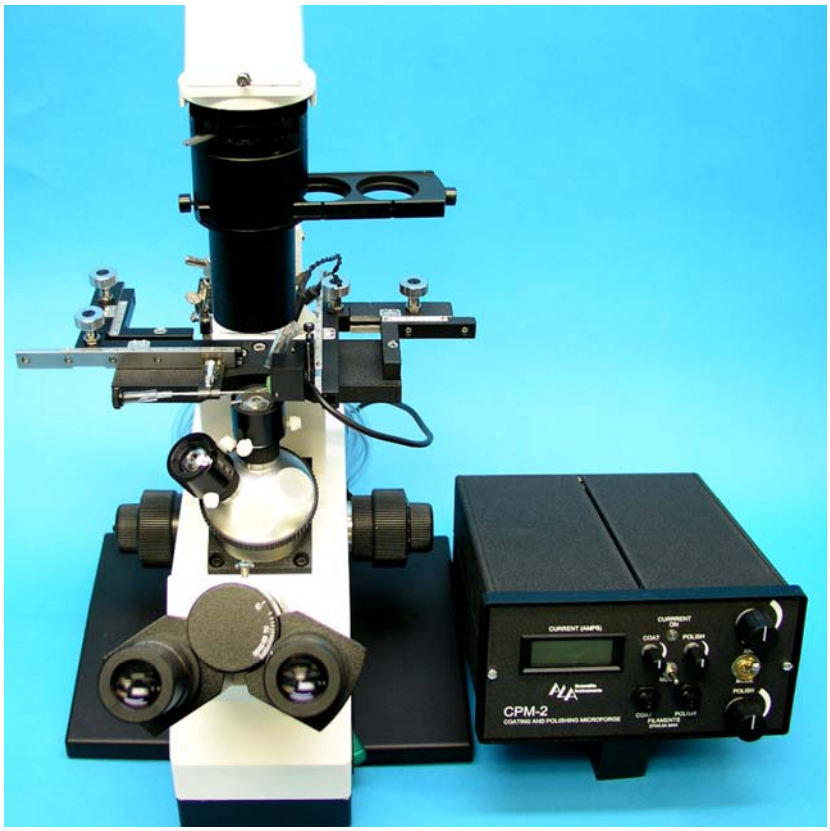
CPM-2

Coating and Polishing Microforge
For
Patch Pipettes

Instruction Manual

V04

Covering CPM-2 kit, CPM-2 and CPM-2 pressure



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Safety Information

CAUTION: This instrument is capable of generating high temperatures with regard to certain external components, care should be taken to avoid contact during operation otherwise burns may result. Parts to avoid contact with are: Hot Air Jet, including the air flow from same and Polishing Filaments (these parts can also ignite other flammables that may come in contact with them). Also, care should be taken not to short the contacts of the filament holder together, this will cause a short and blow the fuse. There is minimal shock hazard for humans, but contact should always be avoided. Filament power is 2-5 Amps at 24 Volts DC.

Never operate the Coating Filament (HOT AIR JET) without air flow. It will over heat and be damaged. Never over-charge the polishing filament, it will burn out. Also, never leave the polishing filament on for more than 10 seconds, excessive heat can result.

European Customers: If your unit bears the CE mark it is in compliance with the directives of the European Council with regard to electrical equipment for the laboratory environment. The unit is in compliance with all safety regulations and the Electro Magnetic Compatibility (EMC) standards. Test certificates are available upon request.

General Safety Information:

This is an electrically operated device. As such there are inherent hazards such as fire and electric shock. The user should take sensible precautions to avoid injury.

Do not use flammable liquids or gases near this product.

If the unit should become wet, do not operate it. Shut it off and contact the manufacturer.

Do not use this product in a manner inconsistent with this manual.

Do not operate the unit with any frayed or broken wires. All connectors must fit securely and a good ground (earth) connection must be present.

Do not operate this product under any other line voltage than what is listed on the power selector on the rear panel.

Do not operate the unit if any loose parts can be heard moving around in the controller.

If the unit fails to operate in any way, contact your representatives or the manufacturer.

NO USER SERVICEABLE PARTS ARE INSIDE THE CONTROLLER

Introduction

Patch Pipettes, made of glass are inherently very sharp at the tip. Not only is the tip at 2-5um less than the width of the edge of a razor, the glass has edges just like a broken piece of glass, right down to molecular sized ridges! The success of a patch experiment is contingent upon the area of high resistance that forms when the membrane of a cell adheres to the fresh clean glass of the patch pipette. It has been found, and one can go back to the papers of Neher and Sackmann, that the proper preparation of the tip, heat polishing it, etc. really increases the likelihood of a good and successful patch. In addition, the coating of Sylgard (® GE Plastics) enables a greater signal to noise ratio. In order to make this process easier, microforges were developed to enable the polishing of the tips of pipettes using heat. Since the dimensions that are involved are so small, tiny filaments and a microscope are employed. ALA has developed a microforge kit that can be placed on any small inverted microscope, as well as a turn-key system where all the parts are mounted on the XDS microscope at the factory.

The CPM-2, Coating and Polishing Microforge kit (CPM-2) is designed to turn virtually any inverted microscope into a coating and polishing microforge system. The CPM-2 kit consists of four components (1, the hot air jet, 2, the polishing filament, 3, the pipette holder and 4, the low power objective cover) that must be mounted on the microscope, and the controller with its foot switch. Various clamps are provided to mount the x-y positioners on the microscope stage

Fabrication of a patch pipette is usually accomplished in three steps: pulling the pipette then coating and polishing the newly formed electrode. The CPM-2 provides an inexpensive, easy and fast way of coating and polishing patch pipettes without the handling and remounting problems associated with other microforges.

