INSTRUCTION MANUAL
FOR
Model OC-725C Oocyte Clamp

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Warner Instrument Corp.
1125 Dixwell Avenue
Hamden, Connecticut 06514 U.S.A.
Phone: 203-776-0664 800-599-4203 Fax: 203-776-1278

1.0 INTRODUCTION

The model OC-725C oocyte clamp is designed for two-electrode, whole-cell voltage clamping of Xenopus oocytes[1] as well as other large cells and cell structures such as squid axons.[2] The instrument has several features that makes it ideal for this purpose.

HIGH VOLTAGE COMPLIANCE

The ±180 volt compliance voltage of the current electrode makes possible clamping of very large cells over a short time course (in many cases, less than 0.5 msec; see figure1).

NOTE: Clamp speed is determined by the RC time constant of the current electrode resistance and membrane capacitance.

BATH CLAMP HEADSTAGE

This is essentially a separate voltage clamp that monitors the current being passed between the current injection pipette and the bath (ground reference), then maintains a stable reference (i.e., creates a virtual ground in the bath) by injecting the appropriate current. Without this circuit, the relatively high currents required to voltage clamp an oocyte would produce a significant voltage drop across the bath resistance. By providing a virtual ground in the bathing solution, the bath clamp eliminates the need for a series resistance compensation circuit.[3]

BUZZ CONTROLS

The "BUZZ" controls facilitate penetration of most cell membranes with a minimum of distortion. These buttons, one for each electrode, activate a square wave oscillation across the electrode that aids insertion through the membrane. This helps to minimize shunt resistance leakage around the electrode.

OVERLOAD ALARM

Another useful feature is the audible "OVERLOAD" alarm. This circuit serves as a reminder when the feedback amplifier reaches its maximum output voltage, a condition which could result in damage to the oocyte under study.

DUAL OOCYTE STUDIES

Studies involving 2 oocytes in a common bath requires two instruments. Current from the individual oocytes must be read from the output leg of each clamp current circuit. If measured with a common bath return, the currents would be summed and impossible to separate. OC-725C is easily configured to accomplish this. Additionally, an optional differential voltage headstage is available for this application. See sections 5.9, 6.9, 6.10 and 6.11 for details.

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[1]Zhou, J., Potts, J.F., Trimmer, J.S., Agnew, and Sigworth, F.J.(1991). Multiple gating modes and the effect of modulating factors on the μl sodium channel. *Neuron*, Vol. 7, 775-785, Nov. 1991

[2]Based on personal communication from W. Gilley.

[3]Changes of solution and/or agar bridges may alter the conductance of the bath and should be tested in advance for their effect.

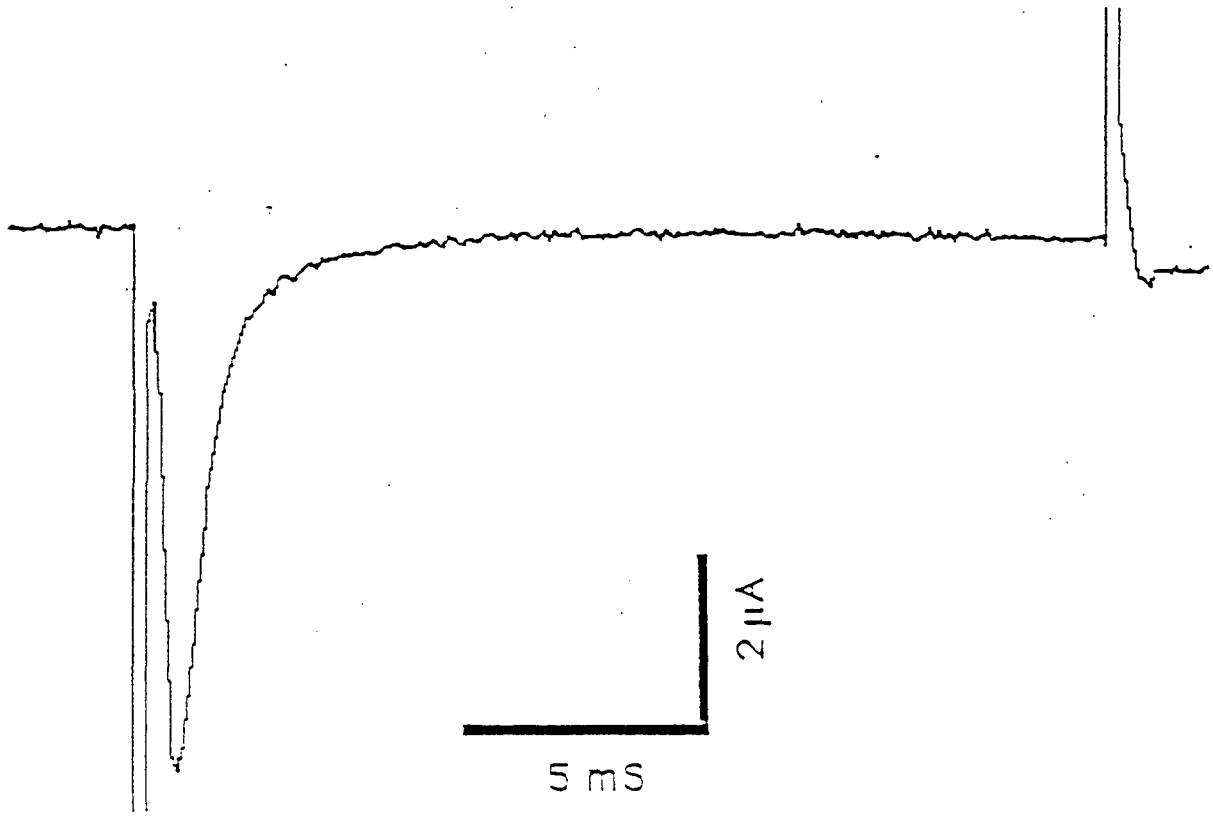


Figure 1 Average current from a Xenopus oocyte that is expressing message for a voltage- sensitive sodium channel and clamped with the OC-725C.

2.0 SAFETY

THIS INSTRUMENT IS FOR INVESTIGATIONAL USE ONLY AND IS NOT FOR USE WITH HUMAN SUBJECTS.

2.1 CONNECTING TO LINE POWER

MODEL OC-725C IS SUPPLIED WITH A 3 CONDUCTOR LINE CORD. ONE CONDUCTOR PROVIDES A CONNECTION BETWEEN THE INSTRUMENT HOUSING AND THE POWER SYSTEM EARTH GROUND. PROVIDED THAT THE POWER OUTLET IS WIRED CORRECTLY AND IS CONNECTED TO EARTH, SAFE OPERATION OF THIS INSTRUMENT WILL BE INSURED. **IF FOR ANY REASON, THE GROUND PIN OF THE LINE CORD IS REMOVED, THE INSTRUMENT CHASSIS MUST BE CONNECTED TO EARTH GROUND WITH A SEPARATE HEAVY GAGE (14 OR LARGER) GROUND WIRE.**

2.2 HIGH VOLTAGE OUTPUT

THE CURRENT CLAMP CHANNEL OUTPUT IS CAPABLE OF HIGH POWER OUTPUT, ± 180 VOLTS @ 10 MILLIAMPERES. WHEN HANDLING THE CURRENT ELECTRODE CABLE, BE SURE TO SET THE GAIN CONTROL FULLY COUNTERCLOCKWISE AND SWITCH THE CLAMP TO OFF.

