



Scientific Systems Design Inc

50 Unit #5 Steeles Ave East
Milton, Ontario, L9T4W9
CANADA

Phone: 1 905 608 9307
ssd@scisys.info
www.scisys.info

Innovative Engineering for Science

BRAIN SLICE CHAMBER SYSTEM

DUAL INTERFACE

BSC2-2

BRAIN SLICE CHAMBER

CAUTION !

YOUR BRAIN SLICE CHAMBER IS A PRECISION ENGINEERED TOOL FOR SCIENTIFIC RESEARCH. PLEASE TAKE A FEW MINUTES TO FAMILIARISE YOURSELF WITH THE CHAMBER AND READ THROUGH THIS SHORT MANUAL BEFORE ATTEMPTING TO USE THE SYSTEM.

DO NOT UNDER ANY CIRCUMSTANCES OPERATE THE PTC03 TEMPERATURE CONTROLLER AND BRAIN SLICE CHAMBER **WITHOUT ADEQUATE WATER IN THE LOWER CHAMBER OR WITH THE SENSOR PROBE REMOVED FROM THE CHAMBER END**. THIS CAN CAUSE OVER-HEATING OF THE HEATER ELEMENT. A THERMAL FUSE IS LOCATED IN THE SLICE CHAMBER TO PREVENT WATER TEMPERATURE RISING ABOVE 70°C. DO NOT LEAVE THE CHAMBER RUNNING UNATTENDED FOR EXTENDED PERIODS OF TIME - PLEASE CONTACT US FIRST FOR DETAILS IF YOUR EXPERIMENTS NEED TO RUN OVERNIGHT AND UNATTENDED.

DO NOT USE ALCOHOL OR SIMILAR SOLVENTS IN ANY CONCENTRATION ON ANY PART OF THE CHAMBER SINCE AS WITH MOST ACRYLICS,™ PERSPEX MAY FRAGMENT OR DEVELOP HAIR-LINE CRACKS.

<u>CONTENTS</u>	<u>PAGE</u>
1) CHAMBER DESCRIPTION	3
2) CONSTRUCTION	3
3) INSTALLATION AND OPERATION	3
SCHEMATIC DIAGRAM	4
LOCATION	4
FILLING LOWER CHAMBER	4
CONNECTION TO GAS MIXTURE SOURCE	5
TEMPERATURE CONTROL	5
Proportional Temperature Controller PTC03	5
Specifications of PTC03	5
CONNECTION TO TEMPERATURE CONTROLLER	6
CONNECTION OF THE PERFUSION FLUID SOURCE	7
Perfusion solution reservoir	7
Gravity or pump?	7
CONNECTION OF EXIT WELL	7
REFERENCE ELECTRODE CONNECTIONS	8
4) SINGLE CHANNEL OPERATION WITHOUT TEMPLATES	9
5) SINGLE CHANNEL OPERATION WITH TEMPLATES	10
SCHEMATIC DIAGRAM	10
6) MAINTENANCE	11

CHAMBER DESCRIPTION

The BSC2-2 Brain Slice Chamber is designed to maintain isolated, living tissues *in vitro* and allow stable electrophysiological recordings to be made from the preparation. This chamber is designed for 'interface' method of maintaining slices. Two completely independent wells with their own feed and exit ports allow two sets of preparations to be used at the same temperature, humidity and oxygen atmosphere. Temperature is maintained by a proportional control heating system.

CONSTRUCTION

The chamber is constructed from two cylindrical blocks of acrylic, and mounted on a base-plate of the same material for securing on to a table. The diameter is 130mm, height is 70mm, base plate diameter is 130mm. The lower section maintains the desired temperature and provides a moistened 95% oxygen, 5% carbon dioxide gas mixture to the upper section. The gas mixture is deflected across the surface of the slices located centrally by an acrylic lid. The slices rest on a sheet of lens tissue in the flat area in the centre of the chamber. In addition it is possible to use acrylic templates provided with the chamber with one channel. The template helps to decrease the dead-space volume of solution and therefore allow more rapid changes of test solutions.

