AIMS Biophysics, 12(2): 164−173.
DOI: 10.3934/biophy.2025010
Received: 23 January 2025
Revised: 18 March 2025
Accepted: 03 April 2025
Published: 10 April 2025

http://www.aimspress.com/journal/biophysics

Research article

A low-cost stage-top incubation device for live human cell imaging using rapid prototyping methods

Michael Worcester[1], Shayan Nejad[1], Pratyasha Mishra[2], Quintin Meyers[1], Melissa Gomez[3] and Thomas Kuhlman[1,*]

[1] Department of Physics and Astronomy, University of California, Riverside, 900 University Avenue, Riverside, CA 92521, USA
[2] Department of Biochemistry, University of California, Riverside, 900 University Avenue, Riverside, CA 92521, USA
[3] Microbiology Program, University of California, Riverside, 900 University Avenue, Riverside, CA 92521, USA

* **Correspondence:** Email: thomas.kuhlman@ucr.edu; Tel: +8585314386.

Abstract: Live imaging of human or other mammalian cells at multi-hour time scales with minimal perturbation to their growth state requires that the specimen's optimal growth conditions are met while fixed to a microscope stage. In general, the ideal conditions include culturing in complete growth media, an ambient temperature of 36−37 °C, and a humidity-controlled atmosphere typically comprised of 5−7% CO_2. Commercially available devices that achieve these conditions are not a financially viable option for many labs, with the price ranging anywhere from $12,000 to $40,000. The advent of 3D printing technologies have allowed for low-cost rapid prototyping with precision comparable to traditional fabrication methods, thus opening the possibility for the in-lab design and production of otherwise prohibitively expensive equipment such as stage-top incubation devices. Additionally, the continued usefulness and widespread availability of single-board computers (SBC) such as Arduino and Raspberry Pi simplify the process by which these devices can be controlled. Here, we report the production of a do-it-yourself (DIY) device for stage-top incubation with temperature and atmospheric control with a cost reduction of approximately 100x.

Keywords: Arduino; 3D printing; human cells

1. Introduction

Human and mammalian cell health in vitro is partly maintained through use of media supplemented with a bicarbonate buffering system. Environmental CO_2 at concentrations typically ranging from 5−7% dissolves into the medium and allows for the regulation of the internal pH, usually between 7.2 and 7.4 [1]. Maintenance of these optimal growth conditions in the lab is typically accomplished via incubators that actively pump CO_2 into the interior. Generally, laboratory incubators are not conducive to facilitating the use of a microscope, thus making it infeasible to perform the frequent microscopy of samples without perturbing the cells unless an environmental chamber for the microscope is purchased or assembled, typically at great financial or labor cost.

Commercially available stage-top incubation devices create a sealed atmospheric environment for the sample that can be quickly accessed and regulated with a series of sensors and controllers [2]. Typically, caution is not taken to make the device airtight, and instead, the system opts for a constant flow of mixed air-CO_2 atmosphere in and out of the device, with humidity control to reduce evaporation from the sample. Purchasing commercially available devices can cost between approximately $12,000 to $40,000 in 2023, depending upon the features and the manufacturer of the device. Such high cost is a significant barrier of entry for many laboratories. The lower cost custom solutions for mammalian cell incubation reported in the literature are generally only suitable for custom built microscopes and stage arrangements and are typically not “plug and play” stage top devices [3,4]. Here, we describe a DIY stage-top incubation device, which we call the “DIYncubator”, that can be simply constructed for approximately two orders of magnitude lower in cost than commercial systems (~ $250 USD) and is compatible with existing commercial or custom microscopes and stages.

