Cancer Toolkit Development

Thesis

Submitted in fulfillment of the requirement of BITS F421

by

Shubham Srivastava (2015B1A40818H)

Under the supervision of

Dr. Kumar Pranav Narayan

Birla Institute of Technology and Science - Pilani, Hyderabad Campus

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Birla Institute of Technology and Science-Pilani,

Hyderabad Campus

Certificate

This is to certify that the project report entitled "**Cancer Toolkit Development"** submitted by Shubham Srivastava (ID No. – 2015B1A40818H) in partial fulfillment of the requirements of the course BITS F421, embodies the work done by them under my supervision and guidance.

Date: 14-03-2019 **Dr. Kumar Pranav Narayan**

BITS- Pilani, Hyderabad Campus

ABSTRACT

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Various researches are being continuously done in order to detect and cure cancer. In the present century, lots of research are being conducted in order to detect cancer cells, whose equipment's cost are too high.

Oral cancer is quite prevalent in India as India accounts for a third of all global oral cancer cases. In about 90% of the cases tobacco use and excessive alcohol consumption are the main causes. Due to low per-capita income individuals aren't able to afford the existing expensive diagnostic services which results in late cancer detection through visible symptoms. My main aim is to build a low cost and effective diagnostic tool using targeted drug delivery system so that it becomes easier and cheaper for individuals to get tested at an early stage.

This tool kit can be used to detect a specific type of cancer cells which will not only be cheap but also be easily accessible by the user. For the same, I will be focusing on the development of a fluorescent microscope

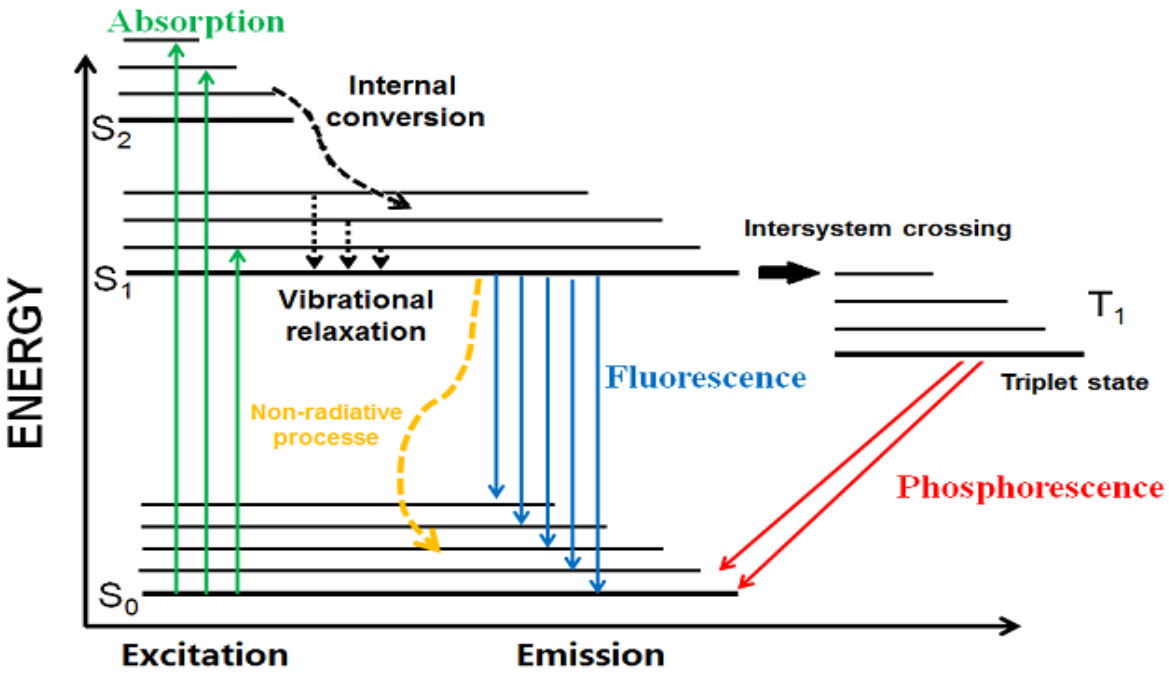
CONTENTS

FLUORESCENCE

It is the emission of light by a substance that has absorbed light. The excitation of electrons will happen only in a fixed wavelength of light and the light emitted will have normally a longer wavelength (less energy).

The basic working is when an electron absorbs wavelength in its excitation range, it will jump to a specific energy level. Since every matter has a tendency to be in a state of minimum energy, the electron will try to come back to its initial lower energy state by the release of the absorbed energy. It is this release of the absorbed energy which is termed as fluorescence due to which we say the material which is excited is now fluorescing.

The possible ways for the relaxation are as follows: - vibrational relaxation, internal conversion, intersystem conversion, fluorescence, delayed fluorescence, phosphorescence and energy transfer to other molecules.



http://www.ibs.fr/research/research-groups/dynamics-and-kinetics-of-molecular-processes-group-m-weik/pixel/photophysics-of-fluorescent/

Figure 1 Schematic Diagram of Energy vs Emission spectrum

1. Vibrational relaxation - This is one of the energy relaxations which occurs through non-radiation processes.

2. Internal conversion - This process brings down an electron from one subset of energy level to another subset of energy level by releasing very low pockets of energy.
3. Intersystem conversion - This type of energy relaxation transfers the electrons to the “forbidden” energy state i.e. triplet state level due to which slow fluorescence is detected. In this case, the material excited will not immediately emit any wavelength but with the passage of time, it will start to emit very low fluorescence.
4. Vibrational relaxation - One more relaxation method in which electrons will reduce their energy by vibrating.
5. Energy transfer - In this, electrons transfer their energy to the nearby matter.
6. Fluorescence - This happens only in a fixed region of wavelength which is different for different materials under different environmental conditions.

Cancer Tool Kit Development can be divided into 3 parts: -

1. Making a dye specific to a cancerous cell.
2. Detecting the fluorescence emitted by the dye upon its excitation.
3. Analyzing the emitted wavelength.

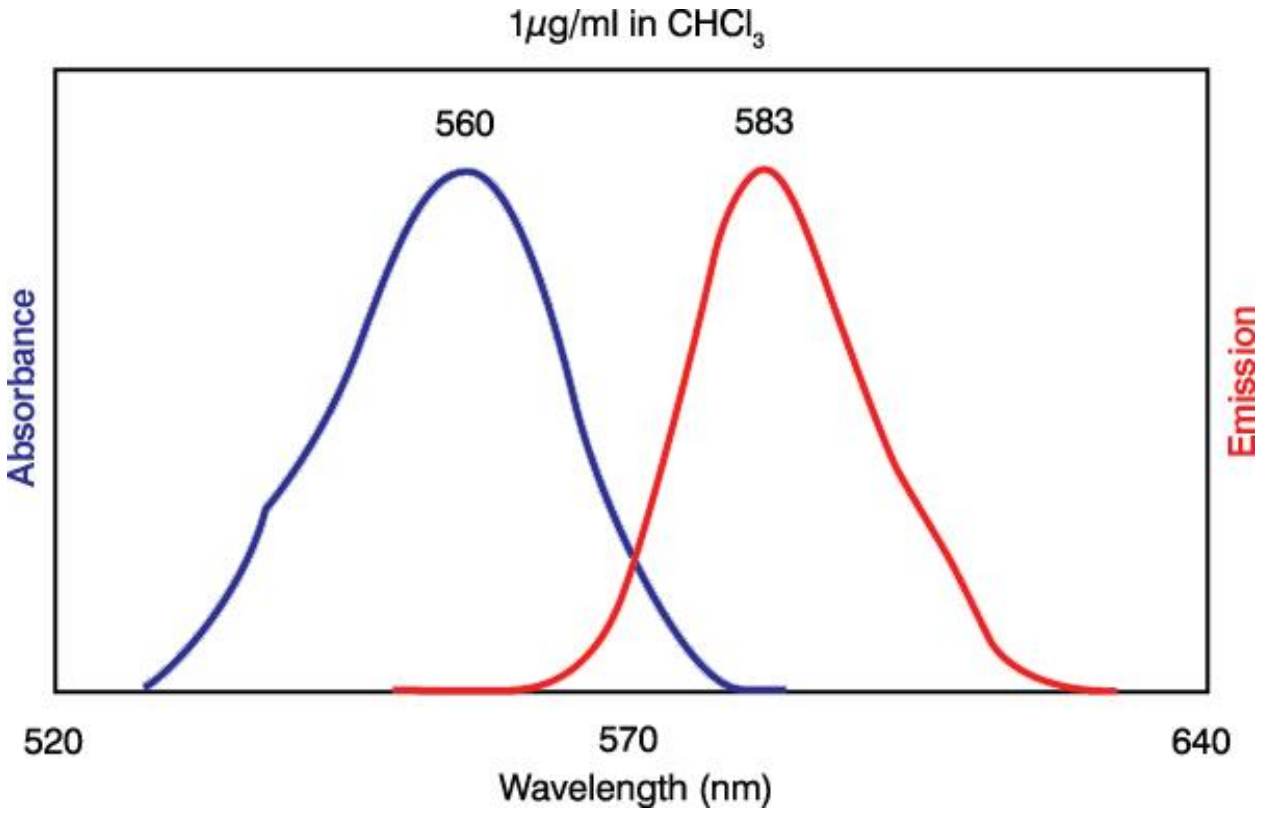
The dye used in our experiment is **18:1 Liss Rhod PE (Rhodamine PE / Rhodamine B)** whose fluorescence is to be detected using the fluorescence microscope followed by the analysis part.

BASIC DESIGN OF FLUORESCENCE MICROSCOPE

Numerous designs can be implemented for basic designing of the fluorescence microscope but the most feasible one is to be chosen considering least cost of preparation and ease of handling.

