

MASTER THESIS

BUENOS AIRES INSTITUTE OF TECHNOLOGY

Cell-lapse

User manual

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The following document consists on a user guide which explains the different functions of the Cell Lapse onstage incubator.

1 Working conditions

Cell Lapse was designed to be used in an EVOS XL Core since its dimensions are adapted to this. However, it is possible to use the device in other microscopes if a suitable platen adapter is designed.



Figure 1: EVOS XL Core Microscope.

The device was designed to meet the requirements of a suitable temperature curve for cell culture. It is a necessary condition that it is used in an environment where the temperature does not fluctuate. It is ideal to avoid places with air drafts.

The device should be used in environments with an ambient temperature between 19 °C and 26 °C and with a relative ambient humidity greater than 20 %. Furthermore, the assays must be carried out with 3 ml of culture medium supplemented with HEPES 1x to reduce the effects caused by evaporation.

Failure to comply with these conditions does not guarantee the correct functioning of Cell Lapse.

2 Installation

Connections

Before starting an assay the user must verify that all connections are properly made. The heater is connected by means of a 5-way cable to the operator module on one side

(sensor information and fan power supply) and with a 2-way cable to the power part by means of a MOLEX type connector; the main cabinet connects via a 4-way cable to the operator module; the switching power supply is connected to 220 V on one side and to the operator module on the other, through a MOLEX type connector.

If the steps in the Manufacturing and Assembly Manual were accurately followed, correct operation is guaranteed since all the connectors have a single way of connecting to the module.

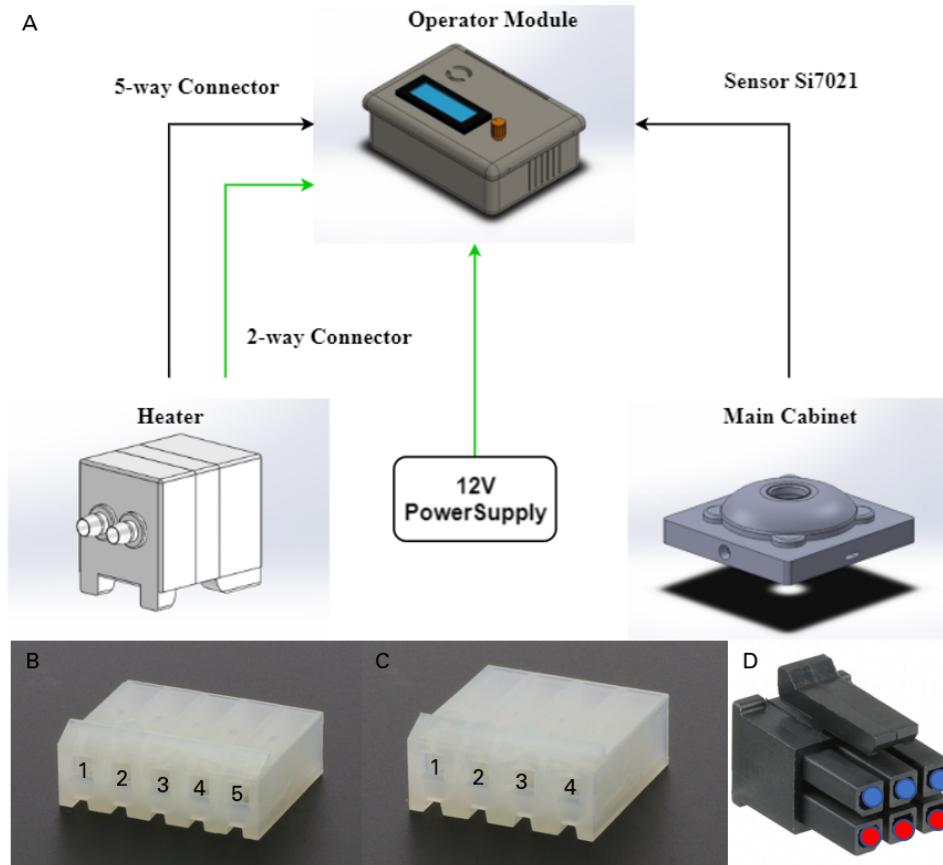


Figure 2: A) Connection diagram between Cell Lapse modules. Connections involving a MOLEX connector are marked in green. B) 5-way connector. See Table 2. C) 4-way connector, Si 7021. See Table 1. D) MOLEX connector. Red - Earth. Blue - 12V.

