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Single Particle Fluorescence and Mass Spectrometry in Various Environments as Observed by Biological Aerosol Mass Spectrometry (BAMS)

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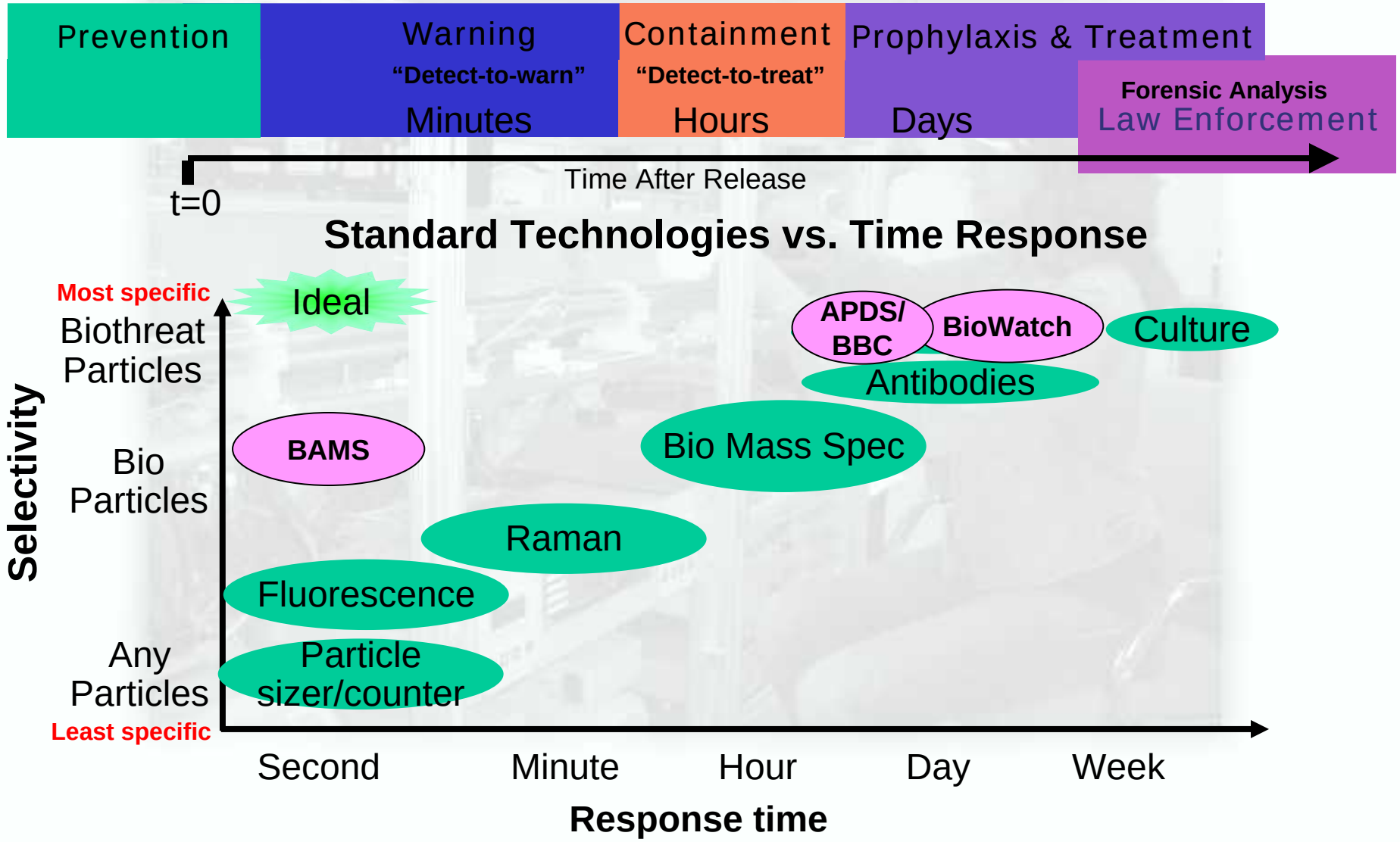
Bioaerosol Mass Spectrometry-Motivation

Environmental detection technologies



Bioaerosol Mass Spectrometry - Goals

Develop a single-particle technique to detect and identify bioaerosols of national security and public health concern in real time (seconds).

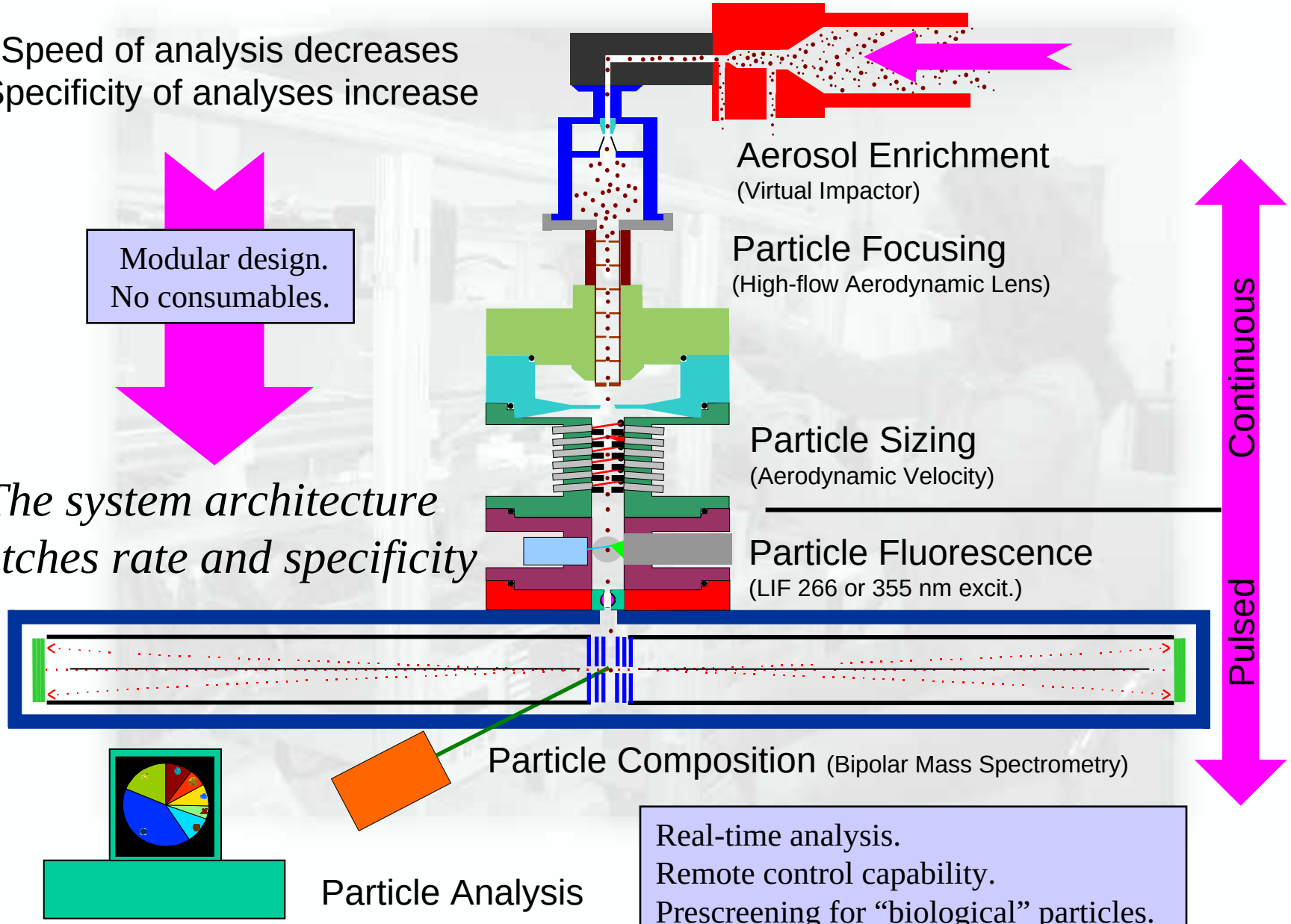
- Single organism detection (e.g. 10^{-12} g material in *Bacillus* spore)
- Discrimination from background and hoax materials ("white powders").
- Broad range of agents (Viruses, Toxins, Vegetative cells, Spores).
  - Capability to also identify backgrounds.
  - No false positives or false negatives. Ideally: At genus or species level.
- Consume only electricity – no reagents.
- Automated, real-time data analysis.

BAMS System Schematic

Speed of analysis decreases
Specificity of analyses increase

Modular design.
No consumables.

The system architecture matches rate and specificity

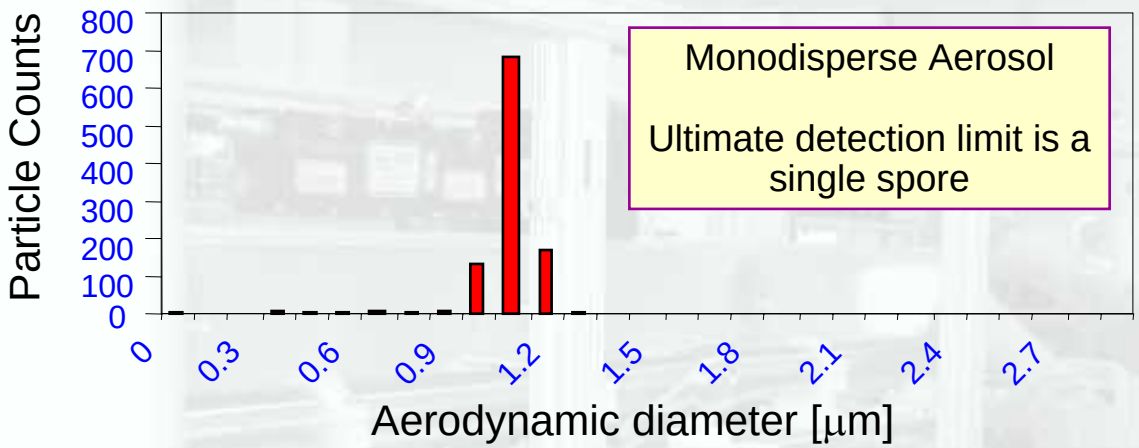


Real-time analysis.
Remote control capability.
Prescreening for “biological” particles.

