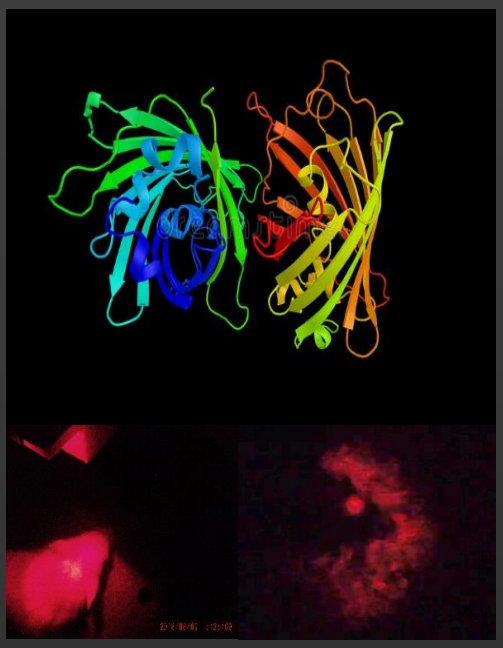

Infrared Florescence & low cost equipment

Research Report

July –August 2018

Bereket Eshete

UST GLOBAL INTERNSHIP 2018 REPORT

Intern: Bereket Eshete (Electrical engineering junior student, KAIST)

Internship period: July 3rd – August 23rd, 2018

Internship Location: IPK, Seongnam, South Korea

Plan after the internship

Return to college to finish my bachelor's degree

Impressions

I enjoyed the internship and grasped important knowledge and experience.

Infrared Florescence

&

Low Cost Imaging Equipment

August 2018

Advisor: Prof. Regis Grailhe

Mentor: Adrien Mesnard

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Introduction

This research report introduces “Infrared florescence & low cost imaging equipment”. In this research, we will see how we can use the current advanced technology to apply to biology alternatively known as biomedical engineering. We will use infrared fluorescence exhibited by special proteins to glow areas of an organism and use the glowing response of these proteins to form a comprehensive image of a specific part of an organism. In addition, we will see how we can use 3D-priting technology for constructing low cost imaging apparatus using infrared fluorescence concept.

KEY WORDS

- Infrared Florescence

- Low cost imaging instrument

- Modeling low cost equipment for fluorescence imaging

- Biomedical Engineering

CONTENTS AND RESULTS OF RESEARCH DURING THE INTERNSHIP

-Flash light

-low cost instrument

-Images from cameras

Infrared Fluorescence

Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation. It is a form of luminescence. In most cases, the emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation.

Optical imaging using near-infrared fluorescence (NIR) light is a new imaging modality (medical imaging) that has recently emerged in the field of cancer imaging. This technique promises high sensitivity and can be used to image a wide variety of molecular entities in vivo, through the promise of versatile fluorescent probe design. The use of near-infrared wavelengths for imaging permits relatively deep photon penetration into tissue, minimal tissue auto fluorescence, less scatter, and higher optical contrast when exogenous NIR fluorophores are introduced. It is of great importance for translational research as future cancer surgery relies on optical imaging during surgery. Larger depth can be probed infrared or near infrared spectral region, as the absorption is as at least one order of magnitude lower than in the visible range. NIR light (700 –1000 nm wavelength) can penetrate several centimeters into tissue. Infrared and NIR imaging auto fluorescence is much lower because tissues do not auto fluoresce under longer wavelength (high energy) light.

In most applications, NIR optical imaging is used together with molecularly targeted fluorescent contrast agents that not only provide enhanced contrast but also, more importantly, reveal specific molecular events associated with cancer formation and progression. In vivo fluorescence imaging with near-infrared light holds enormous potential for a wide variety of molecular diagnostic and therapeutic applications.

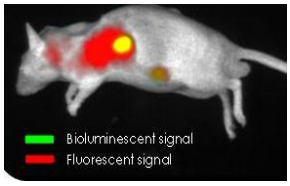


Figure a. NIR fluorescent and bioluminescent signal. Nude mouse with intracardiac injection of PC3 cells (prostate cancer). IV injection of 9 nmol IR780 (0,2mg/kg). Imaging took 24h post injection – signal in the heart. A metastasis can be seen in the intestines on the left side of the mouse. Courtesy of UROLEAD, France

Another application of Near-infrared fluorescence is in the imaging of human lymphatics, which utilizes laser illumination of intradermal injected fluorescent dye.

