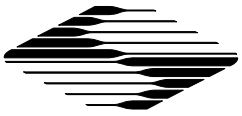
OPERATION MANUAL

REV. 4.13 (20100914)

XENOWORKS™ DIGITAL MICROINJECTOR



SUTTER INSTRUMENT

XenoWorks™

Digital Microinjector

Operation Manual

(Rev. 4.13 ((20100914))

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1. GENERAL INFORMATION

1.1 About This Manual

The XenoWorks Digital Injector is a injection system comprised of three basic parts: the controller/compressor, injection tubing, and the user interface (remote and foot switch).

In the next, few pages you will find a product description to help you become acquainted with the parts, followed by installation instructions, and then detailed operating instructions. Please take the time to read these instructions to assure the safe and proper use of this instrument.

1.2 Technical Support

Unlimited technical support is provided by Sutter Instrument Company at no charge to our customers. Our technical support staff is available between the hours of 8:00 AM and 5:00 PM (Pacific Time) at **(415) 883-0128**. You may also E-mail your queries to **info@sutter.com**.

2. SAFETY WARNINGS AND PRECAUTIONS

Please read this manual carefully before operating the instrument.

2.1 Electrical

- **Use only a properly grounded power source and power cord; both appropriately rated for use with this instrument.**
- **Before operating the instrument, check that the instrument's voltage rating corresponds to the supply voltage. The voltage rating can be found on the power entry module on the rear of the instrument.**
- **Before making electrical connections, ensure that the instrument is switched off.**
- **⚠ Replace fuse only with the same type and rating as indicated in the following table.**

Table 2-1. Mains fuse type and ratings.

Mains Power Source	Mains Voltage Setting	Fuse (Type: Time Delay, 5mm x 20mm, glass tube)	
		Fuse Rating	Manufacturer Examples
110 VAC	"110" (100 – 120 VAC)	4A, 250V	Bussmann: GMC-4A, GMC-4-R (RoHS), GDC-4A, or S506-4A (RoHS) Littelfuse: 239 004, 239.004.P (RoHS), 218.004, or 218.004.P (RoHS)
220 VAC	"220" (200 – 240 VAC)	2A, 250V	Bussmann: GDC-2A or S506-2A (RoHS) Littelfuse: 239 002 or 239.002.P (RoHS)

A spare fuse is provided with fuse holder located in the power input module. Please refer to "Appendix C - Fuse Replacement" for instructions on how to change the fuse.

- **To prevent fire or shock hazard do not expose the unit to rain or moisture.**
- **To avoid electrical shock and exposure to hazardous electrical voltages:**
  - **Do not disassemble the unit. Refer servicing to qualified personnel.**
  - **Always use the grounded power supply cord provided to connect the unit to a grounded outlet (3-prong). This is required to protect you from injury in the event that an electrical hazard develops.**
- **To comply with FDA and CE electromagnetic immunity and interference standards; and to reduce the electromagnetic coupling between this and other equipment in your lab always use the types and lengths of interconnect cables provided with the unit for the interconnection of the remote user interface and foot switch.**

2.2 Precautions

Failure to comply with any of the following precautions may damage this device.

- Please read this manual carefully before operating the instrument.
- Use this instrument only for microinjection purposes in conjunction with the procedures and guidelines in this manual.
- Do not operate if there is any obvious damage to any part of the instrument.
- Do not operate this instrument near flammable materials.
- Do not obstruct the side vents or cooling fan intakes.
- As with all microinjection devices, sharp micropipettes can fly out of their holder unexpectedly. Always take precautions to prevent this from happening. Never loosen the micropipette holder chuck when the tubing is pressurized, and never point micropipettes at yourself or others. Always wear safety glasses when using sharp glass micropipettes with pressure microinjectors.
- This instrument is designed for use with capillary glass micropipettes with an outer diameter of 1mm. For glass with outer diameters of 1.2 and 1.5 mm, please contact Sutter Instrument to order an additional holder.
- Adapters for other capillary diameters are available upon request.
- Use this instrument only for microinjection purposes in conjunction with the procedures and guidelines in this manual.
- Please retain the original packaging for future transport of the instrument.
- Some applications, such as piezo-impact microinjection call for the use of mercury in the micropipette tip. The use of any hazardous materials with any XenoWorks instrument is not recommended and undertaken at the users' own risk.
- Always transport the instrument in its original packaging, and install shipping plate screws before shipping.
- This instrument contains no user-serviceable components — do not open the instrument casing. This instrument should be serviced and repaired only by Sutter Instrument or an authorized Sutter Instrument servicing agent.
- This device is intended only for research purposes.
- Sutter Instrument reserves the right to change specifications without prior notice.

3. PRODUCT DESCRIPTION

3.1 Packing List

The XenoWorks™ Digital Microinjector is shipped with the following components.

If any items are missing or damaged, contact Sutter Instrument immediately.

1. Digital Microinjector cabinet (controller/compressor)
2. Digital Microinjector control box (remote user interface)
3. Cable to connect remote user interface with controller.
4. Foot switch
5. Micropipette holder (2)
6. Tubing kits (2), each including 2.5 m (8 ft) clear ETFE tubing, pressure fitting, 5 spare ferrules
7. Power cord
8. Instruction Manual

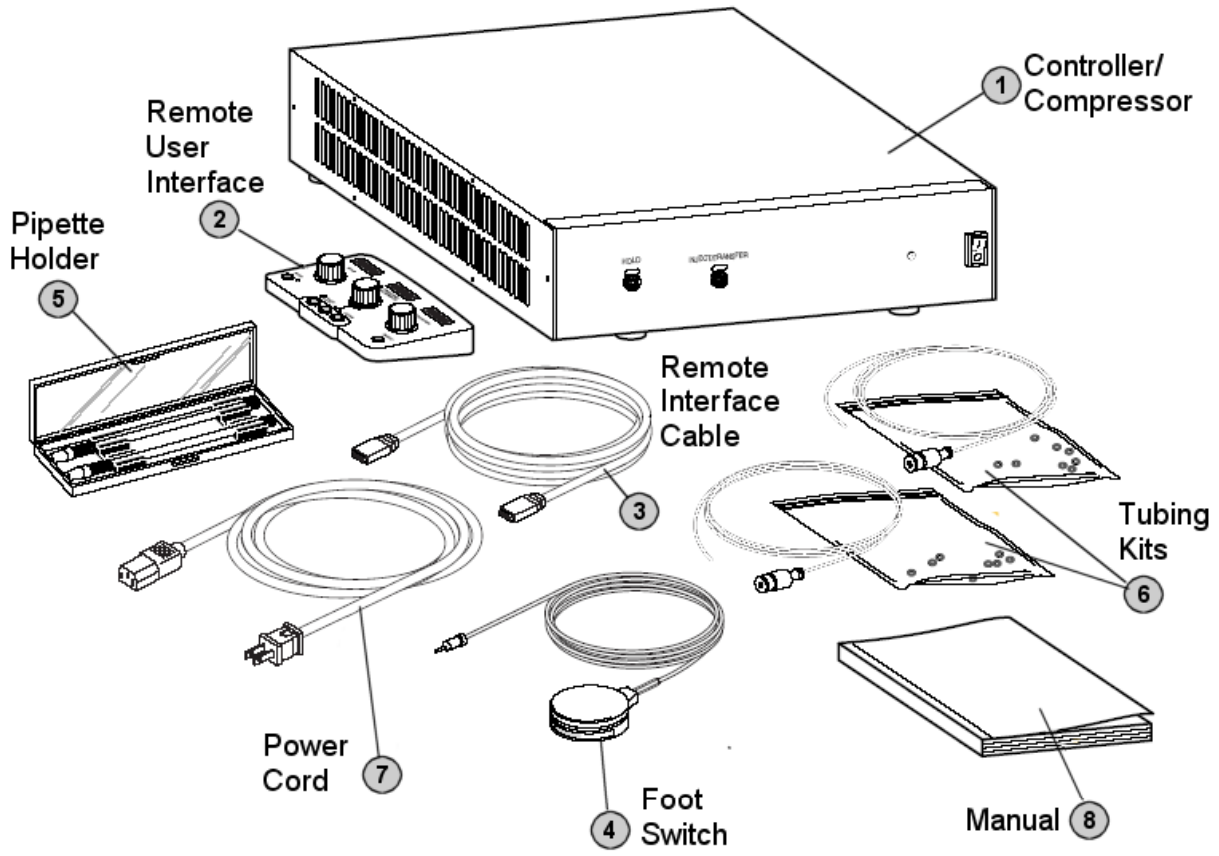

Figure 3-1. Components of the XenoWorks Digital Microinjector.