The basic sequence to be followed in for detection in a fluorescence microscope is as follows:

1. Polychromatic light source - A light source emitting all range of wavelength is used in order to ensure that the region of wavelength required to excite the dye is available.
2. Excitation filter - This is used to filter out unwanted wavelength of light allowing only wavelength having potential to excite the given dye to pass through it which results in increasing the accuracy of the outcome.
3. Dichroic mirror - A dichroic mirror is a device which allows only specific wavelength of light to pass through it and reflecting the leftover wavelength of light.
4. Eye piece - This part will consist of a camera detector which will capture the image of the fluorescent and also analyze its intensity.
5. Excitation Filter - The basic property of the excitation filter is to filter out the unnecessary wavelength of light and let only fixed wavelength of light pass through it. For our experiment, we need a filter of the range 520-570 nm which is the excitation range for 18:1 Liss Rhod PE as can be seen from the following figure: -

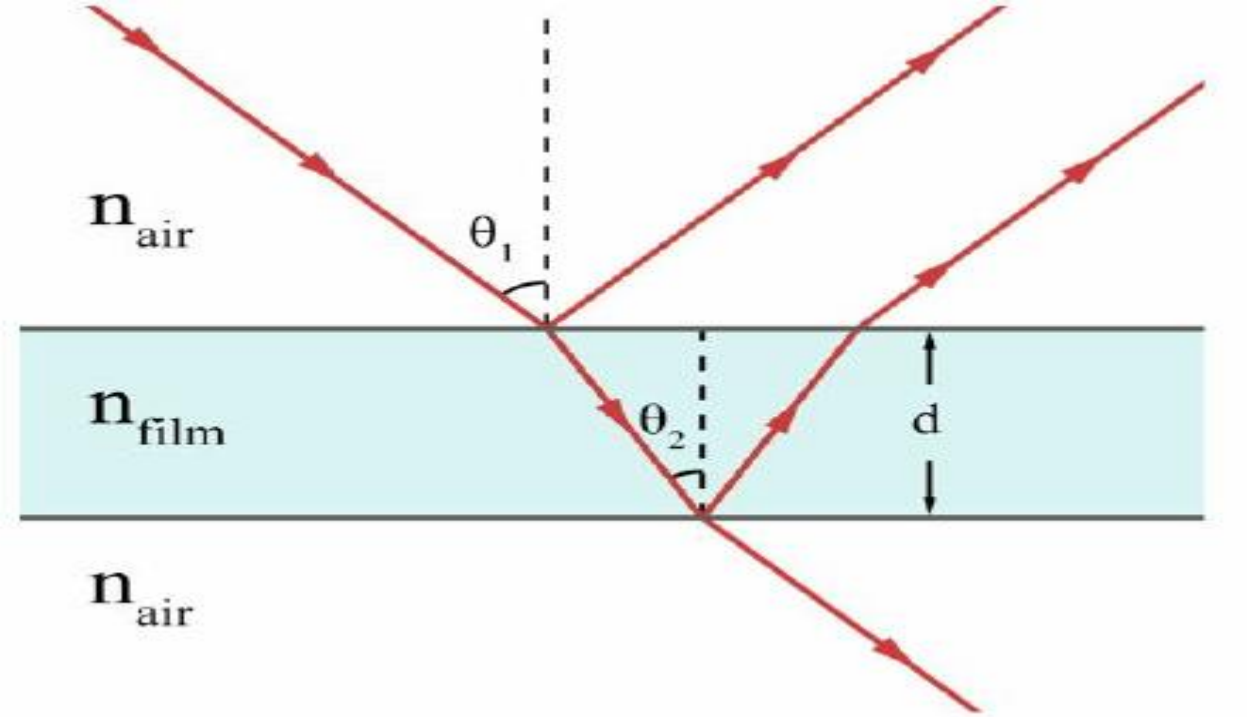


https://avantilipids.com/product/810150

Figure 2 Schematic Diagram of absorbance vs wavelength of Rhodamine PE

6. Dichroic mirror - The dichroic mirror works according to the thin film interference which creates such a condition that only specific wavelength of light is passed though the mirror while the others are reflected from it. It functions as follows: -

   Light is first reflected off from the glass surface, then it reflects off from the film surface which is approximately micro level thick. The design is made in such a way that the reflected light comprises of the desired wavelength to be reflected while the leftover passes through the mirror. The range of emission for the given dye is between 570-640nm.



https://www.researchgate.net/figure/Thin-film-interference-in-the-case-of-eg-soap-bubbles_fig1_241532752

Figure 3 Schematic Diagram of thin film interferance

The basic blueprint of fluorescence microscopy is as follows :-

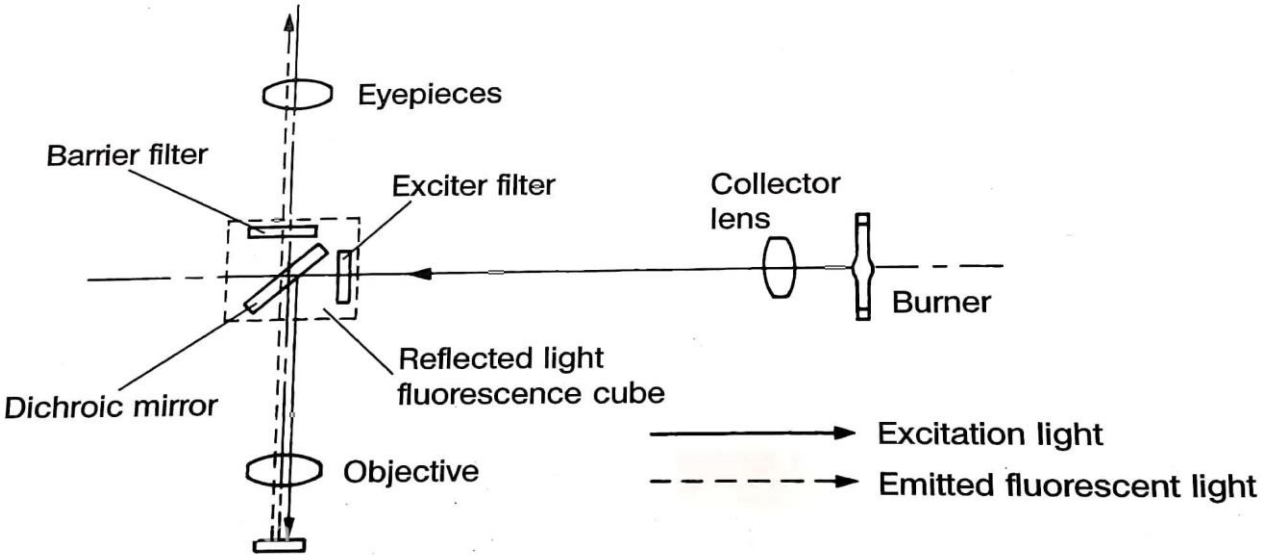


Fig 4 Schematic diagram of a fluorescence microscope.

(http://www.olympus.com.cn/upload/accessory/20114/20114111446116907713.pdf)

SPECIFICATION OF DESIGN COMPONENTS

Terminologies:-

1. Clear Aperture :- Actual diameter or size of an optical instrument or component (excluding the boundary thickness).
2. Center thickness :- Almost all the optical components have different thickness at their center for its proper functioning.
3. Surface Quality :- Signifies surface quality which includes scratches, digs and pits.
4. Surface flatness :- Describes the surface accuracy specification giving out the deviation of the surface from the prescribed dimension.
5. Parallelism :- Extent upto which the two surfaces are parallel with each other. (5 arcminutes – 0.0833 degree.
6. Central wavelength :- The peak value in the range of wavelength to be passed through a filter.
7. Full Width Half Maximum :- Tells spectral width of sources.
8. Optical Density :- Amount of energy blocked by the filter.
9. Working distance :- The distance between the object and the lens of an objective lens.

Link for thorough details :-

1. https://www.edmundoptics.com/resources/application-notes/optics/understanding-optical-specifications/
2. https://www.edmundoptics.com/resources/application-notes/optics/optical-filters/

Specifications :-

1.) Dichoric Mirrors:

Diameter : A rectangle will be most preferable of dimensions 50 x 25 (length x breadth) all dimensions in mm.

Clear Aperture : Greater than 85%

Center Thickness : 5 mm (depth)

Surface Quality : 80-50

Surface Flatness : 1λ

Parallelism : At least 5 arcminutes

Angle of incidence: 0

HR@530 nm % : ---

2.) Excitation filter:

Diameter: 30mm

Clear Aperture: Greater than 85%

Central Wavelength: 560nm

FWHM: 80nm

Blocking Level OD: >4

Blocking Wavelengths: All except green (520 - 570nm)

Thickness: 2-5mm

3.) Barrier Filter

Diameter: 30mm

Clear Aperture: Greater than 85%

Central wavelength: 583nm

Passing Band regions: 570-700nm (yellow, orange, red)

FWHM of Blocking region: 80nm

Thickness: 2-5mm

4) Objective lens :- Magnification of 20x/10x with working distance ranging 4-10mm (tube length 160mm).

5) Eye piece :- Magnification of 10x

OPTICS

The parameters to be considered for construction of any microscope is governed by laws of optics.

Basics:-

Introduction of a lens near to our eyes simply helps in proper focus of image at our eyes. For example, the schematic diagram of a simple microscope i.e. a magnifying glass is as follows:-

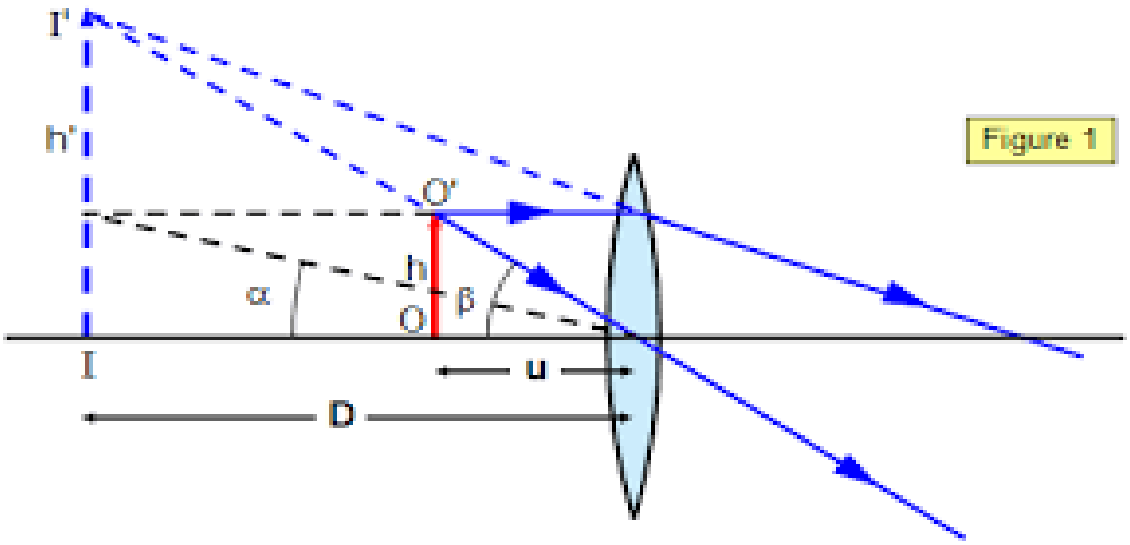


Fig 5 :- Optics of a magnifying glass

http://www.schoolphysics.co.uk/age16-19/Optics/Optical%20instruments/text/Magnifying_glass/index.html

Near point (D) :- It is the point nearest to our eye at which an object can be focused at the retina. Its value is 25cm. If the object is brought inside D, eyes are at strain.

