Heating of Warner imaging and recording chambers: Application fundamentals

Why Heat?

Researchers have long understood the importance of temperature regulation in the study of cellular function. For example, a Medline search using the keywords “temperature” and “cell” produced over 12,000 references since 1995, most addressing the effect of temperature on the biophysical and metabolic function of cells. In addition, the performance of imaging system optics are exquisitely sensitive to temperature as revealed by a shifting focal plane as the temperature of the objective is varied. Therefore, for the scientist studying the function of cells in a recording environment the importance of tight thermal control cannot be overstated.

A number of issues confront the researcher attempting to maintain temperature control. These include maintaining a constant and uniform bath temperature under various flow rates and controlling for changes in the ambient temperature of the environment. In addition, effects due to the addition of cold drugs or solution to the perfusion path and heat sinks due to microscope optics and stage adapters must be addressed.

General Issues

Sources of heat flux

Heat flux pathways can be generally characterized as either conductive, convective, or radiative. Traditionally, the greatest effort is directed towards controlling conductive pathways, followed by convective pathways. We define a conduction heat pathway as one wherein heat energy is transmitted through a continuous intervening material. These include the microscope stage or objective, the perfusion solution, the chamber platform and other physical elements of the working environment. Heat flux through convective heat pathways are generally less significant but include evaporation or condensation and heat transfer due to the movement of air across the imaging chamber. Radiative pathways are, in comparison, easily controlled and will not be considered here.

Space and time considerations in uneven heat conduction

We first consider sources of uneven heat distribution in the spatial regime. These include poor heat conduction across the chamber bath or from the platform to the chamber. Poor heat conduction across the bath is revealed as a temperature gradient from one location to another within the bathing solution. A gradient of this type is usually induced by an immersion objective placed directly under the sample. Heat flux through this pathway can be significant, resulting in as much as a 10°C temperature change within 3-5 mm. Another, less dramatic, temperature gradient can be generated in the bath by restricting the application of heat to just the chamber platform. Since the platform applies heat only along the edge of a chamber, a temperature gradient will be established due to the heat capacity of water coupled with flow of relatively cold solution into the chamber.

Uneven heating in the temporal regime can be generated by varying the rate cool perfusion solution is introduced into a heated platform, or by introducing drugs or solution into the perfusion path at a temperature different than that currently being perfused. These considerations will be addressed in greater detail below.

Outgassing

Another significant issue related to the application of heat to perfusion solutions is termed outgassing. Under standard conditions, atmospheric gas in contact with a liquid solubilizes into solution and tends towards an equilibrium determined in part by the gas' partial pressure and the temperature of the liquid. Generally speaking, cold liquids are capable of solubilizing a greater volume of gas than a warm liquid. Thus, as the temperature of the liquid increases, dissolved gasses come out of solution resulting in the appearance of bubbles.

Heating Methods

A number of approaches are used by investigators to regulate the temperature of a sample under study. These include warming the chamber platform and/or microscope optics using resistive, Peltier, or liquid based application devices, channeling warm air across the imaging chamber and/or optical components, and regulating the temperature of the perfusing solution. Techniques for regulating the temperature of perfusing solutions include preheating the solution reservoir and/or using inline solution heaters in the perfusion pathway. Each strategy has its strengths and weaknesses and are discussed below.

How Temperature is Sensed

In general, temperature is measured using a thermistor or similar device and fed back into a temperature controller which adjusts power to the heating element. When regulating temperature it is important to account for the response time of the feedback loop since this can cause the system to oscillate.

Control and regulation

Warner Instruments temperature controllers utilizes two thermistors per channel to provide monitoring and feedback information to the controller. One thermistor is placed within the heated element (e.g., chamber platform, in-line heater, etc.) to measure the temperature of the heater block. Information from this thermistor is fed back to the control mechanism which applies sufficient power to the heater to maintain the controller set point.

The second thermistor is usually placed in the solution path close to the sample under study and measures the temperature at the sample. Information from this thermistor is used by the researcher to adjust the set point of the controller and to monitor the performance of the system.

