MicroJect 1000A Pico-Injector

USER'S MANUAL

Item No.	Product
45-0750	MicroJect 1000A Basic System
45-0751	MicroJect 1000A Plus System
45-0752	MicroJect 1000A Max System

BTX®

a division of **Harvard Bioscience, Inc.**

5506-001 REV 1.0

Warranty Information

Research Only

BTX

84 October Hill Rd
Holliston, MA 01746, USA

Phone: 1-508-893-8999
Fax: 1-800-429-5732
Web: www.btxonline.com

Warranty

BTX warranties the MicroJect 1000A systems for a period of two years from the date of purchase. At its option, BTX will repair or replace the unit if it is found to be defective as to workmanship or materials. This warranty does not extend to any instrumentation which has been (a) subjected to misuse, neglect, accident or abuse, (b) repaired or altered by anyone other than BTX without BTX express and prior approval, (c) used in violation of instructions furnished by BTX. This warranty extends only to the original customer purchaser. IN NO EVENT SHALL BTX BE LIABLE FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow exclusion or limitation of incidental or consequential damages so the above limitation or exclusion may not apply to you. THERE ARE NO IMPLIED WARRANTIES OF MERCHANTABILITY, OR FITNESS FOR A PARTICULAR USE, OR OF ANY OTHER NATURE. Some states do not allow this limitation on an implied warranty, so the above limitation may not apply to you. Without limiting the generality of the foregoing, BTX shall not be liable for any claims of any kind whatsoever, as to the equipment delivered or for non-delivery of equipment, and whether or not based on negligence. Warranty is void if the BTX MicroJect 1000A instrument is changed in any way from its original factory design or if repairs are attempted without written authorization by BTX. Warranty is void if parts, connections or electrodes not manufactured by BTX are used with the BTX MicroJect 1000A instrument.

If a defect arises within the warranty period, promptly contact BTX, 84 October Hill Road, Building 7, Holliston, Massachusetts, USA 01746-1388 using our toll free number 1-800-272-2775 (US Only) or 508-893-8999 (email: repairs-returns@harvardbioscience.com). Goods will not be accepted for return unless an RMA (Returned Materials Authorization) number has been issued. Please allow a reasonable period of time for completion of repairs, replacement and return. If the unit is replaced, the replacement unit is covered only for the remainder of the original warranty period dating from the purchase of the original device. This warranty gives you specific rights, and you may also have other rights, which vary from state to state.

Out of Warranty Service

Proceed exactly as for Warranty Service above. If our service department can assist you by phone or other correspondence, we will be glad to help at no charge.

Repair service will be billed on the basis of labor and materials. A complete statement of time spent and materials used will be supplied. Shipment to BTX should be prepaid. Your bill will include return shipment freight charges.

Disassembly by the user is prohibited. Service should only be carried out by experienced BTX technicians.

Please contact us at 1-800-272-2775 or by email at repairs-returns@harvardbioscience.com for assistance.

Caution Notice

The MicroJect 1000A is intended for laboratory use only and can be used in research and development applications. These systems have been designed to meet the standards for electromagnetic compatibility (EMC) and safety intended for laboratory equipment applications.

This product should not be used in the presence of a flammable atmosphere such as an anesthetic mixture with air, oxygen, or nitrous oxide.

Safety Information

Please read the following safety precautions to ensure proper use of your equipment. If the equipment is used in a manner not specified, the protection provided by the equipment may be impaired.

To Prevent Hazard or Injury

Use Proper Power Supply and Line Cord

Use only the power supply provided with the instrument (9 V DC) and make sure the line cord is certified for country of use. The operating voltage range for the BTX MicroJect 1000A is 100–240 VAC, 50/60 Hz.

Ground the Product

This product is grounded through the grounding conductor of the power cord. To avoid electric shock, the grounding conductor must be connected to earth ground. Before making any connections to the input or output terminals of the product, ensure that the product is properly grounded.

Make Proper Connections

Make sure all connections are made properly and securely. Any signal wire connections to the unit must be no longer than three meters.

Observe All Terminal Ratings

Review the operating manual to learn the ratings on all connections.

Use Proper Fuse

Use only specified fuses with product.

Avoid Exposed Circuitry

Do not touch any electronic circuitry inside of the product.

Do Not Operate with Suspected Failures

If damage is suspected on or to the product do not operate the product. Contact qualified service personnel to perform inspection.

Orient the Equipment Properly

Do not orient the equipment so that it is difficult to operate the disconnection device.

Place Product in Proper Environment

Review the operating manual for guidelines for proper operating environments.

Observe All Warning Labels on Product

Read all labels on product to ensure proper usage.

Caution Notice

The BTX MicroJect 1000A Systems are intended for laboratory use only and can be used in research and development applications. These systems have been designed to meet the standards for electromagnetic compatibility (EMC) intended for laboratory equipment applications as well as the applicable safety requirements for electrical equipment for measurement, control, and laboratory use. The unit itself does not generate waste, but may be used to treat samples that are hazardous. Please use appropriate PPE and ensure disposal in accordance with local regulations and practices.

This product should not be used in the presence of a flammable atmosphere such as an anesthetic mixture with air, oxygen, or nitrous oxide.

Caution

Protective Ground Terminal

A Message from BTX

Thank you for investing in a MicroJect 1000A Pico-Injector System.

Since its founding in 1983, the main focus of BTX has been gene delivery applications. Because of this, we quickly established a reputation as the technological leader in the fields of electroporation, electrofusion, and microinjection. Our systems have been installed in many prestigious institutes around the globe where they are used successfully for high efficiency transfection, transformation, and cell fusion applications. We offer a variety of instrument and accessory options to provide you with the best tools to achieve your goals.