In order to magnify an object, we use a lens having a focus distance (F=u) less than D (as shown in fig 5). Magnification = **D/F** or **β/α**

A fluorescene microscope has a design similar to that of a compound microscope having one objective lens (of magnification M1) and one eye piece (of magnification M2). When together, the final image formed has a magnification of M1 x M2

The basics of optics followed in a compound microscope is as follows :-

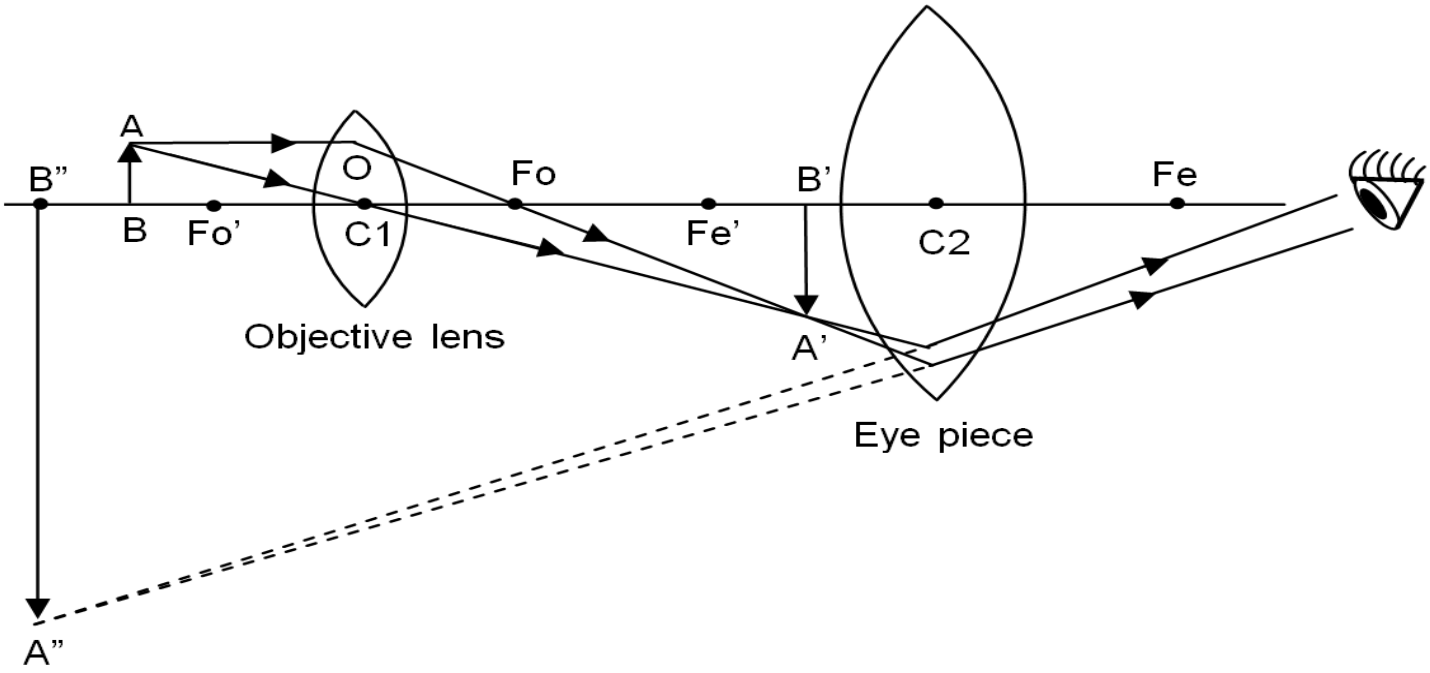


Fig 6:- Optics of a compound microscope

http://www.funscience.in/study-zone/Physics/OpticalInstruments/CompoundMicroscope.php

The image formed by the objective lens should fall on the focus point of the eye piece because our eyes has less strain when it receives parallel rays of lights and since lights passing through the focus points emerges as parallel rays, less strain on eyes will be developed.

OBJECTIVE LENS

It is the first lens through which light emitting from the object passes. The objective lens used in this experiment will have value of 20x and 10x.

According to the Royal Microscopical Society (RMS), by default, the standard tube length and parfocal distance will be 160mm and 45mm respectively.

Tube Length :- It is the distance between the end point of objective lens and the starting of eye piece.

Parfocal Distance :- Distance form mounting point of the lens to the focus point.

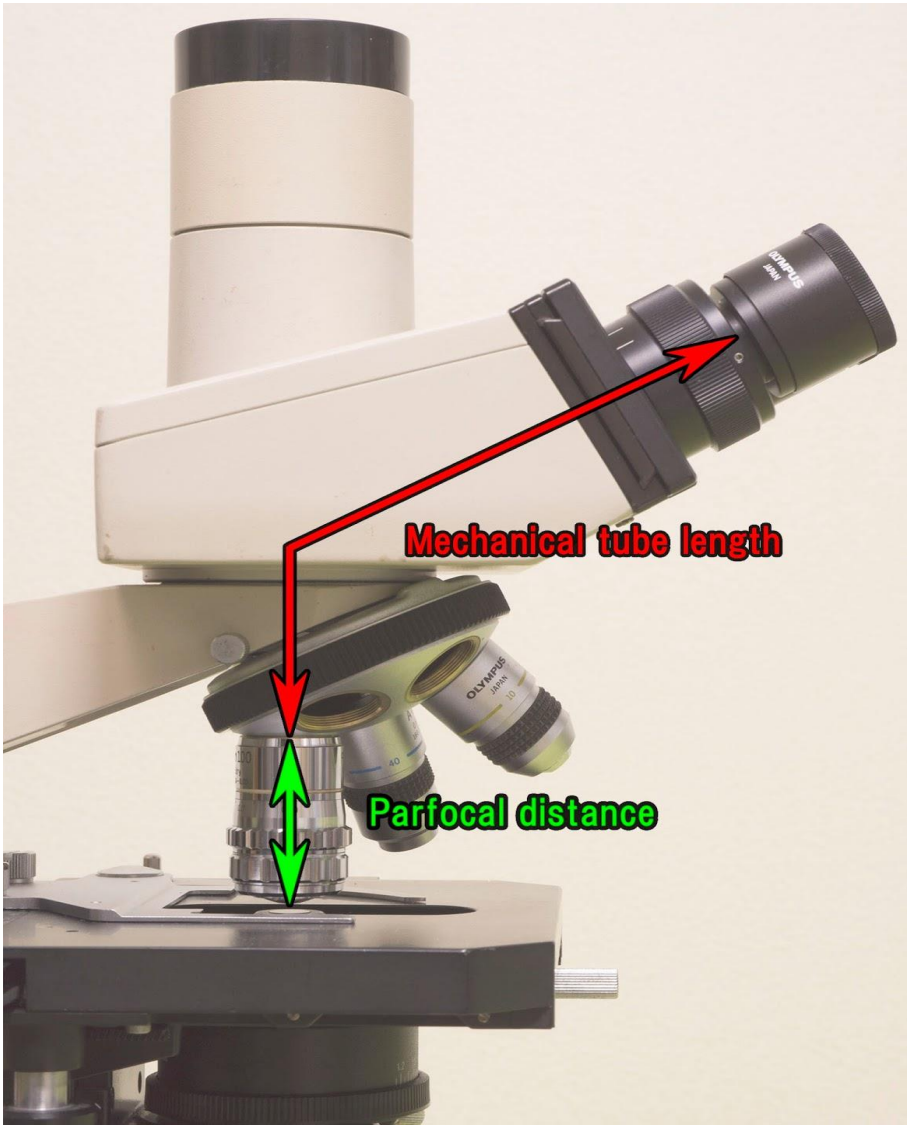


Fig 7 :- Basic diagram of a compound microscope

https://inst-smallworld.blogspot.com/2018/03/objective-lens.html

Since we are using eye piece of 10X magnification, if the objective lens has a magnification of 20X then overall image formed will be 200X magnified and if the objective lens has a magnification of 10X, overall magnification will be 100X.

Numerical Aperture (NA) :- It is a dimensionless number signifying the measure of light collecting ability of a lens.

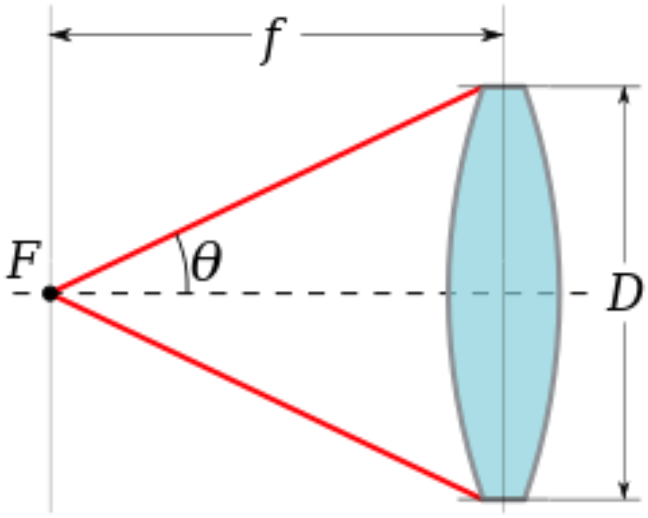


Fig 8 :- Diagram of numerical aperture of a lens

https://en.wikipedia.org/wiki/Numerical_aperture

NA = n.sinθ where n is the index of refraction of the material in which the lens is working (for air, n=1).

As magnification and numerical aperture increases, working distance decreases.

NA in objective lens is usually small to have small focus point.

Intensity of light passing through a lens is directly proportional to NA and magnification of the lens as follows:-

Intensity α $(NA)^4$ / M^2

Numbers on the barrel of the microscope objective :-

Fig 9 :- Numbers meaning on barrel of objective lens

https://www.optics-pro.com/for-microscopes/euromex-10x-0-25-plan-din-bb-8810-microscope-objective-bioblue-lab-/p,56744

- Lower left number :- Tube length in mm
- Lower right number :- Thickness of glass cover slip in mm for best performance
- Upper left number :- Magnification
- Upper right number :- Numerical aperture

MICROSCOPE FILTER CUBE

A filter cube which can hold dichroic mirror, excitation filter and emission filter was to be constructed which can be easily mounted on the microscope. The cube should be designed in such a

way that it should have combined compartments to hold the filters and the mirror. So, following design was chosen to fulfil our requirement :-

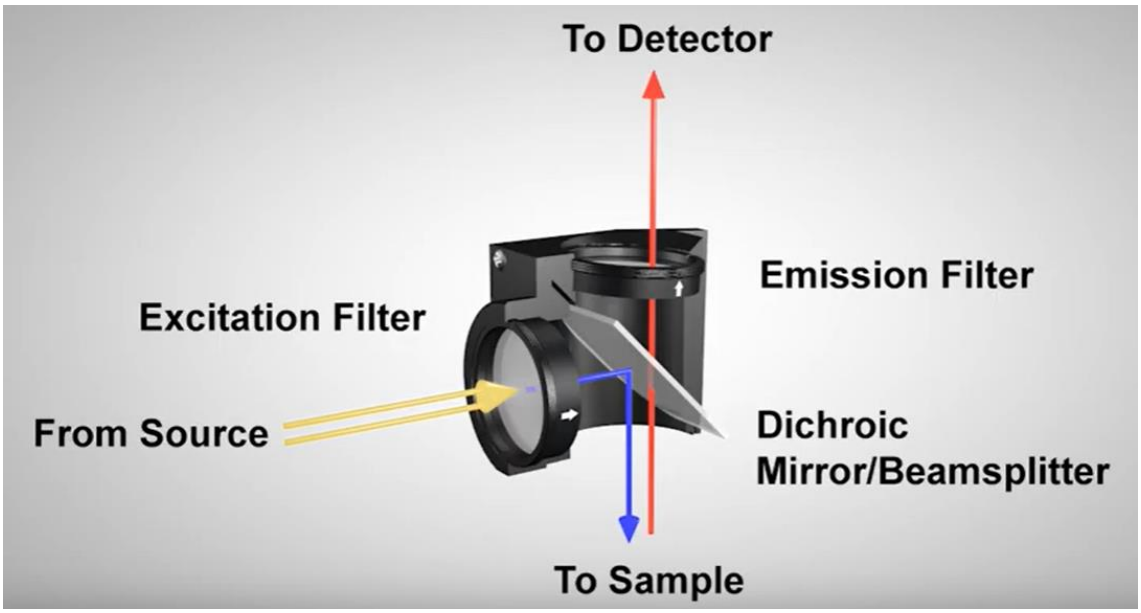


Fig 10 :- Filter Cube

https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_ID=5007

PROBLEMS

After discussion with various vendors for lens and filters, the cost was around 5 lakhs rps which was way above our main aim of constructing a cheap fluorescence microscope. Due to this, we had to formulate new ideas for experiment and design to carry out our project ahead.