Table 1: 4-way connector pinout.

Pin	Connection
1	5 V
2	GNDA
3	SCL
4	SDA

Table 2: 5-way connector pinout.

Pin	Conexión
1	Fan GND to MOSFET
2	Fan to 12 V
3	LM35DZ OUT
4	LM35DZ 5V
5	LM35DZ GNDA

Data collection

Cell Lapse provee dos sistemas diferentes para el usuario que deseé recolectar los datos del ensayo. Tanto mediante una conexión USB como Bluetooth se pueden obtener las curvas de temperatura y humedad. En Windows, se puede utilizar la interfaz de usuario 'Cell Lapse Monitor'.

Cell Lapse provides two different systems for the user who wishes to collect assay data. Both through a USB connection and Bluetooth, the temperature and humidity curves can be obtained. On Windows, the 'Cell Lapse Monitor' user interface can be used.

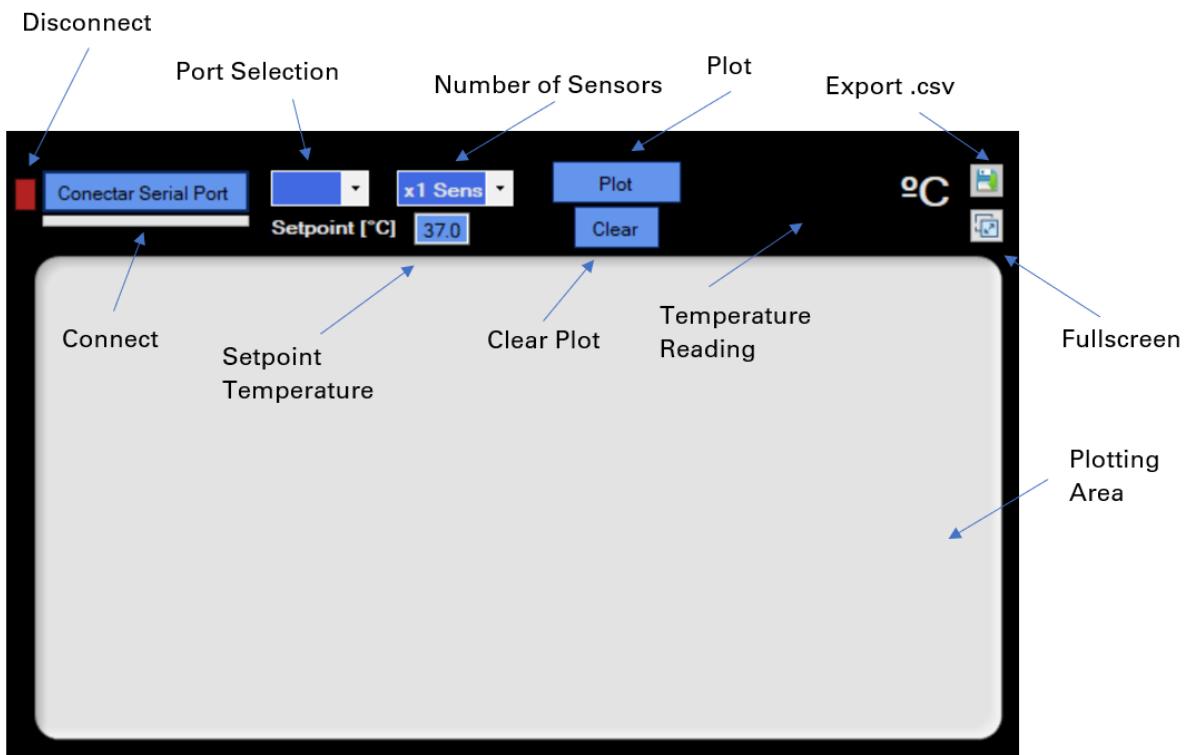


Figure 3: Cell Lapse Monitor. User interface for data collection.

