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Innovative Engineering for Science

MICROSCOPE STAGE CHAMBER

MS3

MICROSCOPE STAGE CHAMBER

CAUTION !

YOUR 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 USE ALCOHOL OR SIMILAR SOLVENTS IN ANY CONCENTRATION ON ANY PART OF THE CHAMBER SINCE AS WITH MOST ACRYLICS, TMPERSPEX MAY FRAGMENT OR DEVELOP HAIR-LINE CRACKS.

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CHAMBER DESCRIPTION

The MS3 Microscope Stage 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 specifically for 'interface' method of maintaining slices on an upright microscope stage. Temperature is optionally maintained by the MH02 in-line perfusion solution heater with a capacity to heat perfusion fluid up to 6ml/minute and is used in conjunction with the PTC03 proportional control heating system.

CONSTRUCTION

The chamber is constructed from a single block of acrylic, and is normally mounted directly onto the moveable stage plate of an upright microscope which may require an adaptor plate. The chamber diameter is 100mm, height is 18mm. The central area of the chamber consists of a glass cover slip with an 18mm diameter work area where the slices are placed. In order for the glass cover slip to rest at the correct working height for the microscope objectives AND the condenser optics from below, the correct stage plate must be utilised with the microscope. This means that the chamber needs to be set within the depth of the existing microscope stage.

In order to provide the necessary humidified carbogen, an external gas humidification vessel is connected via tubing to the lower section of the chamber which channels this to the upper part of the chamber. Here the gas mixture is deflected by an acrylic deflector lid across the surface of the interface slices located centrally. In order to maintain the temperature and increase the humidity, it may be necessary to heat the incoming gas by maintaining the external humidification vessel in a heated water bath.

In addition it is possible to use acrylic templates provided with the chamber. The templates help to decrease the 'dead space' volume of solution and therefore allow more rapid changes of test solutions.

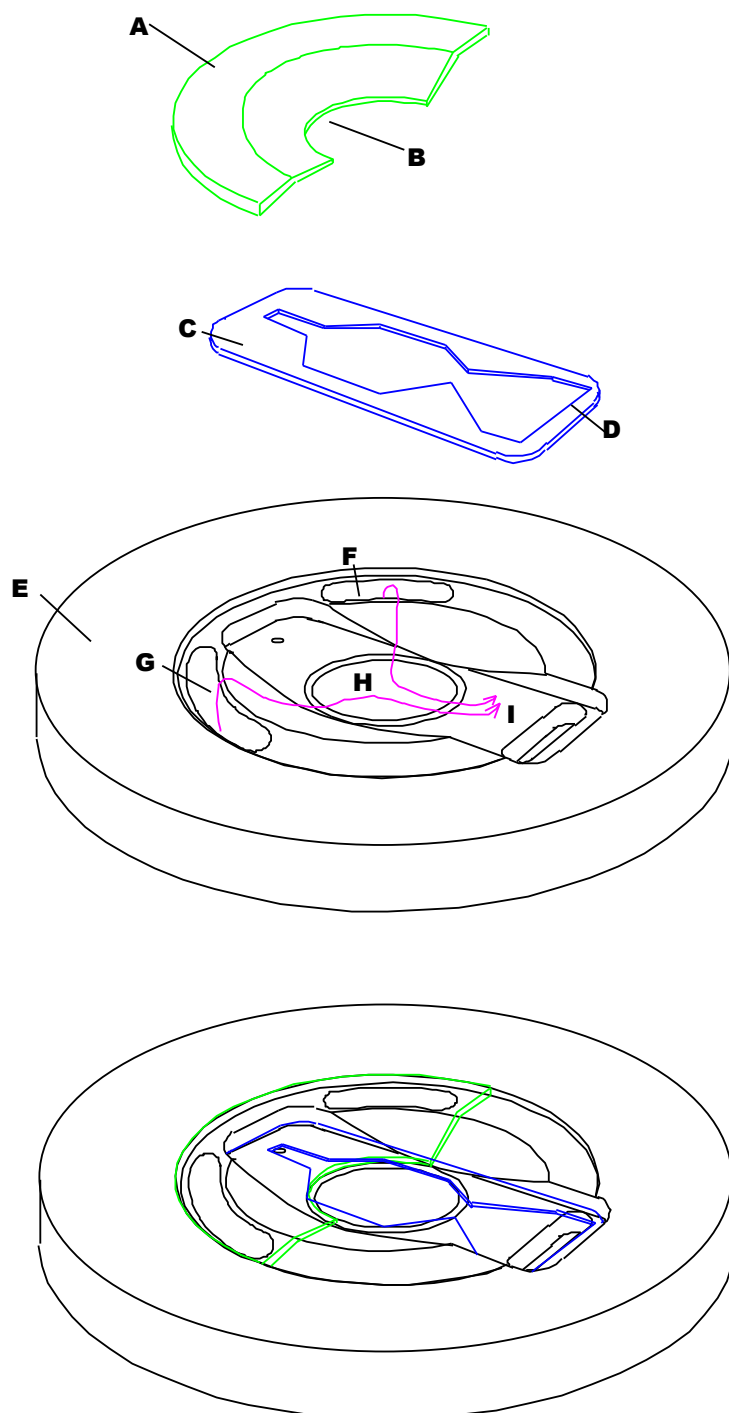
Pre-oxygenated perfusion fluid enters the main body through a fine bore PTFE tube which is pre-heated by the MH02 in-line perfusion solution heater. The heated fluid enters the upper part at the base of the flat slice area. The solution then exits by means of capillary action with lens tissue which is 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 recirculate the perfusion solution.

