UCRL-CONF-204375

LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

Quantitative Evaluation of Bio-Aerosol Mass Spectrometry for the Real-Time Detection of Individual Airborne Mycobacterium Tuberculosis H37Ra Particles

H. Tobias, M. Schafer, M. Pitesky, J. Horn, M. Frank

May 26, 2004

American Society for Mass Spectrometry
Nashville, TN, United States
May 23, 2004 through May 27, 2004

Disclaimer

This document was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor the University of California nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or the University of California, and shall not be used for advertising or product endorsement purposes.

UCRL-CONF-204375

ASMS Wed 05-26-2004

Quantitative Evaluation of Bio-Aerosol Mass Spectrometry for the Real-Time Detection of Individual Airborne *Mycobacterium tuberculosis* H37Ra Particles

Herbert Tobias
David Fergenson, Maurice Pitesky,
Joanne Horn, Matthias Frank, and Eric Gard
Lawrence Livermore National Lab (LLNL)

Millie Schafer
CDC-NIOSH (OH)

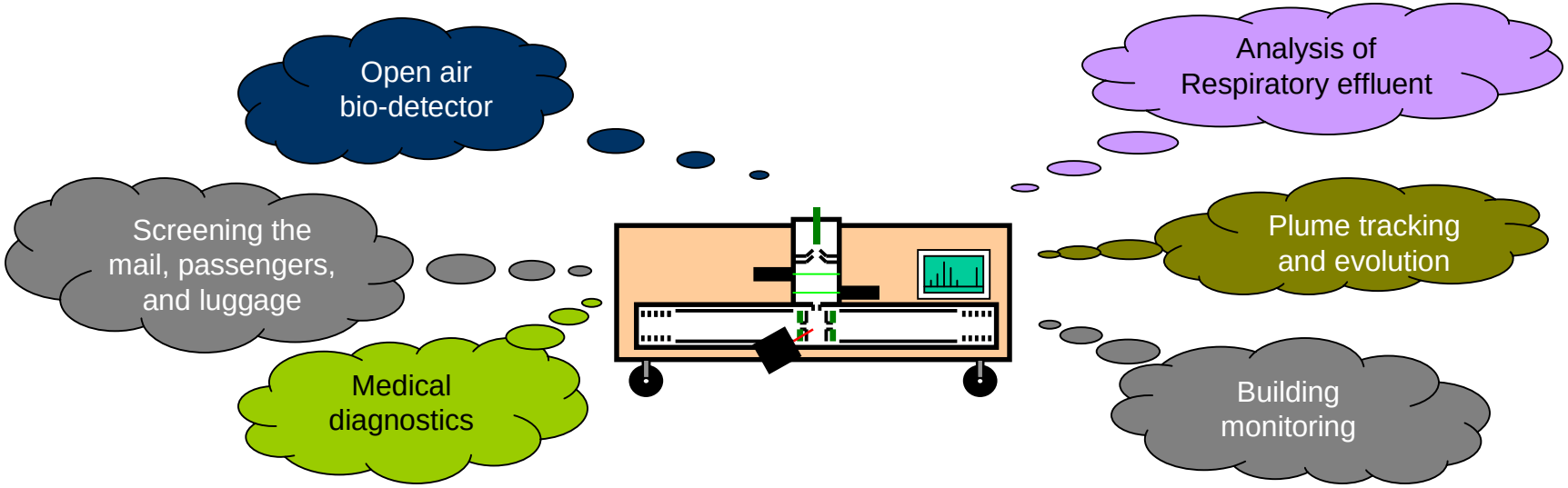
Research Objectives

Overall Group Objective:

Develop a real-time single-particle mass spectrometry technique called Bio-Aerosol Mass Spectrometry (BAMS) in order to efficiently screen and identify bioaerosols of national security and public health concern.

