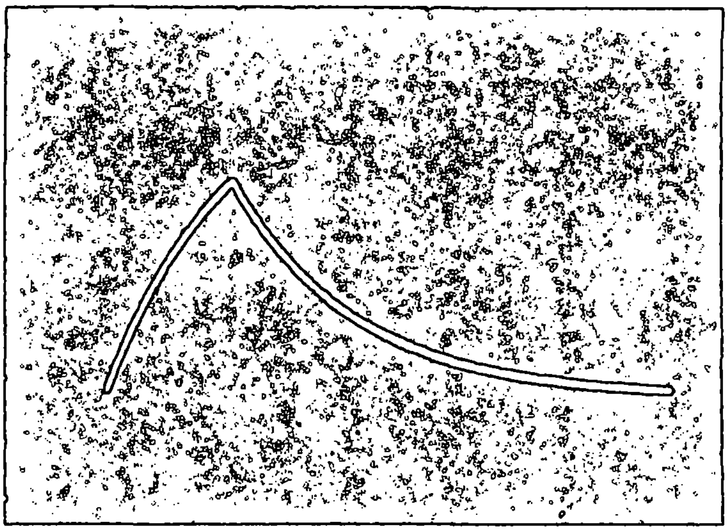
B. Response to +5 nA 1 ms pulse. Cal. bar: 1 ms.

Top trace: No compensation. Fast voltage steps at start and finish of the current pulse are the electrode IR voltage drop.

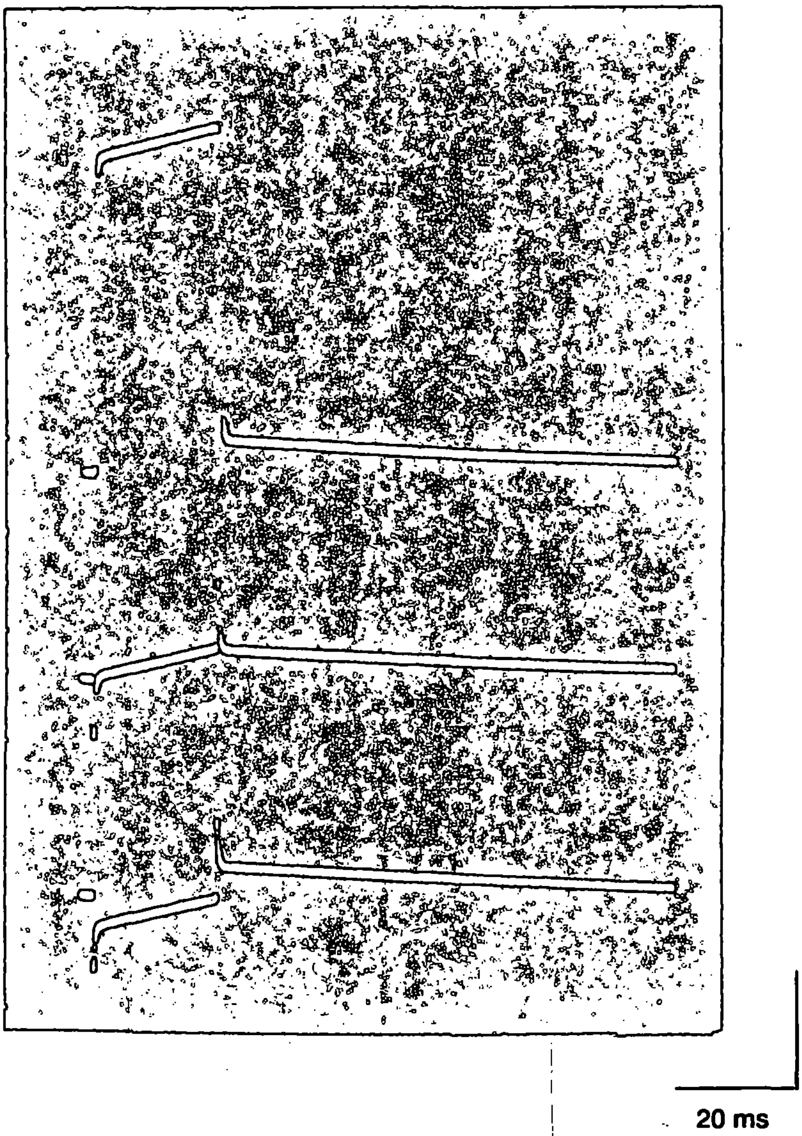
Middle trace: Compensation optimum. Trace is membrane response only. Transient electrode response remains.

Bottom trace: Too much compensation. Negative going step is introduced by the compensation circuit.

A



B



RESISTANCE COMPENSATION PROCEDURE

TEN-TURN POTENTIOMETERS

The ten-turn potentiometers used in the AXOPROBE-1A are high-quality wirewound types.

An inherent problem of wirewound potentiometers is that the wire elements tend to oxidize. This condition is easily cured.

