Quantifying Moss Response to Contaminant Exposure Using Laser Induced Fluorescence

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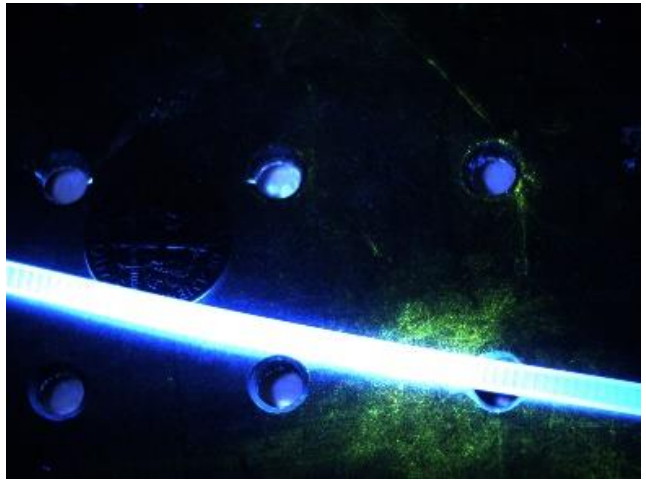
Introduction and Motivation

- TA2: Develop Technology and Methodology Using and Observing Biota
- Goal: Detect contamination - nuclear fallout, mining waste, metals, organics, etc. through its accumulation in biota
- Project developing a non-invasive, remote laser induced fluorescence (LIF) detection technique using the Biofinder (Misra et al., 2018), currently focusing on moss
  - Biofinder characterizes biological material through LIF
  - Moss is robust, simplistic species, used extensively in environmental monitoring – atmospheric accumulation of metals
  - BUT traditional approaches collecting and analyzing moss are labor intensive and destructive – <u>need for remote sensing approach</u>
- Hypotheses:
  - Metal deposition induces a physiological response in moss
  - Response from moss can be detected using LIF
  - Images of LIF can be analyzed to detect/identify contamination



Mission Relevance

- The NNSA mission is focused on detection and prevention of the militarization of nuclear weapons
- The work presented aims to develop a non-invasive remote technique to detect biological response in vegetation after its exposure to metals of interest from:
  - Nuclear event
  - Mining waste
  - Nuclear waste
- End goal: Develop remote sensing technology to survey areas of interest to:
  - Produce maps of metal/contamination spatial distribution and pin-point its source
  - Identify the presence and type of contamination
  - Aid in bioremediation

Technical Approach – Part 1

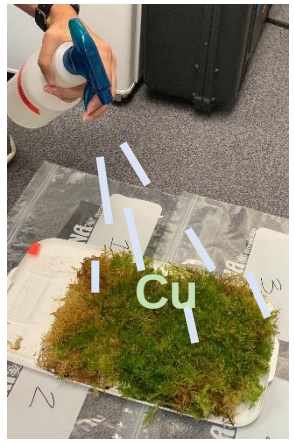
- In collaboration with Wendy Kuhne and Laura Tovo at Savannah River National Laboratory
- Two Part Methodology:
  - Part 1: Cultivate moss, dose with metal of interest ($CuCl_2$)
    - 10-day experiment, 1 control, 3 treated moss mats
    - 5 doses of copper chloride treatment (once every 48-hrs)

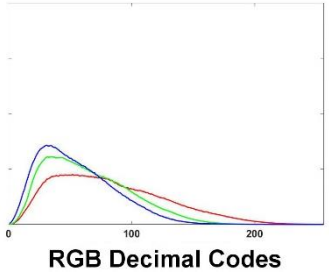
Day:	1	2	3	4	5	6	7	8	9	10
Trial 1	0	0	0	0	0	0	0	0	0	0
Trial 2	0.54	0.54	1.08	1.08	1.62	1.62	2.16	2.16	2.70	2.70
Trial 3	1.08	1.08	2.16	2.16	3.24	3.24	4.32	4.32	5.40	5.40
Trial 4	1.62	1.62	3.24	3.24	4.86	4.86	6.48	6.48	8.10	8.10

