Low-Cost Top Stage Incubator for Live Cell Imaging Applications

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***Abstract*—A top stage incubator is an essential equipment for long term imaging of live cells on a standard microscope. A low cost alternative to off-the-shelf top stage incubators is realized by using 3D printing technology. A closed system is designed for keeping the inner temperature at the desired value and an ITO coated glass plate is employed as a heater. The temperature inside the incubator is stabilized by using PID (Proportional-Integral-Derivative) algorithm that is controlled via Arduino Uno microcontroller. It takes approximately 20 minutes to stabilize the temperature at the desired value. The peak to peak temperature fluctuation after stabilization is ±1°C. Thus, the portable, user-friendly, easily accessible system allows long-term live cell imaging.**

***Keywords*—Top Stage Incubator; Live-Cell Imaging; PID Controller; Biomedical Engineering**

I. INTRODUCTION

Incubators that provide the desired environmental conditions for cells to keep their vital activities are frequently used in various laboratory applications. The real-time observations are critically important in cell biology laboratory activities, and incubators are required for observing living cells throughout their life cycle. Since stage top incubators can be placed directly on top of any standard microscope stage and are ideal for all long-term live-cell imaging applications. Long-term imaging has been applied in numerous research fields including developmental biology, histology, neuroscience, immunology, physiology, functional genomics, stem cell, cancer research, etc. Commercially available long-term live-cell imaging systems are usually equipped with two main components: an optical system for visualization of cells at the microscopic resolution and an incubator for culturing cells [1].

The incubator systems can maintain optimal environmental conditions essential for culturing cells, including temperature and carbon dioxide concentration as well as humidity of the environment. The distinctive property of a top stage incubator is its compatibility with the XY stage of any research microscope. Moreover, they can accommodate microscope slides, dishes, or multiwell plates [2]. In addition to the observation of fixed cells, following the real-time dynamic processes of living cells allows us to have more reliable and comprehensive information about the cell biology. For example, dynamic events such as cell proliferation, motility or reactivities can be observed with temporal resolution. Also, various information about proliferation rate, morphological remodeling, durability can be obtained [3]. The obtained results can provide a solid support for many scientific activities. There are many examples in history, particularly in cell theory, that demonstrate the critical importance of live cell observation. Medical field is one of the fields that most actively uses microscopic imaging. For instance, incubators play an important role in IVF (In Vitro Fertilization) treatment. In IVF, oocytes are fertilized by a sperm under laboratory conditions simulating similar conditions with the body. After that, the fertilized eggs are implanted back to the uterus for full-term completion of pregnancy. The overall procedure is carried out in 4 stages. These stages are superovulation, retrieval, insemination and embryo transfer respectively. Fertilization process is performed in the incubator using the retrieved oocytes and sperms. The conditions are maintained to mimic the in vivo atmosphere [4]. Since each stage has related complications, it is not easy to estimate the success. However, the outcomes of the process can be predicted by daily monitoring at each stage. For example, the success of the first stage which is superovulation can be evaluated according to the number and quality of eggs. Since the output of IVF is affected by the results of this stage, it would give an idea about the outcome of the overall treatment before completion. On the other hand, expensive drugs and daily monitoring associated with each stage increases the cost. Therefore, the use of a well-equipped, low-cost incubator is essential and highly requested for these kinds of treatments. Another application area of top stage incubators is the laboratories where drugs are developed for the treatment of diseases. The top stage incubators are practical to be used in determining the appropriate drug and ideal dose of the chemicals because it is possible to make simultaneous observations [5]. The results of dose-response data can be interpreted, and they lead the way for many small and large-scale scientific developments.

In this study, a portable, lightweight, practical, low-cost prototype of a top stage incubator is developed. The incubator is manufactured using 3D printing technology, designed to be easily mounted on the stage of any microscope. Also, it allows to maintain cells at optimal conditions with the help of well-equipped controllers including Arduino microcontroller and steady PID temperature control. The finalized system is an alternative to expensive commercially available research top stage incubators for long-term experiments, applications in biomedical research and educational field.

II. MATERIALS AND METHODS

A. Mechanical and Electrical Subsystems

1) Mechanical Subsystem:

Since the main goal is to implement a low-cost top stage incubator, 3D printing technology is preferred for manufacturing process. Based on industrial top-stage incubators, the model is designed by using SOLIDWORKS software and presented in Figure 1.

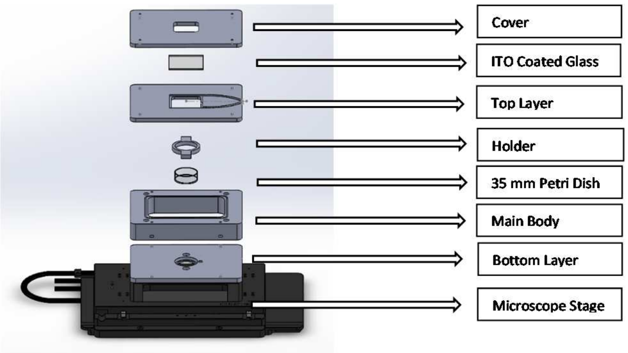

Fig.1. The technical drawing of the designed incubator system.

