Life Cell Imaging: Cultured, Contained and Controlled
ENVIRONMENTALLY FRIENDLY

At the Forefront

Microscopes and imaging systems from Olympus have been used for many years to view fixed and stained preparations, providing a massive pool of data and facilitating the discovery of many important principles. Unfortunately, fixed cells can only give us snap-shot views and as such provide end-point analyses. Thus, changes that occur within cells, tissues and organs to reach an end-point are not very well recorded. This data is also influenced by the fixing methods used and events can vary greatly between different cells, even within the same culture.

Forging Ahead

Microscopes and imaging systems from Olympus are now being increasingly used to look at molecular events, as well as cell components, and this is best done in real-time or by using time lapse footage of the same cells – so called live cell imaging. This type of microscopy requires a very specialised instrumental set-up in order to track movements of molecules or organelles inside the cell or on the membrane. These movements are easily missed if they are not followed closely within the same cell. As a result, Olympus has produced the perfect modular systems for carrying out live cell imaging on any cell, tissue and organotypic or whole organism culture. For complex culture conditions, Olympus can also provide complete solutions. This brochure provides an overview of the environmental control solutions available from Olympus. For a complete list of accessories or to discuss your individual requirements in more depth, contact your local Olympus representative.
CREATE THE PERFECT ENVIRONMENT FOR YOUR CELLS

Everything under Control: from Basics to Systems

Living cells need optimal environmental conditions, and these vary greatly depending on their source tissue. These, combined with the specific requirements of your experiment, define the need to have separate control over temperature, pH, humidity, culture medium and gases such as O₂ and N₂. Moreover, these factors need to be integrated with processes such as micromanipulation. Olympus has made this possible.

With the Olympus expertise comes the competence to ensure that everything is under control – from basics to systems – providing you with the perfect environment for your cells.

Modular Solutions for Optimal Conditions 6–13

Not all cultures need full environmental control, so whatever your requirements the modular units provided by Olympus are perfectly matched so you can pick and choose which parameters you need to integrate.

Complete Solutions 14–20

When you need the ultimate combination of environmental control and microscopy, the all-in-one solutions from Olympus are the perfect choice. The design of these systems still allows a great deal of flexibility in both experimentation and expansion.

Your Partner for Continued Success

As your partner for advanced cellular research and live cell imaging, Olympus is dedicated to making state-of-the-art microscopes and accessories that are the best in their class for live cell experiments. Our capabilities in R&D and quality manufacturing, as well as our attentive and informed customer support, are totally focused on success for your current and future experiments – turning your visions into reality.
MODULAR SOLUTIONS FOR OPTIMAL CONDITIONS

Precise Control: Ultimate Flexibility

Live cell imaging offers improved qualitative and quantitative analysis in molecular and cell biology, physiology, pharmacology and biotechnology. Complex structural and functional aspects of biological processes are being investigated at the molecular level. These advances can only be fully realised if precise environmental control is added without affecting imaging. The modular and innovative systems from Olympus are easy to implement and offer a wide choice of individual components. This maximises the flexibility of environmental control, permitting the full use of advanced microscopic examination techniques.
LIVE ON STAGE

All cultures require regular changes of the specialised media that provide them with the correct balance of nutrients and precise pH control. Once in place on a microscope though, replacing the media will disrupt the experiment. That is why Olympus can supply specialised Cell Cultivation Chambers (CCCs). These meet all the demands of live cell imaging and are capable of open and closed perfusion.

Chambers

The CCCs are available in different volumes and are therefore suitable for any cell or tissue culture experiment requiring time-lapse imaging, such as protein trafficking, drug testing, cell division, cell death, etc. They can be used in either an open configuration, which permits full access to the culture for tools such as manipulators, or a closed configuration, which maintains a sterile system.

Comparison of Cell Cultivation Chambers

<table>
<thead>
<tr>
<th>Features</th>
<th>CCC-58</th>
<th>CCC-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suitable for inverted and upright microscopy</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Works with 0.17 mm cover slips</td>
<td>Ø 32/42 mm</td>
<td>Ø 20/30 mm</td>
</tr>
<tr>
<td>Observation opening</td>
<td>Ø 29 – 32 mm</td>
<td>Ø 17 – 22 mm</td>
</tr>
<tr>
<td>Culture area</td>
<td>6.6 – 8 cm²</td>
<td>2.3 – 3.8 cm²</td>
</tr>
<tr>
<td>Chamber volume (application dependent)</td>
<td>0.3 – 2.8 ml</td>
<td>0.1 – 1.2 ml</td>
</tr>
<tr>
<td>Compatible with high-resolution oil immersion objectives</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DIC-capable</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Permits both open and closed perfusion</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Perfusion

Media changes and the addition of potential pharmaceutical compounds is very easy with the Olympus CCCs since flows are controlled by the multi-channel peristaltic pumps. This helps reduce the impact of metabolite build-up, and assess e.g. the impact of pharmaceutical drugs from the very start through to the end of the experiment. Moreover, perfusion is possible in both the open or closed configurations on high-end fluorescence and even confocal microscopes. This means that highly complex experiments involving Z-stacks from multiple sites can be carried out with ease.
HOT OR COLD — YOU DECIDE!

