

LiveCell³ Users Guide



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The Neue Biosciences' LiveCell³ system is designed to provide users with a simple to use yet precisely controlled environment for the culturing of cells on a microscope stage. You can adjust and precisely control the temperature, humidity and CO₂ level inside of the modest sized microscope stage chamber.

Accurate and precise closed loop control of CO₂ eliminates the need for expensive pre-mixed CO₂ gas or the inconsistencies of flow rate mixing of gases to enrich the environment to the desired level.

Absolute control of the temperature and humidity levels in the chamber enables longer time lapse observations without worrying about evaporative loss or possibilities of condensation on the optical surfaces. You can just simply adjust the temperature, humidity, or CO₂ Set Points through a simple front panel interface, enable the controllers, and the control unit does the rest.

The microscope stage chamber is large enough to allow the user to view cells in larger culture flasks as well as slides and plates without suffering the poor regulation, the need for a separate CO₂ enrichment chamber, and leaks common to the larger whole microscope chambers.

The LiveCell³ was designed to be a closed environmental control system in order to minimize the CO₂ usage and therefore the leakage to the outside working environment (this is particularly critical in small poorly ventilated labs).

OCCUPATIONAL LIMITS FOR AMBIENT CO₂ IS 5000 PPM. THE LIVECELL 3SYSTEM HAS BEEN DESIGNED TO MINIMIZE CO₂ LEAKAGE DURING NORMAL AND EVEN ABNORMAL CONDITIONS, HOWEVER THE USER SHOULD INSTALL A CO₂ MONITOR AND ALARM IN ANY AREA WHERE CO₂ COULD BUILD UP TO DANGEROUS LEVELS.

The temperature is adjustable and controlled from ambient to $50 \pm 0.1^{\circ}\text{C}$ (The system only has heating capability), humidity is adjustable and controlled from ambient to condensation within $\pm 1\%$, and the CO₂ level can be adjusted and controlled from ambient to $10 \pm 0.1\%$.

This document is intended to be a comprehensive guide on the installation, use, troubleshooting, and maintenance of the LiveCell³.

Proper Operation

Proper operation of this equipment relies on the user reading this user guide in its entirety.

CO₂

Use only in a well ventilated area or install a CO₂ Monitor to ensure safety of the operator. Disconnect the CO₂ source when not in use.

Electrical Shock

DO NOT OPEN THE CONTROLLER HOUSING FOR ANY REASON as there are no user serviceable parts inside and potentially dangerous voltages may be present even with the power cord disconnected.

Electrical Shock

Use only properly grounded outlets.

Other Uses

The Equipment is intended to be strictly dedicated to the control of the environment on the microscope stage that the chamber is specifically designed for. Operation of the Equipment for any other purpose or microscope stage may result in permanent damage to the Equipment or may create a harmful situation for the user. THE INSTALLATION ON ANY OTHER DEVICE MAY DAMAGE THE EQUIPMENT, INJURE THE USER, AND VOID ALL WARRANTIES.

Water for Humidification

Use room temperature tap water to fill the bottle to the fill line, DO NOT OVERFILL . Overfilling the bottle may result in damage to internal components of the system. DO NOT USE DI WATER since the system senses the resistance of the water to prevent running dry. THE HUMIDIFICATION BOTTLE MUST BE EMPTIED WHEN NOT IN USE.

Cleaning

The LiveCell³ Controller Unit may only be wiped down with a damp cloth. Avoid the use of solvents and abrasive cleansers. The Chamber itself should never be cleaned with any ammonia based cleaners, solvents, or abrasive cleansers. Use only cleaning products recommended for Acrylic, Plexiglas, Lexan, and coated optical components. DO NOT CLEAN THE CO₂ SENSOR!

Service

DO NOT ATTEMPT TO SERVICE THE EQUIPMENT unless otherwise instructed to do so by an authorized Neue Biosciences representative. Doing so will void the warranty or service contract.

Fuses

Replace only with same type and rating.

The LiveCell³ system is intended to control the temperature, humidity, and CO₂ level within the Stage Chamber. Thereby creating a long term incubation environment right on a microscope stage. The system utilizes CO₂ from an external, regulated, CO₂ source and then meters the CO₂ into the system as a function of the measured concentration and the user selectable Set Point Value in order to maintain the concentration. The system also humidifies and heats the circulating air as a function of the measured temperature and the user selectable Set Point Values in order to maintain the temperature and % relative humidity. The temperature, humidity, and CO₂ concentration are all measured inside of the Stage Chamber in close proximity to the sample.

Enable switches have been provided to allow the user to run the system under temperature, humidity, or CO₂ control alone all at once. The Green LED of the switch will light when the controller is enabled.

