

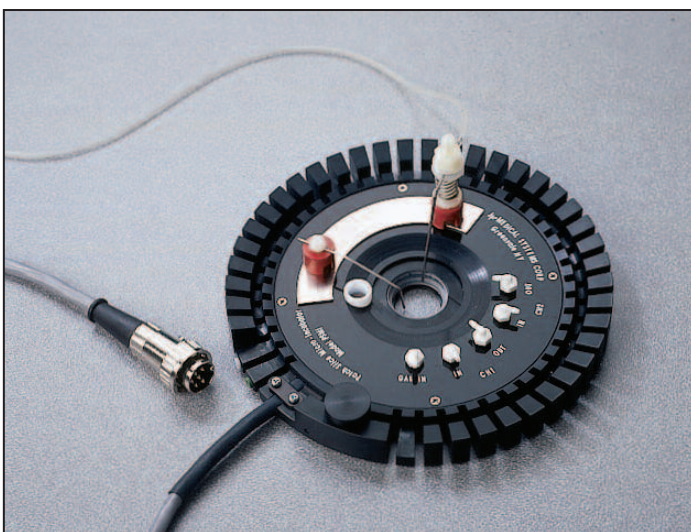
PSMI Patch Slice

Micro-Incubator

User's Manual

PSMI Patch Slice Micro-Incubator

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HARVARD

A P P A R A T U S

Publication 5403-009-REV-A

WEEE/RoHS Compliance Statement

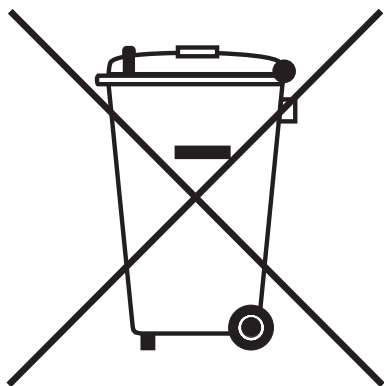
EU Directives WEEE and RoHS

To Our Valued Customers:

We are committed to being a good corporate citizen. As part of that commitment, we strive to maintain an environmentally conscious manufacturing operation. The European Union (EU) has enacted two Directives, the first on product recycling (Waste Electrical and Electronic Equipment, WEEE) and the second limiting the use of certain substances (Restriction on the use of Hazardous Substances, RoHS). Over time, these Directives will be implemented in the national laws of each EU Member State.

Once the final national regulations have been put into place, recycling will be offered for our products which are within the scope of the WEEE Directive. Products falling under the scope of the WEEE Directive available for sale after August 13, 2005 will be identified with a "wheelie bin" symbol.

Two Categories of products covered by the WEEE Directive are currently exempt from the RoHS Directive - Category 8, medical devices (with the exception of implanted or infected products) and Category 9, monitoring and control instruments. Most of our products fall into either Category 8 or 9 and are currently exempt from the RoHS Directive. We will continue to monitor the application of the RoHS Directive to its products and will comply with any changes as they apply.



- **Do Not Dispose Product with Municipal Waste**
 - **Special Collection/Disposal Required**

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General Information

Serial Numbers

All inquiries concerning our product should refer to the serial number of the unit. Serial numbers are located on the bottom plate.

Calibrations

All electrical apparatus is calibrated at rated voltage and frequency. While the flow will stay calibrated, the peak will vary.

Warranty

Harvard Apparatus warrants this instrument for a period of one year from date of purchase. At its option, Harvard Apparatus will repair or replace the unit if it is found to be defective as to workmanship or material.

This warranty does not extend to damage resulting from misuse, neglect or abuse, normal wear and tear, or accident.

This warranty extends only to the original customer purchaser.

IN NO EVENT SHALL HARVARD APPARATUS BE LIABLE FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow exclusion or limitation of incidental or consequential damages so the above limitation or exclusion may not apply to you. **THERE ARE NO IMPLIED WARRANTIES OF MERCHANTABILITY, OR FITNESS FOR A PARTICULAR USE, OR OF ANY OTHER NATURE.** Some states do not allow this limitation on an implied warranty, so the above limitation may not apply to you.

If a defect arises within the one-year warranty period, promptly contact Harvard Apparatus, Inc. 84 October Hill Road, Building 7, Holliston, Massachusetts 01746-1388 using our toll free number 1-800-272-2775. Goods will not be accepted for return unless an RMA (returned materials authorization) number has been issued by our customer service department. The customer is responsible for shipping charges. Please allow a reasonable period of time for completion of repairs, replacement and return. If the unit is replaced, the replacement unit is covered only for the remainder of the original warranty period dating from the purchase of the original device.

This warranty gives you specific rights, and you may also have other rights which vary from state to state.

Repair Facilities and Parts

Harvard Apparatus stocks replacement and repair parts. When ordering, please describe parts as completely as possible, preferably using our part numbers. If practical, enclose a sample or drawing. We offer a complete reconditioning service.

