A Simple Stage Heater / Cooler For Light Microscopy

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INTRODUCTION

Microscopists frequently find that they need to regulate temperature during the course of their experiments. This is especially true for cell biologists working with living cells. Excellent commercial equipment does exist to control the stage temperature of light microscopes, and regulation by such devices is tightly controlled; ± 0.1° C is routinely reported. However, such equipment is often quite expensive and may not provide the investigator with the certainty that the experiment is being performed at exactly the temperature reported by the readout of the thermoelectric control, since the temperature sensor is often at a distance from the actual site of observation. The temperature also varies considerably from the stage heater/cooler to the critical, observed portion of the slide. Since objective, stage and condenser can act as very effective temperature sinks, especially when the slide is fully oiled, the investigator must depend upon a long equilibration time for an accurate temperature readout. Described herein is an inexpensive and easily constructed thermoelectric module that provides very accurate current temperature readout in the Immediate vicinity of the experiment, just outside of the region of illumination and nearly in direct contact with the biological specimen. Although some drift can be expected from the design as described, *the design can be easily modified to provide a negative-feedback* power supply to regulate the local microscale temperature. A typical current/temperature curve is presented. The device described has been used to study the kinetics of dynein/microtubule interactions using a gilding microtubule assay after the method of Moss *et al.*, (1992).

MATERIALS, CONSTRUCTION AND CALIBRATION

Overall Construction:

The assembled system is shown in Figure 1. The design as described, including the Lambda power supply, costs considerably less than $500 but will, of course, vary with local machine-shop expenses and materials cost. The assembly can be separated into three distinct sections: a thermally-conductive aluminum slide block (Figure 2), a plastic insulating block holder (Figure 3), and a removable Sylgard™ film that contains the thermistor and provides a fluid space for the biological experiment (Figure 4).

The Metal Slide Insert:

The thermally-conductive slide component is machined of aluminum, and should be anodized to protect it from corrosion caused by biological salt solutions (Figure. 2). Alternatively, and from the viewpoint of toughness as well as corrosion-resistance, the slide can be machined from stainless steel. It is very important to keep the open viewspace in the slide (a 3 mm wide slot at the end of the aluminum or stainless insert) as small as possible in order to maintain the best temperature control.

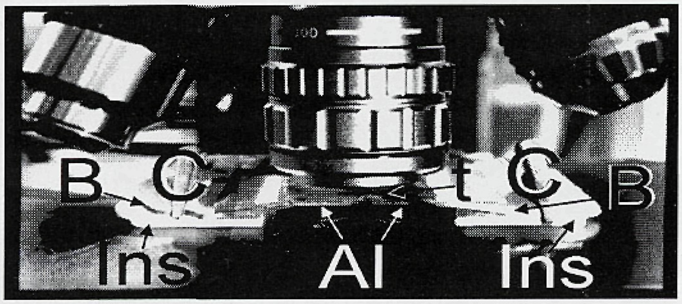

Figure 1: Overview of the thermistor-regulated Peltier-based heating and cooling device. Application is in an upright microscope (Olympus BHS). The flat surface is upward toward the objective, to allow the lenses to freely index in place. Several objectives have been removed for clarity. A high power objective (100X planapochromat) is fitted over the specimen. Code: t, thermistor location; Al, aluminum plate; Ins, plastic insulator plate; C, clip; B, brass thermistor lead extensions.

In the design of the optical and biological chamber, it was important to try to maintain the normal optics and light-path length as close as possible, since the original work was performed at full NA (numerical aperture) under demanding high resolution conditions. The thin end of the thermally-conductive insert was therefore built so that #1 coverslips (22 mm square by approximately 0.1 mm thick) could be fitted to either side of the 0.8 mm-thick section, secured snugly with a tiny dab of vacuum grease, and filled with water to simulate a normal-thickness slide. This satisfys the optical needs of the objective lens. The region of interest in our case could be very small since we were examining microtubule movement, and so a small coverslip chip was further attached to the concave surface of the aluminum slide, again with vacuum grease. The objective viewed the center-most region of the slot, with very little lateral (x-y) displacement possible.