We are vested in your success. To that end, the BTX technical support team constantly tracks published literature for any reference electroporation, electrofusion, and microinjection. We extract the pertinent experimental conditions and yields from these papers to help us in our efforts to enable your success. In addition to tracking publications, we are available to you for support at any time for advice in experimental design, product recommendations, troubleshooting, and any other relevant technical advice.

We thank you again for your investment and we look forward to assisting you in any way we can.

Finally, please read this manual carefully before attempting to operate the microinjection system. If you have any questions about the unit or about particular applications, please contact us:

BTX
84 October Hill Road
Holliston, MA 01746 USA

Toll Free: 1-800-272-2775
International Callers: 508-893-8999
Fax: 508-429-5732
Web: www.btxonline.com
email: support@hbiosci.com

For any customers outside the US or Canada, please call your local BTX dealer or call us directly.

BTX®
a division of Harvard Bioscience, Inc.

Table of Contents

Introduction

The **MicroJect 1000A Pico-Injector** allows small liquid volumes to be delivered precisely through micropipettes by applying a regulated pressure for a digitally set period of time. The pressure is applied pneumatically (compressed gas) to deliver volumes from microliters to femtoliters from the same instrument.

This digital injection pressure is enhanced by three auxiliary pressures:

FILL
Suction can be applied to the delivery pipette to fill it from the tip.

HOLD
A second internal vacuum pump is available to hold a suspended cell (for injection) with a second holding pipette.

BALANCE
A (lower) balance pressure applied to the delivery pipette between injections prevents clogging caused by pipette movement as well as dilution of the injected material by capillary action.

CLEAR
Momentary application of high pressure can be used to clear clogged pipettes.

Triggering of injection is accomplished three ways: panel push button, optional foot switch, or an externally applied electrical signal (TTL pulse).

The duration of injection can be determined by an internal clock set with a digital switch or can be gated by the duration either of an externally applied electrical pulse or of the depression of an optional foot switch.

This manual assumes the reader has no previous experience with microinjection, either extracellular or intracellular. Those planning on larger volume delivery (100 pL and up) can ignore the BALANCE feature. Some reference will be made to a less precise technique for smaller volume delivery to highlight the precision determining features of this instrument. This technique involves pressurizing the gas in a macro-syringe attached to a delivery micropipette by advancing the piston of the syringe with a micrometer screw. Ejection takes place continuously from the pipette: the volume delivered depends both on the unregulated over pressure in the syringe and the poorly known time the pipette is left inside the cell. This continuous ejection technique is thought to reduce the frequency of pipette clogging.

Reliable microinjection also requires skill in making and using micropipettes. Authorities on microinjection state that the most important single factor in microinjection is the micropipette. The MicroJect 1000A Pico-Injector with its reproducibility simplifies the search for the optimum micropipette.

CAUTION: FOR RESEARCH USE ONLY. NOT FOR CLINICAL OR DIAGNOSTIC USE ON PATIENTS.

Specifications

MicroJect 1000A Pico-Injector Specifications	
Input Gas Pressure	70 to 105 psi (480 - 720 kPa)
Injection Pressure	0.2 to 60.0 psi (413 kPa) - regulated, multi-turn control
Balance Pressure	0.1 to 9.9 psi (68.9 kPa) - regulated, multi-turn control
Fill Pressure	0 to -12 psi (-82 kPa) (Vacuum)
Hold Pressure	0 to 1.25 kPa (-12.7 cm of water, vacuum)
Clearing Pressure	Inlet pressure (unregulated)
Injection Time	0.01 to 0.99 s; 1-99 s
Pressure Display Resolution	3.5 digits, 0.1 psi or 1 kPa
Injection Count Display	0-9999
Duration Mode	Internally timed or externally gated
Trigger Mode	Front Panel, Foot Switch, external TTL pulse BNC
Pressure Monitor Out	BNC Connector, 10 mV/psi or 1 mV/kPa
AC Power Lines	External 9V DC@ 400 mA (min) Power Supply universal input 90 to 264 VAC
Accessories Supplied	Input hose, output hose, holding hose and power module
Weight	12 lbs (5.44 kg)
Dimensions (H x W x D)	3.5 in x 17 in x 9.8 in (89 mm x 432 mm x 250 mm)
Optional Accessories	Foot Switch(es) up to four functions, Pipette Holder - up to 2, Input Hose Adaptor
Warranty	Two-year, parts and labor
Atmospheric Specifications:	
Operating Temperature	4˚C to 40˚C (40˚F to 104˚F)
Storage Temperature	-10˚C to 70˚C (14˚F to 158˚F)
Humidity	20% to 80% RH, non condensing
Mode of Operation	Continuous
Classification	Class I
Pollution	Degree 2
Installation	Category II
Supplier Name	BTX
Supplier Address	84 October Hill Road, Holliston, MA 01746
Supplier Phone Number	508-893-8999
Regulatory Certifications	CE, ETL (UL, CSA), WEEE, EU RoHS

Microinjection

Microinjection also involves other skills, several other instruments, accessories, and various supplies. The purpose of this section is to give an overview of these techniques for those new to microinjection; some guidance on equipment selection is also supplied.

Required Auxiliary Equipment for Microinjection

The required equipment includes a pipette puller and micromanipulator(s). Ideally, the puller should be capable of making pipettes with tip diameters in the 0.2 to 1.5 micron range with a short enough taper length for both mechanical strength and low flow resistance. Additionally, 10 micron pipette tip diameter is suitable for microinjection of large cells, such as frog oocytes. The required three dimensional movement can be produced by an inexpensive mechanically linked micromanipulator for large cells. More commonly, a hydraulic one for fine, vibration-free movement is mounted on a coarse mechanical manipulator. Suitable equipment is available from Harvard Apparatus, Inc.