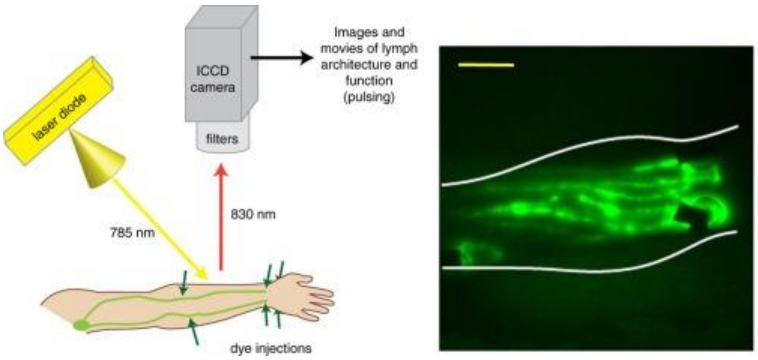
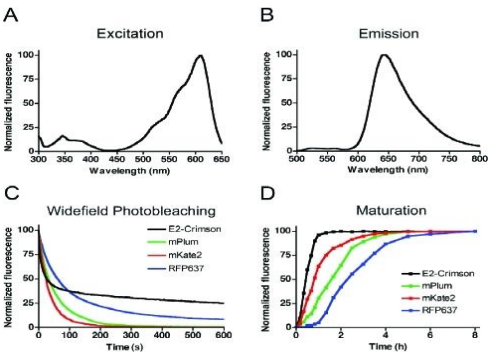


Figure b. (left) fluorophore emissions are captured by an imaging system and ICCD (intensified charge-coupled device) camera, producing real-time images and movies of lymphatic vessel architecture and function, such as the image (right) of ventral forearm lymphatic vessels.

For our experiment, we use E2-crimson protein as infrared fluorescent protein; this protein is in vivo injected in to the DNA of mice. The mice then start producing E2-Crimson from the injected DNA sequence. E2-Crimson is a basic (constitutively fluorescent) far-red fluorescent protein derived from Discosoma sp.. It is reported to be a rapidly maturing protein with low acid sensitivity.



Notes

Researching on laboratory mouse may require permission from the government depending on the country. Make sure you have permission to do so before conducting the experiment.

Figure c. Fluorescence properties of E2-Crimson

Reference

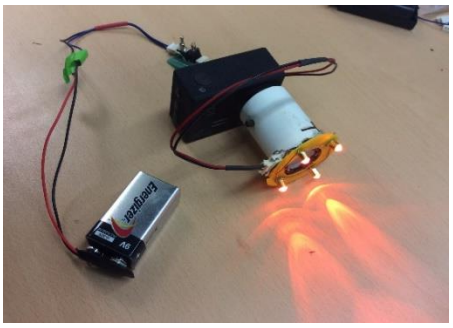
http://www.biospacelab.com/m-107-near-infrared-fluorescence-imaging.html

https://www.researchgate.net/figure/Near-infrared-fluorescence-imaging-of-human-lymphatics-utilizes-laser-illumination-of_fig2_224934183

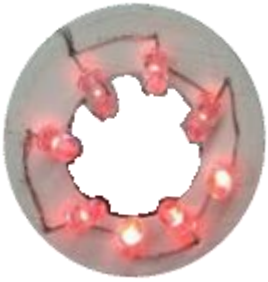
https://www.fpbase.org/protein/e2-crimson/

Flash light and Ring flash light

- 2x2 matrix 610 nm LED flash Light
- Body part made from 3D printing PLA material

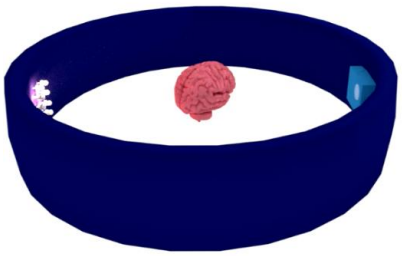


- Four 610 nm LED in parallel
- < 100 ohm resistor in series
- Intended for small camera illumination

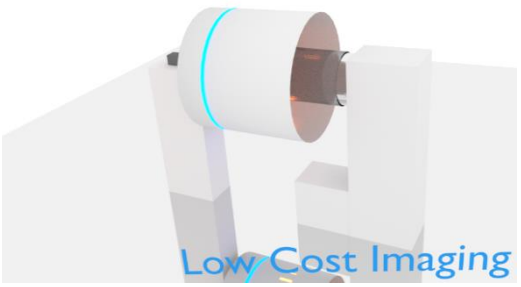


- Eight 631 nm LED in parallel
- < 50 ohm in series
- Intended for bigger camera illumination

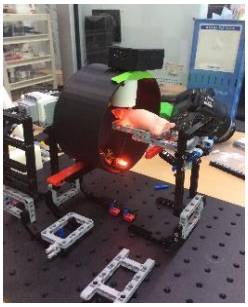
Low cost Instrument



- 3D model for infrared fluorescence image capturing
- Brain image captured at opposite side of the camera



