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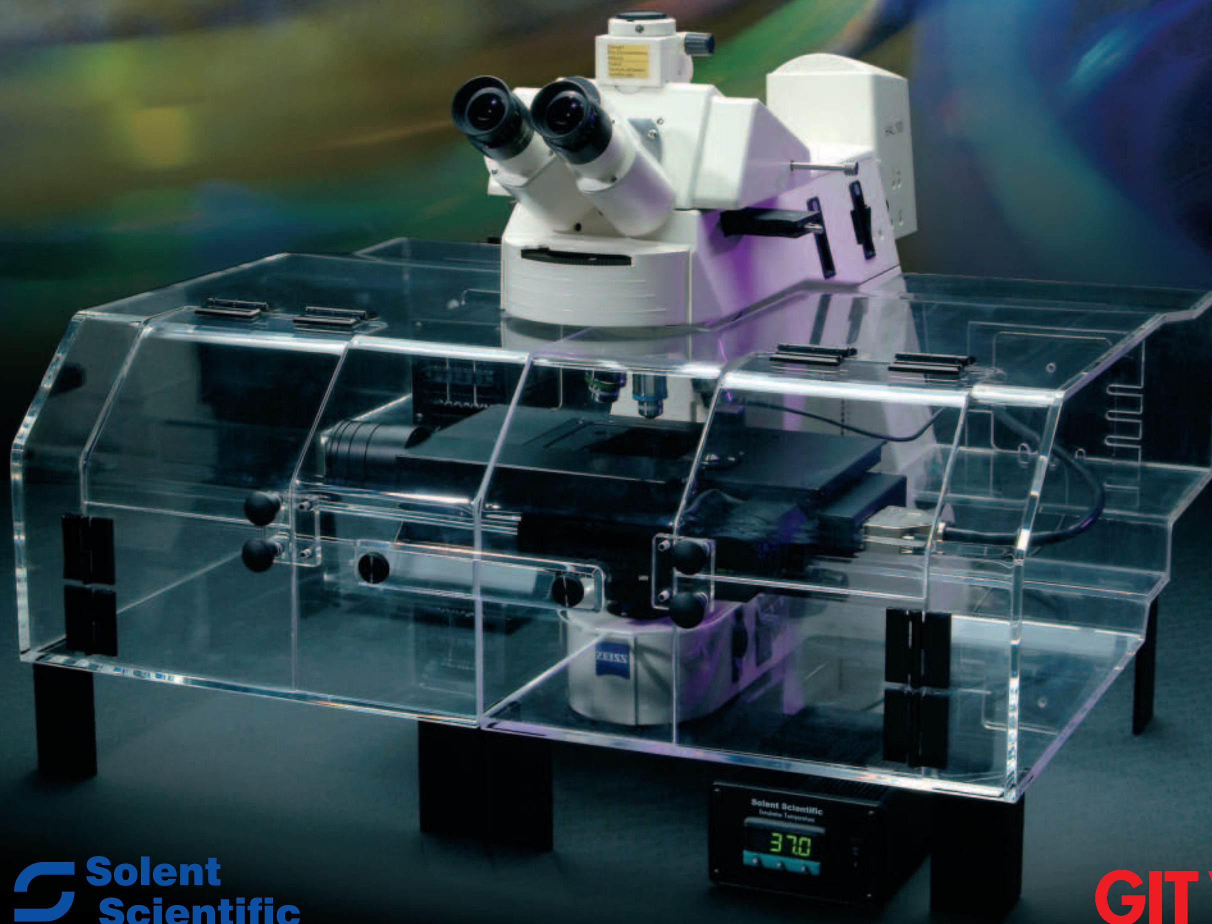

Solent Scientific

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Environmental Control for Live Cell Imaging

Advances in computing power and high resolution cameras have opened many new avenues of biological research over the past 8-10 years. The Solent Scientific full enclosure microscope incubation chamber supports such research by enabling live cell imaging to be studied routinely in the laboratory.
The basic optical experiment has been refined such that high speed, high sensitivity observations can be made, with minimal stress being placed on the specimen. Now, software packages allow the researcher to undertake image analysis and archiving in a fraction of the time taken only five years ago. A variety of image contrast enhancement techniques, such as Differential Interference Contrast, Hoffmann Modulation Contrast, Fluorescence, Confocal and Multi-Photon microscopy have all proved important. Combined with the use of specialised dyes to highlight certain types or parts of the cell, or with the use of electrodes or perfusion techniques to mimic in vivo stimuli, these techniques have created a rapidly expanding area of research into the fundamentals of cell and molecular biology.