ALTERNATIVE

Since by standard conventional equipment, we were not able to reach our main motive of the project, we shifted towards alternative ideas for the construction of the microscope which are :-

- Since we need an excitation filter such that it is able to pass green light through it, instead of excitation filter we opted for 3 alternatives which are :-
    1. Green light emitter (diodes) :- https://www.amazon.in/Flame-Green-Clear-Emitting-100Pcs/dp/B01FLI6086/ref=sr_1_7?crid=U0NSHQD7N5NY&keywords=green+led+light&qid=1573101584&sprefix=green+led%2Caps%2C1216&sr=8-7#detail_bullets_id
    2. Colored plastic sheet :- https://www.amazon.in/Green-Overlays-Transparency-Correction-Lighting/dp/B01LYK6BG3/ref=sr_1_3?keywords=green+filter&qid=1573100740&s=toys&sr=1-3
    3. Color gel filters :- https://www.amazon.in/Green-centimeters-Transparent-Correction-Flashlight/dp/B01N51N0F2/ref=sr_1_20?keywords=green+gel+filter&qid=1573120821&s=electronics&sr=1-20
    4. Green glass slab

These alternatives is still to be tested properly so as to know how much intensity and range of green light is needed for activation of the sample dye.

We are preferring green light emitters (diodes) each of 3.5V for now to carry out the experiment. One diode emits almost 200 mille candela (md) of light. Maxell LR1130 Button cell of 1.5V each will be used to power

the diodes. In order to know how many such diodes are required for excitation of the sample, we need to conduct a separate experiment. The dimensions of the diodes are 0.5 x 0.5 x 3.7 cms (Breadth x Height x Length).

We will be only using an objective lens and an eye piece for the design. The basic diagram is as follows:-

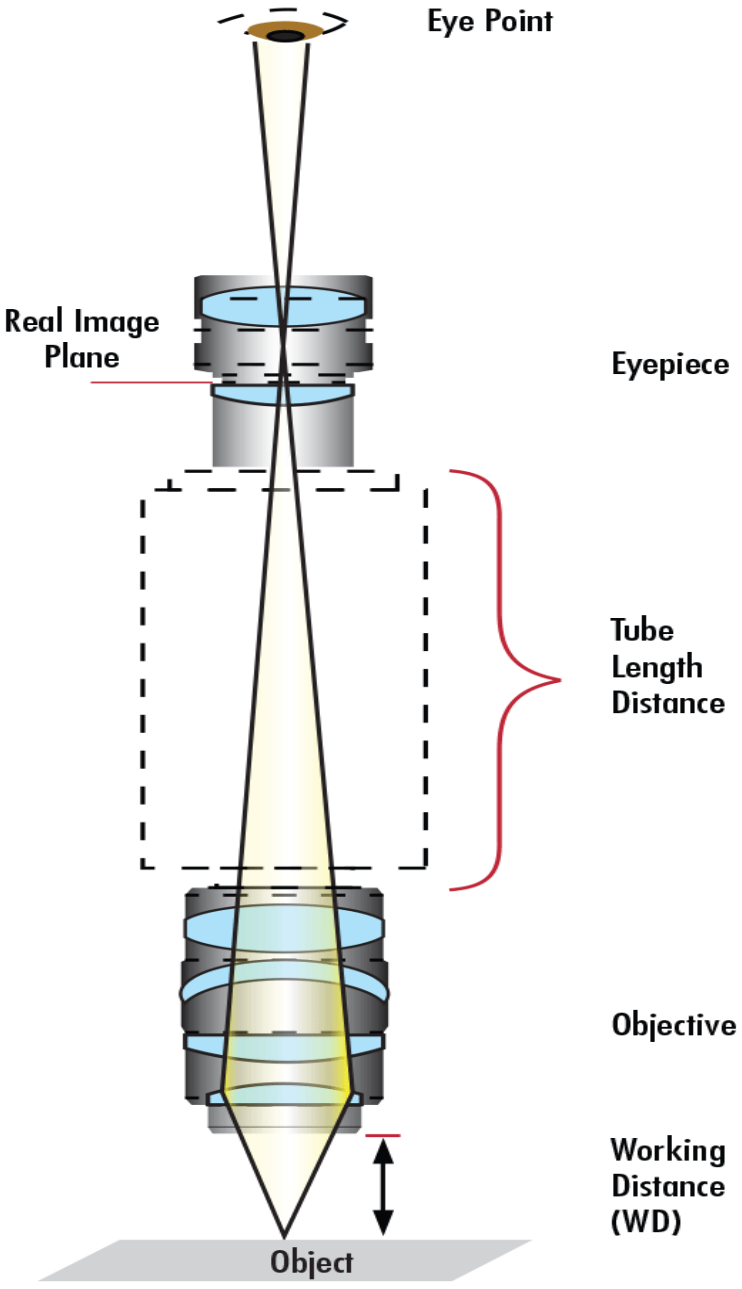


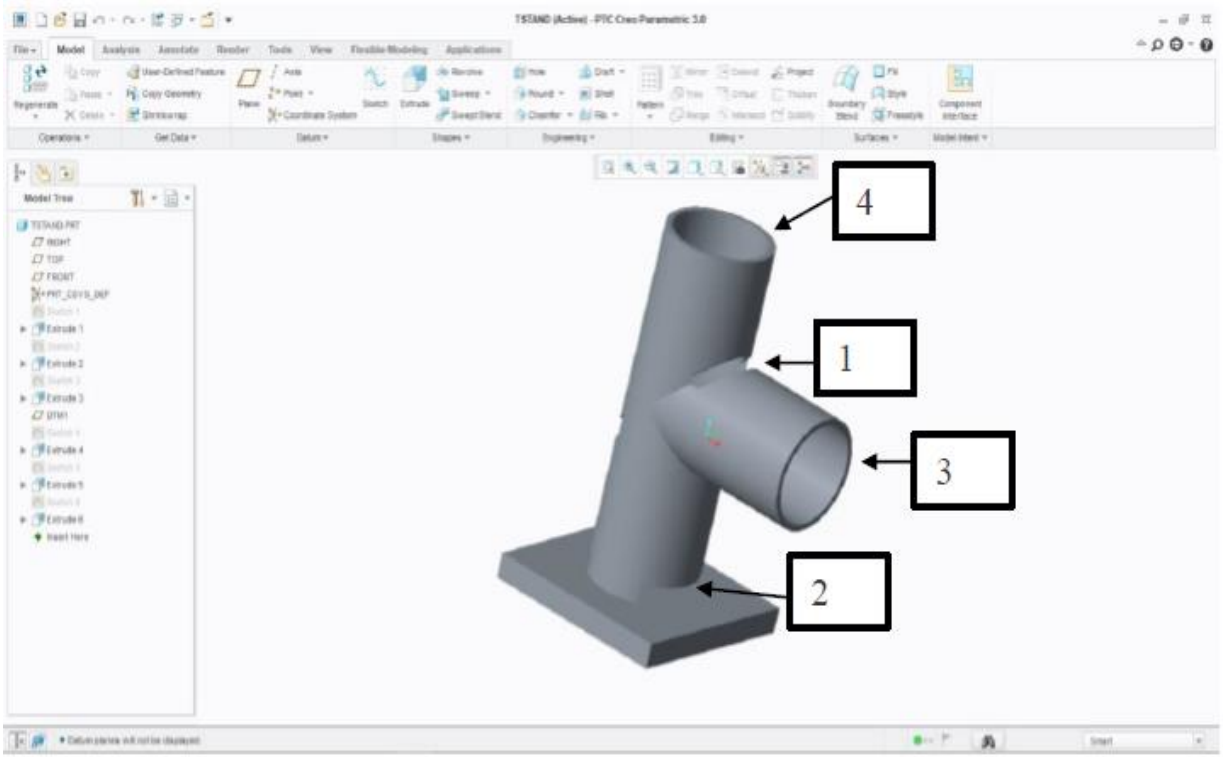
Fig 11 :- Basic of microscope

PREVIOUS DESIGN OF THE MODEL

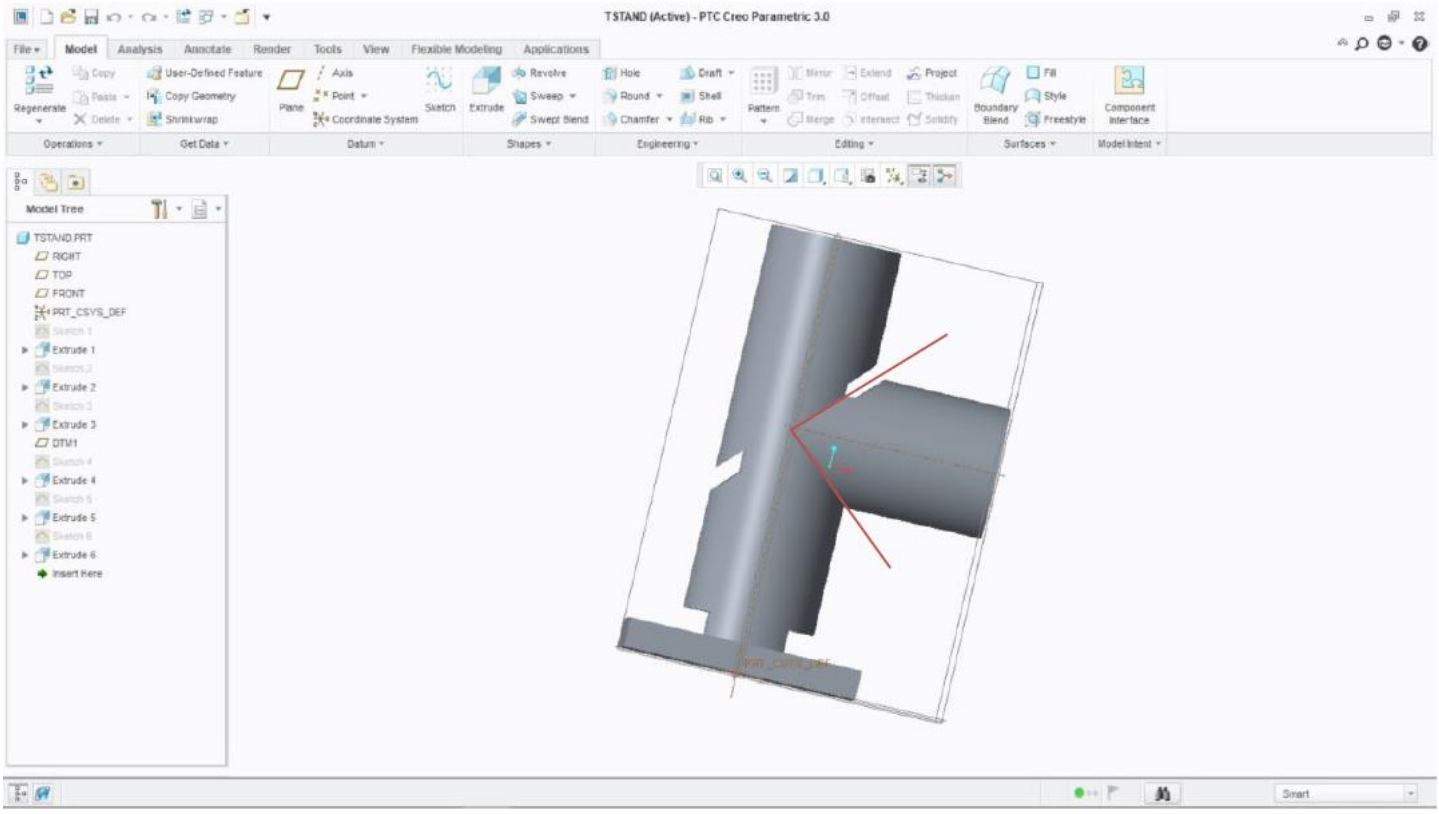
The design has been created using Creo Parametric 3.0. The design was then saved to .stl format which is readable to the 3-D printer software. The software then converts the model to a .vrml file which is readable by the 3-D printer. This file is then fed into the printer either by ethernet connection or by a USB connection.