The CPM-2 is designed as a kit, or as a pre-installed system on a XDS inverted microscope. The design is based on small inverted microscopes so that is what it works best with. The system works quite simply: A freshly pulled pipette is placed in the pipette holder. The tip of the pipette is then brought into focus under low power magnification. Once the tip is in focus a check is made to be sure that the air from the air jet is blowing on the tip and the immediate proximity. The user takes a small amount of RTV silicone or **Sylgard** on a tool and begins to apply it to the pipette. As this is done, the CPM-2 is in the coating mode and a step on the foot switch causes hot air or N2 to be blown onto the pipette in order to cure the silicone compound quickly.

The user then relocates the tip of the pipette, centering it in the field of view. Once this is done, the high power objective is moved into place and the polishing filament is moved into view checking to see that the filament is in the field of view. In the case of the CPM-2 W/scope, the polishing filament moves into place

when the high-power lens is rotated into position. The controller is then switched to the polishing mode. The foot switch is depressed causing the filament to heat up and then the pipette tip is brought near the filament and polished, or just polished in the case of the CPM-2 W/scope. The pipette is then removed and a new one inserted.

Microscope Requirements

For the CPM-2 kit, an inverted microscope of the small variety such as the Nikon TMS or Olympus CK2 for example should be utilized. We encourage the use of an older microscope that might otherwise be idle.

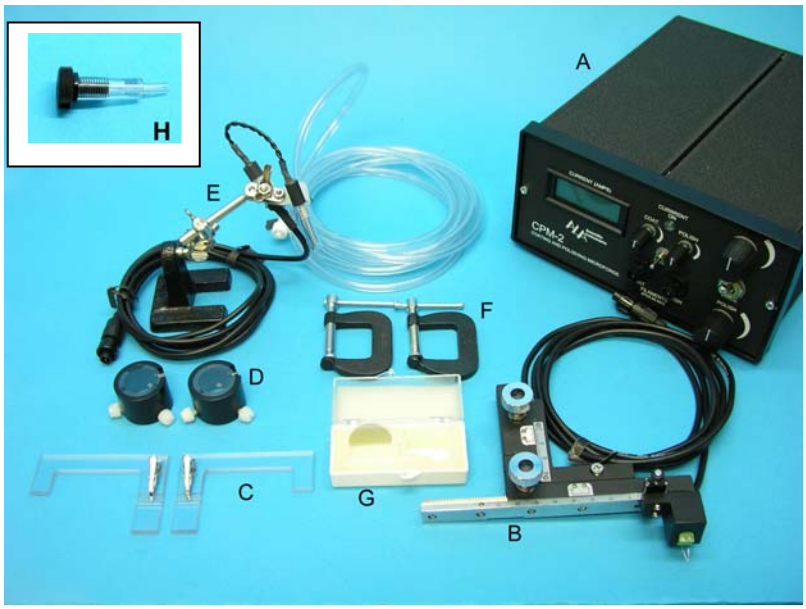
The microscope should be equipped as follows: It should have a fixed stage and an X-Y stage manipulator with a holder for standard microscope slides. The oculars should be 10x or 15x. The objective lenses needed are: 4x to 10x for low power, 20x to 40x for high power. High power lenses should have long working distances. Brightfield lighting is acceptable. If the microscope does not have an X-Y manipulator, the factory can provide one at modest additional cost.

Important Criteria for Microscope Mounting

Virtually any set-up can be used as long as the following criteria are met:

1. The tip of the pipette is held in the light path and can be manipulated easily and the holder sits firmly in the X Y stage manipulator.
2. The polishing filament can easily be brought into position in the light path opposite the tip of the pipette.
3. The low power objective cover fits securely on the low power objective and does not interfere with nose piece rotation.
4. The hot air jet can be pointed at the pipette tip and is no more than two centimeters away.
5. The high power objective must be protected from the heat of the polishing filament. A lens cover that holds a glass cover slip is provided. Alternatively, at the bottom of the Filament Holder a small amount of Fun-Tak™ putty can be used to secure a glass coverslip. The coverslip shields the high power objective lens from heat and potential spattering of the filament. It should definitely be used when a short working distance objective is utilized. (The importance of the coverslip is debatable since heat rises and there is substantial air flow from the air jet—nevertheless it is provided just in case!)

Installation of CPM-2 Kit on a microscope



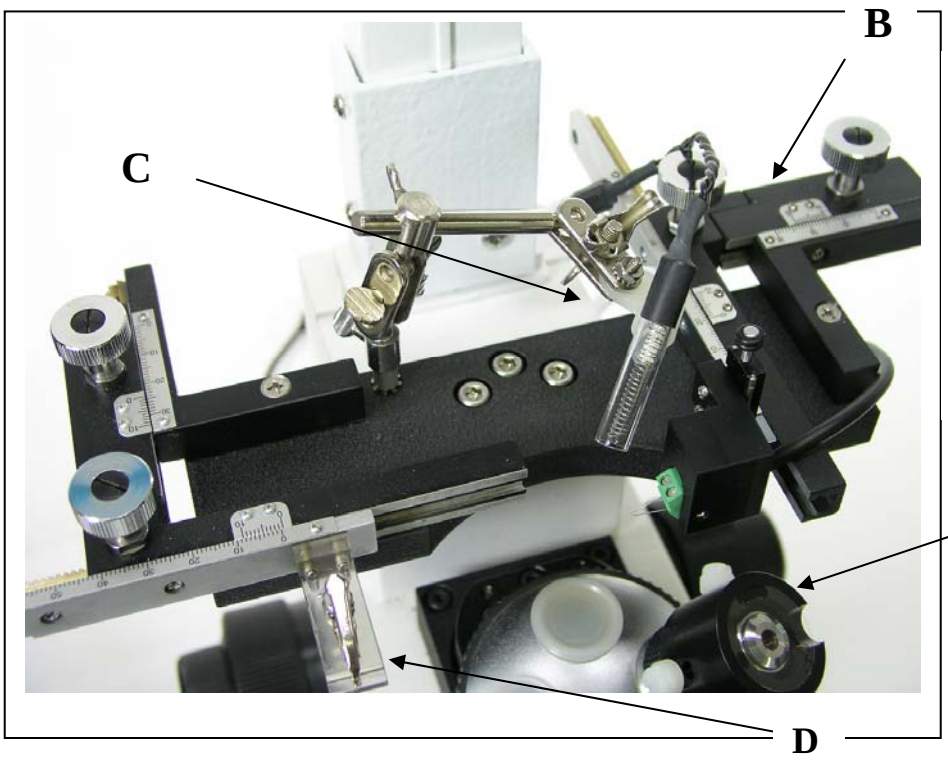
CPM-2 kit for microscope mounting consists of the components shown.