BAMS Data (Aerodynamic Size, LIF, MS)

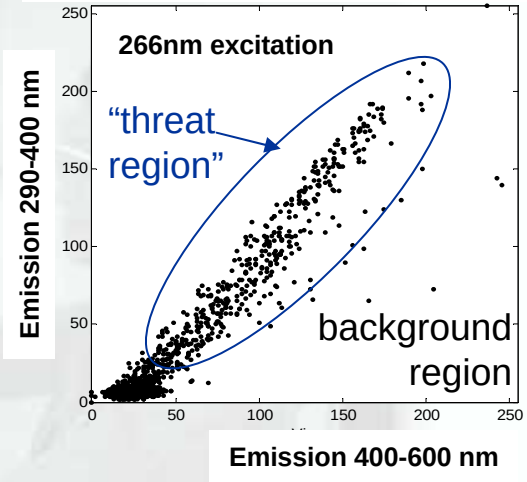
1

B. atrophaeus spore size distribution



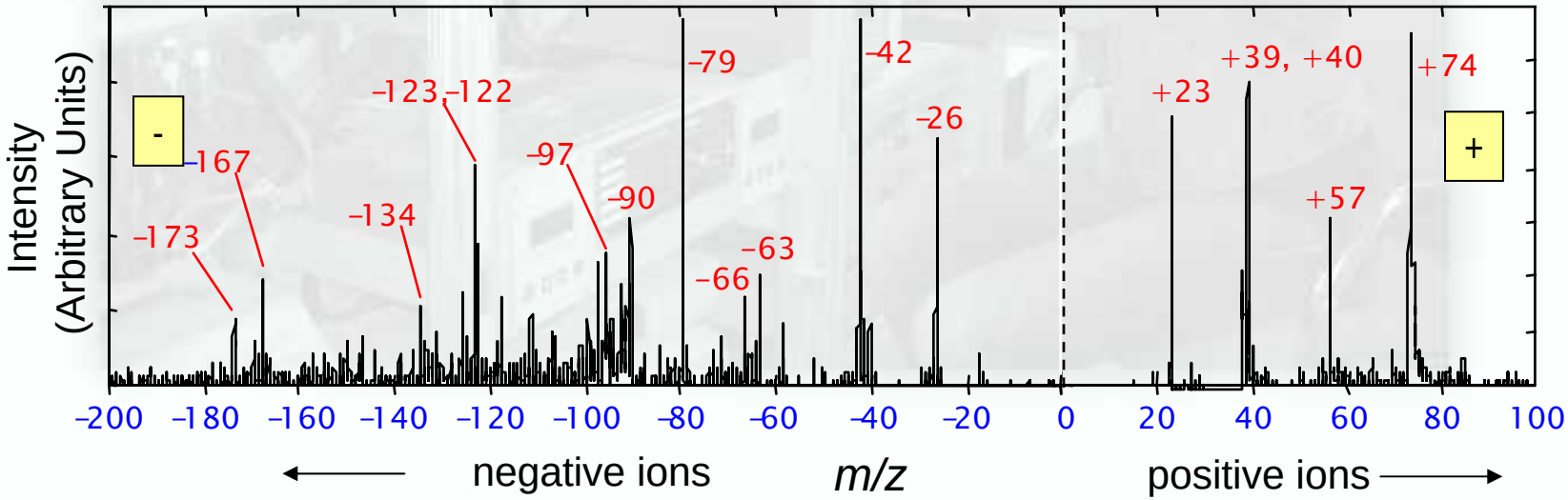
2

UV-LIF *B. atrophaeus*



3

Mass spectrum of <u>single</u> *B. atrophaeus (B. globigii)* spore



Aerosols Under Study

Bio-aerosol/Pathogen Simulants

spore

Bacillus

atrophaeus
cereus
coagulans
megaterium
simplex
sphaericus
thuringiensis

Clostridium

acetobutricum
lituseburense

Fungal

Thelebolus stercoreus

vegetative

Gram +

Bacillus atrophaeus
Bacillus cereus
Clostridium acetobutricum
Mycobacterium smegmatis
Nocardia globerula
Corynebacterium vitaeruminis

Gram -

Escherichia coli
Erwinia herbicola
Pseudomonas putida

virus

RNA

MS2

DNA

M13

Background Aerosols

ambient

San Joaquin soil
Livermore soil
UltraFine particulate matter
Urban particulate matter
Bentonite
Sea salt
Livermore air
Diesel exhaust

white powders

Gold Bond
Equal sweetener
Baby powder
White flour
Gelatin
Pectin
Milk powder
Cocaine

media

LB agar
LB broth
Terrific broth
Peptone powder
Yeast extract
¼ TY sporulation

facilities

Cigarette smoke
Match smoke
Paper dust
Laboratory air

Standards for Signature Characterization

standards

Various amino acids
Various small peptides
DNA
RNA
Dipicolinic acid
Ovalbumin
Casein
Lipopolysaccharides