3.0 DESCRIPTION

OC-725C is comprised of three sections or channels:

1) A high impedance voltage probe with capacity compensation and input offset to measure membrane potential.
2) A current sensing channel with bath clamp to measure the membrane current and clamp the bath to ground.
3) A high voltage amplfier to deliver the clamping current.

The complete voltage clamp system consists of :

1) The main control unit
2) The voltage recording probe with electrode holder
3) The current sensing bath probe with silver wire electrodes
4) The current cable with electrode holder
5) Accessories: Model membraneand rack mounting hardware.

3.1 CONTROL UNIT

The control unit houses the main circuitry and power supply. The front panel is divided into six sections:

1) VOLTAGE ELECTRODE [Vm]
2) BATH ELECTRODES [Im]
3) CLAMP
4) COMMANDS
5) CURRENT ELECTRODE
6) POWER

3.2 VOLTAGE ELECTRODE SECTION contains the voltage probe connector, voltage electrode meter and controls for the voltage recording channel including:

Vm OFFSET provides up to +/- 200mV at the input for offset of membrane junction potentials.

Vm ELECTRODE TEST passes a constant current (10 nA) through the voltage electrode producing a voltage of 10 mV/MΩ read on the meter or 100 mV/MΩ at the V_m x10 output.

Vm BUZZ facilitates penetration of the voltage electrode by producing an oscillation at the pipette tip.

VOLTAGE ELECTRODE METER - reads membrane voltage V_m , full scale range of +/-199.9 mV.

Vm x 10 OUTPUT - The membrane voltage multiplied by 10

NEGATIVE CAPACITY - Capacity compensation has been added to the OC-725B enabling its use as an electrometer for intracellular measurements. Input capacitance up to 45 pF can be neutralized.

Negative capacity will not be useful for oocyte clamp applications because clamp speed is a function of the oocyte's RC time constant (typically 1 MΩ in parallel with 0.5 uF), current electrode resistance and compliance voltage of the clamp current. The current, equal to:

$$\frac{\text{compliance voltage}}{\text{current electrode resistance}}$$

must charge the membrane at the rate: $dt = CdV \div I$.

3.3 BATH ELECTRODE SECTION

This section contains the bath probe connector, current meter, current gain select switches and I MONITOR outputs.

CURRENT METER - Reads the voltage Ve of the current electrode when the CLAMP is OFF. When in clamp (SLOW or FAST), the meter indicates the current (Im) sensed by the bath electrode.

GAIN SELECT - Current ouput is selected by a 7 postion dial (0.1 V/μA to 10 V/μA) and 3 position range switch (x0.1, x1 and x10).

I MONITOR OUTPUTS - Current outputs are available from the I MONITOR output at the full bandwidth (approx. 10kHz) and from the Im MON FILTERED 1 kHz (4-pole Bessel) output.

3.4 CLAMP SECTION includes the CLAMP select switch, GAIN control and DC GAIN switch. The CLAMP select switch has 3 positions; OFF, SLOW and FAST.

OFF - In OFF position, no current is delivered from the clamp amplifier to the current electrode. The voltage of the current electrode [V_e] is read on the meter in millivolts or at the V_ex10 output on the rear panel.

SLOW - The SLOW clamp speed is useful for initial screening of oocytes or where high clamp speed is not required. SLOW clamp speed is approximately 0.5 msec. when measured with the model membrane (1MΩ shunted with 0.47 uF)

FAST - Most clamping is done in the FAST mode. Clamp speed is limited only by the resistance of the current electrode and the oocyte membrane capacitance. Thus to obtain fast clamp speed, the current electrode resistance must be kept as low as possible. Current read on the meter is in microamperes. FAST clamp speed is 350 μsec, measured with the model membrane as above.

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

NOTE: GAIN is turned off by turning the control fully counter clockwise to the detent click off position. Be sure to advance the control until it clicks off before removing electrodes.

3.4 COMMAND SECTION contains the HOLD controls and COMMAND IN ÷ 10 input.

HOLD is set with a digital potentiometer and range switch. Ranges are ± 99 mV and ± 198 mV. Polarity or OFF is selected with the associated toggle switch.

COMMAND IN ÷ 10 - Command signals from an external generator or computer connect to this input are attenuated by 10 [1 Volt at Command Input = 0.1 Volts at membrane Vm)]. Maximum input is +/-10 volts.

3.5 CURRENT ELECTRODE SECTION includes Ve OFFSET, ELECTRODE TEST, BUZZ, OVER VOLTAGE indicator and digital meter and has connectors for both the bath probe and current electrode.

Ve OFFSET - With a range of ± 200 mV (center zero), this control is used to zero the offset voltage of the current electrode. This allows for establishing a zero reference before impaling the oocyte with the current electrode. Once the impalement is completed, the resting potential can be read from V_e X10 output or on the CURRENT ELECTRODE meter.

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

Ve BUZZ - Facilitates penetration of the current electrode.

OVER VOLTAGE - If the voltage at the current electrode exceeds +/-160 volts, the OVER VOLTAGE lamp will turn on. An alarm will also sound when the rear panel ALARM switch is in the ON position..

3.6 POWER SECTION

This section includes the ON-OFF switch and pilot lamp.

ACCESSORIES DESCRIPTION

3.7 VOLTAGE RECORDING HEADSTAGE

3.71 7250V Standard Version

The voltage probe is an active headstage housed in a 12.5mm diameter x 5cm long cylinder. It is nickel plated and epoxy sealed for resistance to corrosion. **The outer shell is electrically driven at the input potential.** The microelectrode holder supplied mates directly to the 2mm dia. input pin on the probe. A mounting block and handle are supplied for attaching the probe to a micromanipulator. The handle can be mounted either axially or at right angle to the probe.

3.72 7255DI Differential Headstage (Optional)

This optional voltage headstage is used for applications involving two oocytes in a common bath or where the voltage drop across the solution resistance needs to be measured and subtracted from Vm. The housing is approximately 2cm longer than 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.

3.73 BATH HEADSTAGE

The bath probe is housed in a 2.8cm x 3.5cm x 4.2cm aluminum enclosure. Inputs are two 1mm pin jacks labeled I SENSE and I OUT. The case is electrically grounded and a pin jack connector is located on the side for connecting to shields. A plastic plate with two mounting screw slots is attached to the probe bottom. The bath probe connects to the control unit with a 6 pin connector.

3.74 CURRENT ELECTRODE CABLE

The 2 meter long shielded cable has a 2mm pin jack to mate with the electrode holder supplied. The holder has a mounting handle for mounting in a micromanipulator. A 3 pin connector mates with the control unit.

3.8 REAR PANEL

The line power connector and fuse are on the rear panel. Operating voltage is specified on the model/serial number sticker applied to the rear. Check to be sure the instrument is wired for the proper voltage before connecting the line cord. The rear panel also contains V_ex10 OUTPUT, GAIN TELEGRAPH OUTPUT, ALARM switch and GROUNDS.

V_ex10 OUTPUT monitors the voltage of the current electrode (x10) when the CLAMP switch is OFF.

GAIN TELEGRAPH OUTPUT provides a dc voltage indicating the gain setting of the I MONITOR gain and range switches for computer monitoring. The output varies from 0.2 to 2.6 volts in 200mV steps as shown in the accompanying chart.

