OPERATION MANUAL

PC-84

Sachs-Flaming Micropipette Puller

SUTTER INSTRUMENT COMPANY

MODEL PC-84

SACHS-FLAMING MICROPIPETTE PULLER

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■ SECTION I

INTRODUCTION

The Model PC-84 Sachs-Flaming Type Micropipette Puller is based on an original design and prototype by Dr. Frederick Sachs. This design was refined and developed for manufacture by Dale Flaming and James Wall of Sutter Instrument Company.

The processes that are required for micropipette fabrication are the same for all pullers, but PC-84 is unique in the flexibility it gives the user in applying these processes. Originally created as a patch-type pipette puller, it is also capable of producing a vast array of pipette shapes for such techniques as microinjection, intracellular recording and microperfusion.

PC-84 is simple to operate, but the 'philosophy' underlying its operation must be thoroughly understood. In addition, PC-84 executes its assigned tasks in an extraordinarily precise and reproducible manner. Thus, the burden for reproducible pipette fabrication falls on the environment, the glass used and, ultimately, the user. Therefore, it is imperative that the user read and understand this manual in its entirety. Section 1 deals with unpacking and setting up, including a discussion of the operating environment and important electrical considerations. Section 2 gives a mechanical description of the puller and the basic maintenance necessary for day to day operation. Section 3 discusses the front panel controls and operation of the puller. Subsequent sections deal with service, warranty, etc.

Reading and understanding this manual will help greatly in realizing the full potential of this unique device.

SUTTER INSTRUMENT CO.

UNPACKING AND SETTING UP

The Model PC-84 is shipped to you in a prefabricated foam mold. Please take note of this packing method, should it ever be necessary to ship the puller to another location, the same method of packaging should be employed. (Additional packing material may be purchased from Sutter Instruments.)

IMPROPER PACKING CONSTITUTES A FORM OF ABUSE, AND MAY BE RESPONSIBLE FOR VOIDING THE WARRANTY WHERE DAMAGE RESULTS FROM SUCH PACKING.

After removing the instrument from its packaging, place it in an area where there is a free flow of fresh air on all sides. Air is drawn in through the vents on the sides of the cabinet, and is exhausted out both ends of the heat sink of the back. Adequate ventilation is vital to the longevity of the various power supplies.

The PC-84 is extraordinarily precise in repeating values of heat and pull strength, thus it is important to consider the stability of the environment in which it is located. In particular, consideration should be given to the stability of room temperature and humidity; these parameters will affect glass 'cooling' and can alter the outcome of a program where changes in said parameters are significant.

Because it is a microprocessor controlled device, the PC-84 is subject to the same electrical power requirements as microcomputers and the like. If line surge protection must be provided for the proper operation of microprocessor based devices already in the laboratory, the PC-84 should also be accorded such protection.

■ SECTION II

BASIC PRINCIPLES OF OPERATION

This section should be read before getting into the specifics of the instrument to acquire an overview of the system.

This puller is designed for maximum control and flexibility. The temperature, the force, the distance pulled and time for a pull are all programmable with a defined sequence of up to 16 steps. A total of ten sequences can be stored for recall by number.

The mechanical structure of the puller should be understood for proper operation. The glass is held by clamps mounted on two movable carriages which ride with ball bearings on precision ground stainless steel rods. The right carriage slides manually for convenience in loading the glass. The left carriage is pulled by a solenoid actuator whose current is programmable. The force is non-linear with distance so that the same current will produce a somewhat greater force when the solenoid is partly seated.

To keep the pipettes symmetrical during this "one-sided" drawing, the filament is automatically repositioned as the left-hand carriage moves. The filament drive is a stepper motor mounted under the right hand carriage.

The distance that the left carriage moves is detected by an optical sensor utilizing a grating which provides a distance resolution of 0.002" (approx. 50u). In the programming section, distance is counted by the number of 0.002" steps. The detector is under the left hand carriage and can be damaged while attempting to retrieve broken pipettes!Vacuum objects out of area only, DO NOT blow out with compressed gas.

The heater is a platinum foil loop which provides enough heat to melt all common glasses with the exception of quartz. The heater temperature is controlled by an AC constant current supply.

A basic program step (called a CYCLE) consists of four entries:

1. Heat. The units represent the amount of current to be passed through the filament. One unit is equivalent to 40 milliamperes of current. The maximum current of 40 amperes is developed at a heat value of 999. **DO NOT INCREASE THE HEAT VALUES IN THE PROGRAMS SUPPLIED WITH THE PULLER UNTIL YOU HAVE READ AND UNDERSTOOD THE SECTIONS OF THE MANUAL DESCRIBING THE SELECTION OF HEAT VALUES.** The use of too much current will melt the filament, although it will not damage the puller.

(The melting point of any type of glass may be determined using a special procedure termed the RAMP TEST which is described below.)

2. Pull (force). The units are arbitrary (ranging from 0-255) with the maximum current set to prevent overheating of the solenoid. In general, when the force is changed from one value to another, it actually ramps from one value to the next at a rate of 1 ms/step. This prevents shattering the glass from a sudden application of force. However, to provide for the high acceleration required to make small tipped (.2u) intracellular pipettes, the pull force must be increased as rapidly as possible. If the puller encounters a pull value of 0, the ramping feature will be disabled on the next line of the program. Thus changes in pull strength which start from 0 are made at maximal speed.

