

ECE496 - Design Project
Final Report
Compact Imaging System for Fluorescence Labeled
Cells
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Executive Summary (Author: Shubham Manchanda)

A healthy human is often supported through reliable diagnoses methods. In particular, understanding the concentration or cellular contents of red or white blood cells is commonly used to diagnose an individual. Today, with an ever-increasing global population, there is a growing need for such diagnostic tools. However, the numerous diagnostic tools available today are often unaffordable, inconvenient, or time-consuming, especially in regions with limited resources. This global issue provided our design team a strong motivation to establish an appropriate means of human health diagnoses.

Therefore, our project explores imaging techniques that can be used to visualize microscopic information, in order to especially enhance the possibilities of medical treatment in environments with limited resources. Specifically, the intended design must be able to display magnified images of labeled cells, given illumination. Furthermore, in order to best tailor harsh environments, our design team has also aimed to synthesize a design that is powerful, accurate, inexpensive, and designed for manufacturability and assembly. With our design, it is also mandatory to avoid contaminating the samples examined in order to provide a loss of information, and be lightweight and compact for high mobility.

Due to the aforementioned project requirements, our design team decided to create a two-lens imaging system, or microscope, using a weighted decision matrix. The two-lens system best met the project requirements that our design was to adhere to. In addition, the two-lens system minimized possible optical issues such as chromatic aberration, and also provided the possibility of digital data capture enabling a user to capture the optically magnified image into a .BMP file or video stream for additional understanding of an examined specimen. The digital data was used by our team to also implement a camera program that automated the detection of cellular contents and their concentrations, providing great convenience for a user and minimizing human error.

Our design team has now completed the imaging system. Additionally, the testing and verification of the design, where the design has successfully met all the aspects defined in the project requirements. Hence, we believe that such a system will be used to appropriately provide health assessments in the future, especially in areas with limited resources. Our design team looks forward to presenting our design in the upcoming design fair and to implement this imaging system for further use and diagnoses in the future.

Group Highlights and Individual Contributions

Group Highlights(author:Xuan Shi)

Since our initial design review, several amendments have been made to our design proposal in order to better convey the aspects that adhere to our design. Our revised goal is to design an diagnostic tool to enable some cellular examinations, such as cell concentrations. As a result, we utilized the optical design tools/components, 3D printer and image sensor to complete the hardware prototype device; we utilized VC++ platform to design the software interface on Windows. In addition, several validation tests have been done to prove that the design goal and objectives are satisfied.

The prototype now is able to examine fluorescent beads around 3 μm and to approximate the cell concentration with appropriate multiplication factor, which is roughly what the project requirements expect. More details will be provided in Validations/Tests section and Final Design section.

Overall, the main group highlight for the final design is that the team was able to realize their own optical design with all the resources available rather than using the existing optical devices.

Each team member is responsible for certain area of the project. Their intended responsibilities are listed below:

Xuan Shi:

1. Optical Design and Simulation
2. Camera Testing and Code Testing
3. Application Integration and Debugging
4. Validation Tests and Sample gathering

Runhong Huang:

1. Interface design
2. Database design
3. Image processing algorithm and function design

Shubham Manchanda:

1. 3D printing
2. Component Purchasing
3. Work Breakdown Structure Update Gantt Chart Update
4. Program Testing, in regards to the camera

Stanley Huynh:

1. Solidworks 3D Model Design
2. Structure Assembly

The team worked according to the updated work plan and work breakdown in the team progress report.

As a result, all assembly and coding are done before Mar 1st, and all tests are done before Mar 11th. Even though the project completion is about one week late according to the work plan, the team still left one week for construction of final report.

Individual Contributions - Xuan Shi(author: Xuan Shi)

As the group representative, I handles the commutation with the supervisor and other individuals/departments we seek help from, and provides regular updates and task assignments to group members.

In addition, my main contributions to the project were mainly centered around the optical design, image processing applicaiton design and testing of code and components. I have finished the design of the optical system to magnify the image of cell sample, The design is verified by the ray tracing simulation using the Zemax Optical Suite. After extensive research on lenses and collimated light source, the optical components are selected and ordered from the supplier, Thorslab. I have also participated in the design and testing of web-camera capture fuction of our application.

After the design project demo, I have intergrated image storage funtion into the original webcam framecapture application to store individual frames. In addition, I have utilized the OpenCv functions and code examples from others to develop a cell counting function which can seperately count cells/light dots with different areas and approximate the concentration.

In addition, I have also handled some modifications to 3D models and some 3D Makerbot print tasks which are originally assigned to Shubham and Stanley due to their tight schedules during midterm period.

After the system assembly, I requested testing samples from Sheeresha, a master student of our supervisor. With the test samples, I then performed the verification tests from the updated test documents. The results shall be discussed in the Testing and Verification section later.

No	task title	Completion date
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1	Optical Design and Simulation	Nov,14,2015
2	RGB Channel Separation Function Design and Testing	Feb 2,2016
3	3D printing of components	Feb 7,2016
3	Cell Identification/Cell counting application	Mar 4,2016
4	Varification Tests	Mar 11, 2016

Individual Contributions - Runhong Huang (author: Runhong Huang)

My main contributions to the project are assisting camera configuration, assisting system assembly, and designing software application.

Before the final report, I finished the design code for the camera interface design and the database design with the assistance of Xuan and Stanley. After the final report, I was able to design the testing and verification, and the design code is able to deliver the expected results.

For the camera interface design, I used online resource(a sample camera initialization code). Edited the sample code such as change the variable name and pointer, I make it compatible to our design project. The detailed design code is listed in Appendix E. To test the code, I used the camera to check if it can capture the picture of a building. (the captured picture is in appendix E) The test is successful.

For the data base design, I used the knowledge from ece297 and online resources. I designed the code that can store and process the captured image by the camera. The detailed design code is listed in appendix E. After I tested the camera interface code, I used data base design code to store the captured image. The code was able to store the image, and the image is shown in Appendix E.

For the last imaging processing and function design, we were able to finish it on time. Our design plan was to make it optional due to the extremely tedious and challenging computer programming. Eventually, we were able to overcome the challenges.

In conclusion, the project is finished. Camera interface design and database design are done which the progress are beyond expectation. The camera and database design are able to deliver expected testing results. In the future, we should try to finish the challenging part.

Task Number	Task Title	Completion Date
1	Camera Interface Design	Feb 28 2016
2	Data Base Design	March 4 2016
3	Imaging Processing and Function Design	Not Completed

Individual Contributions – Stanley Huynh (author: Stanley Huynh)

This subsection outlines my individual contributions to the project of developing a compact imaging system for detecting fluorescent labeled cells. See table below for the list of contributions for the project.

Contribution #	Contribution Title	Completion Date
1	SolidWorks 3D Model Design	November 10, 2016
2	Structure Assembly	March 1, 2016

The first contribution I completed for this project consisted of the design of our project's 3D printed structure. Through the thorough research of different alternatives of 3D CAD (computer aided design) software, the chosen software was SolidWorks, because of the ease of use and wide range of compatibility with commercial 3D printers. After preliminary designs without dimensions and familiarizing myself with the software I was able to produce the final 3D CAD model that was printed and utilized for the final prototype of our project's design.

The second contribution I completed for this project was the assembly of the final prototype with the design's components (camera, physical construct, lens tube, etc) involved in our project. This task of structure assembly consists of merging all the components of our design together and required the consideration different binding material of hot glue and tape. Nearing the design project's deadline, the design team was able to produce all the necessary components and I was able to proceed with the system assembly using hot glue and tape where the level of adhesion was deemed appropriate.

The contributions that I was responsible for made the project a success, because I was responsible for the basis of the design, which includes 3D CAD model and

structure assembly. The 3D CAD model is the underlying structure that maintains the positioning of all the components necessary to produce a stable image outputted from our camera device. Also, the structure assembly is the merging of components to ensure that our final design prototype can be tested, calibrated and verified to be fully functional.

Individual Contributions – Shubham Manchanda (author: Shubham Manchanda)

This following table summarizes my individual contributions to our design team in order to successfully create our compact imaging system.

Contribution #	Contribution Title	Completion Date
1	Purchasing Components	November 14, 2015
2	Software Application Testing	January 24, 2016
3	3D Printing Physical Structure	February 27, 2016
4	Updating Work Breakdown Structure and Gantt Chart	March 13, 2016

My initial contribution to our design team was purchasing the components that were required to proceed with the physical aspects of the project. To do this, I first determined the components that were necessary and purchased the components such that they remained within our budget and at a minimal cost. Specifically, using thorlabs.com and amazon.com I purchased lenses and a lens tube, and a laser pointer, respectively. Upon completion of this task, these components were ready to use and prepared for the physical assembly of the design.

Secondly, I was responsible for the testing of the camera program designed by other team members. During this, I continuously and extensively compiled and tested the software, accounting for possible corner cases. In addition to this, I reported any issues or errors to the respective team members and discussed any solutions I was able to conceive. The test results demonstrate that the software is functional as a concentration-detecting program, successfully able to detect the contents within cells that are 2-8 micrometers in diameter under a 5x magnification.

Next, I was also responsible for printing the 3D framework required for the assembly of our physical design. In order to do this, I first attended a tutorial in the MADLab at

the Gernstein Science Information center, in order to learn how to 3D print. Subsequently, I was required to take an online quiz in order to gain access to 3D print in the MADLab. After successfully passing the quiz, I was able to book a time in the MADLab in order to print the design. Printing the design required converting the SolidWorks framework created by another team member into a .x3g file for the 3D printer to print the structure. After putting the .x3g file onto an SD card and inputting the card into the 3D, the 3D printer was able to print the design as needed. After cooling down, the design was ready for its physical assembly.

Finally, my continual task throughout this project was updating the *Work Breakdown Structure* [WBS] and *Gantt Chart* to reflect the team's progress and make amendments to account for future tasks. Doing so additionally required me to: review completed tasks via collaboration with the team; amend the WBS and Gantt Chart to the schedule of completed tasks using smartsheet.com; determining tasks that needed to be completed, when they needed to be completed by, and how quickly it was feasible for the design team to complete future tasks.

The tasks that I have completed throughout this project have been successful, as all of the tasks were completed and on-time. The design team has been able to continue its tasks accordingly and as planned. We look forward to presenting the design in the design fair in the near future.

Acknowledgements (Author: Runhong Huang)

This research was supported by the University of Toronto and Prof J. Steward Atchison. We thank our team members in team 396 who provided insight and team budget to cover expenses that greatly assisted the prototype design.

Throughout the course, the project supervisor, Prof J. Steward Atchison and the project administrator, Dr Tome Kostaske played critical roles in our design process by providing technical advices for the design documents, solving technical design issues, and providing helpful design guidances and tools.

As the group supervisor, Prof J. Steward discussed the project details and gave clear guidance during regular team meeting. Prof J Steward used different design documents that are similar to our project to help our group to initialize the design. Also, he showed several lens system to our team to help us to visualize the design. During the design process, he consistently helped us to solve technical difficulties. He taught us optical knowledge such as lens diffraction that is related to our design.

As the project administrator, Dr Tome Kostaske helped us to write professional engineering documents. From proposal to progress report, he continued to polish and improve our design documents. He gave us clear instructions on writing different documents. Based on his advices, our documents became more clear, specific, and professional.

Beside our supervisor and administrator, we want to thank MADlab at Gerstein Library for training with 3D printing, and assistance with the use of Makerbot 2. We would also like to express special thanks to CMC.ca for enabling the our use of design tools, and Shreesha Jagadeesh for providing his past experiences, insights and optical test samples.

Thanks to all the people listed above. With all of your help, we were able to finish the final design project. Also, our group had an interesting and helpful experience for the designing.

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Introduction (Author: Runhong Huang)

1.0 Project Introduction

This report summarizes the motivation, design, implementation and testing of a compact imaging system for our final year design project course ECE496. The report concludes with suggestions of improvements of our design and future works.

1.1 Background and Motivation

Identifying the concentration of red or white blood cells and examining the blood cells are commonly used as a diagnostic tool for human health assessments. For example, an abnormally high or low number of white blood cells indicates illness and an increasing or decreasing trend of the white blood cells indicates the possibility of disease progression or improvement. Disease such as anemia is found by identifying sickle red blood cell shape. As such, understanding both cellular content and concentration provides diverse information that can be used to identify and evaluate human health.

Today, common diagnostic tests such as complete blood count such as hemocytometer are time-consuming with high cost instrumentation and a central laboratory. [1] There is a growing need for cheap and portable instrumentation which can allow high precision tests to be carried at the point of care. Recent outbreak such as Ebola and Zika all started in environments with limited resources. If the infective diseases are not found and controlled, they will spread rapidly and cause unimaginable damages. Environments with limited resources and devices are desperate to find alternatives. An example of a compact and cost-effective solution is to put biological cells or micro-objects contained in a chip on the top of the CMOS image sensor surface and the chip is illuminated with a partially coherent light source to generate shadow patterns of the cells or micro-objects. The generated shadow patterns are then captured by the CMOS image sensor and processed with a custom-developed algorithm to extract the cell information. The programmer can write the algorithm accordingly to get cell concentration or cellular content. [1] This is one of many techniques that may be used to provide an inexpensive solution to the medical betterment of especially environments with limited resources.

With this incentive, our project further explores inexpensive and accessible designs and techniques that may be used to visualize microscopic information, particularly for the use of enhanced medical treatment in environments with limited resources.

Using microscopic imaging, harmful and infective diseases can be found and controlled fastly and effectively.

1.2 Project Goal

The goal of this project is to design an imaging system as a tool to enable the human health diagnostics such as a complete blood cell count and identifying cellular content. Ideally, the design should be a cost-effective, portable and stable design for blood tests, enabling accurate observations and best tailoring people in environments with limited resources.

1.3 Project Requirements

This section outlines the the target project requirements, which include the functions, objectives, and constraints that adhere to the design. These project requirements will be used to evaluate the completed design.

1.3.1 Functions

This subsection outlines the tasks that will be performed by the design.

- *Primary Functions* - The following fundamental task will be performed by the design:
 - Using illumination, given cell sample, and computer applications, to display magnified images of the fluorescent labeled cells and cell concentration.
- *Secondary Functions* - The following functions result, enable, or simplify from the primary function listed above:
 - Allows user to focus the image
 - Allows the calculation of cell concentration based on the image
 - Allows cell type identification based on the image

- Illuminate samples or specimen
- *Unintended Functions* - These functions are possible undesired or inadvertent results of the primary function and execution of the design:
 - Loss of thermal energy
 - Alter the appearance and contents of a specimen from which a sample originates

1.3.2 Objectives

This subsection lists what the design should be. In this section the sub-bullets identify the objective goals for each respective objective.

