



PV830

Pneumatic PicoPump

INSTRUCTION MANUAL

Serial No. _____

042712

World Precision Instruments

www.wpiinc.com

CONTENTS

| | |
|---|----|
| ABOUT THIS MANUAL | 1 |
| INTRODUCTION | 1 |
| General | 1 |
| Notes and Warnings | 1 |
| Unpacking | 2 |
| INSTRUMENT DESCRIPTION | 3 |
| Setup | 5 |
| Vacuum Input | 6 |
| Pressure Input | 6 |
| Eject Pressure Port (front panel) | 6 |
| Vacuum Port | 8 |
| OPERATING INSTRUCTIONS | 9 |
| Front Panel | 9 |
| Rear Panel | 10 |
| Techniques In Microinjection | 10 |
| Setting the Hold Pressure | 11 |
| Micropipette Manufacture | 12 |
| Volume Calibration | 12 |
| Multibarrel Microinjection | 14 |
| MAINTENANCE | 14 |
| Cleaning | 14 |
| ACCESSORIES | 14 |
| Replacement Parts | 15 |
| TROUBLESHOOTING | 15 |
| SPECIFICATIONS | 16 |
| Pressure | 16 |
| Vacuum | 16 |
| Timing | 16 |
| Physical Specifications | 16 |
| BIBLIOGRAPHY | 17 |
| DECLARATION OF CONFORMITY | 18 |
| WARRANTY | 19 |
| Claims and Returns | 19 |
| Repairs | 19 |

Copyright © 2012 by World Precision Instruments, Inc. All rights reserved. No part of this publication may be reproduced or translated into any language, in any form, without prior written permission of World Precision Instruments, Inc.

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.

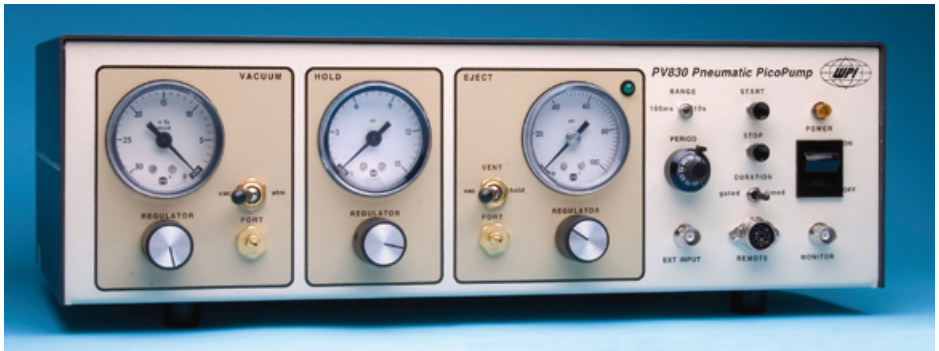


Fig. 1—PV830 Pneumatic PicoPump

INTRODUCTION

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. Cells may be held by vacuum and simultaneously injected using pressure.

Notes and Warnings



WARNING: 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 150PSI (1000KPA). USE DRY AIR, NITROGEN OR OTHER INERT GASES ONLY.



WARNING: THIS INSTRUMENT IS FOR INVESTIGATIONAL USE ONLY IN ANIMALS OR OTHER TESTS THAT DO NOT INVOLVE HUMAN SUBJECTS.

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 23 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 23 of this manual.

