

XENOWORKS™ BRM

MOTORIZED MICROMANIPULATOR SYSTEM

(TOUCHDECLUTCH)

OPERATION MANUAL

REV. 4.10 (20141208)



SUTTER INSTRUMENT

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DISCLAIMER

The **XenoWorks BRM** Micromanipulator is designed for the specific use of moving micropipettes in three-dimensional space in increments of micrometers into and out of an optical pathway of a microscope and no other use is recommended.

This instrument is designed for use in a laboratory environment. It is not intended for use, nor should it 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. Extreme heat and high voltages are present and could cause injury.

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

Any misuse will be the sole responsibility of the user/owner and Sutter Instrument Company 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.

SAFETY WARNINGS AND PRECAUTIONS


Electrical


- Operate the XenoWorks BRM using 110-120 V AC, 60 Hz, or 220-240 V AC., 50 Hz line voltage. This instrument is designed for connection to a standard laboratory power outlet (Overvoltage Category II), and because it is a microprocessor--controlled device, it should be accorded the same system wiring precautions as any 'computer type' system. A surge protector and power regulator are recommended.
-  **Fuse Replacement:** Replace fuse only with the same type and rating as indicated in the following table.

Mains Voltage Setting	Fuse (Type: Time Delay, 5mm x 20mm, glass tube)	
	Rating	Manufacturer Examples
"110" (100 – 120 VAC)	2A, 250V (Time Delay)	Bussmann: GMC-2A, GMC-2-R (RoHS), GDC-2A, or S506-2A (RoHS) Littelfuse: 239 002 or 239.002.P (RoHS)
"220" (200 – 240 VAC)	T1.0A, 250V	Bussmann: GDC-1A or S506-1A (RoHS) Littelfuse: 218 001 or 218 001.P (RoHS)

A spare fuse is located in the power input module. Please refer to the fuse-replacement appendix for more details on fuse ratings and for instructions on how to change the fuse.

Avoiding Electrical Shock and Fire-related Injury

-  Always use the grounded power supply cord set provided to connect the system to a grounded outlet (3-prong). This is required to protect you from injury in the event that an electrical hazard occurs.
- Do not disassemble the system. Refer servicing to qualified personnel.


-  To prevent fire or shock hazard do not expose the unit to rain or moisture.

Electromagnetic Interference






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 type and length of interconnect cables provided with the unit for the interconnection of the controller with the manipulator mechanical and with the joystick (see the TECHNICAL SPECIFICATIONS appendix for more details).




Operational

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

- This instrument is designed for operation in a laboratory environment (Pollution Degree I) that is free from mechanical vibrations, electrical noise and transients.
- This unit is not designed for operation at altitudes above 2000 meters nor was it tested for safety above 2000 meters.
- Before making electrical connections, ensure that the instrument is switched off.
-  **DO NOT CONNECT OR DISCONNECT THE CABLES BETWEEN THE CONTROLLER AND THE MECHANICAL UNITS WHILE POWER IS ON.**

Please allow at least 20 seconds after turning the unit off before disconnecting the mechanical units. Failure to do this may result in damage to the electronics.


- Operate this instrument only according to the instructions included in this manual.
- Do not operate if there is any obvious damage to any part of the instrument.
-  Operate only in a location where there is a free flow of fresh air on all sides. **NEVER ALLOW THE FREE FLOW OF AIR TO BE RESTRICTED.**
-  Do not operate this instrument near flammable materials. The use of any hazardous materials with this instrument is not recommended and if undertaken is done so at the users' own risk.
-  Do not attempt to operate the instrument with the manipulator shipping screws in place - severe motor damage may result.
-  Do not operate if there is any obvious damage to any part of the instrument. Do not attempt to operate the instrument with the manipulator shipping screws in place - severe motor damage may result. When transporting the mechanical manipulator, be sure to install the shipping screws supplied in their correct locations. Failure to do this may result in damage to the motors.
-  Never touch any part of the micromanipulator electromechanical device while it is in operation and moving. Doing so can result in physical injury (e.g., fingers can be caught and pinched between the moving parts of the micromanipulator).

-  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 micropipette holders at yourself or others. Always wear safety glasses when using sharp glass micropipettes with pressure microinjectors.
-  Do not handle the manipulator mechanical while the power is on, and take care to ensure no cables pass close to the mechanical manipulator.
-  Sutter Instrument recommends that the operator touch the microscope equipment base (to discharge any static electricity) prior to using the XenoWorks BRM Micromanipulator system.

Other

- Use this instrument only for microinjection purposes in conjunction with the procedures and guidelines in this manual.
- 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 Sutter Instrument's instrument is not recommended and if undertaken is done so at the users' own risk.
- When transporting the mechanical manipulator, be sure to install the shipping screws supplied in their correct locations. Failure to do this may result in damage to the motors.
- 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.
- Sutter Instrument reserves the right to change specifications without prior notice.
- This device is intended only for research purposes.
- Do not carry the joystick by its joystick paddle, since damage may result.

Handling Micropipettes

 Failure to comply with any of the following precautions may result in injury to the users of this device as well as those working in the general area near the device.

- The micropipettes used with this instrument are very sharp and relatively fragile. Contact with the pulled micropipette tips, therefore, should be avoided to prevent accidentally impaling yourself.
- Always dispose of micropipettes by placing them into a well-marked, spill-proof “sharps” container.

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1. GENERAL INFORMATION

1.1 Introduction

1.2 About This Manual

The XenoWorks Micromanipulator is a manipulator system comprised of three basic parts: the controller (BRM), manipulator mechanical (MP-285/M), and joystick.

In the next, few pages you will find a product description to help you become acquainted with operation, 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.

This manual is continually being updated. If you encounter any areas you feel should be covered in expanded detail we would like to hear from you. Please contact our Technical Support staff with your suggestions (415-883-0128 or info@sutter.com).

1.3 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.

1.4 Product Description

NOTE: Unless otherwise noted, all references to the manipulator mechanical are for the right-handed version.

1.4.1 Packing List

The XenoWorks™ Micromanipulator is shipped with the following components:

1. Manipulator mechanical
2. Controller
3. Joystick
4. Manipulator mechanical base plate
5. Mains power cord
6. Cable for connecting the manipulator mechanical with the controller
7. Cable for connecting joystick with the controller
8. Instruction manual
9. Manipulator mechanical mounting screws (4)
10. Hex key (for manipulator mechanical mounting screws)

If any items are missing or appear damaged, contact Sutter Instrument or your dealer/rep immediately.

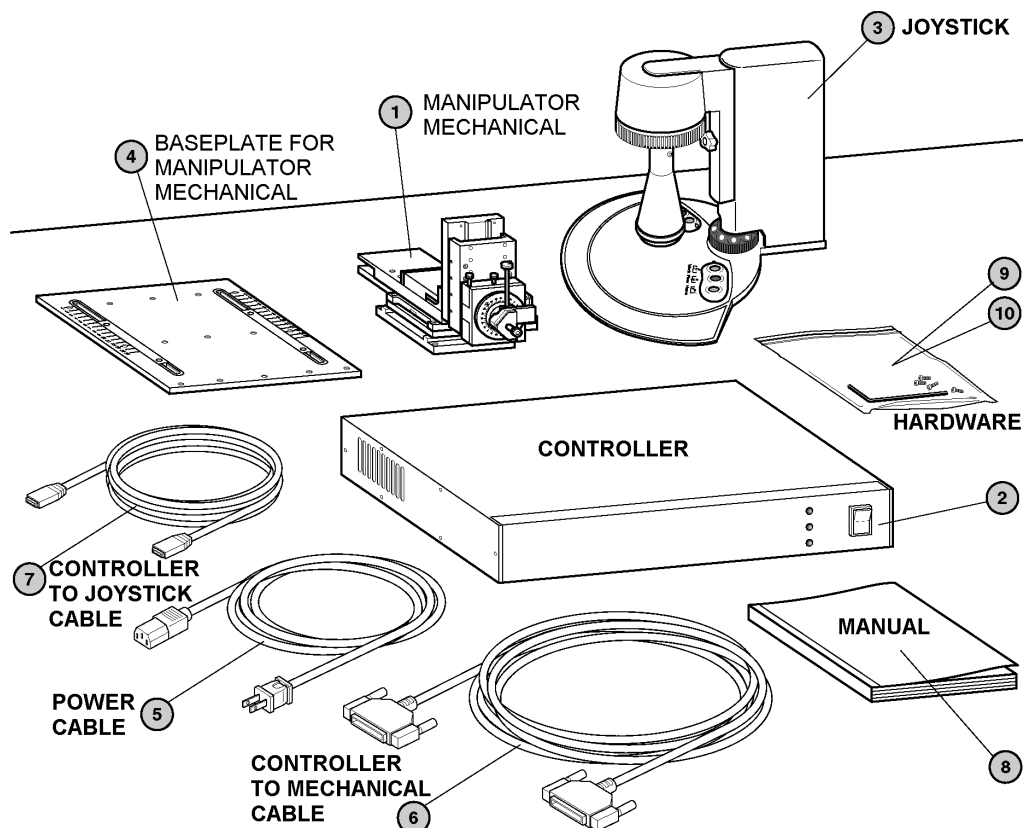


Figure 1-1. Components of the XenoWorks micromanipulator.