A Controller unit, comes with foot pedal (not shown)
B X-Y positioner with polishing filament
C Left and right hand pipette holders to be used on microscope X-Y stage positioner
D Objective lens protectors (2)
E Hot air jet, able and air tube
F C clamp for mounting hot air jet and X-Y polishing filament (2)
G Parts kit, 25mm cover glass, vacuum grease, and two spare filaments.
H Pipette handling tool.

Set the microscope and the control unit next to each other on a counter that is comfortable to work at. We recommend mounting the Hot Air Jet and the Polishing Filament Holder on the microscope stage as shown in the photo below. Other adaptations can also be used. Different microscopes may require different styles, but the user determines what works best for him/her. The manipulators can be mounted to the stage with screws (installed at the factory or by the user) or with C clamps provided with your system. C clamps allow your set-up to be broken down and removed from the microscope quickly. You can set it up quickly on another scope as well. Usually, if your components are screwed down they can be removed quickly as well, but installation on another scope will require drilling and tapping new holes.

Setting up the kit on a microscope

1. The five components (as shown in the photo below.) to be placed on the microscope are: The Low Power Objective Cover and High Power objective cover (2 covers: LPOC, and HPOC) (A), the Polishing Filament Holder with Manipulator (PFHM) (B), the Hot Air Jet (HAJ) (C), and the pipette holder (D).



Protective lens cover

Please Note: The factory mounted CPM-2 will have some slightly different components than the kit.

2. The LPOC is used to protect the low power objective from silicone or Sylgard spills. The LPOC is placed over the low power objective. A 25mm round No. 1 or 2 coverslip is placed atop the holder. The glass should be held in place with three dabs of vacuum grease. The LPOC is held to the low power objective with three Nylon screws. They should be just finger tight. The cover should sit so that the glass is fairly centered over the lens and as close to the top of the lens as possible. If the glass gets soiled it should be replaced. This keeps Sylgard form dripping onto the lens. The HPOC is used to protect the high powered objective from the heat of the polishing filament. It is identical to the LPOC, but another version is available by special request.

3. The pipette holder (C shown above) is placed in the slide holder of the microscope's X-Y stage manipulator (not shown in the photo below). Positioned so that the end of the pipette can reach the optical pathway. An X-Y positioner with pipette holder is also available form ALA, that is the one pictured below.

4. The PFHM (Polishing Filament Holder with Manipulator) is attached to the microscope stage in either of two ways:
    a. it is screwed directly to the stage, or
    b. it is attached with one or two C-clamps that are provided.

5. The PFHM is mounted in whatever orientation works best for the user. The only requirement is that the filament be easy to move in and out of the optical path. The preference is that only one knob be used to move it in and out once it is positioned. This makes it easier for the user.

6. The filament should have a triangular shape and be bent slightly downward at about 15°. When it is brought over to the pipette area, it must be in the viewing field under high power magnification.

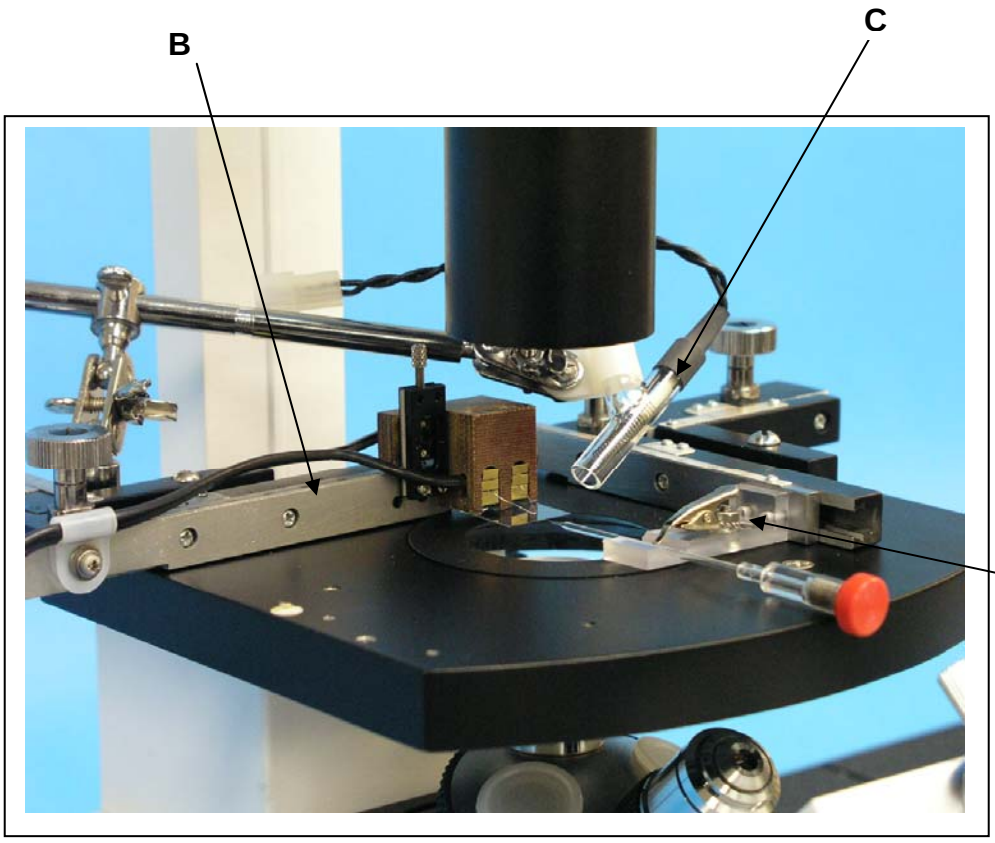
7. The microscope should be focused on the tip of the pipette. The filament should also be in focus. If it is not, use the small micrometer to adjust the filament up and down. If it cannot be moved enough to achieve the proper focus, simply bend the filament into the focal plane and use the small micrometer to get the exact focus. It is possible that after moving the filament out and back in that the filament may shift out of focus. If that happens simply refocus it with the small micrometer.

