Towards an Improved Laser Induced Fluorescence Imaging Technique in Pursuit of a Portable System

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Introduction and Motivation

- TA2: Develop Technology and Methodology Using and Observing Biota
- Goal: Detect contamination - nuclear fallout, mining waste, metals, organics, etc. through accumulation in biota
- Non-invasive, remote laser induced fluorescence (LIF) technique
  - Previous work focused on using the “Biofinder” on moss
  - New experiments focus on optimizing different, chlorophyll focused laser, for field application and potential use with different vegetation
  - Exploration of new laser wavelengths for cost-effective, portable unit

Mission Relevance

- NNSA mission has a focus on detection and prevention of nuclear proliferation activities
- This study is developing a remote-sensing technique for detecting biological response after exposure of plants to metals of interest:
  - Mining waste
  - Nuclear waste
  - Nuclear event
- Remote sensing technology can be used to:
  - Identify presence and type of contamination
  - Produce maps and spatial distributions of contamination
  - Pin-point the source and aid in bioremediation

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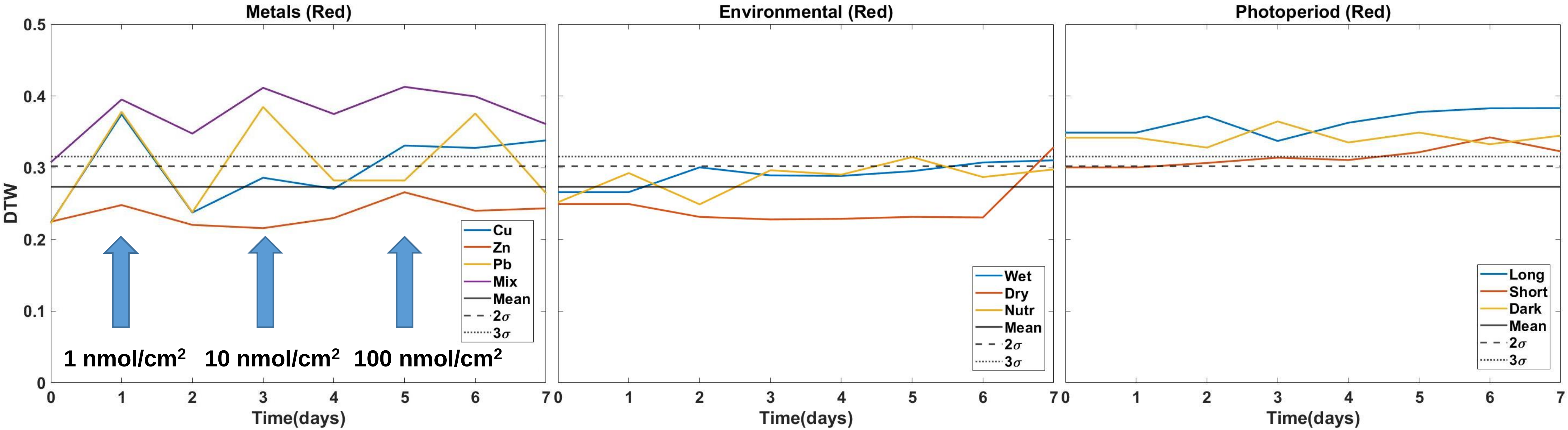
Technical Approach - Stressors

- Designed experiment to test multiple conditions for moss exposure to heavy metals, photoperiods, and environmental stressors

- Copper	- Short photoperiod	- Excess nutrients
- Zinc	- Long photoperiod	- Draught
- Lead	- No photoperiod	- Flood

- Several trials testing the above stressors along with control
- Metal results presented at UPR in Atlanta in 2021
- Currently in pursuit of exploration of best analytical methods for image batch processing
- Experiments inspired by improved need for sensitivity and suggestions by Wendy Kuhne at Savannah River National Laboratory regarding environmental impact and variability

