Warner Instruments
Oocyte Clamp Amplifier
Model OC-725C

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The model **OC-725C** Oocyte clamp is designed for two-electrode, whole-cell voltage clamping of *Xenopus* oocytes, as well as for other large cells and cell structures such as squid axons. The instrument has several features making it ideal for these purposes.

Unique Features

High Voltage Compliance: The **OC-725C** combines high AC and DC gains and a voltage compliance of ±180 V to insure fast, nonsaturating clamp performance under nearly any condition. The AC clamp gain is variable up to 2000. An additional DC gain of 10^6 may be employed for high conductance cells or leaky oocytes.

Two clamp speeds are available. The Slow mode is used for screening oocytes or for applications not requiring rapid response times. The Fast mode is used for accurate voltage clamping of fast whole cell currents. Response time in the Fast mode is 350 µs (10-90% rise time) when applying a 100 mV step to a model cell.

Bath Clamp Headstage: The current measuring range of the **OC-725C** bath clamp headstage is extended at both ends by a 3-position range multiplier. This allows smaller currents to be amplified to usable levels while larger currents (up to 1 mA) can be recorded without saturation.

The unique design of the bath clamp eliminates the need for series resistance compensation. It provides an accurate measurement of bath current by creating a virtual ground in the bath while simultaneously clamping the bath potential at zero.

Dual Oocyte Studies: Studies involving two oocytes in a common bath requires two clamp amplifiers. Traditional bath clamp headstages cannot provide effective clamping because they cannot separate the individual currents from the combined currents appearing in the bath. The **OC-725C** solves this problem by the application of an internal switch permitting measurement of the current in series with the current electrode instead of in the bath. Additionally, an optional differential voltage headstage is available which subtracts the voltage drop across the series resistance in the bath.

Voltage Headstage Probe: The voltage measuring headstage is a single-ended, high-impedance probe. Its small size, convenient mounting rod, and two meter cable make for easy attachment to a micropositioner. Warner electrode holders having a 2 mm jack mount directly onto the headstage.

Voltage and Current Meters: Independent meters provide simultaneous displays of membrane voltage V_m and membrane current I_m. To assure proper impalement of the current electrode, the current meter displays membrane potential, V_e, from the current electrode before the clamp circuit is activated.

Additional Features

Buzz controls for each electrode aid in penetration of cell membranes with a minimum of leakage.

Electrode Test for voltage and current electrodes.

Capacity Compensation for the V_m voltage input.

Overload Alarm serves as a reminder when the feedback amplifier reaches its maximum output voltage, a condition which could result in damage to the oocyte.

DC Offsets for voltage and current electrodes.

NOMENCLATURE

Text conventions

This manual refers to amplifier controls at four functional levels; operational sections, control blocks, specific controls within a block, and settings of specific controls. To minimize the potential for confusion, we have employed several text conventions which are specified below. Since our goal is to provide clarity rather than complexity, we welcome any feedback you may wish to provide.

- Warner Instrument product numbers are presented using a **bold type**.
- References to instrument panel control blocks are specified using UNDERLINED SMALL CAPS. (e.g., COMMANDS, CLAMP)
- References to specific controls within a block are specified using NON-UNDERLINED SMALL CAPS. (e.g., MODE SWITCH, DC GAIN)
- Finally, references to individual control settings are specified in *italic type*. (e.g., *slow, fast*, *100 mV*)
- Special comments and warnings are presented in highlighted text.

Any other formatting should be apparent from context.

THIS EQUIPMENT IS NOT DESIGNED NOR INTENDED
FOR USE ON HUMAN SUBJECTS

CONTROL DESCRIPTION

The **OC-725C** is comprised of three functional channels: A high impedance voltage sensing channel with capacity compensation and input offset to measure membrane potential, a current sensing channel with bath clamp to clamp the bath and measure the membrane current, and a high voltage amplifier to deliver the clamping current.

The complete voltage clamp system consists of the **OC-725C**, the voltage recording probe with electrode holder, the current sensing bath probe with silver wire electrodes, and the current cable with electrode holder.

Front panel

The instrument front panel is divided into six control blocks titled VOLTAGE ELECTRODE [V_m], BATH ELECTRODES [I_m], CLAMP, COMMANDS, and CURRENT ELECTRODE. The instrument rear panel has BNC connectors for GAIN TELEGRAPH OUTPUT and V_e x10 output, an ALARM *on/off* switch, and binding posts for CIRCUIT and CHASSIS GROUND.

Voltage electrode

The VOLTAGE ELECTRODE control block contains the VOLTAGE PROBE CONNECTOR, the VOLTAGE METER, the V_m OFFSET control, the ELECTRODE TEST and BUZZ pushbuttons, and the V_m OUTPUT BNC. Controls for NEGATIVE CAPACITY COMPENSATION are also located in this block.

The VOLTAGE METER reports the membrane voltage (V_m) with a full scale range of ±199.9 mV.

The VOLTAGE PROBE CONNECTOR is a 7-pin DIN connector for attachment of the voltage probe to the instrument.

The V_m OFFSET control is a 10-turn potentiometer providing up to ±200 mV at the VOLTAGE PROBE input for offset of membrane junction potentials.

The V_m ELECTRODE TEST control is used to determine the internal resistance of the voltage probe. When the pushbutton is depressed, a constant 10 nA current is passed through the voltage electrode producing a voltage drop of 10 mV/MΩ of probe resistance. The measured potential is displayed by the meter or reported at the V_m x10 output BNC (at 100 mV/MΩ).

The V_m BUZZ push button facilitates penetration of the voltage electrode by producing a 10 kHz square wave at the pipette tip.

The V_m x10 OUTPUT BNC reports the membrane voltage in mV multiplied by 10.

NEGATIVE CAPACITY COMPENSATION (-C) has been added to the **OC-725C** allowing for its use as an electrometer in intracellular measurements. Input capacitance up to 45 pF can be neutralized using the two associated controls. A lit LED indicates the active status of this circuit.

In general, negative capacity compensation is not useful for oocyte clamp applications since clamp speed is a function of (1) the current electrode resistance, (2) the RC time constant of the oocyte (typically 1 MΩ in parallel with 0.5 µF), and (3) the compliance voltage of the clamp current.

Bath electrodes

The BATH ELECTRODES control block contains the BATH PROBE connector, the CURRENT METER, the GAIN SELECT switches, and the I MONITOR output BNC's.

The CURRENT METER reads the voltage (V_e) of the current electrode when the CLAMP MODE SELECTOR switch is *off* (*see* CLAMP section). A lit LED indicates voltage readings in mV.

When in clamp mode (CLAMP MODE SELECTOR switch set to *slow* or *fast*), the CURRENT METER displays the current (I_m) sensed by the bath electrode. A lit LED indicates current readings in µA.

Instrument gain is set by the two GAIN SELECT controls. Gain is selected by a 7-position GAIN SELECTOR switch, which ranges from *0.1* to *10* in 1-2-5 steps, and a 3-position toggle switch which selects the gain multiplier (*x0.1*, *x1.0* and *x10*). LED's indicate the gain multiplier selection. The combination of these controls allows gain to be set from *0.01* to *100*.

