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IOT-BASED TEMPORARY IMMERSION SYSTEM FOR MICROPROPAGATION

Kasidet Saisudsawat, Kritsana Kumphet and Wansuree Massagram*
Department of Computer Science and Information Technology
Faculty of Science
Naresuan University
Tha Pho, Mueang Phitsanulok District, Phitsanulok 65000, Thailand
{ kasidetsa61; kritsanak64 }@nu.ac.th; *Corresponding author: wansureem@nu.ac.th

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Abstract. *Presented in this article is a student-led project to retrofit a tissue culture temporary immersion system (TIS) for remote operation. This equipment, prevalent in biotechnology research and the agriculture industry, is a great candidate for automation and IoT enablement as the systems are semi-automatically controlled and pose modest hurdles to additional automation. The developed IoT system is capable of being remotely regulated and real-time monitored in real time via an online web application. This system also organizes the data using a cloud platform – allowing researchers to access the records and images for further analysis. Currently deployed at a university laboratory, the IoT-based TIS setup adds remote monitoring and configuring capability to the existing system and provides a base for future expansion.*

Keywords: Internet of Things (IoT), Embedded computing system, Smart laboratory, Temporary immersion system

1. **Introduction.** Micropropagation, also known as "tissue culture", is an artificial plant reproduction technique that multiplies genetic replicas of plants asexually. This process is the common practice for rapidly multiplying plant stocks of great economic interests, e.g., bananas, sugar canes, or orchids. To guarantee sufficient numbers of plants for production purposes, these economic trees require efficient multiplication during their *in vitro* stage. The liquid culture propagation system using the temporary immersion technique has shown advantages over conventional methods using semi-solid media especially in terms of growth and morphogenesis [1].

At Naresuan University (NU), the Plant Tissue Culture Research Unit in the Biology Department is currently using a semi-automatic and reprogrammable temporary immersion system (TIS) in their laboratory. The TIS allows temporary contact between plants and a liquid medium, often providing nutrients. In this TIS, the period of nutrient exposure is hard-coded into a microcontroller; additionally the current system does not allow for remote/real-time monitoring or parameter adjustment.

The Internet of Things (IoT), already available to consumers, provides a platform to connect and control network-enabled devices. This emerging technology presents an ideal solution to untether researchers from their laboratories. Experiment automation and data collection via IoT could potentially increase productivity and accuracy in research and development.

The goal of this study is to strategically retrofit the existing TIS with IoT capability for remote operation. This study proposes creating an IoT system, completing with a web interface and data storage, to control the TIS and keep track of the micropropagation process. An Arduino microcontroller regulates the existing TIS system while a Raspberry Pi

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single board computer provides network connection along with a camera for determining the treatment feeding process.

The following sections of the article provide an overview of automation in plant tissue culture and IoT applications in scientific laboratories, the design and implementation of the proposed IoT-based TIS system, the deployment results, discussion and future directions for expansion, and the conclusion of this study.

2. Background.

The objective of this study is to design and implement an IoT-enabled system that controls the TIS function. The system should be capable of being remotely controlled and should include real-time micropropagation monitoring. The background information related to this work is summarized as follows.

2.1. Temporary immersion systems.

Temporary immersion systems [1] provide the most natural environment for in vitro culture of plant shoots and seedlings. The TIS systems have been used for plant micropropagation, production of plant-derived secondary metabolites, expression of foreign proteins, and potential solutions in phytoremediation. The micropropagation of plant cells, tissue, and vegetative organ cultures are used in production of commercially important crops.

The TIS used at the NU Biology Department, shown in Figure 1, is a “Twin-Flask” setup. Each individual in vitro culture environment consists of two containers connected via a pipe. One container functions as a culture chamber while the other stores a liquid medium as food. The culture chamber also contains glass beads at the bottom for support. Each container is connected to its own pressurized-air line, controlled by two independent timer clocks, and coupled with three-way solenoid valves. The TIS operates on alternating cycles of temporary immersion of the cultured plant tissue into the liquid medium followed by draining and exposing the plant tissue to a gaseous environment. The liquid immersion period is usually short, usually less than 10-minutes, whereas the air exposure period could last several hours. The existing system at the NU Biology Department was used in the works that recently presented these micropropagation studies for orchid [2] and stevia [3].

