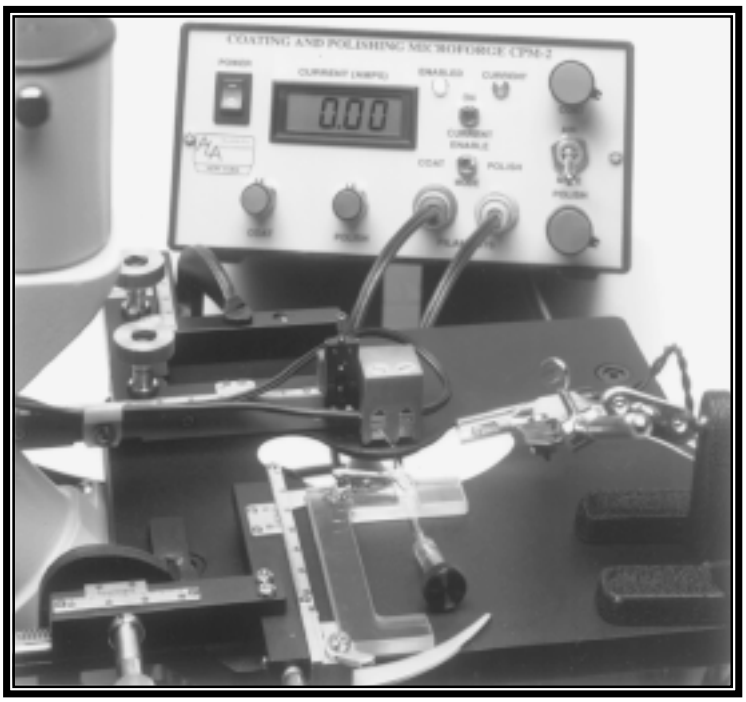
Model # CPM-2
INSTRUCTION MANUAL



ALA Scientific Instruments Inc.
1100 Shames Drive
Westbury, NY 11590-1746
Tel # 516-997-5780
FAX: # 516 997-0528
E-mail: support@alascience.com
www.alascience.com

Table of Contents: Page

Safety Information

CAUTION: This instrument is capable of generating high temperatures with regard tocertain 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 Filament (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 no shock hazard for humans. Filament power is 2-3 Amps at 24 Volts DC.

Never operate the Coating Filament (HOT AIR JET) without air flow. It will over heat and be damaged.

NO USER SERVICEABLE PARTS ARE INSIDE THE CONTROLLER

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 representative or the manufacturer.

Introduction

The **CPM-2**, Coating and Polishing Microforge is designed to turn virtually any inverted microscope into a coating and polishing microforge system. The CPM-2 consists of four components that must be mounted on the microscope, and the controller with its foot switch.

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 and is based on standard inverted microscopes. 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. 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. The pipette is then removed and a new one inserted.

Requirements

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 10 x 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.

Setting Up

1. The four components to be placed on the microscope are the Low Power Objective Cover (LPOC), the Polishing Filament Holder with Manipulator (PFHM), the Hot Air Jet (HAJ) and the pipette holder.

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.

3. The pipette holder is placed in the slide holder of the microscope's X-Y stage manipulator. 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.
4. In either case, the PFHM is mounted off to the left just slightly left of center, at 10-150, This makes it easier to turn the micrometer spindle to move it in and out of position.

5. The filament should have a triangular shape and be bent slightly downward at about 150. When it is brought over to the pipette area, it must be in the viewing field under high power magnification.

6. 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.

7. 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.

8. To help protect the high power lens, a #1 or #2 coverglass can be attached to the bottom of the filament holder with FUN-TAK® (DAP) blue adhesive gum. The edge of the glass should stick out beyond the filament so that the lens has clear focus on the filament and the lens is fully protected from heat and microparticles of glass and platinum.

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 bee's wax, Fun Tack adhesive or clamped with a single 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 toward the user at a 450 angle. (see diagram) 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 for 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 dust contamination.