Individual spore, bacterial cell, virus, and toxin

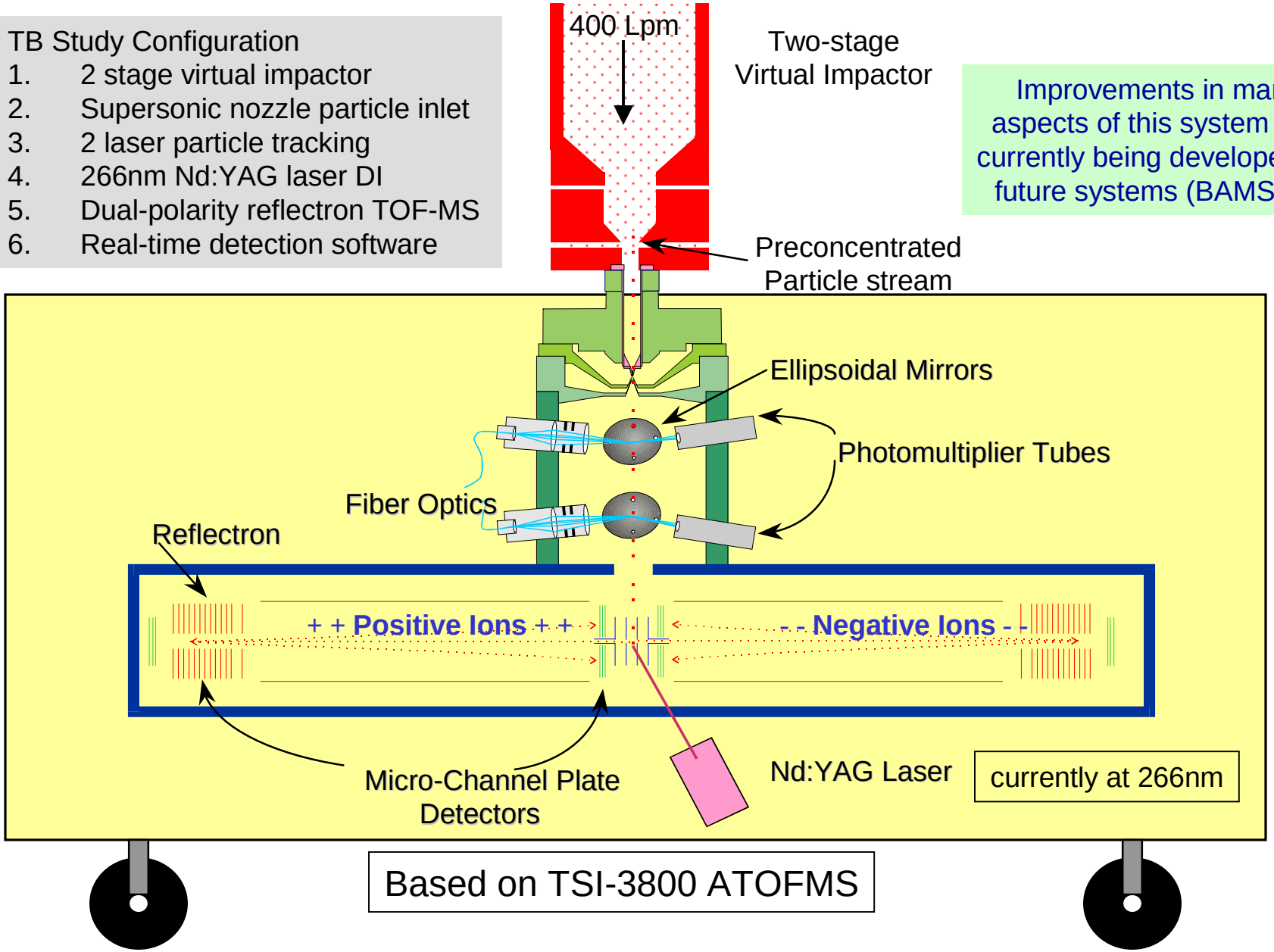
identification → species level



Bio-Aerosol Mass Spectrometer (BAMS 1.0)

