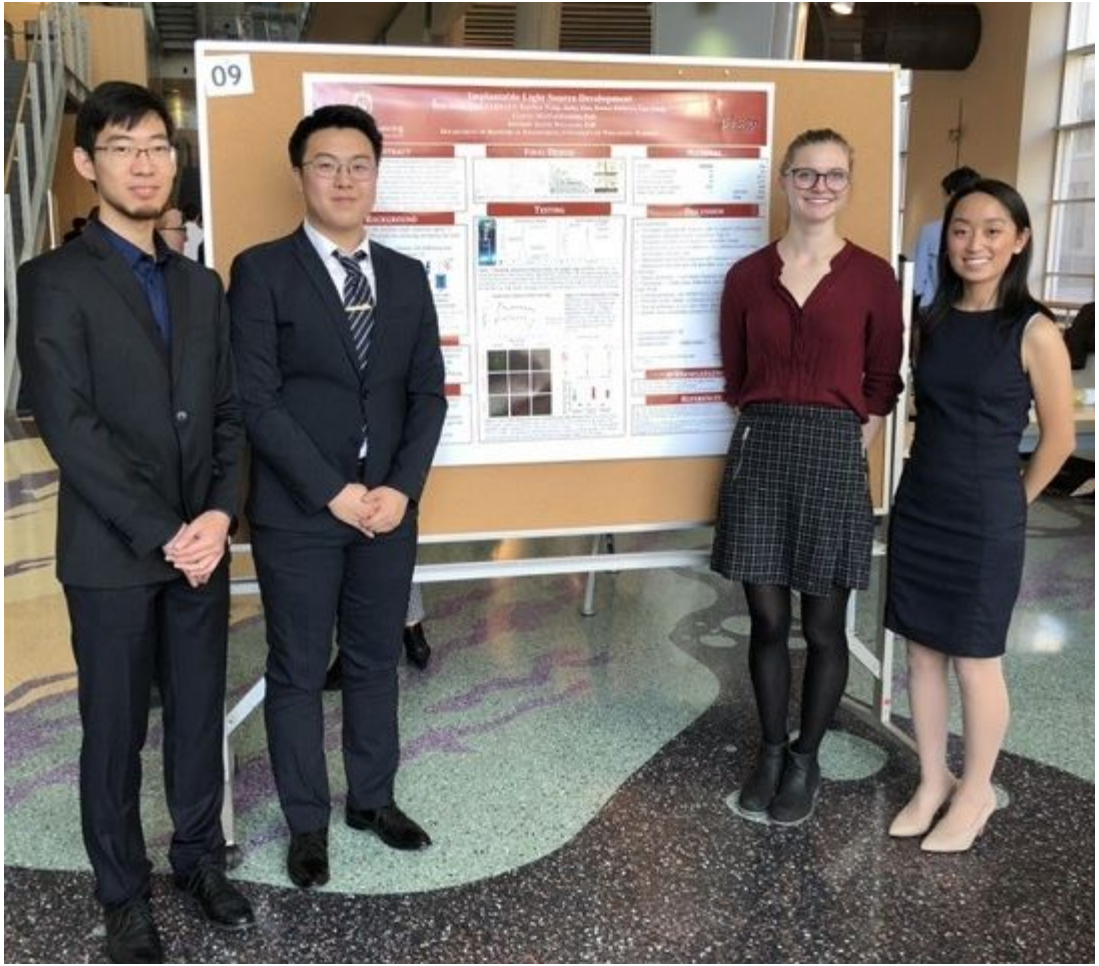
Implantable Light Source

Advisor: Justin Williams, PhD
Client: Matyas Sandor, PhD
Team members: Jacky Tian, Ruochen Wang, Lisa Xiong, and Hanna Rainiero

The Team

Team members from left to right:

- Ruochen Wang (Team Leader)
- Jacky Tian (BSAC & BPAG)
- Hanna Rainiero (Communicator)
- Lisa Xiong (BWIG)



Overview

- Client
- Problem Statement
- Broad Impact
- Design Constraints
- Final Prototype
- Testing
- Future Testing and Analysis
- Final Prototype
- Budget
- Acknowledgements
- References

Client: Dr. Matyas Sandor

- Professor in the Department of Pathology and Laboratory Medicine
- Research on immune response to infectious diseases
- Our projects focus on the creation of implantable light sources for use in tuberculosis and immune response in CNS.

Problem Statement

- Need an efficient, safe, cost-effective method to photoactivate or photoconvert cells *in vivo*
- Current method uses a fiber optic laser (Fig. 1) which has low photoconverting efficiency and too high intensity (Fig 2.)

Fig. 1: Current method of photoconversion uses glass needle with laser attachment.

