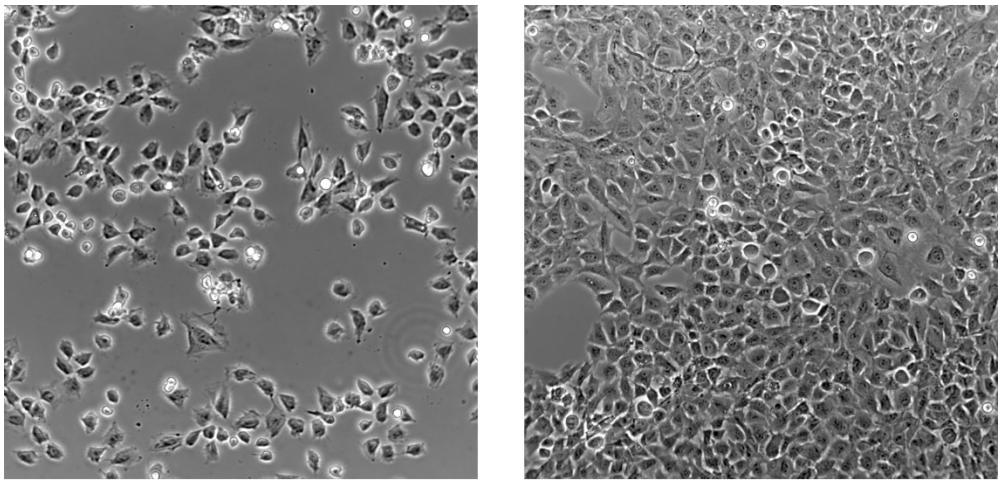
Avoiding Evaporation: Humidity Control in Cell Culture

This Application Note gives an overview of different methods to avoid evaporation from cell culture vessels by controlling the humidity.

Table of Contents



Low humidity: 70% RH | High humidity: 90% RH

Impact of the humidity on cell growth: rat fibroblast cells cultured in full growth medium at different levels of relative humidity (RH) 48 hours after seeding. Phase contrast microscopy, 20x objective lens.

1 Evaporation and Humidity: Important Parameters in Cell Culture

Constant levels of salts, nutrients, and other cell culture medium components are essential for reproducible cell behavior and are crucial for consistent and reliable *in vitro* results. Any evaporation from the cell culture vessels increases the substance concentration in the medium in an undefined way. This concentration change dramatically influences the vital functions of living cells, which leads to impaired cell behavior of all kinds, such as increased or decreased proliferation, up- and downregulation of gene expression, and induction of apoptosis/cell death. Evaporation and these changes happen particularly fast in low-volume vessels.

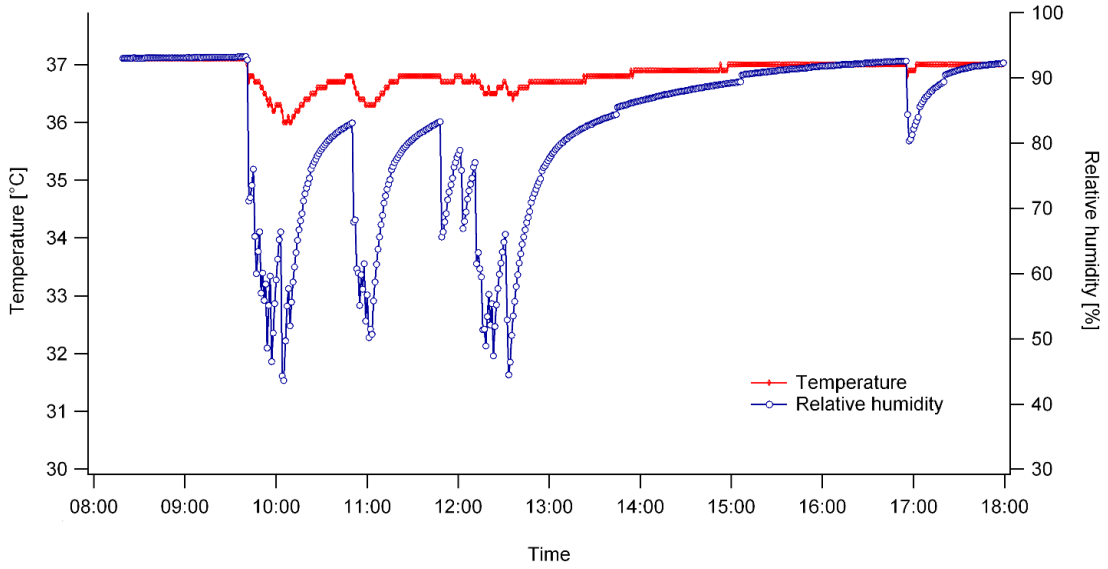
The consequences of evaporation due to insufficient humidity are frequently underestimated. Cells react even more sensitively to concentration changes than to temperature changes, markedly distorting the results. Therefore, evaporation from cell culture vessels during live cell imaging experiments should be absolutely avoided.

