



Duo 773

Dual Microprobe System

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INSTRUCTION MANUAL

Serial No. _____

090211

World Precision Instruments



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ABOUT THIS MANUAL

The following symbols are used in this guide:



This symbol indicates a **CAUTION**. Cautions warn against actions that can cause damage to equipment. Please read these carefully.



This symbol indicates a **WARNING**. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and **TIPS** contain helpful information.

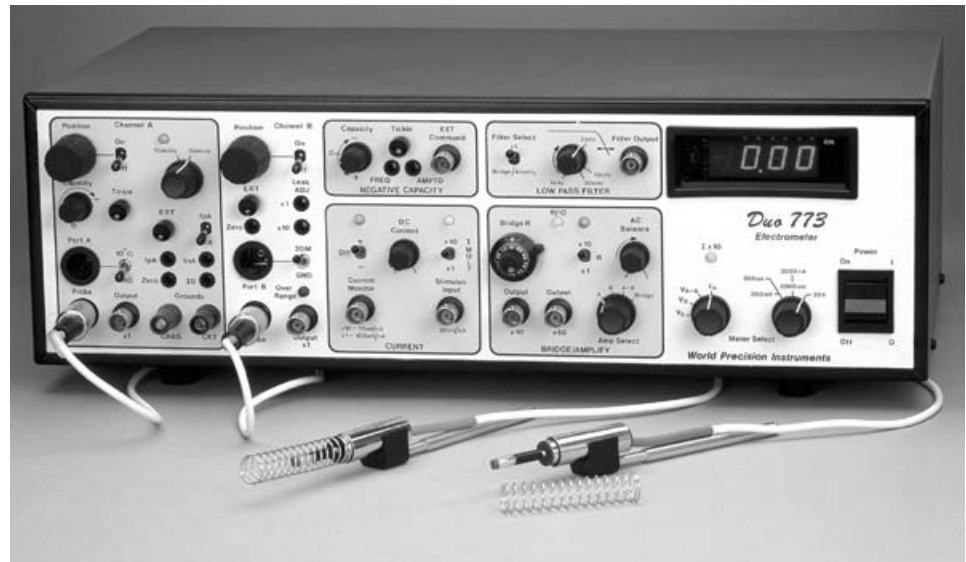


Fig. 1—Duo 773 comes with two headstages.



Duo 773

INTRODUCTION

Duo 773 combines the technologies of WPI's high impedance active probes and wide band electrometers to form a dual channel and/or differential probe system for measuring intracellular ion activities with high resistance, fluid-filled microelectrodes.

Features

- Cell "Tickler" for ease of impaling cells with microelectrodes
- Low and high current injection for either depolarizing or hyperpolarizing cells
- Bridge electrode resistance compensation
- Test ports for WPI's miniature gold plated active probes
- Audio and visual overload warning
- Continuously tuned low pass filter
- LED digital meter showing selectable voltage or injection current
- Capacitance compensation

Normal Configuration

The **Duo 773** has two probe channels that can be used independently or differentially.

- Channel A is an electrometer that is normally configured for use with a high input impedance probe (**715P**) that is used with ion selective microelectrodes. Channel A can also be used for the intracellular measurements of normal membrane potentials.
- Channel B is configured as a electrometer for a lower input impedance probe (**712P**) that can also be used to measure normal membrane potentials using intracellular electrodes. Channel B and its probe can also be used as the reference electrode for Channel A when the microelectrodes on both probes are placed in the same cell.

Optional Configuration

The **Duo 773** can be configured for use with two low input impedance probes (**712P**). This configuration may be special ordered from the factory, or can be set manually by the user. See Appendix A for details and instructions on setting the **Duo 773** to this configuration (page 26).



Parts List

After unpacking, verify that there is no visible damage to the instrument. Verify that all items are included:

- (1) **Duo 773** Dual Microprobe System
- (1) **712P** Low Impedance Probe
- (1) **715P** High Impedance Probe
- (1) **RC1T** Silver/Silver Chloride Reference Cell
- (2) **2547** Driven Guard Shield
- (2) **MEH1SF10** Microelectrode Holder and Pellet - 1.0mm
- (2) **MEH1SF12** Microelectrode Holder and Pellet - 1.2mm
- (2) **MEH1SF15** Microelectrode Holder and Pellet - 1.5mm
- (2) **MEH1SF20** Microelectrode Holder and Pellet - 2.0mm
- (1) **13776** Male Banana Adapter for 2mm Socket
- (1) **13661** Potentiometer Adjustment Tool (Tweaker)
- (1) Instruction Manual

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 30 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 30 of this manual.



Duo 773

INSTRUMENT DESCRIPTION

Hardware Description

Channel A Sector (normally for $10^{15}\Omega$ probe)

This sector has the controls for Channel A, which is normally used with a high input impedance probe:

Position: This knob is operative only when the adjacent **On/Off** toggle switch is set to the **On** position. Over its full range, this control can move the DC level (baseline) of the signal from the probe in Channel A over a range of $\pm 300\text{mV}$. This knob can make ten full rotations. When the **On/Off** switch is in the **Off** position, the voltage level of the real (raw) electrode potential is the signal available for recording.

Standby/Operate: In the **Standby** position, an active electronic clamp keeps the probe tip at or near 0.00mV . The clamp protects the headstage from damage by excessively high voltages (static) when the probe is being handled. When in the standby mode, the yellow LED illuminates. Set the knob to **Operate** before making measurements.

Capacity: As this knob is rotated clockwise, the shunting capacity of the microelectrode on the tip of the probe is effectively counteracted by a compensating negative capacity. Using this control allows the probe tip to respond more quickly to fast membrane signals. However, if the control is moved too far to the right creating a negative capacitance that is too high, the probe system will oscillate and the tip of the microelectrode will vibrate. Initially, the **Capacity** knob should be set to 0 (zero).

Tickle: Push this button to create an electrical oscillation of the probe system, which will cause the tip of the microelectrode to vibrate. This oscillation is often used to help the microelectrode tip to penetrate a cell or to help clean the tip of the microelectrode from cellular debris when the tip is outside of the cell.

TIP: Tickle oscillation can also be effected by applying a shorted input (for example, with a standard foot switch) to the **CHANNEL A TICKLE** input connector on the rear panel.

ERT: When depressed the Electrode Resistance Test (**ERT**) button injects either a 1pA or 1nA DC current through an electrode with which to estimate its resistance. The magnitude of these currents can be adjusted by the screwdriver adjustments marked **1pA** and **1nA** located just below the **ERT** button. See "Channel A Electrode Resistance Test" on page 13.



Fig. 2—Channel A Sector



1pA/1nA: This toggle switch selects which DC current value will flow through the system when the **ERT** button is depressed.