CAUTION

This unit is not registered with the FDA and is not for clinical use on human patients.

CAUTION
NOT FOR CLINICAL USE
ON HUMAN PATIENTS

Introduction

The PSMI Patch Slice Micro-Incubator is a unique environmental control unit that with its matching temperature controller (TC-202A) enables patch clamp recordings to be made from a brain slice using an upright microscope with water immersion objective.

Tissue slices (principally from the brain) are increasingly being used for electrophysiological and optical studies. Although this preparation offers an environment in which the majority of synaptic connections are maintained, less consideration has been given to the microscope's environment where experimental examination takes place. One reason for this is the design problem associated with providing environmental conditioning, without limiting optical or electrode access or reducing the quality of any electrical recordings. This problem is acute with "patch slice recording" in which a water immersion microscope objective is used. The recording and cleaning pipettes need access at very shallow angles with respect to the horizontal axis, and continuous perfusion is required to maintain oxygenation within the slice.

The PSMI permits control of temperature of both a perfused liquid and overlying gaseous medium for the submerged tissue slice. Application of drugs or a change in the ionic composition is achieved without disturbing the set temperature or any electrodes. The tissue chamber for the PSMI is the PS-CSD with two replaceable 22 mm diameter cover slips, one for the floor of the slice chamber and one below it to prevent condensation that would otherwise block/distort condenser illumination from below.

The PSMI uses Peltier devices to drive the heat exchange. APPENDIX 3 describes their operation and identifies their advantages in this application. The design of the PSMI avoids the normal need for Peltier cooling water except at the lowest control temperatures.

Design Considerations:

1. Mechanical access for water immersion objective for its superior optics.
2. Mechanical access for patch pipette & cleaning pipette with access angles as small as 15-20° with respect to the horizontal.
3. Allows temperature regulation both above and below room temperature with perfusant media supplied at room temperature.
4. Maintenance of constant (low) fluid level during flow.
5. Small chamber (22 mm x 5.5 mm) and upstream volume allow rapid media change.
6. Electrical isolation of heat exchange plates from chamber electrical ground. This allows reduction of electrical noise by using a single external connection between signal ground and power ground (see Figure 2, #9 on page 5).

Description

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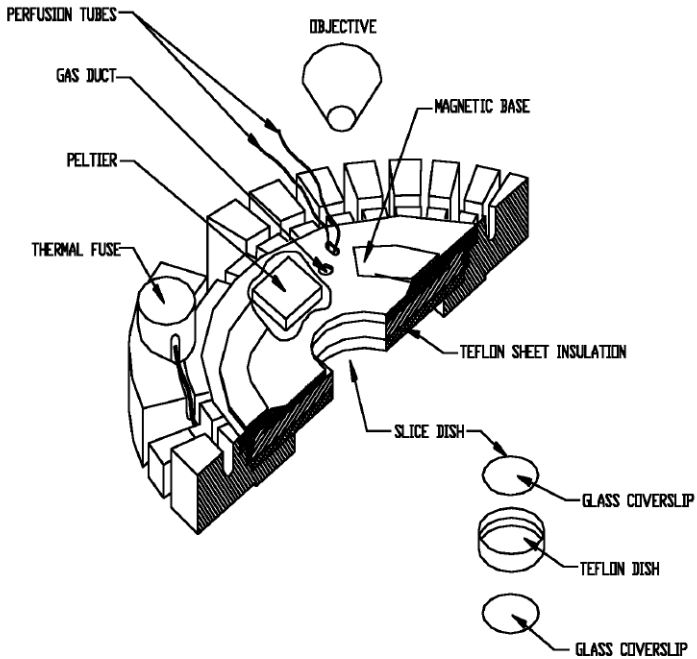


FIGURE 1: PSMI ASSEMBLY

The Open Perfusion Micro-Incubator (PSMI) is an annular shaped assembly surrounding the central chamber (the Patch Slice Cover Slip Dish - PS-CSD).

The PSMI contains two metal annular plate assemblies to effect the heat transfer to the chosen chamber:

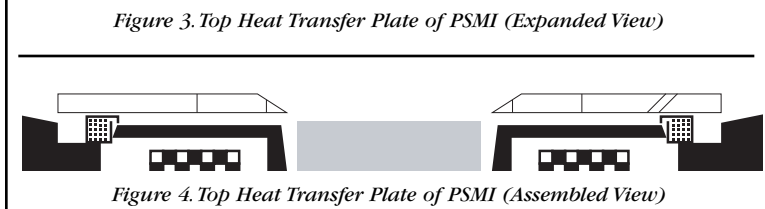
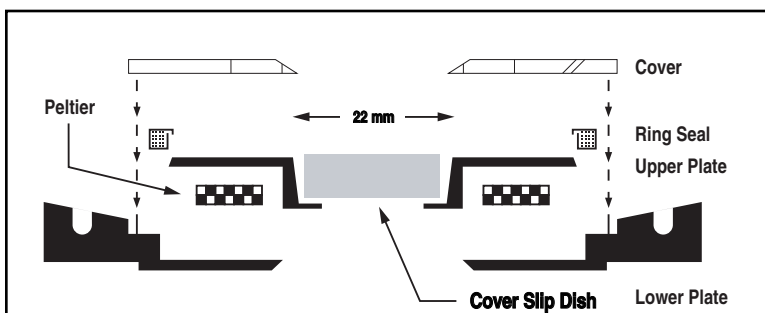
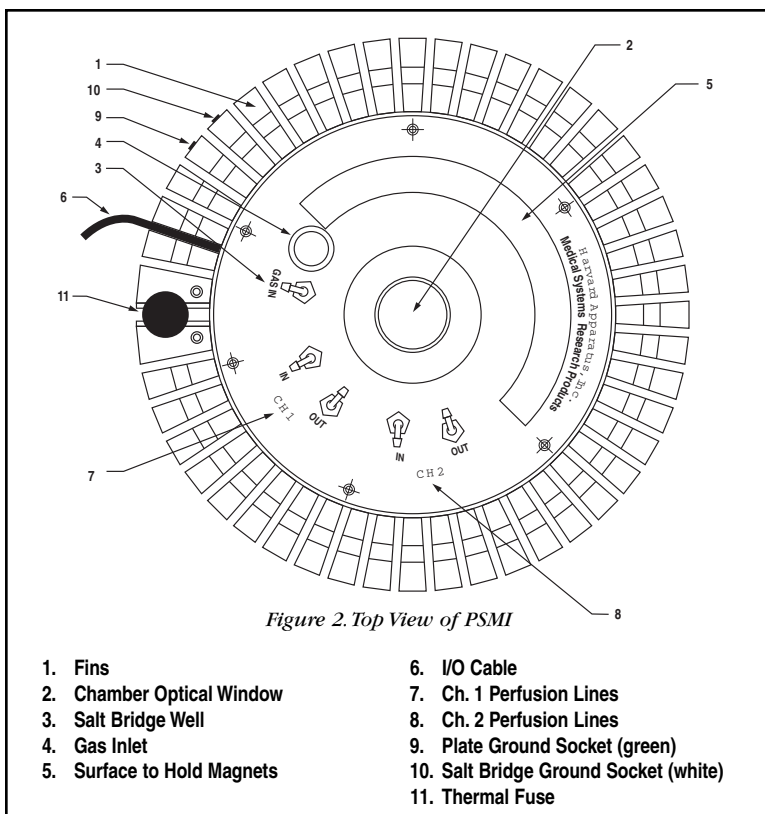
The lower plate assembly - black anodized aluminum plate - has a flat bottom surface to which the appropriate securing ring is attached to form a mating surface with the microscope. This lower assembly serves also as a radiator of peltier waste heat via radial cooling fins (see Figure 1 above) on its outside diameter.

The top plate (see Figure 1 above) is also made from black anodized aluminum. It forms one of the heat exchanges and also serves to support the inserted chamber.

Heat transfer to the chosen chamber is driven by two Peltier thermoelectric devices, which when powered by a suitable feedback controller, can regulate the chamber

Description

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Description

temperature to as close as $\pm 0.2^{\circ}\text{C}$. These Peltier devices are sandwiched between the annular assemblies, through which a central hole (see Figure 3 on page 5) has been milled to provide support for that chamber and an optical window for the microscope. This configuration maintains a controlled temperature at the level of the chamber, by reducing heat loss through the base and maximizing heat transfer to the chamber.

The primary heat transfer to the controlled medium during perfusion occurs through the plastic tubes coiled inside a circling slot (groove) machined in the top heat exchange plate. The extracellular medium first passes through these tubes and is warmed (or cooled) to the set temperature before reaching the chamber. There are also two secondary heat transfers. The first of these is by direct conduction from the support surface and collar to the chamber, while the other is through convection from air or gas flowing over the same controlled plate below a covering annular (black) acrylic cover.

Several different modes of operation are permissible:

1. Continuous perfusion at a constant set temperature.
2. Switching perfusion of extracellular medium at a set temperature.
3. Rapid temperature changes (with perfusion).

Why perfuse the recording chamber?

The ability to change the extracellular environment by perfusing the experimental chamber is important for two reasons: first, excised tissue both requires continued oxygenation and the washing away of substances produced by the excision; second, perfusion is essential for quantitative ionic and pharmacological studies, or for studies of distributed synaptic inputs or network activity, in which drugs need to be applied to a large area. Perfusion is especially useful for the application and subsequent washout of an antagonist at a precise concentration.

Controlling Micro-Incubator

The PSMI has self-contained Peltier heating / cooling elements. Current passed in one direction will heat the enclosed dish, reversed current will cool it. **WARNING:** The maximum sustained current is about 6 amperes. This corresponds to a maximum voltage applied across the Peltier leads of about 4 volts.