In the event the Measured Value of CO₂ concentration, humidity or temperature fall outside of the allowable limits, a red Alarm LED will light indicating a problem.

NOTE: The Alarm LEDs will be illuminated during the initial startup cycle and will turn off when the system has stabilized. OR, when a controller has been disabled.

Humidity is actively added to the system if the water level is maintained within the limits displayed on the bottle holder. A full bottle may provide up to 120 hours of hands off performance.

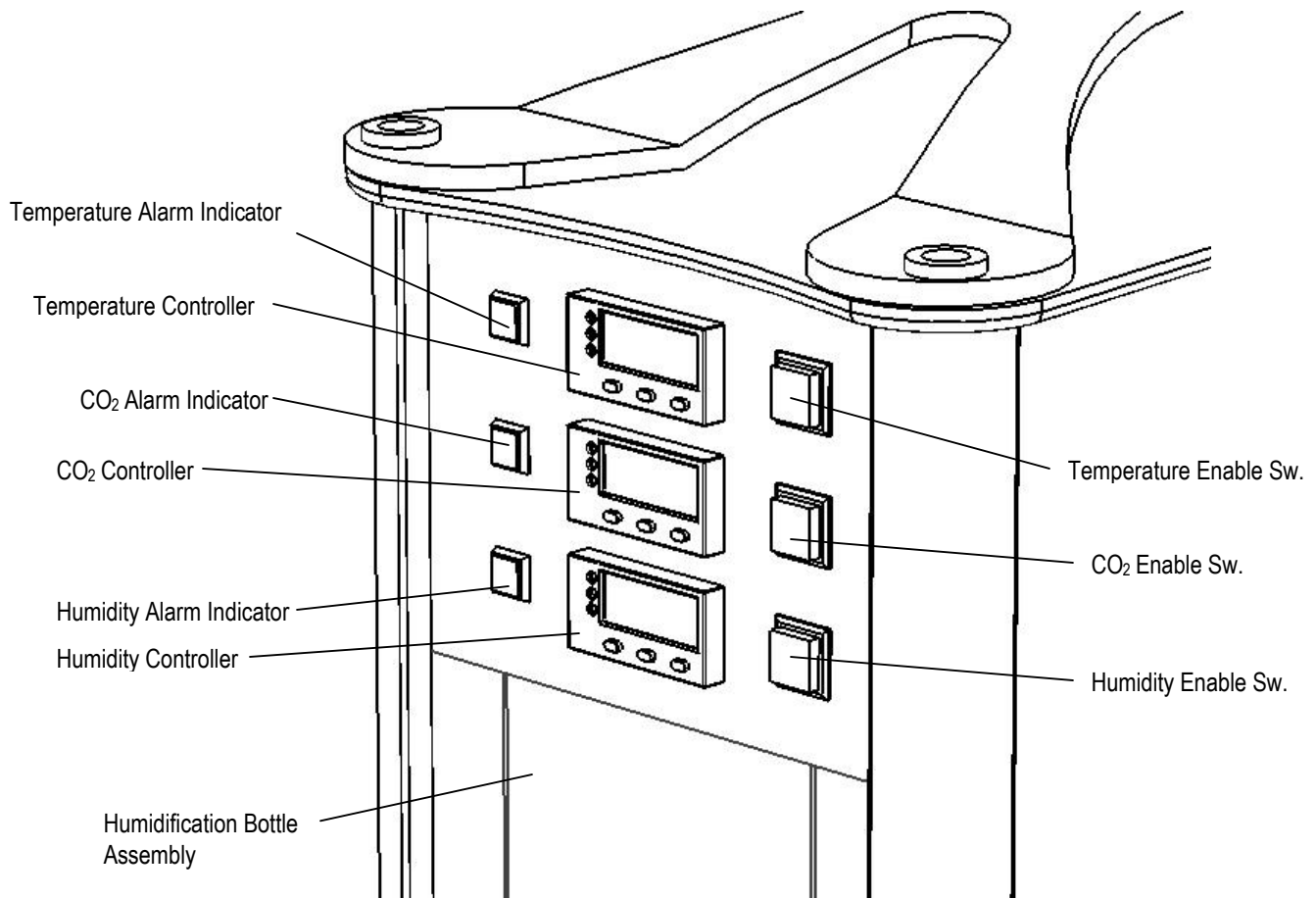
Great care has been taken to minimize the coupling of vibration from the Control Unit to the microscope, however, very high magnification may require the user to relocate the Control Unit if image quality is degraded.

