PARKER HANNIFIN CORPORATION
PRECISION FLUIDICS DIVISION
26 CLINTON DRIVE, UNIT 103
HOLLIS, NH 03049 USA
TEL: (603) 595-1500
FAX: (603) 595-8080

PICOSPRITZER® III Manual

Pressure Systems for Ejection of Picoliter Volumes in Cell Research

PICOSPRITZER® III

SAFETY

Please read this entire set of instructions before attempting to use this manual for intracellular or extracellular ejections. This instrument should only be used as specified by the manufacturer. Use other than as specified may present a safety hazard. It is intended for research purposes only. Not for use on humans or for diagnostic purposes.

Please do not remove the cover as hazardous voltages may be exposed. Use only the electric cord supplied with the instrument or one of equal capacity.

For continuing safe operation of the unit periodic inspections of the connections in the pressure system should be made to ensure they are all still tight, particularly after a period of inactivity or if the system is moved.

This instrument is intended for use in an indoor environment between 10 and 40 degrees Celsius (50 to 104 degrees Fahrenheit) with no more than 90% humidity (non-condensing).

EQUIPMENT SPECIFICATIONS

Power Requirements:	100 to 240 Volts AC 50 to 60 HZ 0.8 Amps @ 115 Volts AC 0.6 Amps @ 230 Volts AC	
Fuses:	1 Amp, 250 Volts, Slow Blow 5 X 20 millimeters	
Maximum Inlet Pressure:	150 PSIG Clean, dry gas Do not use oxygen, corrosive, or combustible gas	
Environmental:	Temperature:	10 C (50 F) to 40 C (104 F)
	Humidity:	90% non-condensing
	Altitude:	0 to 2000 meters
	Overvoltage:	Category II
	Pollution:	Degree 2

The instrument is equipped with a universal power supply which will operate on the full range of voltages between 100 and 240 volts AC.

PICOSPRITZER® III

TABLE OF CONTENTS

1.0 INTRODUCTION

Please read this entire set of instructions before attempting to use this instrument for intracellular or extracellular ejections. These instructions cover the currently produced system (P/N 052-0500-900).

The Picospritzer III is a self-contained, rack- mountable system which supplies repeatable pressure pulses. Volumes dispensed are linear with time and pressure. The system can be initiated three ways: front panel push button, external stimulator (5 volt), or remote foot pedal. The Picospritzer III pressure system was designed for rapid and reproducible ejections of picoliter to nanoliter volumes used in conjunction with intracellular or extracellular studies while avoiding the inherent desensitization of nerve cells which accompanies iontophoretic methodology.

Intracellular applications range from femtoliter ejections of RNA into small cells to nanoliter ejections into oocytes. Extracellular applications range from picoliter applications of dilute neuroactive substances onto neurons during intracellular recording to the presentation of chemical stimuli to whole animals.

The system comes complete with high speed valves and the necessary tubing assemblies (less pipette and holder). It is designed to fit a standard 19" relay rack. The Picospritzer III comes fully adjusted and ready to use.

2.0 SETUP INSTRUCTIONS

1. Position the control unit in the desired location. Position the remote valve box within two feet of the experimental site (VELCRO® is supplied for mounting) and within 6 feet of the control unit.

2. Connect the black ¼ inch pressure tubing to the side port on the front panel regulator and to a source of clean dry compressed gas (air, nitrogen, CO2, or argon) at a maximum of 150 PSI (10bar). DO NOT USE OXYGEN OR COMBUSTIBLE GASES. The fitting on the regulator is a quick- connect type. The end of the tubing should be cut cleanly and inserted fully into the opening in the fitting. An o-ring seals around the outside of the tube. To remove the tubing from the fitting you must first vent the pressure from the line then press the ring on the end of the fitting (around the tubing) toward the body of the fitting while pulling on the tubing.

3. Use the 6-foot long 1/8-inch tubing assembly to connect the front panel regulator to the remote valve box. The connection at the regulator is also a quick-connect type while the remote valve box is threaded (1/4-28 male). There should be a ferrule pressed in to this end of the tubing.

4. The 3-foot long 1/16-inch tubing assembly is used to connect the remote valve box to a pipette holder (not supplied). Both ends of this assembly have ¼-28 nuts and ferrules pre-assembled on it.