The final design should be:

- *Powerful* - Able to reach as 5x magnification. [2][21]
- *Clear* - Able to provide desired images with excellent precision for accurate observations
 - Given the normal cell size between 2-8 micrometer, the sensor chip of system should be able to depict the shape and color of cells under 5x magnification in the image.[2][3]
- *Inexpensive* - Material costs of the design should be of minimal costs
 - Total material costs should be less than \$180 (the price of relay magnification system + the price of image sensor device) for the initial implementation[4][5]
- *Designed for Manufacturability and Assembly [DFMA]* - Product should be easy to manufacture and assemble
 - Construction of structural components should be possible using only a 3D printer;
 - The 3D printer print time should not exceed 4 hours

- Assembly time of the design using the constructed individual components should not exceed four hours, provided with instruction or manual [6][7]

1.3.3 Constraints

This subsection highlights the requirements that the design must meet.

- The design must not contaminate the samples or specimen examined, avoiding loss of information.
- The design must be lightweight and compact in order to achieve high portability in order to carry the product quickly and easily between locations, considering the size and weight of a portable compound microscope.
 - The completed product should weigh no more than 28 lbs [8]
 - Physical dimensions of the design should be no greater than 28cm height, 15cm width, and 19cm length [9]

2.0 Final Design

2.1 System Level Overview

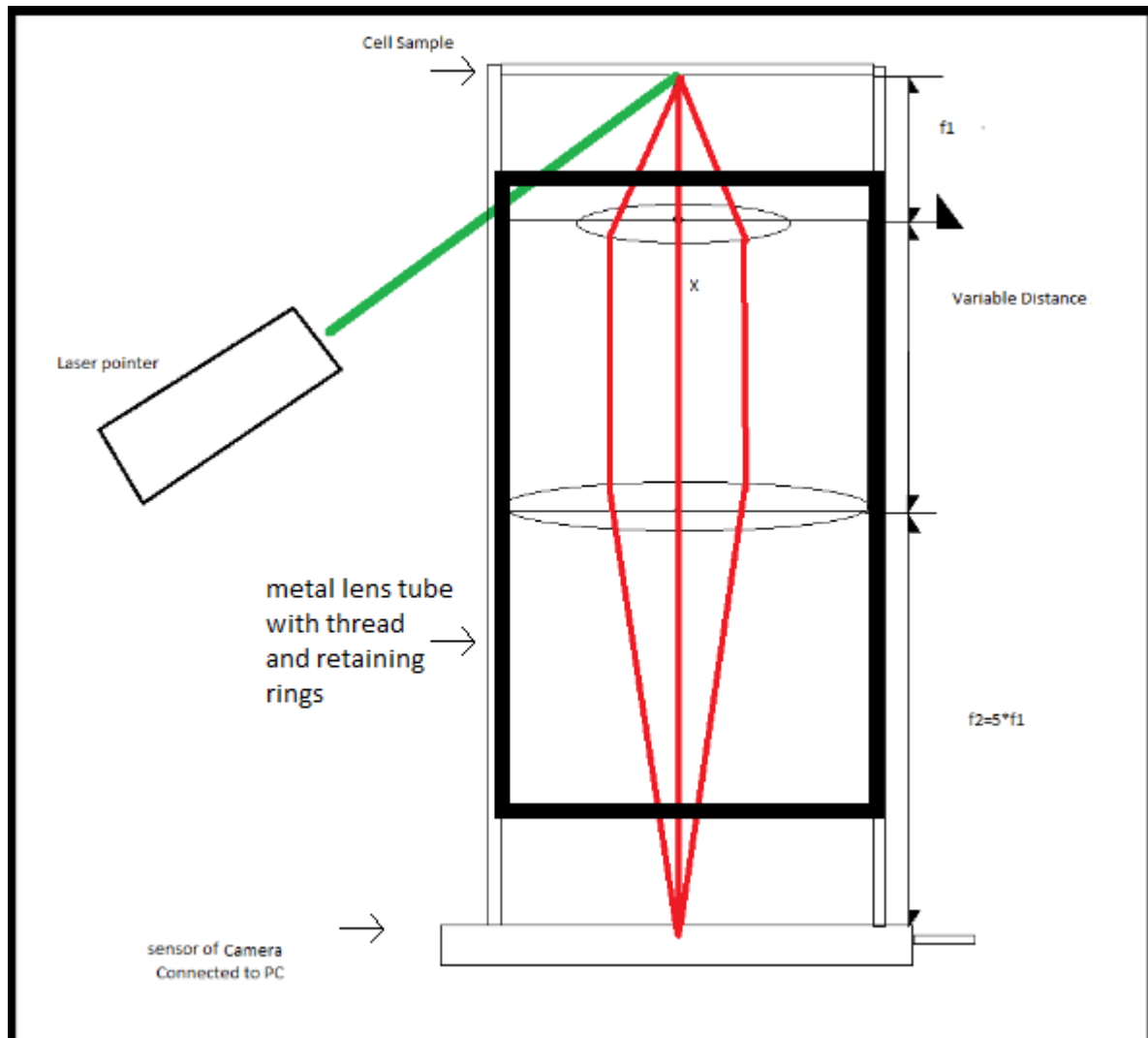


Fig 1. System Structure Diagram

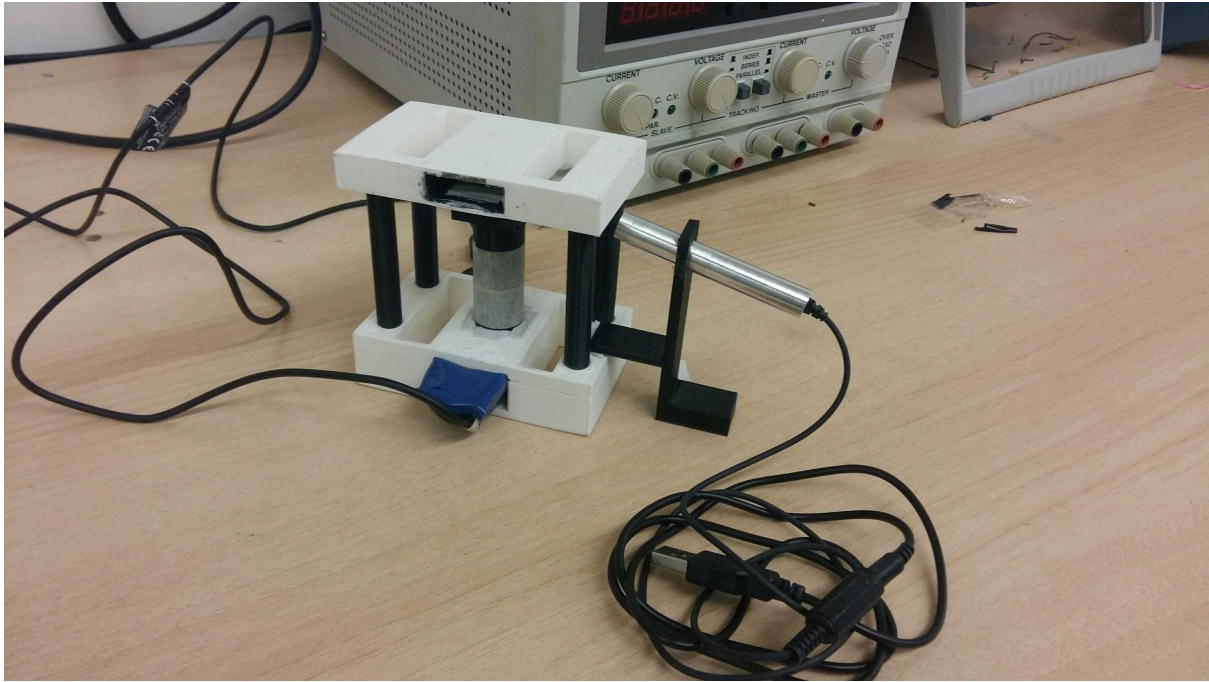


Fig 2. Key modules and the assembled system

Detailed solid work module diagrams are listed in appendix D.

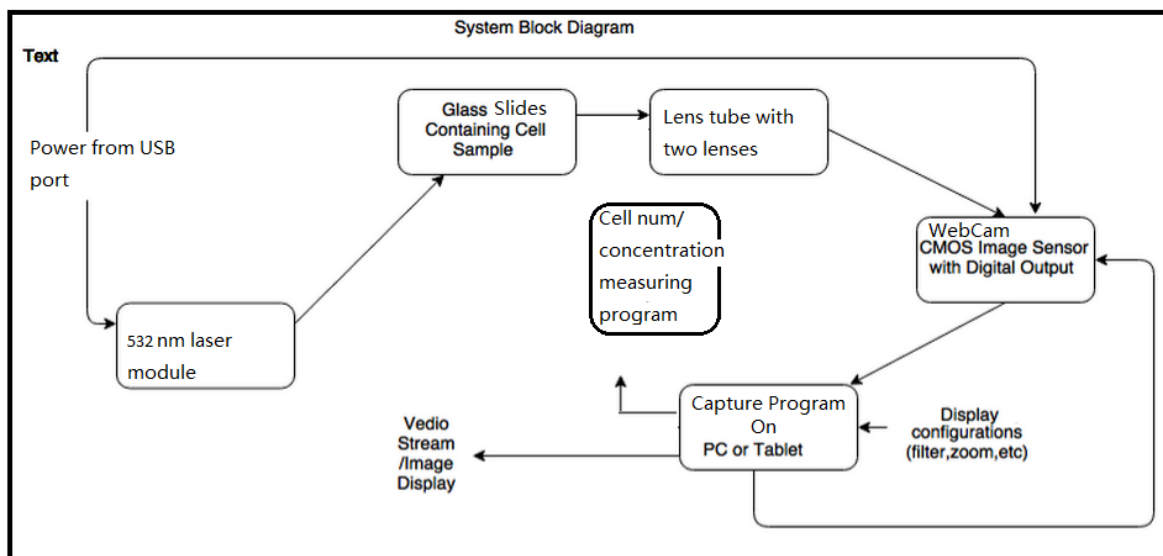


Fig 3. System Block Diagram - Illustrates the processes from power and light input to result generation

As described by the system block diagram, the operation mainly runs through lens, CMOS image sensor and PC, which form a loop reducing errors in calibration. The details of the principle of operation are described below:

1. the light source outputs parallel beams that pass through the glass slides with cell sample and fluorescence.
2. the cells with fluorescent label absorbs the incoming light and radiates rays with specific wavelength. When the rays pass through the lenses, they are projected on the CMOS Image Sensor to generate an image
3. The sensor chip reads the light intensity of each pixel from the image and outputs the data to PC through USB link
4. PC program could also process image digitally through zooming and filtering out unwanted light rays to generate the desired image display on the screen.
5. The captured image can be sent to the cell num/concentration measurement program for further annalysis.

For details of the program and the captured final results, please refer to appendix G.

2.2 Module Level Descriptions

The detailed description of each module from the System Block Diagram:

Module Name	532nm Laser module
Input	3V DC power
Output	532nm Laser
Function	The 532nm Laser module shall generate parallel light to illuminate the Glass Slide Chip(the next module) when it is turned on.

Module Name	Glass Slides
Input	Parallel incoming light
Output	Light rays from the Objects(Fluorescent Labeled Cells)
Function	<ul style="list-style-type: none"> • The glass slide chip shall contain a thin layer of fluorescent labeled cell sample between the two glass slides that could perfectly transmit light without distortion and attenuation.

Module Name	Lens Tube with front lens and rear lens
Input	<ul style="list-style-type: none"> • Light rays from the cell sample • Calibration Specifications
Output	Magnified Image Projection
Function	<ul style="list-style-type: none"> • The lenses shall project the image of the cell sample on a plane parallel and away from the last lens at certain distance to generate 5x image magnification.

Module Name	WebCam CMOS Image Sensor Module
Input	<ul style="list-style-type: none"> • Image data signal from the image sensor plane • Power input from USB cable • Data input (like on/off and captured image size)from PC
Output	<ul style="list-style-type: none"> • Digital data containing the light intensity and wavelength(color) at each sensor unit(pixel) of the sensor chip plane.
Function	<ul style="list-style-type: none"> • The image sensor shall return the light intensities of red light, green light and blue light of the image projection at the pixel level. • The returned data shall be transmitted through an USB data link.(For video stream, the sensor shall read and output the light intensity data for every frame.)

Module Name	Image Capture Program on PC or tablet
Input	<ul style="list-style-type: none"> • Light Intensity Data from the USB link • Instructions from the operator
Output	<ul style="list-style-type: none"> • Image/Video Display on the screen • Saved image in JPG format

	<ul style="list-style-type: none"> • Instructions to Webcam
Function	<ul style="list-style-type: none"> • The PC program shall request the sensor for the RGB light intensity data and combine these data into the color of each pixel which is later displayed on the screen. • The PC program shall configure the image display accordingly when receiving instructions from the operator, such as zooming and filtering. • The PC program shall output the captured image in jpg format

Module Name	Cell number/concentration measuring application
Input	<ul style="list-style-type: none"> • JPG format Image captured • Instructions from the operator
Output	<ul style="list-style-type: none"> • Cell number in image • Cell concentration
Function	<ul style="list-style-type: none"> • The PC program shall display cell concentration and cell number of the cell sample inserted.

2.3 Assessment of Final Design

This subsection assesses the final design which is the two lens imaging system. The detailed comparison for the alternative design and design weighted matrix are included in *Appendix F*.

After the implementation of weighted decision matrix of the key objectives in the design proposal,(appendix F) the Two lens Relay Imaging System scores the highest in our design matrix. Therefore, it becomes the most competitive design solution for our design objectives in all design alternatives.

There is also a major advantage of our final design, in comparison with other alternatives. Since the distance between the front lens and rear lens can be variable, [20] the distance between the object and image can be fixed as long as there is enough room to support our optical system design in the Zemax simulation. As a result, no extendable and movable component is required to be printed using 3d printer, the metal lens tube can suffice the need for optical calibration.

In addition, some tests have been done to verify the performance of the final design regarding the key objectives and the cell number/concentration measuring function. The details are provided in the Appendix C.

3.0 Testing and Verification (Author: Xuan Shi)

Since our design is an integrated system, all the testings are done on the system level. Some of the tests are referred in 'Validation and Acceptance Tests' (in Appendix C). However, due to lack of devices and other complications, some modifications have been made to those tests. Details of modification and documentation are listed in 'Comments and Documentation'.