When using Bluetooth, the user must turn on the Cell Lapse incubator and link the device to their computer. Then through 'Device Manager' the corresponding COM port can be found. If using the USB connection, the user must search for the COM port directly.

Search for COM port in Cell Lapse Monitor and connect. By selecting 'Plot' the system begins to collect the test data. Once finished select 'Stop'. Pressing the 'Export .csv' button generates the file with the information on the user's desktop under the name 'Cell Lapse Assay'.

The Arduino code is made in such a way as to print the data from the sensors on its

serial port once the test has started, so that if the user wants to use another serial port reading terminal, it will not find any problem.

Main cabinet components

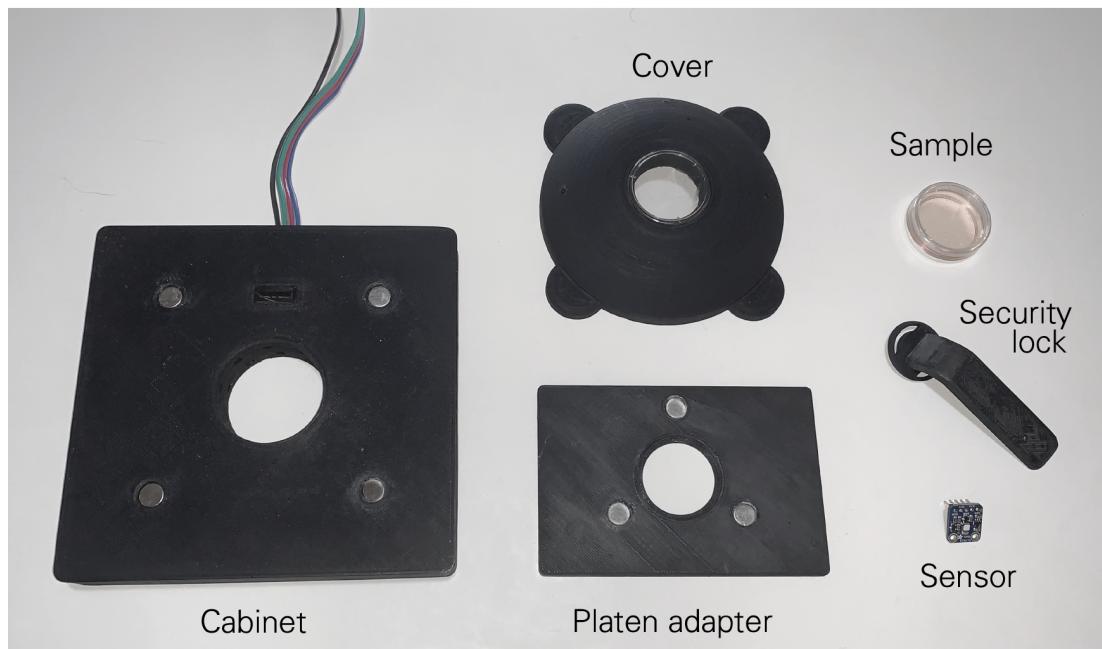


Figure 4: main cabinet components

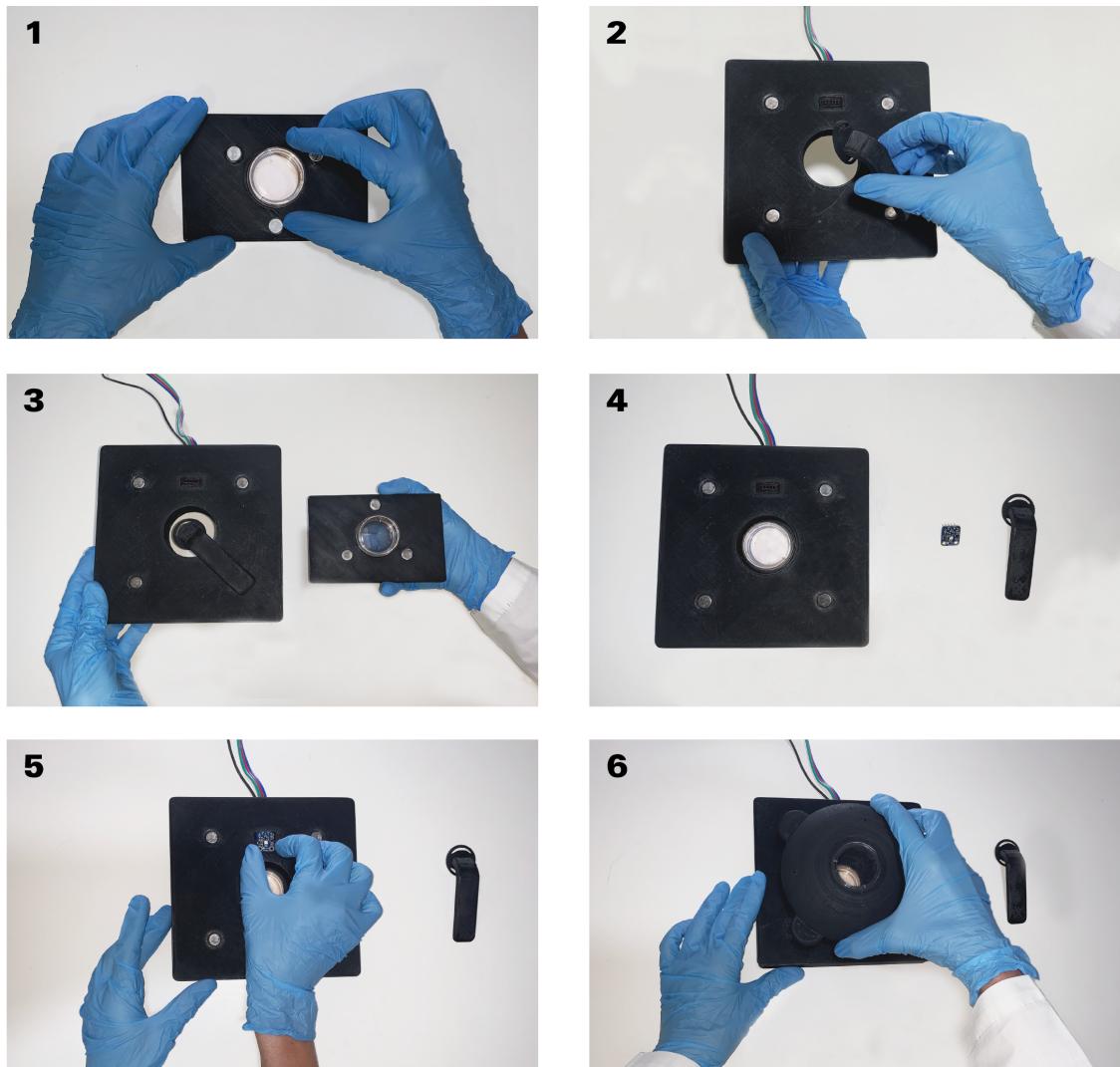
Sample placement

Figure 5: step by step figure of the sample placement procedure.

1. Once the 35 mm Petri dish is ready with the sample, it must be positioned on the platen adapter.
2. attach the security lock to the main cabinet and take it with the hand.
3. Holding the platen adapter with the other hand, slowly bring both parts together until you feel the magnets make contact.
4. Remove the safety lock and insert the Si7021 sensor.
5. Position the cover on the main cabinet.

