2006-2041: DESIGNING, BUILDING, AND TESTING A CLOSED COMPARTMENT STAGE INCUBATOR, CCSI

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Designing, Building, and Testing a Closed Compartment Stage Incubator, CCSI

I. Introduction

The current paper describes the design, construction, and testing of a Closed Compartment Stage Incubator, CCSI. The CCSI was created in order to enable scientists in the Horse Science Laboratory at Middle Tennessee State University, MTSU, to monitor the growth of living cells under a microscope for extended periods. These experiments are referred to as time-course studies. Time-course studies must be performed under very specific and well-regulated conditions, which include controlled temperature, humidity, and ambient gases. The temperature of the CCSI must be maintained to a set value within ±0.1ºC degree over a period of as much as 10 hours or more. In addition, the gas mixture and humidity inside the CCSI must be concurrently controlled to enable the study and viewing of living cells under a microscope. Such an environment in which horse embryo cells can multiply and allows scientists to view this multiplication process under the microscope without impairing their growth is very desirable. The gas mixture, which is composed of N_2 and CO_2, is constantly circulated within a clear acrylic chamber to ensure even temperature, humidity, and gas distribution. The chamber and the control system are shown in Figure. 1.

Fig. 1. CCSI and its Controller.

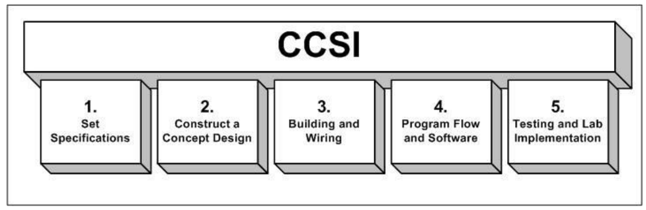
This research is the direct result of an extensive collaboration between the author and his graduate advisor from the Department of Engineering Technology and Industrial Studies on one side and the Director of the Horse Science Laboratory at MTSU on the other. The initial research was partially the result of an Undergraduate Research and Scholarly Creative Activities, URSCA, grant titled "Designing, Building, and Testing a Microcontroller-Based System for Industrial Applications." The research has evolved into the current CCSI project at the graduate level. The authors believe to have found an inexpensive way that allows scientists to monitor the growth of living cells under the microscope. Similar systems are not readily available on the market or are far too expensive when custom made for smaller research facilities. This paper describes the process of designing, building, and testing of a Closed Compartment Stage Incubator. The paper also discusses the importance of a close collaboration, which ensures a high quality and successful research project, between two departments at MTSU; despite the fact that they are very different in nature.

II. The CCSI Design Process

The design of the CCSI project involved five stages. These were:

1. Defining System Specifications and Requirements.

2. Constructing a Design Concept.
3. Building and Wiring.
4. Developing Program Flow and Software.
5. Testing and Lab Implementation.



1. Defining System Specifications and Requirements

All the parties involved met and brainstormed for ideas. The results were defining five key points for the CCSI project: temperature, size, compartment dimensions, completion time, and the materials. These are listed in Figure 2.

CCSI Initial Specifications

Temperature:	Compartment needs to hold samples at 38 ±0.1 degrees Celsius. This accuracy need only be held between 32 and 44 degrees Celsius. The temperature will be held constant within the chamber by: 1) Circulating the medium that is heated in a separate chamber. 2) Circulating heated water in the chamber walls.
Size:	The inside of the chamber will have at least 120x100x40 mm dimensions to place viewing samples. The outside dimensions of the chamber will be roughly 200x150x50 mm.
Compartment:	Compartment is sealed in order to hold the medium that is being circulated in the chamber. In this case, it will be a mixture of CO_2 and N_2. The gas has to be inserted via connections to the CCSI.
Time Frame:	Project needs to be completed by mid December 2005. This includes project and documentation. Documentation needs to be 10-15 pages in length excluding schematics and code.
Materials:	The component materials should be as inert as possible. Great care should be given not to use silicone-based products, which will be exposed to the gas environment within the chamber.