Design has two units. 15

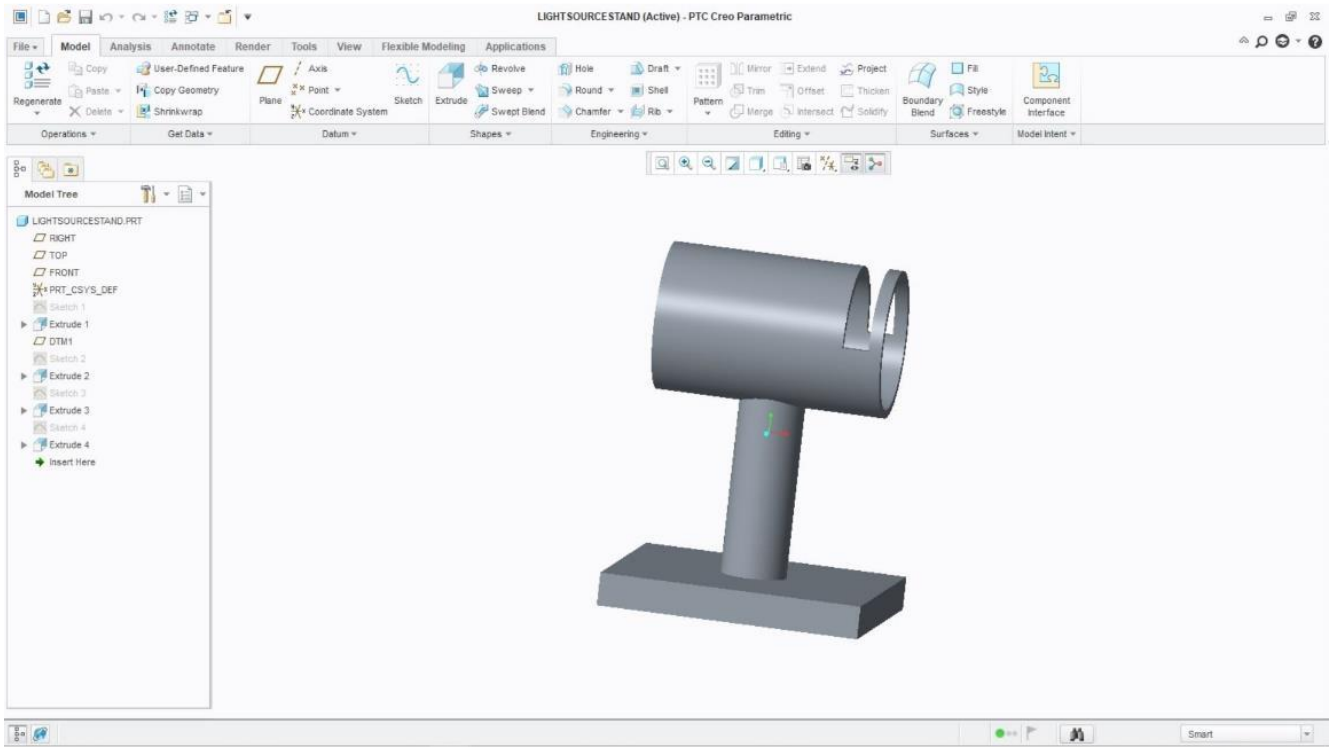
1. Main Unit – This unit houses the sample slide as well as the dichroic mirror. The dichroic mirror is housed in the opening 1 (marked as 1 in the figure) and the slide with the sample is put in the opening 2 (marked as 2 in the figure). The two orifices in the design are for input of light from the source (marked as 3) and the other is for the camera to capture the fluorescent light from the sample (marked as 4).



To reduce the amount of support material used and the time taken to produce the model, the model was cut into three pieces and then assembled after the three pieces were printed separately. The lines (red in color) in the figure depict the place where the model was cut.



2. Light Source Unit – This unit houses the light source and the monochromatic filter



GLASS SLAB HOLDER DESIGN

(All dimensions in cms)

(All design were made in CREO PARAMETRIC software)

- A typical glass slab has a dimension of: - 7.5 x 2.5 x 0.1 (Length x Breadth x Height)
- So, the appropriate dimension of a cell holder: - 8 x 3 x 1.5 (Length x Breadth x Height)
- Thickness (Breadth) of the slab holder due to boundaries: - 0.2 cms from each side = 3.4 cms overall

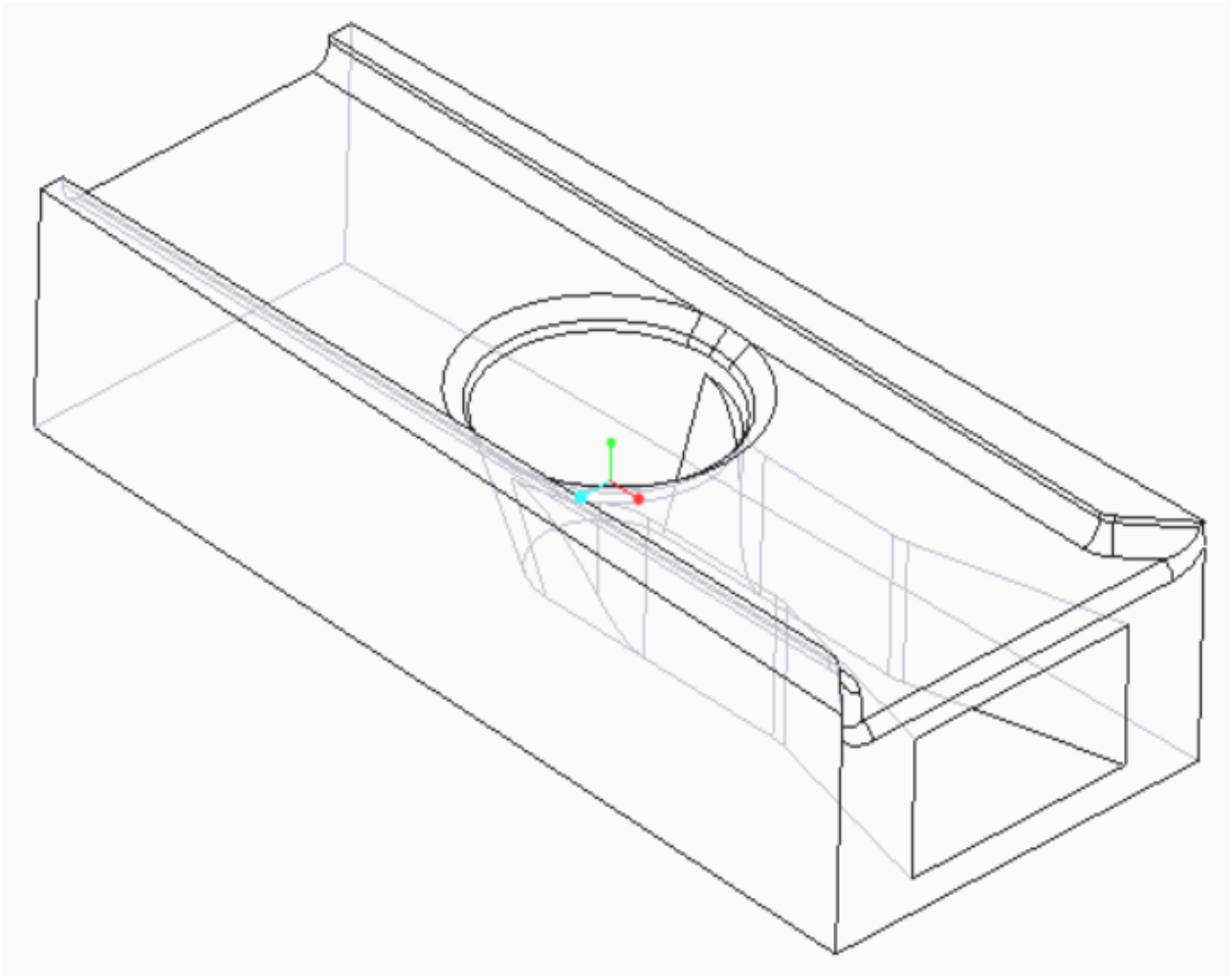


Fig 11 :- Overall design of the glass slab holder

- Front length exposed for proper insertion of the glass slab: - 0.5cms
- Upper space for light source diode emission :- 2 cms diameter with tapering of 20 leading to internal diameter of 10.9cms with the depth of funnel formed 1.25cms

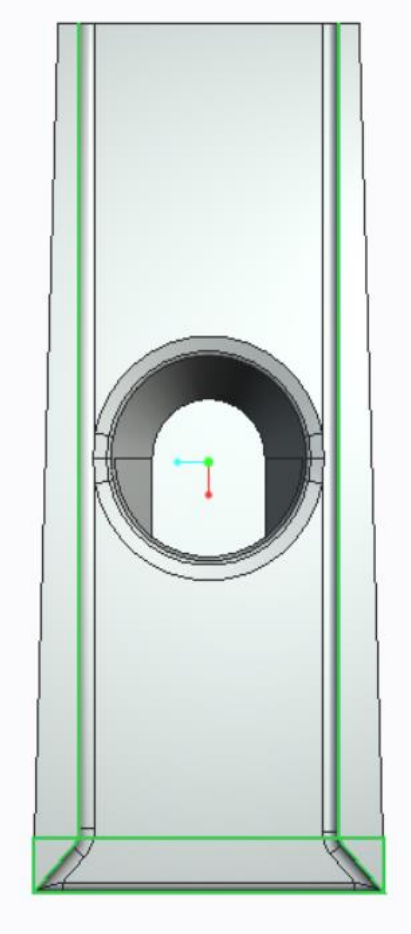


Fig 11:- Top view of glass slab holder showing exposed front length and upper space for light source

- Light source for diodes :- 2 x 1 x 4 (Length x Breadth x Height)

