OPERATION MANUAL

P-77

MICROPIPETTE PULLER

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MODEL P-77, P-77A, P-77B

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P-77 BROWN-FLAMING MICROPIPETTE PULLER

MODEL P-77

P-77A

P-77B

6/1/82

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SECTION I

DESCRIPTION

The Model P-77 Brown-Flaming Micropipette Puller was designed to pull glass pipettes with ultra-fine tips with short tapers.

This manual describes the Models P-77, P-77A and P-77B.

These three models operate essentially in the same manner, with the following exceptions: the original Model P-77 utilized a loop filament and dual air jets; the Model P-77A was an improved version of the original, with a filament length substantially shorter than the original filament, to reduce the possibility of filament sag under high heat conditions. In addition, the Model A incorporated a single jet air supply system, which permitted easier filament alignment.

The Model P-77B represents a significant departure from the previous design in that an entirely new horizontal U-shaped trough filament structure is used to allow the user to pull a much wider range of electrodes than was previously possible.

Secondary advantages of this new filament structure are ease of loading glass and elimination of the possibility of filament deformation due to sagging or accidental contact by the operator. In addition, a greater range of filament widths increases the versatility of the puller.

For a comprehensive description of the puller, refer to the reprints included at the end of this manual.

SECTION II

INSTALLATION AND INITIAL USE

A. GAS SUPPLY TANK AND REGULATOR

The Model P-77 gas supply consists of a tank filled with dry nitrogen of approximately 2½ pounds at a pressure of 1800 psi, a regulator with a high pressure and low pressure meter, and an electronically controlled solenoid valve.

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 as tight as possible.

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

B. POWER REQUIREMENTS

The Model P-77, A, B, operates at 117 volts, 60 Hz. A.C. To operate at frequencies other than 60 Hz., an internal circuit change must be made. To operate at voltages other than 117 volts, an external transformer must be used.

A modification for use at voltages other than 117 volts 60 Hz. A.C. is available. Consult factory.

Operation of this instrument at voltages or frequencies other than those recommended may result in damage to the instrument and/or failure to pull electrodes correctly.

SECTION III

OPERATION

A. FIRST TIME USE

CAUTION: Before turning on the instrument, check the heater temperature setting. Set the heater temperature control knob to "0"(counter-clockwise). Place the heater adjust/operate switch to the operate position. Plug the instrument into a power receptacle of the correct voltage and frequency. NOTE: A power source that is essentially electrical 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 pressure of 50 pounds reading 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.

B. PULL STRENGTH ADJUSTMENT

Turn on the main power switch; set the meter monitors switch to the pull strength position and press down and hold the pull, adjust/operate switch in the adjust position. Rotate the pull strength adjustment knob until a reading of approximately 1250 is seen on the panel meter.

This setting gives the smallest microelectrode tips for a wide range of glass configurations. Low values of pull strength settings in the range of 500-600 will give larger tips, while settings between 1000-1600 give the smallest tips. The pull strength can be set to any value desired with no danger of damaging the instrument.

C. MICROMETER ADJUSTMENT(TRIP POINT)

To adjust the position of the optical switch, which turns on the air and hard pull, use the micrometer, located to the right of the right-hand electrode carrier. To make this adjustment, release the right-hand electrode carrier from the flat spring steel clip used to catch the carrier at the end of its travel. Move the carrier to the left as far as possible; hold the carrier against its stop(in the slot in the center of the carrier). Turn the micrometer knob to lower values until the red L.E.D. "ready" light goes out. Now turn the micrometer counter-clockwise(as viewed from the right side of the puller)until the "ready" light comes back on. Note the reading on the micrometer dial. Then continue to turn the knob two full turns counter-clockwise(.050"). This is the best general value for the trip point.

Settings between .040" to .060" give the smallest tips, while settings below .040" give larger tips. Settings greater than .075" give unreliable results.

D. HEATER SETTINGS(FILAMENT HEAT)

Switch the meter monitor switch to heat index and the heater auto/adjust switch to the adjust position. <u>CAUTION!</u> Because of the large power reserve of the regulated heater power supply, it is very easy to burn out the filament if the heater temperature control knob is at too high a setting. The recommended starting heater value is a panel meter reading of 200. Starting at "0", turn the heater temperature control knob slowly, until the meter shows a stable reading of 200. Now switch the heater auto/adjust switch to auto. Leave the meter monitor switch in the heat index position, to allow checking the operation of the heater filament during each electrode pull.

