Zhejiang University/University of Illinois Urbana-Champaign Institute

Senior Design Final Report

SMARTPHONE-BASED FLUORESCENCE MICROSCOPE

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Abstract

Our project worked on creating a compact, smartphone-based fluorescence microscope to detect the fluorescence effect of green fluorescent protein (GFP) in *Caenorhabditis elegans* (*C. elegans*) sample. By utilizing a reversed external smartphone camera lens as the microscope objective and the built-in smartphone camera as the tube lens, we construct a functional, low magnification (~4X) optical system. This optical system achieved magnification and resolution sufficient to observe the internal structure of *C. Elegans.* We also managed to design and print out a stable casing to couple the microscope system with our smartphone. Our project however, was not successful in delivering fully promising results in the fluorescence microscopy aspect – we could not show fluorescing in the foregut region of the worm, and the overall fluorescing effect is masked by significant background noise.

Key words: fluorescence microscope, *Caenorhabditis elegans*, 4X magnification, green fluorescent protein

Contents

1. Introduction

Fluorescence microscopy is a technology widely used in biomedical field, with its applications including, but not limited to pathological diagnosis, molecular studies, detection of microorganisms and study of genetic expression. However, today's standard benchtop fluorescence microscopes remain a costly laboratory apparatus, with highly specific and sensitive subcomponents, compounded by the fact that benchtop microscopes are often bulky and difficult to move from one place to another. These factors have severely limited fluorescence microscope's potential impact and usability to a wider population. With the rise of smartphone cameras technology and power-efficient light-emitting diodes (LED) that are accessible and relatively low-cost, we will attempt to design a compact smartphone-based fluorescence microscope that aim to detect and observe the presence of green fluorescence protein (GFP). We will aim to integrate smartphones as imaging devices to couple with a miniature, external microscopic device that serves to magnify sample that is otherwise invisible to the naked eye.

One commonly used model organism in fluorescence microscope (FM) technology is *Caenorhabditis elegans (C. Elegans)*, a multicellular round worm organism that has its whole genome fully determined and commonly used in the study of neuroscience. The key reason that makes *C. Elegans* particularly useful is because many genes within *C. Elegans* have corresponding structures that resemble human genes, making the study of this organism insightful. On top of that, *C. Elegans* is a very easy to maintain sample microorganism - they are easily observable and is a sample we could obtain from our neighbor institute – ZJE laboratory. Hence, we will be catering our design towards observing the fluorescence of green fluorescent protein (GFP) in *C. Elegans.* GFP in *C. Elegans* get excited at peak wavelength of 488 nm whereas the emission peak wavelength is 510 nm. Applications of GFP in *C. Elegans* is commonly being used as a reporter gene to signal certain protein locations within the worms.

While a smartphone-based fluorescence microscope system that we set out to design has already have several successful products in the market such as CellScope designed in University of Berkeley, California, smartphone-based microscopes are still a novel design with many possibilities. Therefore, certain approaches, successes and challenges faced in our design may still serve as reference or stepping stones for future, more comprehensive research work. By improving off our design ideas, smartphone-based fluorescence microscope can be highly

impactful in rural, underdeveloped areas to be used as point-of-care (POC) products that serve as biomedical evaluation such as determining quality of water and viral detection.

2 Outline of Subject Matter

2.1 Introduction

A fluorescence microscope can be made up of four fundamental components – an illumination source, an optical system to magnify the sample, excitation and emission filters and an imaging device. Suffice to say, a professional, well-built standard fluorescence microscope comes with many other parts (fine-tuned knobs, condenser, multiple lenses to for aberration correction) that mostly serve as tools to enhance benchmark specifications of the microscope such as magnification, resolution etc. In our design, since magnification factor in 3-5X range is sufficient to observe GFP's structural fluorescence in *C. Elegans*, and we are also limited in terms of funding, we will be designing a simplified system that revolves only around the four fundamental building blocks.

The block diagram of our high level design is constructed based on the trans-illumination model in fluorescence microscope, where the excitation light source faces our imaging device. Figure 1 shows the high level block diagram of our design with its subcomponents.

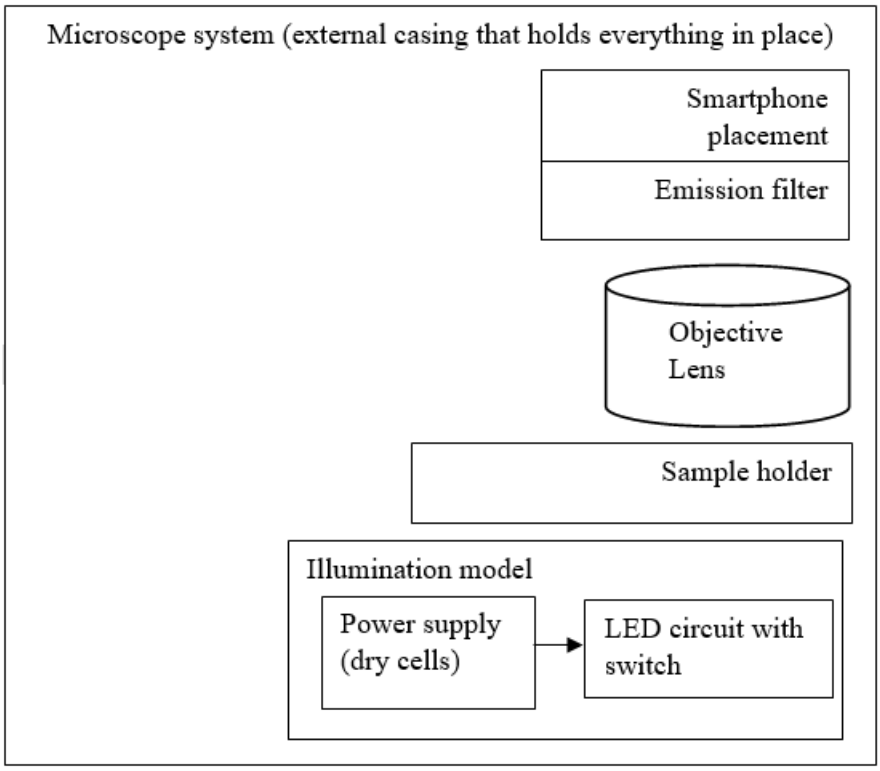

Figure 1. Figure shows the block diagram for trans-illumination smartphone fluorescence microscope design.

As the project is a cross-discipline collaboration, the design of different subcomponents is responsible by different team members. Two team members, Feifan, Xie and Wentao, Ni (both from Mechanical Engineering) are mainly responsible in the computer-aided design of the external casing for the microscope system. The design of the casing includes 3D printed parts to hold the objective lens, the sample, the excitation/emission filters as well as the illumination source. Another team member, Shu Xian, Lai is responsible for the design of the illumination source. Lai is also responsible for coming up with optical model to use in our fluorescence microscope model.