1.4.2 Joystick Controls and Features

The user interface of the XenoWorks™ Micromanipulator is a three-axis joystick. When the micromanipulator is installed correctly, the joystick oriented appropriately on the working surface, and the default axis polarity settings used (see “Axis Polarity”), a displacement of the joystick to the left, for example, will result in concomitant movement of the micromanipulator to the left. The movement of the micromanipulator is directly proportional to the movement of the joystick, however the movement reduction ratio between the joystick and the mechanical can be varied (see “Range” below).

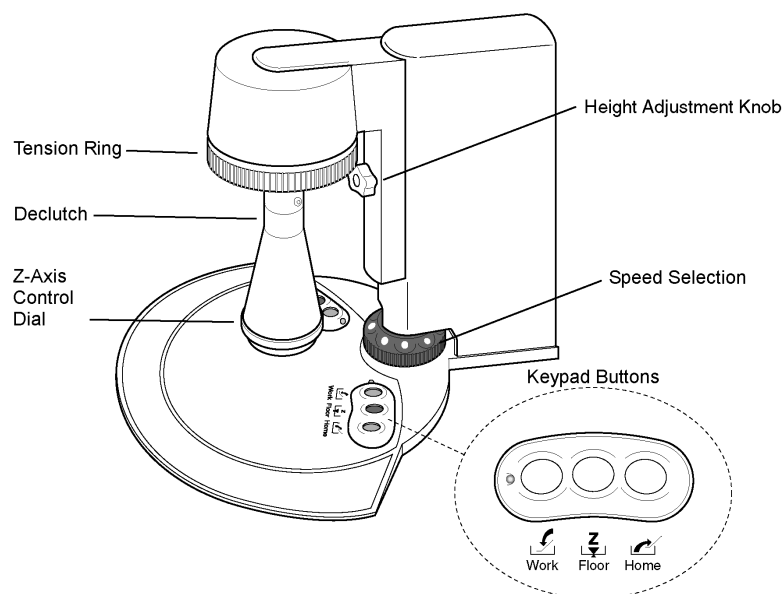


Figure 1-2. Joystick.

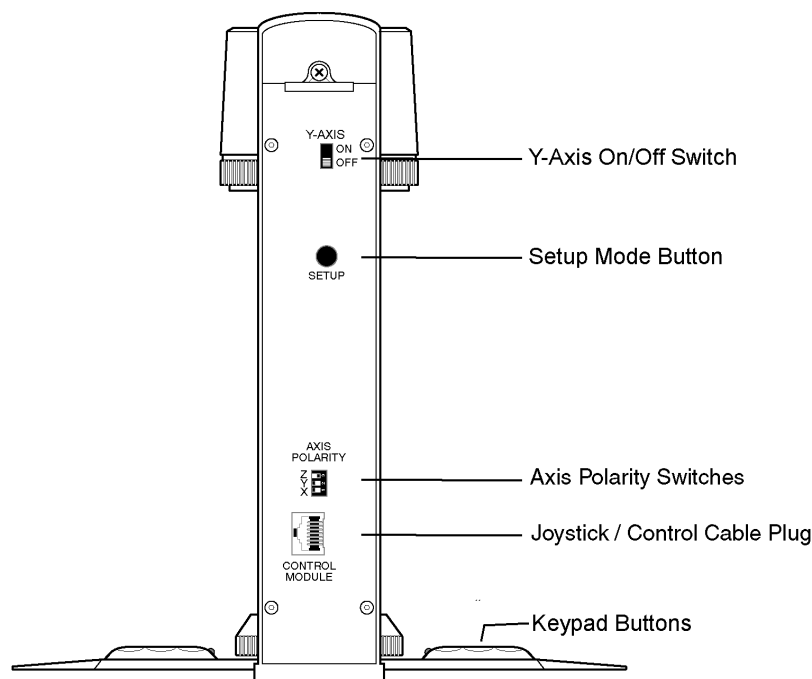


Figure 1-3. The rear panel of the joystick.

1.4.2.1 X- and Y-Axis Control

Movements of the micromanipulator in the X- and Y-axes are achieved by moving the joystick left and right, and forward and backward, respectively (the switches for establishing axis polarity for right- and left-handed manipulator mechanicals are located on the back of the joystick).

1.4.2.2 Y-Axis On/Off

The Y-axis can be turned off to produce pure X-axis travel. This results in a mode that may be preferred for difficult injections/transfers, or when the cell or egg is more sensitive to the withdrawal of the pipette.

1.4.2.3 Z-Axis Control

Movement of the micromanipulator in the Z-axis (vertical) is achieved by rotating the control on the end of the joystick. Looking down on the joystick from above, and using the default axis settings, a rotation of the Z-control in a clockwise direction will cause the micromanipulator to move downwards. A counterclockwise rotation will result in an upward movement of the micromanipulator.

1.4.2.4 Joystick Declutch Mechanism

The electronic link between the joystick and the manipulator mechanical can be disconnected temporarily in order to reposition the joystick without affecting the position of the micropipette. This declutch function is achieved by touching the joystick just above the rubber handgrip - the X and Y movement will remain deactivated for as long as your finger is touching the band. The link is reestablished by releasing the touchpad. See Operating Instructions for more details on how to use the declutch.

1.4.2.5 Height Adjustment

To maintain good ergonomics and operator comfort, the height of the joystick can be adjusted, as shown in the figure below. The bottom of the joystick can be placed anywhere from zero to 45 mm above the black hand-rest pad. The adjustment is made by first loosening the black locking knob located on the front of the joystick column. Move the joystick assembly up and down along its vertical track until a comfortable position is achieved, and then lock the position by tightening the locking knob.

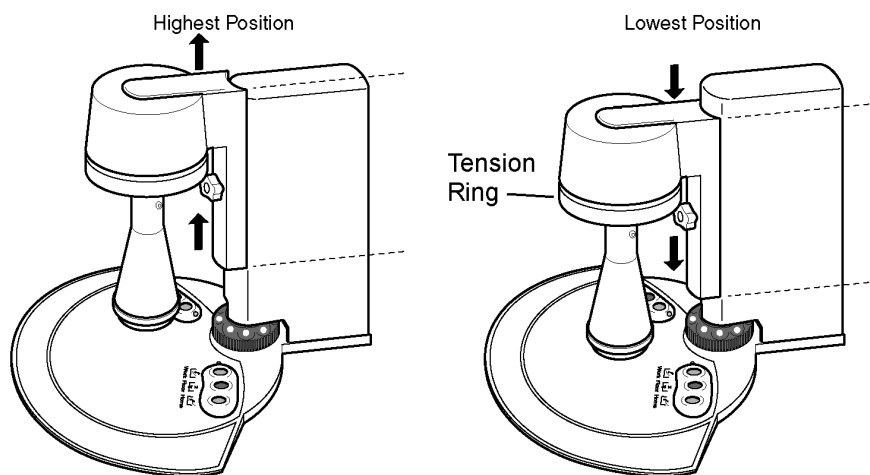


Figure 1-4. Joystick height adjustment.

1.4.2.6 Joystick Tension Ring

There is a friction mechanism in the top of the joystick assembly allowing the X-Y movement of the joystick to be adjusted for comfort and feel. The friction is reduced by rotating the black dial clockwise (looking down from above) and increased by rotating counterclockwise.

Although there is no recommended setting for this friction, it should be noted that if there is no friction at all, the joystick would always fall to the center position under gravity, which may not be desirable. Similarly, if the friction is set too tightly, the joystick may become too stiff to use or may be locked in place and could be damaged if the dial is tightened further. With a little practice, a suitable tension setting can be established.

1.4.2.7 'Work' Position

The users' 'Work' position can be designated as any position within the range of travel of the micromanipulator. It is used primarily to ensure that the micropipette tip is not inadvertently lost from the microscope field of view. It is recommended that the 'Work' position is refreshed at regular intervals during the course of an injection experiment. The micropipette can be returned to the last position memorized by briefly touching the 'Work' key (See Operating Instructions).

1.4.2.8 'Home' Position

Activation of the 'Home' function moves the mechanical to its outermost and uppermost position, with respect to the microscope. The 'Home' position of a right-hand micromanipulator will be all the way up and to the right and the 'Home' position of a left-hand micromanipulator will be all the way up and to the left. It is for this reason that a right-hand micromanipulator should not be used on the left side of the microscope and vice-versa. The 'Home' position is a factory preset and cannot be re-configured. See Operating Instructions for details.

1.4.2.9 Speed

The speed of the micromanipulator can be adjusted to accommodate different microscope magnifications and the differing needs of operators and applications. There are six speed settings corresponding to six different sensitivities of micromanipulator movement. See Operating Instructions on how to set the desired movement range. Speed settings 1 and 2 are good for locating pipette into field of view. Speeds 3, 4, and 5 are ideal for micromanipulation and microinjection.

1.4.2.10 'Z-floor'

The 'Z-floor' is a function which allows the user to define a lower limit (or "floor") of the Z-axis travel of the micromanipulator. In practice, it is used to protect a micropipette from being broken by preventing the user from crashing the micropipette into the bottom of the microinjection chamber. The 'Z-floor' is described in more detail in the Operating Instructions section.

1.4.2.11 Micropipette Holder Clamp

The micropipette holder clamp arrangement on the front of the mechanical is designed to allow many different micropipette types to be used with the maximum stability possible. The clamp can be adjusted for height, reach and angle of attack. See Operating Instructions for details on setting the micropipette holder clamp for the optimum angle of attack of the micropipette.

1.4.2.12 Swing Gate

The swing gate on the front of the mechanical allows the micropipette to be swung out toward the front of the microscope for better access when changing micropipettes. The gate can be released by loosening the fixing screw. The screw should be tightened fully during use.

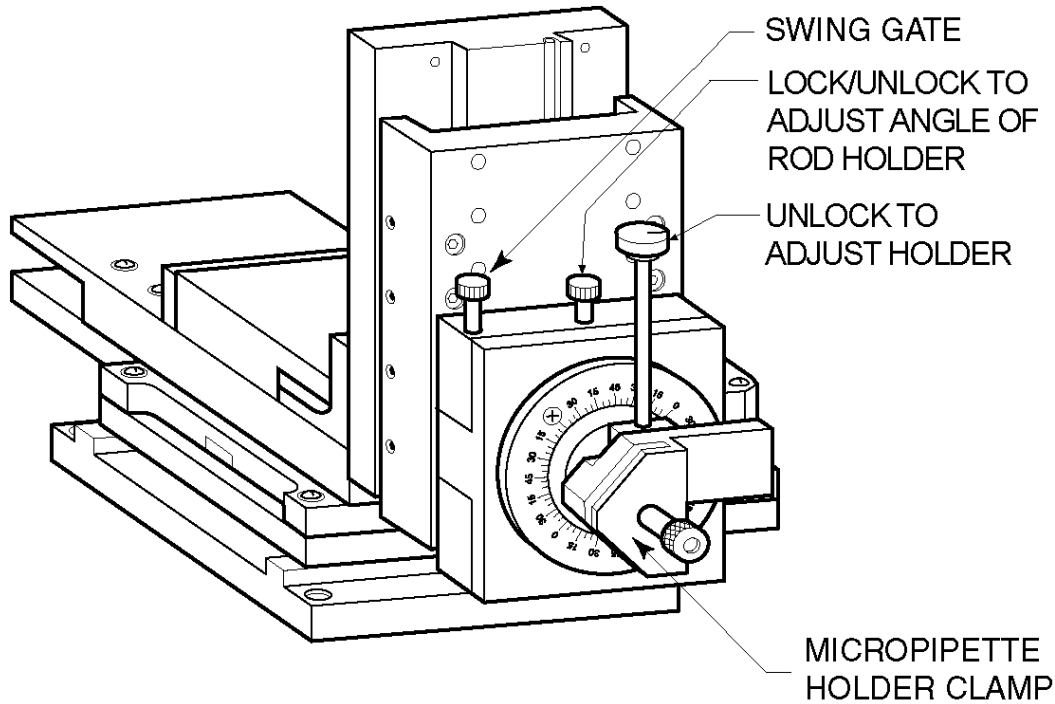


Figure 1-5. Manipulator Mechanical.

1.4.3 Additional Features and Functions

1.4.3.1 'Setup'

When the micropipette is aligned at the beginning of an experiment, the tip should be located in the center of the microscope's field of view and there should be as much movement range as possible in both directions in each axis, X, Y and Z. The 'Setup' function has been designed to easily achieve this and thus protect the user from inadvertently running out of motor travel during an experiment. By following the instructions included in this manual, use of the 'Setup' function will position each of the three axis motors in an optimal position to begin working.

CAUTION: Use the Setup function only when there is no pipette and holder in the manipulator.

The 'Setup' function should only be activated when there is sufficient space available around the mechanical. Activating the 'Setup' function when the manipulator mechanical is too close to a microscope component such as the condenser, may result in damage to the motor or the microscope. Refer to Installation Procedure for details on the use of the 'Setup' function during installation and Operating Instructions for details on routine use.

1.4.3.2 Axis Polarity

Occasionally, it may be desirable to reverse the direction of one, two or all three motors so that the movement of the joystick results in movement of the corresponding manipulator mechanical in the opposite direction. An example of this would be the use of the manipulator with an upright microscope where the image is inverted.

The axis polarity controls are located on the rear of the joystick column.

Each DIP-switch control affects one axis; switch 1 reverses the X-axis, switch 2 reverses the Y-axis, and switch 3 reverses the Z-axis. The figure above illustrates the default axis polarity settings for a left-hand (left image) and right-hand (right image) micromanipulator. Axis polarities can be changed without having to power down the micromanipulator.

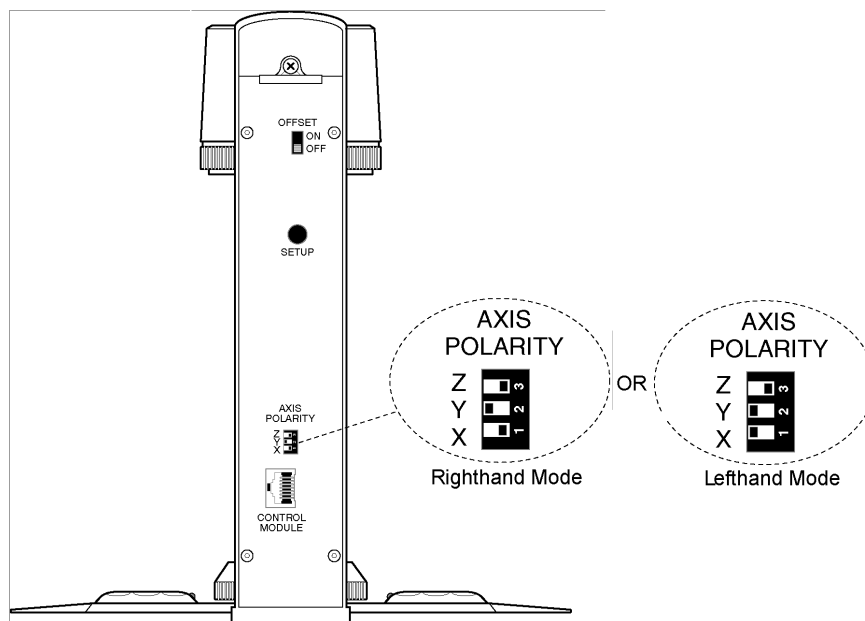


Figure 1-6. Close up of axis polarity switches.

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2. INSTALLATION

The directions below describe the installation procedure common to both left- and right-handed micromanipulators, although the figures illustrate a right-hand micromanipulator (a left hand micromanipulator would be illustrated as a mirror image of these figures). It is extremely important that these installation instructions are followed closely.

1. Unpack and assemble the microscope adapter and attach it firmly to the microscope according to the instructions included with the adapter.
2. Unpack the mechanical manipulator adapter base plate. The Y-axis slide on the bottom of the adapter plate can be adjusted so that the mechanical is positioned correctly with respect to the microscope's optical axis. Each model microscope will require special Y-axis slide settings. To determine the appropriate setting for your microscope, refer to the lookup table contained in the instruction sheet supplied with the microscope adapter.
3. Align the back edge of the slide in the numbered channel with the number in the instructions that corresponds with the microscope being used. Lock the Y-axis slide screws firmly with the hex (Allen) key provided.

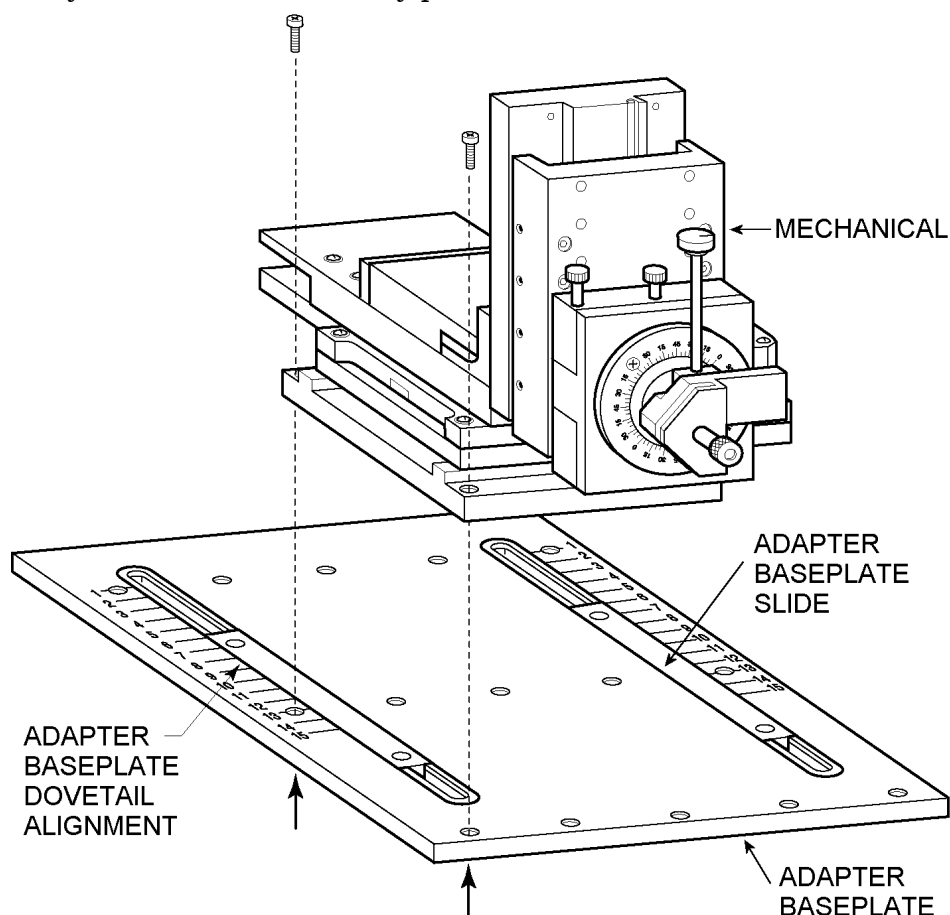


Figure 2-1. Mounting the right-handed manipulator to the far left corner of the adapter plate.

4. Unpack the manipulator mechanical. Take extra care while handling the mechanical - before it is mounted on the microscope adapter, it is vulnerable to impacts which can damage the stepper motors.

5. Remove the six (6) shipping screws (indicated by the red stripes)

IMPORTANT: Retain the six (6) shipping screws and keep them with the instrument packaging in the event that the instrument needs to be shipped to another location.

6. Carefully place the manipulator on the base plate so that the micropipette holder clamp faces toward the front of the microscope, as shown above for a right-hand micromanipulator. The two innermost screw holes (closest to the microscope – arrowed) on the base of the mechanical should be visible. Attach the manipulator to the adapter plate in the position shown, using the screws and tool provided. Do not use shipping screws for this purpose - they are too long.
7. Slide the dovetail on the bottom of the adapter plate into the slide of the microscope adapter (it may be necessary to loosen the locknut on the rear of the microscope adapter). Do not tighten the locknut at this time.

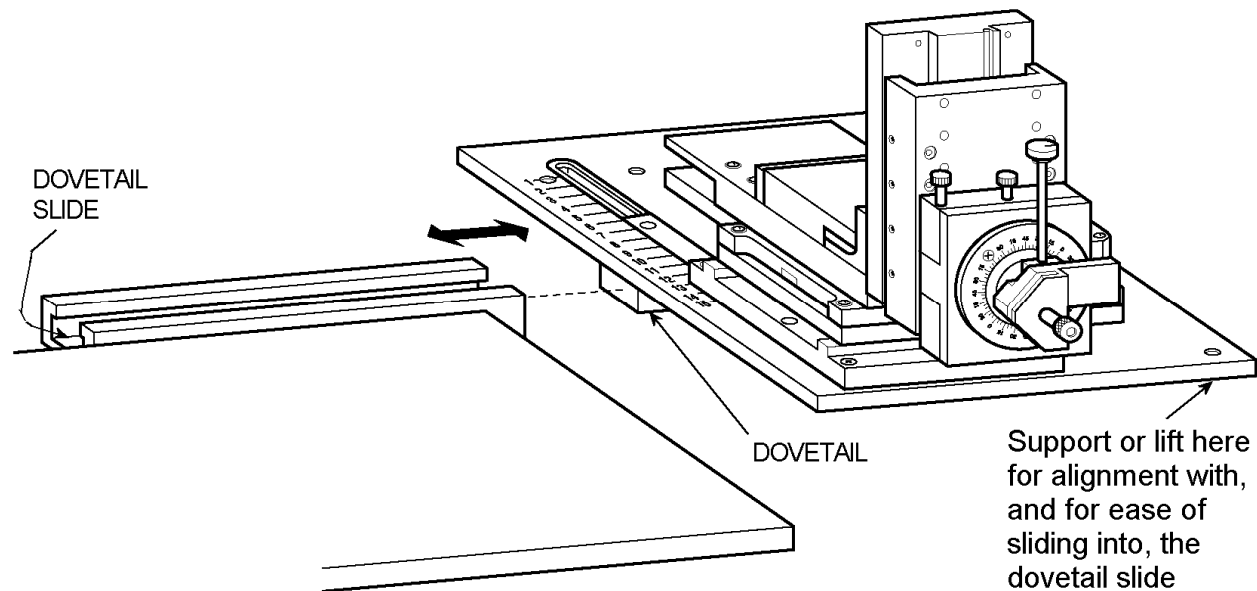


Figure 2-2. Engaging the manipulator base plate in the microscope adaptor.

8. Unpack the controller cabinet and place it on a shelf or flat surface near the microscope.
9. Power and Voltage Selection: The power entry module on the rear of the controller has a voltage-select dial that can be set to either “110” (100 – 120) or “220” (200 – 240) VAC. Ensure that the voltage rating matches your local voltage supply; if not, switch it now.
10. Power Fuse, Ratings, and Selection: The power entry module also contains the fuse. It is very important that the correct fuse rating is used for the voltage selected, as indicated in the table below.

Table A-1. Mains fuse type and ratings.

Mains Voltage Setting	Fuse (Type: Time Delay, 5mm x 20mm, glass tube)	
	Rating	Manufacturer Examples
“110” (100 – 120 VAC)	2A, 250V (Time Delay)	Bussmann: GMC-2A, GMC-2-R (RoHS), GDC-2A, or S506-2A (RoHS) Littelfuse: 239 002 or 239.002.P (RoHS)
“220” (200 – 240 VAC)	T1.0A, 250V	Bussmann: GDC-1A or S506-1A (RoHS) Littelfuse: 218 001 or 218 001.P (RoHS)

11. Using the remaining two cables, connect the mechanical and joystick to the back panel of the controller.
12. Set the joystick height adjustment for comfortable use and adjust the joystick tension ring to achieve a comfortable tightness. Ensure that the joystick is centered (hanging straight down) and that the entire joystick is oriented with the hand-rest to the front and the column to the rear.
13. Connect the power cord to the power input module on the rear of the controller cabinet.
14. Plug the instrument into the electrical supply and switch it on. The green LEDs on the base of the joystick will illuminate and an audible signal will sound to indicate successful initialization of the instrument.
15. Select the coarse range setting by rotating the speed control knob to the ‘C’ position (see Operating Instructions for details).
16. Slowly move the joystick INWARD (toward the microscope), until the end of travel is reached in the X-axis. Use the Declutch if necessary. Take care not to let the mechanical touch any part of the microscope, particularly the condenser. Ensuring that the mechanical base plate is in an outer position on the dovetail slide will help prevent this.
17. Now slide the manipulator mechanical base plate inwards until the manipulator is approximately 5 mm away from the microscope condenser (in the case of inverted microscopes).
18. Since the manipulator is currently at its innermost position, the dovetail of the manipulator adapter plate can safely be locked at this position without danger of the motor running into the microscope condenser during use. Use the locknut on the rear of the microscope adaptor to lock the manipulator mechanical base plate in place.
19. The remaining (outer) two mounting screws should now be visible on the base of the mechanical. Use the final two mounting screws to finish attaching the manipulator mechanical to the base plate.
20. Press the ‘Setup’ button on the rear of the joystick. This will place the motors in the following positions: X motor – $\frac{3}{4}$ of total travel from the outermost position, Y motor – center of travel, Z motor – $\frac{3}{4}$ of total travel from the uppermost position. Using the ‘Setup’ position as a starting point, the user is less likely to run out of motor travel during routine use.

The XenoWorks™ Micromanipulator is now set up and ready for use.

See the next section for details on how to align a micropipette at the start of an injecting session.

3. OPERATING INSTRUCTIONS

3.1 General

3.1.1 Moving the Micropipette with the Joystick

Once the micromanipulator has been installed correctly, the micropipette should follow the movement of the joystick. The higher the number on the range control, the smaller the movement will be for any given deflection of the joystick. When in range settings '2' through '5', the micropipette will follow the movements of the joystick without any delay. Settings 'C' and '1' are for coarse positioning only and should not be used for precision micromanipulation.

3.1.2 Centering the Micropipette with Respect to the Joystick

The Declutch mechanism is used to re-center the joystick with respect to the micropipette tip. This is useful when the micropipette is at the very edge of the microscope field of view but the joystick is centered, or when the micropipette is in the center of the field of view, but the joystick is at the edge of its travel. To center or reposition the joystick without affecting the micropipette, simply touch the declutch ring/band just above the rubber grip as shown in the figure. To re-establish movement, remove your finger from the touch-sensitive band. A red LED lights on top of the joystick whenever the Declutch function is activated.



Figure 3-1. Location of LED and declutch touch band.

3.1.3 Setting the Angle of Approach of the Micropipette

The micropipette holder clamp is capable of rotating around its own axis and the movement can be locked with the thumbscrew (1) shown here. It is also possible to slide the clamp and lock it in place with a separate thumbscrew (2). The clamp itself is spring-loaded, allowing a 4 mm diameter micropipette holder (not included) to be advanced or retracted easily without removing it or risk of it falling out inadvertently.

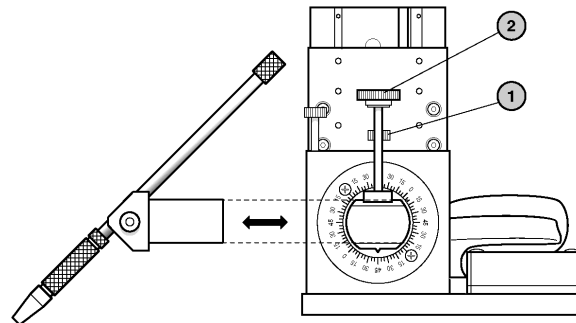


Figure 3-2. Micropipette alignment controls.

These degrees of freedom allow for angles of attack from zero to greater than 45 degrees, while at the same time allowing the micropipette holder to be clamped at any point along its length. This accommodates a large number of different micropipettes, angled or straight, and accessories such as piezo impact drills. When combined with the three fixing patterns on the top plate of the mounting adapter, and the sliding action of the mounting adapter, the XenoWorks™ Micromanipulator is capable of accommodating any application on a large number of microscope platforms.

The following paragraphs describe factors that affect the setting of the micropipette holder.

3.1.3.1 Microinjection Chamber

Some types of microinjection chamber, plastic culture dishes for example, will require a steep angle of approach, so that the micropipette clears the sides of the dish (up to 45 degrees). Other chambers, depression slides, for example, can accommodate a much shallower angle of approach (typically around 10–15 degrees for a depression slide).

3.1.3.2 Applications and Suggested Setups

In general, any application in which suspension cells are to be injected or manipulated (embryonic stem cell transfer, intracytoplasmic sperm injection, pronuclear zygote injection, embryo reconstruction) will require two micropipettes (and thus two micromanipulators), one for holding and one for injecting. In this instance, the micropipette tips must approach the tissue side-on to prevent the injection micropipette from pushing the tissue off the holding pipette. When using a deep dish and angled micropipettes, a 35–45 degree angle of attack is appropriate (shown in the figures on the page opposite). If using a depression slide and straight micropipettes, an almost flat angle is required as shown in the figures below.

Setup for various applications

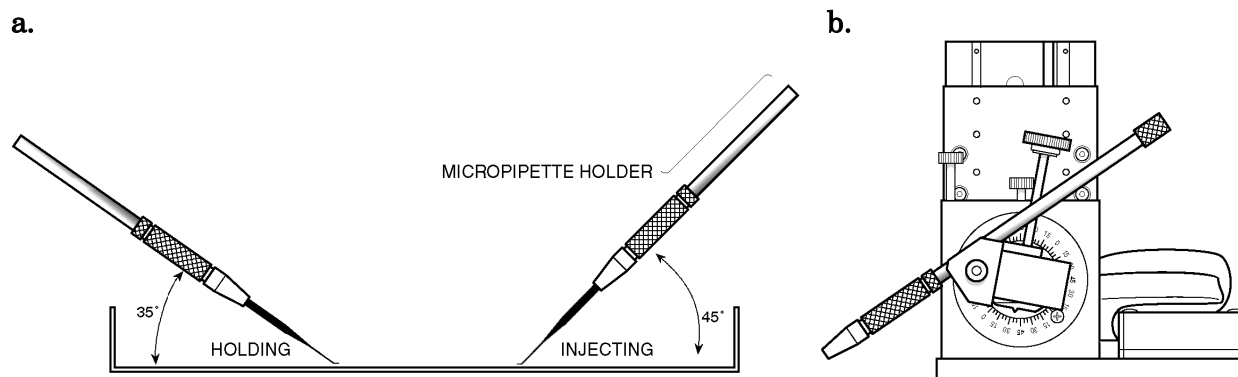


Figure 3-3 (a and b). Suggested approach for **suspended cells with angled micropipettes** (ICSI, Stem Cell, Nuclear Transfer).

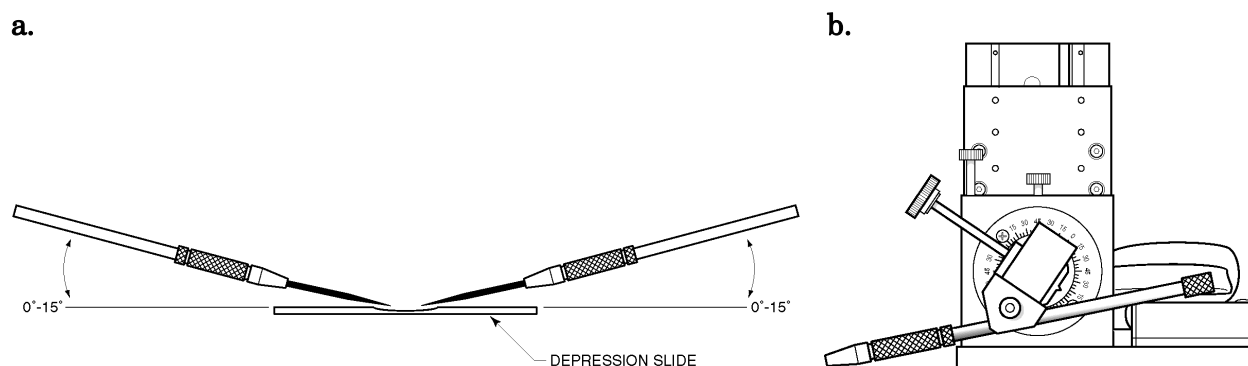


Figure 3-4 (a and b). Suggested approach for **suspended cells with straight micropipettes** (Pronuclear Injection).

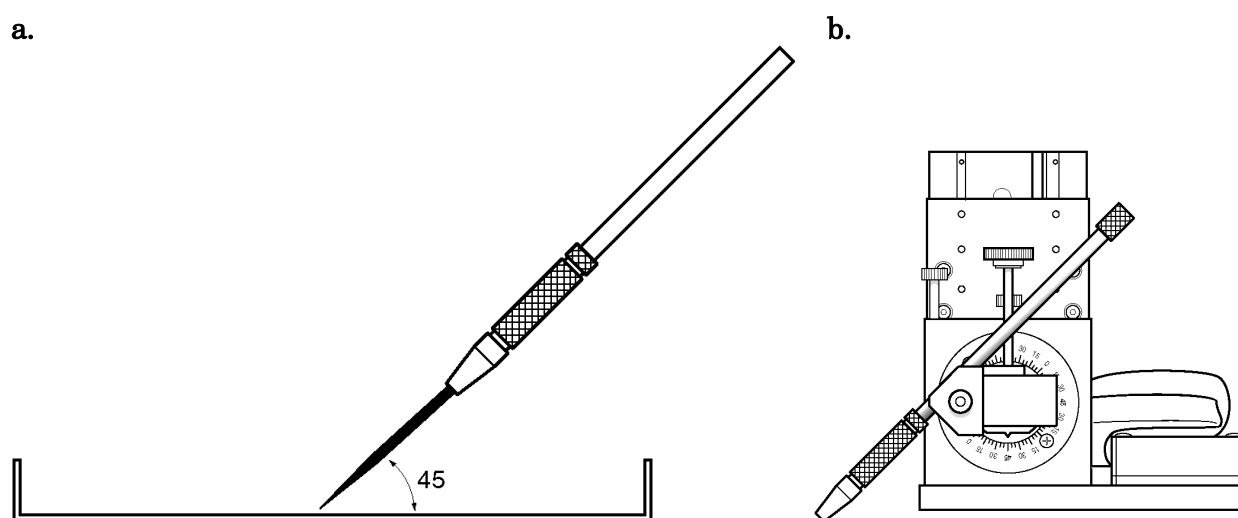


Figure 3-5 (a and b). Suggested approach for **adherent cells and straight pipettes**.

3.1.3.3 Micropipette Type

Some micropipettes (lab-made or purchased ICSI pipettes, for example) are often designed with an angle close to the tip. This is to allow the micropipette tip to approach the injected cell from a trajectory parallel to the bottom of the chamber, and to allow the use of a high-sided dish. Other micropipettes may not have a bend, in which case they must be used with a flat injection chamber (a depression slide for example) of a low angle of approach.

3.1.3.4 Working Distance

Most microinjection procedures are carried out on an inverted microscope where the condenser is located above the stage and the objective lenses below. If the microscope was purchased specifically for microinjection, it was probably supplied with a “long” or “very long” working distance condenser that should provide adequate space above the stage to set up the micropipette and accommodate any type of chamber. When setting up the manipulator adapter plate, the manipulator mechanical and the micropipette holder, carefully observe the relative positions of the micromanipulator, the microscope stage, and the condenser to avoid collision by the manipulator.

3.1.3.5 Piezo Impact Drive

For those applications that require the use of a piezo impact drive, the position of the piezo must be taken into account when aligning the micropipette holder (and also when attaching the mechanical to the microscope mount). Typically, a piezo device is mounted at the back of the micropipette holder, and so the holder can be gripped at the very front. A Prime Tech piezo device can be mounted anywhere along the length of the micropipette holder, either in front, or behind the micropipette holder clamp.

3.1.4 Memorizing the ‘Work’ Position

This is accomplished by first using the joystick to position the micropipette in the desired ‘Work’ position. Then, press and hold down the ‘Work’ button on the base of the joystick for approximately three seconds, until a tone is heard. The beep indicates that the position has been set. A previously set ‘Work’ position can be overwritten with a new position at any time by repeating this action. The micropipette can be moved to the currently set position by briefly touching the ‘Work’ key. The movement can be stopped by quickly pressing ‘Work’ again. Pressing the key once more resumes the movement towards the ‘Work’ position. This process can be repeated until the micropipette reaches the ‘Work’ position. The current ‘Work’ position is preserved in the micromanipulator memory even if it is switched off; it is advisable to establish a new ‘Work’ position when setting up the position of the micropipette.

3.1.5 Using the ‘Home’ Function

The ‘Home’ function is activated by briefly pressing the yellow ‘Home’ button, the outermost button of the group of three on the hand-rest of the joystick.

This function is usually used to temporarily remove the micropipette from the injection chamber (to change dishes, for example), or to exchange micropipettes. Note that a ‘Work’ position must be set (see above) if the micropipette is to be moved back into the position it was in before the ‘Home’ function was activated. When the ‘Home’ function is activated, the micropipette will move from its starting position to the ‘Home’ position. This movement can be halted at any time by briefly pressing ‘Home’ again, or by briefly pressing ‘Work’. Pressing ‘Home’ once more will resume the movement of the micropipette to the ‘Home’

position. Briefly pressing the ‘Work’ key will reverse the direction and move the micropipette to the ‘Work’ position (In this way, a micropipette can be partially raised out of the injection chamber and returned, without having to move all the way out to the ‘Home’ position – see “Cleaning the Micropipette” below). Note that the micropipette tip moves out at approximately 45°, no matter what its angle of incidence.

3.1.6 Cleaning the Micropipette

The ‘Work’ and ‘Home’ functions can be used together to clean material from the tip of the micropipette. Record the ‘Work’ position as the center of the field of view, then activate the ‘Home’ function. As soon as the micropipette tip has passed out of the injection medium, press ‘Home’ again briefly to stop the micropipette movement. By passing the micropipette through the surface of the injecting medium, debris on the outside of the micropipette tip is usually cleared. Press ‘Work’ briefly and the micropipette will return to the ‘Work’ position.

3.1.7 Changing the Working Range of Movement

The range of movement of the micropipette can be varied depending on the desired movement characteristics and the magnifying power of the microscope. The range can be changed by simply rotating the range control knob to the desired setting. The ranges available are listed under Specifications.

3.1.7.1 Setting a ‘Z-Floor’

The ‘Z-floor’ is set in the following manner:

1. Locate the tip of the micropipette in the field of view of the microscope.
2. Slowly rotate the Z-axis control clockwise, lowering the micropipette towards the bottom of the injection chamber, following the micropipette tip down with the microscope’s fine focus. Use slower speeds (3 – 5) when close to the bottom of the dish.
3. When the micropipette touches the bottom of the injection chamber it will appear to move inward as it brushes the bottom of the dish. (**CAUTION: Sharp micropipettes, such as cell injection micropipettes with high angles of approach, may break if they are pressed too hard against the bottom of the injection chamber.**) Raise the micropipette up slightly so that it just clears of the bottom of the chamber.
4. Press and hold the ‘Z-floor’ key down for about three (3) seconds. A tone will be heard indicating that it has been set and a green LED will light on the joystick base indicating that the ‘Z-floor’ is active. The ‘Z-floor’ can be toggled on and off by pressing the key briefly. When the ‘Z-floor’ is active, the Z-axis will not move any lower than the preset position, protecting the micropipette from inadvertent crashes. If the micropipette is moved below the ‘Z-floor’ position when it is inactive, and the ‘Z-floor’ is then activated, the micropipette will only move upwards when the Z-axis control on the joystick is rotated. When the ‘Z-floor’ position is reached, normal movement will resume. A new ‘Z-floor’ can be set at any time to overwrite the previous setting. The ‘Z-floor’ setting is erased when the micromanipulator is switched off.

CAUTION: If a ‘Work’ position is set below a ‘Z-floor’ limit, the micropipette will move below the ‘Z-floor’ when the ‘Work’ key is pressed.

3.1.8 Exchanging the Micropipette

During the course of a microinjection procedure, it may be necessary to exchange micropipettes. One effective method of doing this is to incorporate the 'Home' function in the following manner:

1. Center the micropipette in the microscope field of view and above the dish by 3 mm, and press and hold down the 'Work' key to memorize the coordinates.
2. Press 'Home', and the mechanical will move to the upper and outer limits of travel. The movement from 'Work' to 'Home' can be paused at any time by pressing 'Home' or 'Work' briefly. Movement to 'Home' can be resumed by pressing 'Home' again briefly.
3. Unlock and open the swing gate to access the top of the micropipette holder.
4. Taking care not to disturb the position of the micropipette holder in its clamp, loosen the chuck on the front of the micropipette holder and remove and discard the micropipette safely.
5. Fit a new micropipette into the holder and lock the chuck in place.
6. Return the swing gate to the working position and lock it in place.
7. Press 'Work' to move the micropipette above the injection chamber and in the field of view.

CAUTION: It is important to remember that when activating the 'Work' function, the micromanipulator records the position of the micromanipulator motors, not the micropipette tip. Therefore, if a micropipette is being replaced by one that is longer, the micropipette may crash into the bottom of the injection chamber upon returning to 'Work' from 'Home'. Several measures, taken alone or together, can prevent this: When returning to 'Work' from 'Home', approach the injection chamber with the new micropipette in stages by toggling the 'Work' key. Once the micropipette tip is close to the bottom of the injection chamber, use small movements on low speeds with the joystick.

3.2 Starting a Microinjection Session

3.2.1 Setting up the Microscope

1. Locate the correct focal plane for the microinjection chamber in the following manner:
2. Place a representative microinjection dish, chamber or slide on the stage of the microscope, set the microscope to its lowest possible magnification, and focus on the uppermost surface of the bottom of the chamber (an effective method is to draw a pen mark in the center of the chamber to be used, and focus on that). This establishes the correct microscope focal plane for microinjection.
3. The microinjection chamber can now be removed and, as long as the microscope focus is not disturbed, the micropipette tip can be manipulated into the optical axis, above the bottom of the injection chamber.

3.2.2 Setting up the Micromanipulator

1. Remove the micropipette holder (rod) from the clamp of the mechanical.
2. Activate the 'Setup' key on the rear of the joystick. This ensures that the motors are in their optimum positions prior to starting 'Work'.

3. Load a micropipette (appropriate to the chosen application) into the micropipette holder and install it in the clamp of the mechanical so that the tip of the micropipette is pointing in and down towards the optical axis of the microscope.
4. Adjust the angle setting of the micropipette clamp on the mechanical so that the angle of attack of the pipette is appropriate for the chosen application as described in Operating Instructions.
5. Once an appropriate angle of approach has been set, loosen the micropipette holder clamp slightly and slide the holder inwards along its own axis, until the micropipette tip is level with (almost touching) the plane of the microscope stage.
NOTE: Do this by eye – it is not necessary to look down the microscope at this stage.
6. Place the manipulator in ‘Coarse’ mode ‘C’, and carefully manipulate the joystick until the micropipette can be seen through the microscope eyepieces.
7. Change the speed to 1 or 2, center the micropipette tip in the microscope field of view, and change microscope to a higher magnification. Remember not to adjust the microscope focus at this point. To “focus” the micropipette tip, move it up and down in the Z-axis by rotating the Z-axis dial at the bottom of the joystick.
8. Once the desired magnification has been reached, and the micropipette is still in view, set the ‘Work’ position.
9. To replace the microinjection dish, press ‘Home’ to take the micromanipulator to the ‘Home’ position (make sure that a ‘Work’ position has been set first). Once the micropipette tip is clear of the microscope stage, replace the microinjection dish. Pressing the ‘Work’ button will cause the micropipette tip to return to the ‘Work’ position previously set.
10. At this point, it may be desirable to set the ‘Z-floor’ function to prevent accidentally breaking the micropipette during use. To do this, lower the tip to the bottom of the dish and you will see the micropipette tip “kick-out” slightly as it touches the dish (this is not recommended for sharp micropipettes with a high angle of attack, since the micropipette tip may break). Press and hold the ‘Z-floor’ key until a tone is heard and the green LED illuminates. The ‘Z-floor’ is now active. The ‘Z-floor’ will stay in the micromanipulator memory until it is overwritten with a new limit or until the instrument is powered down.
11. The XenoWorks™ Micromanipulator is now ready for microinjection.

Note that, as the system becomes more familiar, many of these steps (like the Z-limit) can be skipped.

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4. MAINTENANCE

4.1 General

The XenoWorks Micromanipulator requires no regular maintenance.

- In order to avoid restricted movement of the mechanical, make sure that all cables are not taught and have freedom of movement.
- Do not oil the mechanicals.
- If removed for storage or shipping, press the Setup button on the back of the joystick before power down so that the shipping screw holes are in alignment. Then install the shipping screws. If needed, use a screwdriver in the center slot of each motor to fine-tune the alignment of screw holes.

4.2 Troubleshooting

Problem	Cause	Solution
There is excessive vibration at the micropipette tip during movement.	1. The micromanipulator is set on a coarse range of movement setting ("C" or "1").	Change the range of movement setting (Speed) to "2", "3", "4" or "5".
	2. The micropipette is too long.	Use a micropipette that is no longer than about 50mm.
	3. The micropipette has not been pushed far enough into the micropipette holder.	Ideally, no more than about 40mm of micropipette should protrude from the front of the chuck. In practice, micropipettes with wide tip diameters (such as holding pipettes) will be less prone to vibration and so can extend further from the chuck than sharp micropipettes such as those used for intracellular microinjection. Push the micropipette further into the holder if vibration is occurring, but do not seat the back end of the micropipette on the back surface of the holder - pull it back out slightly.

Problem	Cause	Solution
There is still excessive vibration at the micropipette tip during movement.	4. The O-rings in the micropipette holder chuck are missing or damaged.	The micropipette holder has been designed to minimize vibration when used with three O-rings in the chuck, and when the O-rings are the only thing contacting the back end of the micropipette. Less than three O-rings (or the back of the micropipette touching the back of the holder) may lead to excessive vibration. Replace all missing or damaged O-rings.
	5. The micropipette is dragging on the bottom or sides of the microinjection chamber.	If the tip is touching the bottom of the chamber, raise the micropipette slightly by turning the Z-axis control counterclockwise. If the shaft of the micropipette is touching the side of the chamber, consider repositioning the micropipette with respect to the micromanipulator as described in Section 4.
	1. The micropipette holder clamp has not been tightened down sufficiently.	Tighten the micropipette holder clamp.
	2. There is too great a distance between the micropipette tip and the point where the micropipette holder is gripped by the holder clamp on the micromanipulator.	This is a different problem to the one described in 2 and 3 above. Here there is too much of the micropipette holder shaft projecting from the clamp on the micromanipulator. Shorten the micropipette tip-to-clamp distance by moving the holder outboard and clamping the micropipette holder closer to its tip.
	3. The fixing screws of the mounting adapter are not tight.	Tighten the fixing screws.

Problem	Cause	Solution
	4. The swing gate thumbscrew is loose.	Tighten the swing gate thumbscrew.
	5. The mechanical is not screwed securely to the mechanical base plate.	Ensure all four screws fixing the mechanical to its base plate are tight.
	6. The mechanical base plate is not locked to the microscope adapter.	The mechanical base plate is not locked to the microscope adapter.
	7. The micromanipulator is at the extreme limit of one or more of its axes.	Remove the micropipette holder from the micromanipulator. Activate the “Setup” function, which centers the motors. If necessary, adjust the mechanical base plate with respect to the microscope adapter so that the microscope objective intersects the center of the micromanipulator’s Y-axis. Realign the micropipette holder clamp arrangement (X- and Z-axes), so that when the micropipette holder is replaced in the clamp, the micropipette tip will come as close as possible to the microscope’s optical axis. Only now, should the joystick be used to fine-position the tip of the micropipette in the microscope field of view.
	8. The cable attached to the mechanical is under tension.	Release any tension by coiling the cable once on the bench behind, or to the side of the microscope.
	9. The center fitting of the micropipette holder is loose.	Tighten the fitting.
The micropipette has been lost from the field of view.	A too-coarse range of movement setting has been selected, or the joystick resistance is too loose.	Select a finer range setting (Speed) or tighten the joystick friction dial.

Problem	Cause	Solution
One or more motors move in the opposite direction to that commanded by the joystick.	One or more of the axis polarity DIP switches are in the wrong position.	Flip the appropriate DIP switches (see the axis guide in section 2.4). It is not necessary to restart the instrument.
The micropipette will not move any lower.	1. The end of motor travel has been reached.	Set up the micropipette alignment again.
	2. A 'Z-limit' has been set.	Deactivate the 'Z-limit' (press the 'Z-limit' key once briefly. The green LED will go out).
The micropipette appears to move in a direction opposite to that commanded by the joystick, but only in the Y-axis.	1. The Y-axis inversion DIP switch is in the wrong position.	Reverse the Y-axis DIP switch.
	2. A micropipette with an angled tip has been aligned such that the bend or "heel" of the micropipette is touching the dish, but the tip is not. This is usually only noticeable at higher magnifications.	Angled micropipettes should be adjusted from the start so that they are slightly "toe-down", in other words, the tip should touch the bottom of the microinjection chamber before the bend does.
The micropipette does not return to the assigned coordinates memorized by the 'Work' setting.	1. No new 'Work' setting was actually assigned, and so the micropipette moved to a different, previously assigned position.	Relocate the micropipette tip and assign a new 'Work' position.
	2. Repeated rapid, violent movements of the joystick may cause the instrument to miss information coming from the joystick controller to the processing circuitry, and so miscalculate the correct position of 'Work'. Normally, the error is no more than a few tens of microns.	Avoid violent movements of the joystick since they not only confuse the electronics, they can also damage the joystick assembly. To ensure that the 'Work' position is always current, save a new one periodically during the course of an experiment.

Problem	Cause	Solution
	3. The joystick was inadvertently moved during the automatic return to the 'Work' position.	Press 'Work' again.
The movements of the mechanical do not correspond with the movements of the joystick - specifically, movements of the Z-axis control seem to cause "runaway" movements of the Z-axis motor (other anomalous activity may also be observed). Often, the end of the Z-axis travel will be reached but the motor will not stop.	The domestic voltage supply may have dropped below the specification for the instrument.	Investigate the domestic power supply for your facility. It may be necessary to install a transformer to bring the power back to normal. If you have any questions regarding power requirements, please contact Sutter technical support.
The motors "buzz" abnormally during movement, particularly during movements to/from "work" or "home" and particularly at the ends of axis travel.	There is some resistance preventing the motors from moving smoothly.	Do not touch the mechanical during automatic movement. Ensure that the cable connecting the mechanical to the controller is not under tension – coil the cable a few times on the bench surface behind the microscope.

4.3 Transporting the Micromanipulator

When moving the instrument, the entire system should be disassembled and placed in its original packaging. Shipping screws are supplied to prevent inadvertent vibrations damaging the delicate manipulator mechanical. To install the shipping screws, follow this procedure:

1. With the instrument switched on and the mechanical still installed on the microscope adaptor, place the joystick in the coarse ('C' range) setting.
2. Use the joystick to move the mechanical INWARDS, towards the center of the microscope, exposing the outer two screws that fix the mechanical to the base plate.
3. Loosen, remove and retain these two screws.
4. Activate the 'Setup' function by pressing the key on the rear of the joystick.
5. Without touching the joystick further, immediately switch off the power to the instrument.
6. Carefully unplug the connecting cable from the rear of the mechanical.
7. Remove the mechanical from the adapter plate (remove retaining screws and ensure they are kept in a safe place).

8. Locate the shipping screw holes. There are four through-holes in each axis slide, but only two of the holes will line up with the correct tapped holes on the inner slide surface when the instrument is in the 'Setup' position.
9. Locate the original shipping screws that should have been retained with the original packaging, and insert them into the appropriate locations. Do not over tighten the screws - turn them until they are lightly seated in their holes.
10. Place the mechanical in a plastic bag.
11. The mechanical is now ready for transport in the original packaging.
Take care to ensure that the shipping screws are removed before the instrument is operated again (see Section 3 for the correct installation procedure).

APPENDIX A. LIMITED WARRANTY

- Sutter Instrument Company, a division of Sutter Instrument Corporation, limits the warranty on this instrument to repair and replacement of defective components for two years from date of shipment, provided the instrument has been operated in accordance with the instructions outlined in this manual.
- Abuse, misuse, or unauthorized repairs will void this warranty.
- Warranty work will be performed only at the factory.
- The cost of shipment both ways is paid for by Sutter Instrument during the first three months this warranty is in effect, after which the cost is the responsibility of the customer.
- The limited warranty is as stated above and no implied or inferred liability for direct or consequential damages is intended.
- Consumables, PMTs, galvanometers, and Uniblitz^{®1} shutters are exempt from this warranty.
- An extended warranty for up to three additional years can be purchased at the time of ordering, or until the original warranty expires. For pricing and other information, please contact Sutter Instrument.

¹ Uniblitz[®] is a registered trademark of Vincent Associates.

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APPENDIX B. ACCESSORIES

The following accessories are available for the XenoWorks Micromanipulator system.

Extensions

4-in (10.16-cm) Dovetail Extension (X285204)

Z-axis vertical extension (X285305)

Z-axis horizontal extension (X285310)

Rotating Base

Rotating base (225RBI)

Mounts

Hinged headstage mount (285HEA)

Adapters

Mounting adapter plate (X285210). *For use with MT and MD series stands and platforms, or any surface with 1 inch centered holes.*

Right angle adapter (X285300)

Holders

Rod holder (MP-ROD)

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APPENDIX C. FUSE REPLACEMENT

In the event that the controller fails to power up when the power switch is turned on (display and fans do not come on), check the line power fuse to see if it has blown. The fuse is located in the fuse holder on the power entry module on the back of the controller cabinet. To remove the fuse holder first unplug the power cord from the power entry module. This will reveal a slot just under the edge of the fuse holder. Use a screwdriver to pry the holder straight out of the power entry module.

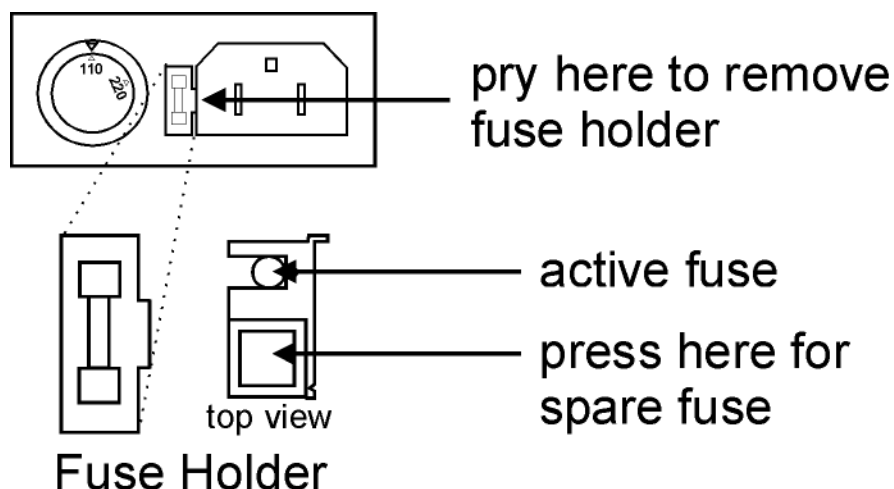


Figure C-1. Fuse replacement.

The fuse that is readily visible in the fuse holder when you take it out is the one that is “active” when the holder is installed. A spare fuse is also stored within the fuse holder. The spare fuse is concealed in a compartment as shown. To remove the spare fuse, press down on the end of the compartment to push it out of the other end. The old fuse can serve as a convenient tool for pushing the spare fuse compartment out. Replace the active fuse with the spare and reinstall the fuse holder and power cord. If the micromanipulator fails to power up with the new fuse installed, call Sutter Instrument technical support for assistance.

Table C-1. Mains fuse type and ratings.

Mains Voltage Setting	Fuse (Type: Time Delay, 5mm x 20mm, glass tube)	
	Rating	Manufacturer Examples
“110” (100 – 120 VAC)	2A, 250V (Time Delay)	Bussmann: GMC-2A, GMC-2-R (RoHS), GDC-2A, or S506-2A (RoHS) Littelfuse: 239 002 or 239.002.P (RoHS)
“220” (200 – 240 VAC)	T1.0A, 250V	Bussmann: GDC-1A or S506-1A (RoHS) Littelfuse: 218 001 or 218 001.P (RoHS)

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APPENDIX D. TECHNICAL SPECIFICATIONS



Manipulator Mechanical:

3-axis stepper motor

X, Y, and Z Axes

62.5 nm per microstep

25 mm (1 in) full travel

Joystick:

Inverted joystick with integral Declutch

Height and resistance adjustable

Independent axis-polarity inversion

6-position rotary range-of-movement control

‘Setup’ function

Position memory:

One user-defined set point (‘Work’),

One user-defined axis limit (‘Z-floor’),

Two preset points (‘Home’ and ‘Setup’)

Ranges of Movement (Speed):

Table D-1. Speed settings and ranges of movement.

Range (Speed) Setting	X & Y (1 joystick swing)	Z (1 rotation)
C	12,700 μm	3,200 μm
1	3,300 μm	800 μm
2	880 μm	400 μm
3	400 μm	200 μm
4	200 μm	100 μm
5	100 μm	50 μm

Electrical:

Input voltage (Mains): 100 – 120 VAC, 50/60 Hz,
200 – 240 VAC, 50/60 Hz

Power consumption: 200 VA max., 88 VA typical

Mains fuse (rear of cabinet):

Table D-2. Mains fuse type and ratings.

Mains Voltage Setting	Fuse (Type: Time Delay, 5 x 20 mm, glass tube)	
	Rating	Manufacturer Examples
“110” (100 – 120 VAC)	2A, 250V (Time Delay)	Bussmann: GMC-2A, GMC-2-R (RoHS), GDC-2A, or S506-2A (RoHS) Littelfuse: 239 002 or 239.002.P (RoHS)
“220” (200 – 240 VAC)	T1.0A, 250V	Bussmann: GDC-1A or S506-1A (RoHS) Littelfuse: 218 001 or 218 001.P (RoHS)

Cables

(Refer to the following two tables for a description of all possible cables.)

Table D-3. XenoWorks BRM Controller cables and receptacles/connectors.

Controller Rear Panel Port Connector/Receptacle	Cable Connector Types	Connects to ...	Cable Type	Cable Max. Length
(Power entry) 3-pin male connector	←3-pin power standard (female) 3-pin male→ (Geographical region dependent)	Mains power source.	10A, 250V, with safety ground plug	3 meters (approx. 10 feet)
DRIVE MODULE (25-Pin DSUB female receptacle)	←DB-25 male DB-25 female→ (straight-through)	MP-285M (25-Pin DSUB male connector)	Minimum of 26 awg stranded wire with 500 Volt (see note)	3 meters (approx. 10 feet)
JOYSTICK MODULE (RJ45 receptacle)	RJ45 male RJ45 male	Joystick CONTROL MODULE (RJ45 receptacle)		3 meters (approx. 10 feet)

NOTE:: A ferrite at the controller end is strongly recommended (Fair-Rite part number 0443164-251). Fair-Rite Products Corp., P.O. Box J, One Commercial Row, Wallkill, NY, 12589, USA

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, CE
EMC - EN61326, CE

Cleaning:

70% alcohol (or e.g., UV)

Dimensions (H x W x D):

Mechanical:	112 x 145 x 185 mm (4.4 x 5.7 x 7.3 in)
Joystick:	265 x 240 x 226 mm (10.4 x 9.4 x 8.9 in)
Controller:	101 x 407 x 280 mm (4 x 16 x 11 in)

Weight:

Mechanical:	174 g (0.38 lb)
Joystick:	254 g (0.56 lb)
Controller:	432 g (0.95 lb)

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