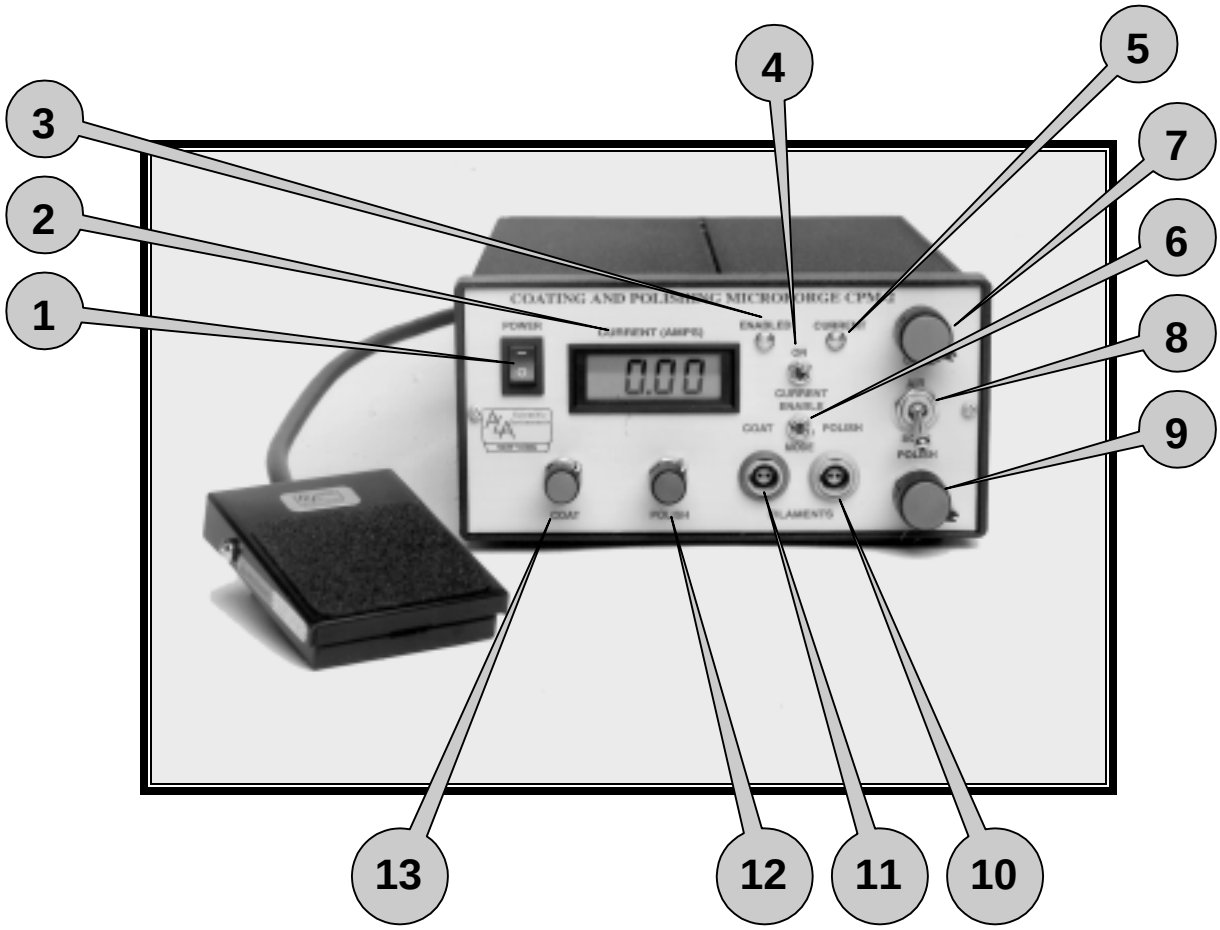
The Controller

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.

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.



CPM-1 Front Panel

The Controller (cont.)