Fig. 2. CCSI Initial Specifications

2. Constructing a Design Concept

With the specifications defined, the authors started by constructing a design concept for the CCSI. A block diagram is shown in Figure 3. The system composed of several individual subsystems. These are:

<u>A. The Chamber</u>
As stated earlier, the chamber should hold different sizes of sample dishes that contained the cells and other substances. The initial idea was to have the gas mixture entering the chamber and then heat the gas in a remote water bath. Therefore, the gas would be circulated from the bath to the chamber. This approach, however, was early abandoned since the sample dishes were actually quite large. Because of the size requirement of the chamber, the gas would not be able to transport enough heat (energy) to the chamber. Not having enough heat meant that the chamber would not be held at the desired temperature. It was then decided that water should be circulated to and from the chamber instead of gas, hence, transporting the heat directly from the water to the chamber. This approach has two main advantages: first, the energy carrying capacity of the water is much larger than that of air or any other gas mixture; and second, it allows the water bath to be heated and cooled more rapidly. The slight disadvantage is that the chamber would have to be more complicated in its design and additional tubing would be required in order to circulate the water and gas mixture in two separate paths. The CAD drawing in Figure 4 shows the left water channel through the chamber wall. Another one is located inside the right wall (invisible in this drawing). The snake-shape design of the channels is intended to maximize heat transfer from the water into the chamber during water circulation. Also visible from the top, is a groove that is fitted with an O-ring for hermetically sealing the top and bottom plates for easy chamber access. This ensures that the gas mixture does not permeate out during the experiments.