The aluminum block was drilled and tapped to accommodate an acrylic strap that secured the Pettier device. Pettier devices transfer heat across their two flat surfaces dependent upon the polarity of the applied current; one side becomes cold while the other becomes warm. Therefore, although the Peltier device must be tightly attached, a heat-conducting strap cannot be used because heat is carried to or from the slide in a small waterjacket mounted to the reverse side of the Peltier device. A thermally-conductive strap would transfer heat back to, or away from, the aluminum plate and would counter the effects of the thermoelectric module.

The Peltier thermoelectric module (we used a Cambion model 806-1006-01 with an integral water jacket, Cambridge Thermionic Corp., Cambridge, MA) was mounted tightly to the surface, with heat-sink compound (GC Electronics, Southern Electronics, Opelika, AL) to intimately connect the Peltier unit and the aluminum slide. Deionized water was used as the working fluid; the water line being a very flexible thin-walled vinyl tubing that ran from a nearby sink, through the water jacket, back to the sink. Water flowed at approximately 100 mL per minute through the system and did not affect the operation of the microscope. Vibration from water flow through the water jacket was not observed.

The Peltier thermoelectric module was powered by a stand-alone DC regulated low voltage, high current power supply, (model 300, Lambda Electronics, Westborough, MA). A Nobotron unit would also work very well. It could alternatively be powered by an automobile battery through a variable resistor (a "Variac") as are found in many laboratory settings, particularly for electrophysiological applications. The power supply must be able to deliver as much as 9 A at 12 V in order to provide full power to the thermoelectric module.

The Insulating Slide Insert Holder:

The aluminum slide was mounted into a machined polyethylene plastic insulating holder (Figure 3). This isolated the aluminum slide from the stage and *allowed the Peltier device to easily displace the slide temperature from the ambi*ent temperature. As a result, the operating temperature of the slide could vary over 0 - 40° C, with temperature changes of 10° C made in less than 2 minutes. Tolerances between the aluminum slide and the plastic insulating holder are not particularly tight, allowing expansion and contraction of the aluminum plate with-

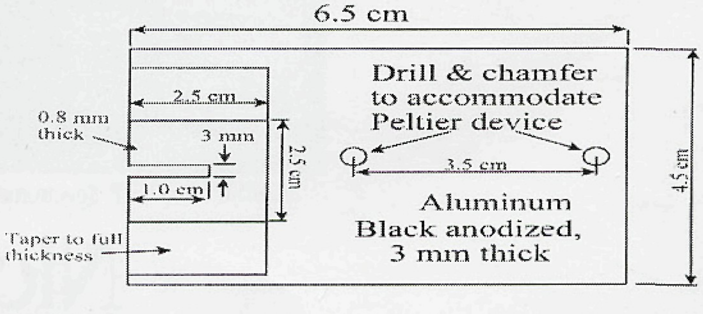

Figure 2: Thermally-conductive aluminum plate. Coverslipped region is in the 2.5 X 2.5 cm square to the left. Peltier device/water jacket (or cooling fins) must be attached to the plate by a nonconductibe strap. In the author's example it was made from acrylic plastic.

out deformation of the plastic assembly, although detectable drift in the aluminum holder must be held to a minimum.