3. Steps (distance). The units are steps of 0.002" (approx. 50u) and range from 0-254.

4. Time. The units are 10ms ticks and range from 0-255 (0-2.55s). When both the step and the time are specified, the condition that is met first takes precedence. It is wise to set time-0 when steps are intended and step=0 when time is intended in order to avoid confusion.

■ SECTION III

MECHANICAL DESCRIPTION AND MAINTENANCE

This section provides a description of the mechanical aspects of PC-84, and describes the few maintenance procedures that are necessary to assure reproducible operation (more extensive maintenance procedures are discussed in later sections).

The puller section of PC-84 consists of the following mechanical elements:

BASE PLATE: The base plate (Fig.1,A) provides the mounting surface for all other mechanical elements of the pull mechanism, as well as allowing access to the interior for the various cables that carry information from the sensors and conduct power to filament, motor, etc.

SUPPORTS: There are a total of three supports. From left to right they are 1) Solenoid Support, 2) Motor Support (Fig. 1, B), and 3) Guide Rail Support (Fig.1, C) (the solenoid and motor supports also provide support for the guide rails).

CARRIAGES: There are three moving carriages. The leftmost carriage is the Solenoid Carriage (Fig.1, D). It is the moving element that is pulled by the solenoid when glass is being drawn. Attached to the bottom of this carriage is one element of the linear encoder. NEVER STICK ANY PROBE OR SIMILAR ITEM INTO THE AREA UNDERNEATH THIS CARRIAGE AS THERE IS A RISK OF DAMAGING THE LINEAR ENCODER. On top of the solenoid carriage is a series of adjustable brackets that culminate in the left glass clamp. (Fig. 1, E)This clamp may be adjusted in both the horizontal(Fig.1, F) and vertical (Fig.1, G)directions to accommodate filament position and the position of the other glass clamp.

The center carriage is the Filament Carriage.(Fig.1, H) It is attached to a linear actuator, and is driven by it at increments equal to one half the distance travelled by the solenoid carriage. Therefore, during a multi-step pull, the filament is automatically repositioned over the heated portion of the capillary glass. Attached to the top of the filament carriage is the filament block support, which in turn has the filament blocks attached to it (Fig. 1, I, I'). The filament blocks are held to their support by two thumb screws positioned in vertical slots in the blocks. This provides an adjustment in vertical position for the filament. Atop the filament blocks are two screws that secure the 'jaws' for holding filaments. The current version of the PC 84 has heater blocks designed to accommodate air cooling. The filament holding blocks have been hollowed out and an air line is connected from the back of the cabinet to each filament block. By connecting a low pressure air line (2-10 psi or .1-.7 bar) the filament assembly is kept cool and more consistent pipettes will be formed. This is an option and does not have to be used to produce good pipettes. Use of this option is particularly recommended when the heat is on for a prolonged period.

The rightmost carriage is the Glass Carriage.(Fig. 1, J) It is so named because it provides the most convenient place for introducing capillaries into the pulling mechanism. On its top can be found a riser to which is mounted the glass guide and right glass clamp assembly. This guide/clamp assembly can be moved in a horizontal plane (Fig. 1, K) to facilitate adjustment. Capillaries are loaded by sliding them along the groove in the guide and underneath the loosened clamp. The carriage is then pushed toward the filament until it stops. The capillary is then pushed through the filament and into the glass clamp atop the solenoid carriage at which point the wing nuts are tightened and a pull program executed.

When loading glass, the solenoid carriage is moved toward the filament carriage until it is stopped by an angle bracket located under the carriage.

GUIDE RAILS: The guide rails (Fig. 1, L, L') are precision ground stainless steel rods on which the various carriages move. TO ASSURE GOOD PULLER OPERATION, THESE RAILS SHOULD BE KEPT CLEAN. Cleaning the rails frequently is important, the frequency of which is dependent on conditions in the individual laboratory. Simply wipe off dust and dirt with a lint free cloth. Occasional use of water displacing cleaner such as WD-40 will remove moisture and the dust that it tends to trap.

LINEAR ACTUATOR: The linear actuator or motor (Fig. 1, M) is a stepper motor of the type used in positioning the 'heads' in computer disc drives. Although the filament carriage can be moved manually when power to the puller has been turned off, this should be avoided as it puts undue stress on the bearings and mechanism of the linear actuator.

Beyond the maintenance already mentioned above, it is only necessary to keep the cover down to minimize the amount of dust settling on the mechanism. It is also advisable to vacuum off the mechanism occasionally to remove glass particles and other debris. NEVER USE COMPRESSED AIR TO 'BLOW OFF' DUST AND GLASS PARTICLES; THIS ONLY CREATES THE DANGER OF DRIVING SAID MATERIAL IN THE MECHANISM.

FILAMENT REPLACEMENT: If the filament wears out or is damaged, it must be replaced. To replace the filament: 1) loosen the screws that hold the 'jaws' to the filament block, 2) remove the old filament or pieces thereof being careful not to drop any material down into the holes in the filament carriage or base plate, 3) reposition new filament and tighten down jaws. It is best to approximate the position of the previous filament with the new one. Then the clamp/guide assembly atop the glass carriage can be adjusted to the new lateral position of the filament, followed by any adjustment of the glass clamp atop the solenoid carriage.