Port A: The tip of the active probe can be inserted into this receptacle for leakage current and zero adjustment.

TIP: When Probe A is not in use, set the **Port A** toggle switch to **GND** and insert the probe into **Port A** for storage.

10¹¹Ω/GND: This toggle switch can set **Port A** to connect to ground (**GND**) or 10¹¹Ω of resistance (10¹¹Ω) when the tip of the active probe is inserted into the test port.

Zero: This calibration screw is used to zero the probe's reading when the probe is grounded and the **Position** toggle switch is set to **Off**. (This unit is calibrated at the factory, and user adjustment is not recommended.)

IG: This is a calibration screw for adjusting the probe's zero position. When the **Meter Select** knobs are set to **V_A** and **200mV**, **Port A** toggle switch is set to **GND** and the **ERT** toggle switch is set to **1nA**, the digital meter display should read 00.0mV. If not, use the potentiometer adjustment tool, also called a tweaker, (WPI# 13661) to adjust this calibration screw.

Probe: Connect Probe A at this input jack. Probe A is marked with a blue band where the cable joins the plug.

Output x1: Use this BNC jack to connect the unmagnified output of Channel A to a recorder.

Grounds (CHAS, CKT): These Panel connectors are for the chassis or mains ground (**CHAS**) and the circuit (**CKT**) ground. The preparation should normally be connected to **CKT** ground via an appropriate electrode. If desired, the grounds may be connected by a grounding lug. This is the normal configuration. Removing the jumper isolates the chassis ground from the circuit ground. Do this **ONLY** when the chassis is independently grounded to a technical earth ground.

Probe B Sector (for 10¹²Ω Probe)

This sector has the controls for Channel B, which is used with a low input impedance probe:

Position: This knob is operative only when the adjacent **On/Off** toggle switch is set to the **On** position. This control can move the DC level (baseline) of the signal from the probe in Channel B. When the **On/Off** switch is in the **Off** position, the voltage level of the real electrode potential is the signal available for recording. This knob can make ten full rotations.



Fig. 3—Channel B sector



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ERT: When depressed, the “Electrode Resistance Test” (**ERT**) button injects 1nA DC current through the Probe B.

Zero: This calibration screw is used to zero the probe's reading when the probe is grounded and the **Position** toggle switch is set to **Off**. (This unit is calibrated at the factory, and user adjustment is not recommended.)

Leak ADJ (x1, x10): These calibration screws are used to adjust controls for probe zero position and probe leakage current for **I MULT** current multipliers of **x1** and **x10**, respectively.

Port B: The tip of the active probe can be inserted into this receptacle for leakage current and zero adjustment. When Probe B is not in use, set the **Port B** toggle switch to **GND** and insert the probe into **Port B** for storage.

20M/GND: This toggle switch can set **Port B** to connect to a ground (**GND**) or 20M Ω of resistance (**20M**) when the tip of the active probe is inserted into the test port.

Probe: Connect Probe B at this input jack.

Over Range: This red LED illuminates if the probe output signal exceeds $\pm 10V$. An audible signal is also heard.

Output x1: Use this BNC jack to connect the unmagnified output of Channel B to a recorder.

Negative Capacity Sector (for Channel B)

This sector controls the capacity compensator for Channel B, which is used to improve the response of the probe to fast potentials:

Capacity: As this knob is rotated clockwise, the shunting capacity of the microelectrode on the tip of the probe is effectively counteracted by a compensating negative capacity. Use this control to allow the probe tip to respond more quickly to fast membrane signals. However, if the control is moved too far to the right creating a negative capacitance that is too high, the probe system will oscillate and the tip of the microelectrode will vibrate. Initially, the **Capacity** knob should be set to 0 (zero).

Tickle: Pushing this button results in an audible electrical oscillation at the tip of the probe. Amplitude (**AMPTD**) and frequency (**FREQ**) of tickler oscillation can be adjusted with the calibration screws below the **Tickle** button.

EXT Command: This BNC connector can be used to attach an external control device that can apply a +5VDC signal, which will trigger the Channel B tickle function.



Fig. 4—Negative Capacity sector



NOTE: A passive shorting footswitch will not activate the tickle circuit via the front panel EXT command BNC.

NOTE: Tickle oscillation can also be effected by applying a voltage of 4VDC or more to the **EXT Command** input connector (front panel) or the **CHANNEL B TICKLE** (rear panel).

Current Sector (for Channel B)

This sector controls the injection of current into a cell through the probe on Channel B:

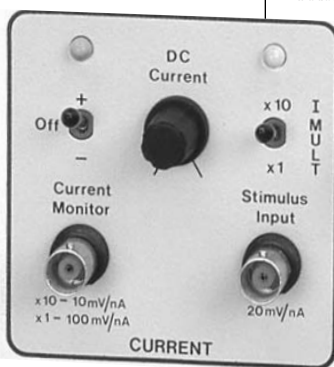


Fig. 5—Current sector

DC Current: This knob controls the level of current delivered to a cell through the microelectrode attached to the Channel B probe. The current is generated from a circuit within the **Duo 773**. When the **Off** toggle switch is set to **+**, a current with positive polarity can be injected into the cell to depolarize it and cause it to fire. When the toggle switch is set to **-**, a current with negative polarity can be used to hyperpolarize a spontaneously firing cell and prevent it from firing.

- If the **I MULT** (current multiplier) toggle switch is set to **x1**, the green LED illuminates. Turn the DC Current knob clockwise to adjust the probe tip current from 0–50nA.
- If the **I MULT** toggle switch is set to **x10**, three yellow LEDs illuminate. Turn the DC Current knob clockwise to adjust the probe tip current from 0–500nA.

Current Monitor: Attach a recording device to this BNC output to monitor a voltage that is proportional to the current passed through the microelectrode. Depending on the position of the **I MULT** switch, 100mV equals either 1nA (**x1**) or 10nA (**x10**).

Stimulus Input: This BNC input permits an external voltage source, like the DAC of a recording unit, to be connected to the **Duo 773**. The current flowing through the microelectrode into the cell is proportional to the voltage from the external source. Depending on the position of the **I MULT** switch, each 20mV from the external source creates either 1nA (at **x1**) or 10nA (at **x10**) of current.

Low Pass Filter Sector (for Channels B or A-B)



Fig. 6—Low Pass Filter sector

This sector has controls for a filter that removes high frequency noise from the output of Channel B or the A-B differential amplifier:

Filter Select: This selector switch determines the signal to which the filter is applied. When the toggle switch is set to **x1**, the filter is applied to the **x1** output of Channel B. When the filter is set to **Bridge/Amplify**, the filter is applied to the **x10** output of the amplifier (A, B or A-B)



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selected with the **Amp Select** switch in the **Bridge/Amplify** sector.