Thermistor Mount:

The thermistor used in this application is an exceptionally tiny and delicate electronic element, and must be protected from damage by embedment in a uniform, tough medium. The black thermistor is encased in a clear glass 350 µm X 500 µm bead with 25 µm Pt-Ir leads. The paradox with the current arrangement is that although rather fragile, it must also be easily removed to allow cleaning of the chamber. Sylgard™, a flexible but tough, polymerizable silicone elastomer (Dow Corning, MI) normally used for a variety of electronic embedment applications, was used to protect the thermistor yet retain ease of removal and remounting. The extremely small, bipolar lead thermistor (Fenwal #GB4IL2; Fenwal Electronics, CT) was embedded in approximately 400 µm thick Sylgard™.The precise overall dimensions of the Sylgard™ film must allow the insertion of the thermistor into the observation chamber end of the aluminum slide near to, but outside of, the illumination path. The thermistor will strongly absorb light and thereby heat up artifactually if placed in the light path. Figure 4 depicts the thermistor/Sylgard™ film assembly.

Brass shimstock (0.001 inch) was used as a tough connecting conductor from the thermistor to the outer edge of the Sylgard™ film. The thermistor leads were carefully soldered in place on the brass, keeping the solder film very hot and thin, and allowing proper cooling characteristics to avoid a cold-soldered joint, while carrying out the process as quickly as possible. Since the thermistor conductors are a 0.001 inch diameter platinum-iridium alloy (and therefore not actually solderable), they were very carefully coiled around the shimstock material prior to attachment, and gently soldered in place. Solder assembly of the thermistor unit must involve great care by the technician since the thermistor is easily damaged by the high heat of the iron. Flat, polished heat sinks must be used between the iron and the thermistor bead to protect the fine wires and the thermistor unit.

A 2 mm-wide channel was cut in the Sylgard™ to allow space for the biological sample, and to allow exchange of fluid via perfusion. The Sylgard™film/thermistor assembly was held in place with a thin film of silicone vacuum grease. The shimstock leads were clamped and stabilized by miniature flat-tip copper-coated spring clips (Radio Shack cat. no. 270-373) mounted to the plastic insulating holder with hot-melt glue. The leads from the clips were made from fine, limp "pillow-speaker" wire to prevent shifting of the slide when mounted on the stage. The leads were then attached to a conventional digital VOM typically found in electronics shops. We used a common model from Radio Shack.

Calibration of the Thermoelectric Module:

Calibration of the thermistor unit was carried out by laying the thermistor/Sylgard™ film assembly in an aluminum boat that floated in the reservoir of a recirculating water bath. The thermistor assembly described here was calibrated at 5 degree intervals over 0 - 40° C. The calibration curve for the described unit is shown in Figure 5.

Operation:

Operation is very simple: The operator attaches coverslips around the thin (0.8 mm) portion of the aluminum assembly, and the aluminum plate is mounted into the plastic insulating holder. The Sylgard™ film is secured with silicone grease into the aluminum plate. The microclips are adjusted to hold the brass shimstock extensions. A third coverslip is mounted in position over the window cut in the Sylgard™ film, allowing the groove to be exposed on either end if the investigator wishes to perfuse the assembly. The entire assembly is mounted onto the stage carrier. Water is applied to the open edge of the 0.8 mm thick region to simulate the full thickness of a glass microscope slide. The author has successfully followed multiple reactivation and microtubule gliding experiments

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over a range of 2 to 40° C using this apparatus.

The design given here fits into a microtiter plate holder for the IM 35. Since the observation region is so small (in order to maintain good control over the chamber temperature), the assembly cannot be moved laterally very much. The operator monitors thermistor resistance while adjusting the current output of the power supply. Thermistor resistance is inversely proportional to the temperature (see Figure 5) and the operator can quickly determine the temperature. Although this procedure might at first sound cumbersome, with a little practice the device can be preset to different power outputs corresponding to specific desired temperatures, and quickly and reliably adjusted to a new temperature.

The Peltier/thermistor apparatus can be set up to drive negative-feedback circuitry to automatically regulate temperature, with a concomitant increase in complexity, time-to-completion, and cost. For many applications this is easy to do and desirable; many such control circuits are available through well-known sources (Horowitz and Hill, 1980). Inoue and Spring (1998) list several appropriate references in which various approaches have been presented. In particular, the circuitry of the designs of Chabala *et al.* (1985) and Datyner (1985) describe, in very great and clear detail, the construction of feedback circuitry designed to regulate temperature using a slightly different scheme, by using thermistor-regulated feedback.