Proceed to insert the Cell Lapse into the microscope ensuring that the stage adapter fits properly. Finally, insert both hoses on the sides of the device, ensuring that the heater rests on a surface and that its fan is not occluded.

3 Functionalities

Below is a flow chart that covers all the functionalities and dynamics of the interface.

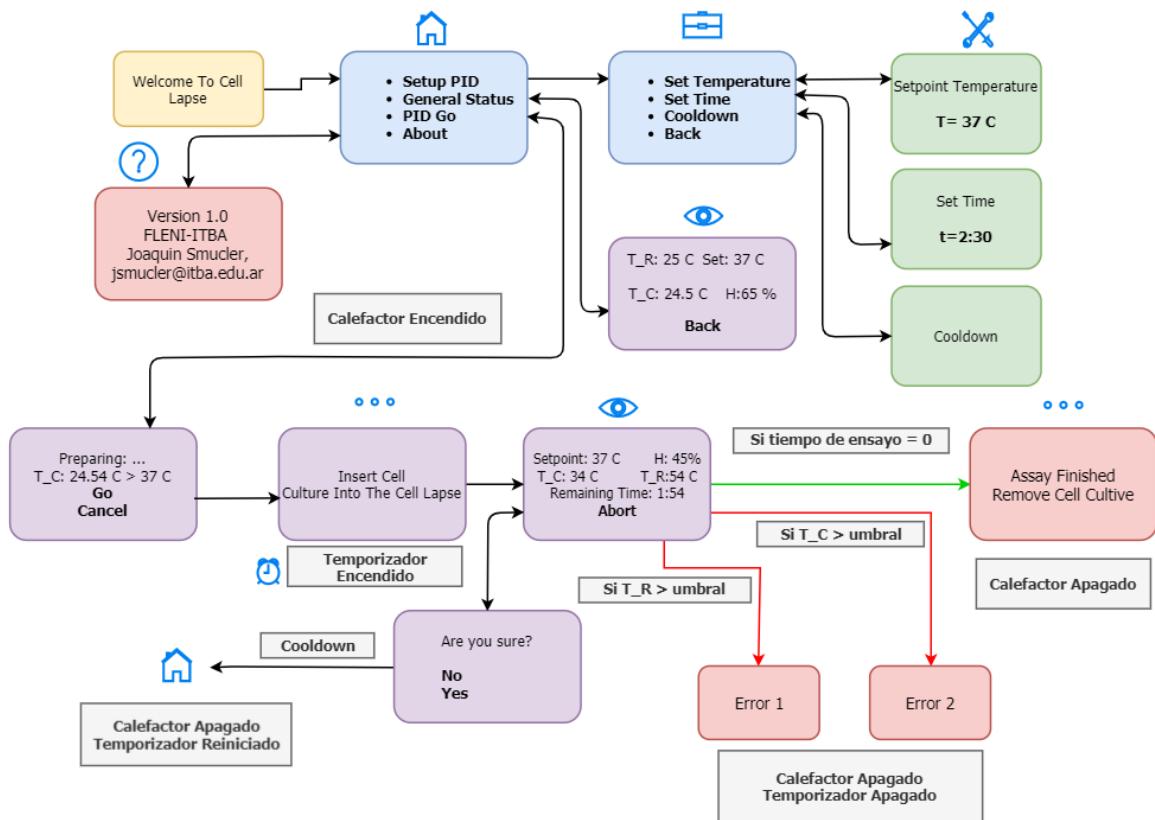


Figure 6: user interface flow chart.

When the system is switched on, a welcome screen will be presented, which is replaced by the main menu when moving the cursor.