PLEASE NOTE: An important detail in arranging the location of the solenoid in relation to the pipette holder is to maintain a loose coil in the interconnecting small bore (1/16

inch) Teflon® tubing to absorb or dampen the pressure pulse, thus avoiding movement of the pressure pipette. DO NOT stretch the tubing out tight between the valve housing and the pipette holder.

3.0 OPERATING SPECIFICATIONS

Standard Pressure Range:
10-100 psig, self-bleeding pressure regulator

Pulse Durations:
3 to 999 milliseconds, 1 ms intervals
0.1 to 99.9 seconds, 0.1 sec. intervals
0.1 to 99.9 minutes, 0.1 min. intervals

Pulse Initiation Trigger:
Pushbutton on panel, remote footswitch, or external stimulator (5 volt TTL input BNC jack).

Time Mark Output:
5-volt TTL signal from front panel BNC jack. References channel one output.

External Inputs:
5-volt TTL input BNC jack per channel.

Remote Valve Box:
24 volt DC high speed enclosed solenoid valve.

4.0 CONTROLS AND USE

The unit is activated when the power switch is in the ON position and the red pilot light is on. The unit must be connected to a source of clean, dry compressed gas at a pressure at least as high as that desired for ejections. Clockwise rotation of the knurled knob on the right of the rack mount panel will increase the pressure (indicated on adjacent gauge) available for injection purposes. This unit contains a self-bleeding regulator so that the pressure may be reduced by simply rotating the pressure control knob counter-clockwise.

The system is rated to operate up to 100 PSI (6.9 bar). One can be assured that all the connections are gas-tight by rotating the knob on the front panel so that the meter indicates approximately 80 PSI of pressure and then shutting off the main source of pressure to the panel. If a gradual reduction of pressure is observed, it indicates that there is a leak somewhere between the remote valve housing and the main pressure source. The site of this leak may be found by carefully listening for a hissing sound or by application of a dilute soap solution (or “snoop”) and watching for the formation of bubbles at various connections. It is essential to have leak proof connections throughout the system to assure reproducible results and to avoid unnecessary loss of gases.

Volume ejected is a linear function of both pulse duration and pressure (see references 1, 3). The pulse duration has a greater dynamic range with more accurate and reproducible settings. Thus, pulse duration will be changed frequently during the course of the experiment while pressure settings will be changed relatively little on a daily basis.

4.1 DURATION SETTING

The pulse duration is indicated by the setting on the three digit thumbwheel switch and the extended range switch (see Extended Range). It may be initiated by pressing the adjacent button on the front panel, by remote foot switch, or by an external input signal. The circuit has a “debounce” control which restricts the action of the push button so that only one pulse is initiated per button press, even if the button is continuously held down. It also restricts the interval between repetitive pulses initiated by the push button to approximately 200 milliseconds. The “PULSE” indicator will light during the course of the pulse.

4.2 EXTENDED RANGE

The Picospritzer is furnished with extended range timing controlled by a three- position toggle switch located at the right side of the “Duration” thumbwheel. The top position (labeled “MSEC”), selects timing in the milliseconds range (2 to 999 milliseconds). In the center position (labeled “SEC”), the range is from 0.1 to 99.9 seconds (note the decimal). In the bottom position (labeled “MIN”), the range is from 0.1 to 99.9 minutes.

4.3 INDICATORS

The Picospritzer III contains four LED indicators. “ON” is the power indicator. The “PULSE” indicator shows that the timer is active; the channel 1 and channel 2 indicators show when each channel is active.

4.4 INPUT TRIGGER

The internal timer now has a separate BNC jack to allow it to be triggered from an external source. A low to high (+5 volts) transition on this BNC will trigger the internal timer just as if the manual pushbutton had been pressed. The Picospritzer will not trigger again until the signal is set to low. If a pulse train is applied to this jack, care should be taken to ensure that the duration setting is less than the period of the pulse. Otherwise pulses will be skipped.

