Temperature controlled Microscopy

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Physiological processes in cells are strongly temperature dependent, they may not occur under and above a certain threshold, but also the duration of e.g. developmental processes or movements may vary strongly with temperature. With a standard preparation in a light microscope it is impossible to keep the sample at a constant temperature during observation. The rather strong microscopic illumination leads to an undefined temperature increase, especially in irradiation absorbing samples. This temperature increase may cause unwanted and uncontrollable effects. In this chapter different attempts to control the temperature during microscopic observation are summarized and a recently constructed new system - the Light Microscope-Temperature Controlled Chamber (LM-TCC) is introduced. The special feature of the LM-TCC is the Peltier-element temperature control of a rather massive metal specimen holder for biological samples. This system works in a temperature range of -10°C to +95°C with an accuracy of $\pm$0.1°C and allows rapid temperature shift rates.

Keywords: Peltier-element; temperature

1. Why control the temperature on a microscope?

The thermal situation on a microscope is generally underestimated and often widely overseen. Temperature shifts may be generated in the microscope room through air conditioning or heating, influencing the temperature of the specimen, which of course must be avoided. To a much greater extend as fluctuation of the room temperature, the illumination system on the microscope and the heat absorption of the specimen contributes to the actual temperature of the specimen.

This is especially problematic because with the development of fluorescent proteins (GFP, YFP etc.) the need for live cell imaging was drastically enhanced [1]. Time course experiments of movements, cytoplasmic streaming, dynamics of cytosekeletal components demand on the control of the temperature during observation and are useless without it. This is because physiological reactions strongly depend on defined temperatures to be fulfilled at all or to be maintained at a certain velocity. A practical example shall illustrate the problem: when a leaf segment in a standard microscopic preparation (cover slip, slide with a liquid volume of 100 µl) is illuminated with 4500 µmol photons$\cdot m^{-2}\cdot s^{-1}$ (a value that is a realistic light demand for proper illumination with DIC optics) a temperature increase δT of 18.0 °C was measured [2].

2. How to control the temperature on a microscope?

There have been several attempts to control the temperature in a microscope. These include tempered microscope stages, perfusion chambers and the control the temperature of especially constructed microscope chambers with Peltier elements.

2.1 Tempered microscope stages

For keeping the temperature of a standard microscopic preparation at a certain preset level, commercially available tempered microscope stages (eg. THMS 600, Linkam, Surrey, UK) are reasonable. These systems are adequate for several demands, however mostly it is not possible to perform rapid changes of

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the temperature during the observation process and therefore commercial systems are not further discussed here.

2.2 Perfusion chambers

A definition of a perfusion chamber can be given as following: it is a vessel on the stage of a light microscope that enables a living specimen to be followed continuously while also allowing the fluid bathing of the specimen to be rapidly exchanged during the observation period. Perfusion with medium from a temperature controlled source may be an effective method for regulating specimen temperature or even to generate temperature shifts. This however can only be achieved when the flow rate and the total volume are large enough for the heat or cold transfer [3].

While in a regular perfusion chamber the temperature is regulated via the temperature of the perfusion medium itself [4,5,6], a rather sophisticated temperature control unit of a perfusion chamber has been constructed by [7]. There, the temperature regulated perfusion chamber consisted of two closely apposed flow assemblies built in a sandwich-like construction forming a functional unit, but containing two separate liquid circuits. The coolant flowed through the lower chamber, whereas the perfusion chamber is a separate unit. This construction was made of several thin glass sheets glued together using pieces of ordinary slides and cover slips. As drain and feed lines synthetic tubes were used. Inside the chamber a heat dependent electrical resistor served as a thermometer probe. A second layer of glass sealed the cooling chamber and served as the bottom of the sample chamber. At this partition the heat exchange took place. The third layer of cover slip glass defined the size and height of the sample chamber. This of course was limiting to the volume the sample chamber could have. If there was a rapid flow of perfusion medium, it took a certain amount of time to fully exchange the temperature.

Moreover in perfusion chambers adequate mounting of the specimen has to be guaranteed, which indeed may be a demanding task. Treating the cover slip with poly-L-lysine may be adequate for some samples, whereas others may be damaged.

