

INSTRUCTION MANUAL

Model 260

Microiontophoresis Current Programmer

Serial No._____

www.wpiinc.com

021216

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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 Model 260 is designed for electroiontophoresis of dyes, drugs and charged substances from micropipettes.

TIP: For an excellent video introduction of the process, see the Jove video at www.jove.com/video/2900/microiontophoresis-micromanipulation-for-intravital-fluorescence.

INTRODUCTION

The Model 260 Microiontophoresis Current Programmer is a floating, battery-operated instrument designed for the electroiontophoresis of dyes, drugs and charged substances from micropipettes. Two identical battery operated current generators are available. In ordinary use, the two current generators are operated in parallel providing two distinct currents; one for preventing substances in the micropipette from outward diffusion (the retain or hold current) and the second for the active ejection of charged material. For pipettes with submicron tips, a hold current may not be necessary if there is little outward diffusion of pipette material.

Model 260 is powered by four standard 9V batteries and 5V DC external power supply. (Units manufactured before 2015 did not require the 5V external power supply.) Their life expectancy can be considerably improved if the power switch is turned off when the instrument is not in use.

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 11 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 11 of this manual.

INSTRUMENT DESCRIPTION

The following controls are available for both the Hold and Eject channels.

POWER ON/OFF–Instrument power switch/external 5V DC. To maximize battery life, turn the power OFF when the channel is not in use.

CURRENT LEVEL–This control varies the current amplitude as indicated on the ammeter. To prevent accidental current flow via the output terminals, place the **OUTPUT** switch in the **PRESET** position while adjusting current amplitude.

CURRENT RANGE-Selects either 100 or 1000nA current range

MODE SELECT-MANUAL operation allows you to apply a DC current when the **OUTPUT** switch is changed from **PRESET** to **ENABLE**. **AUTO** operation requires you to apply a +5V command potential to initiate the flow of current.

OUTPUT POLARITY-NORMAL indicates that the red output connector is positive with respect to the black connector. **INVERT** reverses connector polarity.

AUTO INPUT-If the **MODE SELECT** switch is in the **AUTO** position, the **AUTO INPUT** connector controls current activation. Five volts positive with respect to the outer connector shell maintains the current <u>for as long as this voltage is applied</u>. The **CURRENT LEVEL** control continues to dictate the actual current amplitude.

OUTPUT–In the **PRESET** position, the red and black current output terminals are disconnected from the current generating circuit. **ENABLE** connects the output terminals to the current source. <u>If the **OUTPUT** switch is in the **PRESET** mode, current cannot flow out of the instrument in either **MANUAL** or **AUTO** operating modes.</u>

CHAS GND-This green binding post is connected to the chassis or metallic enclosure of the instrument. It should normally be connected to the ground point of your recording system.

OPERATING INSTRUCTIONS

Preliminary Instrument Check

- 1. Set the **OUTPUT** switch to **PRESET** on either channel. This disconnects the red and black output terminals and internally short circuits the output of the current generator.
- 2. Set the **MODE SWITCH** to **MANUAL**.
- Turn on the **POWER** switch located just below the current indicating ammeter. Notice that as the **CURRENT LEVEL** control is rotated, the ammeter reading changes accordingly.
- 4. Set the current ranges and note the similar ammeter behavior.
 - **NOTE**: You may easily reach and exceed the ammeter's maximum reading. If you cannot reach and exceed full scale on the ammeter, the transistor batteries may need replacement.
- 5. If the **OUTPUT** switch is changed from **PRESET** to **ENABLE** at any current setting, an audible alarm sounds, and a red lamp on the front panel illuminates to indicate that the output current pathway is an open circuit. (At very low nanoampere current settings, the alarm may take several seconds to activate.)
- 6. Check the performance of both channels in the manner indicated above.
- 7. Ground the **CHAS GND** green binding post to assure low noise. The instrument is now ready for use.