3.2 Instrument Design

The XenoWorks Digital Microinjector is a pneumatic pressure control system for single-cell microinjection and manipulation. Two independently controlled pressure channels are available: Hold and Inject/Transfer.

The **Hold Channel** is designed to create negative and positive pressure sufficient to gently hold and release a single cell, usually a mammalian oocyte, zygote or early embryo (including fully hatched blastocyst stage embryos), against a hollow, fire-polished glass micropipette. Ascidian, amphibian and insect embryos can be immobilized in a similar fashion, depending upon their physical characteristics and the type of micropipette used.

The **Inject/Transfer Channel** provides negative and positive pressures required to aspirate single cells (such as embryonic stem cells) into an appropriately fashioned micropipette in preparation for transfer to another location (such as the blastocyst of an early mammalian embryo). The transfer of cells is achieved by reversing the pressure in the transfer micropipette and gently injecting.

There are four range settings available for the Transfer/Compensation channel, allowing for the fine-tuning of the pressure range for a given application.

In addition to these low positive and negative pressures, the **Inject/Transfer Channel** is capable of providing the high positive pressure required to inject solutions through very narrow (typically less than one micron) injection micropipettes (during, for example, the injection of DNA solution into the pronucleus of a mammalian “2PN” stage zygote). Users should refer to later sections of this manual for more information on specific microinjection applications, and to the application note “Introduction to Microinjection” available from Sutter upon request, or for download from http://www.sutter.com.

3.3 Identification of Instrument Components and Controls

The following figures illustrate the features on the front and rear of the controller/compressor and the controls on the remote user interface .

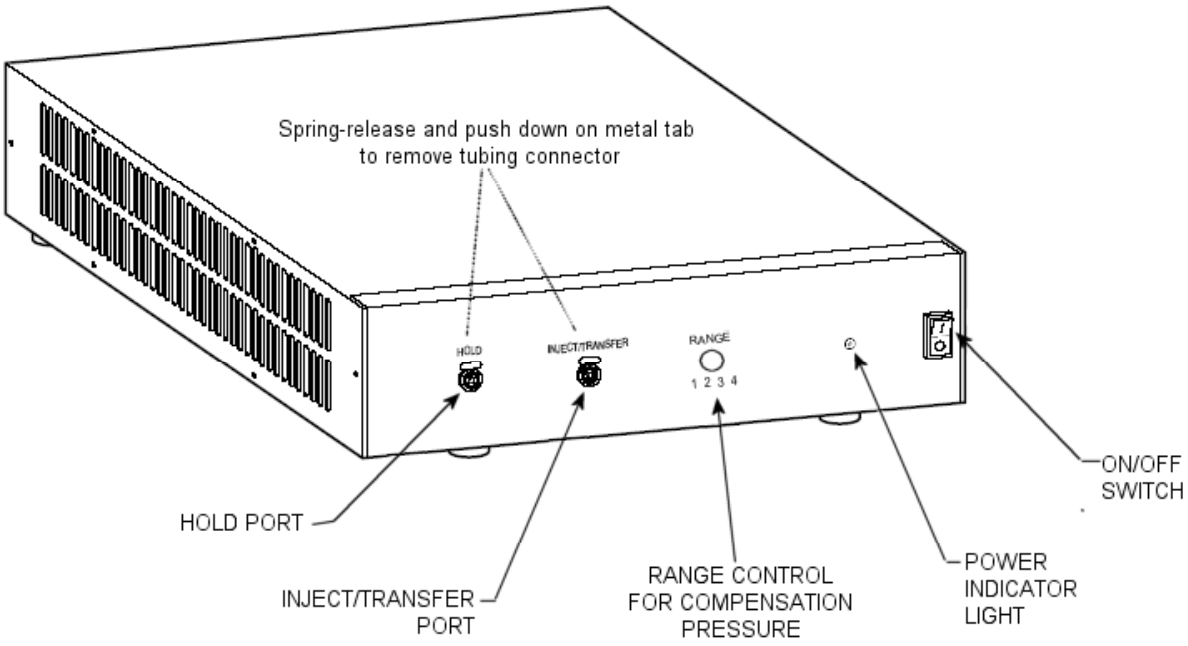

Figure 3-2. Controller/Compressor features (front view).

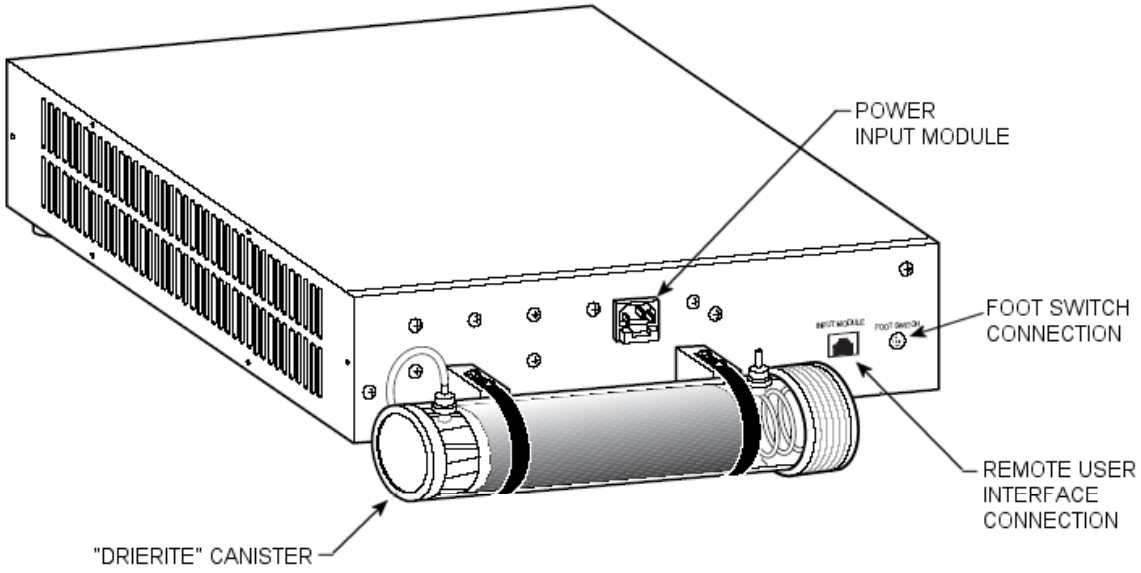

Figure 3-3. Controller/Compressor features (rear view).

3.3.1 Drierite Canister

The XenoWorks Digital Microinjector incorporates an integral compressor, which takes its air supply from the ambient air. The canister of Drierite granules dries the air before it is compressed, to ensure a minimum of condensation and a long working life for the compressor components. The blue Drierite granules slowly turn pink as they absorb moisture from the input air. Once all the granules have turned pink, the Drierite should be desiccated or replaced (described in the Maintenance chapter later in this manual). The approximate life of the Drierite canister is one year under normal conditions.

3.4 Controls and Features

3.4.1 Hold Port

This port supplies a pressure range from –350 hPa to +350 hPa (± 7 hPa) and is typically used to control the pressure in the holding micropipette. The pressure can be varied using the Hold Dial on the remote user interface.

3.4.2 Inject/Transfer Port

This port supplies four possible transfer/compensation ranges. Range setting 1 is the default setting, providing from –175 hPa to +175 hPa (± 7hPa), and is typically used to control the pressure in the transfer micropipette during embryonic stem cell transfer experiments, and to create a compensation pressure for high-pressure injection of solutions. The compensation pressure can be varied using the Transfer Dial on the user interface module. The pressure pulse duration for pronuclear and adherent cell injection can be set with the Pressure Pulse Width dial.

