BME Design-Fall 2021 - SAMUEL BARDWELL Complete Notebook

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Katie Day

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Team Contact Information

SAMUEL BARDWELL - Sep 13, 2021, 3:01 PM CDT

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MAYA TANNA - Sep 11, 2021, 12:17 PM CDT

Course Number: BME 200/300

Project Name: Microscope Cell Culture Incubator

Short Name: Cell Incubator

Project description/problem statement:

Develop a low cost cell culture incubation chamber with interchangeable culture plates that is compatible with an inverted microscope and capable of live cell imaging. This incubation chamber must be able to maintain an internal environment of 37 C, 5% CO2, and 95-100% humidity over long a duration of time, without compromising the integrity of the microscopes optics or functionality. Special consideration should be taken to maintain even heating and humidity across the chamber as gradients can result in evaporation from low volume cultures such as microfluidic devices. Current commercially available systems are prone to these issues are are extremely expensive. Commercial systems also tend to be large and enclose the entire microscope making it difficult to assemble and remove and between uses. Because of their size, they also hinder use of the microscope in general.

About the client:

Client is Dr. John Puccinelli, an undergraduate advisor in BMS, who instructs courses related to biomaterials, tissue/cellular engineering, biomems/microfluids, and design. He is the coordinator of the BME design courses.



Title: Standards and Codes

Date: 10/15/21

Goals: To apply any relevant standards and codes to the project.

Content:

ISO/TS 23565:2021

ISO/TS 23565:2021; Biotechnology-Bioprocessing-General Requirements and Considerations for Equipment Systems used in the Manufacturing of Cells for Therapeutic Use

Notes:

- · Doesn't apply to incubator, but important to note for other aspects of the design
- · Applies for hardware, software, and consumables used in the manufactoring of cells i.e our arduino coding
- Used for tissue engineered product
- tubing, culture vessels or other containors
- also used for monitoring systems intended to control the internal environment.

14:00-17:00, "ISO/TS 23565:2021," ISO. https://www.iso.org/standard/76053.html (accessed Oct. 15, 2021).

ISO Standards Update:

"ISO Update Supplement to ISOfocus," 2021. Accessed: Dec. 12, 2021. [Online]. Available: https://www.iso.org/files/live/sites/isoorg/files/news/magazine/ISOupdate/EN/2021/ISOupdate_August_2021.pdf.

ISO 19090:2018

See link for standard. Note that it is with animal cells, not human.

14:00-17:00, "ISO 19090:2018," ISO. https://www.iso.org/standard/63936.html (accessed Oct 15, 2021).

ISO 24998:2008

- Make note of another standard used for plastic lab ware when completing cell cultures, specifically petri-dishes.

14:00-17:00, "ISO 24998:2008," ISO. https://www.iso.org/standard/42736.html (accessed Oct 15, 2021).

CFR - Code of Federal Regulations Title 21

- To familiarize the team with the code needed to be followed for the incubator.

See attached link:

"CFR - Code of Federal Regulations Title 21," www.accessdata.fda.gov. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=864.2240 (accessed Oct. 15, 2021).

Notes:

Sec. 864.2240 is of most importance, speficially A where they mention the equipment codes for cell cultures.

Make sure Arduino circuitry and tubing materials are in check with **ISO/TS 23565:2021** standard. Check what type of cells Dr. Puccinelli is working with for ISO19090:2018. The team will follow these standards and codes throughout the duration of the microscopic cell culture incubator project.



MAYA TANNA - Sep 18, 2021, 1:00 PM CDT

Title: Client Meeting #1 Introductions to Client/Project Details

Date: 09/17/2021

Content by: Maya Tanna

Present: Sam Bardwell, Katie Mcgovern, Maya Tanna, Caroline Craig, Olivia Jaekle, Ethan Hannon

Goals: To document the discussion with our client, Dr. Puccinelli, as well as the answers to our list of questions prepared for the meeting

Content:

Questions for Dr. Puccinelli

Overview of the Project:

Experimental Teaching Lab \rightarrow Tissue engineering lab needs culture cells for the long term (*what is long term*?) that doesn't have a lot of money. Looking for a smaller, less expensive, and less bulky incubator that doesn't encompass the whole microscope or can be removed. Stage-top cell culture incubator. Grow cells and watch them over the course of time. Have to be able to stay alive with cell culture conditions for at least a week.

1. What is the budget for this project? **\$100**

- a. Will this project be paid for using UW Funds? Departmental teaching funds
- 2. What is the device being used for, industry, research, etc?

a. Used for teaching purposes, but if we get it right we can market this to other researchers

3. What is our margin of error in regards to temperature, CO2 levels, and humidity?

a. 37*C \rightarrow look at industry standard for temp ranges

- b. 5% CO2 \rightarrow helps with buffering from sodium bicarbonate
- 4. Is there a size constraint for the incubation chamber?

a. Has to sit on microscope stage and hold a well plate that also doesn't interfere with the optics

(ideal if both sides are transparent, but bottom must be transparent)

b. Needs to work with inverted microscope

5. What are your preferred dimensions for the incubation chamber?

a. Sits on microscope stage and holds well plate

6. When you imagine the finished product, what color would you want it to be?

a. No preference in color

b. Well plates are clear, black (stops contamination), and white (increases light).

c. Something that blocks out external light would be ideal, but is not required

7. Could we test our design with live cells?

a. Yes, Dr. P will give us some when/if we are ready

b. Use cells that are hard to kill → that's good for us

c. TELL HIM IF WE WANT THEM AFTER THANKSGIVING

8. What are the most important design requirements/specifications (apart from the temperature, CO₂, and humidity level measurements provided)?

a. Optical transparence, microscope stage (google that)

9. How many devices should be created?

a. Just one :)

10. Are there any materials that you prefer we use?

a. Nope :)

11. How long will this device be used in the lab?

a. Could be used up to two weeks, but shoot towards one week at a time.

12. How often do you plan on using this device daily?

a. Device would be used for one week at a time during tissue lab

13. What is the shelf life of this product?

a. Long time \rightarrow 10 years

14. What has been working well for previous projects? What hasn't?

a. Seal insulated box completely?

b. Sterilization is very important $\ {\ \rightarrow\ }$ autoclaving ideal but UV works too

15. Anything particular you would like us to continue with from past projects?

a. Temperature gradients are a large problem for cell cultures (reason for bulky products) look

towards first project insulated box

- 16. What types of cell culture plates do you use?
 - a. What are their dimensions?
 - i. <u>6 Well plate, 24 well plate, 90 well plate</u> → omnitrays?

ii. Standard petri dish

iii. Flasks \rightarrow T25/T75 not really used but her

b. What type of medium do you use?

i. MEM

ii. 10% SPS and antibiotics

17. Will any other microscopes be used with this incubation chamber? Or, should it only be compatible with the inverted microscope?

Mainly inverted microscope

- 18. Should this device be ergonomic(able to move it on your own)?
 - a. Be able to carry it around and store it
 - b. Wires should not be hanging out freely
 - c. Easy to pick up and put away

Notes:

- CO2 humidifiers and such are done using wires and a breadboard
- No team has successfully created an incubator.
- Something that can be easily taken apart would be ideal
- Temp gradients with small amounts of liquid can be evaporated very quickly so humidity is a big issue

Research To Do for Week 9/17-9/24

- Materials
 - What can hold heat?
 - What is transparent?
- Industry Standards
 - What are the industry standards for margin of errors for temp, CO2, and humidity
 - · What is the size of well plates and inverted microscope stages?
- Cells
 - Look up the biology and physiology of MEM
 - When does it evaporate?
 - What temps do we need to stay under?
 - What humidity is best for it?
- Temperature
 - How can we create a better temperature gradient?
 - How can we insulate in a small space?
 - Look towards less industry and more experimental research as to how we can heat things in a small space
- Sterilization
 - Autoclave
 - UV Sterilization
- Past Projects
 - · Check out the older projects to see what other teams did

Conclusions/action items: Tailor research to these specifications and use this information to create the product design specifications document. Look into previous projects and determine what worked well and what led to less successful results.

09/28/2021 Client Meeting #2 Collecting Dimensions and Clarifying Project Details

SAMUEL BARDWELL - Sep 29, 2021, 11:27 AM CDT

Title: Client Meeting #2

Date: 9/28/21

Present: Sam, Caroline, Ethan, Katie

Goals: To get a more in depth understanding of the project, tighten up loose ends, and get dimensions of the inverted microscope.

Content:

1. What is the exact model of inverted microscope for use? (for accurate dimensions)



2. Nikon Eclipse Ti- S

1. Don't want to change the distance sample is from the lens (32.40mm) thickness

2. 310 x 300 mm

- 2. Could we use a laboratory CO2 gas line? Or, will an external CO2 gas supply be necessary to include in materials?
 - 1. Tank with a regulator, hose into incubator
 - 2. Don't need to purchase, readily available with hoses
 - 1. What is the diameter of the hose? 7.16mm wide
- 3. How many cell plates do you need in the incubator?
 - 1. One Prefers just one well plate per incubator
- 4. Would it be possible for us to test transparent materials with the microscope?
 - 1. Optically clear enough?
 - 2. Refraction of light?
 - 3. Bottom of glass on multiwell plates.. Look into

Team activities/Client Meetings/09/28/2021 Client Meeting #2 Collecting Dimensions and Clarifying Project Details

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- 4. YES ALL POSSIBLE
- 5. What is the use of the incubator during the week of class time?
 - 1. AN ENTIRE WEEK
- 6. Do you have any specifications in the margins from industry standard? Or, is the tolerance cells can handle acceptable?
 - 1. pH levels \rightarrow CO2 levels, what is tolerance for a buffer?
- 7. What are the dimensions of the well plates? (Can look up online)
 - 1. length = 127.44 mm
 - 2. Width = 84.91mm
 - 3. Height = 21.60mm
- 8. What would be the ideal recovery time for internal conditions after opening the cell culture incubator "door"? (Flow rates)
 - 1. Five minutes after 30 second opening
- 9. Would you prefer manual CO2 addition, or an automatic regulation with sensors?
 - 1. Incubator itself has a valve and a sensor \rightarrow automatic prefered
- 10. Is the budget for the final design, or does it include materials for preliminary designs?
 - 1. Yes but if the prototype works well then it can be flexible

Notes:

- Current incubator is water jacketed with co2 tank at ~10psi
- · Microscope is able to lift head up so that we can fit the incubator in
- •

Conclusions/action items:

We learned more about the intentions for the project and have a clear understanding of the route we will have to take. The design matrix will be updated with the new information after this meeting. More detailed Solidworks drawings can be made with the new dimensions of the project. A lot of the sensors and parts of the project that we were planning to buy are accessible from past projects and in the BME teaching lab.



11/02/2021 Client Meeting #3 Fabrication Updates

ETHAN HANNON (ehannon@wisc.edu) - Nov 03, 2021, 9:42 PM CDT

Title: Client Meeting #3

Date: 11/2/21

Content by: Sam & Ethan

Present: Sam & Ethan

Goals: To update the client on our position with the project and to receive more feedback on our incubator design.

Content:

- Thermistor to record temperature if the DH22 sensor does not work. Doesn't record humidity. Need a calibration curve

- The lens height is adjustable. He will get back to us with a height at the best refractive value. This will help solidify the dimensions of the incubator box so it can be 3D printed.

- We have the glass plates but they are very small. Will have to update box drawings to account of this change. Intended plan is to have a covering and the set the glass plate on top of the covering to allow transparency.

- Can use any tubing found in the old ECB lab room. Preferably 1/4 to 3/8 inch tubing. 1/4 inch tubing would work best with push adaptors (need to find a way to connect it to heated water incubator). 3/8 may work better for connection to heated water pump.

- He will set aside some cells for us to use to test with in the future.

- He already ordered a new DH22 temperature and humidity sensor to see if the old one was truly faulty.
- Lots of different adaptors to look at. Hose adaptors, push connectors and the gray connector for the heated water bath.





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Figure 1: Different views of gray heated water pump adaptors.

Ethan found links online to order if need be:

For the coupling body: https://products.cpcworldwide.com/en_US/ProductsCat/NS4/NS4D17006

For the valve coupling insert: https://products.cpcworldwide.com/en_US/ProductsCat/HFC12/HFCD22612



Figure 2: Push adaptor for 1/4 inch tubing. Very easy to use.

- Avoid buying from ACE hardware because we can't get reimbursed. If anything needs to be ordered go to Puccinelli and he can have it within a couple of days.

Conclusions/action items:

SOLIDWORKS drawings will be updated to account for the glass dimensions. Testing on the glass can be conducted since some materials have arrived. Sensors will continue to be tested. May have to go a different temperature sensing route. Adaptors will be the main focus for the fabrication team and to figure out the best tubing to use to heat the inside of the cell culture incubator.

09/17/2021 Advisor Meeting #1

MAYA TANNA - Sep 25, 2021, 9:30 AM CDT

Title: Advisor Meeting #1

Date: 09/17/2021

Content by: Maya Tanna

Present: Sam Bardwell, Katie Mcgovern, Maya Tanna, Caroline Craig, Olivia Jaekle, Ethan Hannon

Goals: To document what was discussed at our first advisor meeting with Dr. Melissa Kinney

Content:

Advisor Meeting Notes 9/17/2021

- Prof. Kinney has a lot of experience using cell incubators
- Logistics
 - Find out where we will go for the 2 hours for presentations, show and tell, and final
 - Friday Meetings: 30 minute meetings to productively ask questions, connect to resources, brainstorm ideas. Send questions to everyone in advance for Friday meetings so that we can come to the meeting prepared for the questions we need to tackle. Weekly Recap, Goals, Discussion, and Problems we are running into.
 - Weekly Reports: send to both Prof. Kinney and Dr. P
 - Address the email to Dr. P
- Advice
 - Communication: keep communications open at all times
 - Delegation
 - Fast Paced Class = TIME MANAGEMENT
 - Set concrete goals and intermediate deadlines
 - Make sure that your goals have an actionable concrete outcome and a deadline for that outcome
 - Targeted Research and SMART Goals
 - Be as specific as possible with your PDS
 - Quantitative more than qualitative
- Grading
 - Using Canvas More
 - Final Deliverables weighted most heavily
 - Preliminary Report is graded as if it was a final report (5% of grade)
 - Entire team gets roughly the same grade
 - Individual grades
 - Peer evaluations
 - Lab notebooks
 - Course deliverables
 - Notebooks (preliminary 5% and final 25%)
 - **Oral presentation**(preliminary 5% and final 20%)
 - Written documentation (preliminary 5% and final 25%)
 - Project output and team function
 - **Prototype** construction and evaluation (client satisfaction 5%)
 - Participation (contributions to weekly advisor meetings, group meetings, and team objectives, peer/self assessment 10%)
 - Technical leadership and outreach (for 402)

Conclusions/action items: Make sure to keep consistent communication with Dr. Kinney. It would also be helpful to send out weekly meeting agendas for meetings with her so that everyone on the team is on the same page and questions/clarifications can be dealt with effectively.



MAYA TANNA - Sep 25, 2021, 9:31 AM CDT

Title: Advisor Meeting #2

Date: 09/24/2021

Content by: Katie McGovern

Present: Sam Bardwell, Caroline Craig, Dr. Kinney, Maya Tanna, and Ethan Hannon

Goals: To recap our team accomplishments this week and discuss PDS and design matrix.

Content:

9/24/2021 Advisor Meeting Notes

- · Refractive index in glass optical properties
- · Look into the glass that they use on the bottom of multi-use well plate
- · Maybe 3D print the sides and have optically transparent tops
- Ask about Routine Use
 - · Are we using it for multiple labs for 3 hours only?
 - · Are we using it for multiple days in the same lab?
- · Loosen our variation parameters
 - What level of tolerance will we allow to meet Dr. P's specifications rather than industry standards?
- Size Requirements
 - · Meet on Tuesday with Dr. P to get size requirements
 - More specific size of microscope and well plates as they are all the same size it just depends on the amount of wells
- · Opening and closing the microscope
 - How to keep the gas in when the microscope slides are switched?
 - Sealed?
 - · How long will it take to get back to necessary parameters?
 - Flow rate and time to get to stabilization \rightarrow may need to do during testing
- CO₂
 - Comes in a tank with a regulatory on it, there is a hose on the side that you plug into the incubator; usually with a feedback loop on them
 - Tanks already have regulators on them :)
- · How will we tackle all different pieces
 - Main goal: how to keep temp even
 - Water Jacketed or Direct Heat
- Stage-top Incubators

- $\circ~$ 2 competing designs that have stage-top incubators
 - wet sponge in incubator and whole incubator is placed into conditions for temperature so temp regulated within environment
 - Use outside humidifier to control the inside
- What is the range of pH that we need to keep and will this affect if we heat the incubator manually vs mechanically?
- Design Matrix
 - Figure out where the key parts are and put the weights in
 - Better figure out brainstorming to multi-aspect designs

Conclusions/action items:

- Questions for Puccinelli
 - Ask about Routine Use
 - Are we using it for multiple labs for 3 hours only?
 - Are we using it for multiple days in the same lab?
 - How will flow rates come into play with a very small box? Is there a required flow rate? Should we include a specification for this?
 - Meet on Tuesday with Dr. P to get size requirements
 - Look into materials and equipment already in tissue culture lab

10/01/2021 Advisor Meeting #3

MAYA TANNA - Oct 10, 2021, 8:35 AM CDT

Title: Advisor Meeting #3

Date: 10/01/2021

Content by: Maya Tanna

Present: Maya Tanna, Sam Bardwell, Katie Mcgovern, Caroline Craig, Olivia Jaekle, Ethan Hannon

Goals: To document notes and conversation from our third advisor meeting with Dr. Kinney

Content:

10/1/2021 Advisor Meeting #3

- Recap of weekly events
- Get preliminary report written well!!
 - Prelim report is very similar to final with the exception of testing and results
- Design Matrix
 - Previous Project Extension
 - Heater Pumped Incubator
 - Dr. Kinney likes that idea
 - Water level will be very small to minimize risk of leakage
 - Assuming that with materials we can seal the box
 - Load the plate in from the top
 - Either slot, snap, or hinge
 - Can we do the math to determine how much volume of water needs to be heated to get to 37*C. Depends as well on the tubing.
 - How long does it take to get to that equilibrium?
 - Maybe leave a port or a sensor so that we can measure temp
 - Easy to design ports with 3D printed material
 - Shelving Design
 - Do we brainstorm more based on priority now that we have met with the client?
- Autoclaving will affect material choice
 - How hot does an autoclave get?
 - What is the pressure of an autoclave?
 - Autoclaving doesn't always keep material properties?
 - We can test this in the lab
- How will we seal it?
 - Glass on the bottom will be very secure \rightarrow glue like
 - Glass on the top → **need to discuss how the top will fit together (sliding verus hinge)**
 - Maybe using a rubber casket, like a water bottle cap.
 - Lip in top of box with a cap?
- We can access sensors from old bme labs
 - Still double check that we could build it with cheapo sensors
 - Most incubators do not tell humidity levels → people just put water in and assume that it will be enough
 - Will we get condensation on the inside of the box?
 - NO! → only time they get condensation is when the pan goes dry so as long as there is an equilibrium we should not be getting active condensation

Conclusions/action items: Use this feedback when writing the preliminary presentation and report. Start determining materials and think about how all the design components will come together.

10/08/2021 Advisor Meeting #4

MAYA TANNA - Oct 22, 2021, 12:22 PM CDT

Title: Advisor Meeting #4

Date: 10/08/2021

Content by: Maya Tanna

Present: Maya Tanna, Sam Bardwell, Katie Mcgovern, Caroline Craig, Olivia Jaekle, Ethan Hannon

Goals: To document notes and conversation from our fourth advisor meeting with Dr. Kinney

Content:

10/8/2021 Advisor Meeting Notes

- Comments on general update
 - 3D printing incubator box will be printed
 - Order quickly because shipping is taking a long time
- Design Matrix
 - Next step is figuring out how to put sensors inside th incubator
- Observed Geometry of the box
 - Make sure we include in our presentation of how we will put this together
- Sensors
 - Temp definitely maybe even a CO₂, but less important
 - Temp gage is an output sensor → sensor inside incubator that figures out CO₂, percentage and opens the solenoid when CO₂ levels drop or increase too rapidly
 - Automatic not manual
- Multiple aspects of the project
 - Building the box
 - Figuring out the sensor/
 - nternal environment maintenance
- Q&A
 - Any recommendations to get started on?
 - TESTING PLAN
 - Try to break up the project so that we are never waiting on someone else
 - Send us the preliminary presentation on TUESDAY

Conclusions/action items: Use this feedback when writing the preliminary presentation and report. Start determining materials and think about how all the design components will come together. Also, divide up into subcommittees: 1 for fabrication, 1 for sensor coding, and 1 for ordering materials/writing test protocols.



MAYA TANNA - Oct 22, 2021, 12:22 PM CDT

Title: Advisor Meeting #5

Date: 10/22/2021

Content by: Katie Mcgovern

Present: Maya Tanna, Sam Bardwell, Katie Mcgovern, Caroline Craig, Olivia Jaekle, Ethan Hannon

Goals: To document notes and conversation from our fifth advisor meeting with Dr. Kinney

Content:

10/22/2021 Advisor Meeting #5

- Impressions on the Prelim Presentations
 - Talk more about Client maybe \rightarrow needs of client
 - Bit on on how we picked design criteria
 - Stood out in quantitative data
- Poster Presentation at the end of the semester
 - Still debating whether this will be in person poster or a presentation type thing
- Where we are at in the design process
 - Finalized prelim deliverables
 - Finished the materials purchase request
 - This weekend: Sam and Maya are checking out adaptors for tubing and such
 - Dr. Kinney recommends Ace Hardware in Hildale
 - Split teams up
 - Arduino
 - Materials and Testing protocols
 - Fabrication
- Materials Purchasing List
 - Asked Dr. P if he has any prior materials
 - Follow up email
 - Try to move forward with confidence otherwise
 - There is a way to reimburse if we do choose something
- Next week we will discuss the report
- Show and Tell is in 2 weeks

Conclusions: Reach out to Dr. Puccinelli again to move forward with material purchasing. Take pictures of parts from Ace Hardware, Menards, and Home Depot for more info on adaptors and tubing.



MAYA TANNA - Nov 12, 2021, 1:11 PM CST

Title: Advisor Meeting #6

Date: 11/12/2021

Content by: Katie Mcgovern

Present: Maya Tanna, Sam Bardwell, Katie Mcgovern, Caroline Craig, Olivia Jaekle, Ethan Hannon

Goals: To document notes and conversation from our sixth advisor meeting with Dr. Kinney

Content:

See attachment below.

Conclusions: Edit and execute test protocols. Create instructions for use document. Work on full system printing/assembly as well as ensuring that the code outputs correct values for CO2. Investigate CO2 sensors and go in depth with this component of the project.

MAYA TANNA - Nov 12, 2021, 1:11 PM CST

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MAYA TANNA - Nov 25, 2021, 2:41 PM CST

Title: Advisor Meeting #8

Date: 11/19/2021

Content by: Katie

Goals: To document advice given by Dr. Kinney at our weekly meeting

Content:

See attachment below.

Conclusions/action items: Execute testing and heavily investigate the CO2 tank situation.

MAYA TANNA - Nov 25, 2021, 2:41 PM CST

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Katie Day - Dec 08, 2021, 9:16 PM CST

Title: Advisor Meeting #9

Date: 12/0/2021

Content by: Katie

Goals: To document advice given by Dr. Kinney at our weekly meeting

Content:

See attachment below.

Conclusions/action items: Execute testing and heavily investigate the CO2 tank situation.

Katie Day - Dec 08, 2021, 9:16 PM CST



12_03_21_Advisor_Meeting_Notes_9.docx(7.1 KB) - download



MAYA TANNA - Sep 20, 2021, 5:20 PM CDT

Title: Team Meeting #1 Working/Finalizing PDS

Date: 09/20/2021

Content by: Maya Tanna

Present: Sam Bardwell, Katie Mcgovern, Maya Tanna, Caroline Craig, Olivia Jaekle, Ethan Hannon

Goals: To document the progress we made on the product design specifications document as a team

Content:

- 1. Met to discuss upcoming project deadlines and initial research done by each member of the team
- 2. Everyone read over the PDS and made last edits as well as references
 - 1. Final and submitted draft is below

Conclusions/action items: We will meet next week to start coming up with ideas for the design matrix and go over the team's relevant research. We will also continue to update the PDS if design or client requirements change throughout the semester.

MAYA TANNA - Sep 20, 2021, 5:23 PM CDT

Product Design Specifications



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Product_Design_Specifications.pdf(213.6 KB) - download



Katie Day - Sep 28, 2021, 3:38 PM CDT

Title: Team Meeting #2

Date: 9/27/2021

Content by: Katie McGovern

Present: Katie McGovern, Sam Bardwell, Maya Tanna, Caroline Craig, Ethan Hannon, Olivia Jaekle

Goals: To brainstorm ideas for our preliminary design and create a design matrix.

Content:

See attached File.

Conclusions/action items:

Begin working on preliminary presentation and further research different materials.

Katie Day - Sep 28, 2021, 3:38 PM CDT



Design_Brainstorm_9_27_2021.pdf(1.3 MB) - download



MAYA TANNA - Oct 10, 2021, 8:58 AM CDT

Title: Team Meeting #3

Date: 10/04/2021

Content by: Maya Tanna

Present: Katie McGovern, Sam Bardwell, Maya Tanna, Caroline Craig, Ethan Hannon, Olivia Jaekle

Goals: To finalize our design matrix and start evaluating potential design solutions.

Content:

Conclusions/action items:

Begin working on preliminary presentation and report and further research different materials.

MAYA TANNA - Oct 10, 2021, 9:02 AM CDT



Materials_and_Heating_Brainstorm.docx(598.1 KB) - download



MAYA TANNA - Oct 18, 2021, 5:28 PM CDT

Title: Team Meeting #4 Finalizing Presentation/Organizing Subcommittees

Date: 10/11/2021

Content by: Maya Tanna

Present: Katie McGovern, Sam Bardwell, Maya Tanna, Caroline Craig, Ethan Hannon, Olivia Jaekle

Goals: To finalize our presentation and make revisions according to Dr. Kinney's feedback

Content:

Hi Katie,

Great job - my comments are below:

- Include your advisor/client and the date on your title slide
- · You don't need a presentation overview slide
- Great job with a quantitative PDS!
- Competition: are there other small/low cost incubators that have been developed outside of UW BME design?
- Make sure that the labels on your figures are large enough to read easily (Fig. 5 labels are really small)
- Include a slide describing your design criteria and how they were chosen
- Label the dimensions and points of interest on all of your figures (i.e. Fig 6)
- It might be helpful to include a separate slide describing the workflow for how it will be used

Conclusions/action items:

To finalize the preliminary report and begin compiling materials for purchasing.



MAYA TANNA - Oct 18, 2021, 5:31 PM CDT

Title: Team Meeting #5 Materials Purchasing Organization/Final Edits on Preliminary Report

Date: 10/18/2021

Content by: Maya Tanna

Present: Katie McGovern, Sam Bardwell, Maya Tanna, Caroline Craig, Ethan Hannon, Olivia Jaekle

Goals: To finalize our report and gather all the materials for purchasing together in a document

Content:

Progress is below

Conclusions/action items:

To finalize the preliminary report and purchase materials.

MAYA TANNA - Oct 18, 2021, 5:31 PM CDT

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10/18/21 TeamLab Meeting Summary

SAMUEL BARDWELL - Oct 19, 2021, 1:48 PM CDT

Title: TeamLab Meeting Summary

Date: 10/18/21

Content by: Sam

Present: Sam & Ethan

Goals: To confirm the intended design for the incubator on Solidworks is feasible and what type of adaptors to use between the tubing.

Content:

Notes:

Pipe threading

Rubber Strips

Epoxy is available

Conclusions/action items:

The TeamLab professional saw no problems with our intended design for the project. The biggest questions were surrounding the adaptors between the tubing of the metal and heated water pump. There were a couple ways to go about connecting these and one would be to thread the pipe and the screw on an adaptor to one side and then epoxy the other. The next idea was to just epoxy the metal side of the adaptor and connect the other. The adaptor would have to have a ribbed cone shape for the rubber tubing from the heated water pump to being pushed on. This could then be surrounded with a zip tie to make sure it stays on when the water is being pumped. The professional also said there are different types of epoxy's that would work better for different materials and some research should be done to find which epoxy to use.



MAYA TANNA - Oct 27, 2021, 11:08 AM CDT

Title: Ace Hardware Visit

Date: 10/23/2021

Content by: Maya

Present: Maya & Sam

Goals: To document findings on part specifications from Ace Hardware as well as future action items based on that information

Content:

Rubber water hose heats up to 150 degrees Fahrenheit (we are looking for 98 degrees Fahrenheit) - research if it is effective.

Conclusions/action items: Do more research on vinyl tubing and rubber water hoses (fuel line hose). Look into copper rust specifications to determine feasibility of using copper.

MAYA TANNA - Oct 27, 2021, 11:29 AM CDT





11/05/2021 Show and Tell Feedback

MAYA TANNA - Nov 05, 2021, 2:40 PM CDT

Title: Show and Tell Feedback

Date: 11/05/2021

Content by: Maya

Present: Whole Team

Goals: To document feedback received from other teams regarding sensor and tubing placement

Content:

- Zig zag needs pegs to hold in place
- · Sensors on the top
- · Carbonate water
- Hydrophilic materials
- Just use waterproofed sensors? RESEARCH
- CO2 sensor waterproofing test protocol
- Zig zag best idea, but secure
- Tubing: twice wrap around, tubing coming out of incubator above water
- Waterproof fabric (rain coat material)
- Randomized zig zag
- · Thermistor, coating that works with temperature but waterproof
- · Get curve and calibration stuff from class
- · Snail system with tubing
- · Look into ideas for water proofing the sensors (rubber, styrofoam)
- Test coiled vs. uncoiled tubing (tubing test protocols)

Conclusions/action items: Use a thermistor for measuring temperatures. Write test protocols for tubing and CO2 sensor waterproofing. Use snail system with tubing.



Olivia Jaekle - Oct 11, 2021, 5:03 PM CDT

Title: Design Matrix

Date: 9/28/2021

Content by: Caroline Craig, Ethan Hannon, Olivia Jaekle, Maya Tanna, Katie McGovern, Sam Bardwell

Present: Team

Goals: To document design matrix and provide reasoning for rankings.

Content:

							-	
			Past Project R	efurbished	Heated Water Pu	mp incubator	Sheiving in	oubator
Rank	Criteria	Weight	Score (10 max)	Weighted Score	Score (10 max)	Weighted Score	Score (10 max)	Weighted Score
1	Internal Environment	25	9	23	7	18	5	13
2	Microscope Compatibility	20	10	20	10	20	10	20
3	Accuracy and Reliability	20	7	14	8	16	- 4	8
4	Ergonomics	15	5	8	8	12	4	6
5	Cost	10	2	2	4	4	3	3
6	Life in Service	5	10	5	10	5	10	5
7	Safety	5	10	5	10	5	10	5
	Sum	100	Sum	76	Sum	80	Sum	60

- Internal Environment
 - For this criteria, the Past Project Refurbished scored the highest since the previous BME groups have already done testing on the device's ability to regulate temperature, CO2, and humidity. Our team believed that further work on this system could have improved the device's ability to maintain these conditions by improving the materials. For these reasons, we gave Past Project Refurbished a 9.
 - The Heated Water Pump Incubator scored the next highest because our team believes improving upon previous BME groups' designs by using a heated water tube would benefit the ability to create a better cell culture environment. It scored lower than the Past Project Refurbished design because we would not have the previous testing to use. For these reasons, we gave Heated Water Pump Incubator a 7.
 - Finally, the Shelving Incubator scored lowest with a 5 because the ability of our team to maintain the conditions once the drawers were pulled out had not been completely understood.
- Microscope Compatibility
 - All designs scored a 10 in microscope compatibility because each design was created and could successfully be used with an inverted microscope.
- Accuracy and Reliability
 - For this criteria, our team scored the Heated Water Pump Incubator highest. We believe that the finalized design would have a more reliably designed system for the intended use of the incubator with the materials and external devices we plan to use. For this reason, gave this design an 8.
 - The Past Project Refurbished design scored the next highest with a 7. Like the Heated Water Pump Incubator, the Past Project Refurbished design would have improved upon materials in comparison with previous BME projects, but the mechanics of the system would not be as reliable as the other incubator.
 - The Shelving Incubator received the lowest score of 4 because altering the shape of the environment by opening a drawer would be difficult to maintain accurate internal conditions, and the size of the machine may hinder its reliability in reading accurate conditions. Also, moving components are more susceptible to wear and tear making it less likely to live through its self-life
- Ergonomics
 - Our team scored the Heated Water Pump Incubator highest for this criteria, again because its materials and components would allow it to function the best in comparison with our other designs. For this reason, it scored an 8.
 - The Past Project Refurbished design scored a 5 because the design components implemented by previous BME teams that we planned on keeping the same would not function in maintaining internal environment conditions as the Heated Water Pump Incubator could.

Team activities/Design Process/09/28/2021 Design Matrix

- Finally, the Shelving Incubator scored lowest with a 4 because it would be the most difficult to use with having to pull out drawers each time one wanted to view a sample.
- Cost
 - All the designs scored low for cost because our team's smaller budget will be difficult to stay in range with. The Heated Water Pump Incubator scored the best with a 4 because lots of the components we plan on using will be provided to us. Our biggest difficulty in staying within the budget will be limiting the need to repurchase materials wasted in prototyping.
 - The Past Project Refurbished design scored a 3 because components of the previous design would be reused, but the components we plan on replacing would end up being more expensive than just creating the Heated Water Pump Incubator design.
 - The Shelving Incubator scored lowest with a 2 because its size would increase the cost and create a greater likelihood to go over budget if lots of prototypes are made.
- Life in Service
 - All the designs scored a 10 for Life in Service because they were designed with the intent of functioning for a week period of time every year for 10 years.
- Safety
 - All the designs scored a 10 for safety because the components involved in their designs would not be harmful to the user in any way.

Conclusions/action items:

Based on this design matrix, our team will be moving forward with creating the Heated Water Pump Incubator for our client. This design was ranked the reliable, ergonomic, and cost-effective in comparison with the other designs. The design will include a slot for the well plate, a tube containing heated water to maintain a 37*C temperature and assist in evaporation, and a water well for evaporation water to maintain high humidity. The dimensions of the incubator will match the size of the microscope stand, or it will go over the edges slightly, and the height will not exceed the lowest point of the top light microscope component. Finally, sensors compatible with Arduino will be used to regulate the internal conditions.


SAMUEL BARDWELL - Oct 19, 2021, 1:22 PM CDT

Title: Preliminary SOLIDWORKS Incubator Design

Date: 10/19/21

Content by: Sam

Goals: To create a detailed Solidworks assembly and drawing of the proposed incubator design.

Content:

Item NO.	Item Description	Dimensions (mm)	QTY.
1	Top gias plate	250 x 200 x 5	1
2	Sealed glass plate holder	260 x 210 x 6	1
3	Metal tube for water	d = 7.16	1
4	Outer box of incubator	250 × 200 × 28	1
5	Inner box of incubator to hold cell plate	140×96×18	1
6	Lower glass plate	250 x 200 x 5	1



Figure 1: Exploded view of the Solidworks drawing showing the part names and descriptions.





Figure 3: Solidworks drawing showing more detailed dimensions of all the parts in the incubator.

Conclusions/action items:

This is the preliminary design we are going to continue going forward with. The next step are to obtain the materials needed to fabricate the incubator. Once materials arrive, final touches and dimensions will be updated to the Solidworks design and then the box will be 3D printed at the UW - Madison Makerspace.