The controllers are very sophisticated and have the ability to quickly bring the environment in the Stage Chamber to the Set Points and hold it there with tremendous stability. The Set Point of the controllers can be easily changed at any time by pressing the "SEL" button then using the up or down arrow buttons to change the Set Point. Pressing "SEL" once more changes the display back to the control mode and Measurement Value reading.

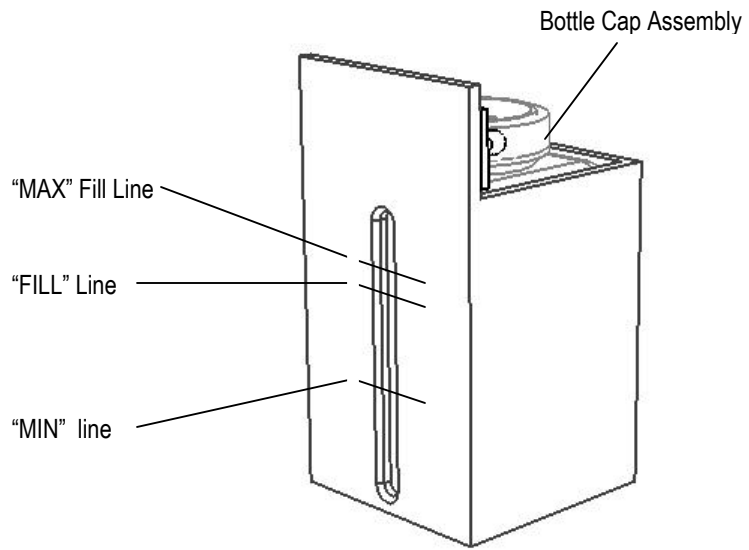
Specifications:

Supply Voltage	110VAC, 5A, 50/60 Hz
Temperature Control Range	Ambient - 50 °C
Temperature Control Resolution	0.1°C
Temperature Accuracy at 37°C	< ± 1.5 %
CO ₂ Control Range	Ambient – 10 %
CO ₂ Control Resolution	0.1%
CO ₂ Accuracy at 25°C	< ± 0.2 %
CO ₂ Non-Linearity	< ± 0.5 %

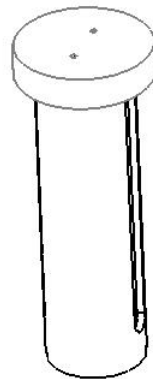
%RH Control Range	Ambient – 85% RH
%RH Control Resolution	1%
%RH Accuracy at 37°C	< ± 2 %
Operational Temperature Range	25 - 35°C
Operational Humidity Range	0 – 85% non-condensing
Warm-up time for stabilization	< 30 min. but depends on room temperature
Storage Temperature	-30 – 70°C