Use forceps to adjust the filament up or down by bending to bring it into focus. Use the Z-axis manipulator to sharpen focus.

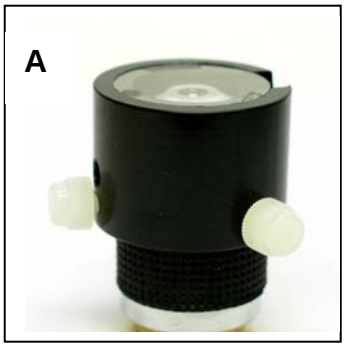
8. Remember that the microscope must always be focused on the pipette and the filament adjusted to meet it. When the pipette tip and the filament are in focus together the best polishing results will be achieved.

9. The HAJ is placed so that the air jet blows over the tip of the pipette. It is best to place the HAJ stand-off to the left side of the microscope stage. It should be attached to the stage with a C-clamp.

10. The extension arm should be extended to the right so that it holds the HAJ pointing at the location of the tip of the pipette. The air should be directed to flow down towards the user at a 45° degree angle (see photo below). The air tube should be connected to the air out connection on the back of the control box. The attachment is a female luer fitting. Two male luer fittings with 1/8th IN. barbs of 1/8th IN ID tubing have been provided. Luers should only be finger tight, over-tightening will damage them. Connect the tube from the HAJ to the Pressure OUT luer connector. BE SURE TO CONNECT A CLEAN POSITIVE AIR OR INERT GAS SUPPLY TO THE INPUT ON THE REAR OF THE UNIT. This air flow helps protect the pipette tip from the dust contamination when not being used to cure the Sylgard.



CPM kit installation on a microscope stage.
Note the position of the pipette holding manipulator vs. the one for the polishing filament. Here the pipette points away from the user, in another installation it can be pointed to the right or the left depending on the user's preference. Notice how the hot air jet is positioned to the side, but very close the working area so that it can blow hot air on the pipette to cure the Sylgard and it can blow cool air on the pipette tip during polishing to prevent contamination. Also visible is the small manipulator that is used to adjust the height of the polishing filament to keep in focus with the



Protective cover for low and high power objective.

Alternative lens protector uses 12mm cover glass for large high-power lenses. Contact factory for details.

Helpful note: 25mm coverslips are attached to the protective covers with three dabs of vacuum grease. When installing on a lens, use the three screws to center the cover as much as possible, position it as far down on the lens as possible. Take advantage of the cut-away on one side to help in tight clearance situations.

During the installation, be sure to move all the components around to see where they fit the best. Remember that the filament tip has to be able to get close to the tip of the pipette. Also, it is wise to try to position manipulators where they can be accessed by hand easiest.

Once all the components are installed on the microscope, the various connections to the controller can be made. A clean source of air for the system is necessary. Use a nitrogen tank or filtered house air. The max and min pressure is listed on the rear panel. The factory supplies a tube, but all that is needed is the proper Luer fitting to connect to the back of the unit. The air hose for the Hot Air Jet should be connected to the rear panel. Simply twist on the Luer until it can no longer turn (finger tight). Then plug in the leads from the hot air jet and the polishing filament in the appropriate pugs on the front panel. Be careful to connect the correct ones. Insert the central part of the connector and twist on the locking cap clockwise until lock. The controller is factory set for operation at 115 VAC for the US and 220 VAC for Europe and elsewhere. Please switch the power connector located on the rear panel if other line voltages are being used. Plug in the footswitch as well, and locate it on the floor.

The CPM-2 can be set up in other ways, such as not on a microscope at all. Also other coils for coating and polishing can be powered by it. Coils should have a resistance of about 2-3 Ohms. Please check with the manufacturer before modifications are made since they may violate the warranty.

The Controller

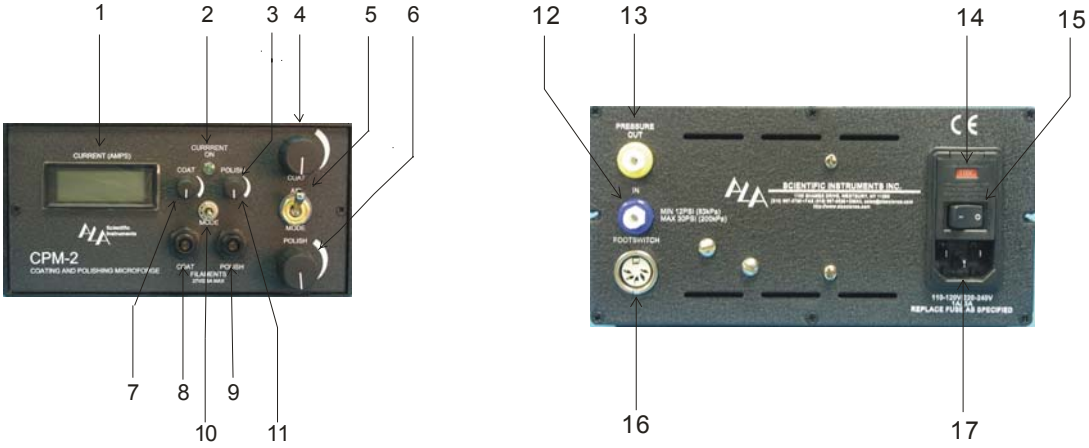
The controller should be located in close proximity to the microscope. Power is brought to the controller via the power input module in the rear. Also on the rear panel is the air input plug. Filtered clean air or nonflammable gas from a tank should be applied. The maximum input pressure should be 40 psi and the minimum 20 psi.