SAMUEL BARDWELL - Oct 19, 2021, 1:24 PM CDT

BME300_Incubator_Drawing_10.19.21.pdf(191.1 KB) - download



11/05/2021 Show and Tell Preparations

MAYA TANNA - Nov 05, 2021, 2:54 PM CDT

Title: Show and Tell Preparations

Date: 11/05/2021

Content by: Maya/Caroline/Katie

Present: Whole Team

Goals: To document work done to prepare for show and tell

Content:

Hi everyone! Our team has been tasked with developing a low-cost cell culture incubation chamber that is compatible with an inverted microscope and capable of live-cell imaging culture plates. The incubator must be able to maintain an internal environment of 37° C, 5% CO₂, and 95-100% humidity without compromising the integrity of the microscope's optics or functionality. Our final design consists of a heated water pump where a conducting plastic tube will be wrapped around the inside of the incubator and connected to a heated water pump that will be set to 37° C. The inside of the incubator will be filled with water, submerging the plastic tubing, allowing the internal environment to be heated by conduction as well as increasing the humidity to 95% or higher. The incubator to allow for wiring and sensors to be inside the internal environment. The sensors will be connected to an Arduino microcontroller where temperature, humidity, and CO₂ levels will be collected and analyzed. Our call to action is to ask for your help on how we can arrange the plastic tubing or sensors in order to achieve a homogeneous temperature environment.

Conclusions/action items: Use feedback from show and tell to drive the remainder of the semester and continue testing/fabrication of device.



MAYA TANNA - Nov 05, 2021, 2:54 PM CDT

Show_and_Tell_Presentation.jpg(54.2 KB) - download



40 of 336

Caroline Craig - Oct 18, 2021, 7:26 PM CDT

Title: Future Expenses Table

Date: 10/18/2021

Content by: Team

Present: Team

Goals: To document and update the expenses table with purchases throughout the fabrication process.

Content:

Hem	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	Link
Category 1 : Incubator	Second Second	later and the second second	Constant and the second second	Sherrow .	- 19 m -			Suc-
3D Printed Casing	for sides of incubator	Makerspace			1	\$20.00	\$20.00	P
Transparent Cover Plates	top and bottom of incubator	Radnor	64005034	1	1.11	51.04	\$2.08	https://www.aintab.com/
Plastic Latches	secure lid to incubator	Cambro	Cambro 60264		4	\$4.69	\$18.76	Cambro 60245 2 Hole Pla
Rubber Lining Tape	create tight seal between lid and incubator	Makenspace	11.000		1	50.00	\$0.00	
Insulating, Waterproof Mat	lining the 3D printed sides of the incubator	Makerspace			1	\$0.00	\$0.00	
Category 2 : Components					-			
3/8x12 Stainless Steel Tube	heated water will flow through	K & S Precision Metals	87119	1		\$6.00	\$6.00	LINK
3/8 in. Compression Brass Coupler	to connect the stainless steel tube to water pump	Everbuilt;	207176323			\$3.65	\$7.30	UNK
1.Smm Tube Connector	connection between CO2 tank and incubator	Fisher Scientific	35031	J.	1	\$14.96	\$14.96	UNK
Arduino 2x16 character Display	STRUCTURE CONTRACTOR STRUCTURE	MIDAS	7773012	6		\$12.71	\$12.71	Alphenumeric LCD
Arduino Operational Amplifier		ONSEMI	LM324ADR2G		1	\$0.28	\$0.28	Tosai Instrumenta Generi
Arduino 5D card logging shield		VELLEMAN	WP(304	1	1	\$4.01	\$4.01	5D card logging shield VM
					1	TOTAL:	\$86.10	-

Conclusions/action items:

The items documented in the table are potential future purchases for our team. A list including these materials has been sent to the client for purchasing, however, the stainless steel tube and 1.5mm tube connector are still being reviewed for potential cheaper or free options through the client. Other components are being reused from previous team's projects, and improved rubber lining tape and insulating mat will be purchased in the future if needed. With purchases in progress, the team is projected to come in under budget for the final design.



Caroline Craig - Dec 11, 2021, 9:44 PM CST

Title: Expenses Table

Date: 10/18/2021

Content by: Team

Present: Team

Goals: To document and update the expenses table with purchases throughout the fabrication process.

Content:

ttem	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	Link
Category 1 : incubator		-20	-12	19 A.				97
3D Printed Casing	for sides of incubator	Makerspace		11/9/2021	1	\$32.32	\$32.32	N/A
Transparent Cover Plates	top and bottom of incubator	Radnor	64005034	10/29/2021	2	\$1.04	\$2.08	https://www.ainpat.com/product
Category 2 : Components								
3/8 and 1/4 in. Polyethylene Tubing	heated water will flow through	USA Sealing	55YU99	11/23/2021	1	\$1.96	\$1.96	LINE
Epoxy glue	to attach loose components	Makerspace	- 25383	10000	- X.	\$1.50	\$0.00	N/A
1.5mm Tube Connector	connection between CO2 tank and incubator	Fisher Scientific	35031	10/29/2021	1	\$14.96	\$14.96	LINK
Vinyl Tubing 3/8" x 1/2"	heated water will flow through	Ace Hardware	4027504	12/6/2021	1	\$8.33	\$8.33	N/A
Barbed Vacuum Connector	connection between tubing	Grainger	SZMH	11/23/2021	2 (of 10)	\$0.95	\$1.90	LINK
		1				TOTAL:	\$61.55	

Conclusions/action items:

The items documented in the expenses table are the items that were purchased for our microscope cell culture incubator. All costs were covered by the client. Other components are being reused from the previous team's projects, so the cost of those materials is not included in the expenses table. If the project were to be reproduced from scratch the total cost would be roughly \$150. Altogether the team came in under budget for the final design.



SAMUEL BARDWELL - Dec 05, 2021, 5:16 PM CST

Title: Box Fabrication: 3D Print

Date: 11/29/21

Content by: Sam

Goals: To 3D print the incubator box and assemble it.

Content:



Figure 1: Top view of incubator box and crown 3D prints



Team activities/Fabrication/11/29/2021 Box Fabrication: 3D Print Figure 2: Bottom view of incubator box and crown 3D prints



Figure 3: Assembled 3D printed incubator box.

Conclusions/action items:

The printed box turned out nicely. There are a couple straggling PLA plastic strings from the 3D printer. Sliding in the crown of the box to the slit printed into the box is a little difficult and not smooth, but it does go all the way in. Next steps are to epoxy the glass to the plastic squares as well as drill holes into the plastic and epoxy adaptors and tubing to the box as well.



SAMUEL BARDWELL - Dec 09, 2021, 1:26 PM CST

Title: Hardware Setups

Date: 11/29/21

Goals: To show photos of the electrical set up for the sensors in the incubator.

Content:







Figure 2: DHT22 sensor hardware set up



Figure 3: CO2 sensor hardware set up

Conclusions/action items:

All of the sensors are up and running. The coding and the schematics will be added to the notebook. Next is to test the sensors and eventually implement them into the incubator box design.



Katie Day - Dec 07, 2021, 8:04 PM CST

Title: Incubator Fabcrication

Date: 12/07/2021

Content by: Katie McGovern

Present: Katie McGovern and Sam Bardwell

Goals: To fabricate the incubator.

Content:

The box was fabricated by first drilling 3/8 inch diameter holes in the front of the box and then using a circular file to expand them so that the barbed connectors could fit in the incubator. They were then hot glued. The glass was hot glued onto the small divot made for them in the design. A 1/4 inch hole was drilled on the bottom right corner for the thermistor and filed with a circular file. A 1/2 inch hole was drilled and expanded via circular file for the CO2 sensor to fit in. The CO2 sensor and the thermistor were hot glued into place. The 3/8x1/4 inch tubing was wrapped in a circular fashion along the interior of the box and connected to the barbed vacuum connectors. They were then secured by zip ties. They were connected to a 1/2x3/8 inch tubing that was secured via zip ties to both the connector and the hot water pump. Then roughly 16 oz of water was poured into the incubator.

Conclusions/action items:

The PLA material needs to be changed as it was difficult to drill into, very brittle, and appeared to be leaking in random places.



IMG_5896.jpg(761.9 KB) - download

47 of 336



IMG_5894.jpg(1.1 MB) - download



IMG_5893.jpg(1.1 MB) - download

Katie Day - Dec 07, 2021, 8:04 PM CST



IMG_5892.jpg(582.6 KB) - download



IMG_5891.jpg(854.1 KB) - download

49 of 336



IMG_5890.jpg(394.4 KB) - download



IMG_5889.jpg(1.2 MB) - download

Katie Day - Dec 07, 2021, 8:04 PM CST



IMG_5888.jpg(761.9 KB) - download

IMG_5895.jpg(676.6 KB) - download



11/01/2021 Testing Protocols Initial Draft

MAYA TANNA - Nov 05, 2021, 2:51 PM CDT

Title: Testing Protocols Initial Draft

Date: 11/01/2021

Content by: Caroline and Maya

Present: Team

Goals: To document the initial draft of test protocols that were sent to Dr. Kinney for review/approval

Content:

See attachment below.

Conclusions/action items: Use feedback from Dr. Kinney to improve test protocols as well as feedback from Show and Tell to add components to test to ensure the most successful final design.

MAYA TANNA - Nov 05, 2021, 2:51 PM CDT

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Testing_Protocols_1_.docx(583 KB) - download



Katie Day - Dec 07, 2021, 8:08 PM CST

Title: Incubator User Manual

Date: 11/15/2021

Content by: Sam Bardwell and Ethan Hannon

Present:

Goals: To establish a user manual to determine how to use the incubator once printed.

Content:

See attached user manual.

Conclusions/action items:

Katie Day - Dec 07, 2021, 8:09 PM CST



Incubator_User_Directions.pdf(46.3 KB) - download



MAYA TANNA - Nov 25, 2021, 2:44 PM CST

Title: Testing Protocols Final Version

Date: 11/19/2021

Content by: Maya/Caroline

Goals: To document the final draft of the testing protocols, which were edited based on the team and advisor's feedback

Content:

See attachment below.

Conclusions/action items: Execute testing wherever possible and investigate CO2 component of the project.

MAYA TANNA - Nov 25, 2021, 2:45 PM CST

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Testing_Protocols_Template_1_.docx(584.9 KB) - download



Katie Day - Dec 07, 2021, 8:05 PM CST

Title: CO2 Testing
Date: 12/3/2021
Content by: Katie, Olivia, Maya, and Caroline
Present: Katie and Olivia
Goals: To test the CO2 sensor to make sure that it is working properly.
Content:
Attached our the results of our testing, testing protocols written by Maya and Caroline, performed by Olivia and me.

Conclusions/action items:

The CO2 sensor is ready for incorporation into the incubator.

Katie Day - Dec 07, 2021, 8:05 PM CST



concentration.csv(2.4 KB) - download

Katie Day - Dec 07, 2021, 8:05 PM CST



concentration_graphs.csv(2.3 KB) - download



Katie Day - Dec 07, 2021, 8:05 PM CST

Katie Day - Dec 07, 2021, 8:05 PM CST

Title: Thermistor Testing
Date: 12/3/2021
Content by: Katie, Olivia, Maya, and Caroline
Present: Katie and Olivia
Goals: To test the accuracy of our thermistor against an incubator.
Content:
Testing protocol written by Maya and Caroline and performed by Olivia and me. Results are below.

Conclusions/action items:

Thermistor is working properly and ready for implementation.

Misty_In_Incubator_10-min.PNG(15 KB) - download



SAMUEL BARDWELL - Dec 11, 2021, 1:53 PM CST

Title: Humidity Testing

Date: 12/3/2021

Content by: Katie and Olivia

Present: Katie and Olivia

Goals: To test the accuracy of our humidity formula against the DHT22 sensor

Content:

Humidity data gathered over time in order to perform ttest to determine statistically significance compared to the DHT22 sensor.

	Variable 1	Variable 2
Mean	12.61830986	12.16718182
Variance	0.090374245	0.424219419
Observations	71	220
Hypothesized Mean Difference	0	
df	255	
t Stat	7.973463829	
P(T<=t) one-tail	2.59912E-14	
t Critical one-tail	1.650851092	
P(T<=t) two-tail	5.19824E-14	
t Critical two-tail	1.96931057	

Figure 1: T-test results comparing the thermistor humidity readings to the DHT22 readings.

Conclusions/action items:

Send data to caroline, olivia, and maya for analysis. The t-test was determined to be significant (significance value of .05). This is not what we expected because the average values are within .5% between the DHT22 and thermistor. We will most likely have to improve the calibration of the thermistor if we want to continue with this project.

Katie Day - Dec 07, 2021, 8:05 PM CST



Misty_Humidity_Data.csv(1.5 KB) - download

Katie Day - Dec 07, 2021, 8:05 PM CST



Combined_Humidity_Data.csv(4.1 KB) - download

Katie Day - Dec 07, 2021, 8:05 PM CST



Katie Day - Dec 07, 2021, 8:05 PM CST



DHT22_Humidity_Data.csv(441 Bytes) - download



Caroline Craig - Dec 11, 2021, 9:47 PM CST

Title: Optical Testing

Date: 12/05/2021

Content by: Caroline Craig and Maya Tanna

Present: Caroline Craig and Maya Tanna

Goals: To determine whether or not the glass being used interfered with the optics of the microscope.

Content:

ImageJ Results of the Optical Testing



Figure 13: Optical analysis from ImageJ of microscopic cells with glass (left) and without glass (right)

Conclusions/action items:

The Optics were not interferred with.

MAYA TANNA - Dec 11, 2021, 8:25 PM CST

	Microscope Image with Glass	Microscope Image without Glass
Red Squares	130	120
Green Squares	54	51
Blue Squares	8	21
Total	192	192

MAYA TANNA - Dec 11, 2021, 8:26 PM CST

Results from this test show that the image with the glass had a slightly higher, yet very similar focus quality compared to the image without glass present.

1

12/07/2021 Attempted Incubator Testing

Katie Day - Dec 07, 2021, 8:04 PM CST

Title: Attempted Incubator Testing

Date: 12/07/2021

Content by: Katie McGovern and Sam Bardwell

Present: Katie McGovern and Sam Bardwell

Goals: To initially determine whether or not our incubator was working as expected.

Content: Data collected during testing.

Conclusions/action items:

- 1. Polyethelene tubing acted more as an insulator than a conductor and would not heat up the water bath to the desired temperature. Need to use a metal tube.
- 2. PLA box was leaking slightly. It is unclear where or how it is leaking as it has been sealed via hot glue and zipties.
- 3. Glass did fog up after about 30 minutes so we will need to figure out how to demist the glass.

Katie Day - Dec 07, 2021, 8:04 PM CST



Incubator_Temp_Over_Time.csv(5 KB) - download







Katie Day - Dec 07, 2021, 8:04 PM CST



Incubator_Temp_Hum_Over_Time.csv(5 KB) - download



Actual_Inc_HUm_Data.csv(2.1 KB) - download



09/24/2021 Product Design Specifications

SAMUEL BARDWELL - Sep 21, 2021, 7:12 AM CDT

Title: Product Design Specifications

Date: 9/24/21

Content by: Everyone

Present: Everyone

Goals: To create a PDS in order to show our intended project in great detail.

Content:

PDF of PDS is attached

Conclusions/action items:

We will follow this PDS throughout the entire project to make sure we create a device that meets the clients needs.

SAMUEL BARDWELL - Sep 21, 2021, 7:13 AM CDT

Product Design Specifications



Macroscop e C of Culture based aster BAG 200109 34 Signador 2011 Class Da His Pacienti University of Viscomic Halman Bignations of Harmonic Tagoneting Tagin Viscomic Bios Databili Maja Tara Olio Janki Candra Filay Elias Hanne

Product_Design_Specifications.pdf(213.7 KB) - download



MAYA TANNA - Oct 10, 2021, 9:11 AM CDT

Title: Design Matrix

Date: 09/27/21

Content by: Everyone

Present: Everyone

Goals: To create a design matrix to evaluate our potential solutions to the project.

Content:

See attachment below.

Conclusions/action items:

We will follow these design specifications to ensure we deliver the desired product to the client.

MAYA TANNA - Oct 10, 2021, 9:11 AM CDT



Design_Matrix_.xlsx(664.8 KB) - download



10/15/2021 Preliminary Presentation

MAYA TANNA - Oct 19, 2021, 4:32 PM CDT

Title: Preliminary Presentation

Date: 10/15/2021

Content by: Katie McGovern, Sam Bardwell, Maya Tanna, Olivia Jaekle, Caroline Craig, and Ethan Hannon

Present: Whole Team

Goals: To present our preliminary findings, goals, and proposed design to our client and advisor.

Content:

Attached is the preliminary presentation.

Conclusions/action items:

Begin ordering materials and prototyping.

Katie Day - Oct 18, 2021, 3:56 PM CDT

Microscope Cell Culture Incubator



Preliminary_Presentation_Slides_1_.pdf(947.8 KB) - download



MAYA TANNA - Oct 19, 2021, 10:04 PM CDT

Title: Preliminary Report

Date: 10/15/2021

Content by: Katie McGovern, Sam Bardwell, Maya Tanna, Olivia Jaekle, Caroline Craig, and Ethan Hannon

Present: Whole Team

Goals: To document our final version of the preliminary report.

Content:

See attachment below.

Conclusions/action items:

Order materials and get feedback on final design/preliminary deliverables from advisor and client.

MAYA TANNA - Oct 19, 2021, 10:04 PM CDT



Preliminary_Report-_Microscopic_Cell_Incubator.pdf(1.4 MB) - download



Katie Day - Dec 11, 2021, 4:32 PM CST

Title: Final Poster Presentation

Date: 12/10/2021

Content by: Katie Day, Sam Bardwell, Maya Tanna, Caroline Craig, Olivia Jaekle, and Ethan Hannon

Present: Katie Day, Sam Bardwell, Maya Tanna, Caroline Craig, Olivia Jaekle, and Ethan Hannon

Goals: To present the work we have done over the course of the semester in a clear and concise fashion.

Content:

See attachment.

Conclusions/action items:

N/A



Katie Day - Dec 11, 2021, 4:33 PM CST

Final_Poster_-_Final_1_.pdf(2.3 MB) - download



Title: Progress Report 1

Date: 9/15/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 08, 2021, 9:18 PM CST



cell_incubator-progress_report-1.docx(11.3 KB) - download



Title: Progress Report

Date: 9/23/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 08, 2021, 9:21 PM CST



cell_incubator-progress_report-2.docx(11.4 KB) - download



Title: Progress Report

Date: 9/30/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 08, 2021, 9:21 PM CST



cell_incubator-progress_report-3.docx(11.6 KB) - download



Title: Progress Report

Date: 10/07/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 08, 2021, 9:22 PM CST



cell_incubator-progress_report-4.docx(11.1 KB) - download



Title: Progress Report

Date: 10/14/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 08, 2021, 9:22 PM CST



cell_incubator-progress_report-5.docx(11 KB) - download



Title: Progress Report

Date: 10/21/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 08, 2021, 9:23 PM CST



cell_incubator-progress_report-6.docx(11.3 KB) - download



Katie Day - Dec 09, 2021, 10:52 AM CST

Title: Progress Report

Date: 10/28/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 09, 2021, 10:56 AM CST



cell_incubator-progress_report-7.docx(11.6 KB) - download



Katie Day - Dec 09, 2021, 10:52 AM CST

Title: Progress Report

Date: 11/04/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 09, 2021, 10:56 AM CST



cell_incubator-progress_report-8.docx(11.8 KB) - download


Katie Day - Dec 09, 2021, 10:53 AM CST

Title: Progress Report

Date: 11/11/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 09, 2021, 10:56 AM CST



cell_incubator-progress_report-9.docx(12.2 KB) - download

11/18/2021 Progress Report 10

Katie Day - Dec 09, 2021, 10:54 AM CST

Title: Progress Report

Date: 11/18/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 09, 2021, 10:56 AM CST



cell_incubator-progress_report-10.docx(11.7 KB) - download

12/02/2021 Progress Report 11

Katie Day - Dec 09, 2021, 10:55 AM CST

Title: Progress Report

Date: 12/02/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 09, 2021, 10:56 AM CST



cell_incubator-progress_report-11.docx(12.1 KB) - download

12/09/2021 Progress Report 12

Katie Day - Dec 09, 2021, 10:55 AM CST

Title: Progress Report

Date: 12/02/2021

Content by: Katie, Sam, Maya, Caroline, Olivia, and Ethan

Present:

Goals: To document our progress over the course of a week in the semester.

Content:

See attached file.

Conclusions/action items:

See attached file.

Katie Day - Dec 09, 2021, 10:57 AM CST



cell_incubator-progress_report-12.docx(12.3 KB) - download



9/13/21 Methods of Heat Transfer

SAMUEL BARDWELL - Sep 13, 2021, 7:58 AM CDT

Title: Methods of Heat Transfer

Date: 9/13/21

Content by: Sam

Goals: To research different types of heat transfer in order to improve the even dispersion of heat in the cell incubator.

Content:

Link: https://courses.lumenlearning.com/boundless-physics/chapter/methods-of-heat-transfer/

Cite: "Methods of Heat Transfer | Boundless Physics." https://courses.lumenlearning.com/boundless-physics/chapter/methods-of-heat-transfer/ (accessed Sep. 13, 2021).

Notes:

- Three different types of heat transfer: conduction, convection, and radiation

Conduction is the transfer of heat through physical contact.

- Best for solids

- Dependent on the temperature difference, the size of the area in contact, the thickness of the material, and the thermal properties of the material(s) in contact.

- Another factor affecting conduction is the thickness of the material through which the heat transfers.
- The rate of conductive heat transfer through a slab of material, such as the one in the figure above is given by

$$\frac{Q}{t} = \frac{kA(T_2-T_1)}{d}$$

where Q/t is the rate of heat transfer in Joules per second (Watts), k is the thermal conductivity of the material, A and d are its surface area and thickness, and (T2–T1) is the temperature difference across the slab.

Convection is the heat transfer by the macroscopic movement of a fluid, such as a car's engine kept cool by the water in the cooling system.

- Convection is driven by the large scale flow of matter in fluids.
- Convection can transport heat much more efficiently than conduction

- Natural convection is driven by buoyant forces: hot air rises because density decreases as temperature increases. This principle applies equally with any fluid.

Sam Bardwell/Research Notes/Biology and Physiology/9/13/21 Methods of Heat Transfer



Convection Cells: Convection cells in a gravity field.

Radiation is the transfer of heat through electromagnetic energy

- The energy of electromagnetic radiation depends on the wavelength (color) and varies over a wide range: a smaller wavelength (or higher frequency) corresponds to a higher energy.

- The rate of heat transfer by emitted radiation is determined by the Stefan-Boltzmann law of radiation

$$\frac{Q}{t} = \sigma \mathrm{AT}^4$$

where

is the Stefan-Boltzmann constant, A is the surface area of the object, and T is its absolute temperature in kelvin.

- The temperature of an object is very significant, because the radiation emitted is proportional to this quantity to the fourth power.

Conclusions/action items:

The three types of heat transfer to look at are conduction, convection, radiation. Radiation is not reasonable at all for this project so the types of ways we could heat up the incubator environment are through conduction or convection. The previous semester project talked about the use of water and convection heating and after researching this appears to be the most reliable way to evenly transfer heat to the environment. Further research can include constant humidity and CO2 levels.

9/13/21 Thermo Scientific SmartNotes

SAMUEL BARDWELL - Sep 13, 2021, 12:03 PM CDT

Title: Thermo Scientific SmartNotes

Date: 9/13/21

Content by: Sam

Goals: To understand the significance of the proper environment conditions for cell incubation.

Content:

Link: https://assets.thermofisher.com/TFS-Assets/LED/Specification-Sheets/PF-CO2-SMARTNOTE-EN.pdf#:~:text=In%20a%20cell%20culture%20incubator%2C%20balanced%20growth%20media,minerals%2C%20etc.%2C%20resulting%20in%20toxicity%20and%20cell%20death.

Cite: Zotero is struggling to cite for some reason.

Notes:

- Proper temperature, gas tension (CO2, O2/N2) and humidity work together to provide optimum growth.
- Mammalian cells will grow best at their native temperature (37 C)
- CO2 gas serves to maintain in vivo pH, similar to CO2 tension in the bloodstream.
- High humidity prevents evaporation of growth media.
- High humidity is the most difficult condition to reestablish but is critically important, as evaporation is 4 times faster at 80% humidity than at > 93%

Conclusions/action items:

This was a very informative PDF. Helps explain the importance of each target characteristic of the desired environment for the cell culture incubator. Some further research can be done on how to keep humidity and CO2 levels at their proper settings. Also, research should be done on how to incorporate a microscope into the incubator.



SAMUEL BARDWELL - Sep 13, 2021, 12:03 PM CDT

PF-CO2-SMARTNOTE-EN.pdf(560.7 KB) - download

9/13/21 Eppendorf CO2 Incubation Chamber Details

80 of 336

SAMUEL BARDWELL - Sep 13, 2021, 4:54 PM CDT

Title: Eppendorf CO2 Incubation Chamber Details

Date: 9/13/21

Content by: Sam

Goals: To understand the optimal conditions for cell incubators.

Content:

Link: https://www.eppendorf.com/product-media/doc/en/853664/CO2-Incubators_White-Paper_056_CellXpert-CO2-Incubators_CO2-Incubator-Temperature-Control-What-Is-Best-Place-Your-Cell-Culture-

 $Vessels.pdf\#:\sim: text=Controlling\%20 temperature\%20 and\%20 levels\%200f\%20 CO\%202\%20 and, approximately\%2095\%20\%20 avoids\%20 desiccation\%200f\%20 the\%20 cultures.$

Cite: J. Wagener, F. Stöhrer, and A. Tacheny, "CO2 Incubator Temperature Control: What Is the Best Place For Your Cell Culture Vessels?," p. 5.

Notes:

- If temperatures in the incubation chamber are not consistent throughout the device, the cells on the top shelf could grow different than the cell on the bottom shelf. Environment should be even throughout to offer the best results.

- Cells with 1 degree Celsius of difference can produce visible differences in the cell cultures.

- Fans can help regulate an even dispersion of temperature and CO2 levels but it does increase the risk for air borne particles and contamination.

Conclusions/action items:

This article had some good information about temperature and gas control. It is very important we have as little error in temperature as possible because even a one degree difference produces physical difference between cell cultures. A couple of things to consider for our project is having multiple temperature probes being inserted into our incubator in order to have temperature readings throughout multiple spots. More research can be done on the best equipment to use to obtain accurate data.

SAMUEL BARDWELL - Sep 13, 2021, 4:50 PM CDT



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CO, Incubator Temperature Control: What Is the Best Place For Your Cell Culture Vessels?

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CO2-Incubators_White-Paper_056_CellXpert-CO2-Incubators_CO2-Incubator-Temperature-Control-What-Is-Best-Place-Your-Cell-Culture-Vessels.pdf(2.7 MB) - download



9/12/21 PHCbi Cell Culture Incubators

SAMUEL BARDWELL - Sep 12, 2021, 8:11 PM CDT

Title: PHCbi Cell Culture Incubators

Date: 9/12/21

Content by: Sam

Goals: To research competing designs for a cell culture incubator

Content:

Link: <u>https://www.phchd.com/us/biomedical/cell-culture-incubators?</u> utm_source=mv&utm_medium=paid&utm_campaign=intent&gclid=5c9dde37c3e11927f99abf0958cdc7c5&gclsrc=3p.ds&

Cite:

[1] America P. C. of N., "Cell Culture Incubators & Biomedical Incubators | PHCbi," PHC Holdings Corporation.

https://www.phchd.com/us/biomedical/cell-culture-incubators?

utm_source=mv&utm_medium=paid&utm_campaign=intent&gclid=5c9dde37c3e11927f99abf0958cdc7c5&gclsrc=3p.ds& (accessed Sep. 12, 2021).

Notes:



Figure 1: Photo of the PHCbi Cell Culture incubators

- Cell-IQ incubators use high-performance (IR) detectors to measure CO2 concentration, CytoGrow incubators use a thermal conducitivity sensor or infrared sensor, depending on model.

- Precise and repeatable chamber environment for cell culture reproducibility
- Easy contamination control options
- Superior CO2 control with rapid recovery after door openings
- In vivo environment replication
- Cost: \$4,000 \$10,000

Conclusions/action items:

These incubators are very well suited for the preservation of cell but fail to include a microscope in order to observe the living cells. Some more research that can be done is how the humidity, temperature, and CO2 levels are so well maintained in these incubators and translate that technology to our design.



9/12/21 Hybrid CMOS/PDMS Microsystem

Title: Hybrid CMOS/PDMS Microsystem

Date: 9/12/21

Content by: Sam

Goals: To research competing cell incubation designs

Content:

Link: https://ieeexplore.ieee.org/stamp/stamp.jsp?tp=&arnumber=4156130

Cite: [1] J. Blain Christen and A. G. Andreou, "Design, Fabrication, and Testing of a Hybrid CMOS/PDMS Microsystem for Cell Culture and Incubation," IEEE Transactions on Biomedical Circuits and Systems, vol. 1, no. 1, pp. 3–18, Mar. 2007, doi: 10.1109/TBCAS.2007.893189.

Notes:

- poly(dimethylsiloxane) (PDMS) has become the mainstay of soft lithography for applications in the life sciences. The low cost (approximately 50 times cheaper than silicon), biocompatibility and its ability to be readily adapted to microfabrication techniques are among the numerous reasons PDMS has become immensely popular.

- Standard incubators for cell culture provide an environment of 37 C, 5% CO , and 100% relative humidity (RH).

- This temperature is the optimal growth temperature for most mammalian cells.

- The humidity is necessary to prevent the evaporation of water from the media, since this would affect the concentration.

- The CO concentration is increased above atmospheric levels to maintain the proper pH, not for cell metabolism which requires atmospheric levels of both carbon dioxide and oxygen.

- A typical research laboratory incubator is the size of a small refrigerator

- Reusable CMOS microheater/solid-state temperature sensor.

- An interface DAQ board that connects to a computer serial or USB port and incorporates a microcontroller, digital to analog converters to read out the temperature in the culture well as well as digital to analog converters to control the temperature in the device.

- Talks about the microcontroller used as well as op amp usage to record analog outputs



Fig. 2. Microelectronics system architecture of the cell culture and incubation microdevice. It is comprised of three components (i) a CMOS die, (ii) an interface DAQ, and (iii) software for closed loop control and data acquisition.

- Talks about testing done with different temperatures and materials

Conclusions/action items:

We may look into the use of having PDMS as our main material for cell incubation. It appears to be a cheap and useful material for our intended project. This is a great article on the scientist attempt to create an effective incubator. A lot of the content can be used toward our project such as the materials that were not good or the electronics and materials used to obtain data in the incubator's environment. Some more things to research or different ways to evenly disperse heat, humidity, and CO2 among a cell culture. Other research is different electronics and coding to be used in order to have an output be printed to analyze for testing.



Design_Fabrication_and_Testing_of_a_Hybrid_CMOS_PDMS_Microsystem_for_Cell_Culture_and_Incubation.pdf(2.5 MB) - download



SAMUEL BARDWELL - Sep 13, 2021, 4:23 PM CDT

Title: Past BME Design Projects

Date: 9/13/21

Content by: Sam

Goals: To research previous project work.

Content:

- Previous projects incorporated a lot of insulation within the final design. This should be an important part of our project as it will keep the temperatures, humidity, and the CO2 levels consistent.

- Previous projects did a lot of theoretical testing using software. This could be important in analysis if we decide to use conduction or convection heating as well as maintaining accurate CO2 levels.

- A lot of the projects included microcontroller in order to output a lot of the numerical data as well as to record data using different testing devices. This will be important to include into our design

Conclusions/action items:

These previous semester BME Projects will be very helpful and should be referenced multiple times. We can incorporate previous ideas into our designs as well as expand on their old work. Some more research that can be conducted is finding the best materials to possibly use for a box as well as how to keep CO2 levels constant.

SAMUEL BARDWELL - Sep 13, 2021, 4:26 PM CDT



Spring_2021_BME_400_MicroscopeCellCultureIncubator.pdf(667.5 KB) - download

SAMUEL BARDWELL - Sep 13, 2021, 4:26 PM CDT



Fall_2020_BME_400_MicroscopeCellCultureIncubator.pdf(667.5 KB) - download

SAMUEL BARDWELL - Sep 13, 2021, 4:26 PM CDT



Fall_2016_BME_400_MicroscopeCellCultureIncubator.pdf(1.1 MB) - download

SAMUEL BARDWELL - Sep 13, 2021, 4:26 PM CDT



Fall_2011_BME_300_200_MicroscopeCellCultureIncubator.pdf(1.6 MB) - download

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Spring_2017_BME_402_MicroscopeCellCultureIncubator.pdf(1.6 MB) - download

SAMUEL BARDV

Title: Humidity & Temp Sensor with Arduino

Date: 9/14/21

Content by: Sam

Goals: To begin thinking about how to record environmental data for the cell incubator.

9/14/21 Humidity & Temp Sensor with Arduino

Content:

Link: https://www.bing.com/videos/search?

Cite: "How to make Temperature and Humidity Sensor with Arduino - Bing video." https://www.bing.com/videos/search? q=temp+and+humidity+probe&&view=detail&mid=67F95D2B70D2182DE53867F95D2B70D2182DE538&&FORM=VRDGAR&ru=%2Fvideos%2Fsearch%3Fq%3Dtemp%2Band%2Bhumidity% (accessed Sep. 14, 2021).

Notes:

- Uses DHT11 sensor, breadboard, and 3 wires



- Has live monitoring of temp and humidity reading in an environment

Conclusions/action items:

The use of an Arduino Uno and breadboard would be fairly simple and helpful for us in order to record live data inside of our incubator. Some possible troubles might occur when trying to feed the high humidity and temperature might be dangerous with the use of electronics. Some further research that could be conducted would be a wireless sensor that uses WIFI to send data to the



SAMUEL BARDWELL - Sep 14, 2021, 12:13 PM CDT

Title: CO2 Sensor for Arduino

Date: 9/14/21

Content by: Sam

Goals: To find a possible CO2 Sensor that is Arduino compatible.

Content:

Link: https://www.aliexpress.com/item/32737298149.html?aff_platform=true&aff_short_key=UneMJZVf&isdl=y&src=bing



Conclusions/action items:

This was a CO2 sensor compatible with Arduino found on the internet. A preliminary plan is to have all of the sensors placed inside of the incubator and have live feedback and data being sent through wire to a Microcontroller which would be connected to a computer. A couple problems that could occur with this is that the device was found on a very sketchy website. I would like to find a sensor on Amazon where it would be more reliable. Also, having a lot of wires and bulky sensors in an incubator could lead to rust and problem. The team might have to CAD draw a plastic case for the sensors to protect the devices from the humid and hot environment. More research can be done on different types of sensors as well preliminary fabrication designs.



9/16/21 Humidifier for Incubator

SAMUEL BARDWELL - Sep 16, 2021, 7:24 AM CDT

Title: Humidifier for Incubator

Date: 9/16/21

Content by: Sam

Goals: To find a possible humidifier for our cell incubator in order to keep humidity level at 95%+

Content:

Link: https://tagdetacher.com/product/12-led-industrial-incubator-humidifier-110v-240v-ultrasonic-mist-maker-fogger-fountain-atomizer-air-humidifier/? v=7516fd43adaa

Source: "Incubator humidifier 12 LED Industrial 110V / 240V ultrasonic atomizer air humidifier," *Tag Detacher Co.* https://tagdetacher.com/product/12-led-industrial-incubator-humidifier-110v-240v-ultrasonic-mist-maker-fogger-fountain-atomizer-air-humidifier/ (accessed Sep. 16, 2021).

Notes:



- This is a possible humidifier for our cell incubator

- A couple negative would be having the LED lights on the humidifier. It also appears that the plug in is not a standard US plugin. It does require the constant addition of water in order to keep evaporating water into the air. Last, it does say that the humidifier works best in 0-40 C temperatures so we would be cutting it close at 37C for our incubator. That could make problems.