Current outputs are available from the I MONITOR BNC at full bandwidth (~10 kHz) and from the I MONITOR FILTERED BNC which is filtered at 1 kHz by an integral 4-pole Bessel filter.

Clamp section

The CLAMP control block contains the MODE SELECTOR switch as well as the GAIN and DC GAIN controls.

The CLAMP MODE SELECTOR switch selects for *slow* and *fast* clamp speeds, or for *off*. These choices are described below.

off - In the *off* position, the clamp amplifier is disconnected from the current electrode. The voltage difference between the

current electrode and the bath electrode [V_e, mV] is read on the METER in the BATH ELECTRODES control block. This information is also available at the V_e x10 output BNC on the rear of the instrument.

NOTE: All CLAMP controls are disabled by turning the CLAMP MODE SELECTOR switch to the *off* position. **Be sure to switch this control to the *off* position before handling electrodes!**

slow - The *slow* clamp mode is useful for screening of oocytes or where high clamp speeds are not required. The *slow* clamp speed is approximately 0.5 ms when measured with the model membrane (1 MΩ shunted with 0.47 µF). In this mode, measured currents are displayed on the BATH ELECTRODES METER in units of µA.

fast - Most oocyte clamping is performed in the *fast* mode. The clamp speed is limited by the resistance of the current electrode and the oocyte membrane capacitance. Therefore, the current electrode resistance must be kept as low as possible to obtain the fastest clamp speeds. Currents are read on the BATH ELECTRODES METER in units of µA. *Fast* clamp speeds are ~350 µs when measured with the model cell as described above.

The GAIN control is a single turn potentiometer which varies the full-bandwidth open-loop gain from *0* to *2000*. A high DC GAIN (10^6) can be switched in with the DC GAIN toggle switch to provide a hard clamp when passing large currents from high expression oocytes.

Commands

The COMMANDS control block contains the HOLD controls and COMMAND IN÷10 input BNC.

HOLD controls - HOLD potential is set with the DIGITAL POTENTIOMETER thumbwheel and RANGE toggle switch. Ranges are ±99 mV and ±198 mV depending on the scale multiplier selected *(x1.0* or *x2.0*). Signal polarity, or *off* is selected with the associated toggle switch.

COMMAND IN ÷10 input BNC- Command signals from an external generator or computer connected to this input are attenuated by 10. Maximum input is ±10 V.

Current electrode

The CURRENT ELECTRODE block includes the V_e OFFSET, ELECTRODE TEST, and V_e BUZZ controls. This section also contains the OVER VOLTAGE indicator and a DIN connector for the current electrode.

V_e OFFSET - With a range of ±200 mV (center zero), this control is used to adjust the offset voltage of the current electrode. Use this control to establish a zero reference before impaling the oocyte. Once the oocyte has been pierced, the resting potential can be read from V_e x10 output BNC or on the current meter (BATH ELECTRODES section).

ELECTRODE TEST - A voltage proportional to the resistance of the current electrode [10 mV/MΩ] will be displayed on the meter by depressing the ELECTRODE TEST push button when the CLAMP MODE SELECTOR switch is in the *off* position.

V_e BUZZ - The V_m BUZZ pushbutton facilitates penetration of the voltage electrode by producing a 10 kHz square wave at the pipette tip.

OVER VOLTAGE LED - If the voltage at the current electrode exceeds ±160 V, the OVER VOLTAGE lamp will light. An alarm will also sound when the rear panel ALARM switch is in the *on* position.

Rear panel

The line power connector and fuse are located on the rear panel. Operating voltage is specified on the MODEL/SERIAL NUMBER sticker applied to the rear of the instrument. The rear panel also contains V_e x10 and GAIN TELEGRAPH output BNCs, the ALARM switch and instrument GROUNDS.

NOTE: Verify that the instrument is wired for the proper voltage before connecting the line cord.

The V_e x10 output BNC monitors the voltage of the current electrode (x10) when the CLAMP MODE SELECTOR switch is *off*

The GAIN TELEGRAPH output BNC provides a DC voltage indicating the gain setting of the instrument. The output varies from 0.2 to 2.6 volts in 200 mV steps as shown in the appendix.

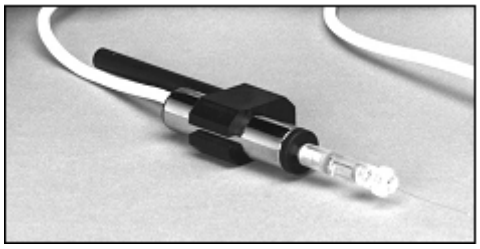
ALARM switch - Activates or deactivates the OVER VOLTAGE current electrode audible alarm.

GROUNDS - Both CIRCUIT and CHASSIS grounding posts are located on the rear panel. CHASSIS is common with the instrument enclosure and connected to earth through the power line cord. A shorting link allows for interconnection of the two grounds. In most experimental setups, separating the grounds will result in minimizing 50/60 Hz signal interference from ground loops. However, trial and error will determine the best results.

NOTE: For safe operation, the ground pin on the power plug must not be removed and the use of "cheater" plugs must be avoided.

Additional components

Voltage recording headstages



7250V PROBE (Standard Version)

The voltage probe is an active headstage housed in a 1.25 x 5 cm cylinder (dia. x length). The probe body is nickel plated and epoxy sealed for corrosion resistance.

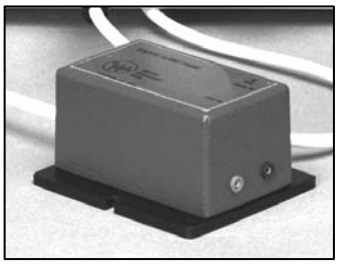
NOTE: The outer shell is electrically driven at the input potential.

Warner microelectrode holders with 2 mm jacks mate directly to the input pin on the probe body. A mounting block and handle are supplied and facilitate attachment of the probe to a micromanipulator. The handle can be mounted either axially or perpendicular to the probe body.

7255DI DIFFERENTIAL PROBE (Optional)

This voltage probe is designed for applications where two oocytes share a common bath or where the voltage drop across the solution resistance is to be measured and subtracted from V_m. The headstage housing is approximately 2 cm longer than that of the **7250V PROBE** and has two additional inputs; CIRCUIT GROUND and V DIFFERENTIAL. When the two inputs are shorted, the probe functions exactly the same as the standard single ended **7250 PROBE**.

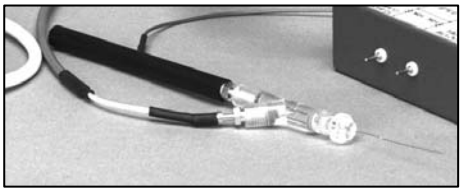
Bath headstage



The BATH PROBE is housed in a 2.8 x 3.5 x 4.2 cm aluminum enclosure. Inputs are two 1 mm pin jacks labeled I SENSE and I OUT. The case is electrically grounded and a pin jack is located on the side for connecting to shields.

A plastic plate with two screw mounting slots is attached to the probe base. The BATH PROBE connects to the control unit with a 6 pin connector

Current electrode cable



A two meter shielded cable is supplied with a 2 mm pin jack on one end to mate with the an electrode holder, and a 3-pin connector on the other end to mate with the instrument. The electrode holder should have a handle for mounting to a micromanipulator. An example is shown to the right.