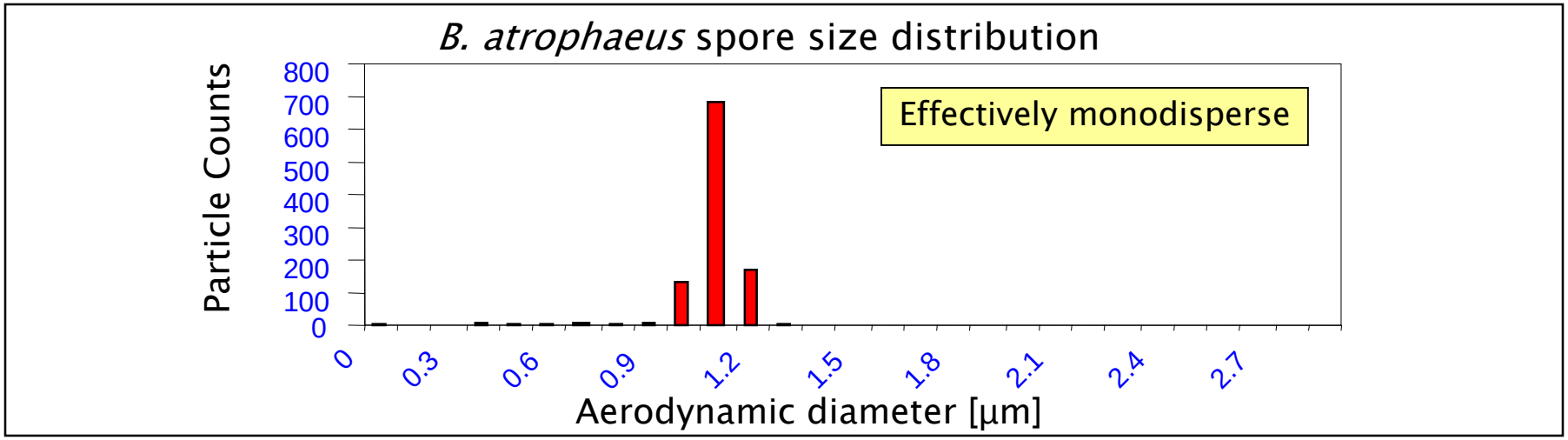
TB Study Configuration
1. 2 stage virtual impactor
2. Supersonic nozzle particle inlet
3. 2 laser particle tracking
4. 266nm Nd:YAG laser DI
5. Dual-polarity reflectron TOF-MS
6. Real-time detection software

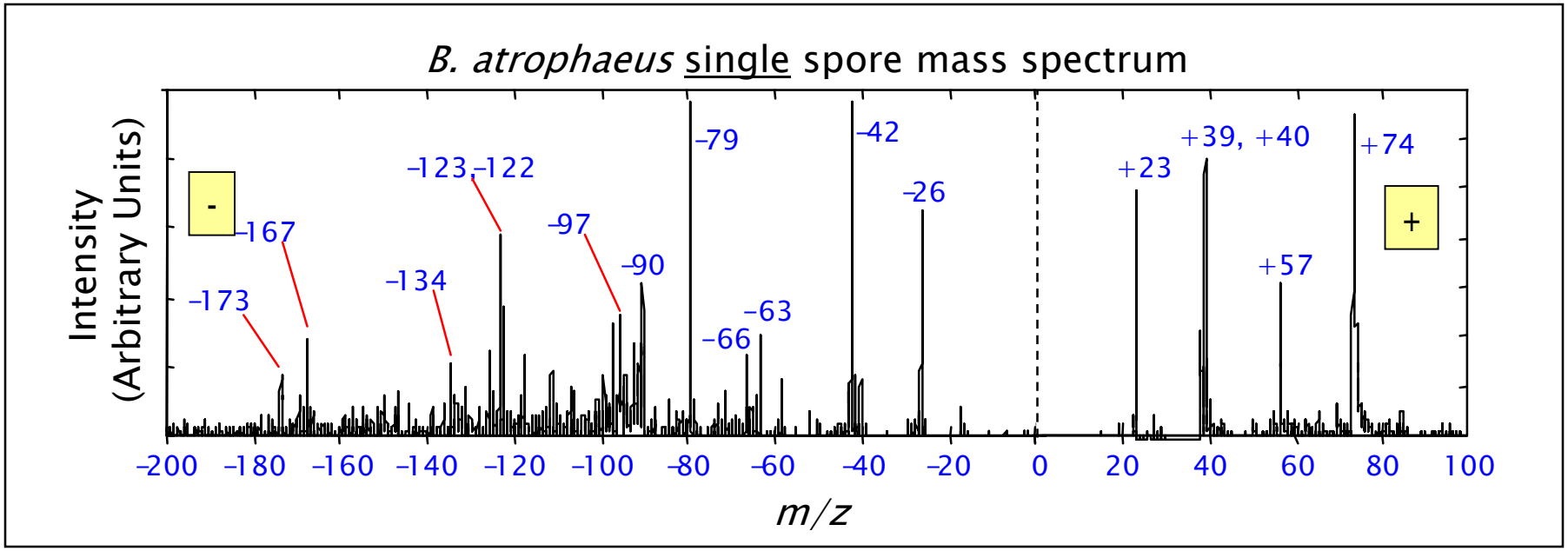
Improvements in many aspects of this system are currently being developed for future systems (BAMS 1.x)