LiveCell Controller Unit

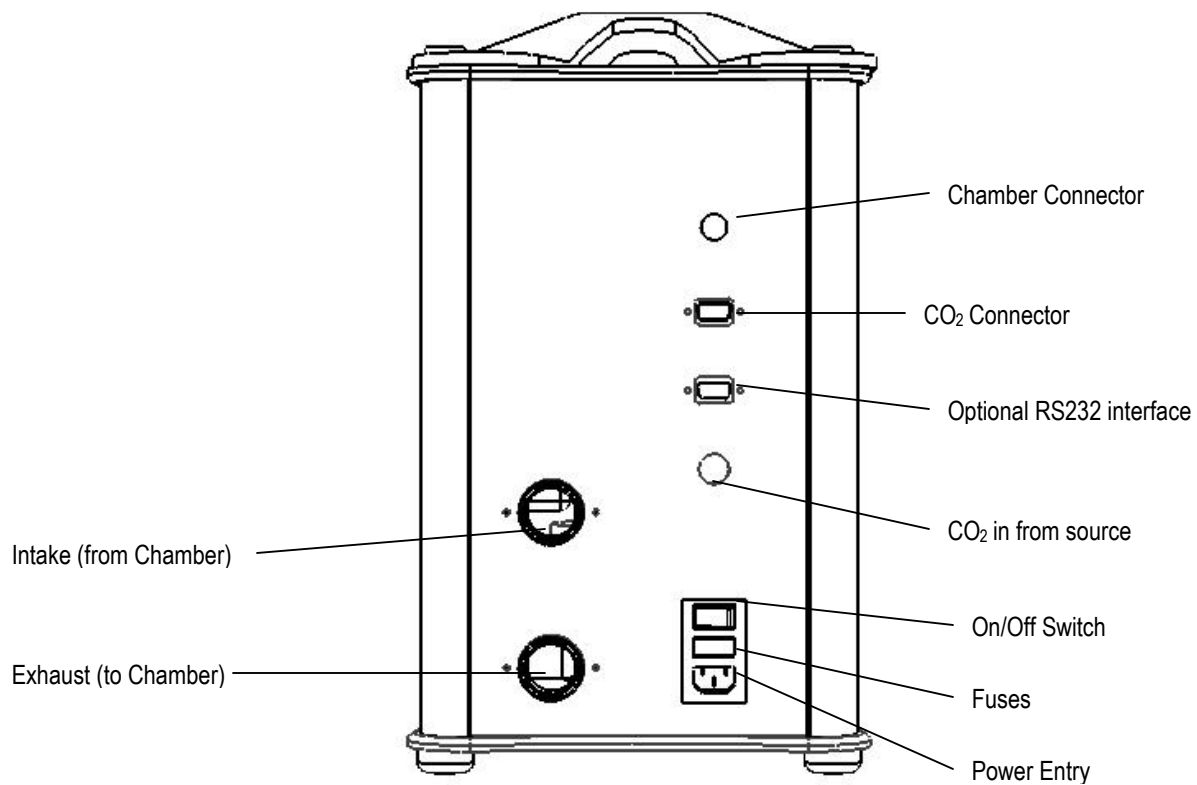


Humidification Bottle Assembly



Bottle Cap Assembly

Temperature Controller	The control unit for the Stage Chamber temperature.
Temperature Enable Switch	Enables and Disables the Temperature control. Illuminated is Enabled.
Temperature Alarm Indicator	An LED that is illuminated any time the temperature differs from the Set Value (SV) by more than 0.5 °C.
CO₂ Controller	The control unit for the Stage Chamber CO ₂ concentration.
CO₂ Enable Switch	Enables and Disables the CO ₂ control. Illuminated is Enabled.
CO₂ Alarm Indicator	An LED that is illuminated any time the CO ₂ level differs from the Set Value (SV) by more than 0.5 %.
%RH Controller	The control unit for the Stage Chamber humidity.
%RH Enable Switch	Enables and Disables the humidity control. Illuminated is Enabled.
%RH Alarm Indicator	An LED that is illuminated any time the %RH differs from the Set Value (SV) by more than 5 %RH.
Humidification Bottle Assembly	A bottle assembly used to introduce water vapor into the system.
Control Unit	The integrated controller for Temperature, Humidity and CO ₂ .



Power Entry

Provides power to the whole system.

CO₂ Entry

Provides CO₂ to the system from an externally regulated source.

Fuses

Two 250V, 5A fast blow fuses type GMA-5ABC.

On/Off Switch

Turns the unit On and Off.

Intake

The return path for the air handling system. This is connected to the Chamber's "Output to Controller" connection.

Exhaust

The output of the air handling system that goes directly to the Chamber's "Input from Controller".

Chamber Connector

This is where the gray chamber cable connects to the Control Unit..

CO₂ Connector

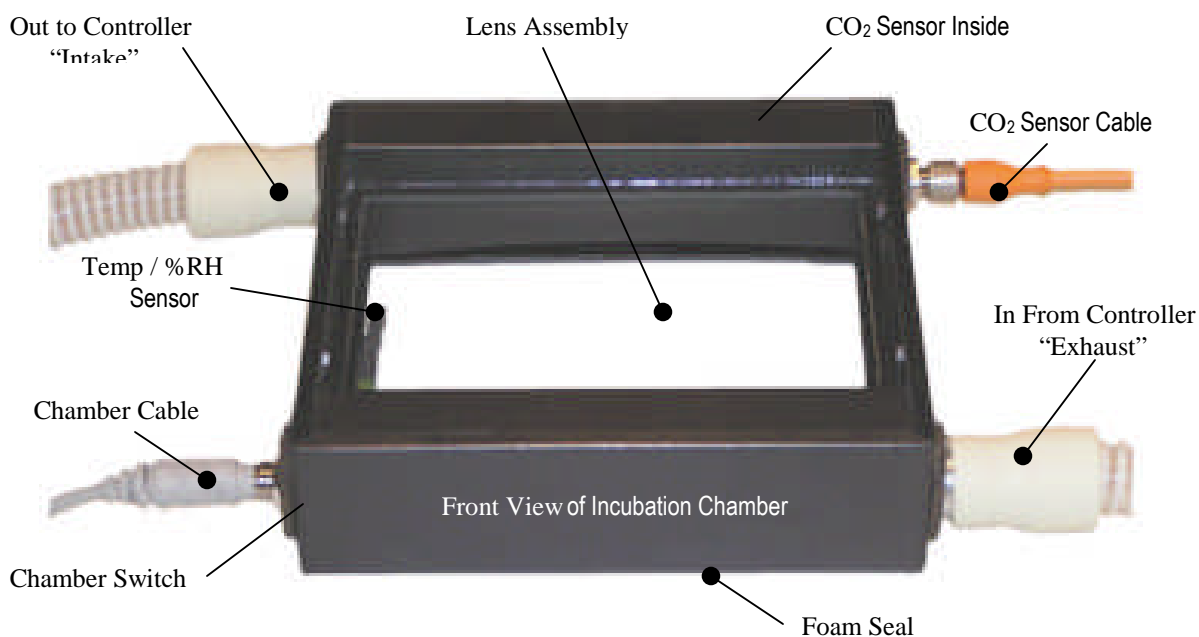
This is where the orange chamber cable connects to the Control Unit.