Two methods of control are possible, manual or feedback. A manual system uses a DC power supply: the direction and magnitude of the current through the micro-incubator is adjusted by hand. For better performance and more stable temperature control, the feedback method is the best choice. In this method, the current supplied is automatically adjusted in magnitude and - with the TC-202A - direction depending on the difference between the actual and the desired temperature. The advantage of feedback is that the calibration curve (chamber temperature versus controller setting) is unaffected by ambient temperature. In the cooling direction, however, the lowest temperature reachable will still depend on the degree of contact with the microscope stage and the ambient temperature.

MANUAL CONTROL:

The power supply should deliver constant current (rather than voltage) and be adjustable up to the maximum needed for the desired temperature range (in any case < 6 amperes). At higher perfusion rates more current will be needed for a given temperature, for static solutions, less current. This data will also be affected by the ambient, the depth of media in the chamber, and the degree of contact of the PSMI with a large metal surface such as a microscope stage. For accurate calibration, produce your own graph of plate temperature (see APPENDIX 5 for converting measured resistance to temperature) versus current (measured either with the current meter of the power supply or with a separate current meter connected in series with the PSMI and the scope. The plate's thermistor will be 1-2° C further from ambient than the chamber's temperature. Due to the low electrical resistance of the PSMI (0.7 ohm), it may be useful to put a power resistor in series with it to spread the operating range out over the control dial of the power supply. (Power supplies with sufficient current capacity tend to have voltages larger than the maximum of the PSMI). (See **Setup and Operation**, pages 9-10, for identification of leads in the control cable of the PSMI).

Controlling Micro-Incubator

FEEDBACK CONTROL:

The control for temperature can be from the thermistor mounted on the top heat exchange plate (built-in thermistor) compatible with the TC-202A temperature controller. (The TC-202A also has a so-called “bath thermistor”, optionally used with the “Open Perfusion Micro-Incubator” for measuring or controlling directly in the chamber. For most patch slice perfusion geometries, it is too large). Due to the fast perfusion rate relative to volume in the chamber, the temperature of the slice relative to that of the plate thermistor will be only 1-2° C closer to ambient. To achieve the feedback control and obtain maximum benefits from your PSMI Micro-Incubator, we strongly recommend that you use the TC-202A matching temperature controller which in summary will:

1. Allow the PSMI to either cool or heat the bath preparation as well as control temperatures near ambient equally well.
2. Automatically switch the current direction when the sensed temperature is higher or lower than the set point temperature.
3. Allow fast changes in temperature.
4. Allow $\pm 0.2^{\circ}$ C regulation.
5. Allow low noise electrical recording.
6. Automatically power shut off when excessive temperatures are reached so that possible system damage during “feed forward failure” is avoided. (***See Troubleshooting on page 12***)

Setup & Operation

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Orientation - Any references to right and left assume the unit is viewed from above with the electrical cable to the left.

CAUTION:

THIS UNIT MUST ONLY BE OPERATED WHILE IN GOOD CONTACT WITH A LARGE THERMAL MASS, such as the metal stage of a microscope or by perfusion of water additionally through the lower plate. For temperatures much more than 5° C below ambient, the stage of a microscope may be insufficient by itself without such additional water perfusion. (See APPENDIX 2).

1. Mounting on the Microscope

The PSMI bottom plate has a flat surface for unrestrained mounting on various inverted microscope stages. Accessories are available to lock the PSMI to the stages of the microscopes from several manufacturers.

The locking devices come in two forms:

- As alignment rings that assemble to the bottom of the PSMI and, in turn, fit on opening in the microscope stage.
- As fixing platforms that fit a locking mechanism of Zeiss or Leica attachable mechanical stages.

Using a stage attachment accessory has the advantage of improved mechanical stability and gives the user the ability to use the microscope stage built in X-Y manipulators to position the chamber in the field of view.

The following microscope stage locking accessories are available:

- PDMI-ARN: Alignment Ring for Nikon Diaphot (old or 300/200) TMD stage
107.75 mm Ø x 1.78 mm H (4.242" Ø x 0.071" H)
- PDMI-ARZ: Alignment Ring for Zeiss Axiovert gliding or rotary stages
102.75 mm Ø x 1.78 mm H (4.046" Ø x 0.071" H)
- PDMI-ARO: Alignment Ring for Olympus IX50/70 or IMT-2 fixed stage
109.73 mm Ø x 1.78 mm H (4.320" Ø x 0.071" H)
- PDMI-FPZ: Fixing Platform for Zeiss Axiovert with attachable mechanical stage.
- PDMI-FPL: Fixing Platform for Leica DAS Microscope DMIL and DMIRB/E with attachable mechanical stage.
- PDMI-ARL: Alignment Ring for Leica Microscope
88 mm Ø x 1.8 mm H (3.52" Ø x 0.072" H)

These rings or platforms are easily attached to the bottom plate with 3 small screws supplied with each ring or platform.

