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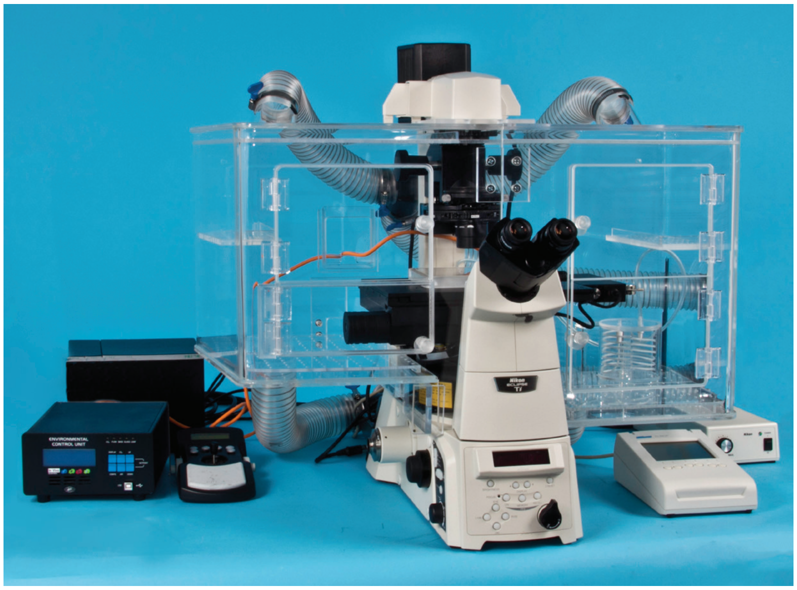
Live Cell Microscopy

Study living cells under a microscope – Stagetop incubator or full system enclosures



Incubator on a Nikon Eclipse TE-2000 Microsocpe. Incubators for Zeiss and Olympus microscopes are also available, as well as versions that accommodate confocal modules. All incubators are compatible with all commercially available cameras, light sources, filter wheels, motorized stages, and motorized nosepieces.

A new, exciting world is opening to cellular biologist. Now, we can witness intracellular and extracellular events as they happen.

In the past, dead cells were stained and examined under a microscope. Now, tiny incubators can be place on a microscope stagetop and live cells in culture can be directly observed.

The living cells are grown in standard culture wells. The well plates are placed in a small stagetop incubator that fits directly on a microscope stage. Or, the entire microscope may be enclosed in a chamber.

WPI, working with a team from InVivo Scientific, has developed a new stagetop environment controller (STEV) for a stagetop incubator or microscope enclosure. The system can control air flow, temperature and CO_2 in the stagetop incubator or the larger microscope enclosure.

Moving to Stagetop

Recent years have seen a trend toward the smaller stagetop environments, because they can provide a more stable condition for culturing cells. Temperature, CO_2 and air flow can be precisely regulated using a simple user interface with a digital readout of the parameters.

This Live Cell Microscope Incubator was extensively tested in laboratories. When compared with other systems, it offers dramatic advantages. For example, other incubators for live cell microscopy rely on passive, random diffusion of heated air from a single source to maintain the desired temperature setpoint.

With no hot air return vent, the heated air escapes from the system through cracks at the microscope/incubator junction in an uncontrolled, random fashion. These systems offer no temperature uniformity, suffer from focus drift and often experience electrical and vibrational interference from the heater. You will also notice dramatic temperature drifts when the imaging environment is disturbed.

Features

- Unique, diffusion grid, combined with air input and return vents provide an air flow pattern for consistent, even heating, with no hot or cold spots in the chamber
- External heater that can be placed far enough from the system to eliminate electrical and vibrational interference from the heater
- High degree of temperature precision and stability
- Minimal focal drift after equilibrium is achieved–Accuracy ±0.1°C at the sample itself, and 0.2°C across the microscope stage (allowing for uniform heating of multiwell dishes)
- Airflow pattern and temperature uniformity eliminate dramatic changes in environmental temperature when the incubator door opens
- Ergonomic design for ease of use– The focus and x/y stage controls are outside of the incubator itself. Large doors allow easy access to the specimen and small ones for cords, tubing, etc.
- Precision, shielded temperature probe
- Simple, one person setup of the system