The most important environmental parameter for cell culture is temperature. Most cultures need to be kept at their optimum conditions during live imaging. Some cultures can withstand short-term imaging with only the temperature controlled. This means that the most basic environmental system consists of a microscope and a temperature control source. Olympus can supply an array of heating and cooling plates and stage inserts, objective heaters and temperature-controlled stages ideal for: a) routine observations; b) in vitro fertilisation; c) long-term imaging and d) analytical experiments. These various products very quickly reach and carefully maintain the chosen temperature, be it above, below, or exactly at 37°C.

Heating Plates
For the greatest flexibility in culture vessel fitting, black anodised aluminium heating plates are available. These laminated plate-based heating modules can be used on standard Olympus microscope stages with long working distance UIS2 objectives, providing direct heating and temperature control from below the sample. Specimens are held by two moveable brackets, and different versions are available based on the culture vessels used or user specified requirements (see page 11, figure E).

Heating Stage Inserts
For more sophisticated live cell experiments, including laser scanning confocal microscopy applications, heating stage inserts are available, which ensure optimum heat distribution to the sample. These modules, besides the heating insert for multiwell plates, ensure precise positioning at higher magnifications for oil immersion objective use. Different options are available to allow simultaneous monitoring and imaging of multiple time-dependent events with various multiwell plates. Temperatures can be accurately controlled from just above ambient to a maximum of 60°C. These stage inserts are an integral part of stage incubator set-ups, as described on page 12.

Transparent Heating Plates and Inserts
For applications requiring heating and a wide observation area, e.g. oocyte checking and intra-cytoplasmic sperm injection (ICSI), special transparent heating plates or heating stage inserts are available.

Heating Stages
Specialised heating stages for the inverted Olympus IX2 microscopes, where heat is dissipated via transistor power, are especially suited for temperature-controlled experiments. The solid, black anodised aluminium microscope stages ensure optimal heat transfer, without generating electrical pulses. What is more, the opening for the UIS2 objectives is the smallest possible to reduce the non heated area.
Exact Temperature Control

Controlling the temperature of the different components within a defined range is important. The control units available from Olympus can maintain any temperature from 3°C above ambient to 60°C, with a temperature stability of ± 0.1°C.

Peripheral Working Plates

Heated peripheral working plates are also available to provide peripheral storage areas when a number of samples need to be screened at once and where different reagents need to be kept at culture temperature. Inserts are available to support smaller tubes.

Expanded Range Heating and Cooling

For physiological heating and cooling from 0°C to 65°C, a series of peripheral working plate, stage and stage inserts are available which use heated and cooled liquids. Temperature is maintained by a thermostatically controlled liquid bath.
Objective Heaters

Microscope components close to or in contact with the specimen, such as oil immersion objectives, may cause problems since they can conduct heat away from the sample. This can make it difficult to maintain a stable temperature. Small heaters can therefore be placed on the objectives to improve the temperature stability and eliminate gradients in the observation area.

Like the other heating elements, they are made from black, anodised aluminium and consist of a ring with a laterally mounted housing for the heating element and temperature sensor. They are available as different options for the entire range of UIS2 objectives. Any spare channel on a standard control unit can be used for their temperature control. However a specially designed controller for use only with objective heaters is available, providing a temperature range of 35-40°C.

Temperature Control (TC) Compatibility Chart

<table>
<thead>
<tr>
<th>Compatible with:</th>
<th>TC for Objective Heater</th>
<th>TC with 1 Channel</th>
<th>TC with 2 Channels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective heater</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heatable working plates</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heating stage</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heating mounting plates</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heating insert</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stage incubator</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Complete stage incubator system</td>
<td>-</td>
<td>-</td>
<td>+*1</td>
</tr>
<tr>
<td>Heating / cooling stage</td>
<td>-</td>
<td>_*2</td>
<td>_*2</td>
</tr>
<tr>
<td>Heating / cooling insert</td>
<td>-</td>
<td>_*2</td>
<td>_*2</td>
</tr>
<tr>
<td>Heating / cooling working plate</td>
<td>-</td>
<td>_*2</td>
<td>_*2</td>
</tr>
</tbody>
</table>

*1 Integrated (see p. 17)  
*2 Need thermostatically controlled liquid bath

Polystyrene Foam Sleeves

To aid in reducing heat loss from the system, four simple and practical polystyrene foam objective sleeves are provided with heating stage inserts to protect the lower specimen area from heat loss.