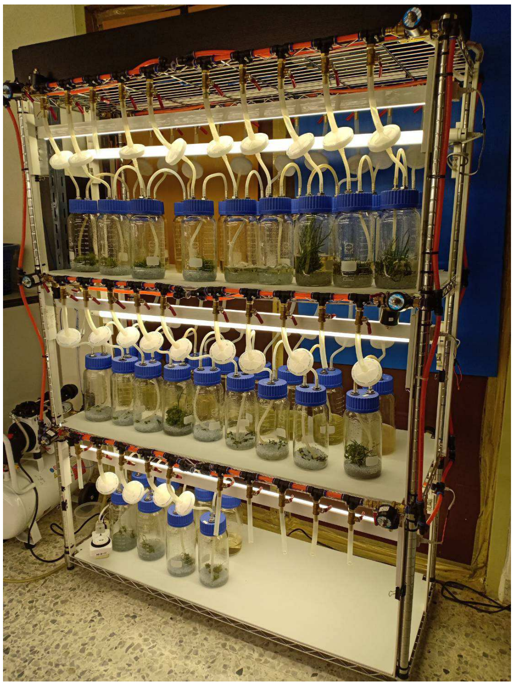


FIGURE 1. Plant tissue cultures grown in Twin-Flask TIS at the NU Biology Department

2.2. **Smart laboratory.** IoT offers various benefits for scientific innovation and discovery, including remote operation, data management, and automation. The fundamental goal of incorporating IoT into the laboratory setting is to effectively conduct experiments and collect research data. Traditionally, researchers have to be physically present in the laboratory to operate the equipment and conduct their studies according to schedules instead of optimizing timing to suite experimental designs. They also have to mediate and manually record the measurements to be later digitized or uploaded.

Smart home and smart city concepts are becoming ubiquitous due to the advancement in IoT technology [4, 5, 6], so the adoption of IoT into laboratories, effectively creating smart labs is not a far-fetched idea. Poongothai et al. [7] developed a smart laboratory system to monitor energy consumption, device utilization, and environmental parameters. Khriji et al. [8] also proposed a real-time IoT-based environmental monitoring system in their so-called "Smart-Lab" concept. Equipment and sensors in laboratories can be connected to the Internet through the Message Queuing Telemetry Transport (MQTT) protocol via devices such as ESP8266, Arduino, or Raspberry Pi. Many IoT platforms such as Blynk, NETPIE, or Thinger.io provide dashboards and APIs allowing a rapid development for IoT interface via web or mobile applications. Most importantly, the control and measurement data can now be saved on cloud storage, e.g., Google Firebase, Amazon S3, or Microsoft Azure.

3. **Design and Implementation.** Temporary immersion systems were initially adopted for micropropagation in the late 1990s and now see routine use in research laboratories and industrial agricultural facilities [9]. The Twin-Flask system, seen in Figure 2, consists of two chambers: the culture tank and the medium storage tank. The process involves four stages: 1) the air exposure stage where the whole liquid medium is contained in the medium storage chamber; 2) the feeding stage where the culture chamber valve is closed and the liquid medium chamber valve is open – pushing the liquid through the tube due to the different pressures in the chambers; 3) the immersion period, where the tissue culture is immersed in the liquid medium; and 4) the draining period, where the liquid nutrient is returned to the storage chamber with the same pressurization principle.

