VE-2 Voltage Clamp User's Guide

Alembic Instruments

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1 Introducing the VE-2

The VE-2 is a versatile, sensitive electronic amplifier designed to measure ionic currents in single cells using the whole-cell patch clamp technique. The letters VE stand for *Virtual Electrode* and refers to a method to compensate for series resistance (Rs) errors outlined by Sherman et. al. (1999) Biophysj. Vol 77. Using this method, the VE-2 achieves 100% Rs compensation to eliminate series resistance voltage errors in < 200us. In other words, the voltage clamp acts as if the effective series resistance of the patch pipette is zero after ~ 200us. In contrast, standard Rs compensation can reduce series resistance errors (generally by no more than 90% before the onset of oscillations) but not eliminate them. This advantage is key to being able to voltage clamp large rapidly activating ionic currents

1.1 Changes from the VE-1

The VE-2 incorporates major changes over it's predecessor, the VE-1. Most significantly, the VE-2 uses a low impedance IV converter headstage whereas the VE-1 uses a high impedance current source headstage. This has the following implications:

stable pipette capacitance compensation

The IV converter headstage allows the pipette capacitance to be fully compensated without oscillation. In contrast, the VE-1 headstage will oscillate if the fast capacitance compensation is over-applied, in the same manner that a high impedance microelectrode amplifier will oscillate when capacitance compensation is over applied.

simplified Voltage clamp mode and Rs compensation

The IV converter headstage functions as a low impedance voltage clamp. Consequently, the loop gain control found on the VE-1 to implement voltage clamp mode is no longer needed and has been eliminated. Furthermore, explicit tuning of the membrane state estimator controls found on the VE-1 which was needed in order to attain full Rs compensation has been replaced by a tunable frequency-selective attenuator (FSA) (US patent No 6,700,427) which performs the same function and is much easier to tune than it's predecessor.

1.2 How to use this manual

This manual is divided into four main sections: an overview, a reference guide, a tutorial, and appendices. The overview outlines the theory of operation of the VE-2 and explains the operating modes in a general manner. The reference section details the specific features of the VE-2, and gives instructions for setting up and operating the VE-2.
The tutorial introduces the user to using the VE-2 with a model cell, simulating the various steps encountered during an actual experiment. Running through the tutorial also performs a check-out of the VE-2 to ensure that everything is in order. The appendices contain background information that is useful to understand the operation of the VE-2.

The manual assumes a familiarity with the electrophysiology techniques associated with voltage clamping ionic current in general and patch clamping single cells in particular. Users unfamiliar with these concepts should other references, such as:

Sakmann, B. and Neher, E. (1983). Single Channel recording. New York: Plenum Press.

1.3 Document conventions

Throughout the manual, pertinent VE-2 panel switches, controls, and connectors are identified using a name and a boldfaced numeral. The name and boldfaced numeral correspond to a unique section in the reference guide where a detailed description of the item is given.

2 Overview

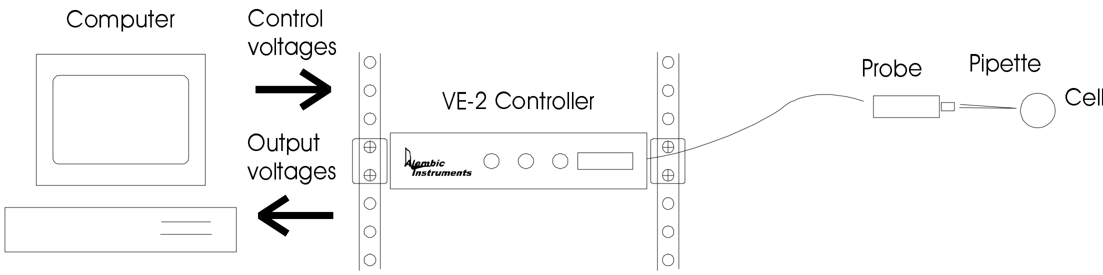


Fig. 1 VE-2 Physical layout

The VE-2 consists of a rack mounted enclosure called the controller, together with a probe, also commonly referred to as a headstage (Fig 1). The controller houses the power supply, the input and output connectors, the signal processing electronics, and all the users controls (knobs, switches, etc.). The input and output connectors on the controller commonly interface with a computer, which simultaneously generates control voltages going *to* the controller while monitoring output voltages coming *from* the controller.

The probe is a small aluminum enclosure housing electronic circuitry that is meant to be mounted physically close to the cell preparation. The probe is connected to the controller with a flexible electric cable. The probe is designed to accommodate a standard BNC glass pipette electrode holder to which is mounted a glass pipette electrode. The pipette electrode is in physical and electrical contact with the cell. The probe, along with the pipette holder/pipette electrode assembly is commonly mounted on a micromanipulator under a microscope.

2.1 VE-2 probe

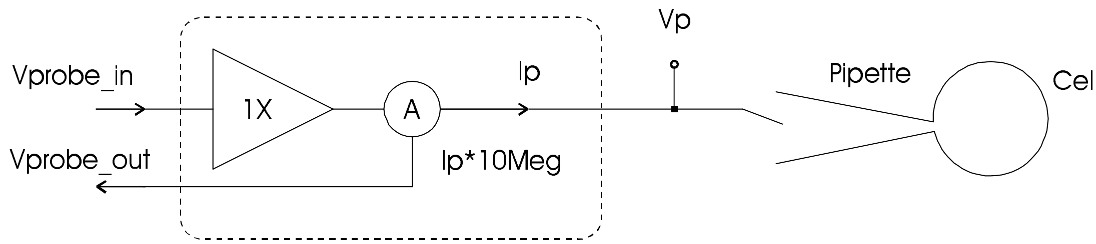


Fig. 2 VE-2 Probe functional diagram

A simplified functional diagram of the VE-2 probe function is shown in Fig. 2. The VE-2 probe (dotted box) is shown connected to a pipette and cell combination. The probe acts as a low impedance voltage source, as symbolized by the unity gain voltage buffer. The low impedance of the probe effectively forces the pipette voltage Vp to follow the probe input voltage Vprobe_in. Simultaneously the probe measures the pipette current Ip, symbolized by the action of the "ammeter" in Fig 2: the ammeter measures Ip and returns an output voltage proportional to Ip given by

$$Vprobe\_out = Ip \times 10M\Omega$$

The VE-2 probe obtains Vprobe_in from the VE-2 controller and passes Vprobe_out back to the controller. How the controller generates Vprobe_in and uses Vprobe_out varies depending on the VE-2 operating modes, as described below.