Adjustment Knob: This knob controls a unity voltage gain amplifier that is used to set a high frequency cut-off value between 1 and 30kHz.

Filter Output: The filtered output of the selected signal can be monitored when this BNC output is connected to a recording device. If the toggle switch is set to **x1**, the filtered signal from Channel B can be monitored. If the switch is set to **Bridge/Amplify**, the filtered **x10** output of the amplifier selected in the **Bridge/Amplify** sector can be recorded.

Bridge/Amplify Sector

Bridge R (Resistance): When a current pulse is injected into a cell through the Channel B probe to cause the cell to fire, the voltage recorded through the microelectrode is the sum of the membrane potential and the voltage drop caused by the resistance of the microelectrode. Since the voltage drop (IR_{drop}) across the microelectrode is the artifact, the Bridge/Amplify circuit can be used to remove this voltage drop from the output signal.

The resulting signal that is recorded from the **x10** output jack on this sector is the membrane potential of the cell. When the **Amp Select** switch is set to **Bridge**, this ten-turn dial is used to set a bridge compensation circuit that subtracts the voltage drop from the overall output signal from the cell. When the bridge compensation circuit is set (balanced) properly, the readings on the ten-turn dial indicate the resistance of the microelectrode (in $M\Omega$) placed inside the cell. The single digit at the top of the dial indicates the number of $M\Omega$ from 0.00 to 10.00. The two-digit dial displays the decimal value of the resistance. For example, if that digit in the window is a 4 and the dial reads 55, then the **Bridge R** dial indicates $4.55M\Omega$. Set the lock on the side of the dial to prevent the dial from turning while making measurements with the same electrode. Using a different electrode, requires the **Bridge R** to be reset.

R/10: The indicator lights for the **R/10** function indicate the multiple/divisor that should be applied to the reading on the **Bridge R** dial.

- When the **R** toggle switch is set to **x1** and the yellow LED is illuminated, the **R** values indicated on the **Bridge R** dial should be divided by 10.
- When the **R** toggle switch is set to **x10** and the green LED is illuminated, the dial reading should be multiplied by 10.
- When both LEDs are on or off simultaneously, the **R** values on the dial are read as face value.

R: This toggle switch multiplies the R factor by 1 (**x1**) or 10 (**x10**).

AC Balance: During the bridge compensation of the current pulse used to

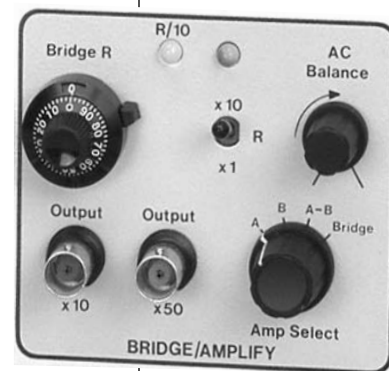


Fig. 7—Bridge/Amplify sector



stimulate the cell, rotating this knob removes the transient voltage spikes that occur at the onset and offset of the square stimulus pulse.

Output x10: The signal from this BNC connection is the output of the selected amplifier (A, B, A-B or Bridge) multiplied by 10. This is the jack that should be used when the low pass filter is applied to a signal from the **Bridge/Amplify** sector.

Output x50: The signal from this BNC connection is the output of the selected amplifier multiplied by 50. This output may be used with **A, B** or **A-B** only.

NOTE: This jack should not be used when the low pass filter is applied to a signal from the Bridge/Amplify sector.

Amp Select: This selector switch determines if the **Bridge/Amplify** sector is to be used as an amplifier for the single probe channel (A or B) or a differential probe setup (A-B) or as bridge compensation circuit.

Digital Panel Meter Sector



Meter Select: These switches select the source of the voltage to be displayed on the digital panel, and the range of the voltages or currents to be displayed.

- The first switch selects whether the voltage from one of the amplifiers (V_A , V_B , or V_{A-B}), or the current from the Channel B probe (I_B) is displayed on the digital display panel.
- The second switch selects the ranges of voltages or currents to be displayed on the digital panel.

Power: THIS IS THE MAINS POWER SWITCH THAT TURN ON THE Duo 773.

Fig. 8—Digital Panel Meter sector



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Instrument Setup (Testing the Duo 773 and Probes)

The instructions in this section should be used to do the following, before working on living tissue:

- Test that the functions of the instrument are working properly.
- Check the calibration of the amplifiers and probes connected to the instrument.
- Demonstrate how to perform amplifier, probe and electrode resistance tests.

Basic Instrument Test

This test will determine if the basic functions of the **Duo 773** are working properly.

1. With **Duo 773** turned off, insert the high input impedance **715P** probe into test **Port A**, and the low input impedance **712P** probe into test **Port B**.
NOTE: The A probe, **715P**, has a blue color marker and identification band. The B probe, **712P**, has a red color marker and identification band.
2. Insert the connectors on the ends of the probes into the appropriate sockets located just beneath the **Port A** and **Port B** test ports.
3. Set both the **10¹¹Ω/GND** toggle switch on Channel A and the **20M/GND** switch on Channel B to their **GND** positions.
4. Rotate the **Capacity** compensation knob on Channel A, which is just below the **Position** knob, completely counterclockwise to 0 (zero). Rotate the **Capacity** for Channel B, which is in the **Negative Capacity** sector, to the center 0 (zero) position.
5. Plug the instrument power cord into the power line (mains) and set the **Power** switch to **On (I)**. All the digits on the panel digital meter should light up.
6. Set the **I MULT** (current multiplier) toggle switch to **x10**. The **I MULT** toggle switch is located in the **Current** sector of the **Duo 773**. If the current circuit is working properly, three yellow lamps will illuminate:
 - One above the **I MULT** toggle switch
 - One in the **Bridge/Amplify** sector
 - One, the **I x10** lamp, below the digital display.
7. After the yellow LEDs are illuminated, set the **I MULT** toggle switch to the **x1** position. All three yellow x10 LED indicator lamps will go out.
8. Push the **Tickle** button located in the center of the **Negative Capacity** sector. An audible tone will be heard.



9. Both Channel A and Channel B have **Position** knobs with **On/Off** toggle switches next to them. Set both **Position** toggle switches to **Off**.
10. There are two **Meter Select** knobs located below the digital display meter. Set the left knob to the V_B position, and set the right knob to a range of **200mV**.
11. The display meter will read very close to 0.0mV. If necessary, use the potentiometer adjustment tool (WPI# **13661**) to adjust the **Zero** calibration screw located in the **Channel B** sector under the **ERT** button.
12. Set the first **Meter Select** knob to the V_A position.
13. Again, the display meter will read very close to 0.0mV. If necessary, use the potentiometer adjustment tool (WPI# **13661**) to adjust the **Zero** calibration screw located in the **Channel A** sector under the **ERT** button.

