PV830 Pneumatic PicoPump

Air pressure microinjection of material into cells or vessels

INSTRUCTION MANUAL

Serial No._____

111505

World Precision Instruments, Inc.

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Introduction

General

The PV830 is designed to inject very small quantities of fluids, such as drugs, into cells or small organelles. Pressure injection is an especially useful alternative to electroionophoresis since it does not mandate the use of charged ions. Two different positive pressures may be applied; one for ejection at high pressure and a second, lower pressure to prevent back filling of the pipette by capillary action or diffusion. Vacuum may also be applied to hold cells or small objects and to load pipettes from the tip. Thus cells may be held by vacuum and simultaneously injected using pressure.

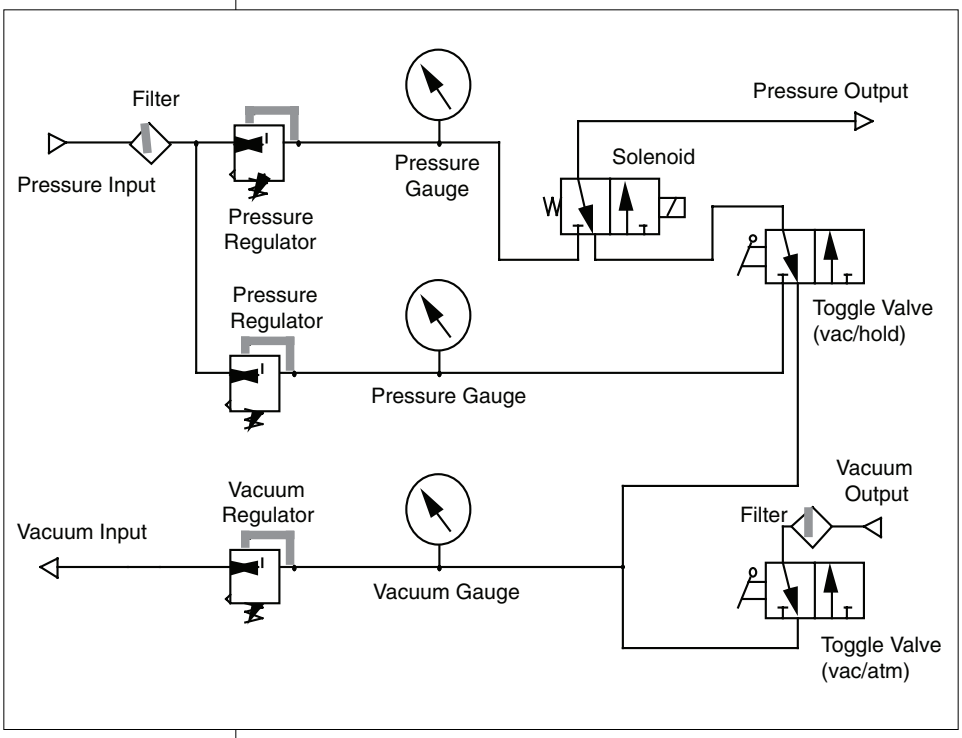


Figure 1

A pneumatic block diagram of the PicoPump is shown in Figure 1. Both pressure lines (Hold and Eject) and the vacuum line pass through precision regulators and are monitored by measuring gauges. A fast solenoid valve controls the application of the Eject pressure to the Pressure Port. When the solenoid is inactive, the Pressure Port is connected to the Hold pressure. Alternatively, the solenoid can be vented to the vacuum/atmosphere line, depending upon the position of the Vac/Hold toggle switch. This feature allows the pressure ejection channel to have a mild sucking action when pressure is not being applied. A manual pneumatic switch also allows the vacuum/ atmosphere line to be used independently of the solenoid-controlled Pressure line.

Quantitative control over the amount of fluid injected is attained by adjusting the pressure and the duration of the pressure pulse. For dispensing aliquots of as little as tens of picoliters

through micron-sized pipette tips, pressures of 10 to 100 psi are used in pulses as brief as 10 ms. Longer pulses, higher pressures and larger pipette tip diameters give correspondingly greater amounts of fluid. For convenience, the pressure is usually kept constant throughout a series of experiments and the pulse width is varied to give different amounts of injected fluid.