NOTE: Trying to force the incorrect cable into a connector will result in bent or broken pins on the cable.

Optional RS232

This is where the RS232 cable would connect for optional serial communications with the controller.



In From Controller

Connection for hose from LiveCell³ Control Unit "Exhaust".

Out To Controller

Connection for hose to LiveCell³ Control Unit "Intake".

CO₂ Sensor

Sensor for CO₂ level feed back to Controller.

Temp and %RH Sensor

Sensor for Temperature and Humidity feedback to Controller.

Chamber Connector

The connector for lid switch signals and temperature signal.

Sensor Connector

The connector to the CO₂ sensor.

Chamber Switch

This switch disables controller outputs if the Chamber is not positioned properly.

Lens Ass'y

The interchangeable optical window assembly for different illumination condensers.

Foam Sealing Strips

Seals Chamber ato the Stage.

CO₂ Control Inside Of The Microscope Chamber

When the system is assembled properly, it controls the CO₂ level in the microscope chamber to the last entered Set Value. The system uses a CO₂ sensor inside of the Chamber to provide information the CO₂ Controller requires for precise and accurate stabilization of the CO₂ level. CO₂ from the eternal source is regulated by the Controller in short bursts based on the self-tuned calculations.

Front panel operations for the CO₂ Controller are:

- Adjustment of the Set Value (SV) – the desired CO₂ level in the Chamber.
- Display of the Process Value (PV) – the actual measured value of CO₂ in the Chamber.
- Adjustment of the Alarm thresholds – the point at which the Alarm LED illuminates.
- Initiation of the Self-Tune process – a function of the controller to maximize the stability of the control algorithm.

All front panel operations of the Controller can be accomplished through the optional RS232 interface as well.

CO₂ Enable Switch

The output of the CO₂ controller can be selectively enabled / disabled by pushing the CO₂ Enable Switch. The green LED in the switch turns ON when enabled.

CO₂ Alarm LED

When the CO₂ Process Value is higher or lower than the Set Value by the Alarm Threshold, then the Red Alarm LED will light as a visual indication that a problem exists. No audible alarm exists. Only when $(\text{Set Value} - \text{Alarm Threshold}) < \text{CO}_2\text{Process Value} < (\text{Set Value} + \text{Alarm Threshold})$ will the LED turn off.

Temperature Control Inside Of The Microscope Chamber

When the system is assembled properly, it controls the temperature in the microscope chamber to the last entered Set Value. The system uses a thermal sensor inside of the chamber to provide information the Temperature Controller requires for precise and accurate stabilization of the temperature. A heat source is regulated by the Controller based on the self-tuned calculations.

Front panel operations for the Temperature Controller are:

- Adjustment of the Set Value (SV) – the desired temperature in the Chamber.
- Display of the Process Value (PV) – the actual measured temperature in the Chamber.
- Adjustment of the Alarm thresholds – the point at which the Alarm LED illuminates.
- Initiation of the Self-Tune process – a function of the controller to maximize the stability of the control algorithm.

All front panel operations of the controller can be accomplished through the optional RS232 interface as well.

Temperature Enable Switch

The output of the temperature controller can be selectively enabled / disabled by pushing the Temperature Enable Switch. The green LED in the switch turns ON when enabled.

Temperature Alarm LED

When the temperature Process Value is higher or lower than the Set Value by the Alarm Threshold, then the Red Alarm LED will light as a visual indication that a problem exists. No audible alarm exists. Only when $(\text{Set Value} - \text{Alarm Threshold}) < \text{Temperature Process Value} < (\text{Set Value} + \text{Alarm Threshold})$ will the LED turn off.

Over Temperature Protection

In the event of a misassembled system, failure of one or more components, or improper operation, the unit has over temperature protection to keep the User from getting burned in the event of a thermal run away condition.