This approach is very effective in providing tight thermal control (± 0.1°C) of the heating element, and consequently the perfusion solution, as long as the feedback loop is sufficiently optimized. Warner Instrument temperature controllers are provided with user selectable feedback response time constants allowing the instrument to accommodate different operating conditions

Methodology

In-line solution heating

The simplest and most direct approach for the application of heat to a sample is to preheat the perfusion solution immediately prior to its delivery to the chamber. The warmed perfusate washes over the sample and maintains a uniform temperature at the sample, even with variable flow rates. This method works well as long as solution continues to flow through the heater into the chamber.

A number of considerations are important when using this approach. First, it is important to minimize the distance between the heater and chamber. If too much heat is lost en route, the user can attempt to raise the heater controller set point above the boiling point of the solution. In addition, care must be taken to maintain flow rates such that the solution temperature reaches equilibrium prior to leaving the heater.

Chamber platform heating

Another strategy is to heat the chamber platform. This is usually achieved by using a resistive element and this approach is provided for in Warner chambers. A slightly more sophisticated tactic would be to supply heat to the platform using either a Peltier or liquid based heating element. Regardless of the method employed, this strategy is best suited for conditions where the solution flow rate is extremely slow or nonexistent.

A number of considerations are important when using this approach. First, the perfusing solution receives heat energy only after entering the chamber, thus generating a temperature gradient within the bath. Second, since heat energy is only applied to the edges of the chamber by the platform, the rate of heat transfer to the solution is very slow. However, this approach excels for conditions where solution flow is nonexistent, such as maintaining a constant temperature while loading cells with indicator dyes.

Solution reservoir heating

Heating the solution reservoir is not generally considered a successful strategy for maintaining temperature in the bath since most, if not all, of the heat energy is lost en route to the chamber. However, this technique is important if outgassing of solutions is a problem. Since the gas load of a solution is dependent on partial pressure and temperature, preheating the solution at atmospheric pressure before delivery to the final heater will minimize the occurrence of bubbles in the bath, even if the solution is allowed to cool en route. Therefore, solution reservoir heating is best used in combination with other heating techniques as described below.

Microscope objective heating

While necessary, the use of immersion objectives in imaging presents a difficult problem for the researcher attempting to maintain temperature at the sample. Since the objective is in direct contact with the solution or chamber and is placed immediately above or below the sample, it presents a significant conduction pathway within the system.

Heated objective collars are commercially available and are designed to minimize heat flux through the optics. However, many researchers indicate that small variations in temperature as the collar cycles off and on induces an oscillating shift in the focus plane. In addition, many researchers express concerns about the effects of deep cycling an objective between significantly different temperatures between uses. Some investigators address this issue by maintaining the objective at a constant temperature, even when not in use.

Heated enclosures

Another strategy currently in use is to encase the imaging chamber and microscope optics within a heated enclosure. The main advantage of this approach is that both the chamber and objective are maintained at a stable and uniform temperature. Heat is usually applied to the environment by the introduction of warmed air to the enclosure. Some disadvantages are slow responses to changes in the thermal set point, possible introduction of airborne contaminates from the blower system, and the requirement for bulky equipment on or about the imaging system.

Discussion

Each heating strategy discussed above has strengths and weaknesses making it an incomplete solution for issues encountered with heating. However, most experimental difficulties can be solved by using these strategies in combination.

For example, platform heating alone is not sufficient to stably heat a perfusing solution, but is necessary if solution flow is to be interrupted for any reason. In addition, the most effective method of heating a perfusion solution is via an in-line heater. Therefore, an optimal combination for experiments where tight thermal control is desired under variable, very slow, or interrupted flow conditions would be a combination of in-line and platform heating.

If solutions are stored cold and used before reaching operating temperature, or if the temperature is changed during an experiment, then outgassing at the sample can be a significant problem. This is addressed by including reservoir preheating in the experimental setup. However, reservoir heating is unable to supply heated solutions to the chamber and is best used in combination with an in-line solution heater.

Of course, all three approaches can be used in combination to provide efficient thermal regulation under virtually any condition. Warner Instruments provides high quality single and dual channel temperature controllers, as well as solution reservoir, chamber platform, and in-line solution heaters. We invite you to call our technical support staff and we will be glad to aid you in determining the best application of these heating strategies to your experimental setup.