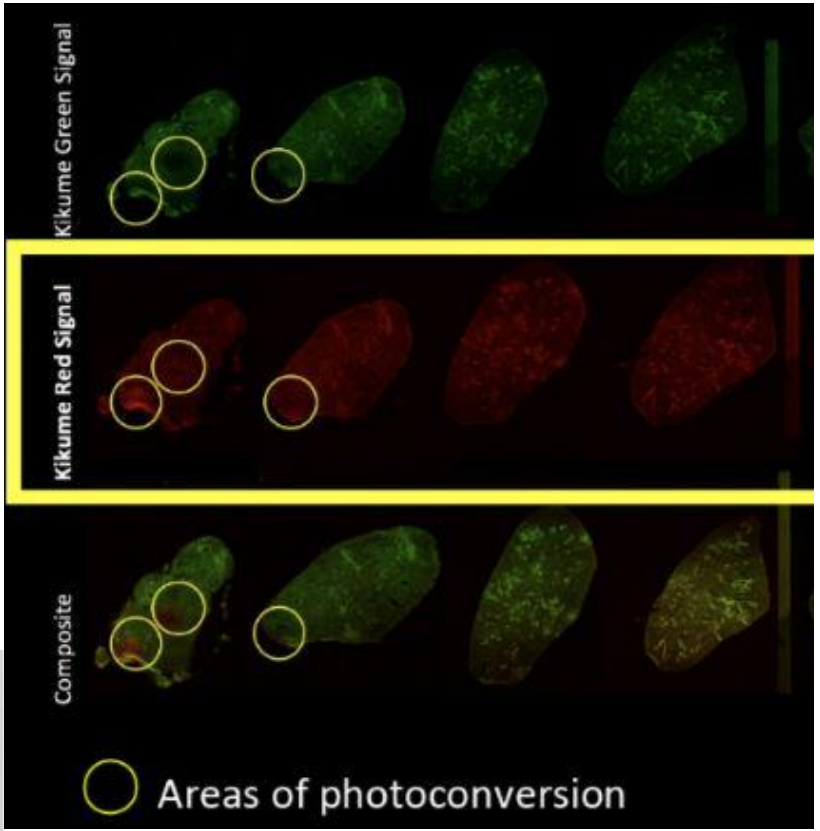


Fig. 2: Photoconverted regions in yellow circles. Notice small area relative to entire lung.

Fiber Optic Light Used

- Light source: ~1000mW 405nm light
- Fiber optic cable adjustments already made
  - Increased Conversion Area
  - Increased output intensity
- Still does not meet client's needs

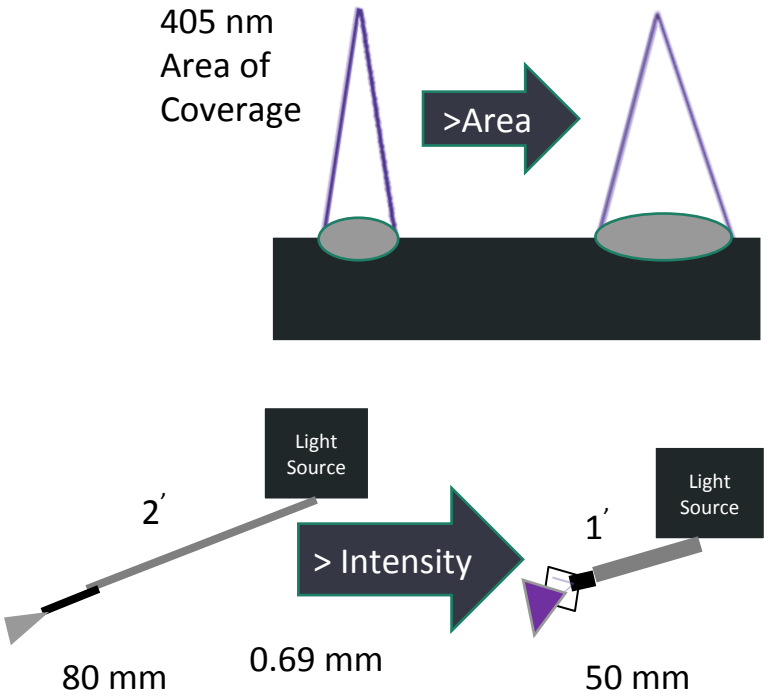


Fig. 3: Previous fiber optic light source testing protocol.

Broad impact

- Tuberculosis (TB) is the deadliest single infectious agent [1]
- Antibiotic resistant TB strains are increasing ➔ need for alternative therapies [1]
- Immune cell manipulation ➔ novel therapies for CNS inflammatory disease [2]
- LEDs > fiber optics for *in vivo* use

Key Design Specifications

Table 1: Design Specifications for the KikGR33 and Ai32 experiments.

	Photoconversion	Photoactivation
Wavelength	405 nm	450 - 490 nm
Intensity	95 mW/cm^2	400 mW/cm^2
Size	1 cm^2	1 cm^2
Heat Output	< 2℃ locally < 1℃ systemically	< 2℃ locally < 1℃ systemically

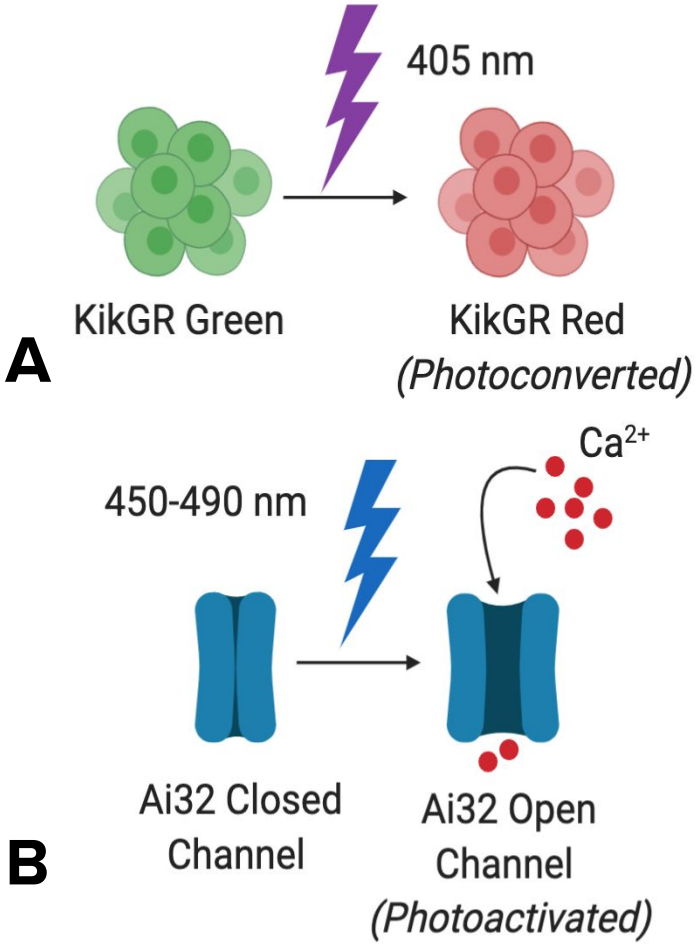


Fig. 4: Wavelength requirements for transgenic mouse models. (Biorender)

Previous Prototype

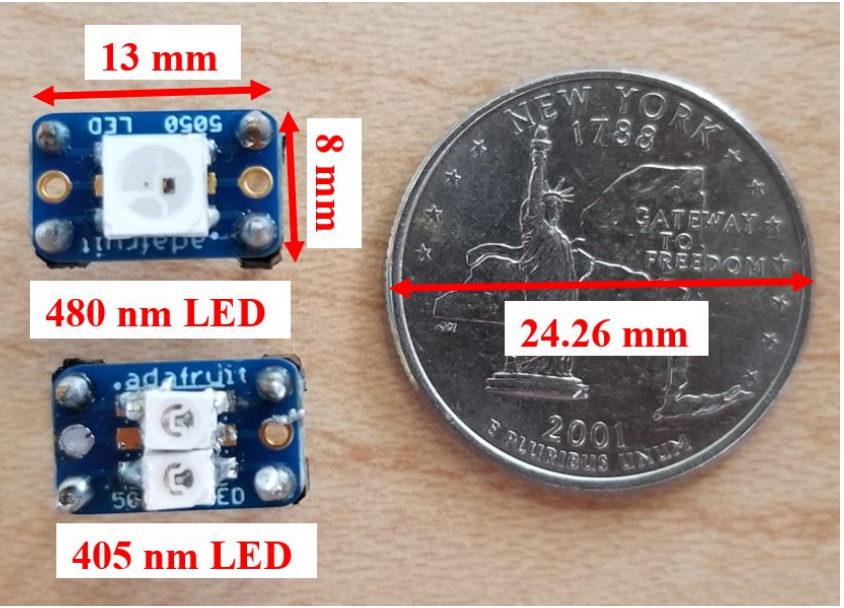


Fig. 5: A 480nm and 405nm LED was soldered onto a breakout board for prototyping on a breadboard to check the Arduino code.

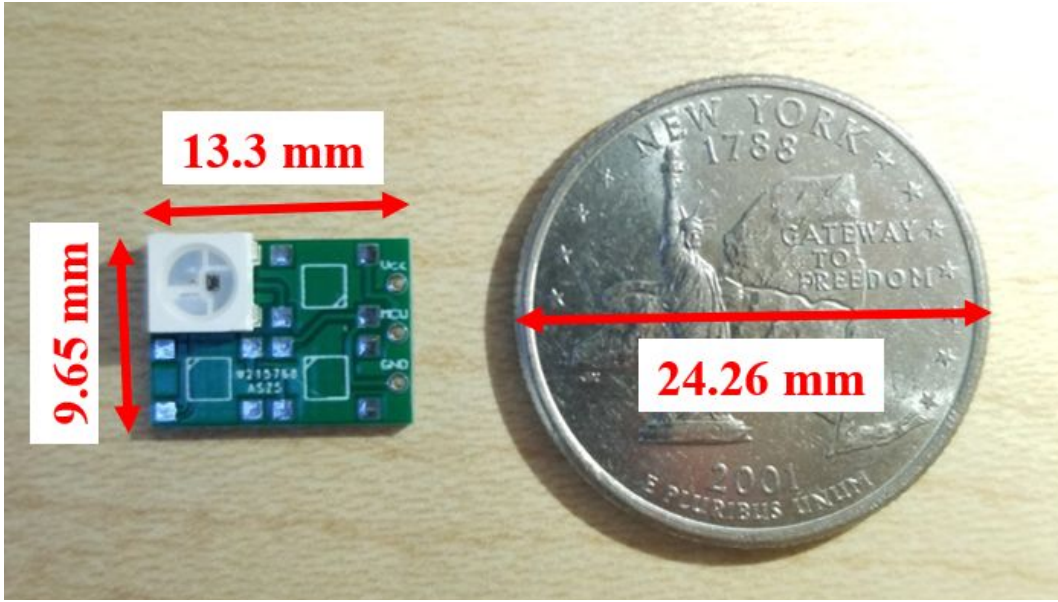


Fig. 6: A 480 nm LED was surface mounted onto our PCB.

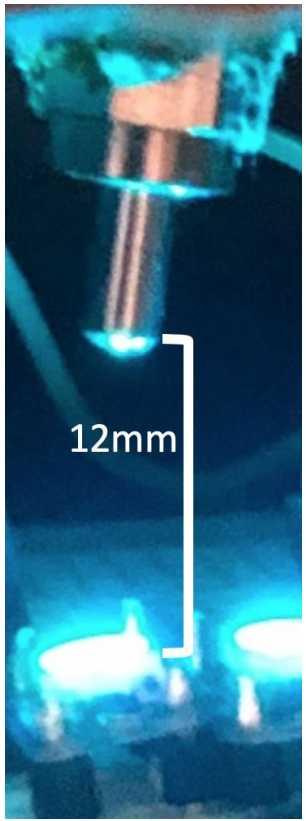
Pulse Width Modulation Control

Color: [<pix_index>/c?<wavelength (nm)>]
Brightness: [<pix_index>/b?<brightness>]
PWM:[<pix_index>/f?<period (ms)>:<duty_cycle>:<no. of cycles>]

Fig. 7: Arduino entry into serial monitor for color, brightness, and pulse width modulation control.

Fig. 8: Microcontroller enabling control over pulse width modulation, brightness, and wavelength. Up to eight separate LED devices can be operated independently from digital pinouts.

Wavelength and Intensity Testing Setup



- The LED is placed in a dark space to avoid background noise
- The light fiber is varied around 12 mm to prevent saturation of the Ocean Optics sensor
- 3 measurements are taken for each wavelength of the LED