Humidity Control Inside Of The Microscope Chamber

When the system is assembled properly, it controls the humidity in the microscope chamber to the last entered Set Value. The system uses a humidity sensor inside of the chamber to provide information the Humidity Controller requires for precise and accurate stabilization of the humidity. Water vapor from the Humidification Bottle Assembly is injected into the system under regulation by the Controller based on the self-tuned calculations. IT IS IMPORTANT TO STABILIZE THE TEMPERATURE BEFORE ENABLING THE HUMIDITY SINCE %RH IS TEMPERATURE DEPENDENT. ALSO, IT IS IMPORTANT TO BRING THE HUMIDITY LEVEL UP SLOWLY TO THE POINT THAT SOME CONDENSATION IS OBSERVED EITHER ON THE LENS ASSEMBLY OR THE STAGE TOP. WHEN THIS OCCURS, ADJUST THE HUMIDITY SET POINT DOWN BY 5% AND THE CONDENSATION SHOULD DISAPPEAR IN A FEW MINUTES, IF NOT ADJUST DOWN ANOTHER 5%. The humidity drops very slowly and to speed the drop in humidity, remove the hose at the Controller Intake Hub for a few seconds.

Front panel operations for the Humidity Controller are:

- Adjustment of the Set Value (SV) – the desired humidity in the Chamber.
- Display of the Process Value (PV) – the actual measured humidity in the Chamber.
- Adjustment of the Alarm thresholds – the point at which the Alarm LED illuminates.
- Initiation of the Self-Tune process – a function of the controller to maximize the stability of the control algorithm.

All front panel operations of the controller can be accomplished through the optional RS232 interface as well.

Humidity Enable Switch

The output of the humidity controller can be selectively enabled / disabled by pushing the humidity Enable Switch. The green LED in the switch turns ON when enabled.

Humidity Alarm LED

When the humidity Process Value is higher or lower than the Set Value by the Alarm Threshold, then the Red Alarm LED will light as a visual indication that a problem exists. No audible alarm exists. Only when $(\text{Set Value} - \text{Alarm Threshold}) < \text{humidity Process Value} < (\text{Set Value} + \text{Alarm Threshold})$ will the LED turn off.

Microscope Chamber

The Microscope Chamber is designed to house various sizes and formats of incubation vessels as well as various working distance illumination lenses. The accommodation of various working distance lenses is accomplished with removable, drop-in, optical window assemblies. The Chamber is designed to create a uniform flow across the optical axis for even control with minimal temperature gradients throughout the Chamber and the temperature / humidity sensor is located in a region representing the average values. For high humidity applications some condensation may occur in the corners or edges of the Chamber, on the stage top, or the Lens Assembly, this is normal and primarily caused by slight temperature gradients in the chamber and ambient temperature. The Chamber has a switch that will disable the CO₂ and Temperature Controllers any time the chamber is repositioned. This is intended to keep the system from over-heating or filling the room with CO₂ in the event the chamber is not sitting properly on the stage while running and enabled.

Typical Usage:

The following description is intended as a summary of the typical usage. All of the following steps will be detailed later in this document.

- STEP 1: The LiveCell³ Controller Unit is turned on with the CO₂, Humidity, and Temperature Controllers Disabled. After the Controllers initialize, the Process Values will be displayed.
- STEP 2: Check the Set Values by pushing the “SEL” button on the front panel of each controller. To make sure the number displayed is the Set Value, the “SV” LED should be illuminated. In order to change the Set Point, use the “up” and “down” arrow key to change the display to the desired Set Point. Once the desired Set Point is displayed, push the “SEL” button once more to accept the value. The “SV” LED should turn off and the display will revert back to reading the Process Value.
- STEP 3: Verify all connections are properly made.
- STEP 4: Enable the desired controllers. For example, If only temperature control is desired then keep the CO₂, and humidity disabled, etc.. Allow the system to stabilize. This will occur in less than 30 min. The Alarm LEDs for the Enabled controllers will be off when stabilized.
- STEP 5: Once stable, the system is ready.

STEP 6: Shutting the system down when humidity is not enabled is as simple as turning the power off, and turning off the CO₂ supply. Shutting down the system when humidification has been used involves running the system with the Humidification Bottle Assembly removed for 15 minutes or until any condensation on the chamber disappears. The bottle should be emptied when not in use, stored, or moved.