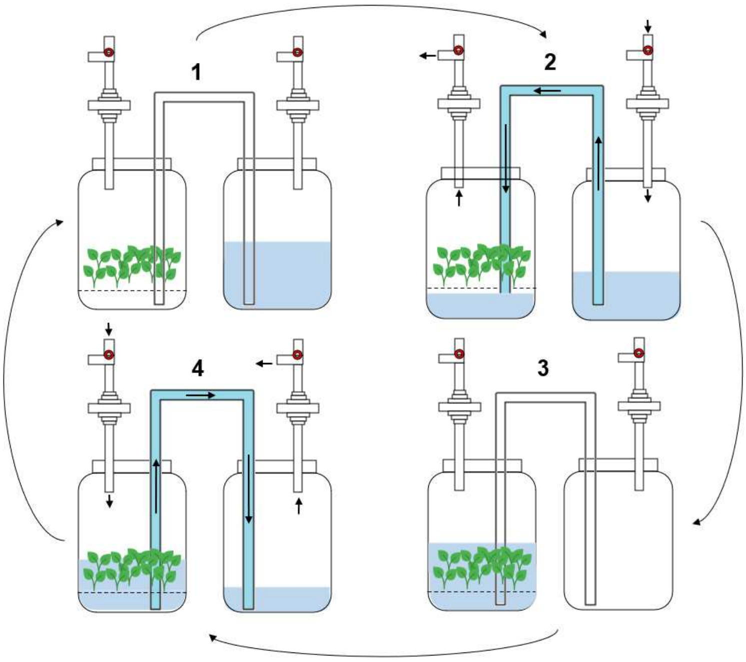


Figure 2. The operational principle of the Twin-Flask TIS (NU Biology Department)

3.1. **Hardware retrofitting.** Each container is attached to an individual three-way solenoid valve for pressurization. These valves are controlled by electrical timers to cycle through the liquid immersion and air exposure periods. The intermittent exposure to liquid medium helps prevent hyperhydricity [10]. Despite the advantages of the TIS, the mass propagation of plants by tissue culture is still labor intensive. Scalable and automatic systems are desirable to minimize production costs, increase reproduction rates, and reduce the amount of manual handling during the micropropagation process. The goal of this study is to enhance the commercially available TIS with IoT capability and improve how a user interacts with and experiences the system, i.e., enhancing its utility, ease of use, and efficiency.

Strategic retrofitting of the existing TIS began with a careful analysis of the routine operations. The components in the dotted line from Figure 3 represent the legacy equipment in the Tissue Culture Lab at the NU Biology Department. The researcher connected an Arduino microcontroller to an off-the-shelf TIS with the Arduino controlling the chamber valves through the TIS relay circuit. The Arduino uses an internal real-time clock (RTC) to control timing of the chamber valves, opening and closing them on a set schedule. This clock is reset every time the board is powered on, starting from a fixed initial date and time. This becomes a crucial problem when the microcontroller loses power. This timing discrepancy leads to errors in immersion and exposure cycles of experiments. Another problem with this setup is that the settings and behavior of the system were fixed in the microcontroller firmware. Configuration updates required the entire system to be taken down to modify the code, e.g., changing the immersion and exposure cycles.

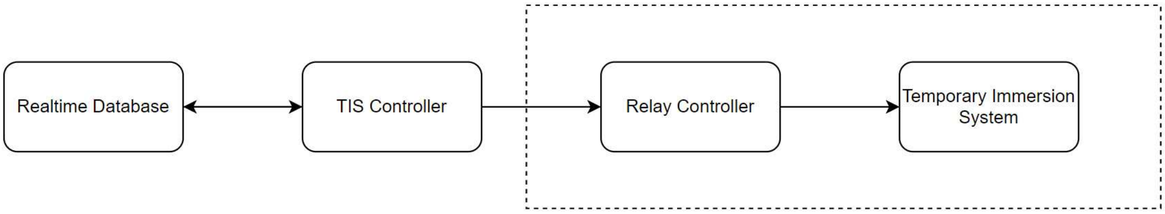


Figure 3. System block diagram of an IoT-enabled TIS

3.2. **Code refactoring.** In addition to retrofitting the existing system, the team refactored the firmware and re-architected the control system. To preserve roll-back capability for the system, the Arduino stayed in place for directly controlling the valves while much of the complexity was migrated to Python running on a Raspberry Pi. The existing firmware suffered from complex interrelations of concerns, repetition, and near repetition. The main problem with the legacy firmware was adding functionality to fit the user's requirements in a changing situation. The early version depended on the on-board clock and a big ladder of if/then for running the treatments while the new version simply runs the commands from the IoT controller.

The existing Arduino firmware was created by a biology researcher without prior programming experience. The firmware contained mostly if-else conditions. While logically sound, the original code suffered readability and complexity problems. With basic refactoring, the Arduino source code was improved in terms of maintainability and extensibility. The code was simpler and cleaner with faster performance and lessened memory requirement.