The power switch is located on the rear panel of the controller. When it is on, three digits will appear on the digital panel meter and slight hissing might be audible. The power supply can be switched to 220V by a switch on the rear panel. Always be sure the correct power setting is used for your area.

The digital panel meter indicates the amount of current in Amperes flowing to the filament selected by the mode switch. In other words, when the polishing mode is selected the current flowing to the polishing filament is shown. In order to see the current, the foot switch must be depressed. Current is activated in both modes for as long as the foot switch is depressed.

Adjacent to the mode switch is the air direction switch. Since the curing of the pipette may require a different rate of air flow than the polishing mode, the air direction switch allows the user to choose between two air flows, one for polishing and the other for coating. The amount of flow is set using the appropriate needle valve knob. Turning them to the right (clockwise) decreases air flow and to the left (counter "clockwise," increases it). Air flow should always be utilized in order to minimize pipette contamination. Never allow the Hot Air Jet to operate without air flowing.

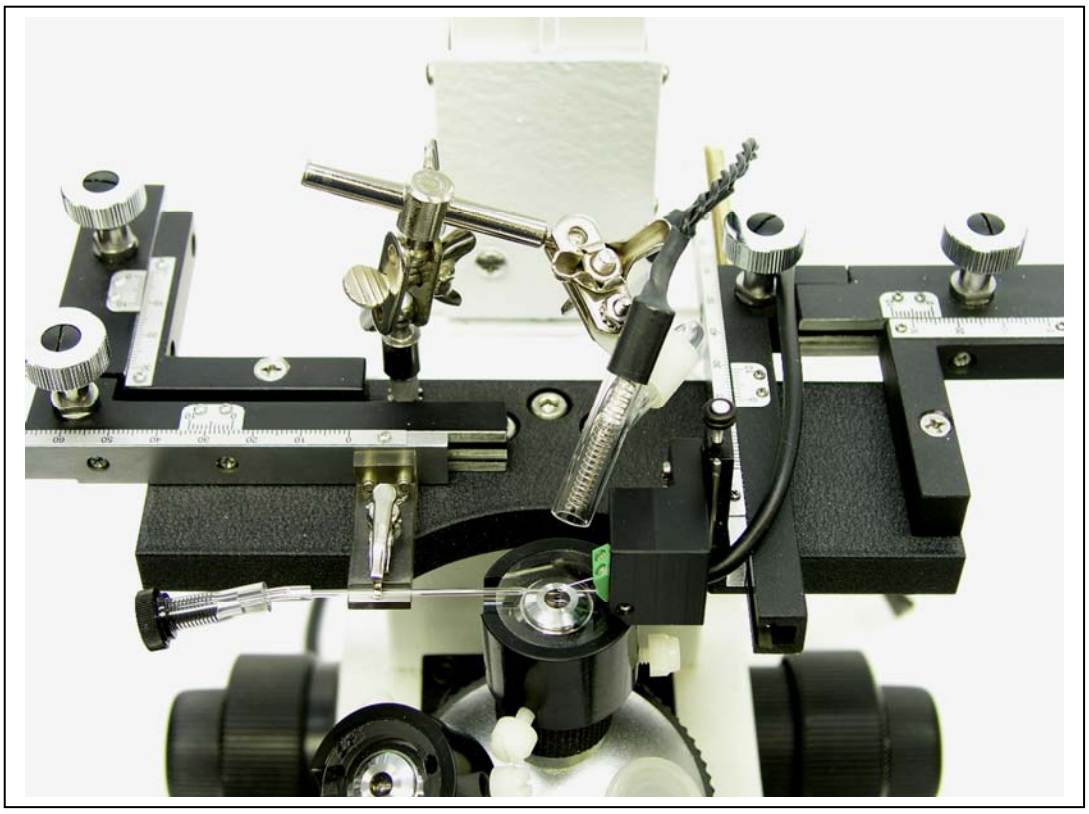
1. Current meter; displays current amount in amps when unit is on and pedal is depressed. (Reading fades out slowly when unit is turned off.)
2. Current light comes on when foot pedal is depressed, stays on until foot pedal is released.
3. Potentiometer for Polishing Filament, turn clockwise to increase current.
4. Coating air adjustment needle valve. Turn counterclockwise to increase flow.
5. Air mode selection switch, allows selection between two previously set air flows, one for coating and one for polishing.
6. Polishing air adjustment needle valve. Turn counterclockwise to increase flow.
7. Potentiometer for Polishing Filament, turn clockwise to increase current.
8. Connector socket for Coating filament. Insert and then twist on lock.
9. Connector socket for Polishing filament. Insert and then twist on lock.
10. Mode switch selects Coating or Polishing mode. Middle position is neutral.
11. Potentiometer for Polishing Filament, turn clockwise to increase current.
12. Input air pressure connection. Luer lock connector need only be finger tight. Observe Min/Max pressures as indicated on the chassis. Air Output to Hot Air Jet.
13. Air pressure out. Luer lock fitting connects tubing which brings air flow to Hot Air Jet.
14. Mains power input and fuse holder. FUSE REQUIREMENT is 1A @110V and .5A @220V.
15. Power Switch
16. Foot switch plug in. Be sure plug is fully inserted. Unit does not operate without footswitch.
17. Mains cable connection. Use only qualified three conductor cable.



