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Apr 6th, 1:00 PM - 2:00 PM

### Non-Destructive Imaging of Phytosulfokine Trafficking Using a Fiber-Optic Fluorescence Microscope

Bernard Abakah

Thomas Ntim

*East Tennessee State University*

Edward Offei

*East Tennessee State University*

Christopher Erb

*University of Maine*

Jessica Morgan

*University of Chicago*

*See next page for additional authors*

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Abakah, Bernard; Ntim, Thomas; Offei, Edward; Erb, Christopher; Morgan, Jessica; Liu, Dian; Jelenska, Joanna; Morrell-Falvey, Jennifer L.; Greenberg, Jean; and Standaert, Robert Frank, "Non-Destructive Imaging of Phytosulfokine Trafficking Using a Fiber-Optic Fluorescence Microscope" (2022). *Appalachian Student Research Forum & Jay S. Boland Undergraduate Research Symposium*. 161.  
<https://dc.etsu.edu/asrf/2022/schedule/161>

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**Author Names**

Bernard Abakah, Thomas Ntim, Edward Offei, Christopher Erb, Jessica Morgan, Dian Liu, Joanna Jelenska, Jennifer L. Morrell-Falvey, Jean Greenberg, and Robert Frank Standaert



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DEPARTMENT *of* CHEMISTRY

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# Non-Destructive Imaging of Phytosulfokine Trafficking Using a Fiber-Optic Fluorescence Microscope

Bernard Abakah

Supervisor

Dr. Robert Frank Standaert

# Introduction

**Plants are exposed to many stresses<sup>1</sup>**

- Hinder growth and development
- Reduce crop yield

**Mobile signals are used to mount systemic stress responses**



**Biotic Stress**

- Pathogens attack
- Insects attack

**Abiotic Stress**

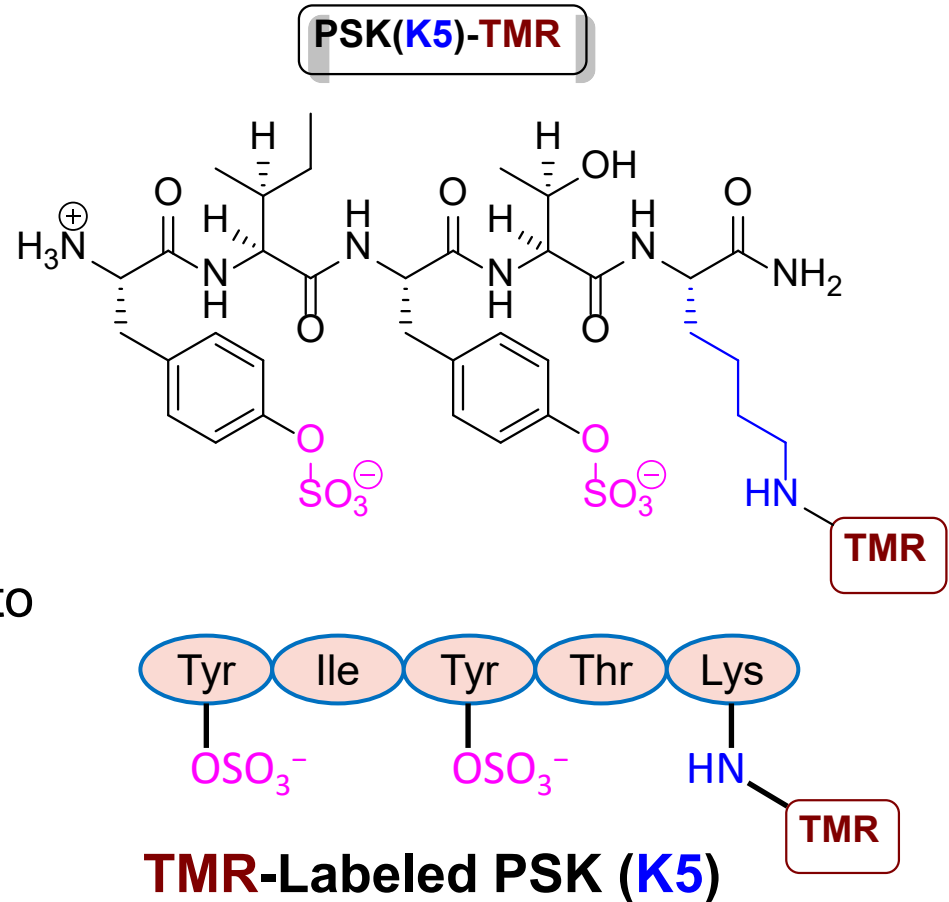
- Drought
- Salinity
- Extreme Temperature

