SAND2015-2354C

The Effects of Atmospheric Aging on Microbial Signatures

DTRA-Threat Agent Science

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Overview

- Background
- Project Objectives
- Technologies
- Technical Work/Experiments
  - Initial Rotating Drum Experiments
  - New Rotating Drum Experiments
  - Hygroscopicity – Modeling Implications
  - Field Studies
- Conclusions
- Future Experiments

A Quick Background on Agent Fate

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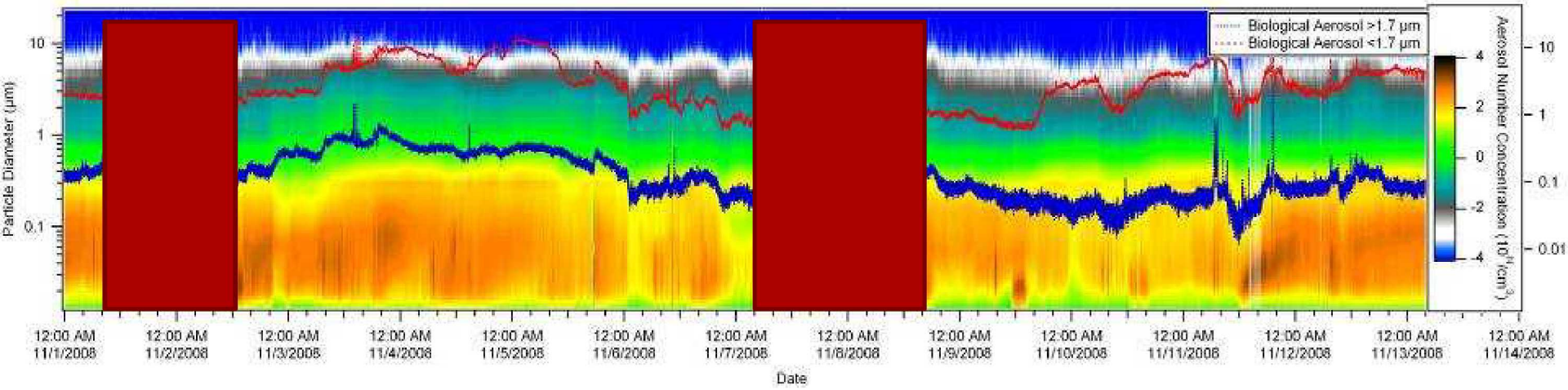
- Primary Biological Aerosols (PBA)
  - May undergo chemical or physical changes in the atmosphere via differing processes
  - Open Air Factor (OAF) that includes ozone, humidity, VOCs, and temperature fluctuations.
  - Study effects in controlled environment and in outdoor natural aging environment.
- Potential benefits include:
  - Determining mechanisms and rates of change to feed into future models
  - Potential forensics for unique reactions with trace, geographically unique atmospheric compounds
  - Better understanding of variables that affect infectivity and detectability of bio-aerosols

Are bioaerosols affected by atmospheric chemistry?

- The data suggests a relationship between the growth of particles smaller than 0.1 microns and increases in biologically fluorescent aerosol
  - Not well quantified
  - May indicate related growth processes
  - Agrees with diurnal variations observed by others (Tong & Lighthart, 1999)



Background particle concentration and geometric diameter over two weeks in MD.

Objectives

1. Gain a mechanistic understanding of the atmospheric processes that affect biological aerosol (bacteria, virus, proteins) transport and fate
    1. Hazard assessment
    2. Detection signature stability
2. Develop parameters to feed into predictive models for short and long-term transport of biological aerosols in the environment
    1. Source terms
    2. Rates of change
3. Better understanding of the biological threat, and better capabilities in fielded biological detection equipment

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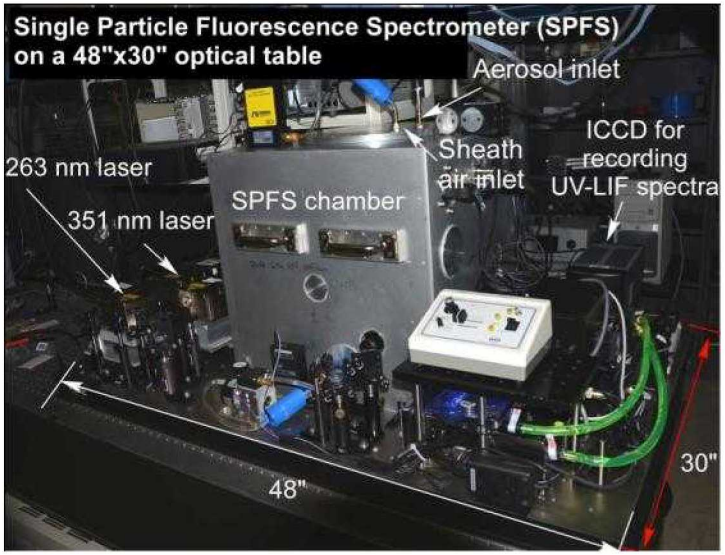
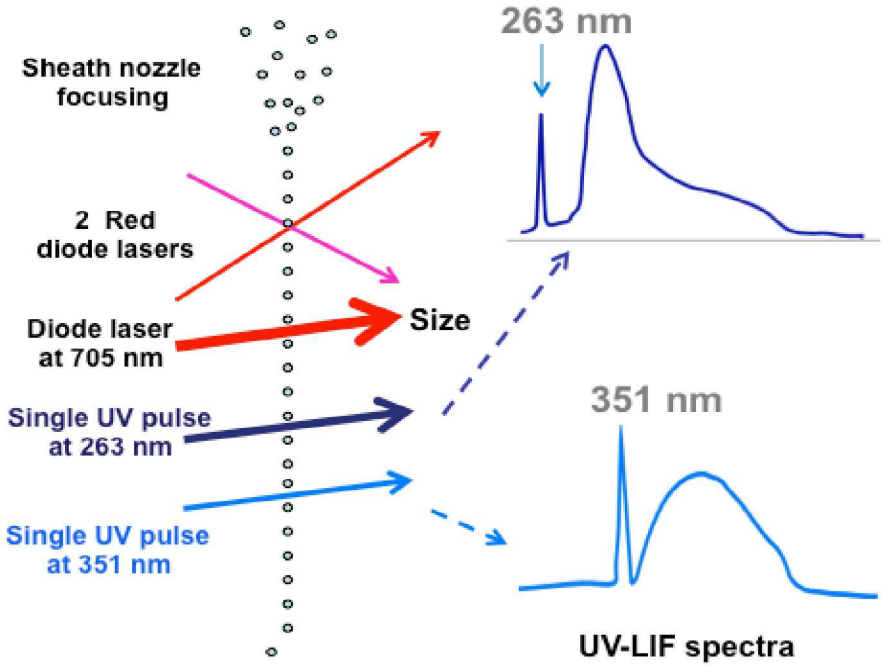
Technologies

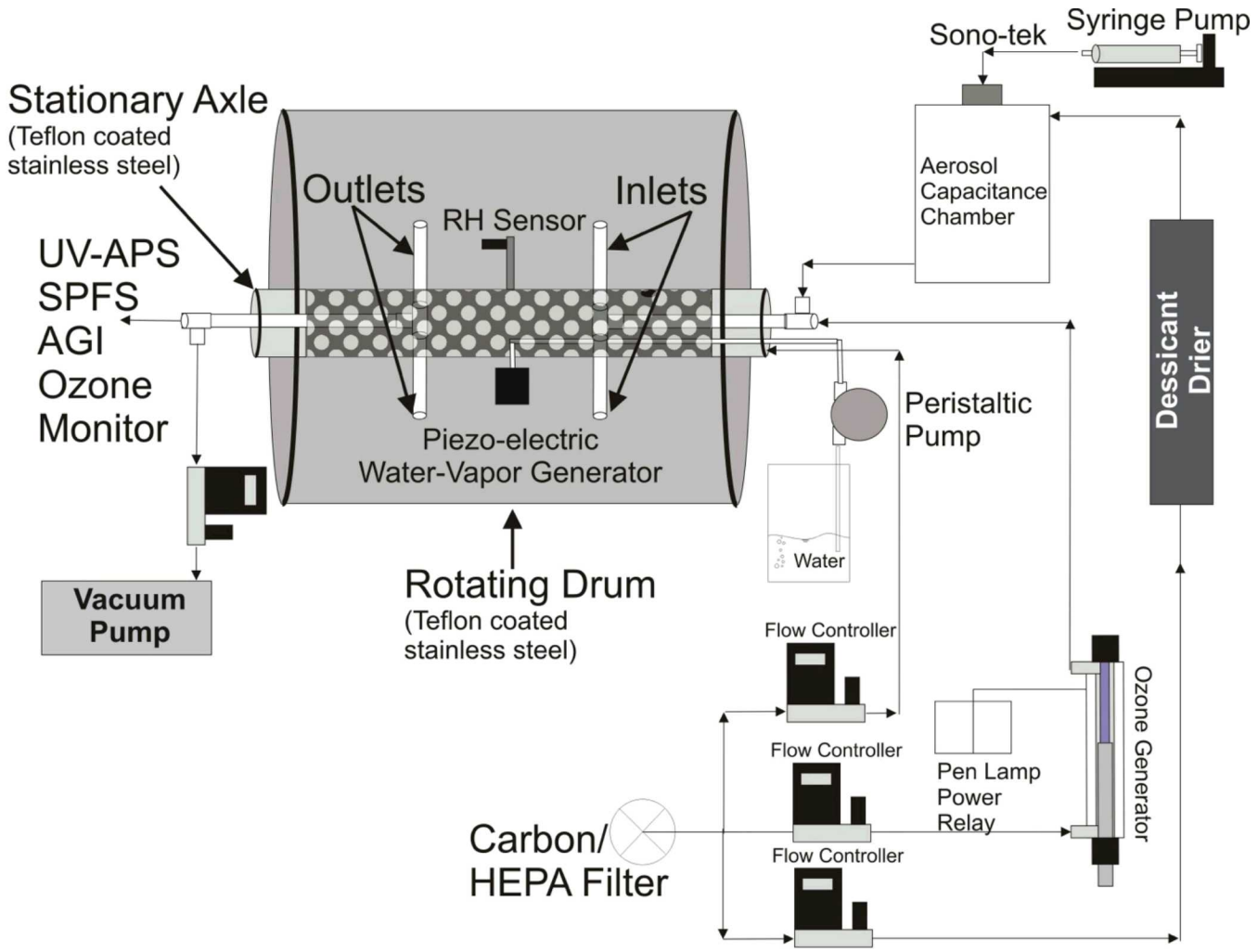
Single Particle Fluorescence Spectrometer (SPFS)

- Army Research Laboratory
  - Current system developed from over a decade of research
  - Has been used for determination of fluorescence cross section of agents and simulants, and ambient aerosol characterization





Goldberg Rotating Drum(s)



Particle Retention Time in a Drum

$$\overline{\tau} = \frac{1}{\sum_i r_i \cdot \Delta r_i \cdot \Delta\theta} \sum_i \tau_i \cdot r_i \cdot \Delta r_i \cdot \Delta\theta$$

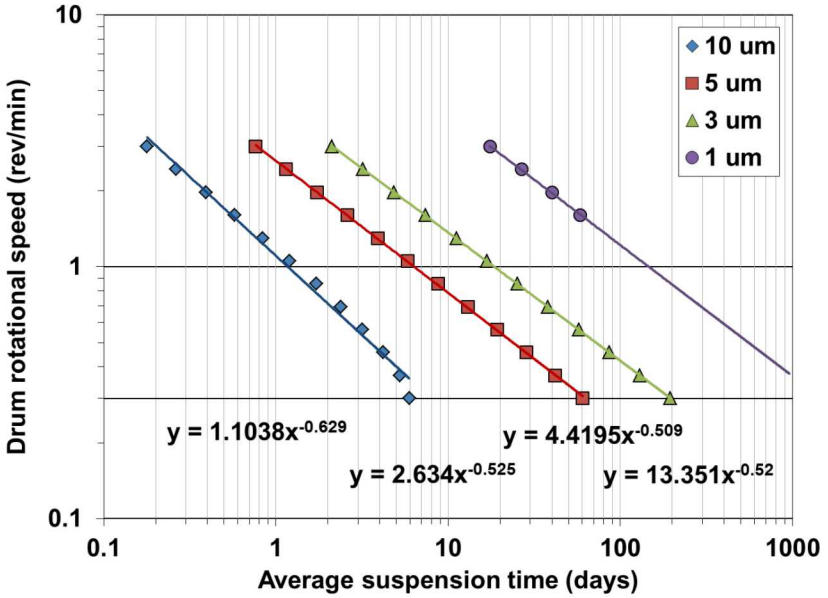
$\overline{\tau}$: Average particle residence time in days, functionally dependent on particle aerodynamic diameter, drum rotational speed, and particle initial position

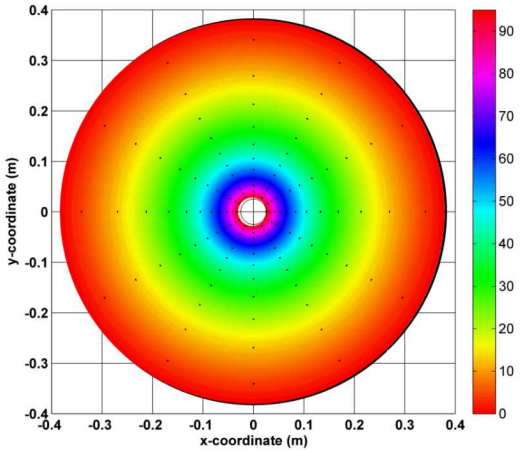
τ_i : Particle residence time in days, for particle initially located in differential area element *i*

r_i : Radial position of differential area element *i*

Δr_i : Width of differential area element *i* in the radial direction, logarithmically spaced for this simulations since the gradient in residence time is highest near the inner axis

$\Delta\theta$: Width of differential area element *i* in the angular direction, constant (2π/20)





Twin CAGE Chambers

- Captive Aerosol Growth and Evolution (CAGE) Chambers
- Evaluate sensitivity of response to perturbations in single variables such as ozone concentration and relative humidity
  - Employ parallel chambers, control chamber and atmospheric chamber