*Image courtesy of zell-kontakt GmbH, Göttingen, Germany.
PERFECTLY BUFFERED

In vivo, a complex system with physiological controls ensures that cells are always exposed to a medium with optimal pH. The key buffer system, for example in blood (CO₂/HCO₃⁻), keeps the pH-value between 7.3 – 7.4. Similar buffer ions, in addition to nutrients and vitamins, are also used in many culture media. CO₂-independent buffers leading to unphysiologically high ion strength, such as Heps, can theoretically maintain reasonable pH control, but also lead to changes in the cellular events, affecting metabolism and thus influence normal cell growth to proliferation or even to cell death.

In physiological in vitro systems, unfortunately the CO₂ escapes from the buffering system and thus destroys the buffer capacity. Additionally, the percentage of CO₂ in the surrounding air is actually too low to maintain a stable pH with physiological ion strength. Therefore CO₂ needs to be provided and controlled at greatly increased percentages.

pH Control

The optimum pH-value varies slightly for different cell lines. For example, fibroblasts prefer a higher pH, e.g. 7.4 – 7.7, whereas transformed cell lines can sometimes grow more efficiently at a lower pH, e.g. 7.1 – 7.2; further on, insect cells prefer pH in the range of 6.9. Lab incubators usually run at circulating CO₂ concentrations of 2 – 7.5%, which allows the HCO₃⁻ buffer to maintain pH within these defined ranges and at physiological ion strength.

Olympus offers stage incubators, which are placed above the heating stage inserts, providing a chamber in which to control airflow and CO₂ concentration within the circulated airflow. Both available CO₂ control units use infrared sensors to maintain the user-defined CO₂ concentration within the closed, air circulating systems. When CO₂ levels drop, extra CO₂ from the attached tank is added.

CO₂ and Humidity Control Compatibility Chart

<table>
<thead>
<tr>
<th>Compatible with:</th>
<th>CO₂ Controller with Air Heating Element</th>
<th>Humidifier System for CO₂ Controller (left)</th>
<th>CO₂ Controller with Integrated Humidifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating mounting plates</td>
<td>-</td>
<td>-</td>
<td>+*¹</td>
</tr>
<tr>
<td>Heating insert</td>
<td>-</td>
<td>+*¹</td>
<td>-</td>
</tr>
<tr>
<td>Stage incubator</td>
<td>+</td>
<td>+*¹</td>
<td>-</td>
</tr>
<tr>
<td>Complete stage incubator system</td>
<td>+*¹</td>
<td>+*¹</td>
<td>-</td>
</tr>
<tr>
<td>Heating / Cooling Stage</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Heating / Cooling Insert</td>
<td>-</td>
<td>-</td>
<td>+*¹</td>
</tr>
</tbody>
</table>

*¹Usable only in conjunction with CO₂ covers  *²Integrated (see p. 17)

The ultimate controller for the Olympus stage incubator system can control not only CO₂, but also provide additional air circulation that can be controlled in conjunction with a temperature controller. Incubation software is available allowing precise management and monitoring of the parameters.

The CO₂ controller with integrated humidifier pumps a defined and controlled CO₂/air mixture to large warm air and mini incubators. The simple mini CO₂ incubators are compatible with the heating plates and heating stage inserts described above in conjunction with the supplied, specific CO₂ covers.

*Image courtesy of zell-kontakt GmbH, Göttingen, Germany.
MODULAR SOLUTIONS FOR OPTIMAL CONDITIONS

SUITABLY SATURATED

One aspect of culturing cells is that the combination of warmth and circulating air may lead to evaporation, which in turn can cause increases in ion concentration causing the media to become toxic and affect the living cells. Also, evaporation can lead to condensation on lids and other components. The amount of water in the gas phase is temperature-dependent and therefore the relative humidity of the circulating air needs to be carefully balanced.

Humidity Control

The Olympus humidity control system can be used with the incubation chambers to increase the relative humidity of the circulating air. This will, in combination with a temperature and CO₂ controlled air supply, greatly reduce evaporation from the culture medium. The system incorporates a humidity trap to ensure that condensation collects in one specified area and not on the microscope incubator or culture apparatus.

Foil Covers

As an alternative to, or in combination with, this humidifier set-up, gas permeable membranes can be fitted over culture wells, which enable the control of CO₂ and O₂ levels, but restrict the loss of water vapour from the cultures. These work in conjunction with specific span frames for easier handling of the different culture vessels. The foil covers are 25 µm thick and available for 35 mm and 60 mm Petri dishes, Lab Tec incubation chambers, Olympus CCC systems and for BD Falcon multiwell plates.