In cloning and microgenics, the removal and replacement of nuclei and the addition of cellular material using laser microbeam or optical tweezer techniques are all commonplace. Meanwhile, outside of the research world, developments in in-vitro fertilisation now means that intra-cytoplasmic sperm injection (ICSI) can be used to inject a single sperm cell directly into an egg. In order to provide useful information for these studies, it is essential that the cell's host environment is reproduced as closely as possible, whilst at the same time ensuring the cells are accessible to microscopic examination.

Cells are routinely cultured in laboratory tissue culture incubators. The characteristics of these enclosures are that temperature is controlled, humidity is kept close to saturation and the atmosphere is maintained at 5 % CO_2 in air. Observation of the living cells under the microscope, however, is not possible with such an incubator. For live cell imaging experiments the conditions maintained in the laboratory incubator must therefore be mimicked in the microscope incubator.

Fig. 1: The iMIC Digital Imaging Platform from TILL Photonics fitted with a Solent Scientific Environmental Chamber

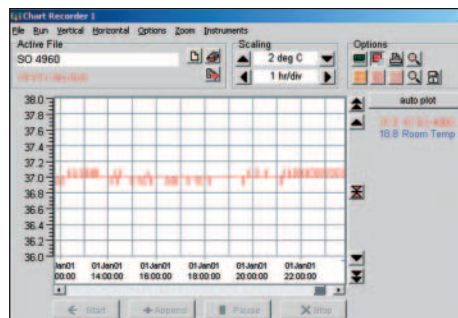

Fig. 2: 4,320 individual temperature measurements taken over a period of 12 hours

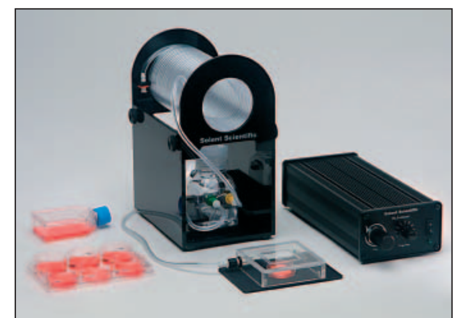

Fig. 3: The CO_2 Enrichment accessory provides humidity and pH control

Temperature Control

The longer the period of time involved, the more precise temperature control must be; the most demanding time-lapse studies being carried out not just over minutes or hours, but in many cases, over days. Consideration of the fractious behaviour of a child whose body temperature has been elevated from 37.0 °C to say 37.4 °C gives us a clear indication that cells similarly heated will be stressed. The precision of the temperature control therefore needs to be in the order of ± 0.1 °C.

The laboratory environment, however, can be a place of considerable heat differentials and drafts, with the high-power laser light sources used in fluorescence and confocal microscopy being a major source of heat. This demands the use of air conditioning systems, which, although designed to stop the lasers overheating, do not take into account the sensitivities of live cells.

Since the introduction of live cell microscopy, a number of approaches has been developed to control the environmental conditions for a specimen under observation.

Warm stages have a heated plate attached to the microscope stage. The cells rest on top of the heated zone and receive heat through the face of the culture vessel. There are, however, three drawbacks to this. Firstly, culture vessels positioned on a microscope stage are generally not placed in direct contact since it is likely that the base of the vessel will become scratched. (There is typically a gap of 0.5 mm between the vessel and the stage.) Secondly, the space directly be-

neath the part of the specimen being examined will not be warmed, since this is open to allow microscope observation. Thirdly, plastic is a poor conductor of heat. The consequence of these effects is that there will be temperature gradients across the culture vessel, from the warmest areas around the periphery, to the area above the observation area in the centre. Temperature gradients of up to 2 °C have been observed across a 35 mm Petri dish and there is equivalent uncertainty about the precise temperature in any part of the growth medium.

For vessels that can be returned to the incubator after quick examination or for basic low resolution microscopy procedures where cells can be placed on a peripherally heated microscope stage, this may be acceptable. However, for more advanced investigations using high resolution imaging techniques, these temperature gradients represent a significant threat to cell viability.