Fig. 9: the light sensor fiber is fixed 12mm away from the light source

Previous Results - Wavelength and Intensity

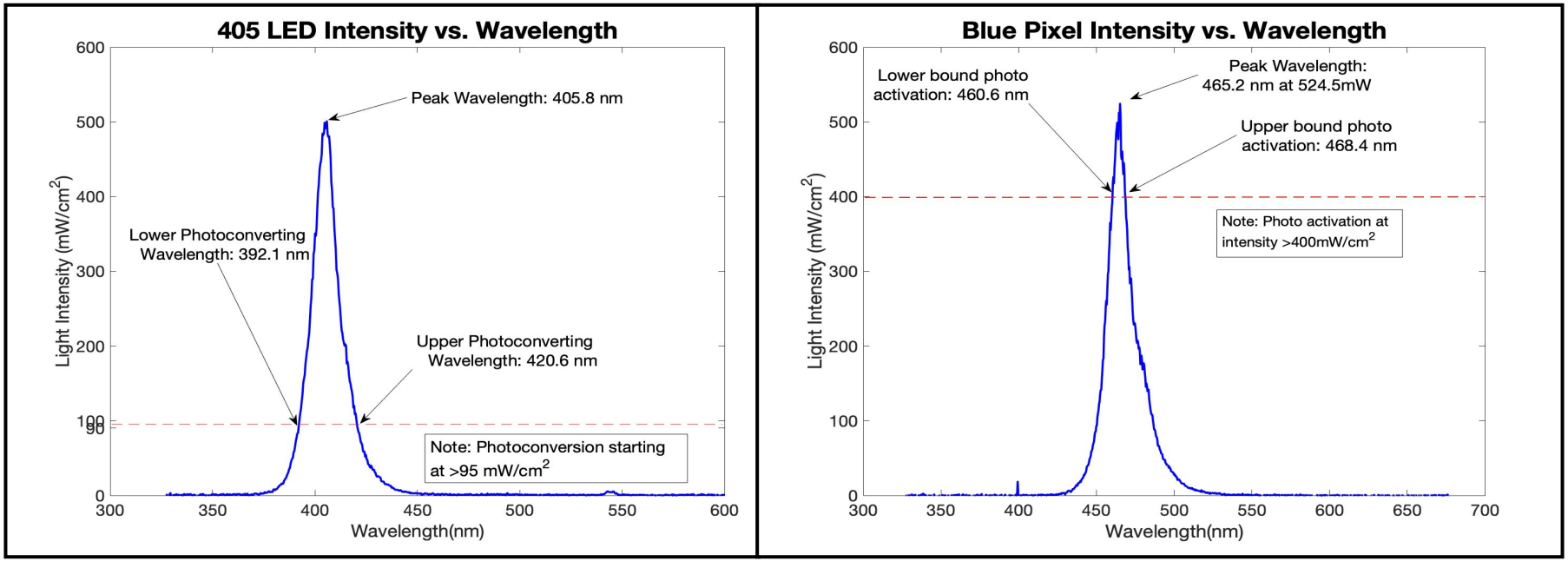
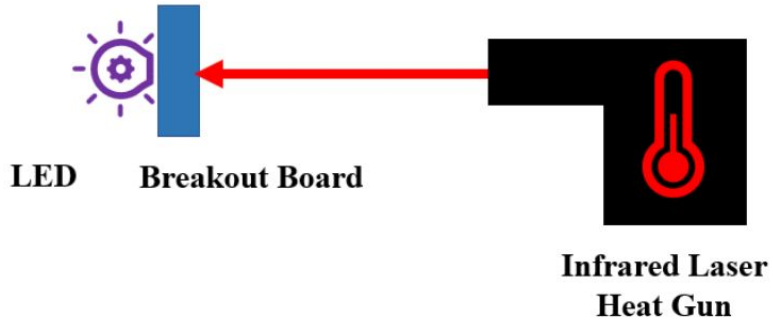
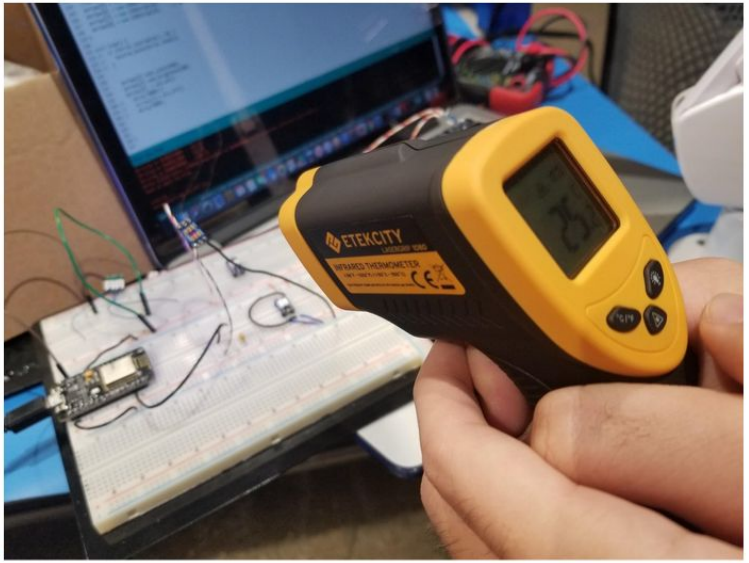


Fig. 10: The LEDs met the intensity and wavelength requirements no significant deviation.

Temperature Testing



A



B

*Fig. 11: **A-B**, The temperature at the bottom of the breakout board was measured using an infrared laser heat gun.*

Previous Results - LED Temperature

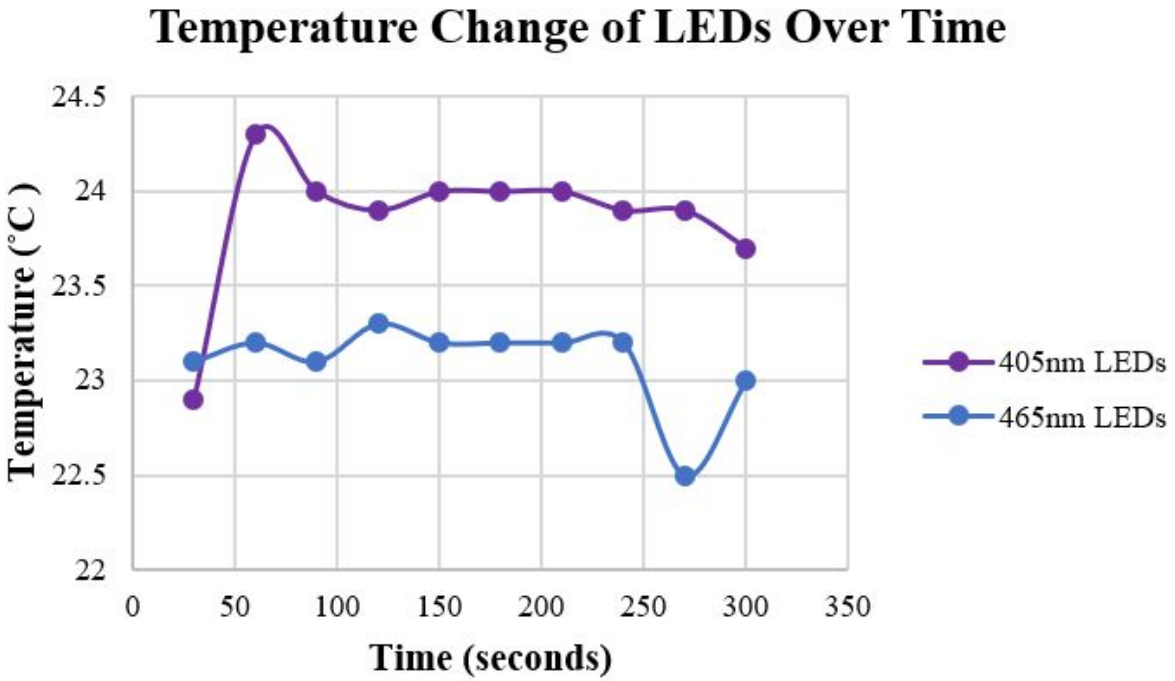


Fig. 12: The temperature of the LEDs over time is neither statistically significant nor correlated to time (p=0.565 and p=0.187). The LEDs maintained a safe working temperature.

Photo conversion *in vitro* testing setup

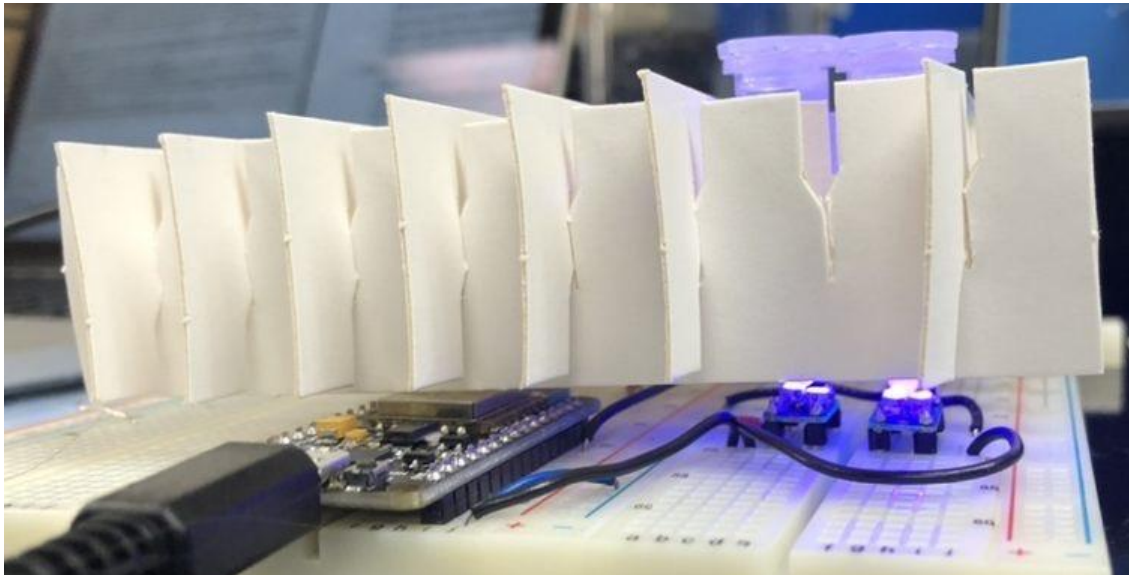


Fig. 13: KikGR cells isolated from murine lymph nodes were pelleted in a 1.7 mL tube and placed directly on the 405 nm LEDs for 0 minutes (control), 5 minutes, and 15 minutes. Cells were analyzed on a confocal microscope for photoconversion.

Previous Results - 405 nm *In Vivo* Testing

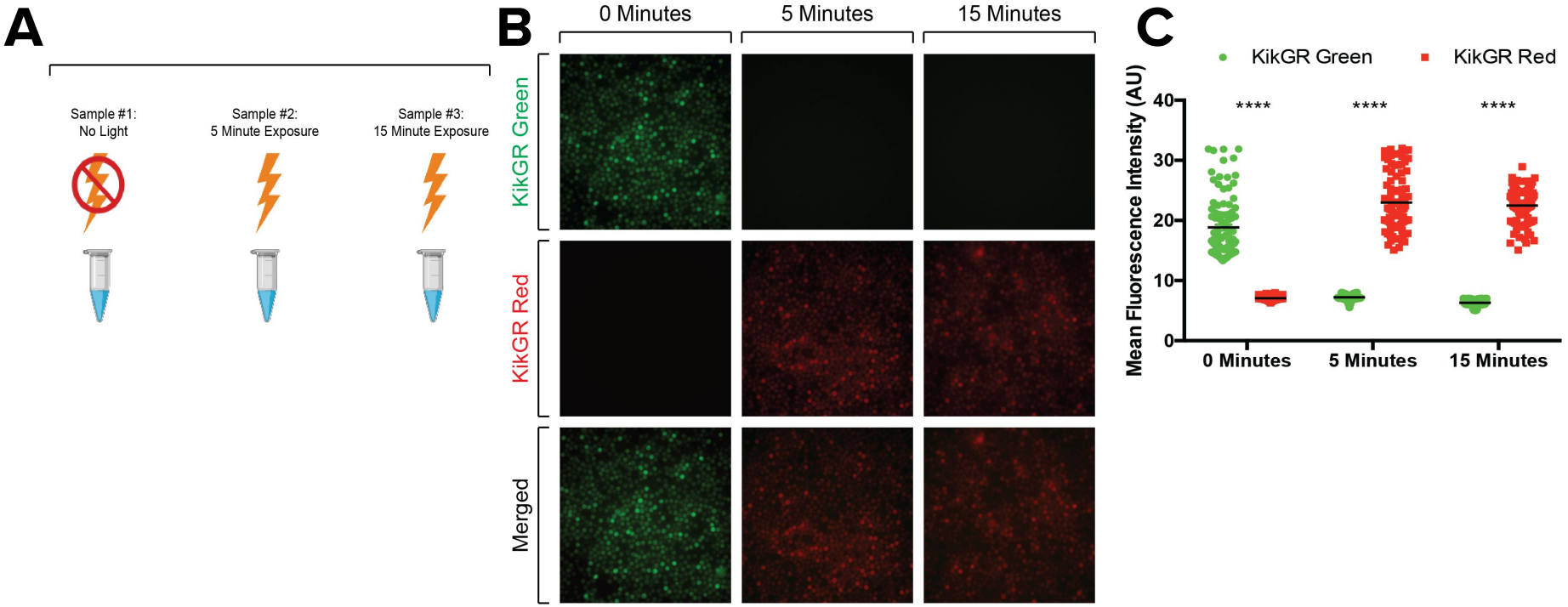


*Fig. 14: **A**, Experimental design. **B**, Fluorescence intensity after 0, 5, and 15 minutes. **C**, Mean fluorescence intensity. Two-way ANOVA, mean +/- s.e.m., n = 100 cells per group, **** $p < 0.0001$.*

Future testing and analysis

- More in vitro testing with client's lab members
  - Cell viability with flow cytometry, photoactivation, PWM optimization

- *In vivo* testing by client's lab members

- Biomaterial testing
  - Ocean optics, safety *in vivo*

Conclusions

- Challenges
  - PCB fabrication is time consuming and expensive
  - Footprint too small and too close
- Future Work
  - PCB Fabrication

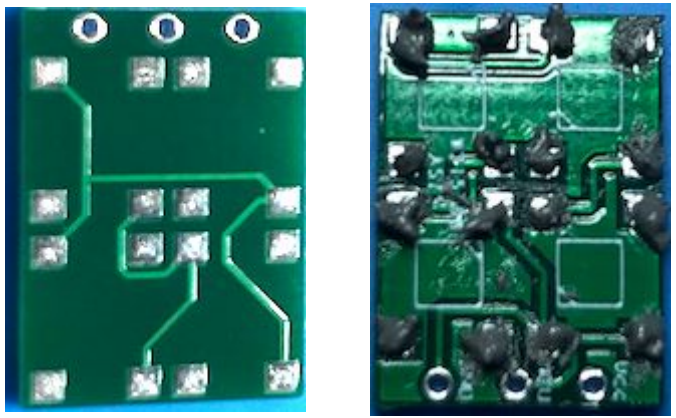


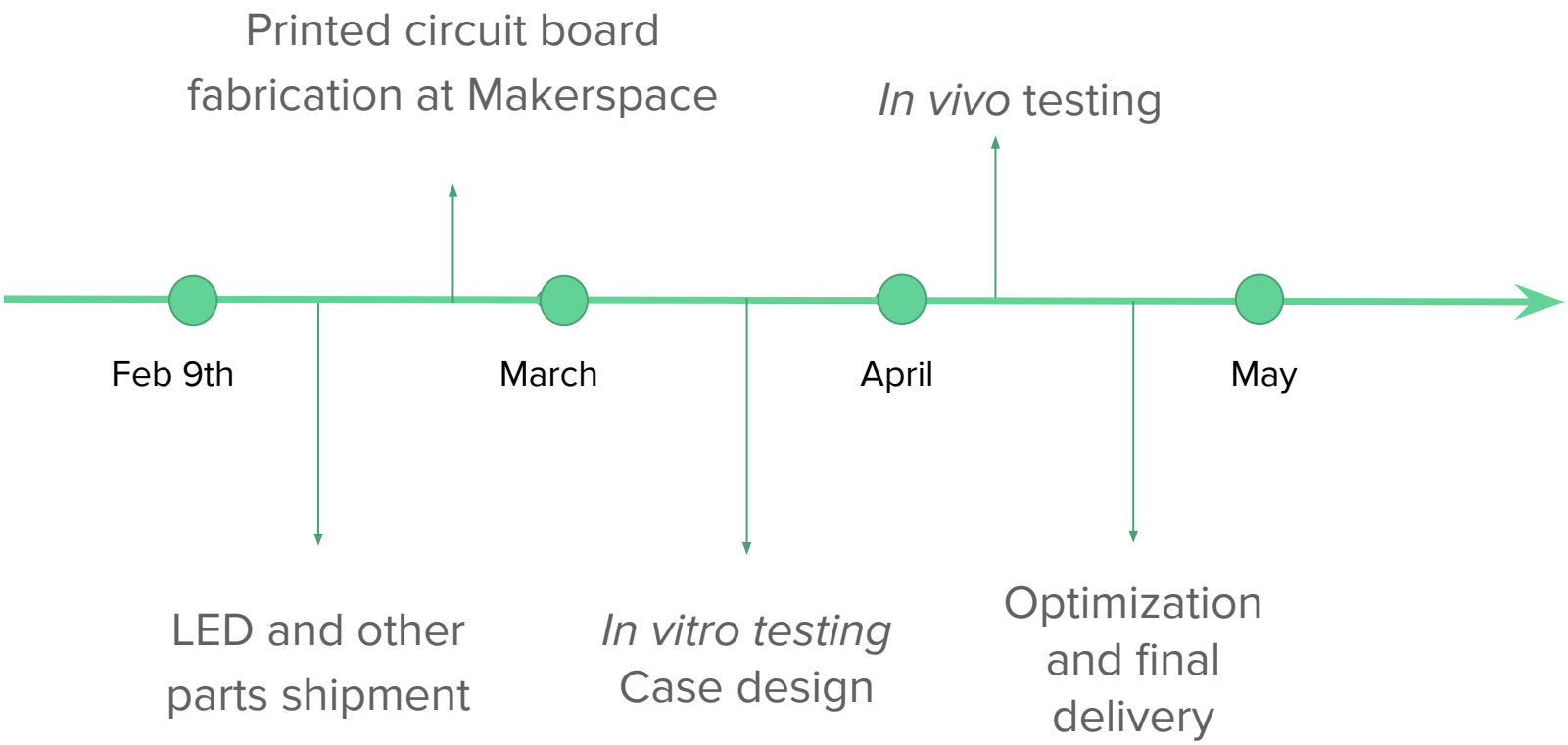
Fig. 15: PCB footprints from our previous prototype were too close together making SMD soldering impossible.