INSTALLATION AND OPERATION

Once all packing material has been removed, please take some time to examine the construction of the chamber. Remove and identify : MS3 chamber, two single channel inserts and one deflector lid.

There is one perfusion solution feed line that enters the chamber. This can be accessed either from above or below depending on the opening in the microscope stage. Another pair of lines accessed from the bottom is the feed for oxygen/carbon dioxide mixture to the upper part of the chamber. The deflector lid then directs this gas mixture over the preparation.

SCHEMATIC DIAGRAM



AN EXPLODED VIEW OF THE CHAMBER COMPONENTS AND HOW IT IS ASSEMBLED

[A] DEFLECTOR LID **[B]** CUT-OUT IN LID FOR ACCESS TO SLICES
[C] TEMPLATE **[D]** EXIT HOLE FOR PERFUSION SOLUTION **[E]**
 MAIN BODY OF MS3 CHAMBER **[F]** & **[G]** PORT HOLES FOR ENTRY
 OF HUMIDIFIED CARBOGEN GAS MIXTURE **[H]** GLASS COVERSLIP
 BASE AND RESTING PLACE FOR SLICES WHERE HUMIDIFIED
 CARBOGEN GAS IS DIRECTED BY LID **[A]** IN DIRECTION SHOWN
 BY ARROWS **[I]**

LOCATION

The chamber should be secured to a microscope stage surface which has the provision to move in X and Y directions +/-10mm. Since large bore tubes are used for the gas input it is important that the chamber is securely fastened to the microscope stage.