2.3 Peltier-Element based temperature control

One early attempt to control the temperature on the microscope stage with a Peltier element has been reported [8].

Recently a system has been introduced that allows to control the temperature of the microscopic preparation, which has been termed LM-TCC (Light Microscope Temperature Controlled Chamber) [2]. This system bears the advantage that it is capable of performing fast temperature shifts, with extremely low overshooting.

The system is basically composed of three different compounds: Specimen holder with integrated object chamber; 2. Temperature generator; 3. Associated facility. The construction and dimensions of the components was described in greater detail in [2]. A schematic representation of the components is given in Fig. 1, the arrangement at the microscope is illustrated in Fig. 2. Here, only the components are briefly listed to give an adequate overview.

The **specimen holder** was constructed from a standard aluminium block, a highly sensitive temperature sensor (Pt-100, W-EYK6, Heraeus Sensor Technology, Hanau, Germany) was integrated in the aluminium block. The block contained a cylindrical hole, which was sealed from the bottom with a cover slip creating a object chamber, which holds approx. 1 mL of liquid. When operated the whole unit was covered with a second cover slip from the top. The specimen holder was insulated against temperature flow to the microscope stage with fibre-glass elements.

The **temperature generator** was constructed of an aluminium contact plate, which can be connected to the specimen holder. The key element of the temperature generator was a PELTIER-element (Seal TEC 1.4-027-145L, Melcor Corp., Trenton, NJ). A miniature connector coupled this element to the associated facility for electric charging (12V/4.5A). To remove incoming temperature energy including

the dissipation loss of the system a custom built copper cooling body was directly connected to the PELTIER element. As coolant hydroxy-phospho(n)-carbon-acid (Antricorro Fluid, Aquatuning Schloss Holte-Stukenbrock, Germany), in an 1:50 dilution with distilled water was used. A pump (Type 1250019, Eheim, Deizisau, Germany) exchanged the coolant continuously in a circuit, removing thermal energy when passing through the cooling body. A ventilation heat-exchanger cooled the coolant.

The **associated facility** consisted of a powerful stabilizing power supply (JWS 100/12, 12V/8A), Lambda, Achern, Germany) and relays (G4F-11123T, Omron Electronics, Langenfeld, Germany).The relays switched the supply voltage of the Peltier element on and off in a timely and polarity dependent manner. The relays were controlled by a PID controller (Model 5120, Drews Electronic GmbH, Kamp-Lintfort, Germany). This unit allowed to preset the temperature or program individual temperature curves through defining cooling or heating velocities. The system worked in a temperature range from –10°C to +95°C with an accuracy of $\pm$ 0.1°C. This was measured at high frequency (8 Hz) with a thermocouple sensor (Type T, solder junction diameter: 0.2 mm, Thermo-Est, Vienna, Austria) and recorded on a data logger (CR10X, Campbell Scientific, Loughborough, UK).

The advantage of this system was the relatively large volume of liquid that can be directly cooled or heated transferring the temperature directly to the sample. With the LM-TCC heating rates of 12.9°C·min^{-1} and cooling rates of 6.0°C·min^{-1} could be reached [2]. This was performed with an accuracy of $\pm$ 0.1°C in the stationary phase and only a relatively small overshooting ($\leq$ 1.9°C) during rapid temperature changes [2]. When returning to the initial example (chapter 1) where a leaf segment was warmed with a δT of 18°C at an illumination of 4500 µmol photons·m^{-2}·s^{-1} this value dropped to δT of 2.5°C in the LM-TCC under the same irradiation conditions [2].