VOLTS/μA at Im OUTOUT	GAIN TELEGRAPH VOLTAGE
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

ALARM - Switch activates or deactivates the OVER VOLTAGE current electrode audible alarm.

GROUNDS - Both CIRCUIT and CHASSIS grounds are located on the rear panel. CHASSIS is common with the instrument enclosure and connected to earth through the power line cord. For safe operation, the ground pin on the power plug must not be removed and the use of "cheater" plugs must be avoided. A shorting link allows for connecting the two grounds. In most experimental set-ups, separating the grounds will result in minimizing 50/60 Hz signal interference from ground loops. However, trial and error will determine the best results.

4.0 SPECIFICATIONS

VOLTAGE RECORDING [Vm] CHANNEL

Input Impedance	5×10^{11} Ohms shunted by 3pF
Output Resistance	100 Ohms
Vm Offset	± 200 mV at V probe input
Noise*	50 uV rms at 1 kHz
Electrode Test	10 mV/MΩ
Negative Capacity	0 - 45 pF
Vm Meter Range, full scale	± 199.9 mV

BATH ELECTRODE [Im] CHANNEL

Ve Offset	± 200 mV
Noise*	
In Clamp	5.5 nA rms at 1 kHz [x1 range]
Open Loop [Clamp Off]	28 pA rms @ 1 kHz [x 1 range]
I Monitor Output	0.01 to 100 V/uA in 3 ranges, 7 steps per range
I Monitor Filtered Output [4-pole Bessel]	Same outputs as above, filtered at 1 kHz
Gain Telegraph Output	0.2 to 2.6 VDC in 0.2V steps
Full Scale Meter Ranges	
Ve [clamp OFF]	± 199.9 mV
Im [x0.1 range]	± 199.9 µA
Im [x1 range]	± 19.99 µA
Im [x10 range]	± 1.999 µA

CURRENT ELECTRODE CHANNEL

Compliance Voltage	± 180 Volts
Alarm	± 160 Volts
Gain	
Variable	0 to 2000 AC/DC
DC	1×10^6 DC, switch selected
Electrode Test	10 mV/MΩ
Commands	
Hold [internal]	± 198 mV in 2 ranges
External Input [attenuated by 10]	1 Volt in = 0.1 Volt command Maximum input 10 Volts

POWER REQUIREMENTS

Standard Models	100 -130 or 220-240 VAC, 50/60 Hz

DIMENSIONS

Enclosure	9 cm high x 42 cm wide x 25 cm deep
Voltage Headstage	12.5 mm dia. x 5 cm long with 1.8 m length cable
Mounting Handle	4.8 mm dia. x 6.3 cm long
Bath Headstage	2.8 cm high x 3.5 cm wide x 4.2 cm deep with 1.8 m length cable

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

5.0 GETTING STARTED

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 micro-injection (for a review of those techniques, see Colman, 1984) and has some familiarity with the basic circuitry of a two-electrode voltage clamp (for a review, see Hille, 1984). For less experienced users or for those who would like to familiarize themselves with the instrument controls, please turn to the "Model Membrane" section 8.0 of this manual.

5.1 PIPETTES

Micro-electrodes 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, though, these pipettes do not require fire polishing nor coating with syl-gard. They will need to be broken off, however, to a relatively large diameter to insure a fast response time by the clamp (see fig. 1 below). 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.

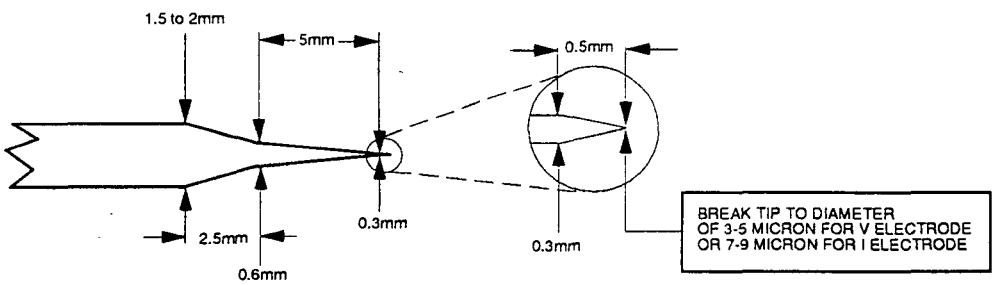


Fig. 2 Approximate shape and dimensions of the recording pipettes used for oocyte clamp studies.

5.2 ELECTRODE HOLDERS

VOLTAGE ELECTRODE - The voltage recording electrode holder ESW-FxxV type uses a silver wire for the electrical coupling between the pipette and holder connector. The portion of wire that is to be in contact with the KCl solution (in the pipette) must be chlorided to reduce junction potentials (see chloriding procedure below). The pipette need only be filled with KCl so that approximately 1/2 inch of the chlorided wire be submerged in the solution. DO NOT FILL THIS HOLDER WITH THE KCl SOLUTION. The pipette/holder assembly is attached to the voltage headstage directly and the assembly is then mounted in a micrpositioner.

CURRENT ELECTRODE - The electrode holder E45W-FxxVH supplied for the current electrode uses a silver wire for coupling. Before inserting a pipette in the holder, the portion of wire that is to be in contact with the KCl solution (in the pipette) must be chlorided to reduce junction potentials (see chloriding procedure below). The pipette need only be filled with KCl so that approximately 1/2 inch of the chlorided wire be submerged in the solution. DO NOT FILL THIS HOLDER WITH THE KCl SOLUTION. The pipette/holder assembly is mounted in a micropositioner with the mounting rod supplied.

5.3 CHLORIDING SILVER WIRE

The silver electrode wires should be handled by the pin connectors or with gloves to avoid tarnishing them. Before use, they will require chloride plating to reduce junction potentials between them and the solutions that they are bathed in. Chloriding a silver wire (electrode) is achieved by making it positive, relative to a solution containing NaCl (0.9%) or KCl (3M) and passing a current through the electrode at a rate of 1 mA/cm^2 of surface area for a minute, or until adequately plated (a 2cm length of 0.25mm wire would require 0.15mA). The color of a well plated wire should be a light grey.[4] Reversal of the polarity while plating the electrode tends to yield a more stable electrode. When replating a previously plated wire, you may find that it does not plate evenly. Removal of the residual chloride plating will be necessary to effect a uniform coat. Then, before making the wire positive to the choriding solution, reverse the polarity for 15 to 30 seconds to kick off the remaining chloride that might be left in small pits on the wire. Then proceed as described above for a new wire.

An alternate method of chloriding is to immerse the wire in Clorox until a light grey color is observed (typically 15 seconds to a minute).

5.4 BATH PROBE

As described in the "Introduction" section, 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.[5] 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 be chloride plated before use, as described in the "Electrode Holders" section above.

5.5 ELECTRODE PLACEMENT AND GROUNDING

Three drawings are included to illustrate the various ways the bath circuit can be configured. Most applications involve only a single oocyte. Figures 3 and 4 illustrate set-ups for these applications. Figure 5 shows the set-up for recording from 2 oocytes in a common bath with the use of two clamps.

5.6 SINGLE OOCYTE SET-UP WITH BATH CLAMP

Single oocyte studies are best accomplished with the set-up in figure 2 using the bath clamp headstage to establish the bath ground. This method 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.