As seen in Figure 1, the incubator is designed to accommodate a petri dish with the diameter of 35mm and is compatible with the XY stage of microscopes. There are O-ring grooves on the top layer and main body to provide a better seal between layers. In addition, the cable guide is located in the upper part to place wires that carry current between the glass and power supply. The design is printed using Zortrax M200 fused filament 3D printerand ABS filament with 30% infill. 3D printed components are shown in Figure 2.

Fig.2.(a) Inside of incubator.

Fig.2.(b) Outside of incubator.

A photo of the interior of the incubator is shown in Figure 2(a). The bottom layer, main body, 35mm petri dish, holder and O-ring that is attached to the main body are visible on this figure. In Figure 2(b), all mechanical parts are assembled and mounted.

2) Electrical Subsystem:

The system includes several different sensors and components: an LCD (Liquid-Crystal Display), a voltage regulator, heating glass, relay, and the controllers. There are two LM35 temperature sensors are placed inside the main body another one above the heating glass. The one inside the main body, is used to monitor the temperature data to the temperature control system and the one above the heating glass is used to limit the glass temperature. Also, there is a DHT11 temperature-humidity sensor which is used as backup temperature sensor and to be used when the humidity control is needed. The real-time temperature data obtained using LM35 and DHT11 sensors are displayed on the LCD. The sensors are positioned inside the incubator as shown in Figure 3. Arduino Uno is chosen as microcontroller for the system. The electrical system is fed by 12V power supply. Moreover, heated glass that can be seen in right side of Figure 3, is connected between the 12V power supply and a relay. The relay that is connected between the power supply and Arduino Uno microcontroller, manages the current that flows through ITO (Indium Tin Oxide) coated glass. The power supply, Arduino Uno, relay, voltage regulator and LCD is packaged as shown in Figure 4.

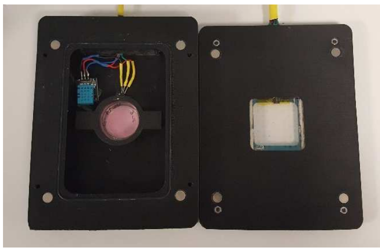

Fig.3. The mechanical and electrical subsystems of the incubator.

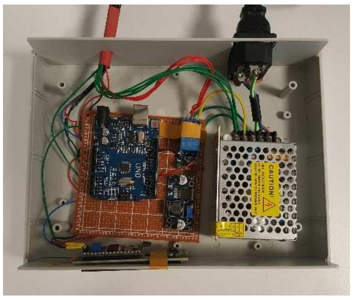

Fig.4. The packaged electrical subsystem.

B. Temperature Control System

1) Indium Tin Oxide Coated Glass:

One of the most important tasks in the development of an incubator is to keep the internal temperature stable at the desired value for cell viability. In this study, ITO coated glass is used as a heater. ITO is a conductive alloy and has a certain resistance value. It means that, when a voltage difference is applied to its two ends, a current is generated. The resistance experienced by the generated current causes the glass to heat up. The uniform resistivity of the ITO coating yields into an even temperature distribution [6]. Busbars at two edges of the glass are connected to the power supply and heated. Besides the main function of the ITO coated glass, it is used to keep the upper surface at a higher temperature than internal chamber. This vertical thermal gradient prevents the condensation on the lid of the dish.

In the arrangement shown in Figure 1, the heating glass is smaller than the system aimed to be heated. This situation increases the standard deviation in the temperature distribution and causes the temperature to be dissipated before reaching the target. Therefore, the location of the heater is important and must be located at the center accordingly. At the same time, since the microscopic imaging is proposed, the cell should be free from outside occluders. Eventually, ITO coated glass is employed because of is transparent nature. On the other hand, if the inside of the incubator is heated by another source and a non-heated glass is placed on the upper part, condensation will occur because the outside being colder than the inside of incubator. Condensation will form water droplets on the upper glass and disturb the imaging performance. Therefore, ITO coated glass is chosen because of preventing the condensation problem.

2) Pulse Width Modulation Signal:

The current passing through the glass is controlled with the help of a relay. DC current will be converted to PWM (Pulse Width Modulation) current as shown in Figure 5 and the duty cycles are determined by the ratio of the relay's on/off times in each period.

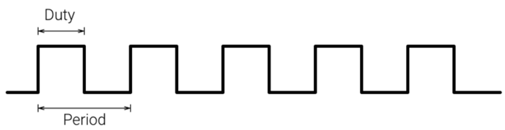

Fig.5. A representation of Pulse Width Modulation signal [7].

The duty cycle of the obtained PWM current will start at zero and gradually become equal to one full period. On other hand, the duty cycle of the source will be reduced when the glass reaches to limit temperature. This method prevents ITO-coated glass from cracking due to sudden temperature changes or deformation due to excessive heat.