Single Channel Operation

Model 260 contains two completely independent floating, battery operated current generators, which may be operated independently or in parallel connection. As an independent current generator, you only need to connect your external electrode pair to the red and black output terminals of channel 1 (HOLD) or 2 (EIECT).

While making these connections and any preliminary control adjustments, the **OUTPUT** switch should be in the **PRESET** position. This will prevent any accidental current flow

- 1. Set the **CURRENT RANGE**. For currents below 100nA, the 100nA range is recommended, because you may adjust these low current values with better resolution in the lower current range.
- 2. Adjust the **CURRENT LEVEL** control until the required DC current value is observed on the channel ammeter.
- 3. Select the **OUTPUT POLARITY** desired. **NORMAL** yields a positive potential at the red output terminal.

 Switch the **OUTPUT** switch from **PRESET** to **ENABLE** to release the required current from the output terminals. To halt current flow, return it to the **PRESET** mode.

Two Channel Operation, Hold-Eject

In "Appendix A: Application Notes" on page 6 is a description of the electrophoretic release of charged substances from micropipettes, which often requires the use of a second current to counter the outward diffusion of material from the delivery pipette. This current has been referred to in the literature as "backing, holding or retaining" current. It cause a small current to flow in a direction that offsets the spontaneous leakage of the active agent from the pipette until it is required.

- 1. Connect a shorting wire or jumper to tie the red **OUTPUT** terminals of the **HOLD** channel and the **EJECT** channel together.
- 2. Connect an identical jumper wire between the black **OUTPUT** terminals. This effectively connects the two current generating channels in parallel. Current does not flow from one channel into the other, but it will sum algebraically in the common electrode pathway, if both channels are operating simultaneously.
- 3. Place both channels in the **PRESET** mode to prevent accidental current flow during set up.
- 4. Set the polarity and current amplitude on the **HOLD** channel to retain the active agent in the electrode.
- 5. Set the polarity and amplitude of the current in the **EJECT** channel.
- 6. Choose one of two operating methods:
 - Leave the hold current on at all times and effect ejection by superimposing the larger eject current by operating the **PRESET/ENABLE** switch on the **EJECT** channel. The ejection current swamps the smaller hold current. Then, algebraically adds the hold current to the eject current to compute the actual ejection current. For example, if the hold current is 5nA subtracted from the eject current of 50nA, the effective ejection current is 45nA. This method has the advantage that you only need to operate a single **PRESET/ENABLE** switch on the **EJECT** channel during the experiment.
 - Alternatively, simultaneously operate the **PRESET/ENABLE** switches on both channels so that the hold current is shut off at the moment the eject current is applied. In the example above, the eject current would then be +50nA.

AUTO Operation

The timing of current flow from each channel of the **Model 260** can be externally controlled by the use of a pulse generator or computer that can supply a logic level of at least +5V to the **AUTO INPUT** terminal. The outer shell of this connector should be connected to your generator's ground. It is not connected to the **260** instrument case.

To use the instrument in this way:

- 1. First place the channel in the **AUTO** mode.
- 2. Place the **OUTPUT** switch in the **ENABLE** position.
- 3. Current amplitude and polarity should be preset as described in "Single Channel Operation" on page 3.
- 4. Upon application of the +5V command potential to the **AUTO INPUT** terminal, the preset current flows from the output terminals for as long as the command potential is maintained.

NOTE: If the instrument is in the **MANUAL** mode, the application of the **AUTO INPUT** signal halts the current for as long as this command is maintained. This is the complement of normal **AUTO** operation.

Compliance Alarms

Each current generator channel contains an audible and visual alarm circuit that is activated when the current amplitude is large enough to cause the voltage compliance to be exceeded. In other words, the load resistance is too high for the amount of current flowing. It lets you know that the instrument cannot deliver the amount of current indicated on the panel ammeter.

The alarm is also triggered if the output path is open-circuited by a loose connection, air bubble or a blocked micropipette. The initial compliance voltage exceeds 100V but will decrease proportionately as the battery voltage falls with age.