2.2 VE-2 controller - operating modes

The VE-2 controller in conjunction with the VE-2 probe implements 4 basic operating modes which are summarized in the table below.

Mode name	Abbreviation	Description
Voltage clamp	VC	Clamp Vp to a user-supplied command voltage. The user monitors Ip
Voltage clamp with Rs compensation	VC w/Rs comp	Clamp Vp to a user supplied command voltage and compensate for the pipette series resistance Rs The user monitors Ip
Current Clamp	CC	Clamp Ip to a user-supplied pipette current. The user monitors Vp
Current clamp to 0	I=0	Clamp Ip at 0 The user monitor Vp

2.2.1 VC mode - Voltage clamp mode

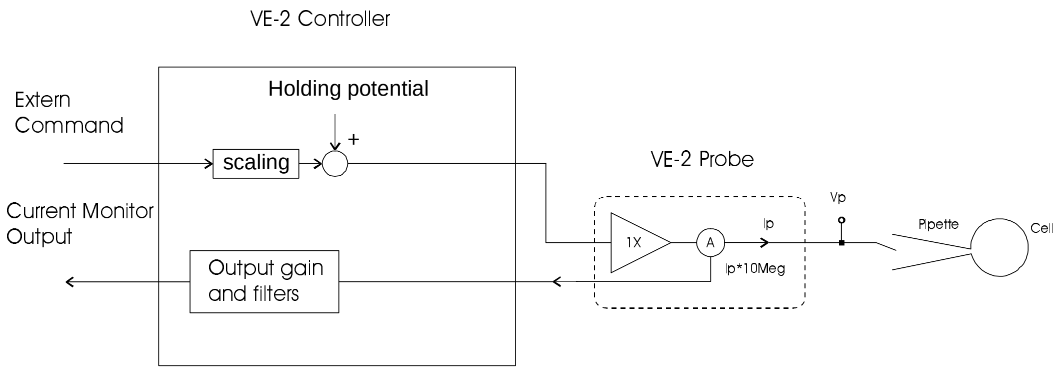


Fig. 3 Voltage Clamp Mode

VC is the usual operating mode of the VE-2 in which whole-cell ionic current is measured. In VC mode, the pipette voltage Vp is clamped to a user-supplied command voltage and the pipette current is monitored. As shown in Fig. 3, the controller scales an external command voltage, adds to this a constant holding potential, and passes this voltage to the probe. The probe acts to voltage clamp the pipette and measure the pipette current. The measured pipette current is then made available as an output voltage by the VE-2 controller.

In voltage clamp mode the series resistance of the patch pipette limits the bandwidth of the recorded currents and of the voltage clamp. For small, slowly-varying ionic currents the effects of Rs can sometimes be ignored. However, for large ionic current (> 1na) and for currents that vary rapidly (< 2ms) the effects of Rs must be compensated in order to accurately voltage clamp these ionic currents, as described below.

2.2.2 VC w/Rs comp mode - Voltage clamp mode with Rs compensation

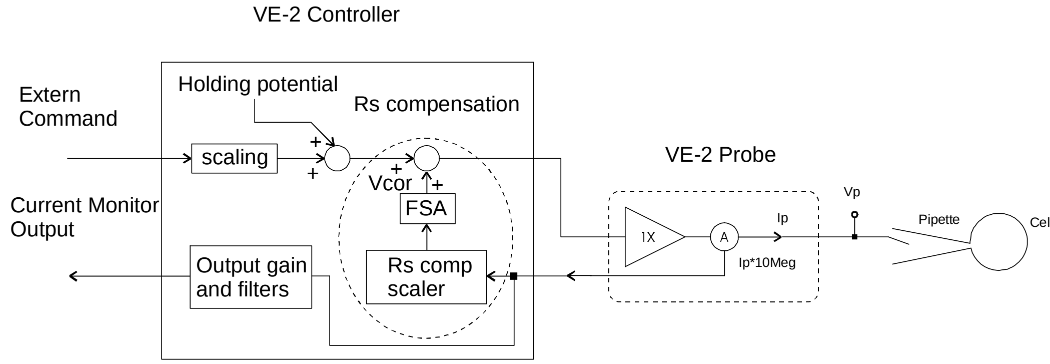


Fig. 4 Voltage Clamp Mode with Rs compensation

As its name implies, VC mode with RS compensation is similar to VC mode but has additional circuitry to compensate for the series resistance (Rs) of the patch pipette. As shown in Fig 4, the Rs compensation circuitry consists of a voltage scaler and a frequency-selective attenuator (FSA) which operates on the measured pipette current signal to form a correction voltage Vcor. Vcor is then used as positive feedback to compensate for Rs.

VE-2 Rs compensation differs from that used on other voltage clamps by the inclusion of the FSA (patent pending) which extends the voltage estimator concept outlined by Sherman et. al. (1999) Biophysj. Vol 77. Using the FSA enables 100% Rs compensation to be achieved while maintaining a voltage clamp bandwidth limit of ~10kHz, sufficient for most rapid ionic currents. Without the FSA the positive feedback can only compensate for ~ 90% of Rs. Attempting to reach higher compensation without the FSA requires achieving very high voltage clamp bandwidths which is not physically realizable. (In the limit 100 % Rs comp would then require infinite voltage clamp bandwidth when not using the FSA)