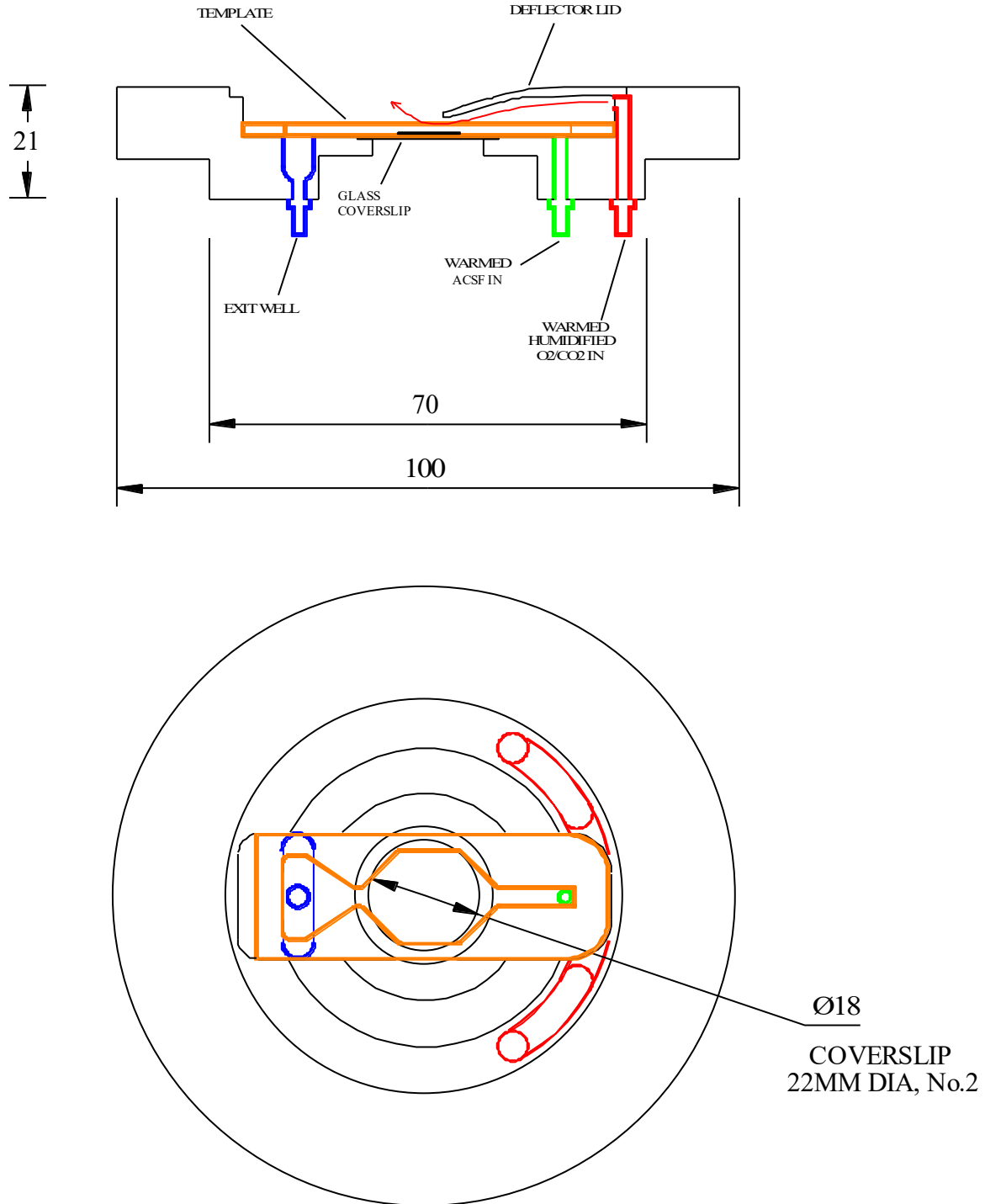
CONNECTION TO GAS MIXTURE SOURCE

The gas mixture source should have a secondary flow regulator for fine adjustments. 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 or a ceramic air stone producing fine bubbles. The wash bottle can be placed in a water bath to increase the temperature and humidity of the gas mixture prior to entering the stage chamber. Use a Y junction to divide the gas to the two gas inlet ports below the chamber. Arrange the tubes in a way that causes minimal interference with the microscope condenser optics. The microscope condenser needs to be able to travel all the way up and almost touch the cover slip base of the chamber.