Required Supplies

Compressed gas and microcapillaries are required. Compressed air is suitable for oxygen insensitive injection material. Nitrogen is a satisfactory inert gas for the general applications. A pressure of 105 psi is sufficient; a regulator will be needed if supplied from a bottle of compressed gas. We strongly recommend the gas supplied is medical grade.

Optional Equipment for Microinjection

This includes a microforge (to bend a micropipette or to polish a pipette tip for holding a cell), a microgrinder (to bevel the pipette tip to increase the delivery rate without additional cell damage), and an micro-incubator (to hold the cells at incubation temperatures during microinjection).

Accessories

A number of accessories are available to enhance your MicroJect 1000A Pico-Injector (see Ordering Information on page 18).

Micropipettes

The micropipettes are made with a pipette puller from a microcapillary (1-2 mm in diameter) by heating some 3-10 mm of its length with a concentric heater while applying a force (gravitational or magnetic) to pull both ends of the capillary apart. Two micropipettes are produced per capillary.

1. Distinguishing Parameters

Two useful distinguishing parameters of the micropipette are the inside diameter of its tip and the angle of taper to the tip. The smaller this angle, the longer the tapered region. The larger the tip, the more material is delivered for the same applied pressure and time. Just a 10% decrease in diameter decreases the delivery rate by over 30%. A 10% decrease in taper angle (longer taper) would decrease the delivery rate about 10%. The extreme sensitivity of delivery rate on tip diameter makes it important to have a reproducible pipette puller. If you use published tip sizes as a starting point, distinguish between the relevant inside diameter and the more visible outside diameter. (The ratio of the two is the same at the tip as for the original capillary glass.)

2. Choosing the Right Pipette

Choosing a pipette size and shape for intracellular injection is difficult. Larger tips deliver more material, but increase the risk of cell damage caused by leakage around the pipette while in the cell or later by incomplete sealing. The smaller the cell, the smaller the pipette tip it will tolerate. The smaller the tip, the more likely it is to clog. For reference, intracellular electrophysiologists routinely record for an hour or so from cells of 10 micron diameter with pipette tips of 0.1 micron inside diameter. Larger tips can therefore be used for brief injection in such cells. For nuclear injections, a smaller taper angle is needed to avoid leakage further up the shank of the pipette at the plasma membrane.

Although intracellular injection can be done from below with an upright microscope, most injections are done from the side or from above the cells. Four different strategies have been used to suitably fix the cell in position for successful intracellular injection.

For suspended cells, a second, larger pipette is used to hold the cell. This pipette's tip is first polished with a microforge (done by placing the pipette within 5 microns of a hot filament for a few seconds). With its axis horizontal, it is moved to hold the cell with applied suction. The injection pipette is also straight and is inserted horizontally from the opposite side. This geometry avoids damage to the cell membrane caused by shearing forces. The optics are straightforward because the pipettes remain in focus as they are advanced.

For cells that can be or are attached to a surface in a closely packed layer, straight injection pipettes can be used. In this case, the pipette axis slopes slightly down from the horizontal. The tendency of the cells to slide when the pipette enters is resisted by the extracellular environment or attachment to the culture surface. The microscope should be focused on the cell's surface. The pipette tip then comes into focus just before injection. If the cell is nearly spherical (the hardest case), the pipette should again enter the cell membrane at right angles to avoid shearing. Non-spherical cells (for example, cultured fibroblasts) have a more robust cytoskeletal structure so the pipette can be pushed in even if not perpendicular to the membrane surface.

For less firmly attached cells, the injection pipette can be bent near the tip after pulling. The pipette's main axis slopes slightly down from the horizontal. The angle of bend should allow the tip to point straight down. With an inverted microscope, the tip is viewed through the cell as it is lowered for injection. The microscope is focused on the cell's top surface and the tip comes into focus just before insertion. Again shearing forces are avoided. (Suitable bends can be made with a microforge: a simple way to do this is to move the pipette near a hot filament at the position of desired bend. The tip will spontaneously bend away).

In all of the above techniques, a three dimensional micromanipulator controls the movement of the injection pipette. If this (straight) pipette is instead attached to a condenser mount (inverted scope), then a one dimensional manipulator can be used. The remaining two directions of manipulation are done with stage micrometers moving the vertical injection pipette over each cell in turn. If the vibrations transmitted with the condenser mounting are manageable, then this approach gives the fastest rate of cell injection.