2.2.3 CC mode - Current Clamp mode

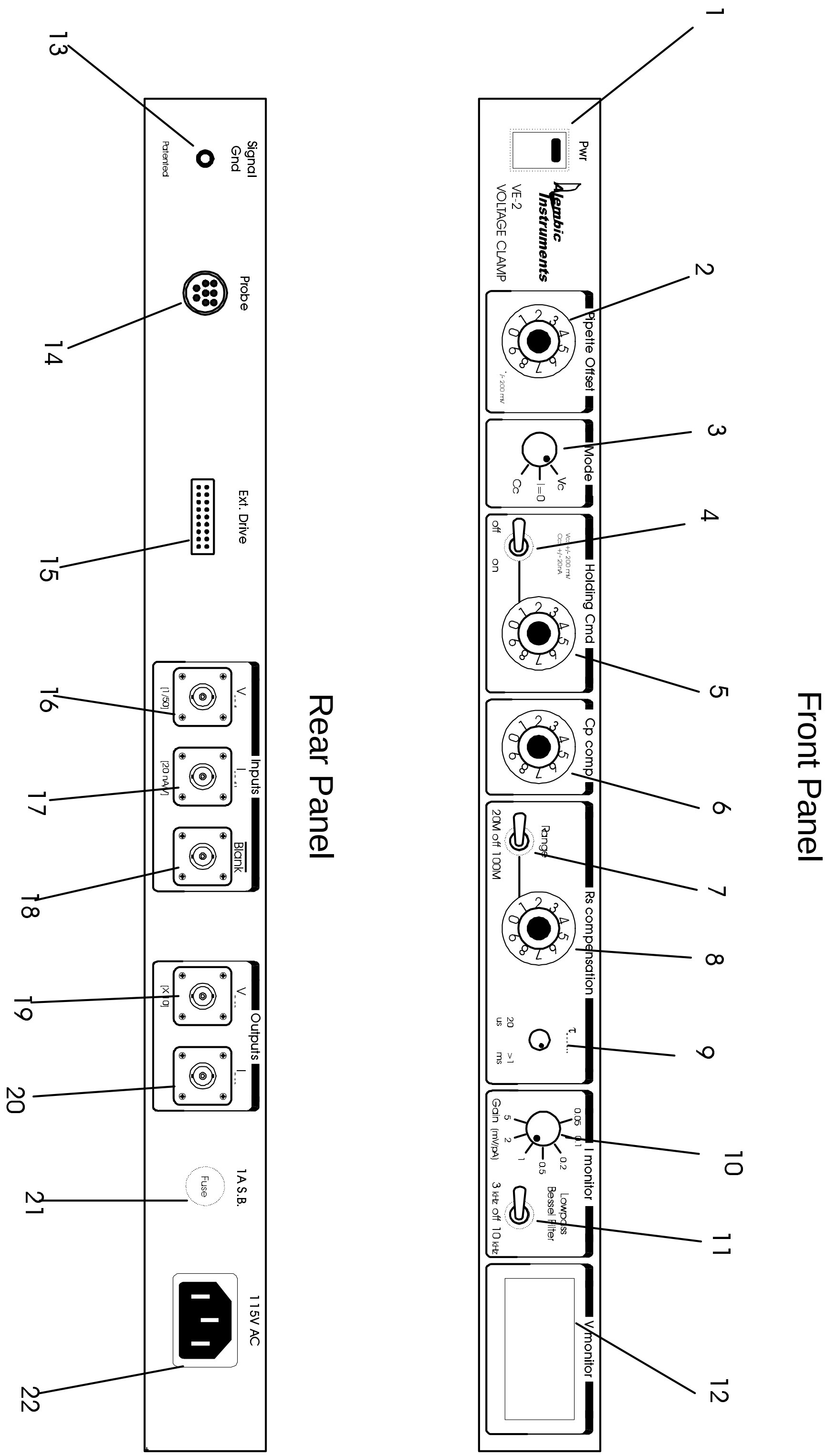
In CC mode the pipette current is clamped to a user-supplied command value while the pipette voltage is measured. This allows spontaneous or evoked action potentials to be measured. The VE-2 controller implements CC mode by using the current monitor output signal of the probe to generate negative feedback. The negative feedback acts to constantly vary the pipette potential in such a way as to keep the measured pipette current equal to the command value.

2.2.4 I=0 mode - Current Clamp to 0

I=0 mode is identical to CC mode except the pipette current is clamped to 0 instead of a commanded value. This mode is useful to measure the membrane resting potential of the cell.

3 Reference section

3.1 VE-2 Panel diagrams



3.2 Front panel

1 On/off switch

3.2.1 Pipette Offset

2 Pipette Offset

Adds a +/-200 mV offset to null the pipette junction potential of the probe. See tutorial section 4.3.2 for the offset nulling procedure.

3.2.2 Clamp Mode

3 Mode switch

Sets the operating mode of the VE-2 to one of the following:

Vc: Voltage clamp mode.

Clamps the pipette voltage Vp to a user-supplied command voltage. The pipette voltage Vp is set according to the following formula:

Vp = (the voltage driving Vcmd **16**) * 1/50 + (constant voltage set by Hold cmd **5)**

CC mode: Current clamp mode

Clamps the pipette current to a user-selected current command. The pipette current Ip is set according to the following formula:

Ip = (the voltage on Vcmd **16**) * 2nA/V + (constant current set by Hold cmd **5**) + (the voltage on Icmd **17**) * 20nA/V

For maximum bandwidth when measuring evoked action potentials in CC mode, it is important for the pipette capacitance to be fully nulled using Cp comp **6**.

NOTE: Over-application ***or*** *under-application of* Cp comp **6** *in CC mode will lead to oscillations*. See tutorial section 4.4.3 for a description of how properly null the pipette capacitance.

I=0 : Current clamp to 0 mode

Similar to CC mode described above, except the pipette current Ip is set as follows:

Ip = (the voltage on Icmd **17**) * 20nA/V

When no signal is driving Icmd **17** selecting I=0 mode has the effect of clamping the pipette current to 0. This is useful when checking the resting potential of the cell under whole-cell patch conditions.

> *NOTE: Over-application* ***or*** *under-application of* Cp comp **6** *in I=0 mode will lead to oscillations*. See tutorial section 4.4.3 for a description of how properly null the pipette capacitance.

3.2.3 Constant Holding Command

4 Holding cmd switch

When Holding cmd switch **4** is toggled to the *off* position (i.e. to the l*eft*) the holding command is disabled. When Holding cmd switch **4** is toggled to the *right* a constant holding command is delivered, as set by the Hold cmd **5**.

5 Hold cmd control

Adds a constant holding command during VC mode or CC mode, when Holding cmd switch **4** is toggled to the right.

In VC mode, Hold cmd **5** selects a constant holding voltage according to the following formula:

Vhold = (# of clockwise turns - 5) * 40mV / turn.

In CC mode, Hold cmd **5** selects a constant holding current according to the following formula:

Ihold = (# of clockwise turns - 5) * 4nA / turn.

The number of clockwise turns is indicated on the turns-counting dial and ranges from 0 to 10. Thus the full range of Hold cmd **5** is +/- 200mV in VC mode or +/-20nA in CC mode.

3.2.4 Capacitance compensation

6 Cp comp

Electronic capacitance compensation. Allows the user to compensate for or neutralize the stray capacitance of the patch pipette.