Model cell

The model cell supplied with the **OC-725C** is useful as a training aid and as a calibration and test device. It has connections for the voltage and current probes and to the bath clamp allowing all aspects of the amplifier's function to be tested.

Comments

Connecting to line power

The model **OC-725C** is supplied with a 3-conductor power cord. One conductor provides a connection between the instrument housing and the earth ground. Safe operation of the instrument will be assured provided that the ground circuit in the power outlet is wired correctly and is connected to earth.

NOTE: If the ground pin of the power cord is removed for any reason the instrument chassis must be directly connected to earth ground using a separate heavy (14 gauge or larger) ground wire.

High voltage outputs

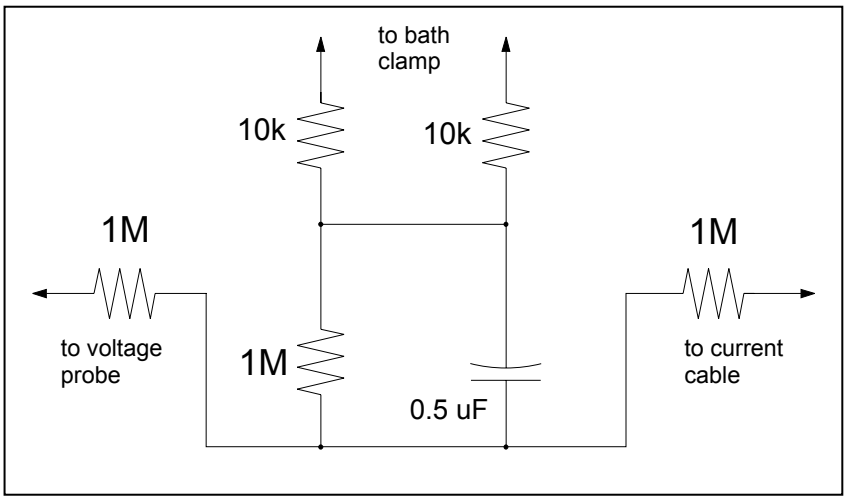
When handling the current electrode cable, be sure to set the GAIN CONTROL ***fully clockwise*** and the CLAMP MODE switch to ***off***.

CAUTION!: The current clamp is capable of high power output (10 mA @ ±180 V) and **can cause serious injury** if not properly handled.

USING THE MODEL MEMBRANE

The model cell supplied with the **OC-725C** can be used for two purposes. First, novice users will find it a convenient tool for gaining experience in the operation of the instrument. Additionally, it is a convenient tool for trouble shooting since the function of the instrument can be quickly checked.

A schematic of the model cell is shown to the right. The oocyte is represented by a 1 MΩ resistor shunted by a 0.47 µF capacitor. The voltage and current electrodes are each represented by 1 MΩ resistances and the bath probes are represented by the 10 kΩ resistors.



Initial instrument settings

Connect the model cell to the **OC-725C** as shown on its cover. Be sure to connect the ground wire to the ground mini-jack on the side of the bath probe. Connect the V_m x10 BNC and the I MONITOR BNC on the **OC-725C** to an oscilloscope.

Set the instrument panel controls as follows and turn the POWER SWITCH *on*.

Control	Control block	Setting
POWER SWITCH		*off*
V_m OFFSET	VOLTAGE ELECTRODE	Center of rotation (approximately 5 turns)
OUTPUT GAIN	BATH ELECTRODES	*0.1 V/μA*
GAIN SELECT	BATH ELECTRODES	*x1.0*
CLAMP MODE switch	CLAMP	*off*
DC GAIN toggle	CLAMP	*out*
GAIN	CLAMP	CCW to detent *off*
HOLD POTENTIAL	COMMANDS	*00 mV*
HOLD POTENTIAL MULTIPLIER	COMMANDS	*x1.0*
HOLD POLARITY	COMMANDS	*off*
V_e OFFSET	CURRENT ELECTRODE	Center of rotation (approximately 5 turns)

Test procedures

In the following testing procedures, allow a tolerance of ±1% on the readings taken. For example, if the test response is indicated as 100 mV, a reading from 99.0 to 101.0 mV would be within tolerance.

Offset controls

V_m OFFSET (VOLTAGE ELECTRODE section): The full range of this control is ±200 mV. This can be verified by rotating the control first fully clockwise and then fully counterclockwise while observing the VOLTAGE ELECTRODE METER. The displayed readings will indicate off scale at the extremes of the control's manipulation since the meter is only capable of displaying ±199.9 mV.

V_m x10 output BNC (VOLTAGE ELECTRODE section): This output can be monitored using an oscilloscope. The reported voltage will swing between ±2 V as the V_m OFFSET control is

manipulated throughout its full range. Set the V_m OFFSET to 0.0 reading on the meter and verify that the V_m x10 reading on the scope also reads 0 V.

V_e OFFSET control (CURRENT ELECTRODE section): The V_e OFFSET control is tested in the same manner as the V_m OFFSET control. This control is adjustable when the CLAMP MODE switch is set to *off*. The voltage of the V_e OFFSET is read from the BATH ELECTRODE METER when the CLAMP MODE switch is set to *off*.

V_e x10 output BNC: This BNC is located on the rear panel of the instrument and reports the setting of the V_e OFFSET control when the CLAMP MODE switch is set to *off*.

Return all controls to their initial settings when done with this test.

Voltage electrode test

In actual practice, the voltage electrode test is used prior to entering the cell and indicates the resistance of the electrode. When used in conjunction with the model cell, it measures both the electrode and membrane resistance (2 MΩ). This test is performed using controls in the VOLTAGE ELECTRODE section.

With the model cell in place, depress the V_m ELECRODE TEST pushbutton and observe a reading of 20 mV on the VOLTAGE ELECTRODE METER. This corresponds to a 2 MΩ reading at a calibrated response of 10 mV/MΩ.

On the oscilloscope, the V_m x10 output will read 200 mV which is x10 the applied test voltage. Since the test current is being passed through 1 MΩ (R_m), the I MONITOR output will indicate 1 mV, which corresponds to 10 nA of current.

With the CLAMP MODE switch set to *off*, the BATH ELECTRODE METER monitors V_e (voltage at the current electrode). In this case, V_e will be a measure of the voltage across R_m and the meter will indicate 10 mV (1 MΩ). The V_e x10 output BNC on the rear panel can also be checked to see that it reads 100 mV (meter reading x10).

Buzz

This test is performed using controls in the VOLTAGE ELECTRODE section.

Set the oscilloscope sensitivity to 5 V/div and depress the BUZZ pushbutton while monitoring the V_m x10 output. A 10 kHz square wave of approximately 24 V p-p will be generated as long as the button is depressed.

Current electrode test

This test is performed using controls in the CURRENT ELECTRODE section.

With the model cell in place, depress the V_e ELECRODE TEST pushbutton and observe a reading of 20 mV on the CURRENT ELECTRODE METER. This corresponds to a 2 MΩ reading at a calibrated response of 10 mV/MΩ.

With the CLAMP MODE switch set to *off*, the BATH ELECTRODE METER monitors V_e (voltage at the current electrode). In this case, V_e will be a measure of the voltage across R_m and the meter will indicate 10 mV (1 MΩ).