2. Materials and methods

2.1. DIYncubator design and assembly

The chassis of the DIYncubator (Figure 1) was designed in Autodesk Inventor 2022, formatted for 3D printing with the Ultimaker Cura software, and printed on an Ultimaker S7 using black acrylonitrile butadiene styrene (ABS) plastic. Machine screws and threaded screw inserts were used to attach the lid to the device. The contact region between the two major halves of the chassis was lined with neoprene to make the device approximately airtight, thus limiting the amount of gas required to maintain a constant atmosphere. It is not necessary to make the device completely airtight; however, a reduction of leakage helps reduce the amount of CO_2 necessary and therefore the cost of operation. Attachment and sealing of other permanent components of the device was achieved with either a polyurethane or cyanoacrylate adhesive and sealed with silicone caulk, which is typically used for kitchen and bath projects. The shape of the DIYncubator was designed to attach to a Prior ProScan III stage on a Nikon Ti-E inverted microscope. Minor design alterations may be necessary when using a different stage design, though these changes are straightforward to accomplish using 3D modeling software.

The top half of the chassis was designed with a large window to provide quick access to the sample. A covering for the window was constructed from a 3D printed ABS black plastic frame, thin transparent plastic recovered from recycled packaging, and refrigerator magnets for easy detachment

from the chassis. Additionally, the borders of this window were lined with neoprene to aid in maintaining an airtight environment. The bottom half of the chassis contained a large opening with the same dimensions as a standard multiwell plate, with a modular adapter capable of being inserted for single dish imaging. Then, we coated both the bottom of the chassis and the adapter in Parafilm, as seen in Figure 1B, which serves both to seal the adapter while in place and to limit movement of the sample when placed on the adapter. Finally, the heating system was placed within a housing at the back of the chassis and covered with a layer of neoprene. To aid in the insulation and temperature control of the system, we also fashioned a rough neoprene cover for the entire unit out of scraps that remained from sealing the other components, as seen in Figure 1D.