Proper setting of the Cp comp ***6*** *is essential when using series resistance compensation. In addition, proper setting of Cp comp* **6** *improves the performance of the VE-2 in current clamp mode.*

3.2.5 Series Resistance compensation

7 Rs comp range

Sets the full-scale range (FSR) of the series resistance compensation circuitry. Can be toggled to one of the following positions:

20Meg: FSR is 20Meg, so that the maximum setting of Rs comp **8** corresponds to a series resistance of 20Meg. This is the usual setting for whole-cell voltage clamping, when the access series resistance is below 20Meg.

off Series resistance compensation is *off*, regardless of the setting of Rs comp

100Meg FSR is 100Meg, so that the maximum setting of Rs comp **8** corresponds to a series resistance of 100Meg. This setting is used when high whole-cell access resistances are encountered, such as with small pipette tip openings, or when using the perforated-patch technique.

When using Rs compensation with an access resistance below 20Meg the Rs comp range **7** *switch should be set to the 20Meg setting, not the 100Meg setting. Doing so facilitates tuning and also increases the performance since the frequency-selective attenuator (FSA) is optimized to work in this range.*

8 Rs comp

10-turn control that scales the Rs compensation circuitry to correspond with the whole-cell access resistance. When Rs comp range **7** is toggled to the *left* each turn equals 2Megs (maximum 20Megs at 10 clockwise turns). When Rs comp range **7** is toggled to the *right* each turn equals 10Megs (maximum 100Meg at 10 clockwise turns).

9 τ_{access}

Tunes the frequency-selective attenuator (FSA) of the Rs compensation circuitry. When set to the approximate access time constant of the cell (Rs * Cm), 100% Rs compensation can be achieved while maintaining ~ 10kHz voltage clamp bandwidth. When turned fully clockwise, the FSA is disabled. (See tutorial section 4.4.4 and 4.4.5 for proper use of τ_{access} **9**).

3.2.6 Output Monitor

10 Imon gain

Sets the gain of the current monitor circuitry that drives the Imon **20** output connector. Ranges from 0.05 mV/pA to 5 mV/pA.

Example: If 1nA (1000pA) of pipette current is being delivered by the VE-2 probe and Imon **10** is set to 1mV/pA, then a 1V (1000mV) signal will be present on the Imon **20** output connector.

11 Output filter

Applies lowpass filtering to the current monitor signal (Imon **20**) present on the rear-panel BNC. Can be set to the following:

10kHz	Imon **20** passes through a 4 pole Bessel filter with -3dB point at 10kHz.
3kHz	Imon **20** passes through two cascaded 4 pole lowpass Bessel filters with -3dB points at 10kHz and 3kHz, respectively.
Out	Imon **20** is unfiltered.

12 Digital panel meter

Displays the pipette voltage in mV.

3.3 Rear panel

3.3.1 Probe and ground connections

13 Ground connector

High quality ground. See reference section 3.5 for proper grounding procedures of the VE-2. Ground connector **13** is isolated from power line ground via a 10 Ω resistor.

14 Probe input

8 pin connector for the VE-2 probe.

Ensure that On/Off switch **1** *is in the* ***off*** *position when connecting or disconnecting the VE-2 probe from the controller*.

3.3.2 External control

15 Ext. Drive

15 pin D-sub connector for external control of the VE-2. Most operating modes and settings of the VE-2 can be controlled and/or queried with suitable digital connections to this connector. See Appendix B for pin-out specifications for this connector.

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3.3.3 Input BNC Connectors

16 Vcmd

External command input. Allows the user to supply a voltage clamp command or a current clamp command, as follows:

mode switch **3** set to VC (voltage clamp mode):

Vp = (the voltage driving Vcmd **15**) * 1/50 + (constant voltage set by Hold **5)**

mode switch **3** set to CC (current clamp mode):

Ip = (the voltage driving Vcmd **16**) * 2nA/V + (constant current set by Hold **5**) +

(the voltage driving Icmd **17**) * 20nA/V

When mode switch **3** *is set to "I=0"* Vcmd **16** *is disconnected.*

17 Icmd

External Current command input. Allows the user to supply a current clamp command as follows:

mode switch **3** set to CC :

Ip = (the voltage driving Vcmd **16**) * 2nA/V + (constant current set by Hold **5**) + (the voltage driving Icmd **17**) * 20nA/V

mode switch **3** set to I=0:

Ip = (the voltage driving Icmd **17**) * 20nA/V

When mode switch **3** *is set to* "VC" Icmd **17** *is disconnected.*

18 Blank

Rs comp Blanking. When this input is pulled low Rs compensation is disabled.

3.3.4 Output BNC Connectors

19 Vmon

Pipette voltage monitor. The voltage on this connector is proportional to the pipette voltage and is given by:

Vmon **19** = (Pipette voltage) * 10

20 Imon

Pipette current monitor. The voltage on this connector is proportional to the pipette current and is given by:

Imon **20** = (pipette current) * (gain as set by Imon gain **10**)

*Vmon **19** and Imon **20** each have an output impedance of 500Ohms and can withstand a continuous short-circuit to ground. Notwithstanding, do not intentionally short either output to ground.*

3.3.5 AC Power

21 Fuse holder

The VE-2 uses a 1A AG slow-blow fuse.

22 Power connector

Either 115V or 230V A/C power, as specified. The VE-2 can tolerate the specified line voltage +/- 10%, with a line frequency ranging from 50Hz to 60Hz. Users should use the shielded line cord provided with the VE-2. The VE-2 is equipped with an RF power line filter and an AC transient suppresser inside the controller.

3.4 The VE-2 probe

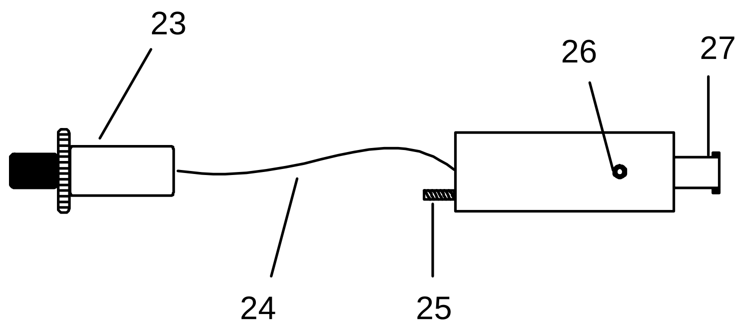


Fig 5 VE-2 probe

23 probe 8 pin connector

This connector mates with probe input **14** on the VE-2 rear panel.

