Analysis of Laser Induced Fluorescence Images of Plants Exposed to Metal Contamination and Environmental Stress

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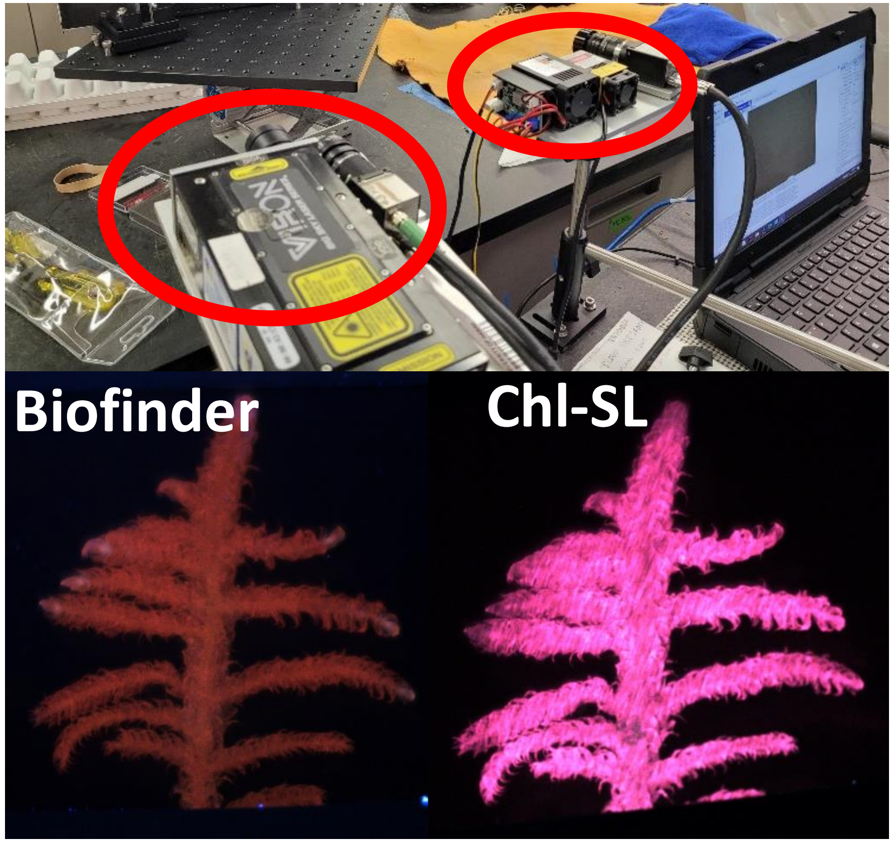
Introduction and Motivation

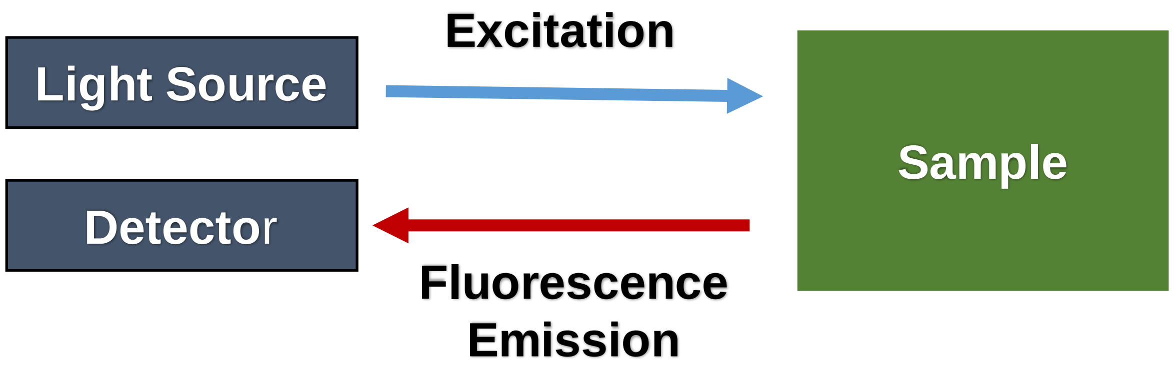
- Thrust Area 2: Tasked with developing a technology and methodology using and observing biota
- Goal: Identify presence and type of metal contamination (nuclear fallout, mining waste, etc.) through monitoring biota
- Non-invasive, remote laser induced fluorescence (LIF) technique
  - Previous work successfully used the "Biofinder" on moss dosed with a single metal
  - New experiments focus on multiple metals, different plants, possible interference from environmental stressors, and testing a new chlorophyll- specific laser (Chl-SL)



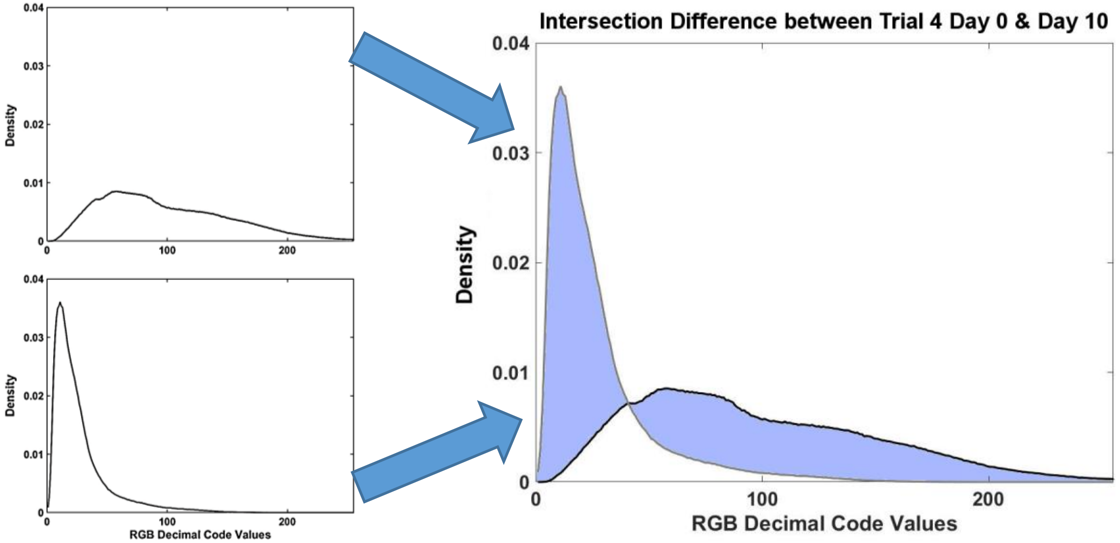
Mission Relevance

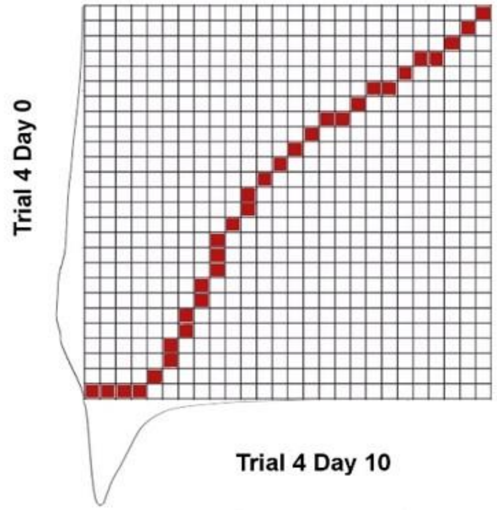
- NNSA mission has a focus on detection and prevention of nuclear proliferation activities
- This study explores the feasibility of a remote-sensing technique for detecting biological response after plants are exposed to metals of interest (e.g. from mining waste, nuclear waste, nuclear event)
- Remote sensing technology can be used to:
    - Identify presence, source location, and extent of contamination (map of spatial distribution)
    - Identify types of contamination (different/multiple metals)
    - Aid in bioremediation (identify plant metal uptake/saturation)

Technical Approach – Moss masses dosed with metals and exposed to variable conditions

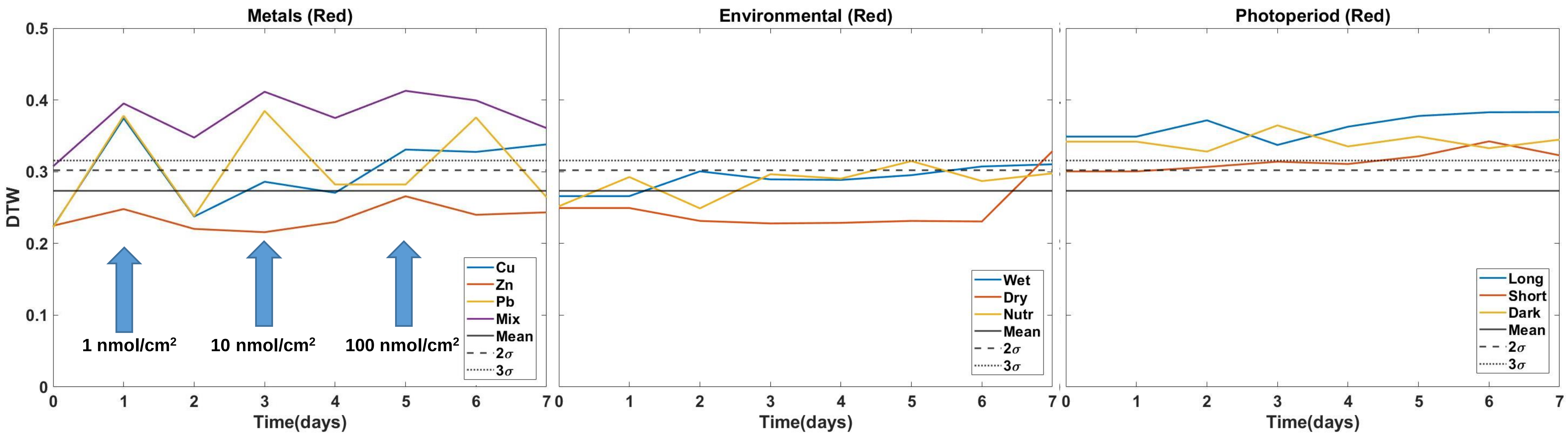


- Use laser to excite molecules to higher energy level which results in an emission of light (fluorescence)
  - “Biofinder” (532 nm green laser and 355 nm UV laser)
  - Chl-SL (445 nm and 462 nm blue lasers)



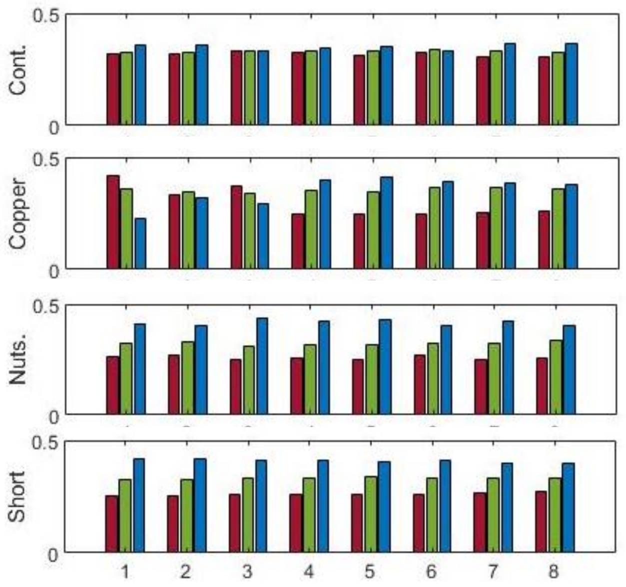


Results – Moss Masses: Metals & Environmental Stressors



- 3 different metals applied:
  - Pb and mixture of metals are clearly identifiable at 1-100 nmol/cm2 levels shortly (minutes) after exposure independently on data processing method, but Zn and Cu require two-color DTW to be identifiable at that level
- Environmental factors:
  - Drought/flood, nutrient rich/poor conditions do not interfere with metal signature (signal undistinguishable from control)
  - Photoperiod length affects LIF image and may impact metal detection

Results – Metals and Environmental Stressors



- Ratios of single-color to all color differences used to assess deviation from control
- "Ratios" developed because other data analysis methods were not successful in differentiating all stressor types (e.g. metal from photoperiod).
- "Ratios" allow not only for identification of plant stress, but help in determining the type of stressor.

*dose	Control			Copper			Nutrients			Short		
day	red	green	blue	red	green	blue	red	green	blue	red	green	blue
1	0.321	0.323	0.357	0.415	0.359	0.226	0.262	0.325	0.413	0.256	0.325	0.420
2*	0.321	0.323	0.357	0.335	0.345	0.320	0.270	0.330	0.400	0.256	0.325	0.420
3	0.334	0.330	0.335	0.370	0.338	0.292	0.250	0.310	0.440	0.261	0.330	0.409
4*	0.322	0.332	0.346	0.249	0.349	0.401	0.260	0.316	0.425	0.260	0.332	0.408
5	0.315	0.332	0.354	0.246	0.342	0.411	0.251	0.318	0.430	0.259	0.336	0.406
6*	0.328	0.338	0.334	0.247	0.364	0.389	0.270	0.325	0.405	0.258	0.333	0.409
7	0.305	0.332	0.363	0.255	0.363	0.382	0.251	0.323	0.426	0.268	0.332	0.400
8	0.306	0.326	0.368	0.263	0.360	0.377	0.258	0.338	0.405	0.273	0.329	0.397

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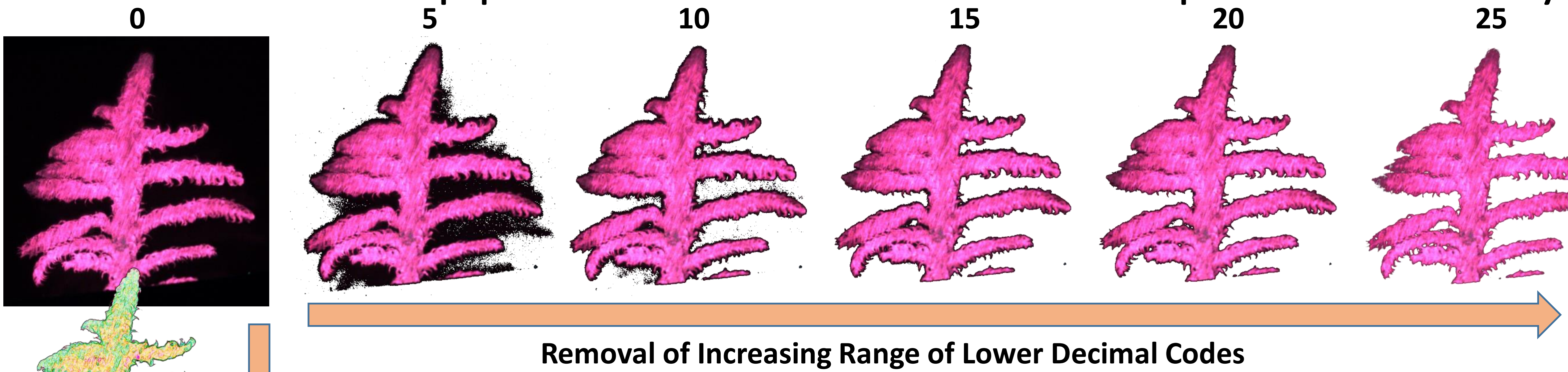
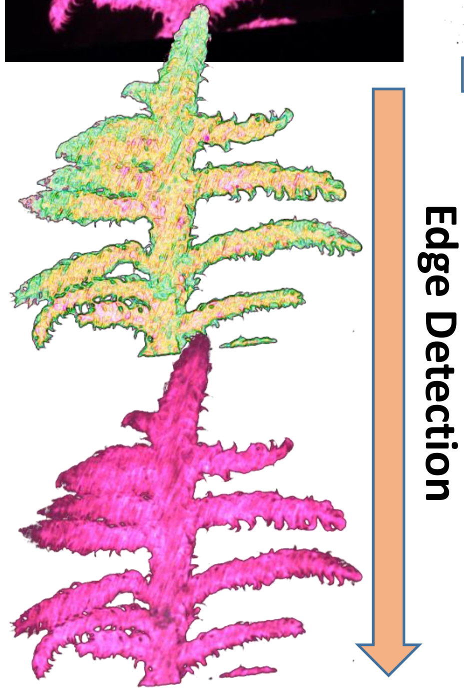
Technical Approach - Moss Frond Specific Analysis

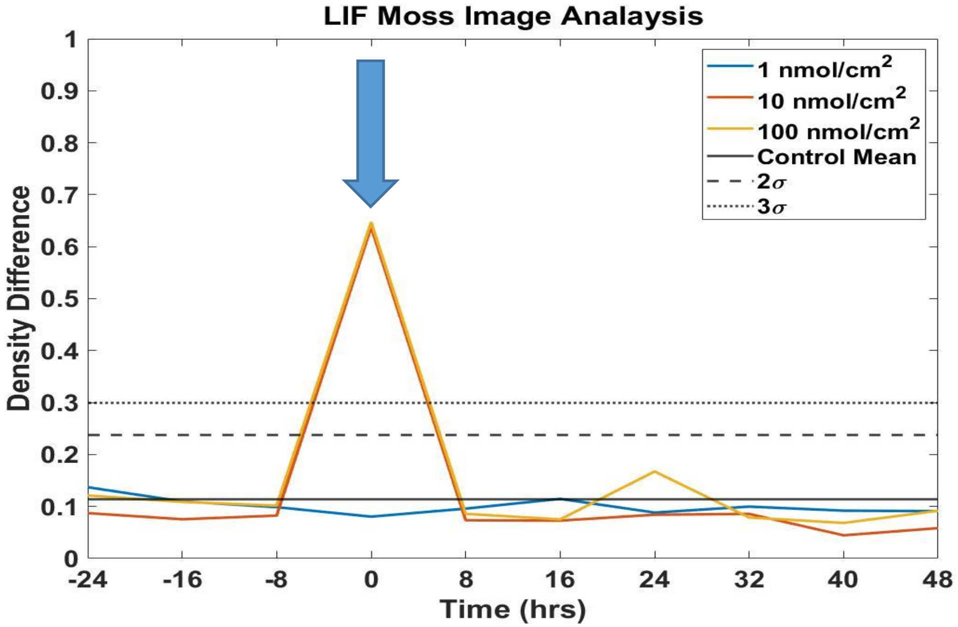


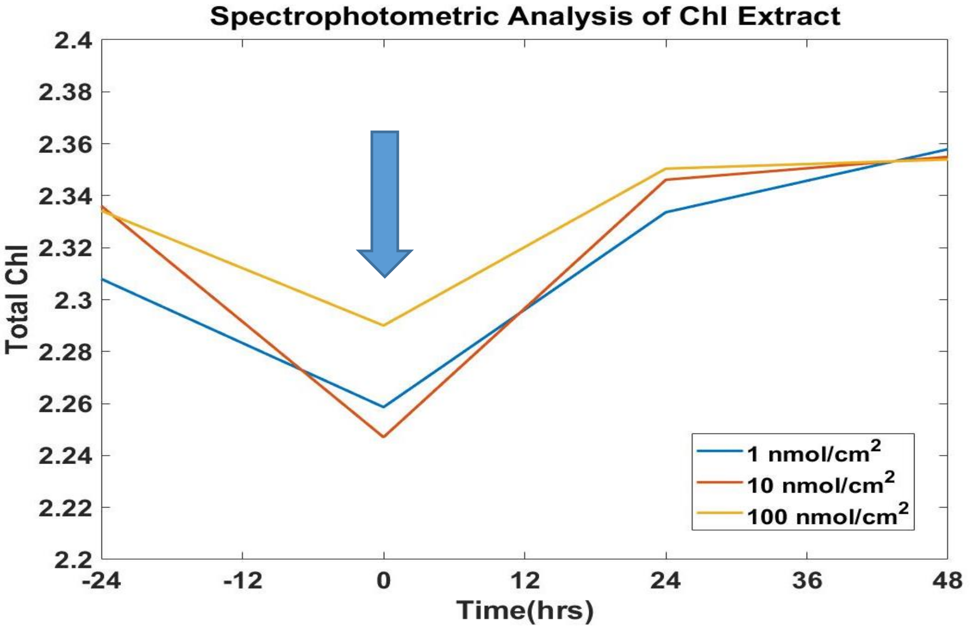


- Objective – remove background/darker decimal code values (DCV; 0-15) not related to plant and plant stress
- Problem - unwanted pixels can show up in the frond data
- Solution - edge detection was used to find the boundary between high and low decimal codes; then edge was extracted to create a polygon mask for creating the layer for data for analysis

Results – Moss Fronds: Single Metal Dosing

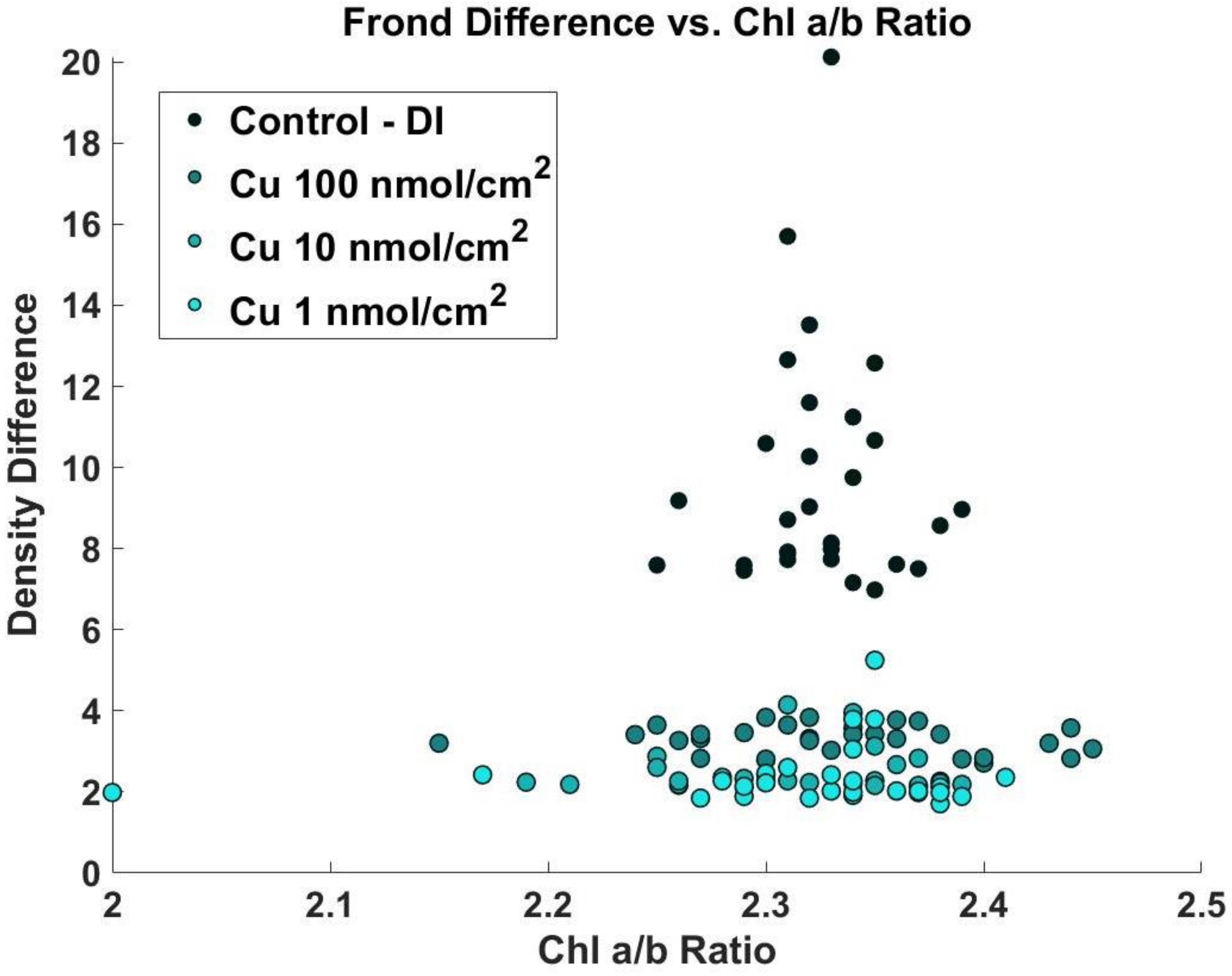
- Cu adsorption confirmed within minutes of dosing through change in LIF (significant difference from control)
- Chemical analysis of fronds shows decrease in chl a/b ratio and overall chlorophyll content with increase in Cu uptake – a/b ratio decrease confirmed by Chl-SL
- Imaging over time before and after dosing reveals immediate and short-term response





Results – Moss Fronds

- New sample preparation methods improved image processing, for 100+ samples with metal and chlorophyll content extracted for each.
- Edge detection is provides better specificity in results than original method of removing DCV
- Correlation between change in Chl a/b ratio and histogram difference creates region of control and contaminated samples.
- Could use second profile as estimate of contamination without need for chemical analysis



Expected Impact

- Possibility of real-time detection of metal contamination in biota
- Helps avoid extensive field 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 (see poster by H. Currier)
- 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

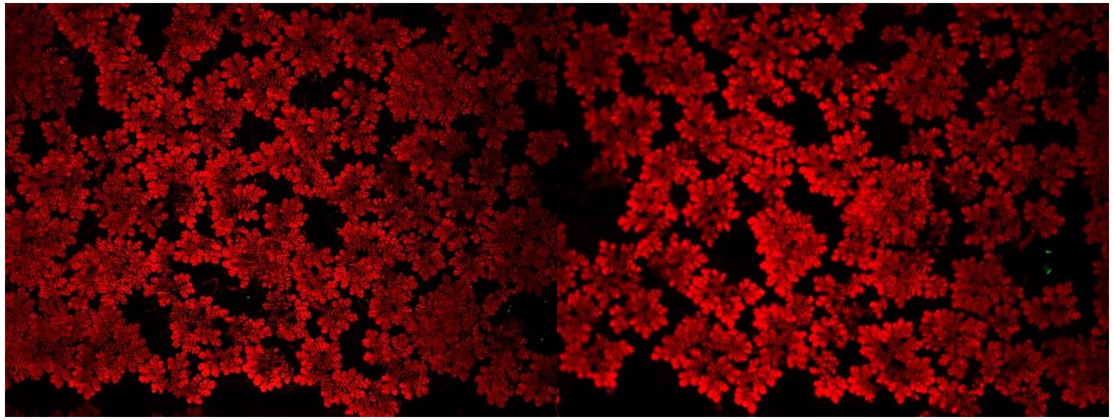
- MTV supports one graduate and one undergraduate student, and has allowed for collaboration with national laboratories
  - Improved experimental design through meetings with LLNL
  - Discussion of future collaborations, and opportunities for site visits SRNL
  - Internship at INL – machine learning classifiers for real time processing of thermal imagery (see poster by K. Truax)
  - Internship at LLNL – image processing user interface for sparse data (SIMS)
  - Previous work with PNNL on machine learning for large sets of gamma spectra
- Presentations: Marc XII, UPR 2022, WOSMIP VIII
- Hosted an International Workshop at UH on alpha spectroscopy
- Methodology for LIF imaging technique published in Applied Sciences

Ongoing Work and Next Steps

MTV Undergraduate: Haley Currier

- UROP proposal to explore Chl-SL limitations
    - Maximum operating distance
    - Impact of external light
    - Using water fern (Azolla)

Control Lead



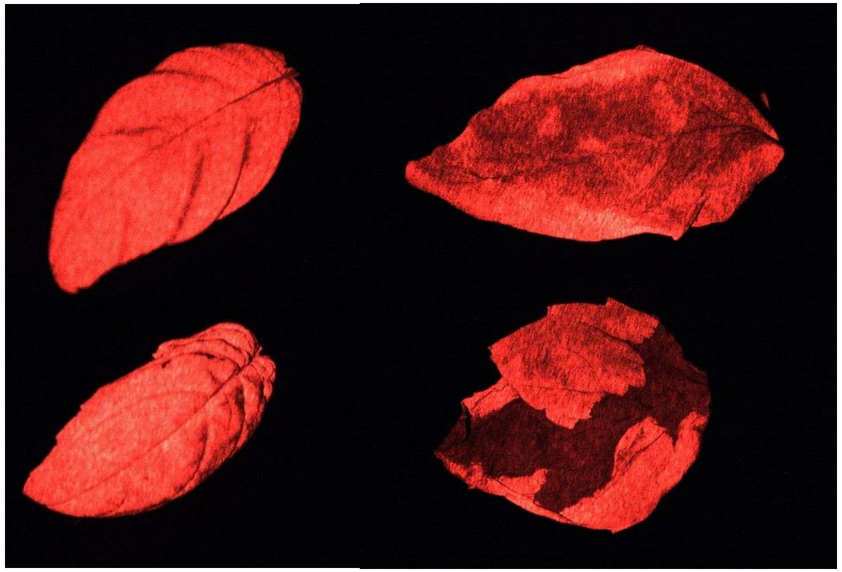
REU Undergraduate: Sarah Obra-Nakata

- Compared basil plants grown hydroponically to those cultivated in soil under draught and heavy metal stress conditions

Normal Draught

Control

Copper



Future work looks to do more testing in the field while coordinating with labs about potential drone applications and implementations.

Conclusion

- Successful implementation of image batch processing for both moss masses and fronds
- Use of ratios of single-color to all color differences results in ability to characterize not only plant stress but type of stress (metal vs. photoperiod)
- Application of new sample preparation plus edge detection provide better results for frond analysis.
- Chemical analysis shows that changes in a/b ratios and decrease in overall chlorophyll content directly correlate with metal uptake, which is also confirmed using Chl-SL image analysis through a shift in Chl a/b ratios or decimal code values when compared to histogram difference
- Chl-SL improved sensitivity to detect changes in plants and successfully applied to multiple types of vegetation
- Testing shows working distance and other limitations for current system – see poster by H. Currier
    - 5-meter working distance with low watt units, works well in lab, also tested as a “hand held”
    - Semi-conductor diode may limit use to low light conditions without time-gating
    - Metal dosed basil leaves removed and imaged after 24 hours have a distinct “metal” signature

Acknowledgements

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