2 Humidity and Evaporation in Different Systems

2.1 Cell Culture in a CO_2 Incubator

Cell culture vessels in the CO_2 incubator are normally cultivated in an open system (e.g., gases can diffuse through the cell culture plastics), and are therefore in an equilibrium with the surrounding air. In standard CO_2 incubators, the humidity is created by evaporation from a water tray. Optimally, the relative humidity (RH) within an incubation system should be within the range of 90%–95%.

Cell culture incubators need a long time to recover humidity, particularly after door openings. While temperature and CO_2 levels recover within minutes, full humidity recovery can take hours. Depending on incubation conditions, small volumes of cell culture medium may evaporate quickly, especially during long-term experiments, requiring complete humidity control.



The graph shows the temperature and humidity inside a typical cell culture incubator, which was opened several times during a 10-hour day. After opening the door, there were only minor decreases in temperature, always between 36°C and 37°C. However, significant differences in relative humidity were measured, with values between less than 50% and the steady state of 93%. This incubator was not opened all afternoon, except briefly around 5 o'clock.

2.2 Live Cell Imaging in a Stage Top Incubator

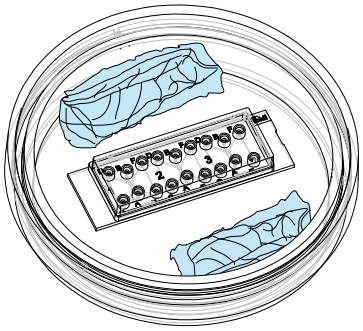
A constant and high level of humidity (>90% RH) in the stage top incubator is one of the keys to artifact-free results in live cell imaging. During a live cell imaging experiment, the evaporation in the stage top incubator should be kept as low as possible, underlining the need for reliable humidity control.

Additionally, in many stage top incubator models, humidity is poorly controlled, leading to a higher relevance for minimizing evaporation.

3 Techniques for Humidity and Evaporation Control in Cell Culture Vessels

3.1 Humidifying Chamber

A simple humidifying chamber can be made by placing wet tissues inside a 10 cm Petri, as shown on the right. Use EDTA-stabilized water and sterile tissues for longer incubation periods. One advantage of this technique is that it allows low resolution microscopy to check the adherence of cells, for example. You can use a similar design to create larger humidifying chambers and boxes.



This technique is recommended for use in CO_2 incubators.

3.2 Parafilm

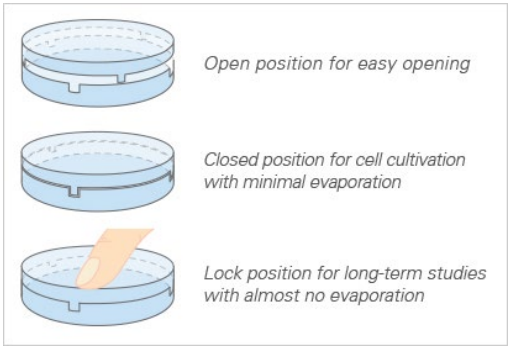
Placing small strips of Parafilm onto the reservoirs/wells of the cell culture vessel will suppress the effects of evaporation. Stretch the Parafilm on top until it fits tightly.