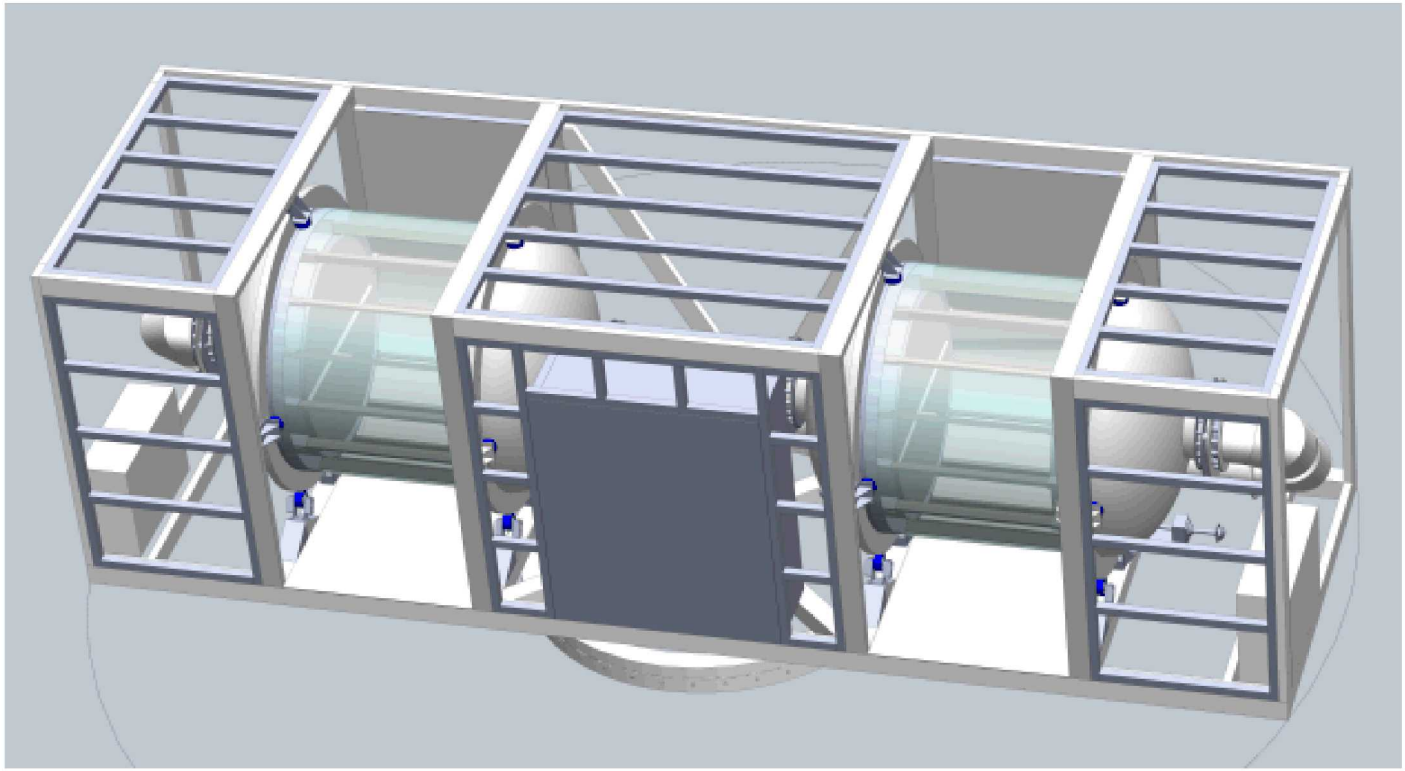
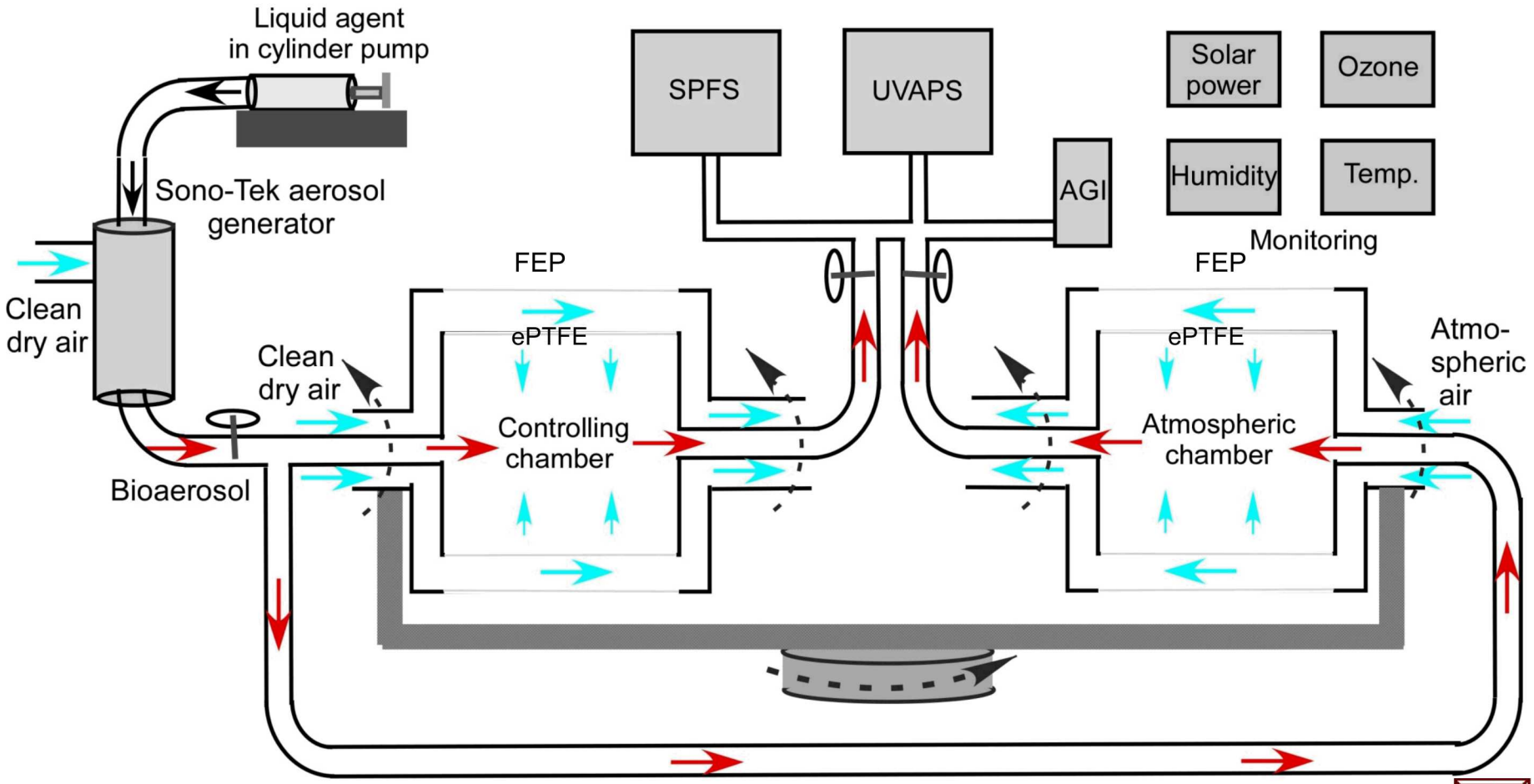


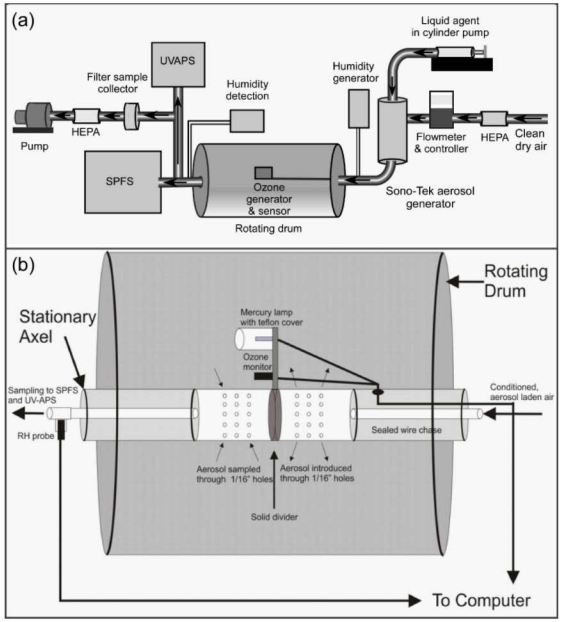
Diagram for Field System



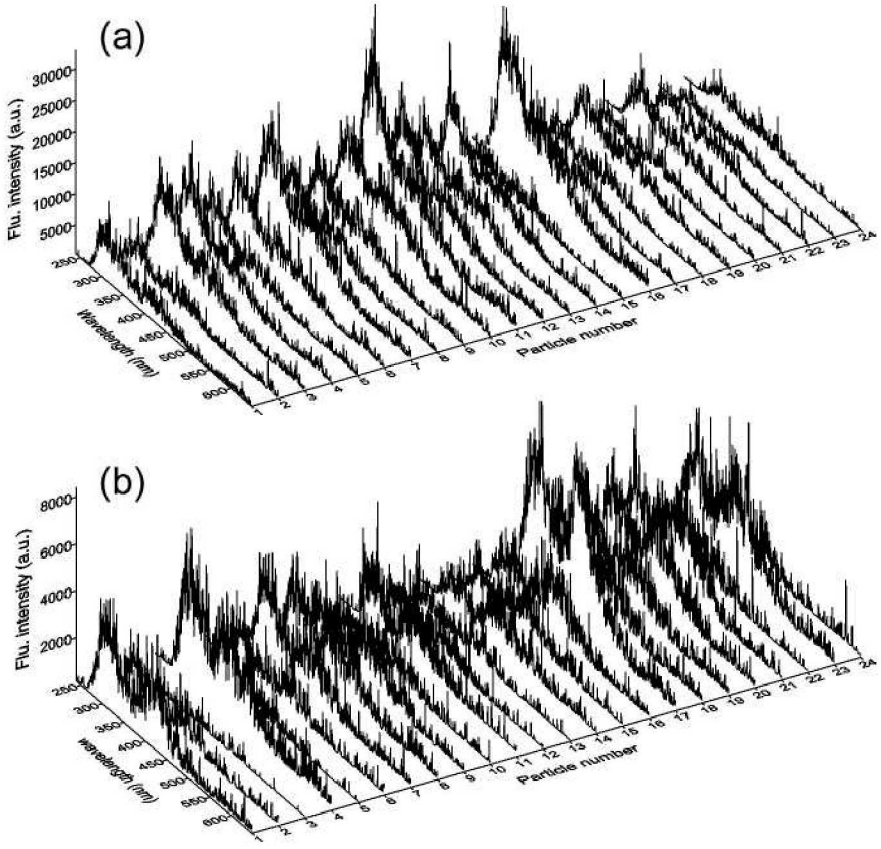
Technical Work/Experiments

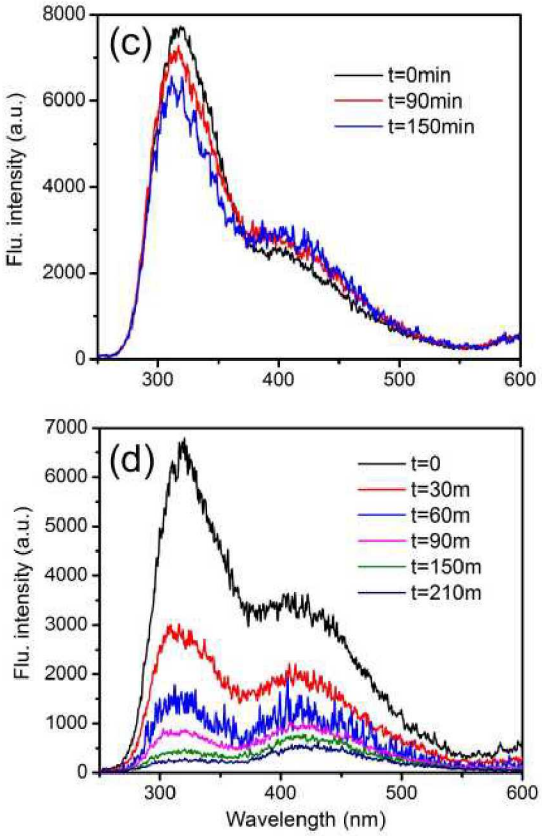
Initial Laboratory Drum Experiments

- Goldberg rotating drum
  - Design based on DSTL, Porton Down, UK
  - Maintain aerosol population for >8hrs for controlled “aging”
- Fill chambers with relevant concentration of ozone, water vapor and bioaerosols
- Measure
  - Bioaerosol size distributions
  - UV-Spectral fluorescence
  - Viability



Potential RH Dependence





RH %

Indicates that water uptake by aerosols may be important to facilitating atmospheric changes in biological aerosols

Fluorophore Oxidation

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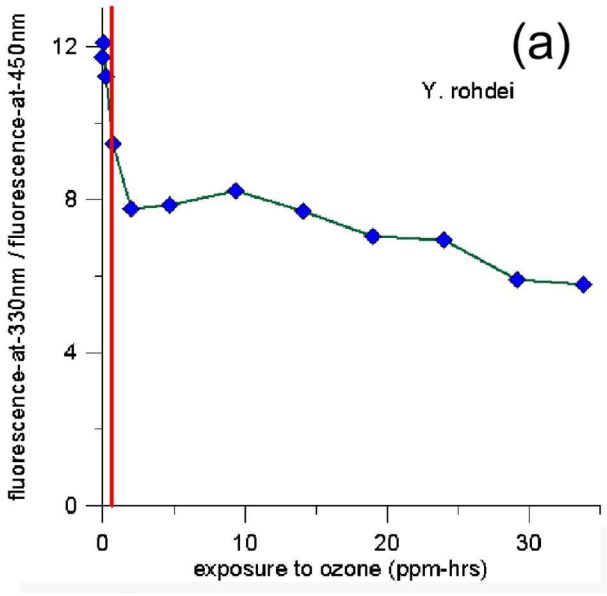
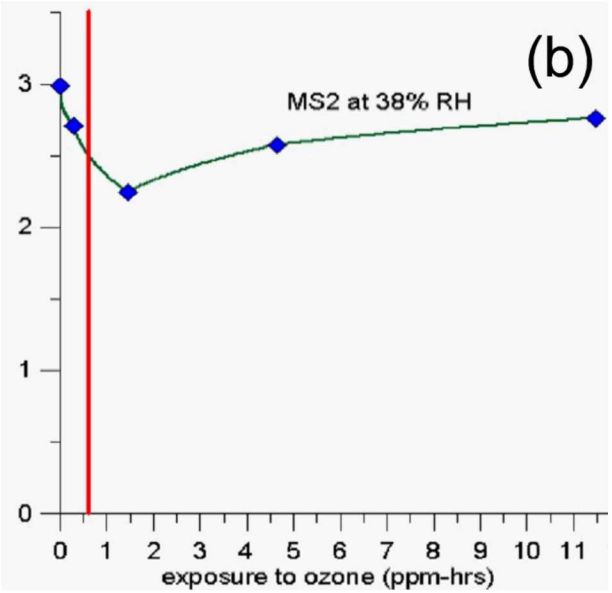
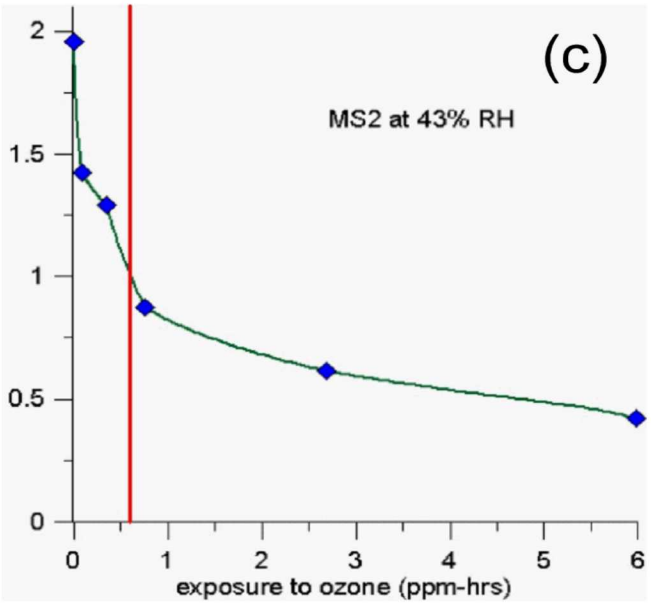
Yr @ 38%



MS2 @ 38%

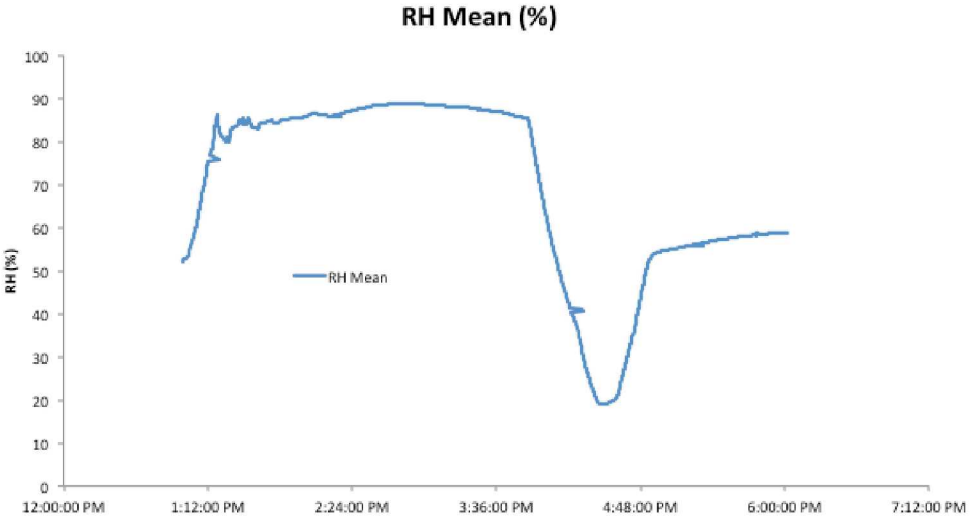


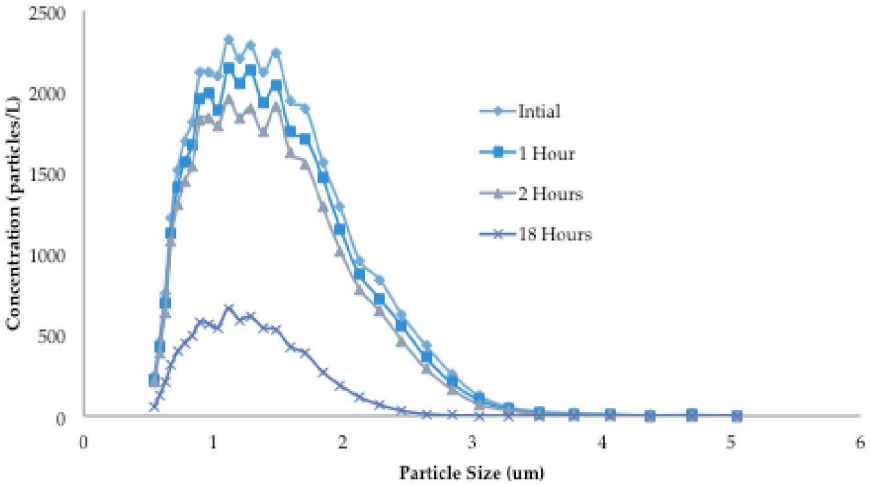
MS2 @ 43%

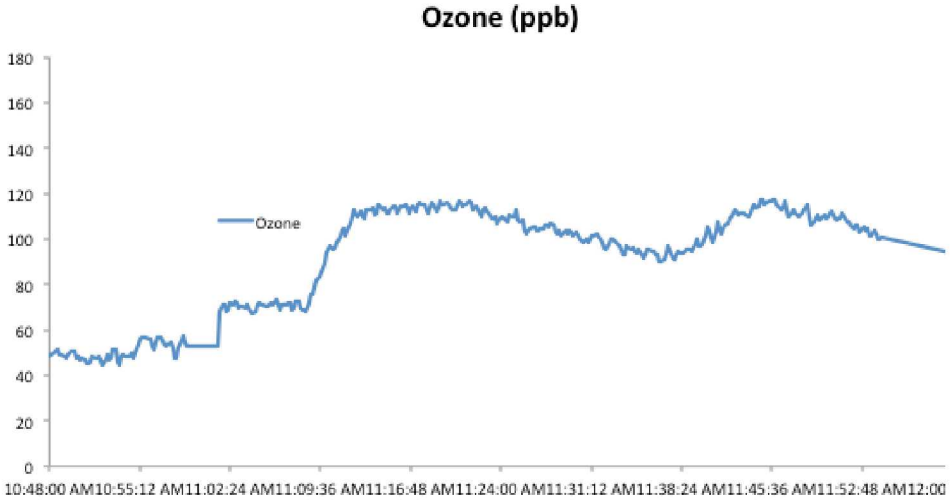


New Drum Performance

- New drum has better RH and ozone control w/ feedback loops for automated experiments
- Experiments focused on exploring the relationships between RH and oxidation of biological aerosols
  - SPFS, UV-APS
  - Viability

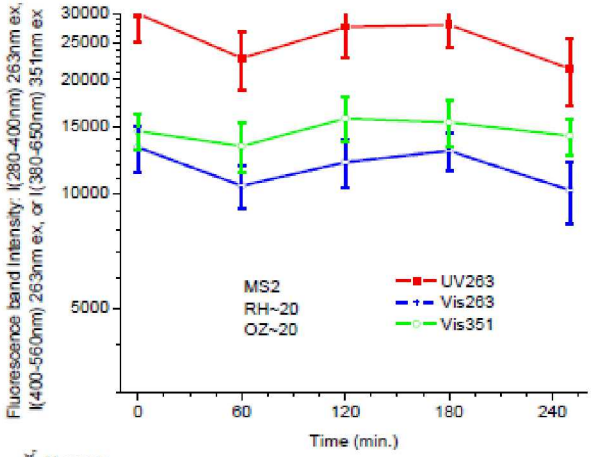


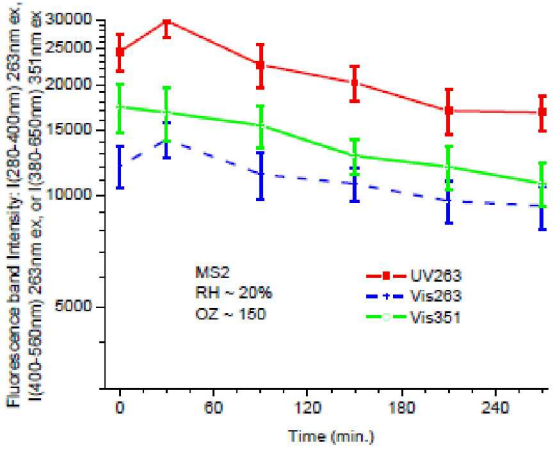


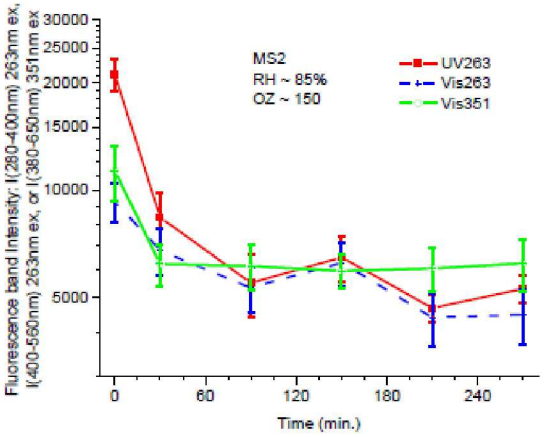


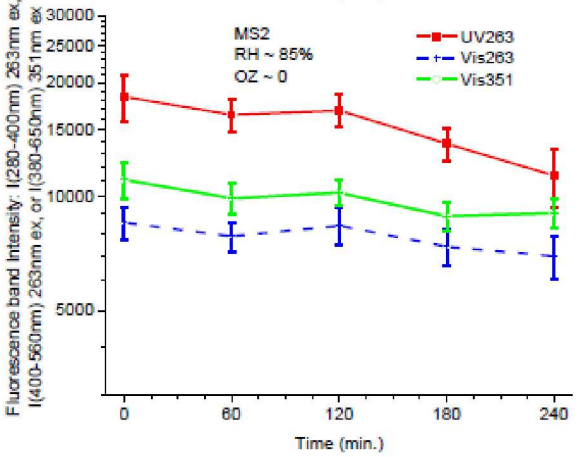
Drum MS2 Experiments

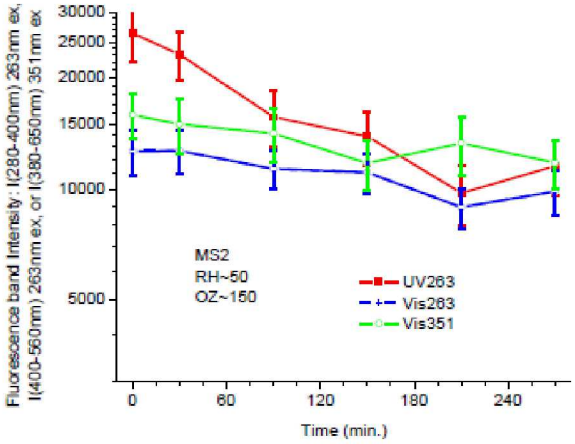
- Individually, both RH and O_3 cause a decrease in observed fluorescence
- Decrease at 150 ppb O_3 and 85% RH is the most profound





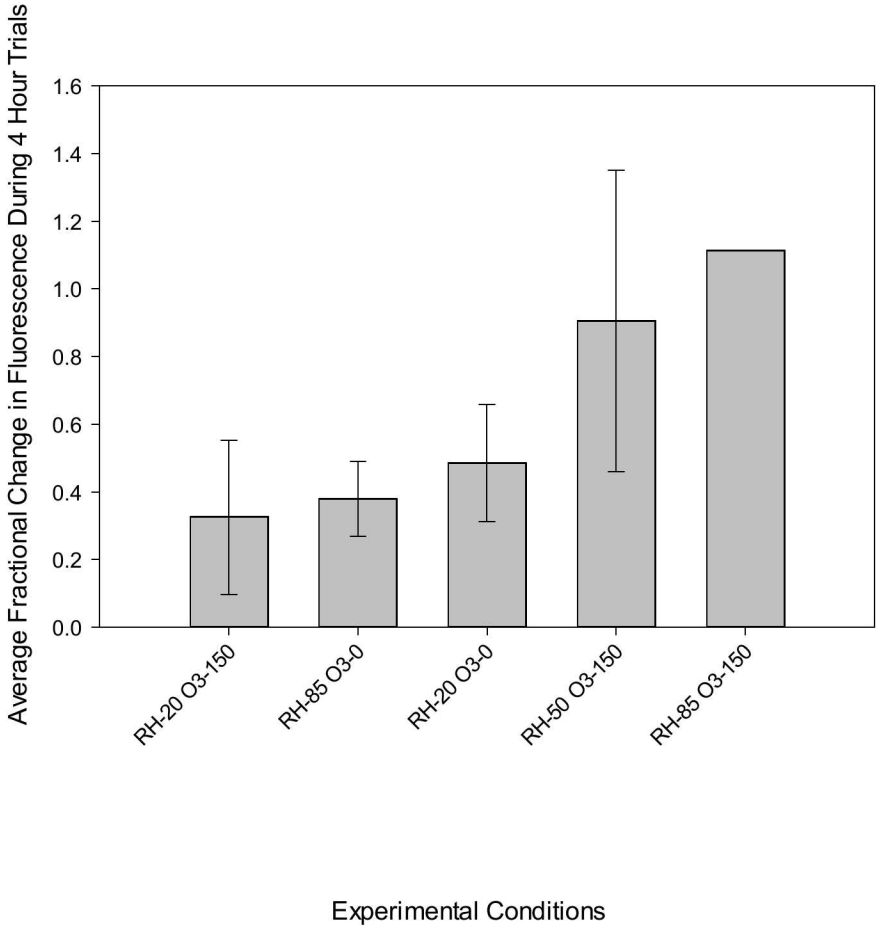






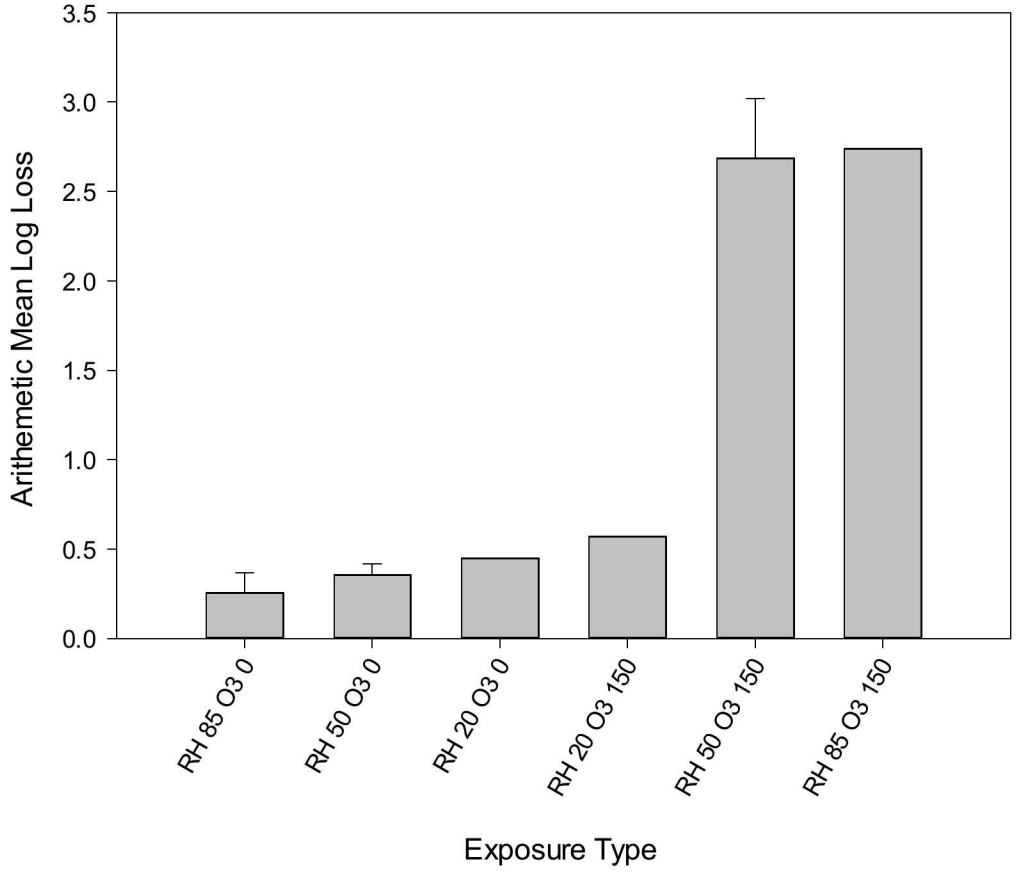
UV-APS 355nm Fluorescence Change-MS2

- Number weighted fluorescence intensity calculated at the peak of the initial size distribution
- Average change $[(Fl_4\text{-}Fl_0)/Fl_0]$ calculated for the three replicates of each experiment
- Change measured UVAPS fluorescence may indicate Kynurenine formation
  - Significant only at elevated RH and high O_3



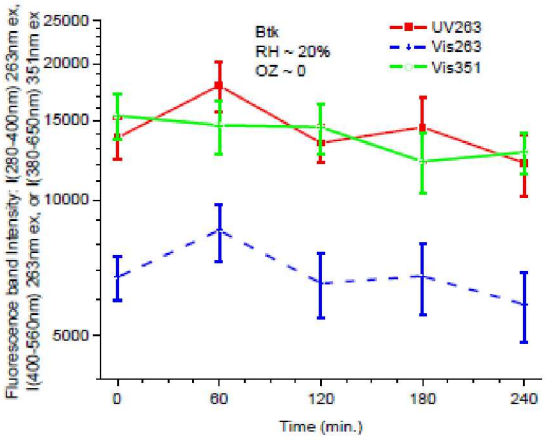
MS2 Viability Due to Ozone

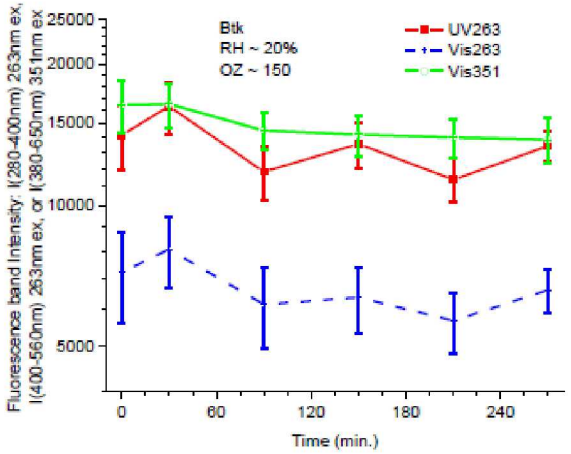
- Log loss in viable fraction calculated from the ratio of PFU divided by the number of genetic equivalents from Q-PCR
- Significant losses in viability observed during elevated RH and high O_3

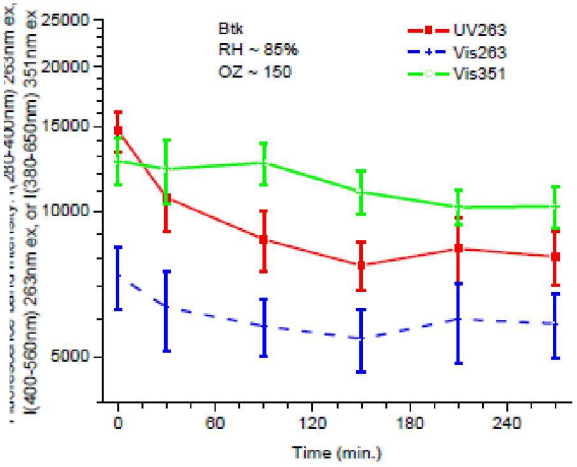


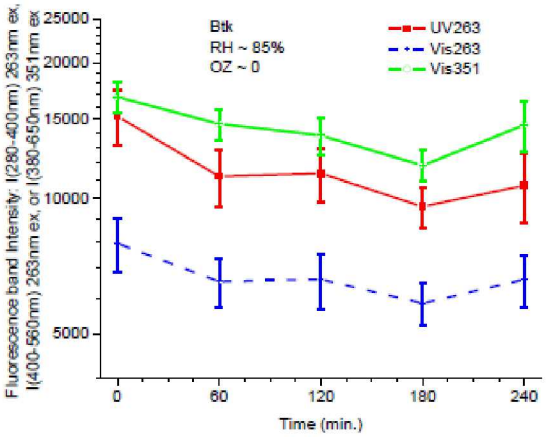
Drum BtK Experiments

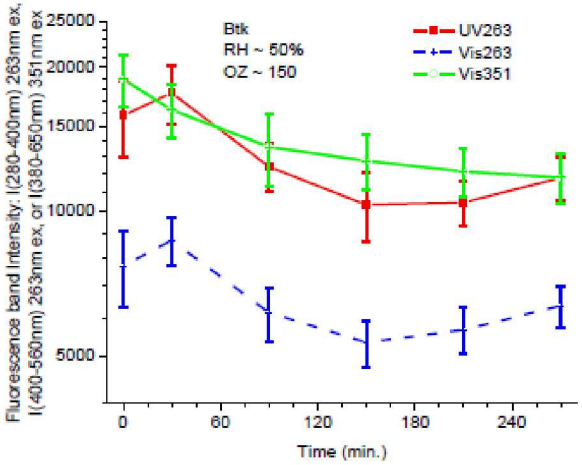
- Individually, both RH and O_3 cause a decrease in observed fluorescence
- Decrease at 150 ppb O_3 and 85% RH is the most profound
- Much less decrease than observed for MS2





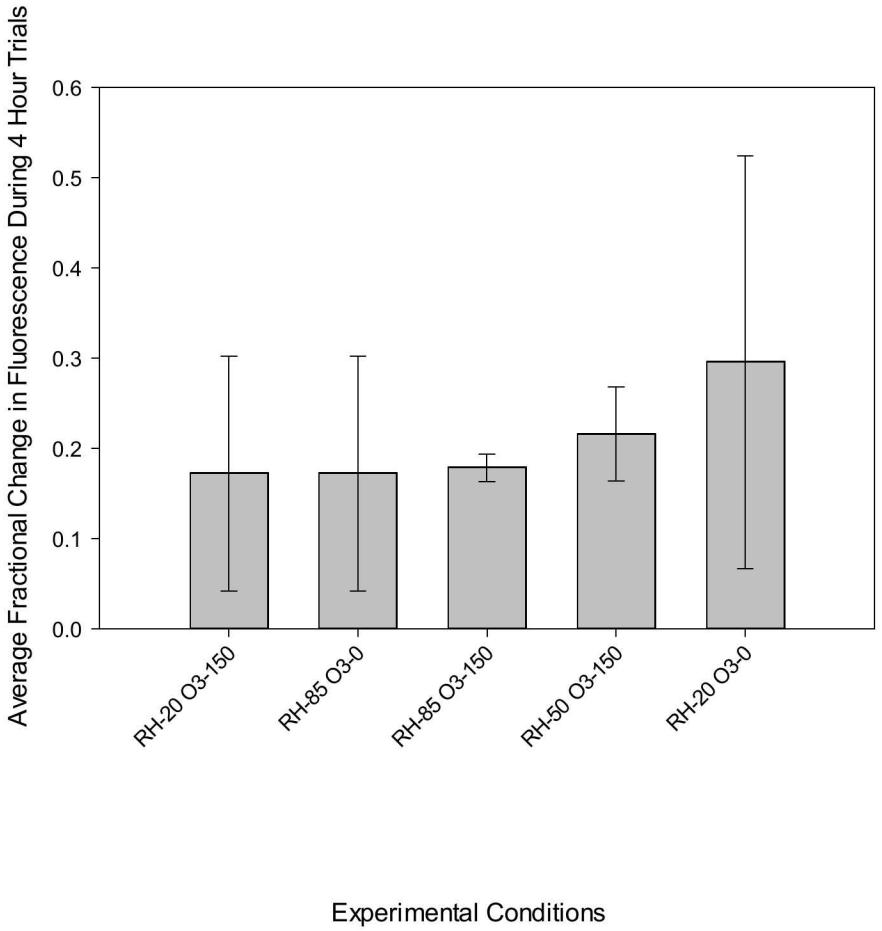






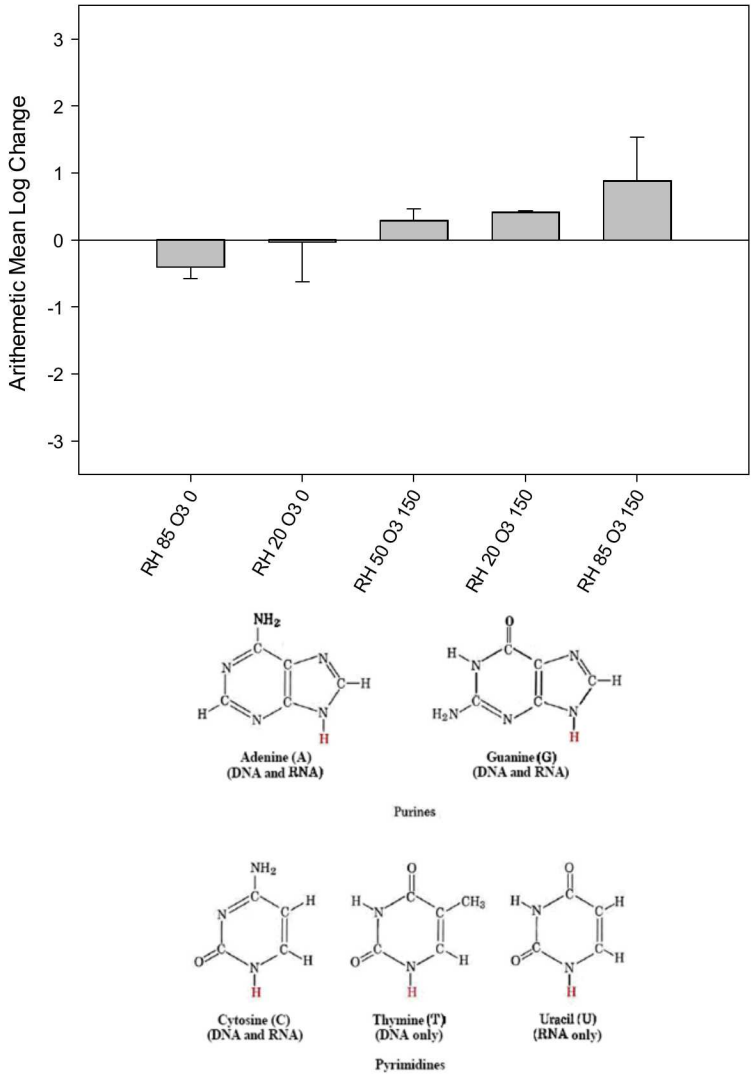
UV-APS 355nm Fluorescence Change-Btk

- Number weighted fluorescence intensity calculated at the peak of the initial size distribution
- Average change $[(Fl_4\text{-}Fl_0)/Fl_0]$ calculated for the three replicates of each experiment
- No consistent, repeatable change in UVAPS fluorescence observed



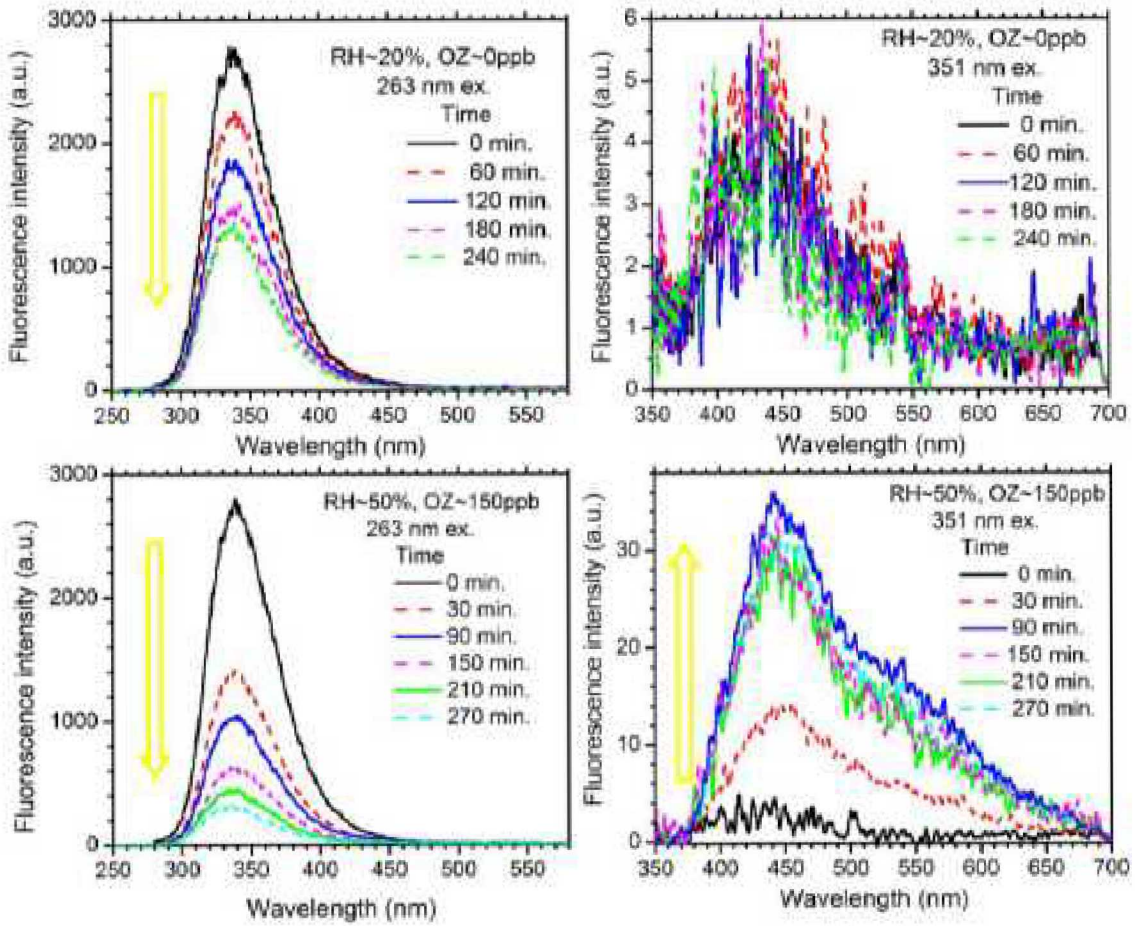
Btk Viability Due to Ozone

- Log change in the viable fraction calculated from the ratio of CFU divided by the number of genetic equivalents from Q-PCR
- Small loss in viability observed during experiments with no O_3 "Apparent" increase in viable fraction observed when ozone was present
  - May indicate that O_3 destroys some of the DNA being detected by the assay
    - Likely extracellular DNA
    - Changes GE/spore therefore appears as an increase in viable fraction
  - Nucleic acids should be susceptible to O_3, but would normally be protected by the cell



Drum Peptide Experiments

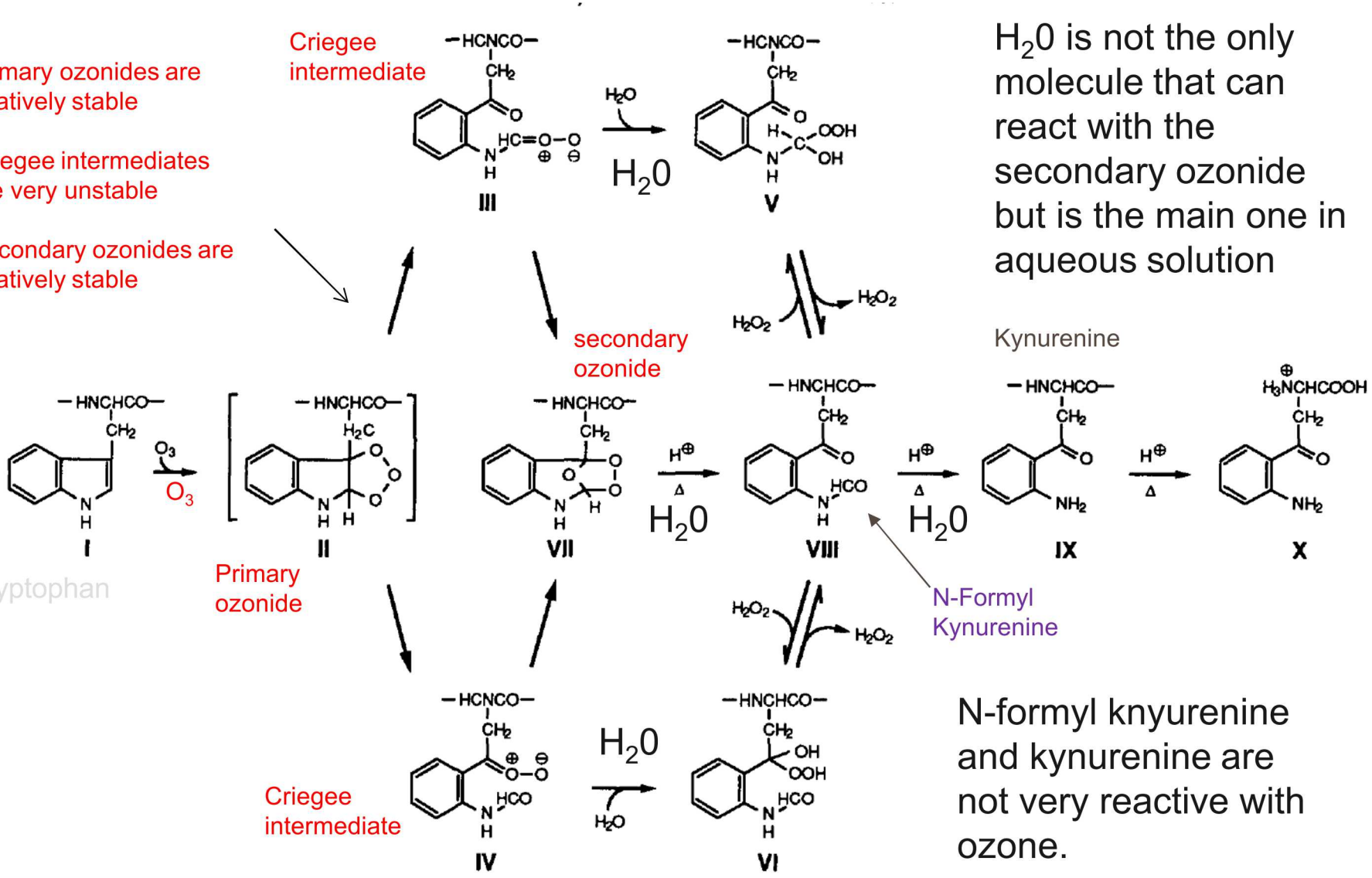
- Clear observations of increase in 351 excited fluorescence when 263 fluorescence is decreasing
  - Elevated RH, 150 ppb O3
- Clearly indicates the formation of Kynurenine through hydrolysis of the product of tryptophan ozonolysis



Primary ozonides are relatively stable

Criegee intermediates are very unstable

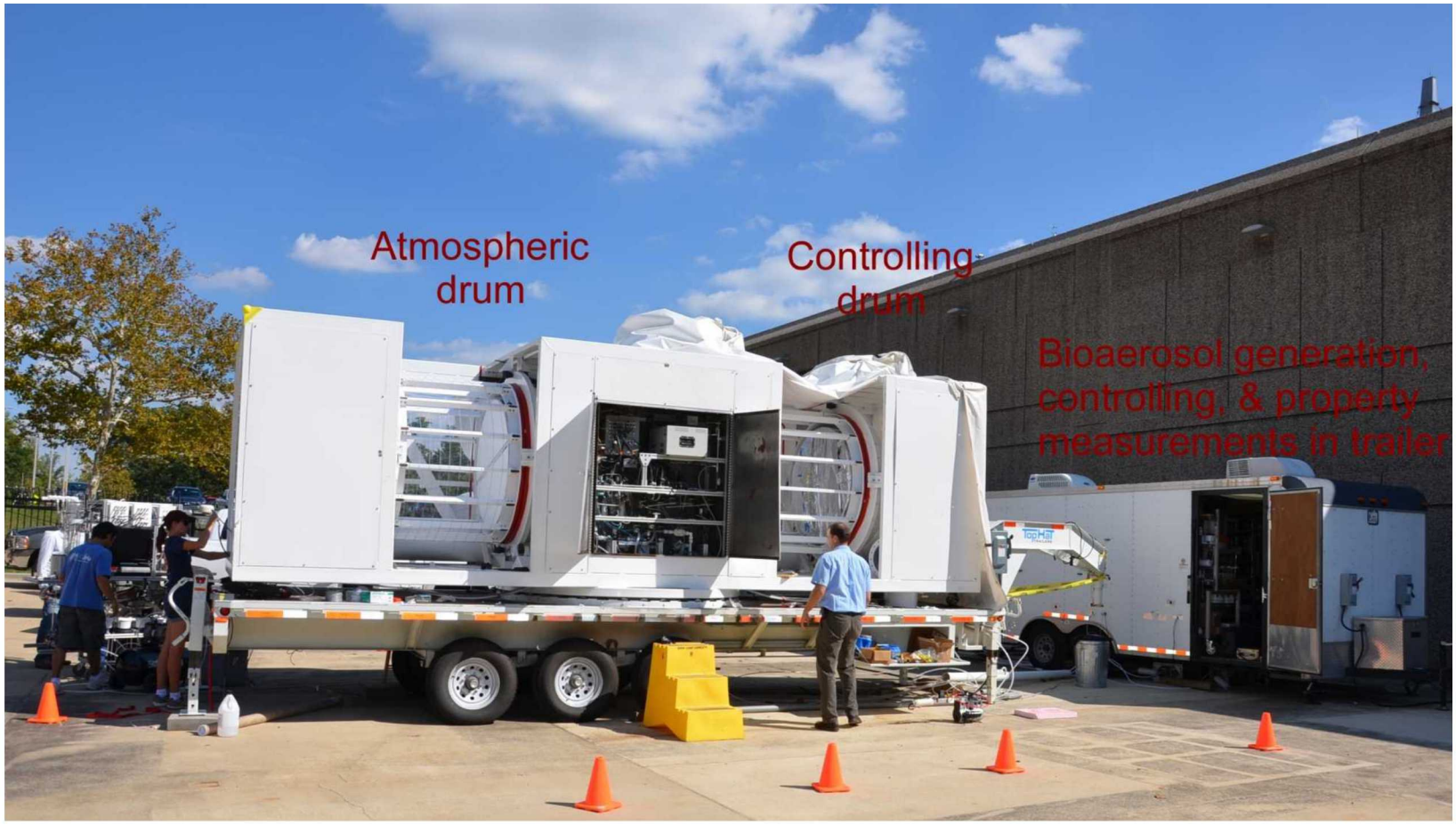
Secondary ozonides are relatively stable



H_20 is not the only molecule that can react with the secondary ozonide but is the main one in aqueous solution

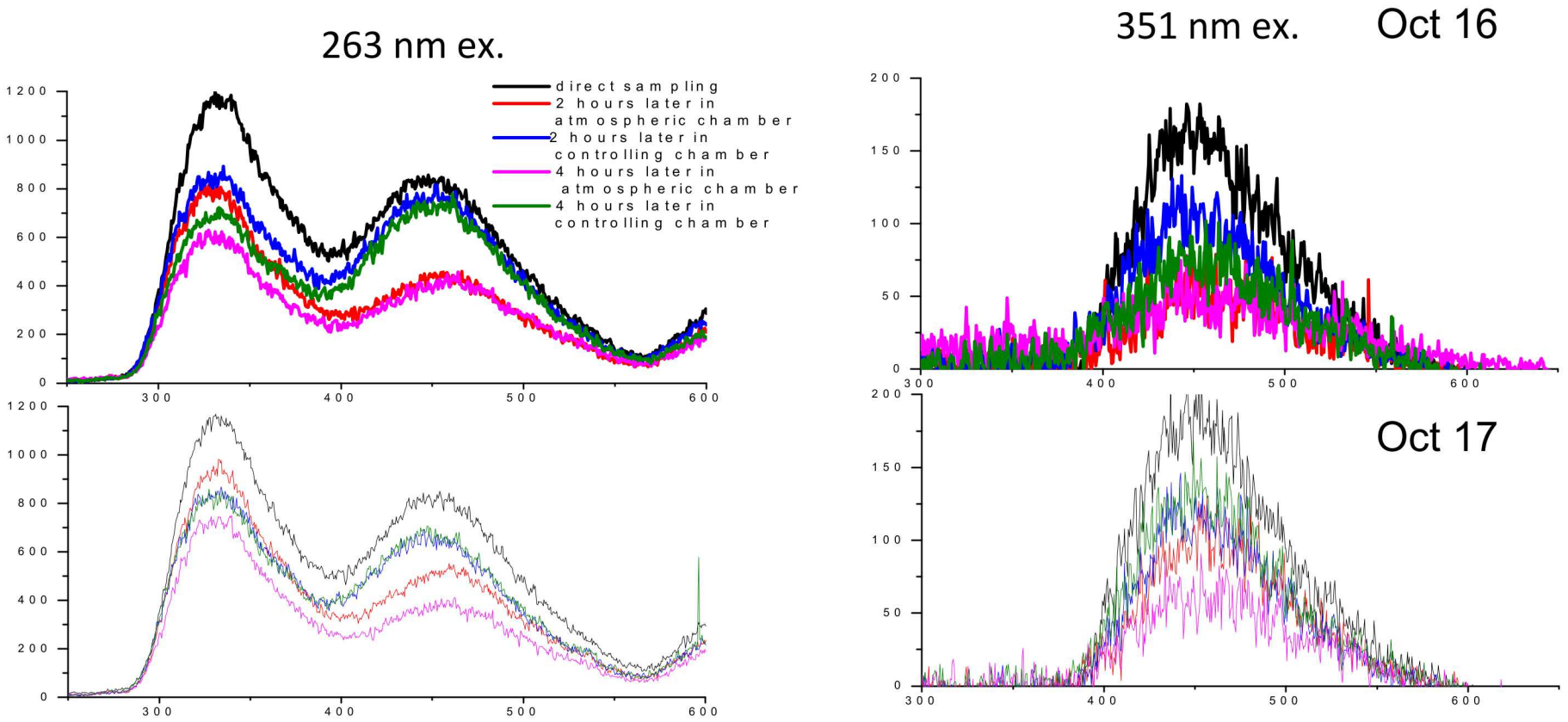
N-formyl knyurenine and kynurenine are not very reactive with ozone.

Field Results at ARL (Adelphi, MD)



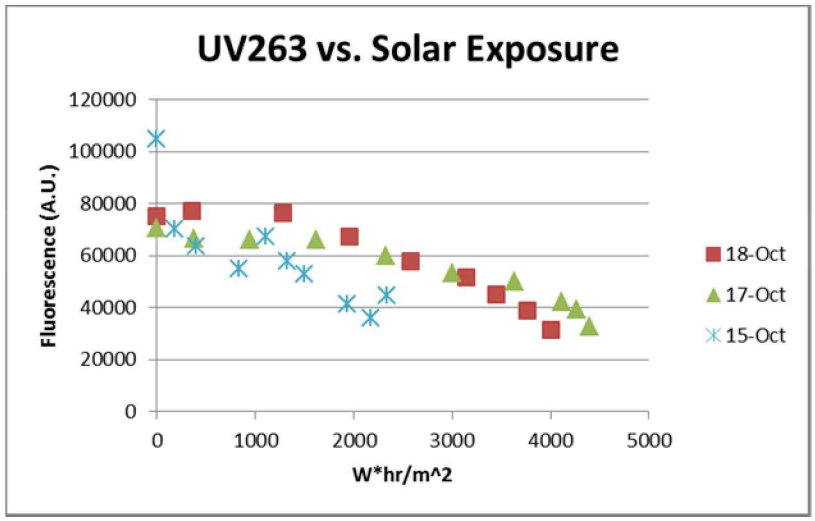
During Field Trial Test in Adelphi, MD - 2013

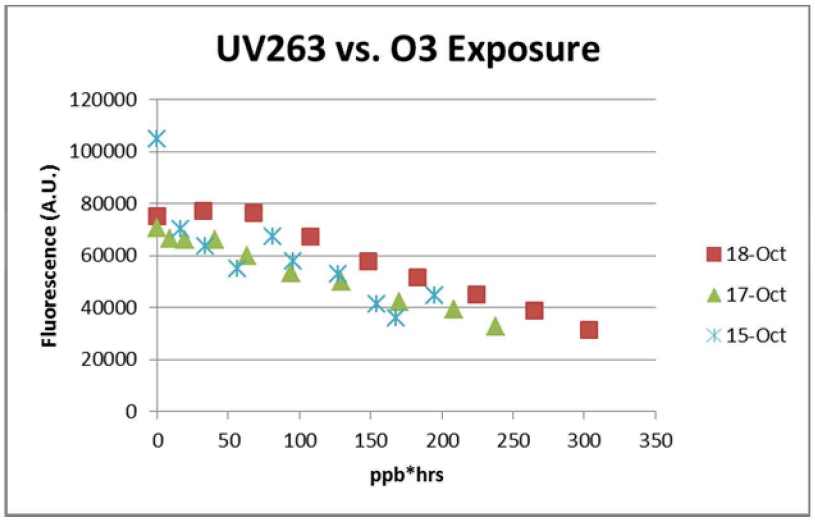
Preliminary MS2 Results

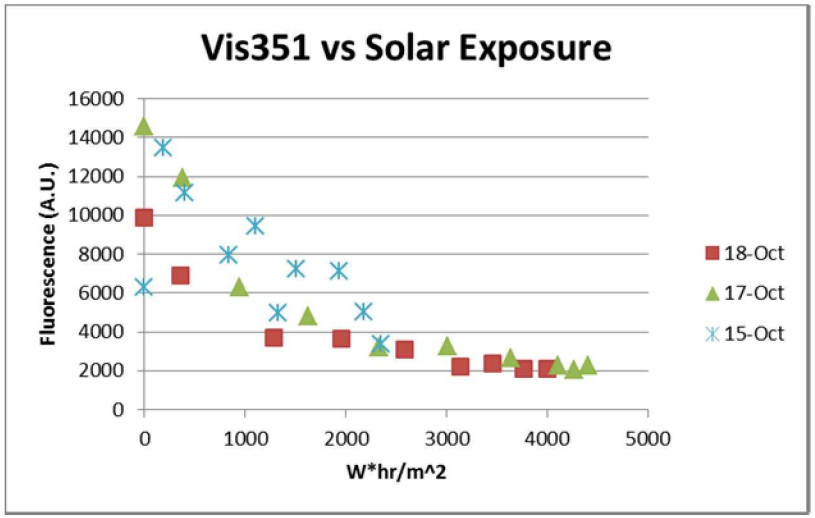


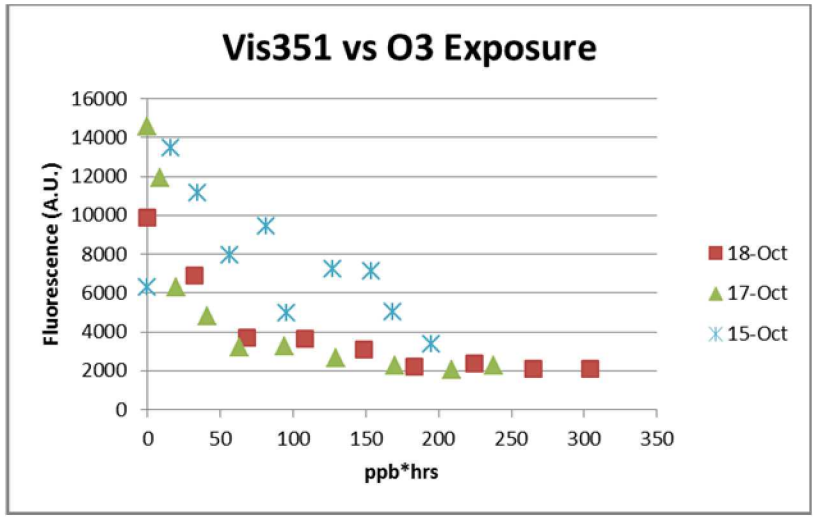
- Control drum was only partially isolated from environment

MS2 Decay

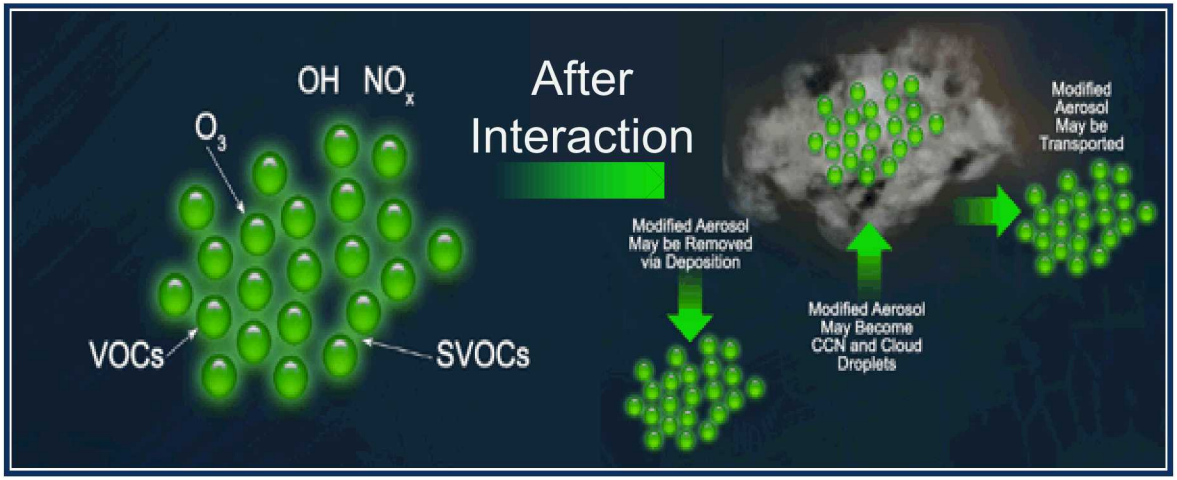

UV263 vs. Solar Exposure
Fluorescence (A.U.)
120000
100000
80000
60000
40000
20000
0
0
1000
2000
3000
4000
5000
W*hr/m^2
18-Oct
17-Oct
15-Oct

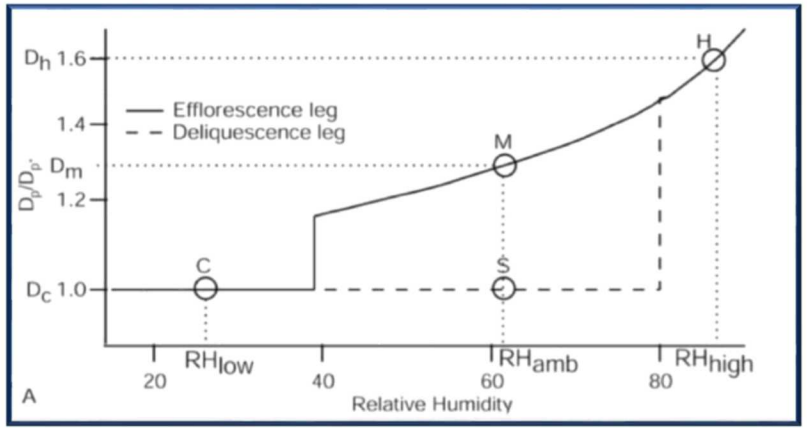

UV263 vs. O3 Exposure
Fluorescence (A.U.)
120000
100000
80000
60000
40000
20000
0
0
50
100
150
200
250
300
350
ppb*hrs
18-Oct
17-Oct
15-Oct


Vis351 vs Solar Exposure
Fluorescence (A.U.)
16000
14000
12000
10000
8000
6000
4000
2000
0
0
1000
2000
3000
4000
5000
W*hr/m^2
18-Oct
17-Oct
15-Oct


Vis351 vs O3 Exposure
Fluorescence (A.U.)
16000
14000
12000
10000
8000
6000
4000
2000
0
0
50
100
150
200
250
300
350
ppb*hrs
18-Oct
17-Oct
15-Oct

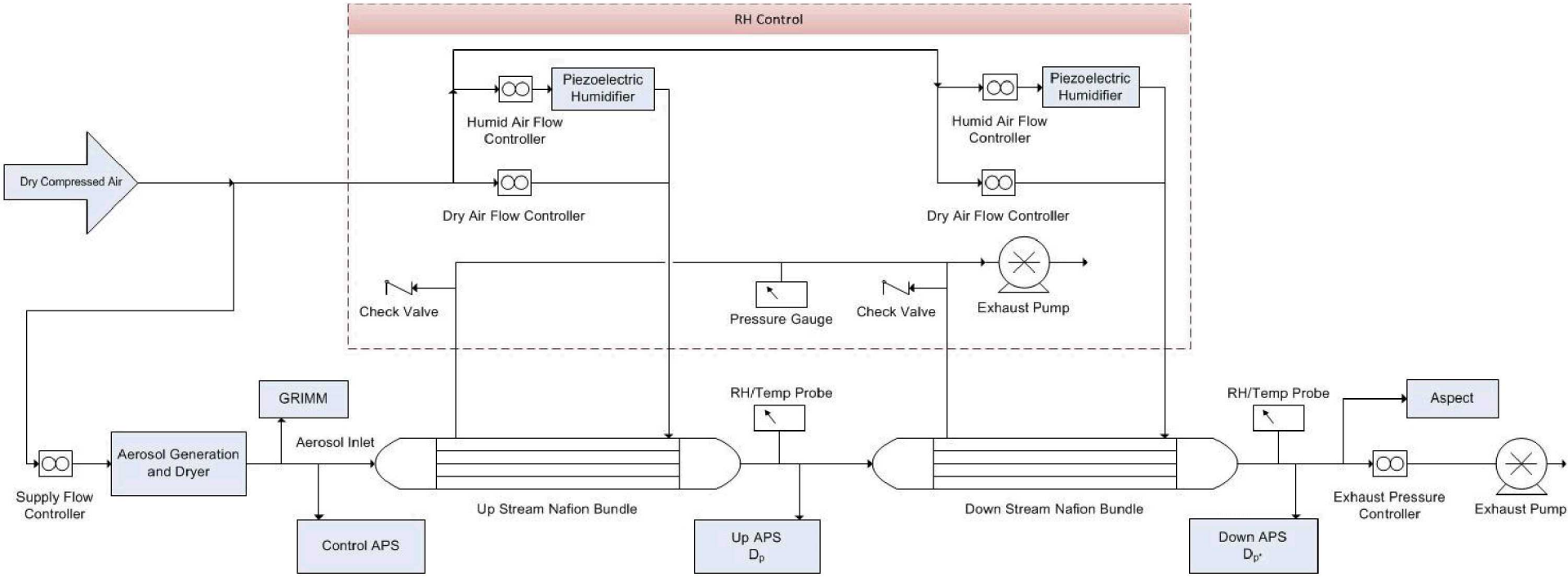
Hygroscopicity Studies





- Changes in RH
  - Lead to size distribution shifts
  - Viability
  - Susceptibility
  - Detectability
- Bioaerosols can serve as CCN or IC
- Effects of media, buffers, additives
- Impacts post agent attack assessments and response

Aerodynamic Hygroscopic Growth Analyzer (AHGA)



Aerodynamic Hygroscopic Growth Analyzer

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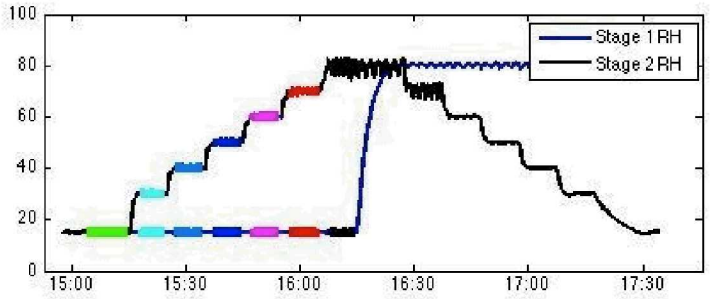
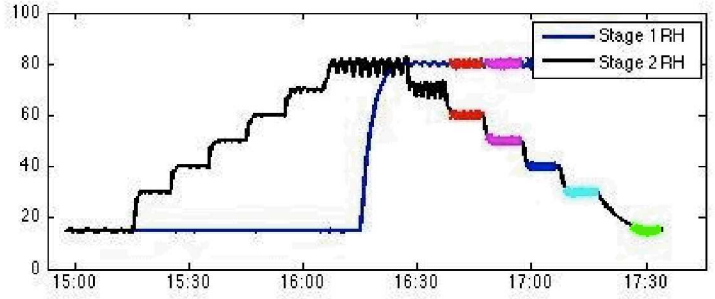
Hardware

Data Analysis

Deliquescence Leg

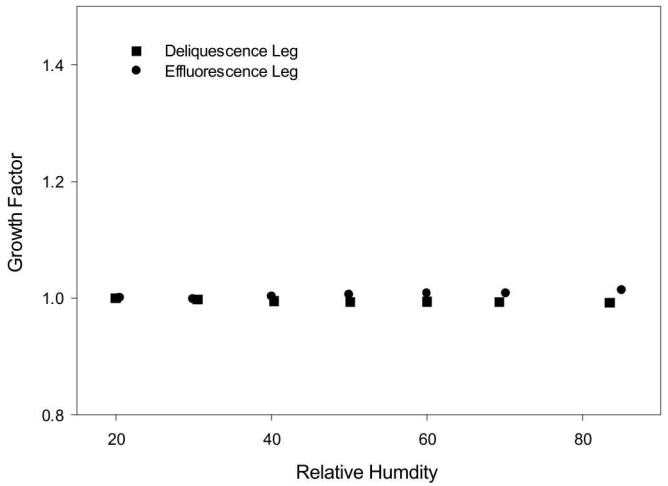


Effluorescence Leg

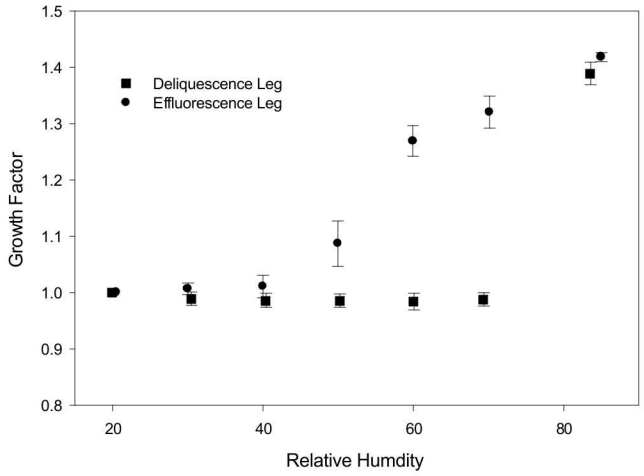


Validation of System Performance

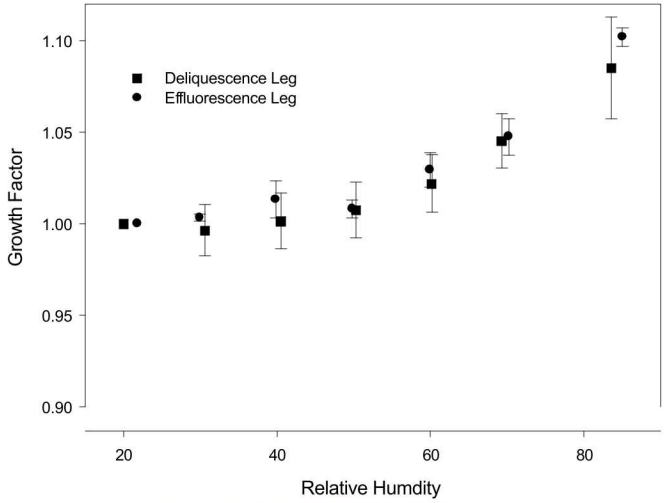
- Using three aerosols with known hygroscopic properties, compared literature reported values with those observed using the AHGA



Polystyrene Latex-DRH, CRH-None



NaCl-DRH 72%, CRH 43%



NaHSO4-DRH-52%, CRH-Unknown/N/A

Processing of Data

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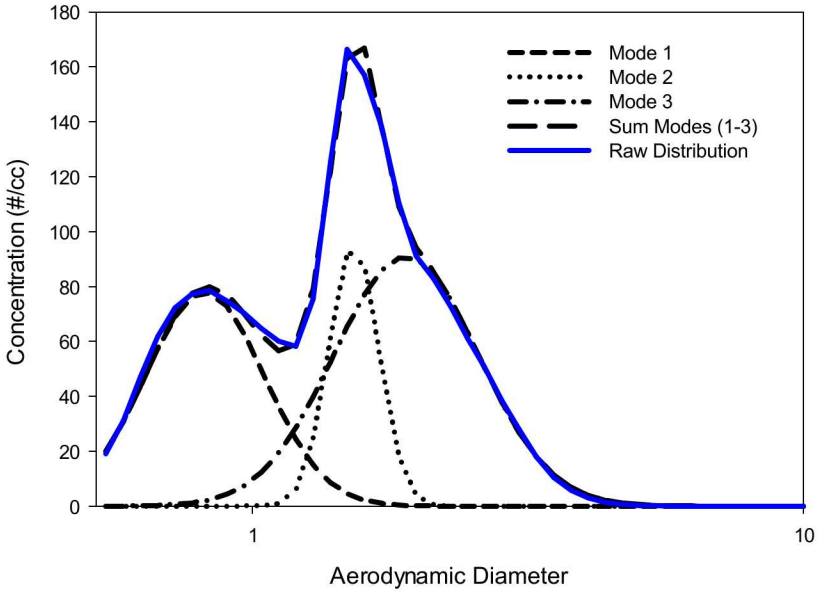
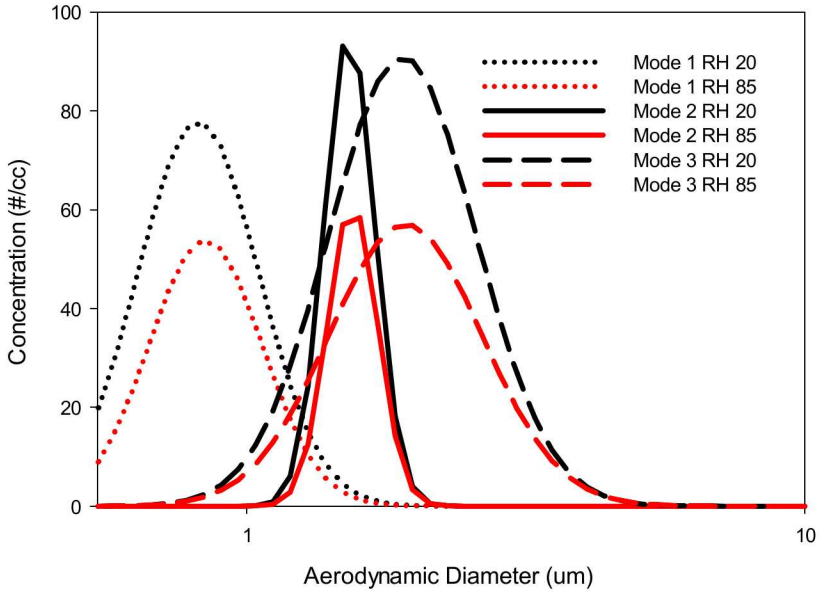
Multiple Modes Exist in a Distribution of Biological Aerosols

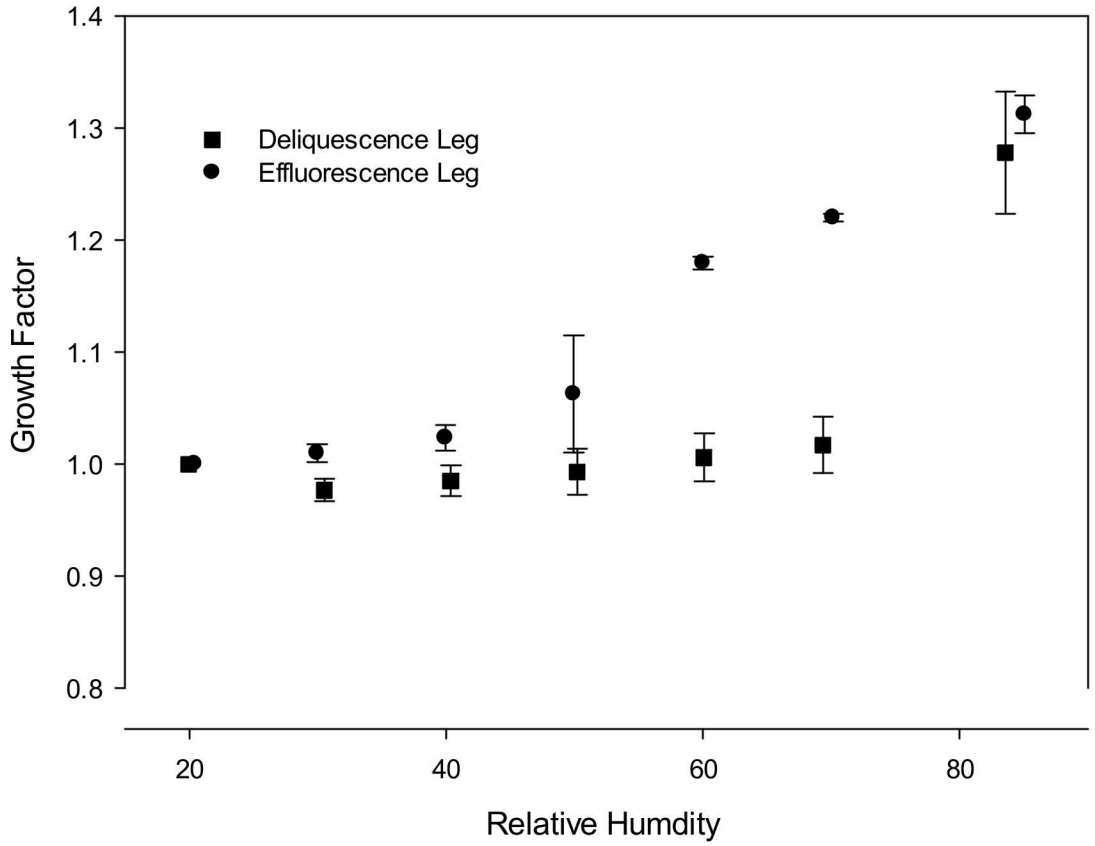


Peak to Peak Shifts Used to Calculate Growth for Each Mode



Phosphate Buffer Solution

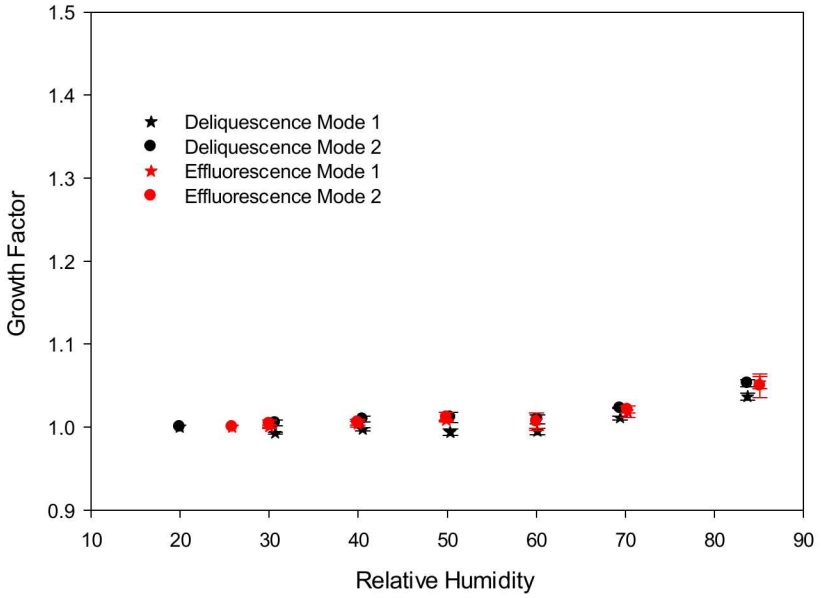
Growth of pure PBS particles behave almost identically to NaCl (the dominant salt)



Bt Al Hakam Spore Bacteria

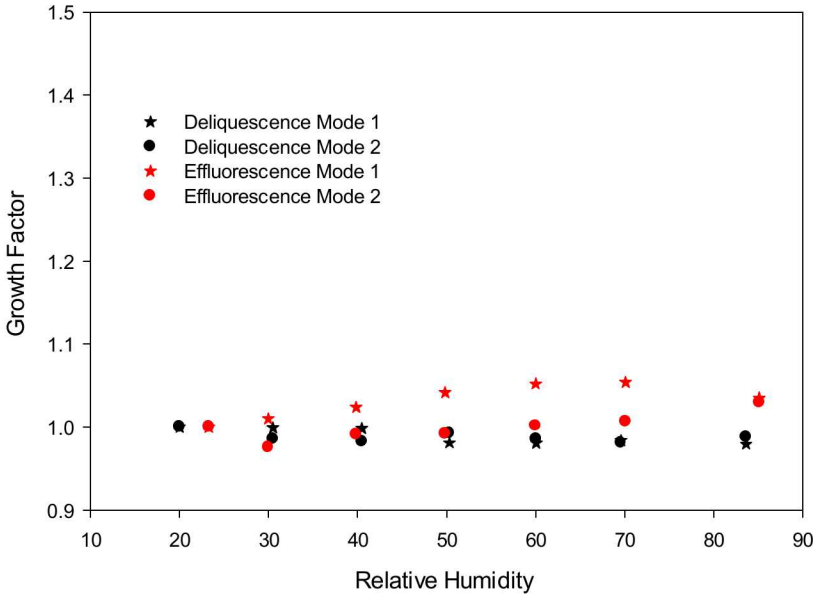
Bt Al Hakam Crude

- Suspended in spent growth media
- Small growth at high RH likely due to the presence of media