2. Electrical Connections

The PSMI main cable has a multi pin connector at its end. This connector matches the TC-202A front panel I/O jack. This cable provides the electrical connections for:

- Power to the PSMI Peltier heat pumps.
- The PSMI built-in temperature feedback thermistor. A table of its electrical resistance versus temperature is shown in Appendix 5, page 17. Note that this temperature is not exactly the same as that in the media in the dish.

Setup & Operation

- c. **System ground.** The micro-incubator's ground scheme is designed to provide the best possible noise shielding for demanding electrical recordings. The PSMI heat exchange plates are anodized and thus not electrically in contact with the microscope stage. These plates are also electrically separated from the Peltier power leads. One of those Peltier power wires is grounded within the TC-202A. The micro-incubator body (heat exchange plates) is grounded to the temperature controller chassis by the metal shell connector on the PSMI cable.

Two independent (separate from the main cable) ground pathways are also available when single channel or similar demanding electrical recordings are to be performed:

- a. **Salt Bridge Ground:** The Ag/AgCl disc in a salt bridge well (see #3, Figure 2, page 5) is connected to a small 1 mm diameter white color socket (see #10, Figure 2, page 5). This allows grounding of the recording chamber via an integral agar/salt bridge.
- b. **Shield Ground:** A small 1 mm diameter green color socket (see #9, Figure 2, page 5) connected to the cable shield and aluminum components of the PSMI. Connecting this to a local ground can sometimes reduce 50/60 Hz

3. Chambers:

The Patch Slice Cover Slip Dish must be assembled before placing it in the central opening. (See Figure 1 on page 4).

NOTE 1: 22 mm glass cover slips are used for the bottom of the slice compartment of the Cover Slip Dish. To prevent leakage of media, yet allow easy replacement of the top one, use stopcock or vacuum grease (sample supplied - both are composed of non-toxic silicone oil/wax). Apply the grease to the inner ledge of the dish on the side where this ledge is closer to the external rim. A glass cover slip is also needed for the other side (see Figure 1 on page 4) to prevent condensation that would impair illumination from the condenser. This one can be attached more permanently with a glue. (Sylgard, used to coat patch pipettes, would be a conveniently available choice).

NOTE 2: The temperature difference between the plate thermistor and the chamber as well as across the chamber itself can be reduced by coating the inside wall on the top surface of the chamber support ring of the PSMI with a good heat conductor - heat compound (messy), silicone oil, stopcock grease. If using perfusion make sure the plastic tubes are inside the dish.

4. Suggestions:

A superfusion micro-incubator system consisting of a PSMI, a matching controller, chamber and fluid control system has three modes of operation (see **Description** section, pages 4-6), each with its own routine procedures and specialized "tricks of the trade". This manual supplies a starting point for your specialized application: allow time to perfect your own procedures. See **Perfusion** section, pages 11-12 for perfusion suggestions. The articles published by the developer 1,2,7 and others 3,4,5,6 for the closely related Open Perfusion Micro-Incubator may also be useful. (Foot notes on page 15)

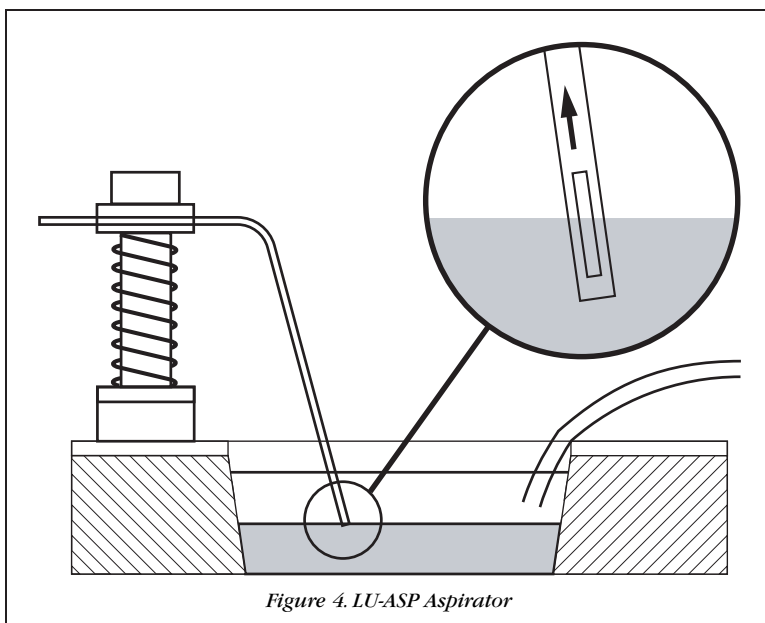


Figure 4. LU-ASP Aspirator

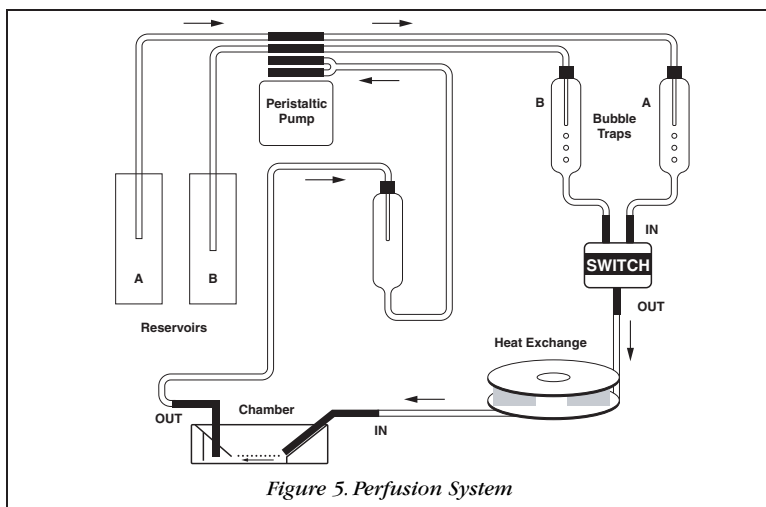
Outlet from the chamber is by direct suction. The included aspirator (LU-ASP) was originally developed by Dr. Can Ince, University of Leiden, for patch clamp applications. The design of this aspirator cleverly avoids fluid level variations (a source of electrical noise) in electrical recording experiments. The aspirator has a magnetic base to grip a matching magnetic surface (see #5, Figure 2, page 5) on the top of the top plastic plate of the PSMI. Connect up a source of suction with a liquid trap to this aspirator. The level of fluid in the chamber is determined by the height of this aspirator and can be changed with its thumbscrew. The oscillations inherent in any peristaltic perfusion are easily damped with bubble-traps. Such traps also allow the independent chamber grounding required for low noise electrical recording.

Fluid Perfusion

For reliability at low flow rates a peristaltic pump is preferable to gravity feed. This also makes it easier to maintain fluid level on changing solutions. A four channel pump is ideal since this allows for perfusion of several different solutions. Two small 90° elbow (white) inputs (CH1 “IN” or CH2 “IN”) are provided in the micro-incubator. In addition, two small 90° elbow (white) outputs (CH1 “OUT” or CH2 “OUT”) are provided for tubes that can be user directed to the desired location in the chamber. Changing the perfusate can be achieved by switching between the two corresponding pump channels attached via a valve to one of the PSMI’s perfusion inlets.

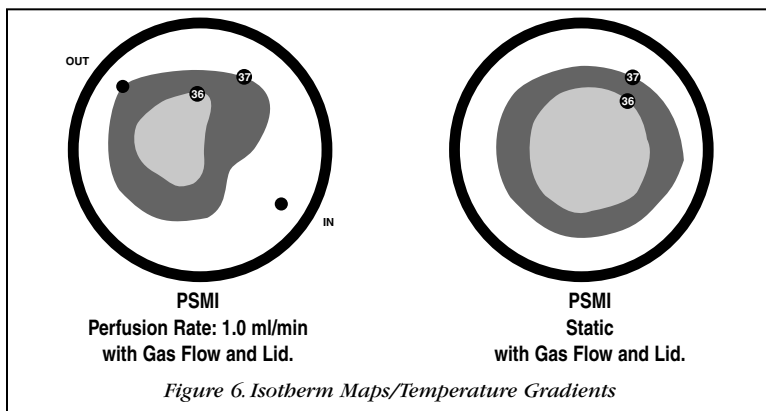
Complete Layout of Liquid Perfusion

For one possible complete layout of liquid perfusion, see Figure 5 on next page.



Temperature Gradients

The temperature at any particular point in the recording chamber is dependent on the mode of operation and the rate of perfusion. The horizontal temperature gradient depends on media perfusion rate, gas flow rate, depth of media and the presence or absence of a cover lid. For absolute determination during a particular experiment it is essential to measure the temperature directly.



Gas Perfusion

This is intended primarily to reduce the heat loss to (or gain from) the local atmosphere, thereby reducing temperature gradients across the dish. It is possible to control the gas mixture above the perfusion chamber. The gas passes over the same heat exchange as the fluids. Any desirable gas mixture may be introduced into the microincubator (e.g., 5% CO₂-air mixture can be used with a bicarbonate buffer medium). An inlet for CO₂ has been incorporated into the PSMI's.

The PSMI requires only a minimum of maintenance. Periodically replace the tubing to avoid clogging by dust particles or growth of micro-organisms - with daily use, every two months. The period between changes can be increased by flushing with distilled water after using followed perhaps by modest heating to dry out the tubing. An occasional perfusion of 70% alcohol helps.

Disassembly for replacement of tubing:

Remove the screws (outside rim) of the plastic lid. Lift off lid, observing carefully how the perfusion tubes are fed through and their entry and exit points from the outer circular “bobbin” of the exposed heat exchange plate.

Remove the tubing. If better heat transfer is needed, use heat sink compound (white paste - zinc oxide - contact an electronics repair shop to obtain some) or silicone oil in the bobbin’s groove. Wind new tubing (APPENDIX 4) through the same slots from the bobbin itself. Cut the tubes to the desired lengths and thread them through those slots. A dab of silicone cement (bathroom caulk - clear- smells like vinegar) to hold the tubing as it goes into the exit slots may be useful. Reseat the plastic ring that circles the outside of the exposed heat exchange plate, (first rubbing a little silicone oil on its top and bottom surfaces) and position the plastic plate.

Reattach the plastic lid with the screws. Be careful in tightening the screws to avoid stripping.

OPTIONAL REASSEMBLY:

For faster perfusion rates, lengthen the tubing on the bobbin by selecting another exit point or else reduce its wall thickness (see APPENDIX 4). Either allows the faster moving fluid to still get enough heat transfer before reaching the chamber. SUGGESTION: Use only one tube and wind it one extra turn (one and three quarters total) for the same exit point. This should allow perfusions of as much as 6 ml./min. or connect ch. 1 “out” to ch. 2 “in”.

WARNING: DO NOT FURTHER DISASSEMBLE THE PSMI. ONCE THE TOP HEAT PLATE IS REMOVED, REASSEMBLY FOR PERFORMANCE THAT MEETS SPECIFICATIONS IS DIFFICULT, AND IT MAY NEED TO BE SENT TO OUR PREMISES FOR ADJUSTMENTS.

1. Slow perfusion rate or excessive fluid build up in the bubble trap.
 - a. Due to excessive perfusion rate.
 - b. Blocked heat exchange tubing. First, flush tubing with 70% alcohol and then perfuse distilled water for one hour. If it is still a problem, then change plastic tubing.

2. Very hot fins/plate - too hot to touch - “feed forward failure”.

TURN OFF THE POWER IMMEDIATELY.

In this condition, too much heat is generated at the Peltier junction nominally at ambient temperature. Attempts to change the control temperature by changing the current have no effect. Resume operation after the device has cooled off preferably after taking actions suggested under one of the following.

- a. You have not placed the device in contact with an adequate metallic thermal mass or are trying to cool to too low a temperature. Check that microscope stage is thermally conducting. Solutions: Apply heat sink compound or silicone oil to the supporting metallic surface of the microscope and/or install water perfusion (APPENDIX 2).
- b. Attempted operation outside the recommended range (10- 50° C) without water perfusion. (SEE APPENDIX 2).
3. Controller is not supplying current. See the manual for the controller in general. Problems specific to the PSMI are listed below:

If “feed forward failure” (see 2) occurs with the TC-202A, it automatically stops delivering current until the Peltier plate temperature cools off. Use one of the solutions given in 2 to avoid repeated lapses in current.

4. Excessive 60 Hz. pickup, or baseline drift if electrically recording.
 - a. Check for ground path between the chamber ground connection and the metal plates. Keep the top (plastic) surface of the PSMI clean, especially of dried saline (salt) solution). Silicone oil may be helpful.
 - b. Drift in baseline when electrically recording is most likely due to one of the Ag/AgCl junctions in the user’s complete recording circuit.
 - c. Ground loops (causing noise) are avoided by using only one grounding point preferably near the chamber. Connect the patch clamp ground, the bath ground and (most likely) the heat exchange plate ground (green pin) here. The source of ground loops can be subtle. If problems remain see Appendix 4.
5. Static discharge during perfusion.
 - a. Check earthing system and fluid level in bubble traps. The simplest way to solve this problem is to use stainless steel needles in the bubble traps and earth them.

1. Forsythe, I.D. et al "A chamber for electrophysiological recording from cultured neurons allowing perfusion and temperature control" J. Neurosci. Meth. 25, 19-27 (1988). This article describes a prototype of the Open Perfusion Micro-Incubator (PDMI-2) without provision for gas flow.
2. Forsythe, I.D., et al "An open perfusion micro-incubator for electrophysiological recording in vitro" J. Physiol. (Lond.) 410, 5P (1989). This article describes the addition of gas flow to the PDMI-2 and the elimination of the need for water cooling.
3. Ince, C. et al "A teflon culture dish for high magnification microscopy and measurements in single cells" Pflugers Arch. 403, 240-244 (1985).
4. "Earthing and Interference" (pages 55-65) in Microelectrode Methods for Intracellular Recording and Iontophoresis. Academic Press(London), ed. R.D. Purves. (1981)
5. DeHaan, R.L. et al. J. Gen. Physiol. 65, 207 (1975).
6. Ince, C. et al "Micro- CO2 Incubator for use on a micro- scope". J. Immuno. Meth. 60, 269-275 (1983). This paper describes the use of gas flow to reduce vertical and horizontal temperature gradients and to control pH in an optically accessible chamber.
7. Forsythe, I, "An environmental chamber regulating temperature and superfusion of tissue cultured neurons during electrophysiological or optical studies", Electrophysiology and MicroInjection, 301-320, volume 4 of Methods in Neuro science, Academic Press, NY Ed.: Conn,P.M. (1991) This article provides a complete description of the final version of the PDMI-2 and its operation.