3.4.3 Range Setting for Transfer and Compensation Pressures

This rotary switch is used to set the compensation pressure range on the Inject/Transfer port. The following table shows the compensation pressure range for each of the four settings.

Table 3-1. Pressure/Vacuum range settings.

Range Setting	Scale	Pressure				Applications
		Range		1 Unit/Transfer Dial (approx.)		
		psi	hPa	hPa	psi	
1	Low	+2.5 to -2.5	+172 to -172	1	0.015	Standard setting for ES Cell, Pronuclear Injection, and Adherent Cell microinjection
2	Medium	+5 to -5	+345 to -345	2	0.03	Used when higher compensation pressures are needed for smaller volumes back-loaded into injection needle
3	Medium-High	+10 to -7	+689 to -483	4	0.06	
4	High	+15 to -7	+1034 to -483	8	0.12	Used for front-loading pipette with very small volumes (femtoliters) and to create higher compensation pressures for these small volumes.

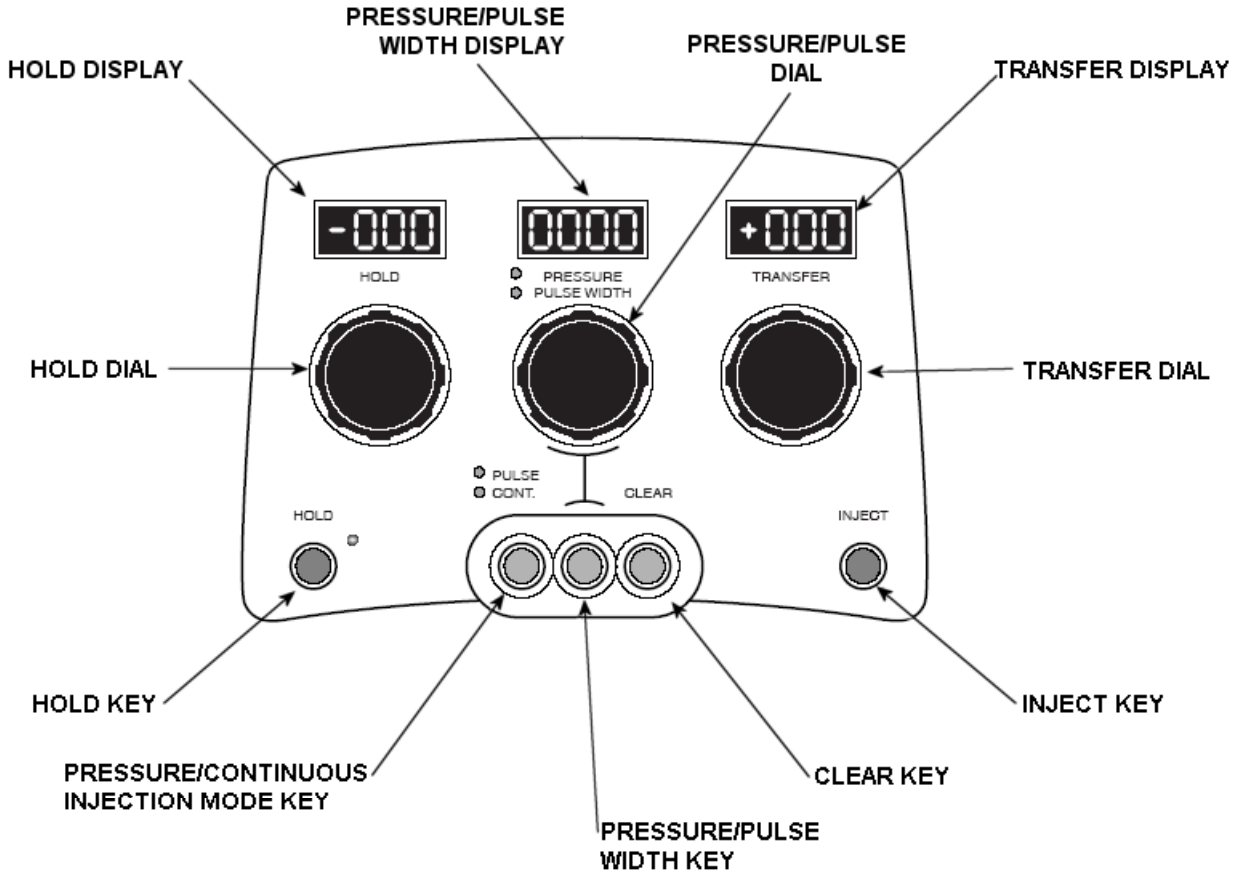

Figure 3-4. Remote User Interface`.

3.4.4 Hold Key

This key is used to toggle the **Hold** pressure on and off. In the OFF position, no pressure is applied to the **Hold Port**. In the ON position, the pressure, in hPa, shown in the **Hold LED Display** (above the **Hold Dial)** is applied to the **Hold Port**.

3.4.5 Hold Dial

Controls the pressure delivered to the **Hold Port** when the **Hold Key** is in the ON position. The range is, continuous from –350 hPa to +350 hPa (± 7 hPa).

3.4.6 Hold Display

Displays the pressure delivered to the **Hold Channel** (in hPa).

3.4.7 Pulse/Continuous Injection Mode Key

This key toggles between the pulse mode and continuous mode used for high-pressure injections. The pulse pressure and duration are set with the **Pressure/Pulse Width Dial.** The continuous pressure is active as long as the **Inject Key** or footswitch is held down. The mode selected and active is indicated with a green LED.

3.4.8 Pressure/Pulse Width Dial

This dial is used to adjust the pressure and duration of injection. Injection pressure can be adjusted from 0 to 5600 (± 50 hPa) in 70 hPa increments. Pulse duration can be adjusted from 0.01 to 10 seconds in 0.01-second increments.

3.4.9 Pressure/Pulse Width Display

This display indicates either the injection pressure (in hPa) or the injection duration (in seconds) when in Pulse Mode. Toggling between these two values is accomplished with the **Pressure/Pulse Width Key**.

3.4.10 Pressure/Pulse Width Key

This key is used to toggle the **Pressure/Pulse Width Dial/Display** between "Pressure" and "Pulse Width".

3.4.11 Clear Key

Applies maximum pressure to the right hand (**Inject/Transfer**) channel and is used to clear blockages of the micropipette. When the key is pressed and held down, the pressure applied increases from zero to maximum pressure (80 psi).

CAUTION: THE CLEARING FUNCTION SHOULD NOT BE USED WITH MICROPIPETTES THAT HAVE TIP INNER DIAMETERS GREATER THAN APPROXIMATELY ONE (1) MICRON.

3.4.12 Inject Key

This key applies the pressure (set by the center **Pressure/Pulse Width Dial**) to the Inject/Transfer pressure port. When the instrument is in **Pulse Mode**, the duration of the pressure will be for the time set by the **Pulse Width Dial**. When the instrument is in **Continuous Mode**, the pressure is applied for as long as the **Inject Key** (or footswitch) is held down.

3.4.13 Transfer Dial

This dial controls the pressure continuously supplied to the Inject/Transfer Port. Range Setting 1 (standard setting) provides –175 hPa to +175 hPa (± 7 hPa) of pressure.

3.4.14 Transfer Display

This display indicates the low pressure being constantly delivered to the **Transfer Channel** (in hPa).

4. INSTALLATION

NOTE: These instructions refer to the use of the XenoWorks Digital Microinjector with the XenoWorks Micromanipulator. While the Digital Microinjector is supplied with micropipette holders that are compatible with a number of different micromanipulators, the setup procedure used with those micromanipulators may vary slightly.

1. Unpack the XenoWorks Digital Microinjector.
2. Remove the (4) shipping screws and plate from the right side of the instrument. (Retain plate & screws for possible future transport.)
3. Connect the microinjector compressor and user interface modules using the cable provided.
4. Place the compressor module on a flat, solid surface, away from the microscope (on a different surface from microscope's is strongly suggested).
5. Ensure that the compressor is located close enough to the microscope that the pressure lines will reach without placing any strain on the micropipette holders or micromanipulators. The user interface module can be located conveniently next to the microscope.
6. Remove the micropipette holders from their cases and connect them to the free end of the pressure tubing as shown below.

