Temperature Control Systems for Light Microscopes

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(Received September 4, 1986)

The development of new techniques in light microscopy has expedited considerable progress of the research of cell motility. By these techniques, even subtle changes in the structure related to the motility can be observed and also recorded from intact cells as well as their demembranated models. It is important for light microscopic observation and recording of the cellular motile events to control the temperature of specimens and their surroundings, because the energy needed for motility is provided by chemical processes which, in common with all other chemical reactions, are directly influenced by temperature.

Small, constant temperature devices using a thermoelectric converter were constructed for the purpose of controlling the temperature of light microscopic specimens lower than ambient temperatures. By reducing the temperature of specimens and their surroundings, and by holding it constant, the cellular motile events especially at low temperatures could be easily observed.

Electric circuits of the temperature control system are shown in Fig. 1. A solid state thermoelectric device (CP2-31-06L, MELCOR Japan, Tokyo) directly cools a copper board on which a microscope slide is placed.

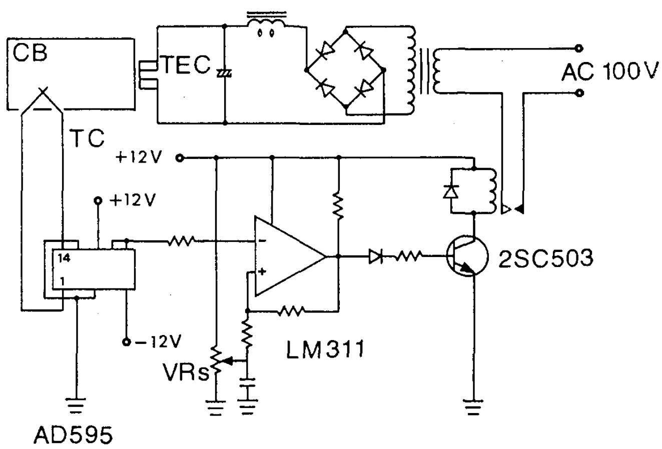

Fig. 1. Electric circuits of temperature control systems. CB; copper board directly cooled by thermoelectric coverter (TEC), TC; thermocouple, VRs; a variable resistor which gives a reference voltage for temperature setting.

The temperature of the copper board is monitored by a chromel-alumel thermocouple and converted to a linear voltage out put (10 mV/degree) by a thermocouple amplifier (AD595, ANALOG DEVICES, Massachusetts, USA). The out put voltage is compared with a reference voltage simply by a comparator (LM311), which drives a transistor (2SC503) to make on and off the supply AC through a relay. It may make some difficulties in driving thermoelectric devices that DC supplied to the devices reaches to a level of several amperes. The circuits demonstrated here are one of the simplest solution for controlling such a large DC. As a result of current control by a discriminator the temperature of specimens changes in a saw-tooth manner, in which the amplitude of variation is dependent on the degree of hysteresis. The comparator IC noted above used for the

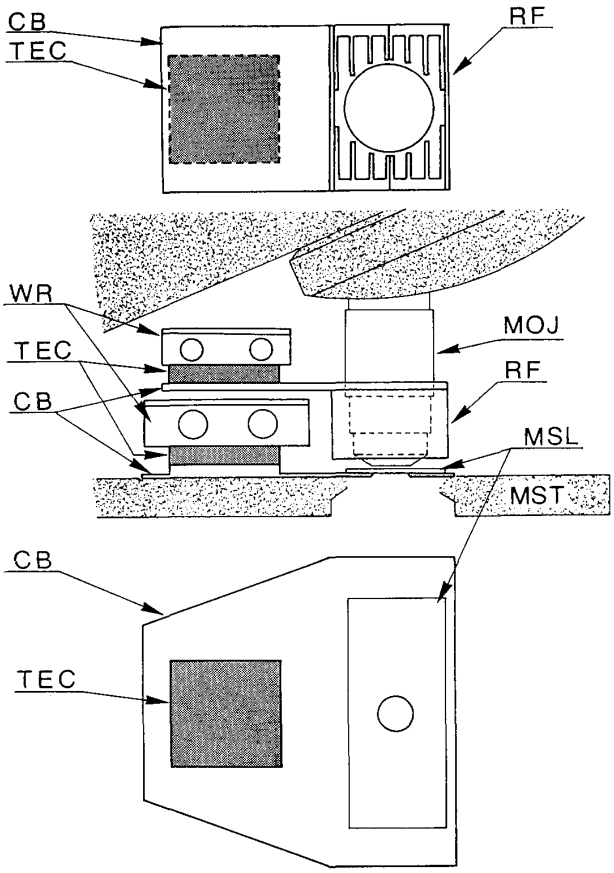

Fig. 2. Illustrations for mounting of temperature control devices on a light microscope. Two different types of devices are shown in the middle part, each of which is shown separately in the upper (device for controlling the temperature of microscope objective) or in the lower part (device for controlling the temperature of microscope slide). The upper part illustrates also the structure of radiator fins (RF) for heat absorption. CB; copper board, TEC; thermoelectric converter, WR; water-circulated radiators for cooling the thermoelectric converter, MOJ; microscope objective, MSL; microscope slide, MST; microscope stage.

purpose of discrimination was effective in controlling temperature with high stability. For operating the thermoelectric devices efficiently, adequate radiation from the devices is required. To minimize the total size of the apparatus, a water-circulating radiator is employed here (Fig. 2). The temperature of circulating water does not necessarily have to be controlled ; conveniently it is useful to circulate tap water directly from the laboratory waterworks.