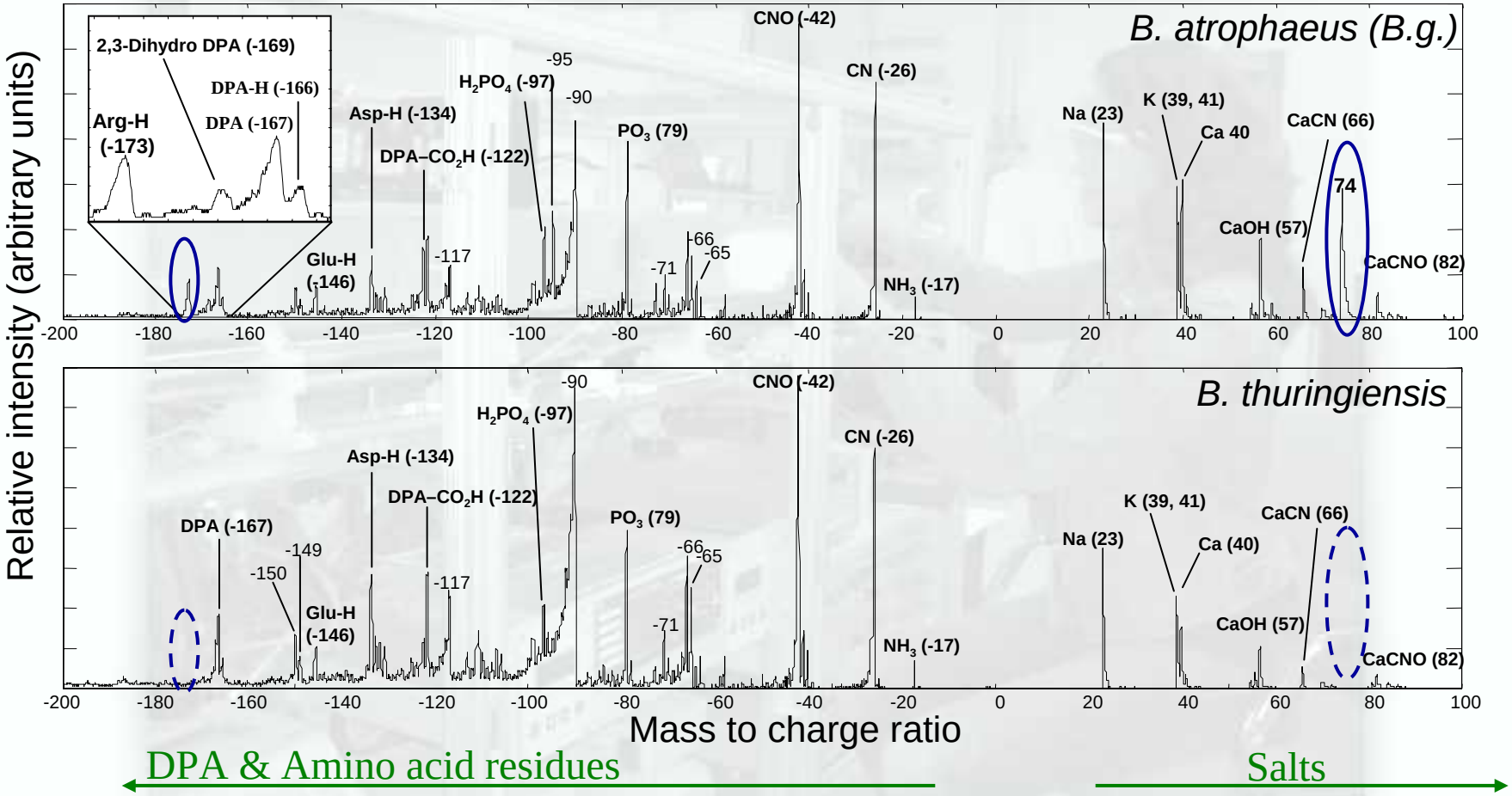
plus more…

Bacillus Spore Spectra

Average of ~1000 individual mass spectra

D. Fergenson, et al., Anal. Chem, vol. 76, 373-377 (2004)

S. Russell, et al., Anal. Chem. (2005), A. Srivastava, et al., Anal. Chem. (2005)



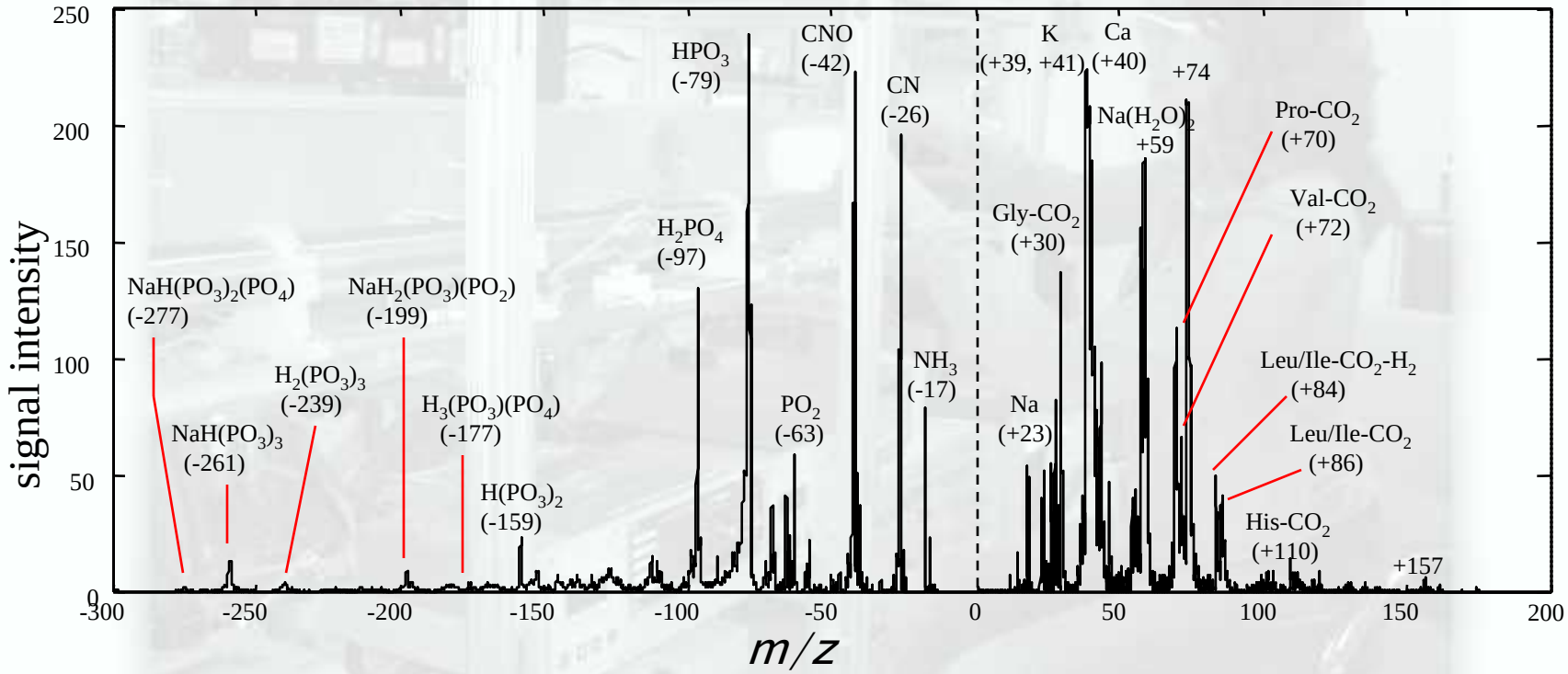
B. atrophaeus (B. *globigii*) spores can be identified at the species level. Spores can easily be differentiated from vegetative cells, viruses, toxins.

Vegetative cell Spectra

Average of ~1000 individual mass spectra

B. atrophaeus (B.g.)

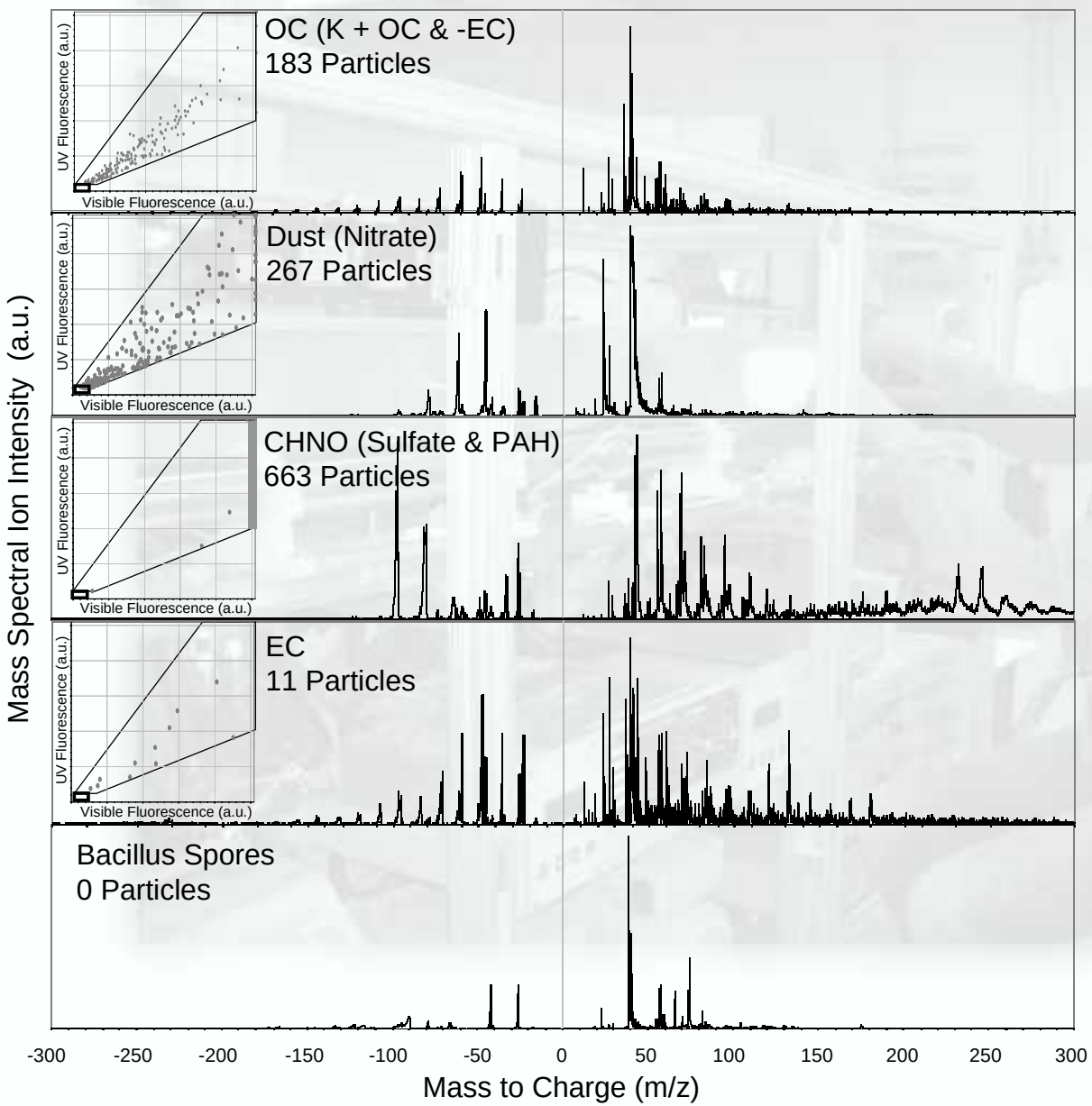
Assignments confirmed using isotope labeling

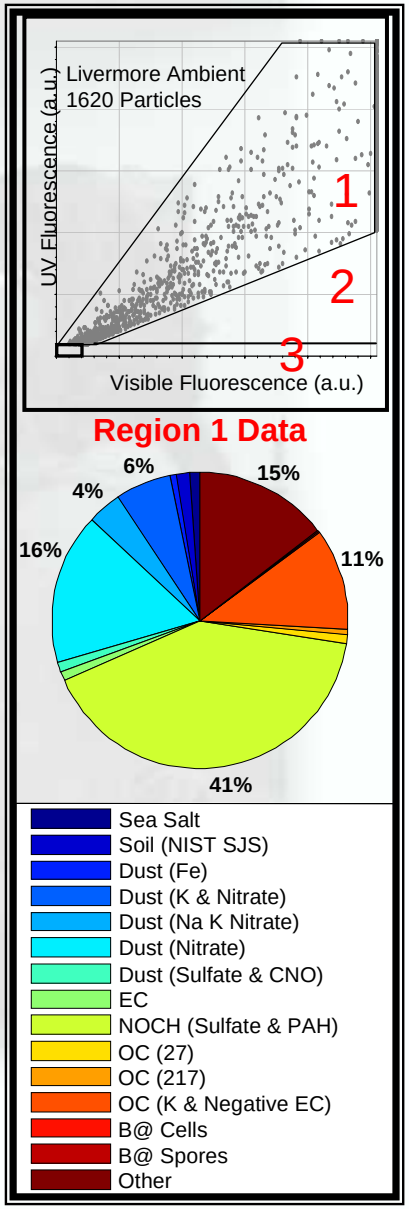


Phosphate clusters ← → Amino acid residues

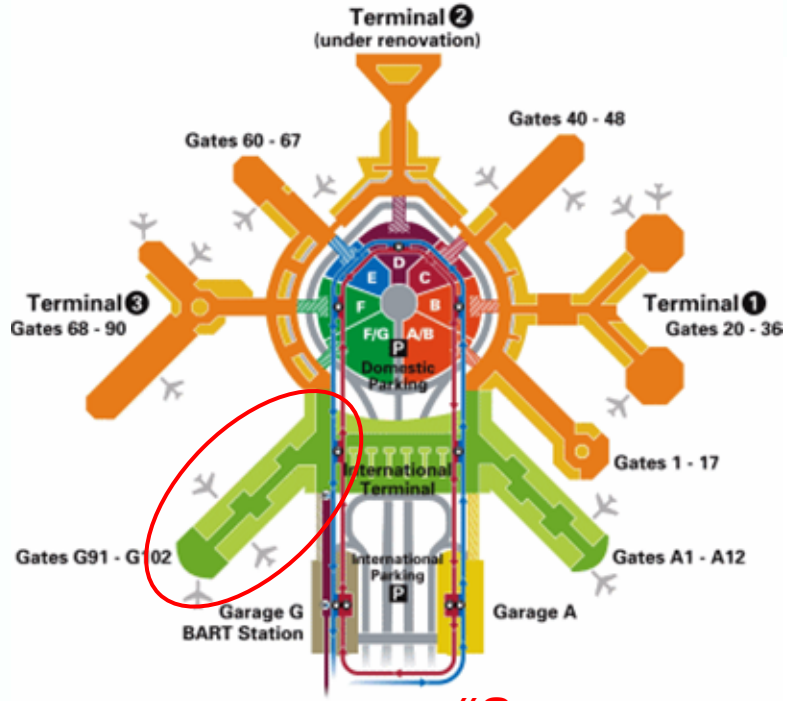
Distinct from the *B. atrophaeus* spore signature