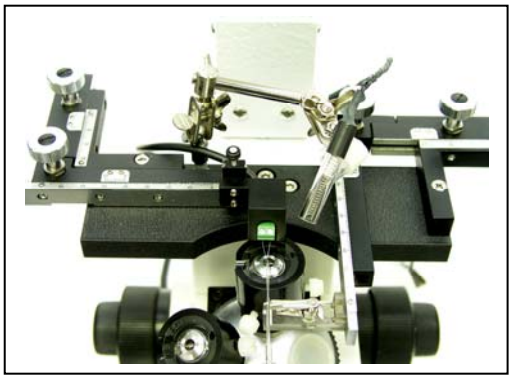
CPM-2 with Microscope

If your CPM-2 came complete with microscope, please see this section.

The stage of the complete CPM-2 with microscope is shown in the photo below.



A complete CPM-2 with microscope will be set up as shown above. The stage is cut in half to allow maximum axis to the pipette. Typically the pipette is held by an X-Y manipulator that is mounted so that the user will use the left hand to position the pipette. The right hand will control the polishing filament which is mounted on an X-Y positioner as well. The polishing filament can be moved up and down with a small manipulator called the "Z axis," it allows the filament to be moved up and down for focusing under high power. In the middle is the hot air jet which points at the front (tip) of the pipette. The hot air jet is mounted to an adjustable post with 5 points of movement. Although this is the standard stage set-up, there is an alternative:



Alternative stage set-up where filament is positioned from the left and the pipette is positioned from the right, however, the pipette lies on the Y axis in this style, which some users might find more convenient.. Notice that the hot air jet is in the same location, it could be set to the left as well.

Some users may find the alternative to be more ergonomic. In this orientation, the application of Sylgard would be in the Y axis, and this can be easier on the wrist.

Users should always use caution when handling the hot air jet, it can be very hot and cause burns.

Both the low power objective and the high power objective come with covers. In the event that Sylgard should drip onto it, the low power objective has a cover. It is exactly like the high power objective cover, which protects the high power lens from the high heat of the filament. There is an alternative cover that can be used for bigger lenses, it is pictured in the "Kit" section of the manual above, and can be special ordered.

It is very important to protect your lenses, at the very least, the cover can be a cover slip that is held in place simply by a small piece of putty.

Alignment of the polishing filament

The polishing filament is mounted on an X-Y positioner so that it can be moved around the stage and brought up to the tip of the pipette easily. A z axis is provided so that it can be brought into focus. First the tip of the pipette is brought into focus with low power. Then bring the filament into view so that the tip is near the tip o the pipette. Switch to high power and move the filament into view. Use the Z axis to focus it so that it is brought into the same focal plane as the pipette tip. (If you can't get the filament into focus with the Z axis adjustment, then use forceps to bend the filament down to bring the tip into the focal plane, see photo above) Switch the controller to Polish, turn the filament power all the way down. Step on the pedal and while observing the filament through the microscope, slowly turn up the power to the filament and you will see it start to expand and then glow. (You will need to adjust the heat as necessary for polishing your pipettes.) Once you see the curve of the apex of the filament in view, it is important to try to move it over to the right side of the field of view. The reason for this is that when the filament heats up it will move to the left as it expands and you do not want it to hit your pipette. Try to get a feel for how much the filament moves when heated so it will not touch your pipette. Use caution with the power control since high power can blow the platinum filament.

Once the filament is aligned, it is a good idea to load a pipette in the holder and touch the tip of the pipette directly to the hot filament so that the glass actually melts on the filament. It is good to have a bead of glass on the tip of the filament. The bead helps prevent particles of Platinum and Iridium that normally fly from the hot filament from striking the tip of the patch pipette. These particles can disrupt the surface of the pipette and affect its performance. Your system is now ready for polishing.

Aiming the hot air jet

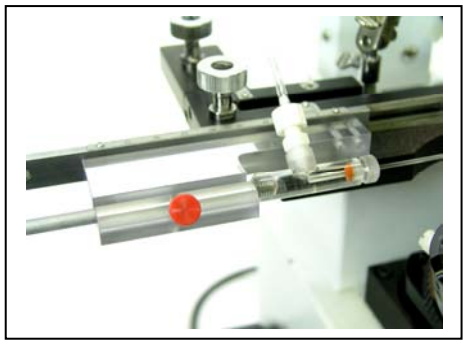
The Hot Air Jet needs to point at the tip of the pipette at all times. For coating, it will provide very hot air (up to 300°C) to cure Sylgard in a matter of seconds. At other times it keeps a stream of air blowing at the tip of the pipette to keep it clear of dust. Be sure its position does not interfere with the position of other components. Bringing the tip in close to the pipette will mean that you can reduce the amount of heat the hot air jet will need, extending its life. Also, always remember that the hot air jet gets very hot and can cause burns. It can take more than a minute to cool off after it has been on. Use caution.

Adjust its position using any and all of the 5 adjustment points on the device. Keep them tight to hold its alignment, but extra tools should not be necessary, hand tight is usually sufficient. **Always use inert gas with the CPM. Flammable gasses can be ignited! Inert gasses such as nitrogen will prolong filament life.**

Pressure Polishing

Pressure polishing patch pipettes allows one to create a pipette with lower resistance by using pressurized air during the polishing phase to expand the tip portion of the pipette. This enlarges the lumen of the pipette, right near the tip, which in turn, results in lower resistance during recording. The complete method for doing this is outlined in the paper: ***Goodman, Miriam B., Lockery, Shawn R., "Pressure Polishing: a method for re-shaping patch pipettes during fire polishing" J. Neuroscience Methods, 100 (2000) 13-15.*** It is recommended that anyone wishing to use that methodology should obtain a re-print as this manual will not go into all the details.