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Consistent Air Flow

Air flow affects the temperature uniformity of incubators. The red arrows on Fig. 1 and Fig. 3 indicate air flow. The Live Cell Microscope Incubator uses a diffuser grid and proper venting to insure consistent air flow. Traditional incubators with poor air flow suffer with hot and cold spots in the incubator, as seen in thermal images (Fig. 2 and Fig. 4). Warmer temperatures are indicated by red and

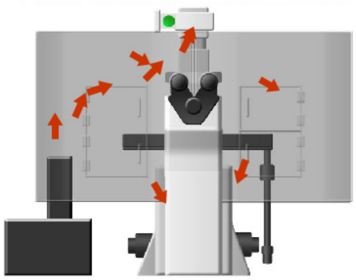

Fig. 1–Single air input and no venting causes random air flow in a traditional incubator

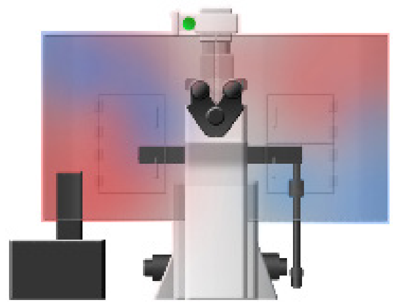

Fig. 2–Hot and cold spots result from inconsistent flow.

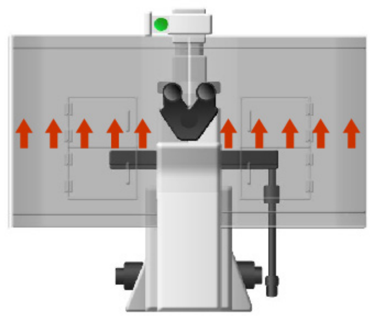

Fig. 3–A diffusion grid with air input and exhaust vents yields consistent air flow.

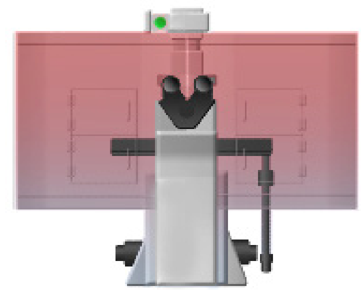

Fig. 4–Consistent air flow means uniform heating.

Temperature Stability

Thermal stability of the Live Cell Microscope Incubator is clearly shown in Fig. 5 (below). Temperatures were recorded with a digital temperature probe placed at the center of the stage. High precision temperature stability is maintained over both short (left) and long (right) durations.

The Environtmental Control Unit is positioned on an inverted microscope for a simple live cell imaging setup.

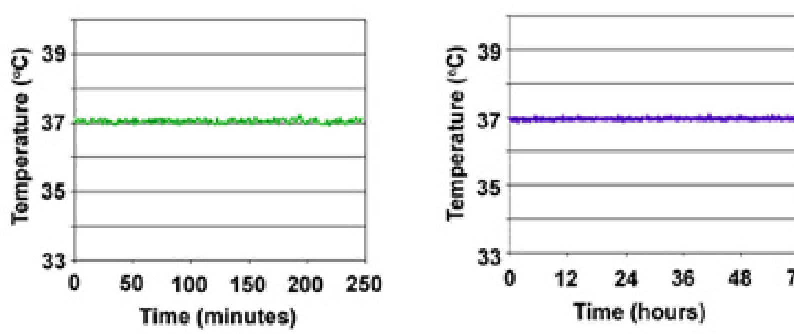

Fig. 5–The temperature remains stable both short- and long-term.

SPECIFICATIONS

Power	110/240V, 50/60Hz
Operating Temperature (ambient)	10 – 50°C (50 – 122°F)
Operating Humidity (ambient)	15 – 70% RH, non-condensing
Warm up Time	20 minutes
Computer Interface	USB via external USB/RS232 converter
Sensor	Non-dispersive infrared (NDIR), dual beam, 20s response time

Systems

Available live cell environment systems include:

- **ECU-H5**: Non-adjustable CO_2 environment
- **ECU-HC**: Adjustable CO_2 environment
- **ECU-HOC**: Adjustable CO_2, O_2 environment

ECU-H5–Static CO_2

Using a static CO_2 concentration, pre-mixed CO_2 gas cylinders that are readily available from any local gas supplier (for example, 5% CO_2, 95% ambient air), **ECU-H5** offers several advantages.