24 probe electric cable

25 headstage mounting bolt

Mounting bolt that attaches to the threaded mounting rod provided. This bolt is electrically connected to signal ground.

26 probe ground connector

High quality signal ground. Use this connector to ground the cell bath electrode.

27 Pipette holder input

Teflon-insulated BNC connector used to mount a standard pipette holder. The center pin is the amplifier input. The BNC shield is driven at low impedance with the amplifier input potential.

Since the BNC *shield is driven it should* ***not*** *be grounded. Therefore,* ***never*** *connect a grounded signal generator or other such device to* Holder input **27** *as this will inadvertently ground the BNC shield.*

3.4.1 Probe handling precautions

1. The probe input can be damaged by static electricity. Before handling the probe, make sure to ground yourself to dissipate any accumulated static charge.

2. Make sure the VE-2 power switch is in the *off* position before connecting or disconnecting the probe to or from the controller.

3. Since the shield of Pipette holder input **27** is driven by a low impedance the shield should *not* be grounded.

3.5 Grounding guidelines for the VE-2

1. The cell bath electrode should *only* be grounded at the probe ground connector **26**.

2. The Faraday cage surrounding the cell preparation and microscope should be grounded to ground connector **13** on the VE-2 rear panel. *Ensure that the Faraday cage does not contact the power line ground*. This can occur inadvertently if the cage touches a metal object, such as the equipment rack, which is itself grounded to the power line ground.

3. Ensure that the probe electric cable **24** is not draped near power transformers, either in auxiliary equipment or in the VE-2 itself. (The power transformer in the VE-2 is located on the left side of the instrument, near on/off switch **1**).

4. Initially make only one connection from the Imon **20** signal to the oscilloscope and verify that no ground loops are present. Add each desired input or output connection to the VE-2 one lead at a time, ensuring that the leads do not pass too close to power transformers in auxiliary equipment.

4 Tutorial

4.1 Tutorial introduction

This tutorial runs through the steps of operating the VE-2 using the model cell, which simulates the following parameters:

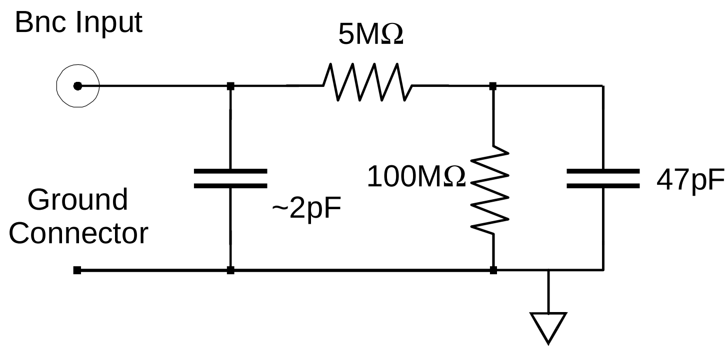


Fig 6 Model cell

<u>Electrode parameters</u>

Rs = pipette series resistance = 5MΩ

Cp = pipette capacitance ~ 2pf

<u>Cell parameters</u>

Cm = membrane capacitance = 47pF

Rm = membrane resistance = 100MΩ

Running through the tutorial allows the user to become familiar with the VE-2 and at the same time performs a functional check-out of the VE-2.

4.2 Equipment connection for Tutorial

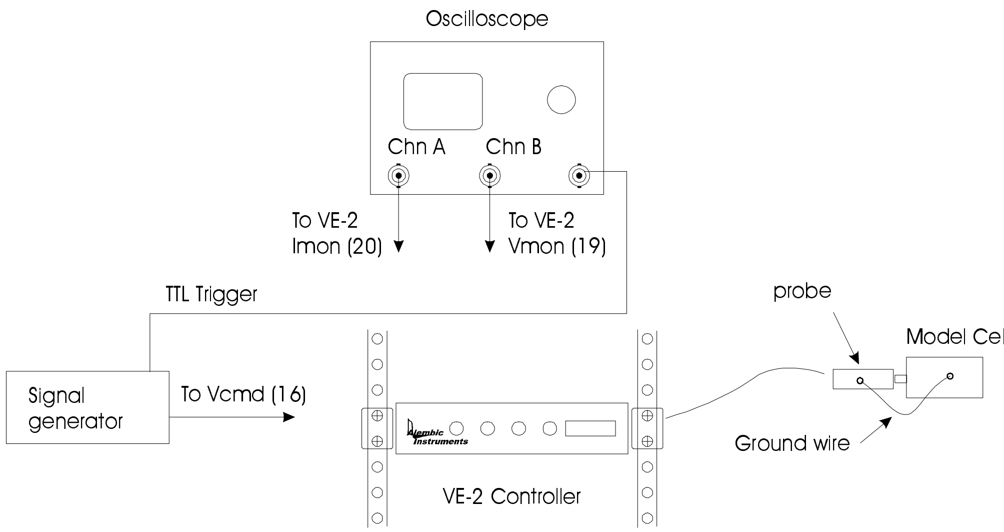


Fig 7 Equipment connection for the tutorial

4.2.1 Connect the equipment as per Fig 7.

➢Follow the headstage handling precautions (section 3.4.2).

➢Make sure the headstage cable does not pass directly over the power supply contained in the VE-2 (located on the left side of the instrument, near on/off switch **1**) so as to avoid inductive 60Hz pickup.

4.3 Voltage clamp (VC) mode tutorial

4.3.1 Set the VE-2 controls to the following:

Cp comp **6** fully counterclockwise (off)
Mode switch **3** set to Vc
Rs range **7** set to off
Output gain switch **10** to 0.05 mv/pA (the minimum value)

4.3.2 Null the "pipette" offset potential

4.3.2.1 Set Mode switch **3** to "I=0"

➢ This enables current clamp mode with 0 command voltage to ensure that no pipette current is flowing.

4.3.2.2 Turn Pipette offset **2** until the digital panel meter **12** reads 0 mV.

➢ This nulls any voltage offset present on the "pipette". If this were a real electrode, the main offset present would be the junction potential formed by the electrode /cell bath combination. Since no current is flowing (I=0 mode), the only potential being displayed on panel meter **12** will be due to this junction potential. This procedure is usually done when the electrode is first inserted into the cell bath.

4.3.2.3 Set Mode switch **3** to "VC"

➢ This enables voltage clamp mode.