DC clamp test

This test is performed using controls in the CLAMP ELECTRODE section.

Place the CLAMP MODE switch in the *fast* position and adjust the V_m OFFSET control for a reading of *100 mV* on the VOLTAGE ELECTRODE METER.

Turn the GAIN control on and slowly turn the control clockwise until the meter reading (V_m) decreases to zero. The CURRENT ELECTRODE METER should read *-0.10 μA*.

Set the HOLD POTENTIAL (COMMANDS section) to *100 mV* (*50 mV* on thumbwheel and MULTIPLIER toggle at *x2*). Select positive *(pos)* polarity. The VOLTAGE ELECTRODE METER should read *100 mV* and the CURRENT ELECTRODE METER should read *0.00 μA*.

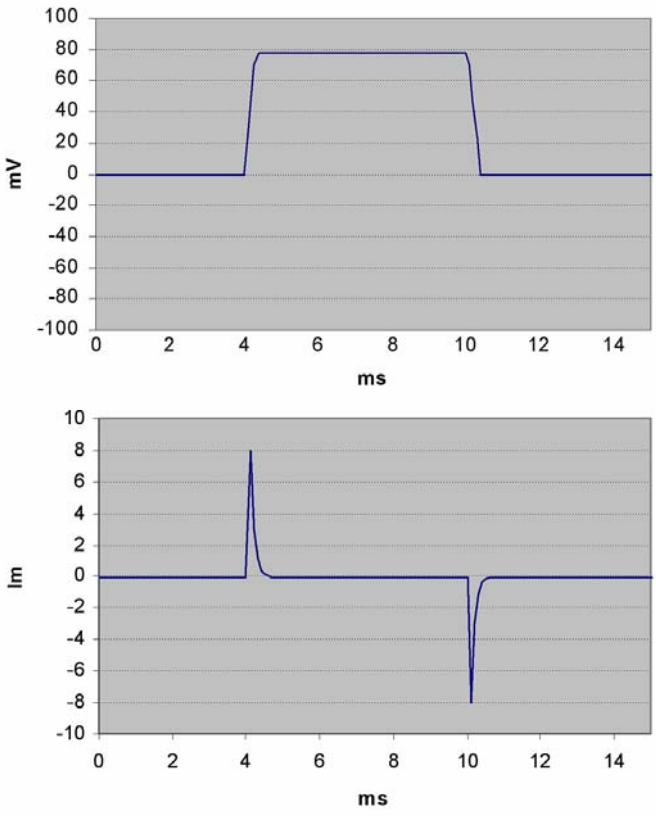
Switch to negative *(neg)* polarity. The VOLTAGE ELECTRODE METER should read *100 mV* and the CURRENT ELECTRODE METER should read *0.2 μA*

Return the GAIN control fully *CCW* and turn the CLAMP MODE switch to *off*.

AC clamp test

Adjust the V_m OFFSET control to *0.0 V*. Apply a 0.8 V, 100 Hz square wave to the COMMAND IN ÷10 BNC. Monitor the V_m x10 and I MONITOR outputs on the oscilloscope.

Switch the CLAMP MODE switch to *fast* and increase the GAIN until V_m reads *80 mV*. Verify that the V_m x10 BNC reports *0.8 V*. As you further increase the GAIN control, you will see the rise time of the oscilloscope trace become faster since the speed of the clamp is limited by the resistance of the current electrode and the capacitance of the oocyte. If ringing (oscillation) is observed, decrease the GAIN setting to obtain the fastest clean waveform as



shown on the previous page. (The current signal (I MONITOR) shown in the figure displays the high current spikes required to charge the oocyte capacitance.)

Set the HOLD control to a reading of *50 mV* and switch the POLARITY toggle to *pos*. The square wave will be displaced 50 mV in the positive direction. Switching the polarity to *neg* will produce a -50 mV offset.

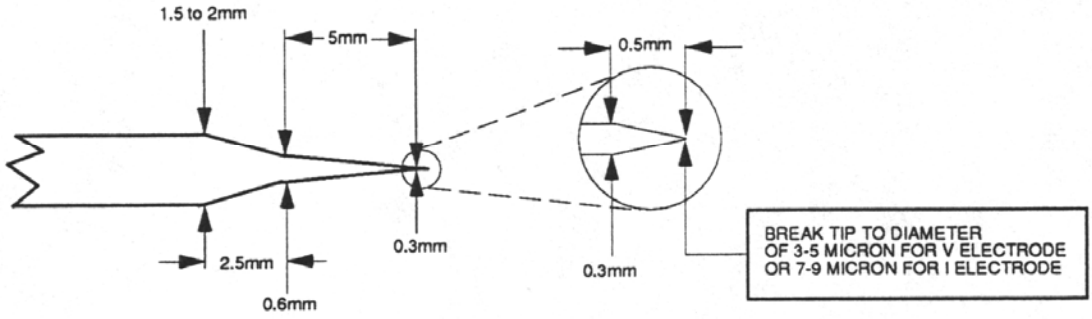
SETUP

The following instructions are designed to guide the user, step-by-step, through a typical recording session involving a *Xenopus* oocyte. It is assumed that the user is already familiar with the techniques of *Xenopus* oocyte excision and microinjection (for a review of those techniques, *see* Colman, 1984). It is also assumed that the user has some familiarity with the basic circuitry of a two-electrode voltage clamp (for review, *see* Hille, 1984).

Pipettes

Microelectrodes can be made using the same glass (tubing and dimensions) as those used for a typical patch pipette and are usually filled with 3 M sterile filtered KCl. Unlike the pipettes used as patching electrodes, microelectrode pipettes do not require fire polishing nor coating with Sylgard. They will need to be broken off, however, to a relatively large diameter to insure a fast response time by the clamp.

For the voltage electrode, the pipette tip should be broken back to an O.D. of 3-5 µm. The current electrode pipette should be broken back to an O.D. of 7-9 µm. The resistances of these pipettes should be about 2 MΩ and 1 MΩ (or less), respectively.



When installed, the current electrode pipette should be shielded from the voltage electrode and that shield should be grounded to the circuit ground. This can be accomplished by wrapping the current pipette with aluminum foil or by mounting a metal screen or plate between the two pipettes. In either configuration, the shield can be grounded by connecting it to the "ground" mini-jack on the side of the bath probe. When using the aluminum foil method, care must be taken to prevent the foil from touching the surface of the bath solution at the bottom end of the pipette or the silver electrode wire at the top end.

Electrode holders

Voltage Electrode - The voltage recording electrode holder uses a silver wire for the electrical coupling between the pipette and holder connector. Any silver wire contacting the KCl solution in the pipette must be chlorided to reduce junction potentials (*see* Chloriding

Procedure in Appendix). The pipette should contain just enough KCl so that approximately 1/2 inch of the chlorided wire is submerged. The pipette/holder assembly is attached directly to the voltage headstage prior to mounting in a micropositioner.

NOTE: Do not fill the voltage holder with KCl solution.

Current Electrode - The current recording electrode also uses a silver wire for coupling. In an manner analogous to the voltage electrode, the current electrode wire must be chlorided prior to assembly and use. The pipette/holder assembly is mounted in a micropositioner with the mounting rod supplied.

NOTE: Do not fill the current holder with KCl solution.