As reported in Table 1, the program storage space and the dynamic memory for global variables were reduced by approximately 50%. Code refactoring is beneficial to embedded design since these devices typically require the optimum processing cycles and memory usage, as there are no extra processing resources available.

Table 1. Arduino firmware sketch size and memory usage before and after refactoring

Firmware version	Program storage space [bytes]	Global variables [bytes]
Legacy	8,288	480
Refactored	4,234	242

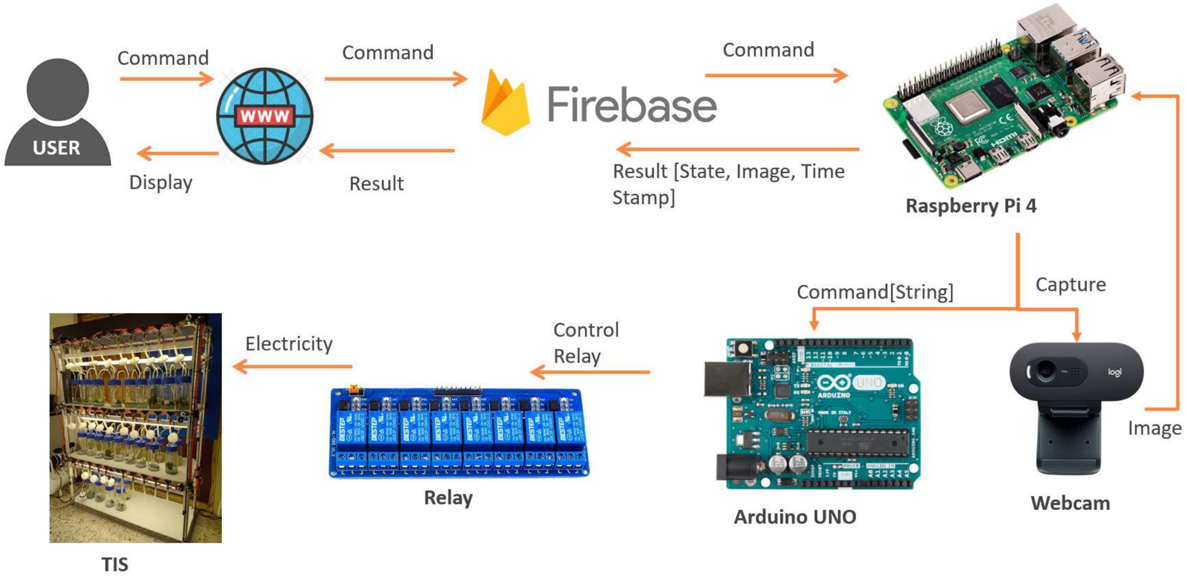


Figure 4. IoT-based TIS system architecture

3.3. **IoT enabling.** The integration of IoT enables the research to control the system on demand in real time. From the system analysis of the existing system block diagram shown in Figure 3, the pressure valves in the chambers open or close according to the Arduino controlled relay circuit. The legacy system inside the dotted lines is retrofitted with networking capability and cloud-based storage. The expansion allows for more enhancement such as additional sensors or automatic feedback control. The proposed architecture for the IoT-enabled TIS is illustrated in Figure 4. A Raspberry Pi board regulates the TIS process while providing the network communication. Users can send commands for different culture treatments, which include number of daily immersion/exposure cycles and their duration, through a web page. This information is stored in the Firebase Realtime Database, a cloud-hosted NoSQL database that allows storing and syncing data between the web page and the Raspberry Pi in real time. The Raspberry Pi retrieves the treatment request data from Firebase and updates the Arduino control operations.

To monitor the TIS process cycles, pictures are captured at the start and finish of each immersion cycle. This additional function allows the researchers to remotely verify the completion of each treatment run. Images from the culture chambers could also be used in biomass detection through computer vision techniques. Computer vision could be applied to images of the culture chambers for biomass detection as a future expansion of this automation system.

4. **Results and Discussion.** For this IoT-based TIS system to be deployed in the Tissue Culture Laboratory, the biology researchers required that the researchers can monitor and update the parameters to control the immersion/exposure periods remotely via a website, log the feeding formula, and search for records from previous experiments. This section presents the system deployment and a plan for future work.