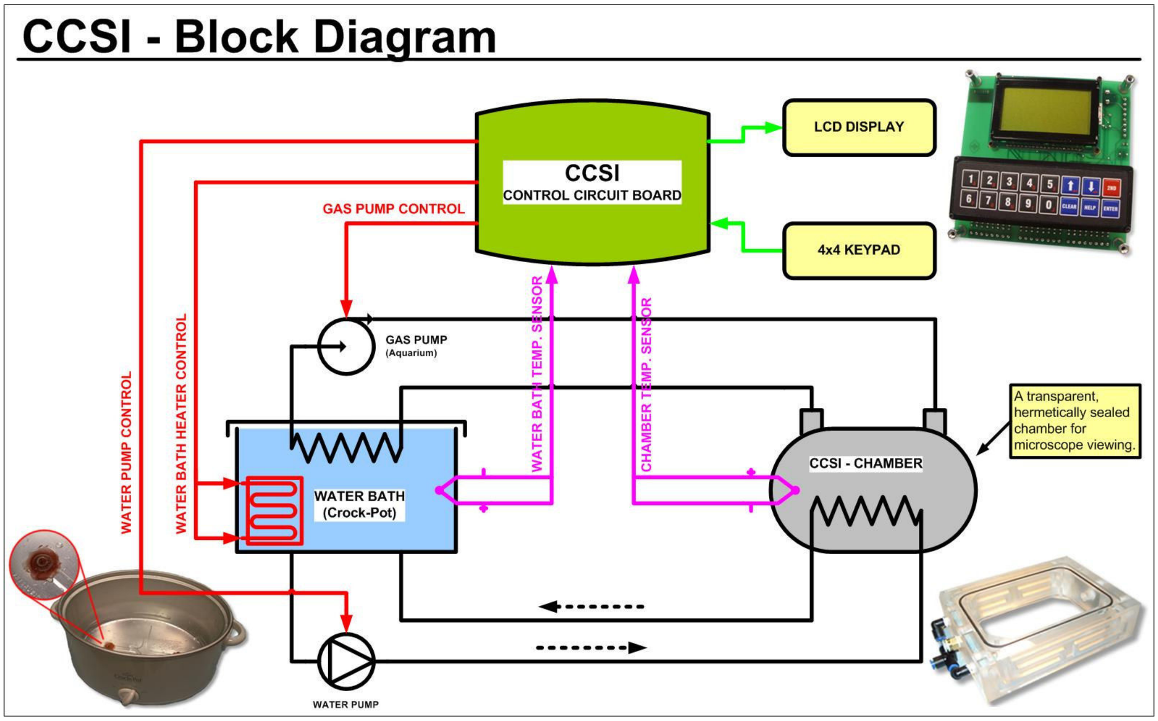


Fig. 3. CCSI Block Diagram

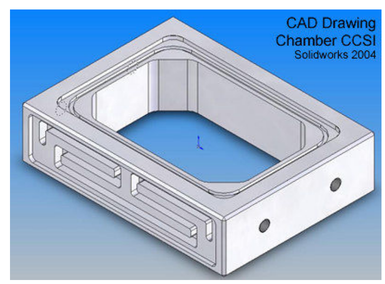


Fig. 4. Chamber Drawing

The entire chamber was constructed from one block of clear acrylic Lexan™, which is a transparent and hard plastic, that can be machined without difficulty in addition to being inert. Since shining a large amount of light into the chamber could interfere with the CCSI temperature regulation system, the transparent material is needed in order to minimize the use of artificial lighting.

B. The Controller Unit

A block-diagram that describes all the logical functions that the controller unit should accomplish was created and is shown in Figure 5. The block diagram was used to ensure that the controller can perform all functions necessary including monitoring, regulating, and controlling the various parameters in the chamber. A key feature to note here includes the LCD Graphics Display, which has a 128x64-pixel dot matrix that allows displaying of characters and graphics. This allows the user to review a temperature versus time graph on the display and make fine adjustments. Another feature of the controlling unit is the use of Reduced Instruction Set Computing (RISC) microprocessor. The RISC AVR from Atmel Corporation was chosen due to its fast and efficient way of computing math functions[3], which guarantees a constant temperature control. A prototype circuit was designed and built based on the above block diagram. After ensuring that the circuit worked properly and satisfied the requirements, a circuit board using Printed Circuit Board (PCB) layout schematics software was designed and fabricated. A software package called Easily Applicable Graphical Layout Editor (EAGLE) was used. The EAGLE Schematic and PCB Layout Editor is a user-friendly software package and a powerful tool for designing printed circuit boards. The program consists of three main modules: a Layout Editor, a

Schematic Editor, and an Auto router. These are embedded in a single user interface. Therefore, there is no need for converting netlists between schematics and layouts[1]. Figure 6 shows a sample circuit editor and PCB design.