Conclusions/action items:

I don't believe we should use this humidifier because of all the possible problems that could arise. It has got a chance to lead to a similar idea or product in order to keep out humidify at very high levels in the incubator. More research could be conducted on other humidifiers or just using exposed water in the incubator to create a humid environment.



9/22/21 Patent for Inverted Microscope

SAMUEL BARDWELL - Sep 22, 2021, 7:38 AM CDT

Title: Patent for Inverted Microscope

Date: 9/22/21

Content by: Sam

Goals: To understand the dimensions and functioning of an inverted microscope in order to have a well-fitting incubator.

Content:

Link: https://patentimages.storage.googleapis.com/15/ad/4d/753d3c2e5125d4/US6404546.pdf

Cite: S. Toyoda and T. Kawahito, "Inverted microscope," US6404546B2, Jun. 11, 2002 Accessed: Sep. 22, 2021. [Online]. Available: https://patents.google.com/patent/US6404546B2/en

- Inverted microscopes have a stage where the incubator will be placed.

- Incubator must be clear on both the top and bottom because cell are viewed form underneath and light is being produced from above the stage to illuminate the cells for better vision.

Conclusions/action items:

The stage will not be extremely big for an incubator so the dimensions of the incubator must be fairly small. This patent didn't provide dimensions of the incubator so more research will be done on inverted microscopes. In order to obtain dimensions, the team will most like have to go to the inverted microscopes currently being used in lab.

9/27/21 Water Pump

SAMUEL BARDWELL - Sep 27, 2021, 8:33 PM CDT

Title: Water Pump

Date: 9/27/21

Content by: Sam

Goals: To find an affordable water pump in order to provide circulation in the water heater throughout the incubator.

Content:



Conclusions/action items:

This is an affordable water pump to use for our project. Might be a little on the excessive side of pump. Could look for smaller. The team also needs to find a way to heat the water to 37 degrees Celsius.



10/18/21 Heated Water Pump Pictures

SAMUEL BARDWELL - Oct 19, 2021, 1:36 PM CDT

Title: Heated Water Pump Pictures

Date: 10/18/21

Content by: Sam

Present: Sam & Ethan

Goals: To have pictures of the heated water pump in order to find adaptors to connect the water pump tubes to the metal tubing inside the incubator.

Content:











Conclusions/action items:

These are photos from the heated water pump we are planning to use for our incubator design. The tubing adaptors should be able to connect to the metal tubing inside of our incubator. The tubing on the water pump appeared to be too big, but it is removable from the pump. This would mean that more rubber tubing would have to be bought, but better dimensions and connectors could be used to connect to the incubator. More specifics will come when parts get delivered.



SAMUEL BARDWELL - Sep 21, 2021, 7:39 AM CDT

Title: Preliminary Design Idea

Date: 9/21/21

Content by: Sam

Goals: To start brainstorming possible design ideas for the design matrix.

Content:



Figure 1: Preliminary design idea for the cell culture incubator.

Labels

1. The petri dishes placed into the incubator in a linear fashion. This would allow the user to slide the incubator along the inverted microscope to examine different sets of cells in the same incubator.

2. The internal environment of the incubator. It would be surrounded by 3D printed plastic most likely and have some plexiglass on top to allow a transparent view. This is where the cell cultures would be kept. The temp of this environment would be 37 degrees Celsius, 95+% humidity, and 5% CO2.

3. This is the box at the end of the incubator where all of the electronics and sensors could be stored. The one problem with this is the the readings would be on the complete opposite side of the incubator and the data might be completely different on the other side. Might have to move later.

4. This is a bottom view of the incubator. The continuous line is a possible heating system for the incubator. This would allow equal temperatures throughout the incubator because the heat is being released at every spot in the incubator. The is also another plexiglass layer in the bottom. In order to see the cells and petri dished, there will have to be a middle layer to hold the dishes in place otherwise the heating mechanism would get in the way or viewing cells.

Conclusions/action items:

This is a very preliminary idea that could be considered for the project. I can already come up with multiple problems that might arise but we could talk as a team and see if we would want to continue with this design and improve upon it or not.

Sam Bardwell/Design Ideas/9/21/21 Preliminary Design Idea



SAMUEL BARDWELL - Dec 05, 2021, 2:09 PM CST

Title: Preliminary Design 2

Date: 9/27/21

Content by: Sam

Goals: To draw a more detailed photo of my intended design for the cell culture incubator.

Content:





- New design includes wells to put the petri dishes or well plates into so they don't shift inside the incubator. The wells will be high enough to put water inside and a tube underneath the water to use for an even heating/humidifying system. The well separation between the water and heating tube will prevent a group of cells experiencing a warmer environment than another one. The top and bottom will have a slit in order for the plexiglass to slide into place. This will help allow a transparent top and bottom cover to view the cell cultures.

Conclusions/action items:

This should be a good design idea. The wells may have to be rectangular because the client uses more well plates instead of petri dishes. One struggle I see is taking the wells plates out and putting them back in. The use of tongs or a needle nose may have to be used. The design can keep being updated and research on convection for the heating tube can be conducted. One other possible problem for the design would be to find the best spot for the sensors inside of the incubator.



SAMUEL BARDWELL - Sep 29, 2021, 11:33 AM CDT

Title: Updated Solidworks Drawing

Date: 9/29/21

Content by: Sam

Goals: To update the Solidworks drawing after the second client meeting.

Content:



Figure 1: Updated Solidworks drawing

Conclusions/action items:

The client provided us with more information about the project during our second meeting and said we only have to fit one cell plate within the incubator at a time. This lead to the same Solidworks design as the sliding design, but there is only one hole for the cell plate. The Solidworks design will continue to be updated and include different features when the logistics of the project are better figured out.

SAMUEL BARDWELL - Oct 05, 2021, 7:44 AM CDT

Title: Updated Solidworks

Date: 10/3/21

Content by: Sam

Goals: To continue to draw a more accurate model of the intended incubator design in solidworks.

Content:



Figure 1: Updated SOLIDWORKS design of the heated water pump incubator design idea.

- This CAD drawing shows the internal tubing that is going to help heat water inside of the incubator via conduction.

- This CAD drawing was also updated to show a crown at the top of the incubator that will hold the top glass piece. This crown will have an inner lining of a rubber material which will later be clamped along with the top glass layer to provide a tight seal inside of the incubator.

- The drawing also shows the inner extrusion being a little shorter to allow for air to circulate about the cell plate.

Conclusions/action items:

The design is starting to come together but there is still a lot of places to improve. The team needs to think of where the placement of sensors and wiring can go. We also need to start testing certain materials we would like to use so even more specific dimensions can be updated on to SOLIDWORKS which we will later 3D print.



SAMUEL BARDWELL - Dec 05, 2021, 5:34 PM CST

Title: Final SOLIDWORKS Drawing

Date: 11/5/21

Content by: Sam

Goals: To show the final SOLIDWORKS drawing before getting 3D printed.

Content:



Figure 1: Collapsed view of the final SOLIDWORKS design before being printed.

Sam Bardwell/Design Ideas/11/5/21 Final SOLIDWORKS Drawing



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Figure 2: Exploded view of the final SOLIDWORKS design.
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Conclusions/action items:

This should be the last SOLIDWORKS drawing upload because the box will get printed. A drawing of the box will be developed for the final report and added soon.



SAMUEL BARDWELL - Sep 29, 2020, 11:55 AM CDT

Title: Woodworking Red Permit 1

Date: 9/29/2020

Content by: Sam

Content:

lmage preview

lmage preview



SAMUEL BARDWELL - Feb 08, 2021, 5:19 PM CST

Title: Biosafety Certification

Date: 2/8/21

Content by: Sam

Goals: To be certified to work with biomaterials.

Content:

University of Wisconsin-Madison

This certifies that SAMUEL BARDWELL has completed training for the following course(s):

Course Name	Curriculum or Quiz Name	Completion Date	Expiration Date
BIOSAFETY REQUIRED TRAINING BIOSAFETY REQUIRED TRAINING QUIZ		2/4/2021	

Oxta Effective: Thu Feb 4 13:40:00 2021 Report Generated: Mon Feb 8 17:06:55 2021

Conclusions/action items:

This will be useful for this semester and future semesters in Biomedical Engineering. It allows me to safely work with biomaterials.



3/12/21 Chemical Safety Certification

SAMUEL BARDWELL - Mar 12, 2021, 3:42 PM CST

Title: Chemical Safety Certification

Date: 3/12/21

Content by: Sam

Goals: To be safe while using chemicals.

Content:

University of Wisconsin-Madison

This certifies that SAMUEL BARDWELL has completed training for the following course(s):

Course Name	Curriculum or Quiz Name	Completion Date	Expiration Date
BIOSAFETY REQUIRED TRAINING	BIOSAFETY REQUIRED TRAINING QUIZ	2/4/2021	
CHEMICAL SAFETY: THE OSHA LAB STANDARD	FINAL QUIZ	3/4/2021	

Data Effective: Thu Mar 4 11:25:00 2021 Report Generated: Fri Mar 12 15:37:01 2021

Conclusions/action items:

Can be used for BME 201 project as well as future classes in BME or at UW Madison



10/28/21 Green Permit

SAMUEL BARDWELL - Oct 28, 2021, 8:12 AM CDT

Title: Green Permit

Date: 10/28/21

Content by: Sam

Goals: To obtain a green permit to utilize if necessary.

Content:

College of Engineering UNIVERSITY OF WISCONSIN-MADISON				
TEAMLAD Technical Educations and Management Management Management makerspace				
Permit No: $LV - 14033 - 6$ Issue Date: $10/27/2021$				
Name: Samuel Barduell User Signed: Sund Barduell				
Display Other Side in Holder				

Figure 1: Front side of the green permit

TEAMLab	Green Shop Permit	t Makerspace
Name: Sou	mel Bardae	
Woodworking 1	Woodworking2:	Woodworking3:
Welding1:	Welding 2:	Welding 3:
CNC Mill 1:	CNC Mill 2: CNC M	Aill 3: CNC Mill 4:
CNC Lathe 1:	CNC Lathe 2: Ha	aas1: Laser1:
Ironworker 1:	Coldsaw1: CNC Rout	ter 1: CNC Plasma1:

Figure 2: Back side of green permit

Conclusions/action items:

This green permit will be used if necessary for BME design projects.
SAMUEL BARDWELL - Dec 05, 2021, 1:50 PM CST

Title: Sam's Progress Report #1

Date: 9/15/21

Content by: Sam

 $\textbf{Goals:} \ \textbf{To update the notebook on my weekly progress and contributions.}$

Content:

Weekly Summary:

- Began researching the ideal conditions for cell incubation.
- Began to brainstorm and research possible design ideas for the sensors that will be placed inside the incubator to help collect data.

Goals:

- Continue to do more research based on client meeting.
- Begin working on the Product Design Specification document for next week.
- Continue to have a welcoming environment for BME 200 students.

Conclusions/action items:

Continue to research the project to develop a good understanding of the task at hand.

SAMUEL BARDWELL - Dec 05, 2021, 2:10 PM CST

Title: Sam's Progress Report #2

Date: 9/23/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- Contributed to the product design specifications.
- Conducted research on different sensors that could be used to record temperature, humidity, and CO2 level data for the incubator.
- Begin brainstorming preliminary designs for the physical appearance of the incubator.

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Figure 1: Preliminary design idea for the cell culture incubator.

Sam Bardwell/Project Progress/9/23/21 Progress Report #2



Figure 2: Preliminary design idea 2 for the cell culture incubator.

Goals:

- Begin brainstorming more ideas about possible designs for the design matrix.
- Conduct more research on inverted microscopes in order to better understand the dimensions of the cell culture incubator.

Conclusions/action items:

Product design specifications should be all ready to be finalized soon. Once preliminary design ideas have been made, I can begin to work on SOLIDWORKS drawings for the project.



SAMUEL BARDWELL - Dec 05, 2021, 2:13 PM CST

Title: Sam's Progress Report #3

Date: 9/30/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- Began creating SOLIDWORKS drawings for possible designs of incubators.



Figure 1: Updated Solidworks drawing

- Researched possible instruments to use along with the incubator to help maintain temperature and humidity levels.

Goals:

- Create more detailed SOLIDWORKS drawings.
- Begin working on the preliminary presentation.
- Research possible tubing options for the inside of the incubator.

Conclusions/action items:

SOLIDWORKS drawings are beginning to look good. I should practice for the preliminary presentation in order to have good commentary.



SAMUEL BARDWELL - Dec 05, 2021, 2:28 PM CST

Title: Sam' s Progress Report #4

Date: 10/7/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- Continued to update the SOLIDWORKS drawing in order to incorporate a tubing and sealing system.



Figure 1: Updated SOLIDWORKS design of the heated water pump incubator design idea.

- Contributed to the preliminary report.

Goals:

- Continue to update the SOLIDWORKS design.
- Finalize the preliminary report and presentation for next Friday.
- Begin ordering materials to test.

Conclusions/action items:

SOLDIWORKS drawing is beginning to look ready to 3D print. Will continue to touch it up as more information and design ideas come in. Materials should be ordered sooner than later.



SAMUEL BARDWELL - Dec 05, 2021, 2:44 PM CST

Title: Sam's Progress Report #5

Date: 10/14/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- Contributed to the preliminary presentation as well as the preliminary report.

- Produced animations of the intended incubator design on SOLIDWORKS to provide a more clear view for the client and readers.

Goals:

- Work with Ethan to begin the fabrication process, primarily begin looking at the heated water pump and how it will connect to the tubing in our design and the thickness of the materials to update the incubator dimensions on SOLIDWORKS.

- Finalize the preliminary report.

Conclusions/action items:

Preliminary report and presentations ended up doing very well. Couple things to touch up on the report once there is feedback. Next steps are to begin fabricating the incubator and hopefully 3D print the box soon.



Preliminary_Presentation_Slides.pptx(5.3 MB) - download

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Preliminary_Report-_Microscopic_Cell_Incubator.docx(1.4 MB) - download

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SAMUEL BARDWELL - Dec 05, 2021, 3:00 PM CST

Title: Sam's Progress Report #6

Date: 10/21/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- Finalized the preliminary report as well as created all the SOLIDWORKS drawings to include dimensions of our proposed final design.

- Met with a Team Lab professional to receive advice on the best route to fabricate the incubator.

- Checked out the heated water pump to figure out how the adaptors will connect.









Goals:

- Begin working on fabrication of the project.
- Go to a hardware store to get a physical sense of the adaptors and tubing most likely going to be used for the project.
- Help with the Arduino code.

Conclusions/action items:

The box is ready to be built and connected to the heated water pump. The Arduino coding will be challenging but we can get a good start on it.



SAMUEL BARDWELL - Dec 05, 2021, 3:27 PM CST

Title: Sam's Progress Report #7

Date: 10/28/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- Maya and I went to a hardware store to find physical parts for our project.
- We did not buy anything because it was more so to see sizes of tubing and adaptors in person.
- Katie, Olivia, and I began working on the Arduino code to output temperature and humidity values.

Goals:

- Continue working on the Arduino code and get that up and running so that part of the sensor testing is ready to go.
- Once materials begin arriving, continue to work on the fabrication of the incubator itself.

Conclusions/action items:

We know the type of adaptors and tubing we want. Next is to order those materials. The Arduino code for the sensors is ready to go which helps the project tremendously. Next is to get the incubator box up and running.

SAMUEL BARDWELL - Dec 05, 2021, 3:37 PM CST

Title: Sam's Progress Report #8

Date: 11/4/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- Worked on the Arduino code for the temperature sensor.
- Meet with Dr. P to receive feedback on the project and asked about different materials we can order and the best way to do it.
- Updated the SOLIDWORKS drawing to include the newly arrived glass.



Figure 1: Updated SOLIDWORKS drawing of incubator box to include glass dimensions.

- Reviewed show and tell.

Goals:

- Continue to update SOLIDWORKS design so once all the intended materials and information have been collected, the box can be printed.

- Once the box has been printed, true fabrication can begin.

Conclusions/action items:

The meeting with Dr. P went very well. Got the team and the client on the same page. The box will be worked on getting printed next.

SAMUEL BARDWELL - Dec 05, 2021, 3:38 PM CST



Show_and_Tell_Presentation.pptx(902.9 KB) - download



SAMUEL BARDWELL - Dec 05, 2021, 3:47 PM CST

Title: Sam's Progress Report #9

Date: 11/11/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- Continued to update SOLIDWORKS drawing of the cell culture incubator.
- Went to the makerspace and developed a file to be able to print the box and the crown of the incubator.
- Continued researching possible adaptors for tubing in the design.

Goals:

- Pick up the 3D prints and make sure they fit to the microscope and are up to scale.
- Begin attaching the glass plates once they meet our testing protocols to the incubator.
- Begin working on attaching the heated water pump to the incubator.

Conclusions/action items:

The box is going to be printed in the next couple of days, just waiting for a printer to open up. The 3D prints will be touched up and checked to make sure the slit works as intended. Next will be to get the materials for the tubing and then to connect them to the box and heated water pump.



SAMUEL BARDWELL - Dec 05, 2021, 5:19 PM CST

Title: Sam's Progress Report #10

Date: 11/18/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- 3D printed the box and the crown for the incubator.



Figure 1: Top view of incubator box and crown 3D prints



Figure 2: Bottom view of incubator box and crown 3D prints



Figure 3: Assembled 3D printed incubator box.

- Worked on a preliminary step by step usage form to have instruction on how to use the incubator.
- Researched different types of adaptors to possibly use for the project.

Sam Bardwell/Project Progress/11/18/21 Progress Report #10



Goals:

- Begin prepping the incubator for tubing and adaptors.

- Hook up the heated water pump to the box to allow for temperature testing.
- Begin working on how to hook up the CO2 tank.

Conclusions/action items:

The box is all ready to be drilled into and have adaptors implemented into it, we just need the materials. We cant go much further with the project until we get the tubing and adaptors seen on this page. Once the tubing and adaptors are connected, the heated water pump will be tested to see if the internal temperature of the box will increase.



SAMUEL BARDWELL - Dec 05, 2021, 5:25 PM CST

Title: Sam's Progress Report #11

Date: 12/2/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- Began working on the final poster.
- Continued fabrication steps for the project.
- Began implementing the heated water pump into the project.

Goals:

- Continue to fabricate the project.
- Begin testing on how the box maintains temperature and humidity.
- Implement the sensors into the incubator box to collect live data.

Conclusions/action items:

The materials still have not showed up yet, and if they don't by the weekend, we will go to ACE hardware and buy them ourselves so we can have a functioning project. We have a lot to get done after being set back from the materials. There has to be statistical testing done on our experimentation and data collected. The sensors are all ready to go and need to be added to the box as well once the heated water pump is functioning. Final poster will be updated and printed last minute. The final report will have to be worked on over the weekend.



SAMUEL BARDWELL - Dec 08, 2021, 5:20 PM CST

Title: Sam's Progress Report #12

Date: 12/8/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- Worked on the fabrication of the incubation box.



Sam Bardwell/Project Progress/12/8/21 Progress Report #12







Figure 1: Images showing the fabrication of the cell culture incubator. Individual fabrication steps are shown as well as complete assemblies including the live outputting of code and the heated water pump.

The box was fabricated by first drilling 3/8 inch diameter holes in the front of the box and then using a circular file to expand them so that the barbed connectors could fit in the incubator. They were then hot glued to the incubator. The glass was hot glued onto the small divot made for them in the design. A 1/4 inch hole was drilled on the bottom right corner for the thermistor and filed with a circular file. A 1/2 inch hole was drilled and expanded via circular file for the CO2 sensor to fit in. The CO2 sensor and the thermistor were hot glued into place. The 3/8x1/4 inch tubing was wrapped in a circular fashion along the interior of the box and connected to the barbed vacuum connectors. They were then secured by zip ties. They were connected to a 1/2x3/8 inch tubing that was secured via zip ties to both the connector and the hot water pump. Then roughly 16 oz of water was poured into the incubator.

Sam Bardwell/Project Progress/12/8/21 Progress Report #12

* See 11/15/2021 Incubator User Manual for testing protocols*



Figure 2: Temperature and Humidity values over 10 minute time interval

- Contributed to the poster presentation and final report.
- Produced SOLIDWORKS assemblies for the poster and drawings for the report.

Sam Bardwell/Project Progress/12/8/21 Progress Report #12





Item No.	Item Description	Dimensions [mm]	QTY.
1	Glass plates to allow transparent viewing	114.5 × 138.5 × 1.3	2
2	Top removeable crown to allow access to interior	Outside: 189 x 250 x 5 Inside Cut: 85.4 x 127.5 x 2.5	1
3	Well Plate to hold cells	85.4 x 127.5 x 22.5	1
4	Innerheated water pump tubing	Outer Diameter: 12.7 Inner Diameter: 9.525	1
5	Incubator box to maintain a controlled internal environment	Outside: 195 x 245 x 40 Inside Cut: 90 x 132 19	1

Figure 3: SOLIDWORKS assembly photos of the incubator with dimensions.

Goals:

- Finish all final deliverables.

Conclusions/action items:

The fabrication and testing process of the project is done. It is time to finalize the poster presentation and the final report. I will also update the drawings including more detailed dimensions of the incubator box. The incubator box did not meet the PDS requriements for a variety of reasons. The first being that the incubator box needs to be made out of a better material rather than PLA. The inner tubing of the incubator box also did a poor job of transfering heat from the heated water pump so this will need to be changed in the future. Lastly, the sensors could be calibrated even more precisely. We are planning on continuing this project next semester to really atttack these little problems and to produce a functioning incubator. CO2 will be a main focus for next semester because we were not able to get to that part of the project due to lack of access to CO2 in the lab.



SAMUEL BARDWELL - Dec 14, 2021, 5:04 PM CST

Title: Sam's Progress Report #13

Date: 12/15/21

Content by: Sam

Goals: To update the notebook on my weekly progress and contributions.

Content:

Summary:

- Contributed to the final poster presentation, specifically the final design section.



ifem No.	Item Description	Dimensions [mm]	QTY.
1	Glass plates to allow transparent viewing	114,5 x 138,5 x 1,3	2
2	Top removeable crown to allow access to interior	Outside: 189 x 250 x 5 Inside Cut: 85.4 x 127.5 x 2.5	1
э	Well Plate to hold cells	65.4 x 127.5 x 22.5	1
4	innerheated water pump tubing	Outer Diameter: 12,7 Inner Diameter: 9,525	1
8	Incubator box to maintain a controlled internal environment	Outside: 195 x 245 x 40 Inside Out; 90 x 132 19	1

Figure 1: Exploded SOLIDWORKS assembly of the final design along with a table explaining the dimensions and parts

- Showed our client, Dr. P, our final design

- Contributed to the final report

Sam Bardwell/Project Progress/12/15/21 Progress Report #13



Figure 2: Drawing of the final design in SOLIDWORKS

- Updated the team notebook

Goals:

- Improve the project next semester.

Conclusions/action items:

The final poster presentation went very well. I think our poster was a very concise representation of the entire semester's work. Dr. P stopped by to see how our final design turned out and said we had the best box he has ever seen with this project. We are already in the works of talking about the continuation of this project next semester. Our group has a very good idea on what improvements can be made and Dr. P seemed to be just as excited about it as we are. The drawings created in SOLIDWORKS should allow for any future semester teams to reproduce the box and its dimensions.

Final_Poster_-_Final.pdf(2.3 MB) - download

SAMUEL BARDWELL - Dec 11, 2021, 1:42 PM CST

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Final_Report_-_Microscopic_Cell_Incubator.pdf(3.5 MB) - download

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Katie Day - Sep 12, 2021, 10:43 AM CDT

Title: Cell Culture Basics

Date: 9/12/2021

Content by: Katie McGovern

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Present:

Goals: To research the basics of cell cultures to better understand how to build our incubator.

Content:

- <u>Cell Culture: Growing Cells as Model Systems in Vitro</u>
 - Cell culture: laboratory methods that enable the growth of cells in physiological conditions
 - most commonly used to study cell biology, replicate disease mechanisms, or investigate drug compounds
 - easy to manipulate genes and molecular pathways
 - culture systems removes interfering genetic or environmental variables
 - Safe Handling of Cell Lines
 - ACDP: national body managed by the Health and Safety Executive (HSE) that advises on hazards and risks to workers from exposure to pathogens during cell cultures
 - consult biosafety levels (BSL) 1-4.
 - Recommended Equipment for Cell Culture Labs Table 9.2

Equipment	Purpose
Biosafety Cabinet	create sterile work surface
Lumid CO2 insubstor	provide a physiological environment for
	cell growth
Inverted light microscope	to asses cell morphology and count cells
fridge/freezers	store cells and cellular materials
Centrifuge	condense cells
Lomontomator	count cells, determine growth kinetics and
Hemacytometer	prepare suitable densities
Autoclave	sterilizator
Coll outure diches	culture cells using flasks, petri dishes, 96
	well plates
Vacuum pump	aspirate cell culture medium

- Cell Cultures in Lab
 - Primary cells: directly isolated from human tissue (ex. fibroblasts from skin biopsies)
 - characterized as finite and rely on continuous supply of stocks since their proliferation ceases after a limited amount of cell divisions and cell expansion is often impossible
 - Transformed cells: can be generated either naturally or by genetic manipulator
 - Self-renewing cells: cells that carry the capacity to differentiate into a diversity of other cell types with long-term maintenance in vitro
 - ex. embryonic stem cells
- Cell Culture Microenvironment
 - The Cell Culture Medium

- create an environment that allows for max cell propagation is achieved through the incubator (i.e. temperature, humidity, O2, and CO2 tensions) and the basal cell culture medium and its supplements
 - Basal Cell culture medium: has carbs, vitamins, amino acids, minerals, growth factors, hormones, and components that control physicochemical properties such as the culture's pH and cellular osmotic pressure
 - serum as fetal bovine serum is added that provides cells with growth factors and hormones and acts as a carrier for lipids and enzymes and transportation of micronutrients and trace elements
- Temperature, pH, CO2, and O2 Levels
 - temp: incubated at 36-37*C
 - can be achieved though tightly regulated and monitoring the temp of the environment
 - pH: 7.2-7.4
 - As the cells propagate, their growth requires energy supplied in the medium, for example in the form of glucose. When metabolized, its by-products include pyruvic acid, lactic acid, and CO₂. Since the pH level is dependent on the balance of CO₂ and HCO₃⁻ (bicarbonate), the addition of bicarbonate-based buffers to cell culture media can equilibrate the CO₂ concentrations.
 - CO2 tensions: 5-7% adjustable
- Subculturing
 - when cell culture vessel reaches ~80% cells need to be transfered

Figure 9.3 Basic Science Methods for Clinical Researchers. 2017 : 151–172. Published online 2017 Apr 7. doi: 10.1016/B978-0-12-803077-6.00009-6

- Applications
 - Drug Development and Drug Testing: used to screen novel chemicals, cosmetics, and drug compounds for their efficacy and asses drug cytotoxicity in cell types
 - Virology and Vaccine Production: using mammalian cells researches can study the growth rates, development, and conditions required for the cycle of infectious diseases
 - Tissue Regeneration and Transplate: cell cultures with hIPSCs, embryonic stem cells, and adult stem cells can be studied for their regeneration properties for use in replacement tissues or organs
 - Genetic Engineering or Gene Therapy: allows for the study of the expression of specific genes and their impact on cells
- Encyclopedia Of Insects (second Edition) Chapter 39- Cell Culture
 - Cell Culture: technique in which cells are removed from an organism and placed in a fluid medium where, under proper conditions, cells can live and even grow.
 - cell growth is characterized by mitosis and differentiation
 - Differentiation: cells can change into specific types that are capable of functions analogous to tissues or organs in the organism

Conclusions/action items:

Cells need a hospitable environment in order to be studied. Incubators are commonly used and we will have to carefully monitor the system we create.

9/12/2021 CO2/Cell Culture Incubator Basics

Katie Day - Oct 03, 2021, 3:30 PM CDT

Title: Cell Culture Incubator Basics

Date: 9/12/2021

Content by: Katie McGovern

Present:

Goals: To understand the physiology of an incubator in order to replicate it at a lower price.

Content:

- Labcompare CO2/Cell Culture Incubator
 - Designed to maintain a constant temp and high humidity under a CO2 atmosphere
 - Temps: 4-50*C
 - controlled by a water bath circulating cabinet or by electric coils that give off heat
 - CO2: 0.3-19.9%
 - · Use non-corrosive stainless steel interiors or antimicrobial copper surfaces
 - Auto decontamination using heat or UV
 - Humidity: 95-98%
 - Features of fancy ones:
 - programmable controls with password protection
 - temp alarms
 - CO2 alarms
 - door opening alarms
- Inexpensive low-oxygen incubators
 - Oxygen tension in mammillian tissues ranges from 1-6%
 - growing normal human diploid cells in 2% o2 extends their lifespance
 - Low Cost Incubator
 - Gas tank with O2, CO2, and N
 - Equipment:
 - Silicone vaccuum grease
 - Nalgene 2117 Stragith-side wide-mouth jars, polymethylpentene with white polypropylene screw-top lids, autoclavable
 - Size 15D silicone rubber stoppers
 - Bubble tubing
 - Procedure
 - First drill two half-inch holes into the clear bottoms of Nalgene 1,000-ml Straight-Side Wide-Mouth Jars (Fig. 2). Although this can be done by a bioengineering department, adequate holes are produced using a home drill press and a flat 1/2-inch wood drill bit.
 - Invert the jar so that the white plastic lid becomes the bottom of the incubator and the holes are at the top. Plug the holes with size 15D silicone rubber stoppers.

- The lid has a small bump in its center that prevents dishes from lying flat on its surface. Form a flat surface by placing the lid from a 10-cm plastic petri dish on the white lid.
- 4 Coat the threads of the jar with silicone vacuum grease so that it closes smoothly and forms a gas-tight seal.
- Bubble tubing provides a very convenient means of connecting the tank to the chambers. Cut one of the expanded sections before it tapers to the small diameter, providing the tubing with a good, snug fit into one of the 1/2-inch holes in order to flush the chambers.
- Connect to a tank containing a special three-gas mix consisting of 2% oxygen, 5% CO₂ and 93% nitrogen.
- 7 Chambers must be re-gassed each time they are opened to observe or feed the cells. There is no need to re-gas unopened chambers (for example, if cloning cells, they can be left for several weeks without regassing).
- Wright, W., Shay, J. Inexpensive low-oxygen incubators. Nat Protoc 1, 2088–2090 (2006). https://doi.org/10.1038/nprot.2006.374
- <u>https://www.businesswire.com/news/home/20201009005417/en/CO2-Incubators-Market-Growth-of-Global-Life-Science-Market-to-Boost-the-Market-Growth-Technavio</u>

Conclusions/action items:

Determine ways in which we can build sensors to deliver CO2 and keep the temperature and humidity in the right spots.

9/12/2021 EU Cell Culture Basics Handbook



Katie Day - Sep 12, 2021, 10:32 AM CDT

Title: EU Cell Culture Basics Handbook

Date: 9/12/2021

Content by: Katie McGovern

Present:

Goals: To learn about how cell cultures work in order to create a low cost incubator

Content: The EU's Cell Culture Basics Handbook

Conclusions/action items:

1. Refer to this handbook for logistics of creating cell plates and for incubator standards.





CellCultureBasicsEU.pdf(4.2 MB) - download


Katie Day - Sep 23, 2021, 9:55 AM CDT

Title: Material Research

Date: 9/22/2021

Content by: Katie McGovern

Present:

Goals: To discover materials that are both insulators and transparent.

Content:

- Mechanical Properties of Zirconia Re-inforced Lithium Silicate Glass-Ceramic
 - Zirconia: enhanced mechanical properties of all-ceramic restorations
 - Lithium dislicate ceramic restoation
 - fabricated with a heat-pressed or CAD/CAM fabrication processes
 - enhanced translucency and different shades of lithium dislicate makes feasible anatomically contoured monolithic restorations --> displays a bluish color

Materials	Fracture Toughness (MPa m^05)	Flexural Strength (MPa)	Characteristic Strength (MPa)	Weibull Modulus	Elastic Modulus (GPa)	Hardness (GPa)	Brittleness index (um^-1/2)
VS (Zirconia reinforced lithium silicate glass- ceramic)	2.31	443.63	460.74	13.41	70.44	6.53	2.84
IC(Lithium disilicate glass- ceramic)	2.01	348.33	361.82	12.49	60.61	5.45	2.72

- Conclusions
 - The VS zirconia reinforced lithium silicate glass-ceramic revealed higher mechanical properties (fracture toughness, flexural strength, elastic modulus, and hardness) compared with IC lithium disilicate glassceramic
 - According to Weibull distribution, VS glass-ceramic appears to be reliable for clinical use; however, clinical assessment is required to give reliable recommendations for dental practitioners
 - IC glass-ceramic revealed lower brittleness index compared with VS glass-ceramic and hence, IC glass-ceramic may have superior machinability.
- Optically Transparent Thermally Insulating Silica Aerogels for Solar Thermal Insulation
 - silica based aerogels coated on black surfaces have the potential to act as simple and inexpensive solar thermal collectors because of their high transmission to solar radiation and low transmission to thermal radiation
 - VTSS technology
 - places a selective surface inside a vacuum to limit convective and conductive losses --> cost of maintance is high
 - OTTI coating: transparent to solar radiation and opaque to IR
 - transmits sunlight to absorber while reducing the reradiation and convection heat losses from the hot absorber to the ambient

- silica aerogels are mostly absorptive in thermal IR spectrum
 - absorption spectra of silica and other gaseous constituents such as H2O and CO2
- Aligned Cellulose/Nanodiamond plastics with high Thermal COnductivity
 - Plastic: orderly layered structure which cellulose is highly oriented along the in-plane direction and Nanodiamond disperses effectively to form an orderly connection with cellulose due to hydrogen bonding
 - Thermal conductivity = 5.37 Wm^-1K^-1 at 5 wt% filler content



- Examining the ROle of Atomic Scale Heterogeneity on the Thermal COnductivity of Transparent, Thermally Insulating Mesoporouus Silica-Titania Thin Films
 - Crystalline materials are often good conductors bc their long range atomic-scale order facilitates heat carrier propagation via lattice vibrations
 - Adding titania to silicate matrix lowers the thermal conductivity of the matrix as a result of introducing additional heatcarrier scattering centers
 - Materials that are the most chemically homogeneous with the most distrubuted scattering sites were more effcient at reducing heat carrier transport



- · Thermally Insulating Nanocellulose-Based Materials
 - Nanocellulose: rod-like partially crystallline cellulose nanparticles with diameters between 3-50nm and lengths from 100-several um, feature a combo of low density, high emodulus, low thermal expansion coefficient, and flexible surface chemistry

Katie Day/Research Notes/Biology and Physiology/9/22/2021 Materials



- Figure: the modes of heat transport in porous materials. Heat transfer by a) convection, b) radiation, c) gas conduction, including the coupling effects at the gas-solid interface, and d) solid conduction, highlighting diffuse and specular photon scattering at interphases
- Replacement of air with water through moisture uptake of hygroscopic materials (wood, cellulose, and CNMs) usually
 results in an increase of the heat conduction bc water has higher thermal conductivity that air

Matarial	Density (kg	λa (mW m^-1	λr (mW m^-1		Т	RH
Material	m^-3)	K^-1)	K^-1)	∧a/∧r	(K)	(%)
Cellulose Iβ	1500-1600	900	240/500	3.8/1.8	298	N/A
CNC	1500-1600	5700	720	7.9	300	-
Partly crystalline cellulose in wood	1500-1600	1040	260	4.0	293	N/A
Wood fibers	1500-1600	766	430	1.8	293	N/A
Birch	680	323	214	1.5	294	30
Oak	753	270	160	1.7	293	30
Shear-oriented CNC films	N/A	530	220	2.4	300	-
TNW nanopaper	1090	2470	290	8.5	298	N/A
TOSNF nanopaper	1100	635	360	1.8	298	N/A

• Table 1: Thermal Conductivity of cellulose-, wood-, and CNM-based films

- a) a-axis of the unit cell
- b) *b*-axis of the unit cell
- c) Under vacuum

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- d) Tunicate nanowhiskers
- e) TEMPO-oxidized Sugi cellulose nanofiber.
- Aerogels with low density and pores smaller than the mean free path of air can display thermal conductivities significantly lower than value for air
- Silica aerogels consist of noncrystalline silica clustors that forms a 3D gel with pores smaller than 05nm and thermal conductivity is the same in all directiors and is sufficient to characterize the heat transfer properties for an isotropic material with a single value for thermal conductivity
- Oven drying fo wet CNM/cellulose-based foams or aerogels is a cost effective way of producing CNM-based thermally
 insulating materials --> can result in strongly distorted porous structures

Katie Day/Research Notes/Biology and Physiology/9/22/2021 Materials



Figure 9: Requirements for cellulose nanomaterial-based insulation materials

- Potentially use solar power insulating glasses --> like a mini-greenhouse for cells
 - Frosted Polycarbonate roofing sheet transparent thermal insulation sheets



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• High Transparent 8mm 10mm Twin Wall Thermal Insulation PC Lexan Polycarbonate Sheet for Home Swimming Pool Cover



• Silica Aerogel 6mm Super Light Isulation Waterproof Sound deadening Mat

Conclusions/action items:

Look into greenhouse glass technology and make sure that we use a crystalline material.