Safety

When using a delivery micropipette, secure the pipette firmly. When high pressure is applied, a loose pipette can be ejected forcefully. Do not apply pressures in excess of 150 psi (1000 kPa). Use dry air, nitrogen or other inert gases only.

This instrument is for investigational use only in animals or other tests that do not involve human subjects.

Specifications

Pressure

Pressure Input (rear)	150 psi (1000 kPa) maximum
Pressure Output	0 to 90 psi (600 kPa) *
Lowest Regulated Pressure	12 in. Water (3 kPa) *
Regulator Accuracy	0.1% (20-turn dial) *
Regulator Repeatability	0.05 psi (0.3 kPa) *
Gauge Accuracy	3% of full scale *
Input Connector	Quick Connect (for 1/4 in. OD Tubing)
Output Connector	Barbed (for 1/16 in. ID Tubing)
Control	Solenoid
Vent	To holding pressure or vac/atm by manual control

** Both Hold and Eject Pressures*

Vacuum

Vacuum Input (rear)	0 to 30.0 in. Hg (101 kPa)
Vacuum Output	0 to 29.9 in. Hg (101 kPa)
Lowest Regulated Vacuum	3 in. water (0.7 kPa)
Regulator Accuracy	0.1% (20-turn dial)
Regulator Repeatability	0.03 in. Hg (0.1 kPa)
Gauge Accuracy	3% at full scale
Input Connector	Quick Connect (for 1/4 in. OD Tubing)
Output Connector	Barbed (for 1/16 in. ID Tubing)
Control	Manual
Vent	Vacuum or Atmosphere

Timing

Duration Modes	Gated (by input signal) or Timed (by internal clock)
Pulse Initiation	Manual, External Input or Foot Switch (optional)
Pulse Width	10 ms to 10 s in Timed Mode (10-turn dial) Lower limit depends upon pressure setting. At 0 psi the minimum pulse width is nominally 8 ms. At 100 psi the minimum pulse width is nominally 3 ms.
Accuracy	0.1% at full scale
External Input	+5 V TTL-compatible (BNC Connector)
	100 μs minimum pulse width
Monitor Output	+5 V TTL-compatible (BNC Connector)

Physical Specifications

Power	95-130 VAC or 190-260 VAC, switch-selectable, single-phase, 50/60 Hz, 20VA
Temperature	10°C (50°F) to 40°C (104°F)
Dimensions	3.5 x 17 x 9.5 in. (89 x 432 x 242 mm) Mountable in standard ANSI/EIA RS310C 19 in. rack.

Operating Directions

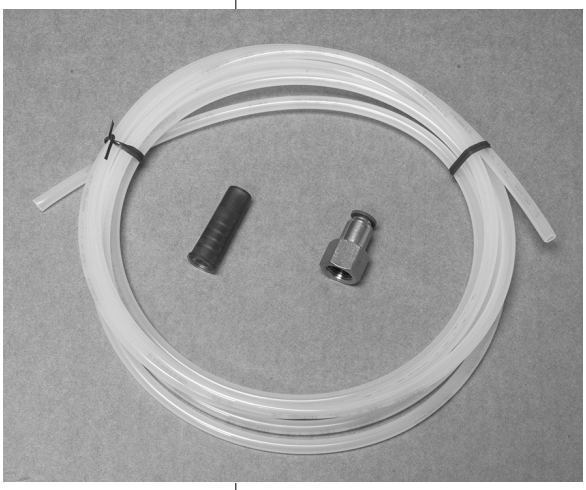


Figure 2 *— PicoPump Startup Kit*

Startup Kit (#3316)

10 ft hard pressure tubing, 1/4-in. OD. (Cut in half to make pressure and vacuum lines.)

Threaded NPT adapter for nitrogen tank regulator.

Soft plastic adapter for barbed vacuum connectors.

