Choosing a Temperature control and Perfusion system

I’ve written this brief overview on how to select a temperature control system and perfusion system based on researchers questions over the years.

Temperature control
Flow rate and inline heating

If you are going to flow solution through a tissue chamber and maintain the temperature in the chamber then you will probably need an inline heater (eg. HPRE2) to heat the perfusate before it enters the chamber. A rule of thumb (based on data from Ref 1.) is that for a 0.25ml tissue chamber when you are flowing at more than 1cc/min most of the heating of the chamber solution is done by the perfusate. Any heater beneath or around the chamber will be providing little heat to the chamber. This is very relevant to situations where the working distance of an objective (for an inverted microscope) is such that you cannot have an extra layer of glass for a transparent ITO heater because the thickness would be too great. If you are using a chamber with a larger volume or slow flow rates you may need heating from below or from an objective heater (see below). Keep in mind that most heat losses occur from the liquid surface so a deeper chamber with the same volume will have lower heat losses. If you want to test whether you need a chamber heater run fluid into the chamber via a pre-heater (eg. HPRE2) at the slowest rate that you want to use and look at the difference in temperature between the outlet and inlet sides of the chamber. If this is less than 1°C then you can probably leave out a chamber heater (see Note 1). You could also consider increasing the flow rate though normally this isn’t practical either because compounds being used are expensive or in some cases the flow will disturb the cells or tissue.

Chamber heater

If you find that you need to heat the chamber then your options depend on the objective working distance and whether you are using an inverted or conventional microscope. With a conventional microscope you could normally use an ITO coated heater (eg. HI-25, HI-24 or the thicker HI-25D etc.). The advantage of a transparent heater is that the heating is uniform and hence the design of the tissue chamber is flexible plus there is no restriction on light from the condenser. Also you can stop the flow of perfusate and maintain the temperature. Indirect heating is possible with a metal stage but in this case you need to maximize the area coupling the heater to the solution in the chamber. If you had a choice you would make the chamber as small as possible. Heat will enter the chamber from the surrounding material usually polycarbonate that has in turn been heated from a metal plate etc. If the side walls in the chamber are also in contact with the heater (and thin enough that you get heat transfer to the solution level) then you will get more heat transfer but it will still take a long time to heat up the chamber often tens of minutes. This compares with times of 10-20secs with direct heating. If you are heating the inflow with a pre-heater then this will help even out the temperature distribution in the chamber and make up for some of the inefficiency of heat transfer by conduction through the chamber.

For an inverted microscope you will often be able to use an ITO heater with most air type objective and some immersion lenses with longer working distances. The HI-25T is about 0.14mm thick (like a #1 cover slide) but you will typically have a #1 or #1 ½ cover slide on top of this. The HI-25 is approx. 0.2mm thick. With an immersion lens there may also be heat losses to the objective. Unfortunately objective manufacturers neither take the thermal properties of the lens into consideration in their design nor do they provide any thermal data on their objectives. One simple way to examine the thermal impact of the objective is to use some liquid crystal sheeting or paint (see Note 1). First set up the immersion lens as for an experiment then either paint the liquid crystal paint on a glass slide (you will need to precoat the slide with a black background-eg. black marker pen) or cut out a piece of liquid crystal sheet to fit the chamber and wet the sheet so that it will be thermally coupled to the bottom of the chamber. You can then flow warmed solution through the chamber and see the 2D
temperature distribution from the color of the material (usually from deep blue through green to light brown). Test the chamber at different flow rates. You should be able find a flow rate at which the temperature distribution is uniform (within <+-1°C temperature difference) as long as the flow pattern in the chamber is reasonable (ie. solution doesn’t just flow down a central channel in the chamber). In all our tissue chambers you get laminar flow patterns with flow rates up to about 2ml/min.

**Objective heater**

Depending on the design of the lens you could use an objective heater (eg. HLS-1P [2cm wide strip] or HLS-8x.8P [0.8cm wide strip]) to compensate for the heat normally taken away by the objective. Ours are simple heaters that wrap around the objective body. The thermistor sensor goes under the wrap of the heater to sense the lens temperature. You should check with the objective manufacturer whether heating the lens to 35-37°C will affect the lens. Because of the large thermal mass of the lens body there is normally little overshoot of the set temperature. Also the objective takes time to heat up so there should not be sudden thermal stresses introduced in the objective. But since objectives are very valuable it is better to check. Most objectives for electrophysiology have the metal body electrically isolated from the lens that contact the tissue chamber bottom but the lenses are still thermally coupled to the lens body and hence to the chamber bottom. If you have an objective heater you can use the same liquid crystal materials to see how effective the heat transfer is from the heater to the lens that contacts the chamber bottom.