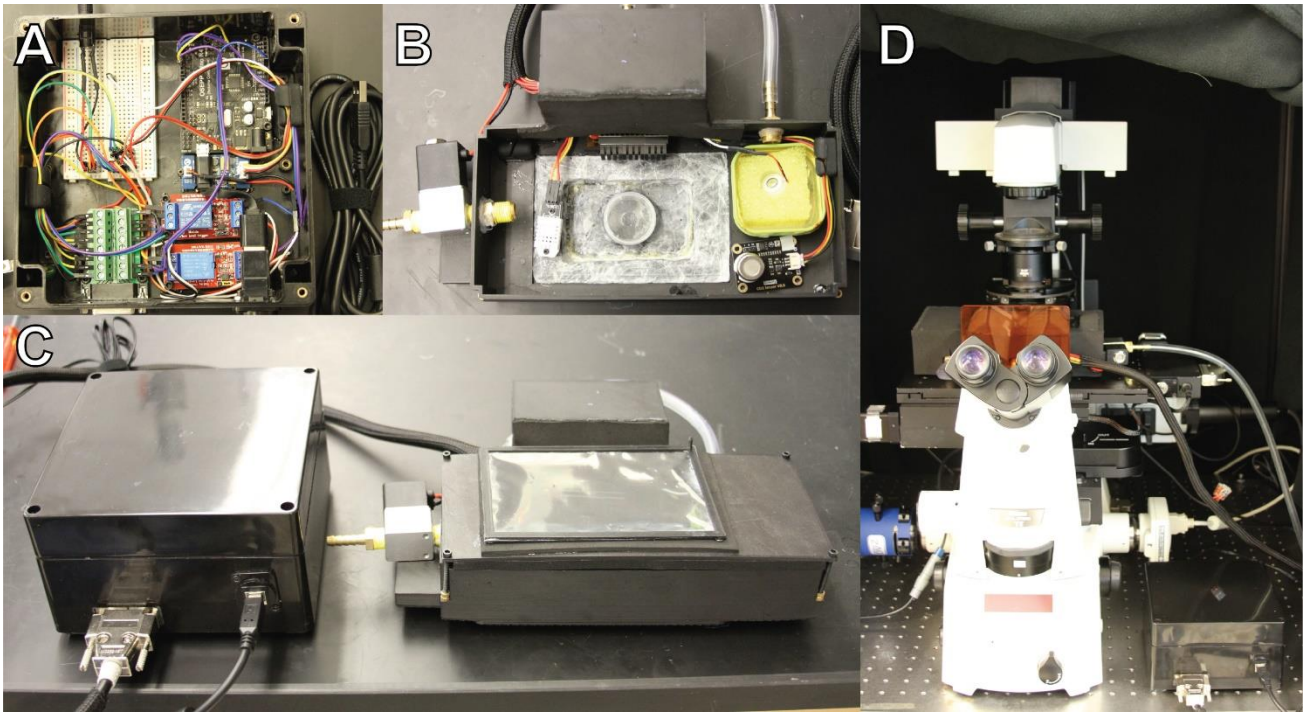

Figure 1. (A) The electronics box, containing the Arduino, relays driving the CO_2 solenoid and heating unit, atomizer control board, a small breadboard for connections, and a female DB15 port. (B) Photograph of the bottom half of the DIYncubator chassis with electric components including the heating unit and fan, CO_2 solenoid, AM2302 humidity and temperature sensor, MG-811 CO_2 sensor, and ultrasonic atomizer with a water reservoir. Note that the bottom of the device and the adapter to hold the sample (here a 29 mm circular dish) is coated with Parafilm to seal the adapter into the chassis and limit movement of the sample. (C) Assembled electronics box and DIYncubator. (D) The DIYncubator deployed with an insulating neoprene cover on our Nikon Ti-E inverted microscope.

2.2. Electronics, environmental measurement, and control

The control system of the DIYncubator was achieved with an Arduino Uno SBC. The CO_2 and temperature/humidity readings were taken with a MG-811 CO_2 sensor and an AM2302 temperature and humidity sensor, respectively. Alternatively, a K30 CO_2 sensor can be used, which will be more

robust in the long term and provide protection against a high humidity. Heat for the system was generated with two flexible polyimide heating film units covered with heatsinks and distributed with a small fan. During operation, the CO_2 and temperature/ humidity sensors were placed as closely as possible to the sample. Gas intake was regulated by a solenoid valve connected to a pure CO_2 gas tank. Humidity control was provided by an NGW-1pc ultrasonic water atomization unit. We used an empty mint tin from Trader Joe's that holds 45−50 mL of water as a water reservoir for the atomizer.

The Arduino was programmed to implement a very simple "bang-bang" control scheme for all parameters, where when the measured value of the parameter fell below/above a set point, the corresponding control device was turned on/off with 100% power. If more precision is required, more complex (and expensive) Proportional-Integral- Derivative (PID) control schemes that employ Metal-Oxide-Semiconductor Field-Effect Transistors (MOSFETs) could be implemented. The device was interfaced with a desktop laboratory computer via USB connection, and an executable program for the user control of the system was developed in Microsoft Visual Studios 2019 for Windows operating systems. The executable program is not absolutely necessary for the device and could be circumvented using the Arduino Uno with an integrated LED display and buttons for operation.