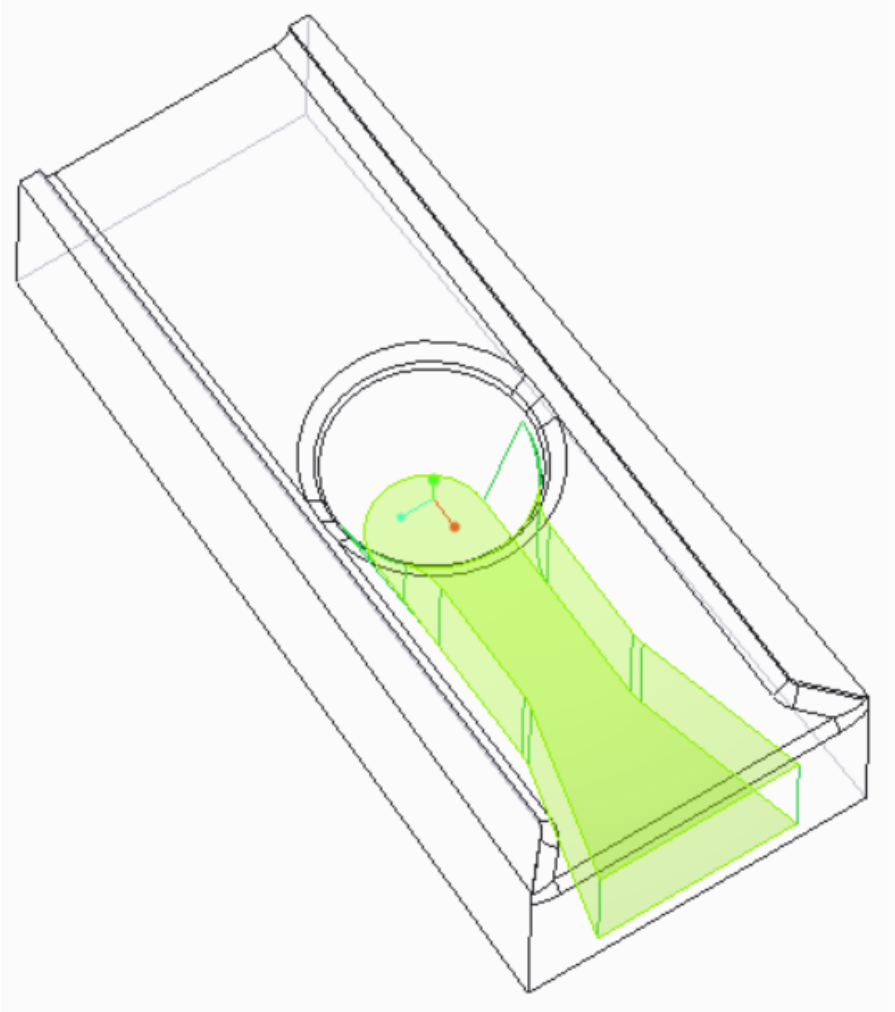


Fig 12 :- View showing the design of light source

Every edges and boundaries are given taper or made round for proper finishing look, decrement of any residual stresses on the structure and removal of any sharp edges.

MICROSCOPE TUBE

(All dimensions in cms)

- Typical dimension of an eye piece used in a microscope: - Inner diameter – 2.3, Middle diameter – 2.8 and Maximum diameter – 3.1cms. Lower length 2.3
- Thus, after obtaining the dimensions of an eye piece, the dimensions of the cylinder formed for the microscope will be :- Inner diameter – 2.45, Outer diameter – 2.9, Inner circle – 2.3(Length) 1.75(Diameter), Length of cylinder :- 16

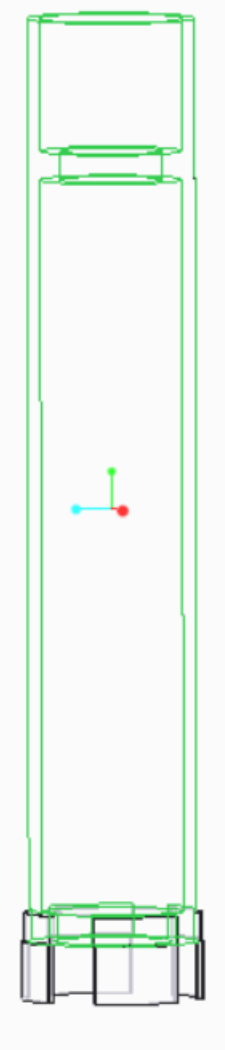


Fig 13 :- Diagram showing eye piece holder and cylinder length

- Objective lens holder in cylinder: - Threads are to be provided on the bottom part of the cylinder for attachment of the lens. Standard convention is Whitworth type of thread prescribed by Deutsche Industrial Norme (DIN). The dimensions to be considered by Royal Microscopical Society is Diameter: - 2.032 cms and pitch of 0.706. **Due to very thin boundaries of the threads, they will wear off when 3D printed.**

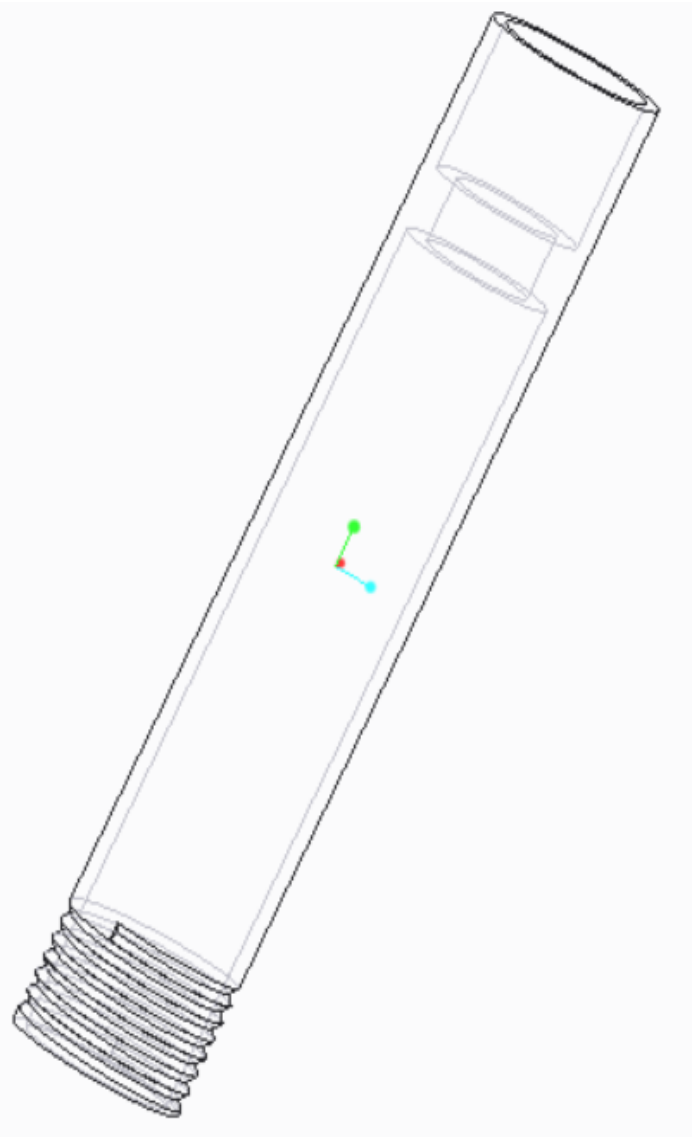


Fig 14 :- Threads on the bottom for attachment of the objective lens

- As an alternative to the threads, a separate objective lens holder was designed which will be discussed later. A separate hollow cylinder (**of 5cms in length**) was made at the bottom part of the cylinder in which the thread portion of the lens can be kept.

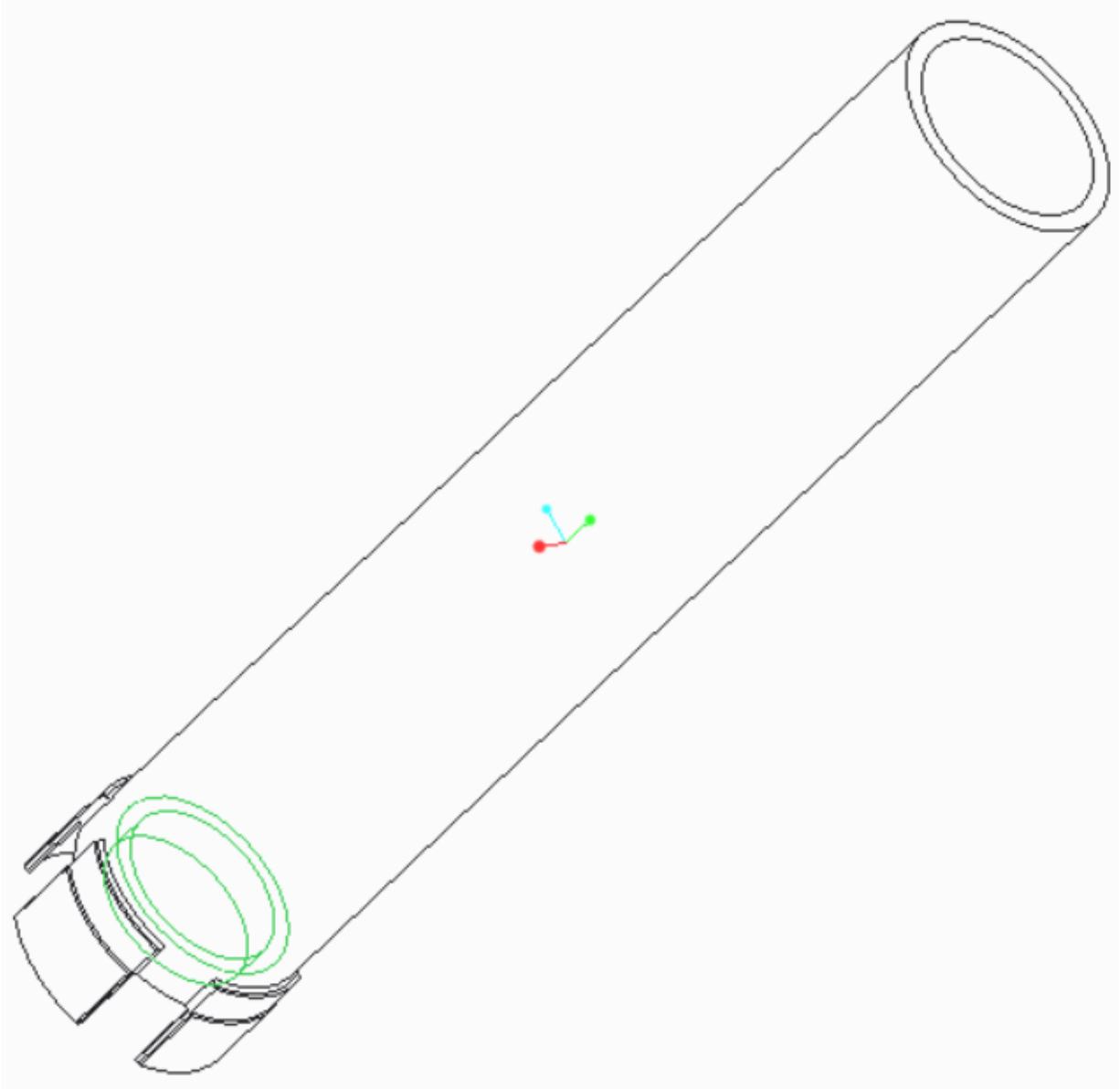


Fig 15 :- Microscope design showing bottom part hollow cylinder for objective lens thread attachement

- Furthermore, extensions (of depth 1cm and thickness of 0.1cm) were provided at the bottom part of the cylinder for the proper attachment of the objective lens holder