DISCUSSION

Utility of the Design. Considerations and Cautions:

The design described here is intentionally simple, and can be easily fabricated by the investigator. There are many far more sophisticated designs reported in the instrumentation literature (In particular, see the elegant design of Delbridge *et al.*, 1990 in which the fluid is also controlled with regard to level, flow rate, and temperature.) However, many investigators have limited resources (and time and money) and this design has worked very well in a particular application - the reactivation of motility in a gliding microtubule assay. Also, many designs involve the use of a considerable amount of a working fluid for heating or cooling (*e.g.*, Inone *et al.*, 1975) and although they work very well, their complexity is likely to result in more leakage and potential difficulty to the investigator. I describe here a scheme to use water to draw off the heat (if the thermoelectric module is used for cooling); however, depending on the application, the user could choose to use a very efficient finned air heat-sink, such as is used on computer CPU chips. Such fins are available at electronics supply houses such as Allied Electronics (Ft. Worth, TX). Such an approach would entirely eliminate potential vibration from water flow, and would also eliminate the danger of a water leak near expensive components. However, the design described here never produced any significant difficulties.

Another approach, suitable for work under constant temperature, is an air-curtain assembly that heats the entire stage and optical path uniformly. Such a system provides the very best stability, and the very best image resolution, at temperatures that are distinctly different from ambient. It also has been used very effectively for culturing cells directly on the microscope stage, and for following cells for an extended period of time (McKenna and Wang, 1989).

The design described here was originally made for an inverted microscope (Zeiss IM 35) but has been used quite effectively on an upright model (BHS Olympus). Depending upon the resolution requirements of the experiment, the operator has the option to oil or not oil the flat condenser surface of the apparatus and the chamber cover glass. Shifting between upright and inverted designs merely requires the relocation of the coverslipped assay space housing the specimen by inverting it. Oiling of course greatly improves the resolution by increasing system NA, but introduces a much greater heat sink problem, especially

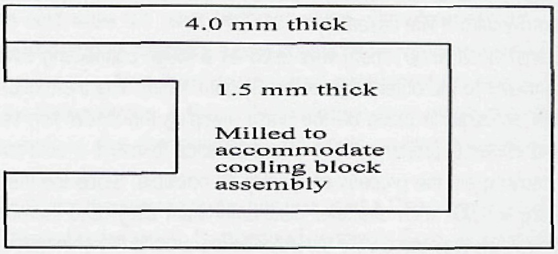

Figure 3: Polyethylene block milled to accommodate the aluminum block of Figure 2. The aluminum block drops into the central area. Dimensions are approximate.

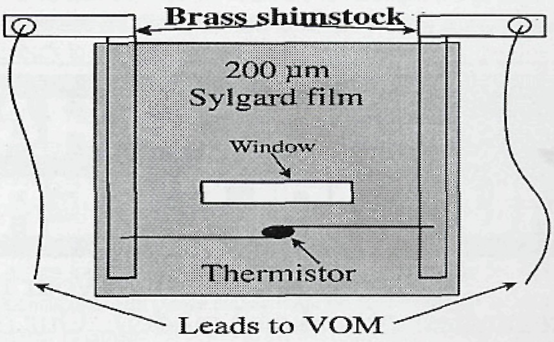

Figure 4: Sylgard™ film embedment of thermistor and brass shimstock assembly. The embedment film was made by building a structure out of plastic coverslips and carefully sealing them to make a flexible mold. Thickness was determined by stacking cover slips and then covering with a glass slide. The assembly was allowed to sit overnight at room temperature to stabilize, then heated for several hours at 60 degrees to assure complete polymerization of the Sylgard™ silicone polymer.