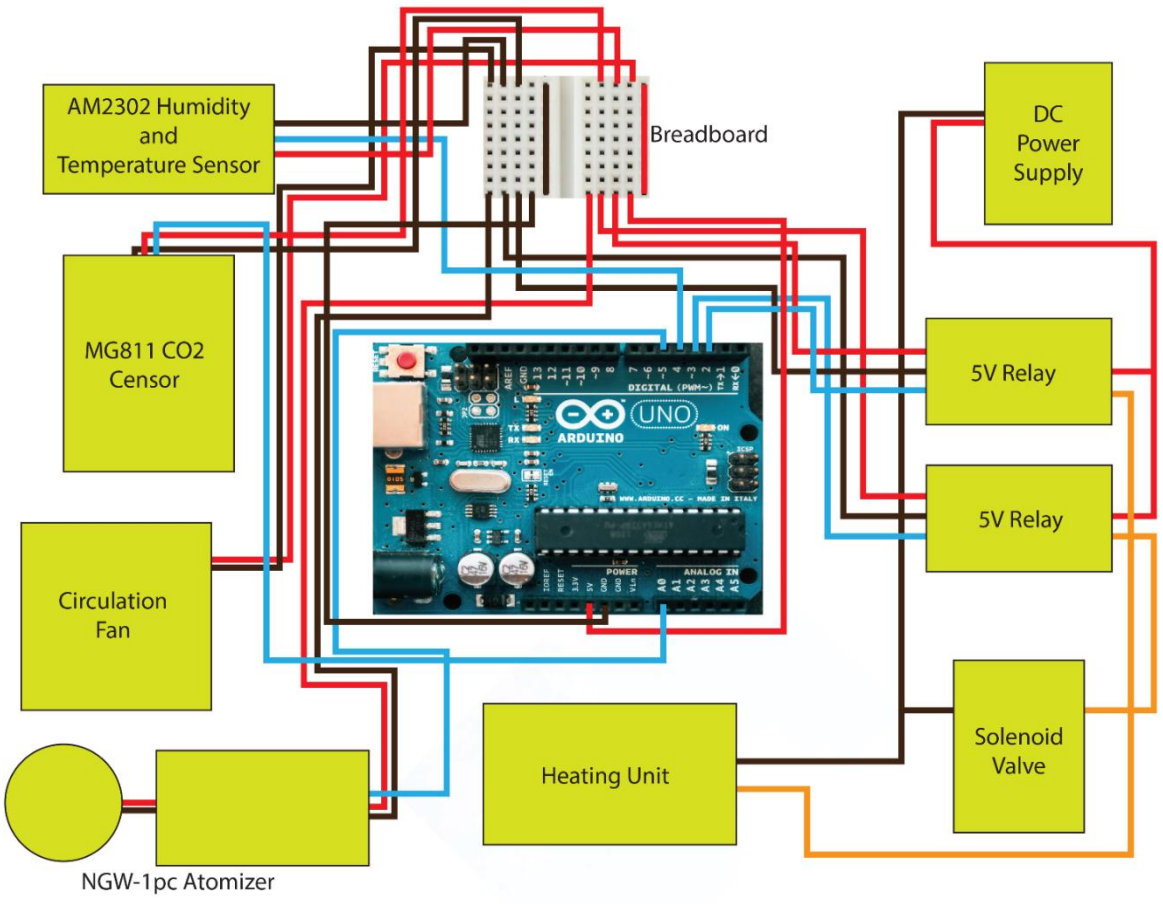

Figure 2. Circuit diagram of the device. Red and orange wires represent positive connections (before and after passing through relays); black wires represent negative connections; and blue wires represent signal connections.

2.3. Human cell culture

HEK-293T cells (Thermo Fisher) were cultured in complete media containing 90% DMEM with high glucose (Gibco), 10% heat-inactivated FBS (Gibco), 200 mM L-Glutamine (100X), 10 mM MEM Non-Essential Amino Acids (100X), 100 mM MEM Sodium-Pyruvate (100X), and conc. Puromycin (Gibco) [5]. Seeding was performed at 25% confluence in 29 mm glass cover slip dishes (Cellvis), and the cells were incubated at 37 °C and 5% CO_2 for 24 hours prior to imaging.

2.4. Sample microscopy and analysis

The cells were imaged using a Nikon Ti-E Inverted Microscope with stage-top incubation, either with the DIYncubator or an Ibidi Silver Line Stage Top Incubator. The fluorescent proteins were excited with a Nikon Intensilight Epi-fluorescent Illuminator and imaged every 10 minutes at either 10x or 100x magnification. The images were analyzed using ImageJ [6], Cell-Profiler [7], and using a custom Python script.

3. Results

3.1. The DIYncubator provides stable environmental control over long periods

The DIYncubator was constructed for a total cost of ~ $250. Some additional, but not required components, such as a plastic project box to house the electronics, DB15 connectors to simplify the connections and deployment, and a cable cover and management sleeve brought the cost to ~ $300. It should be noted that purchasing the materials is up to the user's discretion, and more careful sourcing or building custom circuitry may lead to a further reduction in cost. For example, some of the materials used were recycled from previous experiments and shipping packaging. Creativity is encouraged in building such a device, provided that the solution is still functional.