1. Power Switch, turns on main power, top part of switch in = on.
2. Current meter; displays current amount in amps when unit is on and pedal is depressed. (Reading fades out slowly when unit is turned off.)
3. Enabled Light; signals that current is able to flow to the filaments.
4. Current Enable; when ON, as indicated by #3, current can flow to filaments.
5. Current light comes on when foot pedal is depressed, stays on until foot pedal is released.
6. Mode switch selects Coating or Polishing mode.
7. Coating air adjustment needle valve. Turn counterclockwise to increase flow.
8. Air mode selection switch, allows selection between two previously set air flows, one for coating and one for polishing.
9. Polishing air adjustment needle valve. Turn counterclockwise to increase flow.
10. Lemo connector socket for Polishing filament.
11. Lemo connector socket for Coating filament.
12. Potentiometer for Polishing Filament, turn clockwise to increase current.
13. Potentiometer for Coating Filament, turn clockwise to increase current.

CPM-1 Rear Panel

14. Input power selection switch, be sure this is set to the line voltage for your area, either 120V or 220V.
15. Mains power input and fuse holder; power cord is inserted. Use only qualified three conductor cable. FUSE REQUIREMENT is .500A @110V and .250A @ 220V.
16. Input air pressure connection. Luer lock connector need only be finger tight. Observe Min/Max pressures as indicated on the chassis.
17. Air pressure out. Luer lock fitting connects tubing which brings air flow to Hot Air Jet.
18. Foot switch plug in. Be sure plug is fully inserted. Unit does not operate without footswitch.

Set Up

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 illustrated. Other adaptations can also be used. Different microscopes may require different assemblies. 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) 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—nevertheless it is provided just in case!)

Once all the components are installed on the microscope, the various connections to the controller can be made. The air hose should be connected to the rear panel. Simply screw it 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 spots. Connect an air or inert gas source to the back of the unit, plug in the power and the system is ready. The controller is factory set for operation at 115 VAC. Please switch the power connector located on the rear panel if other line voltages are being used.

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 warranty.

Coating

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

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. Using 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 half a minute to get extremely hot—it does not 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. A small tool consisting of a knob and short length of tubing is supplied to facilitate holding and turning pipettes for coating.

Polishing

To polish the pipette, 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 this 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 actual 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. Just touch a broken pipette tip to the hot filament and the glass will melt on it. We recommend using a 4x to 6x objective for coating and a 20x to 40x long working distance obective 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 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:

1) Remove the filament holder from the microscope with its entire assembly. Loosen the two screws that hold the filament and the contact wires.

2) Push a needle into the space between the two brass plates that clamp the filament. Pull out the old filament and then replace it with the new one.

3) Remove the needles and then tighten the two screws. Be sure that the filament is centered.

4) Replace assembly on microscope.

5) To order new filaments specify part number CPM-PF.

WARNING: MAXIMUM POWER IN **THE POLISHING MODE** WILL **BLOW THE FILAMENT!**

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.

