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Bioaerosol Measurement, Sampling and Analysis

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Overview

- Bioaerosol Collection Techniques
- Offline Bioaerosol Analysis Techniques
- In-Situ Bioaerosol Measurement
- Limitations in Bioaerosol Measurement
- Discussion
- The Holy Grail

Bioaerosol Collection

Common Biological Aerosol Collection Technologies

Sterile does not mean free of all traces of life

Filtration

- Most straightforward/simple
- Most losses come from extraction
- Wide range of downstream analysis
  - Some limitation for fragile organisms (vegetative bacteria and virus)
- Wide range of filter media for different applications
  - Metal filters
  - Membrane filters
  - Gel filters
  - Glass fiber

Filtration – Teflon and Glass Fiber

- Teflon and Glass Fiber filters are both suitable for certain types of downstream analysis
  - Neither are suitable for downstream culture, with the exception of spore forming bacteria and fungii
- Both are suitable for downstream molecular analysis
  - Proteins
  - Nucleic Acids
- Filter holders and filters are generally autoclavable
- May also be chemically treated to remove DNA and RNA
- Teflon more appropriate for microscopy
  - Particles on surface (Baron and Willike, 2001)
- Teflon filters also partially soluble in water (buffers)
  - Improves recovery

Burton, N.C. S.A. Grinshpun, T. Reponen. Physical Collection Efficiency of Filter Materials for Bacteria and Viruses. *Ann Occup Hyg*. 2007; 51 (2): 143-151.
Baron, Paul, Klaus Willike. Aerosol Measurement: Principles, Techniques and Application 2 ed. Wiley-Intersciences Publications. 2001.

Filtration – Gel Filters

- Gel Filters can be useful for viable bioaerosol collection
  - Designed to reduce stress on microorganisms during collection
  - Dessication can still be an issue
  - Sampling times recommended to be 30 min or less
  - Soluble in both water and buffers

Cox, Christopher., Wathes Christopher. Bioaerosols Handbook. CRC. 1995

Impaction

- Two types
  - Standard and Viable
- Standard impaction onto filter material or metal surfaces is generally not suitable for viable analysis
- Impaction onto agar surfaces
  - Slit to agar
  - Viable cascade impactor
  - Can also substitute water or buffer
- Cascade impactors can provide size resolution

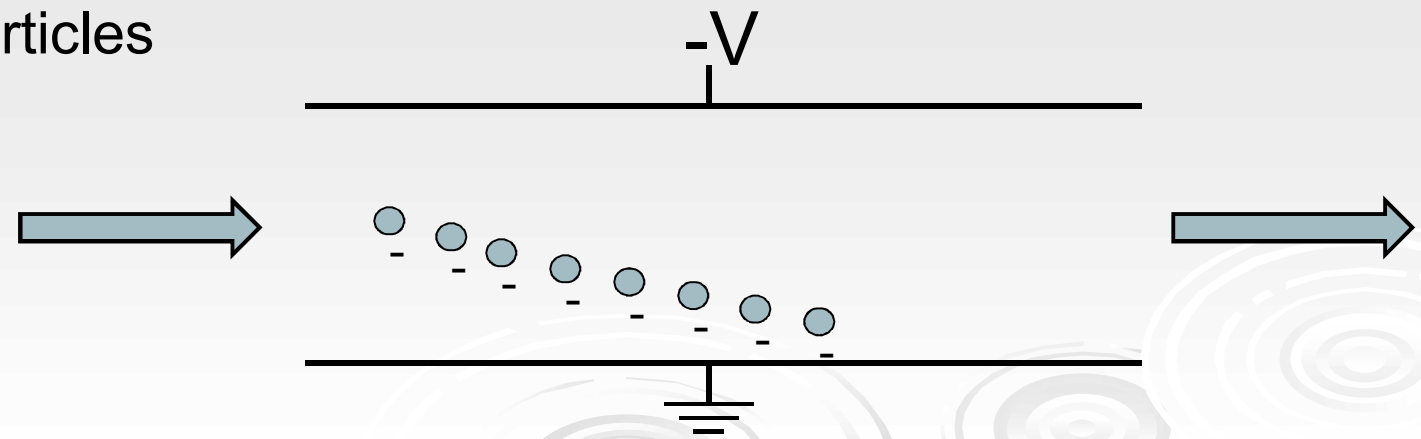
Impingers and Wetted-Wall Cyclones

- Impingers and WWC both collect particles into a hydrosol by impinging an air jet onto a water surface
  - Most impingers are designed to mimic human resting lavage rates
  - WWC are generally designed to operate at high flows
  - Both techniques are effective at preserving viability
  - Multi-stage impingers can provide some size-resolved information
  - Care must be taken as collection liquid may carry proteins or nucleic acid, even if sterile

A.R. McFarland, J.S. Haglund, M.D. King, S. Hu, M.S. Phull, B.W. Moncla and Y. Seo. Wetted Wall Cyclones for Bioaerosol Sampling. 2010. Aerosol Science and Technology, 44(4).

Electrostatic Precipitation

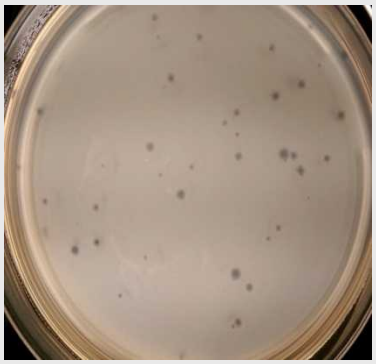
- Electrostatic precipitation is a common technology for particle collection and removal
- Collection of bioaerosol by ESP is less common but could be very promising
- Force of collection could be quite small and controllable by sizing the voltage and collection distance appropriately
- Mainelis has shown that some bioaerosols may carry a preferential charge that could be used for collection/concentration of bioaerosol apart from non-biological particles



Mainelis, G., K. Willeke, P. Baron, T. Reponen, S.A. Grinshpun, R.L. Górny, S. Trakumas. Electrical charges on airborne microorganisms, *Journal of Aerosol Science. 2001*; 32 (9): 1087-1110.

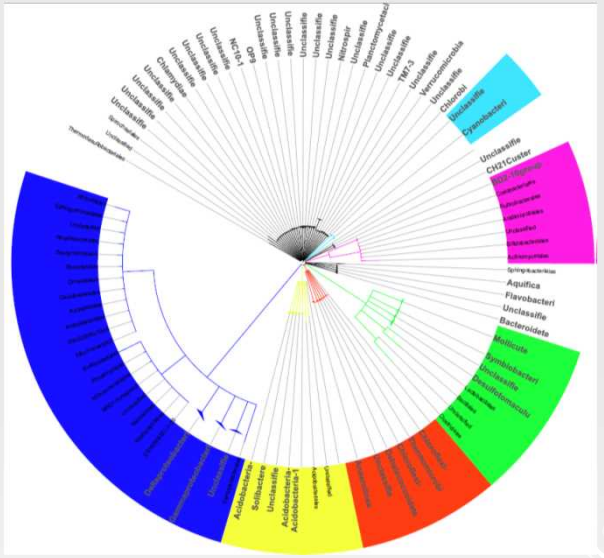
Common Biological Analysis Techniques

Culture

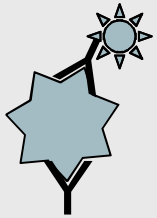


NA Analysis

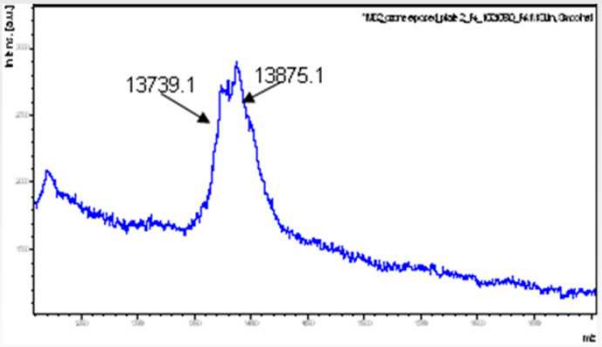
PCR-based Sequencing



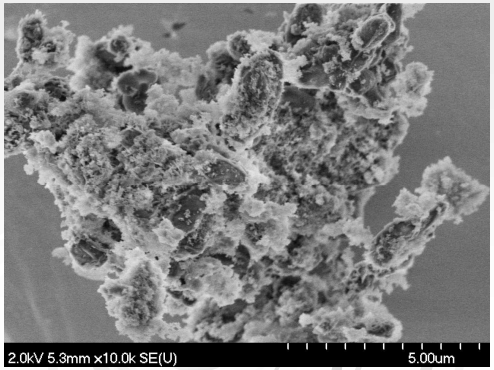
Protein Analysis



Mass Spectra



Microscopy

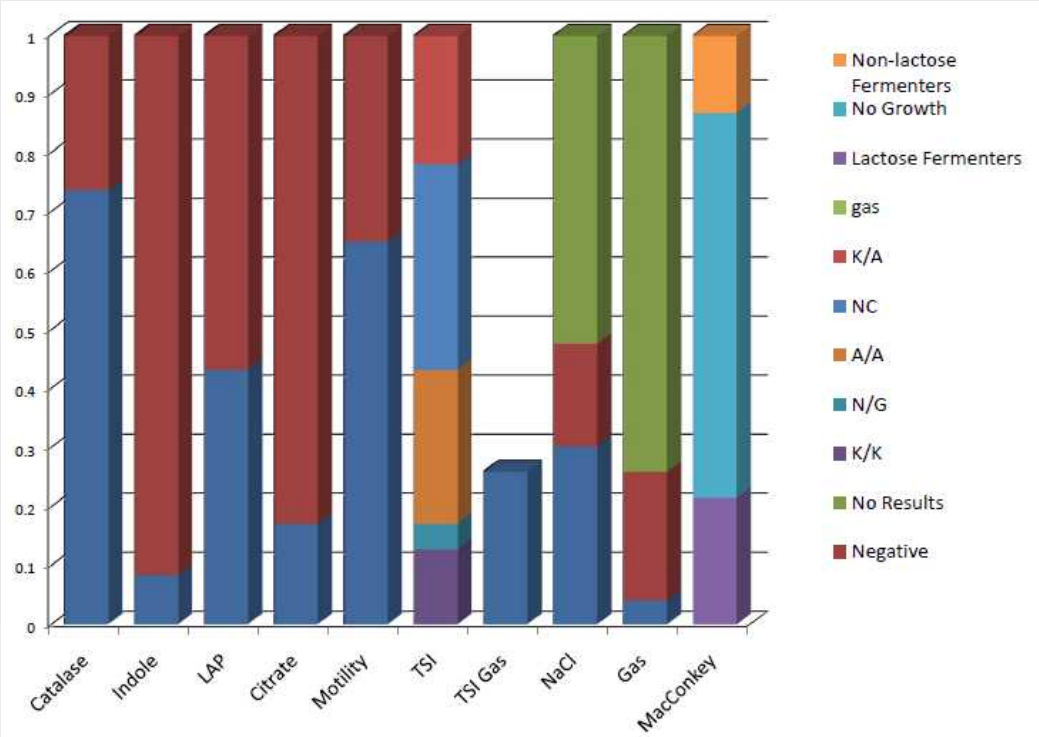


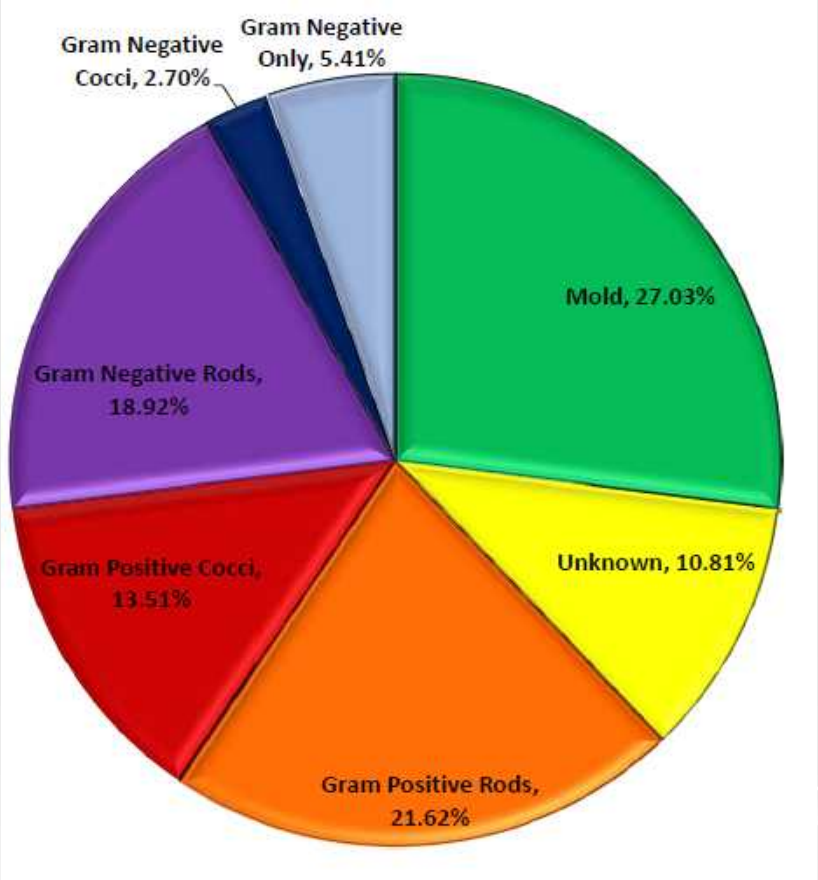
Culture

- Cultivation of collected microorganisms is by far the oldest means to analyze bioaerosols
- Culture comes with significant complications
- Culture can provide some utility that molecular techniques are only beginning to provide
- Organisms cultured from a sample were undeniably viable upon collection, but not all viable organisms can be cultured under known laboratory methods
- It has been suggested that only 1-2% of all environmental bacteria are culturable (Sharma, et al., 2005)

Wagner, M. R. Amann, H. Lemmer, K.-H. Schleifer. Probing Activated Sludge with Oligonucleotides Specific for Proteobacteria: Inadequacy of Culture-Dependent Methods for Describing Microbial Community Structure. Applied and Environmental Microbiology. 1993; 1520-1525.
Sharma, R., Ranjan, R., Kapardar, R. K., & Grover, A. Unculturable bacterial diversity: an untapped resource. *Current Science*. 2005; *89*(1): 72-77.