4.1. **Laboratory deployment.** The existing TIS setup was retrofitted following the circuit diagram depicted in Figure 5. Only two addition serial cables, a Raspberry Pi, and a webcam were required for the augmentation. The Arduino receives the feeding data from the Raspberry Pi via the cable. The relay controllers deliver 5-V electricity to flip the switch to let in the nutritious liquid feeds. Figure 6 compares the differences between the existing setup in (a) and the IoT-enabled setup in (b). If the user prefers to use the original system, the Raspberry Pi can be removed in order to easily return to the existing system.

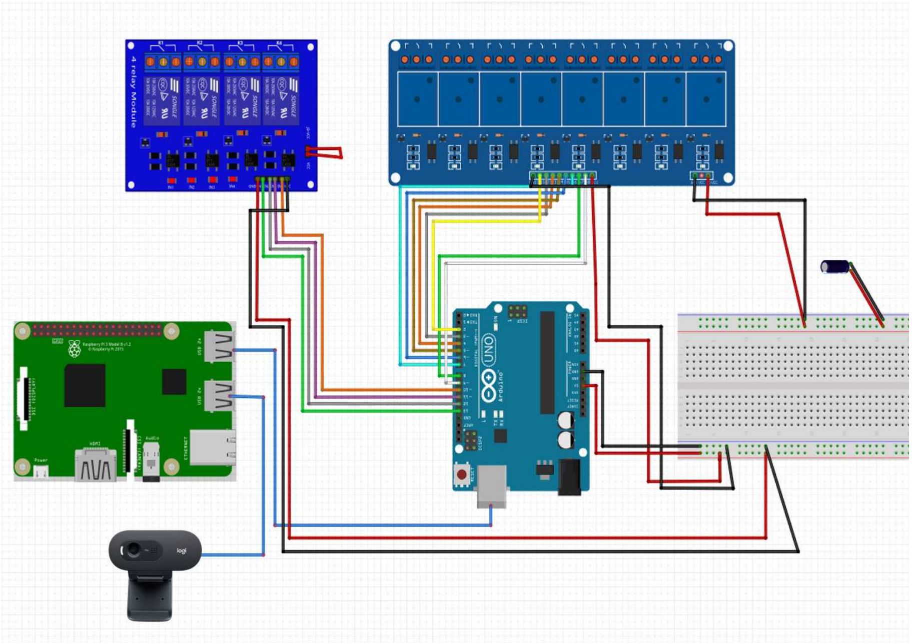


Figure 5. Circuit diagram of the TIS controller with a Raspberry Pi, an Arduino, relays, and a webcam