Should a new shape of filament, or a different diameter of glass be used, the procedure would be as follows: 1) clamp glass in right glass clamp with glass protruding far enough to allow for visu-

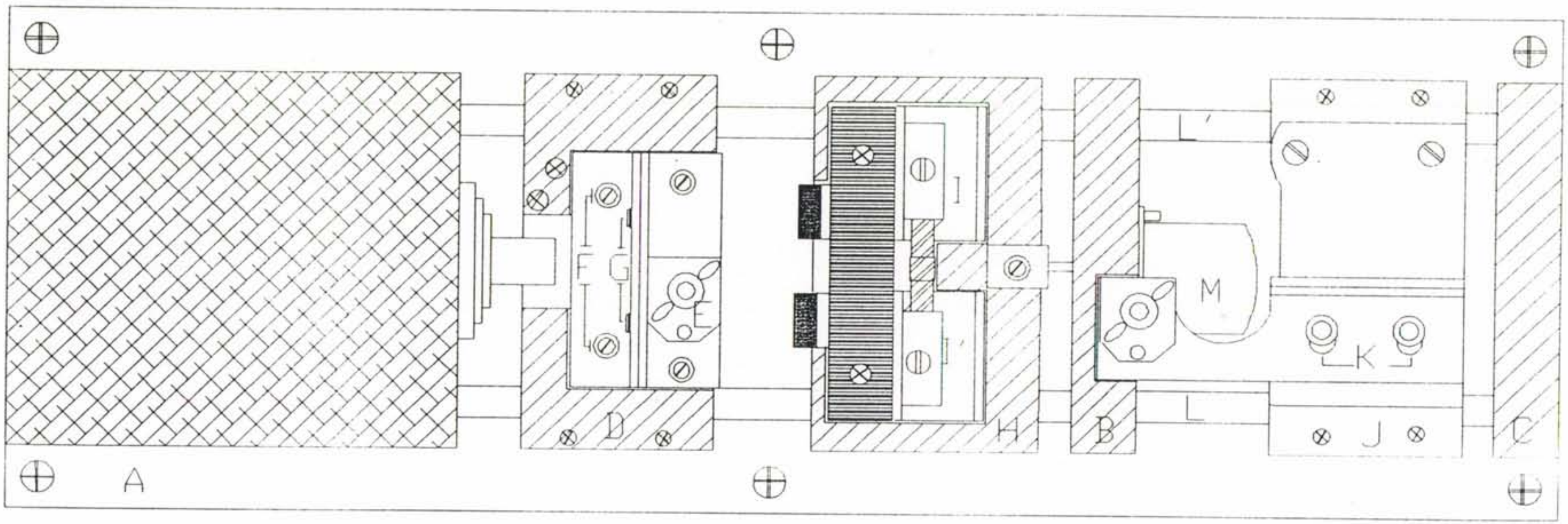

FIGURE 1

alizing new height of filament when glass carriage is moved to leftmost position, 2) loosen cap screws holding filament blocks and move to new vertical position, 3) make any lateral adjustment in glass clamps. NOTE: it is advisable to keep handy a short length of small diameter drill rod or similar item to facilitate 'tuming up' glass clamp position. Donot use a piece of glass, as most capillaries have some curvature to them.

■ SECTION IV

USING YOUR PULLER

FRONT PANEL DESCRIPTION AND OPERATION

The aim of this section is a provide the user with the information necessary to operate the PC-84. 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 programmed cycles in length.

Cycle: A cycle consists of a HEAT value (range 000 to 999), a PULL value (range 000 to 255) and a STEP or TIME value (range 000 to 255 for both).

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 puller, designed to facilitate program alterations when it is necessary to change filaments and/or glass characteristics. This test is discussed in subsequent text.

Ramped Pull: A situation where the pull strength is increased gradually over time as opposed to those instances where the pulling force is applied in a step change manner. Its use 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 to the up position, 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 decisions in certain situations.

Editing (CLR, ENTR, NEXT, LAST): These keys are used for entering 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.

HEAT: A change of one unit (i.e. 333 to 334) represents a change of 40 milliamps in the current through the filament.

PULL: A change of one unit represents a change of 5 milliamps in the current through the pull solenoid.

STEP: One step is the equivalent of .002 inches of movement of the solenoid carriage.

TIME: A change of one unit represents 10 milliseconds.

■ SECTION V

OPERATION TECHNIQUES

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)
```

The PC-84 is shipped with three programs already stored in memory Program 0 is for a patch-type pipette in soft glass. Program 1 is a patch-type pipette in borosilicate or hard glass. Program 2 is a micropipette in hard glass. The first program was created using a standard flint glass of 1.5mm OD/1mm ID. Programs 1 and 2 were written for 1mm OD/.5mm ID borosilicate glass. 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 glass carriage and sliding the capillary through the clamp until it projects about 2 centimeter beyond the clamp. Push the passive carriage toward the filament carriage until it is stopped by the motor support. Now slide the solenoid carriage back toward the filament until it touches the stop located under the carriage. With the two carriages in this position, and the glass clamp on the solenoid carriage loose; slide the glass through the filament and into the other glass clamp. Tighten the two wing nuts on the glass clamps; they 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. Before creating a program we suggest that the user run the ramp test. In order to run the ramp test the user must understand the clear function.

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 few 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 by 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-YES=1, NO=0
```

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

RAMP TEST

A 'NO' response to the above question provides access to the most unique feature of the PC-84, The Ramp Test. Programming of the PC-84 is 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. At this time, it is necessary for the user to keep track of ramp test values.