Pre-oxygenated perfusion fluid enters the main body through a fine bore PTFE tube which spirals in the heated distilled water contained in the lower chamber and enters the upper part at the base of the flat slice area. The solution then exits by means of capillary action with lens tissue which is made to wick the perfusion solution into an exit well. A suction line or the return line from a pump is attached to the exit well to remove or re-circulate the perfusion solution or alternatively the solution can drip into a collecting vessel and pumped out to waste or re-circulation.

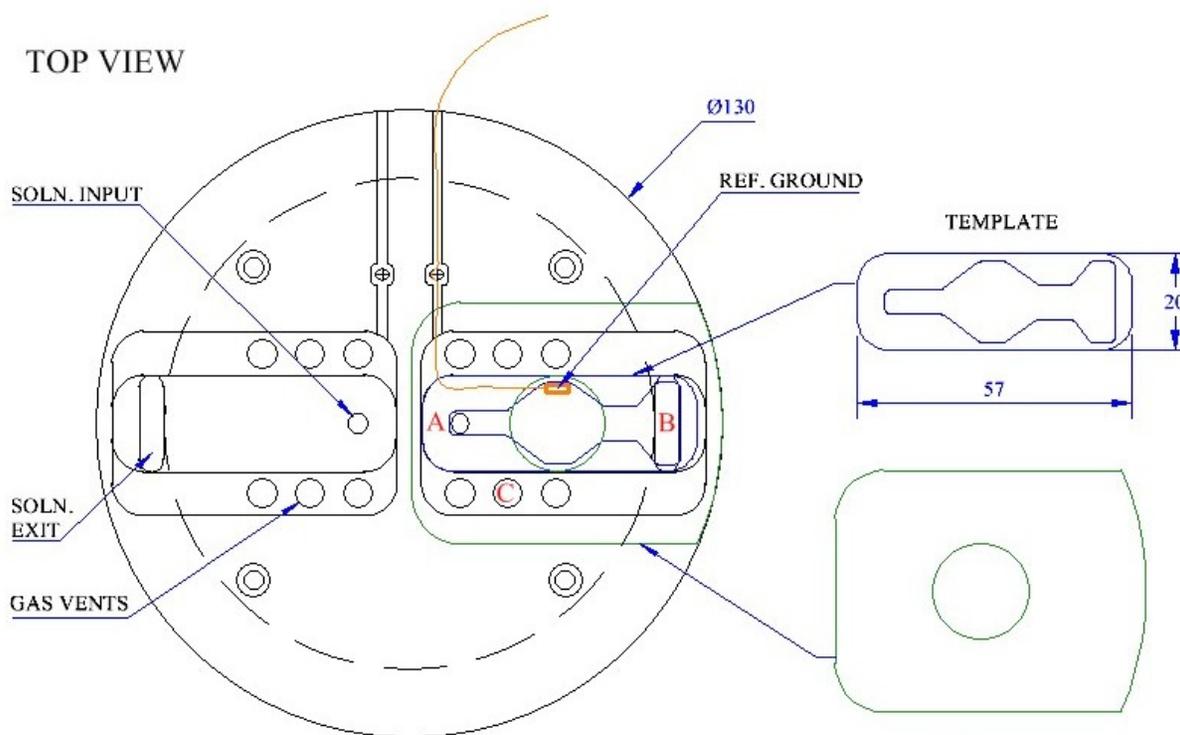
INSTALLATION AND OPERATION

Once all packing material has been removed, please take some time to examine the construction of the chamber. Remove and identify : Two single channel inserts and two lids.

DO NOT CONNECT THE TEMPERATURE CONTROLLER TO THE CHAMBER UNTIL IT IS FILLED WITH DISTILLED WATER — SEE BELOW.