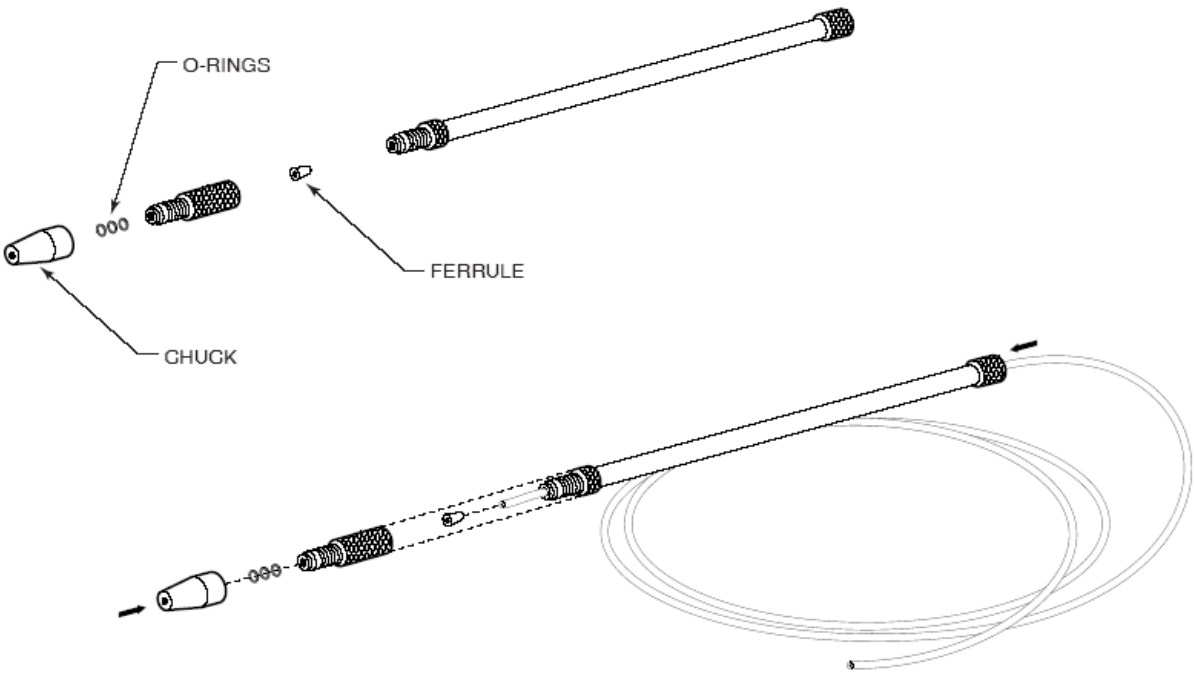

Figure 4-1. Connecting the micropipette holder to the pressure tubing.

9. Attach a pressure fitting to the other end of the pressure tubing as shown in below. Do not use tools to tighten pressure fittings since damage may result.

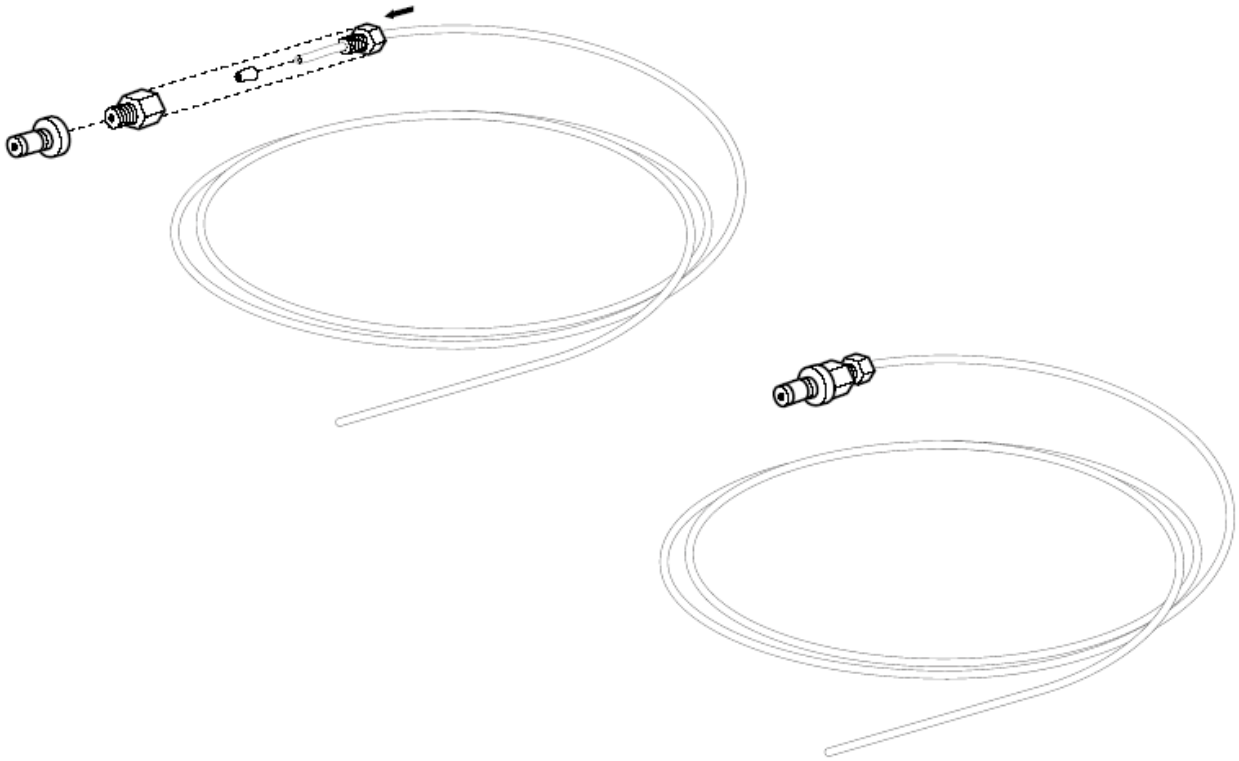

Figure 4-2. Attaching the pressure fitting to other end of pressure tubing.

10. Connect each of the pressure lines to the appropriate ports of the compressor module. You will hear a “click” sound as the fittings engage in the port.
11. Connect the power cord to the power input module on the rear of the compressor and a mains power outlet.

The XenoWorks Digital Microinjector is now set up and ready for use.

5. OPERATING INSTRUCTIONS

5.1 The Hold Function

If injecting adherent cells, or if no Hold Function is required, skip this section and move on to the Transfer Function. The left hand rotary control and its associated LED display correspond to the pressure control of the left-hand port on the front of the compressor module, and of any tubing, micropipette holder and micropipette attached to that port. The pressure delivered by this port ranges from -350 hPa to 350 hPa (± 7hPa), and is designed to allow a typical holding micropipette to hold a mammalian oocyte, blastocyst or zygote, for example. The Hold Function can be used in a similar fashion to that of a traditional micrometer-type air microinjector, by "dialing" the pressure up or down to release or hold the cell in place. A setting of +20 – 30 will advance the meniscus to the top and reducing the pressure to +10 will being to draw in the cell or egg.

Setting up the holding channel and micropipette:

1. First, ensure that the Hold Function is switched off (the default position of the Hold Function after first powering up the unit), then check that the tubing and fitting are properly engaged in the Hold Channel port, (the left hand port on the front of the compressor module) and that all fittings are finger-tight.

2. Fit the holding micropipette into the micropipette holder by loosening the clear plastic chuck ¼ turn, gently inserting the micropipette, and tightening the chuck until the micropipette cannot be moved in or out of the holder.

   CAUTION: Do not over tighten the chuck; doing so could crush the O-rings and prevent them from making a airtight seal around the base of the micropipette. Take care not to force the micropipette into a holder. If the micropipette will not easily slide in, it is because either the chuck is locked down, or there is an obstruction such as broken glass in the holder. Care should be taken to ensure the back end of the micropipette is clean and free of cracks or breakages to avoid damage to the O-rings. Despite these precautions, the O-rings will eventually wear, and they should be inspected from time to time and replaced when necessary.