If a potentiometer becomes noisy, the potentiometer manufacturer recommends rapidly spinning the knob 20-30 times between full clockwise and full counterclockwise. This clears the oxide off the element and restores noise-free operation.

TEST CURRENT

The Test Current switch injects a constant current into the microelectrode so that the microelectrode resistance can be measured. Test Current can be used two ways.

1) To read resistance on the digital panel meter: **Set the Resistance Compensation control to read zero.** Press the Test switch. The electrode resistance corresponds to the **shift** in the electrode potential. Depending on which way the Test switch is pushed the shift is either H mV/MΩ or 100H mV/MΩ (used for increased sensitivity). The current passed is either H or 100H nA respectively.

2) To read resistances by setting the Resistance Compensation control: Press the Test switch. Turn the Resistance Compensation control to eliminate the shift in electrode potential. Read the electrode resistance on the Resistance Compensation Dial.

Cell Resistance

The Test Current switch injects a DC current. If the electrode is intracellular the resistance measured will be the sum of the electrode resistance and the membrane resistance.

TROUBLE SHOOTING

It has been our experience at Axon Instruments that the majority of troubles reported to us have been caused by faulty equipment connected to our instruments.

If you have a problem, please disconnect all instruments connected to the AXOPROBE-1A except for the oscilloscope and one headstage. Ground the headstage through the original test resistor supplied by Axon Instruments. If the problem persists, please call us for assistance.

REFERENCES

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Sachs, F. & McGarrigle, R. (1980). An almost completely shielded microelectrode. J. Neurosci. Meths. 3, 151-157.

Schwartz, T. I & House, Randall C. (1970). A small-tipped microelectrode designed to minimize capactive artifacts during the passage of current through the bath. Rev. Sci. Inst. 41, 515-517.

Suzuki, K., Rohlicek, V. & Frmter, E. (1978). A quasi-totally shielded, low-capacitance glass-microelectrode with suitable amplifiers for high-frequency intracellular potential and impedance measurements. Pflgers Arch. 378, 141-148.

WARRANTY

We warrant every AXOPROBE-1A and every headstage to be free from defects in material and workmanship under normal use and service. For 12 months from the date of receipt we will repair or replace without cost to the customer any of these products that are defective and which are returned to our factory properly packaged with transportation charges prepaid. We will pay for the return of the product to the customer if the shipment is to a location within the United States. If the shipment is to a location outside the United States the customer will be responsible for paying all shipping charges, duties and taxes.

Before returning products to our factory the customer must contact us to obtain a Return Merchandise Authorization number (RMA) and shipping instructions. Failure to do so will cause long delays and additional expense to customer. Complete a copy of the RMA form on the next page and return it with the product.

This warranty shall not apply to damage resulting from improper use, improper care, improper modification, connection to incompatible equipment, or to products which have been modified or integrated with other equipment in such a way as to increase the time or difficulty of servicing the product.

This warranty is in lieu of all other warranties, expressed or implied.

Axon Instruments, Inc.

RETURN MERCHANDISE AUTHORIZATION

RMA No. ________________________ **Date of RMA** ________________________

Shipping check list:

[ ] 1. Package instrument with at least 3 inches of packing material all around.
[ ] 2. Enclose a completed **copy** of this form.
[ ] 3. Write RMA number on outside of package.
[ ] 4. Pre-pay freight for door-to-door delivery.

Model ________________________ Serial No. ________________________

[ ] In warranty [ ] Out of warranty

Customer's purchase order No. ____
(not required for warranty repair)

DESCRIPTION OF PROBLEM: ____
____
____
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Customer's Shipping Address:
Name ________________________
____
____
____
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Phone (___)________________________

Customers Billing Address:
Name ________________________
____
____
____
____
Phone (___)________________________

Send completed form with merchandise to:

Axon Instruments, Inc.
1429 Rollins Rd.
Burlingame, CA 94010
U.S.A.

Write RMA number on outside of package.

POLICY STATEMENT

The aim of Axon Instruments, Inc. is to manufacture electronic instruments for use in research and data acquisition. Emphasis is placed on instrumentation which satisfies specialized and sophisticated requirements to the highest standards of technical excellence. In all cases of conflict, achievement of excellence is held to be more important than containment of costs.

SERVICE

Service will be done at the factory. The usual time till dispatch of a repaired unit is 5-10 days after receipt.

Before returning products to the factory customers must obtain a Return Merchandise Authorization number (RMA) and shipping instructions. Failure to do so will cause long delays and additional expense to customer.

COMMENT FORM

Please use a copy of this form to submit comments about the AXOPROBE-1A, the headstages or the manuals. We will consider your comments carefully and endeavor to make any improvements suggested. You do not have to complete every section.

Features I like: ____

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Features I don't like: ____

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Features I would like to see added: ____

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General: ____

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Name______________________________

Address______________________________

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Phone (___)______________________________