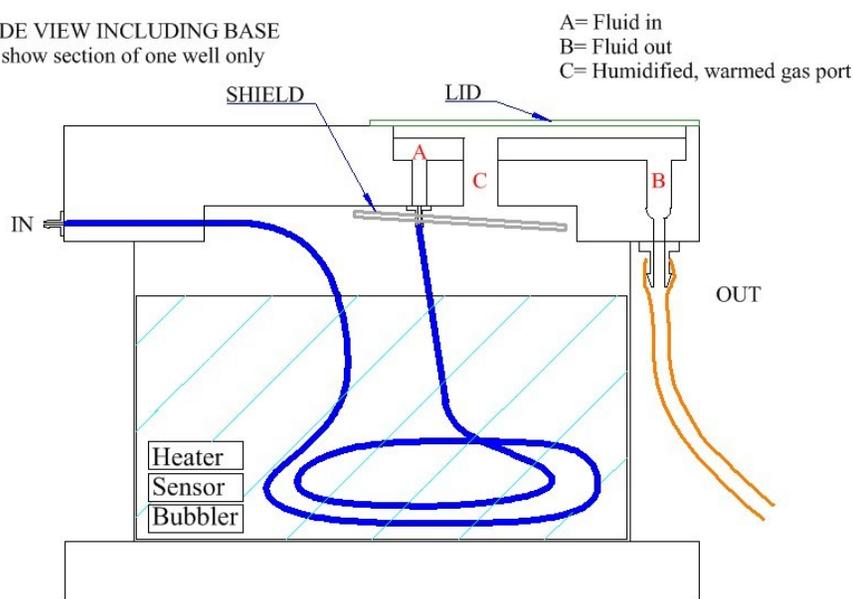
There are two perfusion solution feed lines, one for each of the two wells located on top part of the chamber. A third port is the feed for oxygen/carbon dioxide mixture to the ceramic air stone located in the lower chamber. The heater element connecting cable is fitted with a three pin plug for connection to the PTC03 or PTC04 HEATER socket. The sensor for temperature control is fitted with a seven pin plug for connection to the SENSOR socket.

SCHEMATIC DIAGRAMS



ABOVE: View of BSC2-2 top. Two identical wells have solution input [A], output [B] and gas vents [C], with a 'template' insert having a diamond profile for guiding incoming solution to the exit port. A lid with a centrally located hole helps to maintain the high oxygen concentration above the slices maintained in 'interface' mode'. A reference ground wire or Ag/AgCl pellet can be placed in the well and the wire secured by a screw within a groove located on the upper surface. **BELOW:** View from the side to show pathway of incoming solution at [A] and exit at [B] where a fitting allows connection of tubing to collecting vessel or pump for re-circulation. To prevent atomized distilled water (generated by bubbles breaking in the base) from entering the upper chamber wells, a shield is incorporated under the port holes as shown below.

SIDE VIEW INCLUDING BASE
to show section of one well only



LOCATION

The chamber should be secured to a solid, smooth table surface. The fixing holes in the base can be used to mount small magnetic bases if a steel table is used. The base-plate must not become distorted through securing on to an uneven base. Do not over tighten fasteners on the holes provided.

FILLING LOWER CHAMBER

Once secured, fill the lower chamber with approx 110 mls of distilled water using a syringe fitted with a plastic tube capable of being inserted into one of the vent holes on the upper section. Make a note of the fill level which should be seen to completely immerse the heating element visible in the lower chamber and be 2 to 4 mm below the junction between the upper and lower sections of the acrylic chamber. Check this level routinely on a daily basis before switching on the power to the system. Once a week at the end of the day **switch off the power** and use a fast vacuum line to completely suck out the distilled water in the lower section, rinse and refill with fresh distilled water as before to the correct level before switching on power. This operation prevents the growth of foreign matter.

CONNECTION TO GAS MIXTURE SOURCE

The gas mixture source should have a secondary flow regulator for fine adjustments. Although the warmed distilled water in the bottom of the chamber will provide much of the humidity in the upper chamber, it is very important to have the gas mixture pre-humidified before it enters the chamber. This is usually done with a 'gas wash bottle' fitted with a sintered glass bubbler.

In addition to providing warmed and moistened gas to the upper chamber, this gassing is necessary to keep the lower section distilled water stirred for efficient feed-back from the heater to the sensor connected to the PTC03 Temperature Controller.