4.3.3 Set the holding potential to 0mV

4.3.3.1 For now, make sure that the signal generator connected to the Vcmd **16** input of the VE-2 is outputting a DC voltage of ~ 0 mV.

4.3.3.2 Set holding command switch **4** to *on*

➢ The enables a holding potential to be entered.

4.3.3.3 Turn the Holding cmd **5** until digital panel meter **12** displays 0 mV.

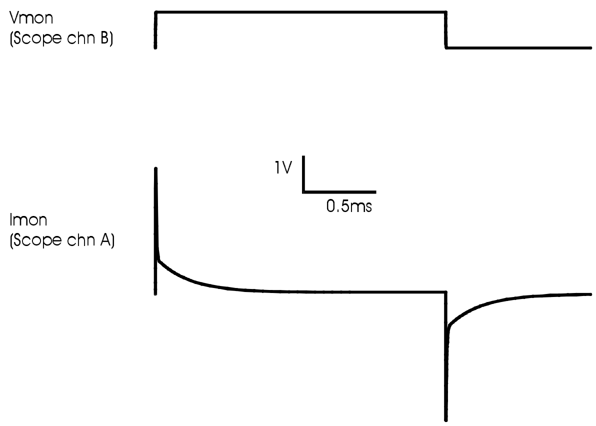
4.3.4 Apply voltage clamp steps

4.3.4.1 Set the signal generator to output a square wave with amplitude of 5V and repetition rate of ~ 100Hz with ~ 0 mV offset.

➢ This signal generator output is attenuated by 50 in the VE-2. Therefore, the VE-2 applies a repetitive voltage clamp steps of 5000mV/100 = 100mV to the model cell.

4.3.4.2 Display Imon **20** and Vmon **19** simultaneously on the dual channel oscilloscope. (Chn A and B 0.5 V/div, timebase 0.5ms/div)

➢ If the oscilloscope is triggered at the beginning of each square wave, the traces should appear as follows:



➢ Voltage monitor (chn B): The pipette potential follows square wave command potential with an amplitude of 1V (100mV *10 since the Vmon **19** has a gain of 10).

➢ Current monitor (chn A): Rapid initial charging transient due to pipette capacitance Cp and then a slow charging transient due to Rs and Cm which decays to its steady state value with time τ =Rs*Cm = 5MΩ * 47pF = 235µs = access time constant. The voltage clamping bandwidth of the membrane voltage Vm is set by this membrane time constant. To increase the voltage clamping bandwidth, use Rs compensation, as described in the next section.

4.3.4.3 Increase the Output gain switch **10** to 1 mV/pA and observe the steady state current flowing through Rm.

➢ Note that the steady state current from the 100mV step should be ~ 1nA (100mV / (Rs + Rm) = 100mV/105MΩ ~ 1nA). This corresponds to a 1V deflection of the Imon **20** channel on the scope since the output gain **10** is set to 1mv/pA = 1V/nA.

4.3.4.4 Toggle output filter **11** and observe the current trace when filtered at 3kHz and 10kHz.

4.4 Voltage clamp mode with Rs compensation tutorial

4.4.1 Set the VE-2 controls to the following:

Cp comp **6** fully counterclockwise (*off*)
Rs range switch **7** to *off*
Rs comp **8** fully counterclockwise
τ_{access} **9** fully *clockwise*
Mode switch **3** to VC
Output gain **10** to 0.05 mV/pA
Output filter **11** to *off* (wideband)

4.4.2 Set a hyperpolarizing holding potential of -60mV. Apply repetitive hyperpolarizing voltage command steps of -30mV.

4.4.2.1 For now, make sure that the signal generator connected to the Vcmd **16** input is outputting a constant DC voltage of ~ 0 mV.

4.4.2.2 Adjust the Holding cmd **5** control until the digital panel meter **12** reads -60mV.

➢ Sets a holding potential of -60mV.

4.4.2.3 Set the signal generator to output a square wave with amplitude of -1.5V and repetition rate of ~ 100Hz with ~ 0 mV offset.

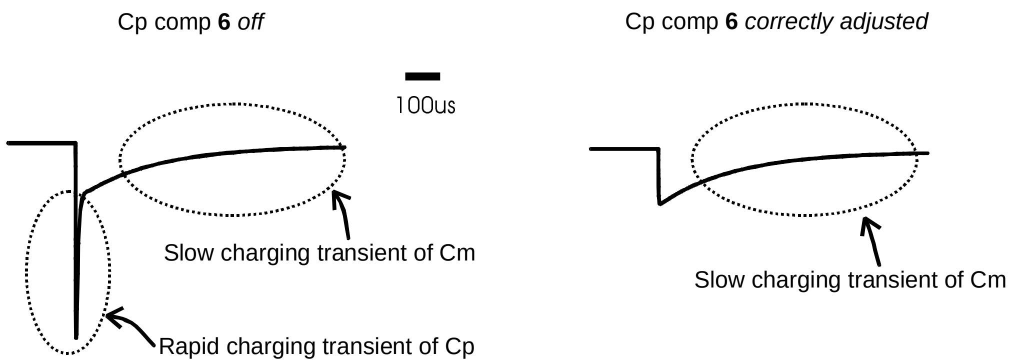
➢ The signal generator output is attenuated by 50. Therefore the VE-2 applies repetitive voltage clamp steps of -1.5V/50 = -30mV superimposed on a holding potential of -60mV to the model cell.

➢ Applying hyperpolarizing test pulses from a negative holding potential is the normal procedure followed when tuning series resistance compensation during whole cell recording. Since this protocol usually does not activate ionic channels in the cell membrane, this protocol allows the passive membrane properties to be observed, which is required to tune series resistance compensation.

4.4.3 Neutralize the pipette capacitance Cp.

4.4.3.1 Increase the oscilloscope timebase resolution to 100µs/div.

4.4.3.2 Turn the Cp comp **6** clockwise until the current trace appears as follows:



- When Cp comp **6** is off the current trace shows two charging transient components: a rapid charging transient of the "pipette" capacitance Cp; and a slow charging transient of the "membrane" capacitance Cm (τ = Rs*Cm). As Cp comp **6** is increased the effective value of Cp is eliminated by the capacitance compensation circuitry, hence the rapid charging transient is eliminated. However, Cp comp **6** does not affect the slow charging transient of Cm.