Microbial Culture





Traditional culture techniques can provide significant information about the phenotypic behavior and function of culturable microns that molecular techniques can only suggest

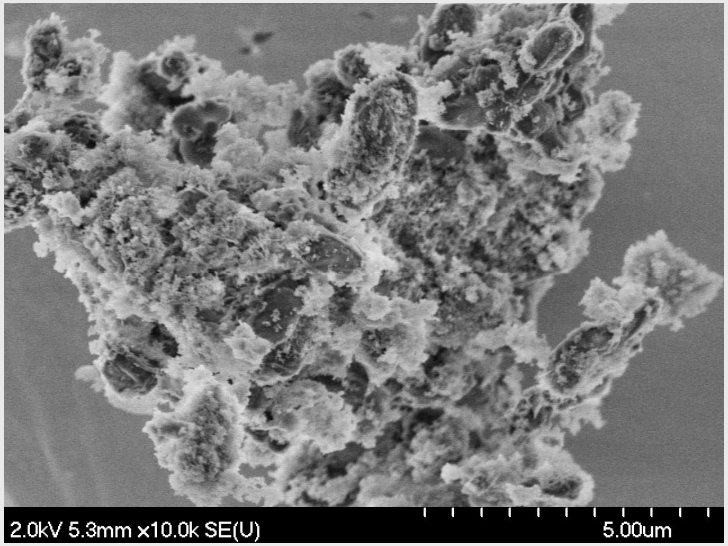
Viral Aerosol Cultivation

- Cultivation of “live” viral aerosols from ambient bioaerosol is extraordinarily difficult unless you know exactly what you’re looking for
- Viruses can be extremely host specific, even cell-line specific
- Broad spectrum live viral assay would be extremely challenging, if not impossible
- It is possible to look for specific “live” virus from a sample by plaque assay or cytopathic effect if the proper cell line has been identified ahead of time (Lindsley, et al., 2010)

Lindsley, W.G., F.M. Blachere, R.E. Thewlis, A. Vishnu, K.A. Davis, G. Cao, J.E. Palmer, K.E. Clark, M.A. Fisher, R. Khakoo, D.H. Beezhold. Mesurements of Airborne Influenza Virus in Aerosol PAticles from Human Coughs. *PLOS One. 2010*; 5(11): e15100.

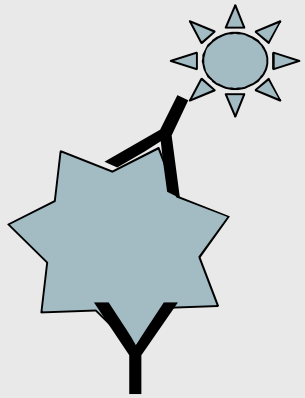
Microscopy

- Visual image of collected particle
- Little or no biological information
- Requires collection that does not damage or distort particle



Protein Analysis

- Immunoassay
  - Very specific to protein/organism
  - Need to know what you're looking for
- Bulk protein analysis
  - Comparable to organic fraction measurement
  - Good quantification of biological mass fraction
  - Doesn't help identify what was there or what they were doing



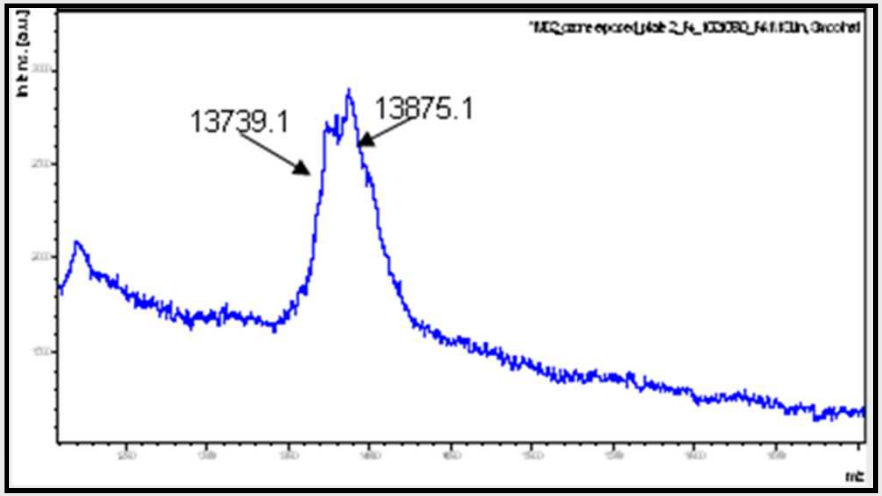
Womiloju, T.O., Miller, J.D., Mayer, P.M., Brook, J.R. Methods to determine the biological composition of particulate matter collected from outdoor air. *Atmospheric Environment. 2003;* 37: 4335–4344.

Hindson, B.J., M.T. McBride, A.J. Makarewicz, B.D. Henderer, U.S. Setlur, S.M. Smith, D.M. Gutierrez, T.R. Metz, S.L. Nasarabadi, K.S. Venkateswaran, S.W. Farrow, B.W. Colston, Jr., J.M. Dzenitis. Autonomous Detection of Aerosolized Biological Agents by Multiplexed Immunoassay with Polymerase Chain Reaction Confirmation, *Analytical Chemistry. 2005; 77* (1): 284-289.

Boreson, J., A.M. Dillner, J. Peccia. Correlating bioaerosol load with PM2.5 and PM10cf concentrations: a comparison between natural desert and urban-fringe aerosols. *Atmospheric Environment*. 2004; 38: 6029–6041.

Protein Analysis – MALDI MS

- MALDI-MS
  - Can be specific to some proteins
  - Relies on mass difference
  - MS-MS can better distinguish
  - Some proteins are hard to characterize with this method



Fenselau,C and PA Demirev. Characterization of intact microorganisms by MALDI mass spectrometry, Mass Spectrometry Reviews. 2001; 20 (4):157-171.
Demirev, P.A. Y.P. Ho, V. Ryzhov, C. Fenselau. Microorganism identification by mass spectrometry and protein database searches. *Analytical chemistry*. 1999; 71 (14): 2732-2738

Nucleic Acid Analysis

- Nucleic Acids (DNA and RNA) code for all the proteins in an organism and therefore, provide a unique representation of every living thing and its function on earth
  - Select small fragments of DNA can be used to ID groups of organisms or even down to the sub-species level depending on which fragments is chosen

Hua, Y. T., R.C. Tong. Use of the polymerase chain reaction for specific detection of type A, D and E enterotoxigenic Staphylococcus aureus in foods. *Appl Microbiol Biotechnology*. 1992; 37:685-690
Hales, B.A., C. Edwards, D.A. Ritchie, G. Hall, R.W. Pickup, J.R. Saunders. Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. *Appl. Environ. Microbiol.* 1996; 62(2): 668-75.
Bacchetti De Gregoris, T., N. Aldred, A.S. Clare, J.G. Burgess. Improvement of phylum- and class-specific primers for real-time PCR quantification of bacterial taxa, Journal of Microbiological Methods. 2011; 86(3): 351-356.

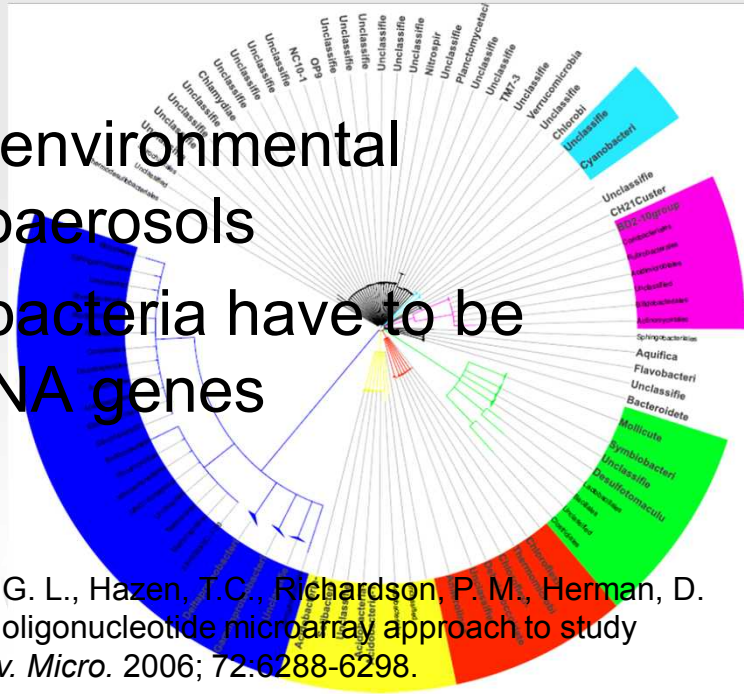
Nucleic Acid - PCR-Based

- PCR uses unique DNA/RNA fragments to determine the presence or absence of an organism
- Bioaerosol characterization by PCR is limited by available assays
- Very sensitive to a specific organism or an organism carrying a specific gene
- Reactions can be multi-plexed to detect several organisms/genes in a sample
  - Multi-plexed reaction require extensive optimization to maintain sensitivity
- Many bioaerosol types can be identified with the same assay (bacteria, virus, etc.)

Peccia, J. and M. Hernandez. Incorporating polymerase chain reaction-based identification, population characterization, and quantification of microorganisms into aerosol science: A review. *Atmospheric Environment*. 2006; 40: 3941–3961.
Elnifro, E. M., Ashshi, A. M., Cooper, R. J., & Klapper, P. E. Multiplex PCR: Optimization and Application in Diagnostic Virology. *Clinical Microbiology Reviews. 2000; 13*(4): 559–570.

Nucleic Acid - Microarray

- A common PCR target for bacteria in the 16s rRNA gene
- The presence of this gene and its unique hypervariable regions in all bacteria has been exploited to develop 16s microarrays
- A microarray is an extensively multiplexed PCR reaction that can identify thousands of genes with thousands of PCR reactions
- This has been applied to numerous environmental microbiology problems, including bioaerosols
- The primary limitation is that target bacteria have to be known and have sequenced 16s rRNA genes



Brodie, E. L., DeSantis, T. Z., Joyner, D. C., Baek, S. M., Larsen, J. T., Andersen, G. L., Hazen, T.C., Richardson, P. M., Herman, D. J., Tokunaga, T. K., Wan, J. M., and Firestone, M. K. Application of a high-density oligonucleotide microarray approach to study bacterial population dynamics during uranium reduction and reoxidation. *Appl. Env. Micro.* 2006; 72:6288-6298.

Nucleic Acid - Sequencing

- No *a priori* information about organisms needed
- Theoretically possible to identify every living thing in a sample
- Whole genome sequencing can offer a broad look at an air sample
- Need to develop better bioinformatics for interpreting previously unsequenced genes and organisms
- Low coverage in air samples complicates analysis
- Only beginning to be used to look at bioaerosol

Amaral-Zettler, LA, EA McCliment, HW Ducklow, SM Huse. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PLoS ONE; 2009;* 4:e6372.
Venter, C.V., K. Remington, J.F. Heidelberg, A.L. Halpern, D. Rusch, J.A. Eisen, D. Wu, I. Paulsen, K.E. Nelson, D.E. Fouts, S. Levy, A.H. Knap, M.W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y.-H. Rogers, H.O. Smith. Environmental Genome Shotgun Sequencing of the Sargasso Sea. *Science. 2004*; 304 (5667): 66-74.
Boissy, R.J., D.J. Romberger, W.A. Roughead, L. Weissenburger-Moser, J.A. Poole, T.D. LeVan. Shotgun Pyrosequencing Metagenomic Analyses of Dusts from Swine Confinement and Grain Facilities, *PLOS One. 2014;* 9(4), e95578.
Hazen, T.C. et al. Deep-Sea Oil Plume Enriches Indigenous Oil-Degrading Bacteria. *Science*. 2010; 330(6001):204-208.

Mass Spectra

- Two types typical for bioaerosol
  - Elemental spectra
    - Typical for single particle analysis
    - Library matching can ID groups of organisms
    - BAMS/SPAMS technology
    - Signatures must be carefully developed in a lab
  - MALDI MS
    - Protein analysis
    - Covered previously

SPAMS from LLNL

Fergenson, D.P., M. E. Pitesky, H. J. Tobias, P. T. Steele, G. A. Czerwieniec, S. C. Russell, C. Lebrilla, J. Horn, K. Coffee, A. Srivastava, S. P. Pillai, M.-T. P. Shih, H. L. Hall, A. J. Ramponi, J. T. Chang, R. G. Langlois, P. L. Estacio, R. T. Hadley, M. Frank, and E. Gard, *Anal. Chem*. 2004; 76, 373-378

Steele, P.T., G.R. Farquar, A.N. Martin, K.R. Coffee, V.J. Riot, S.I. Martin, D.P. Fergenson, E.E. Gard and M. Frank. Autonomous, broad-spectrum detection of hazardous aerosols in seconds, *Analytical Chemistry*. 2008; 80: 4583-4589.