To prevent atomized distilled water (generated by bubbles breaking in the base) from entering the upper chamber wells, a shield is incorporated under the port holes as shown in the diagram on page [4].

TEMPERATURE CONTROL

Proportional Temperature Controller PTC03

The PTC03 is a temperature control unit designed for use with the chamber. A low voltage direct current output with low noise characteristics is used to power the heating element contained within the lower chamber together with a sensor for feedback proportional control. The required temperature is set using the front panel control with a digital readout of set temperature. When the display selector is set to control, the display reads the temperature of the control sensor. Provision is also made to display the temperature from an optional monitor sensor if this is being used. Full control is reached within 20 minutes at a setting of 40°C, with an ambient of 20°C. Set temperature must exceed ambient by 2 °C minimum.

Specifications of PTC03

Readout accuracy	+/- 0.1 degrees centigrade
Control accuracy	0.5°C below set temperature maximum difference.
Control stability	Not more than +/- 0.1°C from control point.
Output power	36 Watts Max.
Output type	D.C. Proportional control
Sensors	Pt100 Platinum Resistance (Control & Monitor)
Power requirements	110V / 240V +/- 10% 50 W, 60/50 Hz
Dimensions	120H x 200W x 280D
Weight	4 Kg

CONNECTION TO TEMPERATURE CONTROLLER

Check that the chamber lower section has sufficient distilled water. Check that the SENSOR probe and HEATER elements are screwed in tight into their holes on the side of the chamber, that there are no leaks here and that the plug ends are connected to the PTC03 Temperature Controller sockets. Connect the mains power lead to a suitable socket **WHICH MUST HAVE AN EARTH CONNECTION** for safety and low noise operation. Turn on the power switch located on the rear of the PTC03. On the front panel the 'LINE ON' light should now be on. Move the selector switch to 'SET', a light above the temperature adjustment knob will turn on to indicate 'SET' mode. Adjust the knob and read the LCD display to set to a desired temperature in °C. Once set, move the selector switch to 'CONTROL'. Assuming you have selected a temperature at least two degrees above ambient, the 'HEATER ON' light will glow brightly or dimly depending on how close the lower chamber temperature is to the set temperature.

NOTE. The temperature shown on the front panel display will be the temperature of the lower chamber distilled water. The temperature achieved in the upper chamber at the location of the preparation depends on a number of factors principally:-

- 1) Ambient temperature
- 2) Incoming gas mixture flow rate
- 3) Perfusion fluid flow rate and initial temperature (e.g. from the refrigerator?)

As long as the above factors are quite stable during the course of an experiment, there is a fixed temperature differential between the upper and lower sections of 3 to 4 degrees centigrade. Given this differential, the PTC03 effectively controls the upper chamber temperature which should be monitored with an independent miniature temperature probe (eg. thermocouple type). Allow at least 10 to 15 minutes for the system to equilibrate, and approximately 5 minutes for a 5°C temperature increase but 20 to 30 minutes for a 5°C temperature decrease.

As part of our program of continual improvements, provision is already made on your PTC03 circuitry for a plug-in monitor temperature sensor (select MONITOR on switch). This sensor will be available in the future.

BASIC TEMPERATURE CONTROLLER

An economic alternative to the low noise PTC03 temperature controller is now available. The new PTC04 performs the same basic function for maintaining a set temperature and has been designed for use where high gain electrical recordings are NOT being performed. It is suitable for use with experiments for incubating slices or performing pharmacology experiments. Connections to the heater and sensor are the same as for the PTC03.

CONNECTION OF THE PERFUSION FLUID SOURCE

The single channel solution enters the chamber from the top part via plastic connectors and emerges at the mid point within the chamber slice area as shown on the diagram earlier.

Perfusion solution feed reservoir

The perfusion solution feed reservoir should be continuously gassed with 95% O₂ / 5% CO₂ gas mixture at a temperature close to but not exceeding the final temperature utilised in the slice recording chamber. The reservoir is usually immersed in a water bath set to the desired temperature, close to or slightly below that set in the slice chamber. This is necessary otherwise bubbling the reservoir at a lower temperature dissolves more gas into solution: when the fluid is heated at the chamber end this gas is liberated in the form of small bubbles in the input line - since warmer solutions hold less dissolved gas. The bubbles can then cause undesirable artefacts such a movement of slices, alteration of fluid levels and 'popping' noises in electrophysiological recordings.