Un-Packing:

The following is a list of the packaged items:

- 1 – LiveCell³ Controller Unit
- 1 – Power Cord
- 1 – Gray 8-pin Mini-DIN cable
- 1 – Orange 8-pin sensor cable
- 1 – Microscope Chamber
- 1 – RS232 Cable (optional)
- 2 – 6 ft. hoses
- 1 – 10 ft. CO₂ 1/4" line
- 1 – Optical Window Assembly Tube
- 1 – Users Guide

Installation:

The installation process is:

STEP 1: Install the Chamber on top of the microscope stage by tilting back the microscope's Illumination Pillar, and positioning the Chamber over the stage until it sets completely over the stage and rests on the Foam Sealing Strips. Note the click of the chamber switch as the chamber is lowered.

STEP 2: The Chamber can be installed with the connectors facing to the right or the left side, but not front or back. When properly installed the Chamber will be level with the microscope stage and should not hang over any edges. Now the Illumination Pillar can be tilted back into position.


STEP 3: Connect one end of the 8-pin gray mini-DIN cable to the connector labeled "Chamber" on the Chamber and the other end to the LiveCell³ Controller Unit connector labeled "Chamber".




NOTE: Trying to force the incorrect cable into a connector will result in bent or broken pins on the cable.

STEP 4: Slide one end of a hose OVER the Chamber connection hub labeled "IN FROM CONTROL UNIT'S EXHAUST" and the other end INTO the LiveCell³ Controller Unit hub labeled "Exhaust".


STEP 5: Slide one end of the other hose OVER the Chamber connection labeled "OUT TO CONTROL UNIT INTAKE" and the other end INTO the LiveCell³ Controller Unit hub labeled "Intake".

 NOTE: The hose connections should slide either into or over the hubs fully to prevent the hose from accidentally disconnecting.

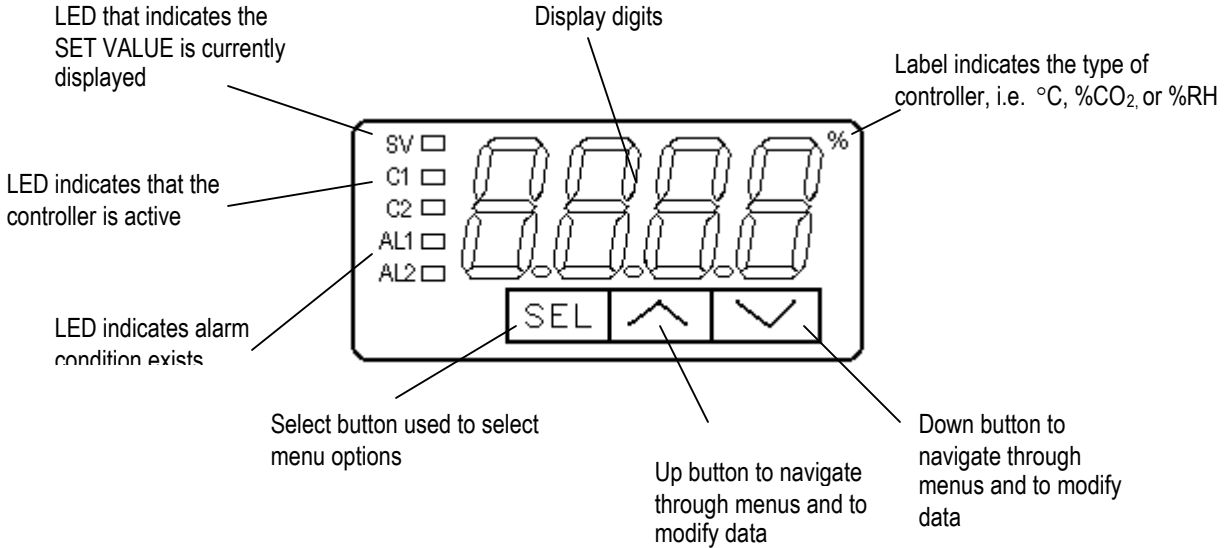
STEP 6: Connect the CO₂ source to the LiveCell³ control unit and adjust the regulator to 10 psi. Do not exceed 10 psi.

 NOTE: When not in use the CO₂ source should be disconnected and turned off.

STEP 7: If humidity is to be controlled, remove the Humidification Bottle Assembly by pushing in on the humidification unit to release, then removing. Once removed, the cap can be removed to add water to the bottle. Add ROOM TEMPERATURE TAP WATER to the bottle's "FILL" line. Once the cap assembly is re-installed the water level will raise but should not be above the "MAX" fill line. During operation make sure the level is within acceptable limits as displayed on the label. DO NOT USE D.I. WATER. D.I. water will disable the automatic shutdown of the humidification unit in the event of running out of water.

 NOTE: Do not attempt to loosen the screws on the cap.

 NOTE: Do not overfill the bottle: damage to the internal components may result.