At this filament setting, a 1 mm o.d., 0.55 mm i.d. glass capillary tube should pull in three to eight seconds after the start button is pressed. If the pull takes longer than eight seconds, increase the panel meter reading about five, i.e.205. Then try pulling electrodes until the pull takes place in less than seven seconds after the start button is pushed.

If the pull occurs in less than three seconds after you start, decrease the panel meter reading by five. For 2 mm 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. This adjustment is discussed in a later section.

The heat setting can also affect the length and size of the tip. Higher heat settings will give longer and finer tips, until you reach a point 10 heat index numbers(as read on the panel meter)below a setting which will burn out the filament. This setting is optimum for producing fine tips and is generally around a meter reading of 220-230.

It should be noted, however, that at this heat setting the filament life is greatly reduced. It is suggested that a setting of 200 be used initially and electrode length be controlled by gas valve adjustment.

E. 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 (4 full turns from the closed position). 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. Long tips can be formed by using wider filaments or by using higher heat settings.

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

F. 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 ward and into the other clamp groove. Center the glass and tighten both clamps.

SECTION IV

HEATER POSITIONING AND REPLACEMENT

A. LOOP FILAMENT POSITION

The loop filament* is used with the P-77 and P-77A models. The position of the filament in relation to the glass tubing and the air jet is extremely important. The major problem caused by improper position of the filament is bending of the electrode tip.

Adjustment of the jet is simple. After replacement or reforming, the filament should be centered over the jet when viewed from directly in front of the puller. The filament is held in place by two screws located to the right and above and below the filament. Loosening these two screws will allow the filament to be moved from side to side, to enable positioning it over the jet.

When the filament is properly centered, tighten the screws. Be sure that the top and bottom legs of the filament leading to the heating loop are not touching each other. The fore and aft adjustment of the jet is made by loosening the screws holding the jet assembly to the filament block. With the screws loosened, the jet is free to move and should be adjusted to the position shown in Figure 1.

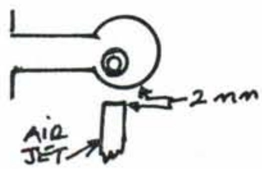

FIG. 1

*All heater filaments are fabricated from 90% Platinum and 10% Iridium, 0.002" thick.

Tightening the screws completes the adjustment.

The position of the glass in the filament loop can be adjusted using the set of adjustment screws to the right of the filament block. See Figure 2.

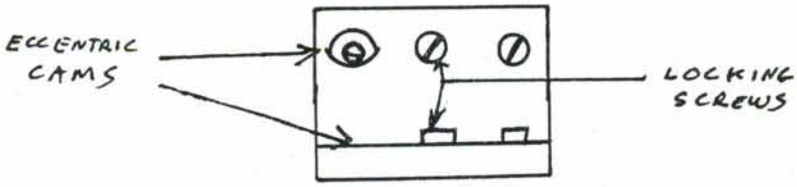

FIG. 2

The filament can be moved in relation to the glass tubing, by loosening the vertical or horizontal locking screw and turning the brass eccentric cam. When small diameter tubing(such as 1 mm o.d.)is used, the tubing will not be centered in the filament(concentric)but will be off center and will sit back and down in the loop as shown in Figure 1. This position works very well and gives better results than when the glass is centered in the filament.

B. LOOP FILAMENT REFORMING

If a loop filament has been deformed but not broken, it is possible to reform it(reshape it)with a tool provided for this purpose.

This reforming tool is a special steel rod with an enlarged brass section. If the filament is completely deformed, use a small screwdriver or forceps to reshape it to the point where the steel reforming tool can be passed through it. Place one end of the steel tool in the carriers and clamp it as if it were a piece of glass capillary tubing. Slide the tubing through the filament until the enlarged brass section is centered over the jet. Clamp the tubing in this position, using the two carrier V-groove clamps.

By using a small screwdriver, you can reform the filament around the brass forming section. After the filament has been reformed into a loop, carefully remove the reforming tool by loosening one of the clamps and sliding the carriers apart.

C. LOOP FILAMENT REPLACEMENT

A loop filament can be replaced by loosening the two screws that hold the filament in place. Remove the old filament by sliding it out to the left. Insert the new filament in from the left side and center it over the jet. Make sure the top and bottom legs of the filament are not touching each other.

Clamp them down firmly by tightening the two filament holding screws. It may be necessary to re-center the jet and/or reposition the filament in relation to the glass tubing as previously described.

A new value for optimum heat setting must now be determined. Use the method described in SECTION III D.