10/8/2021 Optical Properties of Well Plates



Katie Day - Oct 18, 2021, 4:20 PM CDT

Title: Optical Properties of Well Plates

Date: 10/8/2021

Content by: Katie McGovern

Present:

Goals: To determine the optical properties of well plates so that they could be replicated with the incubator materials.

Content:

Optics for Testing:

- 96 Well Plates
 - Material: Polypropylene
 - Young's Modulus = 1.1-1.6
 - Optical Properties:
 - Gloss % = 75-90
 - Haze % = 11
 - Transparency % = 85-90

Conclusions/action items:

Replicate these conditions with the materials for the incubator design.



Katie Day - Sep 14, 2021, 10:32 AM CDT

Title: Competing Incubator Designs

Date: 9/14/2021

Content by: Katie McGovern

Present:

Goals: To discover what other kinds of incubators are on the market and why we are looking to improve them.

Content:

- ThermoFisher Scientific
 - Two chamber Types:
 - Direct Heat CO2 incubators
 - Heracell VIOS 160i CO@ INcubator with Coppor INterior Chambers
 - HEPA filtrations for ISO Class 5 air quality
 - Overnight Steri-Run for total sterilization
 - NOT AVAILABLE IN THE US
 - Forma Steri-Cult CO2 Incubator made of polished stainless steel
 - Water jacketed CO2 INcubators
 - Forma Series 3 Water Jacketed CO2 Incubators
 - Enhanced temp stability and univromite
 - HEPA filtration for ISO Class 5 air quality
 - Intuitive iCAN touchscreen
- NuAire
 - Direct Heat
 - NU-5700
 - Touch panel control and monitoring of temp, CO2, humidity, and O2 lebels inside a 160L stainless steel chamber HEPA filtered to ISO Class 5
 - used for In-vitro cells
 - NU-5800
 - same thing as the 5700 but 200L
 - Water Jacket
 - NU-8600
 - 160L same thing but water jacketed design
- Biocompare
 - New Brunswick Galaxy 48R
 - Water jacketed
 - first to use fan-less, direct heat, and seamless chamber for low gas comsumption
 - 0

Conclusions/action items:

Need to request a quote to see how much these products actually cost and decide between a direct heat or water-jacketed design.



Katie Day - Dec 12, 2021, 2:21 PM CST

Title: ISO/TS 23565:2021

Date: 10/15/2021

Content by: Katie Day

Present:

Goals: To present standards that we must be aware of when creating our design.

Content:

ISO/TS 23565:2021; Biotechnology-Bioprocessing-General Requirements and Considerations for Equipment Systems used in the Manufacturing of Cells for Therapeutic Use

Notes:

- · Doesn't apply to incubator, but important to note for other aspects of the design
- · Applies for hardware, software, and consumables used in the manufactoring of cells i.e our arduino coding
- Used for tissue engineered product
- · tubing, culture vessels or other containors
- also used for monitoring systems intended to control the internal environment.

14:00-17:00, "ISO/TS 23565:2021," ISO. https://www.iso.org/standard/76053.html (accessed Oct. 15, 2021).

Conclusions/action items:

Make sure Arduino circuitry and tubing materials are in check with this standard.



Katie Day - Dec 12, 2021, 2:25 PM CST

Title: ISO Standards Update

Date: 10/15/2021

Content by: Katie Day

Present:

Goals: To make a note of all 2021 updated ISO Standards that may be relevant in our project.

Content:

See link:

"ISO Update Supplement to ISOfocus," 2021. Accessed: Dec. 12, 2021. [Online]. Available:

https://www.iso.org/files/live/sites/isoorg/files/news/magazine/ISOupdate/EN/2021/ISOupdate_August_2021.pdf.



Katie Day - Dec 12, 2021, 2:27 PM CST

Title: ISO 19090:2018

Date: 10/15/2021

Content by: Katie Day

Present:

Goals: To make note of another standard used in cell cultures.

Content:

See link for standard. Note that it is with animal cells, not human. Check what type of cells Dr. Puccinelli is working with.

14:00-17:00, "ISO 19090:2018," ISO. https://www.iso.org/standard/63936.html (accessed Oct 15, 2021).



Katie Day - Dec 12, 2021, 2:30 PM CST

Title: ISO 24998:2008 Plastics Laboratory Ware

Date: 10/15/2021

Content by: Katie Day

Present:

Goals: To make note of another standard used for plastic lab ware when completing cell cultures, specifically petri-dishes.

Content:

See link attached.

14:00-17:00, "ISO 24998:2008," ISO. https://www.iso.org/standard/42736.html (accessed Oct 15, 2021).



Katie Day - Dec 12, 2021, 2:34 PM CST

Title: Code of Federal Regulations Title 21

Date: 10/15/2021

Content by: Katie Day

Present:

Goals: To familiarize myself with the code needed to be followed for the incubator.

Content:

See attached link:

"CFR - Code of Federal Regulations Title 21," www.accessdata.fda.gov.

https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=864.2240 (accessed Oct. 15, 2021).

Notes:

Sec. 864.2240 is of most importance, speficially A where they mention the equipment codes for cell cultures.



Katie Day - Sep 23, 2021, 10:41 AM CDT

Title: Katie and Sam Initial Design Idea

Date: 9/23/2021

Content by: Katie McGovern and Sam Bardwell

Present: Katie McGovern

Goals: To present an initial design idea based on element we have both individually researched

Content:

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Conclusions/action items:

Formalize and present idea to the rest of the team



Katie Day - Oct 27, 2021, 9:06 AM CDT

Title: BME Advising Day

Date: 10/29/2021

Content by: Dr. Puccinelli

Present:

Goals: To understand how to create your design story.

Content:

- Post Graduate Planning
 - General Pointers:
 - Use undergrad experience to "build a story"
 - Gain experience
 - tie them together in the Big Picture
 - REsearch experience = important post-degrees
 - Do your homework
 - What programs are for you
 - location, people, program specifics
 - Prepare for MCAT or GRE
 - Think about letter writers early need at least 3 strong ones
 - Writing your story -- Personal Statement
 - Look for Typical (wrong) statements:
 - Legos --> engineer --> Aunt dies of Cancer --> BME PhD or Md
 - Research/Field interest: will do anything
 - I did this, then that, then the other thing
 - Better story
 - Start with what you want to do
 - e.g. Cancer stem cells and the people who do it
 - Skilled writing
 - Narrow experience and how it applies to broad interest
 - specific to each university to which you apply
 - Reasonable idea of what:
 - You will achieve at University X
 - What you want to do after
 - Defend plan with life experiences most recent first
 - CV to some extent in paragraph form -- be specific
- Grad School at UW (24 Credits)
 - Advisors
 - Bme grad chairs
 - Prof. Ludwig
 - Prof. Kreeger
 - Prof. Suarez-Gonzalez
 - BME Grad Student Services
 - Janna Pollock
 - Options
 - MS, UW BME Masters
 - Stepping stone / change directions / gain depth / expand credentials
 - Med School
 - PHD progrsms
 - Industry focuses
 - one year!
 - PHD programs
 - desire to be an independent researcher
 - write research grants
 - work in academia
 - lead projects in industry, startups, and consulting

- MS as a stepping stone: MD or PHD
 - Researons:
 - Rewrite your story
 - MD: need time to prep for MCAT and apply to Med school
 - PHD: cannot find a funded research assistant ship
 - MS will make you more desirable
 - Higher level of school
 - Fill gaps in resume
 - More experiences
 - Older, more amture
 - Really powerful if you add industry/research experience
- MS for industry oriented students
 - Opens doors
 - higher starting salary
 - Another opportunity for summer for internships
 - can co-op during MS
 - time to find dream job
- Three MS options
 - Research (1.5 2 years)
 - those continuing a phd
 - funded as RA/TA/PA
 - Thesis required
 - Accelerated Program (Accelerated -1 yr)
 - Coursework only
 - Independent study/research allowed
 - Funding RA/TA/PA not allowed
 - Biomedical Innovation, Deisng, and Entrpreneurship (Accelerated- 1 yr)
 - Project based project required
 - Partnership with Bschool
 - Funding as RA/TA/PA not permitted
- Applying
 - Apply online, pay fee, and submit
 - Special for UW BME BS studnet
 - DO not have to submit copy of transcrips
 - Easy to meet deadline of 12/15
 - Application is reviewed separately and given special consideration to BME undergrads
 - HOw to Apply
 - https://www.engr.wisc.edu/department/biomedical-engineering/academic-programs/graduate/
- UW BME One-Year
 - All MS must be 30 credits
 - UW BME BS = special
 - count up to 6 credits of advanced level bs courses to ms
 - 50% of course work must be at grad level (15 credits)
 - Advanced BME courses (400)
 - All courses 700+
 - Research (BME 799)
- Funding your Research (not permitted for accelerated programs)
- Masters Elsewhere
 - Explore opportunities and interests
 - MSEng
 - MS in Global HEalth
 - MS in other EngDept
 - MBA
 - Similar to PhD
 - Find faculty/labs performing research/work in
 - your passion area
 - area that aligns with industry interests
 - Less competive than PhD programs

Katie Day/10/29/2021 BME Advising Day

Katie Day - Dec 03, 2021, 12:23 PM CST

Title: Thermistor Code (Arduino)

Date: 11/14/2021

Content by: Katie Day and Olivia Jaekle

Present:

Goals: To create a code on Arduino that measures temperature and humidity with a thermistor.

Content:

See attached file. Calibration curve for thermistor attached below.



Conclusions/action items: Thermistor is working properly and outputs correct temperatures. Use in testing protocol next week with completed incubator prototype.

Katie Day - Nov 14, 2021, 8:28 PM CST



thermistor.ino(745 Bytes) - download



Thermistor_Circuit_Diagram.PNG(80.9 KB) - download



Katie Day - Nov 14, 2021, 8:32 PM CST

Title: DHT22 Temperature and Humidity Code

Date: 11/14/2021

Content by: Katie Day and Olivia Jaekle

Present:

Goals: To create a code on Arduino that measures temperature and humidity with a DHT22 sensor.

Content:

See attached file.

Conclusions/action items:

- 1. Thank you to Dr. Nimunkar for ordering a proper DHT22 sensor and helping us with code.
- 2. Decide between thermistor applicator or DHT22.
- 3. If going with thermistor check humidity equation with values from the DHT22.

Katie Day - Nov 14, 2021, 8:31 PM CST



DHT-22.ino(885 Bytes) - download



164 of 336

Katie Day - Nov 14, 2021, 8:37 PM CST

Title: DHT22 Sensor Library

Date: 11/14/2021

Content by: Katie McGovern

Present:

Goals: To get the DHT22 sensor to work properly.

Content:

In order for the DHT22 sensor to run properly a library of other files is needed. Attached are those files.

Conclusions/action items:

Katie Day - Nov 14, 2021, 8:38 PM CST



code-of-conduct.md(5.7 KB) - download Download and put into libraries folder in Arduino.

Katie Day - Nov 14, 2021, 8:38 PM CST



CONTRIBUTING.md(1.3 KB) - download Download and put into libraries folder in Arduino.

Katie Day - Nov 14, 2021, 8:38 PM CST

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DHT_U.h(3 KB) - download Download and put into libraries folder in Arduino.

Katie Day - Nov 14, 2021, 8:38 PM CST

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DHT.h(3.1 KB) - download Download and put into libraries folder in Arduino.

Katie Day - Nov 14, 2021, 8:38 PM CST

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Katie Day - Nov 14, 2021, 8:38 PM CST



DHTtester.ino(2.6 KB) - download Download and put into libraries folder in Arduino.



Katie Day - Nov 14, 2021, 8:45 PM CST

Title: Adafruit Sensor Library	
Date: 11/14/2021	
Content by: Katie McGovern	
Present:	
Goals:	
Content:	
In order for the DHT22 sensor library to work, the adafruit sensor library is needed. It is attached.	
Conclusions/action items:	
	Katie Day - Nov 14 2021 8:45 PM CST
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Adafruit_Sensor.cpp(2.3 KB) - download Download and add to libraries folder in Arduino.

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Adafruit_Sensor.h(7.6 KB) - download Download and add to libraries folder in Arduino.

Katie Day - Nov 14, 2021, 8:45 PM CST



library.properties(380 Bytes) - download Download and add to libraries folder in Arduino.

Katie Day - Nov 14, 2021, 8:45 PM CST

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Katie Day - Nov 14, 2021, 8:45 PM CST



README.md(10.1 KB) - download Download and add to libraries folder in Arduino.

Katie Day - Nov 14, 2021, 8:45 PM CST



sensortest.ino(4.1 KB) - download Download and add to libraries folder in Arduino.



Katie Day - Nov 14, 2021, 8:48 PM CST

Title: MH-Z16 NDIR CO2 Monitoring Code

Date: 11/14/2021

Content by: Katie McGovern

Present:

Goals: To create a code in Arduino that allows the MH-Z16 NDIR CO2 monitor to work.

Content:

See attached file.

Conclusions/action items: Test the CO2 sensor using the testing protocols created by Maya and Caroline. Figure out a way to convert ppm to percentage.

Katie Day - Dec 03, 2021, 12:25 PM CST



ReadConcentration.ino(888 Bytes) - download

Katie Day - Dec 03, 2021, 12:25 PM CST



MH_Z16_Circuit_Diagram.PNG(151.2 KB) - download



Katie Day - Dec 07, 2021, 7:43 PM CST

Title: Thermistor Testing
Date: 12/3/2021
Content by: Katie, Olivia, Maya, and Caroline
Present: Katie and Olivia
Goals: To test the accuracy of our thermistor against an incubator.
Content:
Testing protocol written by Maya and Caroline and performed by Olivia and me. Results are below.

Conclusions/action items:

Thermistor is working properly and ready for implementation.

Katie Day - Dec 03, 2021, 12:28 PM CST

Misty_In_Incubator_10-min.PNG(15 KB) - download



Katie Day - Dec 07, 2021, 7:42 PM CST

Title: CO2 Testing
Date: 12/3/2021
Content by: Katie, Olivia, Maya, and Caroline
Present: Katie and Olivia
Goals: To test the CO2 sensor to make sure that it is working properly.
Content:

Attached our the results of our testing, testing protocols written by Maya and Caroline, performed by Olivia and me.

Conclusions/action items:

The CO2 sensor is ready for incorporation into the incubator.

Katie Day - Dec 03, 2021, 3:22 PM CST



concentration.csv(2.4 KB) - download

Katie Day - Dec 07, 2021, 7:42 PM CST



concentration_graphs.csv(2.3 KB) - download

Katie Day - Dec 07, 2021, 7:47 PM CST



Katie Day - Dec 07, 2021, 7:48 PM CST



Misty_Humidity_Data.csv(1.5 KB) - download

Katie Day - Dec 07, 2021, 7:48 PM CST



Combined_Humidity_Data.csv(4.1 KB) - download



Combined_Humidity_Data.txt(2 KB) - download

Katie Day - Dec 07, 2021, 7:48 PM CST



DHT22_Humidity_Data.csv(441 Bytes) - download





Katie Day - Dec 07, 2021, 7:37 PM CST

Title: Group Testing Protocols

Date: 12/07/2021

Content by: Maya Tanna and Caroline Craig

Present: Katie McGovern and Olivia Jaekle

Goals: To create testing protocols and verify that the elements of our design are working as expected, accurately, and precisely.

Content: The Testing Protocols and the parts of the protocol that were able to be evaluated during the semester.

Conclusions/action items:

The temperature, humidity, CO2, and optics are all working as expected.

12/07/2021 Group Testing Protocols



Group_Testing_Protocols.pdf(90.5 KB) - download



Katie Day - Dec 07, 2021, 7:57 PM CST

Title: Incubator Fabcrication

Date: 12/07/2021

Content by: Katie McGovern

Present: Katie McGovern and Sam Bardwell

Goals: To fabricate the incubator.

Content:

The box was fabricated by first drilling 3/8 inch diameter holes in the front of the box and then using a circular file to expand them so that the barbed connectors could fit in the incubator. They were then hot glued. The glass was hot glued onto the small divot made for them in the design. A 1/4 inch hole was drilled on the bottom right corner for the thermistor and filed with a circular file. A 1/2 inch hole was drilled and expanded via circular file for the CO2 sensor to fit in. The CO2 sensor and the thermistor were hot glued into place. The 3/8x1/4 inch tubing was wrapped in a circular fashion along the interior of the box and connected to the barbed vacuum connectors. They were then secured by zip ties. They were connected to a 1/2x3/8 inch tubing that was secured via zip ties to both the connector and the hot water pump. Then roughly 16 oz of water was poured into the incubator.

Conclusions/action items:

The PLA material needs to be changed as it was difficult to drill into, very brittle, and appeared to be leaking in random places.



IMG_5896.jpg(761.9 KB) - download



IMG_5894.jpg(1.1 MB) - download



IMG_5893.jpg(1.1 MB) - download

Katie Day - Dec 07, 2021, 7:52 PM CST



IMG_5892.jpg(582.6 KB) - download



IMG_5891.jpg(854.1 KB) - download

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IMG_5890.jpg(394.4 KB) - download



IMG_5889.jpg(1.2 MB) - download

Katie Day - Dec 07, 2021, 7:52 PM CST



IMG_5888.jpg(761.9 KB) - download



IMG_5895.jpg(676.6 KB) - download


12/07/2021 Attempted Incubator Testing

Katie Day - Dec 07, 2021, 8:00 PM CST

Title: Attempted Incubator Testing

Date: 12/07/2021

Content by: Katie McGovern and Sam Bardwell

Present: Katie McGovern and Sam Bardwell

Goals: To initially determine whether or not our incubator was working as expected.

Content: Data collected during testing.

Conclusions/action items:

- 1. Polyethelene tubing acted more as an insulator than a conductor and would not heat up the water bath to the desired temperature. Need to use a metal tube.
- 2. PLA box was leaking slightly. It is unclear where or how it is leaking as it has been sealed via hot glue and zipties.
- 3. Glass did fog up after about 30 minutes so we will need to figure out how to demist the glass.

Katie Day - Dec 07, 2021, 8:01 PM CST



Incubator_Temp_Over_Time.csv(5 KB) - download







Katie Day - Dec 07, 2021, 8:01 PM CST



Incubator_Temp_Hum_Over_Time.csv(5 KB) - download

Katie Day - Dec 07, 2021, 8:01 PM CST



Actual_Inc_HUm_Data.csv(2.1 KB) - download

09/12/2021 Live-Cell Analysis Within Incubators

MAYA TANNA - Dec 11, 2021, 11:06 PM CST

Title: Live-Cell Analysis Within Incubators

Date: 09/12/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To learn more about live-cell analysis within incubators to gain some initial research on the project

Content:

One of the benefits of live-cell analysis is the ability to keep tabs on the health of cell cultures. CytoSMART offers the Lux2 for online cell culture video monitoring, and the Omni for high-throughput live-cell imaging. The CytoSMART Lux2 is a single position live-cell imager that is commonly used for assessing cell proliferation, morphology, and migration, and evaluating angiogenesis and spheroid-based model systems. The Lux2 generates time-lapse videos that can be accessed on a local PC or remotely via an online environment, preventing unwanted disturbances to the samples.

The compact CytoSMART Lux2 fits inside most standard CO_2 incubators and hypoxia chambers. Its integrated confluence detection algorithm helps researchers to time subculturing more precisely, which is important for setting up reproducible assays and preventing undesirable effects that arise from using over-confluent cultures, such as differentiation and gene expression changes. "Users can set confluency targets and receive notifications when the target is reached," says Joffry Maltha, CEO at CytoSMART.

The CytoSMART Omni, while larger than the Lux2, fits within 150 L CO₂ incubators, and also stores results online for easy access. The CytoSMART Omni can image a wide range of tissue culture vessels and provides whole-well data for multi-well culture plates. "Users can generate time-lapses, which can be analyzed by their own image analysis algorithms, or they can choose from several label-free assays provided by CytoSMART, such as proliferation, wound healing, and colony analysis," says Maltha. For example, at the Heidelberg Institute for Stem Cell Technology and Experimental Medicine, the research group headed by Martin Sprick uses the CytoSMART Omni to investigate the proliferation of pancreatic cancer cell lines, with an aim to overcome drug resistance and improve treatment efficiency of pancreatic cancer subtypes, according to Maltha.

Conclusions/action items: Do more research on live-cell analysis within incubators and start brainstorming project solutions for the client.

Citations: [2]2021. [Online]. Available: https://www.biocompare.com/Editorial-Articles/562305-Live-Cell-Analysis-within-Incubators/. [Accessed: 13- Sep- 2021].



09/18/2021 Minimum Essential Media (MEM) - Earle's Salts

MAYA TANNA - Dec 11, 2021, 11:07 PM CST

Title: Minimum Essential Media (MEM) - Earle's Salts

Date: 09/18/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To learn more about minimum essential media (MEM) and its properties/use

Content:

MEM-Eagle Minimum Essential Medium or Minimal Essential Medium is simply a modification BME containing higher concentrations of essential nutrients. MEM has been utilized for the cultivation of a wide array of cells grown in monolayers. The optional supplementation of non-essential Amino Acids (NEAA) to the formulations that incorporate either Hank's or Earle's Salts has broadened the usefulness of this medium. We offer a variety of MEM modifications for a range of cell culture applications.

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Conclusions/action items: Present findings to team and work to find a solution that involves MEM. Learn more about the biological properties of MEM - relevant temperature properties and humidity.

Citation: E. MEM-Eagle, "Minimum Essential Media (MEM) Eagle - Mammalian Cell Culture", *Bioind.com*, 2021. [Online]. Available: https://www.bioind.com/mem-eagle-earle-s-salts/. [Accessed: 18- Sep- 2021].

MAYA TANNA - Sep 18, 2021, 11:35 AM CDT

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MAYA TANNA - Dec 11, 2021, 11:08 PM CST

Title: Minimum Essential Media (MEM) - Suspension Cultures

Date: 09/18/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To learn more about minimum essential media (MEM) and its properties/use with respect to cell culture media

Content:

Minimum Essential Medium (MEM) is one of the most commonly used of all cell culture media. MEM can be used with a variety of suspension and adherent mammalian cells, including HeLa, BHK-21, 293, HEP-2, HT-1080, MCF-7, fibroblasts, and primary rat astrocytes.

Sectio	n 9 - Physical/Chemical Proper	ties	
9.1	Appearance:	Clear Red Solution.	
9.2	Odor:	Odorless.	
9.3	Physical State:	Liquid.	
9.4	pH:	7.1 - 7.6	
9.5	Boiling Point:	~ 100°C	
9.6	Melting Point:	Not Applicable.	
9.7	Freezing Point:	~ 0°C	
9.8	Vapor Pressure:	Not Available.	
9.9	Vapor Density:	Not Available.	
9.10	Specific Gravity:	Not Available.	
9.11	Evaporation Rate:	Not Available.	

Formulation of MEM

Conclusions/action items: Present findings to team and use this information to make informed decisions about the microscope cell culture incubator.

Citation: 2021. [Online]. Available: https://www.bioind.com/mem-for-suspension-cultures/. [Accessed: 18- Sep- 2021].

MAYA TANNA - Sep 18, 2021, 11:44 AM CDT

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mem_-_cell_culture.pdf(129.6 KB) - download

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09/25/2021 Microplate Dimensions

MAYA TANNA - Sep 25, 2021, 9:53 AM CDT

Title: Microplate Dimensions

Date: 09/25/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To document research done on microplate/well plate dimensions (specifically for 24-well and 96-well) as well as compare/contrast between different brands

Content:



Citation:

- ["Microplate Dimensions, Working Volumes and Packaging | Application Support Knowledgebase | Lab Products &
- 1 Services," PerkinElmer. https://www.perkinelmer.com/lab-products-and-services/application-support-
-] knowledgebase/microplates/plate-dimensions.html (accessed Sep. 25, 2021).

Conclusions/action items: Figure out which brand is preferred and use the well plate dimensions to 3D model on SolidWorks (and potentially 3D print) as well as use for cell culture incubator in general.



MAYA TANNA - Dec 11, 2021, 11:09 PM CST

Title: Microplate Dimensions Date: 10/10/2021 Content by: Maya Tanna Present: Maya Tanna Goals: To document research done on autoclave sterilization Content:

An autoclave is used in medical and laboratory settings to sterilize lab equipment and waste. Autoclave sterilization works by using heat to kill microorganisms such as bacteria and spores. The heat is delivered by pressurized steam. Pressurization allows the steam to reach the high temperatures that are required for sterilization.

According to Centers for Disease Control (CDC) Guidelines for Disinfection and Sterilization of Healthcare Facilities, pressurized steam is the most widely used and dependable method of sterilization. It's nontoxic and inexpensive, it kills microbes and spores rapidly, and it quickly heats and penetrates fabrics.

According to manufacturer Tuttnauer, medical clinics and dental offices typically use tabletop autoclaves, which are about the size of a microwave oven, while hospitals use much larger units that can sterilize many instruments at once.

To work effectively, an autoclave must remove all the air in and around the object that's being sterilized, forcing steam to penetrate its

surfaces, according to Healthcare Purchasing News. There are two basic ways that an autoclave can remove the air and force in steam:

- Gravity displacement autoclaves, also called gravity autoclaves, inject steam into the autoclave chamber and then rely on that steam, which is heavier than air, to force the air to leave the chamber through the drain vent at the bottom, according to the CDC.
- Prevacuum or prevac autoclaves use a vacuum pump to remove air from the chamber before steam is admitted to it, which
 means that steam penetrates even porous objects almost instantly.

What is the temperature for autoclave sterilization?

The most common temperature for autoclave sterilization is 121°C, but many autoclaves allow cycles at higher temperatures, such as

132°C and 134°C.

Citation:

[1]

"How Does Autoclave Sterilization Work? - Grainger KnowHow." https://www.grainger.com/know-how/equipment-information/kh-howdoes-autoclave-sterilization-work.html (accessed Oct. 10, 2021).

Conclusions/action items: Use this information when considering feasible of design solutions.

09/11/2021 Open-Dish Incubator for Live Cell Imaging

MAYA TANNA - Dec 11, 2021, 11:10 PM CST

Title: Open-Dish Incubator for Live Cell Imaging

Date: 09/11/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To do some preliminary research on open-dish incubators and learn more about the project as a whole

Content:

Thermocouples, heating elements, and incubators come in a bewildering variety of types from numerous manufacturers. For these specialized temperature-control components, we ascribe some importance to the particular parts specified here, insofar as other similar parts did not work as well. The incubator is a Fuji Electric PXV3-TAY2-4V fuzzy-logic controller (Fuji Electric, Tokyo, Japan), which can be obtained in versions to accept various line volt-ages for worldwide use. This device is widely available from sales firms specializing in process control.



Figure 2. Drawings of the custom machined metal parts (all dimensions are in inches).

For temperature control of a nonstirred culture dish (the normal state of af-fairs), this characteristic time lag, $\tau(s)$, is related to geometry and material by the following thermal diffusion equation: $\tau = r2/\pi\alpha[Eq. 1]$ where r is the distance between the heat source and the thermocouple, $\pi = 3.14$, and α is the thermal diffusivity of the heated material [α (cm2/s) = thermal conductivity (cal/[cm][s]°C) di-vided by heat capacity (cal/cm3°C)].

Conclusions/action items: Continue doing research on microscope cell incubators and start brainstorming ideas for solutions. Also, have a meeting with the client and start drafting the product design specifications after that.

Citation: [1]"Open-dish incubator for live cell imaging with an inverted microscope | BioTechniques", *BioTechniques*, 2021. [Online]. Available: https://www.future-science.com/doi/abs/10.2144/03354bi01?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed. [Accessed: 11- Sep- 2021].

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Open-dish incubator for live cell imaging with an inverted microscope

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09/25/2021 CO2 Incubators

MAYA TANNA - Dec 11, 2021, 11:10 PM CST

Title: CO2 Incubator

Date: 09/25/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To learn more about CO2 incubators and the different models of them at Thermo Fisher Scientific

Content:



Choose between a direct heat or a water jacketed chamber that can be configured with CO2 or CO2/O2 control.

Direct heat CO₂ incubators



Water jacketed CO₂ incubators



Efficient high-performance heaters located on every chamber surface provide even temperature distribution throughout the entire chamber. Choose the lightweight convenience of direct heat technology with available high temperature sterilization for easy cleaning.

All direct heat models

Unique triple wall construction provides outstanding temperature stability supplied by dual layers of water and high-quality insulation. Choose the added security of a water jacketed chamber for temperature stability and protection against unexpected power outages.

All water jacketed models



Key features to help you achieve your next discovery

Based on 60 years of engineering and design breakthroughs, our expertise is represented in key features that span our portfolio. Together these enhancements provide optimal cell growth, valuable contamination control technologies, and advanced design for highly critical applications, plus simplicity which allows you to spend more time pursuing your goals.

Sterilization cycle	
HEPA filtration (ISO Class 5)	
Active airflow technology	
► CO2 sensors	
O2 control	
Humidity reservoirs	
User Interfaces	

Citation:

"CO2 Incubators - US." //www.thermofisher.com/us/en/home/life-science/lab-equipment/co2-incubators.html (accessed Sep. 25, 2021).

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Conclusions/action items: Look into properties of stainless steel, copper, variable oxygen control, and a cell locker system. Also, look into sensors and how to regulate temperature, CO2, and humidity levels.



10/10/2021 Thermo Scientific Incubator Maintenance

MAYA TANNA - Oct 10, 2021, 11:34 AM CDT

Title: Thermo Scientific Incubator Maintenance

Date: 10/10/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To learn more about incubator maintenance to preserve shelf life/life in service and learn how to calibrate sensors used in the design

Content:

See attachment below.

Conclusions/action items: Share info with team and determine sensor logistics from there.

MAYA TANNA - Oct 10, 2021, 11:34 AM CDT



TNCO2CAREFEED-EN.pdf(2 MB) - download



11/08/2021 CO2 Sensor Waterproofing

MAYA TANNA - Dec 11, 2021, 9:45 PM CST

Title: CO2 Sensor Waterproofing

Date: 11/08/2021

Content by: Maya

Present: Maya and Caroline

Goals: To document the specifications of a waterproof sleeve that could be used for the CO2 sensors

Content:

Specifications are shown in the attachment below.

Conclusions/action items: This could be feasible next semester, when we do more work with the CO2 sensor. At this point in time, I am unsure if this is necessary or not.

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MAYA TANNA - Dec 11, 2021, 9:20 PM CST

Title: Copper Specifications

Date: 10/27/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To document research done on copper rust specifications, specifically how long copper would last if we used that material and if it would be feasible

Content:

Important information from this article

- 1. Properties of Copper
 - 1. Tough, ductile, and malleable
 - 2. Good material for tubing and wires
 - 3. Good heat and electrical conductivity
 - 4. Good corrosion resistance this is useful because our final device has to function 1 week each semester and keep this going for a minimum of 10 years
 - 5. Resist corrosion via fresh water/steam (will be useful for our heated water pump)
 - 6. Resistant to saline solutions, soils, organic acids, etc.

Citation:

[S. B. Says, "Copper - Specifications, Properties, Classifications and Classes," AZoM.com, May 17, 2005. https://www.azom.com/article.aspx?

1 <u>ArticleID=2856</u> (accessed Oct. 27, 2021).

]

Conclusions: Copper could be feasible for tubing because it has high corrosion resistance, good heat conductivity, and is relatively cheap. This would be a very viable option if we need better conductive tubing and don't want to stick with the plastic tubing next semester.



10/27/2021 Copper Corrosion

Title: Copper Corrosion

Date: 10/27/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To document research done on copper corrosion specifications, specifically how long it takes for copper to corrode and the causes of this

Content:

The helpful information from this article on copper was information about rust. According to the article, copper doesn't rust only iron rusts. However, copper can corrode - it's naturally brown but it turns bright green as it corrodes. There is dispute over whether copper's decay over time is tarnish or oxidation. Using copper for tubing would be helpful for the project because copper takes a long time to corrode - up to 20 years. This is pretty good for life in service requirements of the device.

Potentially problematic case: Corrosion that breaks copper tubing is erosion corrosion - only occurs because of exposure to flowing water over a long period of time.

Citation:

["What Metals Rust? | Do Iron & Copper Rust?," *Tampa Steel & Supply*, Jan. 19, 2015. <u>https://tampasteel.com/which-metals-</u> 1 <u>rust/</u> (accessed Oct. 27, 2021).

]

Conclusions: Copper would be a great material for tubing throughout the system if the PVC tubing doesn't work out or causes too much leakage. Only concern is that if water is flowing through the incubator for an extended period of time this could be an issue and cause the copper tubing to decay - we may need to switch out the water in the incubator from time to time to prevent this from happening.



MAYA TANNA - Dec 11, 2021, 9:36 PM CST

Title: Flexible PVC Tubing

Date: 11/03/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To document some specifications on clear PVC tubing from Freelin-Wade

Content:

- PVC Tubing from Freelin-Wade
 - Temperature range: -30 to 150 degrees F (converts to -34 degrees C to 66 C)
 - Hardness: 80A
 - 3:1 factor of safety (very safe tubing to use)
 - Compliant with all necessary guidelines (FDA, USPVI, RoHS, REACH, CA Prop 65)
 - Lots of different packaging options
 - Made in the USA
 - Tubing resists bacterial growth
- They do have 1/2 x 3/8 tubing.

They do have 1/2 x 3/8 tubing. Prices and additional information on specifications is attached.

• Item Number: PVC-705 for exact dimensions

Citation:

["Medical & PVC Tubing - PVC-705 Clear Tubing." <u>https://www.freelin-wade.com/pvc-tubing/clear/705#1</u> (accessed Nov. 03, 2021).

]

Conclusions: Clear and flexible PVC tubing is a very feasible option for our incubator, because the tube fits the right dimensions (1/2 x 3/8) and it has feasible pressure and temperature range specifications. Also, it is very durable and will not contaminate over time whereas, metal may corrode or rust if used in tubing for an extended period of time. There is also a braided option for PVC tubing that is reinforced if we want to achieve higher pressures but I don't think this will be necessary.