- Prototype for capturing mice brain image
- Low cost imaging instrument



- Low cost Imaging device
- Made of 3D printed PLA material

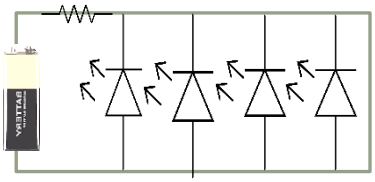
Experiment, Results And Discussion

Experiment 1. Building light source and measuring spectrum of LED

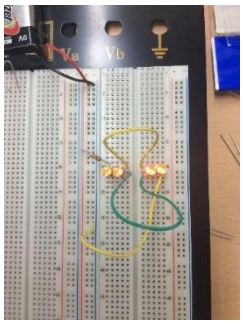
> **Notes**
> Experiment 1 can be skipped if you have good understanding on introduction to circuits LAB in college course

Figure 1.1. Experiment setup

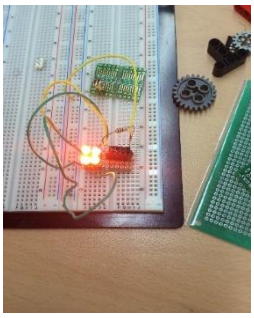
For our first and simple experiment, we start by designing the circuit and mounting the components on a breadboard. For this experiment, we use 610 nm and 631 nm LED (Thor labs)



(a)



(b)



(c)

(d)

Figure 1.2. Experiment procedure, (d) shows our outcome (2x2-610nm LED matrix light source)

We can obtain the spectrum of the LED lights from their data sheets, however to get accurate and reliable measurements. We would like to measure the spectrum of the LED's ourselves and confirm with the data sheet spectrum. For this experiment, we measure the spectrum of 610nm LED; however, you should get similar result with 631nm LED's.

For measuring the spectrum of LED's, we use an equipment called "my spectral lumini". The spectrometer operates by plugging it in to a PC using USB cable and using a dedicated PC application to display the result. (Visit https://myspectral.com/ for more info).

Figure 1.2. “myspectral” spectrometer. This specific spectrometer measures reflected spectrum from four spectrally balanced surfaces.

Now we proceed to measure spectrum of 610 nm LED as shown in the figure below.

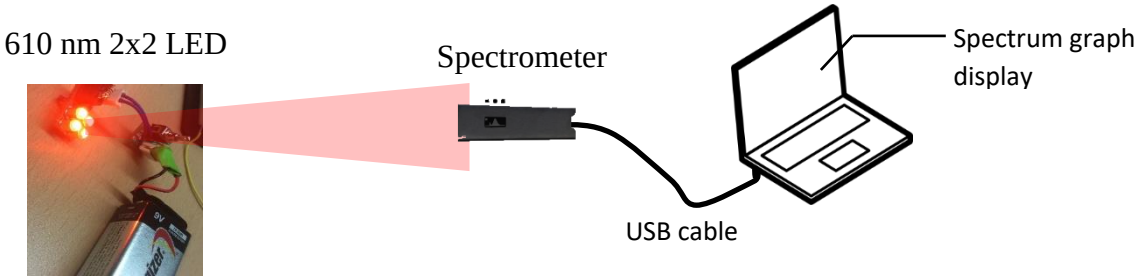


Figure 1.3. Measuring the spectrum of 610nm LED

> **Notes**
> There are many other several methods for measuring spectra’s of LED’s, however we find this method to be the most convenient for this experiment.

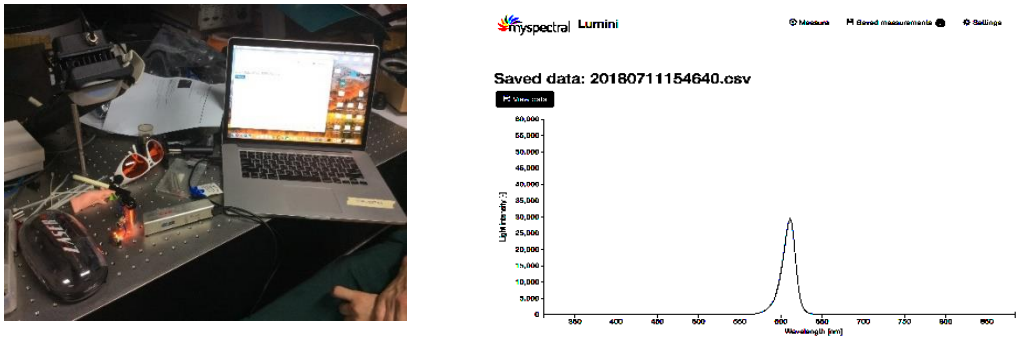


Figure 1.4. Measuring the spectrum of 610nm LED, the right picture shows the spectra graph of 610 nm LED which is Gaussian centered at 610nm similar to the it’s data sheet. Hence, we are now able to use the LED’s with confidence knowing its spectra. This light will later be used as excitation trigger for the crimson.