Bath probe

The bath clamp is designed to maintain a virtual ground in the oocyte perfusate. The bath probe should be positioned so that the silver electrode wires can be inserted into the recording chamber or into the agar bridge wells. Sticky wax or tape is usually sufficient to secure the unit when positioned on a flat surface or, alternatively, the unit can be held in place on a separate stand. The bath probe electrodes should also be chlorided before use, as described above.

Electrode placement and grounding

Three drawings (shown on pages 19 and 20) have been included to illustrate the various ways a bath circuit can be configured. Most applications involve only a single oocyte and Figures A and B illustrate these setups. Figure C shows a setup for recording from 2 oocytes in a common bath with the use of dual clamps

NOTE: Cable routing must be performed with care. Bundle cables together rather than routing them individually and keep them as far as possible from sources of 50/60 Hz interference (e.g., line cords, transformers, etc.).

Bath clamp electrode placement

Proper placement of the bath electrodes (I_{out} and I_{sense}) is important for obtaining optimum performance. The I_{sense} electrode (or the agar bridge associated with it) should be placed as close to the oocyte as possible since this point is the virtual ground node, and on the same side as the voltage recording electrode. The I_{out} electrode (or the agar bridge associated with it), on the other hand, can be placed at a greater distance from the oocyte and should be on the same side as the current electrode

It is recommended that the user not directly expose the electrode wires to the perfusate if the recording session is to last for more than a few minutes. Instead, agar bridges should be employed to provide a circuit between these electrodes and the bath. This protects the cell membrane from the potential adverse effects of the silver wire.

Single oocyte setup with indirect ground

Single oocyte studies are best accomplished with the setup shown in Figure A. This method uses the bath clamp headstage to establish the bath ground and is preferred for two reasons: (1) current readings with the bath clamp will have the lowest noise level, and (2) properly placed bath clamp electrodes will negate the need for series resistance compensation.

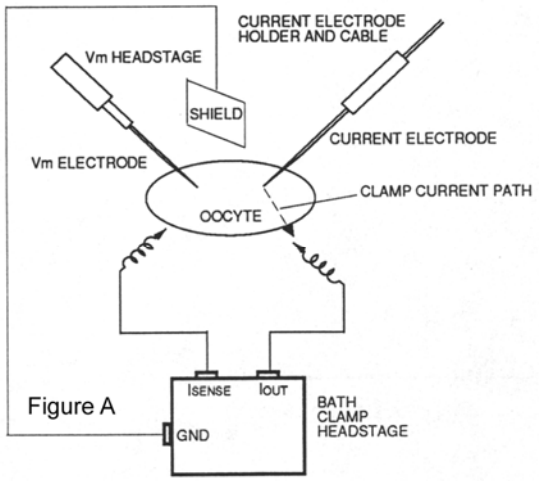


Figure A

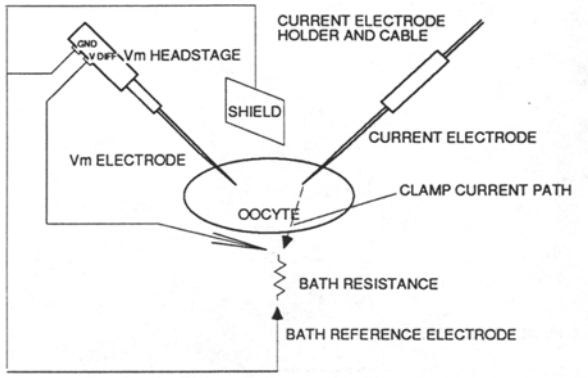


Figure B

Single oocyte setup with direct ground

Applications where use of the bath clamp is not suitable (such as those with a very long solution path to ground) can be configured using the alternate method of directly grounding the bath as shown in Figure B. In this configuration, current is read from the "high side" of the current output leg.

This method also requires the use of the optional DIFFERENTIAL VOLTAGE HEADSTAGE. Two disadvantages are: (1) The noise levels of the current signal measured in the "high side" is approximately double that obtained with a bath clamp, and (2) high levels of clamp current could produce a substantial voltage drop across the solution series resistance.

NOTES: The oocyte clamp has no provisions for compensating the voltage drop described above. The differential voltage electrode should be placed close to the oocyte and in the current path between the V_m electrode and ground.

Dual ooctye set-up

Dual oocyte setup is accomplished using two clamps as illustrated in Figure C. Both clamps must be configured to read current from the "high side" as described in the section titled **HIGH SIDE CURRENT MEASURING** (*see* page 26) and each clamp must be equipped with the optional DIFFERENTIAL VOLTAGE HEADSTAGE.

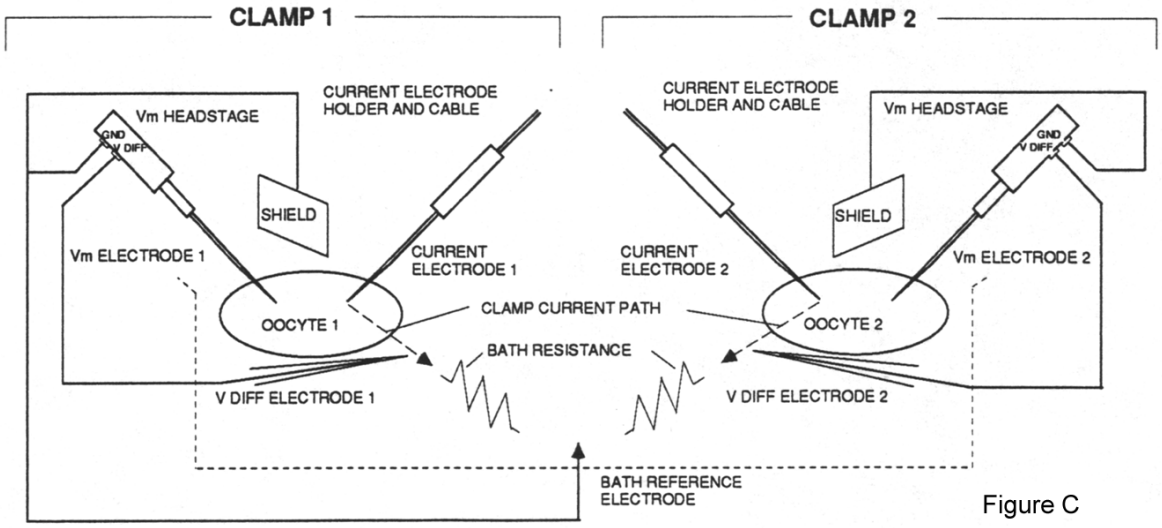


Figure C

Cable connections

Bath clamp headstage: After positioning the probe as described above, connect it to the BATH PROBE socket (BATH ELECTRODES section).

Voltage electrode headstage: The high impedance probe for recording membrane potential should be mounted on a micro-manipulator and connected to the VOLTAGE PROBE socket (VOLTAGE ELECTRODE section).

High voltage current electrode: The holder should be mounted on a micro-manipulator and the cable connected to the I ELECTRODE socket (CURRENT ELECTRODE section).

Command potential: If a computer or external generator is used for controlling the clamp command potential, its signal should be connected to the front panel COMMAND IN ÷10 input (COMMANDS section).