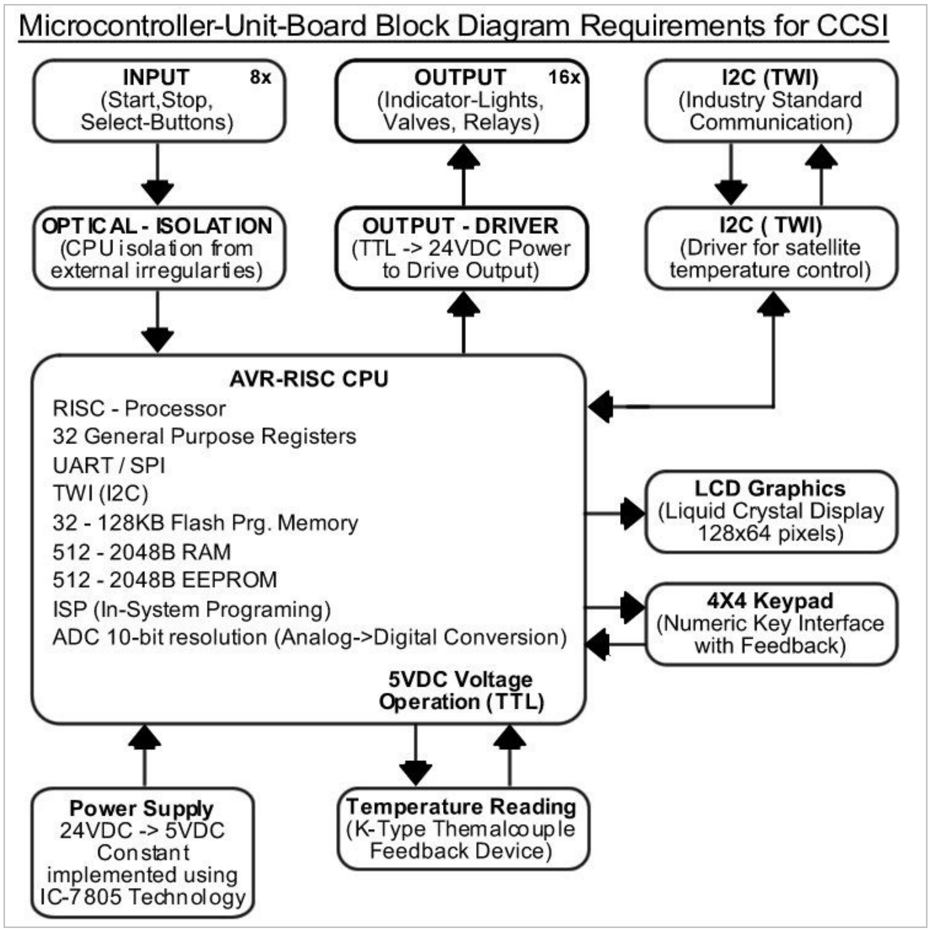


Fig. 5. Controller Block Diagram

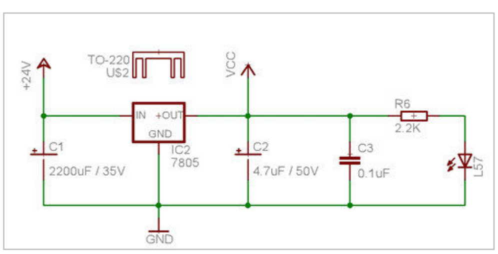
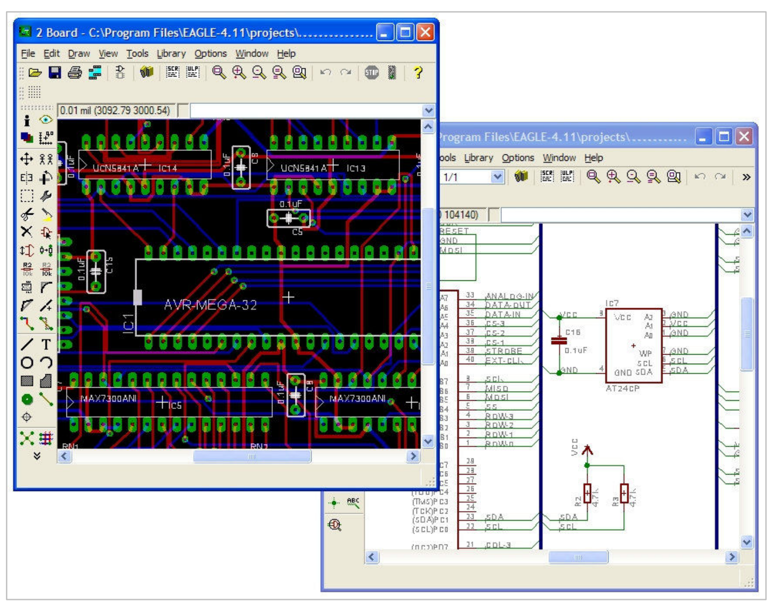


Fig. 6. CAD PCB Design



C. The Water Bath and Gas Heating

A water bath of about1.5 gallons for heating the gas and circulating water was considered to be sufficient. This volume of water would make the heating easier to control and less likely to oscillate. The temperature of the water bath and chamber was measured using specialized low voltage temperature thermometers. The resolution of these devices is 0.065 °Celsius and can be read digitally from the devices. Four such devices were used throughout the chamber and the water bath in order to allow the controller unit to adjust the temperature by controlling the heating element. The precise and non-oscillating temperature was achieved using a Proportional-Integral-Derivative (PID) controller. Figure 7 (top) illustrates the desired temperature curve. It is crucial for the control unit to control the temperature precisely, so it does not rise too much above the set point. Since temperature fluctuations cannot be totally eliminated, the PID controller using Pulse Width Modulation (PWM) must ensure that the temperature oscillation does not exceed the given tolerance of ±0.1 °Celsius. Furthermore, the PID control algorithm should allow the CCSI control system to determine how rapidly the temperature should be raised or lowered. A crock-pot of 1.5 gallons capacity was used for the water bath. To heat, the control unit simply activates an external relay, which in turn allows the line voltage to be applied to the crock-pot. The authors were quite pleased with the heating unit because of its insulation and since its design adheres to Underwriters Laboratories Inc. strict safety codes.