INSTRUMENT DESCRIPTION

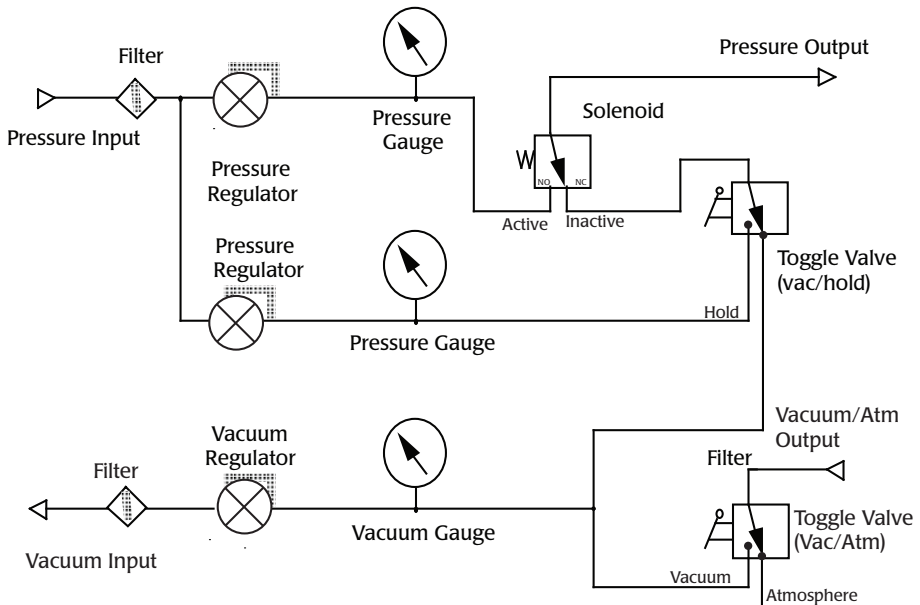


Fig. 2—The pneumatic block diagram of the PicoPump is shown.

Both pressure lines (**Hold** and **Eject**) and the vacuum line pass through precision regulators and are monitored by measuring gauges (Fig. 2). 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 100PSI are used in pulses as brief as 10ms. 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.

The Startup Kit (WPI #3316) includes:

- (1) 0.25" NPT Fitting for nitrogen tank regulators
- (2) 0.25" quick connect fit barbs (use on vacuum side, where applicable)
- (1) 5' hard tubing



Fig. 3—(Left) The PV830 Startup kit includes tubing and several fittings.

Fig. 4—(Right) The threaded fitting is for a nitrogen regulator. Depress the plastic collar to remove tubing.



Fig. 5—The PicoPump Pressure and Vacuum ports are located on the rear panel of the PV830.

To install the hard tubing, push it into the 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

Firmly insert tubing into the quick-connect fittings on the rear of the instrument. To remove tubing, press in on the surrounding collar while pulling the tubing out. Plastic tubing can be disconnected easily, while metal tubing may require more effort. For a diagram of the setup, see Fig. 6.