Results

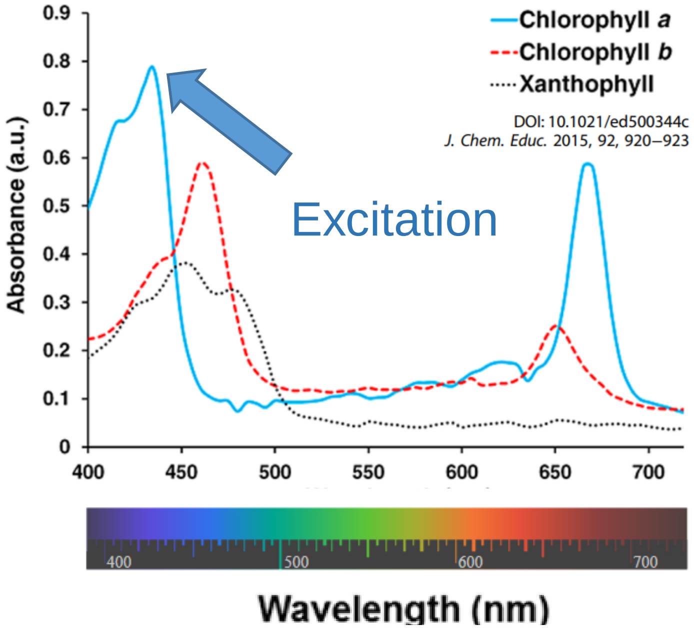


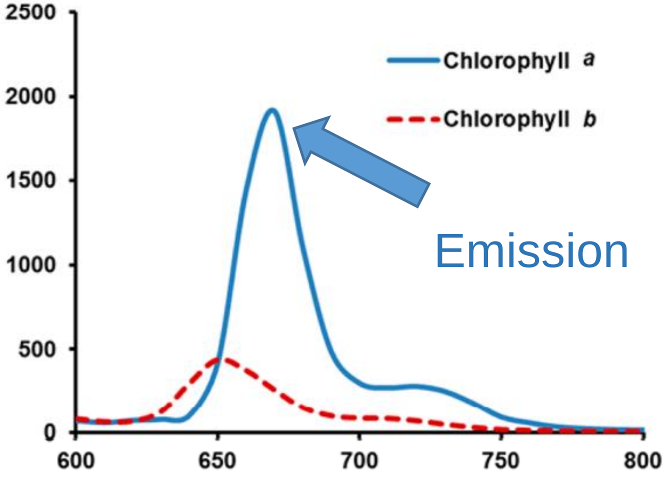
- Improved sensitivity: nmol/cm^2 level metal concentrations detected successfully
- Environmental conditions do not interfere with metal detection
  - Indistinguishable from control
- Photoperiod does produce overlapping color shifts so need to investigate further

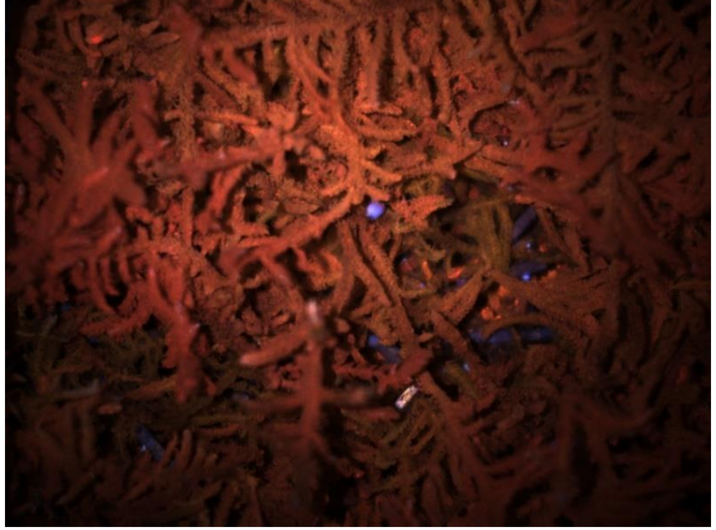
Technical Approach – Chlorophyll

Ayudhya, T., Posey, F., Tyus, J., and Dingra, N. (2015). Using a Microscale Approach To Rapidly Separate and Characterize Three Photosynthetic Pigment Species from Fern. *Journal of Chemical Education, 92 (5),* 920-923.

- Investigate if stress input is chlorophyll related: Designed experiment to test new chlorophyll a and b specific lasers – experimental development with Lawrence Livermore National Lab
    - Lasers: 445 nm and 462 nm blue lasers
    - Filters: 650 nm and 670 nm bandpass filters
- Imaged whole moss masses, individual moss fronds, finally extracted chlorophyll from fronds and measured chl a/b ratios independently
- New system is promising
    - Better specificity and sensitivity
    - Power- and cost-effective portable laser system



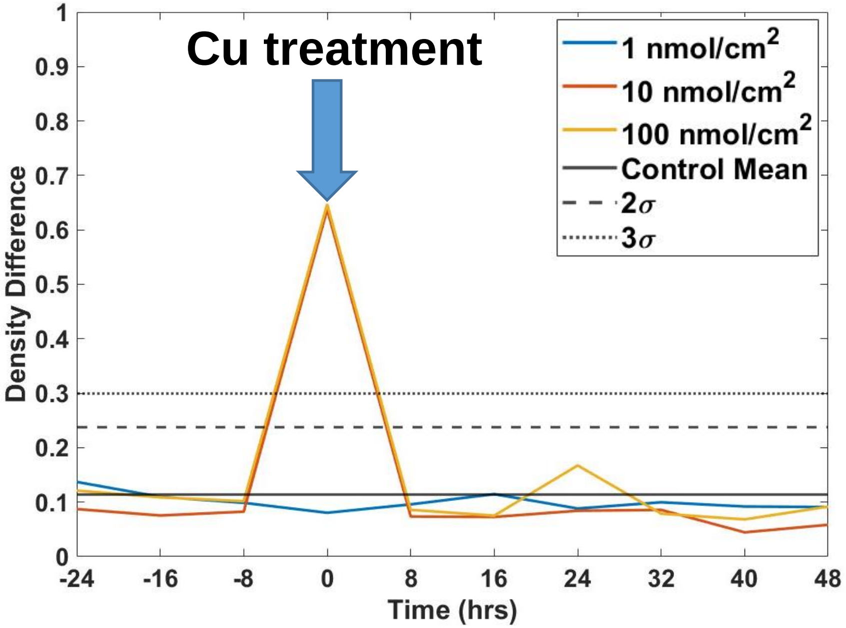


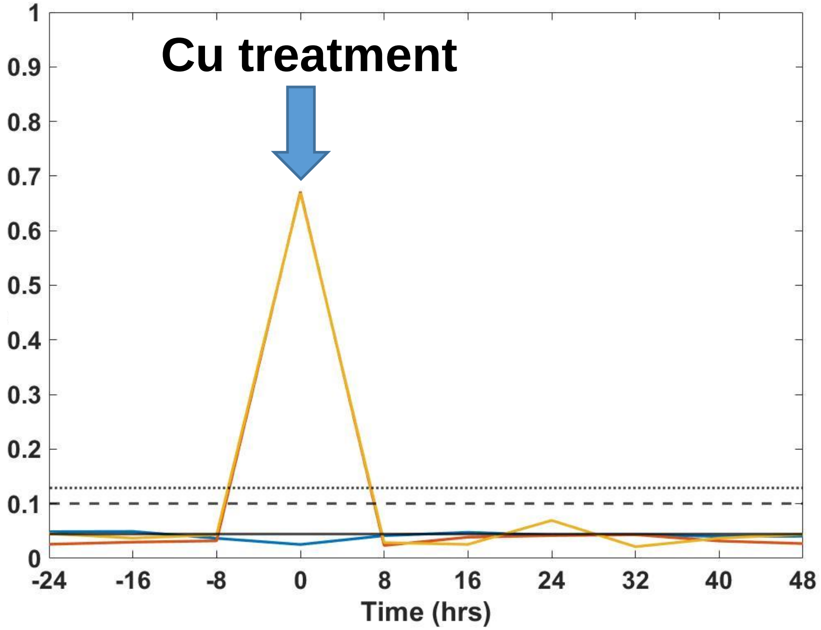


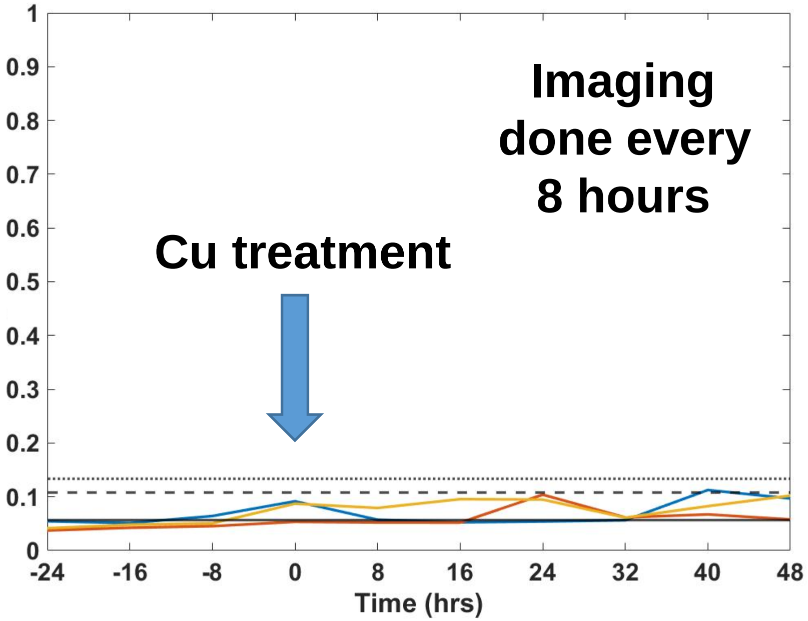
445 nm

462 nm

355 nm + 532 nm

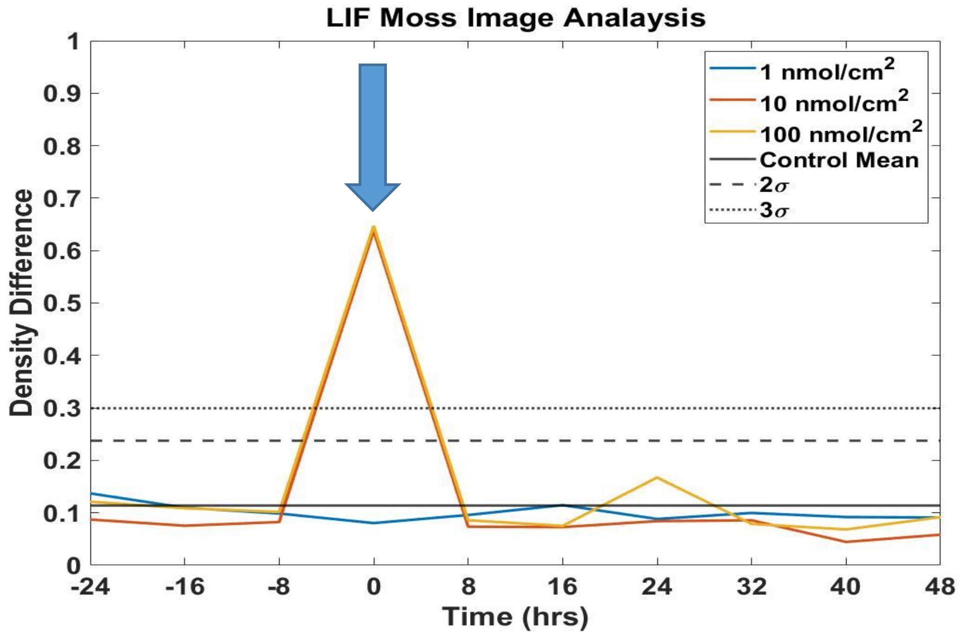


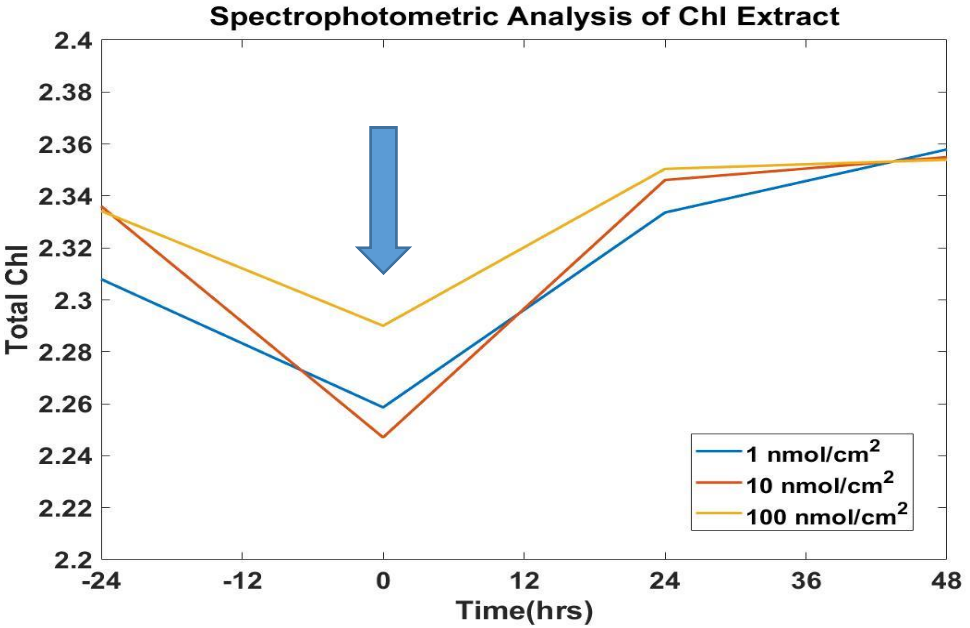




Results – Chl Extraction

- Frond imaging with LIF followed by chlorophyll extraction and extract analysis for total chl content with spectrophotometer and imaging of same extract with LIF
  - LIF of moss after Cu dose significantly different from control.
  - Spectrophotometric analysis showed lower total chlorophyll after Cu dose






ACID

Expected Impact

- Possibility of real-time detection of metal contamination in biota
- Limits need for extensive sampling and laboratory analysis
- Allows targeted sampling and saves on analytical costs
- Not limited by time of day/light conditions (as opposed to NIR) and can be used at m-scale distance
- Low energy use and lower payload – portable designs with in-situ data analysis
- Flexibility in camera type used for capturing images (CMOS, cell phone, and tablet/computer tested so far)
- Field testing on-going for handheld unit with adaptation to remote sensing via drone as the end goal

MTV Impact

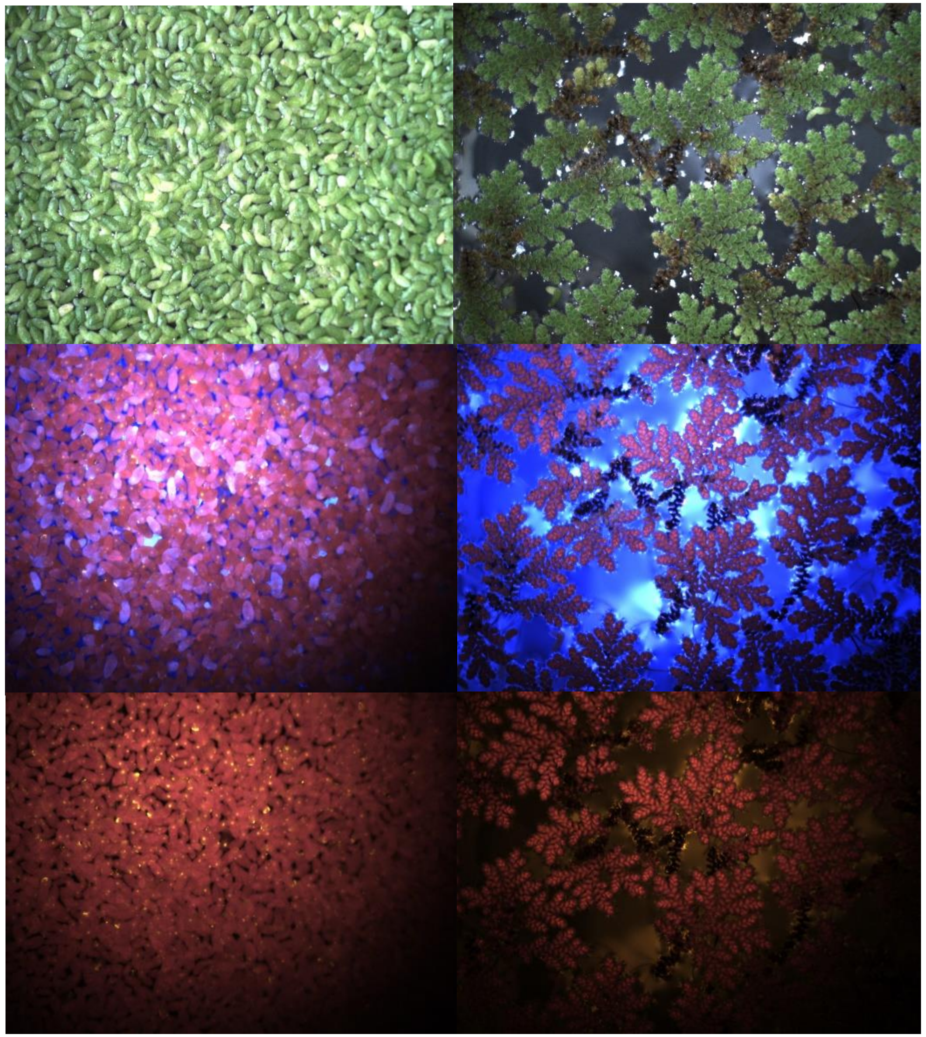
- MTV currently supports one graduate, and one undergraduate student and has allowed for collaboration with national laboratories
  - Impact on experimental design through meetings
  - Discussion of future collaborations, and opportunities for site visits are ongoing with hope of application or internship in the coming year
  - Opportunity to connect and network with other groups at UPR
- Savannah River National Laboratory has continued to be a source of guidance on contamination levels and possible applications
- Lawrence Livermore National Laboratory offered guidance and expertise in experimental design, testing of laser system, and sample preparation
- Collaboration on separate project - Pacific Northwest National Laboratory

Conclusion

- Experiments conducted give us a better understanding of challenges to application in the field – environmental stressors and photoperiod length
- New chlorophyll specific laser wavelengths were able to detect as low as 10 $nmol/cm^2$ levels of Cu.
    - Smaller size and affordable cost make blue lasers ideal for remote applications and testing
    - Need to further test impacts of external light conditions and maximum distance
- LIF results of moss and their chl extracts showed promising results and help explain underlying physiological changes in moss (total Chl and a/b ratios)
- Prototype of portable system travel ready, but more field testing is needed

Next Steps

- Application to different vegetation types
- Finalize portable system tests in lab followed by deployment in the field
- Tests for external light input and impact of distance for possible laser adjustment
- Complete code for field batch processing
- Finalize analysis of frond and chlorophyll extraction images
- Work with National Labs to optimize LIF to meet their analytical needs and field conditions



Acknowledgements

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