Experiment 2. Building flashlight

Notes

Experiment 2 requires intermediate background in 3D modeling. It is recommended to practice with thinkercad before diving into modeling of equipment parts.

Figure 2.1. Experiment setup

This flashlight will be used in a coming experiment as a light source to excite the fluorescent protein in a mouse brain. For building the flashlight, we produce the hard box material utilizing the latest 3D-priting technology. Specifically we use “ultimaker 3”; however, other 3D-printers should also yield similar result. For this experiment, we use mainly “thinkercad” for the 3D modeling, in addition we also use blender for rendering materials, which could not be rendered in thinkercad.

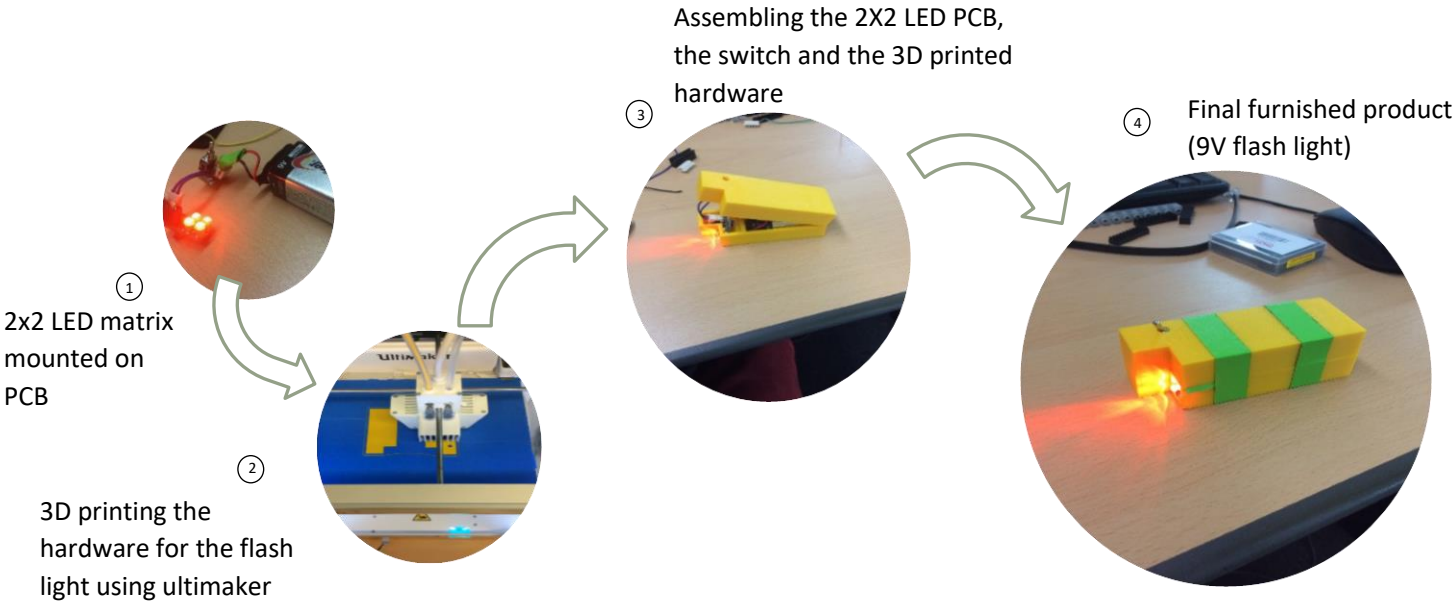


Figure 2.2. Flashlight making process

Experiment 3. Low cost imaging instrument

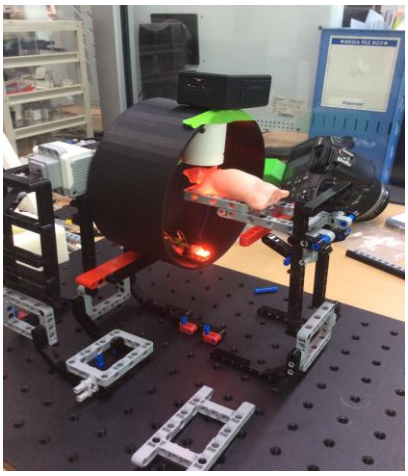


Figure 3.1. Experiment setup

> **Notes**
>
> Experiment 3 may be skipped if you are confident your instrument is ready to test it with living mouse.

The purpose of this experiment is to achieve a 3D scan of mouse brain using infrared fluorescence. However, in this experiment we will be testing the instrument with artificial mouse (phantom) and with a living mouse on the last experiment. The phantom shares similar property with a living mouse and could be used as a substitute for experiment trials. In this experiment, we record a live video 360^0 of the mouse brain and utilize special software to reconstruct a 3D image of the mouse brain.