External monitoring: To monitor the microelectrode's potentials on an oscilloscope, computer or a chart recorder, the following connections should be made:

V_m - Membrane potential may be recorded from the V_m x10 connector (VOLTAGE ELECTRODE section).

V_e - The voltage of the current electrode can be monitored from the V_e connector on the REAR PANEL.

The output will be the same as that reported on the CURRENT ELECTRODE METER, showing the voltage across the current electrode when the CLAMP SELECTOR switch is in the *off* position. (Recall that the meter indicates the current (I_m) when the CLAMP SELECTOR switch is in either the *slow* or *fast* position).

I_m - The current signal is available from the I MONITOR and I MONITOR FILTERED outputs (BATH ELECTRODES section).

Gain telegraph – Automatic monitoring of the I_m gain can be achieved by connecting the rear panel GAIN TELE BNC to the appropriate input on your analog-to-digital converter.

Power: The power cord should be connected to a properly grounded AC receptacle with the line voltage specified on the instrument nameplate (REAR PANEL).

Resting position of the controls

To begin, set the instrument controls to the following positions:

Control	Section	Setting
POWER		*off*
I MONITOR output	BATH ELECTRODE	*1 V/μA*
GAIN SELECT	BATH ELECTRODE	*x1*
HOLD	COMMANDS	*0 mV*
POLARITY toggle	COMMANDS	*off*
MODE SELECT	CLAMP	*off*
CLAMP DC GAIN toggle	CLAMP	*out*
CLAMP GAIN	CLAMP	*off* (fully CCW)
ALARM	REAR PANEL	*off* or *on* (as desired)

Turn POWER *on*.

Using the gain select

A wider range of bath current (I_m) measurements is now possible with the addition of the GAIN SELECT toggle switch located above the GAIN control. The switch has 3 positions; *x0.1*, *x1* and *x10*. Resistance values shown below each LED indicate the bath clamp feedback resistor used for the current measurement.

The chart on the next page shows the effect of the range selection on the I_m output. Note also that range selection changes the sensitivity of the current meter. Typically, measured currents will fall in the *x1* range. The lower and higher ranges are intended to cover those applications where currents are beyond the *x1* range. Currents below 1 μA should be monitored in the *x10* range. Large currents above 100 μA require the *x0.1* range. Since there is overlap in the ranges, the current being measured may be monitored in one of two ranges in which case the choice may be made on the basis of noise or clamp speed.

Gain select	Headstage resistor	I_m output range (V/μA)	I_m max output (μA)	Maximum meter reading
x0.1	10 kΩ	0.01 - 1.0	10 - 1000	199.9 μA
x1.0	100 kΩ	0.1 - 10	1 - 100	19.99 μA
x10	1 MΩ	1.0 - 100	0.1 - 10	1.999 μA

Other gain range selection considerations

The intrinsic noise of the current measuring circuit is a function of the bath clamp feedback resistor with a larger resistor offering lower noise and greater signal resolution. For comparisons, the following measurements were made with a shorted input, with the standard 0.5 μF capacitance model cell, and with a 0.22 μF capacitance modified model cell. All readings were recorded at 1 kHz (8-pole Bessel) and are RMS.

Feedback Resistor	Noise		
	Shorted Input	Standard Model Cell (0.5 μF)	Modified Model Cell (0.22 μF)
10 kΩ	75 pA	6.0 nA	4.4 nA
100 kΩ	28 pA	5.5 nA	4.4 nA
1 MΩ	22 pA	5.0 nA	4.0 nA

A lower value feedback resistor increases the speed of the bath clamp and can handle larger currents without saturating, important when recording from high expression oocytes.

A PROCEDURE FOR RECORDING FROM OOCYTES

Initial electrode placement

1) Make sure that the bath electrodes are submerged in the chamber (or in the agar bridge wells with the agar bridges completing the circuit to the bath) and the oocyte is stable on the chamber floor.

2) Install the voltage and current pipettes onto their respective holders but do not yet place them in the chamber bath solution.

Voltage electrode placement

3) Advance the voltage recording electrode into the bath. The VOLTAGE ELECTRODE METER will indicate (in mV) the potential between the electrode and the bath.

4) If there is no voltage reading and you are sure that the pipette tip is in the bath solution, perform the following checks:

   a) make sure that all cables are connected properly.
   b) inspect the voltage electrode to see if there is a bubble in the pipette which will cause an open circuit.

5) Using the V_m OFFSET control, adjust the VOLTAGE ELECTRODE potential to read 0 mV. If the junction potential of the voltage electrode can not be adjusted to 0 mV, the electrode holder may be at fault. (*See* **Electrode Holders,** page 17.)

6) To test the resistance of the voltage electrode pipette, depress the ELECTRODE TEST button. This passes a 10 nA current across the voltage electrode. The VOLTAGE ELECTRODE METER will display the resulting potential in mV. The resistance of the electrode can be easily calculated by dividing the current into the potential. The resulting answer will be expressed in Ω. For example, if the electrode test indicates that a potential of 25.0 mV is produced by the 10 nA test current, then

$$R = \frac{V}{I} = \frac{25\,mV}{10\,nA} = 2.5x10^6\,\Omega = 2.5\,M\Omega$$

NOTES:

a) A simpler calculation is to divide the voltage readout by 10 and append the units of MΩ. (e.g., 25 mV = 2.5 MΩ)

b) The calculated resistance value may vary widely from pipette to pipette but should be less than 4 MΩ for the voltage electrode.

Current electrode placement

7) Advance the current electrode until the tip is in the chamber bath solution. Adjust V_e OFFSET for a *zero* reading on the CURRENT ELECTRODE METER. This will establish a null reference allowing the resting potential to be directly read.

8) With the CLAMP SELECTOR switch in the *off* position, the resistance of the CURRENT ELECTRODE pipette is tested in the same manner as the VOLTAGE ELECTRODE. Pressing the V_e ELECTRODE TEST pushbutton will cause a 10 nA current to be passed across the CURRENT ELECTRODE. The resulting voltage (in mV) will be displayed on the METER in the BATH ELECTRODE section. From that value, the resistance of the pipette can be calculated exactly as described above (i.e., divide the readout by 10 to get the resistance in MΩ). Since the current electrode has a larger diameter, its resistance should be less than that of the voltage electrode (about 1.0 MΩ or less).

9) If no voltage display is present during the electrode test procedure and you're sure that the electrode is contacting the bath, perform the following checks:

   a) Make sure that all cables are connected properly.
   b) Check to see that the aluminum shield around the current electrode pipette (if used) is not touching the bath solution or the electrode wire.
   c) Check the current electrode to see if there is a bubble in the pipette causing an open circuit.

Impaling the cell

10) Recheck the VOLTAGE ELECTRODE METER to verify that the potential is correctly offset to read *0.0 mV* and readjust the V_m OFFSET control if needed.

11) Advance the VOLTAGE ELECTRODE until its tip is slightly depressing the plasma membrane of the cell and depress the V_m BUZZ pushbutton. This will produce a 1 V, 10 kHz oscillation at the voltage electrode, disrupting the membrane and causing the tip of the electrode to impale the cell with no further movement of the micro-manipulator (this technique will work best with "fresh" oocytes, i.e., 1 or 2 days post-excision). If the buzz technique fails to cause electrode penetration, further advance the voltage electrode until it "pops" through the membrane.