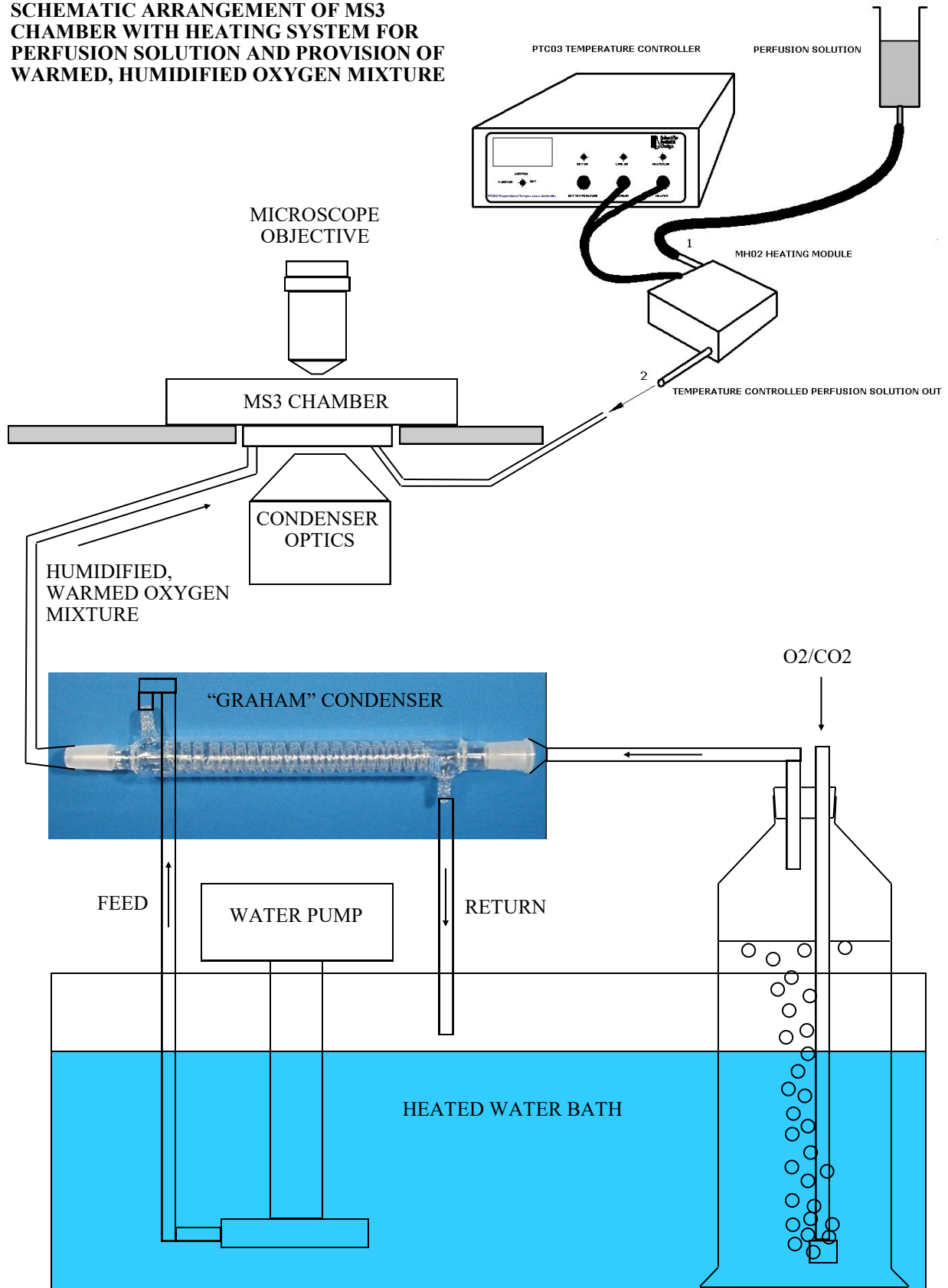
Caution ! Set up a mechanical stop on the condenser to prevent collision with the coverslip. In case of breakage the coverslip type is No. 2 thickness 22mm diameter borosilicate glass.

CROSS-SECTION AND SIDE VIEW OF CHAMBER

The outer diameter of the chamber is 100mm. The lower portion has a diameter of 70mm and is designed to fit through the opening of an upright microscope stage. Silicone tubing is attached to the bottom entry and exit ports and should allow the microscope condenser to move up towards the glass coverslip. The deflector lid must be in place to allow the humidified gas mixture to flow across the centrally located slice preparation, as shown by the red arrow.



**SCHEMATIC ARRANGEMENT OF MS3
CHAMBER WITH HEATING SYSTEM FOR
PERFUSION SOLUTION AND PROVISION OF
WARMED, HUMIDIFIED OXYGEN MIXTURE**



FEED OF HUMIDIFIED OXYGEN TO CHAMBER

As the diagram above shows, a feed of humidified oxygen should go through a Graham condenser or similar arrangement whereby the delivery tube carrying the warmed humidified gas is kept warmed from the feed of a heated water bath. By keeping this delivery tube heated the condensation will be minimised ensuring as much humidified oxygen reaches the chamber on the microscope stage. A concentric tube arrangement all the way up to the chamber will also work to keep the delivery tube heated.

TEMPERATURE CONTROL

MH02 Mini Heating Module and Proportional Temperature Controller PTC03

Your purchase of the MS3 chamber system may have included our optional temperature control system. Temperature control of the perfusion solution can be achieved with the MH02 which is an in-line perfusion solution heater. The PTC03 is a temperature control unit for use in conjunction with the MH02. A low voltage direct current output with low noise characteristics is used to power the heating element contained within the MH02 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. 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	4.5Kg (10lbs)

CONNECTION TO TEMPERATURE CONTROLLER

Connect the two blue plugs from the MH02 to the PTC03 Temperature Controller sockets on the front panel. 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" red 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 temperature is to the set temperature.