Front Panel Connectors & Controls

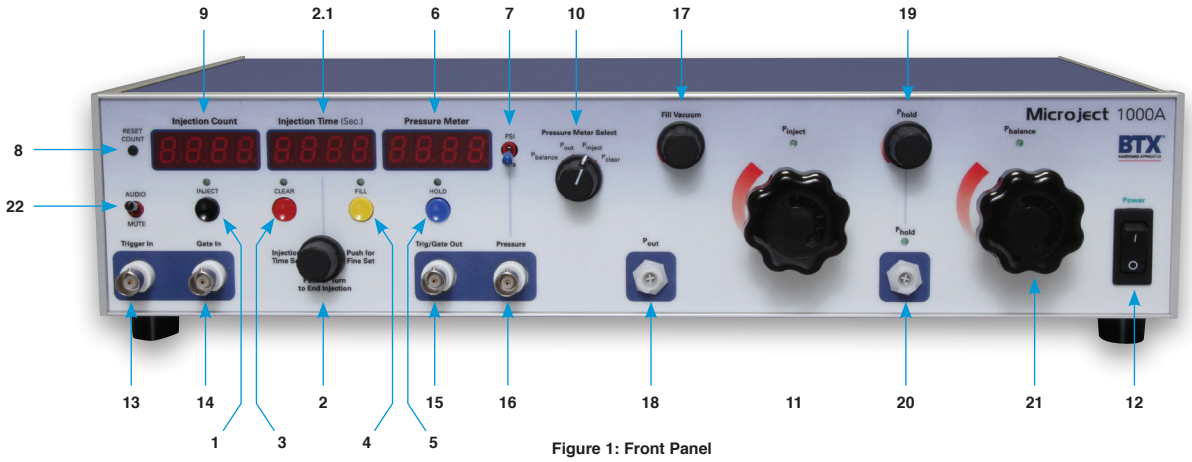

Figure 1: Front Panel

Refer to Figure 1 for the location of controls and connectors. Numbers refer to the order of listing below:

1. INJECT pushbutton

Push this to manually trigger the injection pressure for a time set by the internal timer. The switch remains lit for the duration of the injection.

2. INJECT TIME SETTING DIAL

This dial controls the injection time setting. Turning the dial changes the injection time in seconds; pressing the dial in and turning changes the injection time in fractions of a second.

2.1 INJECT TIME Display

Displays injection time in seconds.

3. CLEAR pushbutton

Push this briefly to deliver a half second pressure surge to clear a clogged pipette. The pressure applied is the supply pressure. If the button is left pushed in for longer than a half second, the clearing surge is extended. The button remains lit for the duration of the clear.

4. FILL pushbutton

Push the button to apply suction to the delivery pipette to fill it from the tip. The suction is applied as long as this button is held and LED is lit.

5. HOLD pushbutton

Push this button to apply sufficiently small suction to holding pipette (LED is lit) to hold a cell for injection. Push this a second time to drop this suction to zero.

6. PRESSURE display

This three digit display gives the “gauge” pressure selected by the PRESSURE METER SELECT switch. A negative (-) sign indicates suction.

7. PRESSURE UNITS toggle switch

Toggle switch controls injection pressure display unit of measure. Injection pressure can be displayed in either pound-force per square inch (psi) or in kilopascals (kPa) 1 psi = 6.89 kPa

8. RESET pushbutton

This button resets the injection count display to zero.

9. INJECTION COUNT display

This four digit display (0-9999) gives the total number of injections triggered manually by the panel pushbutton or footswitch or electrically since the last reset.

10. PRESSURE METER SELECT switch

This switch selects various places inside the Microject 1000A for reading the pressure. It does not change the pressure applied to the output. P_{INJ} is the pressure applied at the output during injection. P_{BAL} is the pressure applied to the pipette when injection is not taking place. P_{CLR} is the pressure externally supplied to the input on the rear panel and used the CLEAR mode. P_{FILL} is the negative pressure applied to fill the injection pipette from the tip. P_{OUT} is the pressure currently applied to the output port.

11. P_{INJECT} regulator

This seven turn control is used to set the injection pressure over the range from about 0.4 to 60.0 psi (about 2.8 to 413 kPa). Clockwise rotation increases the pressure. Locking nut should be loose to avoid damaging the shaft’s threads if changing pressure constantly.

12. POWER rocker switch

Controls power to the unit.

13. TRIG IN connector

This BNC connector is for electrically initiating injection. A positive TTL level pulse is required (baseline 0.0 V, trigger 5 V, lasting at least 50 microseconds).

14. GATE IN connector

This BNC connector is for external timing of the duration of injection. A positive TTL level pulse (see **13. TRIG IN** connector) is required with the duration of injection determined by the duration of the applied pulse.

Front Panel Connectors & Controls

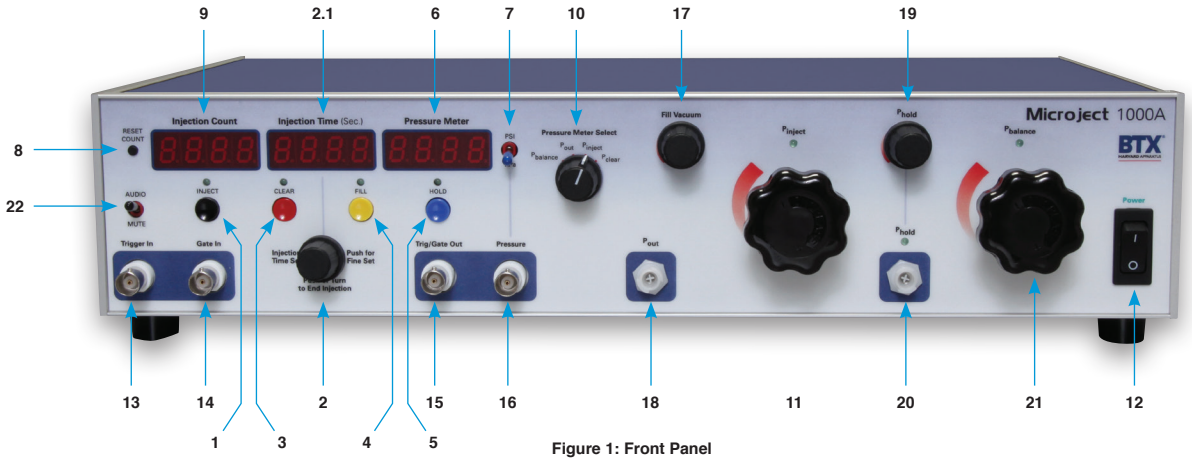

Figure 1: Front Panel

15. TRIG/GATE OUT connector

This TTL output (see **13. TRIG IN** connector) can be used to trigger or synchronize other electrical instruments. Its duration is that of the injection.