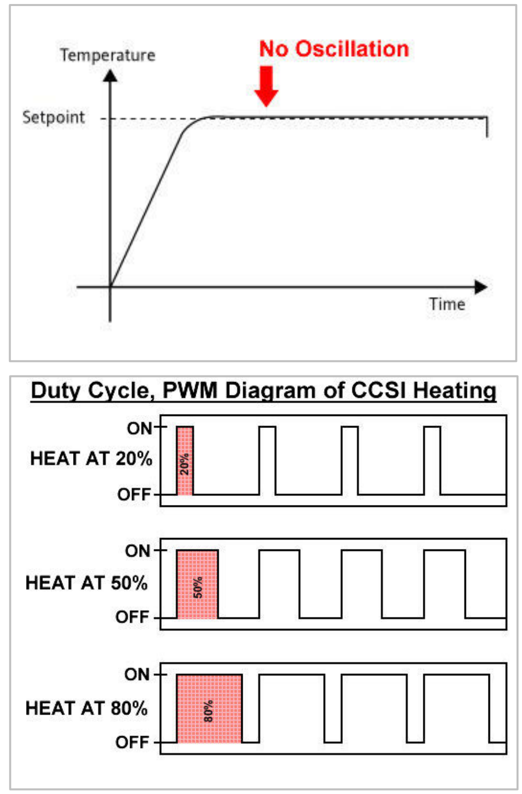


Fig. 7. Temperature Control

3. Building and Wiring

Construction of the CCSI started with the acrylic chamber itself. The pictures in Figure 8 show the actual chamber and its water and gas fittings. The backside of the chamber is identical. The heated water, as it comes into the chamber, splits into the left and right channels that circulate the water through the sides. Then as it merges in the back, the water is returned to the heating bath.

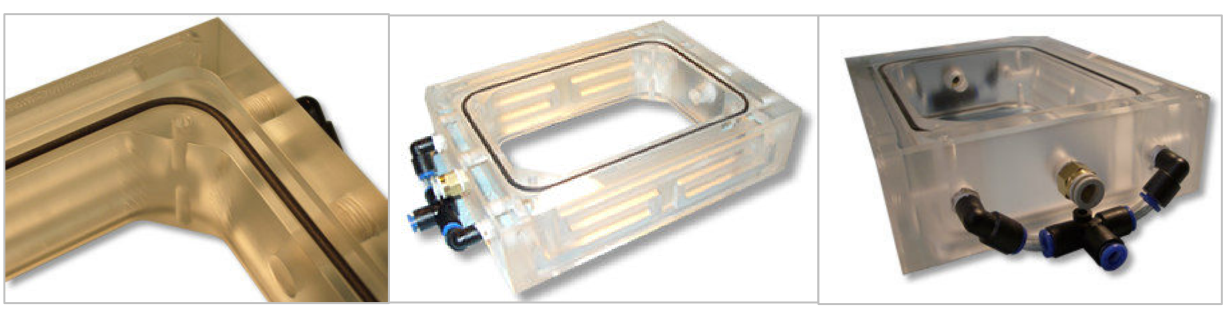


Fig. 8. CCSI Chamber

Sealing the chamber was very essential to the success of the project since each experimental setup can last up to 48 hours.

The assembly and soldering of the controller unit was not very difficult. It was time consuming, however, mostly because the PCB had a few mistakes that were corrected. The red wire spanning over the microprocessor indicates one of these corrections, as shown in Figure 9 below. The red dots on the PCB are LEDs for the inputs and the outputs. The ability to see whether an input or output is active makes troubleshooting of such systems much easier.