Using this feedback and control system (Figure 2), the internal CO_2 levels, temperature, and humidity were stably maintained over long periods (Figure 3), and was primarily limited by the volume of the water container used by the atomizer to supply the humidity; we have found that it is advisable to maintain the humidity for up to ~ 20 hours. However, the removable lid of the box provides quick access to easily refill the container, and the system takes < 1 minute to return to the set humidity and CO_2 levels once the box is resealed. The image focus and quality were sustained throughout each experiment (Figure 4), and there was no noticeable impact of potential warping of the ABS plastic chassis caused by the heating element.

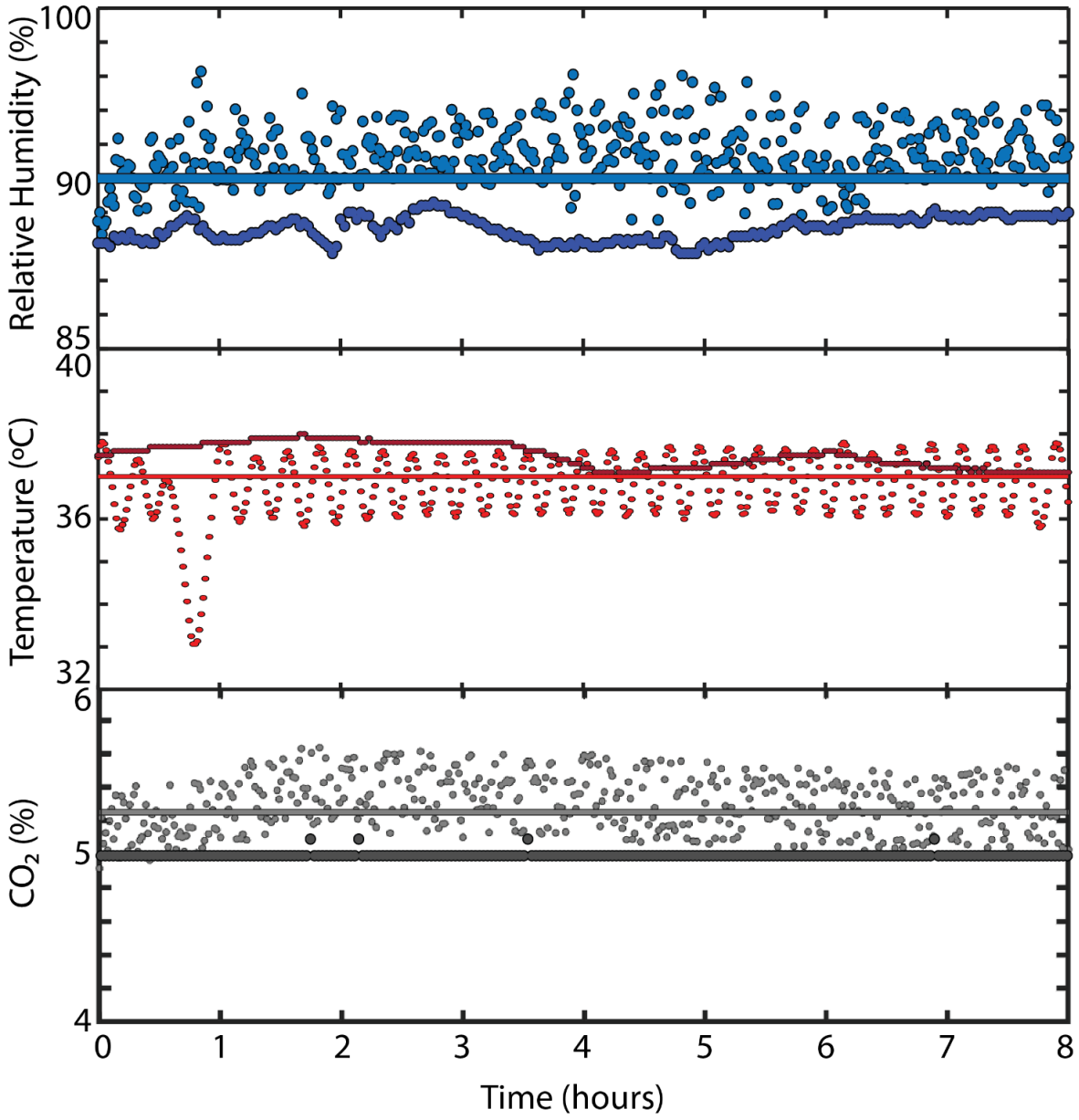

Figure 3. Environmental parameters inside the DIYncubator and Ibidi Silver Line incubator measured at one-minute intervals over eight hours. At t = 0, the device had been running for 1 hour to come up to temperature, at which point the humidity and CO_2 control were engaged. The solid line indicates the set point, with light and dark data points representing the DIYncubator and Ibidi Silver Line readings, respectively. (Top) The percent relative humidity with the DIYncubator had a mean value of 90.8 ± 0.9%. (Middle) The internal temperature with the DIYncubator had a mean value of 36.8 ± 0.7 °C. (Bottom) The internal CO_2 content with the DIYncubator had an equilibrium mean content of 5.3 ± 0.2%.