If one answers '1' as a response to the question above, the following display appears:

```
DO YOU WISH TO PERFORM A TEST FUNCTION?
NO=0    RAMP=1    MEMORY=2    COUNTER=3
```

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 AND PRESS PULL
PRESS RESET TO RETURN TO PROGRAM
```

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 increment at the rate of ten units per second. Events take place as follows: 1) pull strength is turned on to a value of 225; 2) a short delay period occurs to allow the pull to remove all slack from the system, and then 3) the puller begins increment the HEAT at the rate of ten units per second. When the heat output begins to soften the glass, the solenoid carriage will begin to move. When five steps have been taken, the heat and pull are turned off, and ramp test value is shown on the display. In order to run the Ramp Test several times, it is necessary to press RESET, choose any program number, answer 'no' to clearing values, and 'yes' to ramp. The test is run at a speed much faster than the time constant of the filament; this means that there will be a slight variation in the results obtained with the Ramp Test. Assuming no variation in the glass being used, a range of 10 units would be considered normal.

DEVELOPING A PROGRAM

Press reset and the power-on message will appear:

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

Next press a key other than 0, 1, or 2. Assuming that no program has been entered, 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=    STEP=    TIME=
3 02    HEAT=    PULL=    STEP=    TIME=
```

The cursor will be blinking in the leftmost position of the heat value on the first line.

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 50. In order to complete the entry, press the ENTR key. The two digit entry is right justified and the cursor moves to the next position. IF ONE OR TWO NUMBER(S) ARE PRESSED WHEN LOADING A VALUE, THE ENTRY MUST BE COMPLETED BY PRESSING THE ENTR KEY; THE CURSOR MOVES TO THE NEXT POSITION. Continue by entering a value for STEPS (IE 30).

Do not enter a value for TIME, simply press ENTR. PRESSING ENTR WITHOUT FIRST PRESSING A NUMBER KEY WILL CAUSE THE CURSOR TO ADVANCE TO THE NEXT POSITION WITHOUT ALTERING THE VALUE IN THE CURRENT POSITION.

If a value for both STEPS and TIME are entered in the same cycle, the first condition met will cause execution to be passed to the next line of the program.

Enter another set of values, such as 0 for HEAT, 1 for PULL, 0 for STEP, and 100 for TIME. Currently the cursor is poised 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=50	STEP=30	TIME=
3 02	HEAT=0	PULL= 1	STEP=	TIME=100

Now press NEXT; the display scrolls up one position, and the cursor is on the 'next' line of the program. TO MOVE AROUND IN A PROGRAM FOR THE PURPOSES OF EDITING, USE THE ENTR, 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 in line 01; press ENTR 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 ENTR WITHOUT ENTERING A VALUE WILL CAUSE THE CURSOR TO MOVE TO THE NEXT POSITION WITHOUT CHANGING THE VALUE AT THE CURSOR'S PREVIOUS LOCATION.

Assume for the moment that the two line program that has been entered, 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 three-line program:

3 01	HEAT= 590	PULL= 100	STEP= 23	TIME= 0
3 02	HEAT= 0	PULL= 1	STEP= 0	TIME=20
3 02	HEAT= 0	PULL= 100	STEP= 0	TIME=150

Assume that, after loading a piece of glass into the puller and executing the program above, that the filament came on three 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 = 590	PULL = 100	STEPS = 23	TIME =
3 02	HEAT =	PULL = 1	STEPS =	TIME = 20
3 03	HEAT = 0	PULL = 100	STEPS = 0	TIME = 150
3 04	HEAT = 590	PULL = 100	STEPS = 0	TIME = 0
3 05	HEAT = 0	PULL = 1	STEPS = 0	TIME = 0
3 06	HEAT = 0	PULL = 100	STEPS = 0	TIME = 150
3 07	HEAT = 550	PULL = 100	STEPS = 23	TIME = 0

```
3 08   HEAT = 0        PULL =   1    STEPS = 0     TIME = 20
3 09   HEAT = 0        PULL = 100    STEPS = 0     TIME = 150
```

Note the reduction in the heat value in cycle 07. This illustrates how the looping capability can be used to create a multi-step program designed to pull in one program execution. When the puller detects that the pull has been completed the following message will be displayed for one second:

```
PROGRAM LOOPED X TIMES        X=1,2,...
LAST CYCLE WAS LINE Y         Y=1 TO 16
```

This feature is provided to aid in the development of programs that involve multicycle pipette fabrications.

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.

The seven line program above contains an important bit of information. Consider that situation in which a piece of glass has been stretched out by two pulls until it is very thin. If the pull strength were turned on in a step change fashion, the shock to the glass might be such as to break the glass rather than draw it out. To counteract this effect, the PC-84 is capable of 'ramping up' the pull strength. THE SIGNAL THAT A RAMPING PULL STRENGTH IS DESIRED, IS THE INSERTION OF A PULL VALUE OF OTHER THAN ZERO IN THE PREVIOUS CYCLE. Consider the following two excerpts from hypothetical programs.

```
5 02   HEAT = 000    PULL = 001    STEPS = 000    TIME = 250
5 03   HEAT = 550    PULL = 100    STEPS = 050    TIME = 000

6 02   HEAT = 000    PULL = 000    STEPS = 000    TIME = 250
6 03   HEAT = 550    PULL = 080    STEPS = 050    TIME = 000
```

In the first case, the puller recognizes that a ramping of the pull strength is desired when cycle 03 is executed; whereas in the second example, the pull is turned on to 80 instantaneously. Since a pull value of '1' represents such a low pull force as to be nonexistent, there is no difference is the 'effect' of line 02 in both cases above. In practice, it is advisable to ramp the pull strength before turning on the heat. Referring back to the nine line program, note that lines 02 and 03 combine to bring the pull strength up 100 before the heat is turned on in line 04; the same then occurs with lines 05 and 06.