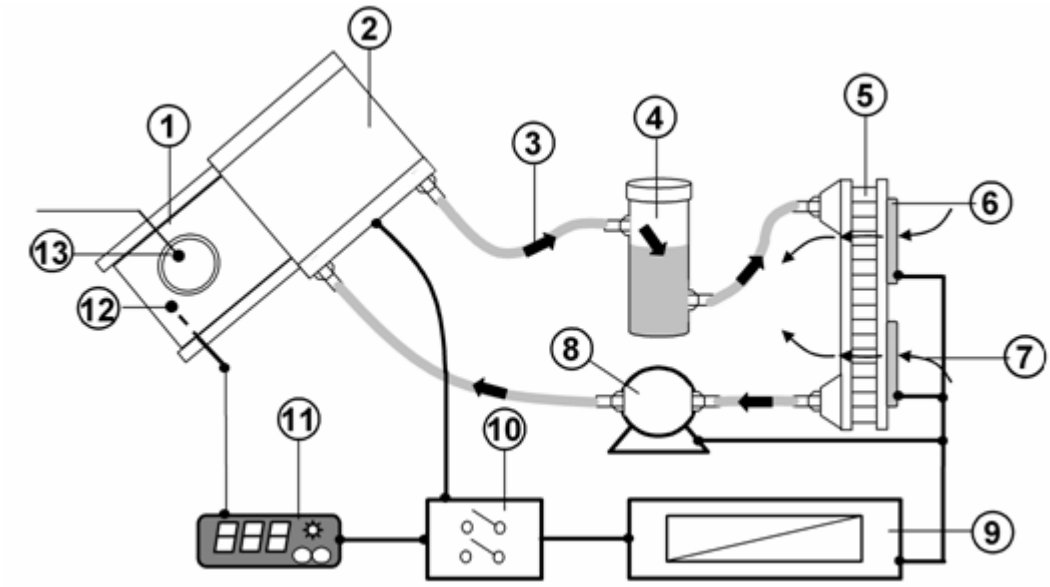

Fig. 1 Diagram of the LM-TCC constructed by Buchner et al. [2] including specimen holder, temperature generator and associated facility. 1 specimen holder, 2 temperature generator, 3 hose for coolant circuit, 4 equilibrium chamber, 5 heat exchanger, 6 fans for directing the airflow (7), 8 pump (Type 1250019, Eheim), 9 power supply (JWS 100/12, (12 V/ 8A), Lambda), 10 relay unit (G4F-11123T, Omron Electronics), 11 control unit (PID-controller), 12 temperature sensor (Pt-100, W-EYK6, Heraeus Sensor Technology), 13 thermocouple sensor (Type T; solder junction diameter: 0.2 mm). The control unit (11) compares the actual measured temperature in the specimen holder (1) with the target temperature. An output signal is produced giving pulses for the relay unit (10) which connects the power supply (9) with the temperature generator (2) which cools or heats the specimen holder (1) according to given values. A pump (8) drives the cooling circuit, heat is removed from the temperature generator (2) and dissipated at the heat exchanger (5). Reproduced from J. Microsc. 225, 183-191 (Blackwell) with permission

Fig. 2 Micrograph showing the arrangement of the specimen holder (arrow) and temperature generator (arrowhead) of the LM-TCC constructed by Buchner et al. [2] at a Zeiss Axiovert 200 M inverted Microscope. The temperature generator which includes the Peltier-Element is connected to the specimen holder on one side allowing proper illumination of the specimens in the specimen chamber

3. Concluding remarks

Other attempts to control the temperature of a microscopic preparation have been the development of objective lens heaters [9] or climate controlled boxes. Such systems depend on resistive coils or circulating warm water or air currents [3]. It can be figured that such systems are capable of maintaining a given temperature, however, rapid temperature chances can not be performed.

One unambiguous advantage of perfusion chambers is the possibility of medium exchange during the microscopic observation and the temperature controlling process [10]. This can be especially important, when it is attempted that the samples are exposed to different chemicals, or when a certain level of dissolved gases has to be maintained. However, there are several disadvantages like slow reaction to changed temperatures, and the problem of adequately mounting the sample without harming it during the preparation. Only a Peltier-element based system, where the liquid medium was directly cooled or heated could overcome these disadvantages. This was allowing a rapid and accurate temperature control directly in the specimen chamber with a rather large volume. Rapid temperature shift rates, with low overshooting could be realized in the LM-TCC [2].

Recently this Peltier-element based temperature controlling system has been successfully applied to elucidate the temperature dependent formation of chloroplast protrusions in the model plant *Arabidopsis* [11] and several high alpine plants [12].

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