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.

CONNECTION OF THE PERFUSION FLUID SOURCE

The two channels enter the chamber from the top via plastic connectors and emerge at corresponding points within the chamber slice area as shown on the diagram earlier.

Perfusion solution reservoir

The perfusion solution reservoir should be continuously gassed with 95% / 5%, oxygen/ carbon dioxide gas mixture at a temperature close to the final temperature utilised in the slice recording chamber. The reservoir is usually immersed in a water bath set to the desired temperature. This is necessary because bubbling at a lower temperature dissolves more gas into solution: when the perfusion solution is heated at the chamber end this gas is liberated in the form of small bubbles in the 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 and smoothest 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 from the drip rate, 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 now many multi-barrel peristaltic pumps available for the control of flow with a wide range of flow rates.

CONNECTION OF EXIT WELL

The perfusion solution 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 one of the exit wells where it guides the solution for removal. A suction line or the return line of a peristaltic pump is attached to the exit well connector.

NOTE: The lens tissue should be positioned in the exit well so that it is all the way down into the exit well. The suction line will then take away the excess fluid from the end of the lens tissue that is very close to the exit hole at the bottom of the exit well. The correct operation will be characterized by a gentle 'crackle' noise as the suction line pulls off the excess fluid from the end of the lens tissue. If the rate of suction is too great, this will be characterized by a 'hiss' and will result in too much fluid being sucked off, leaving the slice surface too dry. The optimum suction rate will be achieved by trial and error, and a perfusion flow rate between 0.5 to 1.5ml/min should be used.

If a vacuum line is being used, it should be connected via a waste bottle to smooth out any irregularity. Typically a high pressure water vacuum-adaptor 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.

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 perfusion 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 solution feed.

REFERENCE ELECTRODE CONNECTIONS

A ground or reference can be introduced into the chamber via the narrow "L" channel. This extends beyond the area occupied by the lid when positioned on the chamber. The ground wire should be covered over with silicone rubber over the channel area so that it can be easily renewed when required but remains firmly fixed during experiments.

Noise problems usually arise from external high voltage sources such as mains power cords, computer monitors, 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 earth 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 earth point of the recording apparatus.