Based on TSI-3800 ATOFMS

B. atrophaeus Spore BAMS Signature



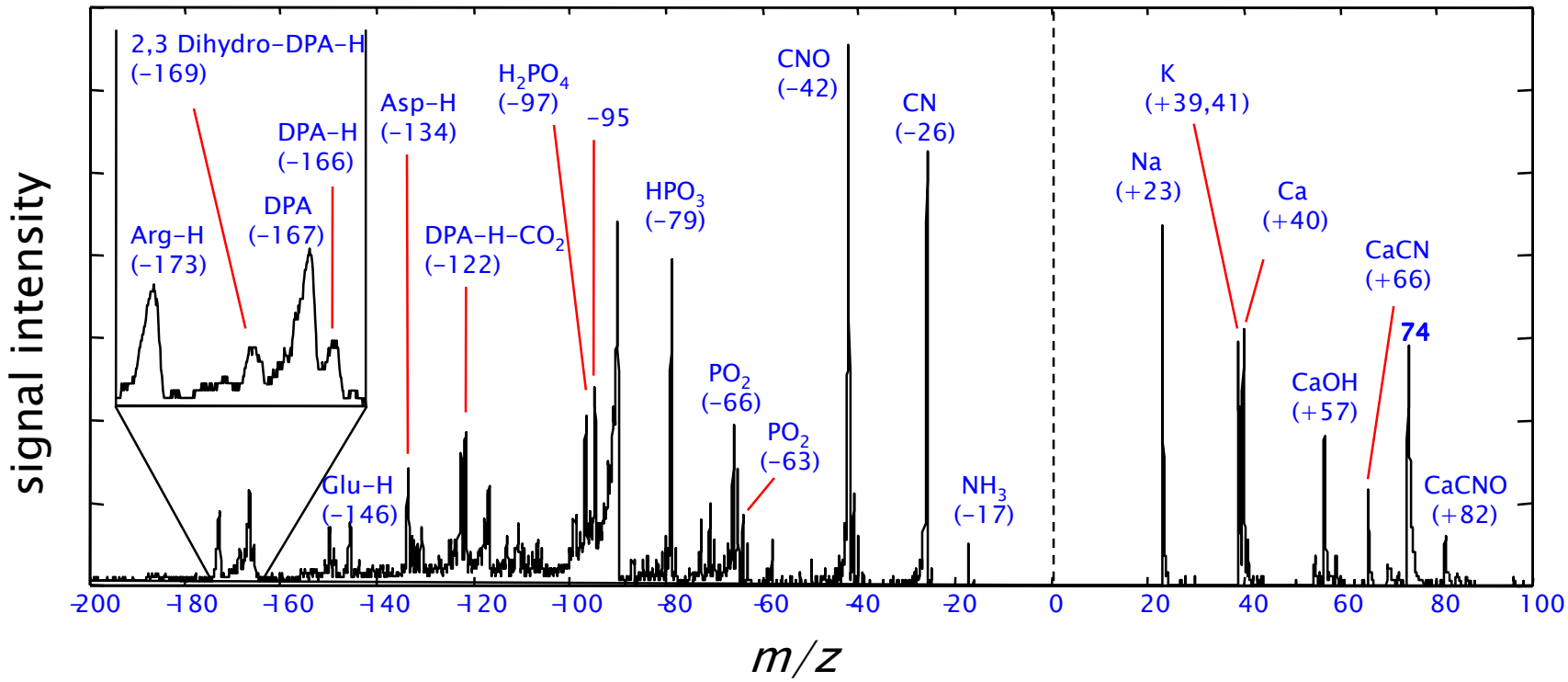


Ultimate detection limit is a single spore

B. atrophaeus spore BAMS peak identifications

Average of ~1000 individual mass spectra

Assignments confirmed using isotope labeling

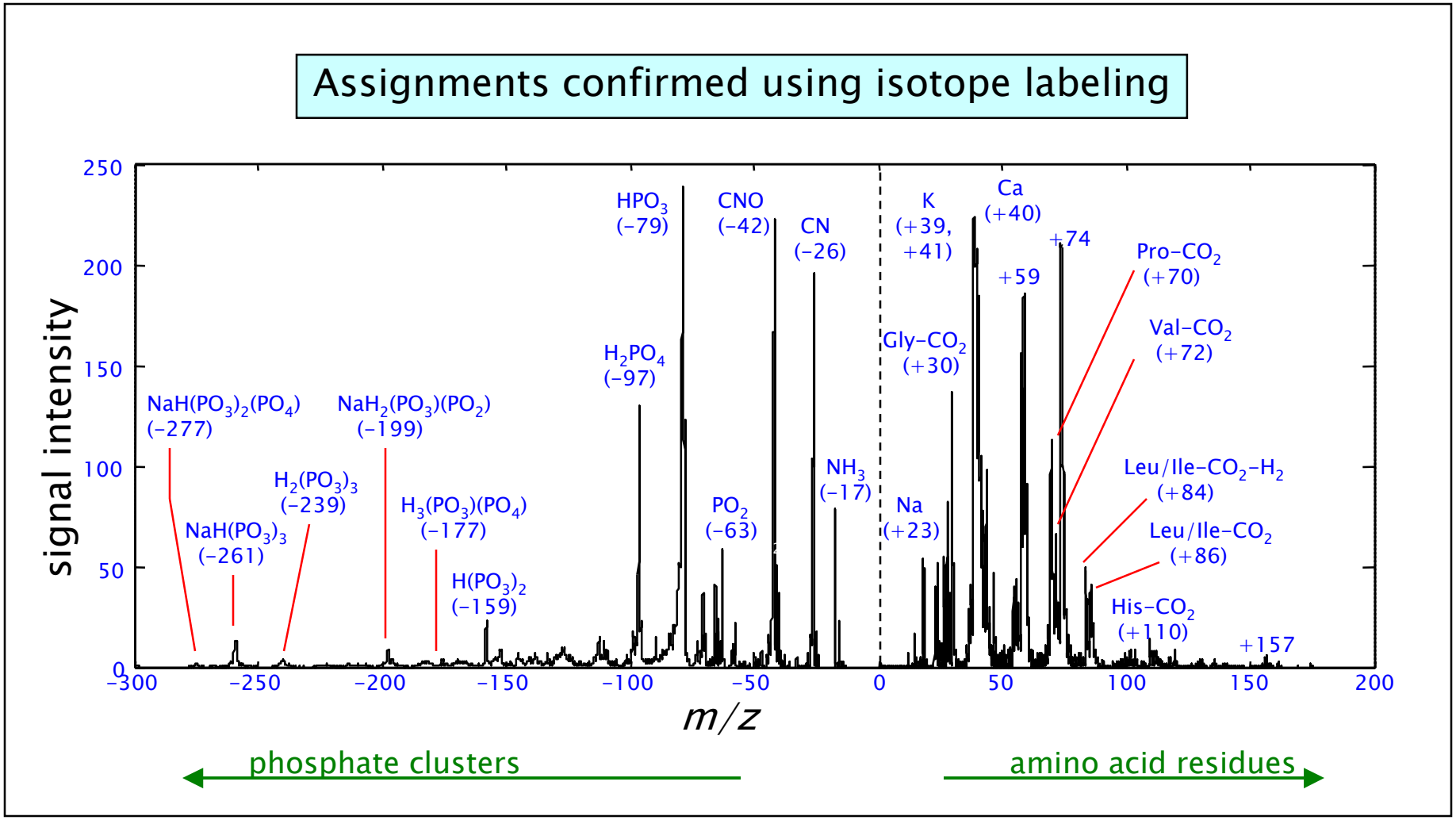


Fergenson, D., Pitesky, M., Tobias, H.J., Steele, P.T., Horn, J., Czerwieniec, G., Russell, S., Lebrilla, C., Frank, M., Gard, E.E. *Anal. Chem.*, **2004**, *76*, 373-378.

Steele, P.T., Tobias, H.J., Fergenson, D.P., Pitesky, M., Horn, J., Czerwieniec, G., Russell, S., Lebrilla, C., Frank, M., Gard, E.E. *Anal. Chem.,* **2003**, *75*, 2480-2487**.**

B. atrophaeus vegetative cell BAMS peak identifications

Average of ~1000 individual mass spectra



Tuberculosis

Evaluate BAMS as a tool for public health applications (in addition to bioterror).

Quantitatively compare BAMS to standard and novel microbial reference techniques, in collaboration with Millie Schafer (CDC-NIOSH).

Mycobacterium tuberculosis

(1) Very infectious respiratory disease.
(2) 15,000 new cases in U.S. each year.
(3) 3 million deaths each year world wide.