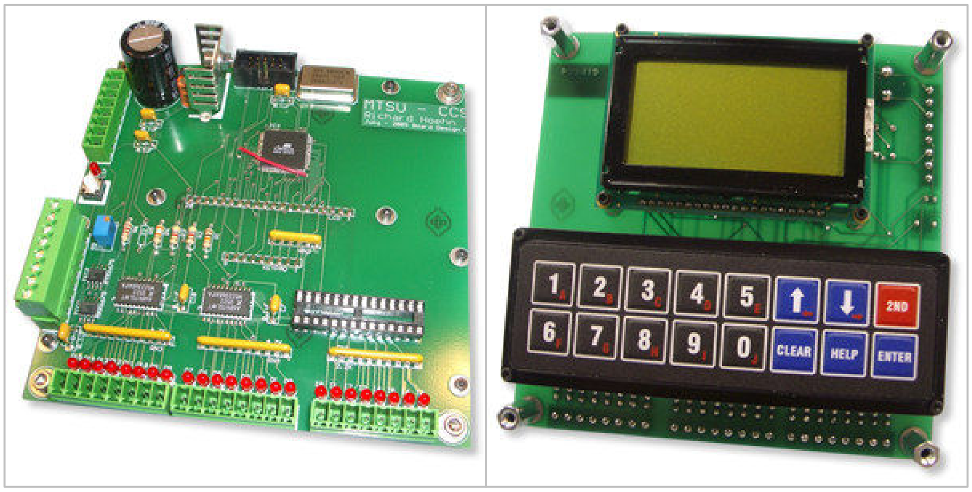


Fig. 9. CCSI Electronic Controller

The building of the water bath was more spontaneous than any other parts of the CCSI project. Very little additional work was necessary to ready the crock-pot for use in the CCSI project. The only tedious part was ensuring that it would not leak water. This was accomplished by sealing the bottom holes with high temperature Plumber-Goop™. The controlling of the crock-pot temperature was achieved via a relay from the control unit. The controlling of the heating elements using a Pulse-Width-Modulation (PWM) system is explained later in the paper. A simple inexpensive pre-made pump was employed to circulate the heated water. The pump that was chosen was actually made for small home use fountains either for indoor or outdoor usage. Again, the most positive aspect was that the water pump was UL/CE compliant, which is a very important safety factor. The pump used is a Flow-Tec™ pump that can move 180 GPH. It operates similarly to a sump pump in that it draws water from the bottom and pushes it out of its top. This system allowed the author to mount the pump directly onto a submerged platform in the water bath. Because the pump already had suction cup feet, its installation was very simple.