4.5 REMOTE FOOT SWITCH (not included)

A jack on the front panel of the Picospritzer is provided for attachment of an optional remote foot pedal for initiating a pulse. This convenience permits the investigator to be some distance from the rack mount panel and to view an ejection through a microscope while initiating the ejection. When using a remote foot switch, the foot switch has the same function as the panel push button

4.6 EXTERNAL INPUT

For additional flexibility in experimental use, the Picospritzer III has a front panel BNC jack for each channel which permits independent activation of the two channels from external sources. The selector toggle switch above each jack determines the source of the control signal for that channel. In the “EXTERNAL” position, it operates by energizing the valve whenever the input signal is high (+5 V.D.C.) and de-energizes it when the signal returns to ground. This allows for pulse durations longer than the capacity of the internal timer. In the “TIMER” position, the channel is connected to the internal timer and will activate whenever that timer is triggered (either manually or externally). The internal timer length can only be adjusted manually.

4.7 SECOND CHANNEL

The Picospritzer III is equipped with a separate BNC jack and selector toggle for each of

the two channels. This allows 2 external signals to be used
to operate the 2 channels independently when the selector toggles are set to the "EXTERNAL" position. Placing a selector toggle to the "TIMER" position connects that channel to the internal timer for operation whenever the timer is triggered (either manually or externally). To prevent a channel from triggering, place the selector switch in the "EXTERNAL" position and do not make a connection to the BNC for that channel.

4.8 MARKER

The time mark provides a convenient indication of the duration of the pulse with respect to a biological signal much like that of the "artifact" associated with iontophoresis. It is a 5-volt TTL signal controlled by channel one. The time mark not only provides a useful indication of the duration of the pulse, but it may also serve as a sync-out signal for triggering the sweep of an oscilloscope, etc.

4.9 PIPETTE CALIBRATION

Pipettes (not supplied by Parker Hannifin) are generally calibrated by ejecting water into oil (generally mineral oil) and then measuring the droplet diameter with an optical micrometer. Smaller droplets may cling to the tip of the pipette. This is the method briefly discussed in the manual and from which the droplet chart is derived.

Since the duration setting is easier to use and more repeatable it is usually the one that is varied. An operating pressure is selected and kept that way for a given experiment. You may need to make a few test droplets at different pressures and durations with a given pipette to determine what operating pressure gives you the desired range by varying just the duration. When setting the pressure, it is important to do so by increasing the value. If you over shoot your desired set point always back down to less than that point and then raise it back up.

The volume ejected during a single pulse depends on the applied pressure, the pulse duration, AND the pipette tip diameter. Every pipette must be calibrated by optically measuring the droplet sizes that result from varying the pressure or duration.

A typical pipette will only hold 1 to 4 microliters of media. Doing one ejection in the microliter range may be possible but multiple ejections are not. Doing multiple ejections to total 1 to 2 microliters is possible but the user still must calibrate the system, know the number of ejections that will be required to reach the desired volume, and keep track of the number of ejections completed

5.0 BACKGROUND

In 1977, Dr. R.E. McCaman and associates provided a complete description (1) of a pressure ejection system that utilized a high speed valve. This valve continues to be the heart of the pressure system offering very precise control of ejection volumes (in the picoliter range) and ejection times (in the millisecond range).

Furthermore, these investigators described a series of holders that permitted ejection through micropipettes with sufficiently small tips that could be used for simultaneous intracellular recordings during ejections.

These systems have been used for intracellular as well as extracellular ejections. In listing advantages of the pressure system, these investigators emphasize that the linear

relationship between ejection volume and either duration of the pulse or of the applied pressure permits a rapid, convenient and reliable calibration of each pipette (1, 3), unlike that for electrophoretic techniques (7-9).

Pressure ejection seems an ideal approach to delivering uncharged substances such as peptides (4, 6), steroids (4), and enzymes (2,5). The solutions used for pressure ejections are usually several orders of magnitude more dilute than those used for electrophoretic ejection (1, 3), thus avoiding receptor desensitization commonly experienced with iontophoresis. The fact that the ejection efficiency of the pneumatic systems is not influenced by solute concentration nor by net charge, makes them ideal for intracellular injections of radiolabeled or tracer substances (13-15). Thus, pressure systems have been used for intracellular injection of radiolabeled precursors or neurotransmitters (10, 11) and [H3] –sugars as precursors of glycoproteins (12) in order to study neuron-specific transmitter biosynthesis, axonal transport and cellular topography. The reproducible and quantifiable ejections obtained with pressure systems make them ideal for neuropharmacological studies of agonist and drug interactions with membrane receptors (1, 3, 4).