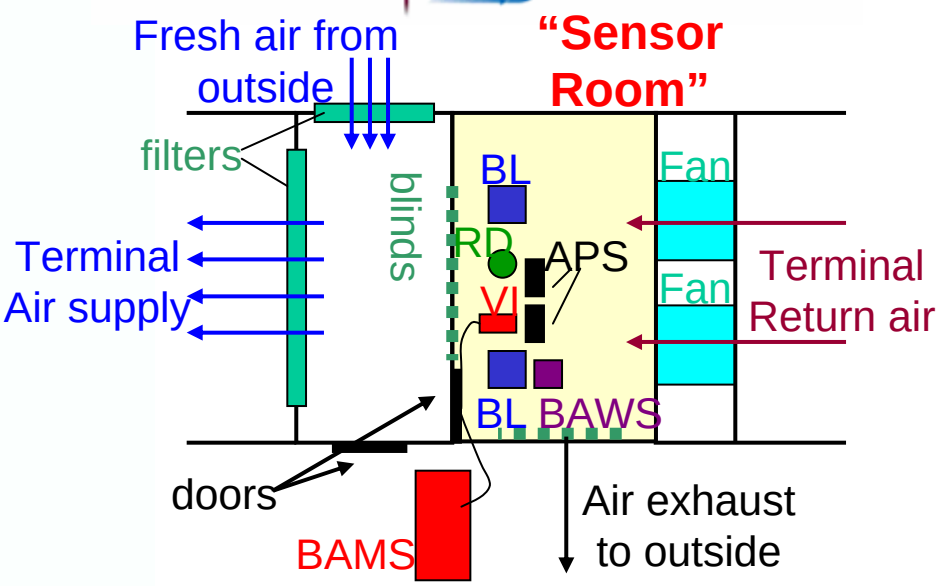
Ambient Livermore Particles

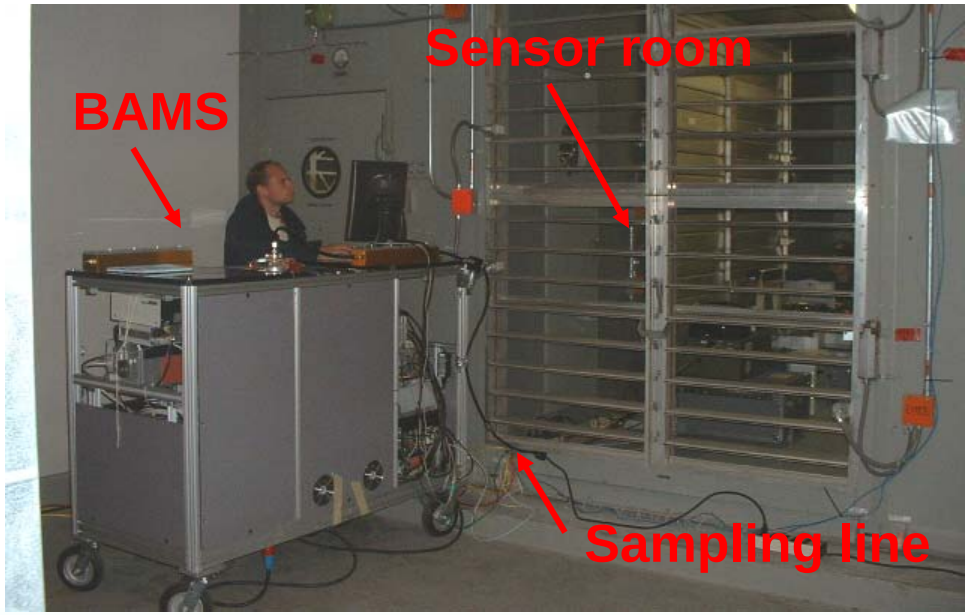




The DHS aerosol test bed at SFO

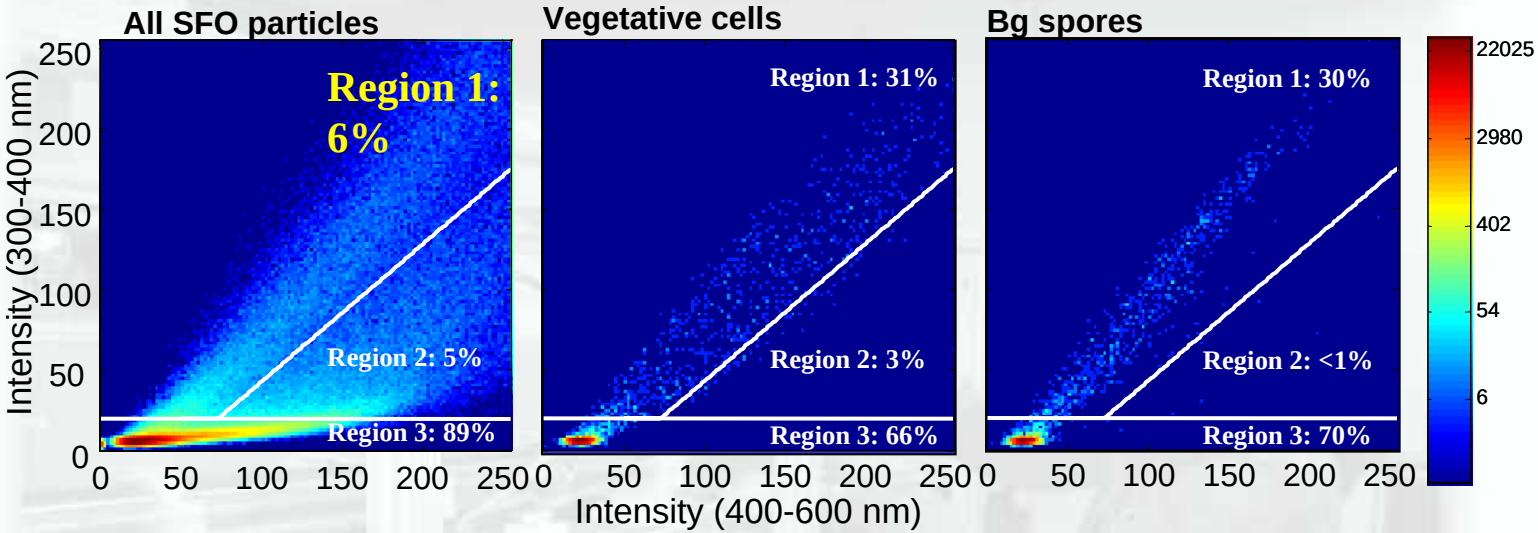




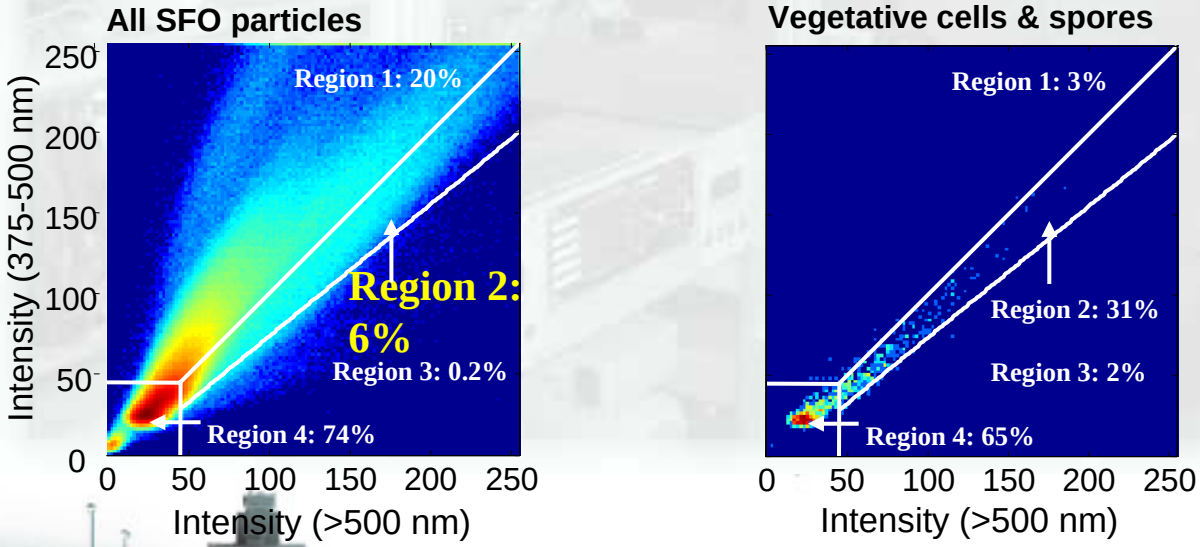


Laser Induced Fluorescence (SFO)