12) The potential across the membrane will now be displayed on the VOLTAGE ELECTRODE METER.

13) Now, advance the current electrode until its tip is slightly depressing the plasma membrane of the cell and depress the V_e BUZZ pushbutton. Similar to the voltage electrode BUZZ, the current electrode BUZZ produces a 1 V, 10 kHz oscillation across the current electrode. This disrupts the cell membrane and causes the tip of the electrode to impale the cell with no further movement of the micro-manipulator. Once again, if

the BUZZ technique fails to cause penetration, further advance the current electrode until it "pops" through the membrane.

Clamping the cell

14) Activate the clamp by switching the CLAMP MODE switch to either the *slow* or *fast* mode. NOTE: *slow* mode is useful for initial screening.

15) Adjust CLAMP GAIN control clockwise as far as possible without illuminating the OVER VOLTAGE LED (located in the CURRENT ELECTRODE section).

    NOTE: If the ALARM signal switch on the back panel is *on*, an alarm will sound when the OVER VOLTAGE LED illuminates.

16) The clamped membrane potential can now be observed over time or it can be manipulated by selecting the desired polarity and amplitude with the controls located in the COMMANDS section. Alternatively, you can control the COMMAND voltage externally from a computer by leaving the POLARITY toggle switch in the *off* position and connecting the appropriate analog output from your computer DAC to the COMMAND IN ÷10 BNC connector. (*See* **Cable Connections,** page 20.)

17) Depending on the amplitude of the response you wish to record, you may adjust the instrument GAIN to a higher or lower position. The CURRENT ELECTRODE METER should now be displaying the current (in μA) that is delivered to hold the cell at the designated command potential.

Clamping high conductance cells

18) DC GAIN mode (CLAMP section) may be required to clamp high conductance (low resistance) cells. This condition will be evidenced by the inability of the instrument to maintain a DC holding potential (to within 1% or better of the set value) and the maximum instrument gain is not sufficient to provide a hard clamp. DC GAIN mode provides an additional DC gain greater than 10^6 while the AC gain remains at 2000 maximum for stability.

Unclamping the cell

19) To unclamp the cell, turn the GAIN control (CLAMP section) fully counter-clockwise to the detent *off* position. This will also disengage the DC GAIN.

    NOTE: If the control is not fully *off* and the DC GAIN is left *on*, the preparation will not be unclamped.

Removing the electrodes

20) It is very important that the CLAMP GAIN be returned to the *off* position (fully counter-clockwise to click *off*) as described above and the CLAMP MODE selector switch be placed in the *off* position before removing the current electrode from the cell.

Failure to perform the above steps will overload the feedback amplifier (due to the large current generated when the membrane resistance between the current electrode and the bath virtual ground goes to zero). This will damage the oocyte! For this reason we recommend that the user enable the audible overload alarm to provide a warning when the potential for such damage exists.

SPECIAL CIRCUMSTANCES

High side current measuring

In studies of single oocytes, current is monitored by the bath clamp headstage. Experiments involving two oocytes in a common bath (such as gap junction studies) requires monitoring currents from each oocyte. This is done in the current output leg (in series with and ahead of the current electrode).

Two disadvantages of monitoring the current in this manner exist.

a) The noise level of this signal is higher. However, this is usually not a serious problem since currents are typically in the μA range.
b) The voltage drop across the solution resistance (from oocyte to bath ground) becomes an error voltage since it is not subtracted out as when the bath clamp headstage is used. This problem is overcome by using the optional DIFFERENTIAL VOLTAGE HEADSTAGE.

Configuration

The **OC-725C** current measuring circuit can be changed to the high side current measuring mode by setting a dip switch on the main circuit board.

1) First disconnect the power cord from the wall.
2) Remove the two screws at the rear of the top cover and it off.
3) Locate **dip switch S10** on the circuit board. For normal operation S4 is *on* and all other 7 switches are *off*.
4) To convert to high side current measuring mode, turn S4 *off* and turn S2, S5, and S7 *on*. All other switches should remain in the *off* position.

Optional voltage headstage

The optional DIFFERENTIAL INPUT VOLTAGE RECORDING HEADSTAGE (**Model 7255DI**) is used in applications where the bath clamp headstage cannot be effectively employed. Two examples are (1) situations where the solution path from oocyte to ground is very long and (2) when recording from two oocytes in a common bath.

Two 1 mm input jacks are located on the side of the headstage, V DIFF and GND. A shorting jumper is supplied and is used for normal single-ended recording. For double-

ended recording, the jumper is removed and a V DIFF ELECTRODE is connected to the V DIFF jack as shown below.

If a shield between the voltage and clamp current electrodes is used, it should be connected to the headstage ground.

COMMENTS AND RECOMMENDATIONS

Membrane damage

Recording from the same cell at a later time requires that the cell remain healthy during the interim incubation. The less damage done to the membrane during handling and impaling the cell, the happier it will be. Use of the BUZZ function should help minimize the trauma from electrode penetration. Membrane damage can be further reduced by properly isolating the experimental platform from vibration. Finally, hydraulically driven micro-manipulators will also reduce membrane damage while the electrodes are in the cell.

Repeated recordings

Most recording sessions will involve repeating the above steps several times with many different cells. Unless there is a concern about contamination of the bath solution by something carried over from previous experiments, the pipettes can also be used repeatedly. They should be free of debris and should have approximately the same resistance as they had in the previous recording. A significantly higher resistance could indicate that the pipette is partially plugged with cellular debris.

Make the following control settings before the next recording is carried out:

Control	Section	Setting
POLARITY	COMMAND	*off*
MODE SELECT	CLAMP	*off*
GAIN	CLAMP	*0* (fully CCW)

Electrophysiology

If you are well versed in setting up electrophysiological equipment, you can safely skip over the rest of this section. If, however, this is your initiation into electrophysiology (as it may well be for some of you molecular biologists), then you may find the following recommendations helpful.

While the whole cell electrophysiological configuration is more forgiving than the patch clamp, it is still important to minimize mechanical motion. The platform for your experimental setup, therefore, should be mechanically well isolated. This will reduce leakage around the electrodes, making the clamp more effective and reducing noise in your recordings. The latter is especially important when recording responses of certain ligand-gated channels where membrane potential changes may only be a few mV.

In addition to mechanical isolation, the setup must also be isolated from external electrical noise sources. These include motors, lamps, and wiring. The platform should be

shielded from these sources of electrostatic radiation with a Faraday cage. All equipment within the Faraday cage should be grounded to the rear panel instrument circuit ground and is best achieved by connecting everything (including the cage) to a ground bus within the cage. Then only one wire is run from the setup to the instrument ground.

You will need to mount the voltage recording electrode headstage and the current injecting electrode on micro-manipulators. They need not be hydraulically driven but such drives will minimize the damage to the cell during and after penetration, and will make for better seals around the electrodes. Another advantage gained by reducing membrane damage by electrodes is to enhance the possibility of making subsequent recordings from the same cell. These suggestions are also important for minimizing mechanical noise in the recorded data

You will need a dissecting scope for viewing the placement of the electrodes. Anything more powerful than 40x will just get in the way. The light source for your scope should be DC and may need to be IR and UV filtered if you plan to use it during recording.