3. Once the micropipette is fitted into the holder, the holder can be locked into the micropipette holder clamp on the micromanipulator drive module and the tip of the micropipette lowered into the injection chamber. Caution: Take care not to let the holder "snap" into the spring-loaded clamp, since this may cause damage to the micropipette tip. Turn the Hold Port on and dial the hold pressure up to +20 – 30. This will prevent oil or media from drawing into the pipette.

4. When the micropipette is lowered into the medium, a small quantity of medium will be aspirated by capillary action into the tip. It is unnecessary to aspirate any more medium than this.

5. With the Hold Pressure Dial set at approximately zero, switch the Hold Function on. The holding channel is now ready to use.

6. Under certain conditions (such as a broken micropipette), it can become possible to aspirate fluid from the injection chamber, through the tubing and into the compressor module through the front port. Particular care should be taken not to allow this to occur. To safeguard against this event, a fluid sensor has been installed inside the controller. If fluid is drawn into the controller, the fluid sensor will be activated and a compensating positive pressure will turn onto expel the fluid. The positive pressure will stay on continuously even if you power off and on the injector.

   To avoid getting aspirating fluid into the controller:

   a. Making sure that the micropipettes are removed from the injection chamber when it is left unattended, by, for example, using the micromanipulator's Home Function.

   b. Switch off the Hold Pressure Port and turn the Transfer pressure to zero when the device is to be left unattended.

   If fluid has been inadvertently aspirated into the Hold Port tubing, it can be purged by connecting the Hold Port to the Inject/Transfer Port. Then pressing the 'Clear' key. Fluid in the Hold Port tubing can also be purged by turning the Hold Dial to maximum positive pressure until the fluid is drained. Never leave the unit unattended when there is fluid in the tubing. If this happens, contact Sutter Instrument for tech support and instructions on how to resolve this matter.

5.2 The Transfer Function

The Transfer Function works in a similar manner to the Hold Function, but is always active – there is no on/off control. Pressure ranges from -175 hPa to 175 hPa (± 7 hPa) (Range 1), and is applied to the pressure port used to control the pressure in the transfer/inject micropipette. A typical application for which this function might be used includes the transfer of embryonic stem cells into a blastocyst.

Caution: Do not shut down and restart the device with a micropipette immersed in the dish, since during the device's initialization process, a small amount of pressure is sometimes released.

5.2.1 Transfer Channel and Settings

1. As with the holding channel, all fittings should be tight and fully engaged, and the transfer micropipette inserted into the holder with care. Remember not to tighten the chuck too far or the O-rings will be crushed.
2. Insert the micropipette holder into the clamp on the micromanipulator. Take care not to let the holder "snap" into the spring-loaded clamp, since damage may occur to the micropipette tip.
3. Adjust the micropipette holder, by sliding it up or down the clamp, so that the tip of the micropipette projects into the optical axis of the microscope. For details on aligning micropipettes, please refer to the instruction manual of the micromanipulator. If using a micropipette with a bend, rotate the holder in the clamp until the bend section is parallel to the bottom of the injecting chamber.
4. Turn the Transfer Pressure Dial +80 and lower the micropipette into the injection medium.

5. A small amount of medium will be drawn into the micropipette by capillary action. Wait approximately 30 seconds for the pressure to equalize, and then slowly turn the Transfer Dial clockwise until the meniscus between air and medium is less than approximately 2 mm from the micropipette tip. The Transfer Dial should read between +060 to +090 at this point.
6. The micropipette can now be loaded with, for example, embryonic stem cells by placing the tip close to the material to be aspirated and slowly turning the Transfer Dial counter-clockwise to lower settings (e.g., between +30 - +70).
7. Expelling cells from the micropipette is achieved by gently turning the dial clockwise, in much the same way as with a standard micrometer microinjector.

5.2.3 Performance Advice

The XenoWorks Digital Microinjector uses precision-regulated air pressure to hold, inject and manipulate cells and other tissue samples. The best control is attained when the interface (meniscus) between the air in the micropipette and the medium in the dish is as close as possible to the tip of the micropipette. The optimum position of the meniscus will depend upon a number of factors including the type, size and diameter of the micropipette, the viscosity of the medium and the depth of the injecting chamber. In general, though, the closer to the micropipette tip the meniscus can be brought, the better the control over the pressure. During the course of an experiment, it may be necessary to ensure that the meniscus has not been allowed to be pulled too far up inside the micropipette. If the meniscus is too close to the micropipette tip, control can become too sensitive, and will not be smooth. This is particularly so with embryonic stem cell transfer micropipettes. If this is the case, simply draw a little more medium into the micropipette by turning the Transfer Dial counter-clockwise.

CAUTION: Care should be taken not to apply too much positive pressure to the micropipette; as with any other microinjection device, it is possible to purge all the medium from the micropipette and stream bubbles into the injection chamber, which may damage tissues and obscure the microscope optics.

NOTE: The pressure reading on the LED display is a guide only; it is possible to maintain embryonic stem cells stationary in the transfer micropipette tip, yet still show a positive pressure in the display. The display is showing only the pressure supplied to the micropipette, even though the pressure effects observed at the micropipette tip are a combination of pressure supplied by the injector and capillary forces.

CAUTION: Under certain conditions (such as a broken micropipette), it can become possible to aspirate fluid from the injection chamber, through the tubing and into the compressor module through the front port. Particular care should be taken not to allow this to happen, since the compressor module could be damaged. Safeguard against such damage by:

1. *Making sure that the micropipettes are removed from the injection chamber when it is left unattended (by using the micromanipulator's Home Function, for example).*
2. *Switch off the Hold Pressure Port and turn the Transfer pressure to zero when the device is to be left unattended.*

Fluid that has been inadvertently aspirated into the Inject/Transfer tubing can be purged by pressing the Clear Key (ensure there is no micropipette in the holder before doing this). Never leave the unit unattended when there is fluid in the tubing.

5.3 The Inject Function

5.3.1 Setting up the High Pressure Injection Channel and Micropipette

If the digital microinjector is being used for the high pressure injection of solutions (such as DNA, RNA, proteins etc.) into cells through narrow microinjection micropipettes (tip sizes less than 1 micron), the Transfer Function should be used as an adjustable positive pressure ("base" pressure) applied to the tip of the injection micropipette. This ensures that there is a gentle, constant flow of solution from the micropipette, preventing dilution of the solution to be injected by the medium in the injection chamber. The base pressure also minimizes, to some degree, blockage of the micropipette tip by foreign objects in the surrounding medium. The wider the tip of the injection micropipette, the less base pressure is required. As a rule, start at 5 hPa and increase slowly until a gentle stream of material can be seen coming from the tip. Under certain microscope optics, this stream is easily visible as a "Schlieren" pattern resulting from the different densities of injected solution and surrounding medium. Once the stream can be seen issuing from the micropipette tip, reduce the base pressure until the stream almost disappears. Two modes of high-pressure injection are available to suit different users' preferences:

5.3.2 Continuous Mode

High pressure of a predetermined value is applied for as long as the Inject Key or the footswitch is held down. This mode is useful when injecting a field of cultured adherent cells, where long durations and/or high pressures will rupture and kill the cells. The pressure can be set low, and the duration short to deliver tiny, discrete and reproducible volumes to each cell.

Table 5-1. Continuous mode actions and controls.

Action	Control Used
Adjust compensation pressure	Transfer dial
Select continuous injection mode	Pulse/Cont. key to "Cont."
Select high-pressure adjustment	Pressure/Pulse Width key to "Pressure
Adjust high-pressure value	Pressure/Pulse Width dial
Inject	Inject key or footswitch

5.3.3 Pulse Mode

Pulse Mode provides high-pressure injection by discrete pulses of a given pressure (in hPa) for a predetermined amount of time. Pressure is applied for a predetermined duration no matter how long the Inject Key or footswitch is pressed. This mode is useful for the injection of DNA into the pronucleus of a mammalian zygote for example, where successful injection

requires a high pressure and a slight inflating (approximate doubling in size) of the pronucleus can be seen.

Table 5-2. Pulse mode actions and controls.