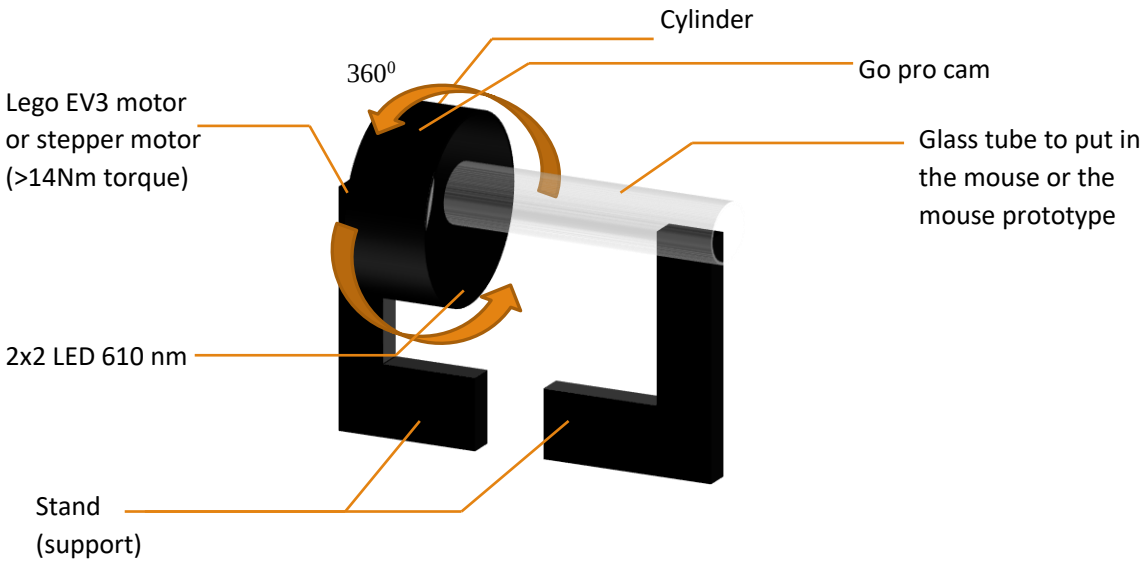


Figure 3.2. Low cost imaging instrument

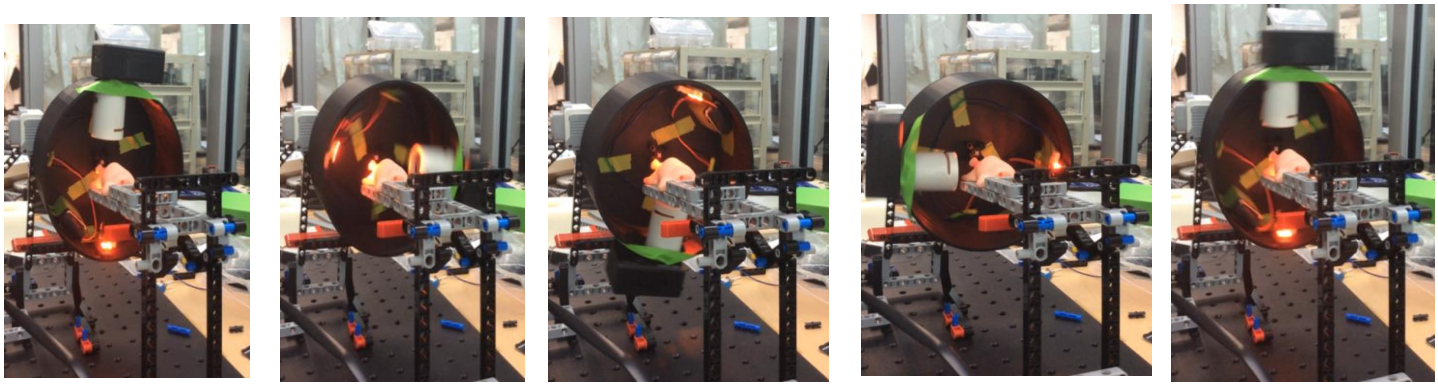


Figure 3.3. Low cost instrument imaging scanning the phantom by rotating the cylinder full cycle.

Figure 3.4 Sample image from the 360^0 video recording of the Go pro of the phantom. (Middle) no crimson and (right) with crimson. (Left) it shows the experiment is carried out in dark condition.