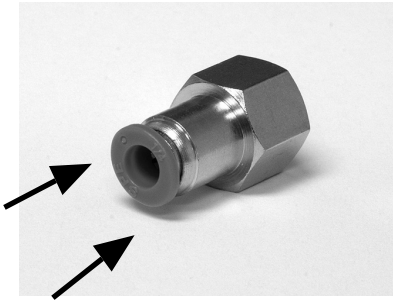


Figure 3 *— Threaded fitting for nitrogen regulator (depress plastic collar to remove tubing)*

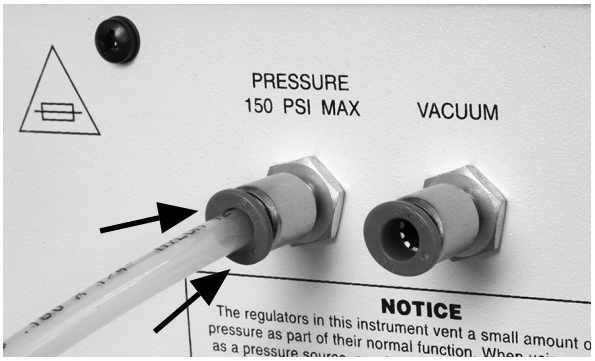


Figure 4 *— PicoPump pressure & vacuum ports: Push hard tubing into port until the tubing engages and cannot be pulled out. To remove tubing, press in on the plastic collar.* Do not remove tubing holders — these parts are intended to remain on the pump.

Setup

Before beginning setup note the quick-connect input fittings on the rear of the instrument. Connection is made by firmly inserting tubing into the fitting. To remove tubing, press in on the surrounding collar while pulling the tubing out. Plastic tubing can be disconnected with little effort, while metal tubing may require the expenditure of more effort. A block diagram of the experimental setup is shown in Figure 5.

Vacuum Input

Connect the VACUUM INPUT (located at the back of the instrument) to a suitable source of vacuum, such as a vacuum pump (see WPI #**LV140**) or aspirator, using 1/4-in. OD hard

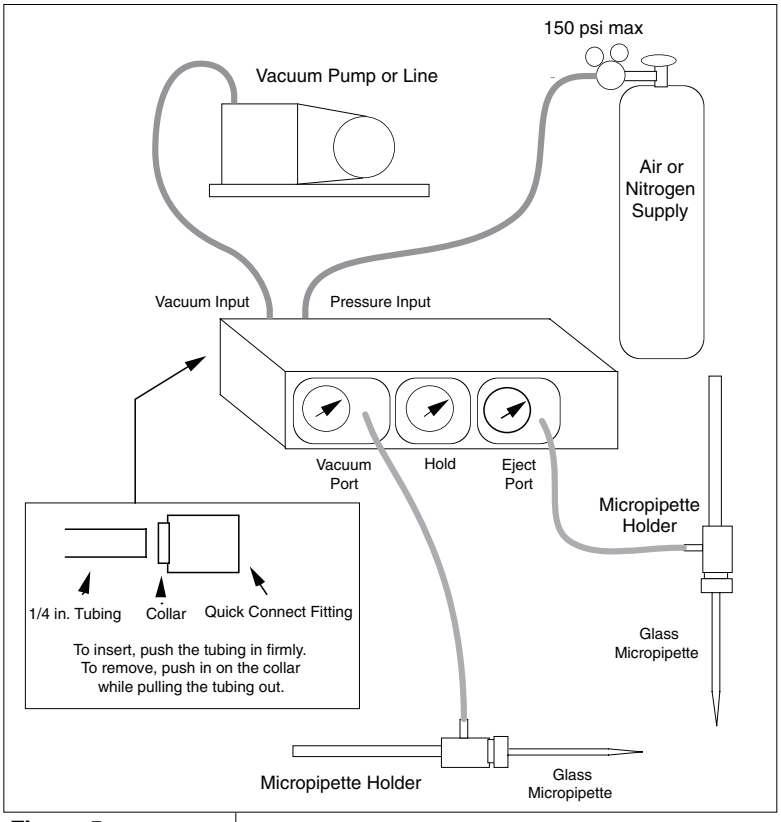


Figure 5

tubing and the soft plastic connector (supplied). Vacuum may be anywhere in the range of 0 to 30 in. Hg. The vacuum line need not be connected if only positive pressure is needed.

Pressure Input

Connect the PRESSURE INPUT (located at the back of the instrument) to a suitable source of pressure, such as a compressed gas tank or an air line, using 1/4-in.OD tubing supplied. Pressure maybe anywhere in the range of 0 to 150 psi. Connect this 1/4-in. OD tubing to the quick connect fitting as indicated in Figure 5.

A 1/4-in. FNPT fitting is supplied for connection to a gas tank. Recommended gases are dry air, nitrogen or argon. Never use corrosive gases. If an air pressure line containing oil or water vapor is used, an external filter is recommended to prevent excessive contamination of the internal pneumatic components.

WARNING: The precision regulators used in this instrument continuously vent a small amount of supply pressure as a part of their normal function. To prevent waste of gas, always turn off the main supply pressure when the PV800 is not in use.

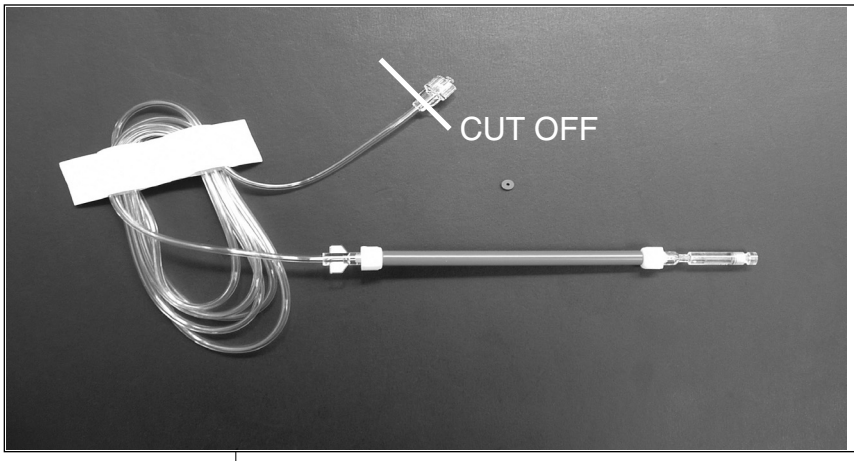
Eject Pressure Port (front panel)

Each PicoPump is supplied with two PicoNozzle Kits plus tubing to conect the holders to the pressure and vacuum ports. Use one kit for pressure, one for vacuum.

***PicoNozzle Version 1* (#5430-xx)**

The microelectrode holder is equipped with a female luer to attach to one end of the 4-in. male/male luer lock adapter. The 5-ft tubing also has a female luer at one end to attach to the opposite end of the 4-in. luer lock adapter. ***The male luer fitting of the 5-ft tubing must be cut off in order to attach it to the pressure port of the PicoPump.***

To mount the micropipette, pulled capillary glass may be inserted in the holder; a screw cap



PicoNozzle Version 1 (#5430-XX)

• 4-in. micromanipulator tube with male luer fittings

• 5-ft tubing, 1/16-in. ID, male and female luer lock connectors (male fitting to be removed for use with PicoPump)

• Holders for 1.0 mm, 1.2 mm, 1.5 mm, and 2.0 mm pipettes (two each)

Figure 6

allows the glass micropipette to be firmly held by a rubber gasket. The luer fittings make changing micropipettes easy by allowing quick removal of the pipette holder from the 4-in. luer lock adapter. Test to make sure the micropipette is firmly held by pulling on it. A firmer hold on the glass can be achieved by using two gaskets in the micropipette holder.

Danger of injury exists if the pipette is insecure. High pressure can cause ejection at extreme velocities.

Figure 7

Long lengths of tubing will tend to decrease the time response of the system due to a loading effect of the increased volume. For fastest possible response, keep the tubing length (and diameter) to the smallest possible value.

PicoNozzle Version 2 (#5430-ALL)

• PicoNozzle tip assembly

• 4-ft Superethane tubing, 1/16-in. ID

• Gaskets for 1.0 mm (green), 1.2 mm (black), 1.5 mm (blue), and 1.65 mm (red) pipettes — two each.

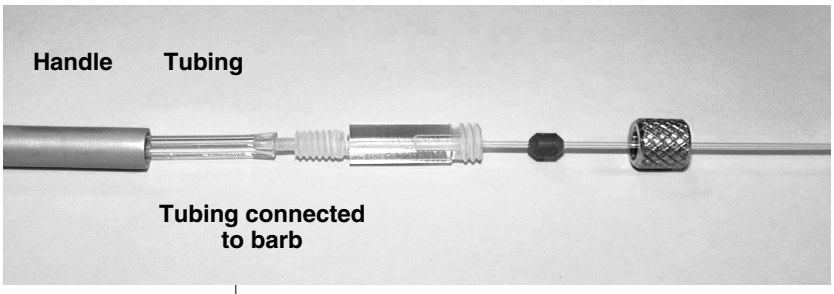


Figure 8

PicoNozzle Version 2 (#5430-ALL)

Before use, the superthane tubing must be slid through the handle and connected to the barb of the body. Be careful so that the barb does not break when attaching or removing the tubing.

Before inserting the pipette, place the correct size gasket must be placed in the cap. gaskets for 1 mm (green), 1.2 mm (black), 1.5 mm (blue), and 1.65 mm (Red) sized pipettes.

Danger of injury exists if the pipette is insecure. High pressure can cause ejection at extreme velocities.

Long lengths of tubing will tend to decrease the time response of the system due to a loading effect of the increased volume. For fastest possible response, keep the tubing length (and diameter) to the smallest possible value.

Vacuum Port

If this channel is being used, connect the other PicoNozzle Kit to it exactly as described above.

Note: Capillary holders can be obtained from WPI which contain Ag/AgCl half-cells. These holders can be easily mounted upon amplifier headstage probes (see WPI catalog) so that potential and/or current, as well as pressure, can be measured or dispensed through the capillary tip.

Instructions for Use

Pressure Adjustment

Pressure is adjusted by turning the REGULATOR knobs located next to the pressure gauges. Turning the knob clockwise increases the pressure, and counter- clockwise decreases the pressure. Because the regulating mechanism is self-venting, pressure is automatically released when the regulator setting is decreased. Although the pressure gauges cannot be read with high accuracy (especially at pressure settings below 2 psi), very reproducible pressures may be obtained because the pressure regulators have a 20-turn dial yielding good pressure resolution.

The pressure regulating mechanism functions by continuously bleeding a small amount of gas. This bleed rate increases as the difference between the input pressure and the output pressure increases. Loss of gas may be minimized by decreasing the difference between the input and output pressures, but some decrease in regulation may be noticed if the input pressure is not at least 10% greater than the output pressure. **WARNING:** To conserve tank contents, make sure to turn off the gas supply pressure when the PV830 is not in use.

Vent

The vac/hold switch selects between venting the Eject pressure port to either the Hold pressure (hold) or to the vac/atm line (vac). If the Vacuum line is not in use and the switch is set to vac, the pressure port is vented to atmosphere.

Vacuum Adjustment

Vacuum is adjusted by turning the vacuum REGULATOR knob located next to the vacuum gauge. Turning the knob clockwise increases the vacuum (lower pressure), and counter-clockwise decreases the vacuum. Because the regulating mechanism is self-venting, vacuum is automatically released when the regulator setting is decreased. Although the vacuum gauge cannot be read accurately at very low vacuum settings (below 2 in. Hg), reproducible settings may still be obtained by means of the 20-turn dial regulator knob.

Vacuum

When the Vacuum Switch is in the vac position, the vacuum output port is connected to the regulated vacuum line. When the switch is in the atm postion, the output port is vented to the atmosphere.

Power

Switching the POWER on allows the solenoid of the Eject Pressure line to be activated. An amber light above the switch indicates when power is on.

Timing

Electronic timing of the Eject pressure solenoid is effected by the control section on the right hand side of the instrument front panel. Operation of the Eject pressure solenoid can be initiated by means of any of three different methods: by manually pushing the START button, by applying a +5 volt level at the EXT INPUT, or by depressing the optional REMOTE footswitch (note that the REMOTE connector is used for accessing the footswitch only). The length of the pulse is determined by the setting of the DURATION switch. In the gated mode the pressure solenoid will open and remain open as long as any of the three commands is maintained. In the timed mode, an electronic timer controls the time duration that the pressure solenoid stays open and the three commands serve only to start the timer. Long pulses in the timed mode may be aborted by pressing the STOP button. A green lamp next to the Eject pressure gauge lights when the pressure solenoid is open (energized).

Period and Range

The duration of the solenoid open time, in the timed mode, is determined by the 10-turn PERIOD dial and the setting of the RANGE switch. In the 100 ms range, solenoid times may be set from 1 ms to 101 ms (every turn of the dial is 10 ms) and in the 10 s range pulses may be set from 100 ms to 10.1 s (every turn of the dial is 1.0 s). The minimum time interval is limited by the speed of the solenoid, which varies from approximately 10 ms at 0 psi to 3 ms at 100 psi.

Monitor

The MONITOR output connector produces a logic-level output (+5 V) corresponding to the time interval during which the Eject pressure solenoid is energized. At all other times the monitor output is low (0 V).

Rear Panel

A polarized, 3-conductor, connector is used for line (mains) power input to the instrument. A removable cordset, terminated with a grounded 3-prong connector, is standard. An alternate cordset may be supplied when local circumstances dictate different mains voltages and connections.

A fuseholder contains a protective fuse in series with the high side (brown or black wire) of the mains. The holder accepts 1/4 by 1-1/4 inch (6.35mm by 31.8mm) fuses of the type indicated on the rear panel.

Techniques In Microinjection

General

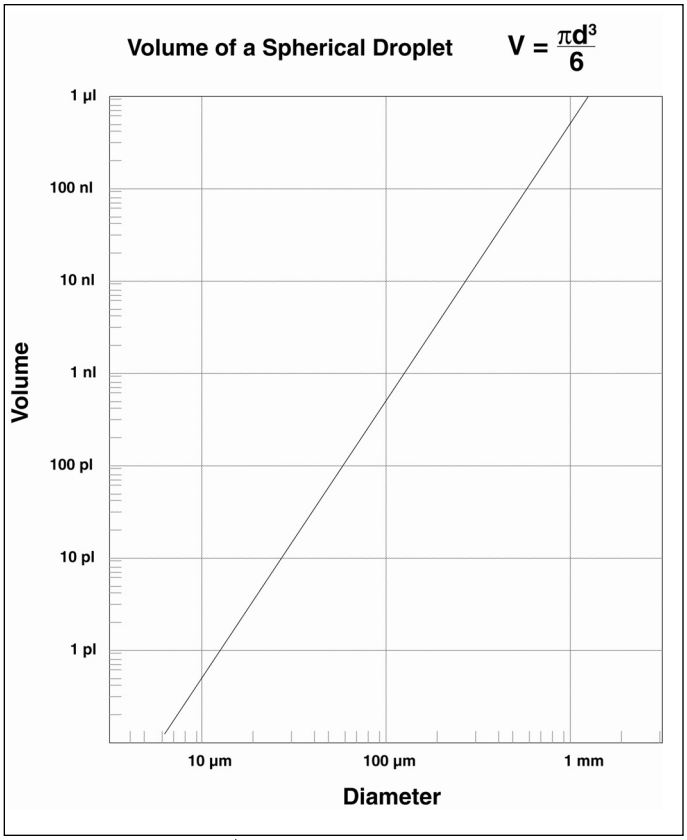


Figure 9

One of the more demanding tasks for which the PicoPump has been designed is the microinjection of fluid into cells. Difficulties encountered will not usually involve the mechanics of the PicoPump, but rather the manufacture of suitable micropipettes. Both care and good hands are needed in making pipettes to take full advantage of the features of the PicoPump. Here we list several important things to keep in mind when working with the PicoPump.

Setting the Hold Pressure

Pressure settings for both the Hold and Eject channels will depend greatly upon the tip size of the micropipette to be used. To set the Hold pressure, i.e. the pressure necessary to prevent the backfilling of the pipette by capillary action, the pipette tip must be inspected visually. When an empty pipette is inserted into fluid, a meniscus can be seen to rise up the capillary tip. The Hold pressure may be set by adjusting the regulator setting (with the hold/vent switch in the hold position) until the meniscus reaches the very tip of the pipette. Alternatively, if the pipette is filled with a small quantity of fluid colored with a dye, the meniscus between the uncolored and colored solutions may be observed.

For tip sizes of 10 to 50 µm (tips broken with fine forceps) typical Hold pressures range from 5 to 10 psi. For smaller tips, 1 to 10 µm, Holding pressures may be as high as 20 to 50 psi. These smaller tips may be conveniently prepared by inserting an unbroken tip into a ball of cotton and then removing the pipette. It should be noted that the high Hold pressures counterbalance capillary action only at the very tip of the electrode, and may be unnecessary if the small volume contained at the tip is not important to the experiment.

Micropipette Manufacture

The volume of fluid ejected is markedly dependent on the micropipette tip size. When using micron-sized tips a reduction in tip-size of a few percent may give an order of magnitude difference in the flow rate. With tip sizes less than 1 μm, pressure ejection becomes increasingly difficult and special steps must be taken.

The most important of these steps is cleaning the glass. Small amounts of dust or grease can easily clog micron-sized tips. Cleaning with chromic acid solutions before pulling the electrode is commonly performed, but care must be taken to thoroughly rinse the pipettes to remove all traces of the chromic acid, which has some affinity for glass. Some researchers prefer hydrochloric or nitric acid.

Silanization of the glass is also recommended for small tips. With 1 μm and smaller tips capillary action becomes prohibitively large, and the hydrophilic surface of the glass greatly limits the flow of fluid through the tip. Silanization decreases the surface tension and allows the fluid to flow smoothly through the tip. For similar reasons use of capillary with an internal filament is contraindicated. Some of the many papers on the art of silanization are listed in the bibliography.

When using the vacuum line to hold and manipulate individual cells a large tip (about 10-20% of cell size) is recommended. To prevent damage to the cell this tip should be fire-polished.

Volume Calibration

For ejected volumes greater than 1 nanoliter visual inspection using a microscope can be an accurate gauge of volume. A single pulse will deposit a drop of fluid on the tip of the micropipette. The volume of this drop may be calculated by measuring the radius of the drop and assuming the drop to be spherical. Figure 3 may be helpful in determining the volume for a given radius. The following table is useful for converting between different units of volume.

Cubic Measure	Volume
1 cm^3	1 mL
1 mm^3	1 μL
(100 μm)^3	1 nL
(10 μm)^3	1 pL (10^{-12} L)
1 μm^3	1 fL (10^{-15} L)

For ejected volumes less than 1 nanoliter, visual inspection in air proves to be difficult due to rapid evaporation. The same technique may be used though if the drop is kept submerged under oil. Droplets may seem to disappear after emergence from the tip. Sometimes this is due to creepage of the aqueous fluid back along the outside shank of the micropipette. This