**Understanding plant signaling mechanisms is essential to develop improved varieties for food, fiber and energy**

# Phytosulfokine (PSK)

PSK is an unusual, sulfated pentapeptide signal<sup>2,3</sup>

- Regulates plants' growth and development
- Regulates how plants respond to stress conditions



# Aims of the Research

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## Approach

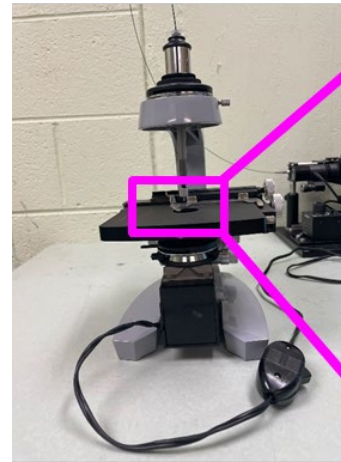
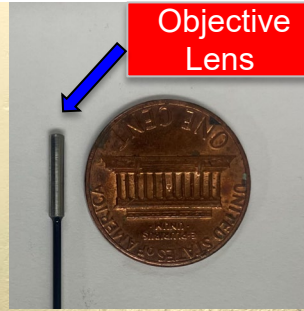
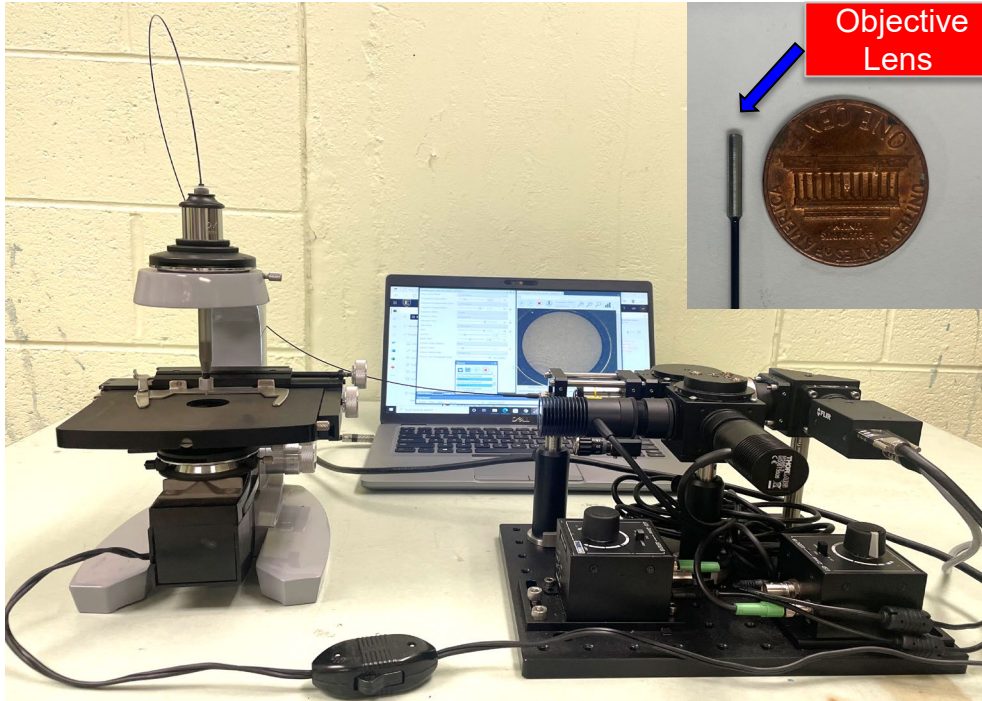
- Deliver both labeled and unlabeled phytosulfokines (PSK) into the roots
- Use fiber-optic fluorescence microscopy to track PSK in live plants non destructively

## Investigate

- If PSK affects the level of its receptor, PSKR, in *Arabidopsis thaliana*
- If PSK moves from the root to the leaves
- How PSK trafficking differs in a receptor-less mutant (pskr1/pskr2)
- Whether PSK movement and/or persistence depend on PSK receptors

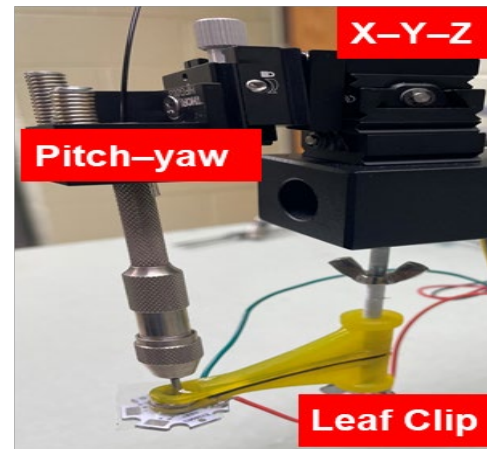


# The Microscope



Gen - 2

Two-color fluorescence imaging  
 1-m Fiber-optic probe  
 2.3× mini objective  
 0.34 mm Field of view (FoV)  
 Color digital camera



Gen - 3

Pierce, M., Yu, D., Richards-Kortum, R. High-resolution fiber-optic microendoscopy for in situ cellular imaging. *J. Vis. Exp.* **2011**, 47, e2306. doi:10.3791/2306

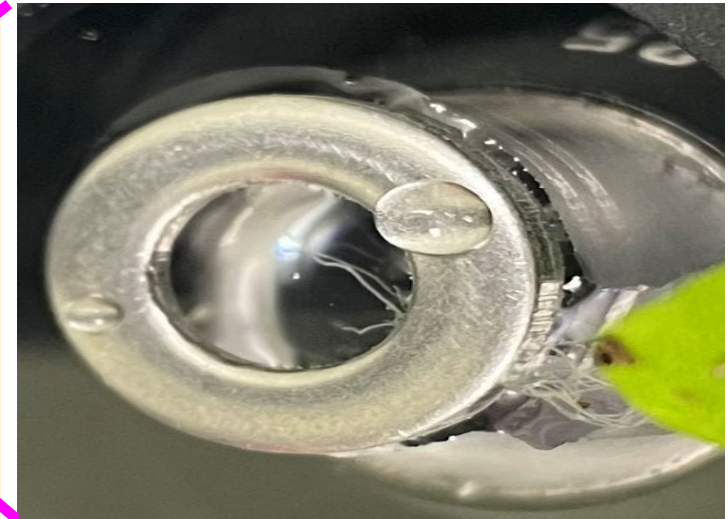
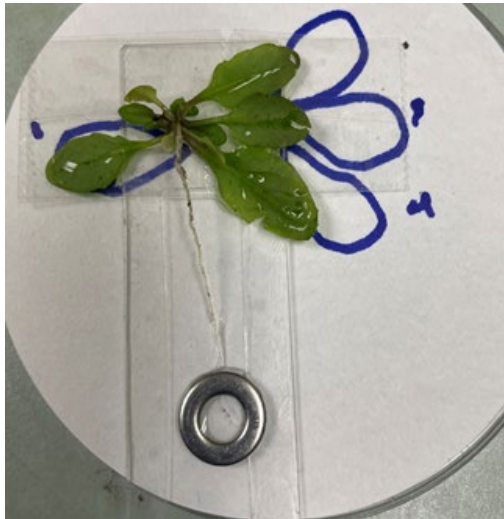
Krstajić, N., Akram, A. R., Choudhary, T. R., McDonald, N., Tanner, M. G., Pedretti, E., Dalgarno, P. A., Scholefield, E., Girkin, J. M., Moore, A., Bradley, M., Dhaliwal, K. Two-color widefield fluorescence microendoscopy enables multiplexed molecular imaging in the alveolar space of human lung tissue. *J. Biomed. Opt.* **21**, 046009. doi:10.1117/1.JBO.21.4.046009



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# Sample Mounting and Peptide Delivery

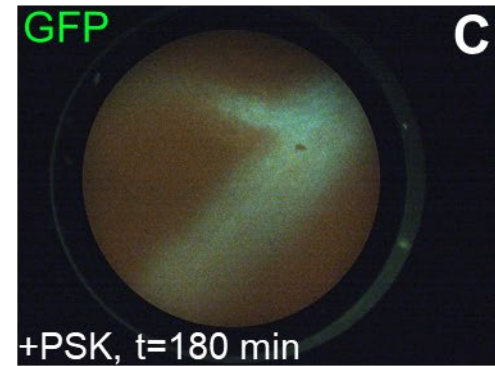
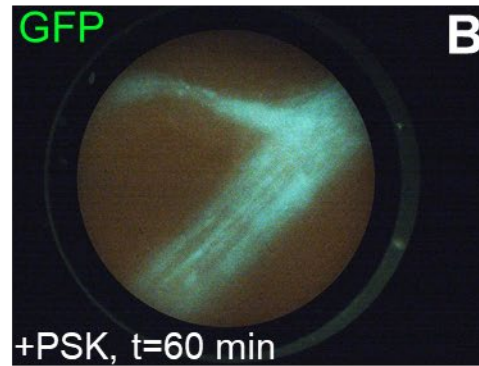
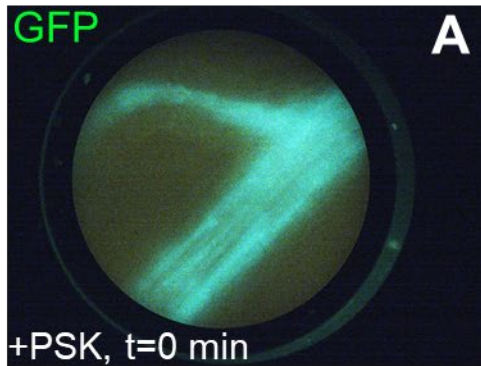
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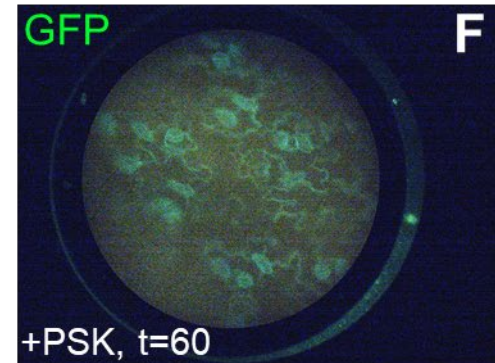
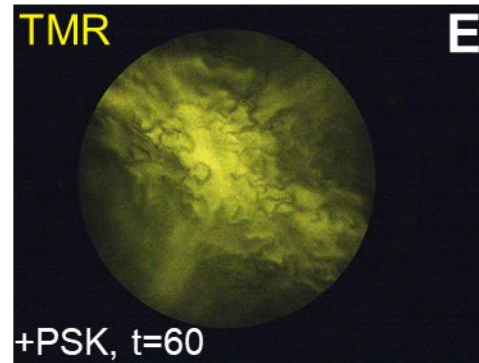
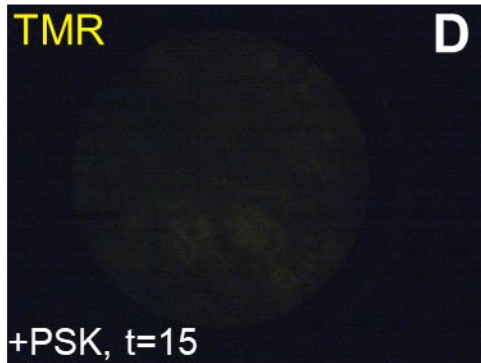