Action	Control Used
Adjust compensation pressure	Transfer dial
Select pulse injection mode	Pulse/Cont. key to "Pulse"
Select high-pressure adjustment	Pressure/Pulse Width key to "Pressure"
Adjust high-pressure value	Pressure/Pulse Width dial
Select injection duration adjustment	Pressure/Pulse Width key to "Pulse Width"
Adjust injection duration	Pressure/Pulse Width dial
Inject	Inject key or footswitch

Once the base pressure, injection pressure and, if used, injection duration have been set, the system is ready to begin injection. Consult the application note “Introduction to Microinjection” for more information on microinjection techniques.

6. TROUBLESHOOTING

6.1 Instrument Power

Problem	Cause	Solution
The instrument does not activate when the power is switched on.	1. The power cord is not connected to an appropriate supply.	Connect the power cord to the mains supply. Be sure that the power rating is matched to the requirements of the instrument. Catalog number BRE110 requires 100-110v AC, BRE220 requires 220-240v AC.
	2. The protection fuse has blown.	Replace the fuse and check that the power supply is appropriate to the requirements of the device.
	3. The user interface module is not properly connected.	Connect the user interface module to the compressor module using the cable provided.

6.2 Hold Function

Problem	Cause	Solution
The holding pressure is insufficient to hold a cell, or the "hold" control seems unresponsive.	1. The holding pressure is not activated.	Activate the holding pressure with the Hold on/off switch (green LED will light).
	2. The tubing is not connected to the holding pressure port.	Connect the tubing to the left hand (holding) port. There will be a positive click when the fitting is engaged.
	3. There is a leak in the tubing, or the pressure fittings are loose.	Check the tubing for kinks, holes and other damage and replace if necessary (order part # BR-DT). Tighten all fittings. Replace ferrules.
	4. The air/medium interface is too far inside the micropipette tip.	Slowly rotate the Hold control clockwise, until the air/medium meniscus is as close to the tip as possible. Note that it is possible to create an effective holding pressure and still show a positive value in the Hold LED display.
	5. The plastic tip of the pipette holder is not tightened down.	Carefully tighten the pipette holder's plastic tip.
	6. The black parts on the rod holder behind the plastic tip are loose.	Carefully tighten the rod holder's black parts located behind the plastic tip.

6.3 Transfer Function

Problem	Cause	Solution
The Transfer pressure control seems unresponsive.	1. The tubing is not connected to the transfer pressure port.	Connect the tubing to the right hand (inject/transfer) port. There will be a positive click when the fitting is engaged.
	2. There is a leak in the tubing, or the fittings are loose.	Check the tubing for kinks, holes and other damage and replace if necessary (order part # BR-DT). Tighten all fittings. Replace ferrules.
	3. The air/medium interface is too far inside the micropipette tip.	Slowly rotate the Transfer control clockwise, until the air/medium meniscus is as close to the tip as possible.
	4. The plastic tip of the pipette holder is not tightened down.	Carefully tighten the pipette holder's plastic tip.
	5. The black parts on the rod holder behind the plastic tip are loose.	Carefully tighten the rod holder's black parts located behind the plastic tip.

Note that the Transfer LED display is a guide only and that it is possible to have a fully equalized micropipette (no net in- or out-flow of material), and still show a positive or a negative (i.e., non-zero) value in the display. If, after performing the above checks, the device is still unresponsive, please contact your Sutter Instrument distributor for assistance.

The Transfer display shows a positive or negative pressure, yet the cells are stationary inside micropipette.	1. Factors such as viscosity of medium and capillary/surface tension forces create a negative pressure in the micropipette. It may take slight POSITIVE pressure from the microinjector to balance this action and even with a net negative pressure at the tip, the pressure applied to the tool may be slightly positive. If the Transfer pressure can be dialed up or down with no effect on the aspirated cells, there may be a problem with the pressure hose connection (see "The Transfer pressure control seems unresponsive", above).	This is normal. Use the display pressure value as a guide only.
	2. Tubing connector not plugged in all the way into the port on the front panel of the digital injector.	Make sure that the tubing connector is plugged in all the way into the port.
	3. Plastic tip of the pipette holder is not tightened down.	Carefully tighten the pipette holder's plastic tip.

6.4 Inject Function

Problem	Cause	Solution
There is no material flowing from the tip of the micropipette, even when positive pressure is applied (80 psi; 5566 hPa on display).	1. The microscope optics are unable to resolve the stream	Switch to suitable optics, if available. Brightfield illumination will be unlikely to resolve the stream, unless the injected solution is colored. Phase contrast will be more effective and Nomarski and Hofmann better still. A constant stream can also be detected by moving the micropipette tip close to a freely floating cell and trying to "blow it away".
	2. The micropipette is blocked.	Press the Clear Key a number of times, holding the key down longer each time until the blockage is cleared. If this does not clear the blockage, the pipette should be replaced.

CAUTION: Some residual pressure may remain in the tubing. Take great care to point the micropipette in a safe direction when removing it from the holder.

6.5 General

Problem	Cause	Solution
Compressor motor runs continuously for more than five minutes when the instrument is on, but not being used.	1. There is a leak in one of the pressure lines.	Check each pressure line for kinks or holes. Replace lines if necessary - order part # BR-DT.
	2. The pressure fitting has not been fully engaged in the pressure port on the front of the compressor module.	Push the pressure fitting into its port until a click is felt. If no tubing is fitted to a port, check that the port is closed by pressing the release tab on top of the port.
	3. There is a loose pressure fitting.	All pressure fittings should be finger tight - no tools are necessary to tighten pressure fittings.
	4. There is no micropipette in the holder.	Insert a micropipette into the holder. If no micropipettes are being used, switch the Hold pressure off and turn the Transfer pressure down to zero.

If, after performing the above checks, the compressor motor continues to run for longer than five minutes, shut the instrument down and contact your Sutter distributor.

The compressor is loud when activated.	The shipping plate and screws on the right side panel have not been removed.	Turn off the compressor. Place the cabinet on its left side (front side up) and remove the hex-head screws and shipping plate.

Problem	Cause	Solution
NOTE: To replace the shipping plate before storage or shipping, place the cabinet on its side again so that the receiving holes inside on the compressor line up with the external holes.		
"ERR" appears on all B displays of the remote user interface.	1. There is an electronic short on the circuit board.	Contact Sutter Instrument.
	2. There is a constant/continuous positive pressure and the compressor is always on. It is possible that the solution is down all the way all the way into the tubing and into the compressor. If this has happened, the fluid sensor has been activated/triggered inside the injector to create continuous positive pressure with protectant fluid used to protect the compressor from water damage.	Contact Sutter Instrument.

7. MAINTENANCE

7.1 Regeneration of the Drierite Desiccant

The XenoWorks™ Digital Microinjector needs no routine maintenance except for periodic replacement or "regeneration" of the Drierite desiccant. The indicating Drierite, found in the canister on the rear of the microinjector, is a desiccant made of calcium sulfate (97%) and cobalt chloride (3%). Drierite is non-toxic and can be handled with few precautions. This material is used to remove water vapor from the air intake of the system. As it absorbs more moisture, it becomes pink in color and must eventually be "regenerated" (dried) or replaced with new indicating desiccant canister. New canisters are available from Sutter (order part number X870700), or the existing granules can be regenerated by following these instructions.

1. Turn off the device and unplug the power cord. To remove the canister from the device, pull off the output (left) air tube from the white plastic connector on the canister.
2. The two black plastic hold-downs that secure the canister to the rear panel can be released by forcing one half of the connector out of the other half at the point where they meet. Remove the canister and unscrew the plastic end cap, being careful not to lose the black rubber-sealing ring that forms the airtight seal within the cap.
3. With the cap off, remove the spring, the perforated aluminum plate and the first filter and second filter. The far aluminum plate can be kept in place. Pour the Drierite out onto a glass or metal tray, spreading it evenly, one granule deep, and heat it for one hour at 200°C. Pre-dry both filters at 100°C for 30 minutes just prior to assembly.
4. Before refilling the canister, the Drierite granules should be cooled in an airtight container. After cooling, replace the Drierite granules, keeper, and filters into the canister in the order they were removed.
5. First, install the filter against the keeper that was left in the canister.
6. If this keeper was removed, slide it in first ensuring that it lays flat against the plastic shoulders at the far end of the canister. With the far keeper and filter in place, pour in the new or regenerated Drierite.
7. Next, insert the second filter followed by the keeper and the spring.
8. Check that the rubber seal is in the proper position and, if you wish, apply a thin layer of vacuum grease on to the rubber seal to ensure a tight fit. There is no need to over-tighten the cap, but a good seal is needed to prevent air leaks.
9. Reinstall the canister on the injector, with the air tube connections to the top.
10. Slide the black plastic hold-downs inside one another and squeeze them together to firmly hold the canister in place.
11. At this point, re-connect the air-output tube (the one which enters into the rear panel of the injector).