Requirement (# & title)	Target specification	Final Result	Compliance? (Pass/Fail)	Comments and Documentation
<i>Powerful</i>	5x magnification	5x	pass	Since the concentration is in #/ml not in #/cm ² , the original method would not work. We used size of 0.5 mm bead in image and size of 0.5mm bead in reality to measure magnification. (See Appendix G.1 for details)

<i>Clear</i>	Clearly identify cellular content under cellular view	Able to distinguish 3.5 um florescent beads from background and other noise	pass	Since the shape and color of the beads are known, traditional microscope is not required for verification.(See Appendix G.2 for details)
<i>Inexpensive</i>	Under \$180	\$175	pass	The cost may be higher due to US/CAD dollar exchange ratio, but the unit material cost could be much lower in mass production, since the optical components purchased are for experiment purpose, which is usually expensive.(See Appendix G.3 for details)
Design for Manufacture and Assembly	Structural components can be printed by 3D printer. Print time less than 4 hours Assembly less than 4 hours with 4 operators.	Yes, all structural components printed by Makerbot 2. Print time 4 hours. Assembly takes less than 30 min with out one operator without optical calibration.	pass	In our design objectives and 'Validation and Acceptance Tests' , we placed too much time and manpower for assembly. The actually assembly would not take that much time, if the optical components were already calibrated. In addition, we also added new specifications like print time to the target objective (See Appendix G.4 for details)

There is also another test that involved the latest developed cell num/concentration measuring application. It is not involved in the project requirement or the 'Validation and Acceptance Tests' because we originally did not finalize our project requirements for the software applications in the design proposal. However, there is still tests to be done to verify its functionality and accuracy.

Accuracy and functionality of the Image processing application	The error ratio of the output should be within 10%	Yes, the actual error ratio is on the edge of passing	pass	<p>For testing and verification, we used two types of florescent bead solution. First one is at a concentration of 5 million/ml, while the second one is at 16 millions/ml. The measured concentration has a 10% error which is on the edge of passing.</p> <p>(See Appendix G for details)</p>
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Summary and Conclusion (author: Stanley Huynh)

In summary, the design team overcame numerous challenges to successfully create a compact two-lens system, which can be used as an effective technique for health diagnoses. Initially, the design team had been overly-ambitious with our goals, such as with the desire to create the same type of system but with the ability to reach far greater levels of magnification and clarity. As the project progressed, the design team moved towards more feasible goals and despite this initial over-ambition of the team, the final design proves to be lightweight, compact, and cost-effective and is able to obtain a clear digital image of cellular content for further microscopic analysis as intended.

The team was able to meet the project goals and requirements by demonstrating the validation and acceptance tests of powerful, clear, inexpensive, and designed for manufacturability and assembly. See Appendix C for the detailed analysis of the validation and acceptance tests. The final testing and verification has been completed, proving that our final design was able to meet the goals of producing a clear image after completing system assembly. The team's design ideas were validated, as the final image was produced and the system produced the magnified image.

In the future, this imaging system has the potential to act as a suitable replacement for current diagnostic tools that display microscopic images, due to its convenience through the automated cell concentration detection and camera software, and its relatively inexpensive cost. These aspects of the design especially allow it to be suitable for environments with limited resources, satisfying our initial motivation for this project. In addition to such environments, our imaging system can also be used in educational environments, particularly due to the automated camera program and its ability to capture images and display them digitally. These features and aspects of the design have the ability to enhance learning in the field of microscopy by automating tedious and repetitive tasks such as cell-counting or determine cellular contents and concentrations.

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Appendix A: Gantt Chart History

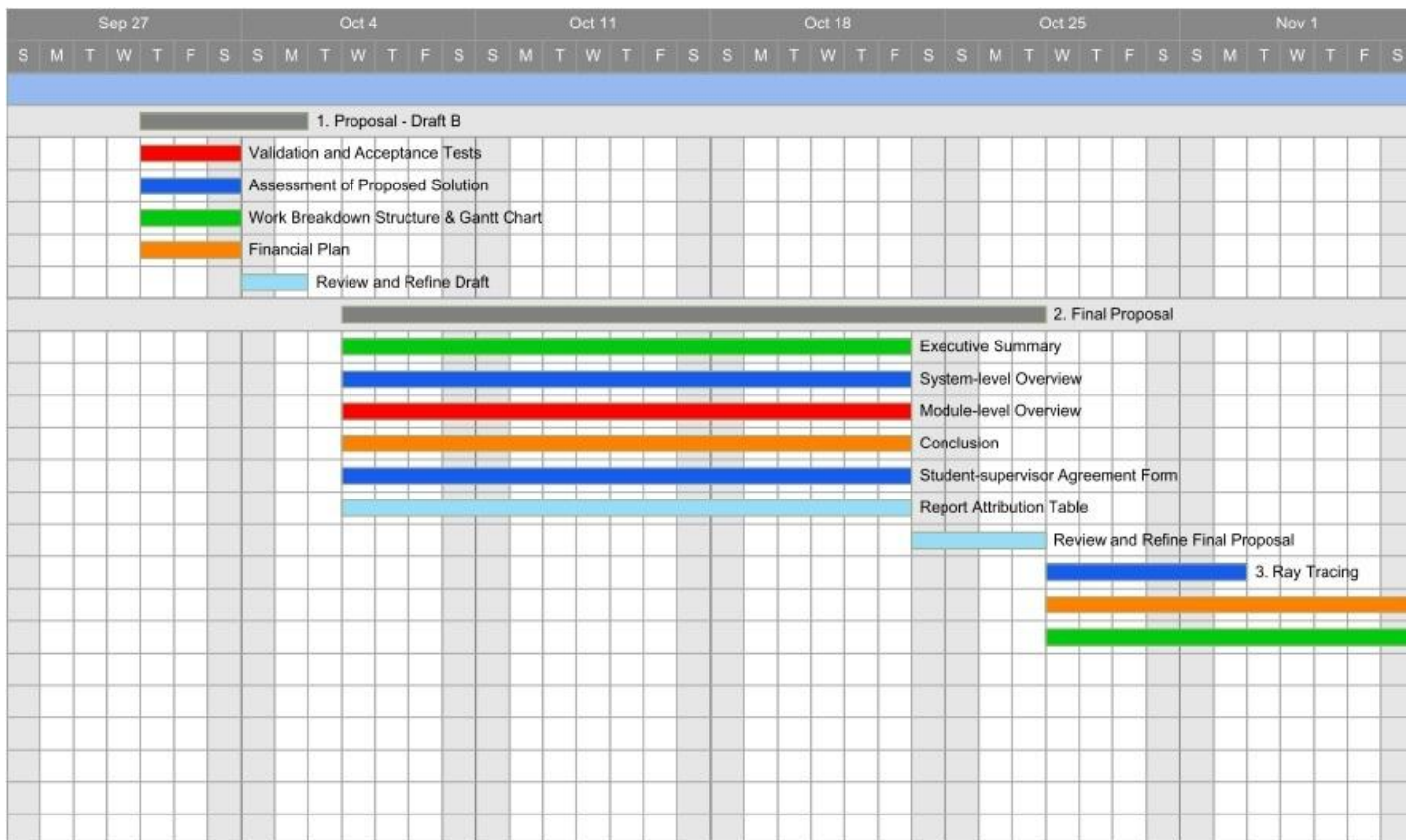
The following charts illustrate all the tasks that will be and have been performed by the design team and their respective delegations, durations, and completion dates. Currently, the presented Gantt Charts are as expected; these Gantt Charts may be subject to change as the project progresses.

The current Gantt Charts presented adhere to the following legend by colour:

- Represents a task that all team members are responsible for
- Represents a task that Xuan is responsible for
- Represents a task that Shubham is responsible for
- Represents a task that Runhong is responsible for
- Represents a task that Stanley is responsible for

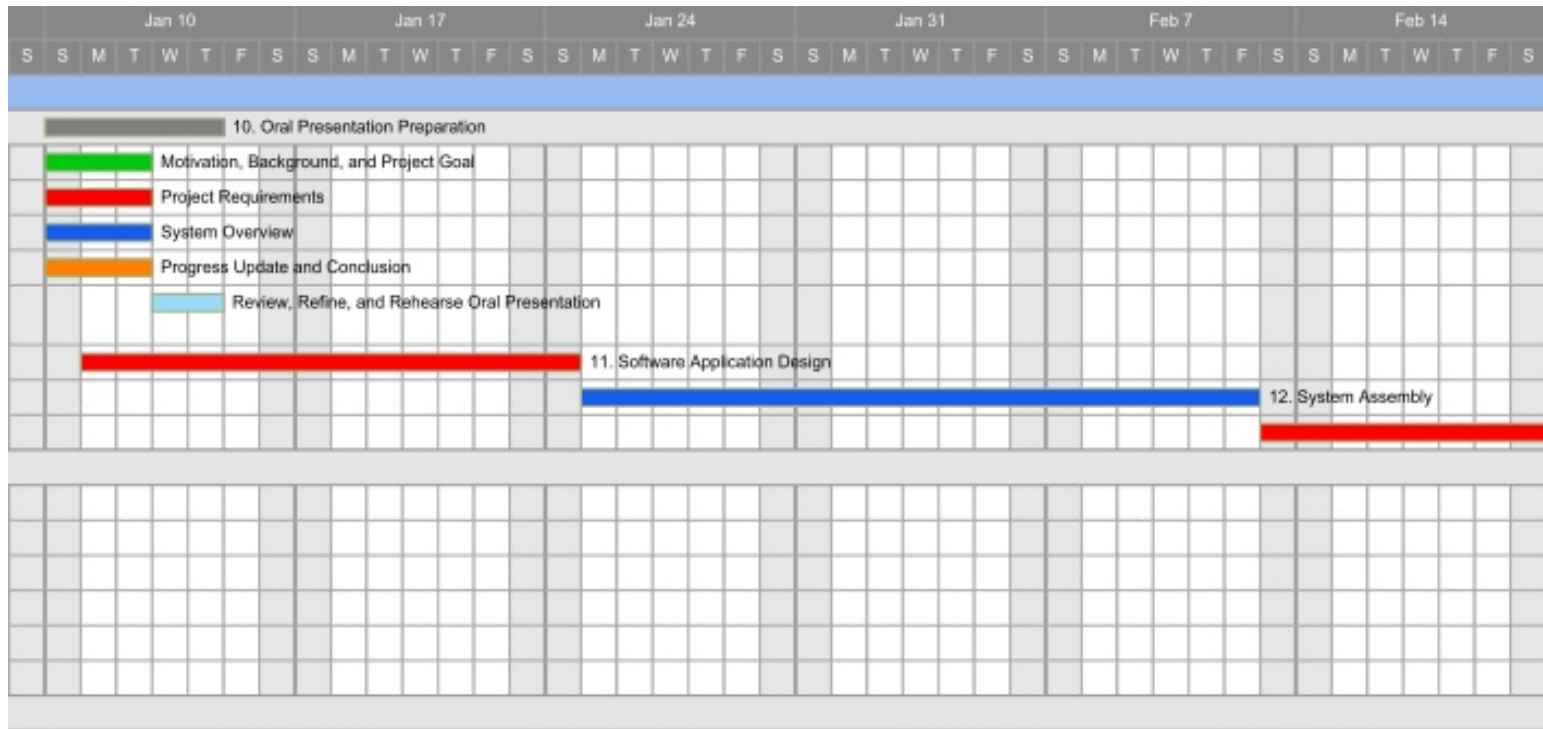
1. Design Proposal Gantt Chart

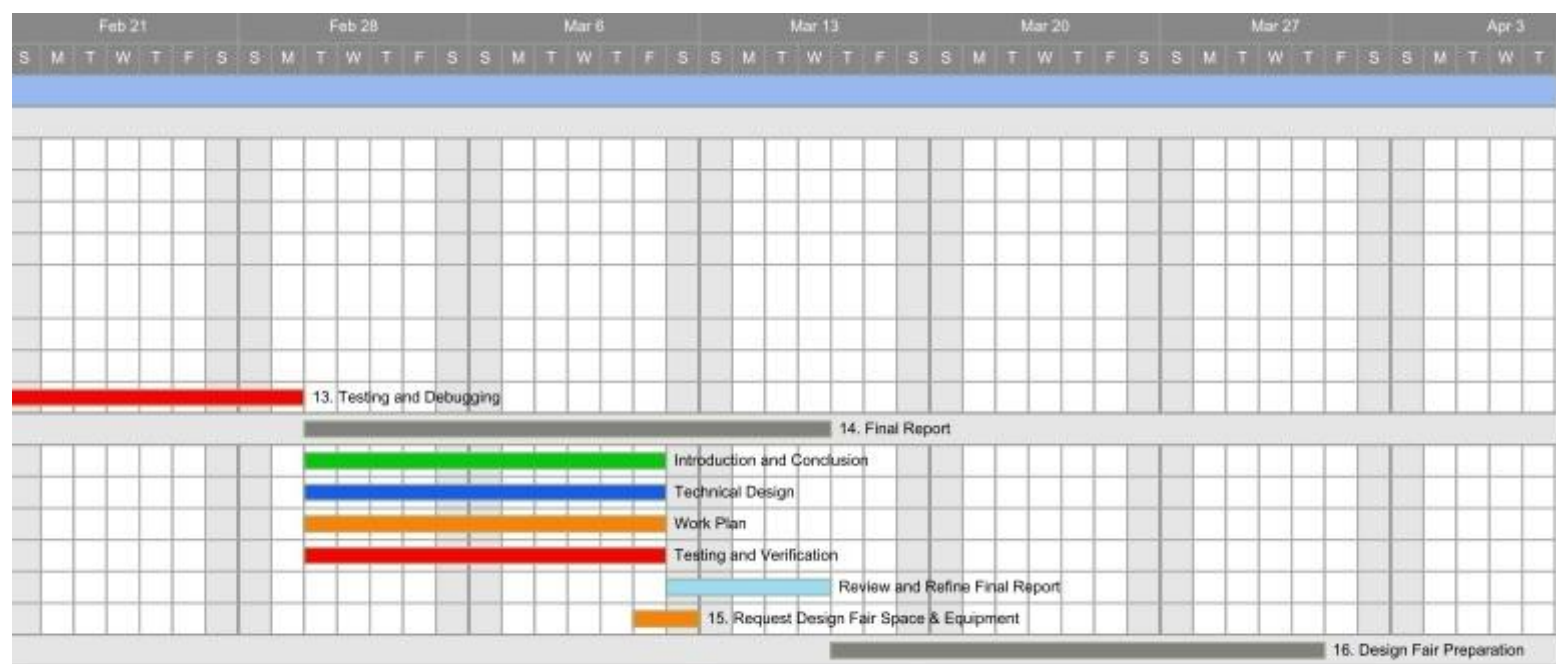
Fall Semester:



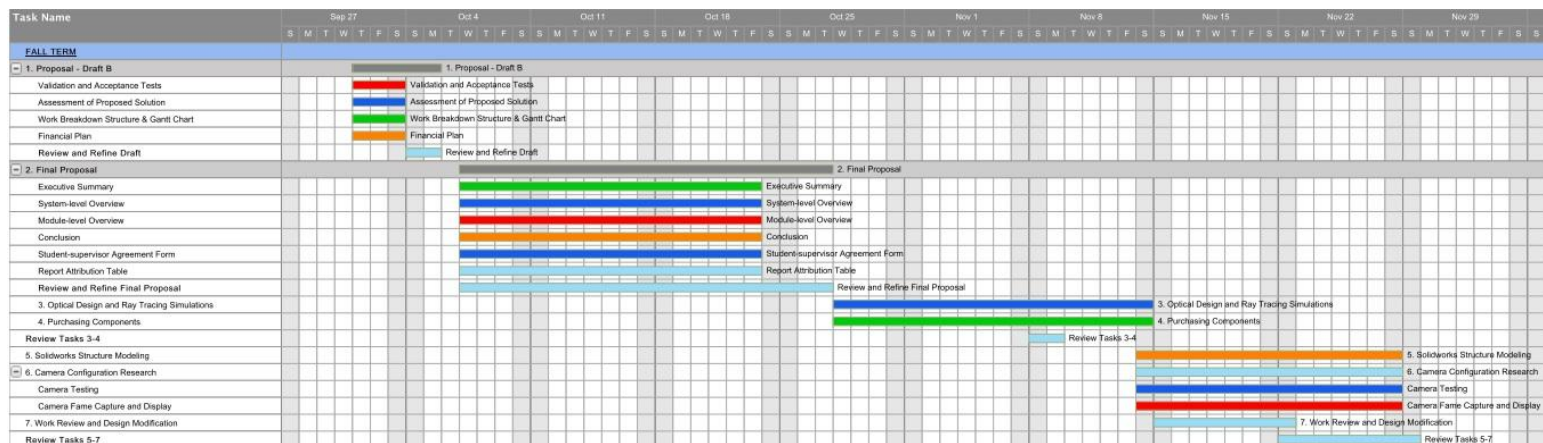
Winter Semester:

It is noteworthy that the components within “16. Design Fair Preparation” will be added after further collaboration within the team.

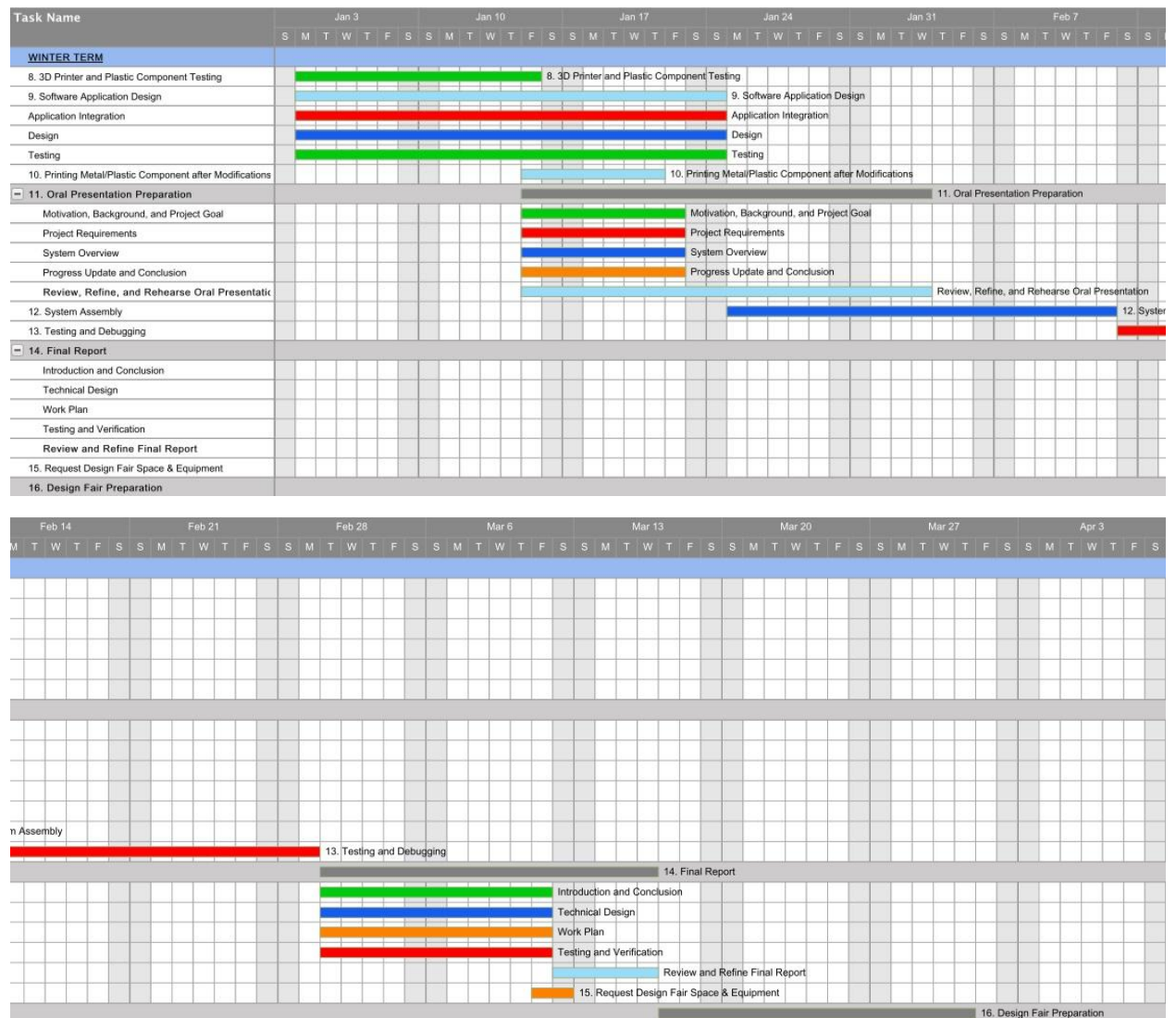




2. Progress Report Gantt Chart Fall Term:

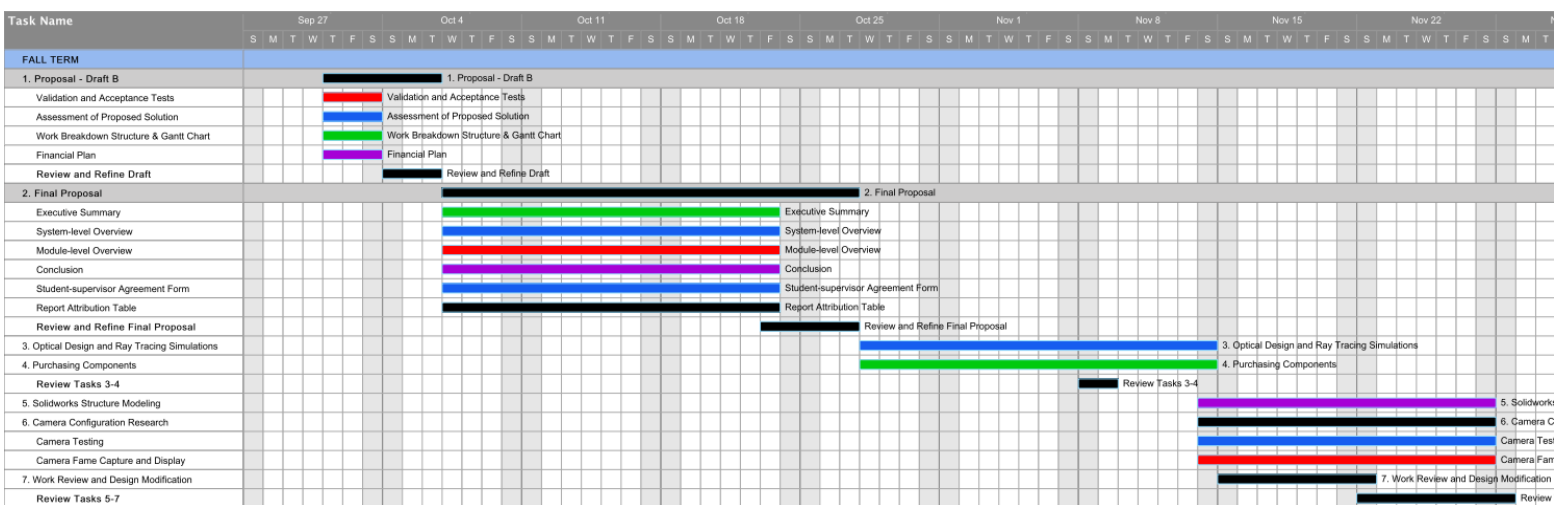


Winter Term:

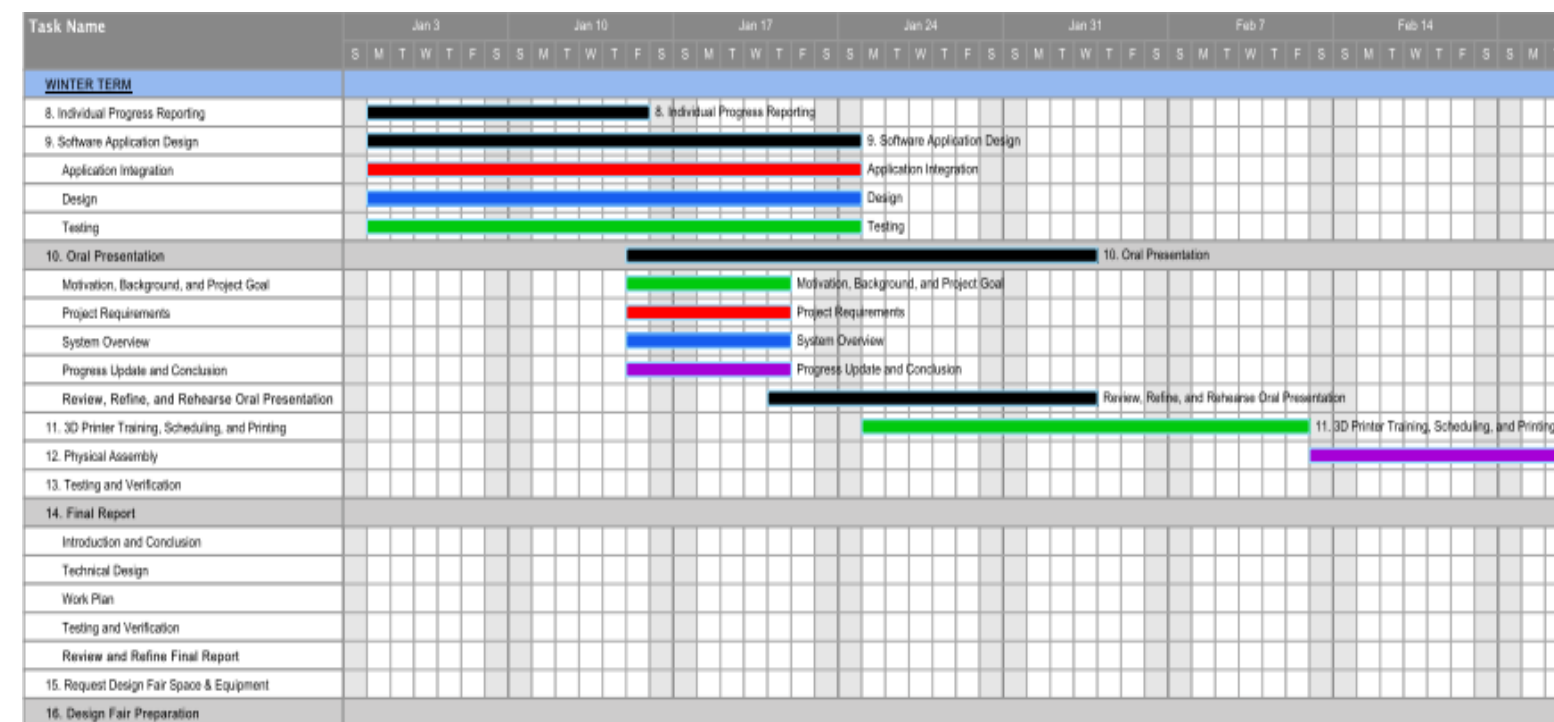


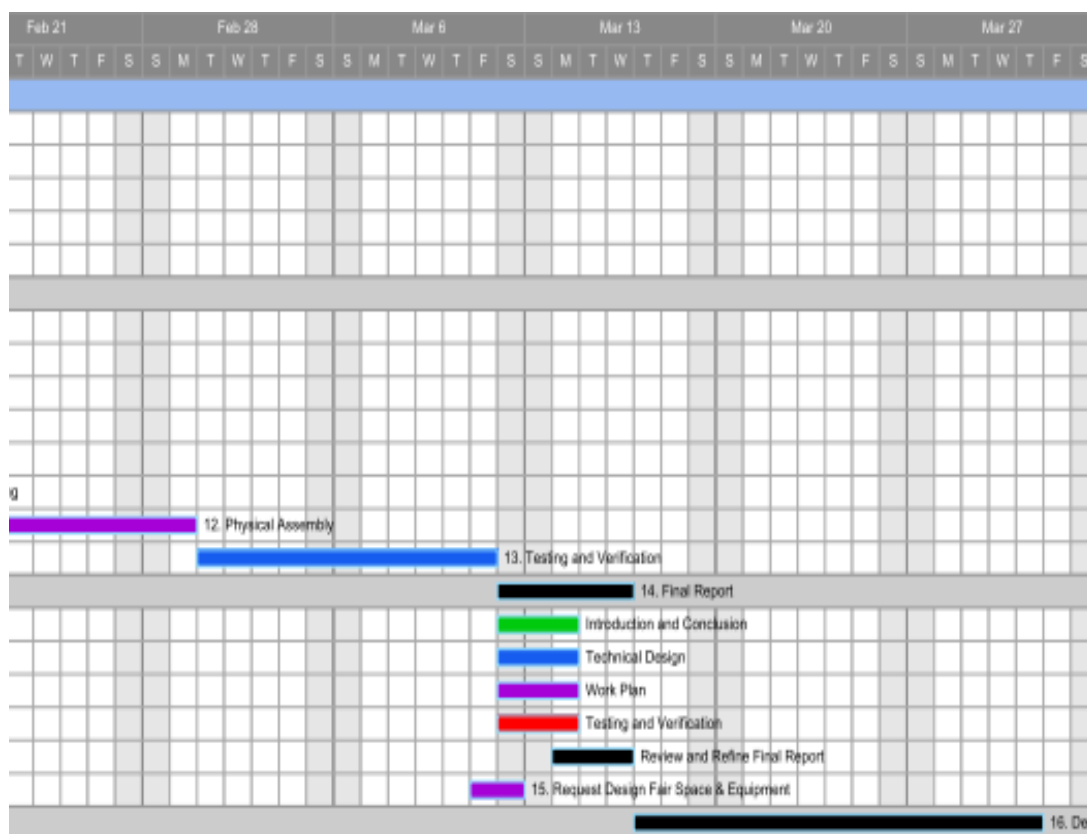
3. Final Gantt Chart

Fall Semester:



Winter Semester:





Appendix B - Financial Summary

Financial Plan

This subsection includes a budget table that outlines the detailed costs and required funding. The items that have costs in the industry but do not require funding in this project would still be listed. The contingency plan will also be provided in case of exceeding the budget limit.