Chen, Q., et al. Mass spectral characterization of submicron biogenic organic particles in the Amazon Basin, *Geophys. Res. Lett. 2009:* 36. L20806. doi:10.1029/2009GL039880.

Appropriate sampling types for different offline analysis methods

		Culture	PCR	Immuno	Mass Spectral	Microscopy	Sequencing
Filter							
	glass fiber	s,f	s,b,f,v	s,b,f,v	s,b,f,v		s,b,f,v
	teflon		s,b,f,v	vs,b,f,v	s,b,f,v	s,b,f,v	s,b,f,v
	gelatin	s,b,f,v	s,b,f,v	s,b,f,v			s,b,f,v
Impactor							
	solid substrate		x	s,b,f,v	s,b,f,v	s,b,f,v	s,b,f,v
	agar	s,b,f	s,b,f,v	s,b,f,v			s,b,f,v
Impinger/WWC		s,b,f,v	s,b,f,v	s,b,f,v	s,b,f,v *		s,b,f,v *
Electrostatic Precipitator		s,b,f,v	s,b,f,v	s,b,f,v	s,b,f,v	s,b,f,v	s,b,f,v

s=spores, b=bacteria, f=fungus, v=virus

* Buffer must be carefully chosen not to interfere with analysis method

*My opinions based on lab and field experience.

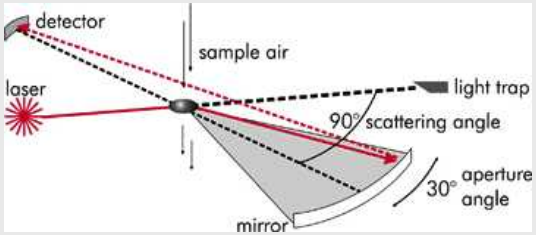
In situ Biological Aerosol Measurement

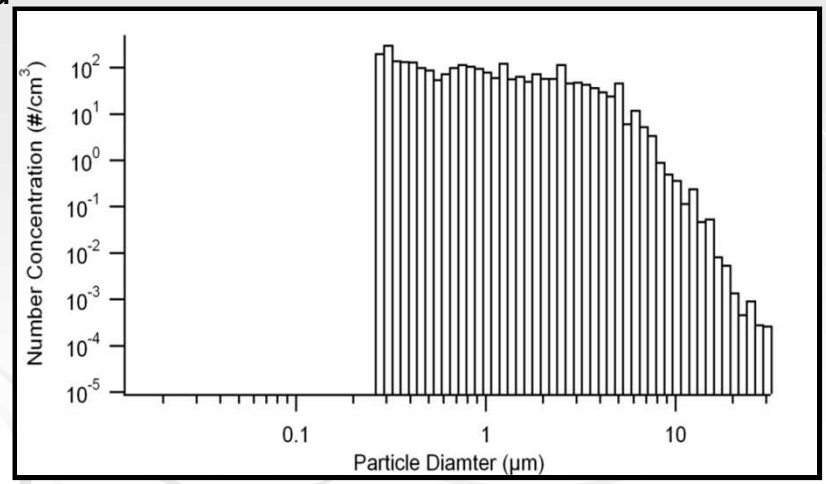
In Situ techniques for bioaerosols

- Optical and aerodynamic particle sizers
  - Often a part of, or used in conjunction with other techniques
- Fluorescence
  - Easily the most prolific technique
  - Several commercial instruments
- Raman
  - Single particle Raman in only recently becoming possible
- Mass Spec
  - Common for other aerosols more complicated to apply to biological

Optical Particle Counter Grimm PAS

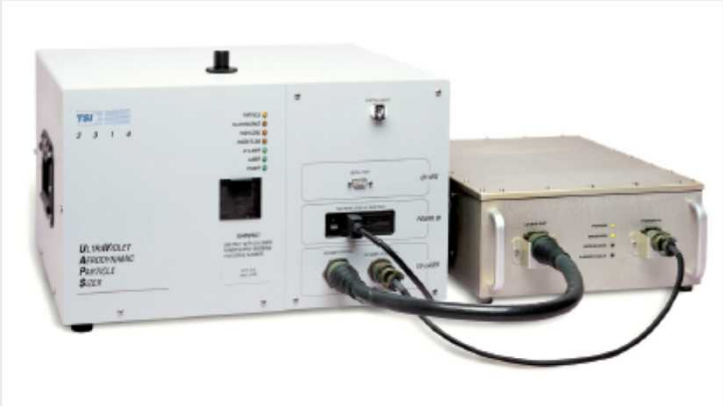
- Grimm Portable Aerosol Spectrometer (Model 1.109)
  - Particle sizing from 0.3-32 µm (Geometric Volume Equivalent Diameter; fine to coarse)
  - Based on 90º optical scattering
  - Flow rate 1.2 lpm
  - Aerosol can be collected on PTFE filter for further chemical analysis
  - Sheath air flow (internally generated) filtered and used for air regulation and maintaining laser optic assembly
  - Minimum measurement period is 6 seconds
  - Communication via RS-232 serial port
  - Can be used remotely
    - Removable data card and battery

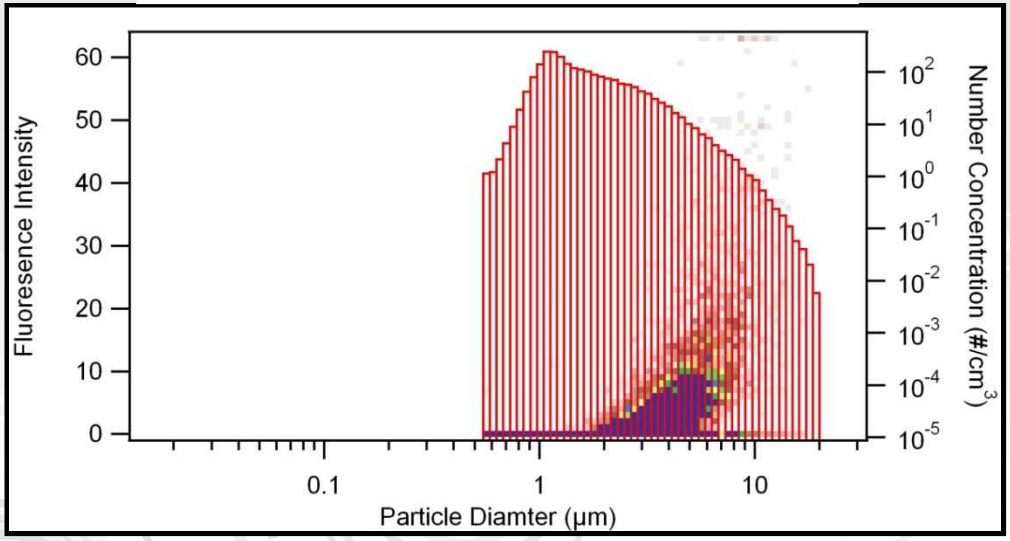




Aerodynamic Diameter Measurement Ultra-Violet Aerodynamic Particle Sizer (UV-APS)

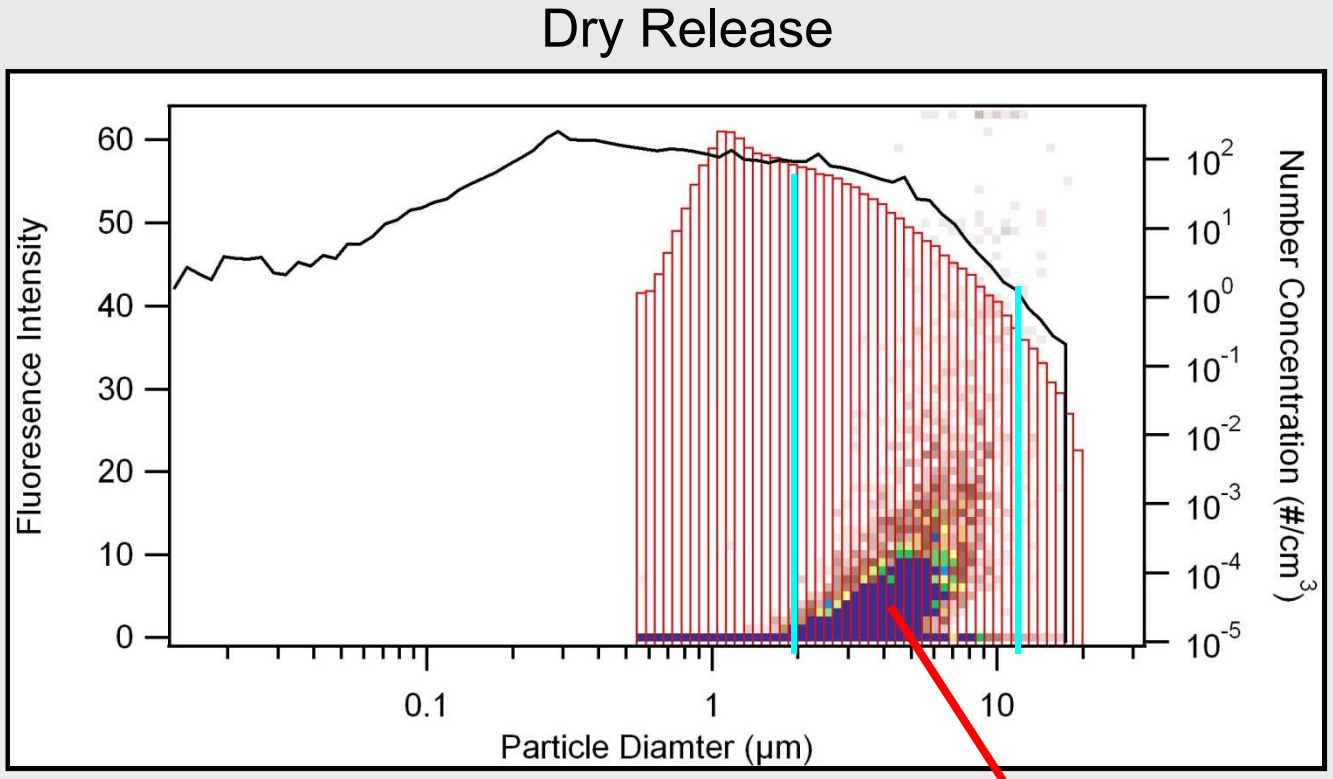
- Characterizes individual airborne particles between 0.5 to 15 μm (fine to coarse)
- Provides instantaneous measurements of aerodynamic diameter and scattered-light intensity
- Measures fluorescence intensity concurrently with above measurements



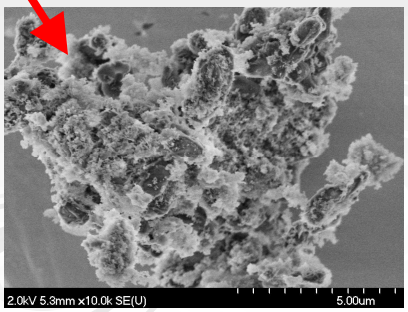


Hairston, P.P., Ho, J., Quant, F.R. Design of an instrument for real-time detection of bioaerosols using simultaneous measurement of particle aerodynamic size and intrinsic fluorescence. *J. Aerosol Sci. 1997;* **28**: 471-482.

Bioaerosol Detection Techniques

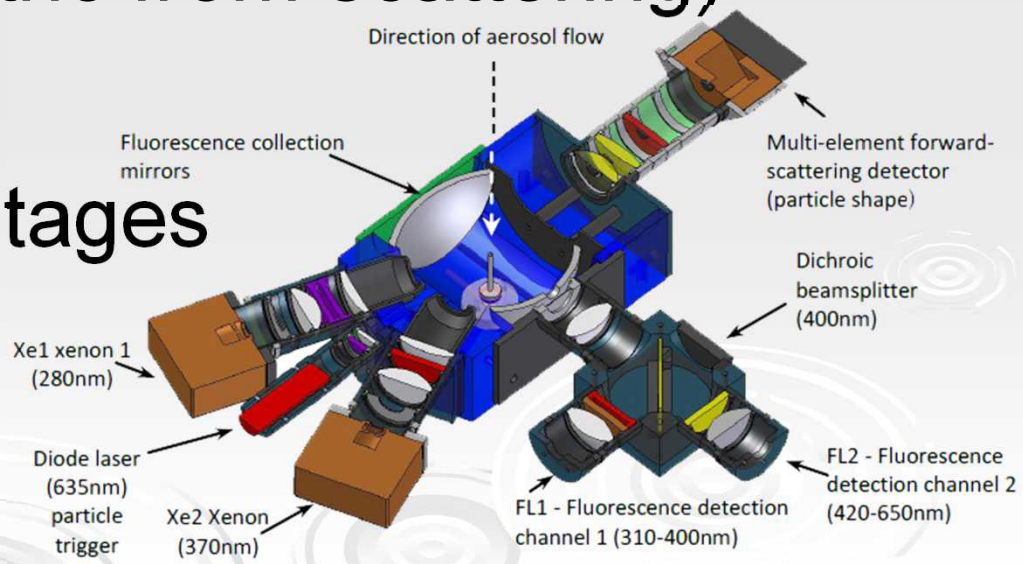


- UV Florescence (UV-APS)
  - Combining 355nm UV laser with TSI APS allows one to distinguish between a biological aerosol plume and a non-bio plume