NOTE: It is recommended that **Duo 773** be allowed to warm-up for at least 30 minutes before adjusting any front panel calibration screws.

Channel B and 712P Probe Tests

The primary function of the low input impedance **712P** probe and the Channel B amplifier is the recording of large changes in cellular membrane potentials caused by the interaction of multiple ion currents, and the injection of current pulses to trigger or prevent changes in cellular membrane potentials.

Channel B Current Leakage and Offset Test

This test will determine if any current is leaking from the probe or if the signal from the probe is offset by a junction potential between the amplifier and the probe.

1. Complete the Basic Instrument Test described on page 10 before beginning this test.
2. Check that the following controls for Channel B are set properly:
 - Set the **Position On/Off** switch on Channel B to the **Off** position.
 - Set the **Capacity** knob in the Negative Capacity sector to 0 (zero).
 - Plug the low input impedance **712P** (red) probe into test **Port B**.
 - Set the **20M/GND** switch to **GND** position.
 - Set the left **Meter Select** switch to V_B .
 - Set the right **Meter Select** switch to **200mV**.
3. Change the **20M/GND** switch to the **20M** position. This setting places a $20M\Omega$ resistance between the **712P** probe tip placed in test **Port B** and ground. This resistance acts as a pseudo-electrode for testing the probe and amplifier.




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- The voltage of the digital display panel may shift a small amount from 000.0mV, because there is a small current leak at probe tip. If the display does not read 000.0V, adjust the **Leak ADJ x1** calibration screw until display reads 000.0mV. This test is analogous to inserting the tip of a fluid-filled microelectrode into a bath chamber filled with physiological buffer that is grounded with a suitable reference electrode.
- Probes and electrodes may also exhibit a potential difference of several millivolts, because junction potentials at the tips of the probe and the electrode.
 - Set the **Position On/Off** toggle switch to the **On** position.
 - Rotate the **Position** knob and notice that voltage displayed on the panel moves over a wide range.
 - Set the **Position** knob so the digital panel displays 000.0V
- Connect the BNC output of Channel B to a recorder and observe the same effects on the recorder trace.

Channel B Electrode Resistance Test

This test will determine if the probe and the amplifier are working properly by using the electrode test resistor to simulate a functional microelectrode.

- Complete the Basic Instrument Test described on page 10 and the Current Leakage and Offset Test (above) before beginning this test.
- Check that the following controls are set properly:
 - Set the **Position On/Off** switch on Channel B to the **Off** position.
 - Set the **Capacity** knob in the Negative Capacity sector to 0 (zero).
 - Plug the low input impedance **712P** (red) probe into test **Port B**.
 - Set the **20M/GND** switch is set to **20M** position.
 - Set the left **Meter Select** switch to **V_B**.
 - Set the right **Meter Select** switch to **200mV**.
- Press the **ERT** button on Channel B. The voltage drop read on the digital display panel should be 20mV. Release the button as soon as the voltage drop can be read.

 **TECH NOTE:** The amplitude of this voltage drop is derived from Ohm's Law ($V = I \times R$). In this case, a 1nA current is driven through a 20MΩ resistor yields a 20mV drop. Since the value of the 20MΩ resistor is approximate, the voltage drop of 20mV is also approximate.

Channel A and 715P Probe Tests

The primary function of the high input impedance **715P** probe and the Channel A amplifier is to record the small, ion-specific changes of the membrane potential of a cell using high resistance microelectrodes.



CAUTION: Since the **715P** probe is extremely sensitive to high voltage charges, like static electricity, Channel A should be placed in **Standby Mode** when:

- The probe is being moved from the test port to the measurement chamber or vice versa. Once the tip of the microelectrode on the end of the **715P** probe is in the bath solution that is grounded to the **Duo 773**, the amplifier can be switched into **Operate** mode.
- The microelectrode is being inserted in the electrode holder, and the electrode holder is being handled.
- The **Duo 773** is being handled while the probe is not in the test port or in a grounded bath.

Channel A Current Leakage Test

This test will determine if any current is leaking from the probe.

1. Complete the Basic Instrument Test described on page 10 before beginning this test.
2. Check that the following controls for Channel A are set properly:
 - Set the **Standby/Operate** switch in the **Standby** position.
 - Set the **Position On/Off** switch on Channel A to the **Off** position.
 - Set the **Capacity** knob to 0 (zero).
 - Plug the low input impedance **715P** (blue) probe into test **Port A**.
 - Set the **10¹¹Ω/GND** switch to **GND** position.
 - Set the left **Meter Select** switch to **V_A**.
 - Set the right **Meter Select** switch to **200mV**.
3. Change the **10¹¹Ω/GND** switch to the **10¹¹Ω** position. This setting places a **10¹¹Ω** resistance between the **715P** probe tip placed in test **Port A** and ground. This resistance acts as a pseudo-electrode for testing the probe and amplifier.
4. The voltage of the digital display panel may shift a small amount from 000.0mV, because there is a small current leak at probe tip. If the display does not read 000.0V, carefully adjust the **IG** calibration screw until display reads 000.0mV. This is difficult to do since each mV is equivalent to 0.01 pA. Some small fluctuation of the reading is normal.

Channel A Electrode Resistance Test

This test will determine if the probe and the amplifier are working properly by using the electrode test resistor to simulate a functional microelectrode.

1. Complete the Basic Instrument Test described on page 10 before beginning this test.



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2. On Channel A, verify the following settings:
 - Set the **Position On/Off** switch to the **Off** position.
 - Turn the **Capacity** knob completely counterclockwise to 0 (zero).
3. Perform an Electrode Resistance Test on the high input impedance **715P** probe and the Channel A amplifier with either 1 pA or 1 nA of current. The other settings required to perform the test are listed in the following table.

	1pA Test	1nA Test
a. Plug 715P probe (blue) into test Port :	A	B
b. Set the GND switch to the right of the test port specified in Step a to:	10¹¹Ω	20MΩ
c. Set the switch to the right of the ERT button on Channel A to:	1pA	1nA
d. Set the left Meter Select switch to:	V_A	V_A
e. Set the right Meter Select switch to:	200mV	200mV

4. Press the **ERT** button on Channel A. The voltage drop appears on the digital display panel. Release the button as soon as the voltage drop can be read.
 - For the ERT Test with 1 pA, the voltage drop shown on the display panel should be 100mV. This is derived from a calculation using Ohm's law, $V = I \times R$. For example, if a 1 pA current is driven through a 10¹¹Ω load, the amplitude of the voltage drop will be approximately 100mV.
 - For the ERT Test with 1 nA, the voltage drop shown on the display panel should be 20mV. This is also derived from Ohm's law. In this instance, if a 1 nA current is driven through a 20MΩ load, the amplitude of the voltage drop will be approximately 20mV.