- Ensure that Output filter **11** is set to *off* (wideband); otherwise the rapid charging transient cannot be observed. See how the charging transient is obscured when Output filter **11** is set to either 3kHz or 10kHz.

- For stable Rs compensation (see below) it is important to *fully* null the rapid charging transient of Cp.

4.4.4 Initiate Rs compensation and tune for 90% Rs compensation (FSA disabled)

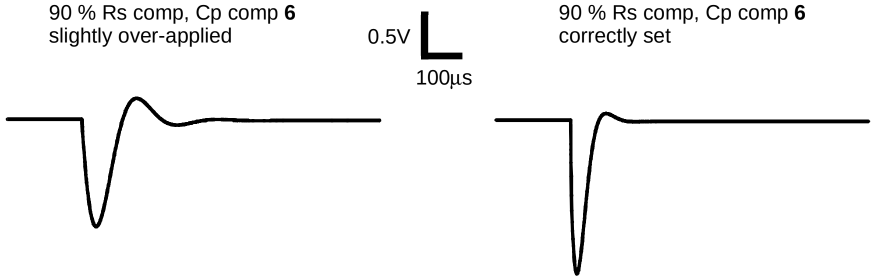
4.4.4.1 Set the VE-2 controls to the following:

Cp comp **6** as set above to completely null the rapid charging transient of Cp

Rs comp **8** fully counterclockwise
Rs range switch **7** to *20Meg*
τ_{access} **9** fully *clockwise* (maximum)
Mode switch **3** to VC
Output gain **10** to 1
Output filter **11** to *off* (wideband)

- When τ_{access} **9** is fully clockwise the FSA is off. In this case, the Rs compensation is equivalent to standard Rs compensation where the effective Rs can be reduced up to a limit of around 90%.

4.4.4.2 Increase Rs comp **8** (clockwise) until the charging transient decay time is minimized, but not so far as to cause large oscillations (below, left)

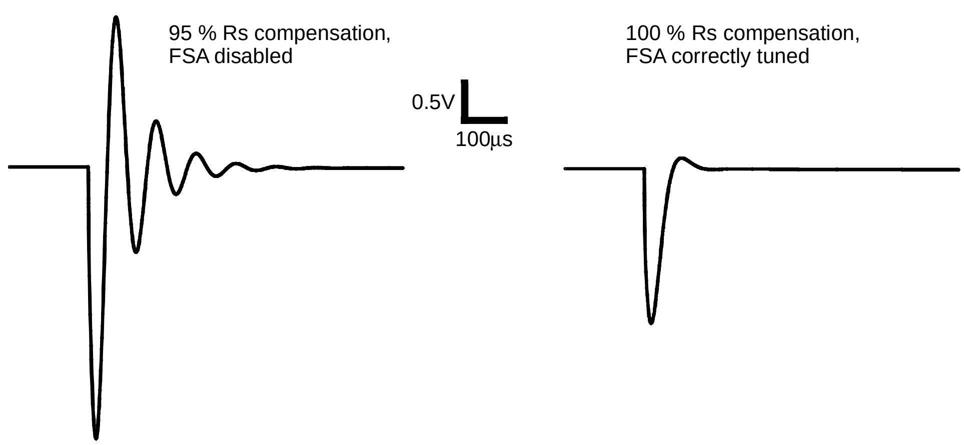


➢ For maximum speed it is important that Cp comp **6** not be over applied. Observe this by slowly increasing and then decreasing Cp comp **6**. Experiment to get the fastest response time by slowly decreasing Cp comp **6** counterclockwise. (above, right). This should be done carefully since decreasing Cp comp **6** too much will lead to instability.

➢ The model cell has an Rs = 5Meg, which corresponds to a reading of 2.5 on the turns counting dial of Rs comp **8**. Note that with the FSA fully clockwise (off) it is not possible to turn Rs comp **8** much beyond 2.25 (90 % Rs compensation) before large instabilities arise.

4.4.5 Use the FSA to enable stable 100% Rs compensation

4.4.5.1 Increase Rs comp **8** (clockwise) until large damped oscillations are present and the turns counting dial reads ~ 2.4, corresponding to 95% Rs compensation (below left). Then turn τ_{access} **9** counterclockwise ~ 1/4 turn to tune the FSA and remove the oscillations. Finally, increase Rs comp **8** until the turns counting dial reads 2.5 (5Meg) corresponding to 100% Rs compensation.



- Only turn τ_{access} **9** far enough counterclockwise to dampen out the oscillations, since turning it further counterclockwise will slow the voltage clamp response. Observe this by turning τ_{access} **9** all the way counterclockwise and seeing how the capacitive transient decay time is lengthened. During an experiment, once full Rs compensation has been set with Rs comp **8** then τ_{access} **9** should be set as far clockwise as possible to minimize the transient decay time, consistent with maintaining a stable damped response.

- If the FSA has been over-applied (turned too far counterclockwise), Rs comp **8** can be increased past 100%. Observe this by turning τ_{access} **9** ~ 1/2 turn counterclockwise and then increasing Rs comp **8** past the 2.5 setting. During an experiment it is important not to overcompensate Rs comp **8** in this manner, since this can result in incorrect activation kinetics of recorded ionic currents (see Appendix A for details).

5 Warranty

Alembic Instruments warrants every VE-2 controller and every VE-2 headstage to be free from defects in material and workmanship under normal use and service. For 24 months from the date of receipt, Alembic Instruments will repair or replace without cost to the customer, any of these products that are defective and that are returned to our factory properly packaged and with transportation charges prepaid. We will pay for the return of the product to the customer if the shipment is to a location within Canada or the United States. If the shipment is to a location outside Canada or the United States, the customer will be responsible for paying all shipping charges, duties and taxes.

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.

6 Appendix A Optimal Rs compensation when recording whole-cell Na+ currents

This appendix outlines the optimal setting for Rs compensation - as set using τ_{access} **9** and Rs comp **8** - in order to voltage clamp whole-cell Na+ currents in excitable cells. In particular, it outlines the effects that can be observed when pipette series resistance is overcompensated, and how to avoid this.

The three possible cases of Rs compensation - undercompensated, fully compensated, and overcompensated - are summarized in the table below, and then discussed in detail.