A program line such as the following is important to understand.

```
3 02   HEAT = 000    PULL = 000     STEPS = 000     TIME = 100
```

The microprocessor does not hesitate between cycles when executing a program, thus it is necessary to program 'cooling' cycles into a program where the glass must be alternately heated and cooled during multi-step fabrications.

INHIBITING MOVEMENT OF THE HEATER BLOCKS

In order to form a very fine tip, the glass must be cooled briefly before the final hard pull. In the PC-84 this is accomplished by stopping the movement of the heater blocks. This allows the soft area of glass to be drawn out of the heater element as the glass is stretched out. To stop the movement of the heater blocks for any programming line, simply enter a step value of 255 on that line. However, you must supply a time value since the step value of 255 is ignored as a criterion for completion of the lines instruction. You will want to use this feature on the last two lines of the typical microelectrode program.

NESTED LOOPS

Programs described above execute line by line until the pull is complete. If the last line of the program is executed but the pull is not complete, the puller will continue by looping back to the first line of the program. Although this allows for unlimited looping through 16 separate lines of instructions, it is sometimes useful to combine many stages of looping with a few program lines that execute only once. We call this a NESTED LOOP. If the puller encounters a line with a heat value of 1 the puller will begin to execute a nested loop. All other values on this line will be ignored, and the puller will continue to execute subsequent lines normally until it encounters a line with a heat value of 2. The puller then checks to see if the step value entered on this line is greater than the total distance traveled since it encountered the heat value of 1. If this is the case the puller will loop back to the line of the program just after the line containing a heat value of 1 to continue execution. If the total distance traveled exceeds the step value on the line containing the heat value of 2, the puller will not loop back, but rather will continue by executing the next line of the program. Here is an example that may prove useful in forming patch pipettes:

```
1. HEAT R+90   PULL  75   STEPS  50   TIME
2. HEAT        PULL   1   STEPS       TIME 200
3. HEAT    1   PULL       STEPS       TIME
```

4.	HEAT	R-10	PULL	75	STEPS	2	TIME	
5.	HEAT		PULL	75	STEPS		TIME	200
6.	HEAT	2	PULL		STEPS	30	TIME	
7.	HEAT	R-20	PULL	75	STEPS	14	TIME	
8.	HEAT		PULL	1	STEPS		TIME	10
9.	HEAT		PULL	75	STEPS		TIME	200

(NOTE: R = THE VALUE OF THE RAMP TEST FOR THE GLASS USED)

This program contains a nested loop, since there is a line with a heat value of 1 and a subsequent line with a heat value of 2. These lines are, respectively, the start and end of the nested loop. The puller will execute lines 1 and 2 to draw the glass out. When the heat value of 1 on line 3 is encountered the puller will begin to execute the nested loop. Thus the puller will execute the program lines within the loop, in this case 4 and 5, over and over until the glass has been drawn out the distance indicated by the steps value on line 6. Although line 4 indicates that the glass will be drawn out only 2 steps per loop, the program will not require 15 loops to meet the distance criterion of 30 steps because the glass will be drawn out in line 5 as well as in line 4. Once the glass has been drawn out 30 steps, the program will continue to execute by advancing to the next line, in this case line 7. Lines 7, 8, and 9 serve to form the final tip and may be adjusted to obtain the desired tip size.

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 steps can be taken. 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.

Lastly, 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 with out 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.

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 above under the heading 'CLEAR'.

This concludes the discussion of operation.

■ SECTION VI

THEORY OF OPERATION

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 80, 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. It should be noted that at high heat settings (filament white hot) the filament life is greatly reduced.

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. For patch-pipettes and injection pipettes a good starting point is the ramp test value plus 30. The time for the first pull will be in the 10 to 20 second range.

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. For the box filament the glass should be centered in the filament.

PULL STRENGTH ADJUSTMENT

Low values of pull strength (below 100) will give larger tips, while settings between 150-250 give the smallest tips. The pull strength can be set to any value as needed for making pipettes, but the user should avoid program errors that might cause the pull to be turned on for prolonged periods. For instance, the use of very low pull strength may not be able to move the carriage and the pull may stall. In particular, do not use a heat value that is too low to soften the glass together with a high pull setting since this could cause the pull to be turned on indefinitely. The pull solenoid has been fitted with a thermal cut-out which will turn off the solenoid if it is over-heated. If this occurs allow the unit to cool for 20 minutes before attempting another pull. The cut-out will reset automatically when the solenoid has cooled.

BOX FILAMENT

The filament which is generally used with the PC 84 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 than trough filaments. This results in faster heating without the necessity of increasing the temperature of the filament. (Note however that higher values for the HEAT setting must be used to produce an equivalent operating temperature. Remember that HEAT is the current through the heater filament, not the actual heat produced.) The box filament also heats the glass in a more symmetrical fashion than trough filaments, so that the pipettes produced tend to be straighter and more concentric than those pulled with a trough filament. The box filament has one primary limitation; 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.

The optimal size of the box filament appears to be 2.5mm wide, 2.5mm high and 2.5mm deep. To produce short, large tips, a box filament of 1.5mm width, forming a box 2mm on each side, may produce straighter tips than a trough filament.