NOTE 1: The 1pA and 1nA currents used in channel A are approximate and somewhat affected by ambient temperature. The 1pA test current can be readjusted by turning the **1pA** calibration screw as the **715P** probe is in test **Port A** while pushing the Channel A **ERT** button. The 1 nA test current can be adjusted when the **715P** probe is in test **Port B** and the **A** calibration screw is turned as the Channel A **ERT** button is pushed.

NOTE 2: If the **CKT** ground has not been connected to a recorder or other grounded instrument, the voltage across the 10¹¹Ω resistor may fluctuate excessively. The fluctuations should cease if the **CHAS** ground is connected to **CKT** ground, or if an externally grounded recorder is connected to any of the BNC connectors on the front panel.

NOTE 3: The **ERT** test in channel A will exhibit some delay in the probe settling due to the additional shielding added to the 10¹¹Ω resistor inside the **Duo 773**. This delay is not normally seen in actual usage.



OPERATING INSTRUCTIONS

Negative Capacity (Capacitance Compensation)

The use of fluid-filled microelectrodes make it easy to measure the changes in cellular membrane potentials. However, these microelectrodes develop a shunting capacitance (capacitance that is in parallel circuit with the electrode resistance) which slows the responsiveness of the probes that are designed to measure fast changes in membrane potentials, like action potentials.



TECH NOTE: It is estimated that 1pF of shunt capacitance is added for each millimeter of microelectrode that is immersed in fluids containing electrolytes.

To compensate for the shunting capacity of the microelectrode, a negative capacitance can be applied to the probe through controls on its amplifier. The proper level of negative capacitance increases the responsiveness of the probe system to rapid changes in membrane potentials so that recordings of these changes replicate the actual events taking place across the cell membrane.



CAUTION: Use care when adjusting negative capacity. Excessive negative capacity can cause the microelectrode to oscillate and disrupt the cell membrane. It can also cause an increased baseline noise level.

- For Channel A, locate the **Capacity** knob in the Channel A sector and rotate it clockwise to increase negative capacity. A safe level of negative capacity begins with the **Capacity** knob set at 10–20° above the 0 (zero) index mark on the panel. To reduce the level of negative capacity, turn the knob counterclockwise towards 0. Channel A is limited to 10pF of negative capacity.
- For Channel B, locate the **Capacity** knob in the Negative Capacity sector and rotate it clockwise to increase negative capacity. A safe level of negative capacity begins with the **Capacity** knob set at 10–20° above the 0 (zero) index mark on the panel. To reduce the level of negative capacity, turn the knob counterclockwise back to 0 (zero).
- The proper level of negative capacity can also be set while changes in membrane potentials are being recorded. The onset of membrane events, like action potential, are quick. If the onset of action potential (AP) is prolonged, try increasing the negative capacity in a stepwise manner between successive recordings of the AP until the onset of the AP is quick.



Duo 773

Current Injection (712P Probe on Channel B only)

To stimulate a cell and cause it to fire, current can be injected into the cell through the microelectrode on the probe tip while the cell is bathed in an electrolytic buffer that is grounded to the **Duo 773**. Current can only be injected into cells that are being measured by the **712P** probe on Channel B. A pulse from an external source, like the stimulator of a data recording unit, can be sent to the probe through the **Stimulus Input** jack in the Current sector. This setup allows the polarity, amplitude and shape of the stimulus pulse to be controlled by an external voltage command applied at the **Stimulus Input** connector.

DC current can also be applied to a cell from an internal DC current source in the **Duo 773**. This current is constant unless the input voltage range of +10V (approximately) is exceeded. That means they are independent of electrode resistance. If the input voltage exceeds +10V, an audible and visual **Over Range** warning occurs.

A voltage signal proportional to injected current can be monitored by connecting the **Current Monitor** output to a recorder.

NOTE: The digital display panel can only be used to monitor the output of internal DC current source.

Injecting Current from an External Stimulator

Before working on living cells, perform the following tutorial to learn how to program the **Duo 773** to inject a stimulus pulse from an external source, like the DAC output of a recorder.

1. Program a stimulator, like a WPI **A310**, to deliver a 100mV square wave with a 5ms duration at 100Hz.
2. Connect the output of the external stimulator to the **Stimulus Input** connector in the Current sector of the **Duo 773**.
3. Set the **I MULT** (current multiplier) toggle switch in the Current sector to the **x1** position.
4. Set the **DC Current** knob to the minimum (counterclockwise) position.
5. Set the **+ / Off / -** switch to the **Off** position.
6. Connect the **x1 Output** of Channel B to a recorder.
7. Insert the **712P** probe into test **Port B**.
8. Set the **20M / GND** toggle switch to **20M**.



9. Turn on the external stimulator and send a series of square waves through the current injection circuit to the probe. Since the **Stimulus Input** conversion factor is 20mV/1nA, the 100mV pulses from the external stimulator will generate 5nA current pulses through the probe tip. Since the probe tip is connected to ground via a 20M Ω resistor, a 5nA current pulse creates a 100mV square wave on the recording from Channel B. Remembering Ohm's Law, $5\text{nA} \times 20\text{M}\Omega = 100\text{mV}$
10. Notice that the edges of the square wave are rounded from the shunting capacity of the 20M resistor in the test port. Slowly rotate the **Capacity** knob to sharpen the edges of the square wave.
11. When finished with this tutorial, turn off the external stimulator.

Injecting Current from the Internal DC Current Source

1. Set the **+ / Off / -** Toggle switch to **+**. The green LED illuminates.
2. Slowly turn the **DC Current** knob clockwise as the output of Channel B is recorded. Note that the DC level of the recording rises in the positive direction.
3. Reverse the polarity by setting the **+ / Off / -** Toggle switch to **-**. Note that the trace goes negative, as well.
4. When the **I MULT** toggle switch is set to **x1**, 1nA is sent through the pseudo-electrode for every 20mV that is applied. The maximum DC current is 50nA. When the current multiplier is set to **x10**, 10nA is applied to the resistor for every 20mV applied. The maximum current is 500nA.

NOTE: The internal current generating circuit will saturate if the product of the current applied to the electrode and the resistance of the electrode ($V = I \times R$) exceeds approximately $\pm 10\text{V}$. When this happens, the **Over Range LED** illuminates to indicate the current applied is no longer proportional to the voltage applied.

Monitoring Injected Current

Current can be monitored directly by recording the signal from the **Current Monitor**. Note that 1nA of injected current deflects the recording trace 100mV when the **I MULT** is on the **x1** range, and 10mV when the **I MULT** is on the **x10** range.