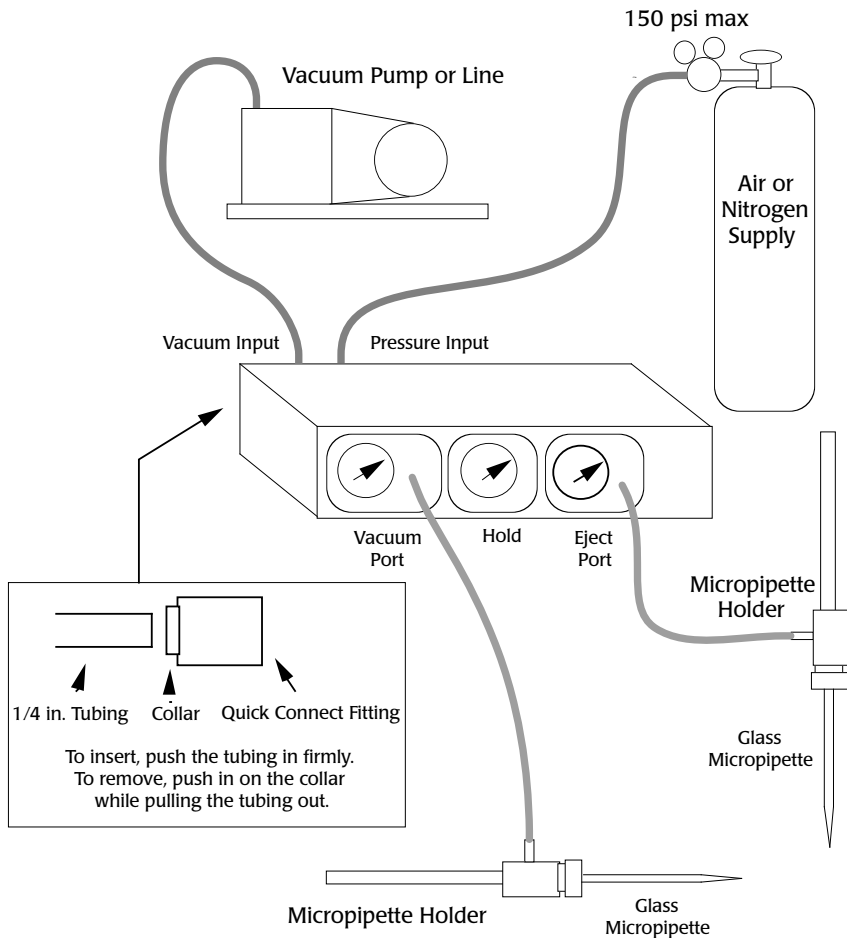


Fig. 6—This diagram show the experimental setup.

Vacuum Input

Connect the **Vacuum Input** (located at the back of the instrument) to a suitable source of vacuum, such as a vacuum pump (WPI #LM-500865) or aspirator, using 0.25" OD hard tubing and the soft plastic connector (supplied). Vacuum may be anywhere in the range of 0–30 in. Hg. The vacuum line does not need to 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 0.25" OD tubing supplied. Pressure may be anywhere in the range of 0–150PSI. Connect this 0.25" OD tubing to the quick connect fitting as indicated in Fig. 6.

A 0.25" FNPT fitting is supplied for connection to a nitrogen regulator on a N₂ 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 PV830 IS NOT IN USE.

Eject Pressure Port (front panel)

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

Version 1 Kit (Optional)

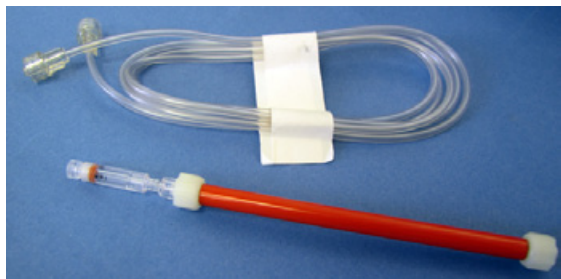


Fig. 7—PicoNozzle Version 1 Kit (#5430-xx) allows micropipettes to be securely mounted in micropositioners for stable axial air delivery. Because air enters the pipette axially, lateral whipping during injection is eliminated.

This kit contains:

- (1) MPH6S microelectrode holder
- (1) Handle for the MPH6S (4" hollow tube with male Luer fitting at both ends-handle diameter is 6.25 x 100mm.)
- (1) 5' tubing (0.060" ID, 0.120" OD, male locking Luer fitting on one end and a female locking Luer fitting at the other end, rated for 200 PSI and 86 durometer shore A)

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

When using a **MPH6S** handle, a firmer hold on the glass can be achieved by using two gaskets in the micropipette holder.

NOTE: Capillary holders can be obtained from WPI which contain Ag/AgCl half-cells. These holders (**MPH6S**, **MPH6R**) can be easily mounted upon amplifier headstage probes so that potential and/or current, as well as pressure, can be measured or dispensed through the capillary tip.

To mount the micropipette, pulled capillary glass may be inserted in the holder. A screw cap 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" luer lock adapter. Test to make sure the micropipette is firmly held by pulling on it.