3) Proportional-Integral-Derivative Controller:

PID (Proportional-Integral-Derivative) is a commonly used control approach. The P parameter represents the proportional, the I parameter stands for the integral and the D parameter refers the derivative. These parameters can be tuned or adjusted for each system and improved empirically. The P parameter adjusts the linear increase or decrease until the desired temperature is reached. In systems, the temperature may be equalized above or below the target temperature by using P parameter. This value can be shifted manually, or it can be stabilized at the desired value with the help of an I parameter. The deviation signal is integrated with respect to I parameter and the proportional band is moved in accordance to adding result of that integral to deviation signal. After reaching the equilibrium temperature with the I parameter, it is possible to move the proportional band, by tuning the D parameter to reach equilibrium point faster [8].The PID controller can result in different signal forms. Time proportional is the most common response signal. According to the response, cycle time determines the percentage of power supplied to the load. As an instance, if the relay is open for 6 seconds and closed for 4 seconds, output would set to 60% for a cycle time of 10 seconds [8]. According to time proportional output form, it is decided to use the relay to implement PID controller. The relay is controlled by Arduino Uno microcontroller.

III. RESULTS AND DISCUSSION

The final form of 3D printed incubator and the project box containing electrical components are presented in Figure 6. Incubator is printed by using ABS plastic with 30% infill density. ABS has two spesific advantges; good thermal insulation as well as being durable material that is easy to use in moulding process.

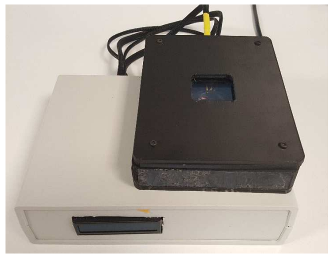

Fig.6. The final configuration of the device.

By using this closed system, several tests were performed successfully. Test results showed that the incubator can stabilize the internal temperature and keep cells alive in the CO_2 independent mediums for 1-2 days. CO_2 - O_2 ratio and humidity distribution control systems are omitted for this particular system. This exclusion minimize the cost with the potential of the future upgrades. The control systems can be enhanced and changed for the future.

PID parameters are tuned to stabilize inner temperature at the desired value, which is adjusted to 37°C for the performed tests. The temperature was stabilized successfully in 100 minutes and time vs inner temperature graph is obtained as presented in Figure 7.

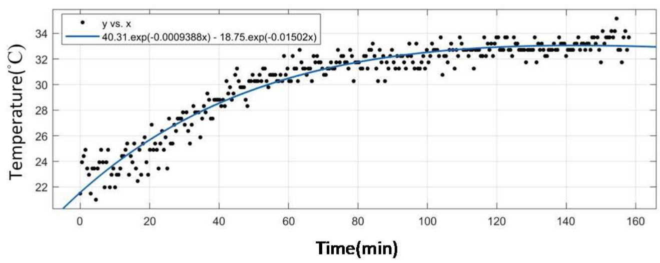

Fig.7. Temperature vs time plot before optimization process.

Also, settling time can be considered as a success criterion. Even though PID parameters are acceptable to reach and stabilize at the desired temperature, the system requires a preparatory lead time. To be able get rid of this preparatory time, PID parameters are iterated. At the end of this tuning process, settling time reached to a value that is less than 20 minutes. The system works with a peak to peak temperature fluctuation of ±1°C and PID parameters are determined as 175, 0.08, 0.10 respectively.

The optimization process is handled, and temperature vs time graph for the first 50 minutes is given in Figure 8.

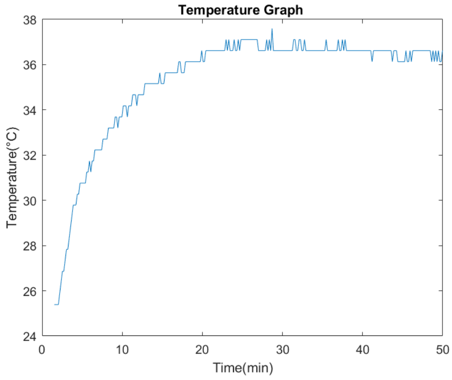

Fig.8. Temperature vs time plot after optimization process.

IV. CONCLUSION

It is known that visualization and analysis of slow dynamic processes in living cells lead the way for many aspects of in vitro cellular studies. However, existing incubator system technologies are often immobile, expensive, and non-practical. In this study, a user-friendly, easily accessible top stage incubator with the cost of 180-200 USD was developed thanks to the recent availability of rapid prototyping technologies particularly 3D printing. Even though it is a low-cost alternative to off-the-shelf top stage incubators, it can succesfully maintain inner temperature at the desired value that can adjust between the room temperature and 40°C. Settling time is around 15 to 20 minutes and it works with ±1 °C peak to peak temperature fluctuation. Therefore, a well-equipped alternative to conventional top stage incubators is proposed. The developed system facilitates many educational and scientific cell-biology laboratory activities where long-term live cell imaging is required.

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