# Results

Prolonged incubation of root in PSK appears to reduce PSK receptor level

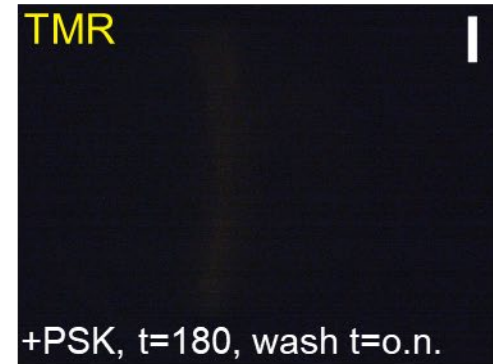
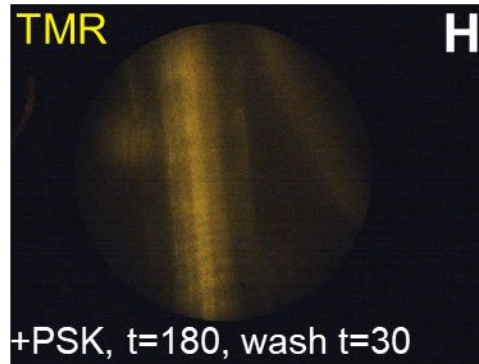
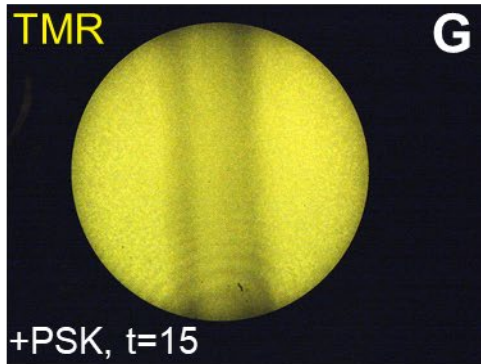


PSK is mobile over time

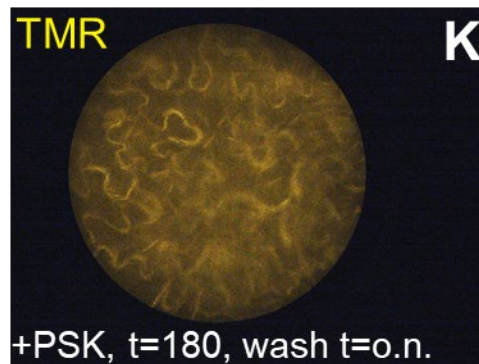
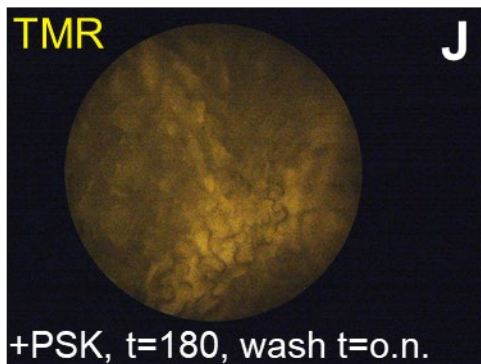


# Results

A receptor-less mutant internalizes TAMRA-PSK, but it is not stable/stationary



Receptor level appears to impact the trafficking level/location of PSK



J: leaf of receptor-less  
(*r1/r2*)

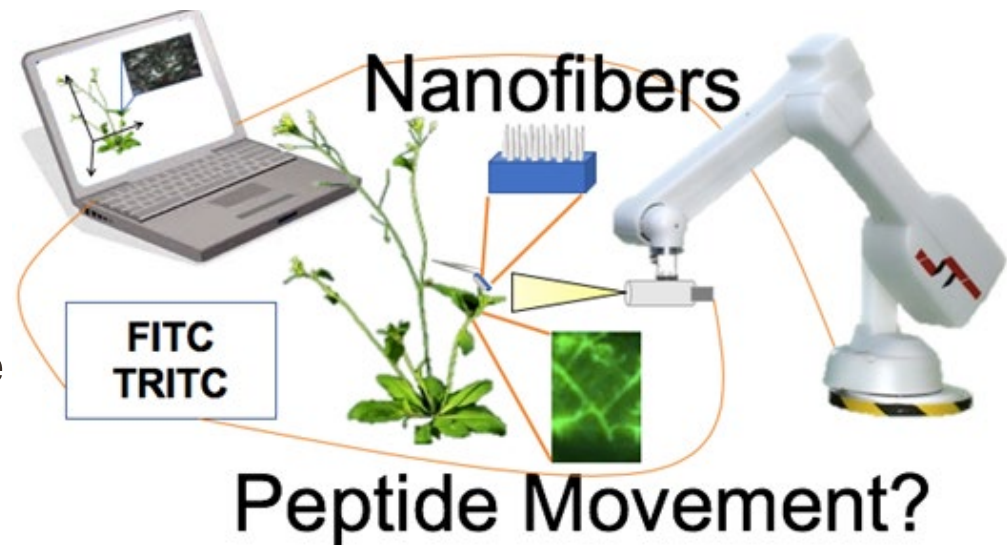
K: leaf of WT



# Conclusion and Future Work

Peptide trafficking was successfully observed in live plants non-destructively, using the fiber-optic fluorescence microscope.

- PSK is mobile.
- Receptor level may change in response to peptide ligand.
- Receptor level may impact the trafficking level of PSK.



Future: These preliminary experiments will be repeated and expanded, and the microscope will be upgraded further.

# Acknowledgments

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- **Dr. Robert Frank Standaert**
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- Thomas Ntim



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- **Dr. Jean Greenberg**
- Dr. Joanna Jelenska
- Jessica Morgan
- Dian Liu



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Bioimaging Science Program**



