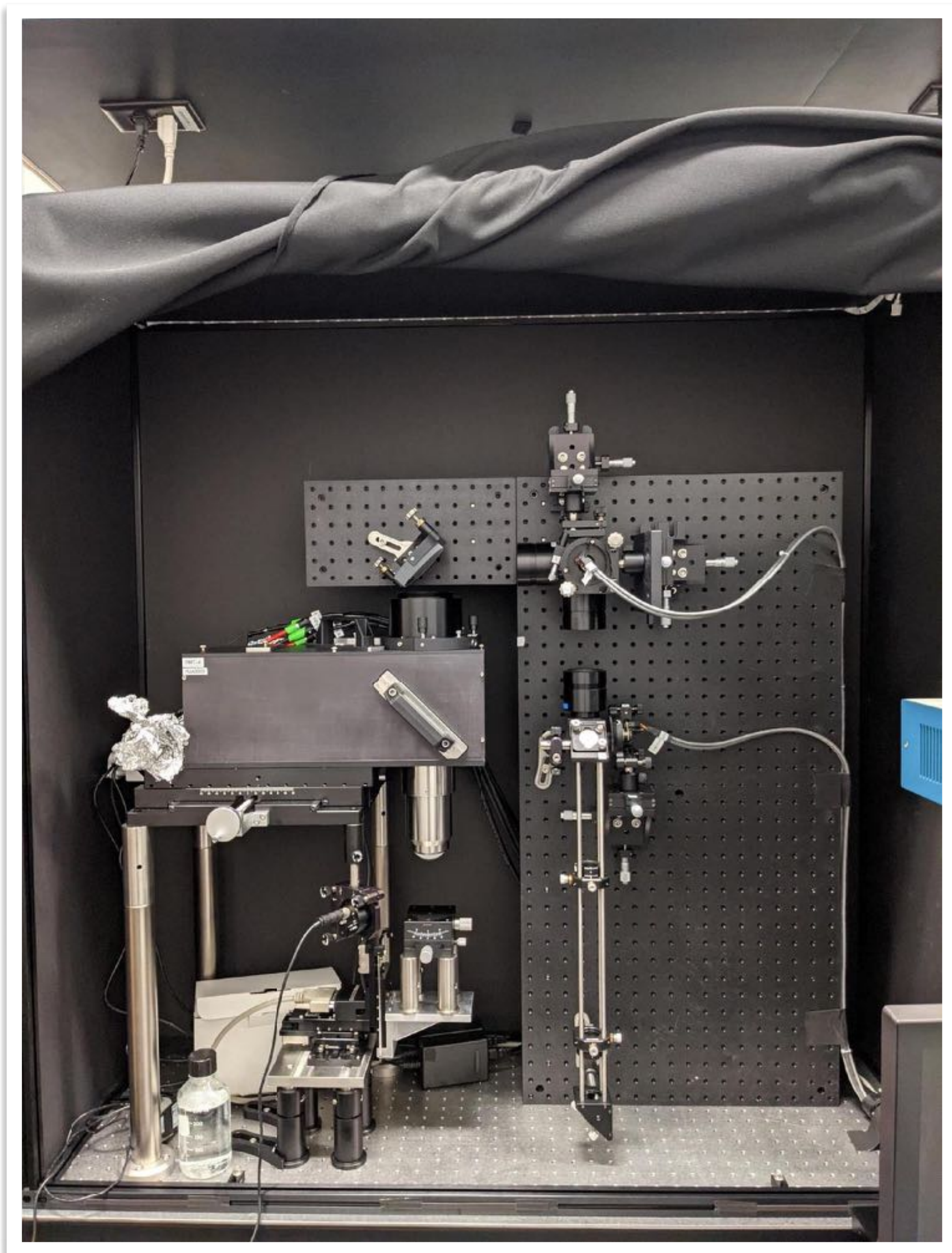


# Development of Three Photon Large Field of View Microscope for Mouse Brain Imaging

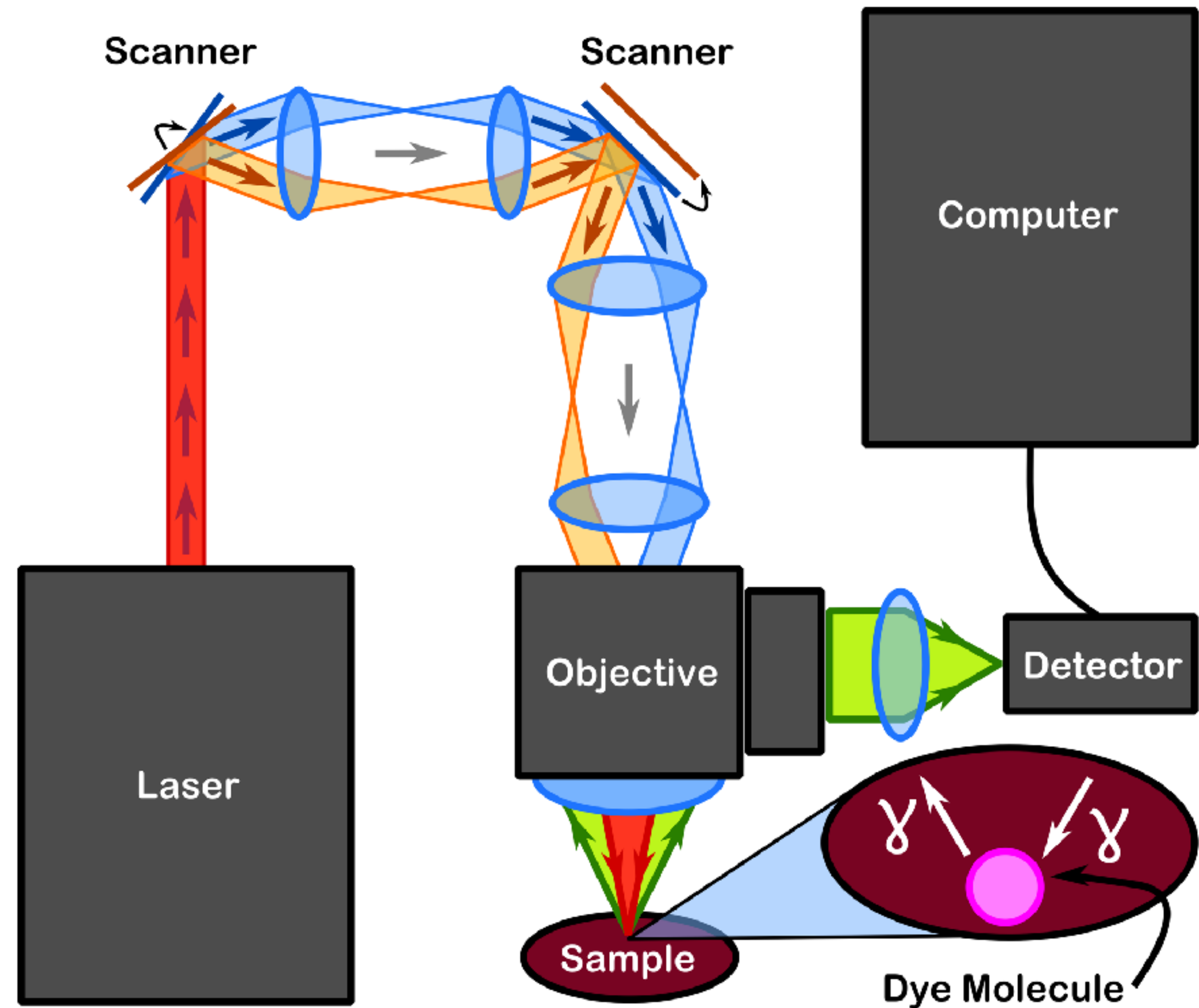
Mariya Sokolova, Cornell University Undergraduate  
Xu Lab  
August 13 2021





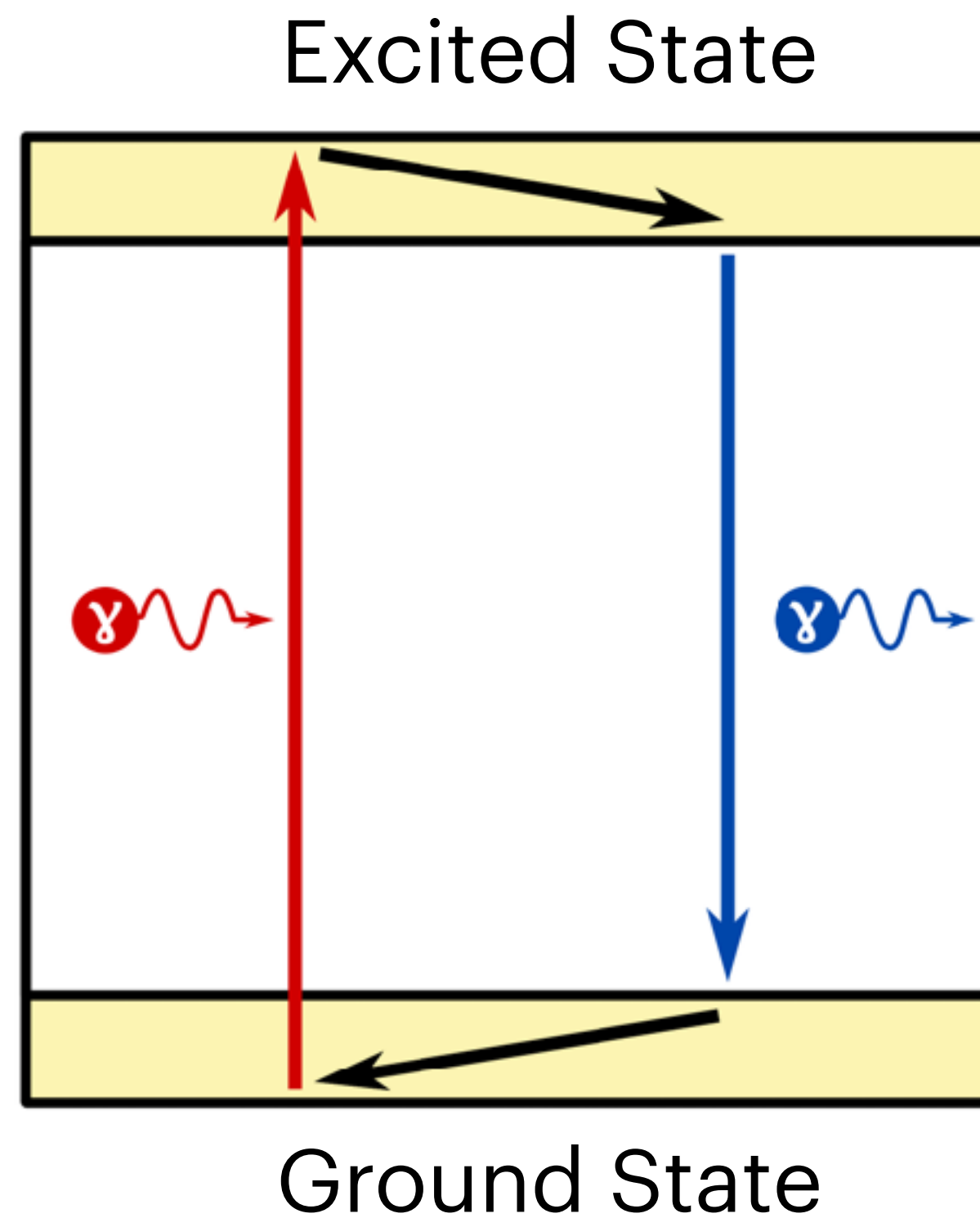
# What Is A Three-Photon Microscope?

- A three-photon microscope is a type of fluorescence microscope.

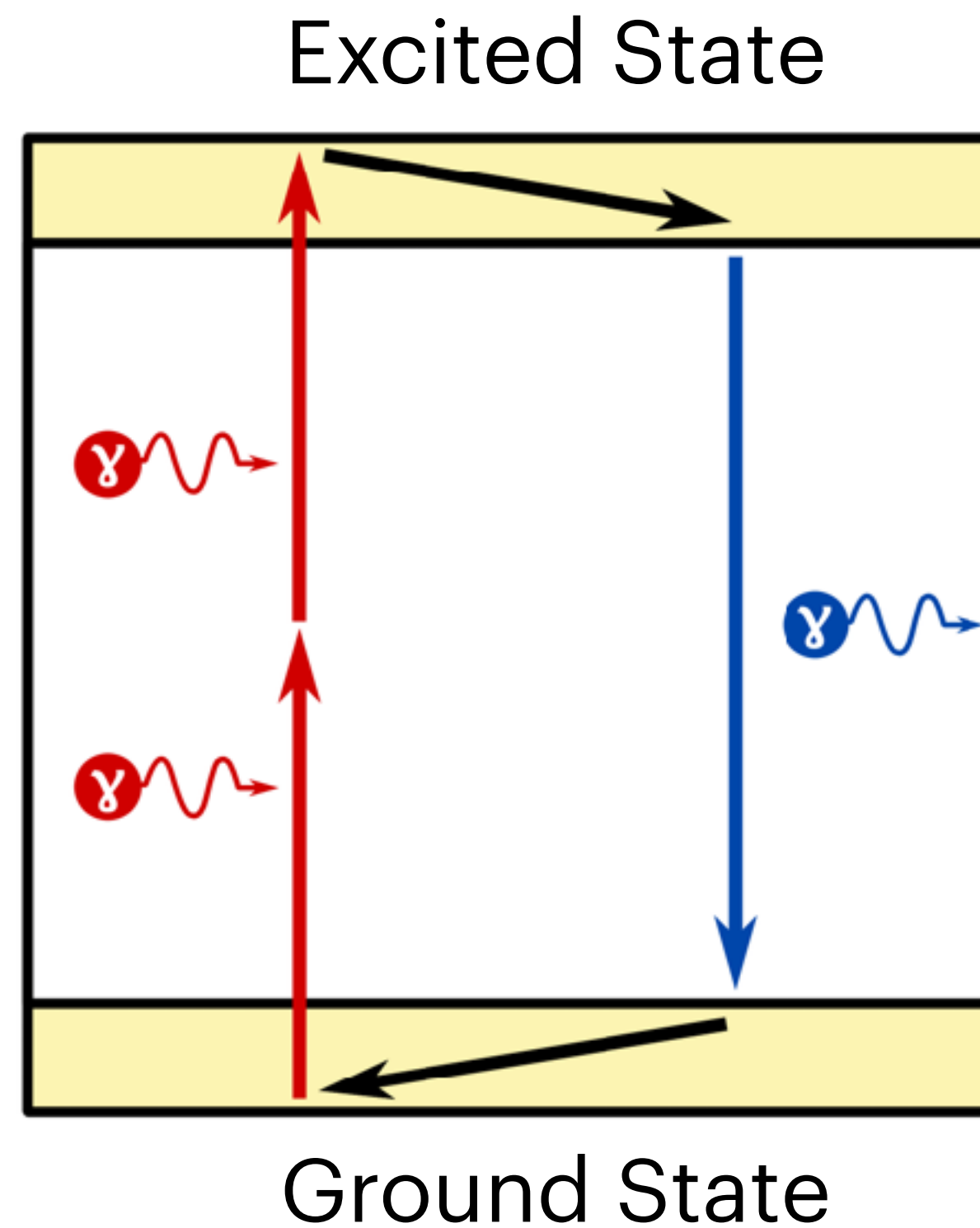


# Kinds of Fluorescence Microscopes

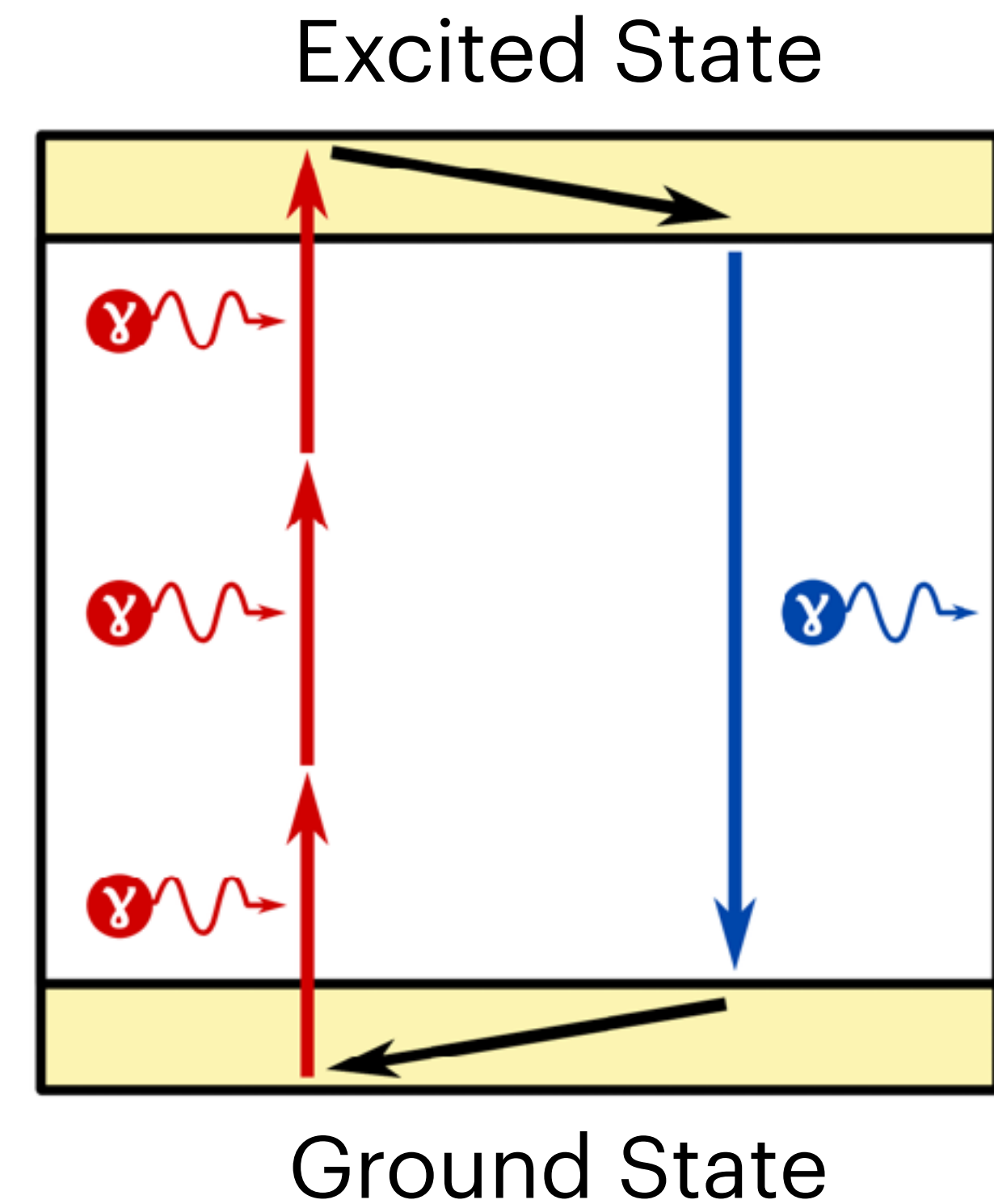
## One-Photon Excitation



## Two-Photon Excitation



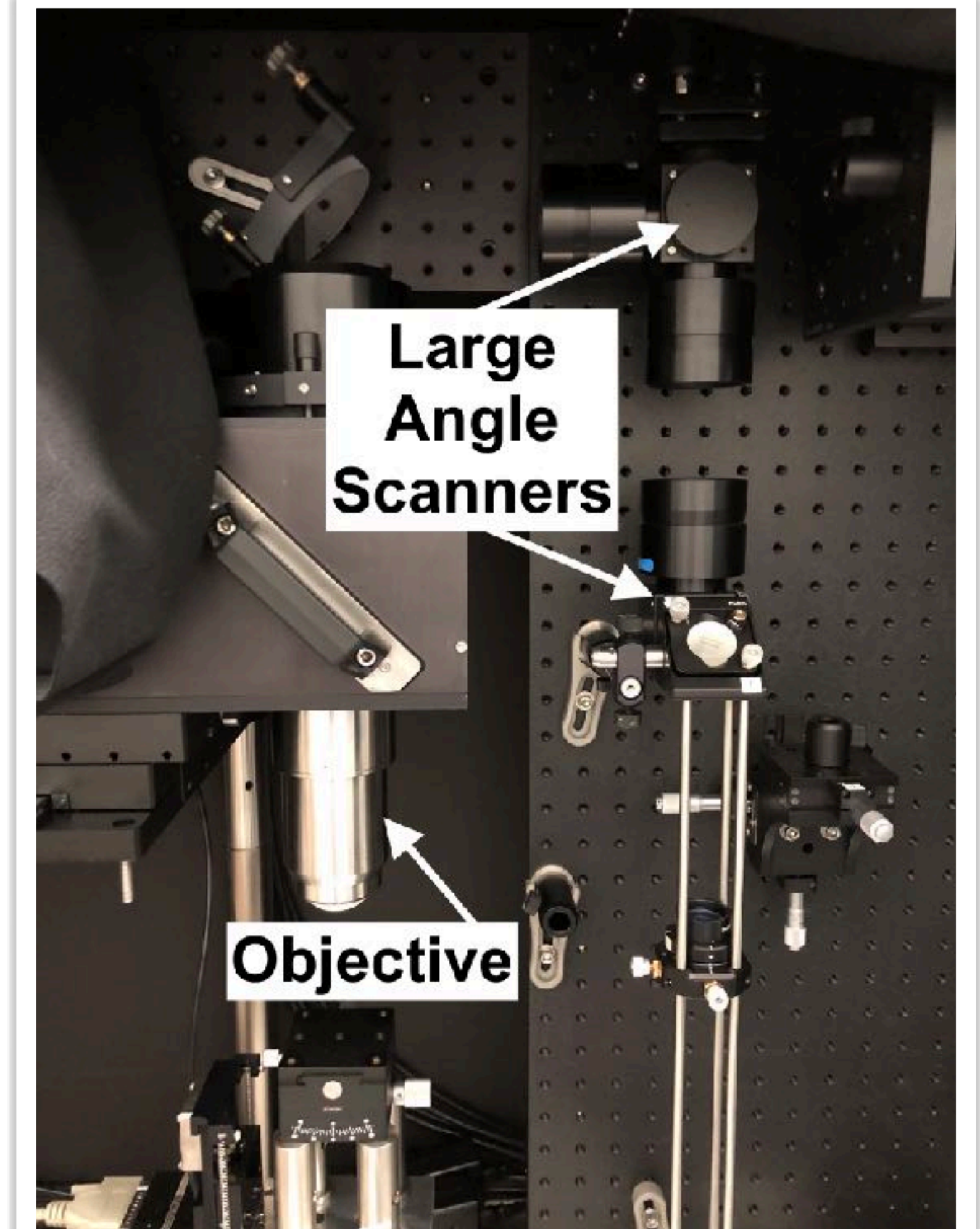
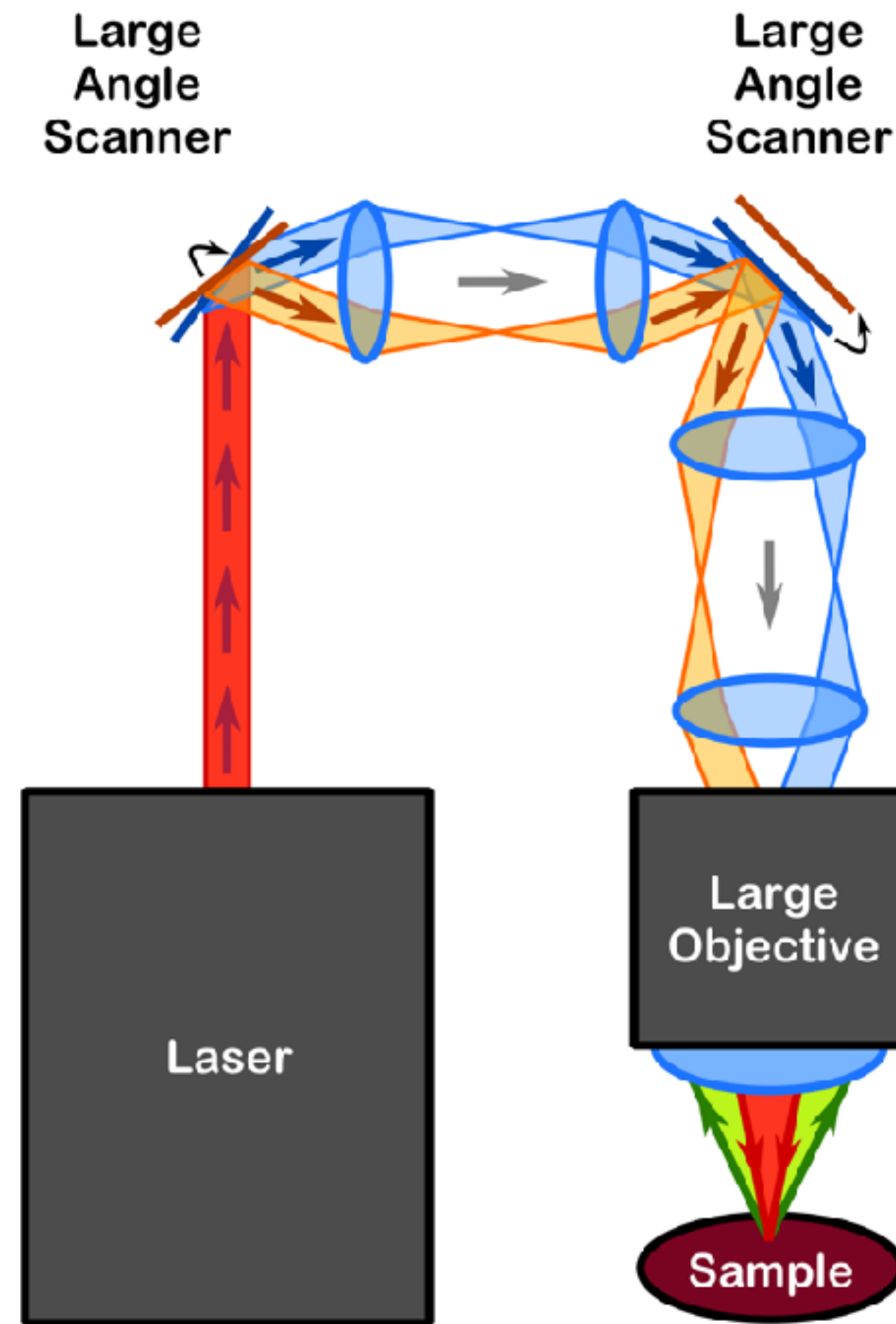
## Three-Photon Excitation





# Large Field of View Microscope

- What is a LFOV microscope?
- A LFOV microscope is a fluorescent microscope (3PM in our case) that uses a large objective lens and large angle scanners to capture a larger area ( $3000 \mu\text{m}^2$  vs  $600 \mu\text{m}^2$ ).



# My Contributions

## Solving a Problem

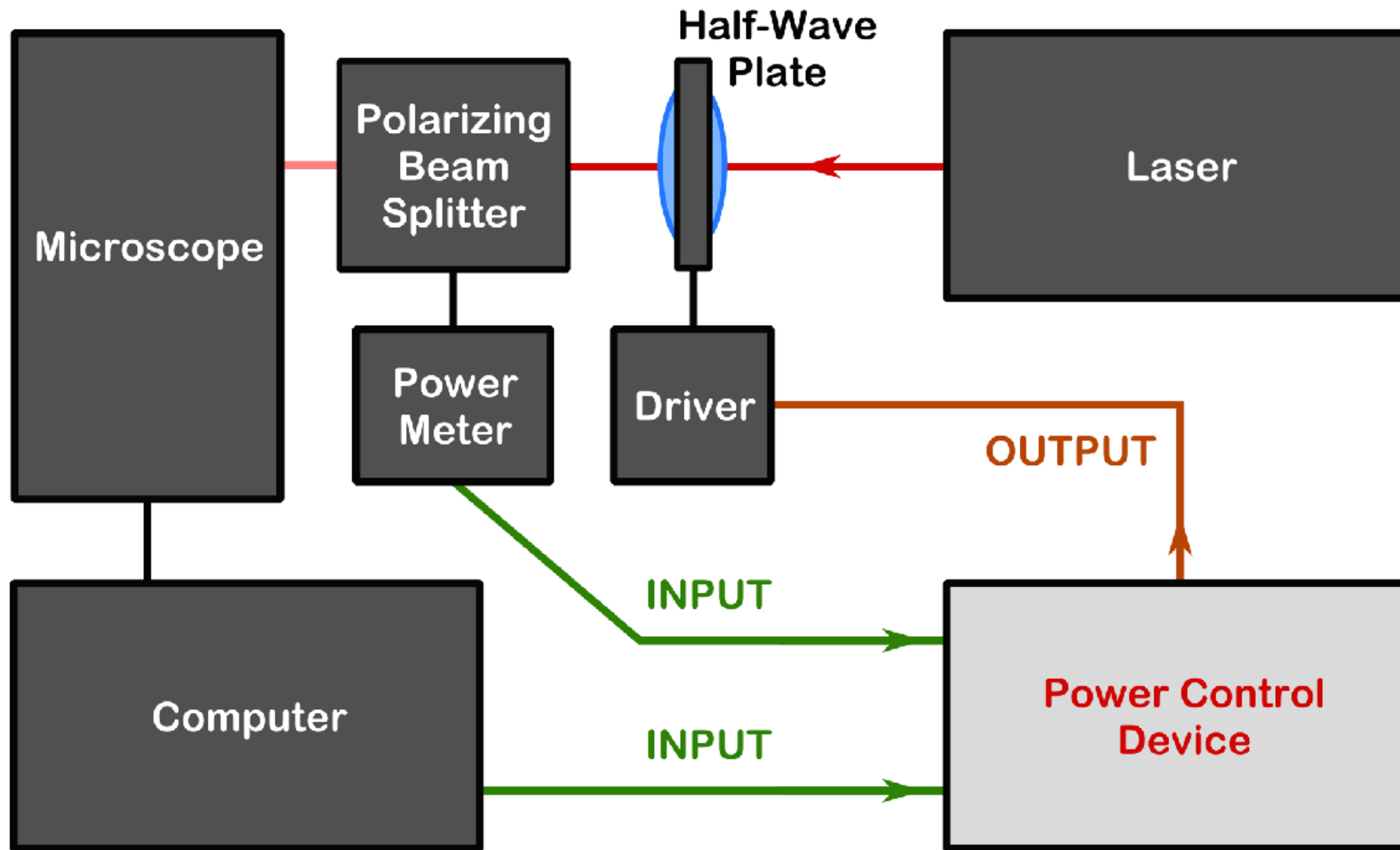
- A LFOV microscope is a major project that involves many different parts, and one of those parts is power control.
- **Problem:** Imaging takes a long time because power needs to be manually adjusted for different depths.
- **My Solution:** Build a device that would do this automatically for a Large Field of View Microscope.

## Learning New Skills

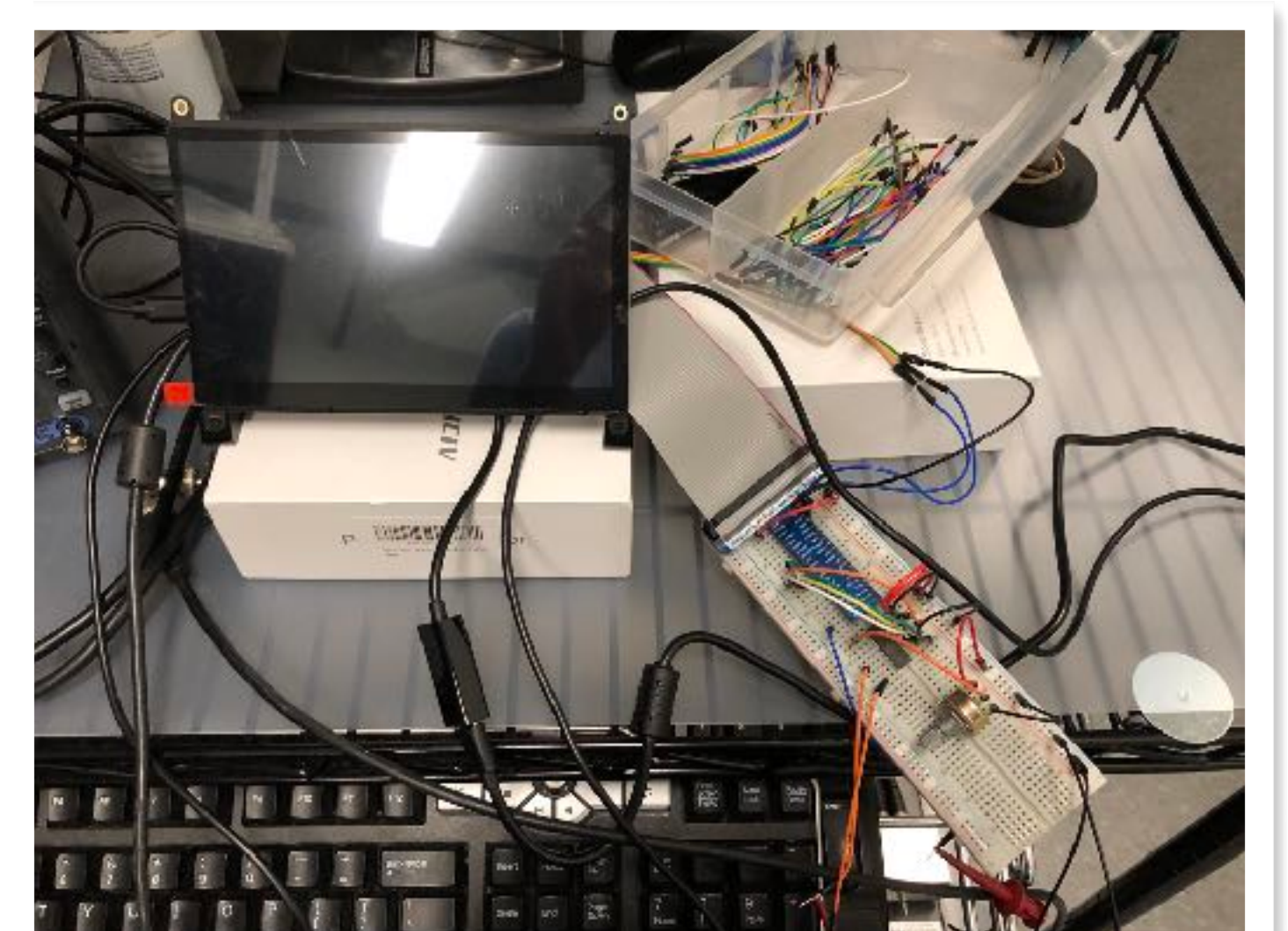
- Hardware Control
- Craniotomy (sample preparation surgery) for mouse brain imaging.



# Power Control Device: How It Works



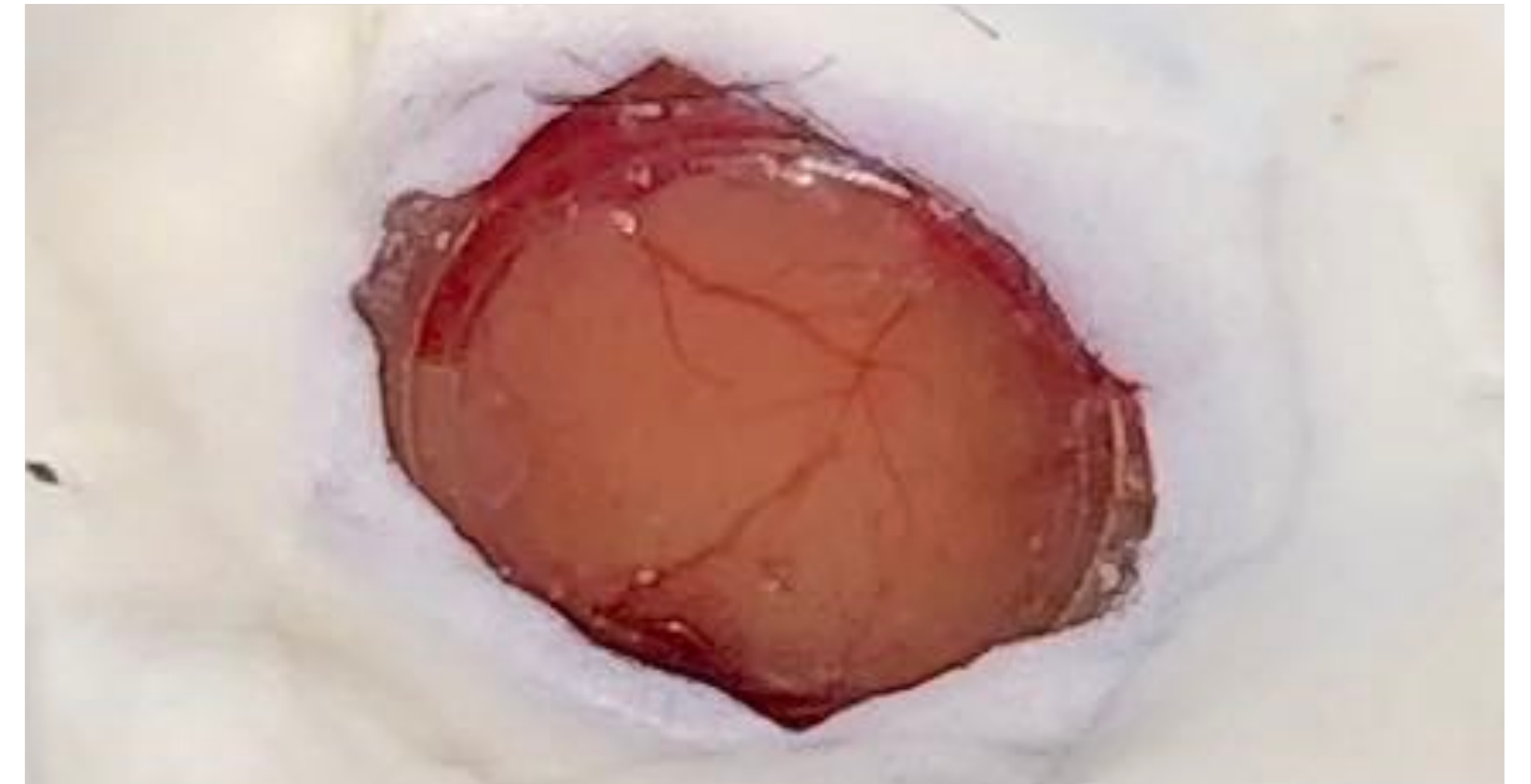
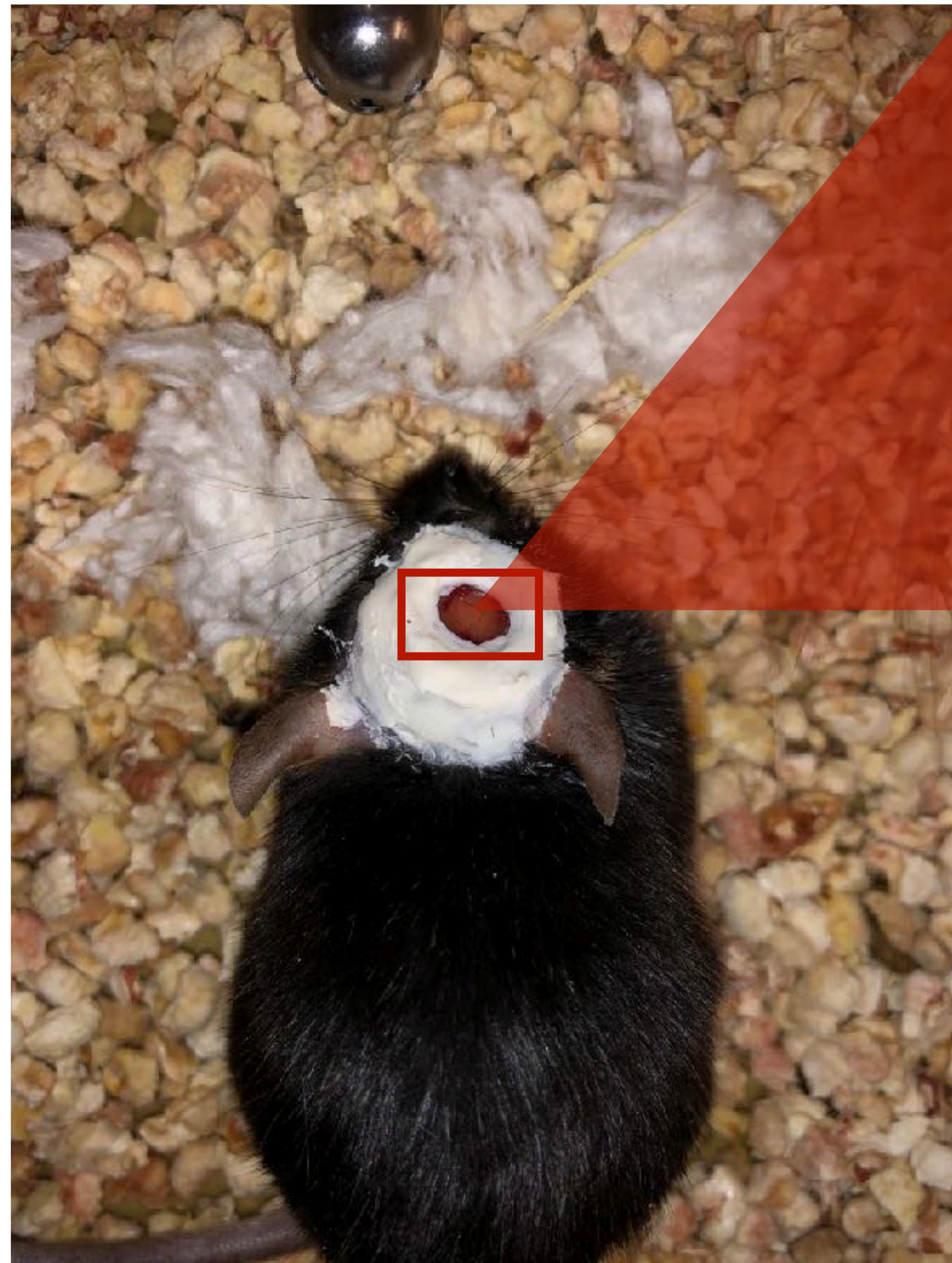
**The Device:**





# Craniotomy

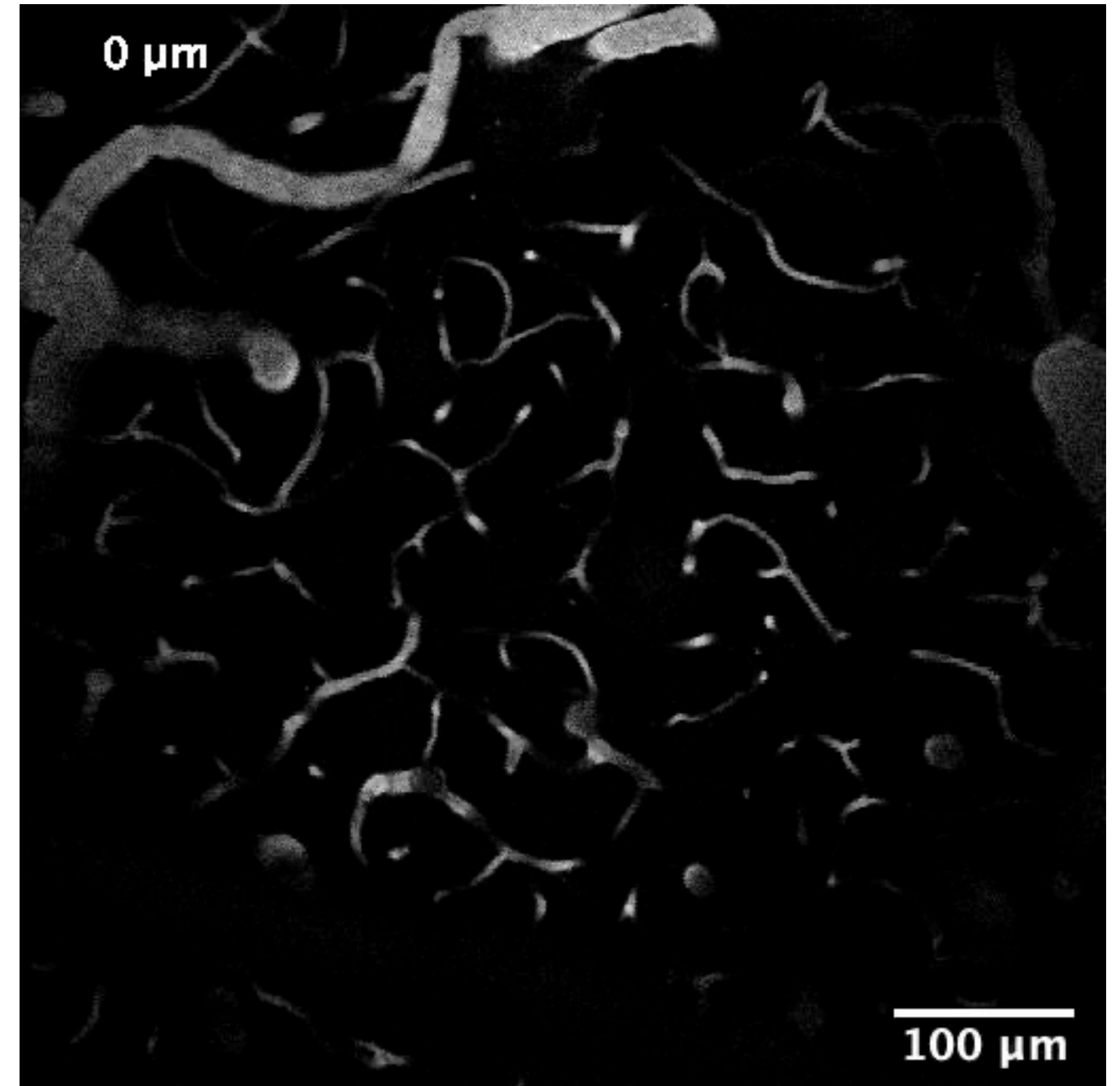
- What is craniotomy?
- Craniotomy is a surgical procedure to prepare mice for in vivo brain imaging.





# Mouse Imaging Data

- We imaged a volume from 0  $\mu\text{m}$  to 1270  $\mu\text{m}$ .





# Thank YOU!

- **Special Thanks To...**

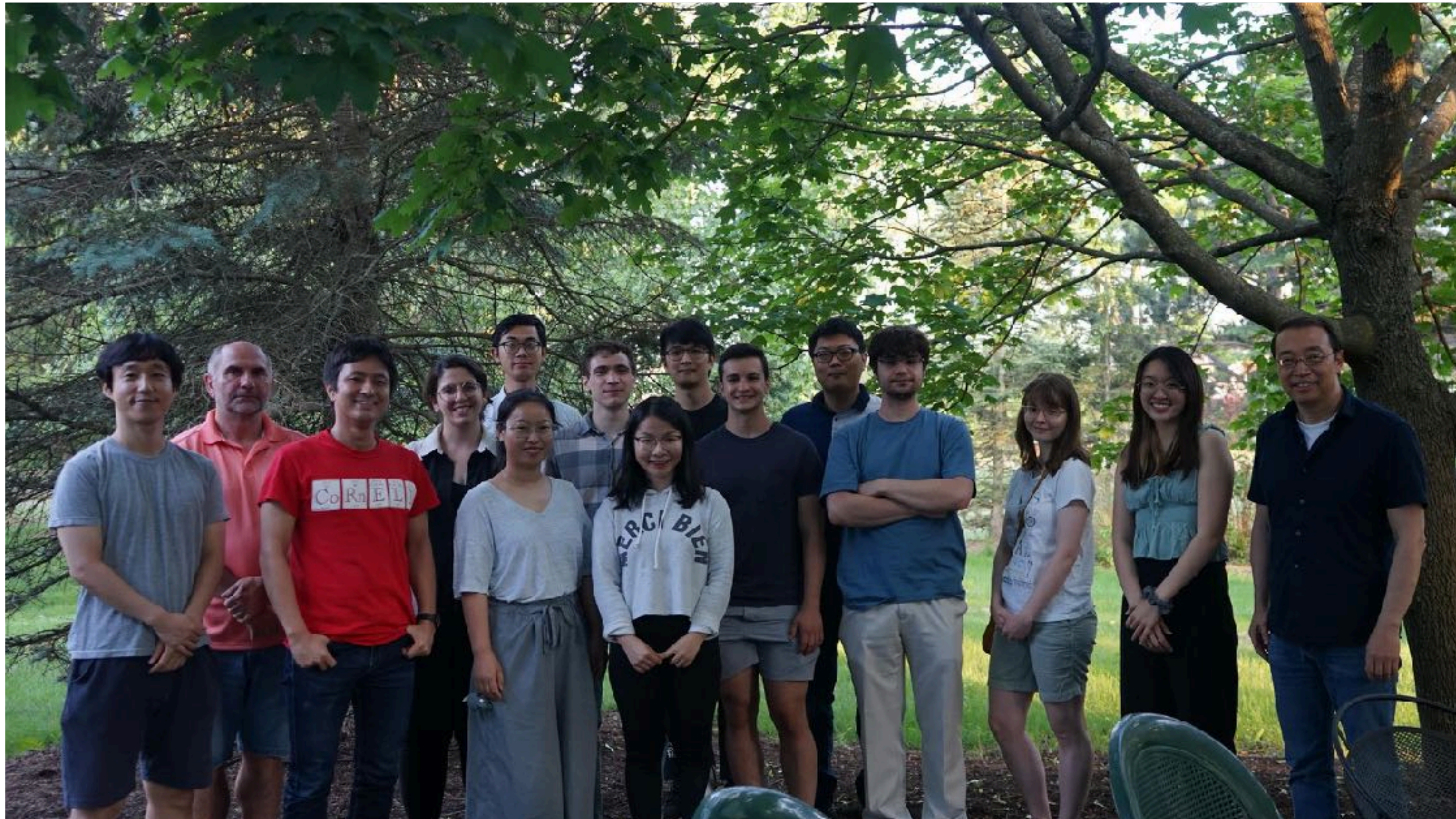


Image source: <https://neuronex.org/projects/9>



**National  
Science  
Foundation**

Image source: <https://neuronex.cornell.edu>





**Any  
Questions?**



# Resources

- Bumstead, Jonathan R et al. "Designing a large field-of-view two-photon microscope using optical invariant analysis." *Neurophotonics* vol. 5,2 (2018): 025001. doi:10.1117/1.NPh.5.2.025001
- Helmchen, F., Denk, W. Deep tissue two-photon microscopy. *Nat Methods* 2, 932–940 (2005). <https://doi.org/10.1038/nmeth818>
- Ouzounov, D., Wang, T., Wang, M. et al. In vivo three-photon imaging of activity of GCaMP6-labeled neurons deep in intact mouse brain. *Nat Methods* 14, 388–390 (2017). <https://doi.org/10.1038/nmeth.4183>
- Tianyu Wang and Chris Xu, "Three-photon neuronal imaging in deep mouse brain," *Optica* 7, 947-960 (2020)
- Two-Photon Microscopy. Lecture by Kurt Thorn, iBiology.org, iBiology, Apr. 2012, [www.ibiology.org/talks/two-photon-microscopy/](http://www.ibiology.org/talks/two-photon-microscopy/).