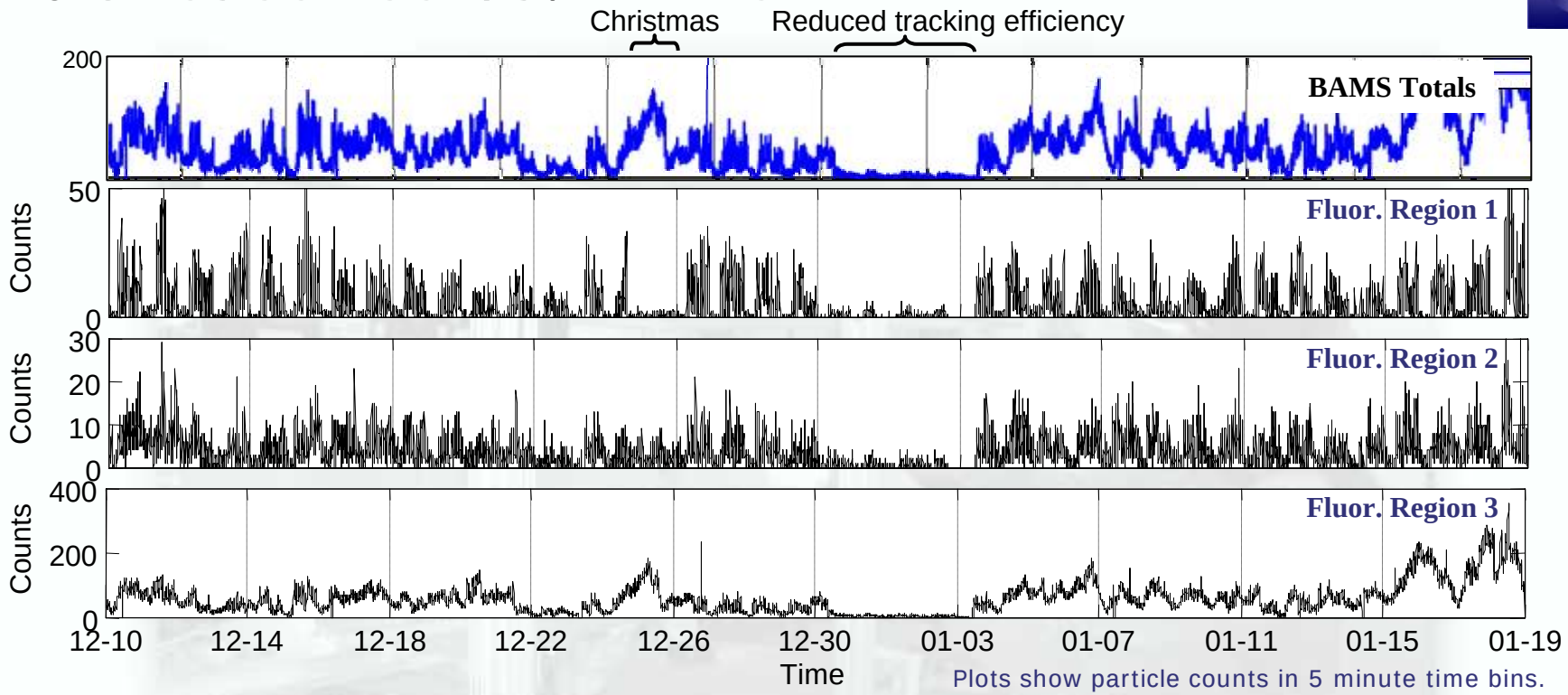
(266nm Excitation)



(355nm Excitation)



Fluorescence vs. Time

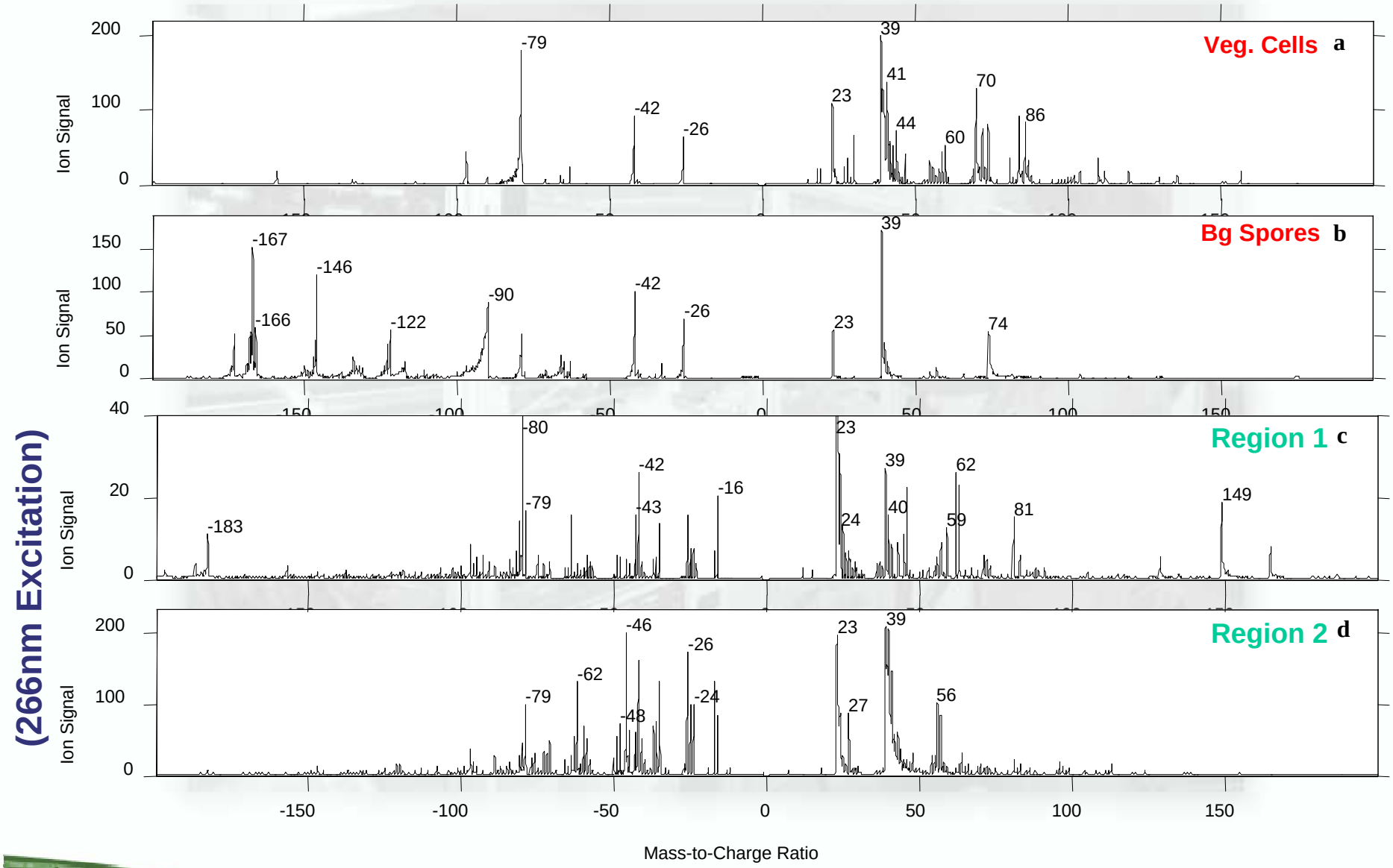


Plots show particle counts in 5 minute time bins.

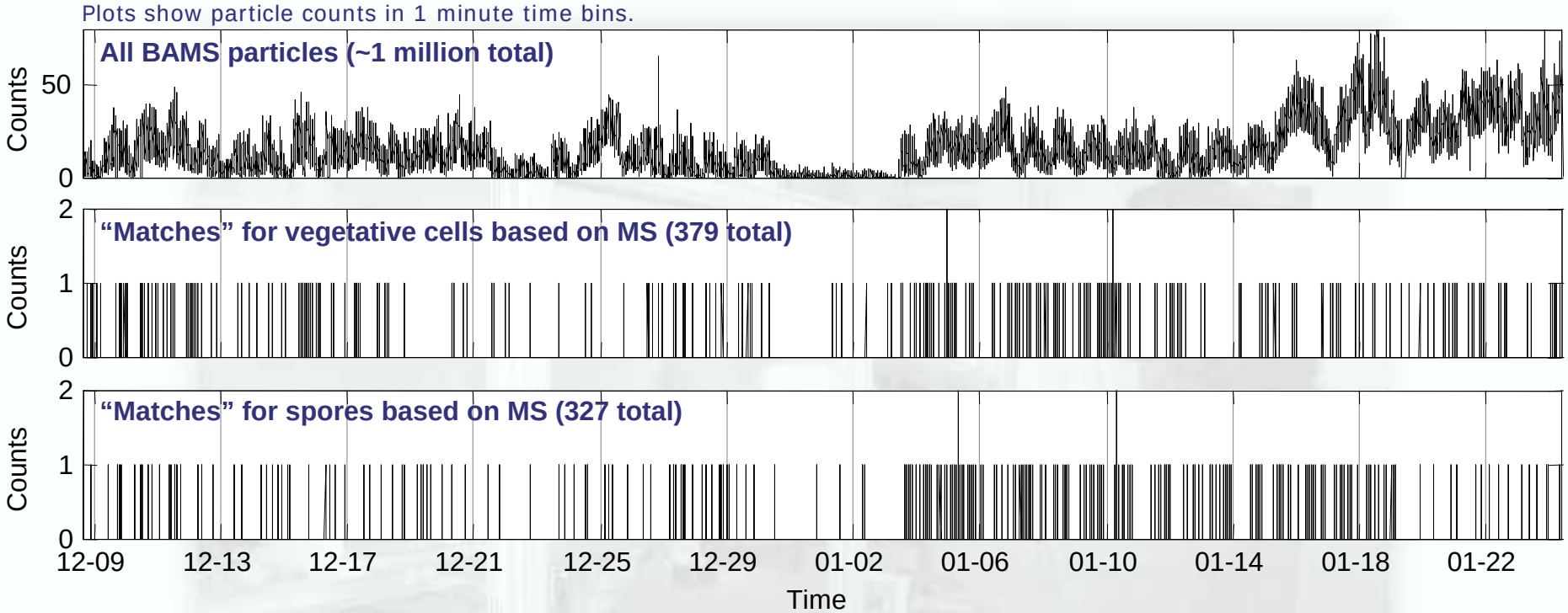
- Region 1 has a clear Diurnal cycle & episoidal behavior.
  Indicating local sources
- Region 3 does not
  Indicating non-local or background particle types
- Region 2 is intermediate in appearance
- The fraction of particles in region 1 varies widely and rapidly

- Christmas
  - Absence of region 1 counts
  - Spike in region 3 counts
- Dec. 30 – Jan. 3
  - Fiber lodged in tracking system reducing efficiency of instrument
  - Easily fix upon access to test bed

SFO Mass Spectral Averages



The Specificity of Mass Spec.



- At no point in ~7 weeks were more than 2 particles identified in one minute as spores or as vegetative cells on the basis of their mass spectra
- The threshold to sound a BAMS alarm is never set below three agent particle identifications in a minute
- **Zero** false alarms would have been sounded!

Mojave Desert

4 locations in 12 days

Mojave Ca.

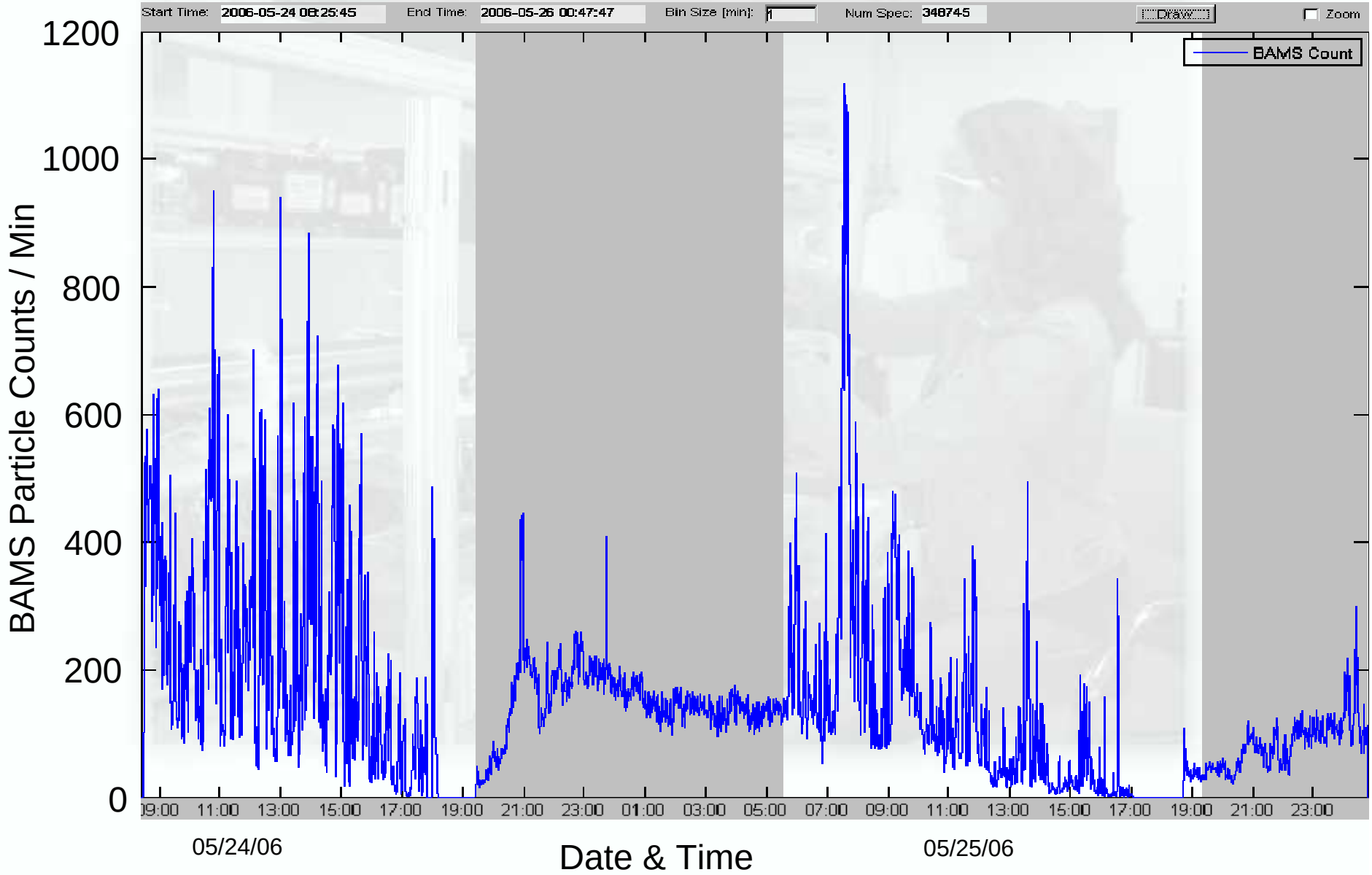
(May 19th to June 14th)

1. Signature Development
   A. Desert Environment
      i. Mineral dust
      ii. Desert vegetation
2. Signature Stability
   A. Comparison w/ previous data
   B. Comparison w/ sampled sources
3. System Ruggedness
   A. Long duration operation
   B. High dust environment
   C. Heat

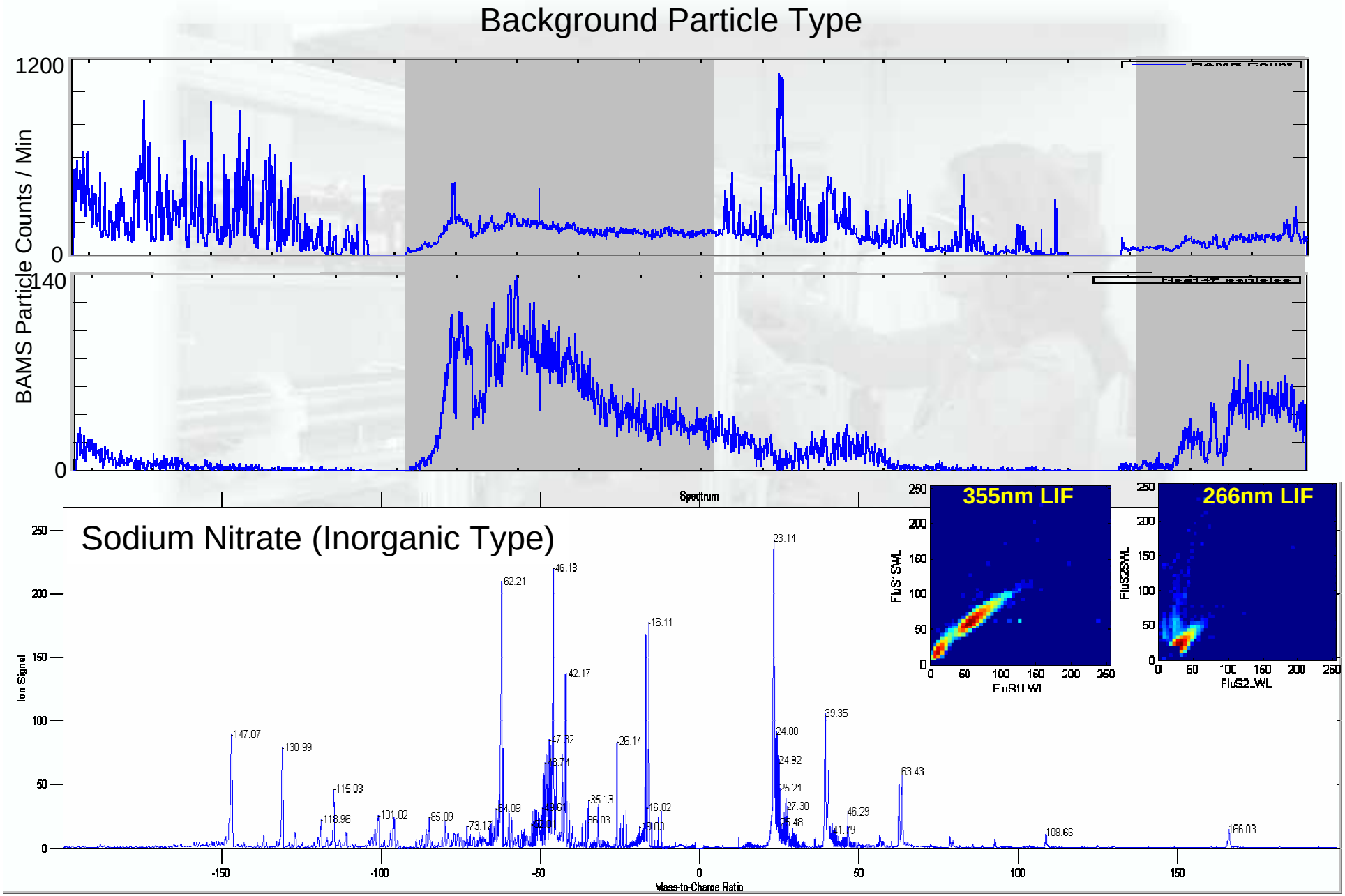
- 3.35 Million particles analyzed by LIF
- 1.36 Million particles analyzed by MS
- Actively sampled particles ~ 90% of the time.