APPENDIX 1 - INCLUDED ACCESSORIES

1. Aspirator (PS-ASP): see Section 4 Perfusion (page 11, figure 4)
2. PS-CSD: Patch Slice Cover Slip Dish. Includes Teflon[®] frame, sample 22 mm diameter glass cover slips, and sample of (vacuum) grease to hold slice chamber cover slip.
3. Teflon[®] perfusant tubing (Teflon[®] may be more appropriate for use of some perfusants.)
4. Two 1 mm plugs (Leads) for plates ground connections.
5. Thermistor holder.
6. Nylon mesh (1pcs of 4in.x4in.)

APPENDIX 2 - USEFUL ACCESSORIES

1. A small, waterproof temperature probe to measure the actual temperature in various parts of the chamber. Only thermocouple type probes will be small enough.
2. Metal tube (loop) for water perfusion- necessary for very low temperature to prevent feed- forward condition-place in circular groove (.45 cm. wide) on top of air fins. (Available from Harvard Apparatus.)

APPENDIX 3 - PELTIER DEVICE OPERATION

A thermoelectric module is a solid state device consisting of a series of semiconductors. Most commercial devices are made from p and n doped bismuth or lead telluride.

The passage of electric current normally generates only heat. The contradictory concept of the Peltier effect is due to Jean Peltier who in the nineteenth century discovered that the passage of current through two dissimilar conductors results in the junction either heating up or cooling down. Each device consists of many couples mounted in series and connected by copper strips; sometimes overlying this is a thin piece of ceramic, an electrical insulator, but thermal conductor. In principle they work as heat pumps; that is when current is passed through the junction, the device pumps heat in one direction, reversing the current reverses the direction of the heat flow. Thus depending on the capacities of the heat source and sink, a temperature gradient is built up across the device. The thermoelectric device is thus sandwiched between the object of interest and a suitable thermal mass or ground.

Use of Peltier devices:

Peltier devices are essential for rapid temperature changes or when the desired temperature is close to ambient temperature (because they can actively correct the actual temperature whether too high or too low instead of relying on the slow passive return of a monopolar system). (These advantages are only available when a bipolar controller is used - see **Description** section, pages 4-6.) Peltier devices also offer the flexibility to cool as well as heat in the same chamber. Such a system further

allows examination of the temperature dependence (Q10) of biological properties by changing the command temperature rapidly during an experiment. Finally, lowering the temperature eases the study of ion channels with rapid kinetics.

APPENDIX 4 - SPECIFICATIONS

Electrical maximum:	6 amperes continuous (approximately 3 volts)
Temperature range:	From 10° C below ambient to 50° C, (on the conducting stage of a microscope but without supplementary water cooling of the heat fins.).
Perfusion rate:	Up to 3 ml. per minute. See OPTIONAL REASSEMBLY page 13 for higher rates.
Thermal Expansion:	Ranges between ± 4 mm between 15-30° C and ± 20 microns between 30-40° C.
Plastic Perfusant Tubes:	One pair of Silastic, one pair of Teflon tubing. (See Figure 1 on page 4)

APPENDIX 5 - THERMISTOR CALIBRATION TABLE

RESISTANCE DATA: PSMI INTERNAL THERMISTOR

Temperature	Resistance	Temperature	Resistance
4o C	271.6 Kilohms	25o C	100.0 Kilohms
5	258.3	26	95.3
6	245.7	27	91.3
7	233.8	28	87.4
8	222.5	29	83.6
9	211.9	30	80.0
10	201.7	31	76.6
11	192.2	32	73.3
12	183.1	33	70.2
13	174.5	34	67.3
14	166.3	35	64.4
15	158.6	36	61.75
16	151.3	37	59.2
17	144.3	38	56.75
18	137.7	39	54.5
19	131.4	40	52.2
20	125.5	41	50.1
21	119.8	42	48.0
22	114.5	43	46.1
23	109.4	44	44.3
24	104.5	45	42.5

From the first release to the market of the PSMI Micro-Incubators to the current design, there have been various design changes. A wiring color code is provided below to aid the wiring of the PSMI cable to the chosen temperature controller's power/control output. The PSMI is provided with a connector which matches the TC-202A output connector. For other options the table below may be used. In any case, we suggest that you contact Harvard Apparatus, Inc. for instructions or more details.

APPENDIX 6 - PSMI Wiring Codes

	<u>Wire Size</u>	<u>Final</u>
Peltier +	AWG 20	Red
Peltier --	AWG 20	Black
Signal Ground (salt bridge)	AWG 26	1 mm White (connector)
AC Ground (60 Hz noise)	AWG 26	1 mm Green (connector)
Thermistor	AWG 26	Green
Thermistor	AWG 26	White