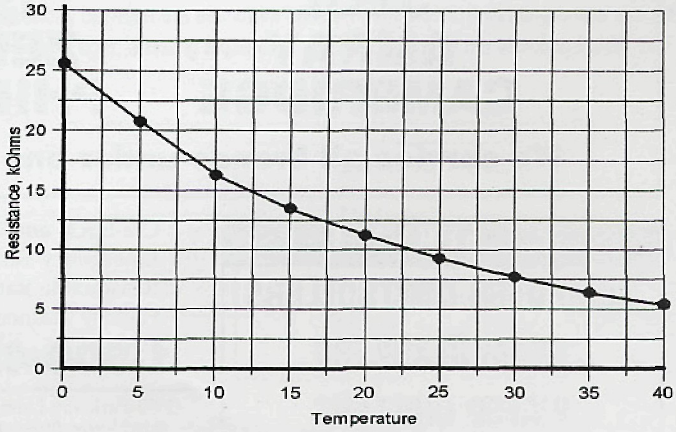

Figure 5: Thermistor response from 0 to 40° C.

if the condenser is also oiled. Furthermore, it is obvious to this author that immersion oils vary in refractive index as the temperature changes; this was, in turn, manifested as changes in focus position and clarity as the temperature was lowered. If the investigator can avoid the use of oil, the temperature control will be much easier with this apparatus. If high numerical aperture is required, substitution of high NA water-immersion lenses, for the more typical oil-immersion objectives, would prevent development of oil/water emulsions that occur in cooled, oiled preparations.

Probably the most difficult aspect regarding day-to-day usage involves condensation of water around the cold objective barrel. Damage to the objective can be alleviated somewhat by sheathing the lens barrel with Parafilm™or a similar wrap. Sufficient insulation to prevent significant condensation requires several layers of tightly-fitting latex glove fingers with a hole cut in the finger tip. This can be difficult and the gloves must be routinely replaced. The opportunity for damage via repeated handlings, the possibility of dropping the lens, etc, make this a less-than-optimal approach.

Long-term exposure of the objective and condenser to fluctuations in temperature can result in loosening of the lens elements, with the unpleasant result that very expensive equipment can be rendered permanently damaged by such experiments. Furthermore, strain-free objectives, such as are used for differential interference and polarization microscopy, that are exposed to temperature fluctuations are likely to develop strain as a function of thermal fluctuations (See Inoue and Spring, 1998 for an in-depth discussion of these dangers) so that their crossed-polars extinction will be greatly attenuated. The investigator needs to balance such effects against the potential payoff of the temperature-control experiment. ■

LITERATURE CITED

Chabala, L. D., R. E. Sheridan, D.C. Hodge, J. N. Power, and M. P. Walsh 1985. A microscope stage temperature controller for the study of whole-cell or single-channel currents. Pfluger's Arch. 404:374-377.

Datyner, N. B., G A Gintant, and I. S. Cohen, 1985. Versatile temperature-controlled tissue bath *for* studies of isolated cells using an inverted microscope. Pfluger's Arch. 403:318-323.

Inoue, S., J. Fuseler, E. D. Salman, and G. W. Ellis, *1975.* Functional organization of mitotic microtubules. Physical chemistry of the *in vivo* equilibrium system. Biophys. J.15:725-744.

Inoue, S. and K. R. Spring, 1998. *Video Microscopy. The Fundamentals*, 2nd ed. Plenum Publishing Corporation, New York, NY. 741 pp.

Horowitz, P. and W. Hill, 1980. *The Art of Electronics*: Cambridge University Press, New York, NY. 716 pp.

McKenna, N.M., and Y-L. Wang, 1989. Culturing cells on the microscope stage. Meth. Cell Biol. 29: 195-205.

Moss, A. G., J-L. Gatti, and G. B. Witman, 1992. The motile β/IC1 subunit of sea urchin sperm outer arm dynein does not form a tight rigor bond. J. Cell Biol. 118 (5):1177-1188.