This method can be used in both CO_2 incubators and stage top incubators.

3.3 Dishes With a Lid Locking Feature

All ibidi µ-Dishes are equipped with the special lid locking feature. The locking position minimizes evaporation, thereby providing excellent conditions for long-term studies in a non-humidified environment. Gas exchange (carbon dioxide or oxygen) during cell culture is maintained thanks to the gas-permeable plastic material of the dish.

Use the locking feature if minimal evaporation is required (e.g., outside incubators or non-humidified microscopy stages).



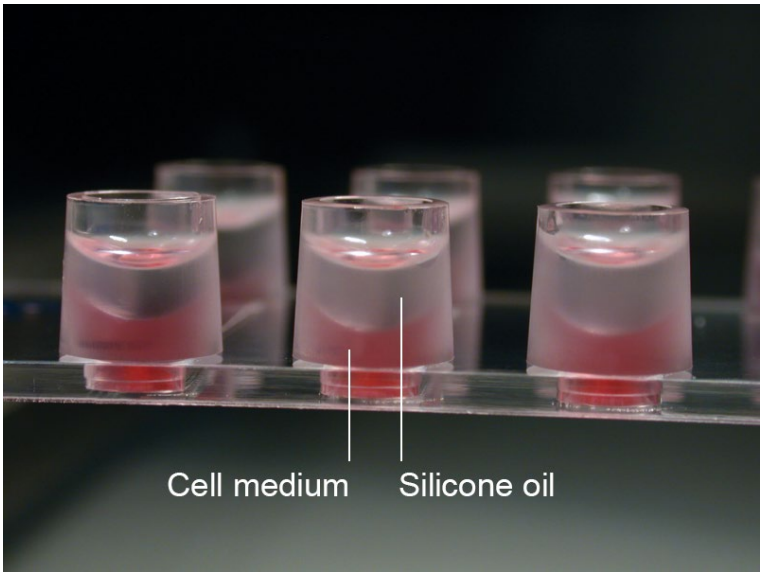
3.4 Silicone Oil (ibidi Anti-Evaporation Oil)

Using silicone oil (e.g., ibidi Anti-Evaporation Oil) can prevent evaporation. This silicone oil is overlaid onto the culture medium, thus decreasing evaporation. Silicone oil is non-toxic, inert, and highly gas permeable for O_2 and CO_2 but effectively blocks humidity loss. Please do not use mineral oil because it is harmful to most cell culture labware.

Procedure:

- Equilibrate silicone oil and cell medium inside the incubator overnight. This step helps avoid the formation of air bubbles and pre-warms all solutions to 37°C.
- Fill your channel/well with cells and medium.
- Overlay the medium surface with an appropriate amount of silicone oil (see Instructions ibidi Anti-Evaporation Oil)
- Do not drip the oil directly onto the surface, rather let it run down the edges by pressing the pipette tip onto the upper side of the chamber. For example, when using the µ-Slide VI $^{0.4}$, fill each Luer reservoir with 50 µl cell-free medium and 30 µl ibidi Anti-Evaporation Oil.

This method can be used in both CO_2 incubators and stage top incubators.



µ-Slide VI $^{0.4}$ with silicone oil (ibidi Anti-Evaporation Oil) inside the reservoirs, which is used to decrease evaporation in non-humid environments (e.g., on the microscope).

3.5 Stage Top Incubator with Active Humidity Control

To prevent evaporation during live cell imaging make sure you use a modern live cell imaging setup, in which the humidity is maintained at a high level throughout the entire experiment. With the ibidi Humidity Control, the ibidi Stage Top Incubation System provides a patented and best-in-class, feedback-controlled humidity regulation.

The ibidi Humidity Control ensures a constant and very high humidity level inside the ibidi Stage Top Incubator—identical to the conditions in standard cell culture incubators. This unique and patent-protected technology actively humidifies the gas mixture in a fast and reliable way before entering the stage top incubator.

The humidity in this system is constant and can be smoothly adapted to specific experimental needs. With a high humidity level, the evaporation within the ibidi Stage Top Incubation System is securely prevented.