Gravity or pump?

Typically the simplest and cheapest system is gravity feed such as a raised bottle with a side exit, filled with the desired perfusion fluid. A blood-drip set arrangement allows the flow rate to be monitored easily, in addition the flow adjustment 'pinch clamp' is usually easy to operate. However the disadvantage is that control of flow rate is affected by the changing fluid height in the bottle and the unreliable nature of the 'pinch clamp' which may cause fluctuations in the flow rate over time. There are many 'multi-barrel' peristaltic pumps available for the control of flow with a wide range of flow rates. Select ones with reduced pulsation (more barrels and/or air buffer and/or narrow gauge tubing).

CONNECTION OF EXIT WELL

The exit well is located to the opposite end of the input to direct flow across the surface. The fluid is made to flow across the flat area of the chamber by the capillary action of lens tissue which is placed on the base area. The lens tissue is then tapered into the exit well where it guides the fluid for removal. A suction line or the return line of a peristaltic pump is usually attached to the exit well connector. Alternatively a piece of cotton thread is inserted into the exit tube to guide the solution out into a collection vessel.

If a vacuum line is being used, it should be connected via a waste bottle to smooth out any irregularity. Typically a house vacuum line is used, electric pumps are equally effective. A bleed valve is recommended when utilising powerful electric pumps to allow adjustments of the level of vacuum, excessive or inadequate levels will cause problems. The correct vacuum level will be found by trial and error, depending on perfusion flow rates. Try pouring a few mls of perfusion fluid into the centre of the upper chamber to see how the fluid behaves with your selected vacuum line. A high suction rate has the potential of reducing the high oxygen tension by pulling external air over the surface of the slice. A slow suction rate is desirable and more stable, it will be characterised by evenly spaced air/liquid columns visible in the exit line.

If a peristaltic pump is being used with flow returning to the feed reservoir, the return flow rate should be at least four or five times the fluid inflow rate. In most cases it is necessary to fit a wider tube in the peristaltic pump for the return flow if the same pump is being used for the fluid feed.

REFERENCE ELECTRODE CONNECTIONS

A ground or reference can be introduced into the chamber via the narrow grooves as shown in the diagram on page [4]. This extends beyond the area occupied by the lid when positioned on the chamber. Each groove has a screw for securing the wire from the Ag/AgCl wire or pellet which can be positioned in the base of the well or within the wall of the diamond shaped 'template' supplied with the chamber.

Noise problems usually arise from external high voltage sources such as mains power cords, CRT monitors, CRT oscilloscopes and fluorescent lights. Relocation of these potential sources may be necessary and/or shielding may be required around the recording electrode.

The heating element in the chamber is driven by a low voltage, low noise direct current power source. If it is found that on switching off the power to the PTC03 (whilst the mains plug is still in the power socket) that noise is eliminated, check the ground connection at the mains plug and socket.

Peristaltic pumps will sometimes also generate very sharp transients due to static discharges along the silicone rubber tubing within the pump mechanism. This may be eliminated by piercing a section of connecting silicone rubber tubing (at a suitable point close to the chamber) with a piece of chlorided silver wire and grounding this to the central grounding point of the recording apparatus. Another way is to spray the external peristaltic pump and tube mechanism with anti-static spray. This increases conductivity around the silicone tubing and prevents build-up of static charges.

OPERATION WITHOUT TEMPLATES

Cut a sheet of lens tissue into a rectangular shape to fit the flat area of the slice chamber. The lens tissue should be positioned close to but not on top of the input solution tube so that any bubbles can escape from the input line. It is sometimes desirable to have the input solution tube protrude slight above the surface so that bubbles breaking the surface do not interfere with electrophysiological recordings. (The chamber top can be opened and the tube pushed up to the desired height). At the other end of the lens tissue, form a taper and extend this into the exit well as described earlier. The flow of the perfusion fluid along the lens tissue is such that it bathes the slice whilst keeping the surface wetted with a thin film of fluid. Some researchers prefer to place a nylon net under the lens tissue. This helps increase the access of fluid from under the slice. The perfusion solution level can be raised slightly if required by adding a second layer of lens tissue around the slice. Changing the slope of the chamber slightly by altering the height of the fixing points on the base of the chamber will also affect flow rates and fluid levels.