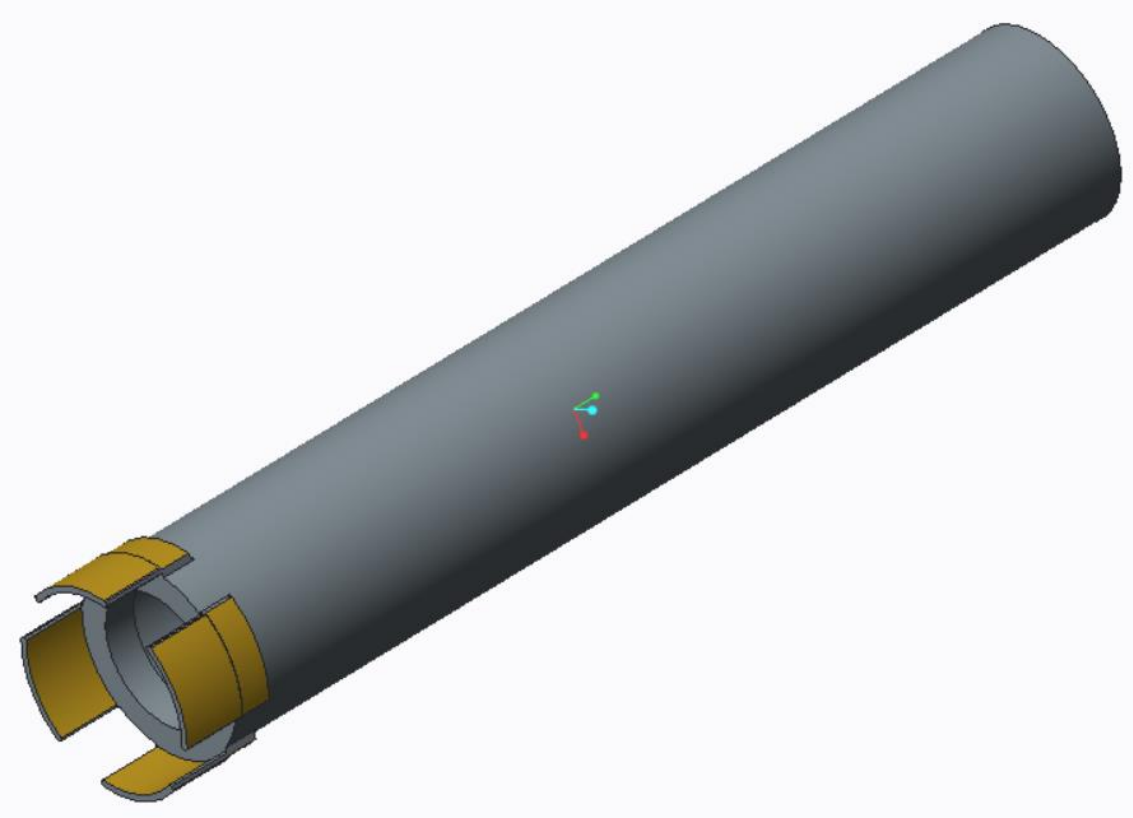


Fig 15 :- Extensions for Objective lens holder

OBJECTIVE LENS HOLDER

The objective lens holder was given the shape of a funnel (internally) for supporting the objective lens inside a cylinder having supporting legs.

Objective lens holder dimensions :-

1. Funnel outer diameter :- 2
2. Funnel inner diameter :- 0.75
3. Outermost diameter of cylinder :- 3.5
4. Second outermost circle diameter :- 2.75
5. Length of funnel :- 0.7
6. Length of cylinder :- 3

Taper given for funnel :- $\theta = 36.869^{\circ}$

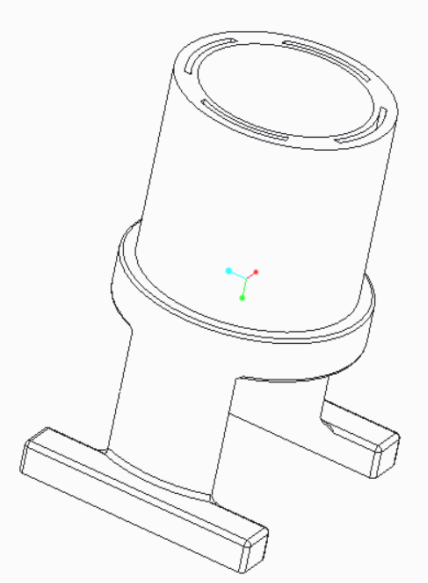


Fig 16 :- Basic representation of objective lens holder

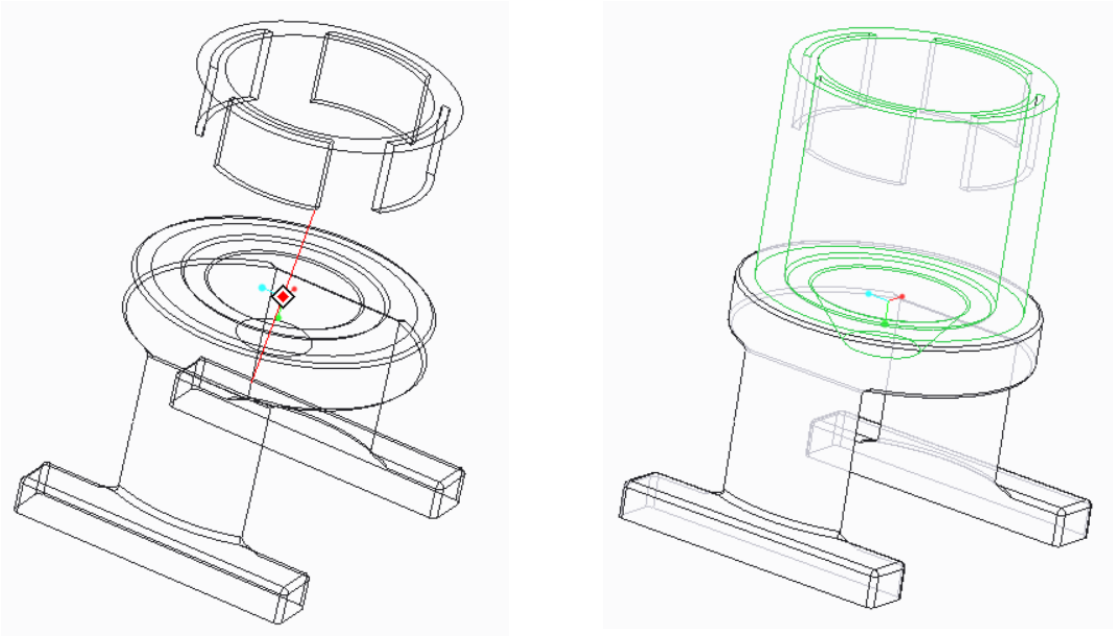


Fig 17 :- Visual representation of top part of objective lens holder

Dimensions of supporting legs :-

1. Thickness (middle most part of the half circle) :- 0.225
2. Length of the side bean :- 1.6
3. Outermost circle diameter :- 4
4. Bottom cubical dimension :- 4 x 0.725 x 5 (Length x Breadth x Height)

The distance between the objective lens bottom most point to the glass slab on the glass slab holder will be 0.5 cms.