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Thank you

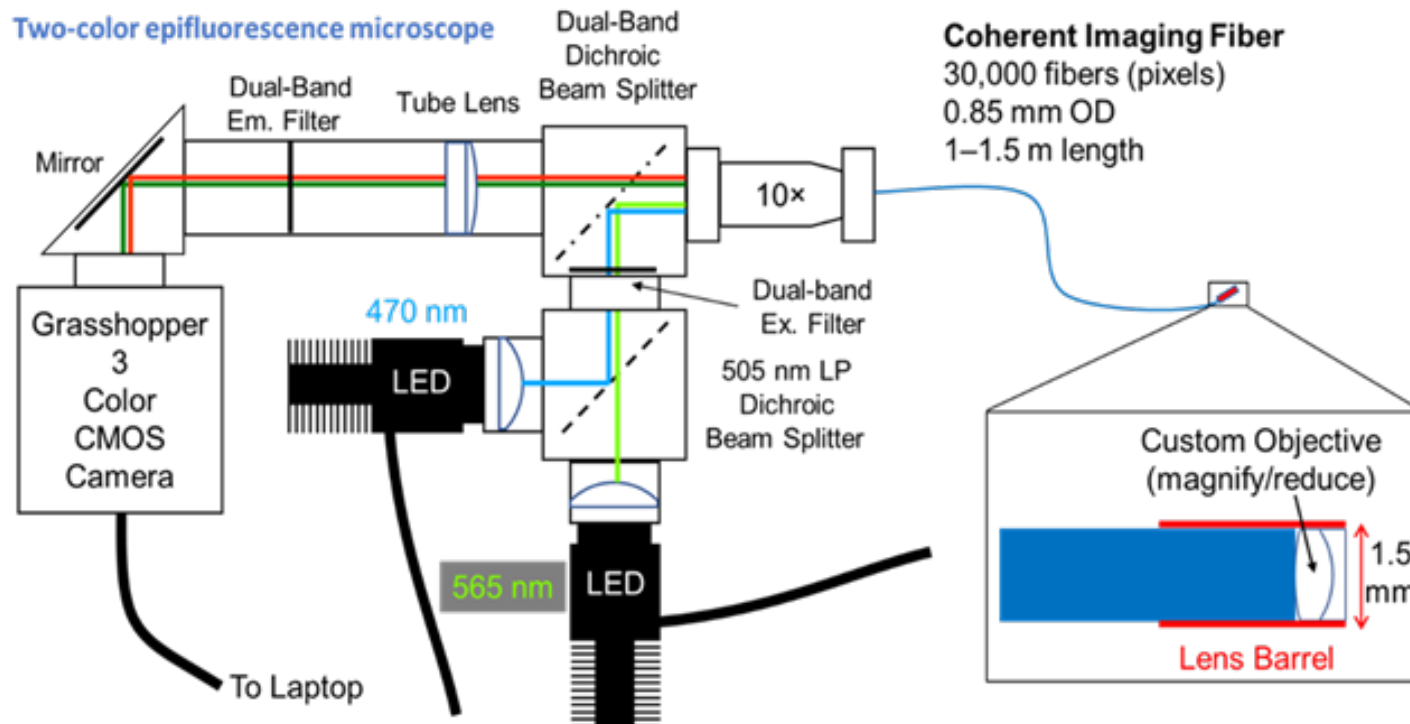


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Questions?



# The Microscope and Imaging



The two-color optical module is an adaptation of published designs<sup>4,5</sup>

## Imaging

GFP: Blue Excitation (470 nm)

TAMRA (TMR): Green Excitation (550 nm)

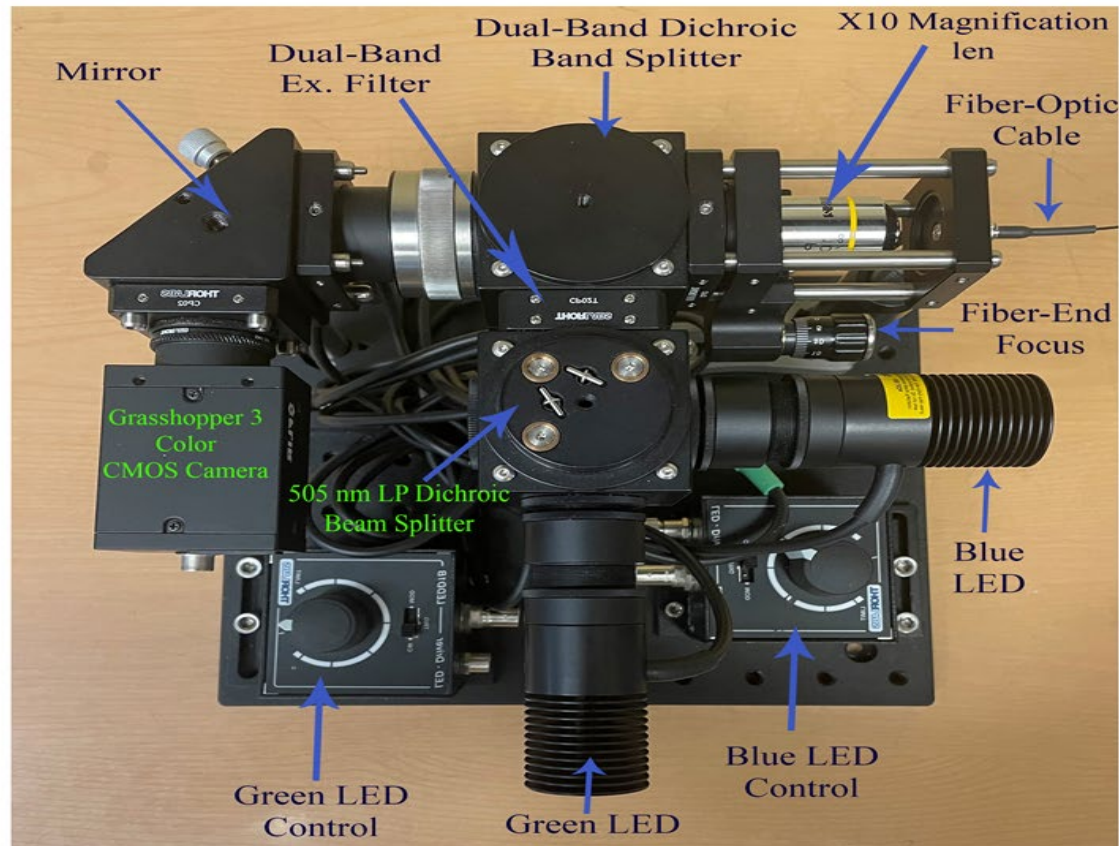
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# The Microscope and Imaging



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