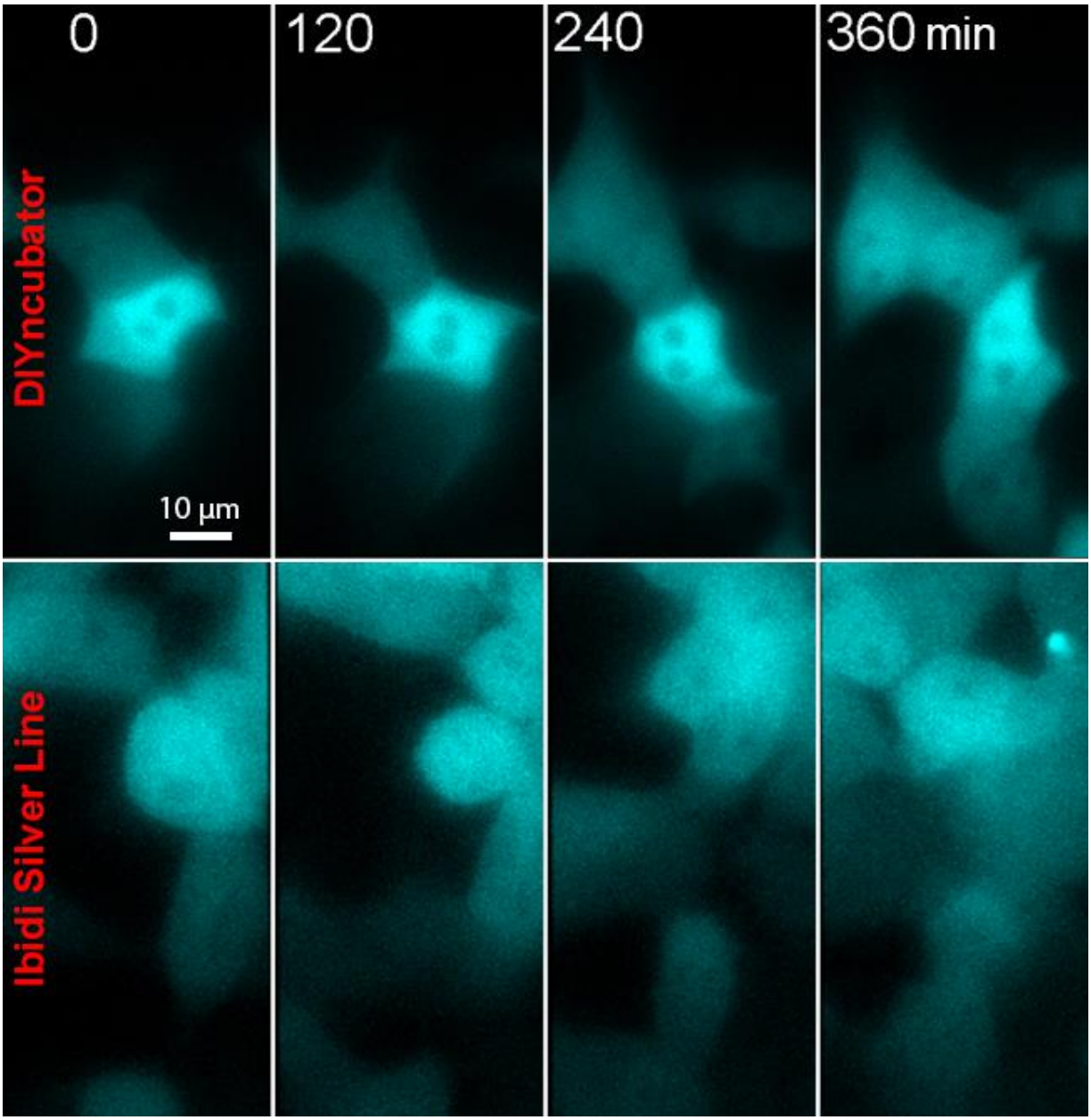

Figure 4. HEK293T cells expressing a fluorescent reporter are imaged inside the DIYncubator (top) and the Ibidi Silver Line incubator (bottom) over an interval of 360 minutes.

3.2. Human cell growth within the DIYncubator compares favorably to commercial solutions

To test the suitability of the device for live cell culture and imaging, we grew HEK293T cells in a 29 mm circular dish for 24 hours in the DIYncubator and compared the doubling time to the same cell line grown with identical conditions in a commercially purchased ibidi Silver Line stage-top incubator (Figure 5). The doubling time of the HEK293T cells inside the DIYncubator was determined to be 39 ± 6.5 hours, as compared to a doubling time of 36 ± 7.5 hours in the ibidi Silver Line incubator.