**Perfusion**

**Bath perfusion**

Although you can use simple rotary mechanical valves and a manifold to change solutions there are many advantages of using valves and a valve controller. One is that you are less likely to cause mechanical vibrations using a valve operated by a controller. Further, with a controller you have more flexibility to determine which solutions are added since typically you will have a choice of 8 solutions while most mechanical valves are limited to 4 solutions. Mechanical valves can also introduce bubbles because of an imperfect seal while in a system using pinch valves there are no seals to let air in. Valve controllers can typically be interfaced to a data acquisition system. For example the cFlow has RS232, analog and digital interfaces to choose from. It isn’t possible to get rapid switches of multiple solutions by turning valves by hand. Pinch valves used in many valve systems (including the cFlow) open and close in about 20ms so you can have applications as short as 50ms, something impossible with hand switching. And these can be synchronized with voltage pulse, light flashes etc. You can log the valve number, opening and closing times using software such as ByteWedge (for Windows XP) from www.fogsoft.com.

**Superfusion**

You can apply local perfusion using a small manifold. Our MPRE8 8Ch Pre-heater differs from many since it is temperature controlled. Normally applying local perfusion is troublesome because the outflowing solution it isn’t heated. This leads to temperature artifacts since the test compound arrives at the cell at an unknown but lower temperature. The MPRE8 plugs into the TC2BIP for temperature control and the cFlow setup for switching the flow in the MPRE8 barrels. Local perfusion saves valuable compounds and permits local application to a cell or region of the chamber. With a laminar flow pattern in our chambers you can also apply a compound downstream first thereby preventing contamination of upstream cells. You can also do this to some extent on cells from front to rear of the chamber.

**Cooling**

We don’t make a dedicated cooling/heating stage because the machining required is costly making the systems very expensive. The CH Heating/cooling module came out of a need for an inexpensive device for cooling solutions down to temperatures below 10°C to study activation kinetics of calcium currents. The article in J. Neuroscience Methods (Ref. 2.) details more of the design and theoretical performance but the device is very simple, relying on a small cross-section passive heatpipe to transport heat to and from a Peltier element.
mounted on a heatsink. This puts the large heatsink away from the setup and makes it adaptable to any microscope arrangement. But you need to be flowing solution since though it is possible to attach metal stages to the heatpipe this isn’t practical in most circumstances. With the CH it is practical to get temperatures as low as 5°C. Virtually all other cooling systems require liquid cooling of the heatsink (heat is transported by the Peltier from the cold side). This has the disadvantage of introducing vibration and/or electrical noise. Plus, do what you will things grow in the tubing. With air cooling you can separate the fan from the heatsink. You only need a modest flow of air to cool the heatsink plus if the fan fails (unlikely) the large heatsink will get hot but not in a range that would damage the Peltier element.

Another advantage of the heatpipe is that the high currents to the Peltier are about 15cm from the tissue chamber plus the heatpipe can be grounded since the glass capillary is an insulator.

**Relatively fast cooling**

You can drive the CH directly from the TC2BIP if you are not concerned about rapid temperature changes. In this case you place a thermistor probe on the heatpipe for feedback. The location isn’t critical because the temperature difference along the heatpipe is typically less than 1°C.

**Fast cooling**

For faster cooling you run the CH continuously using a power supply and rheostat (RH50-50WF) and reheat the flowing solution using the HPRE2. When the setpoint is lowered the flowing solution will cool down rapidly once power is cut off by the TC2BIP to the HPRE2. The rate will depend on the flow rate and tissue chamber volume. Because the rate of heating will be slowed by having cool solution entering the HPRE2 the temperature of the solution flowing out of the CH should be set as high as possible. For example if you wanted to have 10°C as the lowest temperature that you are using then you would set the outflow temperature to be say 8°C rather than 5°C.

**Temperature controller-mTCII (digital) or TC2BIP (analog)?**

We recently introduced the mTCII 2Ch micro-Temperature controller so now we have two different 2 channel feedback controllers. For electrophysiology especially patch clamping you should use the TC2BIP because it was designed and has been used extensively for low noise recording. It can also be extended to add a 3rd channel which is useful if you are superfusing solution over a region of the tissue chamber with the MPRE8. The mTCII is a more general purpose controller suitable for microscopy as well as for controlling a small incubator or in any situation where you need accurate temperature control using heaters of less than 15W on each channel. Because it is a digital device with a reprogrammable microcontroller the firmware can be upgrade and changed to add improvements or adapt it to special applications.

The mTCII was however designed to minimize noise. When the output to the heater is switched (PWM mode) the frequency can be set to 20kHz which is above the frequency in most electrophysiology experiments and so would be filtered out. Also it has an analog mode where the output to the heater is changed continuously. The output power is limited in the analog mode to 2W but this is fine for ITO heaters and inline pre-heaters. In fact it is similar to the default power limit in the TC2BIP.

**References**


**Notes**

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