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Bridge Compensation (Bridge Balance)

Electrode Voltage Drop

When studying the excitability of a cell, it is important to inject current into the cell to measure the voltage gradient across its membrane that results from transmembrane current flow. However, this change in the cell membrane voltage is significantly smaller than the voltage drop that occurs when current passes through a microelectrode. So, the electrode voltage drop must be subtracted from the total voltage signal to observe the cell membrane signal.

This subtraction is accomplished by using the differential amplifier in the Bridge/Amplify sector of the **Duo 773**. This circuit amplifies the difference between the **x1 Output** of either Channel A or B and the voltage that is proportional to the amplitude of the current injected into the cell. When adjusted properly, the electrode voltage drop is accurately subtracted from the total voltage signal.

Adjusting Bridge Compensation for a Current Pulse

Before working on living cells, perform the following tutorial to learn how to program the **Duo 773** to remove the voltage drop, which is caused by the current pulse, from the recording.

1. Using the directions for "Injecting Current from an External Stimulator" on page 16, send additional 100mV pulses through the **712P** probe just as they were before.
2. Set the **Amp Select** knob on the Bridge/Amplify sector to **Bridge**.
3. Set the **R** toggle switch on the same sector to **x1**.
4. View the signal from the **x10 Output** on a recorder. Note that as the **Bridge R** (ten-turn) control is increased from 0, the amplitude of the square wave diminishes from about 1V to 0.0 after about two clockwise turns. The **Bridge R** dial reads the same resistance value measured earlier (approximately 20M Ω).
5. Adjust the **AC Balance** (transient) control knob to reduce the square wave edge artifacts. Note that adjusting the **Capacity** knob may further reduce the transient artifact.

Adjusting Bridge Compensation before Recording from Cells

Before recording membrane potentials from cells, the microelectrode must first be balanced so that the voltage drop from the injected current is canceled out.

1. Prepare the microelectrode by filling it with the suitable electrolyte solution and placing it in the electrode holder on the probe tip.

2. Prior to inserting the microelectrode into the cell, lower the tip of the microelectrode into the bath solution that surrounds the cells being studied. A couple of millimeters of the tip should be immersed in the solution.
3. Using the current pulse needed for the cell and experiment being conducted, balance the electrode resistance as described in "Adjusting Bridge Compensation for a Current Pulse" on page 18.
4. Once the bridge balance is set for the pulse and microelectrode being used, proceed to impale cells and pass current through the microelectrode as designed in the experiment.

NOTE: The bridge compensation must be reset every time a new microelectrode is used.

While current injection is a valuable technique, it is limited by two shortcomings:

- Fluid filled microelectrode resistance does not remain constant as the injection current density increases. Therefore, select the electrode with as large a tip diameter allowed by the experimental protocol.
- The resistance of fluid filled microelectrodes changes slightly when the tip of the electrode is inserted into cells. Therefore, the intracellular electrode resistance is different from the resistance measured outside.

Adjusting Bridge Compensation Intracellularly

Rebalancing the bridge compensation, while the microelectrode tip is in the cell, is possible, because the time constant of the cell membrane is usually much larger than the time constant of the electrode, even when capacity compensation (negative capacity) is used to counteract the shunting capacity of the electrode.

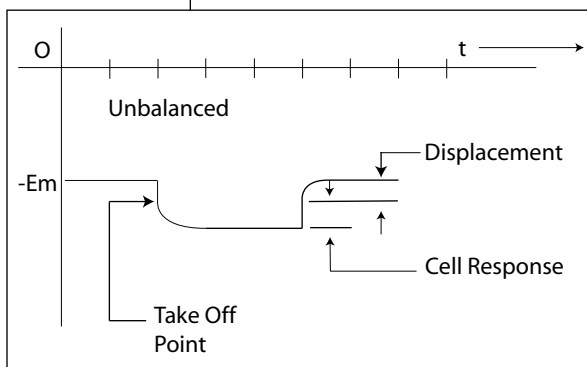


Fig. 9—Step of current through an intracellular micropipette electrode

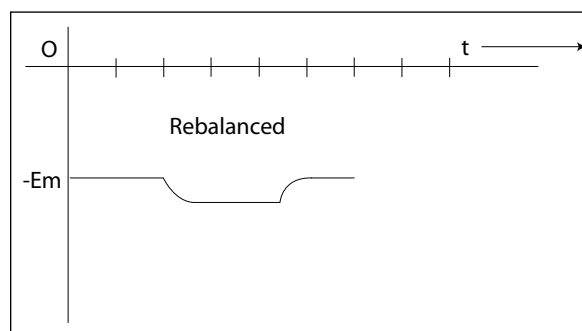


Fig. 10—Bridge R is in balance



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In Figure 9, the effect of a current pulse injected intracellularly through a unbalanced microelectrode is demonstrated by the initial fast vertical step of the signal. The signal slows to an exponentially rounded edge that is caused by the slower time constant of the cell membrane. As seen, the response of the cell membrane begins at a point (Take Off) that is displaced away from the resting cell membrane potential by the amplitude (Displacement) of the current pulse.

If the **Bridge R** control is properly balanced to remove the voltage drop created by the current pulse, the response of the cell to stimulation begins at the resting cell membrane potential, as shown in Fig. 10.

This procedure is performed in the same manner as the balancing of the electrode while it is outside the cell, as described on page 19. It is an acceptable step since the voltage subtracted from the signal is the increment that is caused by the current meeting the resistance of the electrode. The onset of the cell response is identifiable by its rounding caused by the slower time constant of the membrane

Filtering

The **Low Pass Filter** is a variable cut-off filter that can be set at a frequency from 1kHz up to 30kHz. The high frequency roll off of this filter is approximately 40db per decade. The cut-off frequencies are marked on the dial in a nonlinear manner, with the cut-off frequencies between 2 and 3kHz occurring near the middle of the dial's rotation. To use the Low Pass Filter:

1. Set the **Filter Select** switch to:
 - **x1** to filter the x1 output of Channel B
 - **Bridge/Amplify** to filter the output of the amplifier selected in the Bridge/Amplify sector.
2. Rotate the filter setting knob to select the proper cut-off frequency to remove high frequency noise from the recording.
3. To monitor the filtered signal from electrode amplifier, connect a recorder to the **Filter Output** jack. For example, if the **Filter Select** switch is set to **x1**, the output of the Channel B amplifier is filtered. The DC voltage gain of the filter is x1.

NOTE: Low pass filtering is also possible using the **Capacity** knob on the probe amplifier. Move the knob counterclockwise from the 0 index mark. Moving counterclockwise past 0 (zero) adds positive (shunt) capacitance, in parallel with the electrode's resistance, to negate noise.