Clean Bt Al Hakam

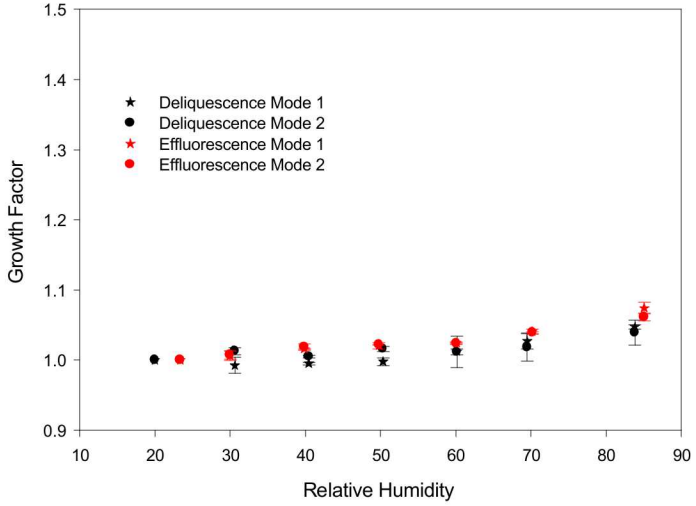
- Centrifuged and pellet resuspended in SFDIH2O
- Negligible water uptake
  - Some growth observed during single efflorescence measurement may indicate incomplete drying



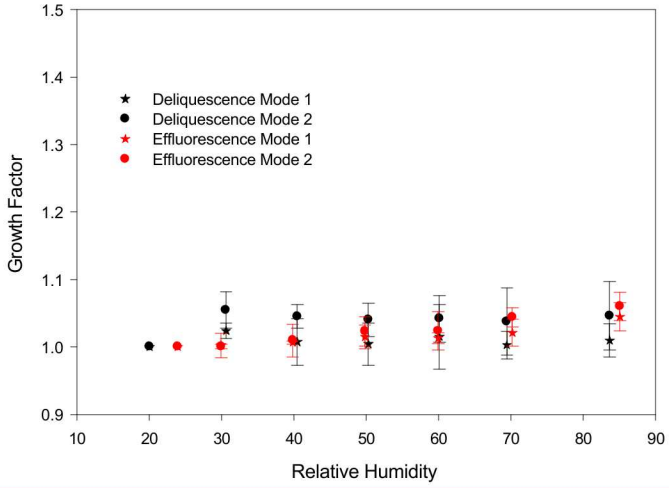
Hygroscopicity of *Pseudomonas fluorescens*

- Pf vegetative bacteria
- Pf is a known ice nucleating bacteria
- However no significant growth shown for both types of preparations

Crude Prep-Dirty w/ Media

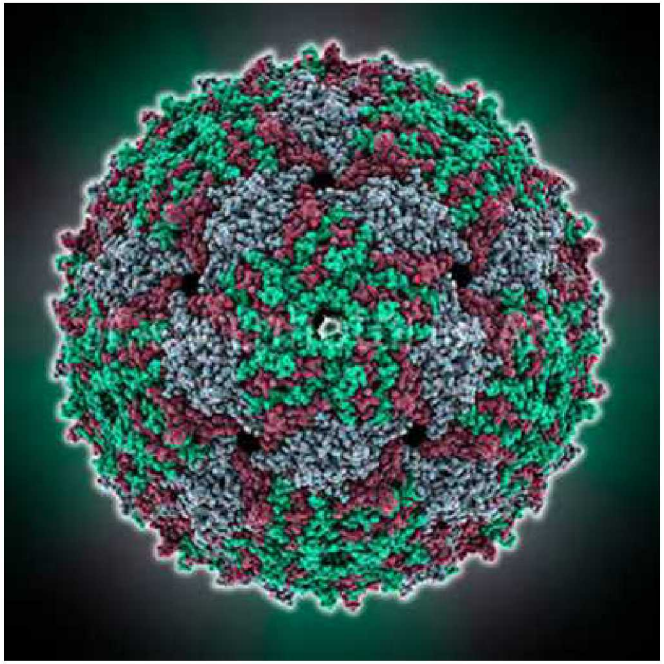


Clean Resuspended in SFDIH_2O

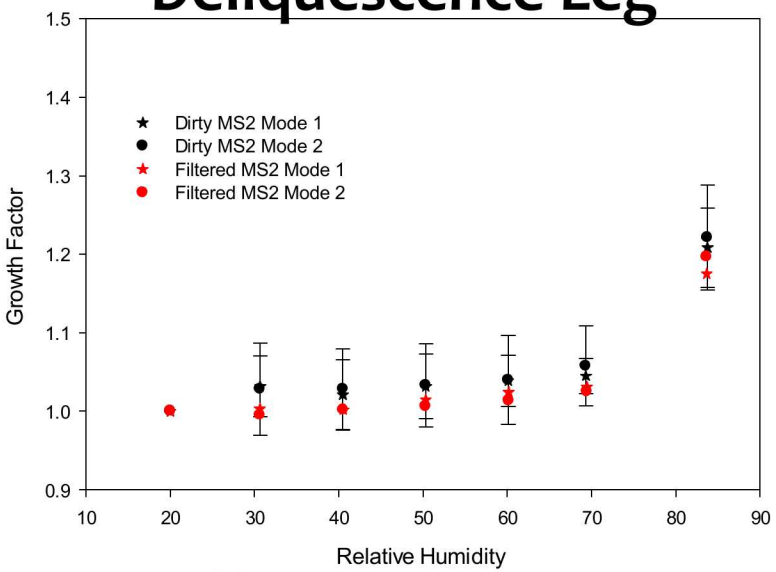


MS2 Phage

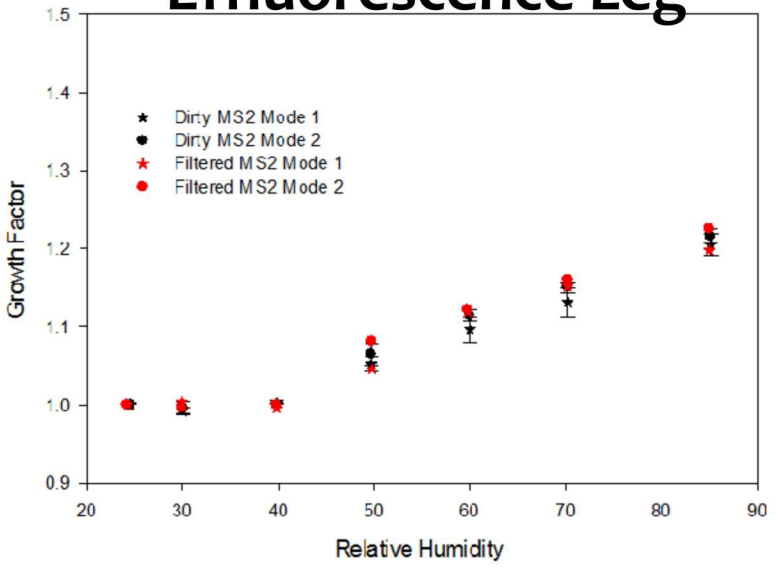
- "Dirty" prep contains E. coli lysate, while "Filtered" has been filtered through a 0.22 um filter
- Deliquescence-Shows significant growth between 70 and 85%
- Efflorescence-Shows hysteresis and return to original size between 40 and 50%
- Behaves very similar to NaCl



Deliquescence Leg



Effluorescence Leg



Conductivity as Salt Indicator

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Aerosol	Average Conductivity (uS/cm)
Bacillus thuringiensis Al Hakam Crude	163.55
Bacillus thuringiensis Al Hakam Clean	5
Pseudomonas fluorescens Crude	21.94
Pseudomonas fluorescens Clean	5.5
MS2 in E.coli Lysate	297.35
MS2 Filtered	301.95
NaCl	
Phosphate Buffer Solution	
Polystyrene Latex Spheres	

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Conclusions and Future Work

Conclusions

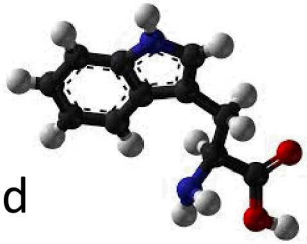
- There is a need for understanding the chemical processes by which biological aerosols are altered in the environment
- Ozone
  - Ozone directly affects specific amino acids even inside complex proteins
    - Tryptophan likely undergoes ozonolysis and is hydrolyzed by water to form N-Formyl Kynurenine and Kynurenine
      - Not reactive with O_3; fluorescent at 355 nm, and not at 263 nm
    - Ozonolysis may destroy microorganism proteins through this process, or affect detectability
  - Ozone appears to destroy free DNA under a similar chemical process
    - Applies largely to extracellular DNA
  - Ozone interactions are most prominent at high RH, and strongly affect resulting viability of virus particles
  - Ozone also degrades NADH/NAD+ under a similar mechanism
    - Apparent in laboratory experiments
    - Not the dominant affect observed in field data

Conclusions (cont..)

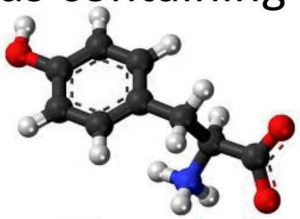
- Solar UV Radiation (300-400 nm)
  - The decay of biological fluorophores under exposure to solar radiation may have several important impacts
    - Fluorescence at 355 nm is degraded through photochemical processes
      - NADH is likely the dominant fluorophore involved
    - Field data indicate that degradation of fluorescence at 355 nm is more closely tied to photochemistry, rather than O_3 directly
- Hygroscopicity
  - The growth of biological aerosol when exposed to high humidity may affect numerous biological aerosol properties
    - Respiratory Deposition, Transport, Mie Scattering Signatures
  - Water uptake by biological aerosols may also change the chemistry that can affect them
    - E.g. Ozone deactivation
  - Spores do not appear to have an affinity for water, however this may be preparation specific
  - MS2 Phage shows growth both in the presence of E.coli lysate and without, again likely preparation specific
  - Media for Pseudomonas and Bt Al Hakam (crude preparations) did not appear to be very hygroscopic

Moving Forward – Increased Number of Simulants

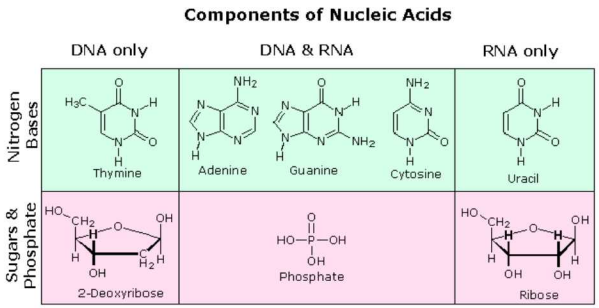
- Protein and Genetic Macromolecule Aerosols
  - Determine specific mechanistic changes due to aging
  - Typical bioaerosols complex mixture of metabolites, proteins, and other macromolecules
  - Isolate the effects of aging on particular amino acids and biological signatures
  - Tryptophan, Tyrosine, Nucleic Acids containing particles
- Gram-negative bacteria
  - Yersinia
  - Escherichia
- Enveloped Virus
  - In addition to previous non-enveloped simulants



Tryptophan



Tyrosine



Increased Assay Support

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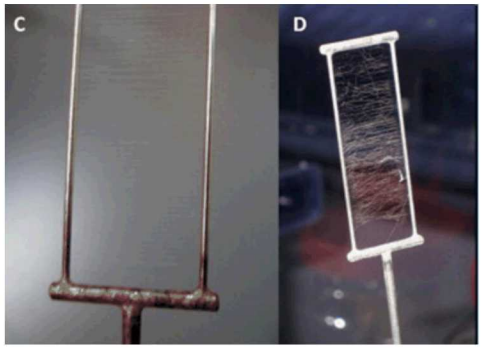
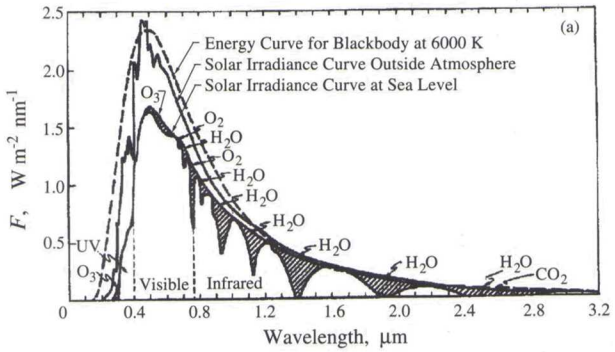
- Infectivity/Effectivity
  - Cultures and plaques previously used
  - Increased use of immunoassays
    - Representative of viral infectivity
    - Useful for
    - Investigate detectability for particles which remain viable
    - Additional data for mechanistic approach to particle aging
- Improved normalization for particle loss
  - Inert substance co-aerosolized with bioaerosol
    - Quantum dots
    - Encapsulated fluorophores
  - Mass mode normalization from aerodynamic particle counter

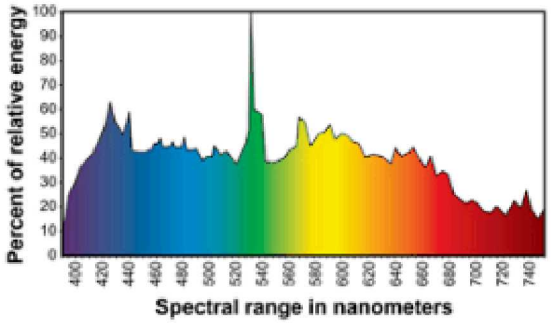
Field Tests, Increased Variables, Webs

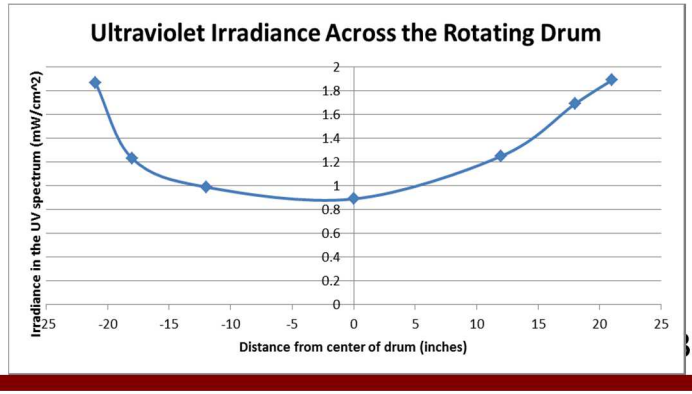
- Increased Variable Control
  - Temperature
  - VOC generation and control (toluene, isoprene, pinene)
  - UV Solar spectrum
- Field Tests – CAGES
  - Methodology established
  - Houston – FY15
    - High Polution, RH
  - Albuquerque – FY16
- Web-based particle entrapment
  - DSTL spider webs to trap particles and age as simulated aerosol.
  - Compare with drum



Solar Spectrum







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