MAYA TANNA - Nov 03, 2021, 11:22 AM CDT



Flexible_PVC.pdf(272.1 KB) - download



MAYA TANNA - Dec 11, 2021, 9:42 PM CST

Title: Clear Vinyl Tubing

Date: 11/03/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To document some specifications on clear vinyl tubing from Curbell Plastics

Content:

- Vinyl tubing properties
 - Depending on which specific brand of vinyl tubing, the typical operating temperature range is -10 to 175 degrees Fahrenheit (this is good because we are aiming for 37 degrees C = about 100 degrees F)
 - The color of the tubing is clear this is helpful because then hopefully we could visualize any contamination or leakage within the tubing system
 - Slight odor
 - Specific gravity/volume is around 1.2 g/cm3
 - Very high tensile strength around 2400 psi
 - Good for withstanding different conditions
 - Tube elongation is around 400%

Citation:

- ["Clear Vinyl Tubing | Flexible, General Purpose | Curbell Plastics." https://www.curbellplastics.com/Research-Solutions/Specialty-
- 1 <u>Products/Tubing-and-Hose/Vinyl-Tubing</u> (accessed Nov. 03, 2021).

]

Conclusions: Clear vinyl tubing/flexible plastic tubing is a highly feasible option for tubing within our incubator system and we will be using this moving forward, because it fits all the design specifications.



MAYA TANNA - Sep 11, 2021, 12:45 PM CDT

Title: Client Question Brainstorming

Date: 09/11/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To brainstorm questions to ask the client, Dr. Puccinelli, at the first client meeting next week and gain clarification about the project expectations/goals

Content:

- 1. What is the budget for this project?
- 2. What is our margin of error in regards to temperature, CO2 levels, and humidity?
- 3. Is there a size constraint for the incubation chamber?
- 4. Could we test our design with live cells?
- 5. What are the most important design requirements (apart from the temperature, CO₂, and humidity level measurements provided)?

Conclusions/action items: Add to this list of questions, schedule first client meeting with Dr. Puccinelli, and do some initial research on microscope cell culture incubators.



MAYA TANNA - Sep 20, 2021, 5:29 PM CDT

Title: Client Questions/Answers

Date: 09/15/2021 Content by: Maya Tanna Present: Maya Tanna Goals: To document questions/answers received from the client Content:

Questions for Dr. Puccinelli

Overview of the Project:

Experimental Teaching Lab \rightarrow Tissue engineering lab needs culture cells for the long term (*what is long term*?) that doesn't have a lot of money. Looking for a smaller, less expensive, and less bulky incubator that doesn't encompass the whole microscope or can be removed. Stage-top cell culture incubator. Grow cells and watch them over the course of time. Have to be able to stay alive with cell culture conditions for at least a week.

1. What is the budget for this project? **\$100**

a. Will this project be paid for using UW Funds? Departmental teaching funds

2. What is the device being used for, industry, research, etc?

a. Used for teaching purposes, but if we get it right we can market this to other researchers

3. What is our margin of error in regards to temperature, CO2 levels, and humidity?

a. 37*C \rightarrow look at industry standard for temp ranges

b. 5% CO2 → helps with buffering from sodium bicarbonate

4. Is there a size constraint for the incubation chamber?

a. Has to sit on microscope stage and hold a well plate that also doesn't interfere with the optics

(ideal if both sides are transparent, but bottom must be transparent)

b. Needs to work with inverted microscope

5. What are your preferred dimensions for the incubation chamber?

a. Sits on microscope stage and holds well plate

6. When you imagine the finished product, what color would you want it to be?

a. No preference in color

b. Well plates are clear, black (stops contamination), and white (increases light).

c. Something that blocks out external light would be ideal, but is not required

7. Could we test our design with live cells?

a. Yes, Dr. P will give us some when/if we are ready

b. Use cells that are hard to kill \rightarrow that's good for us

c. TELL HIM IF WE WANT THEM AFTER THANKSGIVING

8. What are the most important design requirements/specifications (apart from the temperature, CO₂, and humidity level measurements provided)?

a. Optical transparence, microscope stage (google that)

9. How many devices should be created?

a. Just one :)

10. Are there any materials that you prefer we use?

Maya Tanna/Design Process/09/15/2021 Client Questions/Answers

a. Nope :)

- 11. How long will this device be used in the lab?
 - a. Could be used up to two weeks, but shoot towards one week at a time.

12. How often do you plan on using this device daily?

a. Device would be used for one week at a time during tissue lab

13. What is the shelf life of this product?

a. Long time \rightarrow 10 years

14. What has been working well for previous projects? What hasn't?

a. Seal insulated box completely?

b. Sterilization is very important $\,\rightarrow\,$ autoclaving ideal but UV works too

15. Anything particular you would like us to continue with from past projects?

a. Temperature gradients are a large problem for cell cultures (reason for bulky products) look

towards first project insulated box

- 16. What types of cell culture plates do you use?
 - a. What are their dimensions?

i. <u>6 Well plate, 24 well plate, 90 well plate</u> → omnitrays?

ii. Standard petri dish

iii. Flasks \rightarrow T25/T75 not really used but her

b. What type of medium do you use?

i. MEM

ii. 10% SPS and antibiotics

17. Will any other microscopes be used with this incubation chamber? Or, should it only be compatible with the inverted microscope?

Mainly inverted microscope

18. Should this device be ergonomic(able to move it on your own)?

a. Be able to carry it around and store it

- b. Wires should not be hanging out freely
- c. Easy to pick up and put away

Notes:

- CO2 humidifiers and such are done using wires and a breadboard
- No team has successfully created an incubator.
- Something that can be easily taken apart would be ideal
- Temp gradients with small amounts of liquid can be evaporated very quickly so humidity is a big issue

Conclusions/action items: Use these answers to draft PDS and carry on with future research on design ideas.



MAYA TANNA - Sep 18, 2021, 12:40 PM CDT

Title: Product Design Specifications Draft

Date: 09/18/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To work on certain sections of the product design specifications document due next Friday

Content:

Function: Develop a low cost cell culture incubation chamber with interchangeable culture plates that is compatible with an inverted microscope and capable of live cell imaging.

Client requirements:

- Incubation chamber must be able to maintain an internal environment of 37 C, 5% CO₂, and 95-100% humidity
- · Microscope's optics and functionality must not be damaged
- · Maintain even heating and humidity across the chamber
- Create device that stays within a budget of \$100
- · Ensure that the device can be easily assembled and removed between uses

Design requirements:

1. Physical and Operational Characteristics

- a. Performance requirements:
- **b.** Safety: The incubator and the cell culture environment must be in corporation with BioSafety Level 1 Standards. Any material and electrical or mechanical machinery must be sterilizable and waterproof.
- c. Accuracy and Reliability:
- *d. Life in Service:* The device could be used in the lab for up to two weeks, but the device would be used for one week at a time during the tissue lab.
- e. Shelf Life: The shelf life of this product should be about ten years.
- *f. Operating Environment:* The operating environment is a clean room. The incubation chamber must be able to maintain an internal environment of 37 C, 5% CO₂, and 95-100% humidity over a long duration of time, without compromising the integrity of the microscope's optics or functionality. Even heating and humidity across the chamber must be maintained to ensure that evaporation does not occur.
- *g. Ergonomics:* The device should be portable in that one should be able to carry it around and store it easily. Wires should not be hanging freely out of the device, and it should be easy to pick up and put away when needed.
- *h. Size:* The size constraints for this device are that it must sit on the microscope stage and hold a well plate that also doesn't interfere with the optics or functionality of the microscope. It would be ideal if both sides were transparent, but it is a requirement that the bottom is transparent. Overall, the product must be compatible with an inverted microscope.
- *i. Weight:* There are no specific weight requirements. However, minimizing weight would be ideal to promote incubator mobility and usability.
- *j. Materials:* There are no specific materials that are required for development of this device. However, it is important to examine different material properties to determine which materials hold heat effectively and have a transparent appearance.
- **k.** *Aesthetics, Appearance, and Finish:* The client does not have a preference in color. Well plates are clear, black (to stop contamination), and white (to increase light). Using materials that would block out external light sources would be ideal, but this is not a requirement for the device.

2. Production Characteristics:

- **a.** *Quantity:* Only one device is necessary to produce, but ideally, it would have the capacity to be produced on a larger scale to be used repeatedly in the teaching labs.
- *b. Target Product Cost:* The target product cost for this device is \$100. It will be paid for via UW BME departmental teaching funds.

Conclusions/action items: Finish this document with the rest of the team and submit by next Friday. Clarify any additional questions on design requirements with client if necessary. Use these specifications to start brainstorming potential solutions.



MAYA TANNA - Sep 25, 2021, 9:37 AM CDT

Title: Future Questions for Client

Date: 09/24/2021

Content by: Maya Tanna

Present: Katie Mcgovern, Sam Bardwell, Maya Tanna, Caroline Craig, Olivia Jaekle, Ethan Hannon

Goals: To document additional questions the team had after our meeting with Dr. Kinney that we can ask the client

Content:

- 1. What is the exact model of inverted microscope for use? (for accurate dimensions)
- 2. Could we use a laboratory CO2 gas line? Or, will an external CO2 gas supply be necessary to include in materials?
 - a. Tank with a regulator, hose into incubator
 - b. Don't need to purchase, readily available with hoses
 - i. What is the diameter of the hose?
- 3. How many petri dishes do you need in the incubator?
- 4. Would it be possible for us to test transparent materials with the microscope?
 - a. Optically clear enough?
 - b. Refraction of light?
 - c. Bottom of glass on multiwell plates.. Look into
- 5. What is the use of the incubator during the week of class time?
 - a. A few hours a day, or use over the whole week?
- 6. Do you have any specifications in the margins from industry standard? Or, is the tolerance cells can handle acceptable?

a. pH levels \rightarrow CO2 levels, what is tolerance for a buffer?

- 7. What are the dimensions of the well plates? (Can look up online)
- 8. What would be the ideal recovery time for internal conditions be after opening the cell culture incubator "door"? (Flow rates)
- 9. Would you prefer manual CO2 addition, or an automatic regulation with sensors?
- 10. Is the budget for the final design, or does it include materials for preliminary designs?

Conclusions/action items: Set up a meeting to meet the client in-person and look at all the equipment that would be used in conjunction with the device as well as get these questions answered to further provide clarity on certain aspects of the project.



MAYA TANNA - Oct 10, 2021, 9:53 AM CDT

Title: Design Idea - Past Project Refurbished

Date: 09/27/2021

Content by: Maya Tanna

Present: Katie Mcgovern, Sam Bardwell, Maya Tanna, Caroline Craig, Olivia Jaekle, Ethan Hannon

Goals: To document a preliminary design idea that improves a past BME design project

Content:

This semester is the sixth semester a group has worked on this project for the client, however the image below displays a past project that may work with more alterations and/or improvements.. No group has been successful at fabricating a fully functional microscope cell culture incubator. For this reason, continuing work on these designs to further test the product, improve materials, and fix coding errors regarding the sensors was a realistic option. Every previous design involved a rectangular box for the incubation chamber. The design also included a glass top that minimized optical impairment and was able to go through sterilizations while extruding less heat loss. The bottom part of the chamber had a transparent heating element. The CO2 input tub was linked on both sides of the chamber. The disadvantages of this design are finding quality materials that could keep CO₂ levels and temperatures constant while being on a low-cost budget.



Conclusions/action items: Evaluate this design in the design matrix and include in the preliminary report. Look into how to improve various aspects of the circuit design as well as coding errors.

207 of 336



Spring_2017_BME_402_MicroscopeCellCultureIncubator.pdf(1.6 MB) - download



10/04/2021 Materials Brainstorming

MAYA TANNA - Oct 10, 2021, 9:24 AM CDT

Title: Materials Brainstorming

Date: 10/04/2021

Content by: Maya Tanna

Present: Katie McGovern, Sam Bardwell, Maya Tanna, Caroline Craig, Ethan Hannon, Olivia Jaekle

Goals: To identify requirements for the biomaterials as well as general brainstorming for the materials.

Content:

See attachment below.

Conclusions/action items:

Further research materials and start ordering.

MAYA TANNA - Oct 10, 2021, 9:24 AM CDT



Materials_and_Heating_Brainstorm.docx(598.1 KB) - download



10/10/2021 Preliminary Report Draft

MAYA TANNA - Oct 10, 2021, 10:06 AM CDT

Title: Preliminary Report Draft

Date: 10/10/2021

Content by: Maya Tanna

Present: Katie McGovern, Sam Bardwell, Maya Tanna, Caroline Craig, Ethan Hannon, Olivia Jaekle

Goals: To document the first draft of the preliminary report

Content:

See attachment below.

Conclusions/action items:

Further continue updating the preliminary report and add necessary information to make it specific.

MAYA TANNA - Oct 10, 2021, 10:07 AM CDT



Preliminary_Report-_Microscopic_Cell_Incubator.docx(1.1 MB) - download



MAYA TANNA - Dec 11, 2021, 9:47 PM CST

Title: Materials Purchasing Request

Date: 10/18/2021

Content by: Maya Tanna, Katie Day, Caroline Craig

Present: Katie Day, Sam Bardwell, Maya Tanna, Caroline Craig, Ethan Hannon, Olivia Jaekle

Goals: To compile links to purchasing materials and document for Dr. Puccinelli

Content:

See attachment below.

Conclusions/action items:

Further research materials, look for cheaper options that still accomplish the intended goal, and get approval from Dr. Puccinelli for purchasing. Order materials.

MAYA TANNA - Oct 19, 2021, 4:39 PM CDT

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MAYA TANNA - Dec 11, 2021, 10:59 PM CST

Title: Ace Hardware Visit

Date: 10/28/2021

Content by: Maya Tanna and Sam Bardwell

Present: Katie Day, Sam Bardwell, Maya Tanna, Caroline Craig, Ethan Hannon, Olivia Jaekle

Goals: To describe recent progress and Ace Hardware visit with Sam

Content:

Went to Ace Hardware with Sam to look at dimensions and specifications of parts for the project, specifically for the tubing and adaptor aspects. (They had the dimensions we were looking $1/2 \times 3/8$ for tubing)

The guy working there recommended rubber tubing, but that wouldn't give us great visualization if there was any contamination or leakage or anything.

Finished the initial draft of testing protocols with Caroline (includes temperature sensor, humidity sensor, optical, and recovery testing). Did research on properties of copper and copper corrosion specifics to determine if this could be feasible for tubing.

Conclusions/action items: Send test protocols to Dr. Kinney and gain feedback on how to improve them as well as hopefully help with ordering materials (pending Dr. P's approval). Help get everything together for Show and Tell on Friday.



MAYA TANNA - Oct 27, 2021, 11:23 AM CDT

Title: Initial Testing Protocol Progress

Date: 10/27/2021

Content by: Maya Tanna

Present: Maya Tanna and Caroline Craig

Goals: To create testing protocols to evaluate the whole system as well as individual components once the prototype is built

Content:

See attachment below.

Conclusions/action items:

Further edit test protocols based on advice from Dr. Kinney and make them more specific once prototype and sensors are closer to being done.

MAYA TANNA - Oct 27, 2021, 11:24 AM CDT

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11/17/2021 Testing Protocols Draft 2

MAYA TANNA - Nov 17, 2021, 7:30 PM CST

Title: Testing Protocols Draft 2

Date: 11/17/2021

Content by: Maya/Caroline

Goals: To document the second draft of the testing protocols, which were edited based on the team and advisor's feedback

Content:

See attachment below.

Conclusions/action items: Add any last edits to the testing protocols, specifically for the CO2 protocols and execute testing as components of the project are finalized

MAYA TANNA - Nov 17, 2021, 7:31 PM CST

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Testing_Protocols_Template_.docx(585.1 KB) - download



MAYA TANNA - Nov 25, 2021, 2:47 PM CST

Title: Testing Protocols Final Version

Date: 11/25/2021

Content by: Maya/Caroline

Goals: To document the final draft of the testing protocols, which were edited based on the team and advisor's feedback

Content:

See attachment below.

Conclusions/action items: Execute testing wherever possible and investigate CO2 component of the project.

MAYA TANNA - Nov 25, 2021, 2:47 PM CST

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MAYA TANNA - Dec 11, 2021, 10:18 PM CST

Title: ImageJ Focus Quality Research

Date: 12/05/2021

Content by: Maya

Goals: To document research done on the plug-in I need to analyze the two microscope images on ImageJ

Content:

Information Learned from the websites below

- Process for analyzing the images
 - Need to enable the TensorFlow update site by following the directions in the links below
 - · Refresh/restart ImageJ so it can reload the application
 - · Go to Plugins > Classification > Microscope Image Focus Quality
 - Need to click "generate probability image" and "overlay probability patches" (make sure to put thickness of 5 so you can see the squares)
 - This will outline the image into squares with different colors which signify the amount of focus each region of the image has
- Image J Results
 - Red squares = in-focus
 - Green squares = mid-focus
 - Blue squares = out-of-focus

Links Used:

https://imagej.net/plugins/microscope-focus-quality

https://imagej.net/imagej-wiki-static/Microscope_Focus_Quality

Conclusions/action items: Actually process the data in ImageJ tomorrow and count up the squares in each image with the different colors to determine which image had a higher focus quality. Include these results in the final report.



12/06/2021 Group Testing Protocol Results

MAYA TANNA - Dec 11, 2021, 10:01 PM CST

Title: Group Testing Protocol Results

Date: 12/06/2021

Content by: Maya/Caroline/Katie/Olivia

Goals: To document the results of all the testing done for the electronic and optical components of the project

Content:

See attachment below.

Conclusions/action items: Finish testing next semester if the project continues and include all testing results with figures and charts in the final report.

MAYA TANNA - Dec 11, 2021, 10:01 PM CST

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Group_Testing_Protocols.pdf(91.1 KB) - download


12/06/2021 Optical Testing Results via ImageJ Processing

MAYA TANNA - Dec 11, 2021, 10:11 PM CST

Title: Optical Testing Results via ImageJ Processing

Date: 12/06/2021

Content by: Maya

Goals: To document the results of the optical testing and data analysis via ImageJ (I also added these figures and tables to the report, along with the explanation of the test results below)

Content:

Optical Testing Results (Prior and After Installation)



Figure 11: Optical analysis from ImageJ of microscopic cells with glass (left) and without glass (right)

Table 4: Table display	ring the number of	red (in focus), gre	een (mid focus),	and blue (out of focus)	squares shown	in each image above

	Microscope Image with Glass	Microscope Image without Glass
Red Squares	130	120
Green Squares	54	51
Blue Squares	8	21
Total	192	192

The two optical testing images above show boxes around the image that outline the clarity and quality of that part of the image. According to the color scale shown at the bottom of both images, the red end of the spectrum indicates that the image is in focus at a specific region, while the blue end of the spectrum indicates that the image is out of focus at a given region. Results from this test show that the image with the glass had a slightly higher, yet very similar focus quality compared to the image without glass present. Similarly, 100% of randomly selected subjects expressed no difference in clarity between the two optical images. As seen above, the microscope image with glass has slightly more red squares (in focus) and fewer blue squares (out of focus), causing it to have a higher focus quality. However, the two images have very similar values for each color type as demonstrated above. It is important to note that these two images are slightly different because the image with the glass is more zoomed in on a cell than the other image. This may have contributed to a slightly higher focus quality for the image with the glass rather than without the glass. In the future, this test can be improved by identifying the same cell with and without the glass, to ensure a higher accuracy of the results.

Conclusions/action items: These results show that both images had very similar focus qualities, proving that the optical imaging test was successful because the quality of the microscope was not compromised. The image with the glass had a slightly higher focus quality, but this could also be due to the fact that the images are slightly different as you can see just from looking at them. To make this test more successful next semester, we should try to get an image of the same cell so the optics are more comparable.



MAYA TANNA - Dec 11, 2021, 11:00 PM CST

Title: Show and Tell Preparations Prep

Date: 11/05/2021

Content by: Maya/Caroline/Katie

Goals: To document work done to prepare for show and tell

Content:

Drafted the preparation speech for show and tell

Sent testing protocols to Dr. Kinney for review/feedback. Consulted the MakerSpace for materials with Caroline and found that they had epoxy glue. Did research on flexible plastic tubing for the incubator, and discovered that it was a more feasible option than copper/metal tubing. Wrote the call-to-action for Show and Tell.

2-minute spiel:

Hi everyone! Our team has been tasked with developing a low-cost cell culture incubation chamber that is compatible with an inverted microscope and capable of live-cell imaging culture plates. The incubator must be able to maintain an internal environment of 37 °C, 5% CO2, and 95-100% humidity without compromising the integrity of the microscope's optics or functionality. Our final design consists of a heated water pump where a conducting plastic tube will be wrapped around the inside of the incubator and connected to a heated water pump that will be set to 37°C. The inside of the incubator will be filled with water, submerging the plastic tubing, allowing the internal environment to be heated by conduction as well as increasing the humidity to 95% or higher. The incubator box will also include a tube connector to allow CO₂ gas to be pumped in. Lastly, a separate box will be placed inside the incubator to allow for wiring and sensors to be inside the internal environment. The sensors will be connected to an Arduino microcontroller where temperature, humidity, and CO₂ levels will be collected and analyzed. Our call to action is to ask for your help on how we can arrange the plastic tubing or sensors in order to achieve a homogeneous temperature environment.

Conclusions/action items: Use feedback from show and tell to drive the remainder of the semester and continue testing/fabrication of device.



Show_and_Tell_Presentation.jpg(54.2 KB) - download

Feedback from Other Teams

- Zig zag needs pegs to hold in place
- Sensors on the top
- Carbonate water
- Hydrophilic materials
- Just use waterproofed sensors? RESEARCH
- CO2 sensor waterproofing test protocol
- Zig zag best idea, but secure
- Tubing: twice wrap around, tubing coming out of incubator above water
- Waterproof fabric (rain coat material)
- Randomized zig zag
- · Thermistor, coating that works with temperature but waterproof
- Get curve and calibration stuff from class
- Snail system with tubing
- Look into ideas for water proofing the sensors (rubber, styrofoam)
- Test coiled vs. uncoiled tubing (tubing test protocols)



12/09/2021-12/10/2021 Printing and Presenting Final Poster

MAYA TANNA - Dec 11, 2021, 10:25 PM CST

MAYA TANNA - Dec 11, 2021, 10:25 PM CST

Title: Printing and Presenting Final Poster

Date: 12/09/2021-12/10/2021

Content by: Maya

Goals: To document last memories of the project, including a team picture and the final poster versions

Content:

See attachments below.

Conclusions/action items: Present work to Dr. Puccinelli and ask for agreement to continue the project next semester.



Final_Poster_-_Final.pdf(2.3 MB) - download

MAYA TANNA - Dec 11, 2021, 10:26 PM CST



final_team_pic.jpg(83.7 KB) - download

MAYA TANNA - Dec 13, 2021, 6:33 PM CST



Order Details

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iD	45a69786-0d67-4db0-b037-12b819ed3f8e
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PRODUCT NAME	College Library Poster Print
PICKUP LOCATION	College Library
PRODUCT INFORMATION	Width (in inches): 53
	Height (in inches): 40
	Quantity: 1
	Paper Type: glossy
EST. COST	\$58.89



MAYA TANNA - Dec 13, 2021, 5:53 PM CST

Title: Final Deliverables

Date: 12/13/2021

Content by: Maya

Goals: To document my contribution of the final deliverables

Content:

Last Week of Design Contributions

- Final Report
 - Formatting
 - Testing sections
 - Easy copy and paste stuff from preliminary report
 - Results
 - Appendices
- Submitted peer evaluations

Optical Testing Results (Prior and After Installation)



Figure 11: Optical analysis from ImageJ of microscopic cells with glass (left) and without glass (right)

Table 4: Table displaying the number of red (in focus), green (mid focus), and blue (out of focus) squares shown in each image above

	Microscope Image with Glass	Microscope Image without Glass
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Maya Tanna/Design Process/12/13/2021 Final Deliverables

above, the microscope image with glass has slightly more red squares (in focus) and fewer blue squares (out of focus), causing it to have a higher focus quality. However, the two images have very similar values for each color type as demonstrated in Table 4. It is important to note that these two images are slightly different because the image with the glass is more zoomed in on a cell than the image without the glass. This may have contributed to a slightly higher focus quality for the image with the glass rather than without the glass. In the future, this test can be improved by identifying the same cell with and without the glass, to ensure a higher accuracy of the results.





Conclusions/action items: Look forward to working on this project again next semester! Work on getting more conductive tubing, doing more comprehensive optical testing, getting a better box material than PLA plastic to prevent leakage, etc. (relatively easy fixes)



03/09/2021 Biosafety and Chemical Safety Training

MAYA TANNA - Sep 12, 2021, 11:29 PM CDT

Title: Biosafety and Chemical Safety Training

Date: 09/12/2021

Content by: Maya Tanna

Present: Maya Tanna

Goals: To document biosafety and chemical safety training

Content:

See attachment below as proof of training

Conclusions/action items: Do more training and get a Green Pass Certification from the TEAM Lab.

MAYA TANNA - Sep 12, 2021, 11:29 PM CDT

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MAYA TANNA - Sep 12, 2021, 11:29 PM CDT

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Olivia Jaekle - Oct 08, 2021, 3:53 PM CDT

Title: Endothelial Cell Culture

Date: 9/15/21

Content by: Olivia Jaekle

Present: Olivia Jaekle

Goals: Learn about different cell cultures

Content:

Endothelial cell culture: protocol to obtain and cultivate human umbilical endothelial cells

Standard protocol for preparation, maintenance, and quality control control of endothelial cells

These types of cells are used to study the following:

- 1. Leukocyte-endothelial adhesion, which is a key step towards lymphocyte recirculation, immune cells migration towards inflamed tissues of vasculitis (Springer, 1994)
- 2. Leukocyte transmigration through blood vessel (Kaplanski et al., 1994)
- 3. Cross-talk between endothelium and immune cells, resulting in the expression of adhe-Žsion molecules and cytokines (Kaplanski et al.,.1994b, 1997, 1998)
- 4. Atheroma, which is a major pathological phenomenon in developed countries and is largely dependent on both metabolic and inflammatory events (Ross, 1999)
- 5. Metastasis formation (Sheski et al., 1999), which is influenced by adhesive interactions between circulating tumor cells and endothelial

Standard cell culture equipment:

- 1. Laminar flow hood
- 2. CO2 (5%) incubator
- 3. Standard inverted microscope
- 4. Centrifuge
- 5. Water bath

Sterile Material

- 1. Compresses
- 2. Gloves
- 3. String sterilized for 90 min at 180 C or clamps
- 4. Blunt needles
- 5. Scalpel
- 6. Pipettes
- 7. Filters

Quick Procedure

- 1. Cut clamped cord section
- 2. Cannulate vein extremities and tie with string

Olivia Jaekle/Research Notes/Biology and Physiology/9/15/21 Endothelial Cell Culture

- 3. Perfuse vein with M199
- 4. Obturate one vein extremity with a 1-ml syringe
- 5. Perfuse with collagenase
- 6. Incubate about 7 min at 37C in water bath
- 7. Massage the cord and collect the effluent in 50-ml tube containing 10ml culture medium
- 8. Centrifuge and discard supernatant. Resuspend cell pellet in 5 ml culture medium
- 9. Transfer cell suspension in a 25-cm^2 gelatin coated flask
- 10. Replace medium after 24 h, then every 2 days until cell confluence
- 11. For each passage discard medium and wash cells with PBS without Ca2+ and Mg 2+
- 12. Add 3 ml trypsin-EDTA for 30s to 1 min at 37C
- 13. Stop trypsin activity with 10 ml culture medium addition and centrifuge cell suspension
- 14. Discard supernatant and add fresh ECGS culture medium
- 15. Incubate EC in a new gelatin-coated flask

Conclusions/action items:

Figuring out the relation of endothelial cell culture and our design process.

Citations:

V. Marin, G. Kaplanski, S. Grès, C. Farnarier, and P. Bongrand, "Endothelial Cell culture: Protocol to obtain and cultivate human umbilical endothelial cells," *Journal of Immunological Methods*, 08-Jun-2001. [Online]. Available: https://www.sciencedirect.com/science/article/abs/pii/S0022175901004082. [Accessed: 08-Oct-2021].



9/17/21 Basics of animal cell culture: Foundation for modern science

Olivia Jaekle - Oct 08, 2021, 3:24 PM CDT

Title: Basics of animal cell culture: Foundation for modern science

Date: 9/17/21

Content by: Olivia Jaekle

Present: Olivia Jaekle

Goals: Learn about cell culture and the terms used while speaking about cell cultrue

Content:

Notes:

Intro

- "Cell culture is used in cancer research, vaccine manufacturing, recombinant protein production, drug selection and improvement, gene therapy, stem biology, monoclonal antibody production, fertilization tech, cryopreservation and in vitro production of hormones" (Awolowo, 2016)
- Cells can:
 - Expand
 - · Divide into replicas of each other
 - Propagated
 - Purified
 - Preserved (when frozen)
- Cell culture involved isolating cells, tissues and organs from animals and growing them in an artificial environment (in vitro)
- Culture: to keep alive and grow until can simulate natural conditions
- · Lots of different cell types can be grown
- · This process is valuable when studying functions and operations of cells
- · Very important for biotechnology
- · Nutritional factors can be added to the medium to help growth

Procedure of Cell Culture

Primary Culture

- · Primary cultures: freshly isolated cultures
 - No longer a primary cultures if they are subcultures or passaged
- Primary cultures are usually heterogeneous and have low growth fraction
- · First step: isolate cell from tissue or organ
 - Can be done by adding low trypsin to the tissue
 - This degrades extracellular proteases and glycosidase

Subculture culture

• Subculture: new culture taken from a primary culture and grown separately

Olivia Jaekle/Research Notes/Biology and Physiology/9/17/21 Basics of animal cell culture: Foundation for modern science

- Allows expansion of the culture AKA cell line
- · Advantages of this is it allows large amount of consisite materials
- · Does not need trypsinization
- Does not need serum

Monolayer Culture

- · Monolayer: this type is the bottom of the culture plate that is covered by a single layer of cells
- Do not require enzymatic or mechanical dissociation
- The growth of this type can be limited by concentration of cells

Suspension Culture

- This type are cells that are grown within suspension of medium
- The cells are easier to passage rather than detach them
- Do not require enzymatic or mechanical dissociation

Adherent Culture

- · Cells in the adherent culture are dissociated enzymatically
- Do require passaging
- · Super easy to see under inverted microscope
- AKA anchorage dependent cells

Seeding Density and cell propagation

- Good conditions: low cell density, low Ca 2+ concentration, and presence of growth factors
- · Cultures are derived from tissues
 - · Will survive and grow better than those adults tissues

Conclusions/action items: Continue learning about cell culture and getting a better understanding about the project.

Citation:

"Basics of animal cell culture: Foundation for modern science." [Online]. Available: https://academicjournals.org/journal/BMBR/article-fulltext-pdf/7A2154261212. [Accessed: 08-Oct-2021].



Olivia Jaekle - Oct 08, 2021, 3:21 PM CDT

Title: Good Cell Culture Practice for Stem Cells and Stem-Cell-Derived Models

Date: 9/17/21

Content by: Olivia Jaekle

Present: Olivia Jaekle

Goals: Learn more about cell culture practice.

Content:



Fig. 1: Workflow of derivation and differentiation of patient PSC and stage-specific characterization requirements

Figure 1 is a good representation of the workflow of cell culture and the larger overall process of cell culture inhibition.

Conclusions/action items: Continue researching about cell culture and ask the client how this process relates to our project or might influence the design.

9/15/21 Open-dish incubator for live cell imaging with an inverted microscope

Olivia Jaekle - Oct 08, 2021, 3:58 PM CDT

Title: Open-dish incubator for live cell imaging with an inverted microscope

Date: 9/15/21

Content by: Olivia Jaekle

Present: Olivia Jaekle

Goals:

Content::



Picture of the cell incubator

- The overall article is about an inexpensive cell culture incubator for the stage of an inverted light microscope
 - Used for live cell imaging
- Device keeps temp of cell culture at 37° C
- Once reaches equilibrium, device provides focal stability of image for 20-25 minutes with oil immersion lenses
- 2 different types of incubators:
 - Standard 60-mm plastic culture dishes
 - Imaging of cells on glass coverslips
 - Each of these can be made for <\$400
- Only requires 1 wiring and 3 hour assembly

Olivia Jaekle/Research Notes/Competing Designs/9/15/21 Open-dish incubator for live cell imaging with an inverted microscope

- The design is called the ringcubator because the key element is a head, temp-controlled aluminum ring that serves as mini-incubator
- Live cell imaging has helped show growth in green fluorescent protein to allow visualization of target proteins in living cells
- Most incubators cost more than \$1500
- Studies came to show that the ringcubator is quite effective and does a good job at stabilizing the temperature

Source: https://www.future-science.com/doi/pdf/10.2144/03354bi01

Citation:

S. R. Heidemann, P. Lamoureux, K. Ngo, M. Reynolds, and R. E. Buxbaum, "Future science | home." [Online]. Available: https://www.future-science.com/. [Accessed: 08-Oct-2021].



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Olivia Jaekle - Oct 08, 2021, 3:01 PM CDT

Title: CO2 Microscope Cage Incubator

Date: 9/22/21

Content by: Olivia Jaekle

Present: Olivia Jaekle

Goals: Understand the Co2 Microscope Cage Incubator and see how it might be changed to become inexpensive and easy to make

Content:

- This design can maintain temp, CO2/Air Mixture, and humidity at precise measurements.
- The microscope is enclosed in the design so that the cell culture is protected from the changes in temperature or a drift in general.
- Co2 is controlled through a micro-environment chamber on the microscope stage. The CO2 is continuously fed.
- Temp is controlled and maintained by blowing warm air into the cage.
- There are windows on the front and side panels to allow full, easy access to the microscope.



Conclusions/action items: Keep researching different incubators to come up with ideas for preliminary designs.

Citation:

SelectScience, "CO2 microscope cage incubator," *SelectScience*. [Online]. Available: https://www.selectscience.net/product-news/co-2--microscope-cage-incubator/?artID=10278. [Accessed: 08-Oct-2021].



9/23/21 A micro-incubator for cell and tissue imaging

Olivia Jaekle - Oct 08, 2021, 3:47 PM CDT

Title: A micro-incubator for cell and tissue imaging

Date: 9/23/21

Content by: Olivia Jaekle

Present: Olivia Jaekle

Goals: Looking at the different designs of micro-incubators to see how they may be revised for our project.

Content:

A micro-incubator for cell and tissue imaging

- · A low-cost micro-incubator used for imaging living cells and tissues which are being developed
- The micro-incubator provides an environment for the cells that can be easily altered and can last several days/hours.
- · Can be used in any position or direction and used in an inverted microscope
- · Temperature is regulated through a peltier module controlled by a sensor positions close to the sample
- · The reusable modular micro incubator is optimized for mass and heat transfer
 - Has the ability to culture cells in monolayers or on synthetic or natural tissue matrices over extended time periods
 - Image cells on inverted microscope owing to the use of a gas-permeable membrane to separate the culture medium from the ambient environment
 - · Control temperature at the sample level to prevent any light heating or cooling
 - · Perfuse different media onto each side of the tissue samples
 - · Perform micromanipulation on a "open-dish"



- Stainless steel container (height 7 mm, outer diameter 36 mm)
- · Container A conducts heat
- Silicone ring B creates cell growth
- · Syringe needle is used to pierce two small holes in silicone ring through which capillaries are inserted
 - The material silicone ensures sealing

Olivia Jaekle/Research Notes/Competing Designs/9/23/21 A micro-incubator for cell and tissue imaging

• Membrane is clamped between 2 stainless steel rings

Conclusions/action items:

Continue researching and compare the designs to design matrix.

Citation:

C. Picard, "BioSpotlight | biotechniques - future-science.com." [Online]. Available: https://www.future-science.com/doi/full/10.2144/000113643. [Accessed: 08-Oct-2021].



