OPERATION MANUAL

COMPLIMENTS OF

SUTTER INSTRUMENT COMPANY

P-80/PC

Micropipette Puller

SUTTER INSTRUMENT COMPANY

MODEL P-80/PC

FLAMING/BROWN MICROPIPETTE PULLER

by Phone 415-883-0128
Our office hours are
8:00am to 5:00pm
Pacific time from Monday to Friday

by Fax 415-883-0572
24 hours a day

by Mail
Sutter Instrument Company
51 Digital Drive
Novato, CA 94949

by e-mail
info@sutter.com

INTRODUCTION

The Model P-80/PC Brown-Flaming Micropipette Puller combines a proven pulling technology with programmability to produce a very versatile instrument. The pulling mechanism is derived from the P-77/P-80 series of pullers, which have demonstrated the ability to pull a complete range of pipette profiles. Added to this mechanism is the ability to program different pulling sequences; thus, allowing ease of use for pulling a multiplicity of pipettes on one device.

The P-80/PC is a 'velocity sensing' puller. This feature allows the puller to indirectly sense the viscosity of the glass, giving the P-80/PC the ability to pull pipettes from all glasses save quartz. Even difficult to pull formulations, such as aluminasilicate glasses, are handled with relative ease.

The P-80/PC can fabricate pipettes for use in such researches as intracellular recording, patch-clamping, microinjection and microperfusion. However, realizing the full potential of this instrument is dependent on a complete understanding of the way it implements the pulling process. To this end we urge that this manual be read in its entirety. To aid in understanding the function of the instrument, sample programs are already loaded in memory (as discussed in subsequent material).

For that setting in which a number of users must work with one device, or for that single user whose investigations require a variety of pipettes; the P-80/PC is the answer.

SUTTER INSTRUMENT COMPANY

SECTION 1

UNPACKING AND SETTING UP

The Model P-80/PC is shipped to you wrapped in a plastic bubble wrap and surrounded by approximately 6 inches of loose fill on all sides. Please take note of this method of packaging. Should it ever be necessary to ship the puller to another location, the same method of packaging should be employed.

IMPORTANT: Improper packaging is a form of abuse, and as such can be responsible for voiding the warranty where shipping damage is sustained as a result of such packing.

All material shipped with the instrument is contained within or is attached to the plastic wrapping.

After removing the puller from its shipping carton, cut the tape holding the plastic wraps. Be careful not to cut through the power cord at the back of the device. Open the plastic cover by lifting the hinged front and remove any filament containers. These containers are usually located on the left side in front of the main valve on the nitrogen tank. Those pullers ordered without a nitrogen tank will have their filaments shipped in a small container attached to the front of the plastic wrap. While the puller cover is still raised, open the valve atop the nitrogen cylinder by turning it counter-clockwise (as viewed from the valve end of the cylinder).

If you have ordered your puller without a nitrogen cylinder, regulator and cradles it will be necessary to secure a separate cylinder and regulator to deliver nitrogen at 50 psi, via the attached length of tubing, to the gas solenoid. This nitrogen source need not be dry or medical grade; however it should not be a source that is subject to changes in moisture content (as is the case with most piped in sources). Should it be more readily obtainable, compressed air is also suitable, subject to the same moisture considerations.

Place the puller in a location where there is a free flow of fresh air on all sides. The fan draws air in through the vents on the sides and exhausts out both ends of the heat sink. **NEVER ALLOW THE FREE FLOW OF AIR TO BE RESTRICTED.**

Since the P-80/PC is a microprocessor controlled device, it should be accorded the same system wiring precautions as any 'computer type' system. If microprocessor based systems in the lab require line surge protection for proper operation, then the same protection should be provided for the P-80/PC.

FIRST TIME USE

Plug the instrument into a power receptacle of the correct voltage and frequency. NOTE: A power source that is essentially electrically noise free is desirable. The control circuitry of the puller uses digital logic that may be susceptible to transient spikes that can be caused by faulty wiring or noise producing machinery, such as centrifuges or other equipment utilizing SCR control circuitry on the same power lines.

Lift the front of the plastic cover; open the main tank valve located at the left end of the nitrogen tank. Open the valve fully (counter-clockwise). It should not be necessary to turn off this valve between electrode pulling sessions. The high pressure meter should read above 1500 psi. Adjust the regulator to a reading of 50 pounds on the low pressure meter. If this reading is at a value higher than 50 pounds, turn the regulator knob counter-clockwise one turn. No change will be observed in this pressure reading until an electrode pipette is pulled. The pressure will then drop. After several electrodes have been pulled, the pressure will stabilize at a lower value. Now readjust the pressure to 50 pounds.

Pressures greater than 50 pounds are not recommended.

Programs 0 and 1 are preprogrammed into the puller by the manufacturer. Both programs were developed using 1mm O.D. borosilicate glass with an I.D. of .5mm. Program 0 will pull a micropipette of less than .1 micron and program 1 will pull a patch-pipette of about 1 micron.

To change from one program to another press the reset button on the front panel, and then select the program on the keypad. DO NOT USE OTHER PROGRAMS OR CHANGE THE VALUES IN THESE PROGRAMS UNTIL YOU HAVE READ SECTIONS II, III AND IV OF THIS MANUAL.

To execute program 0 or 1, place a piece of glass in the carriers and press the pull key on the keypad.

SECTION 2

MECHANICAL DESCRIPTION, CONTROLS AND ADJUSTMENTS

This section presents a basic mechanical description of the P-80/PC, with particular emphasis on terminology. Knowing the names of the various parts greatly facilitates communication between the investigators and the factory when discussing adjustments or service problems. In addition, various controls and adjustments on the top of the instrument are located and described. Those adjustments which are considered part of maintenance procedures are dealt with in Section 6.

GAS SUPPLY TANK AND REGULATOR: The Model P-80 gas supply consists of a tank filled with nitrogen of approximately 2 1/2 pounds at a pressure of 1800 psi, a regulator with high pressure and low pressure gages, an electronically controlled solenoid valve, a micrometer flow valve, and a nozzle.

The gas should last several years with normal use. Continuous lowering of high pressure might indicate a leaky system, depending on usage. The tank may be refilled by any supplier of compressed gases. Refill only with nitrogen.

When installing a refilled tank, make certain all the fittings are tight.