	Rs compensation setting	Vm in response to +'ve inward ionic current
case i:	undercompensated	depolarization
case ii:	fully compensated	no change in Vm
case iii:	overcompensated	hyperpolarization

case i:

When Rs comp **8** is set below the value of the actual series resistance, there remains some uncompensated series resistance, called the effective Rs. A positive inward Na+ current flowing across the effective Rs will result in membrane depolarization. If there is too much effective Rs and the inward Na+ current is large enough, the resulting membrane depolarization will trigger an unclamped action potential in the cell, resulting in a loss of voltage control. A Na+ current activation series will then exhibit delayed inward inflections and the steady-state IV curve while exhibit a sharp discontinuity.

Case ii:

When Rs comp **8** is set equal to the actual series resistance, the series resistance is fully compensated and the effective Rs is 0 after 200us if the FSA is correctly tuned (see tutorial section 4.4.5) A positive inward Na+ current results in no membrane voltage change, regardless of the current amplitude, consistent with the effective Rs being 0. This is the ideal setting to observe voltage clamped ionic currents. It is important that the voltage clamp response time be minimized by 1. making sure the Cp comp **6** is not too high (too far clockwise) and 2. by making sure that τ_{access} **9** is set to as high a value as possible (as far clockwise as possible).

Case iii:

When Rs comp **8** is set above the value of the actual series resistance the Rs compensation circuitry *over*compensates the actual series resistance present and the effective Rs becomes negative. A positive inward Na+ flowing across this negative effective Rs then results in membrane *hyper*polarization. This hyperpolarization results in rapid Na+ current *de*activation with a similar time course to Na+ current activation. Therefore, a typical Na+ current activation trace in response to a voltage clamp step will show 1. normal rapid Na+ current activation due to the voltage step, and 2. an unexpected and rapid reduction of the current amplitude due to the deactivation induced by the membrane *hyper*polarization, as opposed to the familiar slow *in*activation that would appear had Vm remained constant.

Note that it is only possible to overcompensate for Rs if τ_{access} **9** has been set to a value significantly below the uncompensated access time constant (Rs *Cm) (i.e. too far counterclockwise). Following the tuning procedure outlined in tutorial section 4.4.5 avoids this.

7 Appendix B External Drive Pinouts

Ext. Drive **15** functions as an input/output port allowing most the VE-2 panel settings to be either queried or set digitally.

Querying the panel switches (Using Ext Drive **15** as a status port to read from)
When pin 13 is either driven high or is left unconnected, the remaining connected pins present TTL logic outputs reflecting the state of the front panel switches of the VE-2. See the function table below to decode these logic outputs.

Setting the panel switches (Using Ext Drive **15** as a control port to write to)
When pin 13 is driven low, the front panel selections are disabled and the remaining connected pins present TTL logic inputs. These pins *must* then be driven by TTL logic outputs to control the functions of the VE-2. See the function table below to decode these logic inputs.

Ext drive 15 pinouts

Pin no	VE-2 function	Control inputs (pin 13 = 0)	Status outputs (pin 13 = 1)
pin 1	Rs comp range **7**	1 = Rs comp range is 100Meg 0 = Rs comp is off *if* pin 11 is 0	same as input function
pin 2	Mode switch **3**	1 = CC mode 0 = Vc mode *if* pin 7 = 0	same as input function
pin 3	reserved	reserve	reserved
pin 6,5,4	Imon gain **10**	000 g = 0.05 001 g = 0.1 010 g = 0.2 011 g = 0.5 100 g = 1 101 g = 2 110 g = 5	same as input function
pin 7	Mode switch **3**	1 = I=0 mode 0 = Vc mode *if* pin 2 = 0	same as input function
pin 9,8	Output filter **11**	00 wideband 01 3kHz 10 10kHz	same as input function
pin 10	blank **18**	0 = Rs comp disabled, regardless of the value of pin 1 of pin 11 1 = Rs comp set according to pin 1 and pin 11	same as input function
pin 11	Rs comp range **7**	1 = Rs comp range is 20Meg 0 Rs comp is off *if* pin 1 is 0	same as input function

pin 12	`Hold cmd switch **4**	1 Holding command enabled 0 Holding command disabled	same as input function
pin 13	External drive I/O control	1 or NC panel switches enabled, remaining pins are logic outputs 0 panel switches are disabled, remaining pins are logic inputs	write-only
pin 14	+ 5V		
pin 15	Digital ground		

8 Appendix C VE-2 Specifications

Alembic Instruments **VE-2 Whole-Cell Patch-Clamp Amplifier**

Headstage

Architecture
Wideband IV converter

Current measuring resistor
10MΩ

Maximum deliverable current
1200nA
Large current capability without saturation eliminates the need for whole-cell capacitance cancellation, which simplifies operation during experiments.

Largest measurable current
200nA

Current noise
DC to 3kHz: 3pA RMS

Input connector
Standard BNC

Current Monitor Signal

Gain
0.05 to 5mV/pA, panel switch or external digital control selectable

Bandwidth
50kHz

Current Clamp (CC) Mode

CC command
Main input: 2nA/V (max 1000nA) summed with +/- 200nA user-selectable DC offset
Aux input: 20nA/V (max 1000nA)
Bandwidth
25kHz (Rs=2MΩ, Cm = 50pf)

Pipette Capacitance Compensation

0 to 10pF, manual adjustment, calibrated

Series Resistance Compensation

100% Rs compensation eliminates series resistance voltage errors in < 200µs.

Series resistance ranges
Low range: 0-20MΩ
High range: 0-100MΩ
Rs ranges switch selectable, Rs compensation manually adjusted with a 10-turn calibrated dial.

Applying a TTL low to the rear panel "Blank" input disables Rs compensation.

Pipette offset

+/- 200mV, manual adjustment, 10-turn calibrated dial

Holding Potential

+/- 200mV, manual adjustment, 10-turn calibrated dial

Holding potential can be disabled with a front panel on/off switch.

Filters

Filter 1: 4 pole lowpass 10kHz Bessel filter

Filter 2: 4 pole lowpass 3kHz Bessel filter.

3 position front panel switch directs current monitor signal through Filter 1 or cascaded filters 1 and 2.

Panel Meter

LED panel meter displays pipette voltage

Digital interface

16 pin female D-SUB connector, bi-directional, TTL logic levels

Digital interface allows most front panel controls to be set and/or monitored externally.

Low range:
Rs voltage error < 1mV in < 200µs (10Meg pipette, 33pF cell, 20nA current step)

High range:
Rs voltage error < 1mV in < 800µs (50Meg pipette, 33pF cell, 20nA current step)

Specifications subject to change without notice. VE-2 parts and labour are warranted for 24 months from date of purchase.

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