D. 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 2 mm below the filament.

When using the standard 3 mm 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.

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, and position it over the air jet. Then tighten the two screws.

E. 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 also be changed by using filaments of different widths. Widths of 1.5 mm to 6 mm 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 3 mm is reached. Increasing the filament width beyond 3 mm will produce longer tips with a more gradual taper (which may penetrate better in some cases). However, the tip will not be any smaller.

Utilization of heater filaments wider than 4 mm will cause increased heating of transformers in the puller, with the possibility of electrode variability. This is particularly true if electrodes are pulled in rapid succession.

F. BOX FILAMENT

Another type of filament can be used with the "B" horizontal filament holder. This is the box type heater filament, which is simply two trough filaments, one placed over the other, resulting in a box shape. See Figure 3.

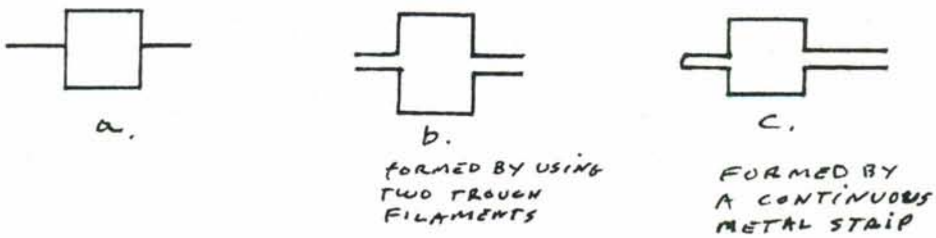

FIG. 3

The box configuration*, when used with thick wall glass tubing or using double-barreled glass, offers advantages over the trough filament. The box filament delivers more heat to the glass as well as heating the glass in a more symmetrical fashion.

The electrodes will start to pull faster for a given filament temperature and in some cases will pull straighter electrodes than electrodes pulled with a loop or trough filament.

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 3 mm wide, 3 mm high and 3 mm deep. To produce short, very large tips, a box filament of 1.5 mm width, forming a box 2 mm 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 P-77B electrode puller. Box filaments wider than 3 to 4 mm may exceed the maximum filament heater current the P-77B can deliver, thus limiting the filament temperature.

SECTION V

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

*Fabricated from 90% Platinum, 10% Iridium, 0.002" thick.

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. 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, ie the carriers are in 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 2 mm 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 and turning the chrome eccentric cam to move the pulley in small increments 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. 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 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 must 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.

C. ELECTRONICS SYSTEM

<u>Description</u>: The electronic control circuit of the Model P-77B Micropipette Puller utilizes a ferro-resonant regulator transformer to stabilize the heater temperature.

Heater temperature settings are adjusted, using a variable transformer, followed by a step-down transformer capable of delivering 80 amperes of current.

Heater current is measured by a current transformer and rectifier system, and current is read out on a digital voltmeter as a numerical index of heat value.

Correct operation of the puller depends on a precise relationship of events during the pull cycle. The sequence is as follows: With the carriers together and the glass clamped in place, the "ready" light should come on. The "ready" light is activated by a trip vane located on the right carrier. The trip vane passes through a photo detector connected to a micrometer used to adjust the trip point.

If the "ready" light is on, pushing the start button latches the filament heater control solid state relay. The filament heats, several seconds later, the glass commences to melt, and the carriers separate under gravitational pull. The trip vane moves away from the photo detector, turning off the heater and activating U-3, a 300 mS one-shot. This can be measured at test point TP-3. This activates the air valve for a 300 mS duration. The trip vane also starts one-shot U-4, which produces a 40 mS delaying pulse(which can be measured at TP-4). After 40 mS, U-5 is triggered, activating the strong pull, which lasts approximately one second. This time may be measured at test point TP-5.

The Model P-77 has several internal power supplies. A +5 volt regulated supply should read +5 volts at test point TP-7. A +85 volt supply should read +85 volts with no load at the plus terminal of capacitor C-7. An unregulated +12 volts, approximately, can be measured at test point TP-8. All of these measurements are made with respect to common(ground). An additional power supply, supplying approximately 20 volts to I.C. U-8 can be measured between pin 7 and 14 of I.C. U-8, using a floating ungrounded volt meter. NOTE: Damage to the I.C. may result if pin 7 or 14 is grounded.

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.

SECTION VI

LIMITED WARRANTY

Sutter Instrument Company, Division of Sutter Instrument Corp., 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 designed to pull glass pipettes for use on experimental animals or animal tissue in vitro. It 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.

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