There is a size limitation on box filaments that can be used with the Model PC84 electrode puller. Box filaments wider than 3mm may exceed the maximum filament heater current that the PC84 can deliver, thus limiting the filament temperature.

The 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 then tighten the two screws.

ELECTRODE LENGTH

Electrode length 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 2mm is reached. Increasing the filament width beyond 2mm will produce longer tips with a more gradual taper (which penetrate better in some cases). However, the tip will not be any smaller. (See Section 4)

■ SECTION VII

SAMPLE PROGRAMS

Programming pipettes is somewhat of an art rather than a science because of the lack of independence of the different parameters, and thermal inertia which causes the effects of earlier cycles to affect later cycles. The basic rules, however, are:

1. Cold glass makes big tips and steep tapers.

2. Hot glass makes long, slow tapering tips.

3. High acceleration makes small tips.

The use of program control is best illustrated with some examples.

A PATCH ELECTRODE

Two patch programs are in memory when the puller is shipped. Program 0 is a looping program for soft glass and Program 1 is a looping program for borosilicate glass. But lets suppose that we have a new glass and start from scratch.

i) FIND SOFTENING POINT OF THE GLASS. This is done by a temperature ramp under constant tension. When the carriage moves 5 steps, the softening temperature is recorded on the screen. To activate this sequence, enter any program, i.e. #1 and press CLEAR. The console will ask whether to clear the current program and reply NO. It then asks you if you want a RAMP and you respond YES. Load the glass and press PULL. The temperature numbers will increase at about 10/s, and after a minute or so it will stop with the softening point displayed. Suppose it comes up 830 which is common for aluminosilicate. Press RESET to end the test, enter a program number to store your program and replace the glass.

ii) THIN THE GLASS. A typical first cycle (Program 9) might be,

```
9  01    HEAT=850    PULL=100    STEP=23    TIME=0
```

This will draw the glass to a neck of about 300u diameter.

iii) COOL THE GLASS. This gets rid of stored heat and prevents drawing out long tips. The time for cooling will need to be adjusted. If it is too long, the glass won't pull (press STOP), and if it is too short, the tips will be too long. The cycle might look like this,

```
9  02    HEAT=0    PULL=1    STEP=0    TIME=5
```

iiii) PULL AND BREAK. This stage will finish the pipette. The force is increased and maintained for enough distance to ensure a break. The lower the force, the bigger the tip. This relationship is due to the inertia of the carriage. Strong forces will accelerate the carriage fast enough to pull the glass while it is still hot. Weak forces will allow the glass to cool and if the force is strong enough to break the glass, it will do so when the glass has not been pulled much and has a large diameter. Extremely large forces may produce large tips by exceeding the plastic limit of the glass before it has cooled. It is possible to produce "fire polished" tips by breaking the glass when it is still plastic. A typical pull cycle might be,

```
9  03    HEAT=0      PULL=100    STEP=0     TIME=150
```

There are many ways to adjust the sequence in case the results are not satisfactory. For example, suppose the program gets stuck in cycle 3 because the glass is too cool to pull. One simple solution is to shorten the cooling time to perhaps 3 ticks. This is a rather dramatic changes since each tick is 20% of the original setting. Another way to accomplish the same thing is to increase the temperature in the first cycle. This is more subtle since the resolution of the temperature scale may be higher.

Another way to prevent getting stuck in cycle 3 would be to increase the number of steps in cycle 1 so that the glass is thinner before the pull so that the force/area is higher. Clearly, more steps could be added for more control.

PATCH PIPETTES BY LOOPING

Rather than use many defined steps, for stubby pipettes it has been proven better to use the looping feature since this permits the glass to be kept as cold as possible. The looping procedure is basically the same as the sequence above, but instead of forcing a break on the last cycle we simply try a break, and if it fails we loop back and try again. (i.e. thin the glass a little, let it cool for a short time and try to break it. If it doesn't break, loop back.)

The trick in constructing loop programs is to remember that you must loop back to the beginning so that what ever conditions you set for the full size glass, must also be compatible with the smaller size which occurs after several loops. Thus, in the following loop sequence for 1.4mm thin-wall borosilicate glass (Drummond Microcaps), the force is increased through the program rather than the reverse so that if a proper breaking force is not achieved at the last cycle, the first cycle will not snap it.

```
A LOOP PROGRAM FOR STUBBY PATCH PIPETTES
    9 01    HEAT=800    PULL=60    STEP=2    TIME=0
```

9 02	HEAT=375	PULL=40	STEP=0	TIME=4
9 03	HEAT=0	PULL=80	STEP=0	TIME=20

The above sequence shows several useful programming features. First, notice in cycle 1 step size is 2, i.e. 0.004". A step of 1 could be used but if there is any play in the carriage, a step size of 1 can often be counted without any net motion of the carriage. (Play can be reduced by gently tightening the small phillips screws on the back side of the carriage which compresses the ball bearing slides. This adjustment is critical and since it is important to avoid any significant drag on the left-hand carriage.)

Second, notice that in cycle 2, the cooling cycle, the heat is not set to 0 but to 375. Adjusting the heat here allows for fine control of the cooling during the allotted 40ms.

Finally, in cycle 3 we don't use a step limit since when the diameter of the glass in large, the force will be insufficient to move the glass and program will stall. Instead, we wait 200ms to see whether it can be pulled.