2.1.1 Alternative Design (Conducted in Parallel by Feifan, Xie)

In the process of designing a suitable working optical model for our system, one of our teammates, Feifan, Xie decided to work on an alternative design that skipped the design steps needed for the optical system. Xie did so by acquiring a commercial smartphone brightfield microscope called "TipScope" (does not have any fluorescence technology), which he then moved on to incorporate the fluorescence aspect on top of "TipScope"'s system and designed a separate, stand-alone housing for it. As our initial effort of building our own optical model eventually proved to work (by using a reversed smartphone camera lens as the objective lens), the main discussion of the paper will revolve around the initial design, since it is a model we built from scratch and has a stronger mathematical standing behind why the model works.

Xie's effort was also hugely beneficial to us throughout the project to compare our original model with a commercialized product and identify strengths and weaknesses between these two systems. Overall, Xie's system provide a higher imaging quality and lower aberration effects compared to the optical model we constructed. However, Xie's system also could not properly capture the fluorescence effect. Xie's efforts, prototype and results will be included in Appendix D, E and F. Our team still greatly appreciate Xie's effort nonetheless.

2.1.2 Performance Requirements

Three performance requirements are listed as checkpoints in our design to ensure our microscope reaches the objective, which is to observe *C. Elegans* sample and detect the fluorescence of GFP. The performance requirements are:

i. The microscope system has a magnification factor of 3-5X, which will allow us to observe *C. Elegans* sample.

ii. The microscope system is able to detect green fluorescence (in the range of 510nm) when *C. Elegans* sample is excited by blue LED light in the range of 488nm.

iii. The dimension of our design casing should be compact and resembles a "mini-box" (base area = 15X15cm, and height = 10cm) such that it will be easy to operate and carry around.

The magnitude of magnification factor required is determined by the size of our sample and our project objective. Since adult *C. Elegans* typically can grow up to 1 mm in length and 50 µm in diameter and we are only interested in the presence of GFP instead of the detailed structural studies, a low magnification suffices.

The dimensions requirement for our casing is based on the typical size of smartphone, since we plan to place our smartphone flat-wise on the system to be able to capture images without having to constantly hold the smartphone.

2.2 Design

2.2.1 Design Procedure

The most crucial design choice to make when designing the miniature fluorescence microscope is which optical model we plan to use. When it comes to imaging system, there are three types of optical systems that are commonly used: finite/finite conjugates, infinite/infinite conjugates and infinite/finite conjugate system. In our case, the infinite/finite conjugate model is the most suitable since it is the model which standard infinity-corrected objectives operate based on. The first advantage of this design choice over the other models are we can utilize the camera lens built within smartphone cameras as the tube lens, and only need to find a suitable component to act as our objective and the microscopy model will be complete. In other models however, a compound lens setup consisting of an eyepiece and an objective is needed on top of our smartphone. Furthermore, the distance between the eyepiece and objective (commonly termed tube length) in a compound lens setup are standardized to 16 cm by the Royal Microscopical Society (RMS), which exceeds our design goal of making the system compact. The second advantage of this design choice is the fact that the magnification factor of an infinite/finite conjugate design does not depend on the distance between the tube lens and the objective lens, but only on the ratio of their focal lengths. Since do not have equipment to produce instruments

with high-degree tolerance control, more flexibility between the components while not losing functionality is a great plus.

In our infinite/finite conjugate model, the magnification factor of our microscope is given by equation 1
(*f* represents the focal length of the lens):

$$M = \frac{f_{tubelens}}{f_{objective}} \quad (1)$$

In the following sections, we will be discussing some of the design specifications of our smartphone microscope by assuming the user is using a HUAWEI P30 ELE-TL00 smartphone released in China. HUAWEI P30 is one of the company's 2019 flagship smartphones, and it is reasonable to assume that most latest mid-range smartphones would be roughly up-to-par with that model.

In our design model, the built-in camera lens inside the smartphone plays the role of tube lens, which means the focal length value of our tube lens is predetermined. Detailed calculations step required to obtain the necessary parameters are expanded in section 2.2.2 below.

After determining which optical model to use, the remaining work will be to align the components as sketched in figure 1 with our smartphone. The external casing will be designed with computer-design software and be 3D-printed out with acrylic material, whereas the illumination module will be designed as a printed circuit board (PCB) using simple electronic components including LEDs, slide switches and current-limiting resistors.

2.2.2 Design Details

2.2.2.1 Optical Train of Infinite/Finite Conjugate Model

To choose an appropriate objective lens that fit into the infinite-corrected conjugate model design, we decided to use a reversed smartphone camera module as our objective. The choice of using a reversed smartphone camera lens is based on the Principle of Reversibility of Light Path, which states that if the traversal direction of light rays is reversed while all other factors remain constant, then the light rays will follow the exact same path as before.

In Figure 2, we show a depiction of how light travels within a smartphone camera lens series until it is captured by the imaging sensor within the smartphone. The key observation to make in

figure 2 is the fact that we can obtain readily detached smartphone cameras, detach the sensors behind those cameras and flip the camera around to couple it with the built-in camera in our smartphones. By doing so, we successfully create a simple but functional model of the infinite/finite conjugate design model, as shown in figure 3.

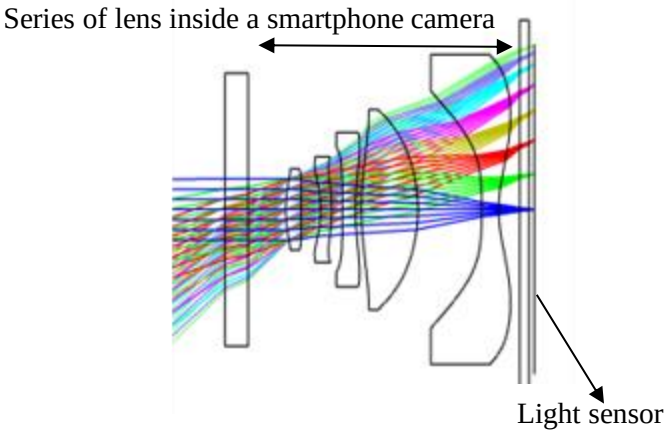

Figure 2. Figure shows different wavelengths of light travelling through the series of lens within a smartphone camera and eventually hit the sensor that is built-in the smartphone.

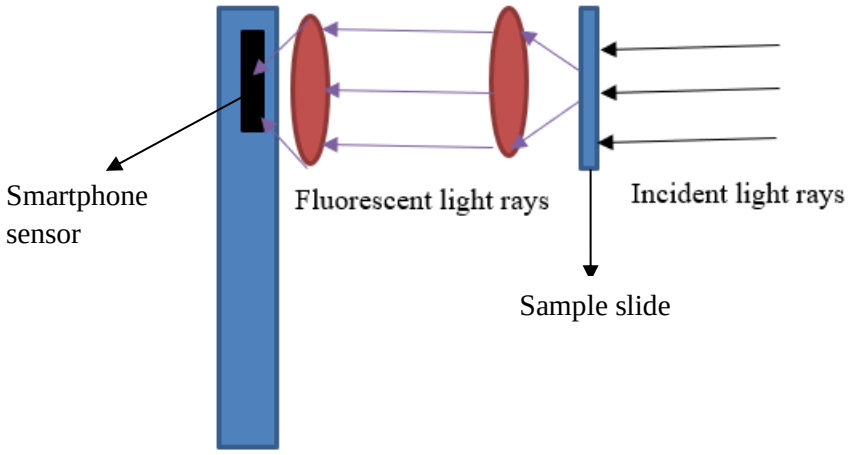

Figure 3. Figure shows a simplified sketch of the ray diagram of our system, where the left oval represents the built smartphone lens working as tube lens and the right oval is the external, reversed smartphone camera module (sensor is removed from the external camera).