CDC-NIOSH (Millie Schafer)

(1) *M. tuberculosis* H37Ra.
(2) BSL 2+ laboratory.
(3) Flowing Bio-Aerosol Chamber.
(4) Collaboration- 2 week field study

Validation of BAMS Quantification with other Techniques

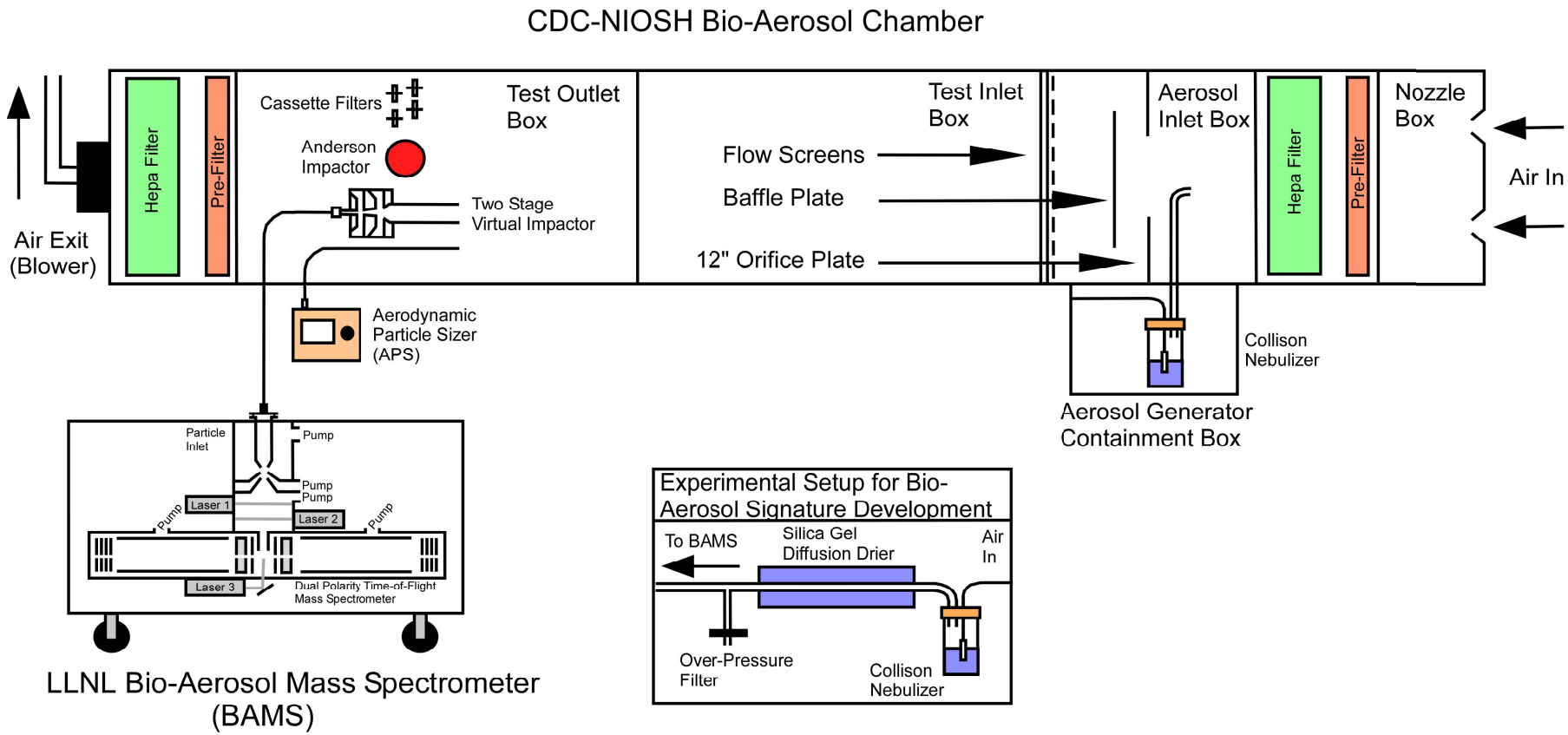
LLNL

CDC-NIOSH

Specificity increases

Technique	particle counter	live & dead cell counter	live cell counter	cell identifier	particle/cell size measurement
APS (real-time)	X				X
SYBR® Green Assay		X			
6-Stage Andersen Impactor			X		X
BAMS (real-time)	X	X		X	X
Quantitative PCR		X		X	

Bio-Aerosol Chamber Experiments

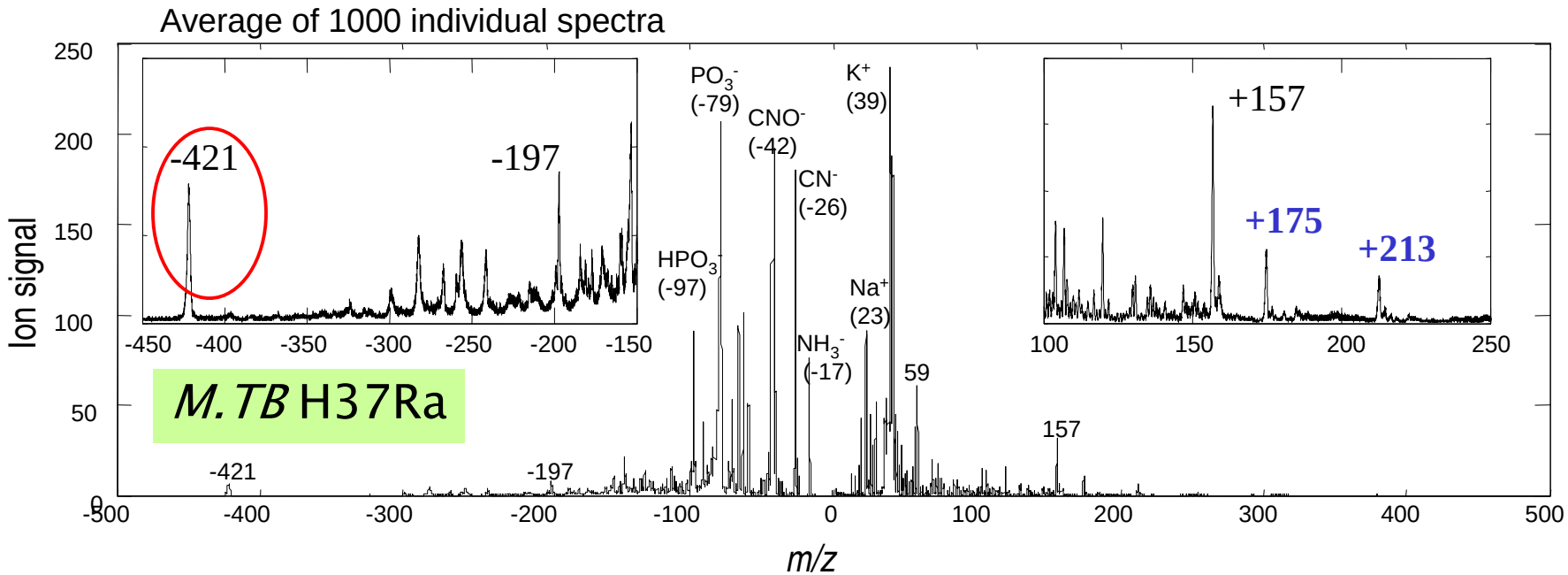


Aerosolize *M. tuberculosis* H37Ra and *M. smegmatis:*

(a) Washed samples at three different concentrations

(b) Unwashed sample

Characteristic BAMS Spectra for *M. tuberculosis* H37Ra

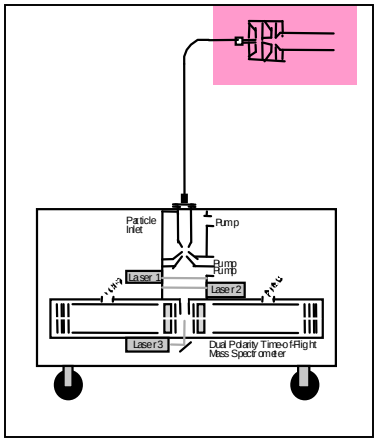


BAMS Calibration and Performance Model

With a characteristic mass spectrum, we can now explore BAMS ability to quantitatively measure the concentration of *M. tuberculosis* H37Ra.