THICK WALL PATCH PIPETTES

It is possible to make patch pipettes with thick wall near the tip to reduce the capacity and noise. The pipettes are thinned and then "cooked" for a while with no applied force. This results in a thickening of the wall. The neck is cooled and then reheated and drawn as a normal patch electrode.

INTRACELLULAR ELECTRODES

The key to making small tipped intracellular microelectrodes programs is the sudden acceleration when the glass is soft. Electrodes can be made with short tapers for work in tissue culture or with long nearly parallel shanks which taper rapidly near the tip for use in deep tissue, such as brain or spinal cord. The higher melting point glasses produce the smallest tips so that aluminosilicate capillaries are preferred for tips in the 0.1u range. A typical microelectrode sequence for borosilicate glass is available in program 2 a the puller is shipped. A sequence to draw 1.4mm thin wall aluminosilicate is shown below.

2 01	HEAT=835	PULL=100	STEP=25	TIME=0
2 02	HEAT=0	PULL=0	STEP=255	TIME=15
2 03	HEAT=0	PULL=250	STEP=255	TIME=100

This program will actually loop if the pipette is not pulled the first time. Note the abrupt change of force in cycle 3. Remember that to obtain maximum acceleration, the force must increase from 0 to the requested value. Note also that a step value of 255 was entered on line 2 and line 3.

This value is a special case of and signals the puller to stop moving the heater blocks. The step value of 255 is ignored as a criterion, so that any line with a step value of 255 will execute as a timed line. The above program makes electrodes of medium taper, similar to those made on traditional two-stage pullers. To make long parallel shanks, we begin with a long draw, cooling to eliminate stored heat and then reheating and drawing with a sequence similar to the above.

PERFUSION PIPETTES

It is often handy to make pipettes with 5-25u tips for use as perfusion pipettes or suction pipettes for holding cells or stimulating nerves. The sequence below makes tips of about 20u diameter form 1.4mm thin wall borosilicate.

```
8 01    HEAT=800    PULL=255    STEP=60     TIME=0
8 02    HEAT=0      PULL=20     STEP=0      TIME=100
8 03    HEAT=630    PULL=130    STEP=100    TIME=0
```

This is a linear program The first cycle thins the glass, the second cools it and third holds a rather low temperature which is below the measured softening point. With sustained tension the glass will flow and break when it reaches about 20u. The tip diameter can be increased with increasing force in cycle3. To decrease the tip size, it would be necessary to raise the heat in cycle 3 since the flow rate is near the minimum possible commensurate with completion of the program.

METAL FILLED PIPETTES

Metal filled electrodes are often used for extracellular recordings from nerves. This puller is able to make glass insulated, gold-filled pipettes in a single step. The basic idea is to place a fine gold wire (0.001" diameter used for wire bonding of semiconductors) inside a glass capillary, and draw the two as a unit. To do this properly, the melting point of the glass must be near the melting point of the gold. Aluminosilicate capillaries work well.

The wire is threaded into the capillary by sucking on the opposite end. The wire is cut off and the concentric pair placed in the puller. There is no need to fix the ends of the wire. The pulling sequence takes advantage of the puller's ability to "cook" the glass without pulling in order to bond the wire to the glass.

```
0 01    HEAT=850    PULL=100    STEP=35     TIME=0
0 02    HEAT=900    PULL=1      STEP=0      TIME=255
0 03    HEAT=850    PULL=1      STEP=0      TIME=255
```

0 04	HEAT=0	PULL=255	STEP=5	TIME=0
0 05	HEAT=0	PULL=1	STEP=0	TIME=2
0 06	HEAT=0	PULL=150	STEP=100	TIME=0

This sequence can be understood as follows.

Cycle 1 thins the glass so that it is close to the diameter of the wire.

Cycle 2 and 3 "cook" the glass and the wire for 5 seconds to bond them together.

Cycle 4 draws the bonded pair to a smaller diameter.

Cycle 5 cools them for 20ms. (The gold wire provides rapid cooling.)

Cycle 6 does the final draw and break.

The result is a pipette in which the glass is bonded to the wire for the last 100u with the exception of the last few micra where the gold rapidly tapers to a submicron tip with a cone angle of about 45 degrees. As usual, the tip can be platinum coated to reduce the interfacial impedance.

We clearly have not explored all the possibilities. In order to take advantage of the variety of expertise of our users and the highly reproducible nature of this puller, we would appreciate receiving favorite programs so that we may test them and distribute the results periodically to the users.

■ SECTION VIII

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 borosilicate glass with an O.D. of 1mm and an I.D. of .50 with program 2 will give tips of .12 to .18 micron as shown in the SEM micrograph included with this puller. Using similar settings borosilicate glass 1mm O.D. and .78mm I.D. will form tips of .2 to .3 micron.

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 too 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 150 will give tips of less then .2 micron. Increasing the pull to 250 will reduce the tips size to about .15u. We recommend a pull of about 250 in most cases.

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. The pull should generally be decreased when pulling large tipped (1-10 micron) pipettes. As the heat or pull strength are decreased the cooling in the next line will also have to be decreased if you do not want to increase the number of loops in the pull.