The DUST BOWL Desert Community

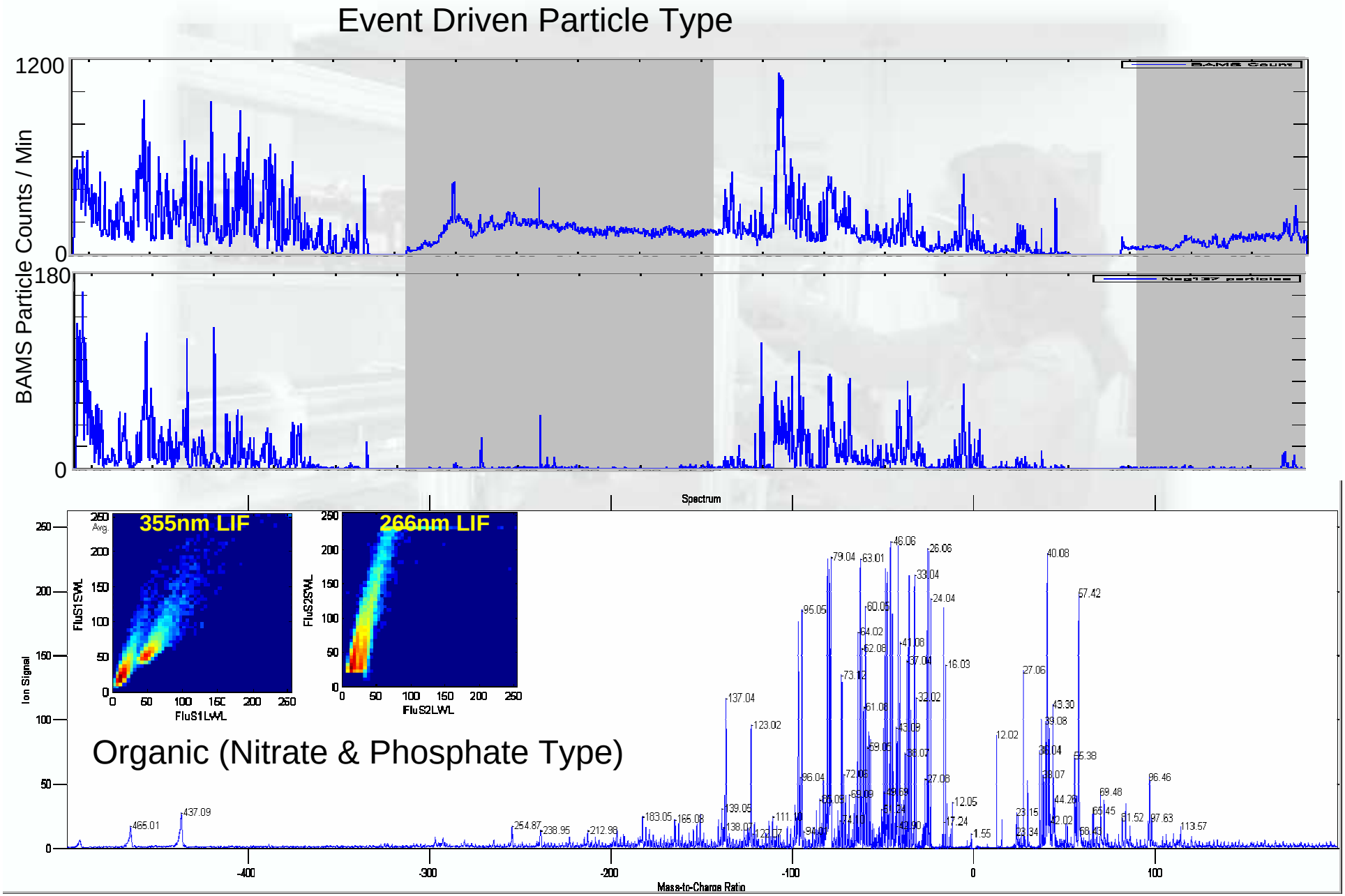
(05/24 to 05/26)



Non-Local Particle Type (05/24 to 05/26)

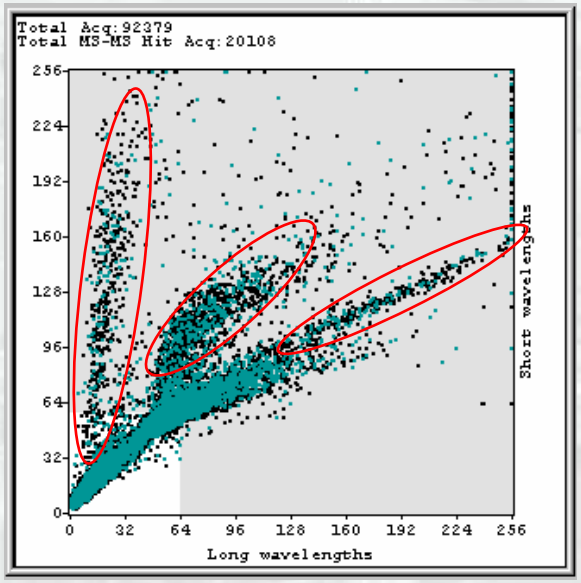


Local Vehicle Particle Type (05/24 to 05/26)

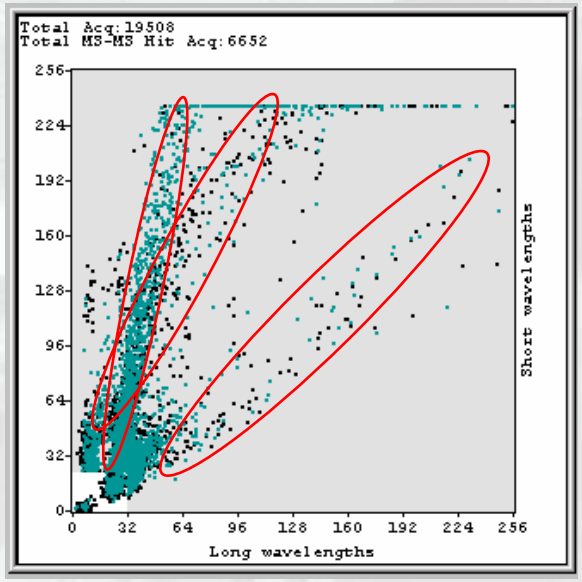


Laser Induced Fluorescence Data (05/18 to 05/26)