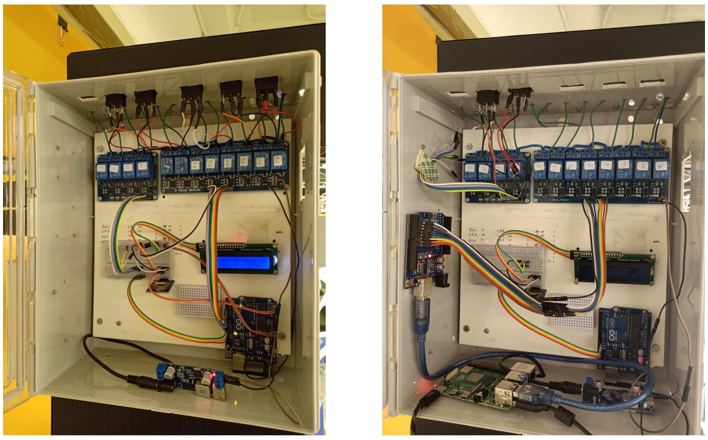
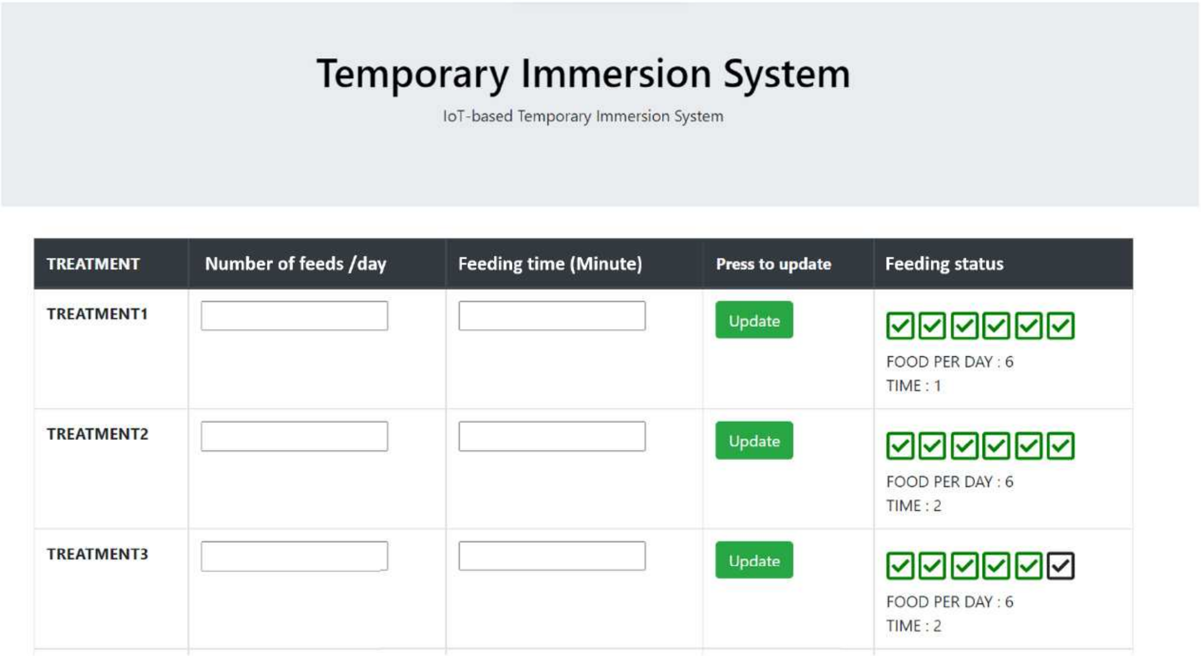

(a) (b)

Figure 6. TIS controller box: (a) Existing setup and (b) IoT-enabled with Raspberry Pi

In preparation for the system roll out, the Arduino software was updated to the refactored version. Once the Raspberry Pi was connected to the Local Area Network (LAN) via Wi-Fi, the new IoT-enabled TIS was up and running. The researchers could then reconfigure the treatments through the web application. Figure 7 shows the partial screen capture of the user interface. There are six available treatment configurations. The first



Temporary Immersion System

IoT-based Temporary Immersion System

TREATMENT	Number of feeds /day	Feeding time (Minute)	Press to update	Feeding status
TREATMENT1			Update	✅✅✅✅✅✅ FOOD PER DAY : 6 TIME : 1
TREATMENT2			Update	✅✅✅✅✅✅ FOOD PER DAY : 6 TIME : 2
TREATMENT3			Update	✅✅✅✅✅☑ FOOD PER DAY : 6 TIME : 2

Figure 7. A simple interface of the web application allowing the researchers to reconfigure the TIS treatment parameters and monitor the process in real time

two treatments (TREATMENT1 and TREATMENT2) regulate the chambers on the top shelf. TREATMENT3 and TREATMENT4 are for the middle shelf. The last two treatments (TREATMENT5 and TREATMENT6) are for the bottom shelf. The two fillable parameters are the number of daily feeds (immersion cycles per day) and the duration of each feed (immersion length in minutes). Once the user presses the "Update" button, the requests are confirmed. The green check symbol indicates the completion of each immersion cycle.

With real-time operations in the microcontroller and the dynamic nature of the IoT system, Firebase, a real-time database is chosen for the implementation to handle the constantly changing workload. The flowchart of the system process is illustrated in Figure 8 where the chart on the left represents the process in the Arduino microcontroller, the middle chart represents the functions in Raspberry Pi, and the chart on the right represents the web application workflow. The Raspberry Pi is the center of the operation. The back-end software was written in Python with its wide range of libraries and functionality. The Raspberry Pi's operation begins with a check of the Internet connection. If connected, it will retrieve data from Realtime Database. The web application receives the treatment configurations from the user and then calculates when each immersion/exposure period should occur. If the wall clock matches the calculated schedule, the Raspberry Pi sends the control command to the Arduino to regulate the valve relay. Images are captured at the beginning and end of the immersion process as seen in Figure 9. The researchers can view these images directly from the web application.