The devices actually mounted on the microscope are shown in Plate 1. Two different types for different uses were developed and simultaneously mounted. One is placed on the microscope stage to control directly the temperature of a microscope slide (Plate 2). Another is mounted just over the slide with a radiator surrounding a microscope objective (Plate 3). Mounting each device on the microscope is illustrated in Fig. 2. The second type of device is used specially for temperature control of the objective to reduce heat conduction from or to the objective when the objective makes contact with the slide by means of oil immersion. The use of this device is also effective in controlling the temperature of the local atmosphere around the slide slip, preventing dew condensation on the surface of both slide and objective when temperature is set far below ambient temperature. When mounting the temperature control devices on the microscope stage, a sheet of Japanese paper is inserted between the

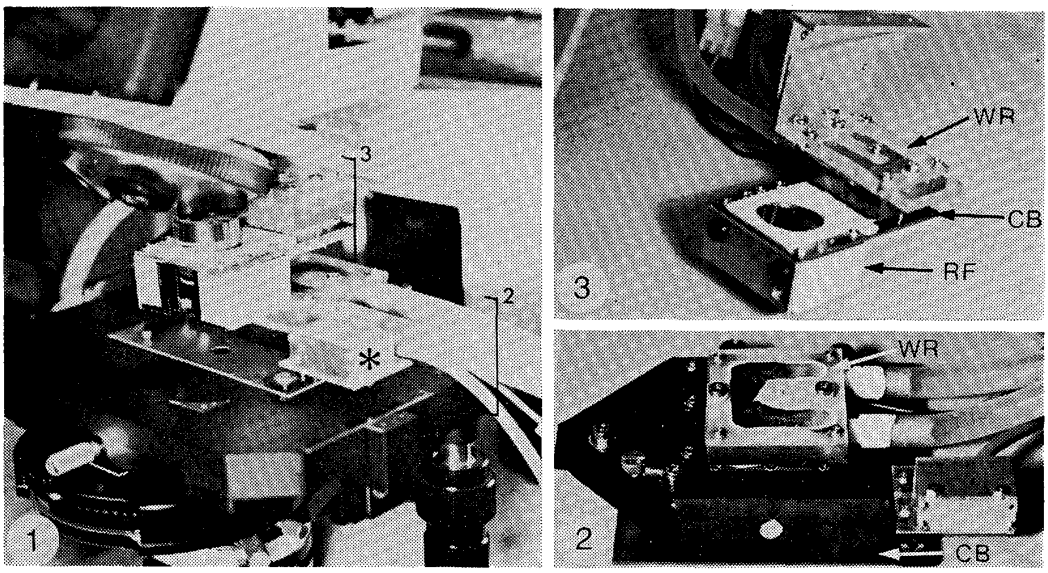

Plate 1. Temperature control devices mounted on a microscope (BHS, Olympus, Tokyo). Two different types of devices are mounted, each of which (indicated by labeled square brackets) is separately shown in Plate 2 or Plate 3. Asterisk indicates the location of a thermocouple amplifier for temperature sensing.
Plate 2. A device for directly controlling the temperature of microscope slides.
Plate 3. A device for controlling the temperature of microscope objectives.
Abbreviations, for Plates 2 and 3, are the same as in Fig. 2.

device and the stage. This material is most suitable as an insulator in this arrangement, since the space where the insulator should be inserted is limited because of the short working distance of microscope condenser. A paper napkin for tea ceremony was used for this purpose in our laboratory.

Fig. 3 shows the cooling ability of the temperature control devices described here. Time of attaining the set temperature is shown as a function of the difference, ΔT, between that temperature and the ambient temperature. The upper limit of ΔT which can be attained is ca. 17°C as indicated by open circles with the first control device set on the stage (Plate 2) is increased up to 23°C by using the second device (Plate 3) in combination with first as indicated by solid circles. The practical working ranges are $\Delta T=0-15$°C and 0–20°C under these two conditions, respectively, where ΔT can be attained within 20 min. The ability of the devices demonstrated here suggests that the specimens for light microscopy can be easily cooled down near 0°C at ordinary room temperatures. Since the temperature far below 0°C is not usually required for the specimens to be investigated in an aqueous environment, it seems likely that the devices described in this paper is useful enough for many of biological researches.

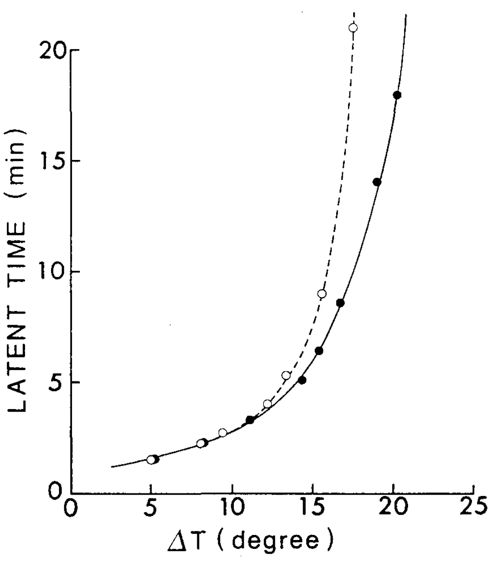

Fig. 3. Cooling ability of the temperature control systems. Abscissa, temperature difference (ΔT), the ambient temperature minus the set temperature of a microscope slide to be regulated by the temperature control devices. Ordinate, time of attaining the temperature difference. Temperature on the microscope slide was measured by a thermocouple-thermometer at the center of the field of view of a microscope equipped with a ×40 objective (NH SPlan, Olympus, Tokyo). The cooling ability of a device when controlling the temperature of the microscope slide (Plate 2) is demonstrated by open circles with the broken line and that of two devices (Plate 2 and 3) when used simultaneously is by solid circles with the solid lines.