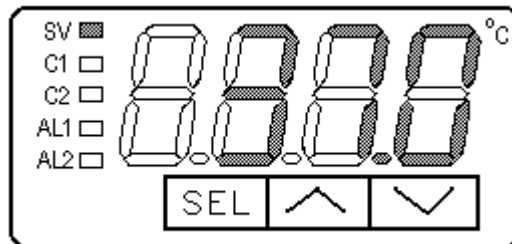


Displaying Current Values:

Upon power up the controllers will display the current values for temperature, humidity, and CO₂ and the “SV” LED will not be illuminated. As the system stabilizes, the current values can be observed.

Displaying and Setting the Set Values:

The previous Set Values are held in memory. To change the Set Values the User must power the unit up, wait for the displays to display actual numbers, then press the “SEL” button once. Now the “SV” LED will illuminate and the current Set Value will be displayed.



Use the Up and Down buttons to change the Set Value. Once the Set Value is correct, the User only needs to press the “SEL” button once to accept. Three seconds later the value will be accepted, stored in memory, and the controller will begin to control the system to that value.

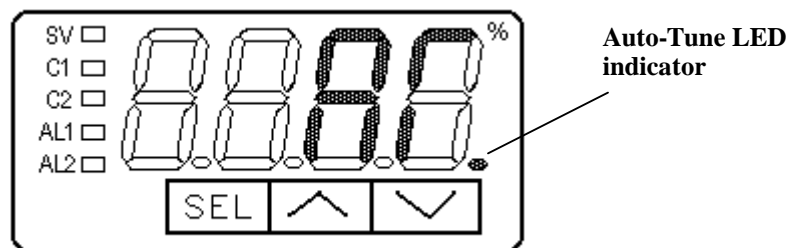
Auto-tuning:

When the system is assembled properly there is no need to run the Auto-Tune feature. However if the system becomes unstable due to changes in the microscope stage or Chamber, the system might require tuning for optimal performance.

STEP 1: Assemble the unit properly, power up the unit and make sure the CO₂ supply is on and regulator is properly adjusted, and that the water level is correct in the Humidification unit.

STEP 2: Enable the controllers with the Enable switches and allow the system to stabilize.

STEP 3: From the Front panel of each controller push the “SEL” button and hold it for ~3 seconds. or until the display looks like:

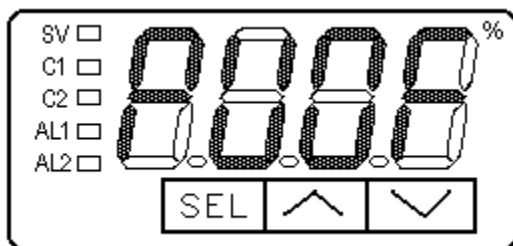


STEP 4: Pushing the “SEL” button once more will display the value of the Auto-Tune value. When the system is normally operating the value is 0. In order to command the unit to self tune, the 0 must be changed to a 1 by using the “Up Arrow”. Once the display shows a 1, the “SEL” button should be held for three seconds until the display returns to the Set Value. One more push of the “SEL” button will display the current reading. During the Auto-Tune process, the Auto-Tune LED will blink.

Calibration:

In the event the system requires calibration, the displayed value can be offset using the PVOF menu. Before entering the adjustment menu, record the difference between the desired measurement and the displayed measurement.

STEP 1: This menu is accessed by holding the “SEL” button down for ~6 seconds or until the display looks like:



STEP 2: Press the SEL button once to transition into the adjustment menu, then using the UP and DOWN buttons, enter the desired adjustment:

STEP 3: Press the SEL button and hold for ~3 seconds or until the Set Value is displayed. The offset is now stored and will be used.

RS232 Interface (special option p/n 40302)

Using **HyperTerminal** software (located in programs/ accessories/ communications in a Windows-based operating system), the following instructions allow for the monitoring and setting of temperature and CO2 data:

Open **HyperTerminal** and start a *New Connection* from **File** pull down menu

Choose following settings: **Com1** port (serial port), **9600** bits/second, **8** data bits, **None** Parity and **2** stop bits. Flow control to **Hardware**. ANSIW format.

Confirm connection of RS232 cable from back of LiveCell to PC Serial port

Type **<enter>** to view the version of the firmware "LiveCell Version: 2"

There are six basic commands:

Type **C?<enter>** to request the current set point and actual data for CO2%

Type **C= 5.0<enter>** to change the set point to 5.0% CO2 (can set up to 10.0%)

Type **T?<enter>** to request the current set point and actual data for Temperature

Type **T= 37.0<enter>** to change the set point to 37.0 deg C (can set up to 50.0C)

For service or technical support contact:

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