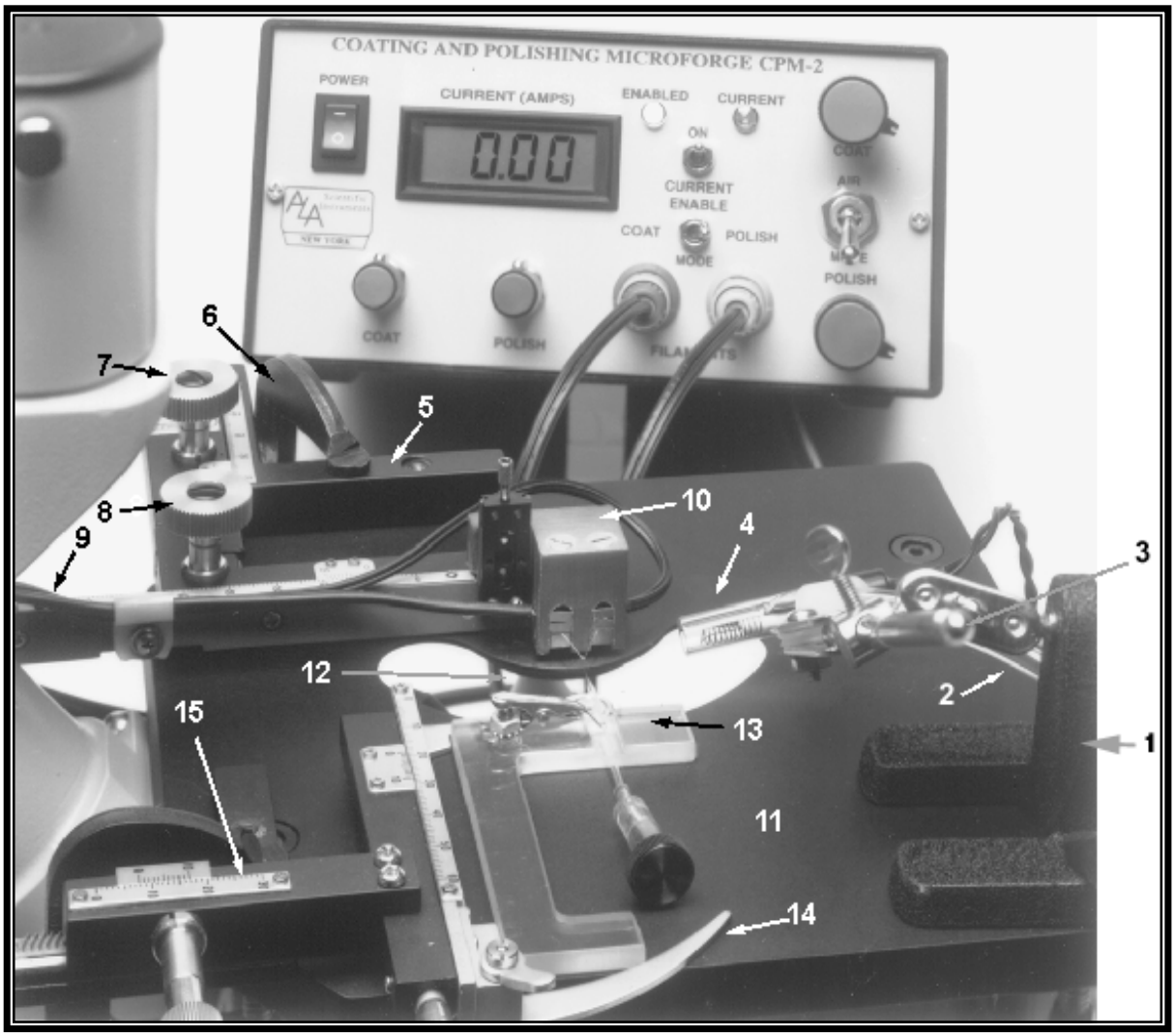
The needle valves that regulate air flow control 12 PSI of pressure. This pressure is set via an internal pressure regulator.

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 cover glass.

The coverglass for the high power should be replaced with a No. 2, 18mm square coverslip. It should be replaced when it appears to blur the image.

Maximum current possible to be generated is 2-3 Amps.

System Overview Diagram



1. Mounting assembly for Hot Air Jet, attached to stage with adhesives or clamps.
2. Air tube.
3. Armature for holding Hot Air Jet.
4. Hot Air Jet
5. Mounting bar for Polishing Filament Holder with Manipulator. (PFHM)
6. C-clamp holding PFHM to stage.
7. “Y” axis adjustment knob for positioning PFHM.
8. “X” axis adjustment knob for positioning PFHM.
9. Power wire for Polishing Filament.
10. Filament holder assembly with “Z” axis mini positioner.
11. Microscope stage.
12. Objective lens.
13. Pipette Holding clamp (whole assembly is clipped into slide holder).
14. Standard microscope slide holder.
15. X-Y stage manipulator for standard microscope slides.

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 defect(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 in 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, care 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.

NO USER SERVICEABLE PARTS ARE INSIDE THE CONTROLLER

Sylgard® is a registered trade mark of Dupont Corp.
Fun-Tak® is a registered trade mark of DAP Corp., Dayton Ohio.