*table displays cumulative Cu concentration in $\mu mol/cm^2$

Technical Approach – Part 2

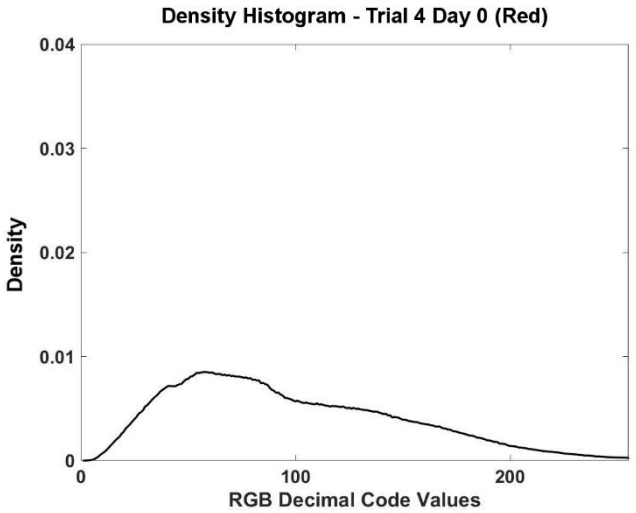
- Part 2: LIF Imaging using Biofinder and Data Analysis
  - Cu treated moss samples were imaged under LIF
    - Every 6-hrs for first 48-hrs, then every 24-hrs
  - Daylight imaging at .5 m distance using CMOS Camera
    - 532 nm green laser and 355 nm UV laser (Nd:Yg pulsed lasers)
  - Images collected for both lasers and each laser individually
  - Images processed in MATLAB – density histograms created from pixel count

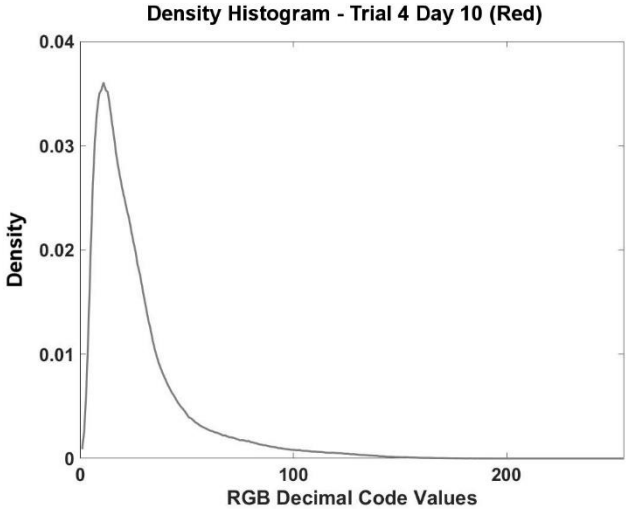




Technical Approach – Part 2 cont.

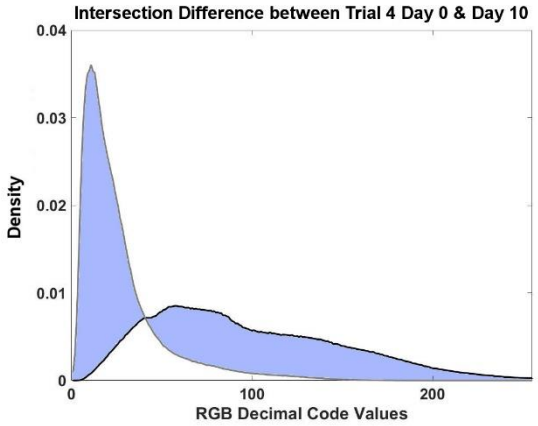
- Day-0 used as reference for comparison
- Shown here is Day-0 of Trial 4 to provide an example

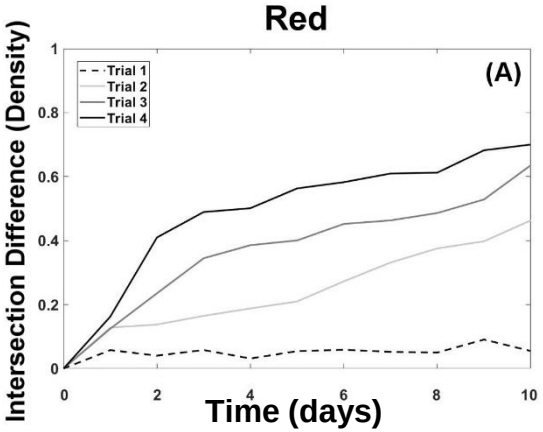




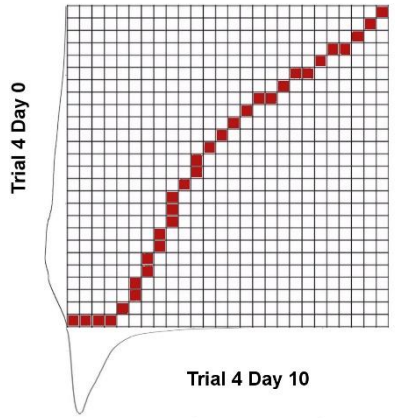
- Each image from each trial is compared to its Day-0 image
- Shown here is Day-10 of Trial 4 to provide an example