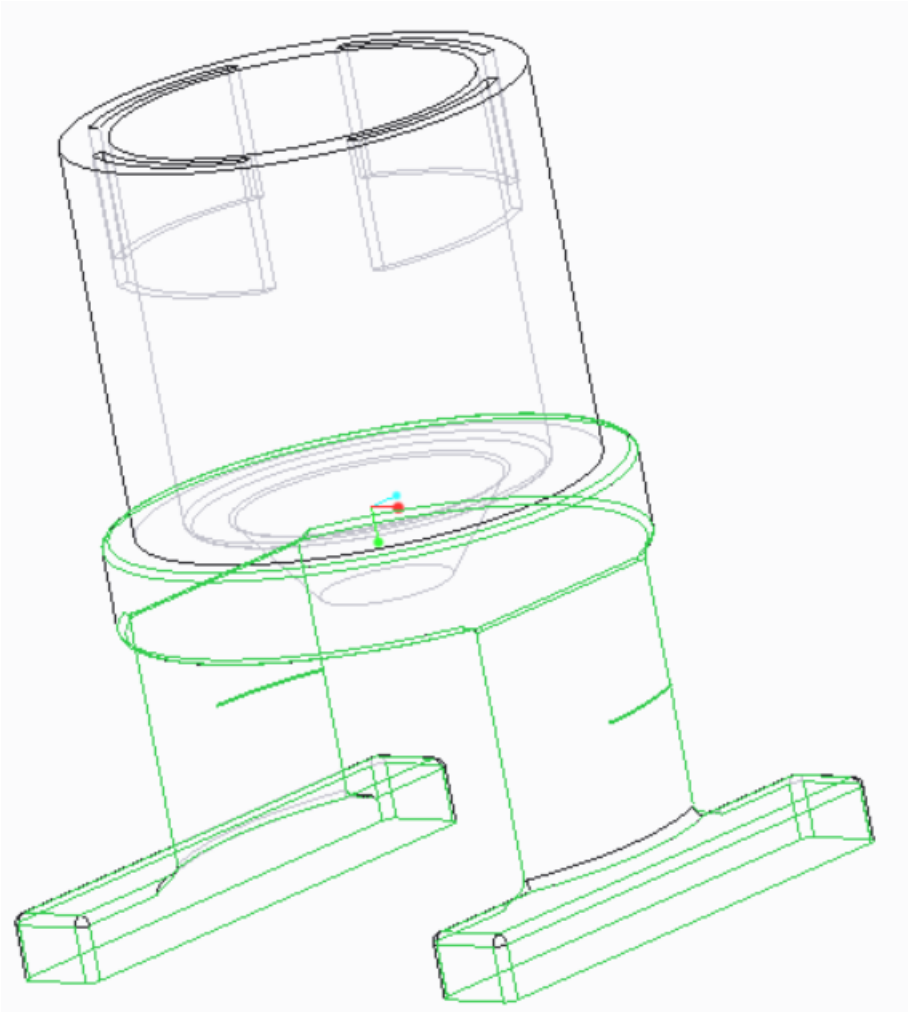
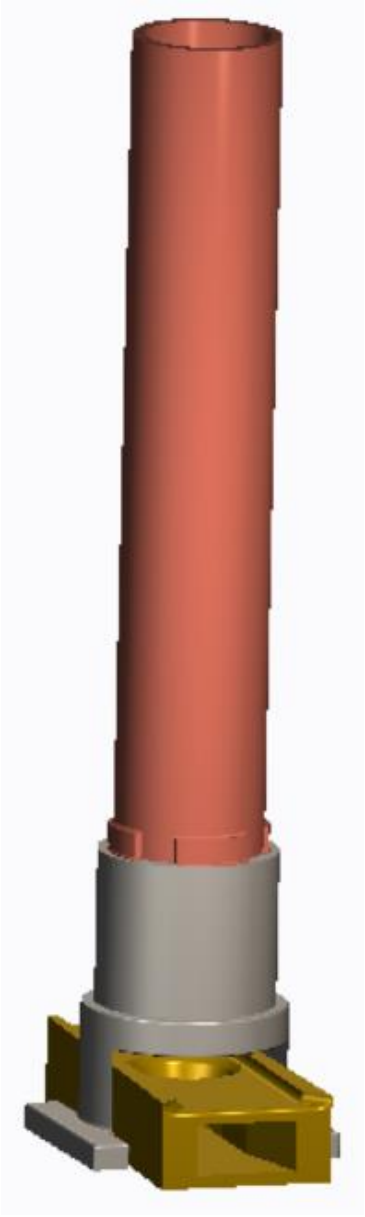
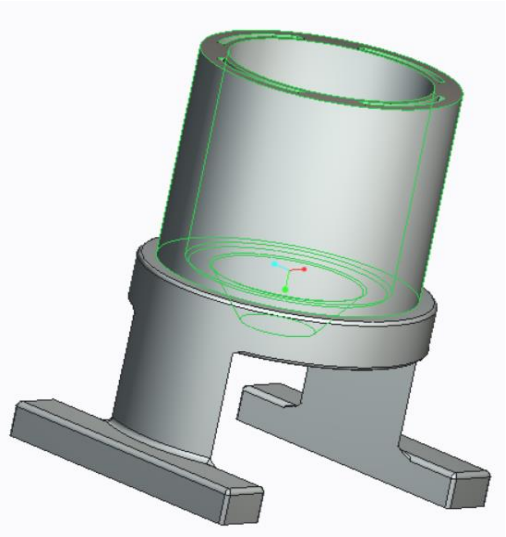
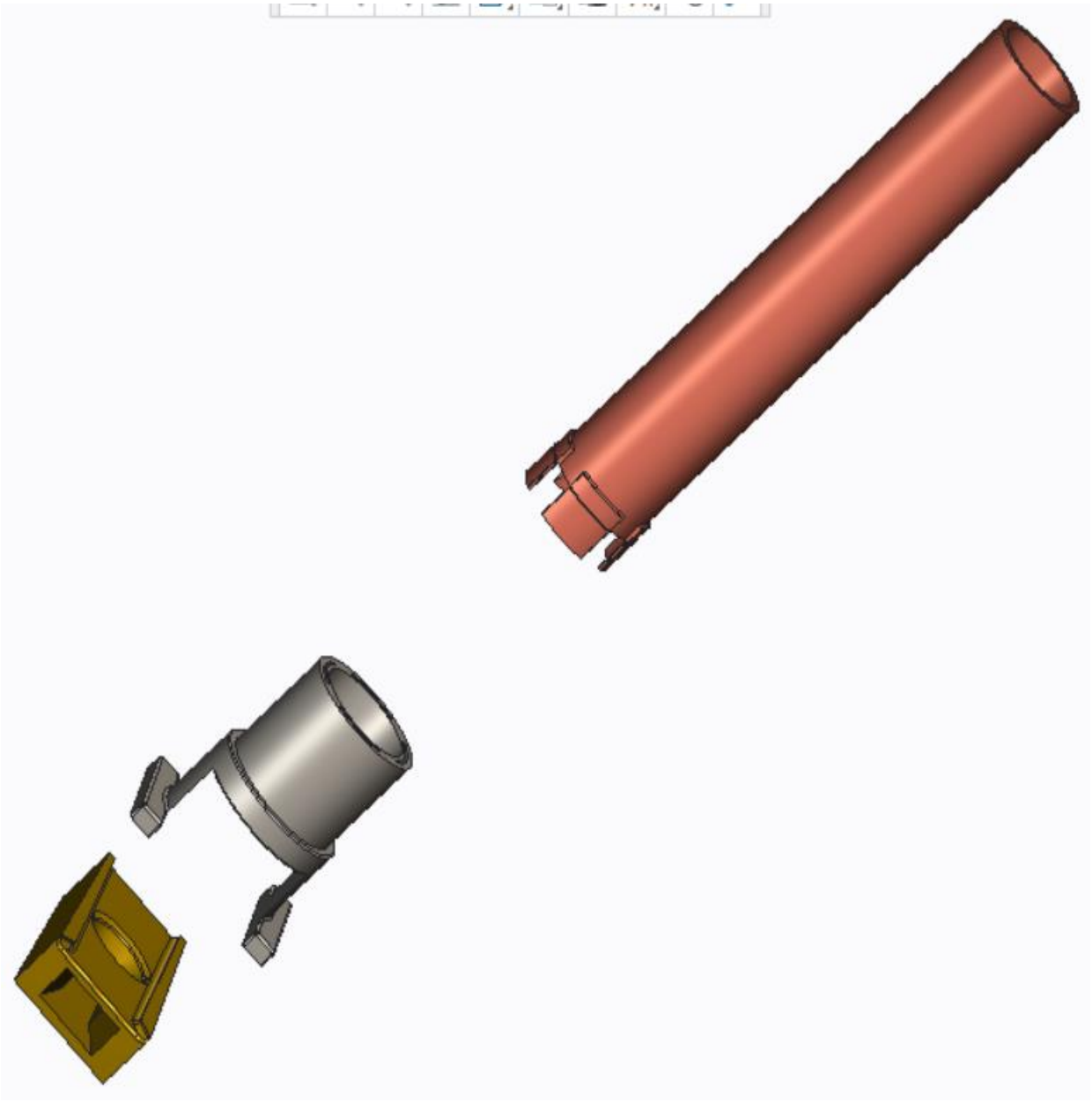


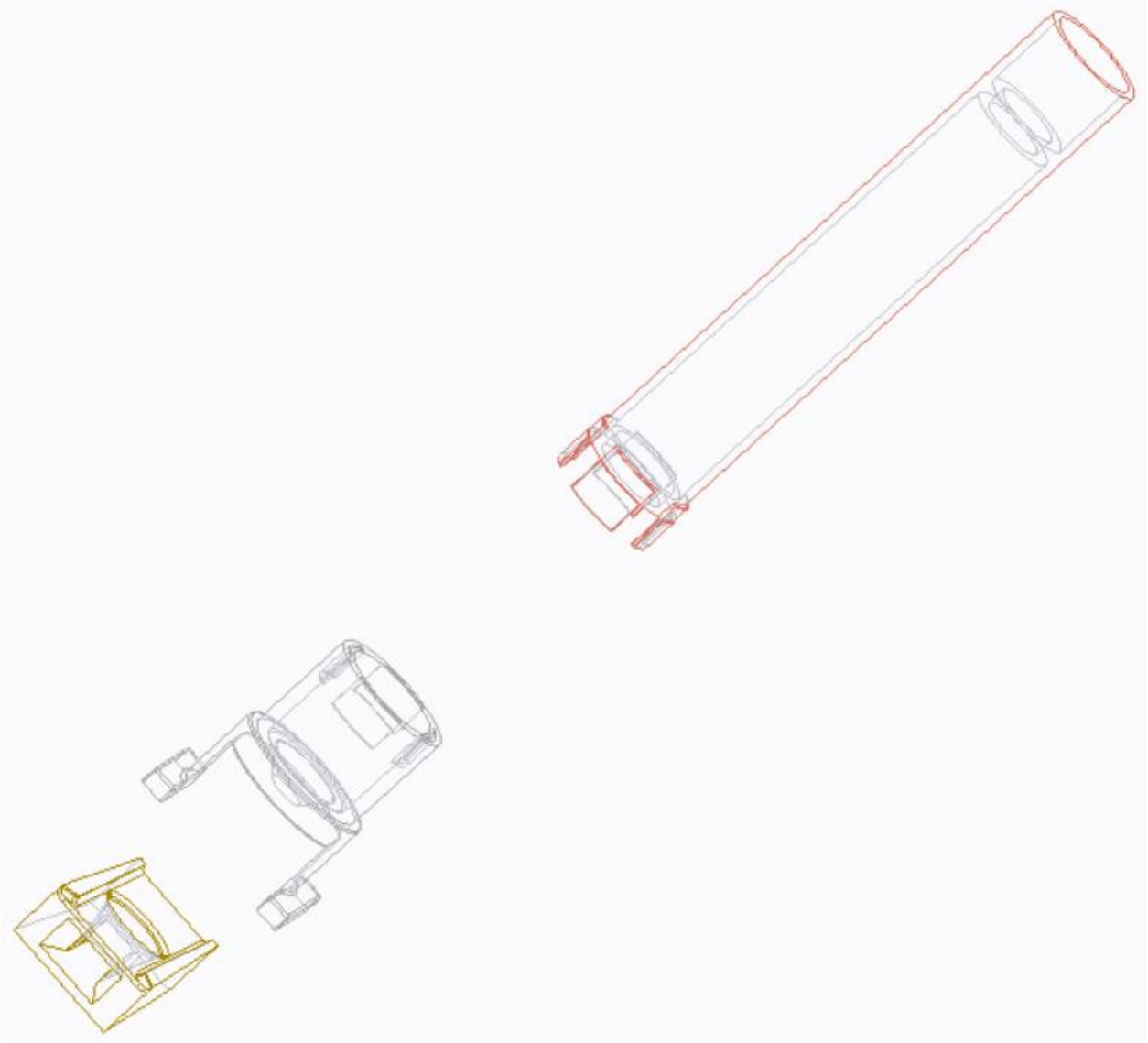
Fig 18 :- Visual representation of side supporting beams

COMBINED VISUAL REPRESENTATION OF THE MICROSCOPE









PROTOTYPING

The design needs to small, compact and easy to fabricate. There are various prototypes involved in the development stage.

1. Paper Prototype – Paper prototyping involves use of printed or hand drawn representation of the concept which makes it easier to visualize the design. This prototype is inexpensive and helps in deciding a design to fabricate. This prototype for our instrument was made using a software named Creo Parametric 3.0.

2. Functional prototype – Functional prototype helps in understanding of the functioning and the appearance of the intended design. It might be made from a different product or on a different scale altogether. This prototype for our instrument is being built using 3D printing technique with PLA material but hasn't reached completion yet.

3. Working Prototype – Working prototype is an initial product which in all sense is as good as the product in terms of functionality but might lack some finishing. This resembles the final product in the working as well the structure and the material. Our instrument hasn't yet reached the working prototype stage.

3-D PRINTING

3-D printing is a process in which material is joined or solidified under computer control to create a three-dimensional object. The material which is added is either liquid molecules or powder fused together. There are different technologies used for this process, like stereolithography (SLA) or fused deposit modeling (FDM). FDM was used for preparation of our model.

FUSED DEPOSIT MODELING (FDM)

FDM is the second most widely used for rapid prototyping throughout the world. It was patented in 1992. FDM is a solid-based prototyping method. It involves extrusion of material which is then laid down layer-by-layer to produce the final model. The system involves a spool of the material (in form of a filament), a head and a table. Filament is generally made from a thermoplastic or wax which has relatively low melting temperature. The head has a heating coil imbibed in it which melts the filament to a semi-solid state and has the ability to move in all the three dimensions to incorporate the various shapes of the models. The table is where the head extrudes the semi-solid filament which solidifies quickly in the desired shape.

Sometimes the design includes some overhangs which require support to be built upon. There are various materials which can be used for support as well but usually the support material is made from a different

material which has lower strength than the material used for the device. This aides in easy removal of the support material after the process is complete. These support structures are generally made as a thin wall section.

There are various materials which can be used for FDM process. Some of them include ABS, PLA, investment casting wax, HDPE, LLDPE, etc. PLA was used in construction of our model.

FDM has notable benefits over other rapid prototyping procedures.

1. The machine is office friendly. The machine and process doesn't release any toxic fumes. The machine is quiet as well.

2. The process is relatively fast as compared to the other prototyping process.

3. The material required for the process is very cost-effective which reduces the cost of the prototyping significantly.

4. The model produced is a finished with very little or almost no need for any finishing process.

5. Different sections of the model can be made from different colors which aide in prototyping.

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