Version 2 Kit (Included)

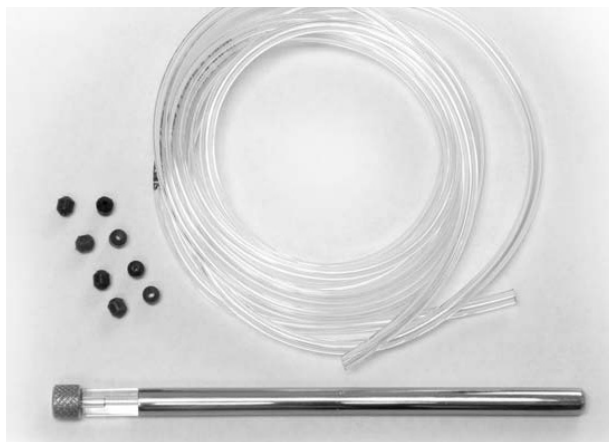


Fig. 8—Each PicoPump is supplied with a 5430-ALL (Version 2) kit.

This kit contains:

- (2) PicoNozzle tip assemblies (Handle diameter is 6.25 x 100mm.)

- (2) 5' tubing
- (4) 1.0mm pipette gaskets (green)
- (4) 1.2mm pipette gaskets (black)
- (4) 1.5mm pipette gaskets (red)
- (4) 1.65mm pipette gaskets (white)

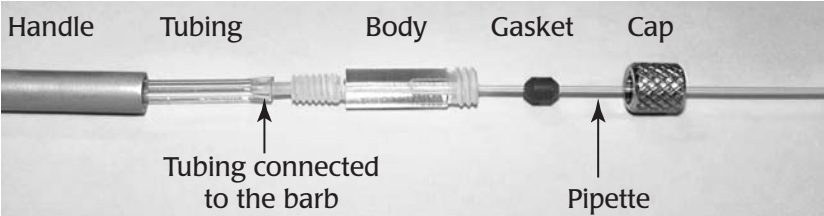


Fig. 9—The 5430-ALL (version 2) must be properly assembled.

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 a gasket of the correct size in the cap.

| Gasket Color | Green | Black | Red | White |
|-----------------------|-------|-------|-----|-------|
| Pipette Diameter (mm) | 1.0 | 1.2 | 1.5 | 1.65 |



WARNING: DANGER OF INJURY EXISTS IF THE PIPETTE IS INSECURE. HIGH PRESSURE CAN CAUSE EJECTION AT HIGH VELOCITY.

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 to be used, connect the other PicoNozzle Kit to it exactly as described above.

OPERATING INSTRUCTIONS

Front Panel



Fig. 10—PV830 Front Panel.



CAUTION: The valves always pass some air. Do NOT apply too much pressure when turning the knobs. They will not reach zero while the **PV830** is running. Over-tightening the knobs will damage the regulators.

Pressure Adjustment—Adjust the pressure by turning the **Regulator** knobs located next to the pressure gauges. Turn the knob clockwise to increase the pressure, and counter-clockwise to decrease it. 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 2PSI), 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.



CAUTION: 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 the 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** position, 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 +5V level at the EXT Input.
- by depressing the optional **Remote** footswitch. (The **Remote** connector is used for accessing the footswitch only).

The length of the pulse is determined by setting the **Duration** switch. In the gated mode the pressure solenoid opens and remains 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 illuminates for as long as 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 100ms range, the solenoid times may be set from 1–101ms (every turn of the dial is 10ms) and in the 10s range pulses may be set from 100ms to 10.1s (every turn of the dial is 1.0s). The minimum time interval is limited by the speed of the solenoid, which varies from approximately 10ms at 0 psi to 3ms 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 (0V).

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 (120V, 0.5A—#6402 or 230V, 0.25A—#6409).

Techniques In Microinjection

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 steady 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

The **Hold** pressure is used to counterbalance the capillary action of the fluid backfilling into the pipettes. When an empty pipette is inserted into the fluid, a meniscus can be seen to rise up from the capillary tip. **Hold** pressure may be set by adjusting the regulator setting (with the **Hold/vent** switch in the **hold** position) until the meniscus stops at the desired position. In many applications, colored dye or fluorescent dye is dissolved in the injection fluid. The capillary effect can be seen with the color change at the tip of the pipette. When the fluid flows into the pipette, the color of the tip becomes lighter. If the hold pressure is higher than the capillary pressure, the fluid oozes out of the pipette. The solution around the pipette will be colored. Adjusting the **Hold** pressure prevents this from happening.

Understanding how capillary action causes the backfilling of the pipette helps you to correctly use the **Hold** pressure. The backfilling flow rate is determined by the pressure of capillary action and the tip size. Since the tip size is often determined by the requirement of the application, controlling the pressure of capillary action becomes the main option to eliminate backfilling. **The pressure of capillary action is determined by the inner diameter of the glass capillary where the meniscus of air/liquid interface is located. It has nothing to do with the pipette tip size, a common misunderstanding.**

If we assume the pipette tip is near a cylindrical shape, the pressure of capillary action can be described by the LaPlace equation:

$$P=4\gamma \cos\theta/d$$

where γ is the surface tension, θ is the contact angle between the water and glass, and d is the inner diameter of the capillary where the meniscus is located. In most cases, we can assume the contact angle for glass and water is zero (unless the glass surface is treated). From this equation we can see that the capillary pressure can vary a thousand times when the meniscus is moved from a 0.5 μ m ID tip to the 0.5mm shank. The pressure at 0.5 μ m tip is about 80 PSI (in aqueous solution) while at the shank will be only 0.08 PSI. Using one regulator to counterbalance the pressure in such a large dynamic range is not practical. The hold regulator in this instrument is optimized to work in the 0.2–10 PSI range. 10 PSI can counterbalance a meniscus at the section of tip where the inner diameter is 4 μ m. In practice, this is the highest pressure ever needed. On the lower pressure end, it becomes difficult to exactly counterbalance the capillary pressure when the meniscus is at the shank of

the pipette. However, a 0.1–0.2 PSI pressure imbalance will not cause a significant problem if the tip is small enough. The gravity of the fluid and the flow resistance caused by friction from the glass wall will both help to stop the solution flow at this pressure level. If the lowest pressure setting still can't stop the fluid from oozing out, try to switch off the hold pressure to see if the gravity and friction are sufficient to counterbalance the backfill. In addition, resetting the regulator could allow the regulator to perform much better in this range (see the "Troubleshooting The Hold Pressure" on page 16).

The capillary action can also be reduced by adding a hydrophobic fluid (such as silicone oil) behind the hydrophilic saline solution. It can be completely eliminated by silanizing the shank of the pipette (silanization increases the θ in the LaPlace equation to 90°).

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 a 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 nL visual inspection using a microscope can be an accurate gauge of volume. A single pulse deposits 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. Fig. 11 may be helpful in determining the volume for a given radius. See "Appendix" on page 19 for a comparison of spherical and cubical volumes.

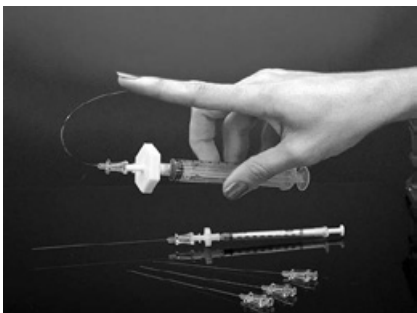
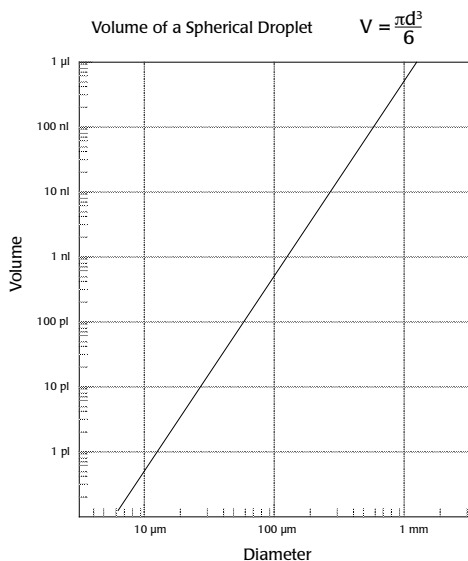


Fig. 11—(Left) The volume of a droplet increases as the diameter increases. See “Appendix” on page 16.

Fig. 12—(Right) WPI’s MicroFil is helpful in backfilling glass pipette tips. Syringe filters (available separately) help prevent clogged micropipette tips.

The following table is useful for converting between different units of volume.

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

For ejected volumes less than 1 nL, 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 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 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 3M KCl and the sample fluid is 0.1M KCl, a significant decrease in resistance of the micropipette occurs 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 0.1M KCl surrounding the tip is replaced by the more highly conductive 3M KCl, decreasing the measured resistance. Likewise, if vacuum is applied, the resistance increases as the less conductive 0.1M 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 1–2µm, we have seen a change in measured voltage of approximately 1mV 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 **PV830** to drive up to six micropipette barrels independently.

MAINTENANCE

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.

ACCESSORIES

| Part Number | Description |
|-------------|--|
| 3260 | Foot Switch |
| 2932 | Rack Mount Kit, 31 2-in. high (PV800 & PV820) |
| 2933 | Rack Mount Kit, 51 4-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 |
| LM-500865 | Vacuum Pump |

Replacement Parts

| Part Number | Description |
|-------------|--|
| 75122-110 | PicoNozzle gasket green 1.0mm, pkg. of 10 |
| 75122-210 | PicoNozzle gasket black 1.2mm, pkg. of 10 |
| 75122-310 | PicoNozzle gasket red 1.5mm, pkg. of 10 |
| 75122-410 | PicoNozzle gasket white 1.65mm, pkg. of 10 |

TROUBLESHOOTING THE HOLD PRESSURE

If the **Hold** regulator can't reduce the pressure low enough to keep the liquid from coming out of the pipette tip, DO NOT try to shut off the pressure by forcing the regulator knob counterclockwise. The regulator is designed so that the flow can't be shut off completely. It should, however, be able to reduce the pressure to below 0.2 PSI, which is low enough for most applications. If the regulator can control the pressure above 0.5 PSI, but not below 0.5 PSI, a simple reset will fix the problem. The reason that the regulator can't reduce the pressure below 0.5 PSI is because the dampening O-ring inside the regulator is stuck. The O-ring is connected to the regulator's diaphragm. It prevents the diaphragm from oscillating in high flow conditions. The diaphragm moves the valve shaft. The diaphragm uses the output pressure to counter-balance the spring force that is controlled by the knob. When the output pressure becomes very low, the force generated on the diaphragm becomes low. Sometimes, it is not enough to overcome the friction of the O-ring to push the valve to the completely closed position. A high pressure from the output port will push the diaphragm and return the O-ring to the seat.

To introduce a high pressure:

1. Remove the 1/4" hard pressure tubing from the back of the pump.
2. Use pieces of tubing with 1/4" OD to make a small 1/16" ID drop down extension.
3. Position the extension inside the end of the 1/4" hard pressure tubing.
4. Place the other end of the extension on the Eject port
5. Then, apply an injection at 20 PSI for 10 seconds.
6. Once the O-ring re-seats, it will remain there even when the output pressure again increases, as long as there is no high flow output. When there is no restriction at the output and the pressure is adjusted too high, the diaphragm has to move a substantial distance to allow the valve to open more to compensate for the pressure loss. This also causes the O-ring to move. Therefore, if low-pressure regulation is very important for the application, you should not set the **Hold** regulator at the high-pressure position when there is no restriction at the output. There are two ways to achieve this:
 - Always reduce the regulator pressure to minimum before assembly and disassembly of the injection pipette.
 - Turn off the **Hold** pressure valve before assembly and disassembly of the injection pipette.

NOTE: If you have a problem/issue with that falls outside the definitions of this troubleshooting section, contact the WPI Technical Support team at 941.371.1003 or technicalsupport@wpiinc.com.

SPECIFICATIONS

This unit conforms to the following specifications:

Pressure

| | |
|---------------------------------|--|
| Pressure Input (rear) | 150 PSI (1000kPa) maximum |
| Pressure Output | 0–90 PSI (600kPa) |
| Hold Pressure Output | 0.2–10 PSI (1.4 to 69kPa) |
| Lowest Regulated Pressure | 12 in. water (3kPa) * |
| 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



CAUTION: The valves will not reach zero while the **PV830** is running. Do NOT apply too much pressure when turning the knobs. Over-tightening the knobs will damage the regulators.

Vacuum

| | |
|-------------------------------|---------------------------------------|
| Vacuum Input (rear) | 0–30.0 in. Hg (101kPa) |
| Vacuum Output | 0–29.9 in. Hg (101kPa) |
| Lowest Regulated Vacuum | 3 in. water (0.7kPa) |
| Regulator Accuracy | 0.1% (20-turn dial) |
| Regulator Repeatability | 0.03 in. Hg (0.1kPa) |
| 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 | 10ms–10s in Timed Mode (10-turn dial)* |
| (Lower limit depends upon pressure setting. At 0 PSI the minimum pulse width is nominally 8ms. At 100 PSI the minimum pulse width is nominally 3ms.) | |
| Accuracy | 0.1% at full scale |
| External Input | +5 V TTL-compatible (BNC Connector) 100s 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.

APPENDIX

Use the chart below to gauge the volume of a droplet and the table to determine the volume a micropipette can hold.

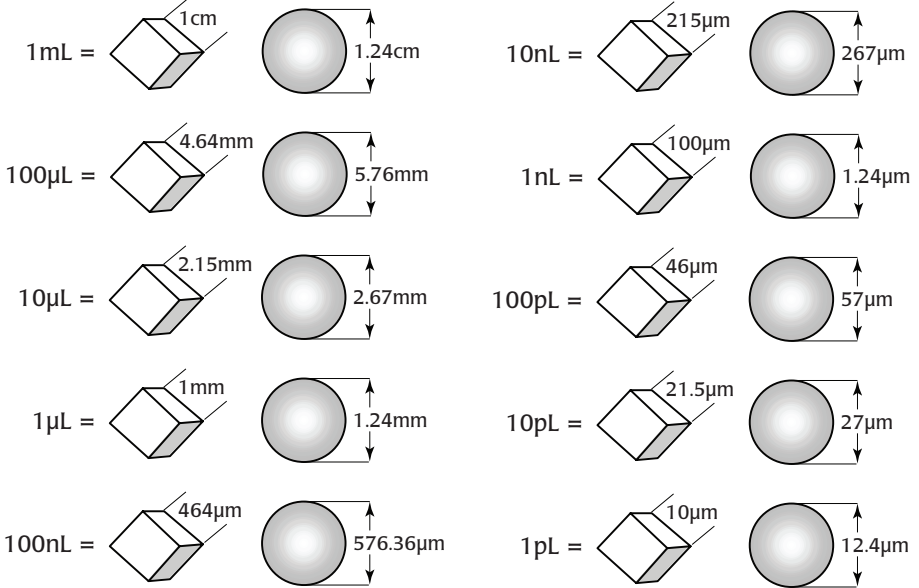


Fig. 13—This graph compares the dimensions of a cube and a sphere with the same internal volume.

