

Live Cell Imaging Setups Under Flow

Live cell imaging is one possible read-out of cell culture assays under flow conditions.

Depending on the duration and temperature requirements of the experiment, there are several possible setups with different advantages and disadvantages, which are shown and discussed in this Application Note.

Related Topics:

[ibidi Pump System instruction manual](#)

[ibidi Heating System](#)

[ibidi Gas Incubation System](#)

[Application Guide Live Cell Imaging](#)

[Application Guide Cell Culture Under Flow](#)

Keywords:

Live cell imaging, cell culture under flow, shear stress, perfusion assay, stage top incubation, pump, microscopy

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1. Introduction

When setting up a flow experiment on the microscope, you might encounter some basic physical challenges to consider.

1.1. Stable Conditions:

Healthy cells need stable conditions such as temperature, pH, ion concentration, etc. Depending on the specific cell type the requirements may vary. Stage top incubators and cage incubators help to ensure homogeneous, stable conditions. Perfusion of media into these incubation setups has the potential to change environmental conditions for the cell.

1.2. Air Bubbles:

Temperature gradients can create air bubbles over time. The physical principle explaining this effect is the solubility of gas in liquids and plastic. At higher temperatures, water and plastic can absorb less gas than at lower temperatures. Every time the medium temperature changes, gas is taken up or released by the medium. This can also happen across the tubing wall, especially when silicone tubing is used. If cold medium warms up along the circuit, it releases gas, which cannot escape because it is trapped in the tubing or channel (Figure 1).

Eventually, the tubing can become clogged by air bubbles leading to a disturbance of flow. This is especially true in adapters, where the cross section is widened. Here the flow velocity goes down and the gas bubbles can gather to form big bubbles, blocking the whole channel cross section (Figure 2).

Favorable Parameters

Some parameters favor stability of the running experiment, and reduce the formation of gas bubbles. To evaluate for the presence of gas bubbles within the system, one must setup and perform a test run of the entire live-cell/perfusion system. This is the only way to verify gas bubble formation over time.

ibidi recommends checking that the following parameters are being met:

- **Homogeneous temperature** over the whole tubing system prevents gas bubble formation.
- **High humidity (>70%)** over the whole tubing system prevents evaporation.
- **Homogeneous flow velocity** rather pushes gas bubbles out, then keeping and enlarging them in regions with lower flow velocity. Adapters are inevitable, and always somehow change the flow velocity. That means in reality an optimal homogeneous flow velocity is not attainable.
- **Overpressure (positive pressure)** creates a pressure drop between inside the tubing and outside, rather pushing the gas out of the tubing. On the other hand, an underpressure (negative pressure) creates a pressure drop sucking in gas from outside the tubing and thus favoring gas bubble formation.
- **Short term** experiments do not give gas bubbles the time to develop.
- **Introduction of a bubble trap** can help catching gas bubbles before they enter the channel with the cells. Bubble traps have a certain capacity until they are exhausted and save time for the experiment.

The principle of a bubble trap is shown in Figure 3. A simple bubble trap can be made joining two syringe parts by melting the edges (Figure 4).

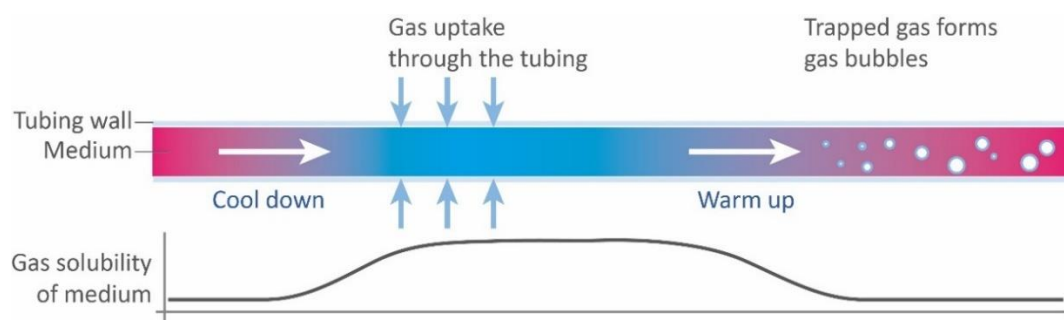


Figure 1 Gas uptake and bubble formation along a temperature gradient caused by different gas solubilities of the medium.