MAINTENANCE

Battery Replacement

Each channel contains two 9-volt transistor batteries. These should be replaced when you can no longer get full scale deflection of the ammeter as described in "Preliminary Instrument Check" on page 3. The instrument will continue to operate satisfactorily for some time after this condition is observed.

- 1. Turn off the power switch for each channel.
- 2. Remove the cover of the instrument by unscrewing the four screws, two on each side, which secure the instrument cover.
- 3. The batteries are mounted in pairs and are held to the printed circuit board by a spring clip assembly. Observe the male and female connectors on the battery mounts, and insert the new batteries so that the connectors mate correctly. Any 9-volt battery will perform satisfactorily, but WPI recommends Duracell 9V alkaline batteries or their equivalent, because of their superior life expectancy and their resistance to leakage.

NOTE: Normal carbon-zinc cells should be replaced when needed or every six months. Alkaline cells should be replaced when needed or every twelve months. To avoid potential problems, replace all the batteries at the same time with batteries of the same brand and lot number.

ACCESSORIES

SPECIFICATIONS

This unit conforms to the following specifications:	
Current Range0 to 100 nar	noamperes 0 to 1000 nanoamperes
Maximum Output Voltage (compliance)	100 volts
Operating Modes	Manual or Auto (external control)
Auto Input	
(external control for current on/off)	+5 volts
Auto Input Resistance	1.5 KOhms, approx.
Auto Input Delay	< 100 microseconds
Output Polarity NORMAL	red terminal, positive
Current Meter Error	+/- 2% max.
Power	
Measurement Circuitry	Four 9V alkaline cells (2/channel)
Measurement Selection	Wall adapter, 5V DC at 300mA*

^{*}Units manufactured before 2015 did not require the 5V external power supply.

APPENDIX A: APPLICATION NOTES

Current Injection in Microiontophoresis

This applications note addresses itself primarily to electrical circuit considerations and is a brief introduction to the fundamentals of WPI's instrumentation for the injection of ions from glass micropipette electrodes.

Constant Current

To pass ions through an electrolyte filled micropipette, a source of current is necessary. Since the current-voltage characteristics of small tipped micropipette electrodes are generally not linear or constant with time and exhibit tip junction potentials, it is necessary to use a large DC potential in series with a very large resistor to generate a current as shown in Fig. 2 The resulting current flow is

$$I = (E + junction P.D.) / (R + R_e)$$

If E is large and R > R_e then the current I = E/R is said to be constant. That is to say, it is independent of the electrode's resistance. The driving potential E is called the "compliance" voltage or the maximum voltage available to force current through the electrode-solution pathway. The compliance is the voltage to which the current generator's terminals rises if the electrode is lifted from the solution or if the electrode resistance is very much larger than R.

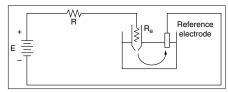


Fig. 2 A basic setup

The basic circuit of Fig. 2 can be elaborated, as shown in Fig. 3, to yield a practical working circuit for the phoresis, for example, of cations.

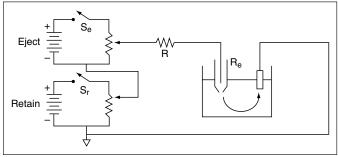


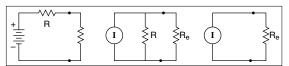
Fig. 3 A practical working circuit for phoresis of cations

When the switch S_r is closed, a small "holding" or "braking" current flows through the electrode to prevent diffusion or hydrostatic pressure from allowing the cations to escape outside the microelectrode.

NOTE: An undesirable consequence of this procedure is that the tip of the micropipette does experience an inflow of some of the external electrolyte, thus diluting the contents of the pipette near the tip opening.

When the switch S_e is closed, the net driving potential becomes positive, swamping the smaller retaining potential difference. A cation current results.