SINGLE CHANNEL OPERATION WITHOUT TEMPLATES

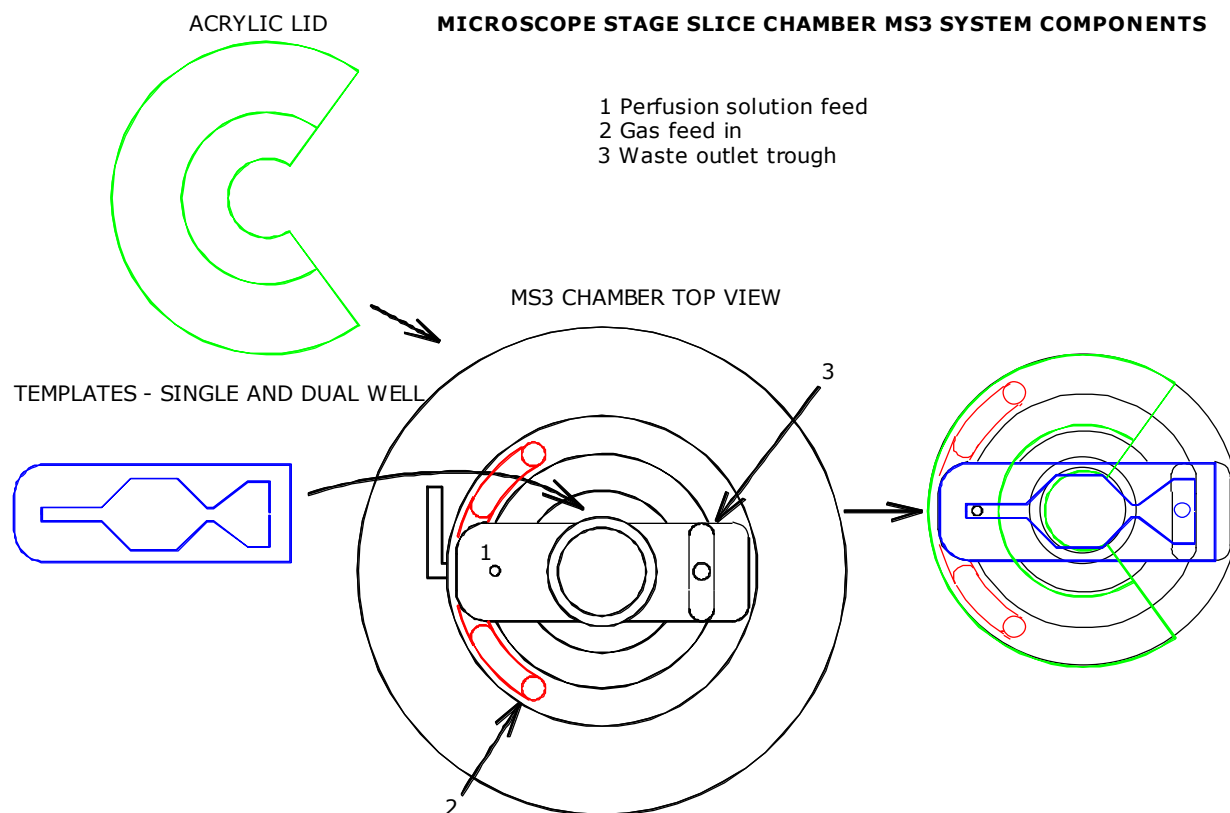
Cut a sheet of lens tissue into a rectangular shape to fit the flat area of the slice chamber. The lens tissue should be positioned close to but not on top of the input solution tube so that any bubbles can escape from the input line. It is sometimes desirable to have the input solution tube protrude slight above the surface so that bubbles breaking the surface do not interfere with electrophysiological recordings. (The tube can be 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 solution. Some researchers prefer to place a nylon net under the lens tissue. This helps increase the access of the medium 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. Use thicker layers of lens tissue/filter paper going into the exit well to increase the flow rate. Tougher filter paper such as that utilised with coffee machine filters also works well instead of lens tissue.

The interface with the humidified, oxygenated atmosphere is a delicate balance of gas flow rates, humidification level, temperature and solution 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.

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 much smaller dead space volumes. 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



Before inserting a template, push the PTFE feed tube down slightly so that it does not interfere with the template. 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. Optionally coat the base of the slice chamber with a thin 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 Cole-Parmer® Micro-90® cleaning solution, Cole-Parmer) used to remove it. **Do not use solvents as they will destroy the acrylic template and chamber surface.** 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 solution to the exit well. Depending on the flow rate, it may be necessary to position the lens tissue all the way down to the tip of the suction pipe within the exit well and/or thicken the layer of lens tissue.

When removing templates, use a pair of curved forceps to lift out from the exit well end of the template. Do not lift close to the glass coverslip as this will break easily.

MAINTENANCE

Alcohol 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.

If the coverslip should break, please follow these instructions:

Wear safety goggles for this procedure. First place the chamber on a flat, soft surface (cork tile) and GENTLY remove all the broken glass, using a small flat ended spatula in a scraping action to remove the old silicone rubber sealant. **DO NOT use any kind of solvent to remove the sealant.** Once completely clear, use a 5ml syringe fitted with a wide needle (10 or 12G) loaded with inert black silicone rubber sealant. Bead this around the circumference of the indentation. Place a clean 22mm coverslip on the fresh sealant and GENTLY press around the circumference ONLY of the coverslip. It is preferable to use a black sealant as it allows the thickness of the sealant to be controlled: darker areas have too much sealant under the coverslip. Try to ensure the final position of the coverslip is central and also level with the surface of the chamber since the acrylic templates need to rest flat on the chamber base area and coverslip. The indentation for the coverslip is approx 300um in depth, the coverslip supplied with the chamber is approx 200um, giving room for a good 100um thickness of sealant. Remove excess sealant whilst still wet, taking care not to displace the coverslip. After about two hours the adhesive will be firm enough to gently polish off any residual adhesive remaining on the glass coverslip central area. Use a cotton wool bud soaked in diluted laboratory detergent (eg Micro 90) to clean off any stubborn adhesive smears. Alternatively return the chamber to us and we will replace the coverslip.