A rich variety of LIF responses for both 355 and 266 nm stages

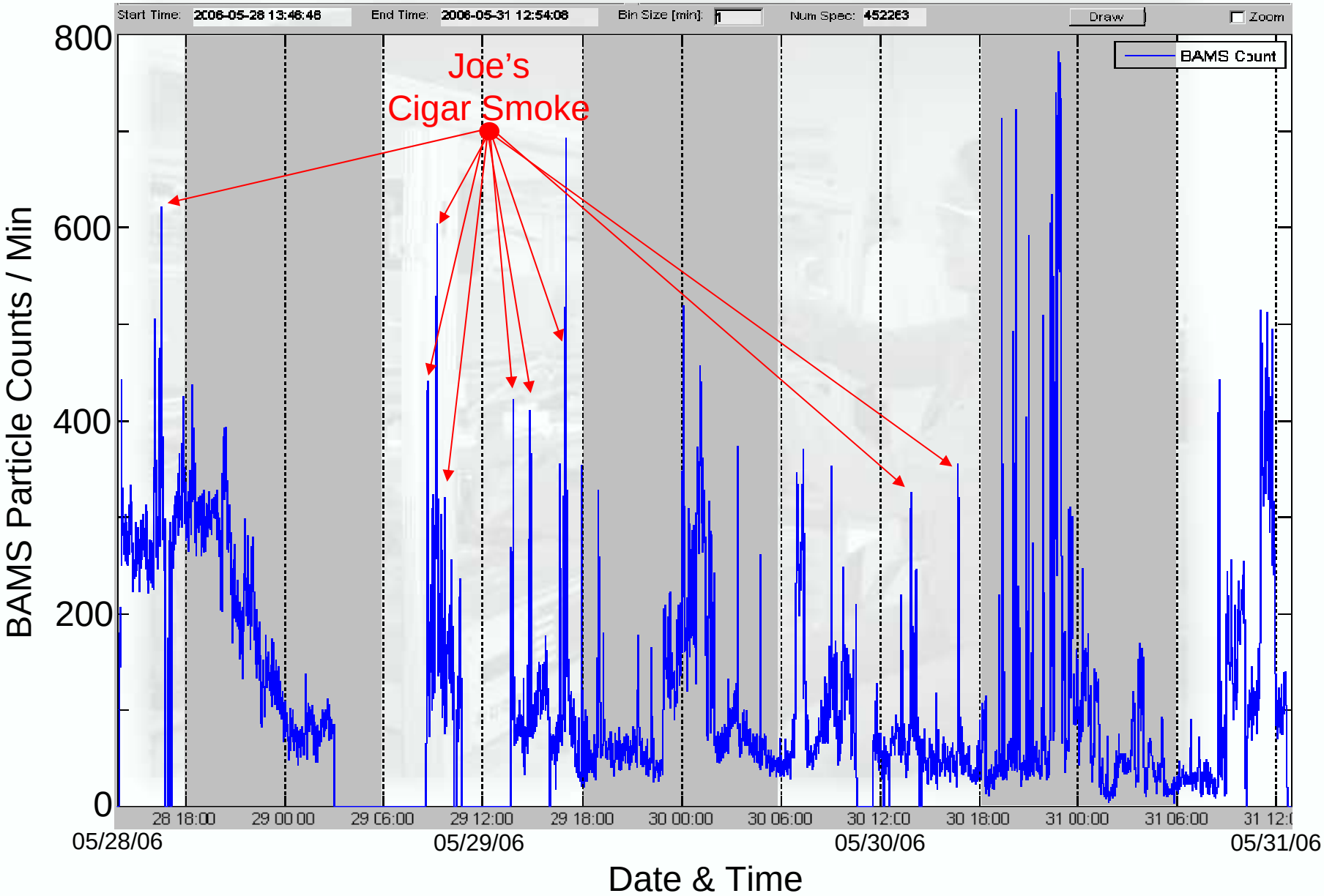


355nm Fluorescence



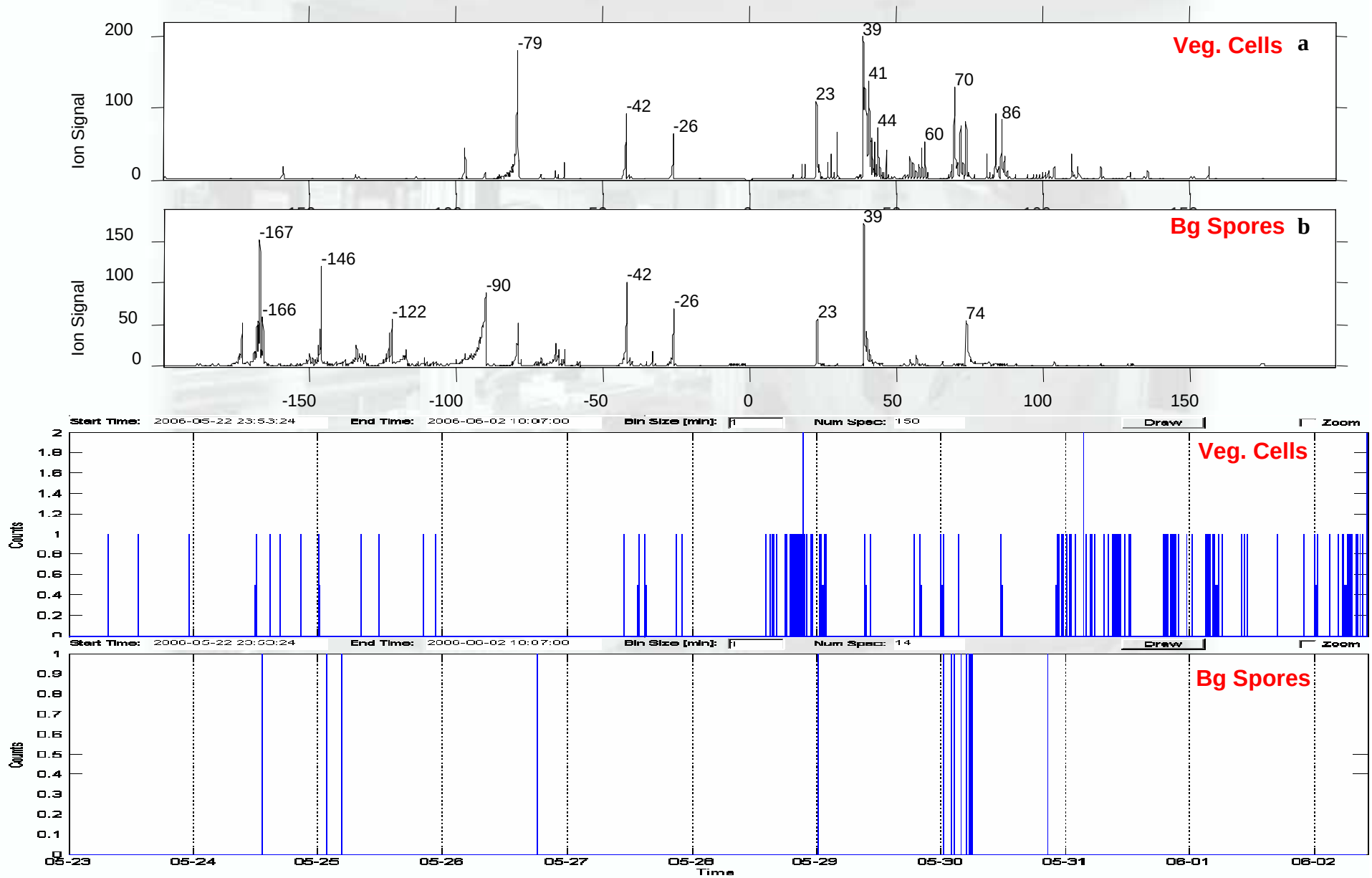
266nm Fluorescence

The 4-Corner Location (05/28 to 05/31)



Bacterial Particles Observed

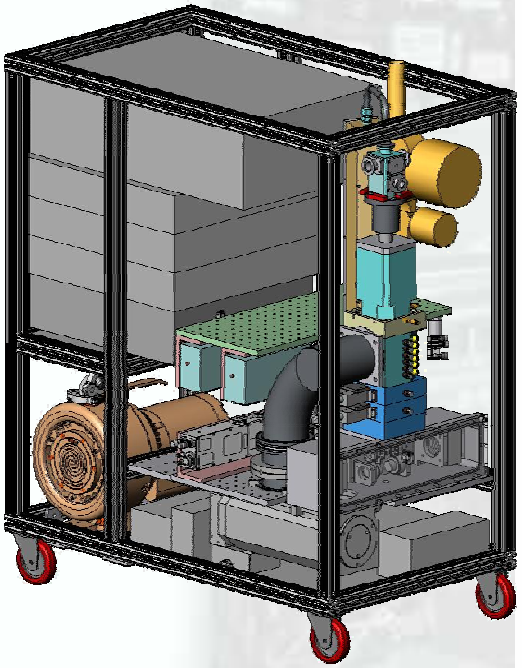
- **Zero** false alarms would have been sounded!

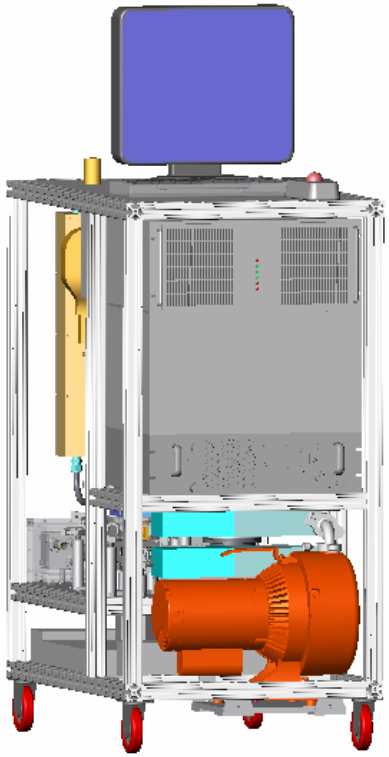


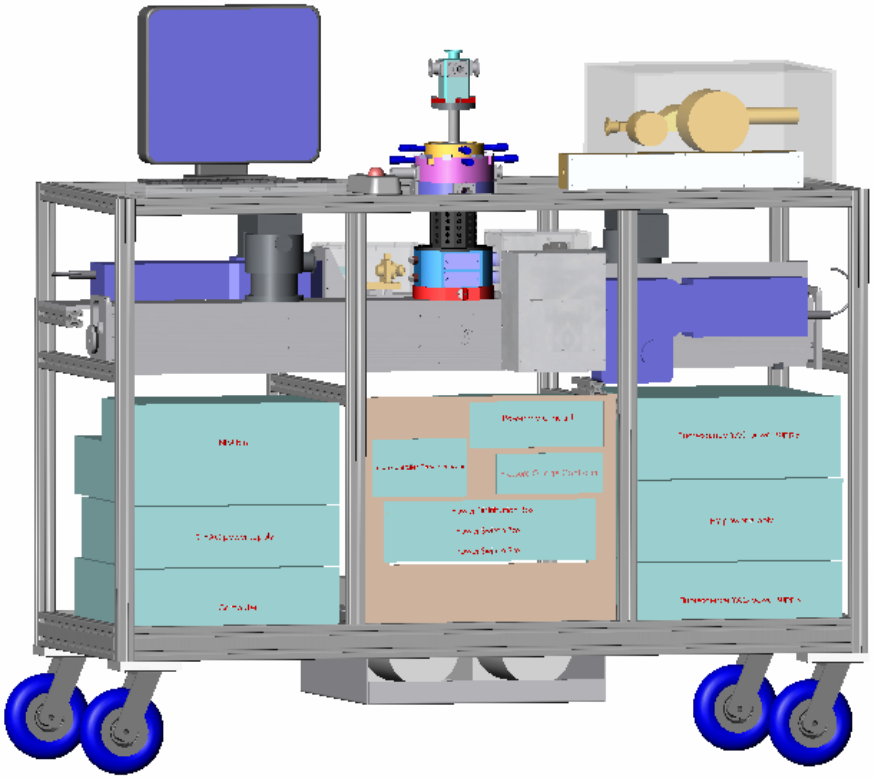
Summary

- Analytically orthogonal techniques coupled into a single system
  - Particle Size
  - Particle Fluorescence (UV & Visible)
  - Particle Composition (mass spectrometry)
  - Full mass spectral analysis of up to 1,000,000 particles per day.
- Fluorescence preselection.
  - Prescreens for particles of interest.
  - Eliminates greater than 90% of ambient background particles.
  - Fluorescence correlates with chemical composition.
  - Fluorescent particles in the "threat region" are from many different chemical types.
  - **Most "threat region" particles are not bacterial nor biological**.
- Long-term, reliable, unattended operation demonstrated at SFO (12 weeks)
  - Quick cleaning once each week.
  - Zero false alarms in 12 weeks indicates very low false positive rate.
- Practicality & Survivability demonstrated in the Mojave Desert
  - > 110° F temperatures
  - Sever dust loads from vehicles and storms.
- Near future efforts will be directed at expanding the signatures and reducing size, weight and power consumption.

BAMS II (just came on-line)







BAMS 2.0 = 0.72 m^3 BAMS 1.3 = 1.6m^3

Acknowledgements

BAMS Team

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