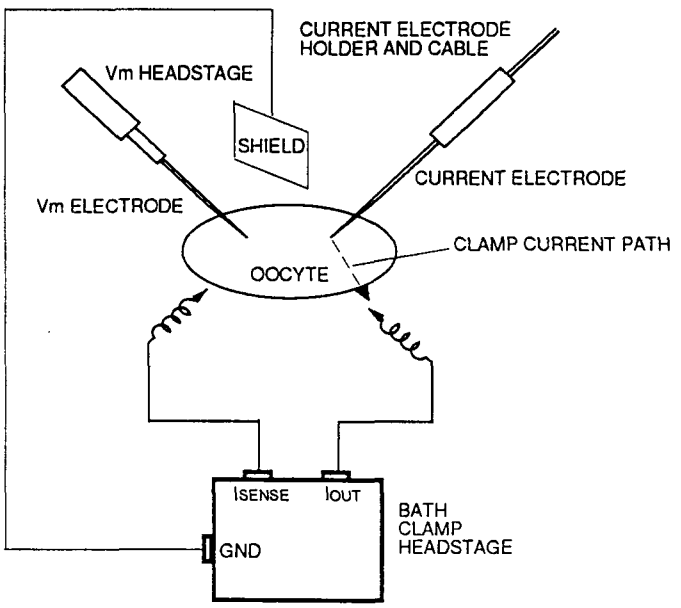


Fig. 3 Single set-up using the bath clamp ground.

5.7 ELECTRODE PLACEMENT

Placement of the bath electrodes (I_{out} and I_{sense}) is important to obtain optimum performance. The I_{sense} electrode (or the agar bridge associated with it) should be placed as close to the oocyte as possible (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.

[4]The longer a wire is plated, the darker and more brittle the chloride will become. These properties are not necessarily desirable if an electrode will be repeatedly inserted into a pipette or flexed.

[5]It is recommended that the user not expose the perfusate to the electrode wires directly 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.

5.8 SINGLE OOCYTE WITH DIRECT GROUND

Applications not suitable for the using the bath clamp such as those with a very long solution path to ground can be configured using the alternate method of directly grounding the bath shown in Fig. 4. Current is read from the "high side" of the current output leg (section 6.10). 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 of those obtained with the bath clamp.

2) High levels of clamp current could produce a substantial voltage drop across the series solution resistance.

The clamp has no provisions for compensating this voltage. V Diff electrode should be placed close to the oocyte and in the current path between the Vm electrode and ground.

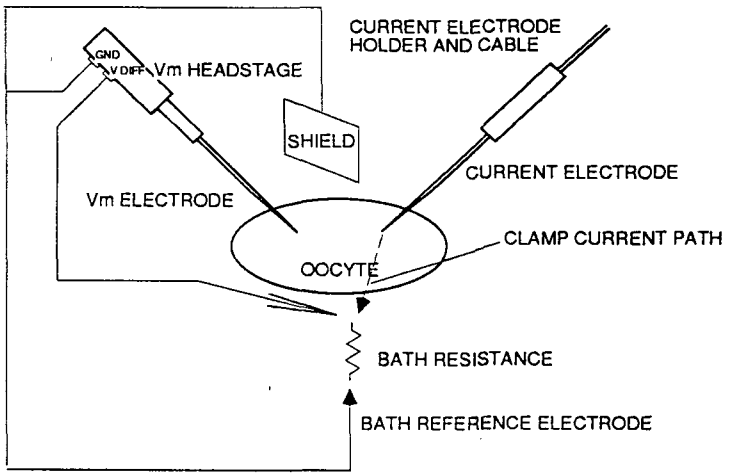


Fig. 4 Single oocyte set-up using a direct ground.

5.9 DUAL OOCTYE SET-UP

This method is accomplished using two clamps. Fig. 5 illustrates the set-up. Both clamps must be configured to read current from the "high side" as in section 6 and each clamp must be equipped with the optional differential voltage headstage.

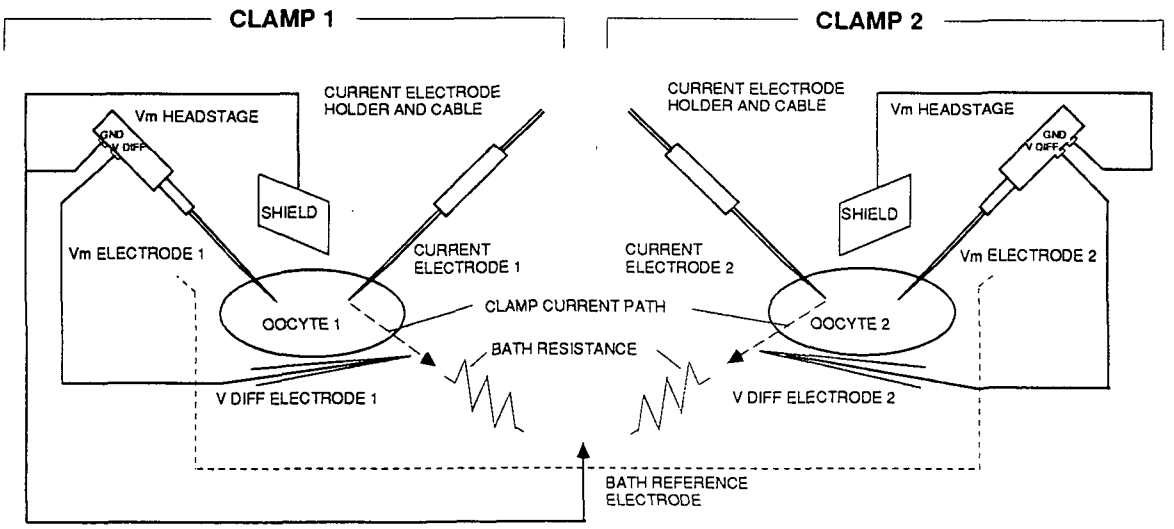


Fig. 5 Set-up for recording from two oocytes in a common bath.

5.10 CABLE CONNECTIONS

Bath Clamp Headstage: After positioning the probe as described above, connect it to the BATH PROBE socket.

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 .

High Voltage Current Electrode: The holder should mounted on a micro-manipulator and the cable connected to the I ELECTRODE socket .

NOTE: The routing of the probe cables must be done with care. Run the cables together as a bundle rather than individually and keep the cables as far away as possible from sources of 50/60 Hz interference (line cords, transformers, etc.).

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

External Monitoring: To monitor the two micro-electrodes' potentials on an oscilloscope, computer or a chart recorder, the following connections should be made.

Vm - Membrane potential may be recorded from the V_mX10 connector in the VOLTAGE ELECTRODE section.

Ve - The voltage of the current electrode can be monitored from the V_e connector on the rear panel. This output will be the same as that read on the current electrode meter, showing the voltage across the current electrode when the CLAMP selector switch is in the OFF position (this meter indicates current (Im) when the switch is in the SLOW or FAST position).

Im - The current signal is available from the I MONITOR and I MONITOR FILTERED 1 kHz outputs in 7 gain settings (0.1 to 10 µA/V) and 3 ranges (x0.1, x1 & x10).

Gain Telegraph - To automatically monitor the Im gain and range switch setting , a cable should be connected to the rear panel GAIN TELE connector.

Power Cord: The power cord should be connected to a properly grounded AC receptacle with the line voltage specified on the nameplate on the rear panel.