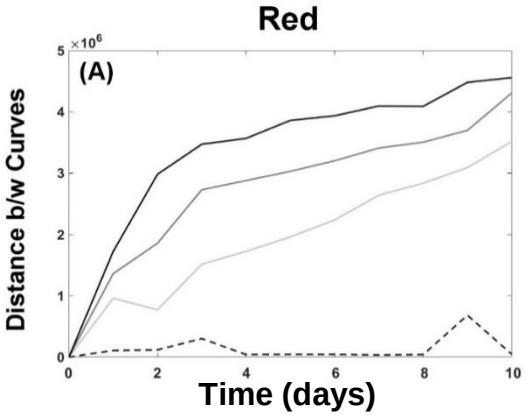
Density Histogram Differences



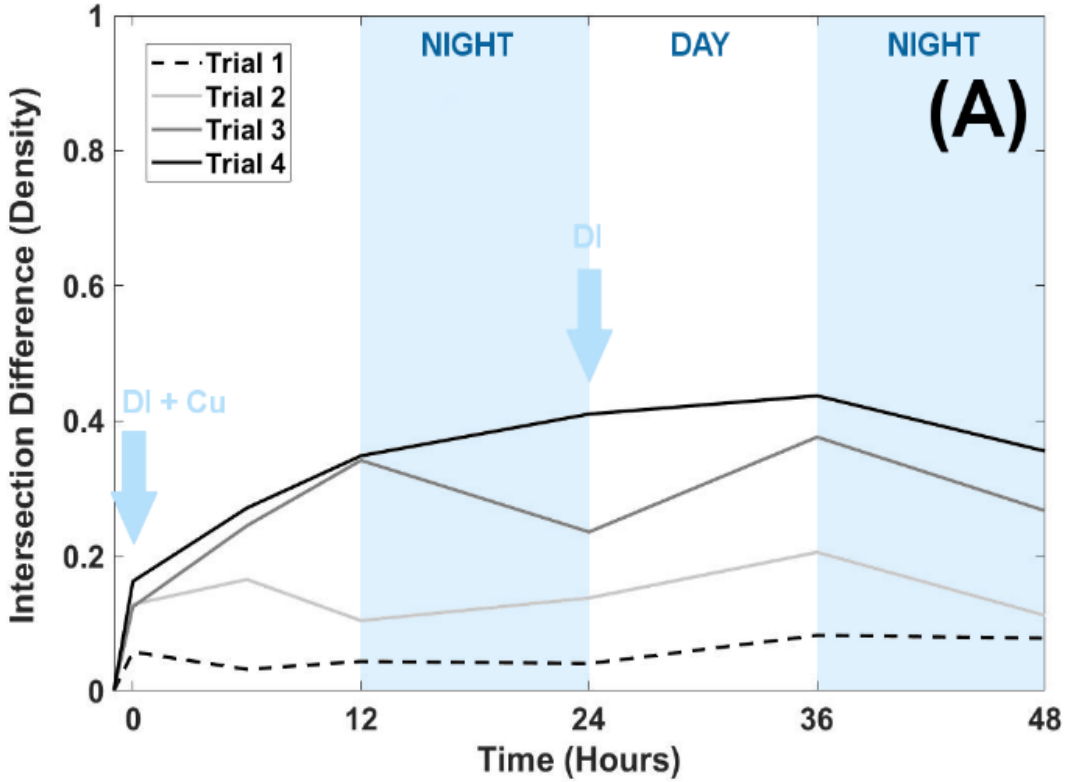


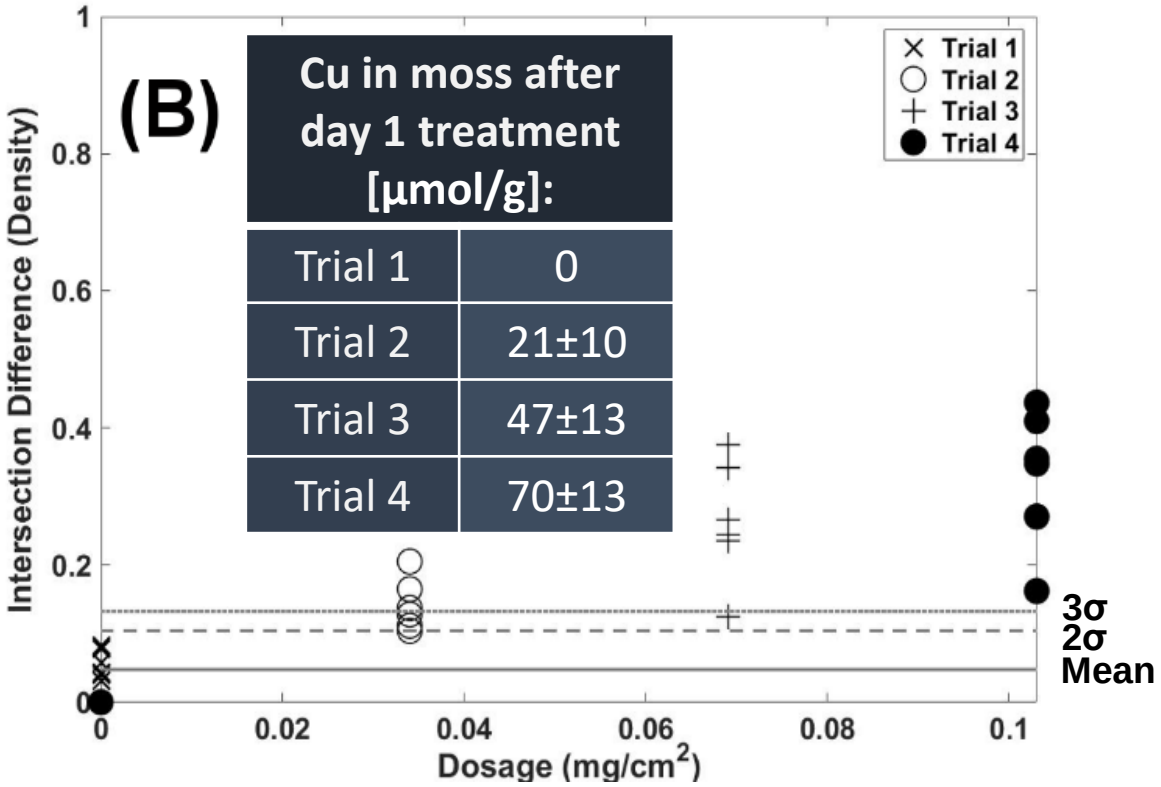
Dynamic Time Warping





Results – 48 Hour Imaging Analysis



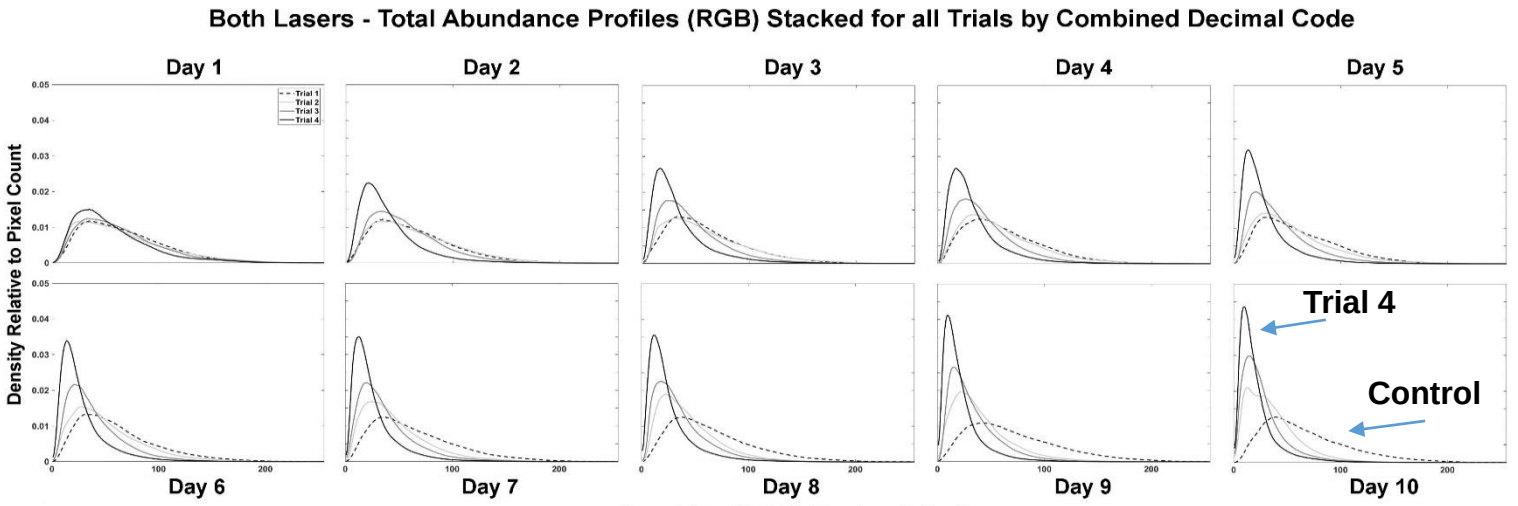


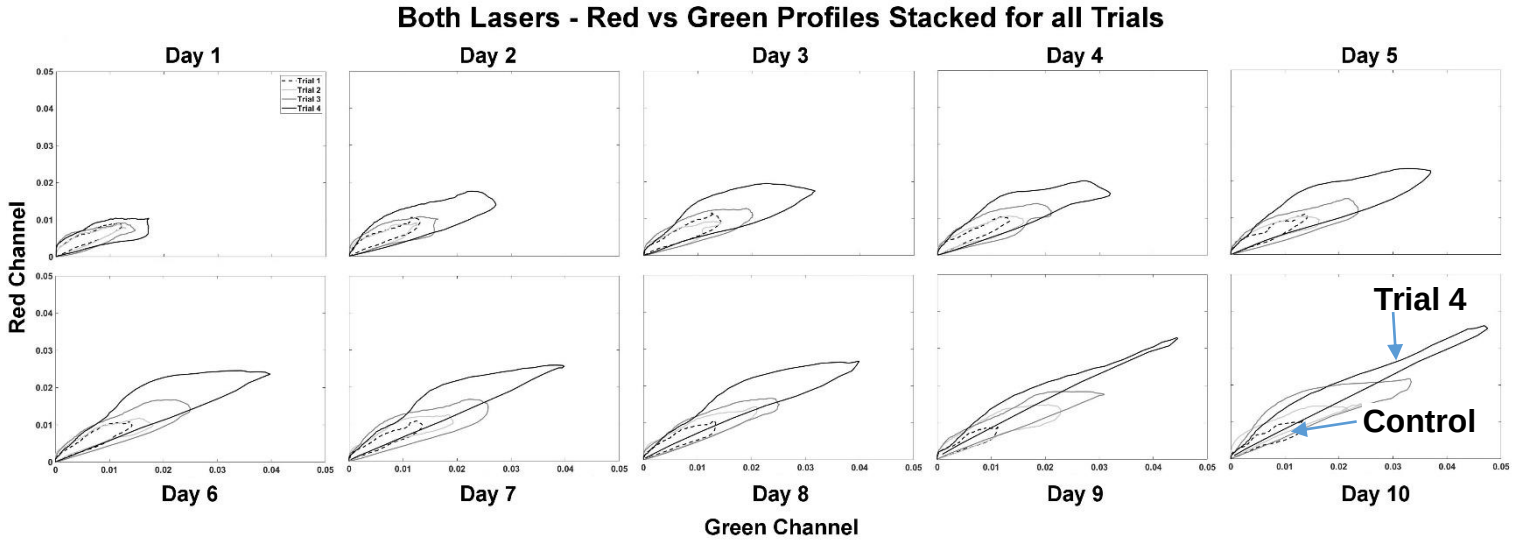
Cu in moss after day 1 treatment [µmol/g]:	
Trial 1	0
Trial 2	21±10
Trial 3	47±13
Trial 4	70±13

- Biofinder imaging within 5 minutes of dosing already produces measurable response
- All treated trials clear control average ± 2σ over 48-hours, never (within 48-hrs) return to pre-treatment level
- Curves show immediate response followed by steady increase and flattening

Results – 10-Day Imaging Analysis

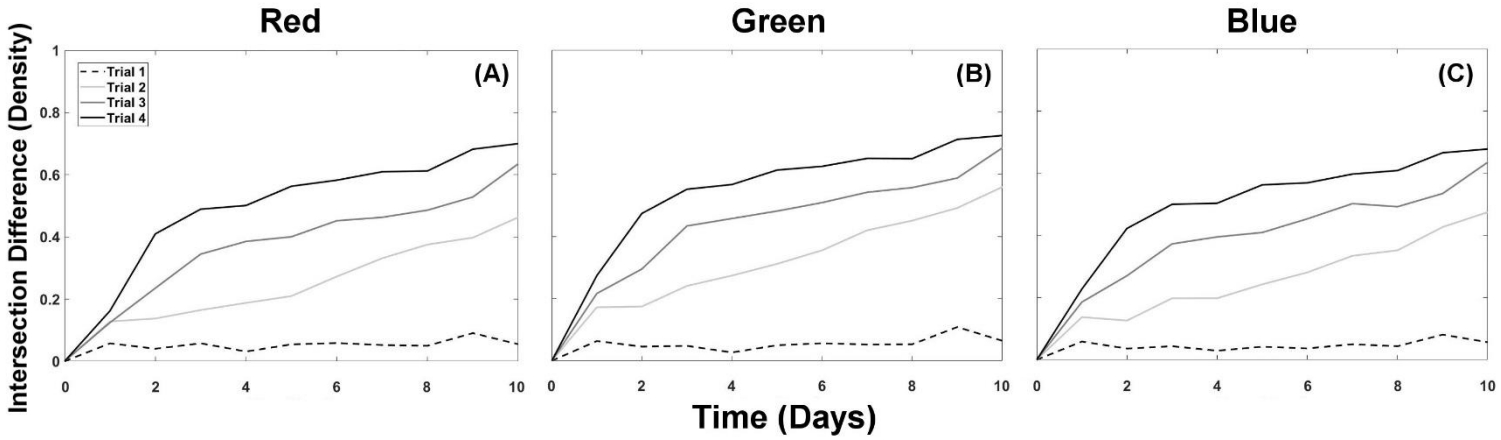
- One color analysis
  - With increased Cu dose shift to “darker,” lower RGB decimal code values
  - Curves more peaked, more right skewed
- Two color analysis
  - Relationship between red and green color channels – shapes change with Cu dose
  - Increased Cu toxicity leads to a longer, narrower “loop” as red and green become more similar, right skewed, and peaked



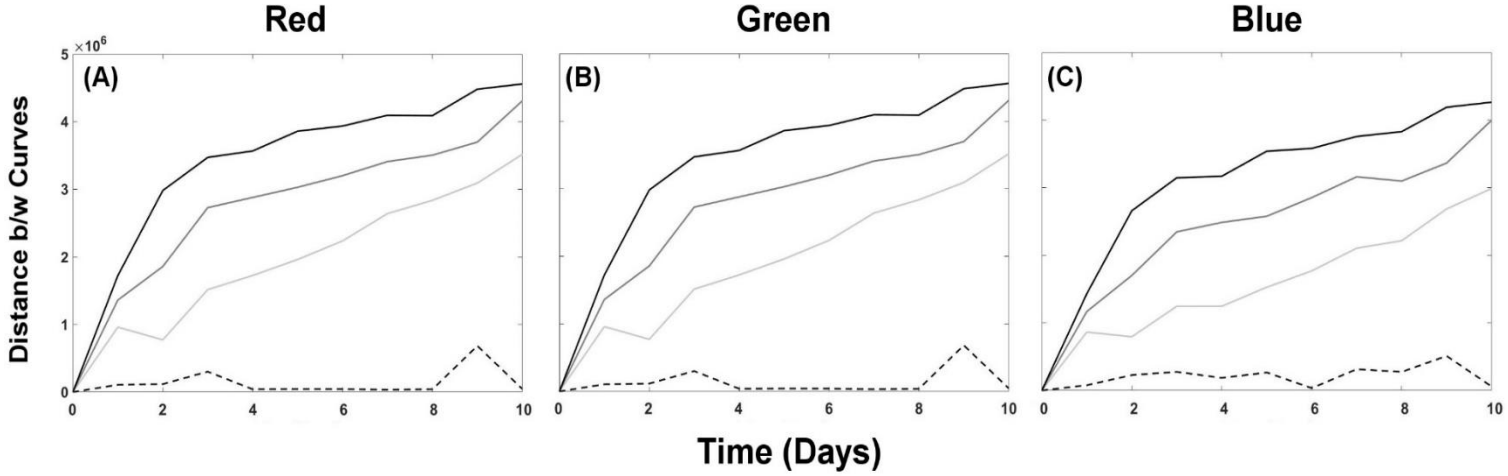


Results – 10-Day Imaging Analysis cont.

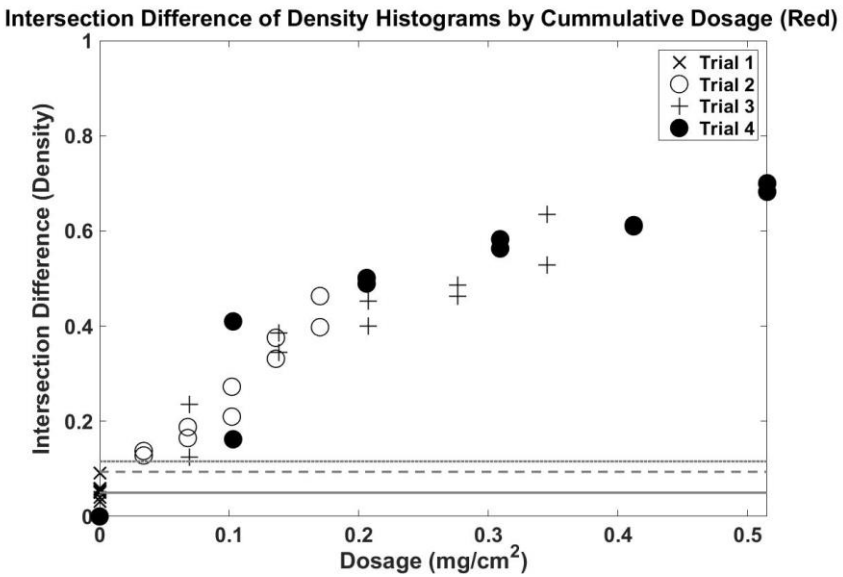
- Density Histogram Difference
  - Intersection difference similar between color channels
  - Increased difference with Cu dose
  - Curves show steep initial response followed by steady increase

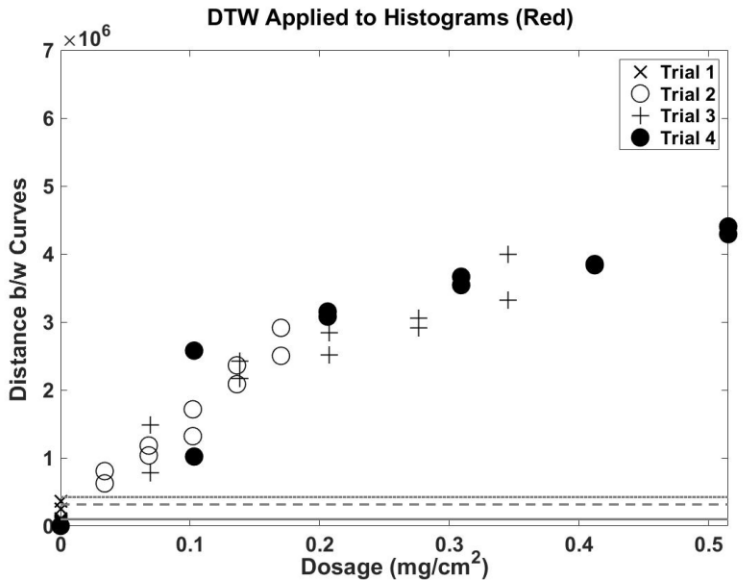


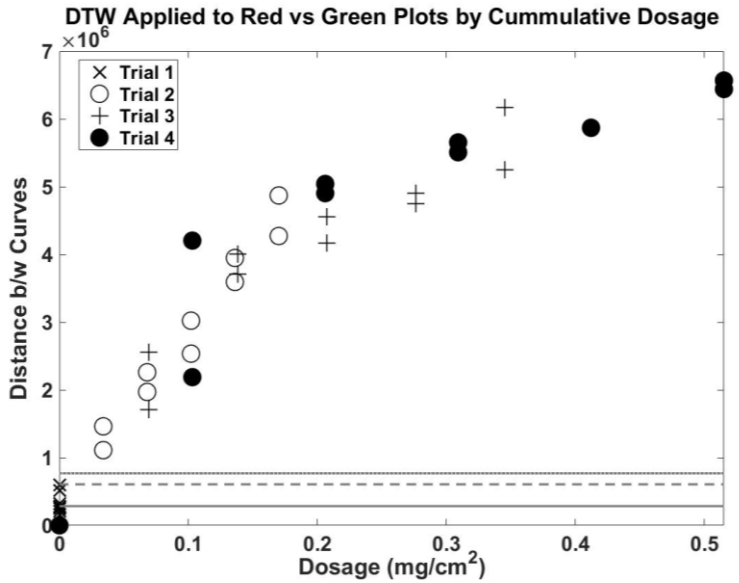
- Dynamic Time Warping (DTW)
  - Alternative method for curve comparison
  - Similar to intersection difference
  - Increase in distance equal to separation from day-0 with increasing Cu



Results – One Color vs. Two Color Analysis







- Trial 1 (control) closely clustered around its 10-day mean
- All treated trials above average ± 3σ of trial 1 starting day-1 treatment
- Appears multiple small or single large dose result in the same difference
- Two-color analysis appears to show greater sensitivity than one-color analysis
- Same trend between curves, greater separation from control average ± 3σ using DTW (two-color analysis)