The Arduino RTC problem mentioned in Section 3, where the microcontroller time is not the same as the actual location time was solved using Internet time synchronization. If the Raspberry Pi is not connected to a network, the TIS can still operate as normally retrieving the commands and saving the captured image files locally.

4.2. **Users feedback and plans for improvement.** Deployment at an active research laboratory provided useful feedback and insight to improve the system. Based on the interviews and observations, the IoT-enabled TIS meets the user original requirements

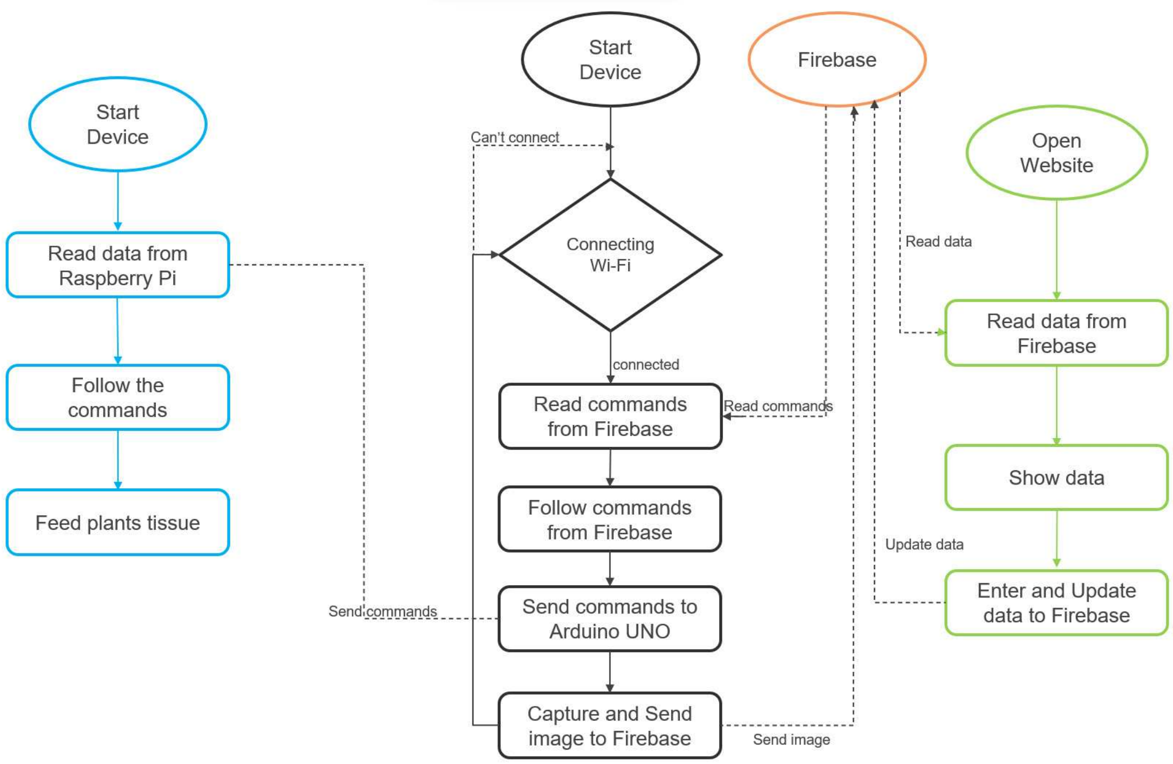


Figure 8. Workflow illustrating how the Arduino, Raspberry Pi, and web application function together