5.11 RESTING POSITION OF THE CONTROLS

To begin, set the controls in the following positions:

	POWER	OFF
BATH ELECTRODE SECTION	I MONITOR OUTPUT	1 V/ µA
	GAIN SELECT	X1
COMMANDS SECTION	HOLD	0 mV (00 set on digital pot)
	+/OFF/-	OFF
CLAMP SECTION	MODE SELECT	OFF
	CLAMP GAIN	OFF (0) fully CCW in the click-off position
	CLAMP DC GAIN	OUT
REAR PANEL	ALARM	OFF or ON as desired

Turn POWER ON.

5.12 USING THE GAIN SELECT

A wider range of bath current (Im) measurements is now possible with the addition of the GAIN SELECT toggle switch located above the 7 position Im OUTPUT (VOLTS/μA) selector. The switch has 3 positions; x 0.1, x 1 and x 10. Resistance values shown below each gain position indicate the bath clamp feedback resistor used for the current measurement.

The chart below shows the effect off the range selection on the Im output. Note also that range selection changes the sensitivity of the current meter. Typicalmeasured currents will fall in the x 1 range. The lower and higher ranges are intended to cover those applications where currents are beyond the x 1 range. Currents below 1 μA should be monitored in the x 10 range. Large currents above 100 μA require the x 0.1 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	Im OUTPUT RANGE	Im MAX OUTPUT	METER MAX READING
X 0.1 [10kΩ]	0.01 to 1.0 V/μA	10 μA to 1000 μA	199.9 μA
x 1 [100kΩ]	0.1 to 10 V/μA	1 μA to 100 μA	19.99 μA
x 10 [1 MΩ]	1.0 to 100 V/μA	0.1 μA to 10 μA	1.999 μA

5.13 OTHER GAIN RANGE SELECTION CONSIDERATIONS

The intrinsic noise of the current measuring circuit is a function of the bath clamp feedback resistor. A larger value resistor offers lower noise and greater signal resolution. The following measurements were made with shorted input, with the standard model cell and with a modified model cell (.22 μF) for comparisons. All readings were recorded at 1 kHz (8-pole Bessel) and are rms.

Feedback R	Shorted Input	Std. Model (.5 μF)	Modified Model (.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.

6.0 STEP-BY-STEP PROCEDURE FOR RECORDING FROM OOCYTES

6.1 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.

6.2 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. 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, causing an open circuit.

4) Using the OFFSET knob, you must now 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. Refer to "Electrode Holders" section.

5) To test the resistance of the voltage electrode pipette, depress the ELECTRODE TEST button that is located under the VOLTAGE ELECTRODE meter. This passes a 10 nA current across the voltage electrode. The meter will display the resulting potential in millivolts. The resistance of the electrode can be easily calculated from this value by dividing the displayed potential by 10 and the quotient will be expressed in MΩ , e.g., if the electrode test indicates that a potential of 25.0 mV is produced by the 10 nA test current, then

$$R = V / I$$

$$R = 25.0 \text{ mV} / 10 \text{ nA}$$

$$R = (25.0 \times 10^{-3} \text{ V}) / (1.0 \times 10^{-8} \text{ A})$$

$$R = 25.0 \times 10^{5} = 2.5 \times 10^{6} = 2.5 \text{ M}\Omega$$

This value may vary widely from pipette to pipette but should be less than 4 MΩ for the voltage electrode.

6.3 CURRENT ELECTRODE PLACEMENT

6) Advance the current electrode until the tip is in the chamber bath solution. Adjust Ve OFFSET for a zero reading on the current meter. This will establish a zero reference and the resting potential will be read directly.

7) 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 except that the ELECT TEST button located in the CURRENT ELECTRODE section. This 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 and from that value, the resistance of the pipette is calculated in the same way as described above in step 6 (i.e., divide the readout by 10 to get the resistance in MΩ). Since the current electrode is larger, its resistance should be less than that of the voltage electrode (about 1.0 MΩ or less). If no voltage display is present during the electrode test and you are sure that the

electrode is in the bath solution, 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.

6.4 IMPALING THE CELL

8) Recheck the VOLTAGE ELECTRODE meter to verify that the potential is correctly offset to read 00.0 mV and adjust the OFFSET knob if needed. Advance the voltage electrode until the tip is slightly depressing the plasma membrane of the cell. Position the current electrode against the cell in the same manner. Now depress the BUZZ switch located under the VOLTAGE ELECTRODE meter. This will produce a 1 V, 1 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.

9) The voltage across the membrane will now be displayed on the VOLTAGE ELECTRODE meter.

10) Next, depress the BUZZ button for the current electrode located in the CURRENT ELECTRODE section. Similar to the voltage electrode BUZZ, the current electrode BUZZ button produces a 1 V, 1 kHz oscillation across the current electrode. This disrupts the membrane and causes the tip of the electrode to impale the cell with no further movement of the micro-manipulator. Once again, though, if the BUZZ technique fails to cause penetration, further advance the current electrode until it "pops" through the membrane.

6.5 CLAMPING THE CELL

11) Turn the CLAMP ON by switching to either the SLOW or FAST position. SLOW is useful for intial screeing.

12) Adjust the feedback amplifier GAIN control (also located in the CLAMP section of the front panel) clockwise as far as possible without illuminating the OVER VOLTAGE light (located in the CURRENT ELECTRODE section). If the ALARM signal switch on the back panel is ON, an alarm will be heard at the same time as the OVER VOLTAGE light in the VOLTAGE ELECTRODE section illuminates.

13) 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 of the front panel. The three-position toggle switch (POS-OFF-NEG) can be used to select the polarity of the command potential and the digital potentiometer above it is used to select the amplitude. If you wish to control the COMMAND voltage externally from a computer, leave the three-position toggle switch in the OFF position and connect the appropriate analog output from your computer DAC to the COMMAND IN ÷ 10 BNC connector, as described above in the "Cable connections" section. Depending on the amplitude of the response you wish to record, you may adjust the OUTPUT selector switch (V/μA) to a higher or lower position. The CURRENT ELECTRODE meter should now be displaying the current (in μA) that is being delivered to clamp the cell at the designated command potential.

6.6 CLAMPING HIGH CONDUCTANCE CELLS

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

6.7 UNCLAMPING THE CELL

To unclamp the cell, turn the GAIN control fully counter clockwise to the detent click off position. This will disengage the DC GAIN also. If the control is not fully off and the DC GAIN is left on, the preparation will not be unclamped.

6.8 REMOVING THE ELECTRODES

14) It is very important that the CLAMP gain potentiometer be returned to the OFF position (fully counter-clockwise to click OFF) as described above and the CLAMP selector switch be placed in the OFF position before removing the current electrode from the cell. FAILURE TO DO SO WILL OVERLOAD THE FEEDBACK AMPLIFIER DUE TO THE LARGE AMOUNT OF CURRENT IT IS DRIVING ONCE THERE IS NO MEMBRANE RESISTANCE BETWEEN THE CURRENT ELECTRODE AND THE BATH (VIRTUAL GROUND). THIS COULD DAMAGE THE OOCYTE! FOR THIS REASON IT IS RECOMMENDED THAT THE USER KEEP THE AUDIBLE OVERLOAD ALARM ENABLED TO PROVIDE A WARNING WHEN THE POTENTIAL FOR SUCH DAMAGE EXISTS.