Wideband Integrated Bioaerosol Sensor

- 280 nm and 370 nm excitation
- 310 to 400 nm and 420 to 650 nm emission bands
- Particle Size (geometric from scattering)
- Asymmetry factor
- Data fusion in early stages

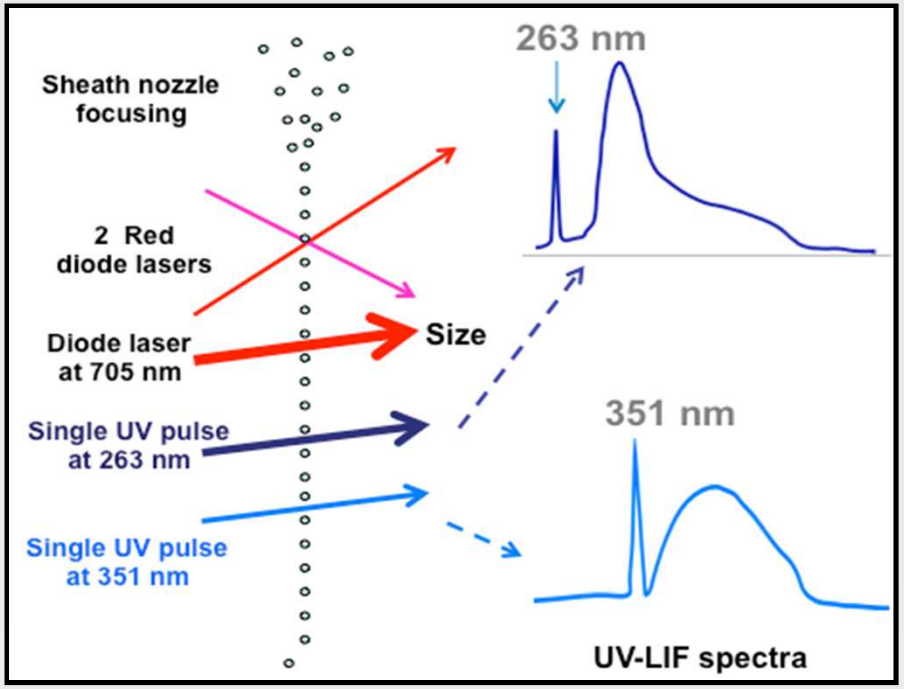


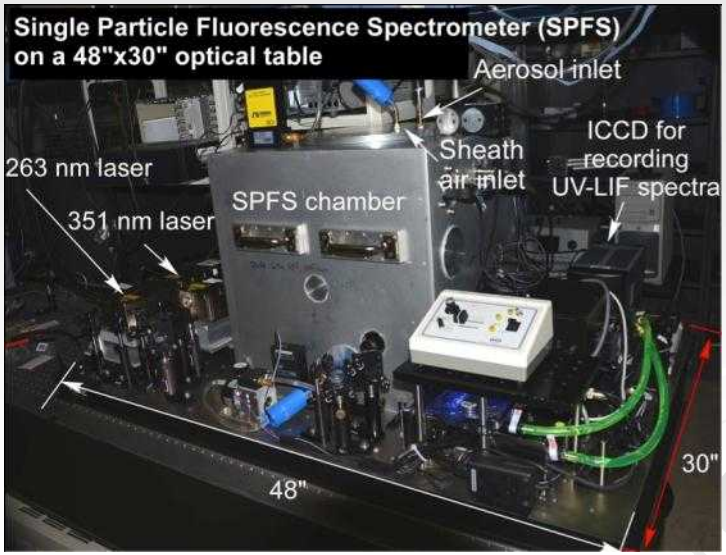
Reprinted from DMT website

Kaye, P.H., Stanley, W.R., Hirst, E., Foot, E.V., K,. L. Baxter, K.L., Barrington, S.J. Single particle multichannel bio-aerosol fluorescence sensor. *Opt. Express. 2005;* **13**: 3583–593.

Single Particle Fluorescence Spectrometer (SPFS)

- Army Research Laboratory
  - Current system developed from over a decade of research
  - Fluorescence excited at 263 and 351 nm
  - Spectra measured each wavelength
  - Particle size from optical scattering





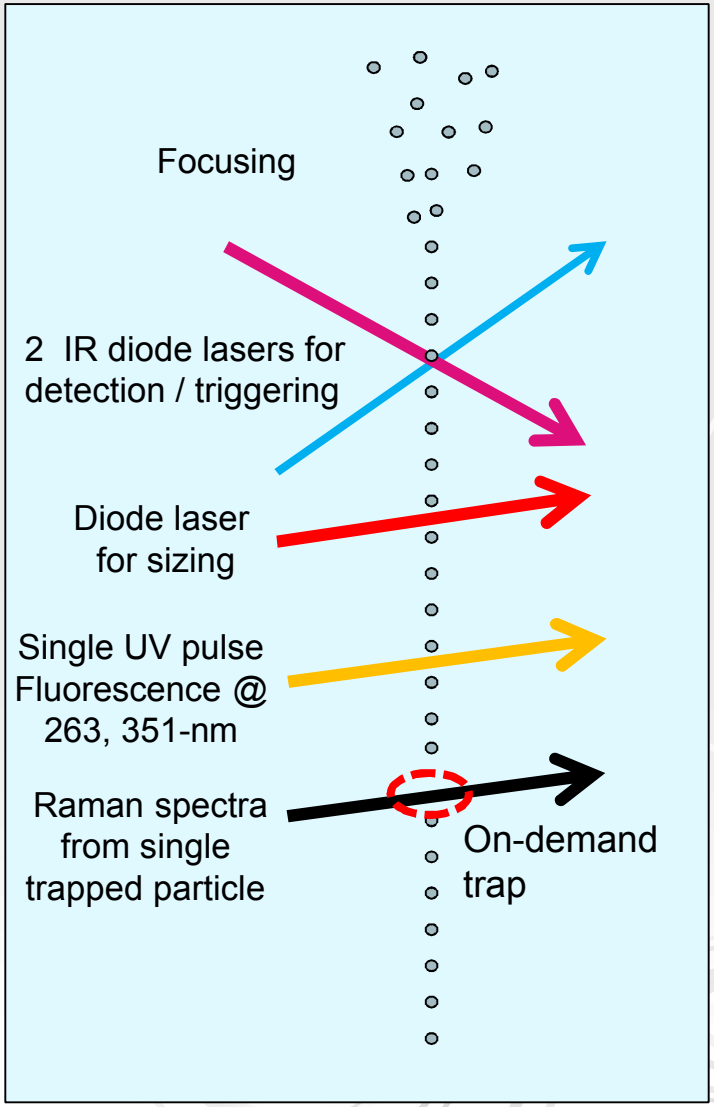
Pan, Y.L., Hartings, J., Pinnick, R.G., Hill, S.C., Halverson, J., et al. Single-particle fluorescence spectrometer for ambient aerosols. *Aerosol Sci. Technol. 2003;* **37**: 628-639.

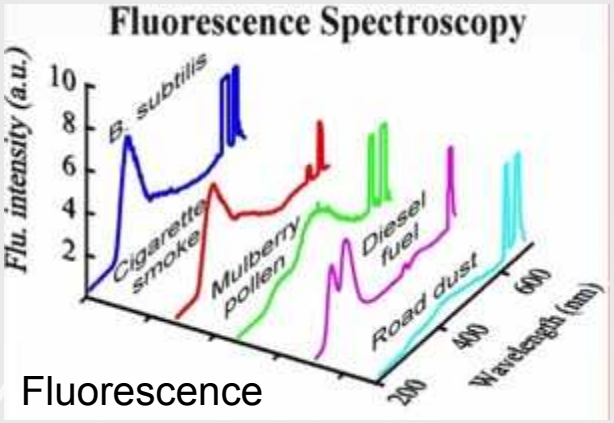
Pan, Y.L., Pinnick, R.G., Hill, S.C., Chang, R.K. Particle-fluorescence spectrometer for real-time measurements of atmospheric organic carbon and biological aerosol. *Environmental Sci. & Technol*. 2009; **43**, 429-434

Pan, Y.L., Hill, S.C., Pinnick, R.G., Huang, H., Bottiger, J.R., and Chang, R.K. Fluorescence spectra of atmospheric aerosol particles measured using one or two excitation wavelengths: Comparison of classification schemes employing different emission and scattering results. *Opt. Express. 2010;* **18** (12), 12436-12457. .

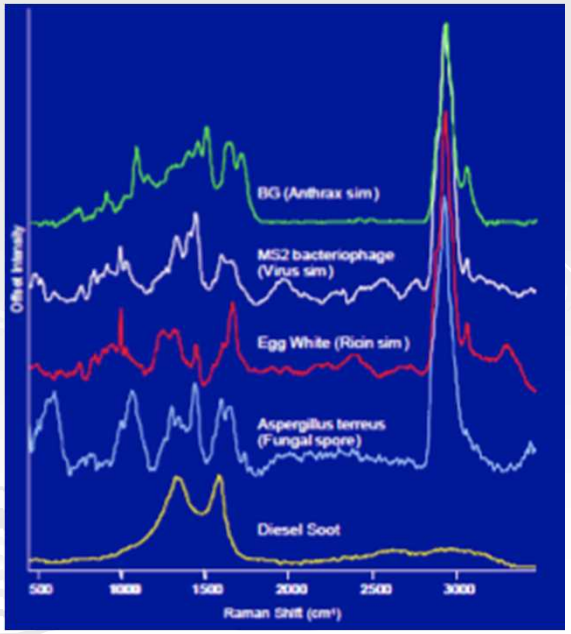
Schematic for measuring optical property of single particles

- Interrogate one particle at a time while it is moving or trapped.
- Elastic scattering for size and morphology.
- Fluorescence spectra for rough classification of bioaerosols.
- Raman spectra for more specific characterization.





Fluorescence



Aerosol Mass Spectrometry

- Real-time aerosol MS for biological particles has only been done by a few groups
- BAMS/SPAMS from LLNL is most common
  - TOF-MS of single particles
  - Developed for Biodefense
  - Uses ratio of elemental composition as a biological indicator
    - Library matching from lab testing
    - Potential significant limitations
- Aerodyne MS has been used by one group
  - Sub-micron only
  - Thermal desorption ionization (softer)
  - Developed for air quality studies of OC
  - Determination of biological material still poorly defined, based on limited lab studies of biological material (amino acids, carbohydrates, etc.)

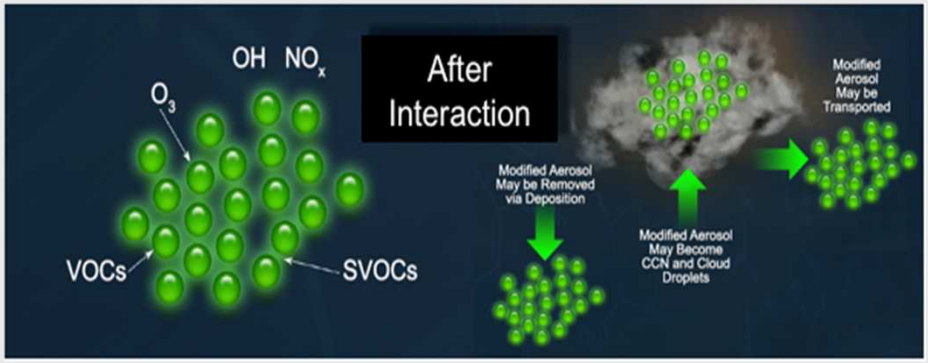
Fergenson, D.P., M. E. Pitesky, H. J. Tobias, P. T. Steele, G. A. Czerwieniec, S. C. Russell, C. Lebrilla, J. Horn, K. Coffee, A. Srivastava, S. P. Pillai, M.-T. P. Shih, H. L. Hall, A. J. Ramponi, J. T. Chang, R. G. Langlois, P. L. Estacio, R. T. Hadley, M. Frank, and E. Gard, *Anal. Chem*. 2004; 76, 373-378

Steele, P.T., G.R. Farquar, A.N. Martin, K.R. Coffee, V.J. Riot, S.I. Martin, D.P. Fergenson, E.E. Gard and M. Frank. Autonomous, broad-spectrum detection of hazardous aerosols in seconds, *Analytical Chemistry*. 2008; 80: 4583-4589.

Chen, Q., et al. Mass spectral characterization of submicron biogenic organic particles in the Amazon Basin, *Geophys. Res. Lett. 2009;* 36, L20806, doi:10.1029/2009GL039880.

Limitations in Bioaerosol Measurement

Background



- Primary Biological Aerosols (PBA)
  - May undergo chemical or physical changes in the atmosphere via differing processes
  - Open Air Factor (OAF)

- Atmospheric Processes
  - Ozone, UV, RH, SOA, Pollutants, Free Radicals
- Changes
  - Viability
  - Size distribution
  - Morphology
  - **Detection and Measurement**
  - Infectivity
  - Resuspension

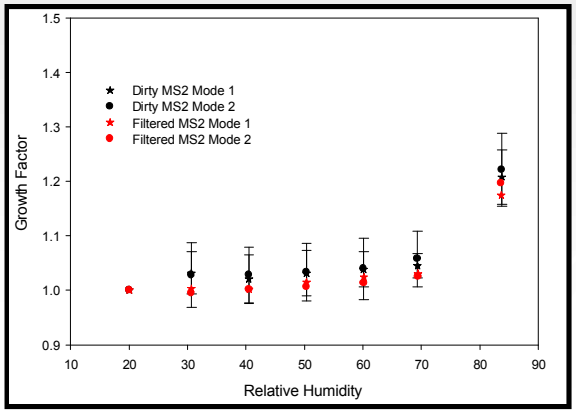
Effects of Aging and Bioaerosol Measurement

- Water uptake
- Interactions with water vapor and ozone
- Many more that haven't been studied
  - SOA
  - Radicals
  - Etc.