Olivia Jaekle - Oct 08, 2021, 3:13 PM CDT

Title: Previous Microscope Cell Culture Incubator BME 400

Date: 9/26/21

Content by: Olivia Jaekle

Present: Olivia Jaekle

Goals: Learn about the previous designs done in BME (200,300,400) and revise their design

- Used an incubation chamber for the final project
- · Schematic of 3D printed casing for final prototype
- 2 separate halves were printed and attached to each other using acetone
- · Ports on the side were created to allow for CO2 gas, sensor, and electronics wiring
- An ABS printed lid with an acrylic insert was used for top imaging surface to keep heat within system
 - Allows users to see the cells
- The cutout on the bottom fit a standard culture plate
- · Power was supplied to all circuit elements from VGD-30-D512 multiple output AC DC converter
- DHt22 temp/ RH sensor was used to sense heat and relative humidity
- · CO2 was measured with MH-Z16 CO2 sensor
- · JFSV00005 gas solenoid value was used to control gas flow from CO2 tank to incubator
- · A small fan was used to provide circulation
- Client wants a team to create an incubator that can fit on a Nikon TI-U microscope stage without blocking the path of light from the microscope.
- Important cells stay at 37C and stay at 5% CO2 concentration
- Important to keep humidity around 95%
- Design:
 - Includes a glass top that will minimize optical impairment and will be able to go through sterilizations while extruding less heat loss.
 - Transparent heating element on the bottom
 - · CO2 input tube will be linked on the side of the box
- As a material they chose plastic because it can withstand the high temperatures, is cheap, easily 3D printed, and can be sterilized
- In specific they chose to use Polypropolene for their material because it was the cheapest and does a decent job of thermal insulation.



Conclusions/action items: Keep researching other designs and compare for design matrix.

Citation:

N. Pauly, T. Madigan, K. Koesser, and B. Meuler, "Microscope Cell Culture Incubator - bmedesign.engr.wisc.edu." [Online]. Available: https://bmedesign.engr.wisc.edu/projects/f20/scope_incubator/file/view/db2b6829-fcc8-4732-8cec-94e60a3cc722/Final%20Report.pdf. [Accessed: 03-Oct-2021].



Olivia Jaekle - Dec 06, 2021, 7:55 AM CST

Title: Design Matrix Evaluation

Date: 9/28/2021

Content by: Caroline Craig, Ethan Hannon, Olivia Jaekle, Maya Tanna, Katie McGovern, Sam Bardwell

Present: Olivia Jaekle

Goals: To document design matrix and provide reasoning for rankings. Will be shared with the team.

							-	
			Past Project Refurbished		Heated Water Pump Incubator		Sheiving Incubator	
Rank	Criteria	Weight	Score (10 max)	Weighted Score	Score (10 max)	Weighted Score	Score (10 max)	Weighted Score
1	Internal Environment	25	9	23	7	18	5	13
2	Microscope Compatibility	20	10	20	10	20	10	20
3	Accuracy and Reliability	20	7	14	8	16	4	8
4	Ergonomics	15	5	8	8	12	4	6
5	Cost	10	2	2	4	4	3	3
6	Life in Service	5	10	5	10	5	10	5
7	Safety	5	10	5	10	5	10	5
	Sun	n 100	Sum	76	Sum	80	Sum	60

- Internal Environment
 - For this criteria, the Past Project Refurbished scored the highest since the previous BME groups have already done testing on the device's ability to regulate temperature, CO2, and humidity. Our team believed that further work on this system could have improved the device's ability to maintain these conditions by improving the materials. For these reasons, we gave Past Project Refurbished a 9.
 - The Heated Water Pump Incubator scored the next highest because our team believes improving upon previous BME groups' designs by using a heated water tube would benefit the ability to create a better cell culture environment. It scored lower than the Past Project Refurbished design because we would not have the previous testing to use. For these reasons, we gave Heated Water Pump Incubator a 7.
 - Finally, the Shelving Incubator scored lowest with a 5 because the ability of our team to maintain the conditions once the drawers were pulled out had not been completely understood.
- Microscope Compatibility
 - All designs scored a 10 in microscope compatibility because each design was created and could successfully be used with an inverted microscope.
- Accuracy and Reliability
 - For this criteria, our team scored the Heated Water Pump Incubator highest. We believe that the finalized design would have a more reliably designed system for the intended use of the incubator with the materials and external devices we plan to use. For this reason, gave this design an 8.
 - The Past Project Refurbished design scored the next highest with a 7. Like the Heated Water Pump Incubator, the Past Project Refurbished design would have improved upon materials in comparison with previous BME projects, but the mechanics of the system would not be as reliable as the other incubator.
 - The Shelving Incubator received the lowest score of 4 because altering the shape of the environment by opening a drawer would be difficult to maintain accurate internal conditions, and the size of the machine may hinder its reliability in reading accurate conditions. Also, moving components are more susceptible to wear and tear making it less likely to live through its self-life
- Ergonomics
 - Our team scored the Heated Water Pump Incubator highest for this criteria, again because its materials and components would allow it to function the best in comparison with our other designs. For this reason, it scored an 8.
 - The Past Project Refurbished design scored a 5 because the design components implemented by previous BME teams that we planned on keeping the same would not function in maintaining internal environment conditions as the Heated Water Pump Incubator could.

Olivia Jaekle/Design Ideas/9/28/2021 - Design Matrix Evaluation

- 238 of 336
- Finally, the Shelving Incubator scored lowest with a 4 because it would be the most difficult to use with having to pull out drawers each time one wanted to view a sample.
- Cost
 - All the designs scored low for cost because our team's smaller budget will be difficult to stay in range with. The Heated Water Pump Incubator scored the best with a 4 because lots of the components we plan on using will be provided to us. Our biggest difficulty in staying within the budget will be limiting the need to repurchase materials wasted in prototyping.
 - The Past Project Refurbished design scored a 3 because components of the previous design would be reused, but the components we plan on replacing would end up being more expensive than just creating the Heated Water Pump Incubator design.
 - The Shelving Incubator scored lowest with a 2 because its size would increase the cost and create a greater likelihood to go over budget if lots of prototypes are made.
- Life in Service
 - All the designs scored a 10 for Life in Service because they were designed with the intent of functioning for a week period of time every year for 10 years.
- Safety
 - All the designs scored a 10 for safety because the components involved in their designs would not be harmful to the user in any way.

Conclusions/action items:

Based on this design matrix, our team will be moving forward with creating the Heated Water Pump Incubator for our client. This design was ranked the reliable, ergonomic, and cost-effective in comparison with the other designs. The design will include a slot for the well plate, a tube containing heated water to maintain a 37*C temperature and assist in evaporation, and a water well for evaporation water to maintain high humidity. The dimensions of the incubator will match the size of the microscope stand, or it will go over the edges slightly, and the height will not exceed the lowest point of the top light microscope component. Finally, sensors compatible with Arduino will be used to regulate the internal conditions.



Olivia Jaekle - Dec 06, 2021, 8:29 AM CST

Title: Design 1: Past Project Refurbished

Date: 10/05/21

Content by: Olivia

Present: Olivia

Goals: Vouch on why we should fabricate this design

Content:

- continuing to work on past design to further test the product, improve materials, and fix coding errors regarding the sensors was a realistic option. Every previous design involved a rectangular box for the incubation chamber. The design also included a glass top that minimized optical impairment and allowed the incubator to go through sterilizations while extruding less heat loss. The bottom part of the chamber had a transparent heating element. The CO₂ input tube was linked on both sides of the chamber . Lastly, sensors that controlled CO₂, temperature, and humidity were connected to an Arduino microcontroller. The disadvantages of this design were finding quality materials that could keep CO₂ levels and temperatures constant while being within a low-cost budg**et**





Olivia Jaekle - Dec 06, 2021, 7:40 AM CST

Title: Thermistor Code (Arduino)

Date: 11/14/2021

Content by: Katie Day and Olivia Jaekle

Present:

Goals: To create a code on Arduino that measures temperature and humidity with a thermistor.

Content:

See attached file. Calibration curve for thermistor attached below.



Conclusions/action items: Thermistor is working properly and outputs correct temperatures. Use in testing protocol next week with completed incubator prototype.

Olivia Jaekle - Dec 06, 2021, 7:40 AM CST



thermistor.ino(745 Bytes) - download

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Thermistor_Circuit_Diagram.PNG(80.9 KB) - download



Olivia Jaekle - Dec 06, 2021, 7:41 AM CST

Title: DHT22 Temperature and Humidity Code

Date: 11/14/2021

Content by: Katie Day and Olivia Jaekle

Present:

Goals: To create a code on Arduino that measures temperature and humidity with a DHT22 sensor.

Content:

See attached file.

Conclusions/action items:

- 1. Thank you to Dr. Nimunkar for ordering a proper DHT22 sensor and helping us with code.
- 2. Decide between thermistor applicator or DHT22.
- 3. If going with thermistor check humidity equation with values from the DHT22.

Olivia Jaekle - Dec 06, 2021, 7:41 AM CST



DHT-22.ino(885 Bytes) - download



Olivia Jaekle - Dec 06, 2021, 7:43 AM CST

Title: Thermistor Testing
Date: 12/3/2021
Content by: Katie, Olivia, Maya, and Caroline
Present: Katie and Olivia
Goals: To test the accuracy of our thermistor against an incubator.
Content:
See attached testing protocol written by Maya and Caroline. Testing performed by Olivia and me.

Conclusions/action items:

Thermistor is working properly and ready for implementation.

Misty_In_Incubator_10-min.PNG(15 KB) - download

Olivia Jaekle - Dec 06, 2021, 7:43 AM CST



Olivia Jaekle - Dec 06, 2021, 7:44 AM CST

Title: CO2 Testing
Date: 12/3/2021
Content by: Katie, Olivia, Maya, and Caroline
Present: Katie and Olivia
Goals: To test the CO2 sensor to make sure that it is working properly.
Content:
Attached our the results of our testing along with the testing protocols written by Maya and Caroline, performed by Olivia and me.

Conclusions/action items:

The CO2 sensor is ready for incorporation into the incubator.

Olivia Jaekle - Dec 06, 2021, 7:44 AM CST



concentration.csv(2.4 KB) - download

Olivia Jaekle - Dec 08, 2021, 10:50 AM CST



Olivia Jaekle - Dec 08, 2021, 10:50 AM CST



Misty_Humidity_Data.csv(1.5 KB) - download

Olivia Jaekle - Dec 08, 2021, 10:50 AM CST



Combined_Humidity_Data.csv(4.1 KB) - download

Olivia Jaekle - Dec 08, 2021, 10:50 AM CST



Combined_Humidity_Data.txt(2 KB) - download

Olivia Jaekle - Dec 08, 2021, 10:50 AM CST



DHT22_Humidity_Data.csv(441 Bytes) - download

12/07/2021 Group Testing Protocols



Olivia Jaekle - Dec 08, 2021, 10:49 AM CST

Title: Group Testing Protocols

Date: 12/07/2021

Content by: Maya Tanna and Caroline Craig

Present: Katie McGovern and Olivia Jaekle

Goals: To create testing protocols and verify that the elements of our design are working as expected, accurately, and precisely.

Content: The Testing Protocols and the parts of the protocol that were able to be evaluated during the semester.

Conclusions/action items:

The temperature, humidity, CO2, and optics are all working as expected.

Olivia Jaekle - Dec 08, 2021, 10:49 AM CST



Group_Testing_Protocols.pdf(90.5 KB) - download



Olivia Jaekle - Dec 06, 2021, 8:04 AM CST

Title: Olivia's Progress Report

Date: 9/15/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Created the team photo for the website. Started to fix up
 our website by updating the project summary. Researched open-dish incubators to get a better understanding on what
 part of the system could be more efficient.
- upcoming Individual goals: Update website with weekly progress report. Meet with the advisor and find out what else needs to be done this week for the website. Continue to do more research after meeting with the client and use that research to draft PDS.



Olivia Jaekle - Dec 06, 2021, 8:06 AM CST

Title: Olivia's Progress Report

Date: 9/23/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Contributed to research for a more thorough understanding of cell incubators. Sorted through past BME 200 projects that are similar to this one and noted important takeaways from each project. Revised product design specifications and uploaded the document to the website and canvas.
- Upcoming Individual goals: Continue to research competing products. Brainstorm at least one idea for the design matrix. Upload progress report to website. Contribute more within team meetings.

Olivia Jaekle - Dec 06, 2021, 8:07 AM CST

Title: Olivia's Progress Report

Date: 9/30/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Researched and created designs for weekly team meeting. Met with the team to share possible designs and then created a design matrix. Read client meeting notes. Started to work on preliminary design presentation.
- Upcoming Individual goals: Continue working on preliminary design presentation. Use client notes to conduct more research on external water heating systems. Also, learn about arduino software and the code used.

Olivia Jaekle - Dec 06, 2021, 8:09 AM CST

Title: Olivia's Progress Report

Date: 10/7/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Researched about water jets and its heating functions. Started learning how to code C++ for the arduino. Looked up tutorials on SOLIDWORKS and started experimenting to get more familiar with the application. Contributed to the preliminary report, specifically preliminary design, design requirements, and references.
- upcoming Individual goals: Continue learning how to use SOLIDWORKS and how to code C++. Revise and finalize preliminary report and presentation.



Olivia Jaekle - Dec 06, 2021, 8:10 AM CST

Title: Olivia's Progress Report

Date: 10/14/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Worked on preliminary presentation and report. Practiced slides for the presentation and learned more about Arduinos.
- upcoming Individual goals: Work with Katie to learn more about Arduinos and how they will be used in the design. See what materials were left from the last group and understand what can be used or revised for our design


Olivia Jaekle - Dec 06, 2021, 8:12 AM CST

Title: Olivia's Progress Report

Date: 10/21/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Katie and I went through prototypes left by previous design teams and analyzed what type of sensors they used/ which sensors would we be able to reuse. I revised the report, submitted the report and the notebook.
- upcoming Individual goals: I will work with Katie and learn arduino code and how to use that code to regulate CO₂, temperature, and humidity. Also, start working on testing protocols for the arduino, arduino code, and sensors.



Olivia Jaekle - Dec 06, 2021, 8:14 AM CST

Title: Olivia's Progress Report

Date: 10/28/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Began to test out the temperature sensor with Katie and Sam. Learned about coding Arduino for temperature sensors. Started to think about ways that might make coding more effective and easier for all sensors.
- upcoming Individual goals: Continue working on Arduino code and thinking about how to test sensors once code is finished.

Olivia Jaekle - Dec 06, 2021, 8:15 AM CST

Title: Olivia's Progress Report

Date: 11/4/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Worked on the Arduino code for temp sensor with Katie and Sam. Researched the code and features for CO₂ sensor. Revised show and tell.
- upcoming Individual goals: Continue to work on code for temp sensor and CO₂ sensor. Take feedback from show and tell and use it for testing arduino code.



Olivia Jaekle - Dec 06, 2021, 8:17 AM CST

Title: Olivia's Progress Report

Date: 11/11/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Worked with Katie to create code for a new temperature sensor. We wanted the code to read the surrounding temperature and humidity. We were also able to calibrate the temperature sensor with the code. Tests were done with extreme heat and cold to see if the temperature sensor would be able to withstand 39°C, to see if the sensor is accurate, and to note that the temperature sensor is waterproof.
- upcoming Individual goals: Start looking at the CO₂ sensor and see how Katie and I will be able to code it for the purposes of the incubator. Start testing CO₂ sensors and finalize all test protocols that have to do with coding.



Olivia Jaekle - Dec 06, 2021, 8:18 AM CST

Title: Olivia's Progress Report

Date: 11/18/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Looked at code with Katie for both temperature and CO2 sensors. Researched more on how to build circuits and the advantages of different sensors we could use.
- upcoming Individual goals: Do more testing for sensors. Finalize code for sensors



Olivia Jaekle - Dec 06, 2021, 8:20 AM CST

Title: Olivia's Progress Report

Date: 12/02/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Worked with Katie to finalize the CO2 sensor. We created a graph that showed the thermistors temperature over a 10 minute period within the incubator. Discussed testing protocols with Caroline.
- upcoming Individual goals: Work on final report and presentation. Also work with Sam, Ethan, and Katie to make sure sensors can fit within the incubator once the incubator is finished.



Olivia Jaekle - Dec 14, 2021, 9:46 AM CST

Title: Olivia's Progress Report

Date: 12/9/21

Content by: Olivia

Present: Olivia

Goals: Update everyone on what I have been working on

- Summary of weekly team member desing accomplishments: Worked on the final deliverables. Analyzed humidity data.
- upcoming Individual goals: Finish all final deliverables.



Caroline Craig - Sep 15, 2021, 10:28 AM CDT

Title: Live-Cell Imaging and Other Light Microscopy Techniques

Date: 9/14/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Learn about how live-cell imaging works with a microscope

Source: https://www.science.org/doi/full/10.1126/science.1082160

- The process of light microscope techniques make studying live/dynamic cells more common
- · Focused on overview of the approaches to live-cell imaging
- Allows for user to have a detailed view of cell interactions and individual cell processes instead of still images
- Environmental Considerations:
 - · Cells sensitive to photodamage, want to reduce that
 - Vital to keep their cellular environment constant (temperature, humidity, CO2, etc.)
- Fluorescence Imaging
 - introducing fluorescent molecules into otherwise non-fluorescent molecules to help identify and see processes better
- Live Cell Imaging
 - Considerations for system to use for imaging live cells:
 - 1. sensitivity of detection
 - 2. speed of acquisition
 - 3. viability of the specimen
 - "Light microscopy of living versus fixed samples is essentially a trade-off between acquiring images with a high signal-to-noise ratio and damaging the sample under observation"
 - Questions to ask regarding the sample:
 - 1. is it thick or thin?
 - 2. is the process to be observed fast or slow?
 - 3. Do you need to image for seconds, minutes, hours, or days, and at how many different wavelengths does the image need to be sampled?
 - 4. How bright is your signal?
 - 5. Will you want to use a specialized techniques such as photobleaching?
 - 6. Are transmitted light images required, and if so, of what quality?
 - Instead, need to understand the pros and cons of different microscope systems since many are likely to be used



- o Things to highlight:
 - Limit Cell Damage:
 - since fluorophores cause cell damaging, everything should be done to limit duration and intensity of illumination
 - remove unnecessary wavelengths of light
 - removing O2 can also help
 - Speed of acquisition:
 - when multiple fluorophores are imaged simultaneously or when a single probe is analyzed ratiometrically
 - Switching between laser lines, filters, or output from a monochromator will slow data acquisition
 - Three- and four-dimensional (3- and 4D) imaging
 - many experiments may be better performed using widefield (conventional) systems with subsequent deconvolution of the data series
 - Widefield microscopes do not exclude light from any plane of focus; they collect it all
 - Most cellular processes occur in three dimensions over time, so to get a complete picture we need to image cells in four dimensions.
 - Multiphoton approaches to in vivo imaging.
 - The two-photon effect excites a chromophore not by a single photon but from two photons being absorbed within a femtosecond time scale
 - enables the use of longer wavelength excitation, which penetrates deeper into samples and reduces photobleaching (cell damage)
- · Other imaging modes

- Bright-field imaging
- Total internal reflection
- Fluorescence correlation spectroscopy
- photobleaching and photoactivation approaches
- Fluorescence resonance energy transfer (FRET)
- Fluorescence lifetime imaging (FLIM)
- Conclusions
 - resolution attainable by light microscopy is being enhanced by recent developments in imaging that break the diffraction limit, and such approaches can be applied to live cells
 - field of live-cell imaging rapidly changing companies and discoveries

Conclusions/action items:

In conclusion, live-cell imaging is crucial for studies that require having a detailed view of cell interactions and individual cell processes, instead of using still images. Keys to consider when imaging to ensure cells aren't damaged are maintaining the cellular environments and reducing damage from too much light exposure. It is also important to understand the pros of using different microscopes, like widefield, scanning confocal, or spinning disk confocal, and their effect on the cells versus the quality of the view. Sensitivity of detection, speed of acquisition, and viability of the specimen are important things to consider for maintaining a quality specimen. "Light microscopy of living versus fixed samples is essentially a trade-off between acquiring images with a high signal-to-noise ratio and damaging the sample under observation." The team should ensure the incubator is compatible with the client's microscope(s) and that it doesn't hinder the view of the cells or damage cells.

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9/15/2021 - Evaluation of Automated Cell Culture Incubators

Caroline Craig - Sep 15, 2021, 12:43 PM CDT

Title: Evaluation of Automated Cell Culture Incubators

Date: 9/15/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Learn about Cell Culture Incubators and their Conditions that maintain cell life

Source: https://doi.org/10.1016%2Fs1535-5535%2803%2900018-2

- Cell culture incubators are important in full automation of cell culture systems, which allow for cell growth without human interaction.
- Source "emphasizes the impact of automation on throughput and environmental controls (temperature, humidity, and CO₂)
- and proposes some basic protocols to check these functions"
- Intro:
 - Cell culture is useful in many labs for many purposes, so successful cell culture is important to allow for research to be done
 - Cell culture can be an intense manual process that requires many steps
 - · main equipment for cell culture is the cell culture incubator
 - enables the reproduction of ideal conditions for cell development
 - decreases human interference and the possibility for error
 - many expensive options on the market
- Cell Culture Automation:
 - mammalian cell cultures rely on media and environmental factors
 - considering cell line is key because different cell lines require different culture conditions
 - · cell culture incubators monitor conditions with internal and external sensors
 - Vessel type is important to consider for different applications
 - automatic incubator specific for one of these
 - · Overall, the keys to consider for an automated cell culture incubator are vessel and conditions for culture
 - Automated incubator features:
 - Traditional manually operated glass external doors.
 - Traditional systems for environmental control, including an input for CO₂, a water tank for humidity, a thermal regulation device for temperature control, and an audio and/or visual warning system, among others.
 - A transport device such as a rotating carousel or Cartesian robot in the storage module.
 - The inclusion of several berths for microplate storage stackers.
 - Whether or not stackers are designed to accommodate different types of vessels.
 - A plate "shuttle" system, such as a robotic arm and tray, that transfers vessels from outside, through an automatic door, and into a stacker (loading); or from a stacker, through an automatic door to the outside (unloading).
 - A microprocessor-controlled automatic door that opens and closes quickly during loading and unloading.
- Evaluation of an Automated Cell Culture Incubator:
 - Loading and Unloading Throughput
 - mean times to load and unload a vessel
 - Environmental Control
 - The accuracy, spatial homogeneity, and stability of environmental conditions are crucial for highquality cell cultures.
 - The main parameters involved are temperature:
 - Temperature
 - 1. Temperature Stability without opening door

- measure using temperature sensors and calculate the mean temperature
- Ensure temperature stability in range and leave room for error



- 2. eventual impact on the temperature of the loading and unloading process
- automation decreases the exchanges between internal and external environments for the culture
 - Common threshold is 95% the previous temperature
 - "recovery time" between opening and closing of the door
 - Trying to make impact of external environmental fluctuation basically negligible.
- Relative Humidity
 - normally high, around 95%
 - created by evaporation from a water tank when heating system is activated
 - Steps required for studying parameter:
 - 1. basic performance without opening a manual door or
 - automated gate
 - only one sensor needed in center of the storage are
 - 2. the eventual impact of loading and unloading on the stability of humidity
 - measure and ensure returns to equilibrium
- CO2
 - often 5%
 - regulated by a CO2 injection device
 - Again, use above steps to regulate conditions in chamber
- Practicability
 - Noise levels should be below 85 decibels
 - continuously record environment and have an alarm if exceeds or dips below
 - often need maintenance and cleaning, set standards and try to prevent contaminations
 - Alarms should be checked regularly
- Conclusion:
 - · Carefully evaluate options and verify specifications to ensure equipment meets cell culture needs

Conclusions/action items:

A fully automated cell culture incubator should be capable of successfully loading and unloading culture vessels and maintaining a stable environment. To maintain a stable environment in the team's incubator, we should include sensors to regulate temperature, humidity, and CO2 levels. A baseline with a margin should be set for these conditions, and if any conditions fall out of equilibrium the user should be notified. These conditions should be constantly regulated with sensors. The team should also ensure our incubator is practical for its intended use and provide maintainece instructions to ensure device longevity.



Caroline Craig - Sep 19, 2021, 6:13 PM CDT

Title: Previous BME Student Cell Culture Incubator (Fall 2011)

Date: 9/19/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: To understand previous UW-Madison BME student's Cell Culture Incubator projects. To take away information that may be useful, and discover areas our team could improve from their projects.

Source: Ian Linsmeier, Tyler Klann, Rebecca Stoebe, Paul Strand

Content:

Fall 2011 Project

- Abstract:
 - similar/same project description
 - · for the client to perform live-imaging of cell cultures
 - for up to a week of use per teaching cycle- same response from our client
 - maintain environmental conditions (37 degrees C, 5% CO2, and 90%-100% humidity)
- Problem definitions
 - Incubation chamber should be compatible with Olympus IX71 inverted microscope
 - · Don't want to compromise microscope funcionality
 - For teaching purposes
- Design Criteria
 - Internal environment of 37*C +- 2*C, 5% CO2 +- 1%, and 90-100% humidity
 - interchangeable with different cell culture plates
 - · Cannot experience interference from condensation, temperature gradients or evaporation of culture liquids
 - Easy to assemble and disassemble
 - Budget is ~\$200 Different
 - All materials used must be biologically compatible

Microscope Cell Culture Incubator Process Diagram



- Humidifier
 - 5x5x6 cm hollow box with 3 circular inlets for fresh gas, recycled air, and fresh water and circular outlet for gas diffusion into incubation chamber
 - Inner basin for water fits inside, made of aluminum coated with ceramic
 - Exterior made of Plexiglas
 - Allows for 0.5 molar flow/min
 - humidified a mixture of 95% air/5% CO2 to 95% humidity using heat delivered from nichrome wires between inner basin and outer chamber
 - exterior water reservoir inputs 0.5 mL of water per minute to compensate for evaporation
- Incubation Chamber
 - .117 in. thick medium impact acrylic
 - allows humidified air and CO2 to enter
 - secures multiple types of culture plates for accurate viewing
 - creates an isolated environment that is easier to regulate and quick to start-up
- Heating housing
 - .117 in. thick medium impact acrylic
 - fits around microscope and allows for full functionality
 - separates into halves for removal and is sealed with silicone gasket
 - edges sealed with WeldOn 4 acrylic adhesive
 - allows incorporation of aluminum stage as heat sink
- Heating element
 - 30 ft. coil of 30 AWG nichrome 60 wire (D=0.010 in.)
 - fan distriutes heat
 - supplied with 189 mA of current to heat to 37*C
 - modulated based on temp. readings
 - coated in thin ceramic with high thermal conductivity and small electrical conductivity
 - possible ceramics include: AIN, SiC, AL2O3, Si3N4, ZrO2
 - Same heating element design for the humidifier
 - 306.3 mA of current --> 19 Watts of power to H2O
- Equations used for heating:

$$\begin{split} N_{Nu} &= \frac{h_{comv}D}{k_{air}} = a(N_{Gr}N_{Pr})^m = a\left(\frac{D^3\rho^2g\beta\Delta T}{\mu^2}\frac{c_p\mu}{k}\right)^m \Rightarrow h_{comv} = \frac{N_{Nu}k_{air}}{D} \\ & h_{rad} = \frac{\varepsilon\sigma\left(T_w^4 - T_a^4\right)}{(T_w - T_a)} \\ q_{total} &= q_{comv} + q_{rad} = (h_{comv} + h_{rad})A(T_w - T_a) = h_{total}\pi DL\Delta T \\ & P = I^2R = I^2\rho L \\ q_{total} &= P \Rightarrow h_{total}\pi DL\Delta T = I^2\rho L \Rightarrow I = \sqrt{\frac{h_{cotal}\pi D\Delta T}{\rho}} \\ & \text{Heating Dement Calculations [3]} \end{split}$$

- Temperature and Air Flow Control
 - two current-controlled solenoid valves control airflow into mixing chamber
 - pressurized CO2 comes from tank and air comes from pressurized wall source
 - regulators control set pressure to 15 PSI feeding into valves
 - K-33 BLG sensor detects CO2 concentration and microcontroller opens or closes valves to correct concentration
 - LPC1768 mbed microcontroller to be used, C++ code
 - N-Type MOSFET used to control current through valves and through heating element
 - Microcontroller detects output from Sensirion SH15 to find incubator temp and adjusts current to nichrome accordingly
- Future Work
 - Integration of the entire design for flow modeling
 - Fine-tune design of individual components to incorporate results of total flow models
 - Development of a control system to allow for recycling humidified air
 - extensive testing of entire system to compensate for sources of error
 - order materials and build physical prototype

Caroline Craig/Research Notes/Competing Designs/9/19/2021 - Previous BME Student Cell Culture Incubator (Fall 2011)

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Most design criteria including incubator conditions, cell plate compatibility, and disassembly ability are the same as our team's project, but our team's budget is much less. The microscope cell culture incubator diagram will be helpful for our team to follow, and the specific types of materials will be useful to compare for our design. The team who created this project, and all the other cell culture incubator teams, were never able to complete their designs. Going off this, our team should strive to be able to at least test a design. The heating/humidifier aspects of this design seemed semi-functional/compatible for the cell culture incubator, so our team should reference this design for our incubator.



Caroline Craig - Sep 19, 2021, 5:30 PM CDT

Title: Portable low-cost long-term live-cell imaging platform

Date: 9/19/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Learn more about imaging for live-cells

Source: https://www.sciencedirect.com/science/article/pii/S0956566314007489

- Abstract
 - current technology for live-cell imaging is often immobile, costly, and requires maintenance and high levels of skill
 - new engineering alternatives
 - discussion of "a fully automated low-cost, portable live-cell imaging system for time-resolved label-free visualization of dynamic processes in living cells"
 - The device is light-weight (3.6 kg), small (22×22×22 cm) and extremely low-cost (<€1250)
 - able to image live-cells with high quality
- Intro
 - · observation of dynamic process of cells
 - · long-term imaging is used in many different fields
 - two main components in commercial systems:
 - an optical system for visualization of cells at the microscopic level
 - an incubator for culturing cells
 - The incubator is capable of maintaining optimal conditions necessary for culturing cells, including temperature and carbon dioxide concentration as well as humidity of the atmosphere
 - Commercial systems are also complicated to use due to their vast range of functionality, bulky/immobile, and expensive
 - Article focuses on developing a low-cost portable live-cell imaging platform that is suitable for maintaining
 optimal culture conditions and parallel time-resolved imaging of mammalian cell lines
- Results
 - The device is made up of a microscope mounted on a motorized stage and an incubator for maintaining optimal culture conditions
 - off-the-shelf components, including a webcam for the microscope, temperature, gas and humidity sensors for environment control and open-source Arduino microcontroller boards for hardware control and data acquisition.
 - A software interface was developed using LabVIEW and LabVIEW interface for Arduino
 - material costs of around €300 including camera, motors, electronics, hard and software for automated and interactive control the fully assembled digital motorized microscope is extremely low-cost and compact at the same time.
 - Includes 3D printed components
 - The device is controlled using a standard laptop computer and requires 220 V power and CO2 gas supply but no additional equipment. CO2 can be provided by either a laboratory gas line or a refillable gas container.
- Incubator characteristics
 - conditions were compared to standard conditions for mammalian cell lines (above)
 - · assessed with regard to the period for reaching equilibrium upon start-up
 - data recorded from sensors every second for 48hr
 - culture conditions were met shortly after start-up
 - 0.55+-0.05*C Standard temperature deviation
 - 1±0.1% Standard deviation in CO2 levels
 - · humidity achieved via evaporation from a reservoir
 - ranged from 70-80% humidity
 - slower process (~2hours)

Caroline Craig/Research Notes/Competing Designs/9/19/2021 - Portable low-cost long-term live-cell imaging platform

- Testing long-term live-cell imaging of mammalian cells
 - prepared HEK293YFPI152L cells as necessary for imaging over 48hr. period
 - · device allowed fully automated time-resolved imaging and culture of mammalian cell lines
 - enabled visualization of the dynamic process
- Discussion
 - · device is 10 times smaller and lighter than conventional technology
 - cost of the portable device is 50-100 less expensive than commercial devices
 - device was prototyped based on a 'Makers' approach, i.e. by using 3D desktop printing, off-the-shelf components and open-source microcontroller prototyping boards and could easily be re-built or modified by individuals
 - · technology could be improved by including more sensors and other condition monitoring devices
 - " the long-term live-cell imaging platform presented in this article improves the classical technologies in terms of portability, user-friendliness and cost and provides an example of low-cost biosensor technology that is compatible with fast and resource-efficient prototype optimization"
- Methods
 - Incubator temperature
 - equipped with a total of 6 high precision thermistors (B+B Thermo-Technik GmbH, Germany) connected to a purpose-built sensor shield plugged on top of an Arduino Mega microcontroller board (Arduino, Italy)
 - measured and controlled temperature
 - two close to dish and two mounted to heating elements
 - heating elements made up of heating wire wrapped around either a frame of stainless steel or four screws arranged in a square
 - The heating elements are connected to a purpose-built actor shield plugged on top of an Arduino UNO microcontroller board
 - Temperature is controlled by a software based feedback loop using the mean temperature reading
 of both sensors installed inside the incubator as actual value and a used-defined temperature as
 set point.

$$T=~\left(rac{1}{(P+B)+\left(P^3 imes C
ight)+A}
ight)-273.15$$

$$P = \; \left(\ln \left(rac{R_{paired} imes V_{supply}}{V_{out}}
ight) - R_{paired}
ight)$$

- *T* is the temperature in degrees Celsius (°C)
- A (1.129148×10−3)
- B (2.34125×10−4)
- C (8.76741×10-8)
 - Steinhart–Hart coefficients taken from the block diagram of the *Thermistor Read.vi* included in the LabVIEW Interface for Arduino toolkit
- *Rpaired* is the value of a paired resistor
- *Vsupply* is the voltage provided by the power supply
- *Vout* is the analogue voltage read from the thermistor.
- Incubator CO2 control
 - CO2 concentration in the incubation chamber is measured using a non-dispersive infrared (NDIR) absorption sensor (Gas Sensing Solutions Ltd., UK) mounted inside the incubator
 - Sensor readings are transmitted wirelessly using a wifi to RS232 UART adaptor module
 - CO2 is injected into the chamber with a solenoid valve in lid
 - The valve is triggered to open for 50 ms when the measured CO2 concentration drops below a used-defined threshold.
 - Gas pressure supplied by a laboratory gas line was set to 0.1 bar.
- Incubator Humidity
 - Relative humidity inside the growth chamber is measured using a HIH-4030 humidity sensor
 - Humidification is achieved passively via evaporation from a printed 10 ml water reservoir placed inside the incubator

Caroline Craig/Research Notes/Competing Designs/9/19/2021 - Portable low-cost long-term live-cell imaging platform

• The analogue voltage read from the thermistor was translated to rel. humidity (%RH) using the following equation:

$$\% RH = rac{(V_{out}/1023 imes V_{supply}) - Zero \ offset}{Slope}$$

•

- Vout is the analogue thermistor reading
- Vsupply is the supply voltage (5 Vdc)
- Zero offset is the analogue voltage measured at 25 °C, 0%RH
- the supply voltage and Slope is the linear output of the thermistor measured during calibration at 25 °C and 5 Vdc
- The values for Zero offset (0.0307) and Slope (0.958) were taken from the sensor's datasheet.
- Amplitude and frequency of temperature and CO2 oscillations were analysed in LabVIEW.

Conclusions/action items:

The device researched was a low-cost portable live-cell imaging platform that is suitable for maintaining optimal culture conditions and parallel time-resolved imaging of mammalian cell lines. This device included an incubator constructed using 3D desktop printing, off-the-shelf components and open-source microcontroller prototyping boards which are similar processes to those our team will likely use for our incubator construction. The incubator monitored and controlled temperature, CO2, and humidity as specified above, and it successfully kept cells in conditions for mammalian cell culture conditions similar to those our team will replicate. Moving forward, the team should verify that we can use a CO2 gas line from the lab to supply gas to the incubator. Additionally, we should determine the appropriate sensors for measuring conditions in the incubator and begin learning to monitor those conditions using various software.



9/21/2021 - Previous BME Cell Culture Incubator (Fall 2016)

Caroline Craig - Sep 21, 2021, 8:00 PM CDT

Title: Previous BME Cell Culture Incubator: Fall 2016

Date: 9/21/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: To understand the Fall 2016 team's vision for the client's project, and to come up with ideas or aspects to include for our team's incubator.