CAUTION: Be certain to shut off the main tank valve before removing the tank or the regulator from the tank. The tank is at high pressure and should be handled with care.

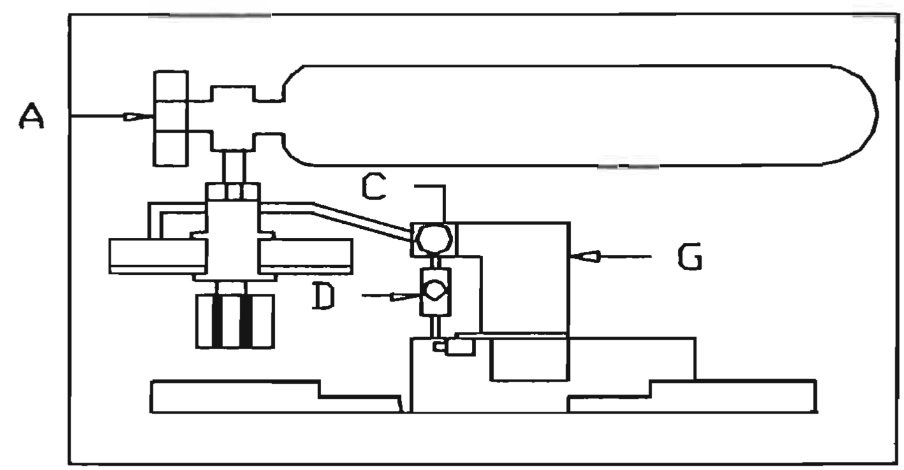

FIGURE 1

CYLINDER MAIN VALVE: (Fig.1, a) This valve is closed at the time of shipment. Upon receipt of the instrument, this valve should be opened all the way. This is a double seat valve and should not be left partially opened; small leaks may occur. If the instrument is to be left unused or stored for any length of time (two weeks or more) this valve should be closed.

NITROGEN REGULATOR: This regulator is fitted with an industry standard CGA 580 connection. The high pressure and low pressure gages read, respectively, cylinder pressure and pressure of gas delivered to the micrometer flow valve. The large central knob adjusts the delivery pressure and is normally set at 50psi.

GAS SOLENOID: (Fig. 1, c) This solenoid is activated during the hard pull phase of the pulling cycle. It admits nitrogen to the micrometer flow valve.

MICROMETER FLOW VALVE: (Fig. 1, d) This micrometer valve (needle valve) controls the flow of gas to the filament. Despite the indicated range of values, this valve has a working range of approximately 100 units from no flow to maximum flow. The micrometer markings refer to thousandths of an inch and are referred to as numerical indication x 1000 (i.e. a reading of .1 inches is called 100).

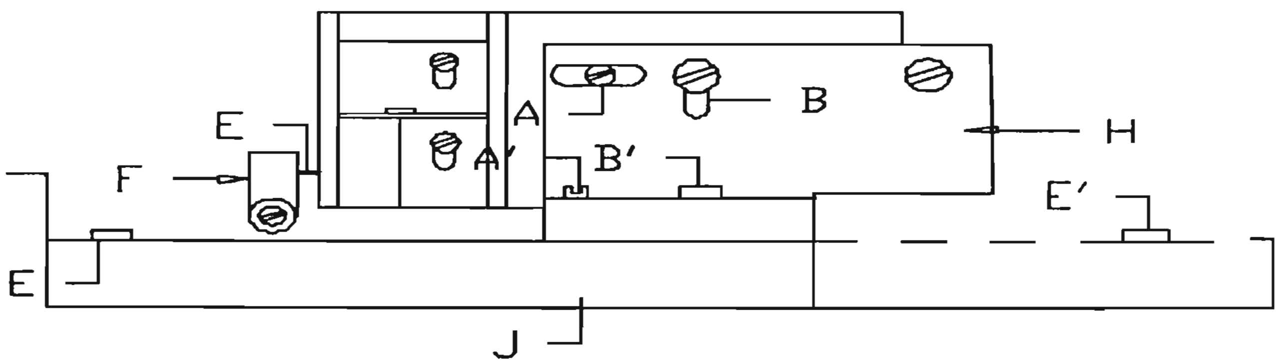

FIGURE 2

NOZZLE: (Fig. 2, e) The nozzle conducts gas from the flow valve to the filament area. It is a press fit into an o-ring located at the front of the flow valve. The nozzle tip is usually located from 1 to 2 millimeters below the filament and centered on it.

NOZZLE HOLDER: (Fig. 2, f) Maintains the nozzle in position below the filament. The holder contains two adjustments. The screw on the top allows the nozzle to be rotated and moved in and out of the front of the flow valve. The screw that secures the holder to the filament block can be loosened allowing the nozzle to move up and down.

FILAMENT BLOCK ASSEMBLY: (Fig. 1, g) The filament block assembly is made up of several pieces of hard black nylon. Wires supplying current to the filament are attached to threaded 'posts'. This current is carried to the filament via the <u>upper</u> and <u>lower</u> <u>heater jaws</u>. Note that these jaws are slotted and may be moved up and down by loosening the screws that secure them to the front of the filament block assembly. If the jaws are moved, make sure that the securing screws have been tightened; otherwise poor current flow can result. It is desirable to keep the gas solenoid/flow valve assembly lined up with the nozzle to prevent leaks at the o-ring. Note the two screws in slots at the back of the filament block assembly. Loosening these screws allows the gas solenoid/flow valve assembly to slide left or right.

ANGLE PLATE: (fig. 2, h) The angle plate secures the filament block assembly to the cover plate; it contains two important adjustments. Note the chrome plated screws in slots at points A and A' and the locking screws in slots at points B and B'. The chrome plated screws are 'eccentrics'; by rotating them with a screwdriver the filament block assembly can be moved up and down (A) or forward and back (A') to adjust the position of the filament. Loosen the locking screw associated with each 'eccentric screw' before turning, and tighten after completing the adjustment.

COVER PLATE: (Fig. 2, j) The cover plate conceals the entry of the pulling cables in to the base of the instrument. It is attached to the top by two screws, in slots, at points E and E'. Loosening these screws allows the filament block/angle plate assembly to move forward and back over large distances.

NOTE: The movements of the cover plate and the jaws constitute the 'coarse adjustments' of filament position, while the eccentric screws are the 'fine movements'.

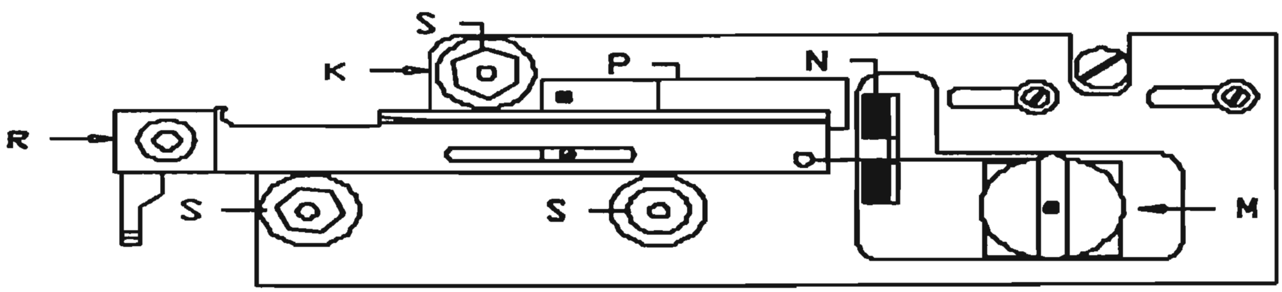

FIGURE 3

PANELS, LEFT and RIGHT: (Fig. 3, k) The panels are the angled surfaces that provide mountings for the puller bars and their bearings, the spring stops, the bumpers, and the upper cable pulley assemblies. Except for minor differences in shape, the left and right panels are identical. Note the three socket-head cap screws that attach each panel to the top. These screws are used to align the pullers bars. Their use, if necessary, is covered in the maintenance section.

UPPER CABLE PULLEY ASSEMBLY: (Fig. 3, m) This assembly conducts the pulling cables from the puller bars to the centrally located (and concealed) lower pulley assembly. Note that this assembly is attached to its panel by two screws, in slots, and contains a large eccentric adjustment screw (G). This eccentric screw is used to adjust cable 'tension' its use is covered in the maintenance section.

BUMPERS: (Fig. 3, n) The bumper stops the motion of its associated puller bar.

SPRING STOP: (Fig. 3, r) This assembly consists of the puller bar, glass clamp, clamp (wing) nut and cable retaining screw. The puller bar is made of mild steel and coated with a controlled thickness of hard chrome. Glass is loaded into the groove near the tip of the puller bar and is held in position by tightening down the clamp nut. The cable retaining screw holds the cable in a shallow groove at the end of the puller bar, and forms the 'resistance' against which the cable ends pull.

V-BEARINGS: (Fig. 3, s) These bearings are the guides for puller bar motion. They are made of stainless steel and should never be oiled (see maintenance section). Note that these bearings are mounted on stainless steel bushings one of which is round with the other two being hexagonal. The hexagonal bushings are used to adjust position and ease of travel of the puller bars (see maintenance section).

PULL CABLE: This cable conducts the pulling force of the solenoid to the puller bars via the upper and lower pulley assemblies. It is made of flexible metal with a nylon coating. NEVER PINCH OR DISTORT THE CABLE. The cable is terminated with crimped-on clamps or 'swages'.

The top is the metal plate on which is mounted the panels, cover plate, etc. Two other items are the cradles which support and hold the gas cylinder and the cover stops to which the plastic cover is secured. The base contains the transformers, circuit board and pull solenoid assembly.

CIRCUIT BOARD REMOVAL

Unplug unit from power. Remove three screws that hold plastic cover in place; one on each side and one on the rear.

Remove two nuts and washers that retain heater wires to the filament holder and remove wires from posts. (Fig. 1, H).

Remove six screws that hold top to base; two along each side edge and two along back edge.

Raise up top, move it forward slightly and rest it atop cabinet supported by solenoid bracket and back edge (Fig. 1)

Reaching around the solenoid bracket, unplug the molex connectors at the front edge of the circuit board and the 26 pin cable. The top may now be lifted clear and set aside.

Remove all other molex connectors.

Remove the eight plastic screws that hold down the circuit board (Fig. 2, locations E and E').

Lift the circuit board clear of the chassis and set aside.

Installation of the board is the reverse of the above procedure. Use extreme care in handling the connection from the velocity transducer. If the instructions are unclear, please contact us via phone or telex for clarification.

SECTION 3

FRONT PANEL DESCRIPTION AND OPERATION

The aim of this section is to provide the user with the information necessary to operate the P-80/PC. It begins with some important definitions and descriptions of the front panel controls.

DEFINITIONS

Program: A program consists of one or more cycles, which when executed in sequence will 'pull' the capillary glass inserted in the instrument. A program can be up to 16 cycles in length.

Cycle: A cycle consists of a HEAT VALUE (range 000 TO 999), a PULL value (range 000 to 255), a VEL.(VELOCITY) value (range 000 to 255) and TIME value (range 000 to 255).

Loop: A loop consists of one or more cycles that are repeated by the instrument until glass separation is achieved.

Ramp Test: A program, resident in the ROM, designed to facilitate program alterations when it is necessary to change filaments. This test is discussed in subsequent text.

FRONT PANEL CONTROLS

Power: When pushed to the up position, this switch applies power to the instrument.

Reset: When momentarily pushed, this switch resets the microprocessor to an initialized condition. It is used to change from one program to another, and in other circumstances as described below.

Keyboard: There are three 'groupings' of keys on the keypad: numerical/decision, editing and control. They function as follows:

Numerical/Decision (0-9): These keys are used to enter the number of the program being chosen, the various values for HEAT, PULL etc. and to make yes/no (1/0) decisions in certain situations.

Editing (CLR, ENT, NEXT, LAST): These keys are used for entering, deleting and editing programs. They allow one to move forward and back through a program, enter new values, and clear out unwanted values. In addition, the CLR key is the access key to the Ramp Test. The function of the various keys will be explained in the context of instrument operation in subsequent text. Control (PULL, STOP): These keys control the initiation and cessation of program execution and control of the Ramp Test.

OPERATION

Apply power to the instrument. After an automatic 'power on' reset, the display will appear as follows:

WHAT PROGRAM DO YOU WANT TO USE (0-9)

he P-80/PC is shipped with two programs already stored in memory. Program 0 is for a micropipette in borosilicate or hard glass. Program 1 is a patch-type pipette in borosilicate or hard glass. Programs 0 and 1 were written for 1mm OD/.5mm ID borosilicate glass (and a gas flow micrometer value of 100). Assuming that 1mm OD/.5mm ID glass is available, proceed in the following manner. Raise the cover and load a piece of glass into position. This is best done by loosening the glass clamp on the right or left puller bar and sliding the capillary through the clamp until it projects about 1 centimeter beyond the clamp. Release both puller bars and pull them to the center until they stop. Now slide the glass through the filament and into the glass clamp on the puller bar and tighten the two wing nuts on the glass clamps. The wing nuts can be tightened quite a bit without breaking the glass, but a tremendous amount of force is unnecessary. In this particular instance the user may wish to leave the top in the up position in order to watch the pulling process; however, in normal use the cover should be down whenever a pipette is being pulled. Now press the number 1 on the keypad; cycles 1 and 2 of program 1 will appear. Press the PULL key and the puller will execute program 1. Whether one obtains a patch-type pipette or not depends on several factors. The programs contained in memory were written for a particular environment (ambient temperature and humidity) and type of glass. Remove the pulled pipettes from the glass clamps, close the cover and toggle RESET.

Once again the display shows the sign on message:

WHAT PROGRAM DO YOU WISH TO USE (0-9)

At this point there are three options: 1) choose a program, load glass and execute the pull; 2) create a new program or edit an old one; 3) run the Ramp Test. The user should now create a program. (please note that the programs that will be written in the following text are not meant to pull pipettes, but are intended as an exercise to help develop an understanding of the programming process. DO NOT USE HEAT SETTINGS GREATER THAN THOSE FOUND IN PROGRAMS 0 AND 1 UNTIL YOU UNDERSTAND THE SIGNIFICANCE OF THE HEAT VALUES.)

First press a key other than 0 or 1. The display should come up with no values for HEAT, PULL, etc. For example, if 3 were pressed, the display would look like this:

3 01 HEAT= PULL= VEL.= TIME=

3 02 HEAT= PULL= VEL.= TIME=

with the cursor blinking in the leftmost position of the heat value on the first line.

If there are already numbers or symbols entered as program values, make sure that this program was not entered by another user of the puller. Unused program areas are usually cleared before a puller is shipped, but occasionally random values or test program values are inadvertently left in memory. Since the program values for heat may be sufficient to damage the heater filament, we recommend that unused programs be cleared completely before proceeding. The clear function is outlined below under the heading 'CLEAR'.

Press a series of three numbers such as '333'. Notice that these numbers are loaded in the HEAT value, and that the cursor has moved on to the PULL value. IF THREE NUMBERS ARE PRESSED WHEN LOADING A VALUE, THAT VALUE IS AUTOMATICALLY ENTERED, AND THE CURSOR MOVES TO THE NEXT POSITION. If three digits are entered for TIME, the cursor moves to the next HEAT value and the display 'scrolls' to the next cycle. For PULL now enter two digits such as 10. In order to complete the entry, press the ENT key. The two digit entry is right justified and the cursor moves to the next position. IF TWO OR ONE NUMBER(S) ARE/IS PRESSED WHEN LOADING A VALUE, THE ENTRY MUST BE COMPLETED BY PRESSING THE ENT(ENTER) KEY; THE CURSOR MOVES TO THE NEXT POSITION. Continue by entering a value for VEL. (ie 30). Enter a value for TIME (IE. 80) and press ENT. Enter another set of values, such as 320 for HEAT, 60 for PULL, 10 for VELO, and 80 for TIME. Currently the cursor is placed in the HEAT value for cycle 03. Press LAST; this will move the display back to the 'last' cycle. Press LAST again, and the display will look as follows;

3 01 HEAT= 333 PULL= 10 VEL.= 30 TIME= 80

3 02 HEAT= 320 PULL= 60 VEL.= 10 TIME= 80

Now press NEXT; the display scrolls up one line, and the cursor is on the 'next' line of the program. TO MOVE AROUND IN A PROGRAM FOR THE PURPOSES OF EDITING USE THE ENT, NEXT AND LAST KEYS. Remember, the cursor only moves to the right. If the cursor were in the PULL value position on line 01 above, and one wished to change the HEAT value; press ENT three times to arrive at the HEAT value on line 02, and then press LAST. The cursor will be in the correct position for entering the new value. PRESSING ENT WITHOUT ENTERING A VALUE WILL CAUSE THE CURSOR TO MOVE TO THE NEXT POSITION WITHOUT CHANGING THE VALUE AT THE CURSOR'S PREVIOUS LOCATION.

It is appropriate at this time to define the units attached to HEAT, PULL etc.

HEAT: A change of one unit (ie 333 to 334) represents a change of 50 milliamps in the current through the filament. Generally changes will be made in steps of about 5 units since in most cases smaller changes will have no effect.

PULL: A change of one unit represents a change of 4 milliamps in the current through the pull solenoid. In the case of pull strength useful changes are 10 units or more to see an effect.

VELO: One unit represents a change of one or more millivolts of transducer output depending on the transducer being used. Useful values for velocity range from 10 to 100 with the lower values being used for patch and injection pipettes and higher values for micropipettes.

TIME: One unit represents .5 milliseconds if the velocity value is 1 or greater. One unit represents 10 milliseconds in the VEL.(VELOCITY) = 0 mode. See subsequent text for further details.

Assume for the moment that the two line program entered above, if executed, caused a glass capillary to stretch but did not cause the glass to separate. What happens next? The puller is 'aware' of the fact that the glass has not separated, and will go back to line 01 of the program and try again; in effect it begins 'looping'. It will continue to do so until the glass separates. This looping capability is very useful. For example, consider the following two-line program:

3 01 HEAT= 350 PULL= 10 VEL.= 25 TIME= 80

3 02 HEAT= 320 PULL= VEL.= 10 TIME=

Assume that, after loading a piece of glass into the puller and executing the program above, that the filament came on five times before the glass separated. This indicates that the puller was into the third time through the program (looping) when the glass separated. Further more, assume that the result of the pull wasn't quite the pipette profile being sought; possibly because there was too much heat on the last pull. Then one might construct a new program that read like so:

3 01 HEAT= 350 PULL= 10 VEL.= 25 TIME= 80

3 02 HEAT= 320 PULL= VEL.= 10 TIME=

3 03 HEAT= 350 PULL= 10 VEL.= 25 TIME= 80

3 04 HEAT= 320 PULL= VEL.= 10 TIME=

3 05 HEAT= 300 PULL= 10 VEL.= 25 TIME= 80

Note the reduction in the heat value in cycle 05. This illustrates how the looping capability can be used to create a multi-step program designed to pull in one program execution. At the completion of the pull the puller will report on the number of times it looped and which line it was on at the time the pull took place. In the two line example above it would report the following.

PROGRAM LOOPED 3 TIMES

LAST CYCLE USED WAS LINE 1

The display then shows the first line of the program, and is ready for another pull. NOTE: IF ONE HAS FINISHED EDITING A PROGRAM AND WISHES TO EXECUTE IT, THE DISPLAY DOES NOT HAVE TO BE RETURNED TO LINE 01 BEFORE EXECUTION. PRESSING THE PULL KEY ASSUMES THAT EXECUTION IS TO BEGIN AT THE FIRST LINE OF THE PROGRAM.

Another user decides to use the program above, but the glass he/she is using is slightly different (thinner wall, different composition). Three possibilities exist: 1) glass pulls in same number of cycles; 2) glass pulls in less cycles, or; 3) glass pulls in more cycles. IF A PULL OCCURS AT A CYCLE OTHER THAN THE LAST ONE IN THE PROGRAM, THE CURSOR WILL BE LOCATED ON THAT LINE AT WHICH THE PULL TOOK PLACE. In the case of our program above, if the pull took place during the cycle on line 03, then the cursor would be on line 03. Likewise, if the pull takes place on a cycle as the puller begins to loop (because pull did not occur by line 05) the cursor will be sitting at that location.

A value of '0' entered for either TIME or VEL.(VELOCITY) has special meaning:

If VEL. = 0; PULL is disabled (= 0); HEAT is on at programmed value for duration of TIME programmed (10 milliseconds/unit) and cycle executes only once, no looping. This allows the one to use the puller as a 'polisher' for patch-type pipettes and the like.

If TIME = 0 and VEL. is <u>not</u> equal to 0; the gas solenoid is disabled (no active or gas cooling). This allows the pulling of special pipette shapes. Most often used to pull long tube-like shapes such as are used for microinjection or micro-perfusion.

If both TIME and VEL.(VELOCITY) are <u>not</u> equal to zero; then the value of TIME is the delay <u>from</u> the simultaneous turning off of the filament and turning on of the gas solenoid, <u>to</u> the turning on of the hard or solenoid pull. Increasing the value of TIME will increase the effectiveness of the active cooling prior to the hard pull. It is, to a degree, the equivalent of changing the micrometer flow valve.

There is always the possibility that the puller will be given a set of values which 'stall' its operation. An example might be where the HEAT value has not been set high enough to melt the glass, thus the glass can not be pulled and no velocity can be achieved. If it appears that a situation of this type has arisen, press the STOP key. This action aborts program execution and allows editing to take place. One could press RESET, but this requires that the program number be reentered.

Finally, it should be mentioned that all programs entered into memory (to a maximum of ten) remain there even after the power is turned off or the RESET switch is toggled. A special memory 'chip' that carries its own battery back-up will retain stored information for as long as ten years without power being applied to the instrument. Miracle that this is, it is strongly suggested that one keep a written record of programs in case of unexpected difficulties.

CLEAR: When a new program is being entered into memory in an area occupied by another program it is helpful to be able to 'clean out' the old program. Also, it may be desirable to remove all the values from the last cycles of a long multi-cycle program to allow for fine tuning of these final cycles. This clearing of program values is accomplished by the CLR key.

The CLR key sets all values to 0 from the line on which the cursor is located to the end of the program. Thus, if the cursor is on line 05 of a seven line program and your response to the query:

DO YOU WISH TO CLEAR ALL VALUES FROM THE PRESENT

LINE TO THE END OF THE PROGRAM YES = 1, NO = 0

is a '1'. Only the values up through line 04 will remain intact. Clearing out a whole program simply requires that the cursor be on line 01 before the 'yes' response.

RAMP TEST

The 'no' or '0' response to the above question provides access to the most unique feature of the P-80/PC, the Ramp Test. If one answers 'no' to the question above, the following display appears:

DO YOU WANT TO PERFORM A TEST?

NO = 0, RAMP = 1

A '0' response returns the user to the current program. A '1' response enters the Ramp Test. The next display after entering '1' is:

LOAD GLASS, CLAMP AND PRESS PULL

A length of capillary glass is loaded, the cover lowered and the PULL key depressed. On the display a number will be seen to be incrementing at the rate of 10 units per second. Events take place as follows: 1) the puller increments the heat at the rate of ten units per second; 2) when the heat output begins to soften the glass, the puller bars begin to move apart. When a certain velocity (the value of which is stored in ROM) is achieved the heat is turned off and the ramp test value is shown on the display. In order to run the Ramp Test several times, it is necessary to press RESET, choose a program number (any number will suffice), answer 'no' to clearing values, and 'yes' to Ramp Test. Glass may then be loaded and the test run again.

Recall that one has been programming the P-80/PC based on the characteristics of the filament that is installed. Since no two filaments are exactly alike, there must be some way to adjust programs when a filament wears out or is damaged and must be replaced. The answer is the Ramp Test. One of the first actions that should be taken is to run the Ramp Test with the glass that will be used for pipette fabrication. An average of several tests gives a number that relates that particular filament to that particular batch of glass. If the filament must be changed, or a new batch of glass is obtained; the Ramp Test can be used to establish a new Ramp Test value to act as a guideline for adjusting program values. It is necessary for the user to keep track of Ramp Test values.

SECTION 4

OPERATION

A. HEATER SETTINGS (FILAMENT HEAT)

CAUTION! Because of the large power reserve of the regulated heater power supply, it is very easy to burn out the filament if the heater value is set too high. The recommended starting heater value is the ramp test value.

At a heat setting of ramp value plus 15, a 1mm O.D., 0.50mm I.D. glass capillary tube should pull in 4 to 6 seconds after the start button is pressed. If the pull takes longer than eight seconds, and you are trying to pull a fine micropipette, increase the heat value by about five. Then try pulling electrodes until the pull takes place in less than eight seconds after the start button is pushed.

If the pull occurs in less than three seconds after you start, decrease the heat value by five. For 2mm O.D. tubing, the pull should occur between 15 and 25 seconds after the start. Make corrections as outlined for the smaller tubing.

The position of the glass within the filament will also affect the time it takes to pull an electrode. When using a trough filament the glass should be about .5mm above the bottom of the filament and centered front to back. The position of the glass with respect to the filament may easily be adjusted with the two eccentrics (A and A' in figure 2). The two locking screws B and B' should be loosened before adjusting the two eccentrics. In the case of a box filament the glass should be in the center of the filament.

The heat setting can also affect the length and size of the tip. Higher heat settings will give longer and finer tips. A heat value of the Ramp Test value plus 15 will generally give a very fine tip.

If should be noted that at high heat settings (filament white hot) the filament life is greatly reduced. It is suggested that a setting of ramp value plus 15 be used initially and electrode length be controlled by gas valve adjustment.

For patch-pipettes and injection pipettes a good starting point is the ramp test value. The time for the first pull will be in the 10 to 20 second range.

B. PULL STRENGTH ADJUSTMENT

Low values of pull strength settings in the range of 40-75 will give larger tips, while settings between 150-250 give the smallest tips. The pull strength can be set to any value desired with no danger of damaging the instrument. The hard pull is turned after the velocity reaches the trip value programmed for the cycle and after the TIME value has elapsed. If the velocity value was greater than 0 the TIME value determines a delay from when the filament is turned off/nitrogen is turned on to when the hard pull begins. This time is in .5 millisecond units and a setting of 80 (40 milliseconds) is recommended as an optimum value for fine tips.

C. VELOCITY ADJUSTMENT (TRIP POINT)

The velocity value is generally between 60 and 120 for micropipettes and 10 to 30 for patch-pipettes. This value is related to the speed at which the two electrode carriers are moving. The lower the velocity value the slower the speed of the carriers at which the trip point will occur. At that time the heat will be turned off and the nitrogen will be turned on. After a delay determined by the time value the hard pull is turned on.

D. GAS FLOW

The gas flow control valve is located above and behind the filament. It is the primary means of adjusting the length of the electrode tip. It is a precision unit and will give reproducible results.

For a first try, set the control valve to a reading of 100. Increasing the gas flow will produce shorter tips, and, conversely, decreasing the flow will produce longer tips.

If the gas flow is decreased too much, the electrode will not form a tip. At air flow settings about five units below the value needed to form a tip, the glass will break and form tips of about one micron. At still lower settings, the glass will form a wispy fiber. The very finest tips for a given pull and heat will be formed at an air setting 10 units higher then where the 1 micron tip was formed. Long tips can be formed by using wider filaments or by using higher heat settings, and conversely still shorter tips can be formed by using narrower filaments or lower heats. Filaments narrower than 2mm can not form as fine a tip as the wider filaments.

Electrodes will not be formed if the gas flow is set too high.

E. CAPILLARY MOUNTING

We suggest the following method of inserting the glass capillary tubing into the carrier clamps, to prevent damage to the filament.

Use either the left or right carrier; move the carrier away from the filament until the carrier is latched by the spring clip. Open the capillary clamp; hold the glass tubing about two inches from one end, and with the two-inch end facing the filament, lower the glass into the clamp and tighten the clamp. The glass should now be in the groove with one end about 1/2-inch from the filament.

Release the spring clip latch and move the carrier toward the filament. If the filament is correctly positioned, the glass will pass through it. Hold the two carriers toward the center by placing two fingers of one hand on the finger bars. Loosen the clamp holding the glass and slide the glass in its groove toward and into the other clamp groove. Center the glass and tighten both clamps.

F. HORIZONTAL TROUGH FILAMENT

The horizontal filament is quite easy to work with. This filament should be centered between the two clamps, and the air jet should be centered under the filament about 2mm below the filament.

When using the standard 3mm trough filament, the glass tubing should be positioned just above the filament and centered between the two sides. This position can be adjusted by using the two eccentric cams, located on the aluminum angle piece which holds the filament assembly.

Slightly loosening the two screws, which lock the filament assembly in place, the filament can be moved in relation to the glass tubing by turning the appropriate cam.

he heater filaments are easily replaced by loosening the two clamp screws holding the filament in place. Slide out the old filament, slip in a new one, and position it over the air jet. Then tighten the two screws.

G. ELECTRODE LENGTH

The length of the electrodes pulled can be varied, as previously stated, by decreasing the heat or by decreasing the air flow. It can be changed by using filaments of different widths. Widths of 1.5mm to 6mm trough filaments can be used. Electrodes pulled using a 1.5mm filament will be very short and will have large tips.

Tips of 1-2u can be formed using a 1.5mm filament, with low filament temperatures and weak pull strengths.

The tip size will decrease with increasing filament width until a width of 3mm is reached. Increasing the filament width beyond 3mm will produce longer tips with a more gradual taper (which may penetrate better in some cases). However, the tip will not be any smaller.

H. BOX FILAMENT

Another type of filament which can be used is the box type heater filament. The box configuration is particularly useful with thick wall or double-barreled glass, since the box filament delivers more heat to the glass. This results in faster heating without the necessity of increasing the temperature of the filament. (Note that the HEAT value must be increased in order to reach an operating temperature.) The box filament also heats the glass in a more symmetrical fashion than trough filaments, so that the pipettes produced tend to be more straight and more concentric than those pulled with a trough filament.

The box filament has two primary limitations. First, it requires more current to heat to a given temperature than the same size trough filament. Thus it is possible to use wider trough filaments without exceeding the maximum current capacity of the puller. Second, the box configuration reduces the cooling effect of the gas jet. For this reason the box filament is not recommended when very short pipettes are to be formed.

The air jet should be centered directly under the box filament. The glass capillary tubing should be centered within the box filament.

The optimal size of the box filament appears to be 3mm wide, 3mm high and 3mm deep. To produce short, large tips, a box filament of 1.5mm width, forming a box 2mm on each side, may produce more straight tips than a trough filament.

There is a size limitation on box filaments that can be used with the Model P-80PC electrode puller. Box filaments wider than 3 to 4mm may exceed the maximum filament heater current that the P-80PC can deliver, thus limiting the filament temperature.

SECTION 5

MAINTENANCE

A. PULLEY ADJUSTMENT

The position of the two pulleys which carry the cables from the solenoid to the carriers is adjustable. This adjustment should be made only if the two electrodes formed from one pull are of quite different lengths. This inequality is generally caused by the jet not being aimed at the center of the filament but may also be caused by unequal cable tensions. (To avoid unnecessary cable adjustment, be certain that the air jet is correctly positioned before proceeding.) The adjustment is made by moving one or both of the pulleys to equalize the tension on the two cables. It should be explained at this point that there are two sets of stops in the system. There are the stops in the carrier slots against which the carriers rest, and a stop to prevent the solenoid from being pulled out of its housing. The adjustment of the pulleys must be made so that the carriers will still come up against their stops while the solenoid is not against its stop. The two cables should not be under tension when the carriers are against their stops, this is the position they would be in just before pulling an electrode.

You should be able to press on either cable between the carrier and the pulley and there should be about a 2mm deflection before the solenoid hits its stop. If the deflection is more or less, the pulley position should be changed. This is done by loosening the two screws above the pulley andturning the chrome eccentric cam to move the pulley in smallincrements until the two cables are of equal tension. If the carrier no longer stops against its stop in the slot, but stops against the cable, then the cam must be adjusted back until the carrier once more hits its stop. It is important that the carriers come up against their stops with no tension on the cables. If there is tension, the initial pull will depend on how tightly you hold the finger stops when the glass is clamped in the carriers. If this happens, the electrodes will not be consistent from pull to pull.

B. CABLE REPLACEMENT

If the cable breaks or gets a bad kink, it will have to be replaced. This is done as follows.

First, take off the plastic cover by removing the three screws which hold it in place. Now the two screws which hold the cover plate down must be removed. The cover plate is the aluminum plate on top of which the filament assembly sits, and below which the two cable wires can be seen coming out on their way to the outboard pulleys. With these two screws removed, the complete filament assembly, including the air solenoid and air micrometer, can be lifted up and moved back out of the way.

Next, with the power cord unplugged, remove the five screws which hold the front panel in place. Swing the top edge of the panel forward so that the panel is face down on the table. The solenoid assembly can now be seen.

The next step is to remove the brass slug on the top of the solenoid. It is held on by two hex screws. The brass slug should now slide up and off of the shaft of the solenoid. The old cable can now be slipped out of the slot in the brass slug. At this point note the path of the cable as the new cable will be strung the same way as the old. To remove the old cable, use a wire cutter to cut the cable near the electrode carriers. Now pull out the cable. Slip the swaged end of the new cable in the brass slug and replace the brass slug. Be sure to get the hex screws tight. Both wires go through the first guide, and then each wire must be fed through a pulley and its accompanying guides.

Using a small screwdriver, loosen the screw at the outboard end of the electrode carrier and remove the short piece of wire and its swage. Feed the wires through the outboard pulleys so that the wires lie across the electrode carriers.

For the next steps, the electrode carriers must be held in toward the center. This can be done with a rubber band around the two finger bars to bring both carriers in to the center. Loosen the two screws which lock down the two outboard pulleys and center the pulleys in their travel.

Now slip a swage on one of the wires. The wire must now be pulled on in order to lift the solenoid. With the solenoid against its upper stop, position the swage over the hole at the end of the electrode carrier and crimp the swage with a swaging tool. Using a wire cutter, cut the excess wire off and tighten the screw down on the wire. This most be done for each wire.

The cover plate and filament assembly can now be replaced. It may be necessary to readjust the filament position in relation to the glass tubing. The instructions for adjusting the outboard pulleys should now be followed to get the correct tension on the cables. This is somewhat easier to do with the front panel down, to see the relationship of the solenoid and its stop.

The final step is to replace the front panel. Be sure that no wires are pinched between the front panel and the cabinet.

SECTION 6

TROUBLE-SHOOTING

A. PIPETTE TIPS

PROBLEM: What glass should I use?

The type of glass and the wall ratio to O.D. (outside diameter) are two of the most important variables in controlling tip size. For example using program 1 which we used to form the pipette in the SEM micrograph included with this puller when shipped from Sutter Instruments; borosilicate glass with an O.D. of 1mm and an I.D. of .50 will give tips of .06 to .07 micron. Using the same settings borosilicate glass 1mm O.D. and .78mm I.D. will form tips of .1 to .12 micron. Aluminosilicate glass with an O.D. of 1mm and an I.D. of .58mm will form tips of .03 to .04 microns again with the same settings.

In general the thicker the wall in relation to the O.D. of the glass the finer the tip will be, and the thinner the wall the larger the tip will be. Thin wall glass will give the best results in most experiments as it will have the largest pore for a given tip size. This means it will have a lower resistance and will allow for easier injection of solutions. However in many cases with small cells the thin wall glass will not form tips fine enough to obtain good penetrations. In this case heavier wall glass must be used.

PROBLEM: The resistance of my pipettes is to low, how do I pull a higher resistance pipette?

The first point to note is that there is very little correlation between tip size and electrode resistance. Most of the resistance of a microelectrode is in the shank of the electrode behind the tip. Electrode tips which are .1 micron in diameter can vary in resistance from 20 Megohms to 1000 Megohms depending on the length of the electrode and what is used for the filling solution. If the same solution is used then resistance may give an indication of how well the electrode will penetrate a cell as the electrode with the higher resistance will probably have a longer shank and a smaller cone angle at the tip. This combination will aid in the penetration of cells where the cell is not a surface cell.

PROBLEM: OK but I still want a smaller tip than I am getting.

The first thing to try in most cases is to increase the heat value. This will generally decrease the tip size but it will also give a longer shank. If the higher resistance is not a problem this is generally the best solution. Continuing to increase the heat is not the final answer as to high a heat can lead to larger tips. In general with 1mm O.D. .5mm I.D. borosilicate glass the finest tips will be formed when the glass pulls in 4 to 5 seconds after starting the pull.

If the electrode is now too long and causes the resistance to go too high to pass the necessary current for example, then the next step is to increase the pull strength. In general a pull strength of 125 will give tips of less then .1 micron. Increasing the pull to 250 will reduce the tips size about 5-10%. We recommend a pull of about 150 in most cases.

The last major variable to adjust is the nitrogen flow. If in the case of 1 by .5 borosilicate glass the pull takes place in 4-8 seconds the tip size will not change with a change in the cooling flow. The only change will be in the length of the shank. If however the heat is such that the pull takes place in more then 8 seconds, increasing the flow will increase the tip size. An increase in gas flow will shorten the tip and a decrease will lengthen the shank.

PROBLEM: How do I increase the size of my patch-pipette?

The first thing to try is to reduce the heat. Try dropping the heat 5 units at a time to see if this will increase the size of the tips. If this does not work increase the air micrometer in units of 5. The pull should generally be set to 0 when pulling large tipped (1-10 micron) pipettes.

PROBLEM: The tips of my patch-pipettes vary in size from pull to pull.

This can happen when a pipette is formed in two or more loops. If the pipette is formed in three loops in one case and then on the next pull it forms in four loops the tips will not be the same. Adding one unit in the velocity value will in most cases cause the pipette to be formed in three loops or subtracting 1 unit should cause the pipette to form in 4 loops. It is always good technique when a program is developed that produces a desired pipette, to try increasing and decreasing the velocity value to be sure that you are in a stable region. The best procedure in developing a very reliable pipette program is to change the velocity value both up and down until the number of cycles to pull the pipette changes. Then pick a value halfway between for the final velocity value.

PROBLEM: I need to form an injection pipette with a 1 micron and 20 to 50 microns long. How do I do this?

Try a program in which the first two lines of the program have a pull value of 0 a velocity value of 10 to 30 a time setting of 80 and use the ramp value for the heat. The third line should have the same heat value, a pull value of 150, a velocity of 30 and the time should be 0.

The idea behind this program is to reduce the size of the glass on the first two cycles and then on the third cycle we give a hard pull with the air turned off. Normally if the air is turned off a long wisp will result, but since we have greatly reduced the size of the glass and with a very hard pull the glass will tend to separate when it is about 1 micron in diameter.

PROBLEM: The electrodes are bent. How do I make them pull straight?

This problem occurs most often when using the trough filament. Going to a box type of filament will produce much more straight pipettes. The bend in the pipette has no effect on the pipettes tip and should cause no problems unless you are penetrating quite deep in the tissue with the electrode and you are aiming at a certain site. Then the bend may lead the pipette to the wrong area. The box filament is not a complete improvement on the trough filament as the gas flow is much less effective with the box filament, and you give up much of the length control that the gas gives with the trough filament.

PROBLEM: The filament does not light up when I press pull.

There are a number of possible reasons why this might happen. First look and see if the filament has burned out. In some cases it may be necessary to loosen the screws holding the filament in place as a very fine break may be hard to see. If the filament is OK, try running the ramp test and see what happens. If you have just changed the filament it is quite possible that the new filament needs a very different heat value than what you have been using. It is always a good idea to run the ramp test each time you change the filament. If you run the ramp test and the heat value reaches 999 without the filament heating up check the screws holding the filament in place and if they are tight then check the two nuts connecting the filament wires to the posts in back of the filament block. If these are tight then the problem is probably on the circuit board.

PROBLEM: One electrode is much longer then the other electrode.

This is caused by one of two things. First check the tension on the two cables as explained in the maintenance section. If the cables have the same tension then the gas jet must be aimed more at one side of the filament than the other. This can be corrected by moving the gas jet until both sides are the same length.

B. ELECTRONICS SYSTEM

The P-80/PC micropipette puller is controlled by a Z-80 microprocessor. Three digital to analog converters control the heat, pull and velocity values. The heat power supply is a precision constant current switching unit which will vary less than 10 millamperes with a plus or minus 10% change in the ac line current. The pull supply is a constant current DC power supply. The velocity trip point is set by a D-A converter. The output of the velocity transducer is compared to the output of the velocity D-A to determine when the trip velocity is reached.

CAUTION: DANGEROUS POTENTIALS EXIST INSIDE THIS INSTRUMENT. SERVICE SHOULD BE PERFORMED ONLY BY QUALIFIED PERSONNEL. THIS INSTRUMENT SHOULD BE UNPLUGGED FROM ITS POWER SOURCE WHEN ANY ADJUSTMENTS OR REPAIRS ARE MADE.

TROUBLE-SHOOTING

PROBLEM: The shape and resistance of the pipette changes from pull to pull.

a. In most cases this is due to one or both of the cables to the pipette carriers being setup to tight. If the cable is adjusted so that the carrier can't come against the stop in the slot in the center of the pipette carrier. In this case the initial pull tension will depend on how hard the carriers are squeezed together when the glass clamps are tightened. To adjust, see the cable adjustment section.

b. A second possible cause of this problem is dirt on the carrier bars or bearings. In this case clean the carriers and bearings with a lint free tissue or cloth.

c. If the problem persists run the ramp test several times. If possible use one long piece of glass and move the glass over after each ramp test (turn the air adjustment up and the glass will not separate). If the ramp values are +/- 4 units or less the problem may be with the glass. If the values are greater than +/- 4 units call Sutter Instruments.

PROBLEM: Display blank, fan not on.

a. Check power cord and wall AC outlet

b. If unit is properly plugged-in and still does not work, remove power cord and check 3 AMP fuse. If the fuse has blown, suspect problems with the large transistors mounted on the heat-sink on the back of the cabinet.

c. If the fuse is still good, suspect the wiring harness.

PROBLEM: Display blank, fan on.

a. Check the 1/2 AMP fuse. If the fuse has blown suspect the main circuit board and transformer T-3, a DMT 6-15.

b. If the fuse is still good, suspect a loose connection between the ribbon cable and display unit or the main circuit board.

c. If the connections are good check the various power supplies located on the main circuit board.

PROBLEM: Display shows a row of blocks.

a. The microprocessor has failed to properly initialize the display.

b. Press reset and the display should show the proper power-up message. Do not turn power off and then on rapidly, as this may cause improper power-up. Always allow at least 5 seconds before turning power back on.

c. If this problem recurs frequently suspect the reset timing capacitor, C 2. You may wish to replace C 2 with a slightly higher value such as 68 microF.

d. If pressing reset fails to produce the proper power-up message check pin 12 of U1 for a clock signal and check the address and data lines of U5 to see if the microprocessor is functioning.

PROBLEM: Displayed program values are not correct.

a. Make sure that values were not changed by another user.

b. Always write down the program values and the ramp test value and keep them in a secure place.

c. If values entered are not held when the power is turned off suspect the zero-power memory, U 6.