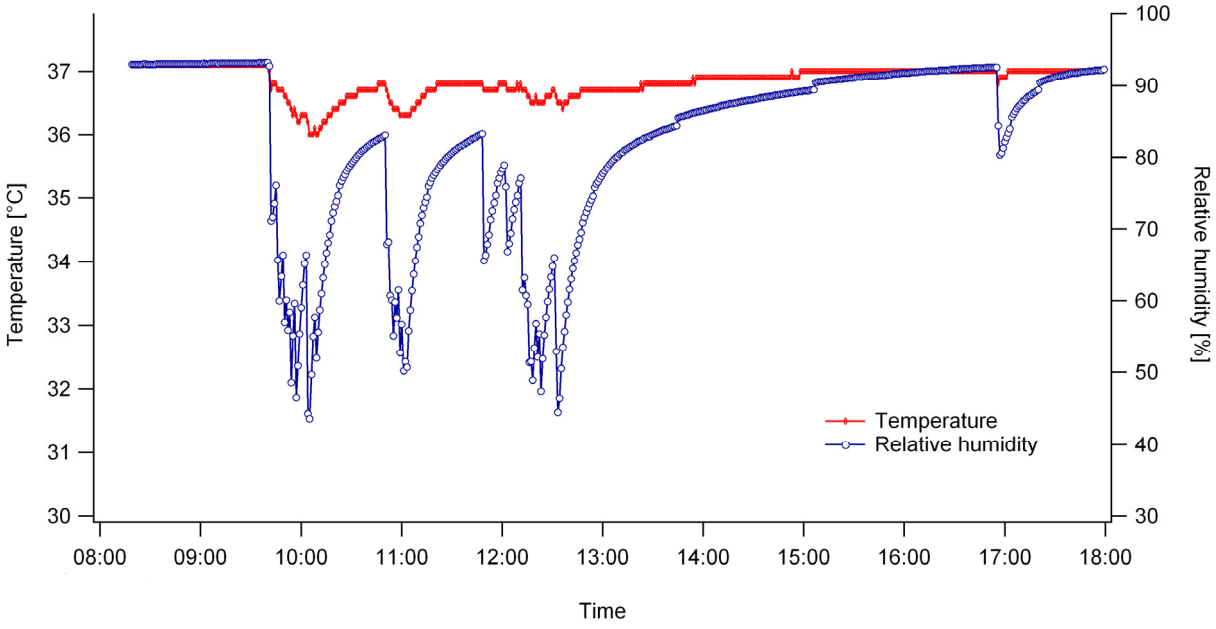
Avoiding Evaporation

0. Introduction

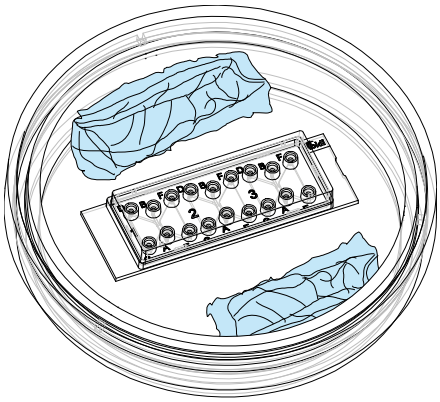
Depending on incubation conditions, small volumes of cell culture medium may evaporate quickly, especially during long-term experiments. Furthermore, all cell culture incubators need a fairly long time to recover humidity, particularly after door openings. While temperature and CO_2 is recovered within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



The above graph shows the temperature and humidity inside a CO_2 incubator over a 10 hour day, during and after briefly opening the door. The graph shows only minor differences in temperature over the day, i.e., between 36°C and 37°C. However, it shows significant differences in relative humidity over the day, i.e., between less than 50 % and the steady state of 93 %. An important fact to note is that this incubator had not been opened all afternoon, except briefly just before 5 o'clock.

Therefore, for controlling and decreasing evaporation we recommend the following techniques:

1. A Petri Dish with Wet Tissues



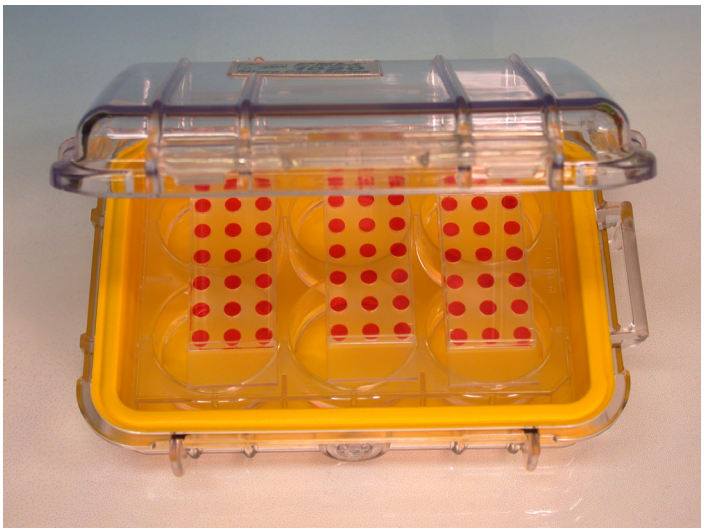
A simple humidifying chamber can be made using a 10 cm Petri dish with wet tissues inside (as shown on the right). Use EDTA (Ethylene-Diamine-Tetraacetic Acid) stabilized water and sterile tissues for longer incubation periods. One advantage of this technique is that it allows access with low resolution microscopy to check, for example, the adherence of cells. This technique is recommended for use inside cell culture incubators.