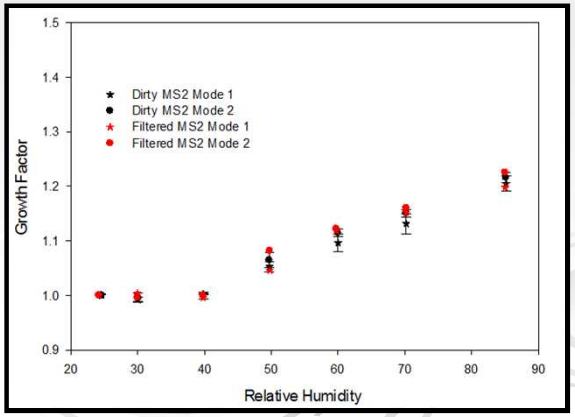
Hygroscopic Growth of Bioaerosols

- 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
- Water Uptake of laboratory generated bioaerosols seems highly media dependent

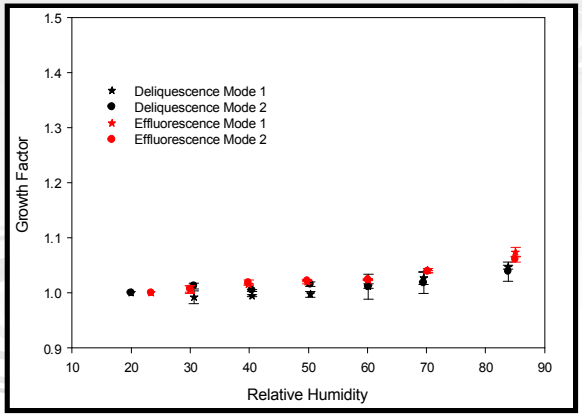
MS2 Deliquescence



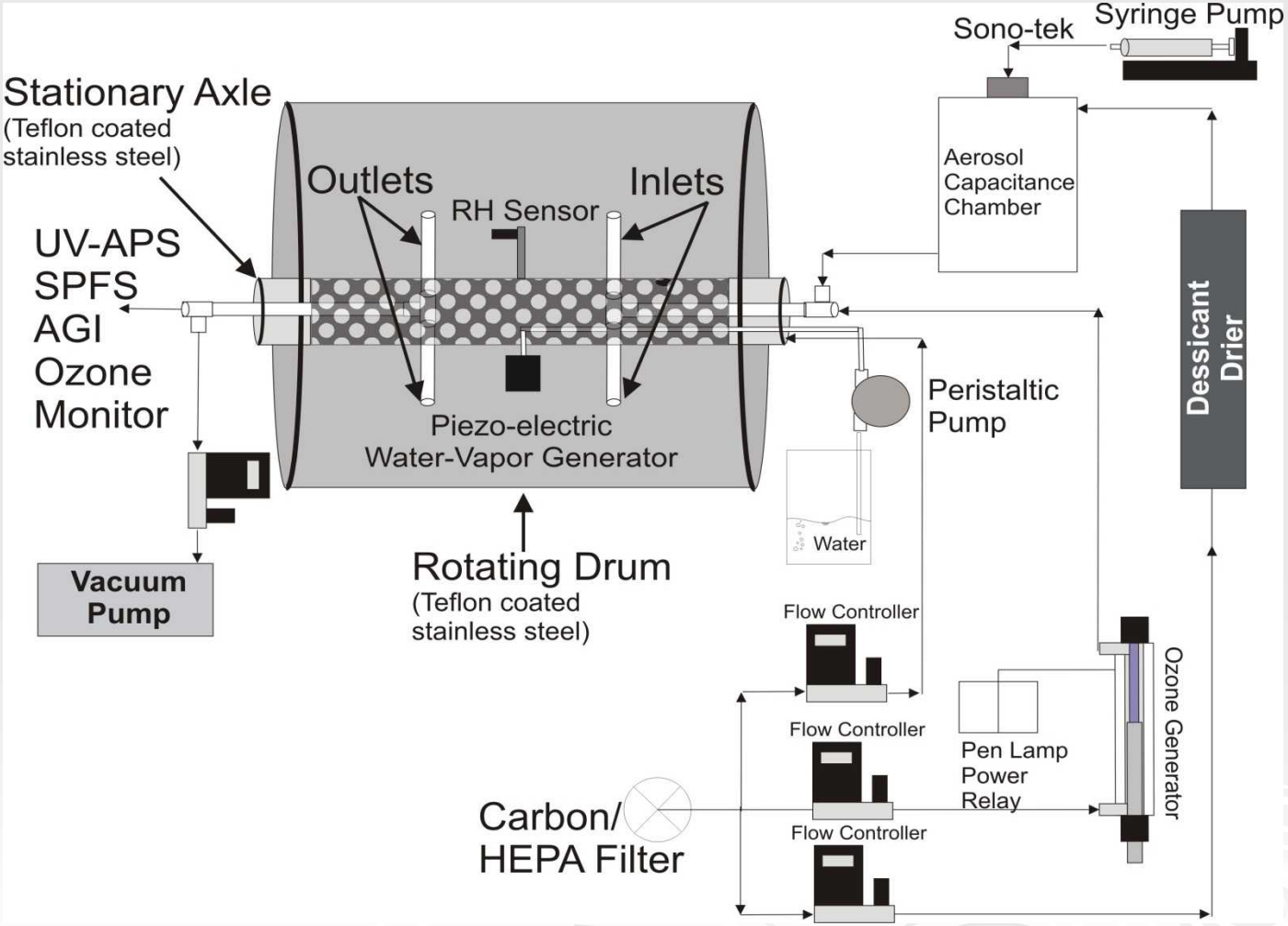
MS2 Effluorescence



Pseudomonas Deliquescence



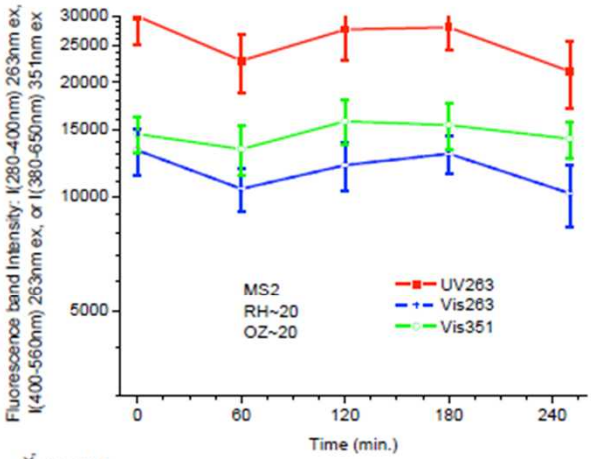
Rotating Drum for Atmospheric Chemistry of Bioaerosols

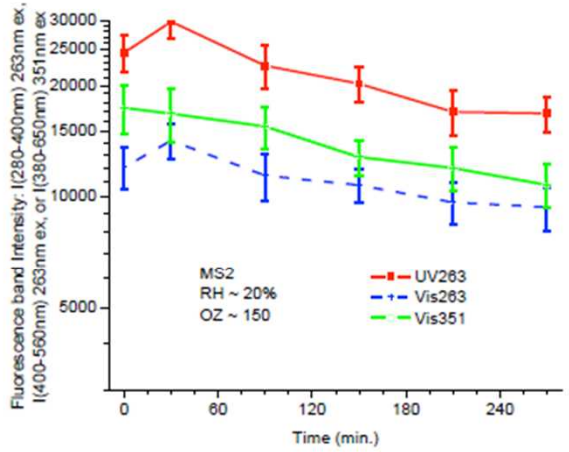


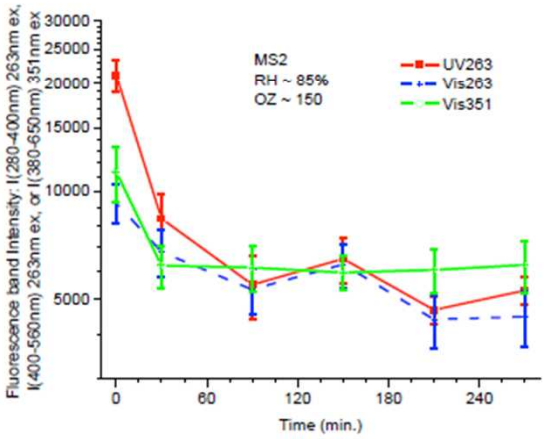
Pan, Y.-L., J.L. Santarpia, S. Ratnesar-Shumate, E. Corson, J. Eshbaugh, S.C. Hill, C.C. Williamson, M. Coleman, C. Bare, S. Kinahan. Effects of ozone and relative humidity on fluorescence spectra of octapeptide bioaerosol particles, Journal of Quantitative Spectroscopy and Radiative Transfer. 2014; 133, 538-550

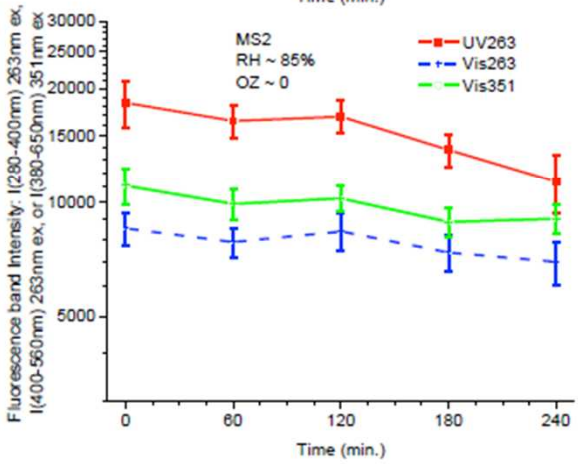
Drum MS2 Experiments

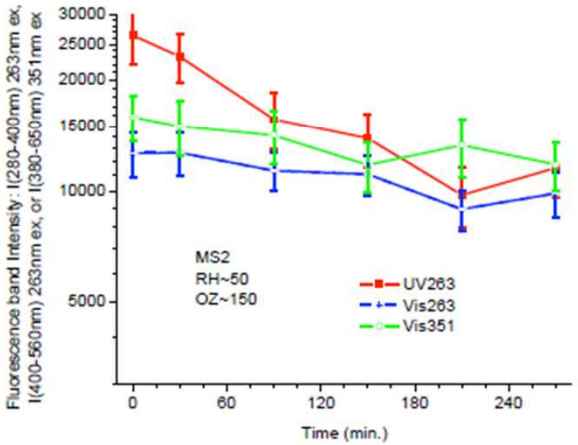
- Individually, both RH and O3 cause a decrease in observed fluorescence
- Decrease at 150 ppb O3 and 85% RH is the most profound







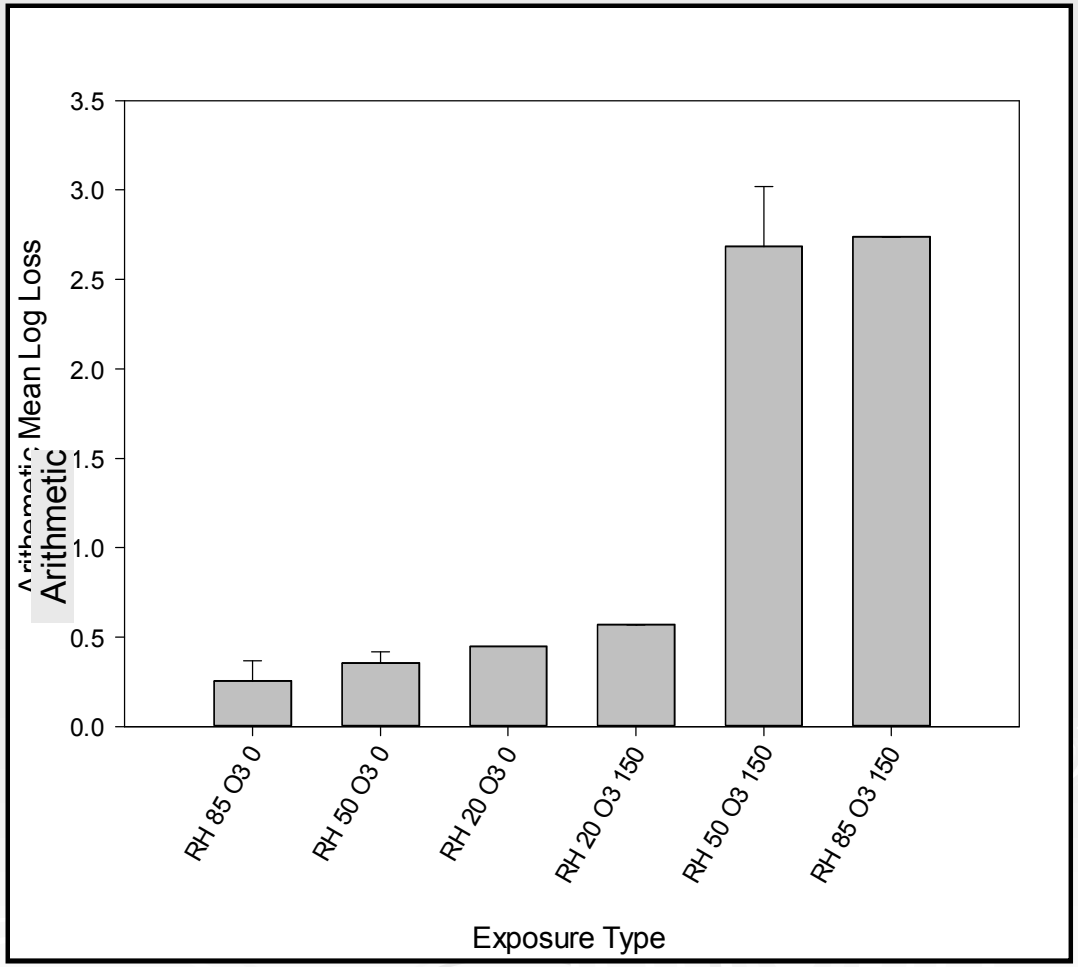




Reprinted with permission from Ratnesar-Shumate, S., Pan, Y.L., Hill, S.C., Kinahan, S., Corson, E., Eshbaugh, J., Santarpia, J.L. Fluorescence spectra and biological activity of aerosolized bacillus spores and MS2 bacteriophage exposed to ozone at different relative humidities in a rotating drum. *Jour Quan Spec & Rad Trans. 2015; 153, 13-28.* DOI: 10.1016/j.jqsrt.2014.10.003

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 O3



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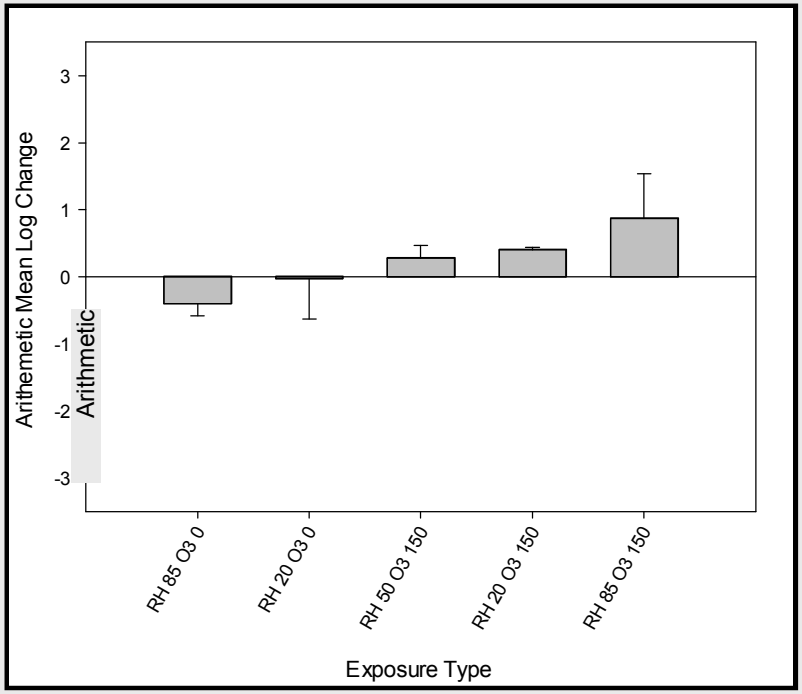
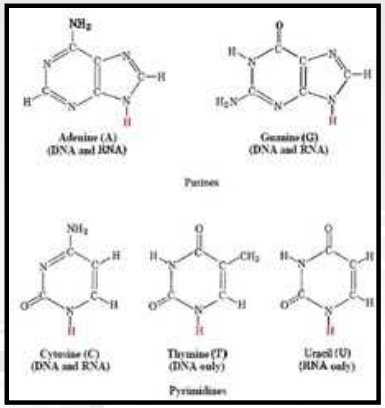
Btk Viability Due to Ozone

- 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 O3
- "Apparent" increase in viable fraction observed when ozone was present
  - May indicate that O3 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 O3, but would normally be protected by the cell





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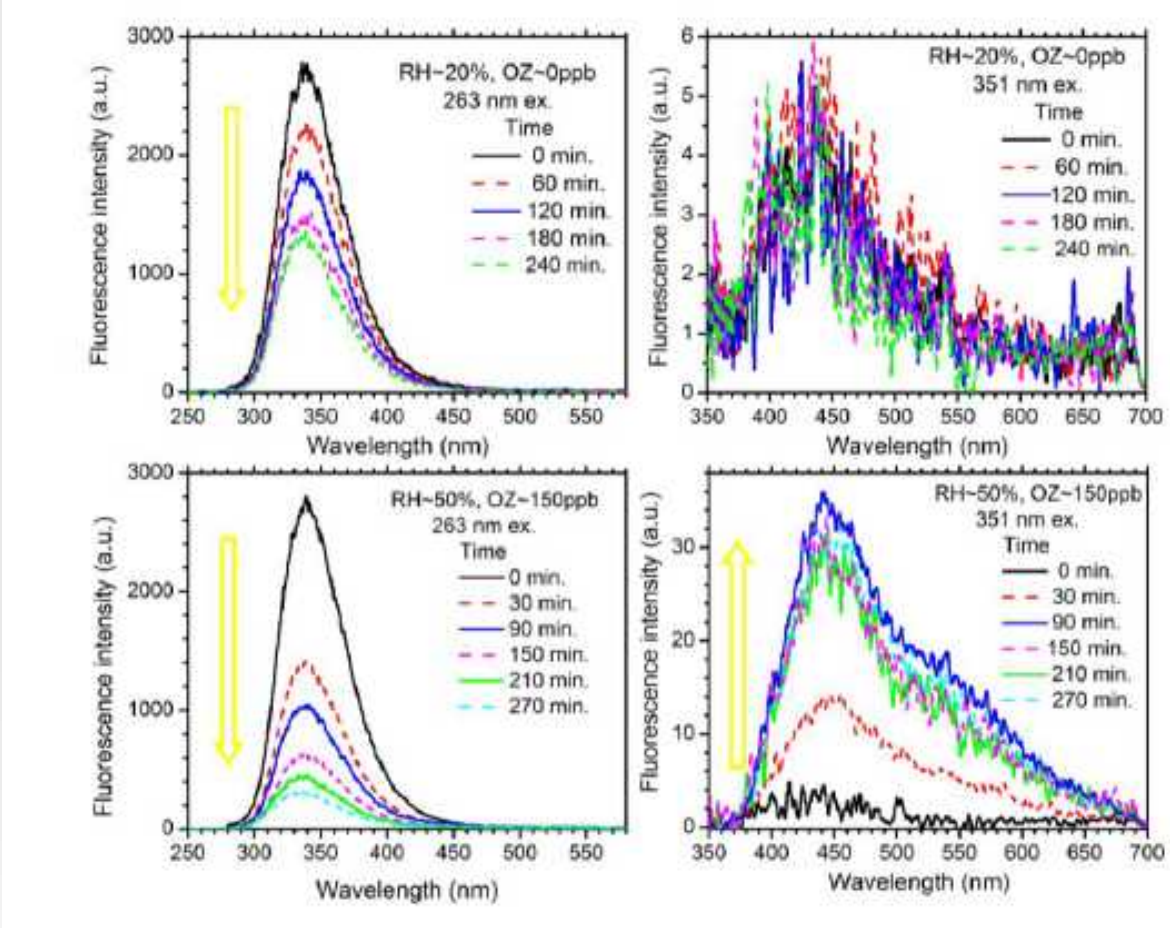
Drum Peptide Experiments

- 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



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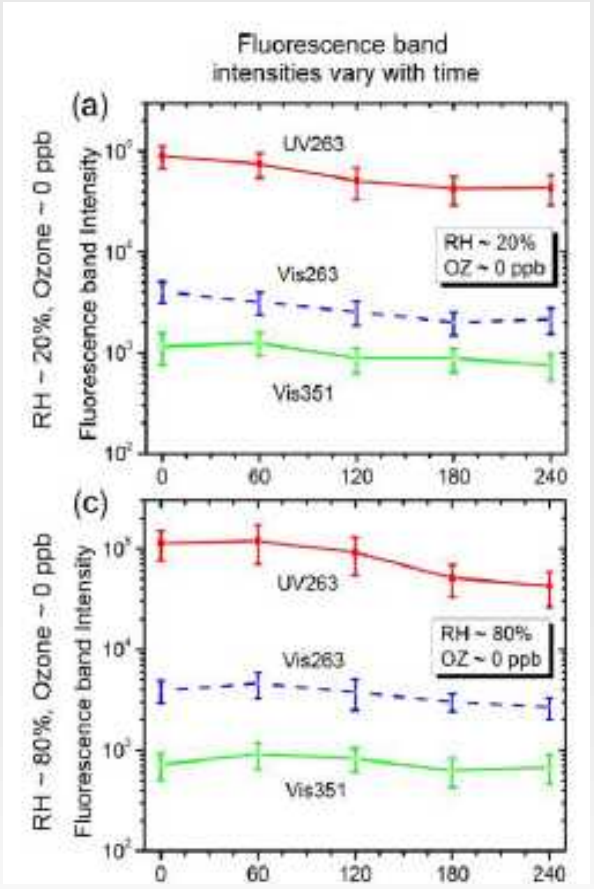
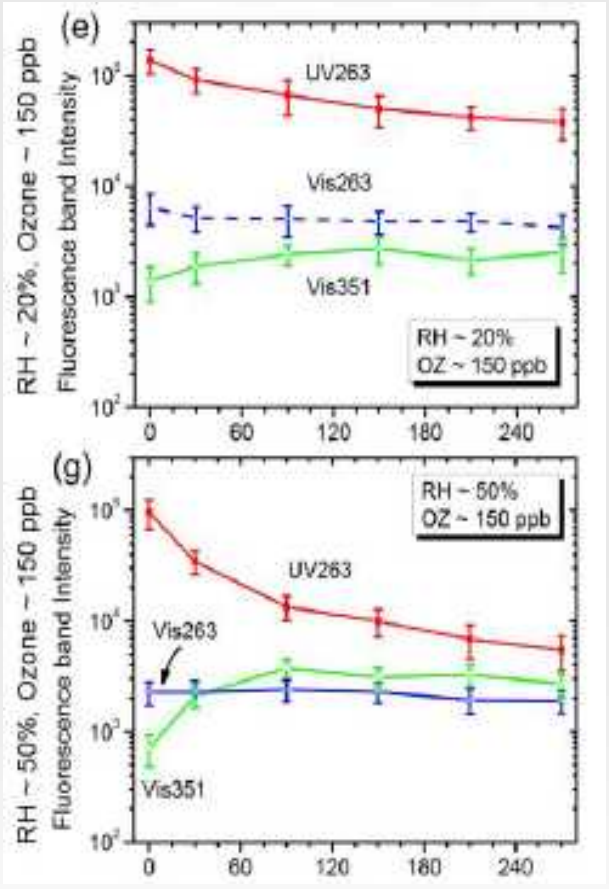
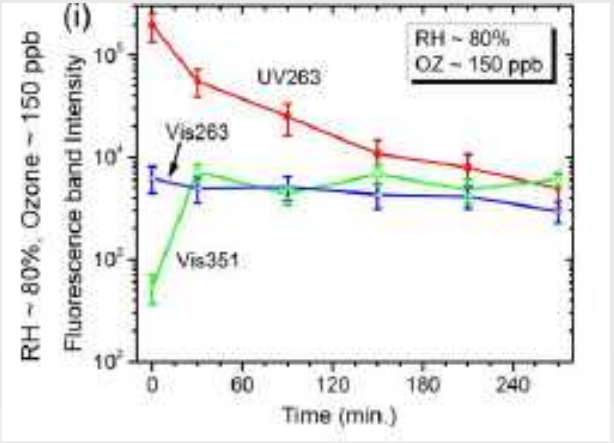
Drum Peptide Experiments







