Temperature Control for Live-Cell Imaging

Importance, Relevance and Reproducibility

Thanks to microscopes, scientists start to observe smaller samples and are now able to observe atoms. In biology, samples might be alive but as a microscope stage is not their natural environment they tend to die after a short period of time. This limiting factor questions the relevance and robustness of the produced results as a results obtained from an organism/organ/cell cultured in a non physiological environment will always remain questionable. The way to improve it was to recreate the optimum sample environment (temperature, pH, nutrient supply...) on the microscope stage.

Why Is Temperature Key for Live-Cell Imaging?

Biological Point of View
At the whole organism level, temperature is a very important parameter as it can affect the complete organism phenotype (e.g. sex of different species such as fish or reptiles is thermo-dependent) [1] (fig. 1). Other organism such as Caenorhabditis elegans, can suffer temporal paralysis due to big drops of temperature [2].

Going deeper, at the cell level, it has been seen that some mammalian cells (e.g. CFPAC-1 cells), are able to reverse their cell cycle between 0 and 22°C [3]. The hypothesis is that temperature induces the depolymerization of microtubules, stopping completely the cell cycle. If no repolarization occurs, the cells collapses and dies [4]. To illustrate this point, Isabelle Vernos group at the CRG, Barcelona Spain, in collaboration with Cherry Biotech inside the DivIDE consortium, study the cellular division and the role of microtubules inside this particular event [5]. They use live-cell imaging techniques to investigate the function and directly track the dynamic movement of different type of microtubules inside the cells. As it can be seen in the figure 2, by controlling the temperature of the sample (Kyoto-HeLa cells), with DNA tagged in red and the microtubules in green, it is possible to completely depolymerize microtubules by changing cells temperature using a temperature control device on a microscope stage.
At the membrane level, temperature variations do not only affect transmembrane proteins such as receptors.

It also has an impact on the medium surrounding the cell, formed by both the cytoplasm and the extracellular electrolytes. The phospholipid bilayer together with the plasma and the intracellular membrane behave differently depending on the temperature conditions. At high temperatures, the bilayer is in a non-lamellar hexagonal phase while at very low temperatures it becomes a very ordered structure acting like a gel. Its diffusion coefficient and the fluidity are very low but the rigidity goes up as the temperature decreases. At the optimal cell temperature, which is higher than 20°C, the membranes behave more like a liquid (less order and structured), allowing the diffusion and the flow in and out of the cell [6].

At molecular level, if temperature is lower than the needed one, the dynamic processes of the cells tend to occur at slower rate [7]. This can also be related with the stability of certain proteins that might denaturize (miss folding, loss of functionality...) if they are not under the correct temperature conditions [8].

**Experimental Point of View**
The purpose of any experiment is to be replicated under the same conditions as many times as desired, or required, to prove the hypothesis. In this context, controlling the environment (such as temperature) at the experiment site is mandatory.

First, ambient temperature at the sample location might change even if the room has climate control, because the equipment themselves gets warmer with the time and thus create a temperature gradient to the sample [9]. Second, heat sink, which is transfer of thermal energy between a higher energy to a lower energy body, may occur while using immersion objective (fig. 3). Indeed, the immersion oil acts as thermal bridge between the objective/microscope and the sample. By monitoring
the temperature of both the objective and the room it is possible to compensate any temperature gain/loss of the sample to guarantee the same thermal conditions between each experimental replicate.

**Live-Cell Imaging and Temperature Control**

Live-cell imaging aims to observe living cells under a microscope in different conditions. There is a wide range of techniques from bride-field, fluorescent and now, super-resolution technology. After the improvement of the image quality and resolution, the next challenge is to recreate the natural environment of the sample on the microscope stage to increase the robustness of the results. In this article, we will focus on temperature as it is a key feature since it plays a big role on keeping the homeostasis of the cell during growth and division steps [10].

Temperature concern during live cell imaging is not recent. Back in 1910s multiple patents by John W Morsbach and Tiodolf Lidberg described a microscope stage heater and incubators, opening the way of external parameters controllers dedicated to live-cell imaging. Their objective was to observe mammalian cells using a microscope. Their main problem was the need of an experimental temperature over the ambient one. To solve it, they developed the first microscope heater system ever. At the beginning of the XXI century many patents with different microscope stage incubator where deposes adding multiple configurations and features. At that time, live-microscopy was a rising field inside the science thanks to the new improvements done in the optics field allowing biologist to observe events at higher resolution and for more time. That is why many companies invested on developing different temperature controllers based either on hot air flows, water close circuits or even on metallic pieces transferring the heat to the sample.

Since that time, it has been clearly established that temperature is a critical parameter in any biological experiments.

**Conclusion**

Temperature is one of the key parameters that live-organism depend on to keep their homeostasis at each scale (organism, cells, molecules). Therefore, while performing biological experiments, temperature control became a necessity, not only to reduce the variability between experiments, but also to guarantee the relevance of the observed results. This concern has been highlighted by the number of temperature control device that have been commercialized in the past decades.

**References**

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Authors
Pablo Salaverria¹, Jeremy Cramer¹, Pierre Gaudriault¹

Affiliation
¹Cherry Biotech, Rennes, France
Contact
Dr. Pierre Gaudriault
Cherry Biotech
Rennes, France
pierre.gaudriault@cherrybiotech.com

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Contact

CHERRY BIOTECH
6 Rue Gurvand
35000 Rennes
Frankreich
Phone: +33 987 047 035