The actual calculations involved in designing our optical train is then to determine the focal lengths required of the external objective lens, so that we could achieve a magnification in the

range of 3-5X. The standard in publishing on smartphone camera lens' specifications uses the 35-mm equivalent focal length instead of the actual focal length of the camera lens.

The conversion to obtain the actual focal length of the camera lens is by using equation (2):

$$F = \frac{f_{35mm-equivalent}}{Crop\ Factor} \tag{2}$$

Where F is the actual focal length of the camera lens, and crop factor is a constant determined by the dimension of the sensor that comes with the camera. In the case of HUAWEI P30 which uses SONY IMX600 sensor (dimension 1/1.7 inches), we obtain a crop factor of 4.5. Using the 35-mm equivalent focal length of P30 rear camera, its actual focal length will be 6 mm as shown below.

$$F = \frac{27mm}{4.5} = 6\ mm \tag{3}$$

Recall equation (1) and the fact that we need a magnification of 3-5X, this means that we need to find an external camera lens that has the focal length in the range of 1.2 to 2 mm. After browsing through a selection of smartphone camera modules that are available to purchase, the closest value that we can get within a reasonable price is HUAWEI's Honor 20 Pro ultrawide camera lens, as it has an actual focal length of 1.7 mm by the same conversion above.

Substituting the actual focal lengths into equation (1), we now have a system that gives us an optical magnification of approximately 3.5X, as seen below.

$$M = \frac{f_{tubelens}}{f_{objective}} = \frac{6}{1.7} \approx 3.5 \tag{4}$$

2.2.2.2 Mechanical Design for Optical System

Based on the arrangement of optical components in an infinite/finite conjugate system, we constructed a setup where our model can achieve a satisfactory performance as a microscope. Wentao, Ni then sketched out a vertical design where the excitation light shone from the top of the device and use HUAWEI P30's front camera to receive the image. The front camera as the imaging camera instead of P30's rear camera as a inverted setup places the sample slide closer to our objective than the setup that utilizes the rear camera. Through trial-and-error, we observe that

a greater distance between the sample slide and the objective will greatly reduce the quality of the image observed (see Appendix C) and therefore using the front camera is a better design choice for our system.

Aside from components that are 3D designed, there are also components in our optical train that we purchased, which are the HUAWEI Honor 20 Pro Ultrawide camera, Daheng Optics light filters, and a optical translation stage. Figure 6 demonstrated the exploded view of the 3D design. In the following paragraphs, a more detailed breakdown of the design of each component will be elaborated.

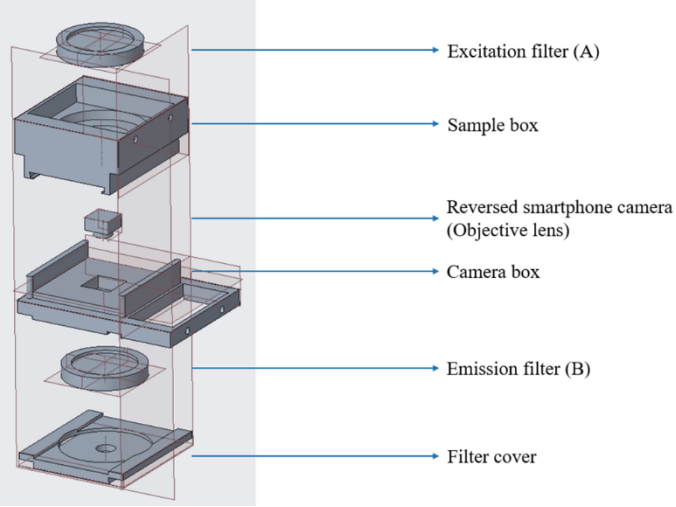

Figure 4. Figure shows the exploded view of the 3D design to hold the optical components in our system.

Filter cover

The function of the filter cover is to hold the emission filter in place. In consideration of resolution and magnification, we want users' smartphone cameras to be as close as possible to the objective lens. So, we embed half of the filter into the camera box and so the cavity for filter in this component is only 3 mm deep. The tenons on two sides are embedded into the camera box and form rigid linkage.

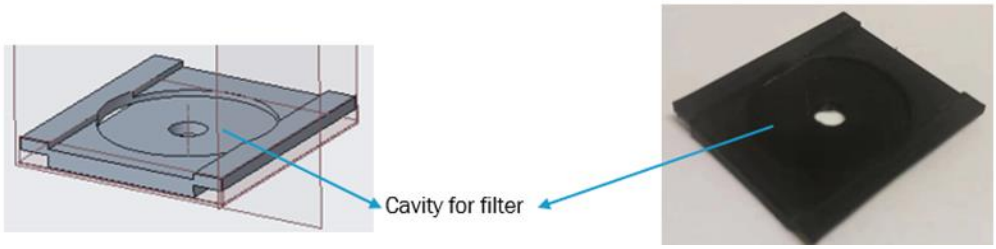

Figure 5. Figure shows the 3D design and the final printed product for the cover to place our emission filter.

Camera box

The main function of the camera box is to hold the reversed smartphone camera from moving around when user is using the system. The cavity for the reversed camera is a cuboid of the size 10 mm × 10 mm × 4 mm and a through hole of diameter 6 mm, just slightly larger than the camera itself in order to ensure the camera can be fully embedded in the cavity. On the side facing down, a cavity of diameter 26 mm and depth 3 mm is to embed the emission filter. The rectangular space extended to the right in figure 6 is used to attach our fine optical translation stage with M3 screws.

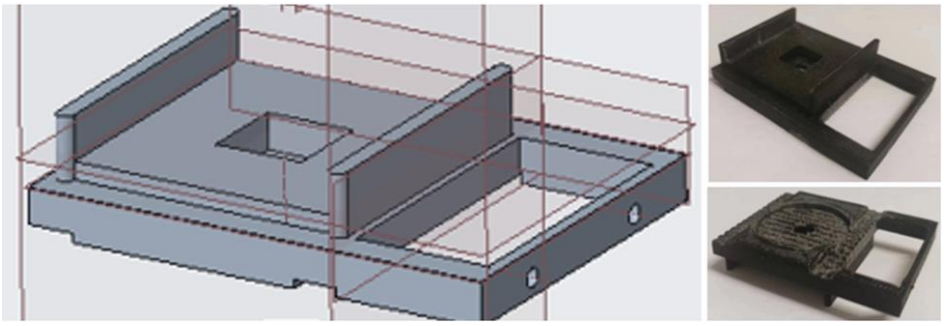

Figure 6. Figure shows the 3D design and the both sides of final printed product of the camera box.

Sample box

This component is supposed to hold the sample slide while users are observing the sample. To enable users to fully and properly examine the sample slide region of interest, we incorporated the capability for rough calibration and fine calibration in moving the sample in our system. The rough calibration is achieved through the ‘slide-able’ slot where the sample slide is inserted, as shown in figure 7, whereas the fine calibration (sensitivity of ±1 mm) is done via the attached optical translation stage, which moves the entire sample box that contains the glass

slide. The circular hollow region facing upwards (as in figure 7) is the space allocated to place the excitation filter.

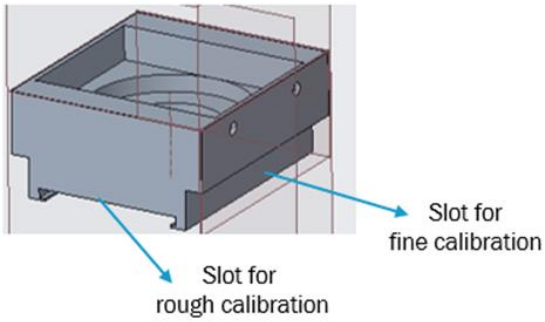

Figure 7. Figure shows the 3D design of the sample box in an inverted manner, the slot for rough calibration is where the sample slide will be placed.

Combining the filter cover, sample box, camera box and the optical translation stage together, we yield the following setup as shown in figure 8.

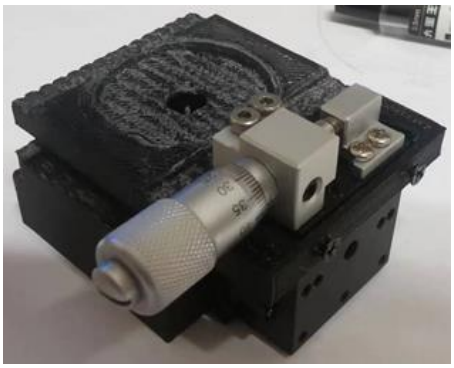

Figure 8. Figure shows the assembled view of filter cover, sample box and camera box with the optical translation stage attached on the side.

Because the front camera provides a similar magnification (the focal length of the front camera is the same as the rear camera in P30) when coupled with our objective but with a higher imaging quality compared to the rear camera, our final setup added a simple housing to hold our optical train upside-down, as shown in figure 9. The external casing has two main functions, first is to hold the illumination module that contains our LEDs, as well as two horizontal support planks to hold the structure in figure 8.

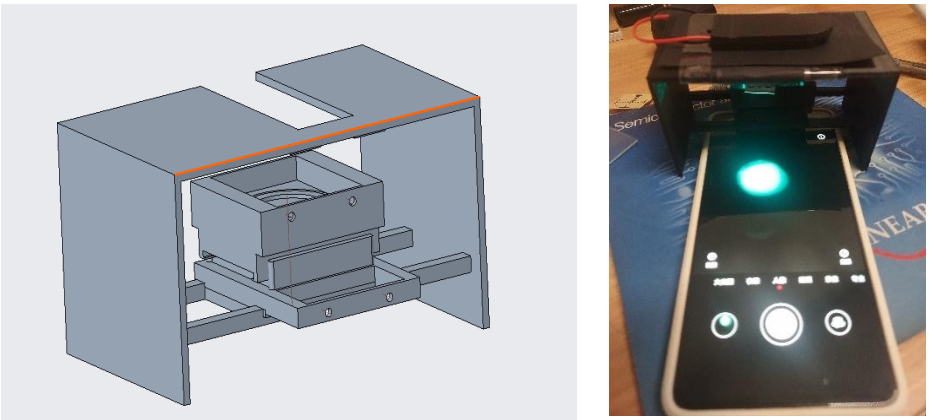

Figure 9. Figure shows the complete 3D design of our microscope system that is 3D printed and the final system will operate when the other modules are integrated.

2.2.2.3 PCB Design for LED-switch Circuit

The illumination module of our fluorescence microscope is made up of LEDs, current-limiting resistors, a switch and a voltage source. The important parameters to consider are the dimensions of the PCB board, and the brightness of the LEDs such that the light is intense enough to excite the *C. Elegans* sample. Since we do not have access to fine calibration equipment to properly measure the brightness of our LEDs, we work around by ensuring we provide our LEDs with its maximum working current (by minimizing resistance) and increase the number of LEDs accordingly when carrying out our tests.

To enable multiple LEDs to lighten up equally and not be affected by the number of LEDs in the circuit, we designed a circuit where LEDs are connected in parallel, as shown in figure 10.

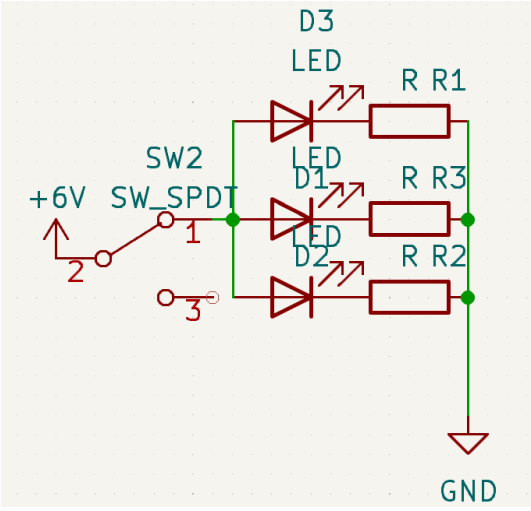

Figure 10. Figure shows the general sketch of the circuit diagram of our illumination module made up of three LEDs connected in parallel.

The maximum working current allowed to pass through each LED is provided in the datasheet (typically in the range of 12-20 mA). The forward voltage and working current of the 490 nm LEDs that we purchased are 3.0 V and 20 mA respectively.

By applying Ohm's Law in equation (5) and Kirchoff's Voltage Law:

$$V = IR \tag{5}$$

Using a 6 V voltage source, we get that the voltage drop across each resistor in each parallel branch of the circuit in figure 4 will be:

$$6V - 3V = 3V \tag{6}$$

Substituting 3 V and our target current to be 20 mA (0.02 A) into equation (5):

$$3 = 0.02R \tag{7}$$

$$R = 150\ Ohm \tag{8}$$

We obtained that we will need resistors with resistance 150 Ohm each to maximize current flow for each individual LED.

After determining the relevant parameters for our electronic components, their individual layouts on the PCB board will be governed by how we design our casing system to hold these components together. One other main design thought on how to arrange the components on a PCB board was to maximize the light intensity by making sure the LED lights are casted onto a small overlapping region. This is done by arranging the LEDs layout on the PCB in a triangular orientation so that they could all be bent slightly inwards, as shown in the PCB design in figure 11 (left), and how the LEDs should be oriented when soldering as in figure 11 (right).

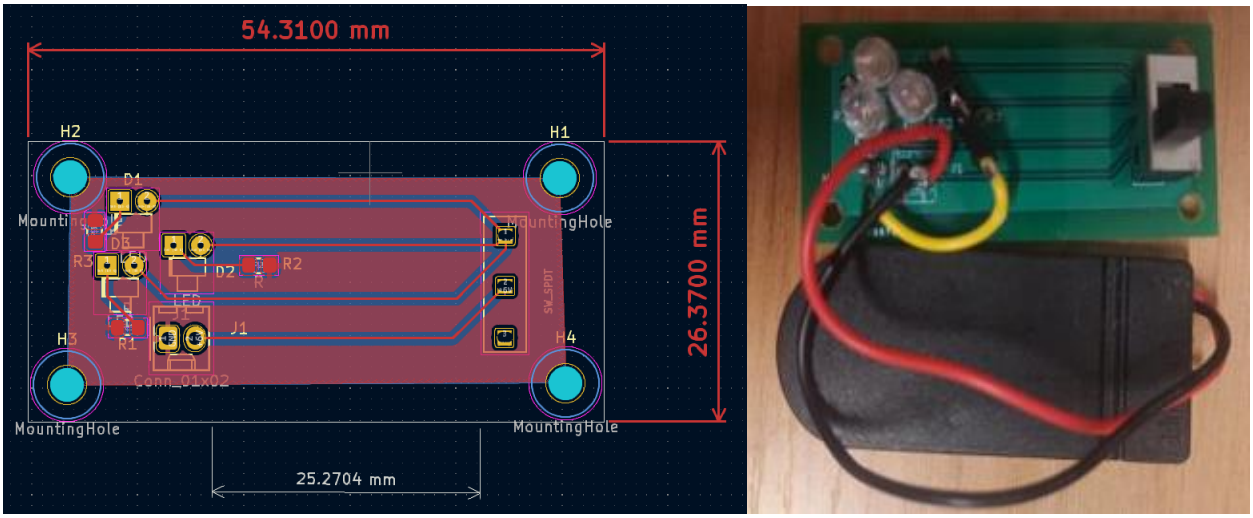

Figure 11. Figure (left) shows the PCB layout of the LED switch circuit. Figure (right) shows the final soldered version of the PCB.

2.3 Verification

A complete, tabulated version of the requirements and verifications is included in the first page of Appendix A for more detailed information of the tests.

It is important to note that due to limited access to professional optical instruments to quantify parameters such as the intensity of our light source, as well as the low-level functionality that our microscope system aims to achieve, most of our verification suites involve direct visual inspection and side-by-side comparison of images captured on different systems.

2.3.1 Magnification Factor of 3-5X to Capture and Observe Clear Images of *C. Elegans.*

2.3.2 Microscope Field-Of-View Capable of Observing Multiple, Full C. Elegans within Single Image.

In order to measure the magnification factor achievable by our reversed smartphone camera lens infinite conjugate design, we manually cut off black paper stripes that are 1 mm in width (as shown in figure 12) to be used as a calibration sample.

Figure 12. Figure shows a simple calibration tool we used to verify the magnification of the microscope. The black paper strips are 1 mm wide whereas the white paper strips are 0.5 mm wide.

We measured the width of the black strip in an image captured by HUAWEI P30 to be 4 mm, which gives a magnification factor of 4X. The slight increase than the theoretical magnification factor we obtained in equation (4) could be due to digital magnification processing within HUAWEI's imaging system. As the magnification factor exceeded our calculation, we tested the imaging directly on a sample slide with *C. Elegans.* Figure 13 (left) shows the image captured under our microscope system with LED light illumination without any filters. The right figure is an image captured using a standard benchtop fluorescence microscope with a magnification factor of 5X in ZJE laboratory. Although we observe relatively more background noise in the image captured by our system, we can verify that the magnification part works as expected and is sufficient for our purpose to detect GFP's fluorescence within *C. Elegans.*

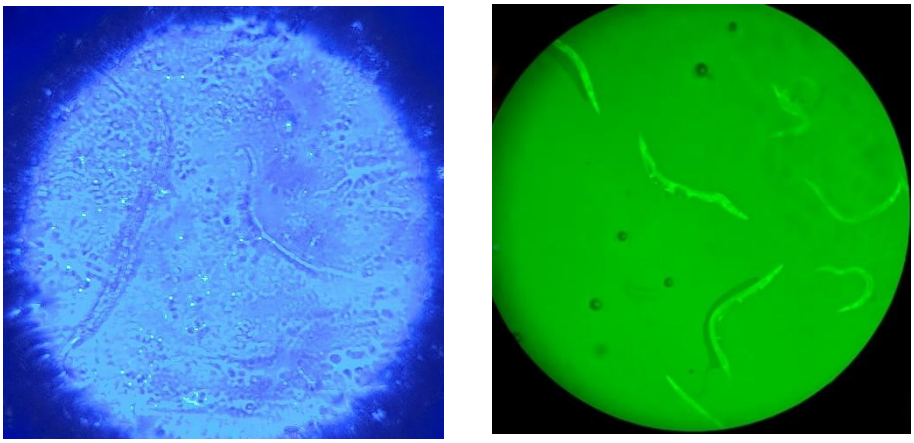

Figure 13. Figure shows a side-by-side comparison of image captured with HUAWEI P30 coupled with our microscope system (left) and image captured with a benchtop microscope (right).

We also captured an image by only using background, natural white light to compare the image quality of our optical model in the brightfield microscopy model. The image is shown in figure 14. The image captured shown in figure 14 also verified another important parameter of our

system – field of view. We successfully designed our microscope system to have a large enough field of view that allows the user to observe multiple complete *C. Elegans* sample in a single capture.

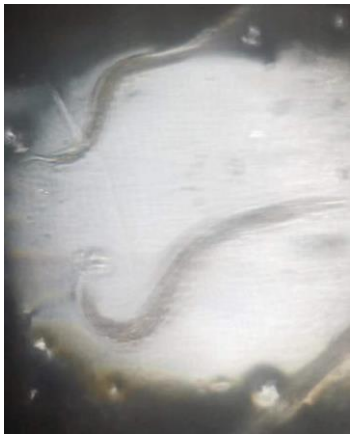

Figure 14. Figure shows an image captured under our smartphone microscope system with white light. The image verified that the system's field of view can capture multiple roundworms in a single image.

2.3.3 Intensity of Light Source Strong Enough to Excite *C. Elegans*.

In order to verify that the intensity of our 480 nm LED light source suffices to excite *C. Elegans* sample, the first test that we did was simply using our light source as a substitute on a standard benchtop microscope and compare the observation obtained when the default microscope light source (mercury lamp) was used. We obtained another side-by-side comparison of images as shown in figure 15.

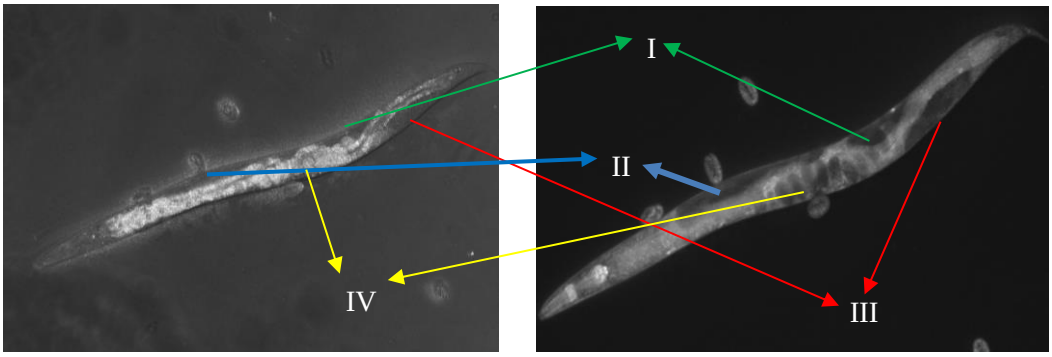

Figure 15. Figure (left) shows the image captured on a microscope camera when our LED light source is used as excitation source while figure (right) shows the image captured when the default mercury lamp light is used.

By visual inspection, we can verify that both light source successfully excite the *C. Elegans* sample on its intestine – which is the long thick gland that makes up most part of the inside of

the worm. By figure 15, we can also verify that the excitation is successful by comparison of the structural shape that exhibits (and does not exhibit) fluorescence in both images. We can see that in all regions labelled I, II and III in figure 8, both images do not exhibit fluoresce, confirming that our LED light source is only exciting specific regions of the sample.

However, figure 15 also shows two underlying problems with our method of verification. The first problem is our light source failed to excite the left tip of the sample (the foregut region), which is a region of interest in the study of protein expression for GFP fluorescence. The other problem is our light source somehow also managed to excite region IV on figure 13, which the microscope mercury lamp light source did not exhibit fluorescence.

We suspected that the first problem occurred due to uneven excitation when we replaced the microscope light source with our own – due to restricted space on how we could position our PCB circuit to shine directly onto the sample. For the second problem aforementioned, the issue was likely due our own LED light source has a broader range of spectrum in the margin of 480 nm. Although the vendor from which we purchased the LEDs stated that the wavelength margin is 480 nm ±5nm, we could not properly verify that property. Besides, our LEDs did not go through excitation filters that targeted the very specific band for GFP fluorescence as compared to the light source within the microscope (seen through the slightly gray background), it is expected that we also excite some other parts of *C. Elegans* that are intrinsically fluorescent.

2.3.4 Fluorescence of GFP Captured in Smartphone Microscope System with Emission Filters

After verifying that our LED light source can at least fluoresce certain parts of *C. Elegans*, we need to verify that fluorescence effect can be properly captured when the light source is integrated with our smartphone microscope system – which is made up of the infinite-conjugate model that we verified as explained in section 2.3.1. Since we are using the trans-illumination orientation in our microscope setup, we need an emission filter that cuts-off the wavelength of our excitation light source (480 nm) and only lets through the emission wavelength of GFP (510 nm).

To achieve that cutoff, we purchase two high pass emissions filter from Daheng Optics (Model GCC-300101 and GCC-300103, specific parameters for filter is included in Appendix B) to test which filter let through the most obvious fluorescence effect. Figure 16 shows the comparison of

two images captured using our system with two different emission filters being used. The image captured on the left shows the image captured when GCC-300101 is used whereas the image on the right is when GCC-300103 is used.

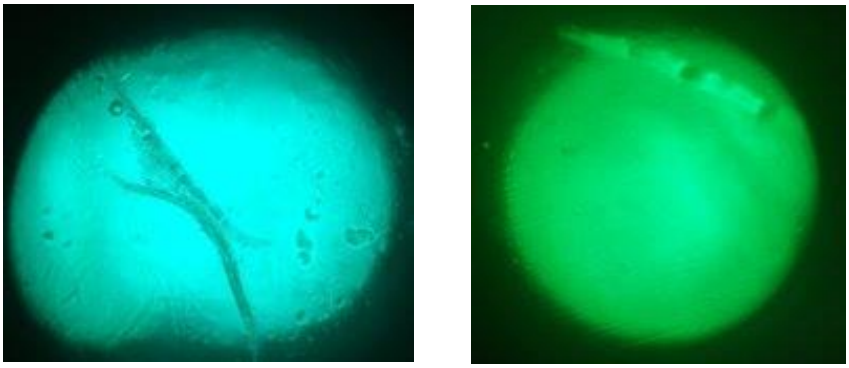

Figure 16. Figure shows comparison when different emission filters are being used in our microscope system. Figure on the left is when GCC-300101 filter is used whereas figure on the right uses GCC-300103.

We can observe directly based on the color difference that the GCC-300103 works best in filtering out the background noise to display a distinctive fluorescence effect (figure 10 right image). This is shown through two aspects, first of which is the bright and dark regions that are distinctive across different segments of the *C. Elegans* and also by the fact that the color of the wavelength being passed through closely resembles the right figure in figure 13, which is the observation under a standard microscope. However, if we then do a comparison between the image captured using our setup and the image captured using a benchtop microscope, as shown in figure 17 below, the contrasting difference of the fluorescence region between the worm (labelled A) and the background noise (labelled B) is not as obvious in the image captured by our system. The image observed in the microscope system on the other hand, is capable of demonstrating that difference distinctively. As such, we failed to conclude with confidence that our system successfully captured the fluorescence effect of GFP in *C. Elegans.*

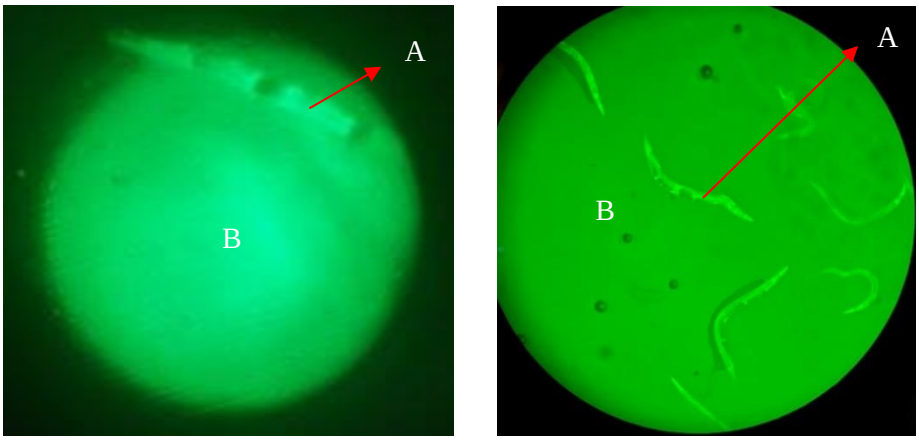

Figure 17. Figure on the left is the image captured by our system and figure on the right is the image captured on a benchtop microscope.

2.4 Costs

We divide the cost estimate for our entire project into labor costs and equipment costs. For the labor costs estimate, we will be using an hourly rate of 50 RMB for each team member. Using the formula suggested by the course as shown in equation (9) for each partner:

$$Individual\ Labor\ Cost = Hourly\ salary\ rate \times hours\ spent \times 2.5 \tag{9}$$

$$Total\ Labor\ Cost = Individual\ Labor\ Cost\ \times 4 \tag{10}$$

Each team member has worked on an average five hours per week on our project for a stretch of 12 weeks, which gives the total labor cost to be:

$$Total\ Labor\ Cost = 50\ RMB \times (5 \times 12) \times 2.5 \times 4 = 30{,}000\ RMB \tag{11}$$

The equipment cost that is required for the successful creation of this project is listed in table 1.

Table 1. Material List and Unit Cost

Material	Cost (RMB)
Optical Translation Stage	210.00
Electronic Components (wavelength specific LEDs, 150 Ohm resistors, mercury cells, battery casing)	50.00
Detached smartphone camera (HUAWEI Honor 20 Pro)	17.00
PCB Manufacture	21.00
High Pass Emission Filter (GCC-300103, Daheng Optics)	160.00

There are a few components that are also used but not included in table 1 as part of the cost calculation because they came as free-of-charge as lab resources. Those materials include the acrylic 3D printing material, soldering tools as well as electrical energy consumption.