Content:

Fall 2016 Project

- Abstract
 - "inexpensive on-stage incubator chamber capable of maintaining temperature, CO2, and humidity"
 - regulation through feedback systems
 - hoped to bridge gap between high-cost, functional systems and cheaper, less effective systems through further development
- Background/Motivation
 - cell imaging useful for research and experiments
 - current market systems are expensive and large
 - mimicking environment for cell culture requires control of temperature, humidity, and CO2 concentration
 - optical compatibility: desired magnification and size limitations
- Design Specifications
 - Temp: 37*C +- 1*C
 - Humidity: 95% +- 5%
 - CO2: 5% +- 0.5%
 - recovery in 6 seconds after 30-second chamber opening
 - demonstrate system stable for at least 2 weeks
 - compatible with various microscopes (different from our project)
- Final Design
 - · control systems independently validated, integrated to regulate CO2, RH, and temperature effectively
 - .08" plexiglass on top and bottom
 - removable for media changes



Figure 3: Systems diagram of final design.



Figure 2: CAD diagram showing the fabrications of the stage enclosure in two components

- Methods and Testing
 - Material testing:
 - quantified with % of relative image focus
 - MATLAB used with Brenner's Law
 - Environmental feedback systems
 - sensors and circuitry
 - integration into one system
- Results and Discussion
 - plexiglass allows for optimal imaging capability
 - · design regulates temperature, CO2, and humidity
 - cost and system recovery were limitations



- Future Work
 - optimize control loops and electrical circuit
 - chamber recovery testing
 - · long-term cell survival and imaging tests
 - tests between various microscopes

Conclusions/action items:

The Fall 2016 team's project description is very similar to our team's project description. Their design specifications are also similar to our team's specifications except for the error margin for environmental controls and compatibility with various microscopes. Our team is planning on designing a cell culture incubator compatible with only an inverted microscope, and our margins of error for environmental controls are narrower margins for error. The Fall 2016 team also highlights recovery for environmental controls which our team still is looking into. The Fall 2016 team's design had a removable plexiglass top and bottom, and their design included sensors to monitor and regulate environmental factors. Finally, the 2016 team wanted to continue work on control loops, chamber recovery, and imaging tests, which our team should do our best to do testing on. The box design with plexiglass top and bottom are good ideas for our team to follow.

9/23/2021 - Hypoxia Chamber for Cell Culture

Caroline Craig - Sep 23, 2021, 8:38 PM CDT

Title: Hypoxia Chamber for Cell Culture

Date: 9/23/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: To look further into possible designs for the team's cell culture incubator, and to gain insight into cell culture incubator design and fabrication.

Source: Wang, Ruoxiang et al. "A novel experimental hypoxia chamber for cell culture." *American journal of cancer research* vol. 4,1 53-60. 15 Jan. 2014

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3902232/

- Abstract
 - inflatable chamber for hypoxia experiments introduced
 - tissue hypoxia is a common pathophysiological process
 - new chamber yielded reproducible results to modular incubator
 - chamber was low-cost, easy to use, and leakage free
 - size of incubator was also adjustable
- Introduction
 - mammalian cells can adapt to oxygen depletion, hypoxia is a distinctive biological response
 - · more recent studies have been on regulating the culture cells
 - reliable experimental device for the cell culture is crucial
 - Other chambers on market include:
 - modular chamber filled with 1% O2, 5% CO2, and 94% N2, leakage was common defect
 - another model uses infusion of N2 with an external high-pressure liquid nitrogen tank
 - third model is a workstation that can offer precise control of O2, CO2, temperature and humidity, but it is expensive
 - The inflatable chamber in this study was successful in cell culture, made of transparent plastic materials, didn't create a pressurized environment, was cost-effective, and was adjustable in size
- Materials and methods
 - Cell lines, culture conditions, reagents
 - cells either cultured either in normal conditions (37*C and 5% CO2) with 21% O2, or in inflatable hypoxia chamber (1% O2, 5% CO2, balanced with N2 and humidified) placed in 37*C
 - Designing and assembling the inflatable chamber
 - Two pieces of transparent polyester barrier membranes of 4 Mil cut and sealed at edges with heat impulse sealer to make the pouch
 - one edge equipped with an airtight "zip-lock"
 - two small holes at corners for gas exchange tubes
 - sterilization by placing under germicidal UV irradiation
 - cell plate placed at center of pouch
 - clean sponge soaked with water placed inside chamber before sealing to get moisture in air
 - after sealing chamber, it was filled with gas using port holes, then once at correct percentages ports were sealed
 - finally, chamber placed in 37*C incubator

Caroline Craig/Research Notes/Competing Designs/9/23/2021 - Hypoxia Chamber for Cell Culture



- Protein extraction and immunoblotting
- Transient transfection and reporter gene assays
- Vascular endothelial growth factor (VEGF) ELISA
- Monitoring GFP-HIF-1a translocation
- HUV-EC-C tubular formation assay
- Results
 - the inflatable chamber effectively created a hypoxia environment



- chamber was applicable in hypoxia experiments for real-time studies
 - good for studying in real-time
 - small size and transparency of chamber was easy to work with
 - successful test results for two different experiments, that were comparable to experiments using modular chamber, show the inflatable chamber can create and maintain cell culture environment

Conclusions/action items:

The inflatable hypoxia chamber had a unique design in comparison to common hypoxia and cell culture chambers. It was easy to use, easy to see cell culture in real-time, small in size, and low-cost, all attributes our team hopes to achieve in our cell culture incubator. A design using characteristics from this design, like including sealed plastic sides and tubes for filtering gas exchanges, could be successful for our project

specifications. A difference from this design that our team would need to account for is that the incubator needs to regulate temperature in its internal environment, not be in an environment of the same temperature.

9/26/2021 - Previous BME Cell Culture Incubator Spring 2017

Caroline Craig - Sep 26, 2021, 6:04 PM CDT

Title: Previous BME Cell Culture Incubator Spring 2017

Date: 9/26/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: To understand the Spring 2017 team's vision for the client's project, and to come up with ideas or aspects to include for our team's incubator.

Source: Peter Hartig, Steven Gock, Jenny Westlund, Jack McGinnity, Trevor Zarecki

Content:

*This project is a continuation from the same team of continuing testing of the project from Fall 2016

- Abstract
 - client needs for incubator are same as for our team:
 - on-stage incubation chamber
 - capable of maintaining temperature, CO2, and humidity
 - prototype for this use was developed by team
 - regulates parameters through feedback systems
 - hope to bridge gap between high-cost, functional systems and cheaper, less effective ones
- Background
 - real time imaging is crucial in performing new assays
 - market need for affordable and more robust system for real time cell imaging
 - mimicking internal environment of incubator requires control of three conditions: temperature, CO2 concentration, and humidity
 - optical compatibility: desired focal length and size limitations
- Design Criteria
 - same internal environmental controls for physiological maintenance as our team:
 - 37*C +- 1*C
 - humidity: 95% +- 5%
 - CO2: 5% +- 0.5%
 - Recovery time of 6 seconds after 30 second chamber opening
 - difference from our project: compatibility with various microscopes
- Market Demand
 - current market limitations in regards to expensive systems being limited to one microscope and less expensive systems
 offering poor environmental control
 - Attributes for 2017 team's design:
 - simplicity
 - affordability
- Design Solution
 - · able to effectively integrate CO2, humidity, and temperature regulation for each control system independently
 - LCD display of environmental conditions
 - 3/32" glass below cell culture to optimize imaging
 - removable lid for fast plate revomal
 - Costs:

Design Area	Product names	Category Cost
Environment Sensors	DHT-22, MHZ -16	\$80.05
Environment Control Components	Fluid valve, Immeserion Heater	\$50
Structural Materials /PCB	ABS filament, acrylic, glass	\$76.79
Testing Materials	CO ₂ and media	\$13.94

Totals to \$220.63 (well over our team's budget)

- Methods and Testing
 - · Past semester testing:
 - sensors and control circuitry for each environmental feedback system integrated into one system
 - 2017 spring semester testing:
 - integrated power supply and PCB
 - longer-term integrated testing
 - Cell imaging tests
 - updated prototype able to capture cell images
 - achieved focus similar to control
 - · Cell culture tests
 - Scratch assay
 - standard incubator
 - final prototype
 - ambient conditions



Figure 6. Images of cells in A) incubator, and it) Figure 7. Images of cells migration in A) on TCPS control at 20X magnification. Focus measure A = 31.8641% 9 = 31.0734%.

standard incubator, 8) final prototype, and C) in ambient conditions. Lines added to show soutch "healing".

Results

0



Figure 8. Humidity and Temperature data from first semester prototype.



- Figure 9. Humidity, temperature, and CO2 data from final prototype. 0
- For final prototype: o
 - CO2 and humidity seemed to have large fluctuations past error margins
 - temperature dipped out of error margins but was more constant than other parameters

- Impact and Future Work
 - · extended testing duration with variety of cell types
 - further optimization of environmental control
 - selection of sensors/components for larger volume production
 - design changes for manufacturing
 - injection molded outer casting
 - robust materials for sterilization

Conclusions/action items:

Further testing of this team's microscope cell incubator over the following semester improved the environmental conditions of the incubator design. The 2017 team did have a larger budget than our team and the microscope was design needed to be compatible with multiple microscopes in comparison with our team's project, but both projects have the same design requirements for internal incubator conditions. This design appears to have good sensor usage and feedback control for the environmental factors, although the team did believe other sensors and components would need to be used for larger volume production. It would be beneficial for our team to use the sensors and feedback systems from this cell culture incubator in our design, just furthering testing and compatibility with our design ideas.

10/1/2021 - Arduino Introduction

Caroline Craig - Oct 01, 2021, 1:35 PM CDT

Title: Arduino Introduction

Date: 10/1/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: To further solidify a basic understanding of the fundamentals of the Arduino software the team will be using for this project.

Source: https://www.arduino.cc/en/Guide/Introduction

Content:

What is Arduino?

- open-source electronics platform based on easy-to-use hardware and software
- Arduino Boards:
 - able to read inputs and turn them into an output
 - · can send set of instructions to the microcontroller on the board using Arduino programming language
- · The tool is easy to use program for fast prototyping aimed at students without a background in electronics and programming

Why Arduino?

- · simple and accessible user experience
- runs on Windows, Mac, and Linux
- can be used to build interactive prototypes
- · inexpensive, cross-platform, clear programming environment, open-source software and hardware

How to use Arduino?

- Getting Started guide: https://www.arduino.cc/en/Guide/HomePage
- tutorials: https://create.arduino.cc/projecthub? _gl=1*vtdrei*_ga*NTY0NzU1NjIwLjE2MzMxMTE5NDQ.*_ga_NEXN8H46L5*MTYzMzExMTk0NC4xLjEuMTYzMzExMjI4OS4w
- Arduino reference: https://www.arduino.cc/reference/en/

Conclusions/action items:

The team will be using the Arduino software in our design since it is simple and aimed at students with no prior programming knowledge. We plan to use an Arduino board in conjunction with the software to program our sensors to read and regulate environmental conditions in the incubator. Other members of the team have more experience using this software/language. I took a Python programming class over the summer with should have some relevance to learning this coding language. Additional practice and tutorials will be needed to fully understand Arduino.



Caroline Craig - Oct 18, 2021, 8:08 PM CDT

Title: Initial BPAG Meeting Notes

Date: 10/1/2021

Content by: Caroline Craig

Present: Caroline Craig and all BME BPAGs

Goals: To fully understand the process of being in the role of BPAG.

Content:

BPAG, Biomedical Purchasing and Accounting Group

- Presentation: Guidelines for BME Design BPAGs (pdf)
- · Document: Guidelines for proposals for design project funding-from the department (pdf)
- Document: Design class invoice (doc)
- Link: <u>BPAG expense spreadsheet template</u> (google sheet)

General Concept/Job:

- Get Client to purchase for you
- · have all expenses approved by your client prior to purchase
- keep track of all purchases
- all original detailed receipts in the notebook
- table of expenses notebook, progress report and report
- SAVE ALL ORIGINAL RECEIPTS!!!
- Contact Susan (or Dr. P) with any problems:
 - susan.sauer@wisc.edu

Client Type: UW Affiliation BME Dept. (Dr. P!!)

- UW Funds? --> Yes. Department teaching funds
 - follow UW purchasing rules
 - Client pays:
 - Shop@UW
 - Procard/no tax
 - Funding string
 - Makerspace, TeamLab, Other UW services
 - Work with client dept accountant for reimbursement (90-day rule)

Vendors available to UW Clients

- Shop@UW
 - complete list: : https://shopuw.wisc.edu/vendors-2/
 - · Guest login: : https://mds.bussvc.wisc.edu/order/shopper_lookup.asp
- DoIT:

Accounting

- Table with ALL of the vital information needed to purchase again
- Put table in:
 - Progress reports
 - team part of notebook
 - Report

Reimbursement

Reimbursement

Only the DPAG will be minimum, when your insurpresentations, neutring, Do nor ALL sock individual minimum-ment from your clients or the

- E.Reinsburgement from UW-Cliente start believ the poster semiond
- BEAG must provide original, hard anyy receipt(s)
- Date, parchaser name, worker details, teening disensity details and quarticly, and easy, and easy.
 Not a screen date. Not obtain Priorid coust in OK, PDP'ss OK, paper register recorder in OK.
- Requires a callel project marries, obtained from the UW class.
- Electronically submitted Takes 3+ washa
- 10 day rule as combursement beyond 90 days of purchase so exceptions (and at limit 10 days for processing)

For Amintanan

- If Chert is a <u>BME Professor</u> Corract Summ Sourr
- If Close is a <u>Non-HWE, UW MD</u>. Work with MD, or their support shall
 Close is a <u>Non-EW</u>: Contact Closet

Conclusions/action items:

The main job in the BPAG role is to record the purchases and expenses for the team to ensure we stay within our budget. Since our client is also the BPAG faculty resource, they can be contacted through the communicator with any purchasing questions. When purchases are made, they should be put into the expenses table which should be put in the progress reports, team part of the notebook, and in the final report. Finally, all materials should be checked for availability in Shop@UW since our client is paying using UW departmental funds.



Caroline Craig - Dec 06, 2021, 9:01 AM CST

Title: Questions for Client

Date: 9/14/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: To come up with meaningful questions to ask the client

Content:

- 1. Will this project be paid for using UW Funds?
- 2. Will you be paying for this project?
- 3. When you imagine the finished product, what color would you want it to be?
- 4. What are your preferred dimensions for the incubation chamber?
- 5. How often do you plan on using this device daily?
- 6. What types of cell culture plates do you use?
 - What are their dimensions?
 - · What type of medium do you use?
- 7. Will any other microscopes be used with this incubation chamber? Or, should it only be compatible with the inverted microscope?

Conclusions/action items:

Add questions to shared team document. Following the preliminary client meeting, update with clients anwers/prefernces for the project. Finally, add information gained from client meeting regarding specifications to the PDS document.



Caroline Craig - Oct 18, 2021, 8:09 PM CDT

Title: Responses from Client

Date: 9/19/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: To come up with meaningful questions to ask the client

Content:

1. Will this project be paid for using UW Funds

• The project will be paid for using Department teaching funds

- 2. Will you be paying for this project?
 - Same as above

3. When you imagine the finished product, what color would you want it to be?

- No preference in color
- Plates are clear, black (stops contamination), and white (increases light)
- · Something that blocks out external light would be ideal, but is not required
- 4. What are your preferred dimensions for the incubation chamber?
 - Sits on the microscope stage and holds a well plate

5. How often do you plan on using this device daily?

- The device would be used for one week at a time during tissue lab
- 6. What types of cell culture plates do you use?
 - What are their dimensions?
 - 6 well plate, 24 well plate, 90 well plate --> omnitrays?
 - Standard petri dish
 - Flasks --> T25/T75 not really used but her
 - What type of medium do you use?
 - MEM
 - 10% SPS and antibiotics
- 7. Will any other microscopes be used with this incubation chamber? Or, should it only be compatible with the inverted microscope?

• Focus on compatibility with an inverted microscope

Conclusions/action items:

The client was very helpful with responses to our questions, and their information introduced new research topics for the project. New research topics to look into include industry standards, cell culture, sterilization, and past projects. Looking into industry standards and sterilization will help the team to ensure our incubator is up to quality and cleanliness standards for product longevity. Research on cell culture will help to team to understand how the incubator will help maintain cell life. Finally, research on previous team's projects will help our team to establish a starting point and improve upon other designs.

Caroline Craig/Design Ideas/9/19/2021 - Responses from Client



Caroline Craig - Sep 27, 2021, 5:02 PM CDT

Title: Design Idea 1: "The Box"

Date: 9/21/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: To brainstorm an idea for our microscope cell culture incubator that can maintain the cell growth environment and allows for viewing.

Content:



- The inspiration for this design idea is from previous BME 200 projects for this client
 - the two I have researched so far use this design shape
- The design includes:
 - heating coils between the exterior and interior layers to alter the temperature and keep internal conditions near 37*C
 - Humidifier input to connect a tube from external humidifier to incubator interior and keep internal conditions near 95% humidity
 - CO2 input to connect a tube from CO2 tank or lab spigot to incubator interior and keep internal conditions near 5% CO2
 - · CO2, temperature, and humidity sensor inputs to connect to a laptop for internal incubator condition monitoring
 - 3D printed exterior case
 - · Removable side for Cell Culture Plate removal and insertion with cell plate stabilizers to prevent extra movement
 - Transparent plexiglass top and bottom to allow for viewing and light
- · The design is imagined to rest on the microscope stand and have cord and tubes connecting to external devices off of it

Conclusions/action items:

This preliminary microscope cell culture incubator is entitled "The Box" representative of its shape. The design is inspired by the previous BME 200 projects for this client from 2011 and 2016 design shapes and is intended to sit on the microscope stage. The inputs and sensors for CO2, temperature, and humidity are for maintaining the internal environment. The top and bottom are plexiglass for viewing and to let in light, and the

side removes for inserting cell plates. Future work from this design would be to decide on sensors, tubes, and eternal devices for the incubator. Also, design dimensions need to be added.



Caroline Craig - Sep 27, 2021, 5:03 PM CDT

Title: Design Idea 2: "Inflatable Box"

Date: 9/24/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: To build off of design 1, but including ideas from hypoxia chamber research



- The inspiration for this design idea is from previous BME 200 projects for this client and research on the hypoxia chamber for cell culture
 - The box shape is used by previous BME 200 project groups
 - The inflation aspect is used in the hypoxia chamber for cell culture to promote gaseous flow and limit pressure on the cell culture
- · The design includes:
 - heating coils between the exterior and interior layers to alter the temperature and keep internal conditions near 37*C
 - Humidifier input to connect a tube from external humidifier, or water source, to incubator interior and keep internal conditions near 95% humidity
 - CO2 input to connect a tube from CO2 tank or lab spigot to incubator interior and keep internal conditions near 5% CO2
 - · CO2, temperature, and humidity sensor inputs to connect to a laptop for internal incubator condition monitoring
 - 3D printed exterior frame case for edges
 - Removable side for Cell Culture Plate removal and insertion with cell plate stabilizers to prevent extra movement
 - Transparent plexiglass top and bottom to allow for viewing and light
 - Three Inflatable, insulated sides
 - the sides of the incubator not connecting to external devices would be made from a flexible PVC plastic coated with an insulating material on the interior (black or white internal material)
 - these sides would allow gases to flow through the interior environment of the incubator and reduce internal pressure that may affect cell culture growth
- · The design is imagined to rest on the microscope stand and have cord and tubes connecting to external devices off of it

Conclusions/action items:

Design 2 is largely based off of Design 1, but Design to includes an inflatable sides aspect. The inflatable sides will be included on the three sides of the incubator not connecting to external devices and will be made of a flexible PVC plastic coated with an insulating material on the interior. The other modification on this design from Design 1 is that the 3D printed box component will be more of a box frame for the edges, and it will have one fully 3D printed side for connections to external devices. The final component to this design is dimensions which will be added once we meet with the client and see the microscope on 9/28/2021.


Caroline Craig - Oct 18, 2021, 8:10 PM CDT

Title: Design Matrix Evaluation

Date: 9/28/2021

Content by: Caroline Craig, Ethan Hannon, Olivia Jaekle, Maya Tanna, Katie McGovern, Sam Bardwell

Present: Caroline Craig

Goals: To document design matrix and provide reasoning for rankings. Will be shared with the team.

Content:

			Past Project B	18 shuthished				
Rank	Criteria	Weight	Score (10 max)	Weighted Score	Score (10 max)	Weighted Score	Score (10 max)	Weighted Score
1	Internal Environment	25	9	23	7	18	5	13
2	Microscope Compatibility	20	10	20	10	20	10	20
3	Accuracy and Reliability	20	7	14	8	16	4	8
4	Ergonomics	15	5	8	8	12	4	6
5	Cost	10	2	2	4	4	3	3
6	Life in Service	5	10	5	10	5	10	5
7	Safety	5	10	5	10	5	10	5
	Sun	n 100	Sum	76	Sum	80	Sum	60

- Internal Environment
 - For this criteria, the Past Project Refurbished scored the highest since the previous BME groups have already done testing on the device's ability to regulate temperature, CO2, and humidity. Our team believed that further work on this system could have improved the device's ability to maintain these conditions by improving the materials. For these reasons, we gave Past Project Refurbished a 9.
 - The Heated Water Pump Incubator scored the next highest because our team believes improving upon previous BME groups' designs by using a heated water tube would benefit the ability to create a better cell culture environment. It scored lower than the Past Project Refurbished design because we would not have the previous testing to use. For these reasons, we gave Heated Water Pump Incubator a 7.
 - Finally, the Shelving Incubator scored lowest with a 5 because the ability of our team to maintain the conditions once the drawers were pulled out had not been completely understood.
- Microscope Compatibility
 - All designs scored a 10 in microscope compatibility because each design was created and could successfully be used with an inverted microscope.
- Accuracy and Reliability
 - For this criteria, our team scored the Heated Water Pump Incubator highest. We believe that the finalized design would have a more reliably designed system for the intended use of the incubator with the materials and external devices we plan to use. For this reason, gave this design an 8.
 - The Past Project Refurbished design scored the next highest with a 7. Like the Heated Water Pump Incubator, the Past
 Project Refurbished design would have improved upon materials in comparison with previous BME projects, but the
 mechanics of the system would not be as reliable as the other incubator.
 - The Shelving Incubator received the lowest score of 4 because altering the shape of the environment by opening a drawer would be difficult to maintain accurate internal conditions, and the size of the machine may hinder its reliability in reading accurate conditions. Also, moving components are more susceptible to wear and tear making it less likely to live through its self-life
- Ergonomics
 - Our team scored the Heated Water Pump Incubator highest for this criteria, again because its materials and components would allow it to function the best in comparison with our other designs. For this reason, it scored an 8.
 - The Past Project Refurbished design scored a 5 because the design components implemented by previous BME teams that we planned on keeping the same would not function in maintaining internal environment conditions as the Heated Water Pump Incubator could.

Caroline Craig/Design Ideas/9/28/2021 - Design Matrix Evaluation

- 290 of 336
- Finally, the Shelving Incubator scored lowest with a 4 because it would be the most difficult to use with having to pull out drawers each time one wanted to view a sample.
- Cost
 - All the designs scored low for cost because our team's smaller budget will be difficult to stay in range with. The Heated Water Pump Incubator scored the best with a 4 because lots of the components we plan on using will be provided to us. Our biggest difficulty in staying within the budget will be limiting the need to repurchase materials wasted in prototyping.
 - The Past Project Refurbished design scored a 3 because components of the previous design would be reused, but the components we plan on replacing would end up being more expensive than just creating the Heated Water Pump Incubator design.
 - The Shelving Incubator scored lowest with a 2 because its size would increase the cost and create a greater likelihood to go over budget if lots of prototypes are made.
- Life in Service
 - All the designs scored a 10 for Life in Service because they were designed with the intent of functioning for a week period of time every year for 10 years.
- Safety
 - All the designs scored a 10 for safety because the components involved in their designs would not be harmful to the user in any way.

Conclusions/action items:

Based on this design matrix, our team will be moving forward with creating the Heated Water Pump Incubator for our client. This design was ranked the reliable, ergonomic, and cost-effective in comparison with the other designs. The design will include a slot for the well plate, a tube containing heated water to maintain a 37*C temperature and assist in evaporation, and a water well for evaporation water to maintain high humidity. The dimensions of the incubator will match the size of the microscope stand, or it will go over the edges slightly, and the height will not exceed the lowest point of the top light microscope component. Finally, sensors compatible with Arduino will be used to regulate the internal conditions.



Caroline Craig - Sep 28, 2021, 10:35 PM CDT

Title: In-person Client Meeting Notes

Date: 9/28/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: To document dimensions of components obtained from meeting with the client and cite the answers to our team's most recent questions.

Content:

1. What is the exact model of inverted microscope for use? (for accurate dimensions)



- 1. Olympus IX71
- 2. Nikon Eclipse Ti- S

1. Don't want to change the distance sample is from the lens (32.40mm) thickness

- 2. 310 x 300 mm
- 2. Could we use a laboratory CO2 gas line? Or, will an external CO2 gas supply be necessary to include in materials?
 - 1. Tank with a regulator, hose into incubator
 - 2. Don't need to purchase, readily available with hoses
 - 1. What is the diameter of the hose? 7.16mm wide
- 3. How many cell plates do you need in the incubator?
 - 1. One Prefers just one well plate per incubator
- 4. Would it be possible for us to test transparent materials with the microscope?
 - 1. Optically clear enough?
 - 2. Refraction of light?
 - 3. Bottom of glass on multiwell plates.. Look into
 - 4. YES ALL POSSIBLE

5. What is the use of the incubator during the week of class time?

1. AN ENTIRE WEEK

- 6. Do you have any specifications in the margins from industry standard? Or, is the tolerance cells can handle acceptable?
 - 1. pH levels \rightarrow CO2 levels, what is tolerance for a buffer? (Can look up online)
- 7. What are the dimensions of the well plates?
 - 1. length = 127.44 mm
 - 2. Width = 84.91mm
 - 3. Height = 21.60mm
- 8. What would be the ideal recovery time for internal conditions after opening the cell culture incubator "door"? (Flow rates)
 - 1. Five minutes after 30 second opening
- 9. Would you prefer manual CO2 addition, or an automatic regulation with sensors?
 - 1. Incubator itself has a valve and a sensor \rightarrow automatic prefered
- 10. Is the budget for the final design, or does it include materials for preliminary designs?
 - 1. Yes but if the prototype works well then it can be flexible

Notes:

- Current incubator is water jacketed with co2 tank at ~10psi
- · Microscope is able to lift head up so that we can fit the incubator in

Conclusions/action items:

After meeting with the client in the lab, our team obtained the dimensions of the microscope that the incubator will need to be compatible with. The stage is 310x300 mm and the distance from the top of the stage to the component above it was 32.40mm. This means our team will not want to exceed a 32.40mm height on our incubator. The well plates are 127.44mm in length, 84.91mm in width, and 21.60mm in height. Also, our original plan was to design an incubator that could hold multiple well plate, but the professor prefers that the incubator only holds one at a time. We will be able to access the lab for optical clarity of the transparent components of our design, and we learned that the incubator will need to reliably be used 24/6 for a week during the semester. Finally, we learned that the client would prefer the incubator have a 5 minute recovery time are a 30-second opening, and the CO2 should automatically be regulated.



10/4/2021 - Spreadsheet for Expenses Layout

Caroline Craig - Dec 12, 2021, 8:23 PM CST

Title: Spreadsheet for Expenses Layout

Date: 10/4/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Create a spreadsheet that will be used to record all purchases for the project

Content:

Rem	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	Link
Category 1 : Incubator	Concerning of the	Warmoures		jan series	1.00	Street of		
				-		-	-	-
Category 2 : Components								
			-	-	-	-	-	-
						TOTAL:	\$0.00	1

Conclusions/action items:

This spreadsheet is a template. Throughout the semester all purchases for the project will be recorded in this spreadsheet. The two purchase categories include purchases for the incubator itself and purchases for incubator components. The goal for the team is to keep the total fabrication cost below \$100.



Caroline Craig - Dec 11, 2021, 8:00 PM CST

Title: Preliminary Purchasing Request

Date: 10/4/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Create an initial materials list

Content:

Microscope Cell Culture Incubator

Team: Maya Tanna, Sam Bardwell, Katie McGovern, Ethan Hannon, Olivia Jaekle, Caroline Craig

Lab: 27694

Advisor: Melissa Kinney

Project Summary: Develop a low cost cell culture incubation chamber with interchangeable culture plates that is compatible with an inverted microscope and capable of live cell imaging.

Supplies/Materials for purchasing:

- 1. Polycarbonate Transparent Thermal Insulation Sheets
 - https://www.airgas.com/product/Safety-Products/Head%2C-Eye-%26-Eace-Protection/ Welding-Lens/Welding-Lens--Passive/p/RAD64005012 (\$0.50 ea.)
 - b. <u>https://www.airgas.com/product/Safety-Products/Head%2C-Eye-%26-Face-Protection/</u> Welding-Lens/Welding-Lens---Passive/p/RAD64005034
- 2. Plastic Latches (x4)
 - a. Cambro 60246 2 Hole Plastic Kit (\$4.69 ea.)
- 3. Epoxy Glue (Makerspace) (\$1.50 a packet)
- 4. Rubber Strips
- 5. 3/8x1/2 Plastic Tubing
- 6. Tube Connector
 - https://www.fishersci.com/shop/products/hamilton-luer-lock-adapters-hamilton-valves-2/ p-201613#?keyword= \$14.96
- 7. Metal Tube Connectors
- 8. Pre-Existing Materials from Past Projects 1
 - a. CO₂ Sensor
 - b. AOSONG DHT22 Humidity and Temperature Sensor
 - c. Rubber Lining
 - d. Alligator Clips
 - e. TIP120 Arduino Transistor x3
 - f. ED1543-ND Rectangular Male Connector

Implementation of Materials into Design:

- The transparent insulation sheets will be used on the top and bottom of the incubator to allow the microscope optics and lighting to be used properly while also maintaining a 37°C temperature.
- The plastic latches will be used on the longer sides of the incubator near each corner to seal the removable lid to the incubator and prevent leakage of gases or heating.
- 3. Epoxy glue will be used on various parts of the incubator to securely connect components.
- The rubber lining will be used on the inner rim of the lid and upper part of the incubator to ensure a tight seal to maintain internal conditions of the incubator.
- The plastic tubing will be snaked through the water bath in the incubator to allow the heated water running through it to heat the water to maintain heat and humidity conditions in the incubator.
- The tube connectors will be used to connect the external tubing to the internal parts of the incubator.
- The metal tube connectors will connect the heated water pump to each end of the stainless steel metal tube in the incubator.
- Existing components will be reused and possibly implemented after their functionality and compatibility to the design have been evaluated.

Timeline and Upcoming Goals:



Conclusions/action items:

This preliminary purchasing request was created based on materials needed for design from an initial discussion with the team. This preliminary list will be first sent to the client to see if he has any materials available before another request is sent for purchases to be made. Throughout the semester changes will be made to the purchasing request based on changes to the design or new materials needed by the team. Discussion of the use for each material is included. All purchases expect to be completed before October 22nd.



Caroline Craig - Dec 11, 2021, 8:50 PM CST

Title: Initial Purchases

Date: 11/3/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record initial purchases made by the client for the project

Content:

Item	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	Link
Component	I: Incubator		6 				· · · · ·	
Transparent Cover Plates	top and bottom glass of incubator	Radnor	64005034	10/29	2	\$1.04	\$2.08	link
Component :	2: Components							
1.5mm Tube Connector	connection between CO2 tank and incubator	Fisher Scientific	35031	10/29	1	\$14.96	\$14.9 6	LINK
TOTAL:	\$17.04			÷	1	82	12-12	

Conclusions/action items:

This is the expenses table from the team's weekly progress report #8. Recorded are two purchases made by the client ahead of approval from the team. The tube connectors in components may not be exactly what the team needs dimension-wise for the project, but there may be a way to implement them into the final design. The current total expense for the project is only \$17.04.



Caroline Craig - Dec 11, 2021, 9:31 PM CST

Title: Updated Purchases

Date: 11/10/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record updated purchases made by the client for the project

Content:

ltem	Description	Manufacturer	Part Number	Dute	QTY	Cost Each	Total	Link
Component I	: Incubator				22	192. 		
Transparent Cover Plates	top and bottom glass of incubator	Radnor	64005034	10/29	2	\$1.04	\$2.08	lick.
3D Printed Case	Sides and removable top of incubator	UW Makerspace	N/A	11/10	1	\$30.00	\$30.00	N/A
Component 2	: Components				-			-
1.5mm Tube Connector	connection between CO2 tank and incubator	Fisher Scientific	35031	10/29	1	\$14.96	\$14.96	LINK
TOTAL:	\$47.04							

Conclusions/action items:

This is the expenses table from the team's weekly progress report #9. Recorded is the 3D printed case purchase from the UW Makerspace. The casing is the base for our incubator and different components will be added to in for functionality purposes. The current total expense for the project is \$47.04.



Caroline Craig - Dec 11, 2021, 9:34 PM CST

Title: Client Purchases

Date: 12/1/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record recent purchases made by the client for the project

Content:

ltem	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	1.ink
Component 1	Incubator							
Transporent Cover Plates	top and bottom glass of incubator	Radnor	64005034	10/29	2	\$1.04	\$2.08	liek
3D Printed Case	Sides and removable top of incubator	UW Makerspace	N/A	11/10	1	\$32.32	\$32.32	NA
Component 2	Components							
.5mm Tube Connector	connection between CO2 tank and incubator	Fisher Scientific	35031	10/29	1	\$14.96	\$14.96	LINK
3/8 and 1/4 in. Polyethylene Tubing	heated water will flow through	USA Sealing	55YU99	11/23	i	\$1.96	\$1.96	LINK
Barbed Vacuum Connector	connection between tubing	Grainger	<u>SZMHI</u>	11/23	2	\$0.95	\$1.90	LINK
TOTAL	\$53.22			1	1.			

Conclusions/action items:

This is the expenses table from the team's weekly progress report #11. Recorded are the polyethylene tubing and barbed vacuum connectors purchases. The tubing and connectors will be used in conjunction with a heated water pump to create the necessary temperature condition for cell culture. The current total expense for the project is \$53.22.



Caroline Craig - Dec 11, 2021, 10:03 PM CST

Title: Optical Testing - Imaging

Date: 12/05/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: To image cells under the microscope, with and without the transparent, polycarbonate sheets, so the team can determine whether or not the glass being used interfered with the optics of the microscope.

Content:

Images acquired of cells under the microscope are attached below.

- The images attached below were acquired in the lab using an imaging software and the microscope
- Images "Captured Bright 1" and "Captured Bright 2" will not be assessed since the images are hard to depict due to their dark nature
- Images "#1" and "#2" were then assessed to determine if there was a difference in optical clarity between the two

Location: Team Activities > Testing and Results > Experimentation > Optical Testing

Conclusions/action items:

The two attached images were acquired in the lab. Both images were taken using a Nikon Microscope and the "NIS-Elements" software. Image #1 was taken with the transparent, polycarbonate sheet and Image #2 was taken without the sheet. These images will then be assessed using ImageJ and the microscope focus quality plugin.