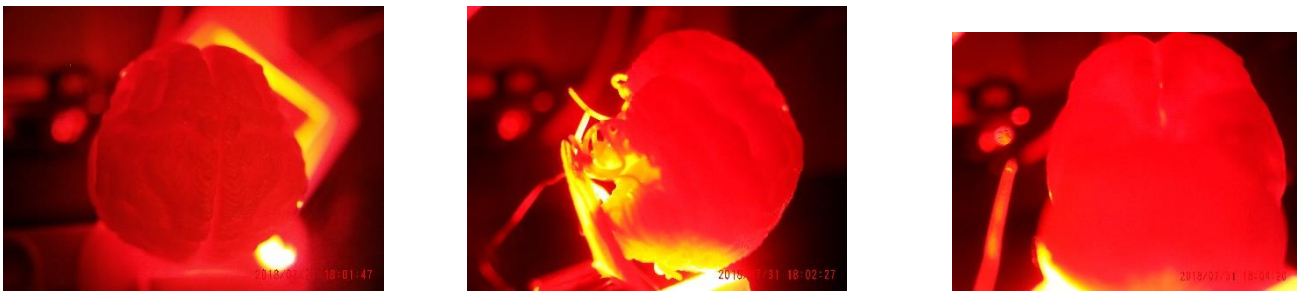


Figure 3.5. Sample images of 3D printed prototype mouse brain from the 360^0 video recording of the Go pro.

Experiment 4. Building a ring flashlight

Notes

The PCB we use here are 3D printed, however flexible PCB are more suitable to make ring flashlight.

Figure 4.1. Experiment setup

In this experiment, we will build a ring flashlight. The ring flashlight can replace the job of flash light. We will attach the ring flash light to the camera; hence, we will be no more needing flashlight to excite crimson protein.

4-pair pin hole

8-pair pin hole

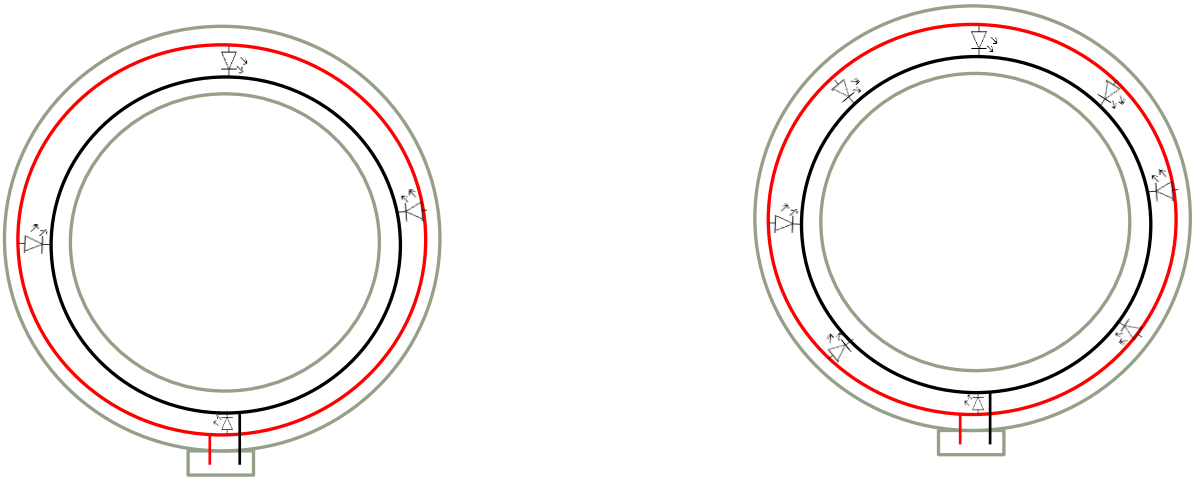


Figure 4.2. Schematic diagram for ring flash light

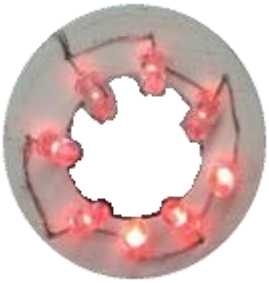


Figure 4.3. 8-pair pin hole ring flash light, 9 LED's in parallel, 9V and 50 ohm in series.