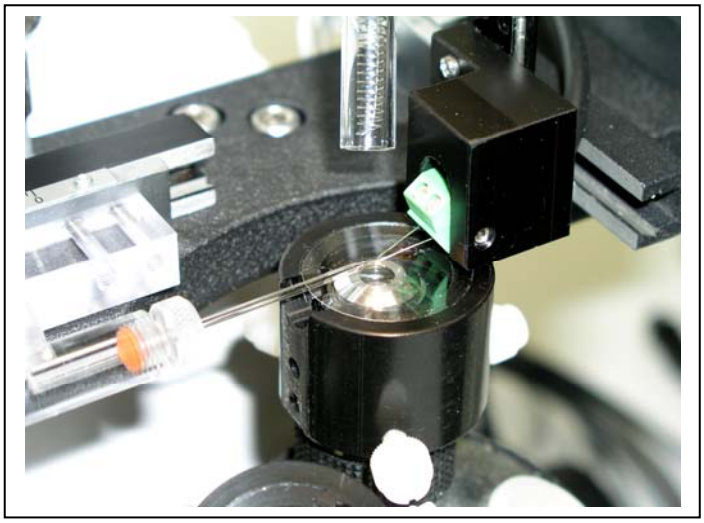
Besides the microforge, the user will need a positive pressure source and a special pipette holder that holds a glass pipette under pressure. The positive pressure is provided for by the PR-60 pressure regulator from ALA. The PR-60 is a supped up version of the PR-10 that includes a 0-60psi regulator instead of the standard 0-10.



LEFT: Pressure pipette holder in special bracket for CPM-2. **RIGHT:** PR-60 pressure regulator for pressure polishing.

The technique simply involves applying pressure to the pipette while the tip is being heated. Similar to the way that the pipette tip would normally be polished, it is expanded. The pipette tip is brought near the filament, the filament gets hot, softens the glass while it is under pressure, then it expands. It is just like glass blowing in miniature. The filament, which is on its own manipulator can be moved next to the pipette wall rather than across from the tip. The air pressure can be left on and the user can observe the glass expanding until the desired size at which point the pipette can be moved away from the heat source, or the pressure turned off. The PR-60 allows the pressure that is set on the gage to be conducted to the pipette, or vented to atmosphere at the flick of a switch.

One specialized piece of equipment necessary for this technique is a special pipette holder that can be pressurized. For safety, this specially designed holder connects the pipette with the pressure source. This helps prevent the pipette from becoming a projectile if it is not held correctly.



The above photo shows the typical pressure polishing configuration. The special pressure pipette holder sits in its own holder as well, to attach it to the X-Y stage. The pressure polishing technique requires the PR-60 Pressure Regulator from ALA.

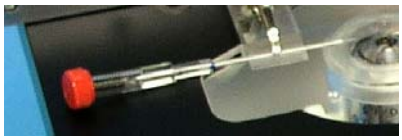
The pressure to be applied to the pipette is set from 0-60PSI using the knob and the gage. When the pressure is ready to be applied, the user switches the selector switch to 'PRESSURE' and the selected pressure flows to the pipette. 'BLEED' allows the pressure to bleed to atmosphere, de-pressurizing the pipette. The PR-60 should be connected to a clean source of air or nitrogen**. Flamable gases can never be used!** Input and output connections are on the back of the PR-60. The output is connected via small bore tubing to the pipette holder.

Operational Modes

Coating

Typically patch pipettes are coated with Sylgard® (GE) or silicone and then they are polished, so we begin with the coating mode.

The parts of the system necessary for coating are as follows: You will need the Hot Air Jet, to blow hot air on the pipette to quickly cure the Sylgard, the pipette must be held, so that involves the pipette holder (note that for pressure polishing, the pipette is held in a special pipette holder that allows it to be pressurized.) and the pipette must be viewed with the low power objective, which in turn needs to be protected by its cover in case any Sylgard drips onto it. Also, the Pipette Handling Tool, visible in the photo below is handy for turning the pipette while the Sylgard is applied.



Place the pipette into the pipette holder so that a good portion of the shank extends past the edge. Look through the microscope and bring the pipette into focus using the low power objective. Use a tool with a small wire tip (preferably stiff wire), take a small dab of silicone and begin to spread it on the pipette shank. As you do this, step on the foot pedal. Adjust the air regulator for the coating mode to full flow (turned all the way counter clockwise to start). Then turn up the current on the air jet filament (do not adjust this one all the way up) until the silicone cures quickly and to your satisfaction. It should be noted that maximum current combined with maximum air flow does not necessarily produce the most heat at the pipette. A more moderate air flow can allow much hotter air to reach the pipette. One should experiment with different current and air flows until the desired speed of curing is reached. No further adjustment should be necessary.

Use caution when working with the air jet since the heated air/gas can be as hot as 300°C and the glass casing also gets quite hot!

The air jet will take about 10 seconds to get extremely hot—it does not reach full heat the instant the pedal is depressed. After you find settings that are to your satisfaction, they should be used each time you coat. That is why a separate control is provided for air flow and current for coating and polishing.

As you apply the silicone, be careful not to cover the tip. It may be desirable to apply the silicone completely and then cure it rather than curing as you go. This

is merely a matter of comfort. After the silicone has dried, the pipette can be polished.

Polishing

For polishing the pipette you will need the following features: The high-power objective, the Polishing Filament, the Hot-Air Jet.

To polish the pipette, using the CPM-2 kit or the Pressure Polishing set-up: Bring the pipette tip over to the right side of the field as you look at it under high power. Carefully bring the filament into the field of view. When the filament becomes visible, check to see that it is in focus with the pipette tip. Focus on the pipette tip with the microscope focus control, and then bring the filament into focus using the tiny micropositioner on the filament holder bracket designed for this purpose. (It may be helpful to place marks on the micrometer slides of the filament holder bracket so that it is easy to re-orient the filament each time it is brought into use.)