CAUTION: Failure to maintain the Drierite may result in impaired instrument performance and can damage the instrument.

7.2 Fitting New O-rings

Routine use will create wear on the three black O-rings located in the tip of the micropipette holder chuck. Occasionally, the O-rings will need to be replaced. To do this, gently remove the old O-rings with an appropriate tool (a bent paper clip, for example). Discard the old O-rings and insert three new ones, taking care to ensure that they are flat against the back of the chuck. The new O-rings can be pushed down inside the chuck by gently screwing the chuck onto the knurled black aluminum pressure fitting. Take care not to damage the chuck during this procedure.

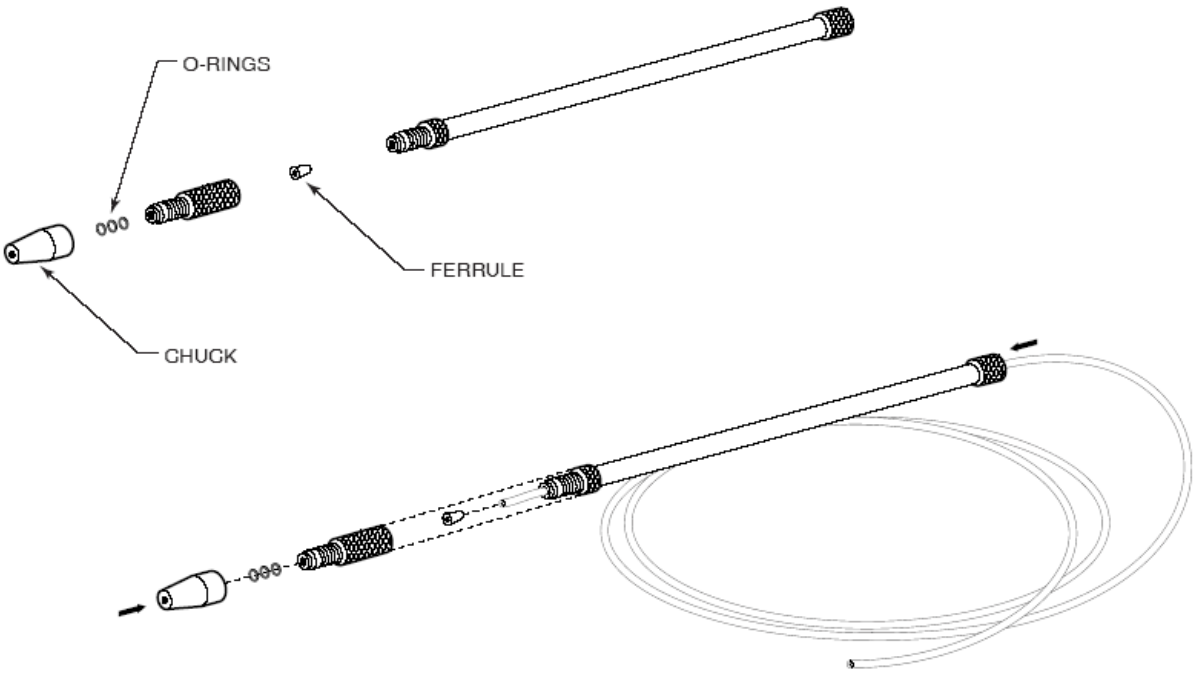

Figure 7-1. Connecting the micropipette holder to the pressure tubing.

APPENDIX A. SPARE PARTS

BR-MH	**Micropipette holder,** includes micropipette holder body, 9 O-rings
BR-DT	**Digital tubing kit,** includes 2 x 2 m ETFE tubing, pressure fitting, 6 ferrules
X870700	**Drierite canister**

APPENDIX B. PRESSURE CONVERSION

XenoWorks Digital Microinjector displays pressure in hecta-Pascals (hPa). The following conversion factors should be used for other pressure units:

1 hPa = 100 Pascals (Pa) 1 Pascal = 0.01 hPa

1 hPa = 1 millibars 1 millibar = 1 hPa

1 hPa = .001 bars 1 bar = 1000 hPa

1 hPa = 0.0145 psi 1 psi = 68.9655 hPa

Table B-1. Pressure/Vacuum range settings.

Range Setting	Scale	Pressure				Applications
		Range		1 Unit/Transfer Dial (approx.)		
		psi	hPa	hPa	psi	
1	Low	+2.5 to -2.5	+172 to -172	1	0.015	Standard setting for ICSI, NT, ES Cell, Pronuclear Injection, and Adherent Cell microinjection
2	Medium	+5 to -5	+345 to -345	2	0.03	Used when higher compensation pressures are needed for smaller volumes back-loaded into injection needle
3	Medium-High	+10 to -7	+689 to -483	4	0.06	
4	High	+15 to -7	+1034 to -483	8	0.12	Used for font-loading pipette with very small volumes (femtoliters) and to create higher compensation pressures for these small volumes.

APPENDIX C. FUSE REPLACEMENT

In the event that the instrument fails to power up when it is switched on, the line power fuses should be checked to determine whether they have blown. The fuses are located in the fuse holder in the power entry module on the rear of the control module.

To replace a fuse, first unplug the power cord from the power entry module, revealing the fuse holder below. Remove the fuse holder. Note that both fuses in the fuse holder are used simultaneously. Either one or both may have blown. Discard the blown fuse(s). Insert appropriately rated replacement fuses (see below). Replace the fuse holder in the power entry module and reconnect the power cord.

Replacement Fuse Ratings:

Table C-1. Mains fuse type and ratings.

Mains Power Source	Mains Voltage Setting	Fuse (Type: Time Delay, 5mm x 20mm, glass tube)	
		Fuse Rating	Manufacturer Examples
110 VAC	"110" (100 – 120 VAC)	4A, 250V	Bussmann: GMC-4A, GMC-4-R (RoHS), GDC-4A, or S506-4A (RoHS) Littelfuse: 239 004, 239.004.P (RoHS), 218.004, or 218.004.P (RoHS)
220 VAC	"220" (200 – 240 VAC)	2A, 250V	Bussmann: GDC-2A or S506-2A (RoHS) Littelfuse: 239 002 or 239.002.P (RoHS)

APPENDIX D. TRANSPORTING THE MICROINJECTOR

When transporting, use the original packaging and the supplied shipping screws and shipping plate. If the packaging and shipping screws have been misplaced, contact your local Sutter distributor.

Install the four shipping screws and the shipping plate to the right side of the compressor module using the following procedure:

1. Align shipping plate holes with holes in the right side of the instrument.
2. Mount the shipping plate using the four 10-32 socket-head shipping screws provided by placing screws first through plate, then through instrument side panel and finally engaging screws into interval motor bracket.
3. Slowly advance the shipping screws in evenly to secure the compressor before storage or shipping.
4. Tighten the (4) shipping screws using a 5/32" allen wrench.
5. The controller/compressor is now ready for transport, and can be placed into the original foam packaging for shipping.

CAUTION: Do not transport the compressor module without using the provided shipping screws and plate or instrument damage may result. If screws and plate are misplaced and unavailable, please contact Sutter Instrument to obtain the proper hardware.