2. Olaf Humidifying Chamber

The Olaf Humidifying Chamber (Cat No. 80008) prevents problems caused by evaporation, especially during long term assays. Olaf is a humidifying chamber that can be completely sealed, which prevents loss of humidity while inside an incubator. The Olaf chamber can be sterilized with a mixture of 70% ethanol and water, and is recommended for use inside cell culture incubators.

Procedure:

- Fill a standard 6 well plate with sterile water (i.e., 4 ml per well) and place the plate into an Olaf Chamber. Use EDTA stabilized water for longer term experiments.
- Put a µ-Slide on top of the 6 well plate, close the Olaf chamber and place the chamber into an incubator.



An Olaf Chamber with a µ-Slide 18 well inside. A water-filled 6 well plate provides humidity.

Another option is to place a µ-Slide Click Rack (Cat No. 80007) onto the water-filled plate. This option allows convenient handling of up to 4 µ-Slides in parallel.
Please note, however, that the µ-Slide 18 well is not compatible with the µ-Slide Click Rack. In this instance we recommend placing a µ-Slide 18 well directly on top of the 6 well plate.

3. Parafilm

Placing small strips of Parafilm (produced by the Pechiney Plastic Packaging Company) onto the reservoirs of the slides will prevent the effects of evaporation. Just fill the slide as recommended and then stretch the Parafilm on top until it fits tightly.

The Parafilm technique is recommended for use with stage top incubators and with non-humidified incubated microscopes.

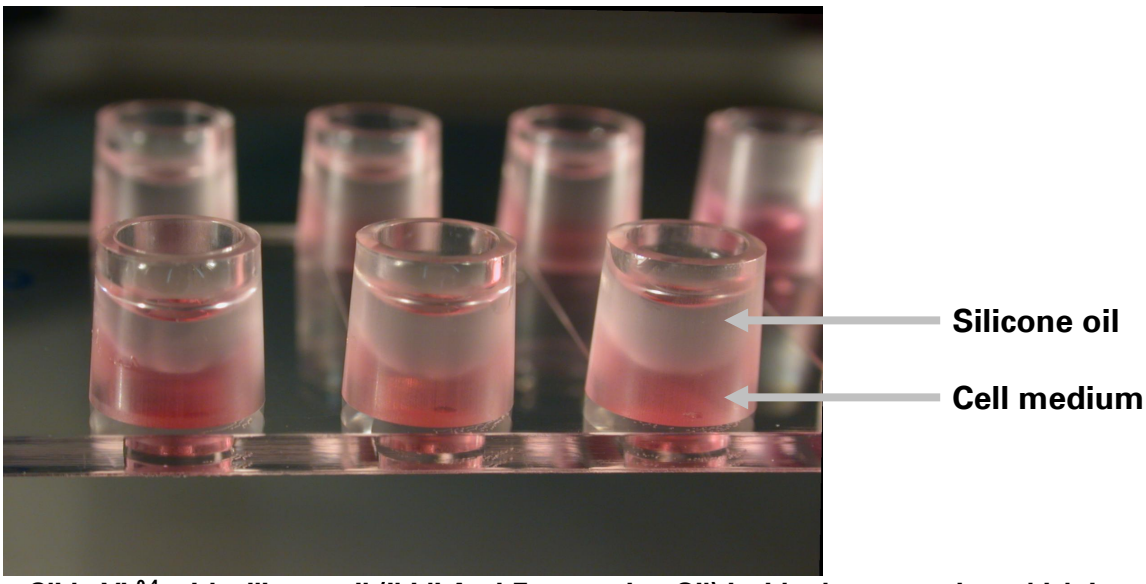
4. Silicone Oil (ibidi Anti-Evaporation Oil)

To prevent evaporation effects one can overlay the cell culture medium with silicone oil available from ibidi (#50051). This silicone oil is gas permeable and non-toxic. Please don't use mineral oil as this is harmful to the µ-Slides.

Procedure:

- Equilibrate oil and cell medium inside the incubator overnight. This step helps in avoiding the formation of air bubbles and pre-warms all solutions to 37°C.
- Fill your slide with cells and medium.
- Overlay the medium's surface with an appropriate amount of silicone 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 reservoir. For example, when using the µ-Slide VI $^{0.4}$, fill each reservoir with 50 µl cell-free medium and 30 µl Anti-Evaporation Oil.

The silicone oil technique is recommended for use with stage top incubators and when imaging for several hours using non-humidified incubated microscopes.



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.