When the pipette tip and the filament tip are in focus, set them apart on opposite sides of the viewing field. Turn down the power to the polishing filament (just during the initial set up) and step on the foot switch. Hold it down and begin to turn up the current to the polishing filament until it glows pink near the tip.

Next adjust the air flow for the polishing mode. The flow should be gentle and you will notice it cooling the filament. Adjust the current so that the filament glows slightly even when the air blows.

After all adjustments are made (these settings should work every time as long as the pipettes are the same), step on the foot switch and move the pipette tip over to the filament. As you bring it closer, watch for a change in color near the tip. This indicates that polishing has occurred; though you may not observe the actually change in shape of the tip.

A few things to note: When the filament heats up, it expands, so be careful not to let it hit the pipette. You may wish to melt a micro-glob of your favorite glass on the filament tip to insure that no platinum splatters on your pipette tip. This should be done any time you replace the filament and it is recommended. Just touch the broken pipette tip to the hot filament and the glass will melt on it. We recommend using a 4x to 10x objective for coating and a 20x to 40x long working distance objective for the polishing.

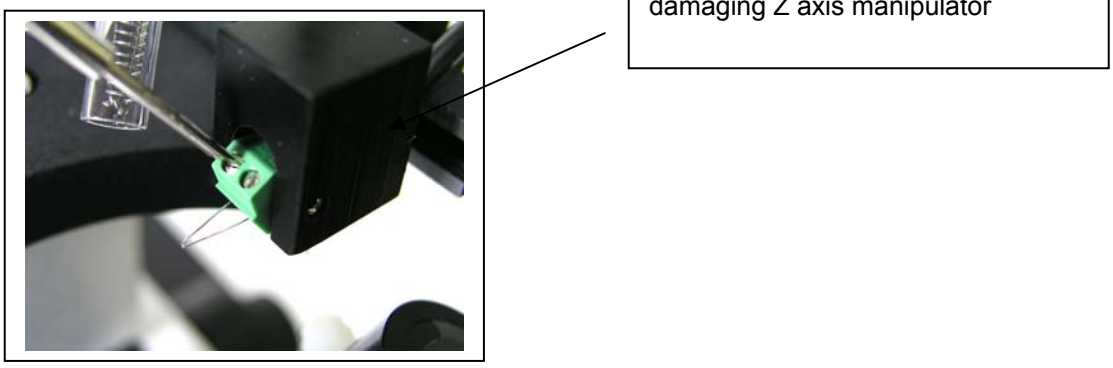
Maintenance and spare parts

The CPM requires very little maintenance. There are a few things to remember though.

The hotter and the longer the intervals the filaments are used the shorter their life-span. The Hot Air Jet is more likely to get broken then burn out—it should last many years. The polishing filament may burn out after a while. It is not hard to replace:

If your system has an X-Y stage mounted polishing filament with the filament held in a green screw terminal:

1. Loosen the two screws on the green screw terminal. (use caution and hold the black head piece securely so that a minimum of force is transmitted to the Z axis manipulator when loosening and tightening the screws.)
2. Remove the old filament
3. Insert new filament
4. Tighten screws



WARNING: MAXIMUM POWER IN THE POLISHING MODE WILL BLOW THE FILAMENT; adjust carefully!

Another area of concern is that only clean air or gas is used. Internally, a 5um filter protects the regulators etc. from the occasional particle that gets in. If house air is used an external filter should be employed. Remember that the purpose of the air flow is to keep the pipette tip free of dirt and dust; therefore clean air is essential.

The Needle valves that regulate air flow control 12 PSI of pressure. This pressure is set via an internal pressure regulator.

The coverglass on the low power objective should be replaced when it becomes hard to see. Simply replace it with No.1 or No.2 round 25mm coverglass.

If your objective cover is a small black ring, then 12mm coverslips must be used.

Maximum current possible to be generated is 2-3 Amps DC at 24V.

Specifications

power output	27VDC / 3.5A
controller dimensions	7.65x9.85x3.85 inches
weight	6lbs 0.2 oz (2.82kg)
polishing filament	Pt/Ir(90%/10%), 0.25mm diameter / 0.4Ω
input pressure (Min/Max)	12/30 psi (83/200kPa)
power input	110-120/220-240VAC - 0.5/0.25A, 60/50Hz
polishing manipulator travel	70mmx40mmx3mm
output pressure	(Min/Max) 0/5 psi (0/34.6kPa)
footswitch push	current on

Selected References

- Practical electrophysiological methods: a guide for invitro studies in vertebrate neurobiology. Editors: Kettenmann H. & Grantyn R. Wiley Liss, Inc. 1992 (Chapter 4.6 “Production and Calibration of Ion-Sensitive Microelectrodes”, Heinemann U, Arens J, 206-212

- Microelectrode Techniques: The Plymouth Workshop Handbook. Edited by Ogden, D, National Institute for Medical Research, Mill Hill, London, The Company of Biologists Limited 1987

- Signal-Channel Recording, Second Edition. Edited by Sakmann, B Neher, E, Plenum Press 1995 (Chapter 4 “Pipette Fabrication”, 17-20

Warranty

ALA Scientific Instruments, agrees to warranty this instrument for a period of one year from date of shipment. The warranty covers all parts and labor necessary to correct defects(s). ALA will at their option repair or replace nonworking components. This warranty does not apply to heating or polishing filaments. ALA is not responsible for damage resulting from the improper use of this product.

Installation of this system is a manner inconsistent with this manual will render this warranty null and void. No other warranties are expressed or implied. Your rights may vary from state to state.

CAUTION: This instrument is capable of generating high temperatures with regard to certain external components, car should be taken to avoid contact with these during operation. Parts to be avoided are: Hot Air Jet and Polishing Filament. Also, take care not to short the filament holder to other metal parts while in use.

NO USER SERVICEABLE PARTS ARE INSIDE THE CONTROLLER

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