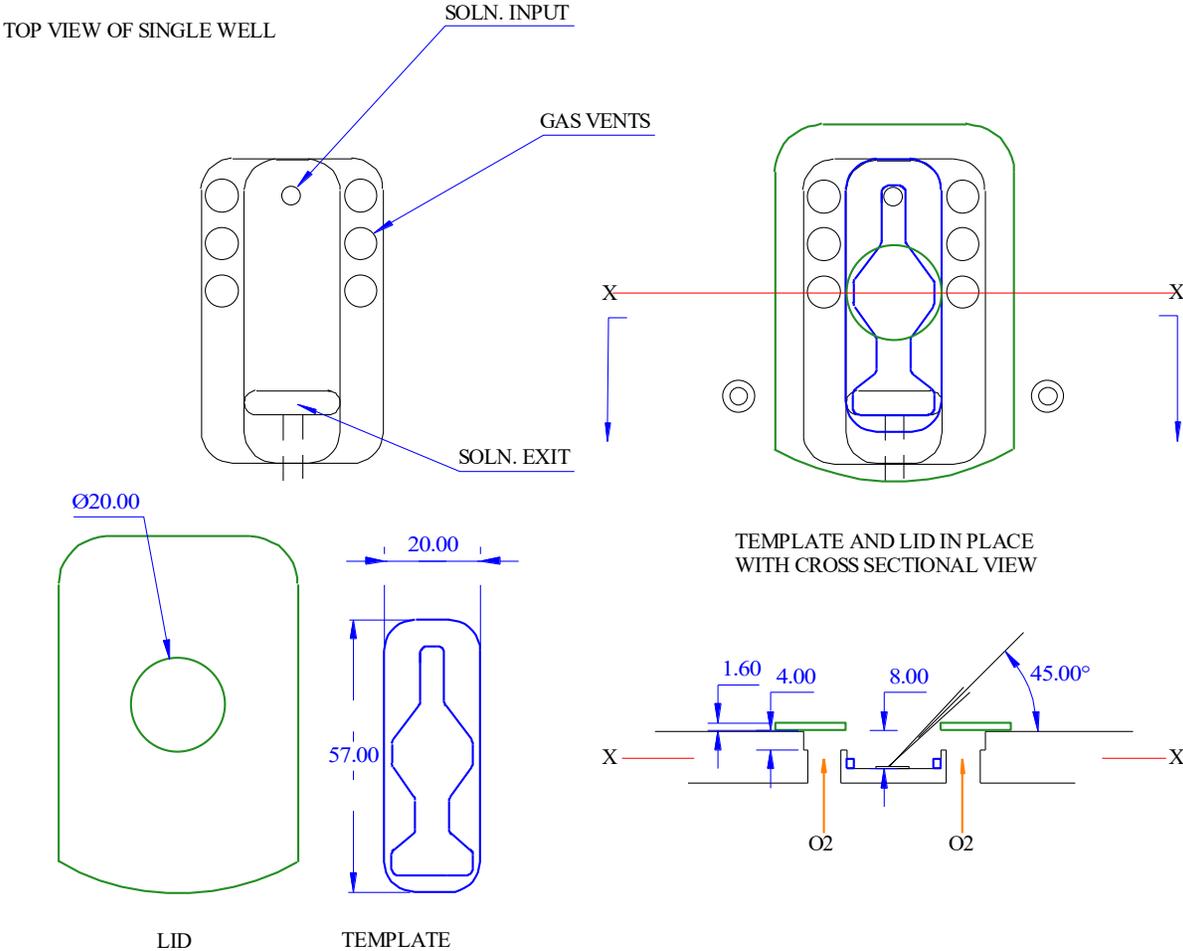
The interface with the humidified, oxygenated atmosphere is a delicate balance of gas flow rates, humidification level, temperature and fluid flow rate. Excessively high gas flow rates will cause the surface of slices to become dry and to appear shiny or 'glazed' and the slice surface will be seen to resist the penetration of recording/stimulating electrodes. (Microscope illuminating lamps with high infra red heat output can produce the same effect).