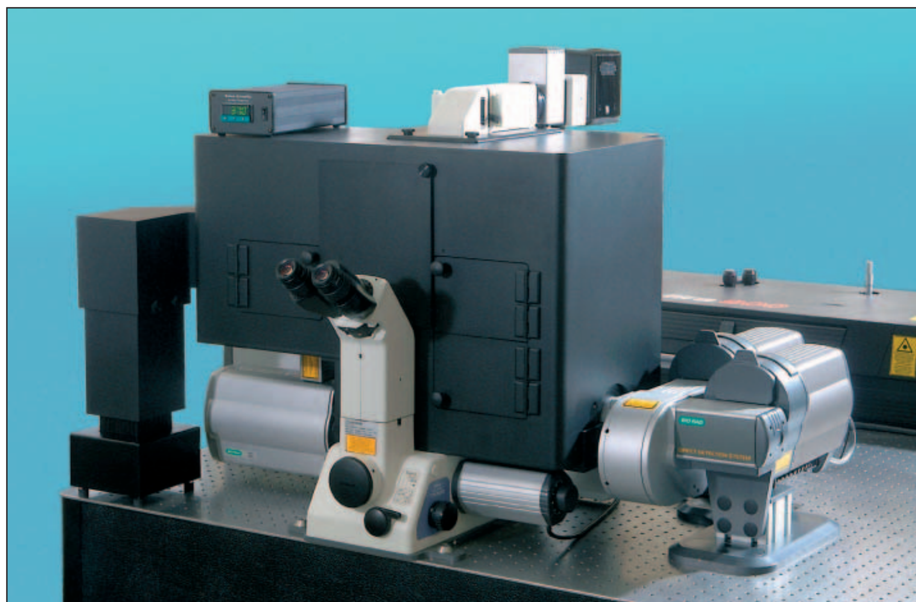

Fig. 4: A multi photon microscope fitted with a light tight enclosure

Perfusion chambers can provide a heated "jacket" around the living cells, or in some cases, provide heating below. They provide the living cells with a flow of culture medium, which in many cases is the primary reason for their use. As well as removing waste products from the cells, the technique also allows experiments to be performed that involve the introduction of reagents to alter cell physiology. While the pumped supply of fresh medium helps maintain temperature and control pH, it has not been designed specifically for this purpose, and as a consequence, heat loss and consequent temperature gradients can be considerable for many applications.

Stage top incubators provide improved control because the circulating warm air eliminates heat losses above the growing cells. Heat loss through the observation port in the centre of the stage still remains. This means that the part of the specimen under observation will be at a lower temperature than that at the periphery of the culture vessel, and as such, temperature differences across the specimen can be as much as 2 °C.

In high NA imaging using water immersion or oil immersion objectives, these temperature gradients are increased as the objective which is at room temperature acts as a heat sink.

It is only the full enclosure incubation chamber which provides a heated environment all around the specimen. The entire stage, nosepiece and a large part of the microscope frame are at physiological temperature so there is no need for supplementary objective heaters. Temperature gradients are minimal and typical temperature stability is better than ± 0.1 °C (Fig .2).

For confocal microscopy, the issue of cell viability may be secondary to the drift in focus when changes in temperature cause expansion of the stage or frame. Shifts of the order of 40 µm in focus position have been observed. When microscopy at this level is required, the only realistic solution is to keep the whole microscope warm.

Humidity and pH Control

The role of the 5 % CO_2 atmosphere is to maintain the pH of the growth medium. The CO_2 interacts with the surface of the growth medium so, provided that the headspace immediately above the liquid is maintained at 5 % CO_2, pH is controlled. Humidity does not need to be 'controlled' as such. It simply needs to be maintained at, or close to, the saturated vapour pressure of water at 37 °C. Under these conditions the growth medium cannot evaporate and the cells remain free from stress.

The Solent Scientific CO_2 enrichment accessory simultaneously controls both temperature and humidity (Fig. 3).

The workhead provides a mini environment. Heat is derived from the circulating air and the stage, which is at 37 °C and in intimate contact with the workhead. The blanketing gas is humidified and then trickled into the workhead. The flow rate is low so there is little need for creating the CO_2/air mixture in situ. It is more economical to use premixed 5 % CO_2 in air from a laboratory gas cylinder.

Ambient Light Control

Maintaining living cells not only under physiological conditions of temperature and pH, but also in the dark can further help keep them stress free. In multi photon microscopy there is another consideration; the observed signal is very weak. The background noise from the detectors is significant and the signal to noise ratio is a matter of serious consideration. Current technology indicates that there is little that can be done to enhance the signal strength, but if the background noise is reduced by lowering the ambient light level, there will be a significant increase in the signal to noise ratio. A light tight enclosure (Fig. 4) generally means that good experimental data can be obtained in a laboratory illuminated under red light conditions rather than total darkness.

Environmental control for live cell microscopy is central to the study of cell growth, life and death, as well as to the many cell manipulation techniques and high resolution imaging work involving live cells. The Solent Scientific semi customised full enclosure incubator controls temperature, humidity, pH and ambient light level for all types of microscopes.

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