Tickle

Investigators often succeed in penetrating living cells by briefly oscillating the tip of the microelectrode while the tip is gently pressing on the outside of the cell. The process is called “tickling,” and it is controlled by an electrical circuit that is connected to the electrode when the **Tickle** button in the Negative Capacity sector is pushed. The frequency and amplitude of oscillation can be adjusted using the **AMPTD** (amplitude) and **FREQ** (frequency) calibration screws in that sector. An external voltage command of +5V, applied to **EXT Command** can also initiate tickling. The Tickler’s oscillation is audible. Since cell membranes have different degrees of stiffness, the frequency, amplitude and duration of the tickle oscillation needs to be adjusted accordingly.

Grounding

Proper grounding of the experimental preparation is essential in order to obtain accurate recordings with minimal interference from power line induction. Normally, a silver/silver chloride electrode with a salt bridge (such as WPI # **RC1T**) is placed in the bath solution that surrounds the cell. The lead wire of the ground electrode is connected to the **CKT** ground terminal on the **Duo 773** to create a stable electrochemical reference potential with respect to the bath solution that contains electrolytes.

Differential Recording

Matching Probe Time Constants

When recording differentially from a cell with two intracellular microelectrodes of unequal resistance, large transient artifacts may be generated during the rapid changes in cell membrane potential. The artifacts are caused not only by the unequal electrode resistances, but also by the unequal electrode time constants. The two electrodes track more closely in time if the inequality is compensated by using:

- Less negative capacity on Channel B to slow down its faster, low resistance electrode.
- More negative capacity on Channel A to speed up its slower, high resistance electrode.
- The Low Pass Filter to slow the faster, low resistance electrode in Channel B.

To match the electrodes:

1. Inject a square pulse through the low input impedance (**712P**) probe on



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Channel B, while the bath solution is grounded to the **CKT** terminal and both microelectrodes are touching the bath solution.

2. Increase the negative capacity on Channel A and decrease the negative capacity on Channel B to minimize the amplitude of the transient artifact spikes.
3. If using filtering, it is applied to Channel B. Use a differential preamplifier to compare the **x1** output of Channel A to the **Filter Output** of Channel B (Filter Select x1) to determine if filtering is more effective.

NOTE: A **Duo 773** may come equipped with two **712P** (red) probes. In this case, the Channel A probe would have a resistance of 10^{12} rather than the $10^{15}\Omega$ cited above. An internal switch has been toggled to effect this modification (See Appendix A).

Differential Amplifier

To program the differential amplifier that will subtract the output of Channel B from the output of Channel A, set the controls of the **Duo 773** as follows:

1. Rotate the **Amp Select** switch on the Bridge/Amplify sector to select **A-B**.
2. Set the left **Meter Select** switch to V_{A-B} . The digital display panel will display the DC differential voltage. The **x10 Output** in the Bridge/Amplify sector amplifies the differential voltage ten (10) times. The **x50 Output** amplifies the differential voltage fifty (50) times



ACCESSORIES

712P	Replacement probe (includes calibration)
715P	Replacement probe (includes calibration)
2933	Rack Mount kit
2547	Driven Guard Shield
15790	Replacement Probe Handle
TW100F-4	Glass capillary with filament
TW150F-4	Glass capillary with filament



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SPECIFICATIONS

Channel A:

Active Probe Input R	$10^{15}\Omega$
Voltage Gain	x1 - 0.1%
Output Resistance	100 Ω
Output Voltage Range	$\pm 10V$
Max. Input Voltage*	$\pm 15V$
Probe Input Leakage Current	$10^{-14}A$ (Adjustable to zero)
Input Shunt C Compensation	0 to -10pF
DC Position Adjust Range	$\pm 300mV$
Electrode Resistance Test	1pA, 1nA test currents
Cell "Tickler"	>8V peak to peak at 1000Hz

* Higher input voltages permissible if input current is limited to less than 0.5mA.

Channel B:

Active Probe Input R	$> 10^{11}\Omega$
Voltage Gain	X 1.0 -0.2% X 10 $\pm 2\%$
Output Voltage Range	$\pm 10V$
Output Resistance	100 Ω
Max. Input Voltage*	$\pm 15V$
Probe Input Leakage Current	5pA max. Adjustable to zero
Input Shunt C Compensation	+10 to -50pF
Risetime, 10 to 90%, typ	< 1 μs , direct input, small signal < 25 μs , 20M Res.w.neg.C comp.
Noise Level, 10 kHz BW	< 50 μV , probe input shorted < 200 μV , input 20M Ω to GND
DC Position Adjust Range	$\pm 300mV$, 10 turns
Electrode Current**	50nA per V, I max.=50 nA 50nA per 0.1V, I max.=500nA
DC Electrode Current	+/- variable 0 < I < 50 and 500nA
Electrode Resistance Test	1mV/M Ω (IX1); 10mV/M (IX10)
Electrode Current Monitor	100mV per nA, I MULT X 1 10mV per nA, I MULT X 10
Electrode Resistance Bridge Range	0 < R < 100M Ω ; 0 < R < 1000 M
Resistance Bridge Amplification	X 10, X 50
Differential Amplifier Gain	X 10, X 50
Low Pass Filter	Cont. variable, 1 to 30KHz Roll off = 40 dB per Decade
Cell "Tickler"	0 to 15V variable, 1-8 KHz, variable, External gate feature included

** Maximum current limited by (I x Electrode R) < 10V.



LED Digital Panel Meter:

Display	3.5 digit LED display
Ranges	200mV, 2000mV and 20V 200nA, 2000nA (channel B)
Accuracy and Resolution	±1 digit
Power	120/240V, 60/50Hz, 20VA
Physical Dimensions:	
Probe	Body: 12mm (D) x34mm (L); tip 2mm
Instrument	W=43.2cm, H=13.4cm, D=25.4cm

Notes

1. **Duo 773's** active probes have been accurately calibrated for operation with this instrument. If probe replacement becomes necessary, return the entire instrument to an authorized repair facility for re-calibration.
2. These probes can be damaged by static electricity. Connect the probe tip to an electrical ground terminal (for example, the test ports A and B) when not in use.
3. Allow the instrument to warm up for at least 30 minutes before making any screwdriver adjustments on the front panel.



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APPENDIX A: USING THE 712P (LOW IMPEDANCE PROBE) IN CHANNEL A

The normal configuration of the **Duo 773** allows the use of one high input impedance (**715P**) electrode in Channel A and one low input impedance (**712P**) electrode in Channel B. An optional configuration of the **Duo 773** allows for the use of low input impedance balanced probes (**712P**) in both Channel A and Channel B. There are limitations with this optional configuration:

- Channel A will not stimulate when a **712P** probe is used on it. Only Channel B can stimulate.
- The Channel A tickle function only operates at a voltage greater than 8V peak to peak at 1,000Hz. It is not adjustable.