Caroline Craig - Dec 11, 2021, 9:52 PM CST



_1.jpg(549.3 KB) - download



_2.jpg(503.6 KB) - download

Caroline Craig - Dec 11, 2021, 9:52 PM CST



Captured_Bright_1.jpg(634.9 KB) - download

Caroline Craig - Dec 11, 2021, 9:52 PM CST



Captured_Bright.jpg(657.7 KB) - download



Caroline Craig - Dec 06, 2021, 9:08 AM CST

Title: Research and Client Questions

Date: 9/15/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Research: documented under Research Notes > Biology and Physiology
 - Live-Cell Imaging and Other Light Microscopy Techniques
 - Evaluation of Automated Cell Culture Incubators
- Client Questions: documented under Design Ideas > Questions for Client
- · Looked over the BPAG role at: https://bmedesign.engr.wisc.edu/course/resources#preliminary-presentations

Conclusions/action items:

This week, our team picked our roles, began researching relevant topics for the project, and came up with questions for our client. My contributions to the research included looking into Live-Cell Imaging and Automated Cell Culture Incubators to get an idea of what the concept behind this project is. I then came up with some questions for the client related to what I had researched. Finally, I looked over the instructions for my role as BPAG on the BME website.



9/22/2021 - Competing Designs and Design Idea 1

Caroline Craig - Dec 06, 2021, 9:25 AM CST

Title: Competing Designs and Design Idea 1

Date: 9/22/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Research: documented under Research Notes > Competing Designs
 - Previous BME groups (2011, 2016)
 - Portable low-cost long-term live-cell imaging platform
- Design Ideas: documented under Design Ideas
 - Design Idea 1
- · Brainstorming for Product Design Specifications: based on meeting with the client
 - meets necessary environment for successful cell culture
 - fits under microscope
 - no necessary specifications for the appearance
 - easy to use/assemble

Conclusions/action items:

This week the team met with the client and discussed their preferences for the product design specifications. Based on this discussion, I then conducted research on previous BME teams' projects since the client referenced some of their strengths and weaknesses during our meeting. Then, I came up with, and sketched, my ideas for some possible microscope cell culture incubators. Finally, I began brainstorming requirements that should be included in our PDS.



Caroline Craig - Dec 11, 2021, 7:37 PM CST

Title: Research and Design Idea 2

Date: 9/29/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Research: Research Notes > Competing Designs
 - Previous BME groups (2017)
 - Hypoxia Chamber for Cell Culture
- Design Ideas: documented under design ideas
 - Design Idea 2
- Client Meeting:
 - learned about how microscope for incubator functions
 - learned more about the lab equipment (CO2 tanks, etc.) and large cell culture incubators in the lab

Conclusions/action items:

This week the team met with the client in the lab and created/evaluated our design matrix to decide what design we will be fabricating this semester. Neither of my design ideas were evaluated in the matrix, but other designs had similar qualities to mine. Before the meeting, I continued research on previous BME group's incubators and another competing design that had an "inflatable" feature. Finally, I learned more about the lab the cell culture incubator would be used in with other team members.



10/6/2021 - Arduino, SolidWorks, and Preliminary Deliverables

Caroline Craig - Dec 11, 2021, 8:11 PM CST

Title: Arduino, SolidWorks, and Preliminary Deliverables

Date: 10/6/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Research: Research Notes > Materials and Software
 - explored the Arduino website to learn more about the software and its possible applications to our project
- Designing in SolidWorks: Review
 - Got a brief refresher from Sam on how SolidWorks works
 - Reviewed:
 - Extruded Base
 - Extruded Cut
 - Fillet
 - Part versus Assembly
- Preliminary Deliverables
 - Added design matrix and evaluation to team notebook and preliminary report
 - See: Team Activities > Design Process > Design Matrix
- BPAG Meeting: Research Notes > BPAG
 - officially learned about BPAG role
 - created spreadsheet for expenses and a preliminary purchasing request
 - Preliminary Purchasing Request: BPAG Resources > Preliminary Purchasing Request
 - Spreadsheet for Expenses: BPAG Resources > Spreadsheet for Expenses Layout

Conclusions/action items:

In the past week, the team has shifted from the design to the fabrication process for our project. In preparation for weeks to come, I learned more about Arduino coding and SolidWorks since the team will be using these two things. Additionally, we began working on the preliminary deliverables so I contributed to the Design Matrix and evaluation portions. Finally, I had a BPAG meeting where I learned more about my role for the semester, and I created a purchasing request and expense spreadsheet that will be used by me throughout the rest of the semester.

10/13/2021 - Preliminary Deliverables and Small Groups

Caroline Craig - Dec 11, 2021, 8:27 PM CST

Title: Preliminary Deliverables and Small Groups

Date: 10/13/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Preliminary Presentation
 - Worked on Design Matrix, Discussion of Design Matrix, Future Work, and Upcoming Project Goals slides
 - Added information about research I conducted to competing designs sections
 - Edited layout and added figures/dimensions where applicable
- Preliminary Report
 - Created Design Matrix and Discussion of Design Matrix based on team notebook notes from meeting
 - made edits to grammar, formatting, and figures as needed
- Small-Group: Testing Protocols and Testing
 - broke into small groups for the second part of the semester
 - will be working with Maya on testing protocols and conducting testing

Conclusions/action items:

This week the team worked heavily on preliminary deliverables for the upcoming preliminary presentation. I contributed most to the Design Matrix portions of both the preliminary presentation and the preliminary report. Additionally, I discussed with the team finalizing the materials to send to the client. Finally, Maya and I began discussing our plans post preliminary presentation regarding testing protocols.

10/20/2021 - Preliminary Presentations and Materials Ordering

Caroline Craig - Dec 11, 2021, 8:33 PM CST

Title: Preliminary Presentations and Materials Ordering

Date: 10/20/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Materials Ordering
 - Sent needed materials from the Preliminary Purchasing Request to the client for discussion
- Preliminary Presentation
 - Made final edits
 - Practiced part for the presentation

Conclusions/action items:

Work this week was a continuation of work from last week in preparation for the preliminary presentation. I made any final edits to the slides based on the advisor's feedback, and I practiced my part alone and with the team. Additionally, I finalized the Preliminary Purchasing Request with Maya and Katie to send to the client for feedback before any purchases are made. Finally, I completed peer/team preliminary evaluations.



Caroline Craig - Dec 11, 2021, 8:39 PM CST

Title: Testing Protocols

Date: 10/27/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Small-Group: Testing Protocols
 - Worked with Maya on testing drafting test protocols for:
 - glass optics
 - temperature/humidity sensor
 - CO2 sensor
 - incubator recovery
 - · Reviewed client preferences for specific components to ensure they are included in test protocols
 - Location: Team activities > Testing and Results > Protocols > Testing Protocols Draft

Conclusions/action items:

Following the preliminary presentations, the team began work in the fabrication process. Maya and I worked in our small group on the testing protocols. The areas we plan to conduct testing on are listed above. Additionally, we created a draft document where we will record results from the testing.

11/3/2021 - Materials Purchasing, Protocols, and Call-to-Action

Caroline Craig - Dec 11, 2021, 8:57 PM CST

Title: Materials Purchasing, Protocols, and Call-to-Action

Date: 11/3/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Materials Purchasing
 - Went to Makerspace to review available materials to purchase off our Preliminary Purchasing Request
 - Updated the expenses table with purchases the client made ahead of approval from our team
 - Location: BPAG Resources > Initial Purchases
 - Discussed with the team our plans for completing necessary purchasing
- Protocols
 - Sent testing protocols draft to client for feedback
- Small-Group: Show and Tell
 - Drafted a Call-to-Action with Maya to present
 - Hi everyone! Our team has been tasked with developing a low-cost cell culture incubation chamber that is compatible with an inverted microscope and capable of live-cell imaging culture plates. The incubator must be able to maintain an internal environment of 37°C, 5% CO₂, and 95-100% humidity without compromising the integrity of the microscope's optics or functionality. Our final design consists of a heated water pump where a conducting plastic tube will be wrapped around the inside of the incubator and connected to a heated water pump that will be set to 37°C. The inside of the incubator will be filled with water, submerging the plastic tubing, allowing the internal environment to be heated by conduction as well as increasing the humidity to 95% or higher. The incubator box will also include a tube connector to allow CO₂ gas to be pumped in. Lastly, a separate box will be placed inside the incubator to allow for wiring and sensors to be inside the internal environment. The sensors will be collected and analyzed. Our call to action is to ask for your help on how we can arrange the plastic tubing or sensors in order to achieve a homogeneous temperature environment.

Conclusions/action items:

This past week the team worked in small groups on different aspects of the project. Maya and I created a Call-to-Action to share at the Show and Tell, and we reviewed available materials to purchase at the Makerspace. Additionally, I updated the expenses table with some purchases made by the client. Finally, Maya and I sent our protocols draft to our advisor for feedback.



Caroline Craig - Dec 11, 2021, 9:23 PM CST

Title: Editing Testing Protocols

Date: 11/10/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Small-Group: Updated testing protocols
 - · Based on our advisor's feedback, Maya and I updated our testing protocols
 - Protocols including feedback attached below
 - "One thing to spend some time on is getting familiar with the CO2 tank and how the regulator works. I
 don't think you can use the pressure from the CO2 regulator (which just tells you how much gas is left
 in the tank and how fast it flows out of the tank) to directly compare the percentage of CO2 in your
 incubator."
 - "One thing that you might want to do in the meantime is to spend some time getting comfortable with the CO2 tanks and regulators in the BME lab space. Generally, regulators provide 2 pieces of information: 1) the total pressure of gas left in the tank, and 2) the output pressure. When you adjust that output pressure above zero, the gas will flow out of the tank at a constant pressure. So, my questions for the team are: how will you control the gas input to your device? Manual control with the regulator could work, but you will need to use real time sensor information to know when to turn the gas flow off. For reference, our incubators in lab have the output set at a constant output pressure (~10-15 psi) and there is a solenoid in the incubator that opens and closes to let gas in. Secondly, what types of readings do you get from the CO2 sensor? What units does it measure in? Does it need to be calibrated?"
 - Sent updated protocols to the advisor for final feedback
 - Show and Tell
 - Reviewed recorded feedback from peers
- BPAG
 - Updated expenses table with 3D printed casing purchase
 - BPAG Resources > Updated Purchases
 - · Created invoice to send to client for the purchase
 - attached below

Conclusions/action items:

This week the team continued working in our small groups. Maya and I updated our testing protocols with feedback received from our advisor. Then, we sent another draft of the testing protocols to the advisor for finalized feedback. Additionally, I reviewed the feedback we received from the Show and Tell so that I could keep it in my mind during the upcoming weeks. Finally, I updated the expenses table with our most resent purchase and created and invoice to send to the client regarding it.

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Caroline Craig - Dec 11, 2021, 9:24 PM CST

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Microscope_Cell_Culture_Incubator_Invoice_1.docx.pdf(37.9 KB) - download



Caroline Craig - Dec 11, 2021, 9:17 PM CST

Title: Finalized Testing Protocols

Date: 11/17/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Small-Group: Finalized Testing Protocols
 - Updated Testing Protocols with most recent feedback from our advisor and following team meeting regarding them
 - "In terms of the CO2 sensor reading, one of the quickest ways to confirm that it's working correctly (in the right ballpark of CO2 ranges), would be to stick the sensor into one of the big incubators in the teaching lab. That way you can just make sure that you see a change in the CO2 reading and confirm that it is close to 5%. If you want a true calibration, the standard way to calibrate CO2 is with a fyrite"
 - https://www.mybacharach.com/wp-content/uploads/2021/01/fyrite_gas_analyzers.pdf
 - "Optical testing step #6: "Using Image Properties on a desktop computer, compare and record the horizontal and vertical resolution of the two images in the comments. The higher the dpi value, the higher the resolution." – the resolution is typically set by the camera, so you will see the same resolution across all of your images. There is a microscope focus quality plugin in ImageJ that might be helpful to quantify your optical properties"
 - https://imagej.net/plugins/microscope-focus-quality
 - Location: Team Activities > Testing and Results > Protocols > Testing Protocols Final Version

Conclusions/action items:

Again, the team continued working in small groups on different aspects of the fabrication process. Following our team's weekly advisor meeting, Maya and I finalized our testing protocols with the feedback we received. The protocols cover temperature and humidity sensors, CO2 sensors, CO2 feedback loop, optical testing, and incubator recovery. In the following weeks, these protocols will be put to use in the lab and results from our testing will be recorded.



Caroline Craig - Dec 11, 2021, 9:36 PM CST

Title: Testing and Materials Purchases

Date: 12/1/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Small-Group: Optical Testing
 - Went into the lab to learn how to use the microscope for imaging
 - · ran into issues with not having the computer password to practice using the microscope for optical testing
 - tested thermistors ability to increase and decrease with changes in temperature
- Sensor testing
 - Discussed with sensor coding team how to use our protocols for testing
- Material Purchases
 - updated the expense spreadsheet and expenses table with all recent purchases
 - Location: BPAG Resources > Client Purchases

Conclusions/action items:

This week the team continued the fabrication process for the incubator. Maya and I worked on optical testing and temperature testing in the lab. Additionally, we discussed with the sensor coding small group how to use our testing protocols. Finally, I updated the expenses table and spreadsheet with the most recent purchases of tubing and connectors.

12/8/2021 - Optical Testing and Final Deliverables

Caroline Craig - Dec 11, 2021, 9:46 P

Title: Optical Testing and Final Deliverables

Date: 12/8/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Optical Testing
 - Learned how to use the microscope and image capturing software to take pictures using the microscope
 - Captured images under the microscope with and without the transparent, polycarbonate sheet of cells
 Location: Testing > Optical Testing Imaging
 - Transferred the images from lab computer for analysis
- Final Deliverables
 - Worked on the following parts of the final poster:
 - Motivation
 - Competing Designs
 - Methods and Testing
 - Results
 - Discussion

• Practiced my part before the presentation

- Expenses
 - Finalized expenses spreadsheet
 - Team Activities > Materials and Expenses > Expenses Table

Rem	Description	Manufacturer	Part Number	Cute	QTY.	Cost Each	Total	tirk.
Category 1 : Invulsator		101		í		16		S
30 Printed Casing	for sides of incubator	Makerspace	- Karana - K	11/9/2021	1	\$32.32	\$32.32	N/A
Transparent Cover Plates	top and bottom of incubator	Radivor	64005034	10/29/2021	2	\$1.04	\$2.08	https://www.akpan.com/product
Category 2 : Components								
3/8 and 1/4 in. Polyethylene Tubing	heated water will flow through	USA Sealing	55Y099	11/23/2021	1	\$1.96	51.96	1298
Epoxy glue	to attach loose components	Makerupace		Louis and		\$1.50	\$0.00	N/A
E.Smm Tube Connector	connection between CO2 tank and incubator	Fisher Scientific	35031	10/29/2021	1	\$14.96	\$54.96	LINK
Viryl Tubing 3/8" x 1/2"	heated water will flow through	Ace Hardware	4027504	12/4/2021	1	\$8.31	\$8.83	04/6
Barbed Vacuum Connector	connection between tubing	Grainger	52504	11/23/2021	2 (of 10	\$0.95	\$1.90	LINK
						TOTAL:	\$61.55	

Conclusions/action items:

This week the team worked on testing and final deliverables before our final presentation. I went into the lab and captured images using the microscope for our optical testing. Additionally, I we on different aspects of the final poster and practiced my part. Finally, I updated and finalized the expenses spreadsheet.



Caroline Craig - Dec 11, 2021, 10:08 PM CST

Title: Final Deliverables

Date: 12/15/2021

Content by: Caroline Craig

Present: Caroline Craig

Goals: Record weekly contributions in the design process.

Content:

- Final Deliverables
 - worked on the following:
 - Final Report
 - Peer Evaluations
 - Team Notebook
 - Client Evaluation

Conclusions/action items:

The worked on the final deliverables in this final week of our project. I contributed to the Testing, Results, Discussion, and Conclusion portions of the final report. Additionally, I completed my notebook, worked on the team notebook, and completed my peer evaluations. Finally, I worked with the team on our client evaluation.



ETHAN HANNON (ehannon@wisc.edu) - Oct 19, 2021, 11:13 PM CDT

ETHAN HANNON (ehannon@wisc.edu) - Sep 14, 2021, 11:20 PM CDT

Title: Temperature Sensitive Cell Growth Mutants

Date: 9/14/2021

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To find information and data regarding cell incubation and regulation control.

Content:

[1 Y. Nishina, M. Kosaka, K. Matsumoto, A. Matsushiro, and M. Sakuda, "ISOLATION OF MUTANTS SEOWING TEHPERATURE433NSITIPE] CELL GROWTEFROMEMBRYONAL CARCINOMACEUS: CONTROOLF STEM CELL DIFFERENTIATION BY INCUBATION

TEMFERATURES," BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 165, no. 1, p. 8, 1989.

"We have isolated three novel mutants with temperature-sensitive (ts) cell growth that were able to differentiate at a non-permissive temperature for cell growth."

Study identifies possible genes within certain Stem Cell (Embryonal Carcinoma) were affected in their population growth through temperature sensitive genes. This reinforces the factor that proper temperature control within an incubator is key in ensuring an efficient cell growth.

Conclusions/action items: Continue to research further information regarding cell incubators and the science behind cellular growth.

between the momentum sector transmission $h_{\rm CM} \approx 0.75$ where he will Investor 20, 1919 privation of endance interference additional class consists was assored interference and a consistence of page oper provide the performance of page operation of the performance of the performance operation of the performance operation of the performance operation operat THE R. LEWIS CO., LANSING (a) From Historica, Tobic Horizon, Tobics, March McC, Minde Linner, Result Parally, Johns Managers, Alas Menaders on Respirat Social and president for Resident Streamen, Backgorth and Midd Surgery, Sector Residing Jonates the Solite, Sector 905, 405. ото реализион об работаку. Помоте об секотов¹⁶, бани от селоторудство, боло кото, е село Relation Science 1, 1981 Reverse approval terrange for inter- to exclusion and prove terra-electronic methods where the provide terration of the provide terration of the second provide terration of the second terration of the second methods are represented to the second second terration of the second second terration of the terration of the second second second second terration of the second second second second second second second terration of the second The grow make of the resonant sector we while he indifferentiates in some ever anale existing and the events of restant estimates by analyzing the detroiting generates for sold differentiation as will as the entropy efferentieter vogelijt. Strikke analeste die seine hie offisieringen if missief werken: other near new word to sharp of the restantions of size and reflected and the first the use of such associate and suppliers the WRITEWARDER OF CONTRACT ADDRESS THAT MANAGEMENT IN ANY NAME OF OTHER OF ADDRESS stile. The period exception we optimize they, while not period the dist-tions, or any mechanical maps concrete in some tradigited glasses and

Temperature-Sensitive_Cell_Growth_Article.pdf(1 MB) - download This is the article related to the information stated above.

10/18/2021 - Effects of Temperature and Atmospheric Perturbation During Cell Culture: The Silent Variables

ETHAN HANNON (ehannon@wisc.edu) - Oct 19, 2021, 11:14 PM CDT

Title: Effects of Temperature and Atmospheric Perturbation During Cell Culture: The Silent Variables

Date: 10/18/2021

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To better understand and utilize the science behind biological species in different climates/environments

Content:

- [1 "The Effects of Temperature and Atmospheric Perturbation During Cell Culture: The Silent Variables."
-] https://www.essenbioscience.com/en/resources/articles/temperature-atmospheric-perturbation-cell-culture/ (accessed Oct. 19, 2021).

Temperature:

- Mammalian cell growth is most efficient at 37°C
- Too high temperatures cause cells to denature while too low causes cells to remain in the G1 phase of their cycle (both slow cell culture growth)
- Constant removal of cells from an incubator temperature to a laboratory's room temperature (usually around 20-25°C) will ultimately
 disrupt cell growth over time
- Higher volume with less surface area means heat is better retained (well plates cool faster than flasks)

Atmosphere:

- Almost all ambient atmosphere has nearly 80% nitrogen, over 20% oxygen, and less than 0.5% CO2
- · Too much oxygenation to a cell can result in it being damaged to too much reactivity to oxygen species in the atmosphere
- Incubators of 3% oxygen typically have the best cell proliferation
- Humidity of at least 95% is best for cell proliferation (too little humidity results in more evaporation which can alter the osmosis around cells)

pH Levels:

- Mammalian cell growth is best at around 7.4pH
- Buffers in a cell environment work best in maintaining a certain pH (typically done with a CO₂ buffer to control water and carbonic acid equilibriums)
- · Removal of cell cultures from incubators can increase alkalinity in the atmosphere which drives up pH
- · Alkalinity can be harmful to cell cultures and their continued growth

Other Factors:

- · Light can be harmful to cells because they can be photosensitive which can result in the eventual death of the cell
- · Cells work best in darker environments that are cut off from most sources of concentrated light

Conclusions/action items:

Utilize this information when looking for the right conditions to set incubator to in order to maintain the best cellular growth



10/5/2021 - Pressure on Cell Cultures

ETHAN HANNON (ehannon@wisc.edu) - Oct 19, 2021, 11:11 PM CDT

Title: High pressure conditions promote the proliferation of rat cultured mesangial cells in vitro

Date: 10/5/2021

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To research and understand cell cultures within incubators when under certain conditions

Content: "In conclusion, we demonstrated that pressure-load itself could promote cell proliferation by enhancing cell cycle progression, especially by enhancing G1/S progression induced by low concentration of serum and promoting DNA synthesis rate at S phase. What type of stress occur to cell by pressure-load and what type of signal transduction pathway is activated by stimuli derived from pressure-load are not yet known, so further examination will be needed."

The study aimed to understand the effect of different pressure loads on cell cultures and discovered the an increased pressure load resulted in a greater rate of cell growth and DNA synthesis. They carried out an experiment using hydrostatic pressure to conclude and prove these points.

Y. Kawata, Z. Fujii, T. Sakumura, M. Kitano, N. Suzuki, and M. Matsuzaki, "High pressure conditions promote the proliferation of rat cultured
 mesangial cells in vitro," *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1401, no. 2, pp. 195–202, Feb. 1998, doi: 10.1016/S0167-4889(97)00112-2.

Conclusions/action items:

The better understanding of what pressure delivers to cell cultures means that it is all the more important for the final design of the incubator to maintain a constant, higher pressure level consistent with the information and data provided in this study. The incubator must be air tight and able to withstand higher pressures without any leakage or cracking in the entire structure. Research into stronger materials for the design will have to be researched then in order to oversee this does not happen. However, the level of pressure within the incubator should not be significant enough to be anywhere near this unless specified otherwise by the client.

9/14/2021 - Cell Culture Incubator CHINA INNOVATIONS

Katie Day - Sep 20, 2021, 4:47 PM CDT

Title: Cell Incubator Patent from China Innovations INSTR CO LED

Date: 9/14/2021

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To research current models and information regarding cell incubator models and their functions.

Content:http://https://worldwide.espacenet.com/patent/search?q=pn%3DWO2018086563A1

Model utilizes a 3 box design of the incubator allows faster heating conditions, heat regulation, and condensation control. Patented model was meant to resolve issues regarding poor heat control, condensation build up, and poor gas circulation from older incubator models that resulted in bacterial populations to either stagnate in growth or be hard to control in said growth.

Conclusions/action items: Use of a three box system or similar design could prove beneficial to overall model design. Heating regulation is important to ensure proper control of cell growth. Will continue to research proper methods of cell cultivation and growth regulation to properly control population levels in incubators.

ETHAN HANNON (ehannon@wisc.edu) - Sep 14, 2021, 11:01 PM CDT



WO2018086563A1_Original_document_20210915051311.pdf(905.9 KB) - download This is the original document of the patent. However, the original text is in Chinese which is why a separate link above is provided for a translated copy of the patent.

Katie Day - Sep 20, 2021, 4:49 PM CDT



US2019218498A1_Original_document_20210920234853.pdf(538.6 KB) - download



11/02/21 Valve-Hose Coupling Insert

ETHAN HANNON (ehannon@wisc.edu) - Dec 14, 2021, 1:38 PM CST

Title: Valve Coupling and Adaptor Pieces

Date: 11/02/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To find and research similar adaptors from current ECB lab hose adaptors to implement on the team's incubator design in order to properly attach a heated water pump.

Content:







For the coupling body: https://products.cpcworldwide.com/en_US/ProductsCat/NS4/NS4D17006

For the valve coupling insert: https://products.cpcworldwide.com/en_US/ProductsCat/HFC12/HFCD22612

The figures and links listed above provide the direct route to the manufacturer webisite on the intended hose adaptors needed and shown in the lab. These adaptors come in either 3/8" or 1/2" sizes allowing for the team to choose which one is overall best for the final design. However, the company is not found in the UW online Market which might complicate purchase of the adaptors as they need to be looked over and accepted by Dr. Puccinelli beforehand.

Conclusions/action items:

Further research into other possible ways on purchasing the product will be undertaken as well as messaging Dr. Puccinelli on whether or not the parts can be purchased. Further research on other adaptors will also be undertaken as alternatives.





ETHAN HANNON (ehannon@wisc.edu) - Oct 19, 2021, 6:47 PM CDT

Title: Shelving Incubator Design

Date: 9/18/2021

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To create different preliminary designs that tackle the issue given by client

Content:

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Microscopa
Tiack
i ait
Luelving Fulls Out
Sin O
A Lott
or plastic
A sliding glass door will open the incubator where
multiple shelves can be pulled out. Microscope con adjust
to shelf height and be moved around the plate
U un proc.

As said in the picture, there is a multiple shelving system inside the incubator that offers the ability to hold multiple well plates within it. This is beneficial when it comes to larger class or research objectives since the users don't have to purchase multiple incubators. A track is situated on each shelf that allows the microscope to be slid into place as per the desired well plate.

Potential Pros:

- · Good at holding multiple well plates
- Designed to be compatible with microscope
- Easy to understand and use

Potential Cons:

- Internal environment is difficult to maintain if incubator constantly opened for viewing purposes(solve that by closing off each shelf with plastic latches)
- · Might be more costly than other designs based on size alone
- · Larger size means maintaining specific heat levels more difficult

Conclusions/action items:

All in all, this device holds some promise. It ability for quantity mixed with intent on quality makes in the lines of usability. However, areas listed above showcase potential concern that must be addressed if it is to be used. This device will need to be further analyzed and critiqued for overall practicality. Continue to refine preliminary design and research better ways at controlling inter environment.





ETHAN HANNON (ehannon@wisc.edu) - Oct 19, 2021, 6:56 PM CDT

Title: Tub Incubator

Date: 9/18/2021

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To create different preliminary designs that tackle the issue given by client

Content:

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2	1 agence ce ce
3	Rung (7) (1) (1) (1) (1) (1) (1) (1) (1) (1) (1
9	Haarted Water
3	Light panel
3 3	Flat, board-like design allows for easy movement of microscope to distributed well plates. Eight panel sits on bottom of Newborr while water for heart is filtered above.

This design utilized a horizontal aspect contrary to the vertical features of the shelving design before. This design was capable of holding multiple well plates in one large tub-shaped basin where a sliding/hinged clear plastic(or glass) door would seal off the top. Water would be situated and filtered below the basin in a separate container where the heat would transfer through conductive material to then heat the well plates above. Other atmospheric and humidity regulators would also be installed within the well plate section to provide the rest of the desired atmospheric conditions needed for the incubator. Also, the clear doorway above made it possible for a well plate to be observed using a microscope provided it only contained the magnification lenses while the illumination for observation would be situated at the bottom of the tub basin.

Potential Pros:

- · Holds multiple well plates for larger group research
- Easy to operate and observe
- · Easy to clean and control internal environment

Potential Cons:

- · Complicated design means that maintenance might be difficult
- · Complicated parts means more expensive design
- · Need of a specific microscope might not be acceptable for client's needs

Conclusions/action items:

This design carries some potential but doesn't seem as feasible as the first idea in this folder. Future research and tweaking to device will need to be done in order to make this idea feasible.



ETHAN HANNON (ehannon@wisc.edu) - Dec 13, 2021, 10:36 PM CST

Title: Ethan's Progress Report 1

Date: 9/15/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: We conducted preliminary research on the project as well as setting up a meeting with our advisor and client to begin working on the project.

Individual: Started to research current design on microscopic cell culture incubators as well as questions for the client and advisor meetings.Began formulizing how to solve current problems for our project's success.

Conclusions/action items:

Team: Work on the PDS for the client.

Individual: Continue research into microbial enrivonment control as well as possible design ideas for the project. Continue to look into other current design models.



ETHAN HANNON (ehannon@wisc.edu) - Dec 13, 2021, 10:42 PM CST

Title: Ethan's Progress Report 2

Date: 9/23/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: Conducted more research regarding the project. Met with the client and advisor to get a greater sense of the overall project as well as answering some early questions. Worked on the PDS for the project.

Individual: Worked with team on completing the PDS. Worked on coming up with new preliminary design ideas for the project. Research a 3 layer gaseous incubator patent that I found earlier to help with new preliminary design drafts.

Conclusions/action items:

Team: Complete the PDS with the feedback given from the client. Brainstorm preliminary design ideas as a team and create a design matrix for the evalutation of said design ideas.

Individual: Continue researching design ideas and sketching them as well. Study the heat control methods of incubators to incorporate into the preliminary and eventually final design.
ETHAN HANNON (ehannon@wisc.edu) - Dec 13, 2021, 10:50 PM CST

Title: Ethan's Progress Report 3

Date: 9/30/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: We carried out more project research and met with the advisor and client to help gain a better understanding of the project. Created a design matrix off of the mixture of design ideas the team had created/found and brought together. Began figuring out what would be the final design of the project.

Individual: Finished and showcased my preliminary designs to the team and helped come up with a design matrix. Met with client on provided devices for the project as well as some follow up questions.

Conclusions/action items:

Team: Concluded the design matrix to find our final design and began work on the preliminary design presentation.

Individual: Started to work on next week's prleliminary design presentation. Begin research on air filtration and possible water heating methods for the design. Brainstorm any more questions or concerns for the client or advisor.

ETHAN HANNON (ehannon@wisc.edu) - Dec 13, 2021, 11:08 PM CST

Title: Ethan's Progress Report 4

Date: 10/7/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: Continued to work on the preliminary report and design presentation. Came up with materials and material requirements for the project. Began a 3D design of the project using SolidWorks.

Individual: Continued to work on finsihing the preliminary design as well as working on the preliminary design presentation. Practiced how to use SolidWorks from Sam and researched possible methods to seal and contain the incubator.

Conclusions/action items:

Team: Finish the preliminary presentation, conintue working on the report, finish the final design prototype method.

Individual: Continue to work on preliminary presentation and report. Practice SoidWorks and continue research on sealing incubator



ETHAN HANNON (ehannon@wisc.edu) - Dec 13, 2021, 11:14 PM CST

Title: Ethan's Progress Report 5

Date: 10/14/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: Completed the preliminary presentation, finalized materials, and continued to work on the preliminary report.

Individual: Worked on both preliminary presentation and the report. Continued to research better incubation methods for improvement of the overall design.

Conclusions/action items:

Team: Looked at beginning list of ordering materials. Started work on testing protocols. Updated SolidWorks design to a more accurate 3D model of the current incubator design.

Individual: Worked on beginning fabrication process with Sam. Look into how to properly create current designs of the project or improve them if needed to



ETHAN HANNON (ehannon@wisc.edu) - Dec 13, 2021, 11:20 PM CST

Title: Ethan's Progress Report 6

Date: 10/21/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: Finalized and submitted all preliminary deliverables (report, peer evaluations, and LabArchives Notebooks). Sent full materials list to Dr. Puccinelli for approval.

Individual: Went with Sam to the old makerspace to look for left over parts from previous or other projects and met with a profession int he TeamLab for advice on how to better fabricate the incubator. Finsihed peer and team evaluations and made final updates to the lab archives.

Conclusions/action items:

Team: Order the materials for fabrication. Begin to create Arduino codes for sensors and create test protocols.

Individual: Follow up with research given by the professional and help with early stages of fabrication.



ETHAN HANNON (ehannon@wisc.edu) - Dec 14, 2021, 12:55 PM CST

Title: Ethan's Progress Report 7

Date: 10/28/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: Arduino code for CO2 and temperature sensor written. Team went to Ace Hardware to look for more parts and gain a better understanding about what to use.

Individual: Worked on fitting the sensors better within the incubator and tried to find better ways to improve space and efficiency in the overall design.

Conclusions/action items:

Team: Team is splitting into different sub groups for the project: SolidWorks and 3D printing, coding for sensors, ordering materials and fabricating, and improving test protocols. Work on preparing for Show and Tell.

Individual: Continue helping with fabrication and improving the overall design like last week

ETHAN HANNON (ehannon@wisc.edu) - Dec 14, 2021, 1:01 PM CST

Title: Ethan's Progress Report 8

Date: 11/4/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: Continue working in sub groups for the project.

Individual: Went with Sam to ECB and met with Dr. Puccinelli to follow up on material and ordering updates. Researched supplier for a water hose adaptor to help connect water pump to incubator.

Conclusions/action items:

Team: Continue working in sub groups for the project.

Individual: Help Sam and others with the fabrication whenever possible in the future (when the materials arrive). Continue to research a compatible hose adaptor and possible ways to control water that exceeds the desired temperature.



ETHAN HANNON (ehannon@wisc.edu) - Dec 14, 2021, 1:06 PM CST

Title: Ethan's Progress Report 9

Date: 11/11/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: Team worked on testing protocols, sensor research, 3D printing the design, and sensor calibration.

Individual: Helped Sam with 3D printing the main box for the incubator. Continued to figure out and research how to better incorporate the sensors within the design of the box.

Conclusions/action items:

Team: Finish material purchasing and finish testing based on finalizing protocols.

Individual: Continue helping with 3D printing of any other parts to the design. Continue helping Sam and others with any other fabricating needs.



ETHAN HANNON (ehannon@wisc.edu) - Dec 14, 2021, 1:12 PM CST

Title: Ethan's Progress Report 10

Date: 11/18/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: Continue to work on sub groups projects.

Individual: Started work on user manual of the incubator. Continued to work on better implementing the sensors to the incubator.

Conclusions/action items:

Team: Start testing, finalize sensor coding, buy the needed tubing and adaptors, start fabricating design.

Individual: Start drilling into incubator to attach the tubing, adaptors, and other parts of the incubator. Help Sam or others with any other fabrication needs. Update user manual list



ETHAN HANNON (ehannon@wisc.edu) - Dec 14, 2021, 1:16 PM CST

Title: Ethan's Progress Report 11

Date: 12/2/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: Team continued to work on sub group projects.

Individual: Helped with working on the final poster. Made updates to the final report. Continued with the fabrication process.

Conclusions/action items:

Team: Practice on teh final presentations and finalizing all final deliverables. Complete testing and fabricating as much as possible.

Individual: Continue with fabricating the incubator. Update design fabrication with incoming parts. Finish working on final poster.



ETHAN HANNON (ehannon@wisc.edu) - Dec 14, 2021, 1:27 PM CST

Title: Ethan's Progress Report 12

Date: 12/9/21

Content by: Ethan Hannon

Present: Ethan Hannon

Goals: To record weekly progress of team and individual goals

Content:

Team: Seperate group projects were finalized for the end of the project.

Individual: Worked on finshing the poster and preparing for the final presentation. Helped Katie with drilling and filling the incubator box in the TeamLab.

Conclusions/action items:

Team: Finish all final deliverables.

Individual: Finish all my and the team's final deliverables.



John Puccinelli - Sep 05, 2016, 1:18 PM CDT

Use this as a guide for every entry

- Every text entry of your notebook should have the **bold titles** below.
- Every page/entry should be **named starting with the date** of the entry's first creation/activity, subsequent material from future dates can be added later.

You can create a copy of the blank template by first opening the desired folder, clicking on "New", selecting "Copy Existing Page...", and then select "2014/11/03-Template")

Title: Descriptive title (i.e. Client Meeting)

Date: 9/5/2016

Content by: The one person who wrote the content

Present: Names of those present if more than just you (not necessary for individual work)

Goals: Establish clear goals for all text entries (meetings, individual work, etc.).

Content:

Contains clear and organized notes (also includes any references used)

Conclusions/action items:

Recap only the most significant findings and/or action items resulting from the entry.

John Puccinelli - Nov 03, 2014, 3:20 PM CST

Title:

Date:

Content by:

Present:

Goals:

Content:

Conclusions/action items: