A Project Report

On

Designing affordable kits for oral cancer diagnostics.

BY

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Birla Institute of Technology and Science-Pilani,

Hyderabad Campus

Certificate

This is to certify that the project report entitled "**Designing affordable kits for oral cancer diagnostics.**" submitted by Mr Shrinivas Reddy (ID No. 2018B1A40761H), Mr Akash Rajeev (ID No. 2018B1A30491H), Mr Athul V. (ID No. 2018B1A30860H), Mr Vedant Agrawal (ID No. 2018B1A40904H) in partial fulfillment of the requirements of the course BIO F376, Study Project Course, embodies the work done by him under my supervision and guidance.

Date: 15/12/2021

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ABSTRACT

Oral cancer detection and the prototyping of sensor modules for Cheap and Effective Fluorescence Microscope.We look into the photosensors and their application in Cancer Detection.We also look into basic principle of fluorescence imaging and alternatives in the market.Also compare cost to optimize the most efficient and cost effective methods available in the market.Oral cancer or oral squamous cell carcinoma is one the most common type of carcinoma and it develops due to environmental factors. The idea of use of fluorescent dye, fluorescein, on the lesion region to differentiate the cell types is pondered on. As well as the idea of use of antibody capture of CD44, and color change of total protein, to determine the over-expression of CD44, indicating the presence of carcinoma was researched.

After looking at different methods we looked into more. We also looked at ways to improve the light supply system of the techniques, and thereby it led us to build a better prototype. The new and improved design consists of using a 3d printed model surrounded by light around the viewport. The key difference for fluorescence microscopes is the light supply. Switching between brightfield imaging and fluorescence is possible. The model was designed in Blender 3D.

CONTENTS

Need for the Study

Oral cancer or oral squamous cell carcinoma is one the most common type of carcinoma and it develops due to environmental factors. It is so abundant that it affects nearly 0.35 million people yearly. Oral cancer is mostly caused by external factors such as chewing of tobacco, betel nuts, consumption of alcohol, or by factors such as human papillomavirus, premalignant lesions etc. India, being one of the major consumers of tobacco and betel nut, is majorly affected by oral cancer. Affording proper detection and diagnosis support in a developing country like India can be expensive and time-consuming. Hence, new methods and techniques, prototypes and models, need to be devised which are quite affordable and accessible to the public, improving health care of the nation.

Outline

- Available Technology
- Fluorescent sensors
- Photo Diodes
- Idea
- Cost Comparison
- Alternatives
- Review Papers

Introduction

Cancer is a term used to describe a group of diseases characterized by abnormal cell proliferation that has the potential to infiltrate or spread to other parts of the body. Many studies are being carried out in order to detect cancer cells, but the expense of the necessary equipment is prohibitive. Oral cancer is very common in India, accounting for a third of all oral cancer cases worldwide.

Tobacco usage and excessive alcohol use are the main factors in roughly 90% of instances. Individuals with low per-capita income are unable to pay the currently available pricey diagnostic tests, resulting in late cancer identification through visual signs.

Background

The development of a fluorescent microscope prototype by Shubham Srivastava It is a cheap and effective prototype of Fluorescent microscope

Since the lens used in the microscope is expensive we then then have to look into alternative solutions

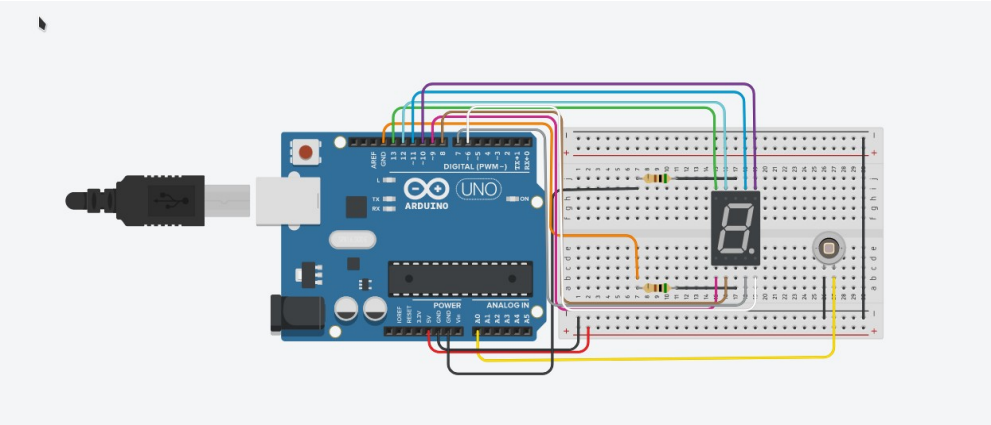
Our Aim : To build an effective sensor module for the detection of Fluorescence.

Proposed Idea

A good camera or a sensor would detect EM radiation in the visible light spectrum(between 400nm and 700nm) ?

Substance	Excitation (nm)	Emission (nm)
Chlorophyll a	400-450	650-750
Phycoerythrins	545-565	565-585
Phycocyanins	615-650	640-660
CDOM (typical)	360-390	450-470
Hydrocarbons	250-350	300-450
Fluorescein	410-510	500-600
Rhodamine B	490-590	510-690

Photodiode Arduino in the market



A photodiode can be used to detect the specific wavelength of light that has been emitted. An microprocessor can be used (Arduino) to detect and display the specific wavelength of light.

Individual Units and Price of Arduino And Photosensor

Quantity	Component	Price (Rupees)
1	Arduino Uno R3	1 x 499
1	Photodiode	1x400
1	Cathode 7 Segment Display	1x12
2	500 Ω Resistor	2x129 = 260

Total price = 1170 ~1200 Rupees

Raspberry Pi Camera Module

The second option would be to use a Raspberry Pi Camera Module as Image Sensing Unit and connect it to a server or a Image processing unit to detect specific wavelengths

Total Cost = 4700 - 5000 Rupees , A bit costly for our application

Phone Camera

Our Next Idea is to use a Phone Camera with a Mount attached to the microscope

Build a phone mount for the microscope.Use the phone camera as sensor

Everyone has a phone today. The Cost of the idea is virtually nil. We 3d model a mount for keeping the phone

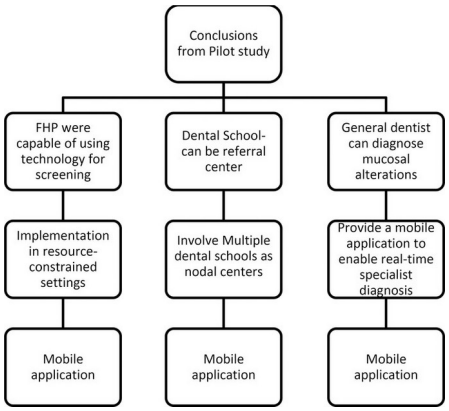
Detecting Specific Wavelengths of Light using phone camera and OpenCV

Roll over image to zoom in

Comparison: Pros,Cons and Costs

Arduino - Photodetector	Raspberry Pi - Camera Module	Phone Camera Light Sensor using OpenCV Color Detection
Cost - Rs 1200	Cost - Rs 5000	Cost - Rs 500(Max)
Cheap	Highly versatile	Cheap and Get the job done approach
Non Configurable, Unable to work on Detection of Specific wavelength.	Cost (High)	Questionable Accuracy(?)

A Novel Mobile Health Approach to Early Diagnosis of Oral Cancer



Fluorescent Sensor

A fluorescent sensor is the complete optical sensing device:

- the light source
- the analyte-responsive (supra)molecular moiety properly immobilized
- the optical system
- the light detector (photomultiplier or photodiode) connected to
- appropriate electronics for displaying the signal.

Working of a Photodiode

To comprehend Photodiodes, we must first grasp what a diode is. I won't go into great detail here, but a diode is a component that only enables current to flow in one way (only in forward bias). When the current is blocked by a reverse biassed diode.

When it comes to photodiodes, they have the similar property of allowing current to flow when forward biassed. When reverse biassed, however, it resists current until light strikes it. When light strikes a photodiode, current begins to flow through it, which is proportional to the intensity of the incident light. When current passes through the Photodiode, a voltage is created across it, which we will use in our circuit to detect incident light.

Designing a PhotoDiode Circuit

The circuit you'll need to build will be determined by the application. A light sensor is one of the most basic photodiode circuits. The output of a photodiode is linked to the inverting input of an operational amplifier, and a feedback resistor is connected between the output and the op-input. The current from the photodiode is effectively converted to a voltage, with the output voltage equal to the photodiode current multiplied by the feedback resistance. In this situation, the photodiode does not need to be biassed. Place all analogue circuitry away from (noisy) digital circuits when constructing a photodiode circuit.

Cost

The cost of making a Photodiode circuit will depend on the following factors :-

The photodiode parameters:

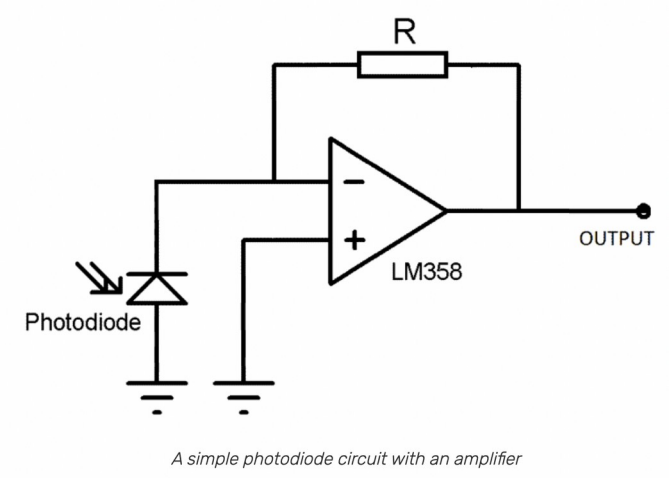
Responsivity:

Saturation current:

Terminal capacitance:

Responsivity temperature coefficient:

Shunt resistance



A simple photodiode circuit with an amplifier

Diagnostics with alternative and cheap methods (without use of expensive equipment like microscope)

Use of Fluorescein dye for Oral Cancer Detection

India has one of the highest oral cancer deaths in the country annually. Early detection can help to reduce the mortality of cancer patients and especially for oral potentially malignant disorders (OPMD) and thereby prevent malignant transformation.

Fluorescein dye has not been explored much yet when it comes for diagnosis of oral cancer while being it has been used before for other tumour detections like breast,brain, colon and stomach.

Tumour biomarkers, saliva based oral cancer diagnostics and fluorescence spectroscopy has been explored before but these types of technologies are very expensive and not feasible for a developing country.

What are Oral potentially malignant disorders?

- These disorders have a very increased chance of malignant transformation which is when a normal tissue turns into a cancer tissue.

The lesions which were showing autofluorescence were excluded in the experiment after examining them under blue light.

Fluorescein dye is used and applied to a lesion and then the blue light (480nm) is used to find out the fluorescence and record it in a dark room.

The lesions might not show dysplasia which is a very important predictor for malignant transformation.

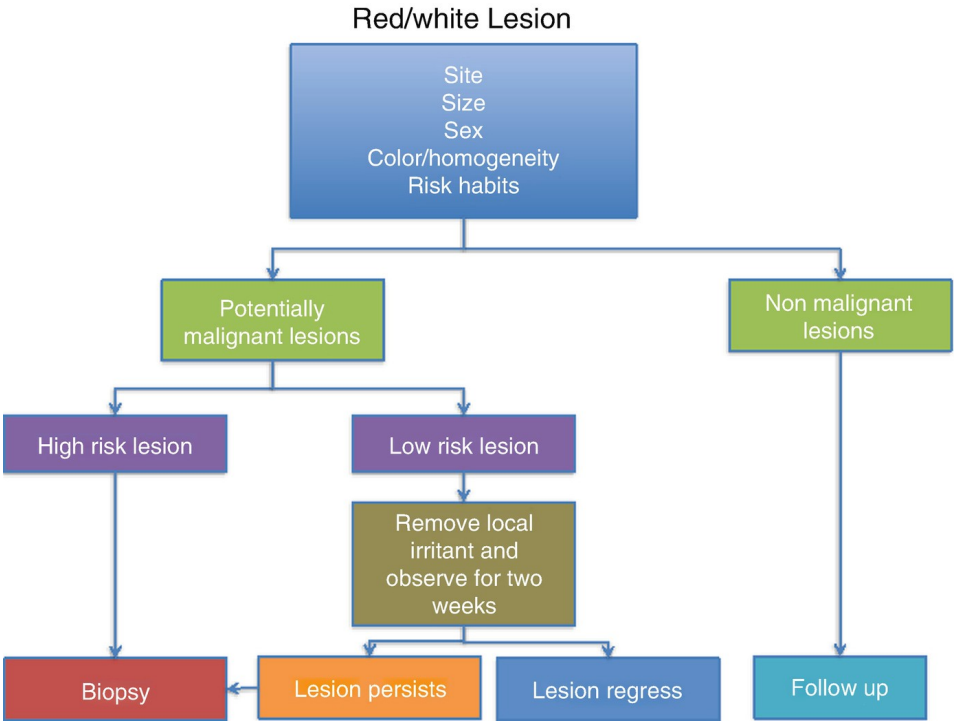
The major difficulty might be to screen oral cancer is that before the experiment the lesions which were showing autofluorescence were excluded and further research must be to screen for healthy people and high risk patients.

The results showed that 95 % of cancer and dysplasia cases showed fluorescence and also without dysplasia 47.6 percent of cases showed fluorescence. It also showed that only 11 % of inflammatory lesions were showing fluorescence.

Sensitivity for fluorescein detection method for OPMDs was 96.6%.

Fluorescein has been showed in various studies to be safe in administration to a large number of patients for dermal use. It is a non toxic and vital dye which in alkaline to slightly acid solutions produces green fluorescence.

This test will be able to bring down the costs of oral cancer associated tests as due to limited resources fewer people have access to expensive screening and diagnostics for oral cancer.



Comparing all the different dyes

There are some dye options which can be used to stain and then hence detect fluorescence. These dyes are:-

1. Fluorescein dye
2. Methylene blue

3. Toluidine blue dye

4. Rhodamine

In a study methylene blue was tested to find its use in a clinical setting and find out its usefulness and shortcomings when it comes to tissues which have undergone malignant transformation and these were carried out in the study. The study consisted of 39 cases of non homogenous leukoplakia and two other types which were 4 cases of erythroplakia and 5 cases of oral squamous cell carcinoma.12 cases were stained positively for methylene blue out of the 25 cases which were of homogenous leukoplakia and also there were 13 cases which were stained negatively for methylene blue.

This study led to a positive predictive value which was higher than any other study which was done previously.This means that 55 out of 60 cases were able to stain positively which is 91% and is very high when compared to 74% which was found out in previous studies.
It is also cheaper than toluidine blue and hence due to its very positive qualities and a very high predictive rate much higher than described in the previous studies and can be a great substitute.

- Methylene blue was found to have a sensitivity of 91.4 percent in detecting dysplastic and carcinomatous alterations, a specificity of 66.6 percent, and a positive predictive value of 97.7%.
- Toluidine blue currently has no evidence that it is a cost effective way to be used as a dye for oral cancer screening. The amount of false positives it has makes it a not very suitable dye when it comes to a primary care setting. Using it as a first approach might work but a cost effective tool to be built can be quite difficult.
- Rhodamine can be further explored to check its effectiveness when it comes to cancer screening and being used as a dye and fluorescein dye is the only other option which can be compared due to the false negatives of toluidine blue.

Salivary diagnostics – without the use of expensive equipments like microscope

Saliva is a extracellular fluid mixture which comprises of gland secretions, mucosal cells, nasal secretions, proteins and macromolecules, as well as a spectra of microbial species.

Saliva can be used for diagnosing a disease as it is easily obtained and is a non-invasive way to collect specimen at the site of lesion.

For cancers specific to oral and oropharynx region, salivary biomarkers have been prominently used for early detection and prevention of cancer. Collecting salivary samples is usually done in three methods –

1. Salivary rinse
2. Unstimulated saliva (whole)
3. Stimulated saliva (whole)

Oral rinse allows the sampling of parts of larynx, hypopharynx, ie entire oropharynx, and is not restricted to anterior oral cavity, hence it is a better method of saliva collection.

TP53 is a gene which encodes instructions for production of tumor protein (p53). **P53** is a protein that suppresses tumor. It is responsible for the regulation of cell division and avoiding production and proliferation of cells in an uncontrolled way. Mutations, either inherited or somatic, to the p53, can lead to the loss of control of cell cycle.

Numerous studies have discovered several salivary proteins like **CD44**, IL-8, CyclinD etc and some auto-antibodies that work against the abberently expressed TP53 and antigen proteins associated to melanoma. Along with the proteomic profiles, total DNA, DNA variants of TP53, microsatellite instability etc. are also present. To evaluate relevance in assessment of disease, around 100 different potential biomarkers have been identified, relying on comparative studies between cancer patients and healthy human controls.

CD44 transmembrane protein family have a important roles in biochemical process such as organogenesis, hematopoiesis, and the immune system. In a healthy epithelium, expression of CD44 is mainly observed in the suprabasal and basal region, but with advancement of dysplasia the expression is noted to migrate to the superficial layers. This indicates the involvement of CD44 in changes during early stages of carcinoma. Cells with CD44 have been found to flourish on the relapsed tumor surface, in comparison to primary tumor.

Mellatoproteinases, found in saliva, cleaves and releases CD44 (in soluble form SolCD44) from surface of cell. SolCD44 is detectable in oral rinses, with inexpensive assays and detects HPV +ve and –ve oral and oropharyngeal. Prognosis can be determined to be worse if higher levels are found. It was found that early stage and advanced HNSCC (Head and neck squamous cell carcinoma) patients were differentiated from healthy controls on the basis of SolCD44 with the test having **specificity of 88% and sensitivity of 62%.**

High levels of expression of CD44 protein and total protein indicated a **25 times** more chance of oral squamous cell carcinoma. When the combined concentration of SolCD44 and total protein was assessed and analysed from oral cancer specific specimen of oral rinse saliva, irrespective of consumption of alcohol and tobacco, age, gender, it was found to have a score of **0.7.** This indicated that the use of CD44 can diagnose both HPV and non HPV related type oral cancers.

POC (Point of care) model for diagnostics

POC device is made of two test strips, one for CD44 and one for total protein, respectively, encased in a single plactic molding case. The oral rinse sample is collected through a sample collection cup and saline solution. The poc device is inserted into the cup, until the strips are wet and is placed on a absorbant pad for a small period of time. Capillary action results in rise of the sample through the tubes, as it rises a monoclonal antibody that labels human sCD44 is rehydrated, while sample flows through detection membrane. Another anchored monoclonal antibody which binds to a different domain of CD44 is stripped on the membrane, and it captures the CD44 protein as it passes. A visual line is seen, when a certain threshold of concentration of CD44 exceeds. In the second total protein strip, changes color, in variance the concentration of protein, ie yellow (minimal protein) to green (moderate conc. of protein), and to dark green/blue (High conc. of protein).

Potential presence of cancer can be accurately indicated through this dual marker system.

The test is a point-of-care device that detects human sCD44 and total protein in oral rinses, as well as other clinical factors, to help diagnose oral and oropharyngeal cancers.

Cost of the test kit is identified to be around the same as of a pregnancy kit, hence this test is affordable.

Performing the test is additionally simple as it does not demand any previous training or hands-on-experience. Additional equipment is also not required, instead of blotting paper and timer, which are fairly cheap.

Fluorescence and microscopy

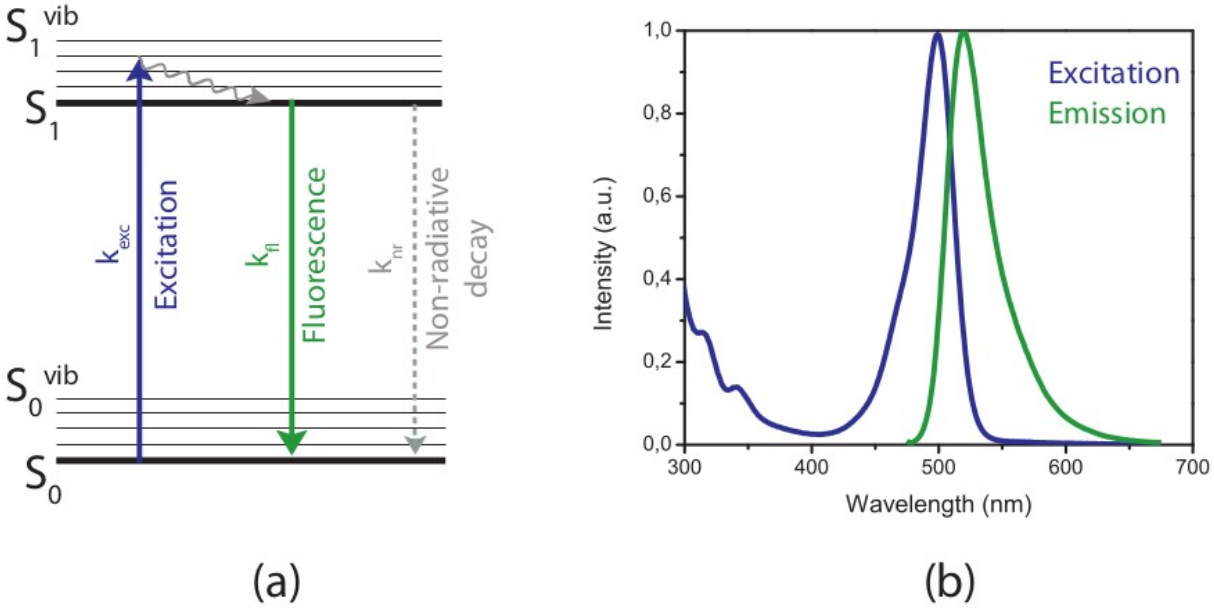
Fluorescence or phosphorescence is the phenomenon which occurs due to absorption of smaller wavelength electromagnetic waves resulting in excitation of electron(s) and subsequent emission of longer wavelength electromagnetic waves due to the vibrational relaxation to its ground state. It is a form of luminescence and takes place when there is an emission of low energy photon, without a change in the electron spin. The microscopy takes advantage of it by scanning across a single plane, the fluorescence intensity of cells, tissues, or other intra-cellular structures. The fluorescence is specifically produced by using a fluorophore (present in the fluorescence dye) to label an antibody, which subsequently targets and attaches to it’s specific antigen in the sample. Multiple channel method is used for visualization of numerous targets in an image, using different antibodies, specific to their non-identical antigens, attached to multi-colored fluorophores having significantly different wavelengths.

A very bright light source, such as a xenon or mercury arc lamp, is required for fluorescence microscopy. The mercury lamp emits light which is 10-100 times more intensely brighter than a conventional incandescent lamp, also producing the electromagnetic waves ranging from wavelengths of ultraviolet to infrared light. The excitation wavelength of the emitted light is selected by the exciter filter through which the light passes through, after leaving the arc/ lamp. A

unique mirror called the dichroic mirror is placed so as to reflected the light specifically of the desired wavelength on the sample. A barrier filter is used to filter the emitted light and the emission wavelength is analysed for fluorescence.

The filter cube consists of all three components essential for the fluorescence microscopy, ie the exciter filter, dichroic mirror, and the barrier filter.

Stoke's Shift is the difference between the peak of the absorption, or excitation curve, and the peak of the emission curve. The larger the gap between the two wavelengths, the easier it is to distinguish them. In order to eliminate background and increase image quality, the components of the filter cube must also remove any overlapping spectrum.



(a) Energy level change in electron (b) Stokes shift and emission spectra

Ray Optics and Fluorescence Microscopy

Ray optics can be used to model fluorescence microscopes.

The thin lens imaging and magnification equations are the two most important equations to remember:

1/si + 1/so = 1/f

-si/so = M

The letter f stands for the focal length of a lens. The focusing power of a lens with a shorter focal length is greater.

Also crucial is the object distance, or the distance between the lens and the object (e.g. a tree)

The image distance (si) is the distance between the lens and the picture creation point.

Magnification is represented by the letter M.

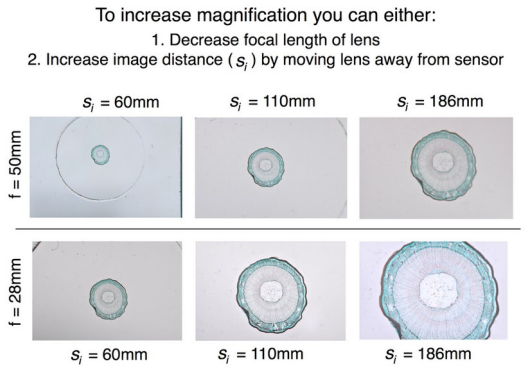


Fig. Different magnification for Si and f.

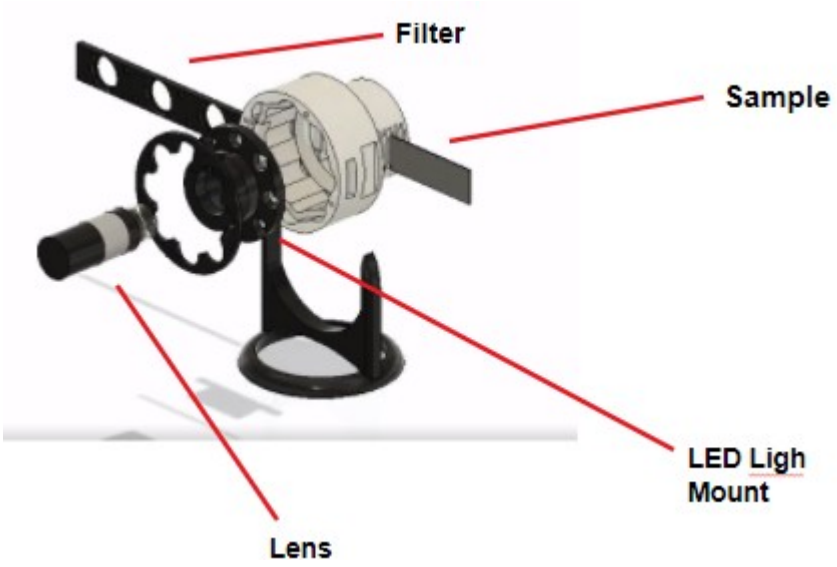
Improvement on the Light Supply system

So we took it to ourselves to build a better prototype

One with multiple LED Functionality and Lens mount perfect for a phone. And the source of

illumination is also small.We have extended by using multiple LEDs3D model Previous Model(Left),Our Model (Right)

New Prototype Design



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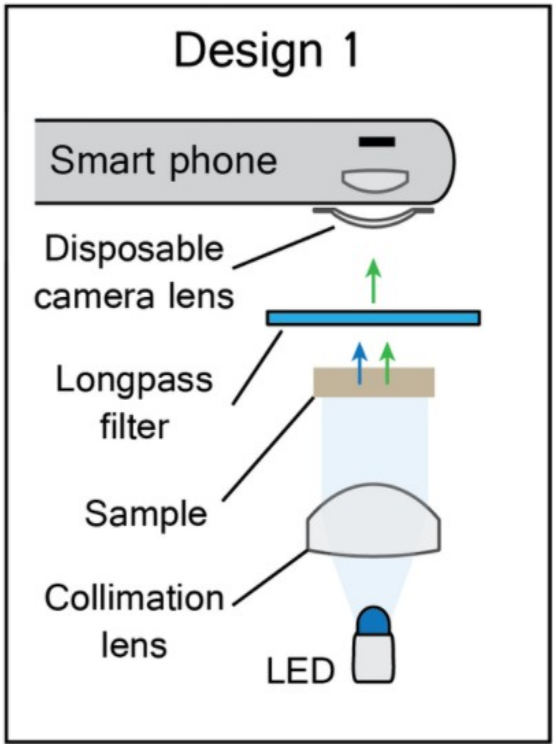
The new and improved design consists of using a 3d printed model surrounded by light around the viewport. The key difference for fluorescence microscopes is the light supply. We can switch between brightfield imaging and fluorescence. The model was designed in Blender 3D.

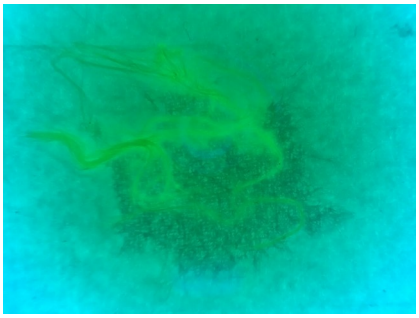


The light from the LED excites the fluorescence sample and the sample re-emits an excitation spectrum. We then magnify the light and block it out by a long pass filter. Smartphones uses an OpenCV program to differentiate between the light that must be occluded out. .

Supplies and Tools

S.No	Supplies	Cost
1	3d Printer	-
2	Excitation LEDs	1000-2000 Rupees
3	Longpass filter	700 Rupees
4	Prepared microscope slides	165 Rupees
5	Arduino Nano	400- 500 Rupees
6	Fluorescent dye	-
7	Macrolens	500-1000 Rupees
*	Total	~ Rupees 2500 Min

Macrolens Images



Images of some samples captured in macrolens images.

Conclusion

Cancer cell diagnostics is a part of wide research around the globe. New methods and technologies are being developed with pace to bring advancements to the field of cancer cell detection. Economic viability of a cancer diagnostics kit is a major drawback as the newer technologies are either expensive or are rare, which makes them unavailable and unaffordable. In this project the group has compiled and improved upon the previous methods/version of oral squamous cell detection and has improved the light supply system, as well as, formulated the use of cell-phone with a camera for smart detection of cancer cells. The project has included compilation of information on fluorescent dyes and fluorophores and also other diagnostic methods for cancer cell detection.

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