Expected Impact

- Promise of laser methodology for field application
- Real-time detection of metal contamination in biota
- Eliminate need for extensive sampling & allows targeted sampling and laboratory analysis
- Help identify source of contamination and its distribution
- Not limited by time of day (as opposed to NIR)
- Low energy use – portable in current state
  - Further development of programming and housing needed
- End goal is remote sensing via drone

MTV Impact

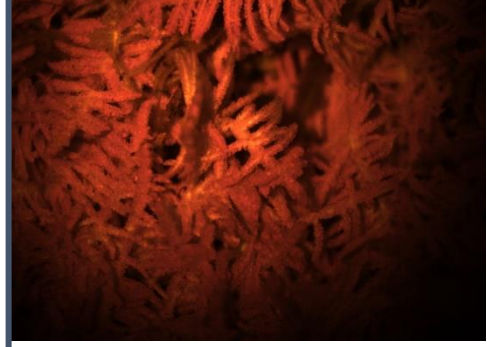
- MTV currently supports one graduate, and two undergraduate students and has allowed for collaboration with national laboratories
  - Co-developed experimental design
  - Discussed future collaborations, opportunities for site visits and internships
  - Next-Gen AI for Proliferation Detection: Domain Aware Methods Workshop
  - NSSC Alumni Speaker Series
- Potential technology field application - Savannah River National Laboratory
- Other collaboration and internship opportunities discussed - Pacific Northwest National Laboratory, Lawrence Livermore National Laboratory

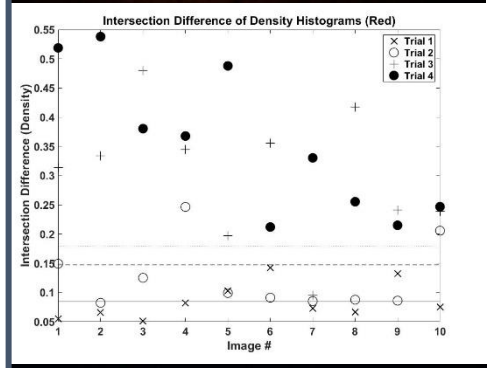
Conclusion

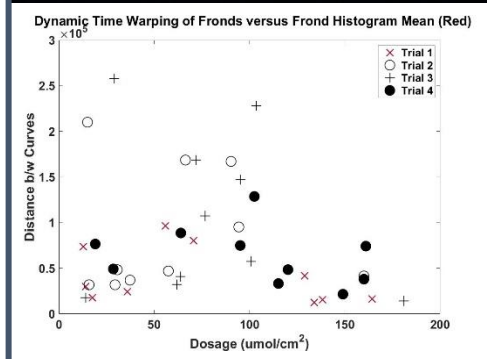
- LIF is a viable remote sensing method for detecting metal exposure in moss:
  - Moss physiological response to Cu results in measurable changes in LIF using the "Biofinder"
  - Single-color (red) and two-color (red and green) analyses of treated sample images show separation from control - All treated trials above 3σ of trial 1 control mean
  - This was a proof-of-concept study, lowest dose (340 mg/m^2) detection sensitive enough for extremely contaminated sites (Murgul Cu mine, Turkey) but need to improve sensitivity to <1 mg/m^2 to monitor environmental levels
- Preliminary data on other metals (Zn, Pb) showed promise, U method in development
- Continued work towards improved sensitivity will make methods field applicable
  - work with National Labs to establish desired sensitivity levels
- Develop methods for real-time processing, remote sensing, and field tests (e.g., at SRNL)

Next Steps

- Improved Sensitivity of Biofinder
  - Detection in the 1-10 nmol/cm^2 range needed for environmental levels
  - Establish sensitivity-to-distance relationship, optimize remote sensing
- Test Biofinder with other metals and biota
  - Do full experiments with Cu, Zn, and Pb; Begin development for U experiments
  - Application to other vegetation (bioaccumulators)
  - Chlorophyll tests to determine chl-a/b ratios effect on images
  - Moss and other plant stress tests: environmental, temperature, nutrients, etc.
- Undergraduate projects:
  - Cultivation of moss and new plants
  - Image analysis (moss mats vs fronds, other plants)
  - Chlorophyll analysis
- Foster Collaborations with National Labs
- Further Develop Machine Learning Technique
  - Assist with real time image analysis and contaminant identification







Acknowledgements

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