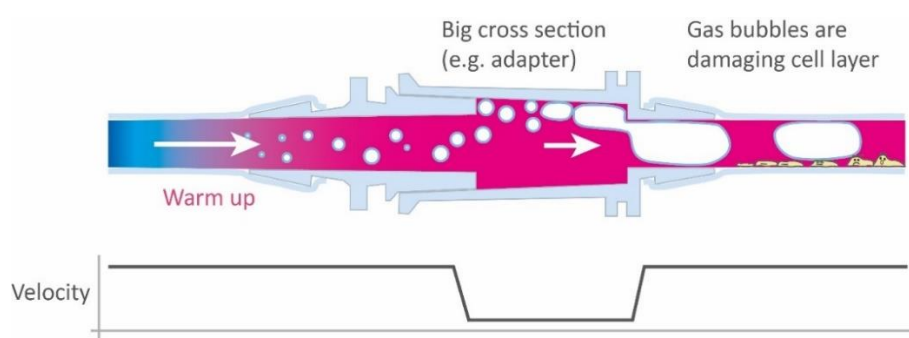


Figure 2 Formation of large bubbles at widening cross section of the tubing.

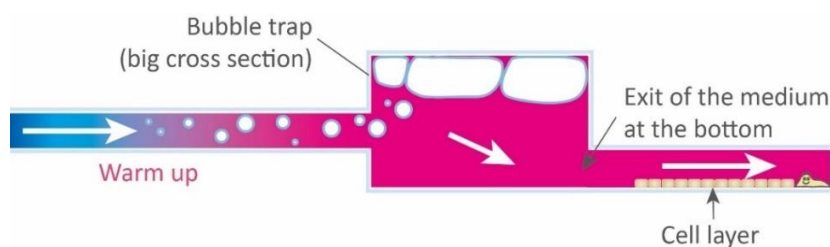


Figure 3 Principle of a bubble trap

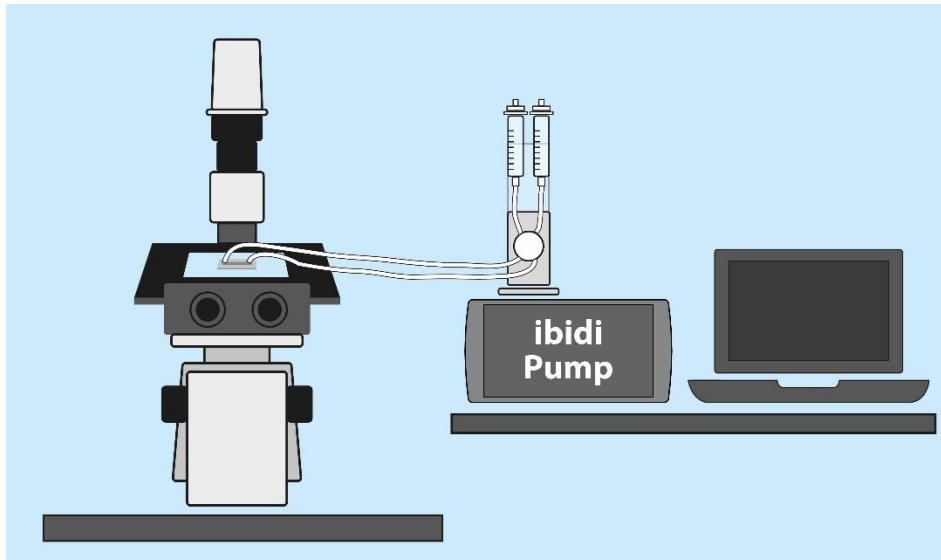


Figure 4 Example of a simple DIY bubble trap.

2. Setup Without Incubation

Material:

- ibidi Pump System placed next to the microscope
- Inverted microscope



Room temperature, no CO₂, no humidity

Pro:

- Homogeneous medium temperature along the perfusion tubing.
- Low probability of gas bubble development.
- Very stable over longer time periods.
- Very low costs and easy handling.

Con:

- Cells are at room temperature.
- No CO₂ supply to the cell culture medium (carbonate buffers are not functional).