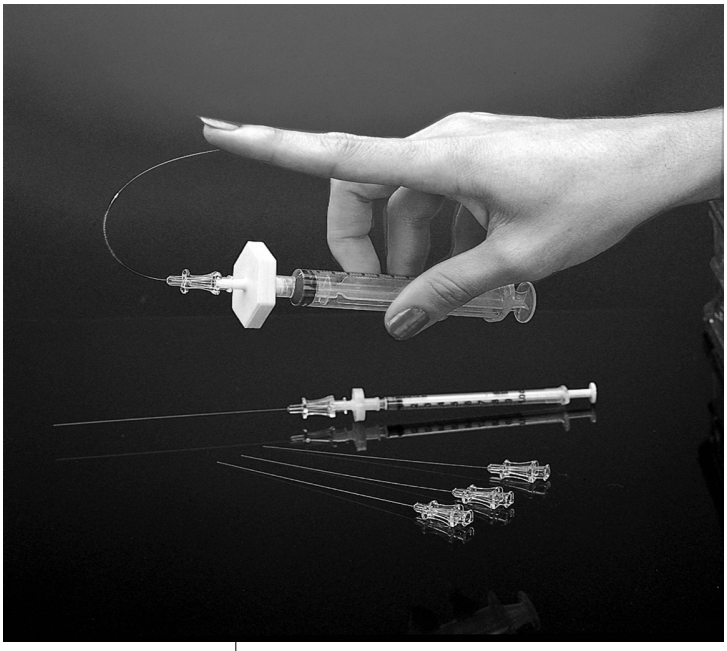


Figure 10 *— WPI's* ***MicroFil™*** *is helpful in backfilling glass pipette tips. Syringe filters (available separately) help prevent clogged micropipette tips.*

creepage may be decreased by silanizing the outside of the pipette. If it is desired to silanize only the outside of the pipette, the PV830 may be used to good advantage by applying air flow through the pipette during the silanization.

Precise assays of ejected volume may also be obtained through various radioisotopic methods. See the bibliography for further information.

A slight deflection of the micropipette tip may be noticed during the application of the pressure pulse. This deflection may be eliminated by ensuring that the micropipette is firmly seated in the holder (two gaskets may be necessary) and that the holder is firmly held by a manipulator.

When pumping electrolytes, some indication of flow may be obtained by monitoring the resistance or the voltage of the micropipette. If, for example, the micropipette is filled with 3 M KCl and the sample fluid is 0.1 M KCl, a significant decrease in resistance of the micropipette will occur when pressure is applied. This happens because the resistance measured is determined primarily by the conductivity of the solution at and within the tip. As pressure is applied the 0.1 M KCl surrounding the tip is replaced by the more highly conductive 3 M KCl, decreasing the measured resistance. Likewise, if vacuum is applied, the resistance will increase as the less conductive 0.1 M KCl fills the tip.

Note: The above technique will not work with exterior and interior solutions of the same concentration. However a voltage change can be measured as a function of the pressure applied. Working with tip sizes of 1 to 2 microns, we have seen a change in measured voltage of approximately 1 mV for every 10 psi of applied pressure.

Multibarrel Microinjection

For injection with multibarrel micropipette, the **PolyFil** multibarrel micropipette coupling kit can be purchased from World Precision Instruments. This multibarrel micropipette coupling kit allows easy and secure coupling of a multi-barrel micropipette to a pressure source. Kits include a five-port manifold which allows use of a single PV800 to drive up to six micropipette barrels independently.

Maintenance

General

The PicoPump has been designed to yield reliable performance. However particular laboratory conditions may require occasional replacement of the pressure and vacuum filters. Should this become necessary, return the instrument to the factory for filter replacement.

Cleaning

Do not use alcohol, aromatic hydrocarbons or chlorinated solvents for cleaning. They may adversely react with plastic materials used to manufacture the instrument.

The exterior of this instrument may be cleaned periodically to remove dust, grease and other contamination. There is no need to clean the inside. Use a soft cloth dampened with a mild solution of detergent and water. Do not use abrasive cleaners.

Optional Accessories

3260	Foot Switch
2932	Rack Mount Kit, 3½-in. high (PV800 & PV820)
2933	Rack Mount Kit, 5¼-in. high (PV830)
5430-10	PicoNozzle 1 Kit (MPH6S for 1.0 mm pipette & 5-ft tubing assembly)
5430-12	PicoNozzle 1 Kit (MPH6S for 1.2 mm pipette & 5-ft tubing assembly)
5430-15	PicoNozzle 1 Kit (MPH6S for 1.5 mm pipette & 5-ft tubing assembly)
5430-20	PicoNozzle 1 Kit (MPH6S for 2.0 mm pipette & 5-ft tubing assembly)
5430-ALL	PicoNozzle 2 Kit
MPH6S	Micropipette Holder (specify 1.0, 1.2, 1.5 or 2.0 mm)
MPH6R	Micropipette Holder (specify 1.0, 1.2, 1.5 or 2.0 mm)
3316	Replacement Input Kit
LV140-Y	Vacuum Pump (120V)
LV140-Z	Vacuum Pump (240V)

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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 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.

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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: PV800/PV820/PV830

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.

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