- Low flow gas used
- Highly effective humidifier
- Virtually no evaporation from the sample and eliminated contamination
- Stagetop incubator has an optical grade glass lid suitable for DIC applications, and is designed to fit under all long working distance condensers
- Microscope enclosure ensures that the sample is always exposed to the perfect environment, even when working inside the stagetop chamber.

1. Dry, pre-mixed 5% CO_2 exits the gas cylinder, asses through a pressure regulator set to 100mmHg.
2. The gas passes through an adjustable flow regulator (usually at 30-50mL/min.).
3. The low-flow gas enters the microscope chamber. It is humidified using a proprietary water vapor humidifier (without changing the CO_2 concentration). It significantly increases the water saturation of the gas compared to "bubble" systems (>99% saturation vs. ~80% saturation). This leads to almost no evaporation from the sample over prolonged periods, as well as reduces the chance of contamination (since the gas is not directly in contact with water at all).
4. The humidified gas passes through a temperature controller on top of the microscope stage.
5. The gas enters a stagetop incubator that fits into our stage adapter. This mini-chamber can be custom designed to fit any sized dish/plate, as well as stage. Glass lids can be customized for users who require open access for micromanipulators.

ECU-HC–Adjustable CO_2

In addition to the features of the **ECU-H5, ECU-HC** also allows for precise CO_2 concentration control.

This system allows for more customization and a higher precision in CO_2 control for more demanding applications, or for labs that need more flexibility than pre-mixed gas cylinders can provide (like, multiple cell lines with different CO_2 requirements). This system requires a 100% CO_2 cylinder, as well as an ambient air gas supply (via pressurized cylinder or a ambient air house line).

1. The stream of ambient air passes into the controller at a defined rate (40 mL/min).
2. This air enters a mixing chamber, along with a second stream of 100% CO_2 through a variable flow, feedback controlled valve.
3. The percentage of CO_2 can be precisely adjusted at any time using a digital controller.
4. This gas mixture leaves the controller and enters the heated microscope chamber. As in the first system, it is humidified using our special tubing humidification system and the temperature is equilibrated to the stage temperature.
5. The gas enters the same stagetop incubator that surrounds the sample.

ECU-HOC–Adjustable CO_2, O_2

In addition to CO_2 control from a 100% CO_2 source, the **ECU-HOC** controls oxygen levels from ambient (20.7%) to 0% by mixing in bottled nitrogen gas. Like the other CO_2 systems, the **ECU-HOC** uses specialized humidification tubing and gas temperature equilibration.

1. A stream of nitrogen gas passes into the controller at a defined rate to mix with ambient air to reduce the O_2 concentration.
2. This air mixture enters a mixing chamber along with a controlled stream of CO_2 through a variable flow, feedback controlled valve.
3. The percentage of CO_2 can be precisely adjusted at any time using the digital controller.
4. This gas mixture leaves the controller and enters the heated microscope chamber. As in the first system, it is humidified using our special humidification system and the temperature is equilibrated to the stagetop temperature.
5. The gas enters the same stagetop incubator that surrounds the sample.

The Satellite AirTherm can be controlled by the ECU to regulate temperature in an incubation chamber.

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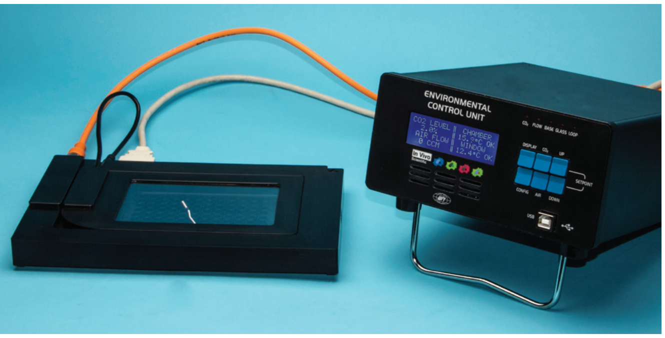


(Above) The ECU system consist of the stagetop environment base unit and the electronic controller

Environmental Control Unit

This system offers precision control of temperature, carbon dioxide and oxygen, as well as remote control and data logging via a USB connection. The system is flexible and easy to configure for a variety of experimental conditions.