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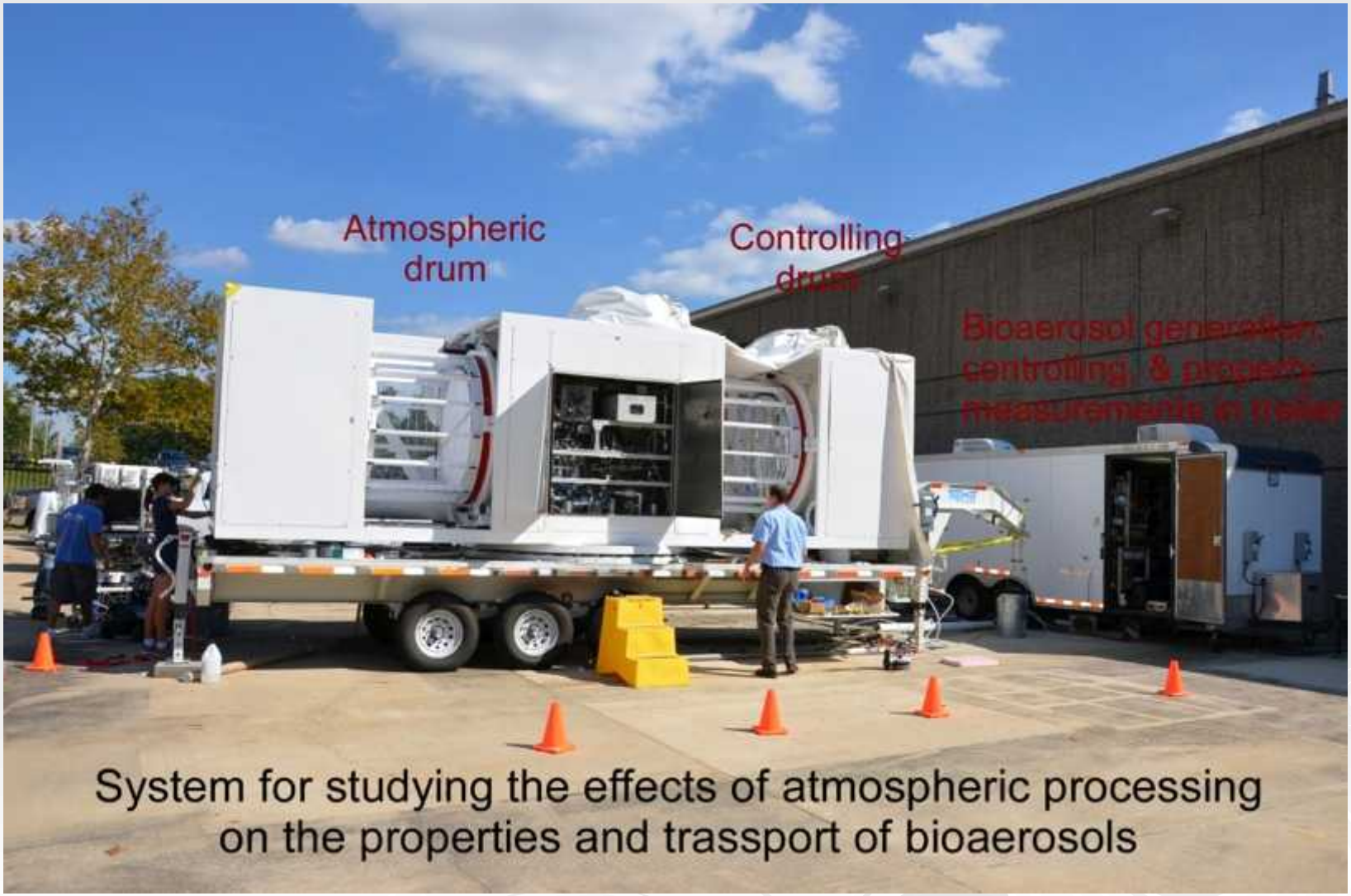
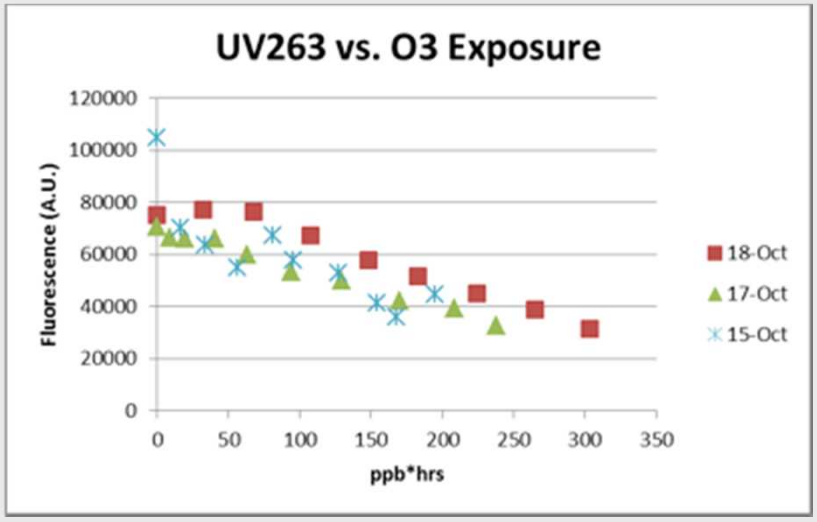
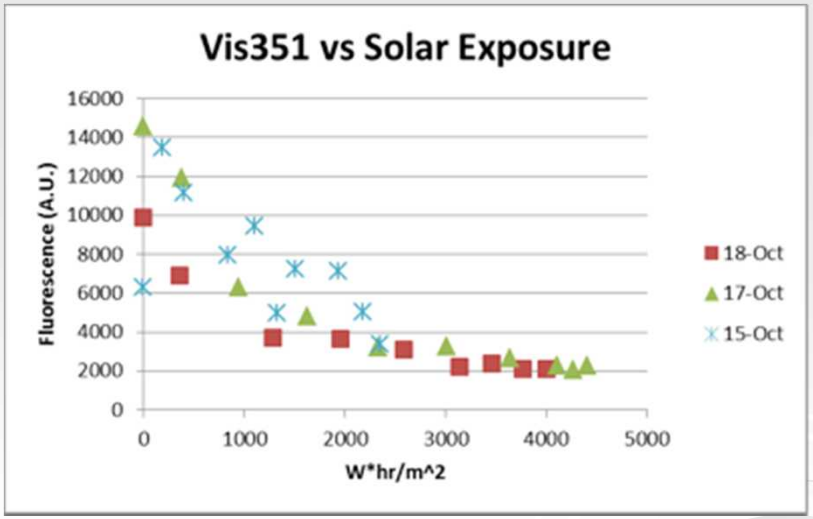
CAGE Chambers



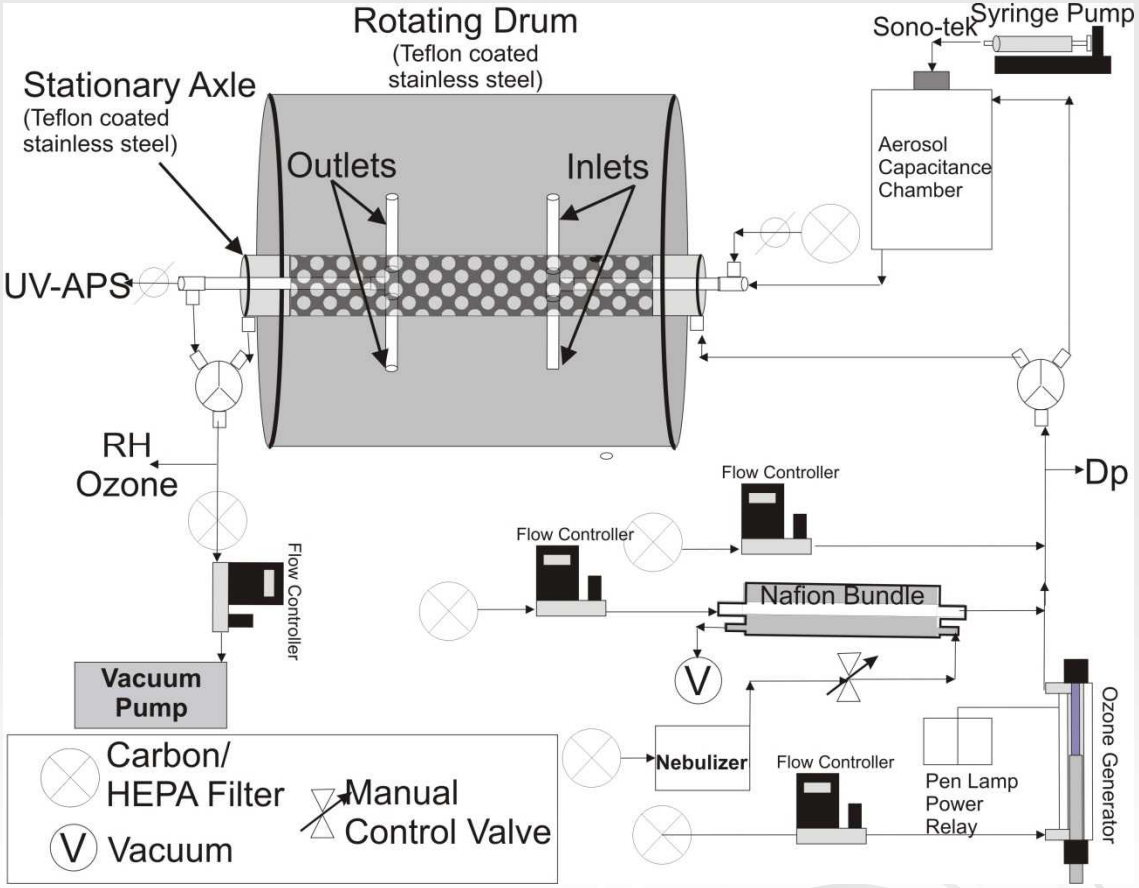
Aging of MS2 Bioaerosol



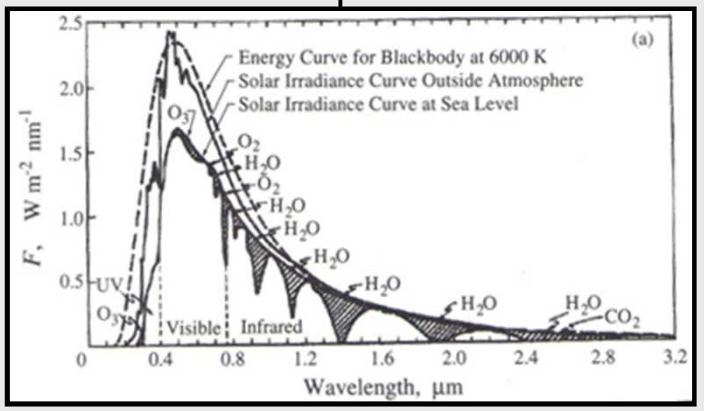


- Tryptophan undergoes ozonolysis causing the observed decrease in the UV band when particles are excited at 263 nm
- NADH and other molecules are photobleached by the same band of Solar UV (300-400 nm) that causes tropospheric ozone production
  - NADH may also be affected by O3 (as in lab results) but photobleaching likely dominates outdoors