Item	Priority	Cost/Unit	Quantity (Hours)	Total Cost	Requires Funding (Y/N)	Kept/Paid by students (Y/N)
Student Labour						
Student 1		\$12	200	\$2400	N	N
Student 2		\$12	200	\$2400	N	N

Student 3		\$12	200	\$2400	N	N
Student 4		\$12	200	\$2400	N	N
Total Unfunded Student Labor				\$9600	N	N
Materials and Components						
Laser Pointer	2	\$30	1	\$30	Y	Y
3D Printer Plastic	1	\$7/inches^3	20	\$140	N (free resource provided by the Design Center)	Y
WebCamera	1	\$35	1	\$35	Y	Y
Battery for the laser pointer	2	\$2	5	\$10	Y	Y
Lens Tube	1	\$20	1	\$20	Y	Y
Retaining Ring	1	\$4	2	\$8	Y	Y
Plano Convex Lens	1	\$30	2	\$30	Y	Y
Total Cost of Materials and Components	\$303					
Budget Required	\$163					
Capital Equipment						
Ray Tracer Software	1	\$0	1	\$0	N (free edition)	N

Total Cost of Capital Equipment	\$0
Budget Required	\$0
Total Budget Required	\$163

Actual Costs

Item	Priority	Cost/Unit	Quantity (Hours)	Total Cost	Requires Funding (Y/N)	Kept/Paid by students (Y/N)
Student Labour						
Student 1		\$12	180	\$2160	N	N
Student 2		\$12	180	\$2160	N	N
Student 3		\$12	180	\$2160	N	N
Student 4		\$12	180	\$2160	N	N
Total Unfunded Student Labor				\$8640	N	N
Materials and Components						
Laser Pointer	2	\$30	1	\$30	Y	Y
3D Printer Plastic	1	\$3/hour	4	\$12	Y(price from gerstein MADlab)	Y
WebCamera	1	\$35	1	\$35	Y	Y
Battery for the laser pointer	2	\$2	5	\$10	Y	Y

Lens Tube	1	\$20	1	\$20	Y	Y
Retaining Ring	1	\$4	2	\$8	Y	Y
Plano Convex Lens	1	\$30	2	\$30	Y	Y
Total Cost of Materials and Components	\$303					
Budget Required	\$175					
Capital Equipment						
Ray Tracer Software	1	\$0	1	\$0	N (free edition)	N
Total Cost of Capital Equipment	\$0					
Budget Required	\$0					
<u>Total Budget Required</u>	<u>\$175</u>					

The actual cost is almost identical to the financial plan except the cost of 3d printing is significantly lower than planned. It costs \$3 each hour, and we used 3 hours only. However, it is free from design center, so it does not contribute to the budget. Also, we were able to finish the project in a shorter time than planned which reduced the total costs of the student labour. Eventually, we were able to finish the project, and we reduced the expenses of the project.

Appendix C - Validation and Acceptance Test

In this subsection, tests to evaluate the design with each objective and its objective goal will be discussed. Success throughout each of these tests will exemplify the level of success with our completed design. Below, the objectives are listed and their respective validation tests are outlined.

- *Powerful*
 - The magnification level will be increased up to the design's maximum; if this imaging system is able to reach a magnification level of 100x, it will be considered as a success in terms of its power.
 - Since the dimensions of the image sensor plane are known, the concentration of the cell sample could be verified through the equation, $(\text{concentration} = \# \text{ of cells in the image} * \text{magnification}^2 / \text{area of sensor plane})$.
 - The technique above could be used to calculate the maximum magnification level by inputting the known concentration, the number of cells in image and area of sensor at the maximum magnification level.
 - If the results of the concentration at all magnification levels are within 10% error range of the given cell concentration value, it means the magnification levels are calibrated correctly as what is expected in the optical calculation and the simulation.
- *Clear*
 - Regarding this objective, two tests will be hold.
 - In the first test, the observable distance between the two shortest points of a specimen will be measured and recorded:
 - At 20x magnification, this distance should be at most 1 micrometers
 - At 100x magnification, this distance should be at most 0.20 micrometers

If the design is able to meet this resolution, the design will be considered to have partial success in terms of clarity

- The second test will require the design team to make observations on the cellular content of a fluorescent labeled cell sample with known concentration.
 - The design must be able to differentiate cells with fluorescent label from the non-labeled cells at certain magnification level, if there are non-labeled cells to be compared with.
 - The shape and color of the cells should also be verified by the cellular view of a traditional two lenses microscope. Generally, the cells observed through the microscope and the cells displayed by the design system should have the same shapes and colors, if the light sources are the same.
- If the design is able to pass all tests, the design will be considered to have complete success in terms of clarity.
- *Inexpensive*
 - The team will make a comparison of costs and components between an optical microscope and the design in order to illustrate its cost-effectiveness. Additionally, if the total material cost of the design is lower than \$150, then it will also be considered to have success in this field.
- *DFMA*
 - Success in design for manufacturability will immediately be achieved if all individual components are printed using a 3D printer.
 - In regards to designing for assembly, a two-page manual will be created to assemble the design using all the individual components. The manual and the individual components will be given to 3 volunteers to assemble the design; if all three volunteers are able to assemble the design within four hours, the design will be considered to have success in its design for assembly.

With success in all these fields, our design will be able to help people living in environments with limited resources.

Appendix D: SolidWorks Models

Figure 1. Assembled Solidworks Model

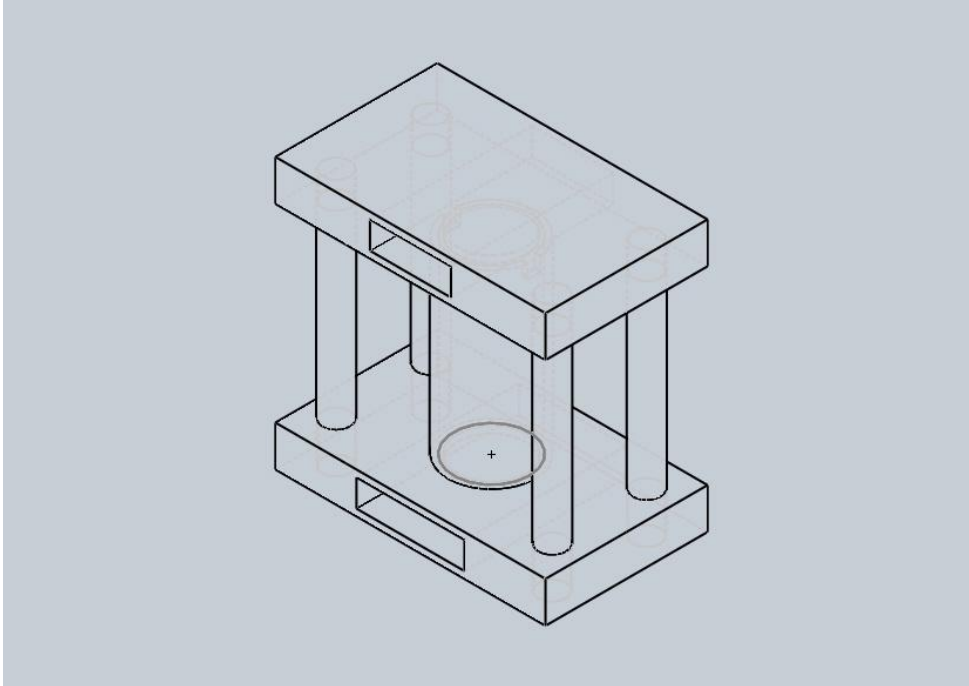


Figure 2. Base for camera (in mm.)

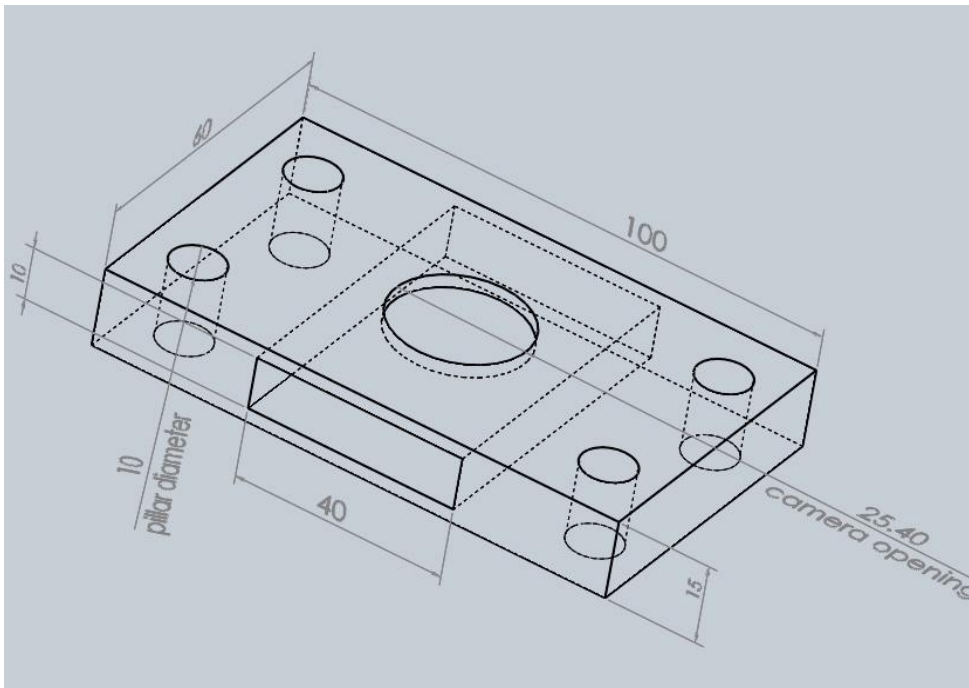


Figure 3. Pillar (in mm.)

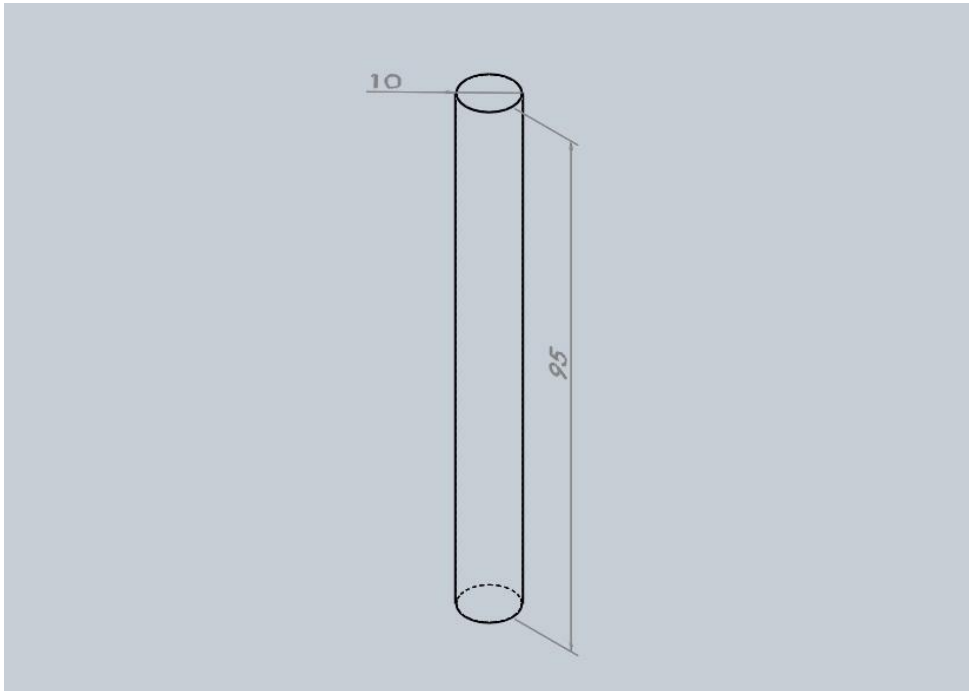


Figure 4. Top cover for test sample (in mm.)