As you find additional uses for your Picospritzer, please send us a reprint for addition to our reference section so that others may benefit from your experience.

N.B.; H3=radioactivity (tritium) label substance.

6.0 REFERENCES

References describing the use and unique advantage of pressure systems in several types of experimentation in the field of neurobiology, cell biology, and biophysics are:

1. McCaman, R.E., Mc Kenna, D.G. and Ono, J.K. “A pressure system for intracellular and extracellular ejections of picoliter volumes.” Brian Research 136:141 (1977).

2. Sakaki, M., Sakai, H. and Woody, C.D. “Intracellular staining of cortical neurons by pressure micro-injection of horseradish peroxidase and recovery by core biopsy.” Exp. Neurol. 58:138 (1978)

3. Sakai, M., Swartz, B.E. and Woody, C.D. “Controlled micro release of pharmacological agents: measurements of volume ejected in vitro through fine tipped glass microelectrodes by pressure.” Neuropharmacol. 18:209 (1979)

4. Dufy, B., Vincent J-D. Fluery, H., Pasquier, P., Gourdji, D., and Tixler- Vidal, A. “Membrane effects of thyrotropin-releasing hormone and estrogen shown by intracellular recording from pituitary cells.” Science. 204.509 (1979)

5. Tauc, L., Hoffman, A., Tsuji, S., Hinzen, D. and Faille, L. “Transmission abolished in a cholinergic synapse after injection of acetylcholinesterase into the presynaptic neuron.” Nature, 250, 496 (1974)

6. Chang, J.J., Gelperin, A., and Johnson, F.H. “Intracellulary injected aequorin detects transmembrane calcium flux during action potentials in an identified neuron from the terrestrial slug.” Brain Research 77:431 (1974)

7. Krnjevic, K., Mitchell, J.F., and Szerb, J.C. “Determination of iontophoretic release of acetylcholine from micropipettes.” J.Physiol. 165:421 (1963)

8. Zieglgansberger, W., Southmann, G., and Herz, A. “Iontophoretic release of substances from micropipettes in vitro.” Neuropharmacol. 13:417 (1974)

9. Kuffler, S. and Yoshikami, D. “The number of transmitter molecules in a quantum: an estimate from iontophoretic application of acetylcholine at the neuromuscular synapse.” J. Physiol. 251:465 (1975)

10. Eisenstadt, M., Goldman, J.E., Kandel, E.R., Koike, H., Koester, J. and Schwartz, J. “Intrasomatic injection of radioactive precursors for studying transmitter synthesis in identified neurons of Aplysia.” Proc. Nat. Acad. Sci. 70:3371 (1973)

11. Schwartz, J.H. “Synthesis, axonal transport and release of acetylcholine by identified neurons of Aplysia.” Proc. Nat. Acad. Sci. 70:3371 (1973)

12. Thompson, E.B., Schwartz, J.H. and Kandel, E.R. “A radio autographic analysis in the light and electron microscope of identified Aplysia neurons and their processes after intrasomatic injection of L-H3-Fucose.” Brain Research.112:251 (1976)

13. Baux, G., Simonneau, M. Tauc, L. “Transmitter release, Ruthenium red used to demonstrate a possible role of sialic acid containing substrates.” J. Physiol. 291, 161-178 (1979)

14. Sakai, M., Sakai, H., and Woody, C.D. “Sampling distribution of morphologically identified neurons of coronal- pericruciate cortex of awake cats following intracellular injection of HRP.” Brain Research. 152: 329-333 (1978)

15. Amaral, D.G. and Price, J.L. “An air pressure system for the injection of tracer substances into the brain.” Jrnl. Neuroscience Methods. 9:35-34 (1983)

16. Waitzman, D.M., Sliakov, V.L., DePalma-Bowles, S. and Ayers, A.S. “Effects of reversible inactivation of the primate mesencephalic reticular formation. I. Hypermetric goal-directed saccades.” Journal of Neurophysiology (2000) https://journals.physiology.org/doi/full/10.1152/jn.2000.83.4.2260