6.9 REPEATED RECORDINGS

15) Finally, 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 the previous cell's bath, the pipettes can be used repeatedly. They should be free of debris and they 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 up with cellular debris. Make the following control settings before the next recording is carried out:

COMMAND

(+/Off/-) OFF

CLAMP

Select OFF

Gain 0 (fully CCW)

Now, repeat the procedures listed above, starting with step 4.

Although it is not necessary for the operation, a computer can be employed to control the command voltage. This is possible using just about any computer through an appropriate interface. Acquisition and display of data can also be handled by computer, storing it on whatever medium is most convenient. The OC725C is fully compatible with all commercially available software packages that are designed for electrophysiological research.
If desired, data can also be recorded directly by a chart recorder or simply observed on an oscilloscope.

You will need a micro-electrode puller for making the appropriately sized voltage and current electrodes. Whatever puller you are using to make micro-injection pipettes should be capable of making the micro-electrodes, as well. You will also need to use a microscope to break off the tips of the pipettes, as described below.

All equipment within the Faraday cage should be grounded to the rear panel instrument circuit ground. This is usually best done by connecting everything (including the cage) to a ground bus. Then only one wire needs to be run from the set-up to the instrument ground.

6.10 HIGH SIDE CURRENT MEASURING

In studies of single oocytes, current is monitored by the bath clamp headstage. Experiments involving 2 oocytes in a common bath such as gap junction studies requires monitoring the individual currents. This is done in the current output leg (in series with and ahead of the current electrode).

Two disadvantages to monitoring the current in this manner exist.

1) Noise level of this signal is somewhat higher. However, this is usually not a serious problem since currents are typically in the microampere levels.

2) The voltage drop across the solution resistance (from oocyte to bath ground) becomes an error voltage since it is not subtracted out as it is when the bath clamp headstage is used. This problem is overcome by using the optional Differential Voltage Headstage (section 6.12)

6.11 CONFIGURING MODEL OC-725B FOR HIGH SIDE CURRENT MEASURING

The current measuring circuit can be changed to HIGH SIDE CURRENT MEASURING by setting a dip switch on the main circuit board. First disconnect mains power, remove the two screws at the rear of the top cover and slide top cover off. Locate dip switch S10 on the circuit board. For normal operation S4 is ON and all other 7 switches are OFF. To convert to HIGH SIDE CURRENT MEASURING, turn S4 OFF and turn S2, S5, and S7 ON. All other switches should be in the OFF position.

6.12 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) In situations where the solution path from oocyte to ground is very long.

2) When recording from two oocytes in a common bath.

Two 1mm 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. The jumper is removed and a V Diff electrode is connected to the V Diff jack as shown in fig. 3. If a shield between the voltage and clamp current electrodes is used, it should be connected to the headstage ground.

7.0 SOME RECOMMENDATIONS

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. The BUZZ should help minimize the trauma from electrode penetration. Further reduction of membrane damage can be realized by properly isolating the platform used for the recording set-up from vibration. The use of hydraulically driven micro-manipulators will also reduce membrane damage while the electrodes are in the cell.

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

While the whole cell configuration is more forgiving than patch clamping, it is still important to minimize mechanical motion. The platform for your experimental set-up, therefore, should be isolated from vibrations. This will reduce leakage around the electrodes, making the clamp more effective, and will reduce mechanical noise in the recordings. The latter is especially important when recording responses of certain ligand-gated channels where the membrane potential changes may only be a few millivolts. 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, again, this will make for better seals around the electrodes. Another advantage gained from reducing membrane damage by the electrodes is to enhance the possibility of making subsequent recordings from the same cell, if desired. These latter two suggestions, of course, are also important for minimizing mechanical noise in the recorded data.

The platform should be shielded from electrostatic radiation with a Faraday cage. This can be built from metal window screens and should be connected to the instrument circuit ground.

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 filtered if you plan to use it during recording.

Minimally, the recording chamber need only be a stable surface on which the oocyte will not roll around. A disposable petri dish with a piece of nylon mesh in the bottom will do nicely. The dish can be stabilized by some sort of holder or just some wax around its perimeter. The diameter need only be large enough to accommodate the oocyte and the two bath electrodes. The walls of the recording chamber should be low enough so as not to interfere with the placement of electrodes. Perfusion of the chamber, if desired, can be accomplished with a gravity fed plumbing system, evacuating the perfusate from the dish with a gentle vacuum line (use as small an aperture as possible to avoid disturbing the surface of the perfusate in the dish). NOTE: Chambers suitable for oocyte studies are available from WARNER INSTRUMENT CORP.

8.0 TESTING WITH THE MODEL MEMBRANE

8.1 DESCRIPTION

The model membrane supplied with the OC-725B serves two purposes:

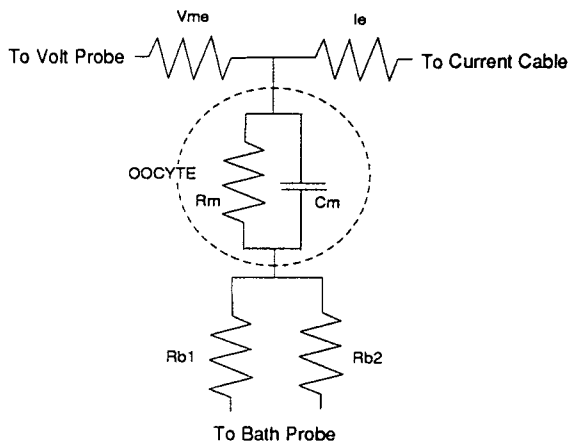
1) First time users will find it a convenient model for gaining experience in the operation of the clamp.

2) As a trouble shooting tool the proper functioning of the OC-725B can be quickly checked by connecting the model membrane and running through the following procedure.

The schematic of the model membrane is shown in the accompanying figure. The oocyte is represented by a 1MΩ resistor shunted by a .47uF capacitor. The voltage and current electrodes are each 1MΩ and the resistances to the bath probe are 10KΩ each.

Model membrane circuit.

Vme	Voltage Electrode	1MΩ
Ie	Current Electrode	1MΩ
Rm	Membrane Resistance	1MΩ
Cm	Membrane Capacitance	.47uF
Rb1,Rb2	Bath Resistance	10KΩ each



8.2 USING THE MODEL MEMBRANE

Connect the model membrane 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_mx10 and I_m outputs to an oscilloscope. Set the panel controls as follows:

V_m OFFSET - Center of rotation (approximately 5 turns)
V_e OFFSET - " " " " " "
CLAMP - OFF
GAIN - CCW to detent OFF
HOLD - 00 mV
HOLD POLARITY - OFF
OUTPUT GAIN - 0.1V/uA
POWER - OFF

Turn the power switch ON.

8.3 OFFSET CONTROLS

The range of the V_m OFFSET is +/-200mV. This can be verified by rotating the control first fully clockwise and then fully counterclockwise while observing the VOLTAGE meter. The reading will indicate off scale at the extremes of the control because the full scale meter reading is +/-199.9mV. Monitoring the V_mx10 output on the scope, the voltage will swing from +2 volts to -2 volts. Set the Vm OFFSET to zero (00.0 reading on the meter). Check the Vmx10 reading on the scope for zero also.

Check the Ve OFFSET control in the same manner. The voltage of the Ve OFFSET is read from the current meter when the CLAMP switch is OFF. Ve x10 is available at the rear panel when the CLAMP switch is OFF. Set Ve OFFSET to 00 mV.