By the information provided in table 1, the total cost of the materials sums up to 458 RMB. Combining both labor costs and equipment costs, the total expenditure for the project will be:

$$Total\ expenditure = 30{,}000\ RMB + 458\ RMB\ \approx 30500\ RMB \quad (12)$$

2.5 Conclusion

To summarize, our team managed to design and create a functional miniature smartphone-based microscope that achieves low power magnification of 3.5X, which is sufficient to capture and observe images of *C. Elegans* and their general structure. The optical model that we used in constructing our microscope system is an infinite/finite conjugate system that utilizes a reversed smartphone camera lens as our objective lens, and the user's smartphone built-in lens as tube lens. We also successfully designed a stable and compact external casing that aligns the necessary optical components together. The external casing also integrates both rough and fine calibration (in the x-axis) for the user to adjust their field of view of the sample slide. For the fluorescence microscope aspect, we managed to design a simple circuit that is capable of fluorescing the intestinal region of *C. Elegans*, but failed to capture that fluorescence effect within our imaging system.

Moving forward, continuous effort should be done in coming up with an excitation light source that is more targeted and has higher light intensity to only show the fluorescence of GFP. This can be done by integrating LEDs with higher power output specifications that correspond to brighter light output, or using lasers instead of LEDs as the light source. A more matured approach of determining the luminance needed to excite *C. Elegans*, if possible, is preferred, instead of the trial-and-error approach.

Aside from the fluorescence aspect, we believe there is still huge room for potential in terms of the robustness of the design of our microscope system. Several important directions to consider include the possibility of adjusting the sample slide in all three axis for better observation instead of only x-axis in our design, how to design a system that can accommodate fluorescence targets

of different wavelengths, as well as to design a system that could observe cell cultures that are in petri dishes instead of glass slides.

2.5.1 Ethical Considerations (IEEE Code of Ethics)

Throughout the process of working on the project, there are certain ethical considerations that we have to consistently bear in mind that closely align with IEEE Code of Ethics. For the sake of brevity, we will choose to expand the most relevant ones in this section. The code of ethics are:

i. "to seek, accept and offer honest criticism of technical work, … and realistic in stating claims… and to credit properly the contributions of others"

This code of ethics is important to us because the field that we are exploring is a field that many has contributed excellent working ideas on. Knowledge in the field of optics and biology are not aspects that we have learned in-depth, which is important because we have to be careful that we proceed with realistic goals, and always be humble to ask questions upon facing uncertainties and credit all sources that offered us insights.

ii. "…to undertake technological tasks for others only if qualified by training or experience…"

In the middle stages of our project, many testing and verification procedures involve the usage of biological samples, electronic components with bright light as well as glassware. These materials and apparatus can pose hazard to ourselves as well as others if treated recklessly. For instance, when working on biological samples such as *C. Elegans,* we must thoroughly clean our hands before and after using the sample and wear lab gloves at all times when working with the sample.

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Appendix A Requirements and Verifications Table

Table 2. Table for Requirements and Verifications (with verification status).

Requirement	Verification	Verification status (Y or N)
1. Microscope system able to achieve magnification factor in the range of 3-5X that allows user to clearly observe the presence of *C. Elegans* and the general structure of the sample. a. The resolution of the image captured by the smartphone through the microscope system should be able to clearly differentiate worms that are close to each other, as well as differentiating background noise (between water droplets and worms) 2. The field-of-view (FOV) of our system is capturing multiple worms in a single image, given the *C. Elegans* worms typically grow up to 1 mm in length and 50 microns in diameter. a. To allow more than one worms, we set our minimum field of view to have a FOV with a diameter of 2 mm.	1. Factor of optical magnification can be determined by capturing images of black paper strips that we cut off with 1 mm thickness and measuring the thickness of the strip in our captured image. a. Visual inspection of the captured image when using a sample slide with *C. Elegans* whether the roundworms can be observed. b. When using sample slide with multiple *C. Elegans*, smartphone can still observe full structure of multiple worms. 2. Field-of-view of our microscope system can be measured by manually cutting out a small circle paper sample with 1 mm markings on it. a. The border value readings of the cut-out circle will be the diameter of our system's FOV.	Y
3. Fluorescence effect of the *C. Elegans* sample can be observed when the sample is excited by blue light in the range of 480-490 nm. a. When excitation and emission filters are applied in the microscope system, we should see distinction of a dark background where *C. Elegans* are not present (black in color), whereas the worms should be fluorescing	3. Use the PCB circuit of our illumination module to act as the light source in a proper benchtop fluorescence microscope and compare the images captured when the built-in light source of the microscope is used. a. The images captured when using two different light source (one that is designed by us and the default in the benchtop microscope) shows similar regions of the worm fluorescing.	Weak Y

	4. After ensuring that our light source is intense enough to excite the sample on benchtop microscopes, images with similar fluorescence effect should be able to be captured via smartphone cameras as well.	

Appendix B Daheng Optics Emission Filter Models Specifications

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Table 2 Snapshot of Wavelength Cut-off and Transmission Range for GCC-300 series

GCC-300 Long-Pass Filters

Part No.	Transmission Band	Blocking Band	Cut-off Wavelength	$\lambda_{T=80\%}$-$\lambda_{T=10\%}$	Diameter
GCC-300101	500-2500nm	200-360 nm	380nm	33nm	ø25.4
GCC-300201	560-2500nm	200-430 nm	450nm	33nm	ø25.4
GCC-300301	600-2500nm	200-490 nm	510nm	33nm	ø25.4
GCC-300401	650-2500nm	200-530 nm	550nm	33nm	ø25.4
GCC-300501	680-2500nm	200-580 nm	600nm	33nm	ø25.4
GCC-300601	720-2500nm	200-630 nm	650nm	33nm	ø25.4
GCC-300701	800-2500nm	200-665 nm	685nm	33nm	ø25.4

Appendix C Image Quality Comparison between HUAWEI P30 Front Camera and Main Rear Camera

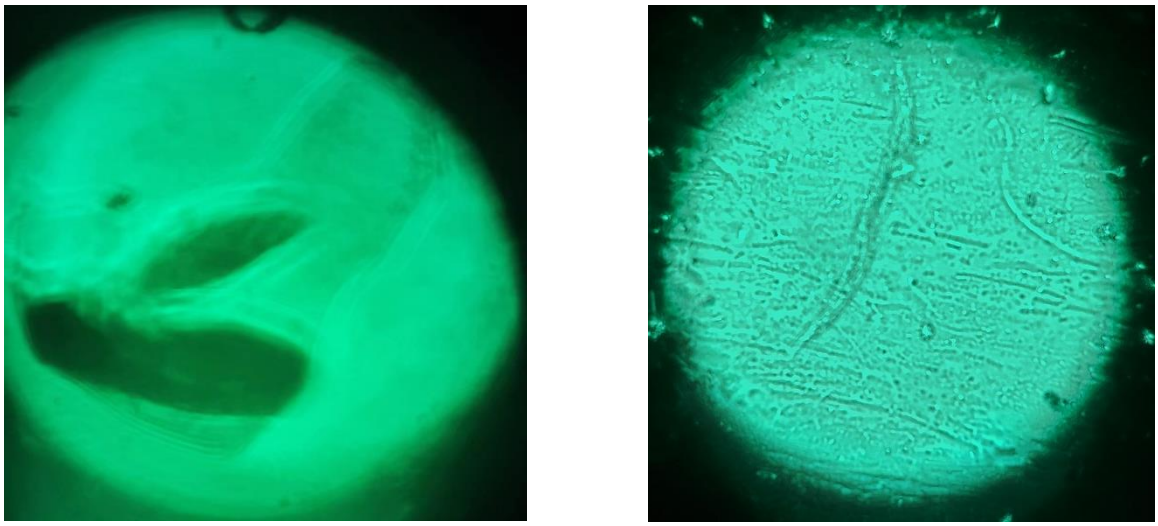

Figure 18. Figure shows the side-by-side comparison when using rear camera and front camera to capture images on our system using HUAWEI P30. Figure on the left is captured with P30's rear camera whereas figure on the right is captured with front camera.

Appendix D Alternative Approach of Fluorescence Microscope by Using Commercialized Smartphone Microscope (TipScope): CAD design

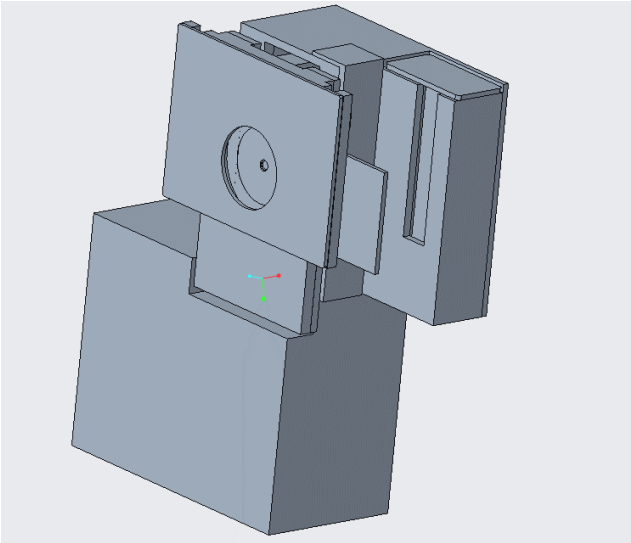

Figure 19. Figure shows the assembled view of our alternative design by our teammate, Feifan, Xie.

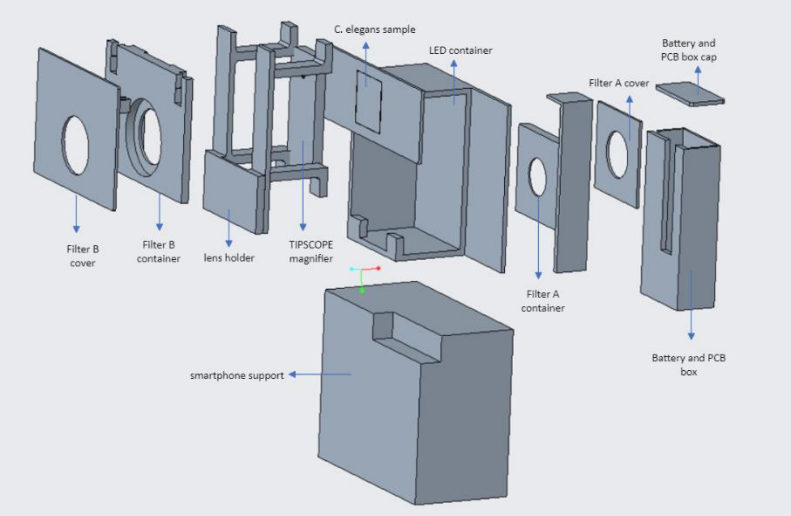

Figure 20. Figure shows that exploded view of Xie's alternative design to our project.

Figure 21. Figure shows the TipScope product, a commercialized brightfield microscope, and how it is used with a smartphone.

Appendix E Alternative Approach of Fluorescence Microscope by Using Commercialized Smartphone Microscope (TipScope): Final Product

Figure 22. Figure shows the final, 3D printed product designed by Feifan, Xie, where the TipScope, PCB and filters are enclosed within the casing.

Figure 22. Figure shows how a smartphone will be placed (user hands-free) on Xie's system.

Appendix F Images of *C. Elegans* Captured Using Alternative Design

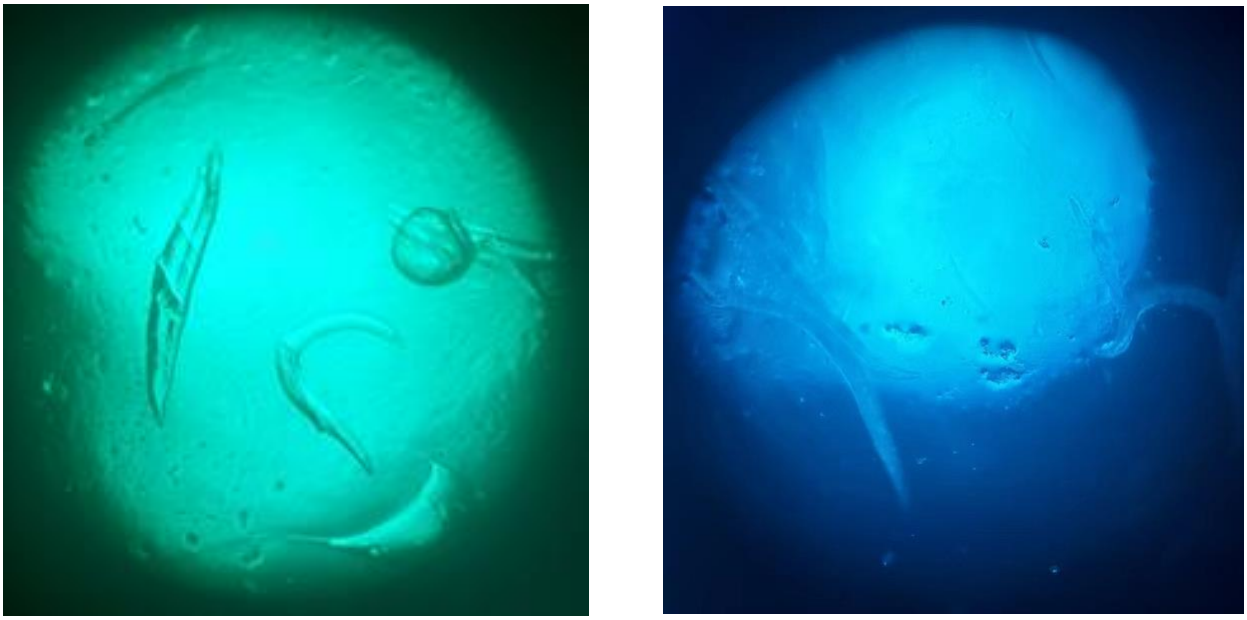

Figure 23. Figure shows two images captured using Xie's system under different wavelengths. The insides of the worm structure can be viewed much clearly and less background noise is present compared to the images we present in the main section.