17. Pham, L.N., Dionne, M.S., Shiarsu-Hiza, M. and Schneider, D.S. “A specific primed immune response in drosophila is dependent on phagocytes.” PLOS https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.0030026 (2007)

18. Kim, L.K., Choi, U.Y., Cho, H.S., Lee, J.S., Lee, W., Kim, J., Jenog, K., Shim, J., Kim, J. and Kim, Y. “Down-regulation of NF-κB target genes by the AP-1 and STAT complex during the innate immune response in drosophila.” PLOS https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.0050238 (2007)

19. Kuhlman, S.J. and Huang, Z.J. “High-resolution labeling and functional manipulation of specific neuron types in mouse brain by cre-activated viral gene expression.” PLOS https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0002005 (2008)

20. Gibbs, G.M., Orta, G., Reddy, T., Koppers, A.J., Martinez-Lopez, P., Vega-Beltran,

J.L., Lo, J.C.Y., Veldhuis, N., Jamsai, D., McIntyre, P., Darszon, A. and O'Bryan, M.K. "Cysteine-rich secretory protein 4 is an inhibitor of transient receptor potential M8 with a role in establishing sperm function." University of Hawaii https://www.pnas.org/content/108/17/7034.figures-only (2011)

FIGURE 1: DROPLET CALIBRATION CHART

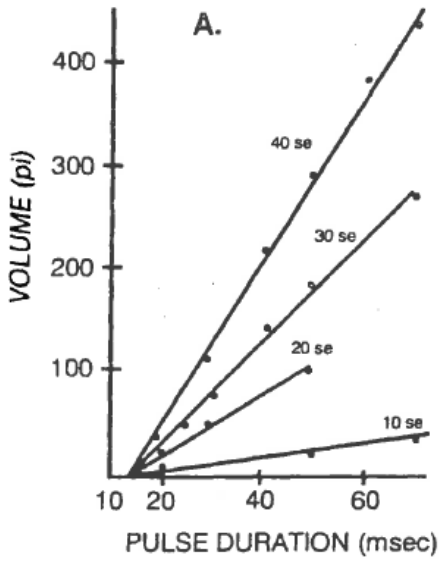
(4/3) 4.2 x r3 DIAM.	RADIUS	PICOLITER VOL(F1)
10 μm	5	.52
20	10	4.2
30	15	14.2
40	20	33.6
50	25	65.6
60	30	113
65	32.5	144
70	35	180
75	37.5	221
80	40	269
90	45	383
100	50	525
110	55	699
120	60	907
125	62.5	1025
140	70	1441
150	75	1772
160	80	2150
170	85	2579
175	87.5	2814
180	90	3062
200	100	4200
225	112.5	5900
250	125	8203
275	137.5	11037
300	150	14175
325	162.5	18022
350	175	22509
375	187.5	27685
400	200	33600
450	225	47840
500	250	65625
600	306	113,000
625	312.5	128,000
750	375	221,480

Calibration chart provided courtesy of Dr. Joyce K. Ono, Department of Biological Science, California State University

FIGURE 2: **CHARACTERISTICS OF THE PRESSURE EJECTION SYSTEM**

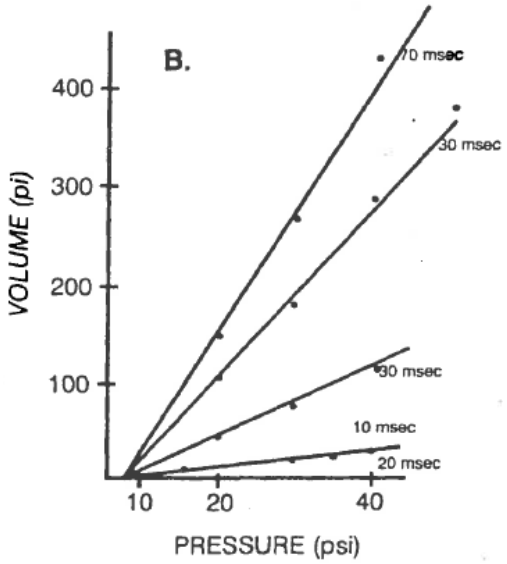
The diameter of the droplet ejected in air was measured with an ocular micrometer in a dissecting microscope. The volume was calculated for droplets formed by varying pressure or pulse duration parameters. The following graphs demonstrate that each pipette can be calibrated by varying these two major determinants of the volume ejected.

FIGURE 2A: LINEARITY OF VOLUME EJECTED WITH VARYING PULSE DURATION AT CONSTANT PRESSURE



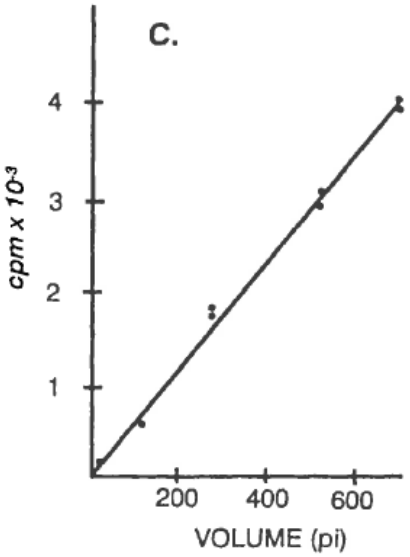
The X-intercept (15 MSEC) represents the mechanical lag time of the particular solenoid valve.

FIGURE 2B: LINEARITY OF VOLUME EJECTED WITH VARYING PRESSURE AT CONSTANT PULSE, DURATION



The X-intercept (7.5PSI) represents the minimum pressure necessary for ejection and is a characteristic of a particular pipette.

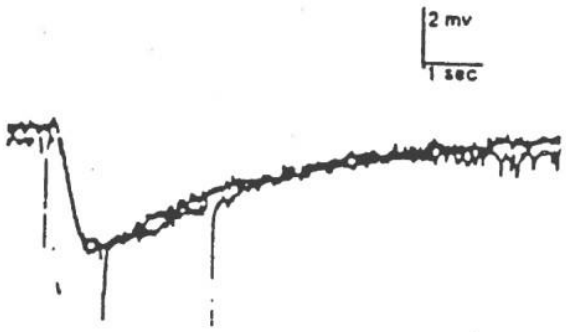
FIGURE 2C: LINEARITY OF THE AMOUNT OF AN H3 STANDARD WITH VARIOUS EJECTED VOLUMES



The points of this graph are highly correlated (R=.97) with the independently determined specific activity (6.5 x 106 CPM/mL) of the radioactive solution.

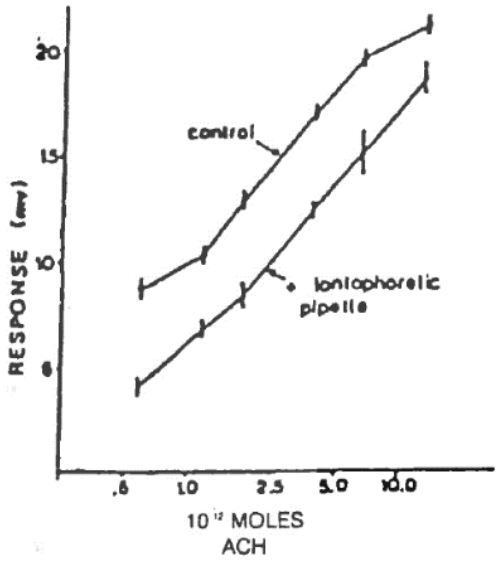
FIGURE 3: **COMPARISONS OF RESPONSES OF APLYSIA CALIFORNICA NEURONS TO IONTOPHORETIC AND PRESURE APPLICATION OF COMPOUNDS**

FIGURE 3A:



Superimposed traces of Aplysia buccal neuron responses to ACh delivered by an iontophoretic pulse (1µ amp, 80 MSEC) and a pressure pulse (40 PSI, 60 MSEC, 60 µm diameter droplets of 10-3 M ACh). The amplitude and polarity of the pressure artifacts (negative square pulse in this case) can be manipulated.

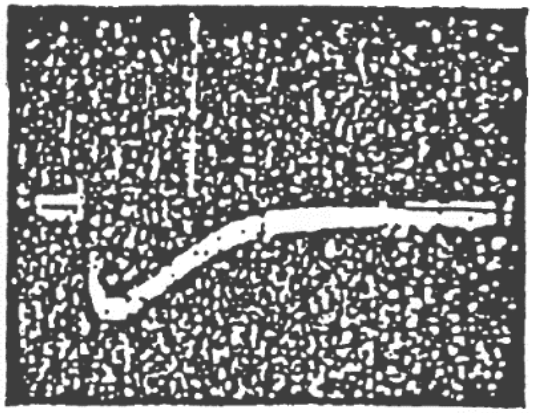
FIGURE 3B:



Comparisons of responses to pressure ejected acetylcholine (ACh) in Aplysia neuron in the absence (control) and presence of an iontophoretic pipette (70 MEGOHMS) containing 1 M ACh. The dose-responsive curve is shifted to the right because of desensitization from ACh leaking out of iontophoretic pipette. Each point is the mean response + standard error of the mean.

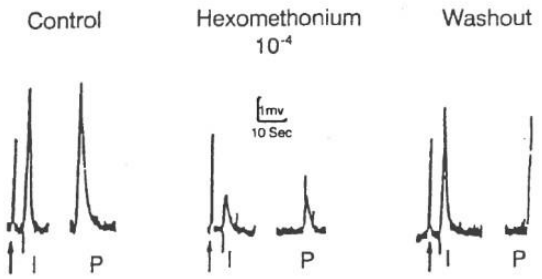
Problems of desensitization can be circumvented with a pressure pipette since the pipette is usually filled with agonists in the concentrations of 10-6 to 10-3 M in contrast to iontophoretic pipettes. Braking current is not necessary for the pressure pipette, thus avoiding inconsistent ejection of compounds.

FIGURE 3C:



Reproducibility of 12 consecutive responses to acetylcholine (ACh) delivered by a pressure pulse to an Aplysia (Sea Hare) neuron. A 10-4M solution of ACh was ejected by 22 PSI for 5 MSEC to produce a droplet estimated to be 10 PL (=1 Femtomole of ACh). Calibration: 2 mV, .02 Sec.

FIGURE 3D:



Comparison of response to iontophoretic (I) and pressure (P) applied ACh in a drug study. Both responses are equally antagonized by hexamenthonium, even though the ACh in the pressure pipette is ejected in a droplet of normal artificial seawater. Similar results are obtained during substitution experiments. The arrows in the figure point to an input resistance test pulse.

7.0 **PICOSPRITZER III FAQ** (Frequently Asked Questions):

1. I used to purchase the vacuum loading unit is that still available?

The vacuum loading unit is no longer available for purchase.

2. I used to purchase a 0-10, 0-30 or 0-60psi regulator, are those options still available?

The 0-10, 0-30, or 0-60psi regulator options are no longer available, only the 0-100psi configuration is available. Please see Appendix 1 for alternative regulator information.

3. Can I trigger the two channels independently using two separate footswitches?

The Picospritzer III can only handle one footswitch (the footswitch controls the internal timer). You can trigger them independently using an external trigger. Please see Section 4.7 Second Channel for more details.

4. Can I provide separate pressures to the two valve boxes?

The Picospritzer III will only provide the same pressure to both valve boxes.

5. What can I do if my system is experiencing an issue with backflow?

Check the capillary tip size. Typically, we recommend a tip size between 1 and 5 microns. Once the resistance of the tip is higher than the capillary effect of the pipette, material will no longer be drawn into the pipette. Keep in mind that other factors such as pressure, timing, and the nature of the material being ejected, may come into play.

6. What is the shortest pulse duration the Picospritzer III can support?

A minimum of 3 milliseconds should be used to ensure the valve in the valve box can fully actuate for injection.

7. What are the approximate shipping weights and dimensions of the Picospritzer III

The Picospritzer III typically ships in a box with dimensions of 24" x 14" x 9" and weighs approximately 10 lbs. Actual shipping weights and dimensions may vary.

APPENDIX 1 – PART INFORMATION

Available from Parker Hannifin

PICOSPRITZER® III Two Channel Unit (0 – 100 psi regulator)
PARKER HANNIFIN P/N 052-0500-900

VALVE BOX & CABLE ASSEMBLY
PARKER HANNIFIN P/N 051-0009-401-1

Commercially Available from Parker Hannifin Distributors (except as noted*)

ALTERNATIVE REGULATORS:

Regulator Range	Regulator Vendor	Regulator P/N	Pressure Gauge Vendor	Gauge P/N
0-100 psig	Parker Watts	R374-01C	Wika*	9690234
0-60 psig	Parker Watts	R374-01B	Parker Wilkerson	K4515N18060
0-30 psig	Parker Watts	R374-01A	Parker Wilkerson	K4515N18030
0-10 psig	Parker Watts	R374-01AX43	Ametek*	146010

Commercially Available (Not Sold by Parker Hannifin)

PIPETTE HOLDERS:

Available from Warner Instruments (www.warneronline.com)

PIPETTES:

Available from Eppendorf (www.eppendorf.com)

TUBING AND FITTINGS:

Picospritzer to Valve Boxes
Tubing: PTFE Teflon 1/8” OD, .062” ID
Nut: 1/4-28 THD for 1/8” OD Tubing
Ferrule: for 1/8” OD Tubing

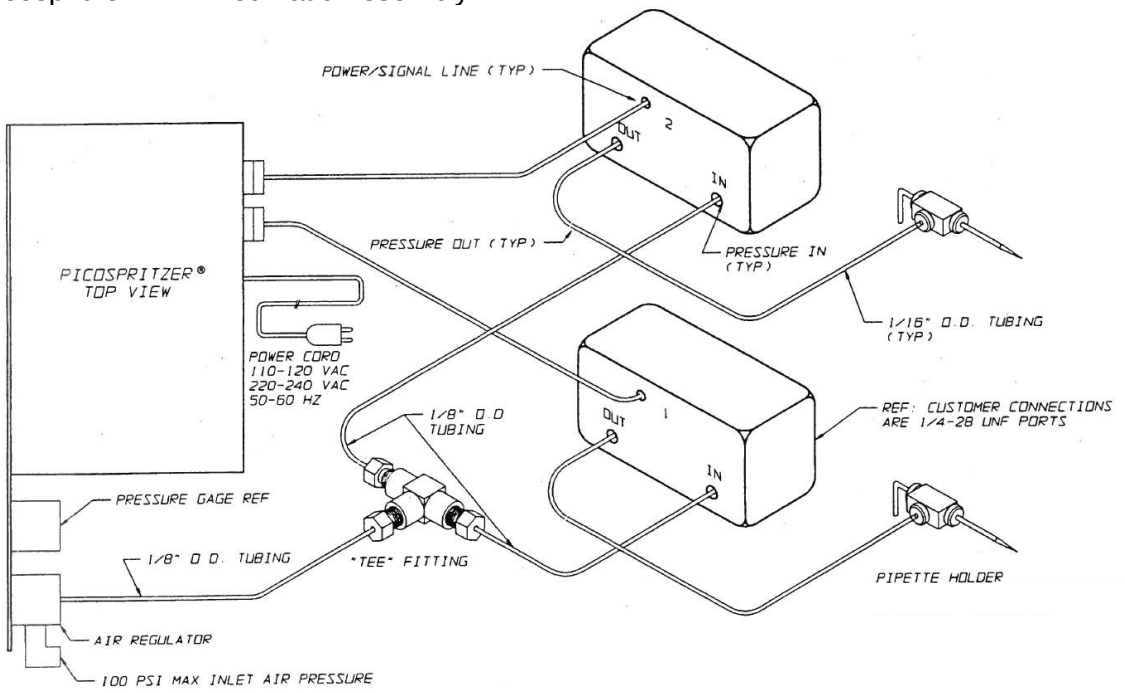
Valve Boxes to Pipette Holders
Tubing: PTFE Teflon 1/16” OD, .031” ID
Nut: 1/4-28 THD for 1/16” OD Tubing
Ferrule: for 1/16” OD Tubing

FOOTSWITCH:

StealthSwitch FS-2 available from StealthSwitch (www.stealthswitch.com)

APPENDIX 2 – DRAWINGS

Picospritzer III – Pneumatic Assembly



Regulator Assembly Schematic