*Images courtesy of Dr. Dagmar Malun, Berlin, Germany.
CHAPTER II

COMPLETE SOLUTIONS
All-in-One Systems: Ultimate Control

Studying the biological processes of living cells requires accurate imaging and documentation. Olympus systems ensure you always achieve the best results possible – whatever your experimental aims. More than this though, we can help you break through into uncharted territory.
Many cultures need the utmost care and attention during growth and differentiation – the slightest change in any of the environmental parameters can lead to undesirable side-effects and irreversible changes. For these cultures Olympus can provide the most complete combinations of incubator and microscope, taking the pressure off of you.

Universal Warm Air Incubators

The Olympus uniform warm air incubators enclose all relevant components of the microscope, including the various available micromanipulation and microinjection tools. The temperature of the entire space within the incubator housing, including the area around condenser, stage, objective and the nosepiece, is carefully controlled using the same or similar units described above. Different versions are available depending on the detailed microscope configuration – providing the most flexible warm air environmental control available.

Large doors on the front of the incubators permit easy access to the specimen, condenser, or other components mounted above the stage. In addition, microscope components below the stage remain easily accessible to manipulate items such as: reflector turrets, objectives or DIC sliders. Furthermore, the warm air circulating inside the incubator creates a similar air-flow to that used on clean benches. The pressure generated inside the incubator is slightly higher than that outside and therefore, if the doors are opened, micro-organisms and other particulate matter will not penetrate.

The control units for this system are compatible with a PC-based software program, and therefore even the most complex experimental requirements become very easy to monitor.

*Images courtesy of zell-kontakt GmbH, Göttingen, Germany.
CO₂ and Humidity Enrichment

Due to the nature of the components used, uniform warm air incubators are not totally sealed. For experiments requiring accurate CO₂ and humidity control, additional small covers inside the incubator can be used to create precise micro-environments inside the temperature-controlled atmosphere. Several different CO₂-covers are available depending on the control devices used.

The Oxygen (O₂) System

All too often, the oxygen concentration of the air circulating around a culture is not controlled. Ideally, the O₂ concentration should be the same as that of the organism being observed. Therefore, less is definitely more, depending on the cell type the oxygen content of the ambient air should be reduced from ambient to approximately 5%. Olympus offers a complete system solution that can accurately achieve this through displacement with nitrogen. Moreover, this can be done in addition to the temperature, CO₂ and humidity control detailed above.

This oxygen system requires an improved sealing of all relevant components and is therefore only available as complete “all-in-one” solutions.

Components:
- Heating stage insert
- Special version of the stage incubator
- Air temperature, CO₂, and humidity controllers
- O₂ controller and sensor
When you need the best possible environmental control with the highest quality optical components, Olympus is your ideal solution provider. Our attention to detail means that your cultures are in safe hands and your experiments will run extremely smoothly. Olympus has combined the best possible microscope, optics, illumination and software with excellence in incubator design.

The cell
cubator

The cell
cubator

The cell
cubator

The cell
cubator

The cell
cubator

The cell
cubator

The cell
cubator

Specifications

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm-up time</td>
<td>30 – 40 minutes</td>
</tr>
<tr>
<td>Temperature control</td>
<td>ambient to 42°C ± 0.1°C</td>
</tr>
<tr>
<td>CO₂ control</td>
<td>ambient to 10%</td>
</tr>
<tr>
<td>Humidity control</td>
<td>ambient to 60%</td>
</tr>
</tbody>
</table>

All achieved within the warm-up time
The cellBox enables the observation of cultured cells in a temperature, CO₂ and humidity-controlled environment with active closed-loop regulation. The incubator box is fully autoclavable and when fitted, encloses the condenser, stage objective and nosepiece, ensuring no temperature gradient between those components.

The air flux between the box and controlling unit is performed in a closed loop with HEPA-filter (= 99.995% of all particles > 1µm filtered). Software packages to control and set the parameters are available, thus they can be logged in an Excel compatible format for further analysis.

The Experiment

Uta Joos and colleagues at the Fraunhofer Institut have used a cellBox system on an Olympus IX71 microscope to produce time-lapse images over a 48-hour time period. The L929 fibroblast cells, as shown on the right, were visualised using a 10x objective and relief contrast. The images show the last 18 hours of the experiment, where cells can clearly be seen to be proliferating and taking up positions along parallel gold lines. These lines are approximately 45µm apart and were coated onto a glass slide. This experiment demonstrates the stability of the cellBox system to easily show a process not previously visible without continuous manual adjustment.

"Images from a time-lapse study taken at the time points t = 30, t = 36, t = 42 and t = 48h after seeding and start of time lapse series; 10x relief contrast, width of lines group 45 µm.

Courtesy of Uta Joos, Fraunhofer Institut für Biomedizinische Technik, Berlin, Germany.