Final prototype

- Neat, enclosed case to hold microcontroller and connect to power
- Fine tune PCB design and fabrication
- User manual with safety documentation
- Biocompatible coating for *in vivo* work

Fig. 16: Our final prototype will consist of a box to house the microcontroller, connection to a power source, and controls to change light brightness. (Biorender)

Timeline


Printed circuit board fabrication at Makerspace
In vivo testing
Feb 9th
March
April
May
LED and other parts shipment
In vitro testing
Case design
Optimization and final delivery

Budget

Fall 2019

Material	Quantity		Cost
Printed Circuit Board (PCB)	10		$43
480nm LEDs	20		$47.10
Breakout PCB	10		$15.97
Microcontroller and Circuitry	N/A		$0.00
Ocean Optics Spectrometer	1		$0.00
		Total	$106.07

Spring 2020

Material	Quantity		Cost
Circuit Box	1		$25.00
Flexible PCB	1		$50.00
480nm LEDs	10		$23.55
405nm LEDs	30		$13.41
		Total	$111.96

Acknowledgement

Our team would like to thank Dr. Williams for his guidance and thank Dr. Sandor’s Lab for providing us the opportunity to work on this project.

Sources

1. http://www.fluorocarbon.co.uk/news-and-events/post/18/what-is-ultra-high-molecular-weight-polyethylene-uhmwpe
2. https://www.azom.com/article.aspx?ArticleID=2630
3. Schmidt A., Westendorf C., Ridelis I. “Photoconversion.” Internet: https://www.leibniz-fmp.de/fileadmin/user_upload/Cellular%20Imaging/pdf/Photoconversion.pdf [Oct. 2, 2018]
4. Fabry Z, Schreiber HA, Harris MG, Sandor M. Sensing the microenvironment of the central nervous system: immune cells in the central nervous system and their pharmacological manipulation. *Curr Opin Pharmacol*. 2008;8(4):496–507. doi:10.1016/j.coph.2008.07.009
5. Turkowyd B., Balinovic A., Virant D., Carnero H., Caldana F., Endesfelder M., Bourgeois D. “Photoconversion of Green-to-Red Fluorescent Proteins Based on Blue and Infrared Light.” Internet: https://www.ncbi.nlm.nih.gov/pubmed/28574633 , 2017 [Oct. 3, 2018]
6. M. Tomura, A. Hata, S. Matsuoka, F. H. W. Shand, Y. Nakanishi, R. Ikebuchi, S. Ueha, H. Tsutsui, K. Inaba, K. Matsushima, A. Miyawaki, K. Kabashima, T. Watanabe, O. Kanagawa, "Tracking and quantification of dendritic cell migration and antigen trafficking between the skin and lymph node," Scientific Reports, vol. 4, no. 6030, Aug. 2014. [Online] Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4129424/
7. M. Tomura, N. Yoshida, J. Tanaka, S. Karasawa, Y. Miwa, A. Miyawaki, O. Kanagawa, "Monitoring cellular movement in vivo with photoconvertible fluorescence protein "Kaede" transgenic mice," Proceedings of the National Academy of Sciences of the United States of America, vol. 105, no. 31, pp. 10871-10876. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2504797/
8. J. Heisterkamp, R. V. Hillegersberg, and J. N. Ijzermans, “Critical temperature and heating time for coagulation damage: Implications for interstitial laser coagulation (ilc) of tumors,” Lasers in Surgery and Medicine, vol. 25, no. 3, pp. 257–262, 1999.
9. Dr. Sandor and his team

Questions?