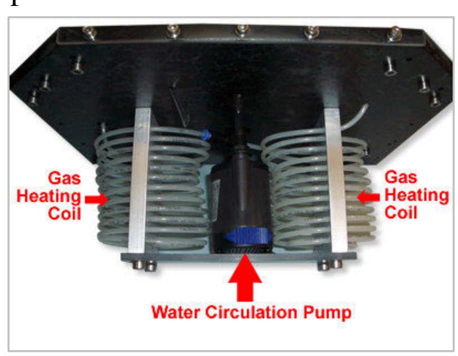


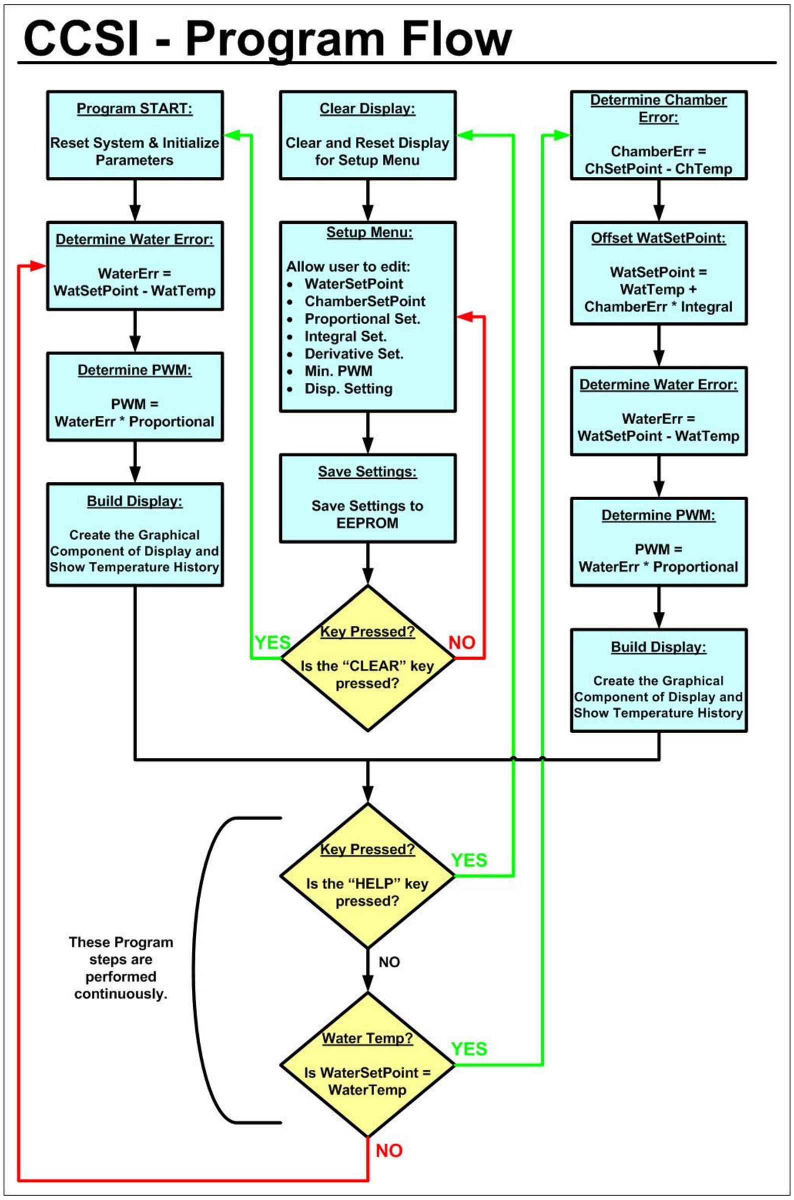
Fig. 10. Gas Heating Coil

Mounted on the bottom of the CCSI platform are the gas heating tubes as shown in Figure 10. These are placed in a circular fashion, coiling inside the crock-pot to ensure even gas heating. The gas mixture has to travel through fifteen feet of tubing within the heated water bath, which guaranteed that the gas mixture is approximately at the same temperature as the water bath.

4. Developing Program Flow and Software

Many hours were spent reviewing different concepts of how to heat the water and the chamber. These included the simple ON-OFF, also known as the two-position control system. With this system, the controller would read the temperature and would turn the heat either on or off depending on the temperature it read. The two-position control system, however, tends to oscillate around a given set point. According to Maloney[4], this is a universal characteristic of the ON-OFF control. This oscillation would have a “shocking” effect on the cells within the chamber. Therefore, it was decided to implement an adaptation of a PID control system.
In this PID control system, the heating element is not just on or off, but rather the amount of energy used to heat the water bath is regulated by time intervals and determined by measuring the difference between the set temperature and the actual water bath temperature. The actual amount of energy released into the water bath remains the same as with the simple ON-OFF control, but a change in energy levels is achieved by controlling the length of time the energy is induced into the water bath. By multiplying the error factor by a proportional factor, the control unit can determine the amount of energy to inject into the water bath in the form of a percentage. This percentage factor is used then to create a Pulse-Width-Modulation (PWM) factor, which is used to turn the heater on and off for certain time intervals as shown in Figure 7. With the proportional control, the author also used an integral part for the temperature adjusting. The integral is a time integral of the error signal, which means that the magnitude of the error signal/factor is multiplied by the time that it has persisted[4]. Because of this ability, the CCSI automatically adjusts the water temperate to the set point without the user’s intervention. For time-cycle experiments that require 24-48 hour durations, a non-interactive approach of temperature control such as the CCSI system provides is advantageous. As noted beforehand, the energy that is induced into the water bath is based on the PWM value that is calculated from a proportional offset. The use of a PWM factor allows for a controlled amount of energy to be transferred into the water bath within a specific period. The actual temperature measurement takes place every 1/10 of a second. These ten samples are saved then averaged every second to determine the temperature curve that is taking place. With this approach, it is possible to ensure that the controller unit uses an accurate temperature reading.