APPENDIX E. SPECIFICATIONS

CE

Pressure channels	2, independently controllable
Maximum pressure	5600 hPa (+/- 50 hPa)
High pressure	0 to 5600 hPa (+/- 50 hPa) in 7 hPa increments
Injection time	0.01 to 10 seconds in 0.01 sec increments
Inject activation	Transient; hand or foot switch
Transfer pressure	-175 to +175 hPa (+/- 7 hPa) in 7 hPa increments
Hold pressure	-350 to +350 hPa (+/- 7 hPa) in 7 hPa increments
Clear function	Ramp from Inject value to maximum pressure
Display	3 x 7-segment LED
Controls	Tactile keys, rotary optical encoders
Tubing	2 m ETFE
Micropipette holder diameter	4 mm
Micropipette compatibility	1 mm capillary glass* other sizes available upon request
Piezo-impact drive compatibility	Prime Tech, Burleigh
Storage Environment	
Temperature:	0 – 70°C (32 – 158°F)
Humidity:	0 – 95% (non-condensing)
Operating Environment	
Temperature:	3.5 – 35°C (38.3 – 95°F)
Humidity:	0 – 80% (non-condensing) (80% @ 31°C (87.8°F), decreasing linearly to 67% @ 35°C (95°F))
Regulatory	
Safety:	EN61010-1, TUV, CE
EMC:	EN61326, TUV, CE

Cleaning	70% alcohol (or e.g. UV)
Dimensions (H x W x D)	Compressor module - 407 x 440 x 150 mm (16 x 17 x 6 inches) User interface module - 164 x 123 x 70 mm (6.5 x 4.8 x 2.8 inches)
Weight	
Controller/compressor:	1504 g (3.36 lb (3 lb, 5 oz))
Remote user interface:	42 g (0.093 lb (1.48 oz))
Electrical:	
Mains voltage	100 - 240 V, 50/60 H
Power consumption	440W
Power cord	10A, 250V, with safety ground plug
Mains fuse (rear of cabinet)	Time delay (or time lag) 5 x 20 mm glass tube.
Input power (BRE110)	100–120 VAC (±10%), 50/60 Hz Fuse rating: 4.0 A. Power: 450 VA
Input power (BRE220)	220–240 VAC (± 10%), 50/60 Hz Fuse rating: 2.0 A. Power: 280 VA
	For manufacturer examples, refer to **Table C-1 Mains fuse type and ratings.**

APPENDIX F. DRIERITE MATERIAL SAFETY DATA SHEET

IDENTITY: INDICATING DRIERITE **DATE PREPARED** 1-3-96
DESCRIPTION: 1/16" TO 1/4" BLUE GRANULES

SECTION I

MANUFACTURER'S NAME: W.A. HAMMOND DRIERITE CO. LTD.
ADDRESS: P.O. BOX 460,
138 DAYTON AVE.,
XENIA, OH 45385
EMERGENCY PHONE NUMBER: (513) 376-2927
INFORMATION PHONE NUMBER: (513) 376-2927

SECTION II

INGREDIENTS

CHEMICAL IDENTITY	%	OSHA PEL	ACGIH TLV	UNITS	C.A.S. #
CALCIUM SULFATE	97	15	10	mg/M^3	7778-18-9
COBALT CHLORIDE	3	0.05*	0.05*	mg/M^3	7646-79-9

*(AS COBALT METAL)

HAZARDOUS MATERIALS IDENTIFICATION SYSTEM (HMIS)

HEALTH	FLAMMABILITY	REACTIVITY	PROTECTIVE EQUIPMENT
1	0	1	E

SECTION III
PHYSICAL/CHEMICAL CHARACTERISTICS

SPECIFIC GRAVITY: (H_2O=1): 1.87
SOLUBILITY IN WATER: 0.25 GRAMS PER LITER
MELTING POINT: 1450° C DECOMPOSES
APPEARANCE: BLUE GRANULES; NO ODOR

SECTION IV
FIRE AND EXPLOSION HAZARD DATA

FLASH POINT: NONE
EXTINGUISHING MEDIA: NOT COMBUSTIBLE
SPECIAL FIREFIGHTING PROCEDURES: NONE
UNUSUAL FIRE AND EXPLOSION HAZARDS: NONE

SECTION V
REACTIVITY DATA

STABILITY: STABLE
INCOMPATIBILITY (MATERIALS TO AVOID): STRONG ACIDS
HAZARDOUS DECOMPOSITION BYPRODUCTS: Cl_2 @ 318°C; SO_3 @ 1450°C
HAZARDOUS POLYMERIZATION: WILL NOT OCCUR

SECTION VI
HEALTH HAZARD DATA

EYES: PARTICLES MAY CAUSE IRRITATION.
SKIN: THIS MATERIAL IS NOT TOXIC. MAY DRY OR IRRITATE SKIN
INHALATION: MAY CAUSE AN IRRITATION OF RESPIRATORY ORGANS OF SENSITIVE PERSONS RESULTING IN THE OBSTRUCTION OF AIRWAYS WITH SHORTNESS OF BREATH.
INGESTION: MAY CAUSE VOMITING, DIARRHEA AND SENSATION OF WARMTH
SIGNS AND SYMPTOMS OF OVER EXPOSURE: EYE, NOSE, THROAT, OR RESPIRATORY IRRITATION

CARCINOGENICITY OF INGREDIENTS:

MATERIAL	IARC	NTP	OSHA
CALCIUM SULFATE	NOT LISTED	NOT LISTED	NOT LISTED
COBALT CHLORIDE	YES*	NO	NO

*(COBALT & COBALT COMPOUNDS ARE CLASSIFIED AS GROUP 2B)

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY EXPOSURE:

PRE-EXISTING UPPER RESPIRATORY AND LUNG DISEASE SUCH AS, BUT NOT LIMITED TO, BRONCHITIS, EMPHYSEMA & ASTHMA

EMERGENCY AND FIRST AID PROCEDURES:

EYES: FLUSH WITH WATER
DUST INHALATION: REMOVE TO FRESH AIR
SKIN: WASH WITH WATER
INGESTION: NONE KNOWN

SECTION VII
SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED:

SWEEP OR VACUUM MATERIAL INTO APPROPRIATE WASTE CONTAINER FOR DISPOSAL. AVOID DUSTING CONDITIONS.

WASTE DISPOSAL METHOD: THIS MATERIAL CAN BE DISPOSED OF AS AN INERT SOLID WASTE IN AN APPROVED LAND FILL OR BY OTHER PROCEDURES ACCEPTABLE UNDER FEDERAL, STATE AND LOCAL REGULATIONS.

PRECAUTIONS TO BE TAKEN IN HANDLING AND ST0RING:

KEEP CONTAINER CLOSED
STORE IN A COOL DRY PLACE
AVOID GENERATING DUST

SECTION VIII
CONTROL MEASURES

RESPIRATORY PROTECTION: NIOSH/OSHA APPROVED FOR DUST
VENTILATION: TO MEET TLV REQUIREMENTS
EYES: SAFETY GLASSES OR GOGGLES
OTHER PROTECTIVE EQUIPMENT: GLOVES OR PROTECTIVE CLOTHING ARE NOT USUALLY NECESSARY BUT MAY BE DESIRABLE IN SPECIFIC WORK SITUATIONS.

SECTION IX
• REFERENCES

U.S. DEPARTMENT OF LABOR – OSHA FORM APPROVED OMB NO.1218 -0072.
OSHA HAZARD COMMUNICATION STANDARD 29 CFR 1910.1200
U. S. GYPSUM CO.

APPENDIX G. LIMITED WARRANTY

Sutter Instrument Company, a division of Sutter Instrument Corporation, limits the warranty on this instrument to repair or replacement of defective components for one year after the date of shipment, provided the instrument has been operated in accordance with the instructions outlined in the instruction manual.

Abuse, misuse or unauthorized repairs will void this warranty.

Limited warranty work will be performed only at the factory, and the cost of shipment both ways is to be borne by the user.

The limited warranty is as stated above and no implied or inferred liability for direct or consequential damages is intended.

APPENDIX H. DISCLAIMER

The XenoWorks Digital Microinjector (Models BRE110 and BRE220) should only be used in a laboratory environment for use on animal tissues. It is not intended for use, nor should be used, in human experimentation, or applied to humans in any way. This is not a medical device.

Do not open or attempt to repair the instrument without expressed and explicit instructions from Sutter Instrument Company. Extreme heat and high voltages are present and could cause injury.

Do not allow unauthorized and or untrained operatives to use this device.

Any misuse will be the sole responsibility of the user/owner and Sutter Instruments assumes no implied or inferred liability for direct or consequential damages from this instrument if it is operated or used in any way other than for which it is designed.

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