16. PRESSURE MONITOR out

This BNC connector delivers an electrical level whose amplitude gives the actual pressure applied to the meter. The conversion factor is 10 mV/psi or 1 mV/kPa.

17. FILL VACUUM regulator

This regulator sets the vacuum applied to the P_{OUT} connector. The range is 0 to -12 psi (0 to -82 kPa, regulated).

18. P_{OUT} connector

This connector is attached to the injection pipette using the supplied output hose. Without the hose attached the port is closed. In use, the injection, balance, fill, and clear pressures are delivered here.

19. P_{HOLD} regulator

This regulator sets the (low) suction pressure applied to the P_{HOLD} connector. Counterclockwise increases the pressure. Like the other pressure controls the current rotational position is uniquely related to the pressure. The range is 0 to 1.25 kPa (-12.7 cm of water).

20. P_{HOLD} connector

The supplied holding hose is attached to this connnector. The suction set by the P_{HOLD} regulator is applied here to hold a suspended cell for injection.

21. P_{BAL} regulator

This regulator sets the balance pressure over the range from 0.1 to 10 psi (about 0.7 to 70 kPa). Clockwise rotation increases the pressure.

22. AUDIO / MUTE switch

Controls injection sound.

Rear & Bottom Panel Connectors & Attachments

Figure 2: Rear Panel

1. Foot Switch Inputs (Connectors)

An optional footswitch (45-0770) can be connected to any one of these connectors. When connected to HOLD, pushing the footswitch once turns on the suction for holding a cell. Pushing it a second time turns that suction off. When connected to INJ, pushing the footswitch starts injection. When connected to FILL, the filling suction is applied as long as the footswitch is depressed. When connected to GATING, injection pressure continues as long as the footswitch is depressed.

2. Pressure Input Connector

To have all the functions working, the instrument needs 70 to 105 psi. To have only injection, 70 psi is needed. This connector is the input for the compressed gas. See PRELIMINARIES for recommendations on the gas. A maximum of 105 psi can be safely applied; 100 psi is optimal. At lower pressure gas will be used at a slower rate. Avoid input pressures less than 100 psi if the FILL function is being used.

3. Input Filter

The MicroJect 1000A is supplied with an input filter. It traps particles larger than 0.5 micron as well as the liquid often present in a building's compressed air lines. Drain by pushing the button on the underside of its transparent case through a hole located in the bottom case.

4. Filter Window

Allows for easy viewing of filter to determine when it needs to be drained.

5. Power Input Connector

Jack for universal AC input 9 volt 6 watt DC output power supply.

Figure 3: Bottom Panel/Input Filter

General Considerations

It is much easier to reliably inject large volumes than small ones. For “large” volumes, the balance pressure capability is not needed. Pipettes seldom clog so the clear capability is also not needed.

The dividing line between large and small is not rigid; it depends on how quantitative a delivery is required. That volume line would typically be in the 10-100 pL range. For convenient visualization and approximate geometric measurement, 1 fL is a cube 1 micron on a side or a sphere 1.24 microns in diameter, 1 pL is a cube 10 microns on a side or a sphere 12.4 microns in diameter, while 1 nL is a cube 100 microns on a side or a sphere 124 microns in diameter. Visual estimates are useful because volume goes as the cube of a linear dimension.

Extracellular delivery is nearly always “large”. Intracellular is often of “small” volumes (except for frog oocytes). A more quantitative way to distinguish between “large” and “small” is given in the balance section on page 14.

Interconnections and Initial Set-Up

Connect the gas input hose to the rear panel input connector of the MicroJect 1000A Pico-Injector. Connect the other end (with optional input hose adapter, if needed) to the gas supply. Turn on the POWER switch and verify the input gas pressure with the digital meter by setting the PRESSURE METER SOURCE switch to P_{CLR}. Practice with the inject and balance pressure controls by first turning the METER SELECT switch and then adjusting each in turn.

With non zero values of inject and balance pressure set the PRESSURE METER SOURCE switch to P_{OUT}. Set the inject time to five seconds and push the panel INJECT switch to see the temporary change in pressure from balance to inject and back. A buzzer will sound during injection. If this is not wanted, turn off the switch on the rear panel.

NOTE: *The PRESSURE METER SELECT switch must be set to P_{OUT} to read changes in pressure on the display or MONITOR output.*

Two output hoses are supplied with the MicroJect 1000A Pico-Injector. These are designed for any of the three optional pipette holders described in the Ordering Information section. Connect the hoses to the chosen holder(s) as follows:

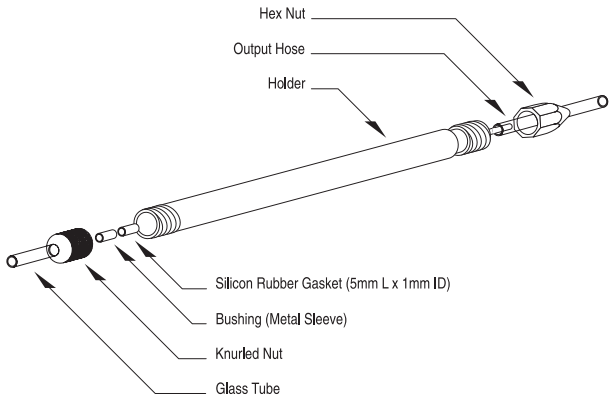

Figure 4: MicroJect 1000A 1.0 mm OD Glass Pipette Assembly

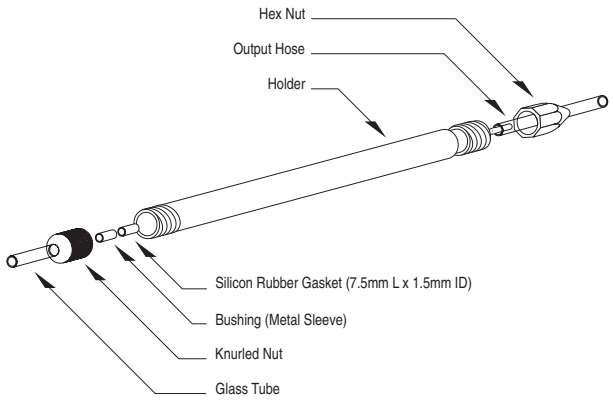

Figure 5: MicroJect 1000A 1.5 mm OD Glass Pipette Assembly

Glass Pipette Assembly

The micropipette is shipped with a cleaning wire in place. This wire is supplied in the case that a glass capillary breaks inside the micropipette. Use this wire to push out all the pieces of glass.

Unscrew the end of the holder with the 2 mm diameter hole and place it over the output hose. Reattach this fitting securely with the hose inserted as far as it will go. The 1.5 mm OD capillary is similarly attached to the other end of the holder through its “O” ring.

Attach output and holding hose to the appropriate front panel gas ports. Tighten them securely so that the valves within these ports are open, allowing pressure to be controlled in the hose and connecting micropipette.

Micropipette Filling

Filling can be done from the tip of the micropipette, or from the barrel of the pipette. Filling from the tip is most suitable if a filling fiber is deliberately not being used due to irregularities in the balancing (see BALANCE below). Simply prepare a bolus of injection material on a glass slide and maneuver the tip into it using a micromanipulator. Depress the FILL button to apply about 12 psi (82 kPa) of suction continuously as long as needed. If evaporation of a small bolus is a concern, it can be prepared under oil and the micropipette tip similarly moved into it.

Filling can be done from the back end (barrel) of the pipette using a syringe with a thin hypodermic needle inserted so its tip is down in the tapered section. If the capillary has a filling fiber attached to the inner wall the pipette tip will then fill by capillary action without air bubbles, at least for larger tips. Variations on this procedure involve filling a smaller capillary first and inserting it for the back fill.

Balance

The inflow into the pipette (caused by capillary forces) prior to or in-between injections should be a small percentage of that being injected. As the volume desired gets smaller, the relative inflow gets larger and faster due to the smaller tip size being used.

To avoid this problem, set the balance pressure before placing the pipette in the cell's external medium. Ten percent of the injection pressure is a good starting value. Exact balance is difficult to determine, often the fastest way to handle this is to set the balance high enough that slight outflow is observed. Balance is assessed by watching the movement of the liquid meniscus in the pipette. The outflow left is still small compared to the continuous streaming used in the continuous ejection technique.

If a filling fiber is used, the capillary inflow is larger so a higher balance pressure is needed. In general we do not recommend a capillary with filling fiber when using the balance module. If a user does use a glass capillary with filling fiber the injection material could run out spontaneously or the pressure needed to balance will abruptly change with time. These issues can be caused by particles lodging on the fiber.

An estimate of the inflow can be made visually. Focus the microscope on the liquid meniscus in the loaded pipette while its tip is still in air and with the balance pressure set to zero. Use an eyepiece reticle to measure the meniscus movement when liquid is then raised to cover the tip. The inflow volume is approximately the difference between the volume of two cones. The estimate is more precise for smaller initial volumes in the pipette.

Clear

To clear a clogged micropipette, push the CLEAR button momentarily once the tip has been removed from a cell. Watch the tip region while this is done to see the brief movement of liquid denoting that the tip is clear. Repeat if needed. The clearing pressure pulse has a preset duration of 500 ms to avoid excessive release if the clog clears immediately. Repeat as needed.

Hold

To hold a suspended cell for injection, attach a suitable holding pipette to the pipette holder/holding hose. Momentarily attach to the P_{OUT} port. Fill the holding pipette (using the fill function switch) with fluid so that tube length is half to three quarters filled.* Disconnect the holding hose connector from the P_{OUT} port and attach to the P_{HOLD} port. Set the hold pressure control to minimum and move the micropipette to the cell using a micromanipulator. Turn on the HOLD panel switch and increase the hold pressure as needed. When finished with that cell turn off the hold pressure with the panel or footswitch. If more cells of the same kind are being injected then subsequent holds require only the ON/OFF control of the switch. If the cell doesn't drop off the holding pipette tip when hold is turned off, a brief, gentle squeeze of the rubber bulb located on the holding output hose should release it.

***NOTE:** *Be careful not to overfill the hose to avoid liquid going into the pneumatic system inside the unit.*

Footswitch(es)

Optional footswitches can be used for inject, fill, and turning on and off the hold function. These switches are plugged into the rear panel, each is wired in parallel with the corresponding front panel switch. See the instructions for each panel switch to see how they operate. When a footswitch is used with the GATE connector, the duration of injection is manually determined by the duration of pressing the footswitch. In some applications, users may prefer to set up the duration of injection manually: connect the MONITOR output to an oscilloscope or fast enough strip chart recorder to measure this time and then switch to the internal timer for subsequent injections.

Electrical Inputs/Outputs (Items 13-16 in Figure 1)

These connectors allow additional control, especially when synchronizing injection to other stimulations or recording. In particular, the PRESSURE MONITOR output signal is a precise measure of relative injection volume (for a given pipette). Since the volume delivered is linear in the net injection pressure (P_{INJ} -P_{BAL}) and injection time, the area under a graph of this signal versus time is a measure of the relative volume.

Injection Volume Adjustment

Adjust injection volume by changing either injection pressure, injection duration, or the micropipette (inside) tip diameter or taper. The dependence on (net) injection pressure, duration and pipette taper angle is linear; the dependence on tip diameter is cubic.

Micro-Injection Substances/Cell Types

A wide range of substances have been micro-injected onto or into a large number of cell types, such as germ cells, embryos, cultured mammalian cells and plant protoplasts.

Setting Pressures and Time Pulses

1. **Balance Pressure**

   Set the balance pressure while viewing the pipette under magnification. This method will help the user to stabilize the solution within the pipette easily.

2. **Injection Pressure and Time Pulse**

   Setting the initial injection pressure low prevents the loss of solution. To easily obtain the desired pressure setting, set the time pulse on (1) one second with the injection pressure set at its minimum. Trigger the time pulse while viewing under magnification. Increase the injection pressure until the solution within the pipette begins to flow out the tip opening. The pressure shown on the LCD can now be used as the initial injection pressure setting. Adjust the injection pressure and timing to obtain the desired injection.

3. **Holding Pressure**

   Set the holding pressure at the minimum. This will prevent possible damage to the cell. Fill 3/4 of the pipette's capillary tube with a solution (medium). This serves as a buffer to prevent a constant vacuum at the pipette tip.

Gas Usage Warning

To provide finely controllable output pressure, the internal gas regulators are of the "bleeding" type. Such regulators use gas even in the absence of ejections.

To eliminate this consumption and as a good safety practice, turn off the gas supply at the source when the MicroJect 1000A Pico-Injector is not in use.

The suction functions (HOLD and FILL) use much more gas than INJECT and BALANCE. Plan accordingly. Notice, that the BALANCE is on continuously once an output hose is attached to the front panel: without a micropipette attached, this gas usage can be significant.

Hose Connections

The input, output, and holding hoses should be attached to their respective connectors. If each connector's needle valve, located in the micro-injector body, is not fully opened, the airflow will be restricted or blocked. To prevent this from happening, check each connector for tightness by turning clockwise. This will ensure needle valve depression.

Volume Calibration Chart

Formula

Volume in Nanoliters = .17952(*) x (tip I.D. in micron)^3 x (Pressure in psi) x (Time in sec)

Example

Volume = .17952 x (5)^3 x (10) x (1) Nanoliters = 224.40 Nanoliters

Pressure (psi)	Time (seconds)	Pipette Tip I.D. (µm)	Femtoliters	Picoliter	Nanoliter	Microliter	Milliliter
1	1	0.1	179.52	0.18	-	-	-
10	1	0.1	1795.20	1.80	-	-	-
20	1	0.1	3590.40	3.59	-	-	-
30	1	0.1	5385.60	5.39	-	-	-
40	1	0.1	7180.80	7.18	-	-	-
50	1	0.1	8976.00	8.98	-	-	-
60	1	0.1	10771.20	10.77	-	-	-
1	1	1	-	179.52	0.18	-	-
10	1	1	-	1795.20	1.80	-	-
20	1	1	-	3590.40	3.59	-	-
30	1	1	-	5385.60	5.39	-	-
40	1	1	-	7180.80	7.18	-	-
50	1	1	-	8976.00	8.98	-	-
60	1	1	-	10771.20	10.77	-	-
1	1	5	-	-	22.44	0.02	-
10	1	5	-	-	224.40	0.22	-
20	1	5	-	-	448.80	0.45	-
30	1	5	-	-	673.20	0.67	-
40	1	5	-	-	897.60	0.90	-
50	1	5	-	-	1122.00	1.12	-
60	1	5	-	-	1346.40	1.35	-
1	1	10	-	-	179.52	0.18	-
10	1	10	-	-	1795.20	1.80	-
20	1	10	-	-	3590.20	3.59	-
30	1	10	-	-	5385.60	5.39	-
40	1	10	-	-	7180.80	7.18	-
50	1	10	-	-	8976.00	8.98	-
60	1	10	-	-	10771.20	10.77	-
1	1	75	-	-	-	75.74	0.08
10	1	75	-	-	-	757.35	0.76
20	1	75	-	-	-	1514.70	1.51
30	1	75	-	-	-	2272.05	2.27
40	1	75	-	-	-	3029.40	3.03
50	1	75	-	-	-	3786.75	3.79
60	1	75	-	-	-	4544.10	4.54

(*) Constant includes unit conversion factors, and effects of water like viscosity and needle taper for a cone half angle of 10 (medium taper).

Power Entry Module (PEM)

The Microject 1000A is equipped with a universal AC input 9 volt 6 watt DC output power supply with interchangeable input blades.

1. Slide the blade lock in the direction of the arrow with one hand and lift out the contact protector with your other hand.
2. Align the rounded corners of the input blade being installed with the rounded corners on the adapter.
3. Lower the square corners of the blade into the adapter.
4. Pull back on the blade lock and lower the rounded corners into adapter, release the blade lock.

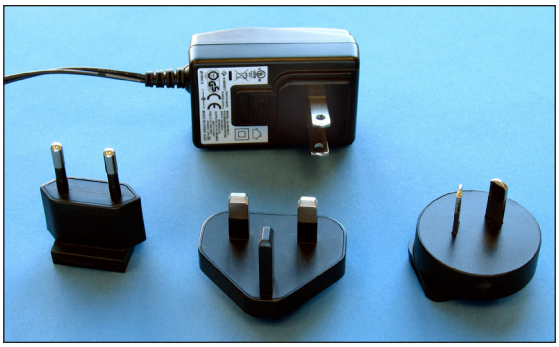

Figure 1: Wall adapter with interchangeable input blades

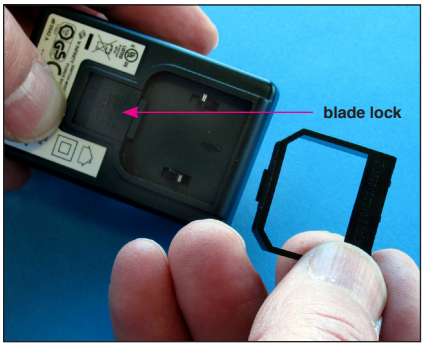

Figure 2: Remove contact protector

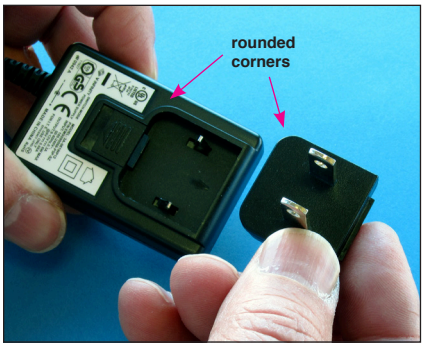

Figure 3: Install input blade

Figure 4: Complete assembly

Ordering Information

Item #	Description
45-0750	**BTX MICROJECT 1000A BASIC SYSTEM Includes:** MicroJect 1000A pico-injector with Injection, Balance, Clear/Fill and Hold Pressure and Power Cord
45-0751	**BTX MICROJECT 1000A PLUS SYSTEM Includes:** MicroJect 1000A pico-injector with Injection, Balance, Clear/Fill and Hold Pressure, one Footswitch, Input/Output Hoses, Holding Hose, one Pipette Holder and Input Adaptor for Hoses and Power Cord
45-0752	**BTX MICROJECT 1000A MAX SYSTEM Includes:** MicroJect 1000A pico-injector with Injection, Balance, Clear/Fill and Hold pressures, two Footswitches, Input/Output Hoses, Holding Hose, two Pipette Holders and Input Adaptor for Hoses and Power Cord
Accessories	
45-0762	Acrylic Pipette holder for 1.0 mm pipettes
45-0763	Acrylic Pipette holder for 1.2 mm pipettes
45-0764	Acrylic Pipette holder for 1.5 mm pipettes
45-0765	Acrylic Pipette holder for 2.0 mm pipettes
45-0766	Stainless Steel Pipette Holder, 130 mm length, for 1 to 1.5 mm OD pipettes
45-0767	Stainless Steel Pipette Holder, 80 mm length, for 1 to 1.5 mm OD pipettes
45-0768	Pipette Holder Hardware Replacement Kit for use with Stainless Steel Pipette Holders, Includes 1 each Stainless Steel Bushing Replacement, Stainless Steel Cap Replacement, Stainess Steel Hose Nut Replacement, and 1.5 mm Silicone Rubber Gasket Replacement
45-0769	Stainless Steel Pipette Holder Adapter for Eppendorf ECET FEMTOTIP
45-0770	MicroJect 1000A Footswitch, compatible with Inject, Hold, Fill, and Gate Foot Switch Inputs
45-0771	MicroJect 1000A Input Hose Adaptor
45-0772	Output Hose (Tygon Tubing) 9 ft L x 0.071 in (1.80 mm) OD x 0.039 in (1 mm) ID x 0.016 in (0.41 mm) Wall Thickness; Connects to Pipette Holders, Brass Screw Fitting
45-0773	Gas Input Hose (Tygon Tubing) 9 ft L x 0.250 in (6.35 mm) OD x 0.125 in (3.18 mm) ID x 0.062 in (1.57 mm) Wall Thickness; Connects to MicroJect 1000A Gas Input, Quick Connect/Disconnect Type
45-0774	Output Hose (Tygon Tubing) 9 ft L x 0.071 in (1.80 mm) OD x 0.039 in (1 mm) ID x 0.016 in (0.41 mm) Wall Thickness; Connects to Pipette Holders, Brass Screw Fitting
45-0775	Output Hose (Tygon Tubing) 9 ft L x 0.071 in (1.80 mm) OD x 0.039 in (1 mm) ID x 0.016 in (0.41 mm) Wall Thickness; Connects to Pipette Holders and MicroJect 1000A Gas Output, Quick Connect/Disconnect
45-0776	Holding Hose with Cell Release Bulb 9ft L x 0.071 in (1.80 mm) OD x 0.039 in (1 mm) ID x 0.016 in (0.41 mm) Wall Thickness. Pipette Holders and MicroJect 1000A Hold Output, Brass Screw Fitting
45-0777	Holding Hose with Cell Release Bulb 9 ft L x 0.071 in (1.80 mm) OD x 0.039 in (1 mm) ID x 0.016 in (0.41 mm) Wall Thickness. Pipette Holders and MicroJect 1000A Hold Output, Quick Connect/Disconnect
45-0780	Silicone Rubber Gasket Replacement for use with 1.0 mm pipettes
45-0781	Silicone Rubber Gasket Replacement for use with 1.5 mm pipettes
45-0782	Bushing Replacement for Pipette Holders
45-0783	Knurled Nut Replacement for Stainless Steel Pipette Holders
45-0784	Replacement Hex Hose Nut for Stainless Steel Pipette Holders
Related Products from Harvard Apparatus	
PMP-102	Model PMP-102 Programmable Single-Barrel Micropipette Puller
PMP-107	Model PMP-107 Programmable Multi-Barrel Micropipette Puller
MFG-5	Microforge Grinding Center-for glass micropipette polishing, shaping, tipping, bending, beveling and grinding