Control System Configurations

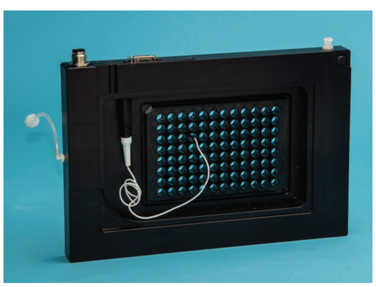
ECU-H5	Controller with heat only
ECU-HC	Controller with CO_2 and heat
ECU-HOC	Controller with CO_2 and O_2

The **Environmental Control Unit (ECU)** houses all the electronics for:

- Regulating the CO_2 and O_2 flow and temperature in the chamber
- Controlling the temperature of our **AirTherm** Satellite or an auxiliary heater
- Monitoring the air flow, CO_2 level, O_2 level and temperature

The system comes in a variety of configurations:

- **ECU-H5**–Pre-mixed CO_2 gas of the desired concentration is pumped into the system, and this **ECU** regulates the airflow at the desired level. It also controls the heating and monitors the environment chamber.
- **ECU-HC**–This control unit mixes the air and CO_2 gas to the desired concentration. An internal CO_2 sensor (inside the **ECU** controller) monitors the concentration of the gas. The **ECU-HC** regulates the airflow at the desired level, controls the heating of the system and monitors the environment chamber.
- **ECU-HOC**–This unit performs like the **ECU-HC**. In addition to controlling the temperature and CO_2, this unit also controls the O_2 level. However, it offers no auxiliary heating options. Nitrogen is used to displace oxygen from the background air, which generally has about 20.7% oxygen. The O_2 level of the background gas can be regulated down to as low as 1%.



The base unit above has the lid removed, showing the placement of the temperature sensor in one of the wells of a 96-well plate.

Features

- USB-based remote control and data logging
- Electronic flow meter
- Programmable alarm for out of tolerance condition
- Compact and lightweight
- Adjustable setpoints for parameters, including:
  - Temperature PID controls for the environmental chamber with ±0.1ºC precision
  - CO_2 and O_2 digital PID control with ±0.1% precision
  - Airflow digital PID control from 0–900 SCCM

SPECIFICATIONS

CO_2 Sensor (ECU-HC)

Sensor Range	0 – 20% CO_2
Control Range	0 – 20%
Control Precision	0.1% CO_2
Control Accuracy	0.1 – 3% of reading
Drift	<2.5% reading/year

O_2 Sensor (ECU-HOC only)

Sensor Type	Zirconium Dioxide, diffusion, 4s response time
Sensor Range	0–25%
Control Range	0–25%*
Control Precision	0.1% O_2
Control Accuracy	±0.5% (2% of the full scale)

*The upper limit of the **ECU-HOC** oxygen control range is constrained by the oxygen content in the background gas. For example, if the background air has 20.7% oxygen, the **ECU-HOC** can only control up to 20.7% oxygen.

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USA: International Trade Center, 175 Sarasota Center Boulevard, Sarasota FL 34240-9258 USA
Tel: (941) 371-1003 • **Fax:** (941) 377-5428 • **E-mail:** wpi@wpiinc.com • **Internet:** www.wpiinc.com

UK: 1 Hunting Gate, Hitchin, Hertfordshire SG4 0TJ England • Tel: 44 (0)1462 424700 • E-mail: wpiuk@wpi-europe.com
Germany: Saarstraße 23, D-61169 Friedberg (Hesson) • Tel: +49 (0)6031 1602171 • Fax: +49 (0)6031 1602180 • E-mail: wpide@wpi-europe.com
China & Hong Kong: Rm 25e, No8 Donfang Rd., Pudong District, Shanghai 200120 PRC • Tel: +86 688 85517 • E-mail: ChinaSales@china.wpiinc.com
Brazil: Conselheiro Nabias, 756 sala2611, Santos-São Paulo 11045-002 Brazil • E-mail: info@brazil.wpiinc.com