Ring flashlight

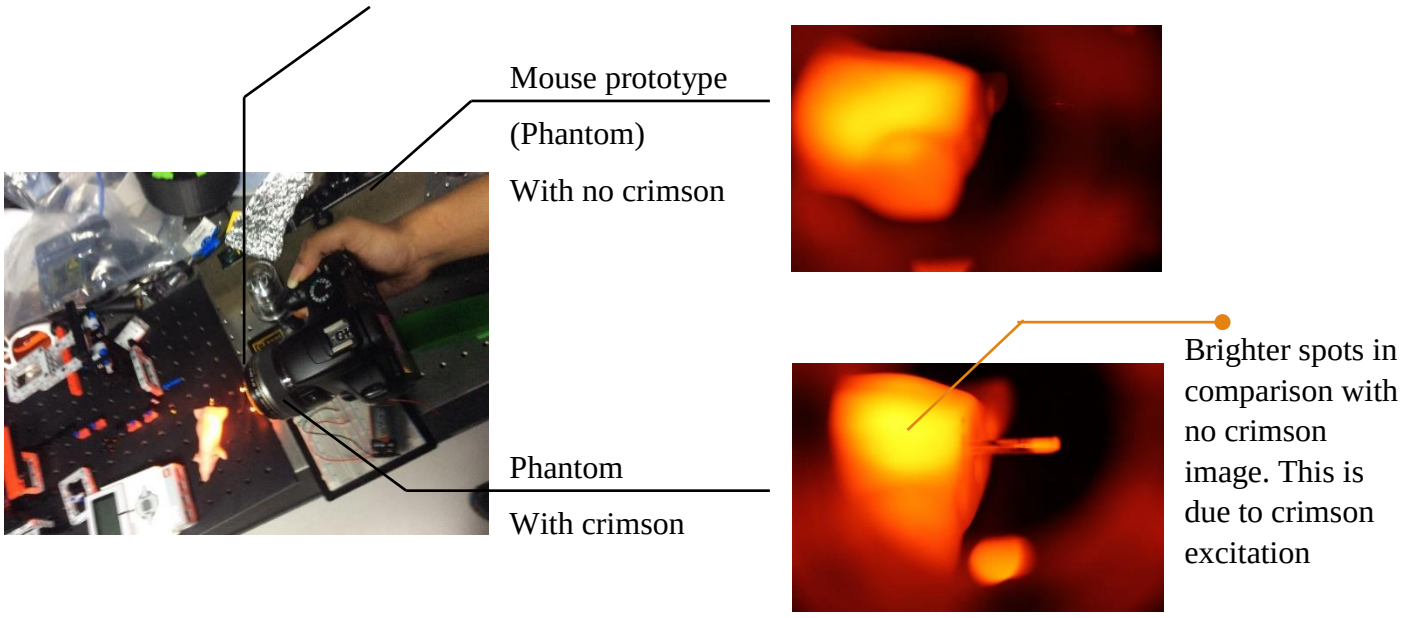


Figure 4.4. Testing the performance of ring flashlight on mouse prototype (Phantom)

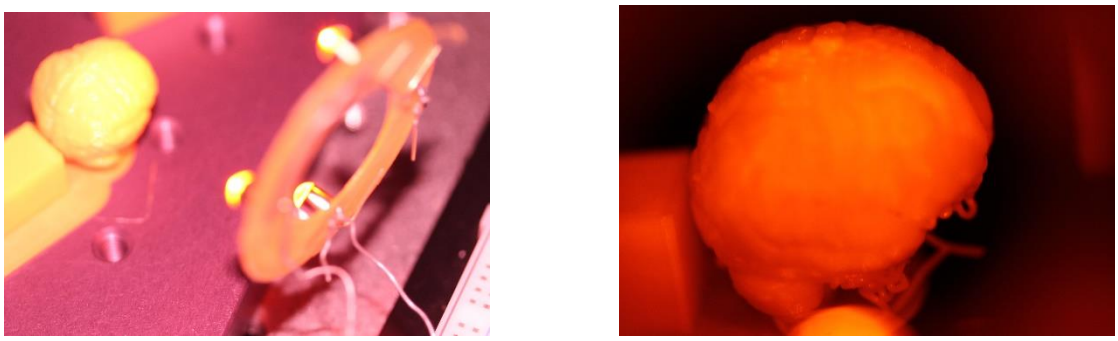


Figure 4.5. Testing the performance of the ring flashlight with 3D printed mouse size brain.