8.4 ELECTRODE TEST (VOLTAGE ELECTRODE)

In actual practice, the electrode test is used prior to entering the cell and indicates the resistance of the electrode only. When used with the model cell, it will measure both the electrode and membrane resistance (2MΩ). Depress ELECRODE TEST and observe a reading of 20 mV on the VOLTAGE ELECTRODE meter(10mV/MΩ). The Vmx10 output will read 200mV. Since the test current is being passed through Rm, the Im output will indicate 1mV (10nA), the test current.

With the CLAMP switch OFF, the CURRENT meter monitors Ve (voltage at the current electrode). In this case, Ve will be a measure of the voltage across Rm and the meter will indicate 10 mV (1MΩ). Vex10 output at the rear panel can be checked to see that it reads 100mV.

8.5 BUZZ (VOLTAGE)

Set the scope sensitivity to 5V/Div. and depress the BUZZ switch while monitoring the Vmx10 output. A 1KHz square wave of approximately 24 volt p-p will be generated as long as the button is depressed.

8.6 ELECTRODE TEST (CURRENT ELECTRODE)

The procedure for the current ELECTRODE TEST is the same as the voltage electrode section 8.4. Depressing Ve ELECTRODE TEST will produce a 20 mV (2MΩ) reading on the CURRENT meter and 10mV (1MΩ) on the VOLTAGE meter.

Set the HOLD control to 50mV and switch to + polarity. The square wave will be displaced 50mV in the positive direction. Switching to - polarity will produce a -50mVoffset.

Switch HOLD off, return the GAIN control fully CCW and switch CLAMP to OFF.

This completes the model membrane test procedure

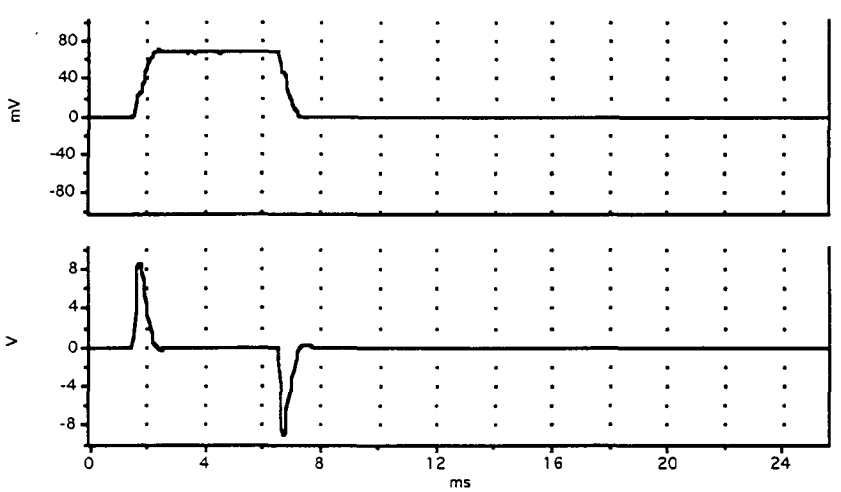


Fig.6 Scope waveforms with model membrane; Vm x 10 upper and Im lower.

8.7 DC CLAMP TEST

Place the CLAMP switch in the FAST position and adjust the Vm OFFSET control for a reading of +100mV on the VOLTAGE meter. Turn the GAIN control clockwise slowly and continue until Vm decreases to zero. The meter reading (CURRENT meter) should be -100nA (-0.10uA). Now, set the HOLD control to 100 mV (50 mV x2) and switch on in the positive (+) position. Vm should read +100mV and the current Im=0.00. Switch to negative (-) polarity and Vm should indicate -100mV with the current Im = 0.2 uA.
Return the GAIN control fully CCW and turn the CLAMP switch to OFF.

8.8 AC CLAMP TEST

Adjust the V_m OFFSET control to 00.0V. Apply a 0.8 volt 100Hz square wave to COMMAND IN÷10. Monitor V_mx10 and I_m on the oscilloscope. Switch the CLAMP to FAST and increase the GAIN (CW rotation) until V_m=80 mV (0.8V at V_mx10). As you further increase the GAIN control, you will see the rise time become faster (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 wave form as shown in figure 4.
The current signal (I_m OUTPUT) also shown displays the high current spikes necessary to charge the oocyte capacitance.
Set the HOLD control to a reading of 50 mV and switch polarity to +. The sqare wave will be displaced 50 mV in the positive direction. Switching the polarity to - will produce a -50 mV offset.

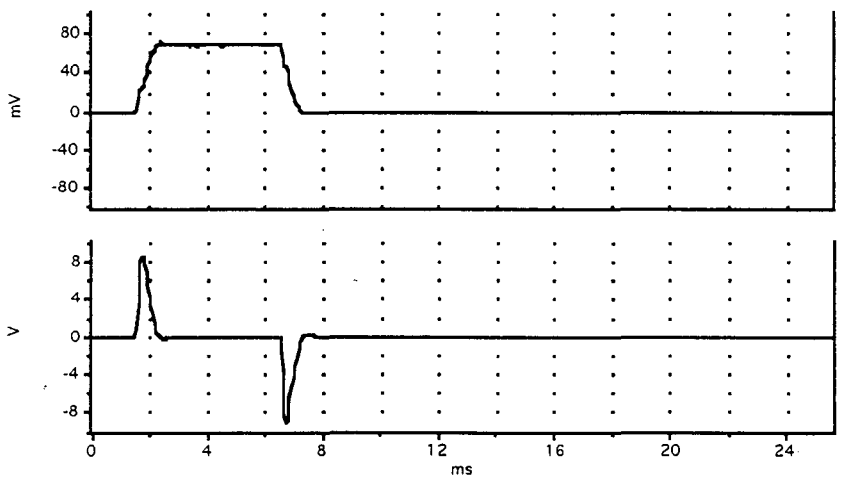
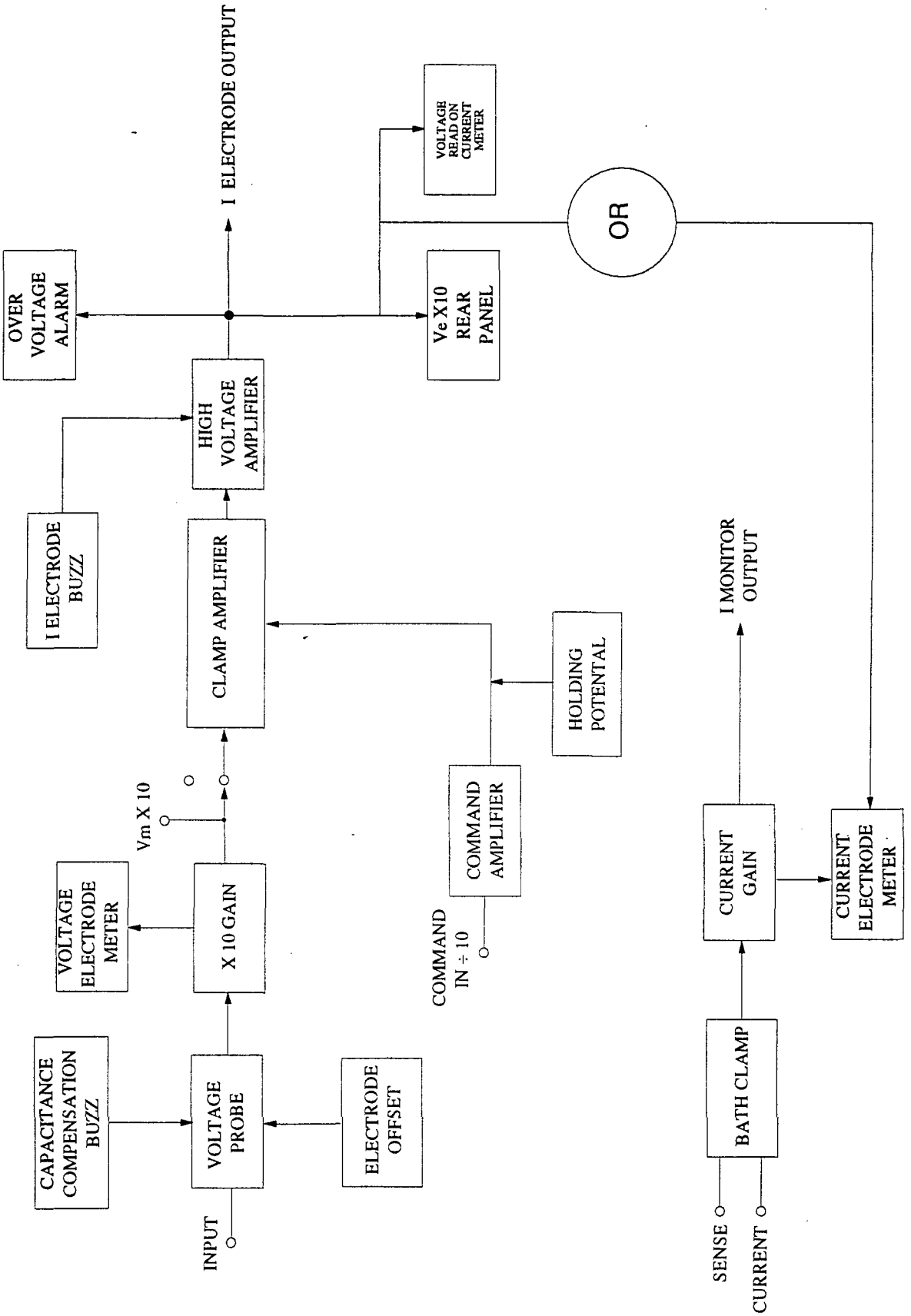