Setup

By pressing the cursor on the 'Setup' option, the user is presented with a new menu to configure, on the one hand, the setpoint temperature at which he/she wants the device to reach and, on the other, the total test time (in hours and minutes). The 'Cooldown' option turns on the fan for 1 minute in order to cool down the heater in case a restart

is needed in the event of an error. While the system is in this mode, a 'C' will appear in the upper right corner of the screen and the user will not be able to use the device.

General Status

By pressing the cursor on the 'General Status' option, the user is presented with an informative screen in which they can observe:

- T_r : Heater Temperature.
- T_c : Main Cabinet Temperature
- H: Main Cabinet Relative Humidity
- Set: Setpoint Temperature.

About

This screen contains information regarding the version of the device and a contact email is provided.

Start!

By pressing the cursor on the 'Start!' option the user is presented with a screen informing the main cabinet temperature and the setpoint temperature. By pressing this option the system begins to heat up. This stage serves as a preliminary step in the case in which the user wishes to preheat the device before introducing the sample. If you don't want to, press the 'Go' option to continue. It should be noted that at this stage the assay timer has not yet been initialized.

After the user clicks on 'Go', the timer starts to work and a test screen is displayed where:

- T_r : heater Temperature.
- T_c : main Cabinet Temperature.
- H: main Cabinet Relative humidity.
- Set: setpoint Temperature.
- Assay Time: remaining test time (hours: minutes).

In both instances, the option to cancel the test is provided, asking for confirmation only in the case of having reached the assay screen. If you press the 'Abort' option and confirm, both the heater system and the timer turn off and restart. In addition, the fan continues to run for 30 seconds to cool the heater.

There are 3 conditions for which the assay can be stopped. If the assay time is met, the control system stops and a screen is presented to the user informing the end. On the other hand, when facing errors 1 or 2 (exceeding T_r threshold or T_c threshold,

respectively) after the system stops, a screen is presented to the user informing the type of error. If this is the case, the user must manually restart the equipment (disconnecting it).

3.1 Humidifier

The Cell-Lapse system is prepared to maintain a temperature functional to cell culture for a maximum time of eight hours. In the case of wanting to increase the test time (up to sixteen hours), the user must add the humidifier module. The steps for its use are as follows:

1. Load 50 ml of warm water into a 50 ml Falcon tube.
2. Using a syringe, load the water from the Falcon tube into the container through the rear holes of the same.
3. Gently fit the two connectors of the heater into the two holes of the container.
4. Avoiding sudden movements, fit the hoses to the container connectors.

When using the humidifier the user will notice a decrease in the maximum temperature in the main cabinet. To reduce this effect, the use of warm water is recommended.

4 Troubleshoot

Err 1

‘Err 1’ corresponds to a high temperature in the heater (greater than 95 °C). This may be due to control system or fan problems or high ambient temperature. Faced with this error, the user will be informed of this code and they must disconnect and connect the device to use it again. If the problem persists, use a voltmeter to analyze the voltages across the heater fan and across the heater resistors.

Err 2

‘Err 2’ corresponds to a high temperature in the main cabinet (3 °C above the setpoint temperature). This may be due to problems in the control system or a high ambient temperature. Faced with this error, the user will be informed of this code and they must disconnect and connect the device to use it again. If the problem persists, the controller configuration must be analyzed.

5 Common questions

Can I choose to use hoses longer than 0.5m?

The user can vary the length of the hoses if the arrangement is uncomfortable. It will be necessary to readjust the controller parameters.

Can I use less than 4 ml of culture medium?

The correct operation of Cell Lapse is given so that it maintains a constant temperature in the main cabinet for up to eight hours. When working with a volume less than 4 ml, the maximum test time will be reduced due to possible medium evaporation problems.

Plastic odor is felt when the assay begins. Is it normal?

If the device has not been used for a long time, dirt is likely to accumulate on the heater resistors. It is normal that when starting an assay this smell is felt for about 15 minutes. If this persists, suspend the test and review the module.

6 Contact information

In the event of any inconvenience with the use of the device or inquiries regarding its operation, contact jsmucler@itba.edu.ar.