Once the acrylic lid is in place and the flow within the chamber is stable, slices can be placed on the lens tissue. The dead-space volume within the PTFE tubing inside the chamber is approximately 0.5 ml. This can be reduced by reducing the length of tubing in the lower chamber but it will affect heat transfer to the solution when high flow rates are used.

SINGLE AND DUAL CHANNEL OPERATION WITH TEMPLATES

The chamber system is supplied with acrylic templates that fit into the flat area of the slice chamber. These allow operation with dead space volumes in the hundreds of micro litres range, excluding the volume of the PTFE feed tube which is approx 400ul. The templates have machined profiles in a diamond shape so as to encourage efficient flow around the slice preparation. Other design layouts can be supplied upon request.

SCHEMATIC DIAGRAM



The templates have an opening where the PTFE feed tube emerges from the base of the chamber and another opening at the location of the exit well. The area in between the two openings forms the feed and exit routes with a diamond shaped area in the centre for the slice preparation. The template fits into the base of the chamber and should not require sealing. Once lens tissue is laid on the base the solution will flow towards the exit well where the lens tissue over-hangs. If a seal is needed, lightly coat the base of the slice chamber with a layer of laboratory stopcock grease (inert silicone grease). Avoid applying excess amounts around the input PTFE tubes and prevent any grease spreading into the exit well as this can cause the suction to oscillate. Do not allow the grease to spread on to the top surface of the acrylic templates as this alters the way the perfusion solution flows in the channels of the template.

If unwanted grease gets on to the top surfaces of the template, it should be wiped clean and an approved laboratory detergent (eg Micro-90, Cole-Parmer *) used to remove it. **Do not use solvents as they will destroy the acrylic template.** The same detergent can be used to clean the rest of the chamber at monthly intervals.

The template can now be positioned on the silicone coated base of the chamber, pressing down firmly as this forms a mechanical bond with the slice chamber base and is required for stability.

Cut a piece of lens tissue in the shape of the diamond to fit into the profile of the template. Cut another long thin section to lay from the diamond shape all the way into the exit well. This will guide the fluid to the exit well. Depending on the flow rate, it may be necessary to position the lens tissue all the way down to the bottom of the exit well. The optimum setting will be found with experience. Note that with new chambers the acrylic surfaces are clean and hydrophobic. After a few days the surfaces will adapt and a negative meniscus will form that assists stable flow characteristics.

MAINTENANCE

Alcohols should never be used on the slice chamber for cleaning purposes even at low concentrations because it de-hydrates and produces hair-line cracks in acrylic. Templates are also made of acrylic and therefore must be treated with the same care. A laboratory detergent which completely rinses out should be used. Heavy deposits of salts should be washed out with distilled water overnight and carbonate salts treated with mild acids such as citric acid. The most common contaminant is fungal growth in the upper section tubes and cavities. This can be avoided by agitated washing i.e. suck out plenty of distilled water intermittently with air bubbles through the tubes and holes of the chamber by use of a powerful vacuum line at the end of each experiment. Continue to dry out by using the vacuum line around all the tubes and also below the templates. Leaving the chamber dry will prevent the growth of foreign matter. Cover the chamber with a sheet of clean medical wipes to prevent dust settling on the surfaces. Before the start of each experiment rinse with perfusion fluid.

Remove and clean templates and surrounding areas at least once per week. At the end of each experiment, flush through the system with an agitated stream of distilled water or suck through the tubes with a powerful vacuum line ensuring agitation by allowing air to intermix with the stream of distilled water. The bubbles will assist in removal of growth lodged in the tubes of the chamber.

****Cole-Parmer and Micro-90 are registered names and products of their respective companies***