NOTE: If this optional configuration is required, the standard **Duo 773** amplifier and its probes may be returned to WPI for re-configuration or the instructions below can be used for re-configuring the **Duo 773**.

User Re-configuring for Two 712P Probes

1. Disconnect the power cord of the **Duo 773** from the MAIN.
2. Remove the four screws on the sides of the **Duo 773** case.
3. Lift the case cover to remove it.
4. When looking at the case from the front, the LOW-Z switch is located on the left side behind the Channel A sector. See Figure 11. Set the switch to the desired position:
 - LOW-Z for using a **712P** probe in Channel A.
 - NORMAL for using a **715P** probe in Channel A.

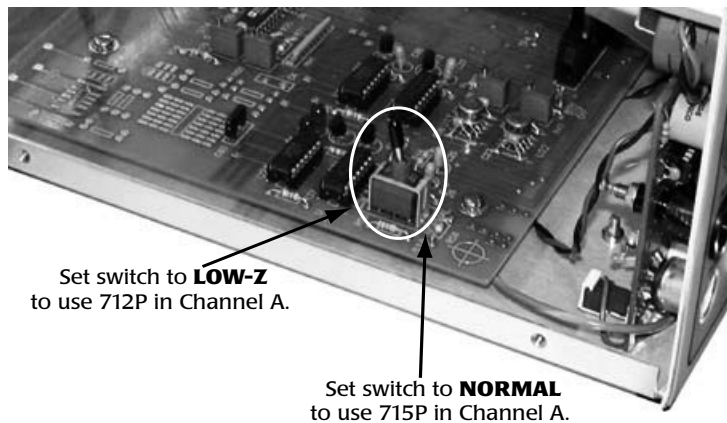


Fig. 11—LOW-Z switch set for use with a **712P** in Channel A.

5. Slide the case cover back into place and secure it with the four screws again.



Using the 712P in Channel A

If your **Duo 773** is set to the optional configuration, you may use the **712P** probe in Channel A. To set up Channel A:

1. Test a low input impedance (**712P**) probe on Channel B to make sure the probe works properly.
2. Turn off the **Duo 773**. Remove the probe connector from **Channel B Probe** socket and place it in the **Channel A Probe** socket.
3. Place the headstage of the Channel A **712P** probe into **Port B**. The rest of the calibration tests will be performed with this setup.
4. Turn the Channel A **Mode** switch to **Standby**.
5. Turn the **Duo 773 Power** switch to **On**.
6. Set the Channel A **Position** control to **Off**.
7. Set the **Port B** to **GND**.
8. Rotate the **Capacity** control knob on Channel A counter-clockwise as far as it will go.
9. Turn the Channel A **Mode** switch to **Operate**.
10. Set the left **Meter Select** knob to V_A .
11. Set the right **Meter Select** knob to **200mV**.
12. Monitor the **X1** current output with a recorder. If necessary, increase the range setting temporarily to make these first adjustments.
13. Using a small screwdriver, adjust the Channel A **Zero** control screw until the meter reads zero.
14. Switch the **Port B** toggle switch to **20M**.
15. Using a small screwdriver, adjust the Channel A **IG** control screw until the meter reads 0mV.
16. Depress the **ERT** push button on Channel A. The meter should read 20mV. If it does not, use a small screwdriver and adjust the Channel A **1nA** control screw until the meter reads 20mV.
17. The 1pA function does not require adjustment, because it does not function when the probe connected to Channel A is plugged into the test port on Channel B.
18. Turn the Channel A **Mode** switch to **Standby** to prevent any damage to the Channel A amplifier when the probe is handled.
19. Remove the probe being used on Channel A from test **Port B**. The **Duo 773** in this optional configuration is now ready for laboratory use.



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WORLD PRECISION INSTRUMENTS, INC.

175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA
Telephone: (941) 371-1003 Fax: (941) 377-5428
e-mail wpi@wpiinc.com

DECLARATION OF CONFORMITY

We: World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota FL 34240-9258
USA

as the manufacturers of the apparatus listed, declare under sole responsibility that the product(s):

Title: 773

to which this declaration relates is/are in conformity with the following standards or other normative documents:

- Safety:** EN 61010-1:1993 (IEC 1010-1:1990)
- EMC:** EN 50081-1:1992
EN 50082-1:1992

and therefore conform(s) with the protection requirements of Council Directive 89/336/EEC relating to electromagnetic compatibility and Council Directive 73/23/EEC relating to safety requirements.

Issued on: 18th February 2000

Dr. Mark P. Broderick
President and COO

World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA

Mr. Glen Carlquist
Production Manager

World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA



Duo 773

WARRANTY

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of one year* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

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Claims and Returns

- Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within 10 days after receipt of shipment. Claims for lost shipments must be made within 30 days of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim settles. In some instances, photographic documentation may be required. Some items are time sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container.
- WPI cannot be held responsible for items damaged in shipment en route to us. Please enclose merchandise in its original shipping container to avoid damage from handling. We recommend that you insure merchandise when shipping. The customer is responsible for paying shipping expenses including adequate insurance on all items returned.
- Do not return any goods to WPI without obtaining prior approval and instructions (RMA#) from our returns department. Goods returned unauthorized or by collect freight may be refused. The RMA# must be clearly displayed on the outside of the box, or the package will not be accepted. Please contact the RMA department for a request form.
- Goods returned for repair must be reasonably clean and free of hazardous materials.
- A handling fee is charged for goods returned for exchange or credit. This fee may add up to 25% of the sale price depending on the condition of the item. Goods ordered in error are also subject to the handling fee.
- Equipment which was built as a special order cannot be returned.
- Always refer to the RMA# when contacting WPI to obtain a status of your returned item.
- For any other issues regarding a claim or return, please contact the RMA department

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

Warning: This equipment is not designed or intended for use on humans.

World Precision Instruments, Inc.

International Trade Center, 175 Sarasota Center Blvd., Sarasota FL 34240-9258

Tel: 941-371-1003 • Fax: 941-377-5428 • E-mail: sales@wpiinc.com

UK: 1 Hunting Gate, Hitchin, Hertfordshire SG4 0TJ • Tel: 44 (0) 1462 424700 • Fax: 44 (0) 1462 424701 • E-mail: wpiuk@wpi-europe.com

Germany: Liegnitzer Str. 15, D-10999 Berlin • Tel: 030-6188845 • Fax: 030-6188670 • E-mail: wpide@wpi-europe.com