A basic flowchart of the program sequence can be seen in Figure 11. This describes how the program finds and adjusts the PWM settings constantly throughout the running cycle of the CCSI. The CCSI also has an extensive setup menu that allows an operator to adjust the temperature set points of the water and chamber. It is also used to adjust the proportional, integral, and derivative gain of the system. A basic block diagram of the menu system can also be seen in Figure 11. The entire CCSI program was written in C, a standard language for commercial embedded design projects. The software was written and compiled with Open-Source tools and compliers, which can be used for a wide array of embedded as well as conventional computer projects.



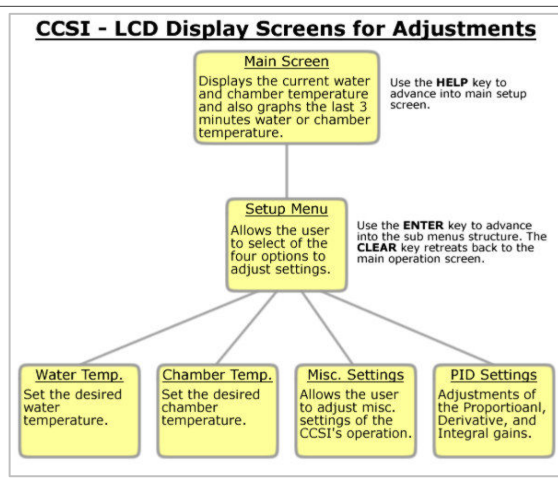


Fig. 11 CCSI Program Flowchart

5. Testing and Lab Implementation

Current experiments have indicated that the CCSI is functioning well and has met all the specification requirements. The heating system in particular is working in excellent manner such that it does not heat too fast. It does induce enough energy into the water to enable the control unit to read the changes and act on them as needed. The use of a water pump allowed the water circulation system to function well, which was a concern at the design stage. In order to keep the chamber at a specific temperature, the water temperature was held slightly above the desired temperature, as expected, due to the energy loss while transporting the heated water to the chamber. The circulation of the gas mixture has been functioning well too. Current consumption for the CCSI is under 1.2 A during the heating cycle and less then 0.4 A when not heating. The CCSI is powered by 100-120 VAC 60 Hz and consumes approximately 150 W, which is within a safe range of power consumption in a lab environment.

III. Summary and Conclusions

A close collaboration between a graduate student (the lead author) and his advisor from the Department of Engineering Technology and Industrial Studies and the Director of the Equine Reproduction Laboratory at Middle Tennessee state University has resulted in the design, construction, and testing of a Closed Compartment Stage Incubator for the study and monitoring of living cells under a microscope.

The implementation of the CCSI has shown to be very promising and generated good results to date. These include the preliminary tests that were performed with limited gas mixtures and fully incorporated experiments over extended periods. The authors were pleased with the outcome of the PID control system, which demonstrated excellent temperature regulation abilities. Software enhancements will further the capability and quality of the CCSI system. The integration of the CCSI with a personal computer (PC) that allows a digital camera to take pictures of cell cultures every half hour is under consideration. The author believes very strongly to have not only learned a great deal about temperature control but also about the importance of interacting with different departments within the university. This invaluable collaboration on the CCSI project is definitely an experience that the author has never encountered during his scholarly career. Although it was frustrating at times not being able to use specific terminology, the author and his collaborators worked together to overcome this obstacle through ongoing discussions. Consequently the CCSI project moved forward quickly and relatively smoothly, and after six months, the author produced a fully working Closed Compartment Stage Incubator that the Horse Science Department at MTSU is using to further its research.

IV. References

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