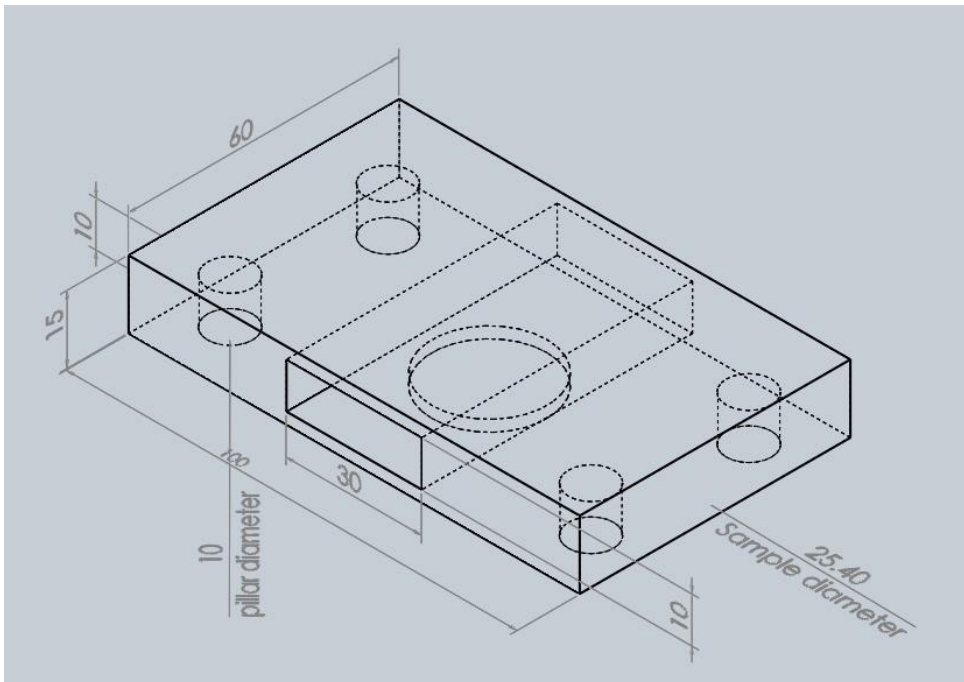
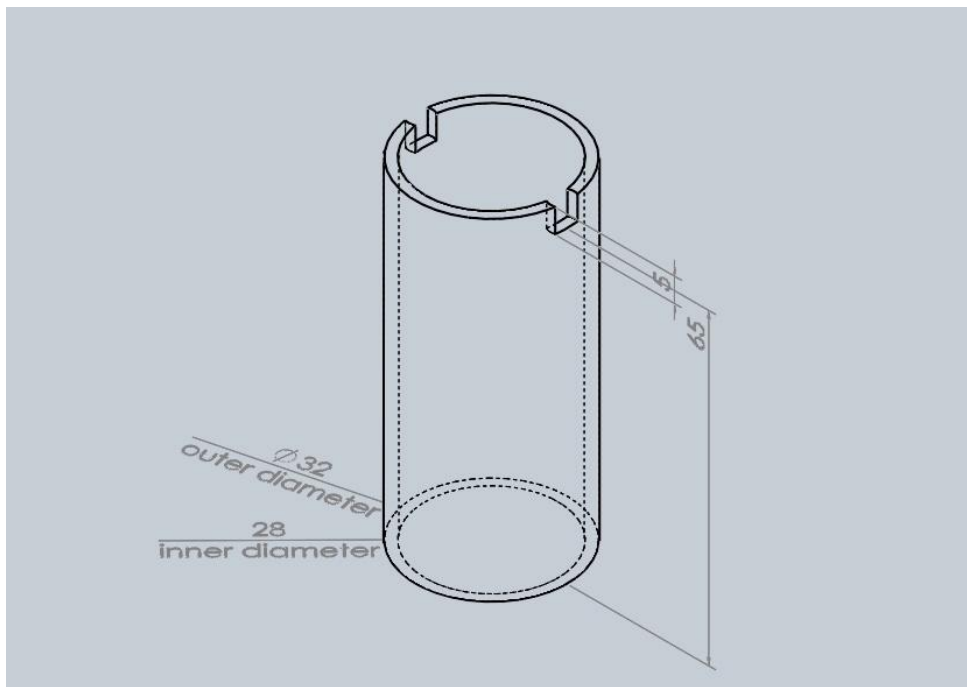


Figure 5. Lens Tube Cover (in mm.)



Appendix E Camera Capture Function and Camera Testing

Camera interface

```
#include "stdafx.h"
#include <cv.h>
#include <cxcore.h>
#include <highgui.h>
int main( int argc, char** argv )
{
    p[0] = CV_IMWRITE_JPEG_QUALITY;
    p[1] = 10; //quality value
    p[2] = 0;
    char* filename="D:\\1.jpg";
    //declare IpIImage pointer
    IpIImage* pFrame = NULL;
    //acquire camera
    CvCapture* pCapture = cvCreateCameraCapture(-1);
    //create new window
    cvNamedWindow("video", 1);
    //show screen
    while(1)
    {
        pFrame=cvQueryFrame( pCapture );
        pSaveFrame=cvCreateImage(cvSize(768,1280),pFrame->depth,pFrame->nChannels);
        if(!pFrame)break;
        cvShowImage("video",pFrame);
        char c=cvWaitKey(33);
        if(c==27)break; //press esc to exit
        if(c==13) //press enter to store image
        {
            cvResize(pFrame,pSaveFrame,CV_INTER_LINEAR);
            cvSaveImage(filename,pSaveFrame,p);
            cvReleaseImage(&pSaveFrame);    }
    }
    cvReleaseCapture(&pCapture);
    cvDestroyWindow("video");
}
```

Data Base Design

```
protected void butSubmit_Click(object sender, EventArgs e)
{
    SqlConnection connection = null;

    try
    {
        Byte imgByte = null;
```

```

if (FileUpload1.HasFile && FileUpload1.PostedFile != null)
{
    HttpPostedFile File = FileUpload1.PostedFile;
    imgByte = new Byte[File.ContentLength];
    File.InputStream.Read(imgByte, 0, File.ContentLength);
}

connection = new SqlConnection(ConfigurationManager.ConnectionStrings
["ConnectionString"].ConnectionString.ToString());
connection.Open();
string sql = "INSERT INTO Table1(title,image) VALUES(@theTitle, @theImage) SELECT
@@IDENTITY";
SqlCommand cmd = new SqlCommand(sql, connection);
cmd.Parameters.AddWithValue("@theTitle", txtTitle.Text);
cmd.Parameters.AddWithValue("@theImage", imgByte);
int id = Convert.ToInt32(cmd.ExecuteScalar());
lblStatus.Text = String.Format("ID is {0}", id);
Image1.ImageUrl = "~/DisplayImg.ashx?id=" + id;
}

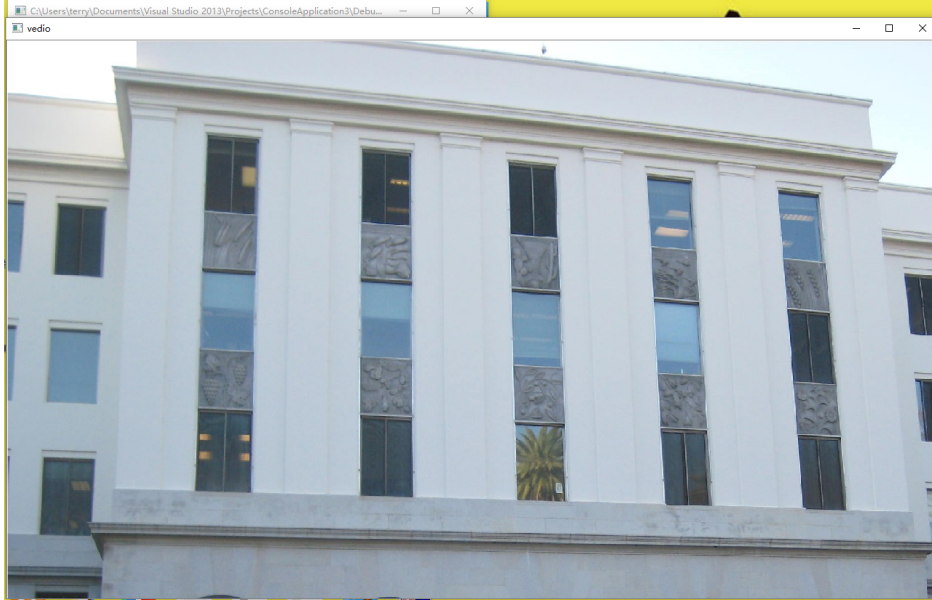
catch
{
    lblStatus.Text = "There was an error";
}

finally
{
    connection.Close();
}
}

```

Camera Captured Image

Figure 1. Test result of the camera capture. Video stream captured from the camera is in the window. Press ESC to close window and exit. Press enter to store image to 1.jpg



Appendix F - Weighted Decision Matrix

- The Single Lens Imaging System and the Lensless Imaging System are the solutions with most mobility, most accessibility of materials and lowest cost because of their simple structure and lower prices for certain components, such as the image sensors. However, their optical designs restrict them from generating colour images.[10][17][18]
- The last two design alternatives could not adjust the magnification for different purpose.[10][18][19]
- The fiber taper in the third design is much more expensive than the lenses in other alternatives.[14][16]
- The CMOS sensor in the fourth design requires the highest resolution and the smallest CMOS size.[17][18] Therefore, the overall costs of the respective two designs is expected to be the highest.

Recall that “DFMA” is an acronym for “Design for Manufacturability and Assembly”.

Weighted Decision Matrix

Weighted Decision Matrix								
Objective:	Weighting		Notes:					
Powerful	32.5%		This weighted decision matrix weighs all the objectives using collaboration and consensus within the team. From this, the team evaluated each design alternative with their level of compliance to the weighted objectives in order select the most appropriate design. The highest score reflects the selected design. The weightings of the objectives in this decision-making processs were also made via consenses within the team. The objectives were rated in order of importance earlier using a pairwise comparison and can be					
Clear	27.5%							
Inexpensive	22.5%							
DFMA	17.5%							
Total	100.0%							
Alternative Design:	Single Lens		Two Lens		OFT		Lensless	
Objective:	Percent	Weighted	Percent	Weighted	Percent	Weighted	Percent	Weighted
Powerful	80.00%	26.0%	100.00%	32.5%	60.00%	19.5%	50.00%	16.3%
Clear	80.00%	22.0%	75.00%	20.6%	75.00%	20.6%	80.00%	22.0%
Inexpensive	80.00%	18.0%	70.00%	15.8%	50.00%	11.3%	90.00%	20.3%
DFMA	80.00%	14.0%	80.00%	14.0%	50.00%	8.8%	90.00%	15.8%
Total		80.00%		82.88%		60.13%		74.25%

Appendix G - Testing and Verification results and documentation

1. Powerful

For this criterion I have compared the size of florescent beads in reality and the size of the beads in the image to obtain the level of magnification.

First of all, the diameter of beads is between 500-600um. This type of beads absorbs green light to emit orange light. We choose to take the median 550um as the diameter of the bead of interest. The captured bead is shown below:



The measured diameter is approximately 900 pixels, while the size of image is 1280X720 and the size of the sensor plane is around 4 mmX2.3 mm. The level of magnification could be obtained using the following equation.

Magnification= Width of the sensor plane * diameter(pixels) / width of image(pixels) / actual size of bead

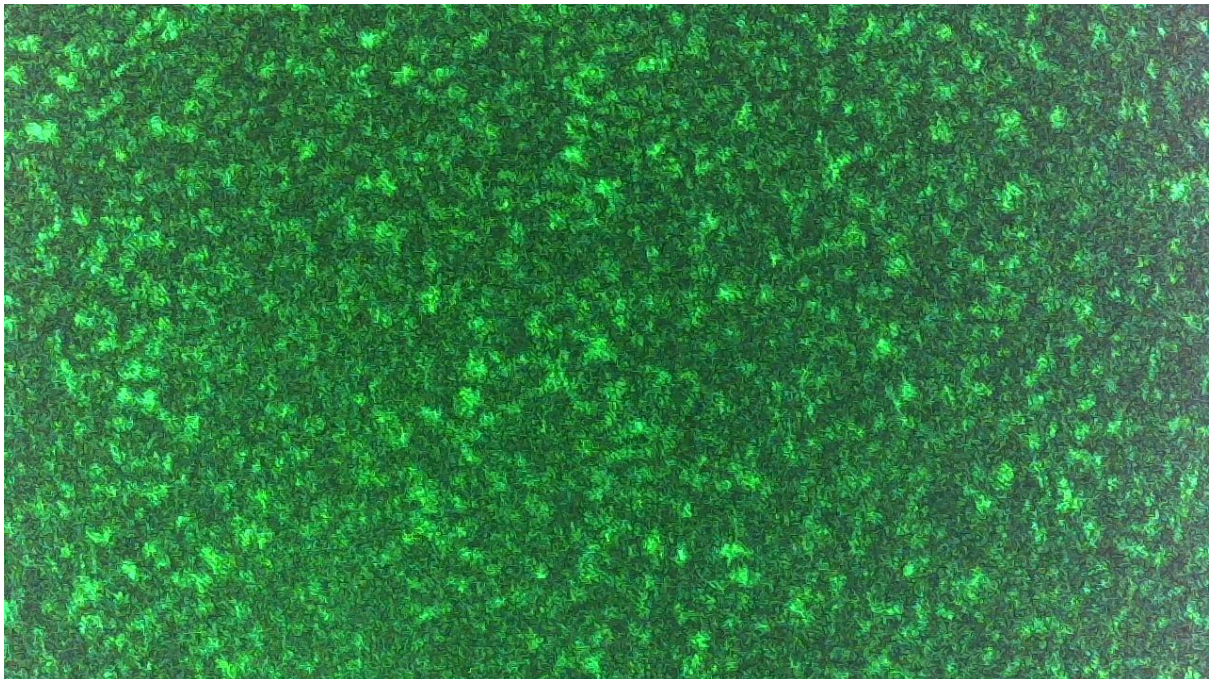
$$=4\text{mm} * 900 \text{ pixels} / 1280 \text{ pixels} / 0.55\text{mm}$$

$$=5.1$$

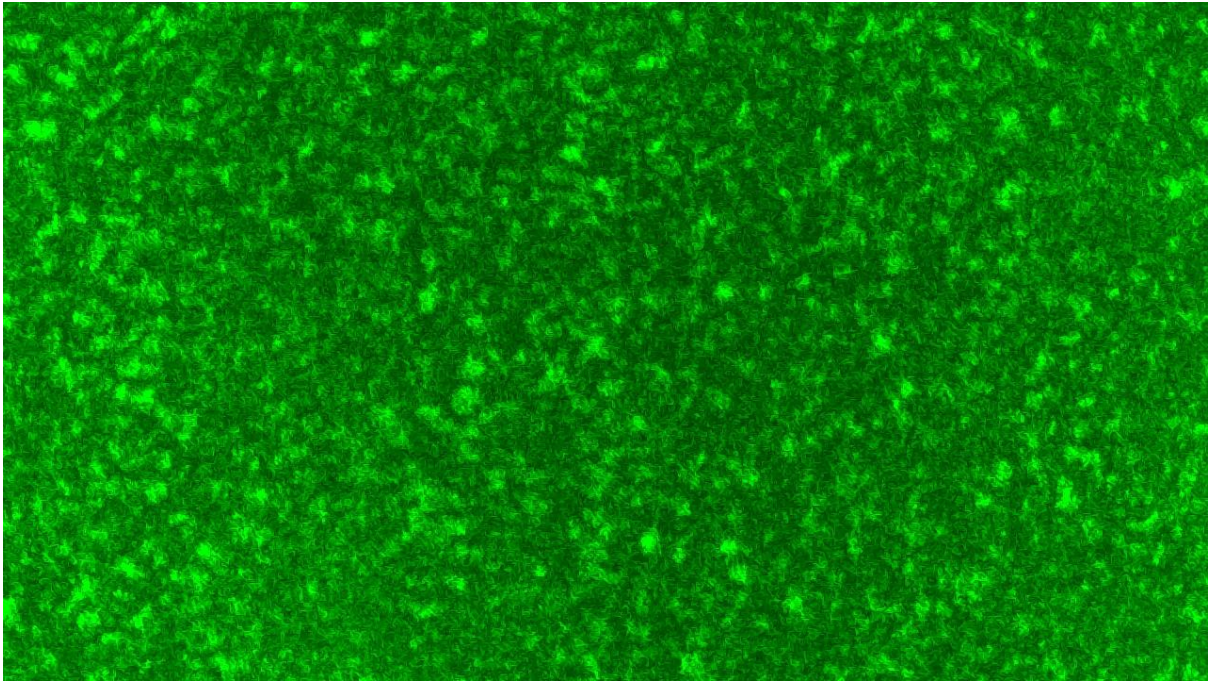
The level of magnification reaches the target magnification level 5x. Therefore, our system is deemed successful for this criterion.