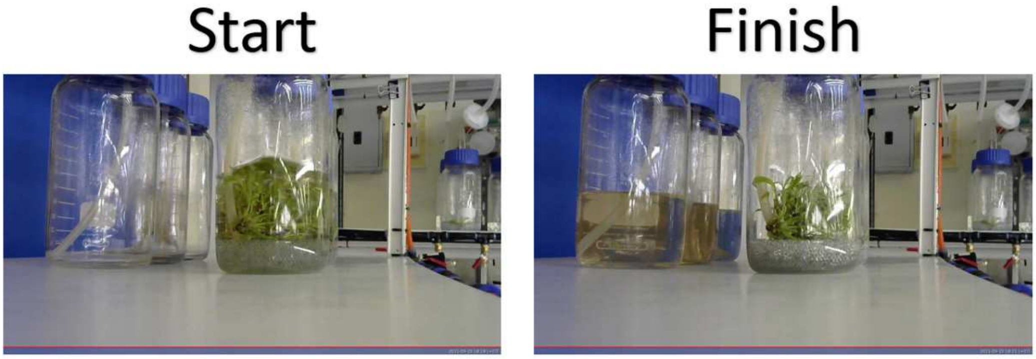


Figure 9. Tissue culture chambers at the start and finish of the immersion period, automatically captured via Raspberry Pi (access these information via the web)

which are 1) updating the control parameters without reprogramming the Arduino, i.e., no downtime, and 2) monitoring the chambers to see if the cultures are properly immersed in the liquid medium. During the two months of deployment, the researchers at the Tissue Culture Lab needed to reconfigure the treatment parameters three times without having to change the Arduino firmware as they were previously accustomed to doing. One area of hardware improvement would be having a keypad or a touchscreen for onsite input. With the responsive nature of the web application, this extension should be straightforward.

Another extension to the project towards automation and data collection is to enhance the system with image analysis capability as suggested in Section 3. To analyze biomass and micropropagation, as well as the liquid medium level for IoT feedback, computer vision is a technique that provides quantitative performance metrics. The steps involved in setting up an image analysis tool to monitor the TIS system are as follows: 1) standardize stage environment and provide calibration, 2) set up an image capture device, 3) digitize and process the image, and 4) display visualization and interactive measurement. The TIS can be staged with a calibration marker to guarantee the consistency of the specimen orientation. Markers such as the chessboard pattern could be utilized both as camera calibration and feature extraction [11, 12]. Visible features of a culture should be in the camera's field of view. Well-designed staging and presentation of the specimen along with high resolution images allow for the maximum possible information to be preserved and analyzed. Additionally, the collected tissue culture imagery data could provide valuable training sets for future machine learning and for further understanding of applying computer vision to micropropagation assessment. This system functions without automated feedback from sensors (e.g., water levels), but such sensors could be added for closed-loop control and adaptability or just to provide alerting in the case of abnormal conditions.

4.3. **Computer vision enhancing.** Computer vision for image analysis allows the collection of numeric information from the image of a microculture subject or specimen. By carefully calibrating the digitized images to the physical dimensions of a specimen, spatial and spectral features can be extracted. Thus, augmentation of cameras in the TIS systems could lead to future imagery analysis with any of the following approaches.

From the numerical representation of spectral information, callus density, pigmentation, leaf chlorosis, or tissue hyperhydricity can be derived [13]. Evaluation of growth during micropropagation using video clips and still images was proposed as early as 1989 [14]. For micropropagation image analysis, there are two main approaches: color-based image processing and neural networks. [15] built a robotic arm for a pan-tilt system equipped with a compact color zoom camera and environmental sensors to detect subtle red-green-blue (RGB) color changes and analyze them with RH, CO_2, and temperature data. [16] utilized a graphics processing unit (GPU) with compute unified device architecture (CUDA) to evaluate several hue-saturation-lightness (HSV) histogram-based image processing algorithms for plant growth analysis in a performance metrics. The 3-D topography reconstruction of microalgal biofilms proposed by [17], while maybe on a microscopic scale, could potentially be applicable for the biomass quantification of micropropagation in tissue culture systems. Both supervised and unsupervised learning models have been applied to analyzing the images from tissue culture micropropagation [18, 19, 20].

5. **Conclusions.** By successfully creating this platform, we were able to decrease the system downtime for reprogramming (hard code) the microcontroller, allowing the researchers to easily and systematically study the micropropagation with the existing TIS. The Twin-Flask system is controlled by the user's request. The level of liquid medium and the micropropagation of biomass is monitored via captured images, which can be further analyzed for growth using computer vision techniques. This system provides remote/real-time access to their experiments, allowing researchers to work from home or keep socially distanced during this COVID-19 pandemic.

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