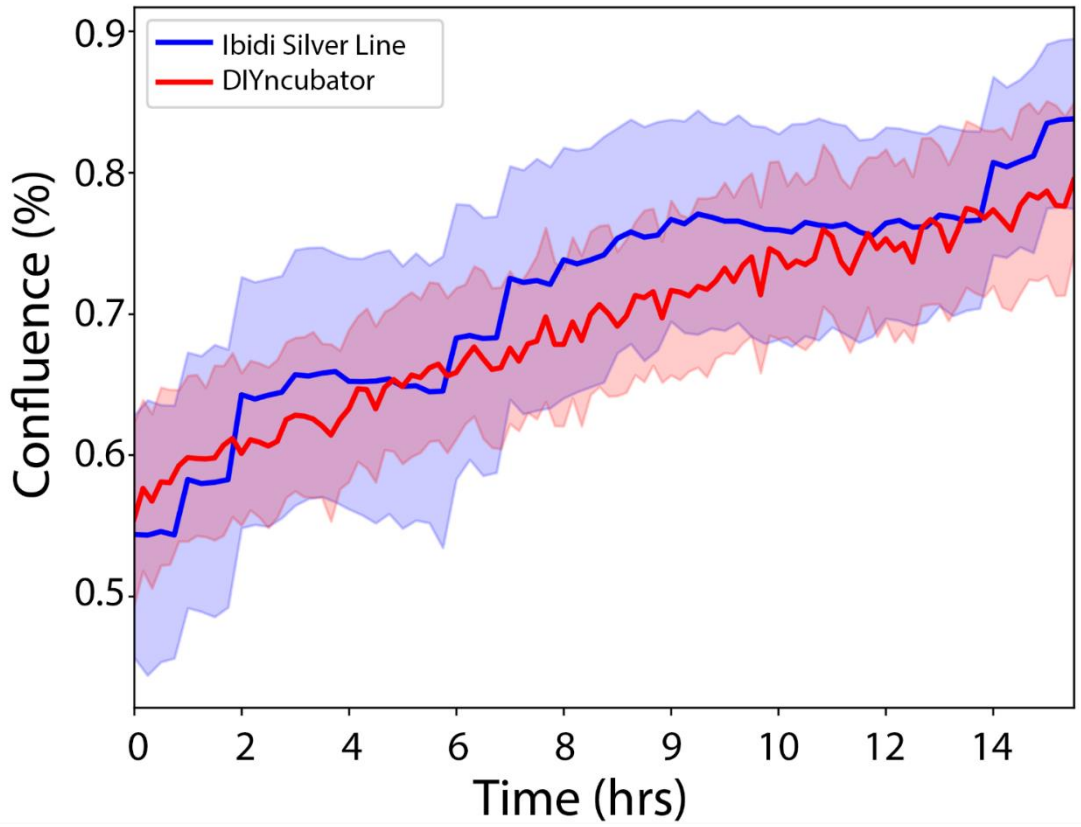

Figure 5. Comparison of the growth rates between HEK293T cells cultured in an ibidi Silver Line stage-top incubator (blue) vs. the DIYncubator (red). The shaded regions indicate 95% confidence intervals, with solid lines indicating the mean over ~ 200 cells.

4. Conclusions

We have described the design, construction, and implementation of a low cost, easy to build stage top incubation device called the "DIYncubator" for the growth and imaging of human and other mammalian cells. The DIYncubator can maintain an environment with a controlled temperature, humidity, and CO_2 content over long, multi-day time scales, thus allowing for fluorescent and brightfield imaging during that span [8−10]. This performance favorably compares to commercially available solutions at ~ 100x the cost.

However, the advantage of the low cost comes a variety of disadvantages that should be kept in mind. The DIYncubator, as described here, was fabricated using ABS plastic, which is a low-cost material but is relatively flexible and a poor conductor of heat. Additionally, care must be taken with the heating approaches, as ABS can considerably warp when exposed to high temperatures. Furthermore, the interior of the DIYcubator can become quite wet over long time periods, as the atomizer sprays moisture into the box and condensation forms on the surfaces. As a result, the DIYcubator described here is ideal for fluorescence imaging where illumination is provided through the objective under the sample, though brightfield imaging accomplished by illumination through the removable lid may somewhat suffer as a result of the accumulation of condensation. However, the lid is easily removable and can periodically be wiped clean with minimal perturbation to the sample.

Additionally, the accumulation of condensation and moisture may wreak havoc with the electronic components over long times. However, after long periods of use, we have not had significant failures of electronics, and, in any case, the electronics can easily be replaced many times over before the cost even begins to approach a fraction of the cost of commercially available systems.

As an alternative, the DIYcubator could be machined out of aluminum, which would yield several advantages. First, aluminum is more rigid and would aid in creating a more airtight environment that would aid in reducing the costs of the operation by reducing the amount of CO_2 required for a constant environment. Furthermore, the high thermal conductivity of aluminum could be utilized such that the entire body of the box could be used as a heating element by adhering the heating film directly to the chassis in a variety of locations to provide a more stable and homogeneous temperature profile across the unit. Finally, heating the chassis of the box in this way, and potentially combined with transparent heating film to make the removable lid, would substantially reduce condensation and provide an environment more amenable to long-term brightfield illumination and imaging. However, acquiring and machining the requisite materials for this approach would add significantly to the financial and time cost and the skills required to fabricate the DIYncubator, the suitability of which is left to the discretion of the user.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Acknowledgments

The authors acknowledge the University of California, Riverside Department of Physics and Astronomy for providing seed money that funded the execution of this project.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization, M.W.; software, M.W.; formal analysis, M.W.; investigation, M.W., S.N., P.M., Q.M., and M.G.; writing-original draft, M.W.; writing-review & editing, M.W.; supervision, T.K.; funding acquisition, T.K. All authors have read and agreed to the published version of the manuscript.

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