2. Clear

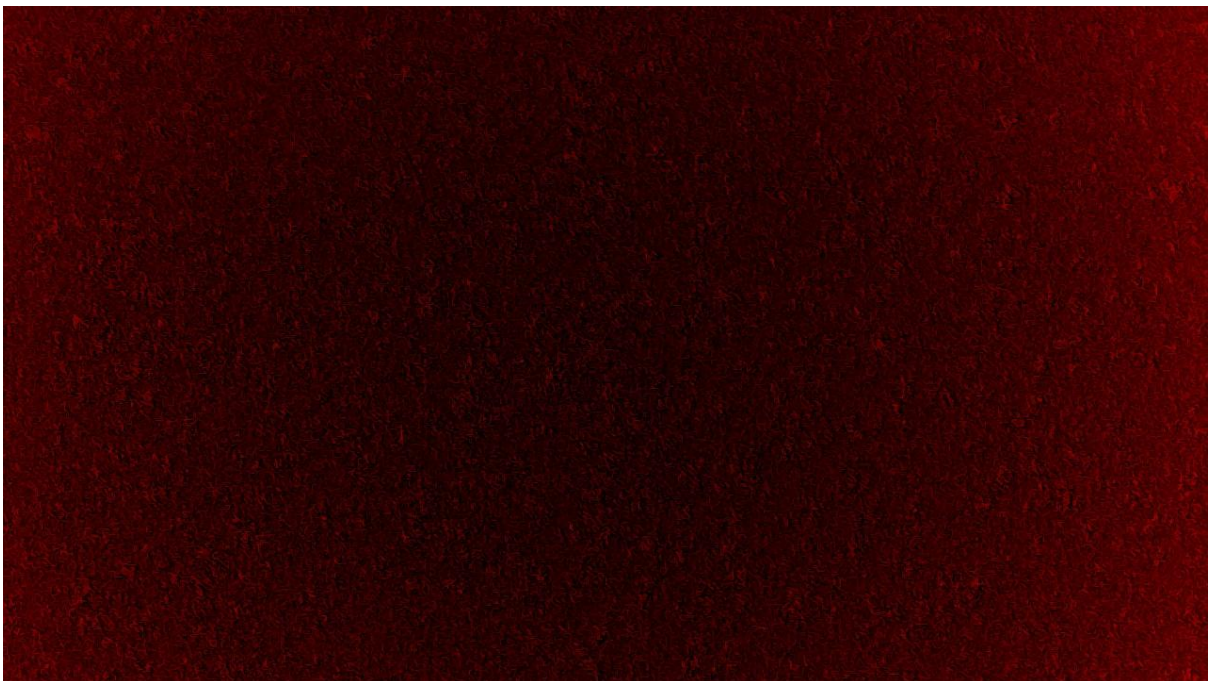
For the design objective, we set the standard as indentifying cells between 2um to 8um. In our case, due to lack of biological test sample and lab environment, we decided to use spherotech FP-3058-2 Fluorescent Pink Particles as test sample with diameter between 2.5um-3.4um. The following sample solution is at concentration around 16 million beads/ml.



Original Channel

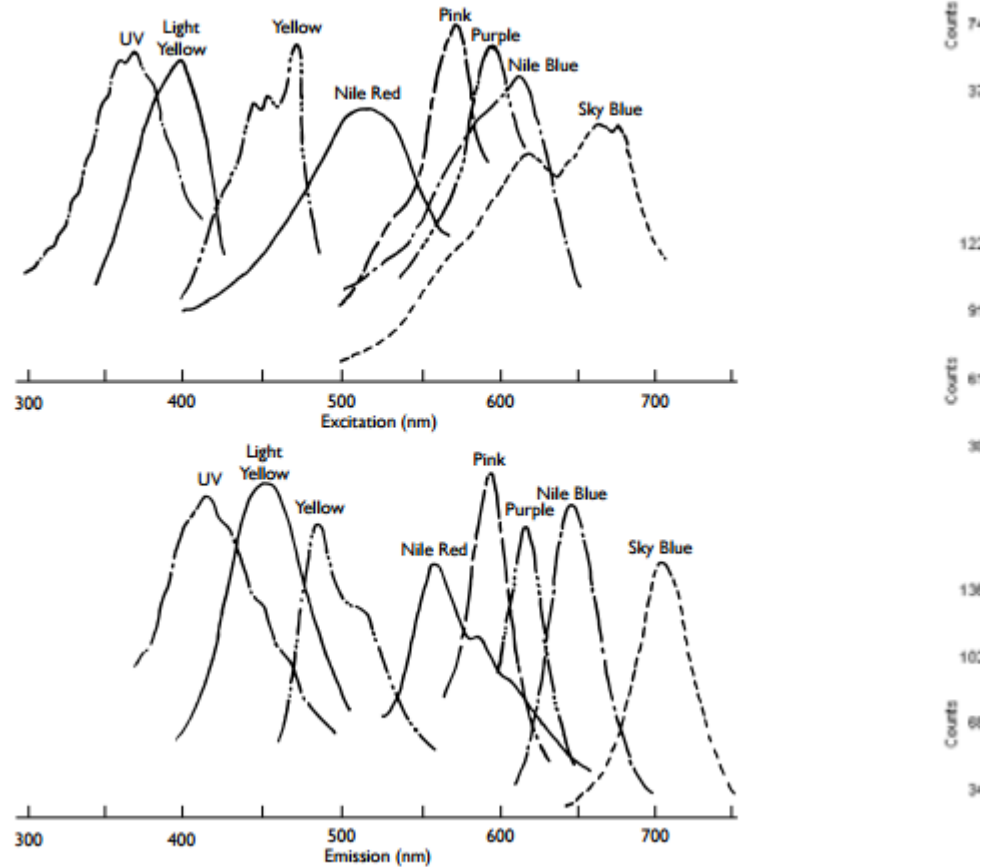


Green Channel



Red Channel

Figure 1 Excitation & Emission Spectra of the fluorophores used in SPHERO™ fluorescent particles



NOTE: All spectra are taken with fluorescent particles in aqueous

As we can see, the particles are emitting pink light which has intensity peak at 590nm. Therefore, it is possible to see those particles in both green channel(spectrum peak at 530nm) and red channel(spectrum peak at 660nm). However, since 532nm green laser is used as excitation for fluorescence, there is lots of noise in green channel that we are not interested in.

Thus, we can see some "halos" around the particles in green channel. In red channel, the shape and light intensity of the particles could be clearly distinguished from the background, but the light intensity is quite low for naked eye to observe. For the future development, we can add filter in our system to isolate the pink light we are interested in.

3. Inexpensive

In our project requirements and “Tests and Validation” section, the target standard for ‘inexpensive’ is material cost below \$180. Here is the latest financial plan for the materials and components.

Materials and Components						
Laser Pointer	2	\$30	1	\$30	Y	Y
3D Printer Plastic	1	\$3/hour	4	\$12	Y(price from gerstein MADlab)	Y
WebCamera	1	\$35	1	\$35	Y	Y
Battery for the laser pointer	2	\$2	5	\$10	Y	Y
Lens Tube	1	\$20	1	\$20	Y	Y
Retaining Ring	1	\$4	2	\$8	Y	Y
Plano Convex Lens	1	\$30	2	\$30	Y	Y
Total Cost of Materials and Components	\$303					
Budget Required	\$175					

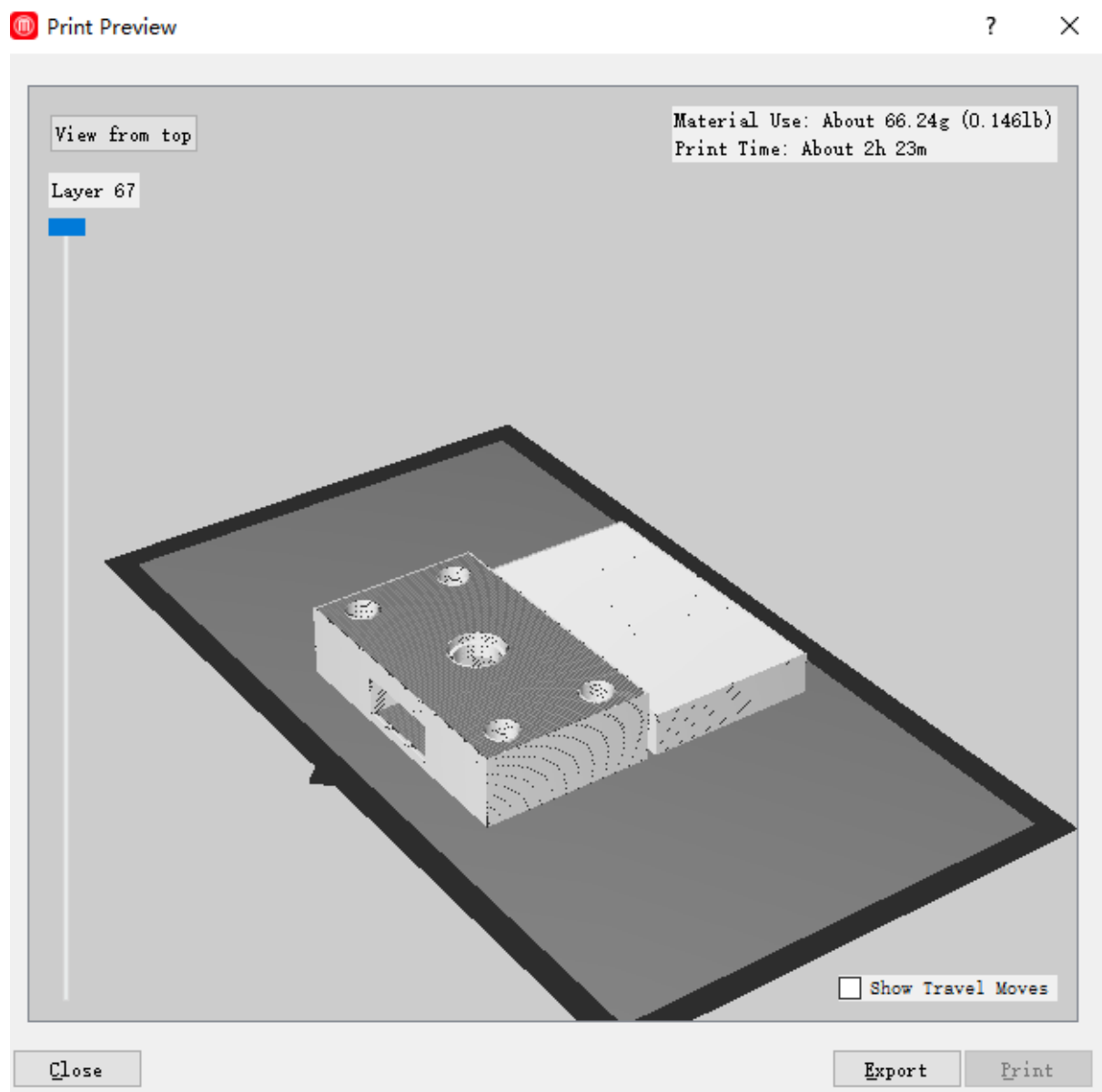
The overall cost is \$175 which is lower than our target cost. There is a issue with US/CAD currency conversion in the purchase optical components. In this case, the US dollar is not converted back to CAD dollar.

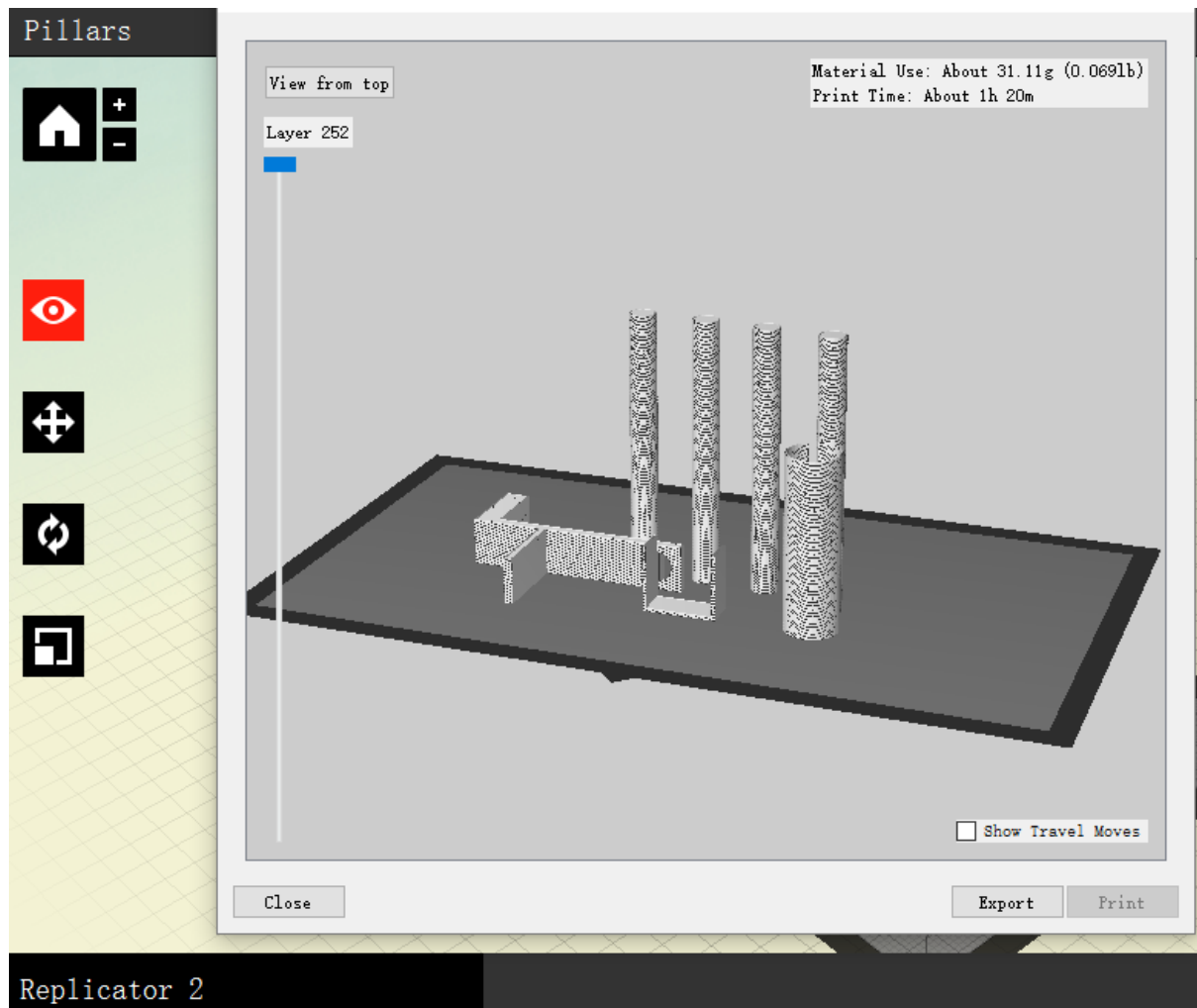
4. Design for manufacturability and Assembly

As can be seen from the Makerbot desktop preview. All components of our system can be printed with Makerbot Replicator 2.