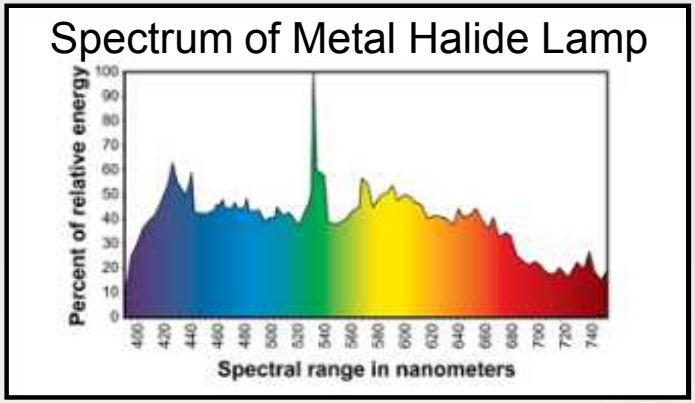
Solar Simulation in Large Rotating Drum

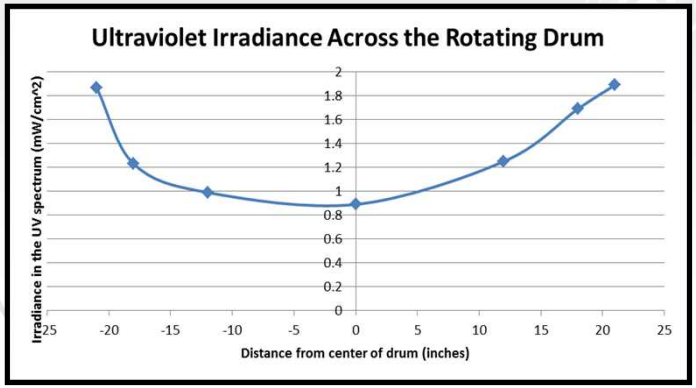


Solar Spectrum

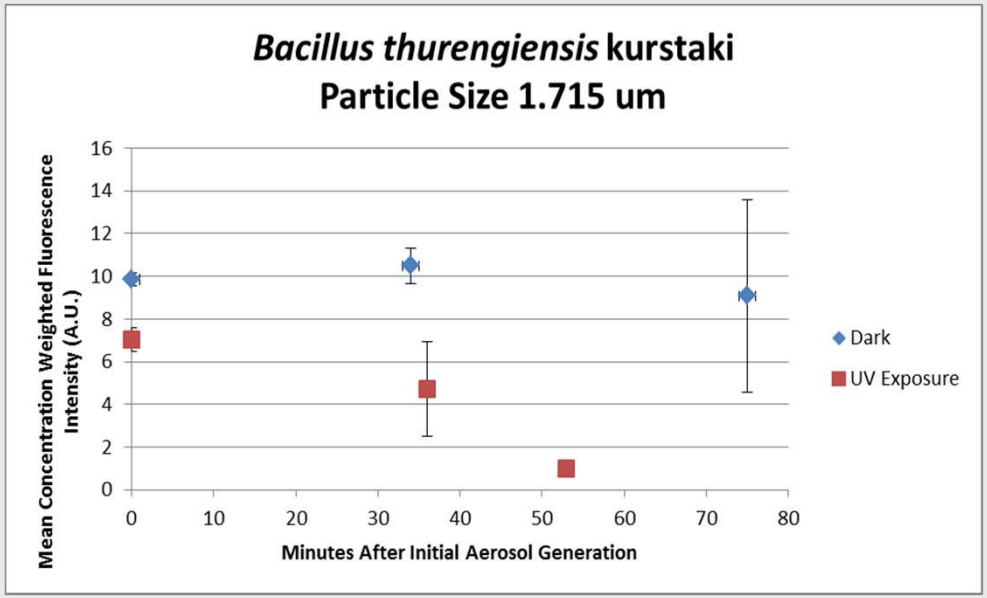


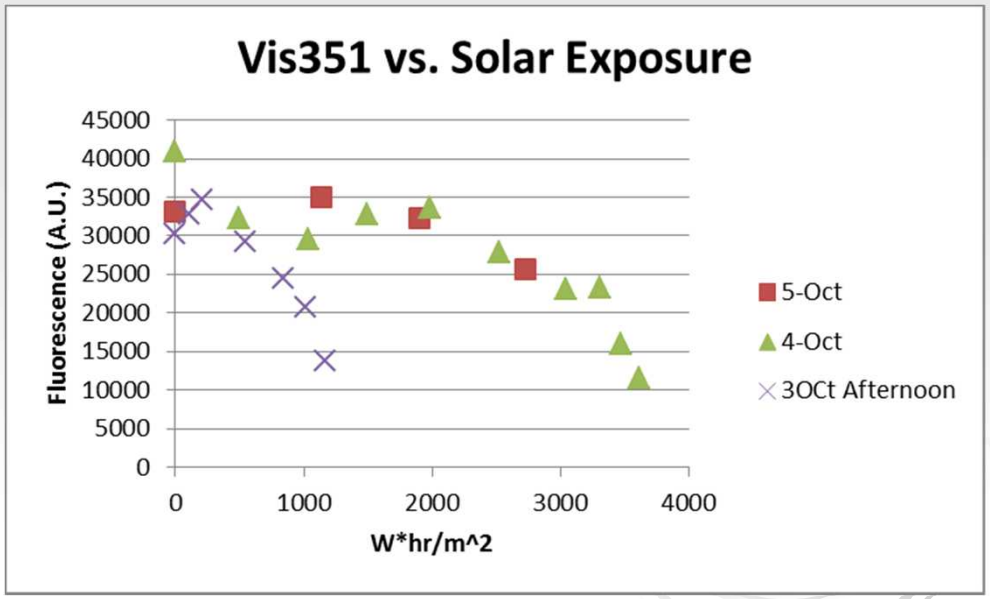
Spectrum of Metal Halide Lamp





Solar Simulation

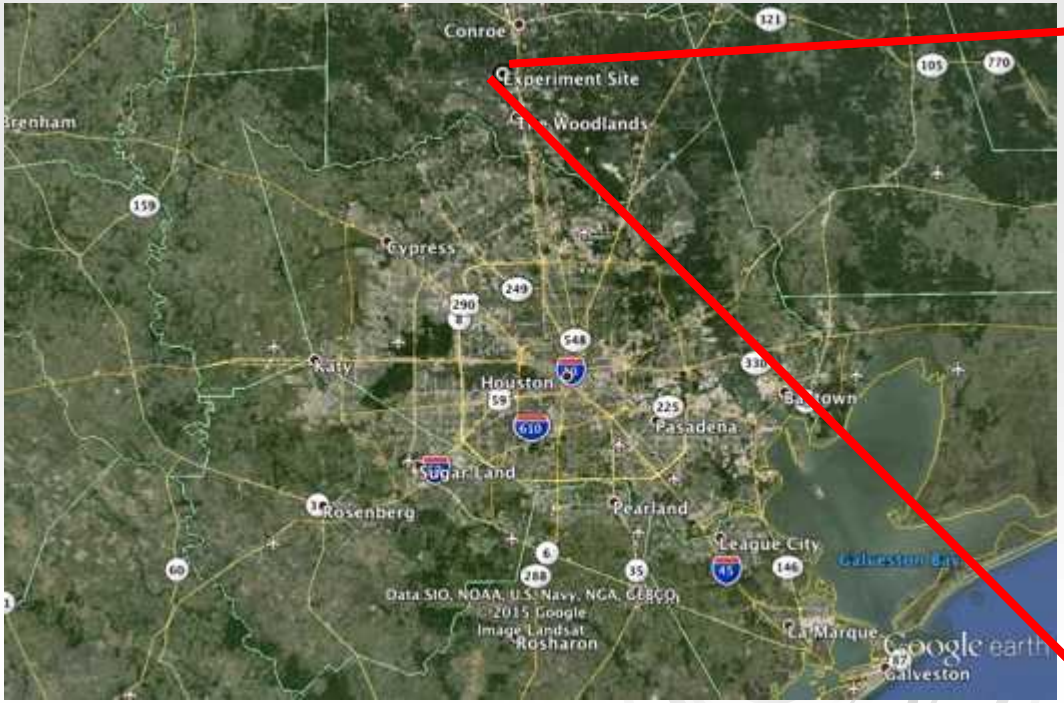




- Preliminary results indicate the UV produced by the metal halide lamps is sufficient to cause degradtion
- Although not directly comparable with field data, the data indicate that representative degradation may be simulated in the laboratory system

Experiment Site

- WG Jones State Forest
- Managed by Texas A&M Forest Service
- Location of Texas Commission for Environmental Quality monitoring site

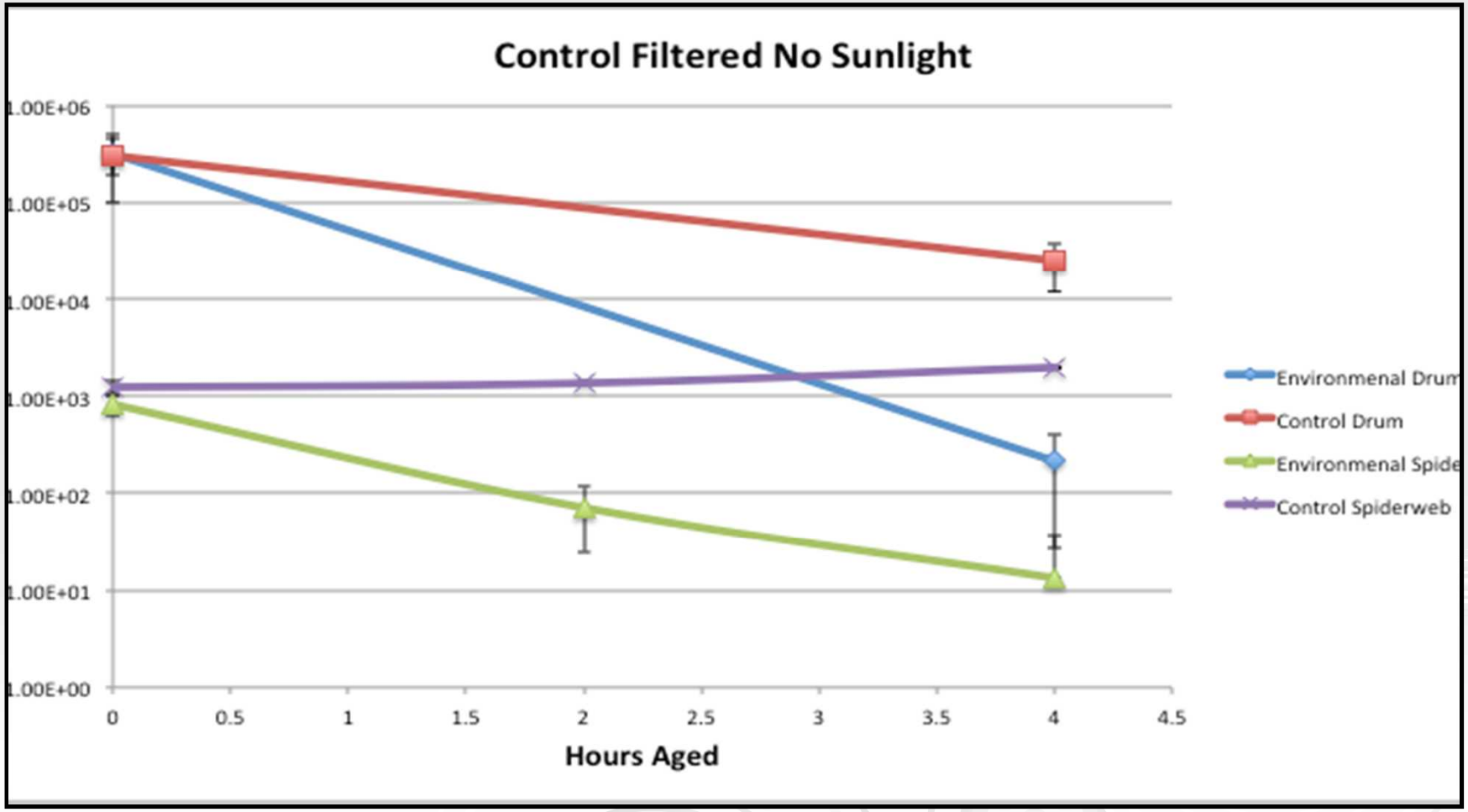


Set up of Field Study

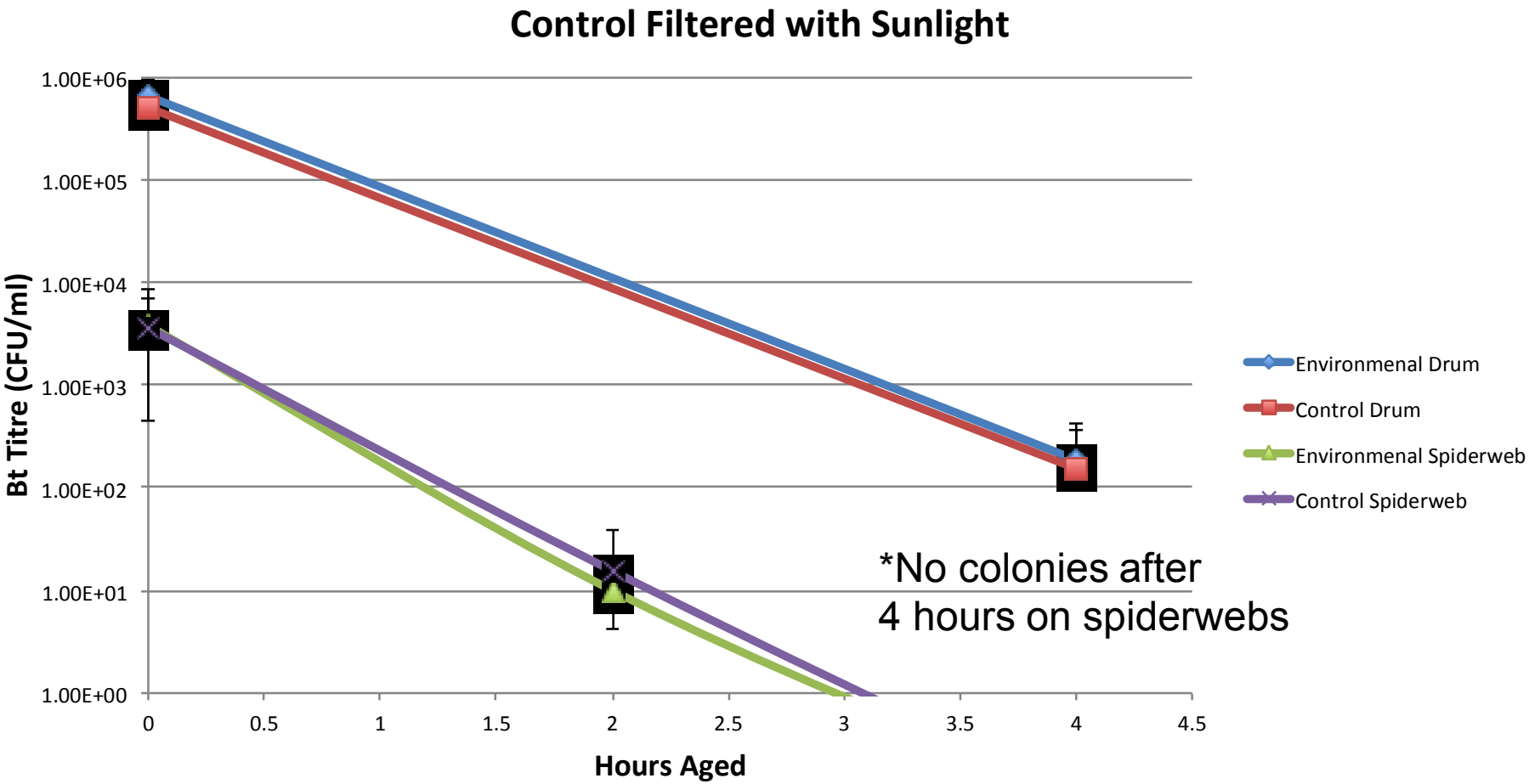
- Two chambers
  - Can limit sunlight and/or gas phase species in one or both
- Two spiderweb boxes
  - Can limit sunlight and/or gas phase species in one or both

- Co-located to Environmental Sampling Site
  - PTR-MS
  - AMS
  - Ozone
  - Temp and RH
  - Nox, Sox, etc.
- Local pyranometer (Solar)

Aging of Bt in Ambient vs Controlled Conditions



Aging of Bt in Ambient vs Controlled Conditions (with Sunlight)



Conclusions

- Atmospheric Processing can have an affect on many aspects of bioaerosol measurement
- Ozone
  - Ozone directly affects the specific amino acids even inside complex proteins
    - Tryptophan undergoes ozonlysis and is hydrolyzed by water to form N-Formyl Kynurenine and Kynurenine
      - Not reactive with O3, and fluorescent at 355 nm, and not at 263 nm
    - Ozonolysis may destroy microorganism proteins through this process
  - Ozone appears to destroy free DNA under a similar chemical process
    - Applies largely to extracellular DNA
  - Ozone is 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 O3 directly
  - Solar radiation can profoundly reduce the viabilty of bioaerosols
- 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
  - Media effects dominate the hygroscopic properties of lab generated bioaerosols

The Holy Grail

The Holy Grail of Bioaerosol Measurement

- Real-time (even pseudo) molecular identification of single bioaerosol particles
  - Immuno
  - PCR
  - Sequencing
- Why hasn't it been done?
  - Complexity of assays difficult for treatment of single particles
- Can it be done
  - Probably