Volume of a Micropipette

| OD | ID | Approx. Volume/cm | Approx. Volume/inch |
|--------|---------|-------------------|---------------------|
| 1.0 mm | 0.58 mm | 2.6 μ L/cm | 6.7 μ L/in |
| 1.2 mm | 0.68 mm | 3.6 μ L/cm | 9.2 μ L/in |
| 1.0 mm | 0.75 mm | 4.4 μ L/cm | 11.2 μ L/in |
| 1.5 mm | 0.84 mm | 5.6 μ L/cm | 14.1 μ L/in |
| 1.2 mm | 0.90 mm | 6.4 μ L/cm | 16.2 μ L/in |
| 1.5 mm | 1.12 mm | 9.8 μ L/cm | 25 μ L/in |
| 2.0 mm | 1.12 mm | 9.8 μ L/cm | 25 μ L/in |

BIBLIOGRAPHY

- R.E. McCaman, D.G. McKenna and J.K. Ono, A Pressure System for Intracellular and Extracellular Ejections of Picoliter Volumes , *Brain Research* 136 141-147 (1977)
- T.W. Stone, Microinjection and Pressure Ejection (1985)
- Y. K Ilstrom and S. Lindstr m, A Simple Device for Pressure Injections of Horseradish Peroxidase into Small Central Neurons , *Brain Research* 156 102-105 (1978)
- R.E. Hammer, et al. Production of Transgenic Rabbits, Sheep and Pigs by Microinjection , *Nature* 315 680-683 (1985)
- R.J. Griesbach, Advances in the Microinjection of Higher Plant Cells *BioTechniques* 3 (5) 348-350 (1985)
- Morikawa, H. and Y. Yamada, Capillary microinjection into protoplasts and intranuclear localization of injected materials *Pl. Cell Physiol.* 26 229-236 (1985)
- Ansorge W. Improved system for capillary microinjection into living cells *Exper Cell Res* 140 31-37 (1982)
- Munoz, J.L., F. Deyhimi, and J.A. Coles, Silanization of glass in the making of ion-sensitive microelectrodes , *J. Neuroscience Methods* 8 231-247 (1983)
- Deyhimi, F. and J.A. Coles, Rapid silylation of a glass surface: Choice of reagent and effect of experimental parameters on hydrophobicity , *Helv. Chim. Acta* 65 (6) 1752-1759 (1982)
- Capecchi, M.R., High efficiency transformation by direct microinjection of DNA into cultured mammalian cells , *Cell* 22 479-488 (1980)
- Diacumakos, F.G., Methods for micromanipulation of human somatic cells in culture *Methods in Cell Biology*, edited by D.M. Prescott, pp. 287-311. Academic Press, New York (1973)
- Gordon, J.W., G.A. Scangos, D.J. Plotkin, J.A. Barbosa and F.H. Ruddle, Genetic transformation of mouse embryos by microinjection of purified DNA , *Proc. Natl. Acad. Sci. USA* 77 7380-7384 (1980)
- Graessmann, A., M. Graessman and C. Mueller, Microinjection of early SV 40 DNA fragments and T antigen *Methods Enzymol.* 65 816-825 (1980)
- Kopchik, J.J., G. Ju, A.M. Skalka and D.W. Stacy, Biological activity of cloned retroviral DNA in microinjected cells , *Proc. Natl. Acad. Sci. USA* 78 4383-4387 (1981)
- A. Crossway, H. Hauptli, C.M. Houck, J.M. Irvine, J.V. Oakes and L.A. Perani, Micromanipulation techniques in plant biotechnology , *BioTechniques* 4 320-334 (1986)

DECLARATION OF CONFORMITY



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: 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.

Issued on: 18th February 2006

Dr. Mark P. Boudrick
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

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.*



World Precision Instruments, Inc.

USA

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

Zossener Str. 55, 10961 Berlin

Tel: 030-6188845 • Fax: 030-6188670 • E-mail: wpide@wpi-europe.com

China & Hong Kong

WPI Shanghai Trading Co., Ltd.

Rm 20a, No8 Dong Fang Rd., Lu Jia Zui Financial District, Shanghai PRC

Tel: +86 688 85517 • E-mail: chinasales@china.wpiinc.com

Internet

www.wpiinc.com • store.wpiinc.com • www.wpichemistry.com

www.wpi-europe.com • www.wpiinc.cn