Experiment 5. Experiment with transgenic mouse

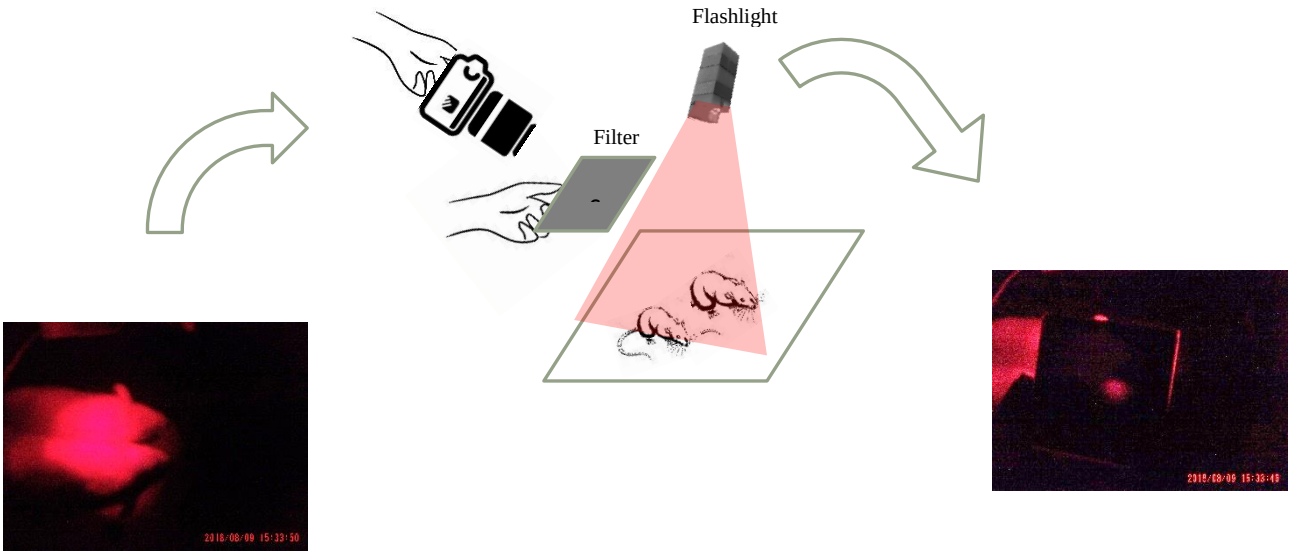
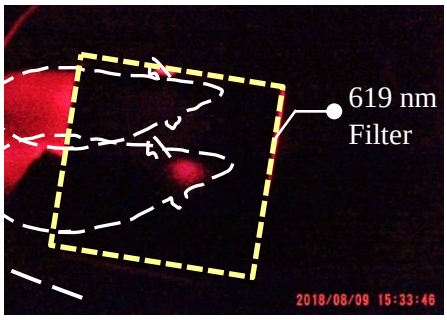


Figure 5.1. Experiment setup. A pair of transgenic (mouse injected with DNA sequence to produce E2-crimson protein) and non-transgenic mouse (normal mouse, control variable) being seen under a 619nm low wavelength pass filter.



Notes

The duration for this experiment is usually less than 5.minutes since the mice wake up from sleep in short time.

Figure 5.2. A pair of transgenic and non-transgenic mouse being seen under a 619 nm low wavelength pass filter and a cannon 110D camera. The transgenic mouse that produces crimson can be seen glowing red in the brain area, while nothing glowing is observed on the non-transgenic mouse.

In this experiment, we use laboratory mouse to demonstrate infrared fluorescence. For our experiment, we use laboratory mouse that are kept in animal and control lab facility. In doing this experiment, it is recommended to shave mouse's hair to avoid absorption of the light source.

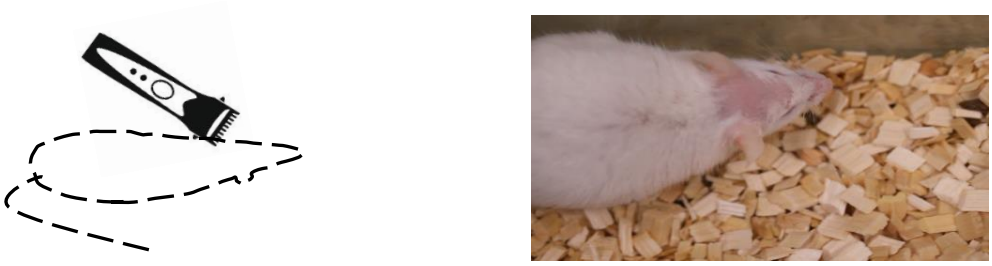


Figure 5.3. This figure shows mouse that are shaved to counter the effect of light absorption.

In addition, it is hard to carry out the experiment while the mice are running around. Hence, it is recommended to put the mouse inside anesthesia chamber for 7 minutes before carrying out the experiment.

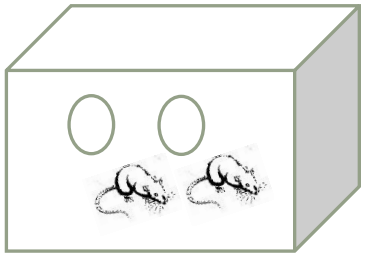


Figure 5.4. Mice are put inside anesthesia chamber to put them to sleep before experiment.

Experiment 6. 3D scan of mouse brain

This is similar to experiment 2, except that instead of a prototype mouse we will use living mouse.

(ON progress)

Summary

- E2-Crimson is excited when illuminated by 610 nm LED flash light. This excitation is recorded on a camera using low wavelength pass filter.
- A transgenic mouse that produces crimson can be glowing red in the brain area under infrared fluorescence, while nothing glowing is observed in non-transgenic mouse.
- Low cost imaging instrument can be built for in vivo infrared fluorescence using tools such as 3D printing and specific LEDs that match the desired excitation wavelength.
- Improving infrared fluorescence can help cancer patients be diagnosed easily at early stage without surgery, which could help save lives of thousands of people.