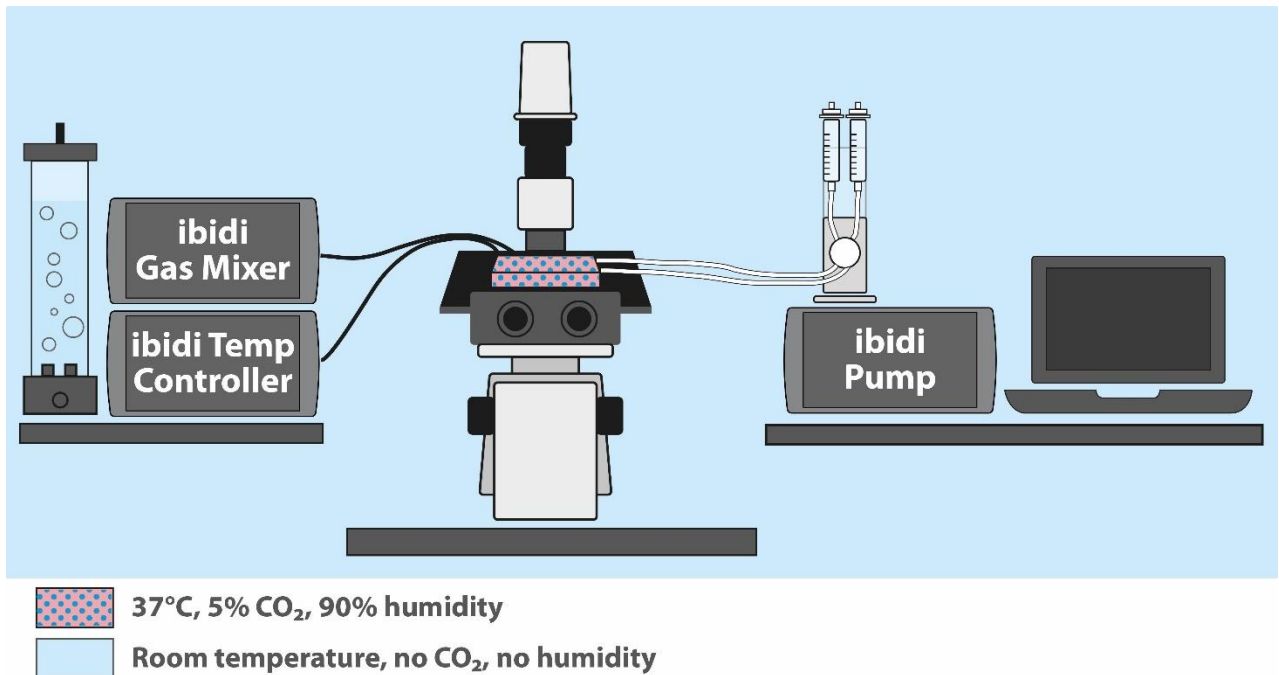
Suited for:

- Cells or organisms that can be cultivated at room temperature.
- Experiments without cells.
- Potentially for short term imaging, if cells tolerate the temperature drop.

3. Setup With Stage Top Incubation

Material:

- ibidi Pump System placed next to the microscope
- ibidi Stage Top Incubation System (Gas Mixer and Temperature Controller) controls temperature, CO₂, and humidity in the stage top incubation chamber.
- Inverted microscope



Pro:

- Cells are at warm temperature.
- Low costs.

Con:

- The temperature gradient along the perfusion tubing promotes the generation of air bubbles.
- Low CO₂ input to the culture medium. The exposure time in the incubation chamber (supplied with CO₂) is very short. It helps to suck in the atmosphere from the stage top incubator to the pump back port. Like this CO₂ enriched gas is applied on top of the medium reservoirs.
- Not stable over long time periods.

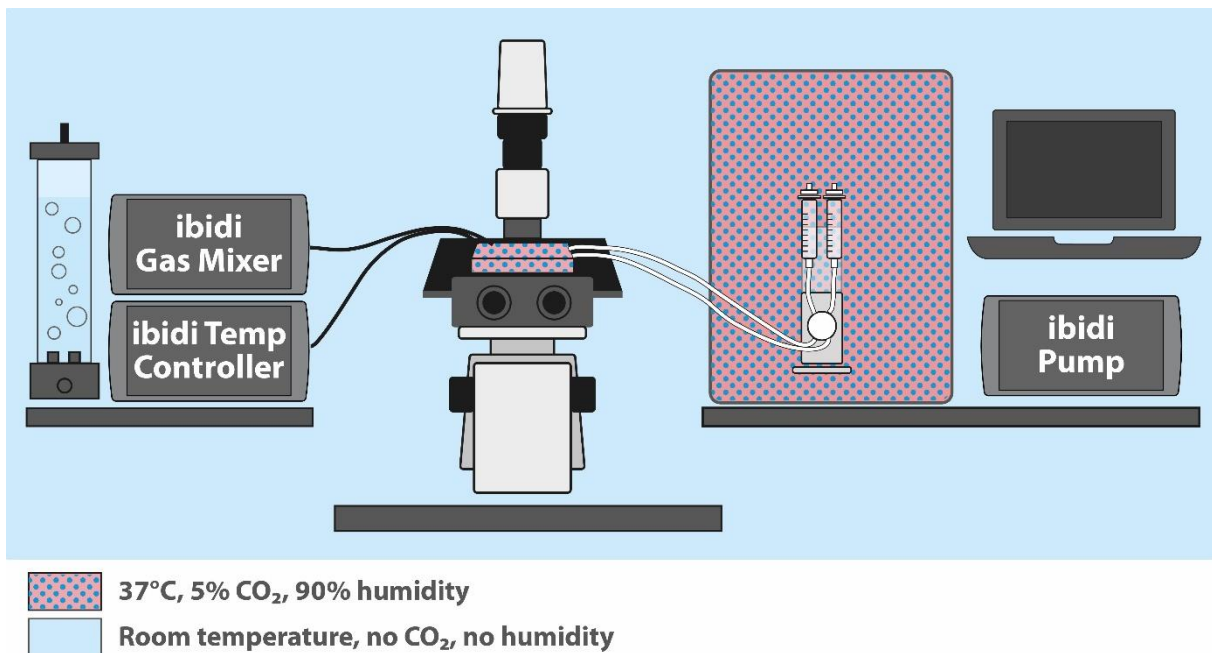
Suited for:

- Short term experiments.

4. Setup With Stage Top Incubation and Bench Top Incubator

Material:

- ibidi Pump System
- Bench top incubator
- ibidi Stage Top Incubation System (Gas Mixer and Temperature Controller) controls temperature, CO₂, and humidity in the stage top incubation chamber.
- Inverted Microscope



Pro:

- Cells can be kept at 37°C since cooling of the medium only occurs in the tubing leading from the incubator to the stage top incubation.
- CO₂ supply to the medium is provided to the medium reservoirs and channel slide. For optimal CO₂ supply, the pump back port must be connected to the small incubator's atmosphere.
- Stable over medium time periods.

Con:

- The temperature gradient along the perfusion tubing favours the generation of air bubbles during long time periods.

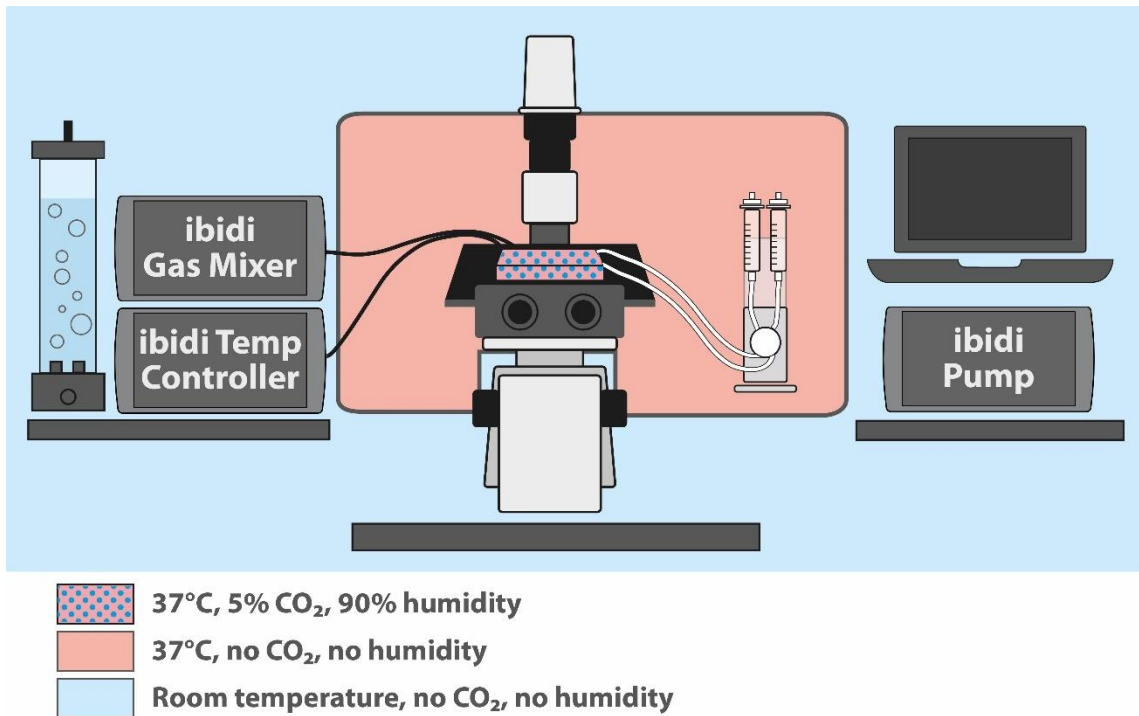
Suited for:

- Medium time periods with cells that need to be kept warm.
- Longer time periods with an additional bubble trap.

5. Setup With Cage Plus Stage Top Incubator

Material:

- ibidi Pump System placed next to the microscope
- ibidi Stage Top Incubation System (Gas Mixer and Temperature Controller) controls temperature, CO₂, and humidity in the stage top incubation chamber.
- Inverted Microscope equipped with heated cage



Pro:

- Homogeneous temperature along the whole perfusion tubing and reservoirs prevents air bubbles.
- Optimal temperature at the cells.
- Stable over long time periods.

Con:

- Dry atmosphere inside the cage incubator could favor the generation of air bubbles.
- Low CO₂ supply to the culture medium. The dwell time in the incubation chamber is very short.
It helps to suck in the atmosphere from the stage top incubator to the pump back port. Like this, CO₂ enriched gas is applied on top of the medium reservoirs.
- High costs.

Suited for:

- Long term experiments.