Fig. 6 Scope waveforms with model membrane; Vm x10 upper trace and Im lower trace.

Switch HOLD off, return the GAIN control fully CCW to the click off "0" position and switch CLAMP to OFF. This completes the model membrane test.

NOTE: 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 will be intolerance.

9.0 OC-725B CIRCUIT BLOCK DIAGRAM



10.0 REFERENCES

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Zhou, J., Potts, J.F., Trimmer, J.S., Agnew, W.S. and Sigworth, F.J. (1991). Multiple gating modes of the μl sodium channel, *Neuron*, Vol. 7, 775-785, November 1991, Cell Press.

11.0 APPENDIX

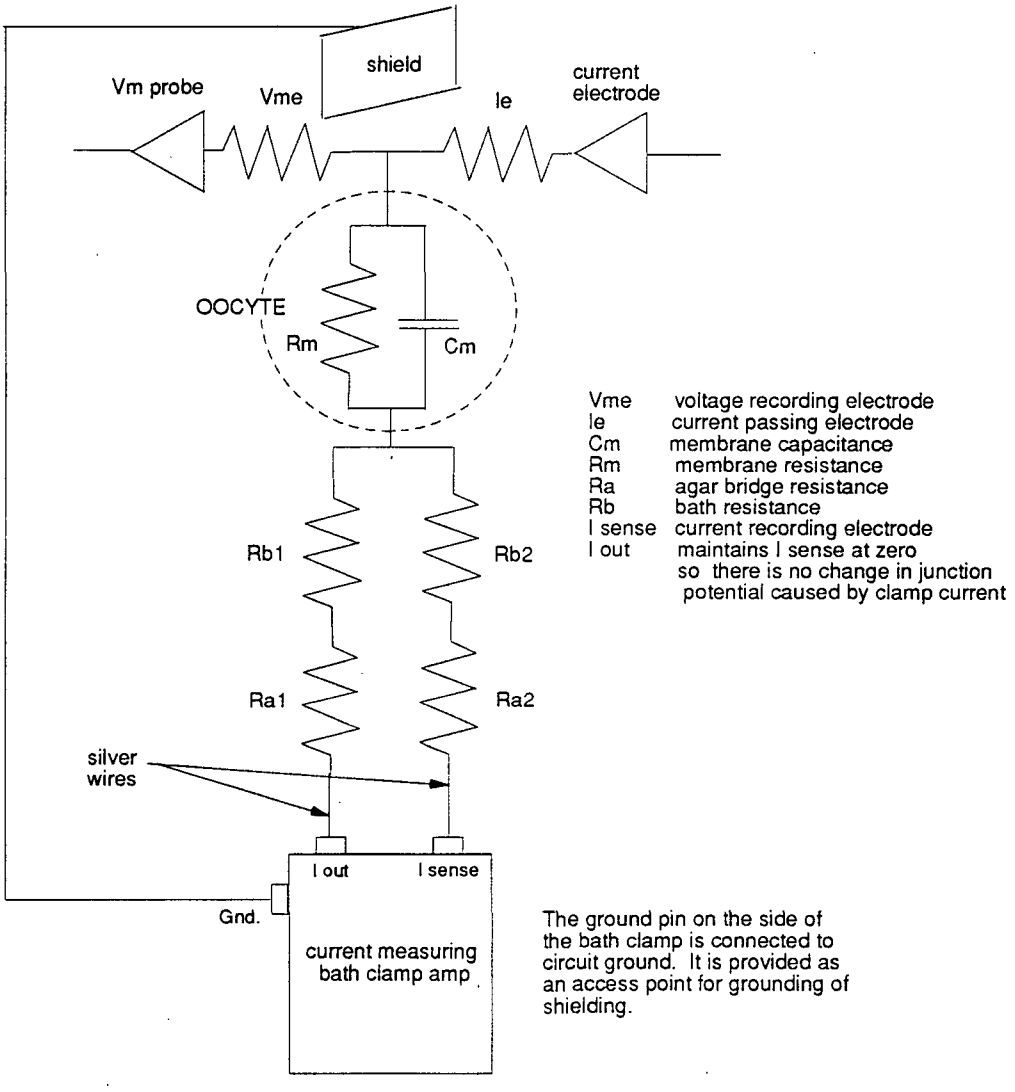


Fig. 6 Scematic diagram of preparation and connections.

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: Manufacturer's Address:	Warner Instrument Corp. 1125 Dixwell Avenue Hamden, CT 06514 Tel: (203) 776-0664
Equipment Description:	Oocyte Clamp
Equipment Class:	Laboratory measurement & Control Class A
Model Number:	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

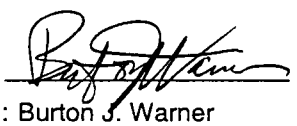
CE MARKING (LVD)

Application of Council Directive: 73/23/EEC

Standards To Which Conformity Is Declared	EN61010-1:1993
Manufacturer's Name:	Warner Instrument Corp.
Manufacturer's Address:	1125 Dixwell Avenue Hamden, CT 06514 Tel: (203) 776-0664
Equipment Description:	Oocyte Clamp Safety requirements for electrical equipment for measurement and laboratory use
Equipment Class:	Class I
Model Number:	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