The total estimated time to run those two tasks is respectively 2 hour 23 min and 1 hour 20 min. The total print time is 3 hour 43 mins which is less than our target, 4 hours.

The assembly time is usually around 30 mins, including time for cutting and gluing, but not optical calibration, since calibration is usually time-consuming.





5. Accuracy and functionality of the cell num/concentration measuring application

In this verification process, we used two types of florescent particle solution, respectively, 16 millions/ml solution and 4 millions/ml solution. The former is 4x more concentrated than the latter.

Firstly, 10um of each type of solution is held between the two glass slides for observation.

Then, the captured image of 16 million/ml sample is transformed into to binary image below to seperate particles from the background color. The threshold is set, so pixels below curtain intensity is converted to black, while the rest is converted to white.

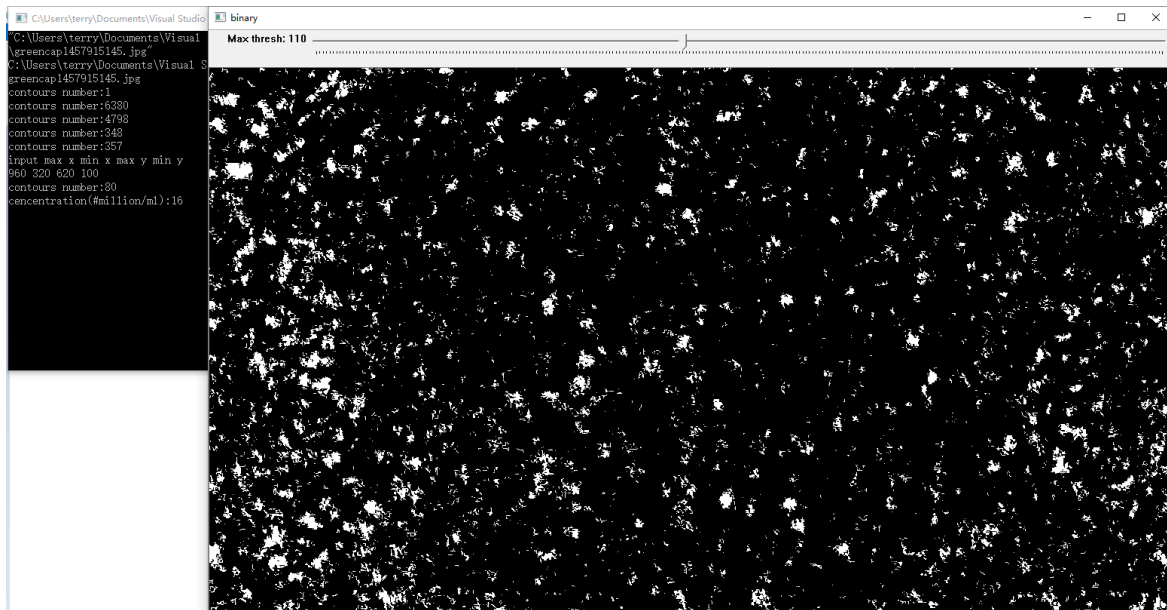


Fig. Setting image threshold to obtain binary image(black/white) (16million/ml)

After setting restrictions on the area of white dot, the number of particles(contours) and can be obtained. In the flowing graph, the red rectangles enclose the contours of florescent particles. The area of interest is set as the rectangle with x axis range 320 to 960 and y axis range form 100 to 620 due to the light intensity aberration on the edge of image. Thus, contours of particles outside the area of interest is not counted. In the end, the particle number is 80. To obtain 16 million/ml concentration, the multiplication ratio is preset to 0.2 .

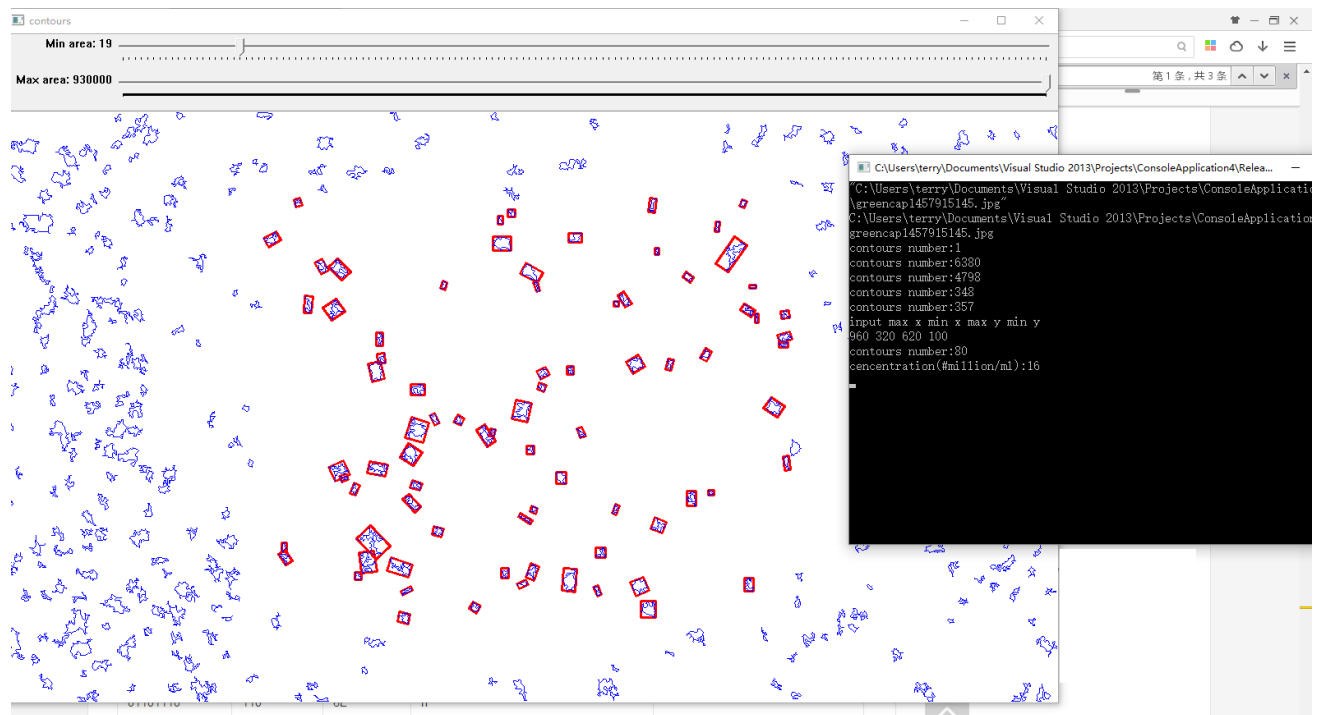


Fig. Setting contour restrictions and ratios to obtain the number of contours and concentration (16million/ml)

For the 4 million/ml sample, we obtained particle number 22 with the same area of interest and particle contour area restrictions. After setting the same multiplication ratio 0.2, we obtain the concentration- 4.4 million/ml. Comparing to the 4 million/ml concentration obtained after dilution, the measured concentration has an error of 0.4 million/ml and a error percentatge of 10%

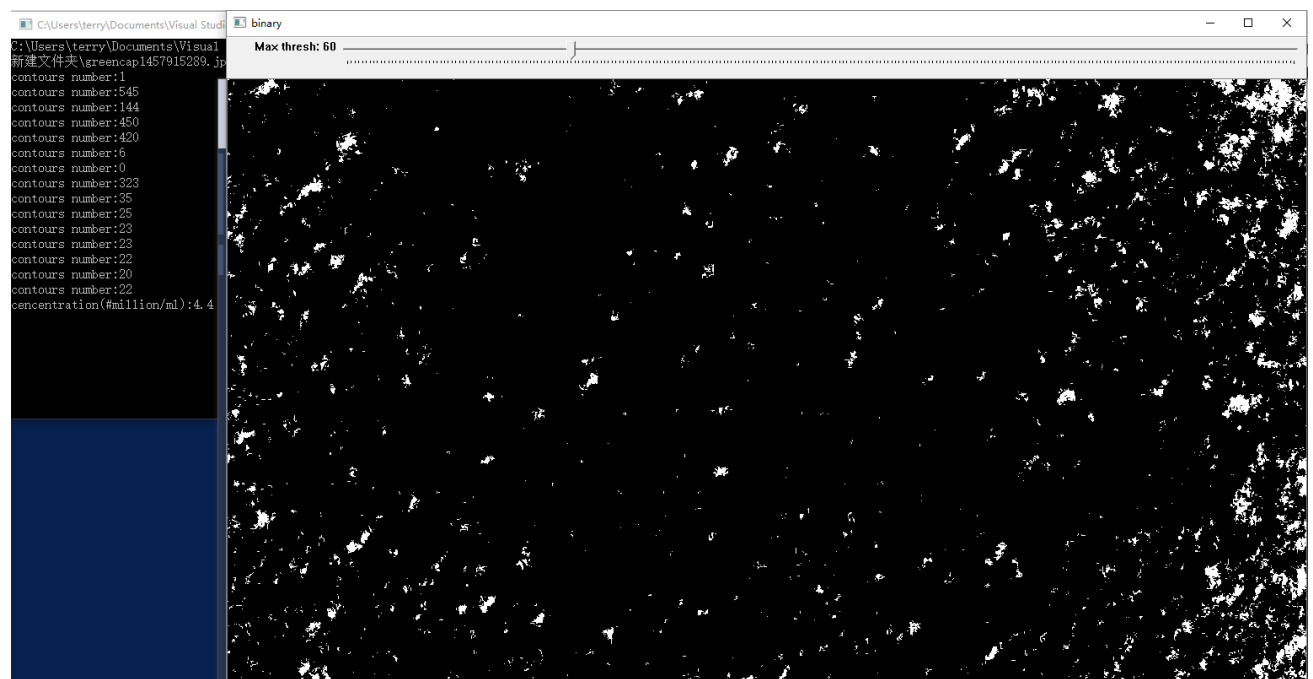


Fig. Setting image threshold to obtain binary image(black/white) (4million/ml)

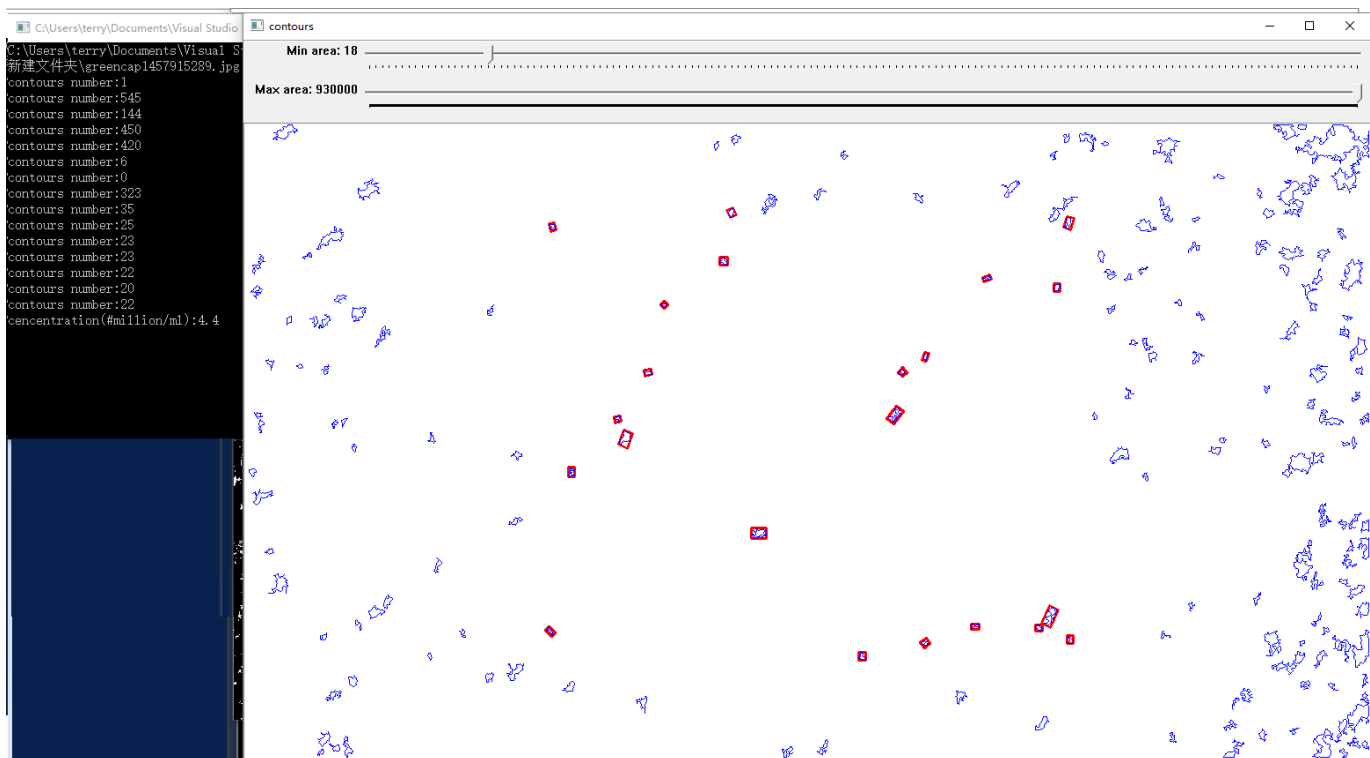


Fig. Setting contour restrictions and ratios to obtain the number of contours and concentration (16million/ml)