ECE445/ME470

Project Proposal

A Portable, Smartphone-based Fluorescence Microscope

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Part 1: Introduction

1.1 Background and Problem Statement

Be it introductory level usage such as for high-school students to perform simple field experiments, or advanced, professional applications including (but not limited to) diagnostic imaging and single molecule studies, microscopes give us the ability to see and analyse things at detailed levels that are otherwise limited to our naked eye. However, conventional microscopes are mostly benchtop devices that one can only access under laboratory settings. On top of that, benchtop microscopes are usually bulky, expensive and require personnel with expertise knowledge to operate. These limitations hinder microscopy technology from reaching a wider population, especially those living in rural areas who need it urgently.

With the advancing of smartphone technology, the demand for light, mobile microscopic devices has been on the rise. Utilizing low-cost but power-efficient LEDs coupled with robust smartphone cameras, we aim to deliver a pocket-size, single-lens fluorescence microscope (FM) device that is attachable to smartphones.

1.2 Goal and Functionalities

The first problem that our prototype aims to solve is to provide people with a relatively affordable and easy-to-carry-around microscope system. Instead of having limited devices in science labs, our device gives more people the opportunity to use a microscope wherever they go.

Limited by the size, we expect our single-lens microscope system to deliver a magnification ~20X-30X, which is sufficient for education use to perform structural studies on simple tissues, plant and environmental samples [1].

In the recent decade, there has been successful prototypes and products of smartphone microscopes that provide magnification and resolution capable of imaging tissue samples and observing cell structure. However, most smartphone FM prototypes are highly specific due to the specific excitation and emission wavelengths of the samples. We aim to design a prototype that has a the mechanism to help users efficiently perform fluorescence imaging across multiple excitation/emission wavelengths.

1.3 High-level Requirement List

The necessary components to build a pocket-size FM system include: an objective lens, a LED illumination source, a sample stage, excitation and emission filters, and the microscope casing.

1. We are still in the process of finalizing the methodology to calibrate our microscope's magnification and resolution. This is because standard calibration materials such as fluorescent beads are too expensive (~6000 RMB).

   After discussing with our instructor (Prof. Weeliat Ong), we decided to set the first verification milestone (qualitatively) at being able to "magnify and see clearly" simple samples like water/thin slice of onion skin. After that, we will progress to using the microscopes in ZJE laboratories to observe more specific samples (we have been told that there is readily available *C. Elegan* - roundworm samples) and compare those results with the results obtained by our smartphone microscope.

We will need to find out more about specific tools and methodologies on quantitatively defining our microscope specifications in further discussions with our project instructor. The research that we have done on calibrating suggests tools (other than the aforementioned expensive fluorescence beads) including Ronchi ruling and hemocytometer, which we still lack knowledge in.

2. The size of the external casing should approximate the length and width of a standard smartphone, but definitely has more depth (thicker) than a smartphone. Our goal is to limit the casing within 7cm wide, 14cm tall and depth of 7 cm. This is set with reference to Apple iPhone 13's dimensions, which is 7.2cm wide, 14.7 cm tall and 0.7cm depth. The mass of the entire microscope system should be within $3kg \pm 5\%$ for portability and mobility.

   These measurements of the casing dimensions can be pre-determined as we will be designing with CAD before 3D printing it. The depth measurements (7cm) is done by taking into consideration the height of the objective lens that we bought, which is ~3.5cm.

3. The optical path for our transillumination model should work as expected when coupled with excitation and emission filter. For example, if we use an excitation filter of 485nm (blue light) and emission filter of 515nm (green light) [2], with a tissue/blood sample stained with carboxyfluorescein diacetate, succinimidyl ester (CFSE), we expect to see green dots on our image, because that is the range where CFSE fluoresces when it interacts with the amine groups in live cells [3].

   This verification can be done by capturing images with no staining and with the LEDs turned off (these images will act as control variables). We can then capture images with the LED turned on with the sample stained. The comparison can be done first with naked-eye comparison, and if unsatisfied, image processing by analysing the pixel values of the images can be done.

Part 2: Design and Requirements

2.1 Block Diagram

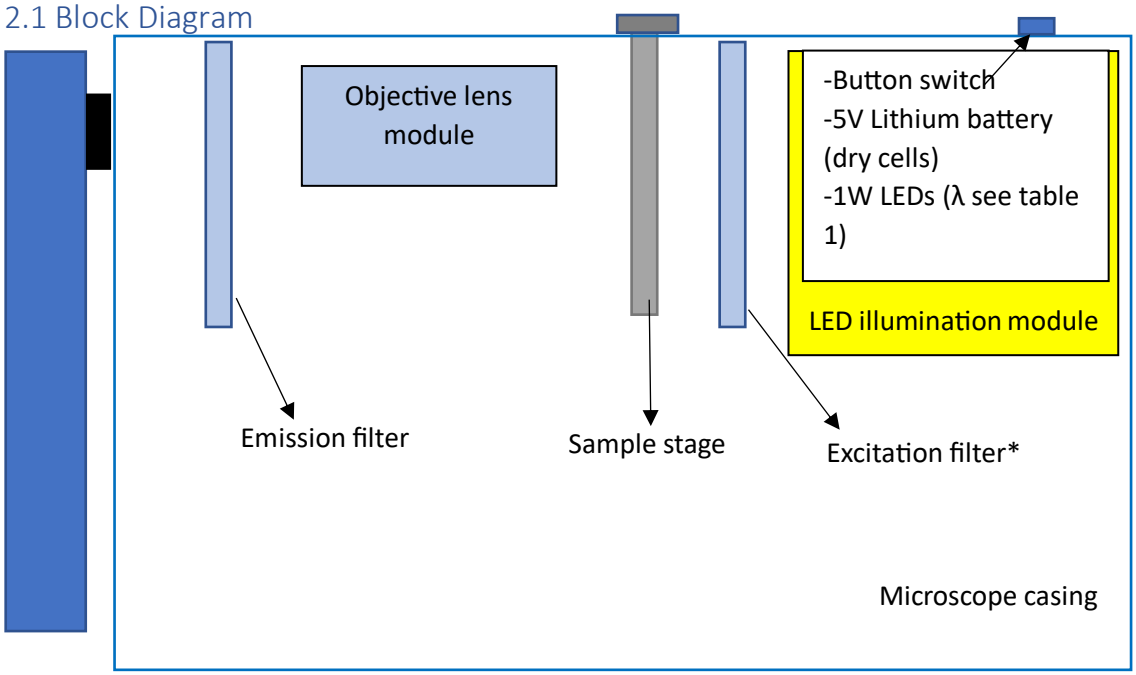

Diagram 1 shows the high-level block diagram for our smartphone-based microscope.

2.2 Description & Requirements of components

1. Excitation/Emission filter
    a. The parameters for our excitation/emission filters are currently set at:

Stain	Excitation wavelength (nm)	Emission wavelength (nm)
CFSE	485	515
Acridine Orange	460	600
Rhodamine-B	556	580
Auramine-O	450	500

Table 1: Table shows the excitation/emission wavelengths for corresponding bio-chemical stains to fluoresce [5], [6].

*Excitation filter is optional if we use LED specific to the exciting wavelength (emission filter will still be necessary in this case). This limits the flexibility and robustness of the microscope. If a high-power white LED is used, we can have a rotary excitation/emission filter to accommodate multiple wavelengths combinations.

The reason we chose these stains are application-based – these stains are commonly used in fluorescence microscopy. CFSE stain can be used in tracking live cells and lymphocytes. Acridine orange can be used to detect human cells, prokaryotic cells, DNA and RNA. Rhodamine-B and Auramine-O are two widely used stains as well, and are sometimes being used together. Rhodamine-B can be used as a dye-tracer in water to analyse water flow or to detect microplastics. Auramine-O is commonly used in detecting acid-fast bacilli (e.g. Mycobacterium tuberculosis).

2. LED illumination module
    a. Because the lack of a collector and condenser system in our microscope to do the light focusing, we set our specifications for LEDs to be driven by 5V battery and an output power of 1W $\pm 5\%$. This is set with reference to existing prototypes that utilize a collector-condenser setup, which uses LED providing 300mW [4].
    b. As a prototype, our current power source is set as a 5V dry cells, and we will use a button switch to control the switching on/off of the LEDs.
    c. As shown in the block diagram, the button should be external to the casing for user to be able to turn the LEDs on/off.
3. Objective Lens
    a. We will be using readily acquired objective lens purchased online, we will be testing out objective lens from 20X to 40X. The specifications that we obtain from the merchant include:

Objective magnification	NA	Working Distance (WD) (in mm)	Cover slide thickness (mm)
20X	0.40	3.1	0.17
40X	0.65	0.53	0.17

Table 2: Table shows the objective's relevant parameters to help design our optical system.

    b. The main parameter that we are left to figure out is the distance between the camera lens of our smartphone to the objective.
    This is currently work in progress as we are setting up a stable system to hold our components together and manually controlling the different parameters of our smartphone to control auto-focusing etc.
4. Sample stage/holder
    a. The sample stage will be made of sample slide (bottom of sample) and thin cover slide (on top of sample).
    b. The more complicated part about the sample stage is enabling a control-knob that could move the sample stage closer/away from the objective, this is more of a feature of the casing.
    c. Since we are using a low-power objective lens (20X and 40X objective), we will set aside a stage working distance of $10mm \pm 1\%$ and $2mm \pm 1\%$ respectively to adjust our sample, approximately 3 times the working distance.
5. External casing
    a. We will be using computer-aided design (CAD) software and 3D-printing to design and printout the casing for our microscope system.
    b. The casing should have clamps to hold the objective lens and the filters stable.
    c. The casing should also have a slot for the user to insert sample holder into the casing and a knob to adjust the sample holder position.

2.3 Tolerance analysis

The first major challenge in setting up our optical train is to determine the distance (and its margin) between our smartphone camera lens to our objective lens to capture a sharp image. In the model below, we will be assuming a HUAWEI P30 camera (because one of our teammates uses this model) and an objective lens of 40X (see table 2 for other specs).

There are several limitations that could lead to potential errors in setting up our microscope model for tolerance analysis. The two main limitations are:

- Incomplete information we manage to get from the optics system in the objective lens we purchase online and the smartphone camera lens specifications. For example, we do not know the focal length of our objective lens, whether there are multiple optical elements inside the objective, or simply a single lens.
- The uncertainty on variable naming conventions and theoretical knowledge in the camera/lens industry to be confident in our calculation.

Using formula for the objective NA,

$$NA = nsin(\alpha) \quad (1)$$

Where n = the refractive index of the medium between the cover glass and the front lens of the objective, which in our case is glass-air = 1.0

And α = half-angle of light cone entering the objective lens (see diagram 1).

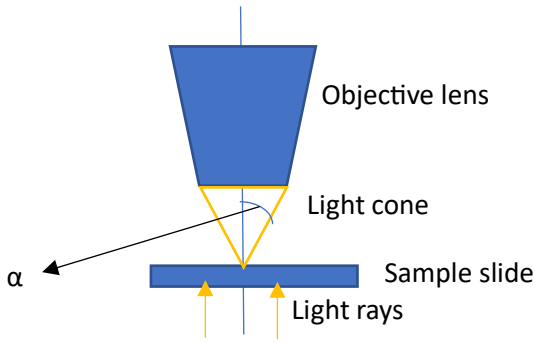

Diagram 2 shows the ray diagram that corresponds to the variables in calculating NA.

Using NA = 0.65 (from table 2), and n = 1 we get that

$$\alpha = 40.54°$$

By simple trigonometry using distance between sample and lens to be WD (0.53mm), we can get the 'half-object' height to be 0.45mm. After magnifying by 40X, the image height will be ~18mm (inverted image on the other side of the objective lens, not shown in diagram 1).

By considering the thin-lens formula for magnification, M, where

$$M = \frac{v}{u} \qquad (2)$$

v = image distance, u = object distance.

By substituting M = 40, u = working distance (WD) = 0.53mm, we get:

$$v = 21.2mm$$

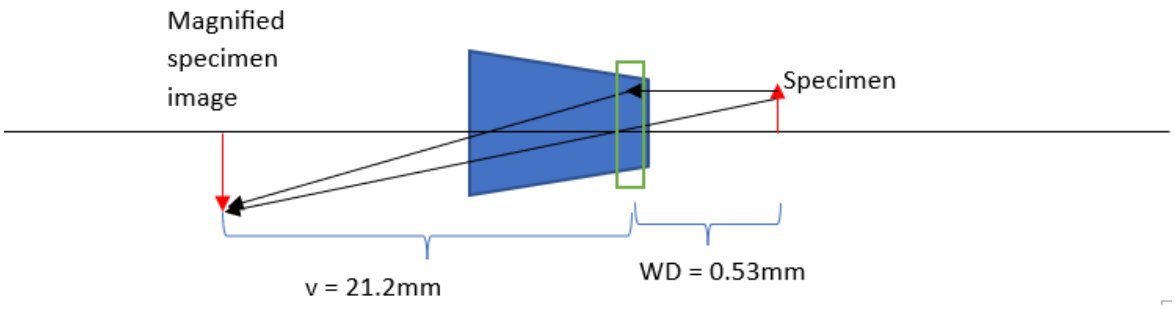

Diagram 3 shows the ray diagram assumption we used to calculate the inverted, real, magnified image formed through the objective lens. The green rectangle is our assumed position of the magnifying lens in the objective.

The calculation in diagram 3 however, leads to a complication in determining our image position because our objective lens is 35 mm long in real-life (diagram 3 is not drawn to scale). In other words, according to our calculation and measurement, the magnified image must be formed *within* the objective (as shown in diagram 4).

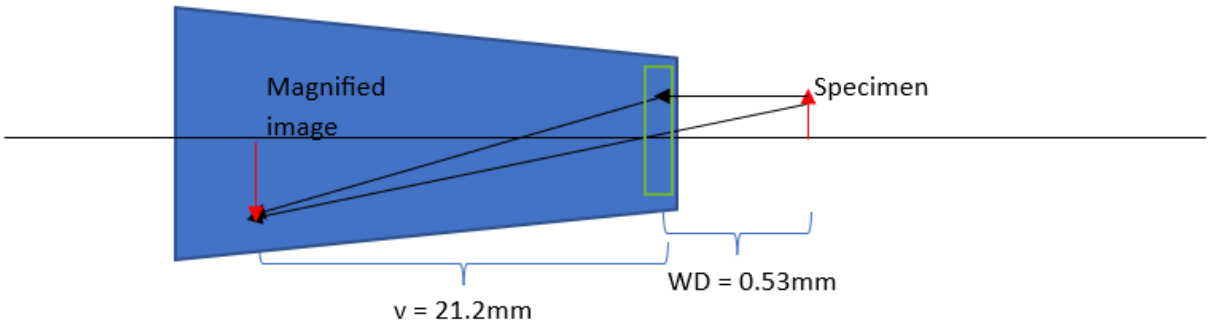

Diagram 4 shows the corrected image after considering the actual measurements of our objective lens in real life.

This is the first uncertainty we have to confirm with further study and discussion with our professor because we do not fully understand the lens setup within the microscope.

The second uncertainty is directly linked with the first uncertainty and is the final clarification we need to setup a mathematical model to determine the distance between our smartphone camera and objective lens.

The second uncertainty involves some of the formulas and calculations we obtained to determine the variables required to calculate the angle-of-view for our smartphone camera, α_{view}.

The reason we are interested in α_{view} is shown in diagram 5.

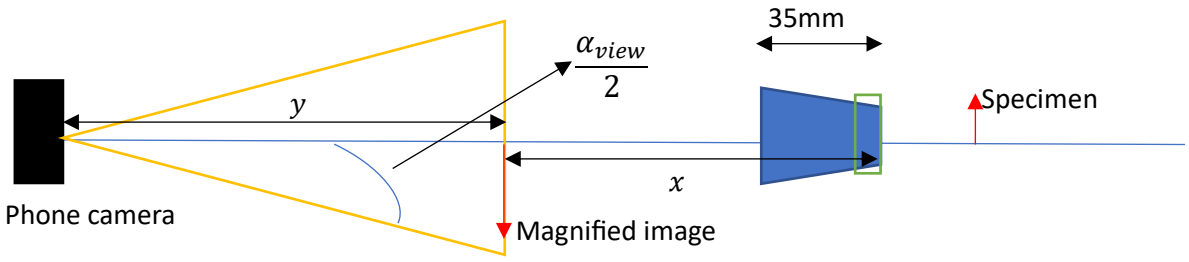

Diagram 5 shows the trigonometry needed to calculate the distance between our phone camera and the objective lens.

From diagram 5, we can see that if the magnified image is formed outside of the objective lens (as opposed to figure 4), then we can easily determine the distance, *D* needed between our phone camera and the objective lens to obtain the full magnified image, where

$$x = image\ distance \tag{3}$$

$$\tan\left(\frac{\alpha_{view}}{2}\right) = \frac{image\ height}{y} \tag{4}$$

$$image\ height = 18mm \text{ (from previous calculations)}$$

$$D = (x - 35) + y \tag{5}$$

For now, the calculation we performed are based on formulas obtained online that do not have formal proofs or derivations.

Obtained from a third-party website, the P30 wide-angle camera lens has specifications: 40 MP, f/1.8 (aperture), 27mm (focal length), 1/1.7" (sensor size). (Note: we have to resort to external websites because such information is not available on HUAWEI's official site.)

Assuming the 27mm focal length of the camera is the "35 mm equivalent focal length", the actual focal length of the lens is 6mm.

Based on the formula for angle of view ($\alpha_{view,general}$):

$$\alpha_{view,general} = 2\arctan\left(\frac{d}{2f}\right) \quad (6)$$

Where d = size of the sensor in the direction measured (can be vertical, horizontal or diagonal) and f = focal length.

We also found that a $\frac{1}{1.7"}\ inch$ sensor size corresponds to $7.6mm\ (width) \times 5.7mm\ (height)$, which corresponds to a diagonal measurement of 9.5mm.

Assuming the correctness of the parameters above, we can determine the angle of view across the vertical axis (height) because we are using vertical axis in diagram 5.

$$\alpha_{view} = \alpha_{view,vertical} = 2\arctan\left(\frac{5.7}{2(6)}\right) = 50.82° \quad (7)$$

But D still cannot be determined because we are still stuck in the first uncertainty, where we cannot get the value for x where x extends out of the objective lens.

Part 3: Ethics and Safety

3.1 Code of Ethics (IEEE, ACM)

There are specific code of ethics that we need to adhere to that relate closely to our project and align with IEEE [10] and ACM Code of Ethics. We choose to elaborate the most relevant ones as listed in Table 3.

IEEE Code of Ethics	ACM Code of Ethics and Professional Conduct
I.1 "to hold paramount, the safety, health and welfare of the public, …"	1.1 Contribute to society and to human well-being, acknowledging that all people are stakeholders in computing.
I.5 "to seek, accept and offer honest criticism of technical work, … and realistic in stating claims… and to credit properly the contributions of others"	1.5 Respect the work required to produce new ideas, inventions, creative works, and computing artifacts.
I.6 "…to undertake technological tasks for others only if qualified by training or experience…"	2.6 Perform work only in areas of competence.
II.9 "to avoid injuring others…"	

Table 3: Table shows the list of IEEE and ACM Code of Ethics that closely relate to our project that we will follow throughout this class.

As mentioned in the proposal's introduction, microscope applications have huge impact on rural and underdeveloped areas where access to laboratory technology is severely limited. Besides, most conventional microscopes are not easily accessible to the general public, therefore limiting the possibility of new discoveries and explorations. Therefore, the success of our project could be useful in increasing microscope usage among our society, and be utilized in rural areas for simple healthcare and clinical uses. This aligns with IEEE code of ethics (I.1) and ACM General Ethical Principles (1.1), both placing the welfare of humanity as the utmost priority.

Besides that, as there are already several successful, existing prototypes, models, and products on smartphone-based microscope, our project will be taking references to some of those accessible work. Therefore, we must always bear in mind to credit all the work that we borrowed and include them in the correct documenting format. This aligns with IEEE Code of Ethics (I.5) and ACM General Ethical Principles (1.5).

Significant parts of our project touch upon knowledge that our team members do not have in-depth knowledge about, specifically on optics system of a microscope, calibration of microscope imaging quality, and the process of preparing and using biological samples with chemical stains. Because of this, we have to always be ready to accept criticism and seek help and ask questions across different parties (we talked to one of the ZJE instructors in the first week to see have a brief walkthrough on how current microscopes work). This is important for us to be realistic in terms of our

project and to prevent causing any danger/harm during the project. This also aligns with IEEE Code of Ethics (I.5, I.6, II.9) and ACM General Ethical Principles (1.2) and ACM Professional Responsibilities (2.6).

3.2 Safety

The main safety hazard in creating the design of our smartphone-based microscope is during the preparation of the biological samples for imaging. For samples such as the *C. Elegan* (roundworm), it is important that we perform the imaging steps in a closed laboratory settings (with lab gloves and coats) such that the samples are strictly contained and do not get ingested/consumed/brought out the lab in any form. The other safety concern is also with the preparation of chemical stains and specific biological samples. We must do some preliminary research on our end, clarify with our project instructor, and further confirmation with ZJE lab personnel (when necessary) because some chemical compounds can be harmful in different ways (carcinogenic, skin-irritating etc.). Any usage of chemical compounds and laboratory procedures must be formally approved by our instructor before we proceed on our own.

The other safety hazard is with the setting up of our LED illumination module. Although the circuit is a relatively simple switch circuit, we must not be reckless with the power source as LEDs can get over-burnt (or even explode) when not limiting power and current supply. When using the LEDs (especially with excitation and emission filters), we have to be careful of the range of the wavelength and prevent direct contact and long-term exposure to the light, since certain wavelength ranges can be harmful (e.g. UV exposure).

There are also minor hazards with glassware, since we will be using some objective lenses and microscope slides. These apparatus should be handled with care and stored properly after using. It is important that we work on a clean bench during any setup so that if we break anything, the glass residue can be cleaned up thoroughly.

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