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

Variations in pipette size and shape usually can be attributed to a particular combination of parameters that tend to over emphasize variations in the glass used. For example, consider the case of a program intended to produce a patch pipette in two loops. If one were to decrease the heat enough, the glass would not separate after completing two loops and the puller would begin to execute a third loop of the program. Because there is considerable variation in the size of glass from even the best sources, it is possible to find a heat setting that will execute correctly in two loops for some pieces of glass and yet, for other pieces of glass from the same batch, separation will not occur until the first line of the third loop. This will result in dramatic variations in the tip size and shape since the tips will be formed with the heat on rather than after a period of cooling. This is the most extreme of several possible problems that can occur when the program values are not properly adjusted. The cure for this problem is adjustment of the parameters. For example, increase the cooling in line two until the pull always takes one more loop than desired. Next, decrease the cooling until the pull takes one fewer loop than desired. Select a cooling time midway between these values and the program should be very stable. It may sometimes be desirable to use values that are close to causing a change in the number of loops. For instance, very large tips (5 micrometers) may be obtained by decreasing the heat so that the glass begins to set-up with after being greatly attenuated. As the pull in the last line increases the glass may break near the desired diameter on some pulls and may continue to the next loop on other pulls. Programs of this sort will be intrinsically more variable.

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

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 less than normal cooling. Normally low cooling results in a long wisp , 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 straighter 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. Check to make sure there is not excessive play in the carriages. This can result in abrupt bends near the tips of patch type pipettes.

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. If they are tight then check the allen screws that hold the heater wires in place. If these are tight then the problem is probably on the circuit board or the main heatsink.

B. ELECTRONICS SYSTEM

<u>CAUTION</u>: 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.

The PC84 micropipette puller is controlled by a Z-80 microprocessor. Two digital to analog converters control the heat and pull 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.

TROUBLE-SHOOTING

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

a. In most cases this will be caused by improper adjustment of parameters as noted above. Changes over the course of a day or more may also be due to changes in humidity.

b. A second possible cause of this problem is dirt on the carrier rods or bearings. In this case clean the carriers and bearings with a lint free tissue or cloth. Check the leftmost carrier for free operation. Although these units are shipped with free operation over the entire range of travel, any binding that might develop after the carrier has traveled sufficiently to separate the glass will have no effect.

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 . 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 heatsink on the back of the cabinet.

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 10 seconds before turning power back on.

c. 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 U6 (U5 if circuit board is Rev. E or earlier) to see if the microprocessor is functioning.

d. Make sure stopper retracts on reset. Failure to interrupt the right side optical will prevent proper sign on.

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. Use memory test below to look for defective memory.

d. Make sure that the power supplied to the puller is not subject to spikes or brown-out. Do not turn the puller off then on again without waiting at least 10 seconds. Any of the above could produce a corruption of the data in the zero-power memory. Before re-entering extensive programs check the puller's ability to retain a simple test program overnight.

e. If values entered are not held when the power is turned off suspect the zero-power memory, U7 (U6if circuit board is Rev.E or earlier).

TEST FUNCTIONS

Section 4 of the manual has instructions on the use of the ramp test. As indicated in section 4, there are 2 other tests that may be performed in addition to the ramp test. These tests are the memory and counter tests. As with the ramp test, these tests are started by pushing the clear button will in a program. Next, push 0 indicating that you do not want to clear the program. You will then be given the options:

```
DO YOU WANT TO PERFORM A TEST FUNCTION?
NO=0        RAMP=1      MEMORY=2      COUNTER=3
```

Press 1 for the memory test. You will then be asked:

```
DO YOU WISH TO DO A NON-DESTRUCTIVE
MEMORY TEST NO=0 YES=1
```

Press 1 and the puller will report:

THE MEMORY IS OK

or

THE RAM IS BAD.

In the event that the second message is obtained, consult Sutter. The message will appear for a few seconds and then you will be returned to the program. Program values will not be changed.

To test the optical encoder, press 3 for the counter test. This should be done with the left-hand carriage moved all the way to the right. When this test is selected, the display will go blank except for the blinking cursor. Now slowly move the carriage to the left and you should see numbers displayed that correspond to the number of steps the carriage has been moved. After moving the carriage about 0.5 inch or 12.5 mm the reading should approach 255 and then drop down to a low value. Further movement to the left will cause the numbers to increase again until the scale moves beyond the encoder unit. If the counter does not reflect the first 0.5 inch of movement of the carriage, consult Sutter.

■ SECTION IX

CIRCUIT BOARD REMOVAL

Unplug unit from power. Remove the screws that hold the top white cover in place; three on each side and two on the rear. Lift the top cover and plastic cover out as one piece.

Remove six screws that hold the front of the cabinet to its base; two along each side and two under the front edge of the puller.

Lift the front of the cabinet with the attached mechanism slightly and slide it forward until the circuit board is revealed. Unplug the various connectors on the circuit board and note the position and orientation of each. We have tried to insure that the connectors can only be put on in the proper position and orientation, but care on your part will eliminate possible mistakes.

Remove all other molex connectors.

Remove the plastic screws that hold down the circuit board. Lift the circuit board clear of the chassis and set aside.

Installation of the board is the reverse of the above procedure. If the instructions are unclear, please contact us via phone, FAX, or telex.

LIMITED WARRANTY

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

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

This instrument is not intended to be used, and should not be used, in human experimentation or applied to humans in any way.

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

SUTTER INSTRUMENT COMPANY

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Novato, CA 94949

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DISCLAIMER

The pipette puller Model PC-84 is designed for the specific use of creating micropipettes and no other use is recommended.

This instrument creates items which should only be used in a laboratory environment for use on animal tissues. It is not intended to be used and should not be used in human experimentation or applied to humans in any way. This is not a medical device.

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

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

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