Minimally, the recording chamber can be a stable surface on which the oocyte will not roll around. A disposable petri dish with a piece of nylon mesh on the bottom has been successfully used for this purpose. The dish can be stabilized by a holder or by some wax placed around its perimeter. The diameter of the dish needs to be large enough to accommodate the oocyte and the two bath electrodes. The walls of the recording chamber should be low enough to not interfere with electrode placement. Perfusion of the chamber can be accomplished using a gravity fed system. Perfusate from the dish can be evacuated using gentle vacuum. Use as small an aperture as possible to avoid disturbing the surface of the perfusate in the dish.

NOTE: Specialized equipment suitable for oocyte studies (oocyte chambers, perfusion systems, a Faraday cage, a noise-free DC lamp, and micro-manipulators) are available from Warner Instruments.

Although it is not required, a computer can be employed to control the command voltage. Acquisition and display of data is also usually handled by computer. The **OC-725C** is fully compatible with all commercially available software packages designed for electrophysiological research.

Finally, a microelectrode puller is necessary for making appropriately sized voltage and current electrodes. Usually, the microinjection pipette puller can also be used to make microelectrodes. You will need use of a microscope to break off the pipette tips.

APPENDIX

Specifications - Equipment is intended to be operated in a controlled laboratory environment

Voltage recording channel (V_m)	
Input Impedance	5 x10^{11} Ω, shunted by 3 pF
Output Resistance	100 Ω
V_m OFFSET	± 200 mV at V probe input
Noise*	50 μV RMS at 1 kHz
Electrode Test	10 mV/MΩ
Negative Capacity	0 - 45 pF
Vm Meter Range, full scale	± 199.9 mV
Bath electrode channel (I_m)	
V_e OFFSET	± 200 mV
Noise*	
I_m Clamp (clamp *on*)	5.5 nA RMS at 1 kHz [*x1* range]
Open loop (clamp *off*)	28 pA RMS at 1 kHz [*x1* range]
I MONITOR	0.01 - 100 V/μA in 3 ranges, 7 steps per range
I MONITOR FILTERED (4-pole Bessel])	Same as above, filtered at 1 kHz
Gain Telegraph	0.2 - 2.6 VDC in 0.2 V steps
Meter Ranges (full scale)	
V_e (clamp *off*)	± 199.9 mV
I_m (*x0.1* range)	± 199.9 μA
I_m (*x1.0* range)	± 19.99 μA
I_m (*x10* range)	± 1.999 μA
Current electrode channel (V_e)	
Compliance Voltage	± 180 V
Alarm	± 160 V
Gain	
Variable	0 - 2000 AC/DC
DC	1 x10^6 DC, switch selected
Electrode Test	10 mV/MΩ
Commands	
Hold (internal)	± 198 mV in 2 ranges
External input (attenuated by 10)	1 V in = 0.1 V command
Maximum external input	10 V
Power requirements	
100 -130 or 220-240 VAC, 50/60 Hz	
Dimensions	
Enclosure	9 x 42 x 25 cm (H x W x D)
Voltage Headstage	1.25 x 5 cm (dia. x length); with 1.8 m cable
Mounting Handle	4.8 mm x 6.3 cm (dia. x length)
Bath Headstage	2.8 x 3.5 x 4.2 cm (H x W x D); with 1.8 m cable

* All noise measurements made with an 8-pole Bessel filter.

Gain telegraph outputs

I_m Output (V/μA)	Gain Telegraph
0.01	0.2 V
0.02	0.4 V
0.05	0.6 V
0.1	0.8 V
0.2	1.0 V
0.5	1.2 V
1	1.4 V
2	1.6 V
5	1.8 V
10	2.0 V
20	2.2 V
50	2.4 V
100	2.6 V

Gain select settings

Gain select	Headstage resistor	I_m output range (V/μA)	I_m max output (μA)	Maximum meter reading
x0.1	10 kΩ	0.01 - 1.0	10 - 1000	199.9 μA
x1.0	100 kΩ	0.1 - 10	1 - 100	19.99 μA
x10	1 MΩ	1.0 - 100	0.1 - 10	1.999 μA

Noise from bath clamp feedback resistor

Feedback Resistor	Noise		
	Shorted Input	Standard Model Cell (0.5 μF)	Modified Model Cell (0.22 μF)
10 kΩ	75 pA	6.0 nA	4.4 nA
100 kΩ	28 pA	5.5 nA	4.4 nA
1 MΩ	22 pA	5.0 nA	4.0 nA

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Certifications

Declaration of Conformity

CE MARKING (EMC)

Application of Council Directive: 89/336/EEC

Standards To Which Conformity Is Declared:	EN55022 Class A EN61000-3-2 EN61000-3-3 EN50082-1:1992 EN61000-4-2 EN61000-4-3 ENV50204 EN610000-4-4 EN610000-4-8 EN610000-4-11
Manufacturer's Name:	Warner Instruments, LLC
Manufacturer's Address:	1125 Dixwell Avenue Hamden, CT 06514 Tel: (203) 776-0664
Equipment Description:	Instrument Amplifier
Equipment Class:	ITE-Class A
Model Numbers:	OC-725C

I the undersigned, hereby declare that the equipment specified above, conforms to the above Directive(s) and Standard(s).

Place: Hamden, Connecticut USA

Signature:

Full Name: Burton J. Warner

Position: President

Declaration of Conformity

CE MARKING (LVD)

Application of Council Directive: 73/23/EEC

Standards To Which Conformity Is Declared:	EN61010-1:1993
Manufacturer's Name:	Warner Instruments, LLC
Manufacturer's Address:	1125 Dixwell Avenue Hamden, CT 06514 Tel: (203) 776-0664
Equipment Description:	Instrument Amplifier Safety requirements for electrical equipment for measurement and laboratory use
Equipment Class:	Class I
Model Numbers:	OC-725C

I the undersigned, hereby declare that the equipment specified above, conforms to the above Directive(s) and Standard(s).

Place: Hamden, Connecticut USA

Signature:

Full Name: Burton J. Warner

Position: President

WEEE/RoHS Compliance Statement

EU Directives WEEE and RoHS

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Harvard Apparatus is committed to being a good corporate citizen. As part of that commitment, we strive to maintain an environmentally conscious manufacturing operation. The European Union (EU) has enacted two Directives, the first on product recycling (Waste Electrical and Electronic Equipment, WEEE) and the second limiting the use of certain substances (Restriction on the use of Hazardous Substances, RoHS). Over time, these Directives will be implemented in the national laws of each EU Member State.

Once the final national regulations have been put into place, recycling will be offered for those Harvard Apparatus products which are within the scope of the WEEE Directive. Products falling under the scope of the WEEE Directive available for sale after August 13, 2005 will be identified with a "wheelie bin" symbol.

Two Categories of products covered by the WEEE Directive are currently exempt from the RoHS Directive – Category 8, medical devices (with the exception of implanted or infected products) and Category 9, monitoring and control instruments. Most of Harvard Apparatus' products fall into either Category 8 or 9 and are currently exempt from the RoHS Directive. Harvard Apparatus will continue to monitor the application of the RoHS Directive to its products and will comply with any changes as they apply.

- **Do Not Dispose Product with Municipal Waste.**
- **Special Collection/Disposal Required.**