It can be shown that the equivalent circuit of Fig. 4, which is a simple version of Fig. 2, is shown in Fig. 5. An "ideal" current generator with infinite source resistance generates a current of E/R amperes. Further, if $R > R_{\rm e'}$, Fig. 5 can be simply represented by Fig. 6.



- Fig. 4 (Left) A simplified drawing of the circuit in Fig. 2
- *Fig.* 5 (Center) This is the same drawing represented a different way.
- Fig. 6 (Right) If R > Rww in the center drawing, it may be show this way.

The concept of an ideal current generator is useful. Practical electronic circuits have been constructed using photocells and transistors to achieve current sources which are virtually independent of electrode resistance. WPI has used its own field proven solid state constant current generators for years. The actual circuit details are omitted here but the manner in which they are used is suggested by Fig. 7. The current sources are arranged in a paired parallel combination. A switch changes the current from I, (hold) to I_o (eject).

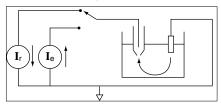


Fig. 7 Simple circuit drawing of an ideal current generator

Current Return

Up to this point, we have assumed that the resistance to the ground terminal of the external solution is negligible. The current return path through the solution for one or more current passing electrodes includes the sum of all currents flowing from the electrodes to ground, as shown in Fig. 8.

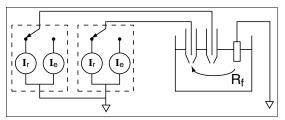


Fig. 8 The sum of all the currents flowing from electrodes to ground equals the total current.

As shown, the current generators are "single-ended," that is the current return path is via the ground pathway. Some applications cannot tolerate the resulting potential at the current electrode site owing to the $\rm I_r$ voltage drop across the fluid resistance $\rm R_r$. This current flow to ground is found undesirable in some cases because:

- The voltage of cells being measured near the current release site may include an error potential owing to the current induced local potential.
- The current may be large enough to cause local stimulation of excitable membrane.

To prevent the flow of current from the phoresing electrodes to ground, it is necessary to introduce a common or current return electrode which will "sink" or absorb all of the current locally generated by ionophoresing electrodes. As shown in Fig. 9 the effect will be to maintain the site of the multi-current electrodes a net zero potential with respect to the ground electrode.

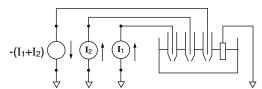


Fig. 9 Create a sink to prevent the flow of current from the phoresing electrodes from going to ground.

WPI's floating current generators are differential as shown in Fig. 10. This means that if the common terminals of the current generators are all connected to the return electrode, the net ground current is zero. If the generation of local potentials is negligible, then we may connect WPI's floating current source as a ground referred current source. Then, these generators can be used as ground referred or floating current sources.

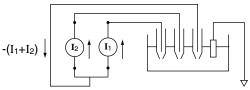


Fig. 10 The net ground current is zero.

Measuring Current

WPI's floating, battery-operated current generator contains a built in ammeter. External electrodes may be disconnected by operating a PRESET switch. This allows you to preset the desired levels of eject and retain currents while observing the ammeter. Current may also be measured by two other techniques as shown in Fig. 11. As shown in Fig. 11 (left side) the normally grounded side of the current generator pair is connected to ground through a known resistance. A recorder or oscilloscope may then monitor the potential drop across this resistor as a measure of the current. An alternative method shown in Fig. 11 (right side) replaces the preparation ground return with a "current to voltage" converter. This instrument maintains the ground (zero) potential and simultaneously displays a voltage at its output proportional to current flow.

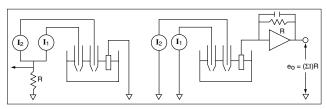


Fig. 11 Two methods of measuring the current

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Krnjevic, K., Mitchell, J.F. and Szerb, J.C., Determination of lontophoretic Release of Acetylcholine from Micropipettes, J. Physiology, London, 165 1963, pp 421-436.

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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.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

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 ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. 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

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

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

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