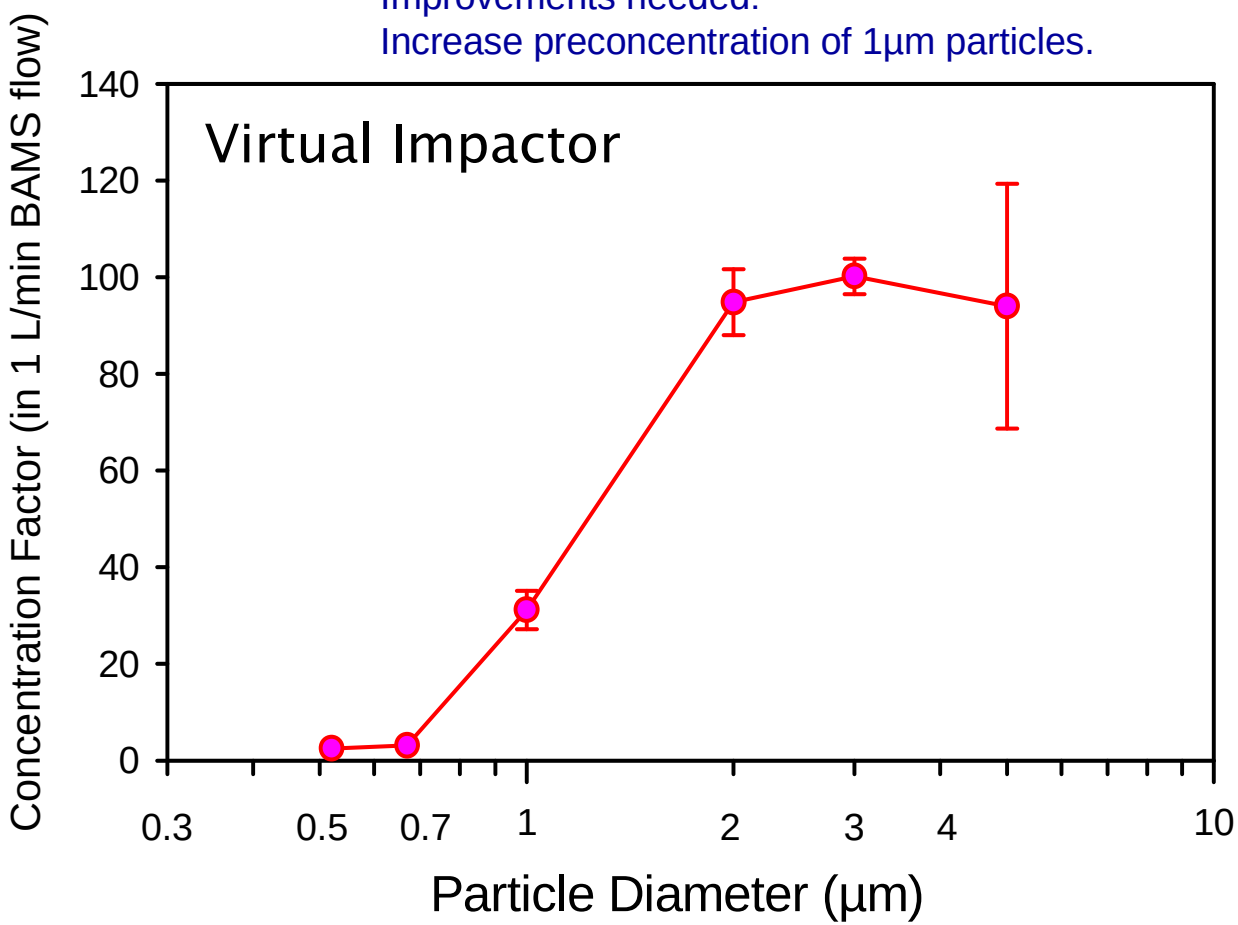
(Relate BAMS particle counts to actual airborne particle concentrations)

BAMS 1.0 Aerosol Preconcentration



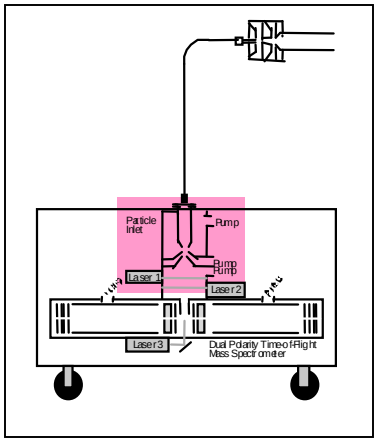
(1) 2-stages sample 400L/min into 3 L/min outflow.
(2) Concentration effect experimentally measured using particles sampled into an APS with and without virtual impactor in-line.
(3) Polystyrene Latex Spheres (PSLs).
(4) Assume spore concentration effect similar.

Improvements needed:
Increase preconcentration of 1µm particles.



95

BAMS 1.0 Aerosol Sampling & Tracking: Particle Inlet

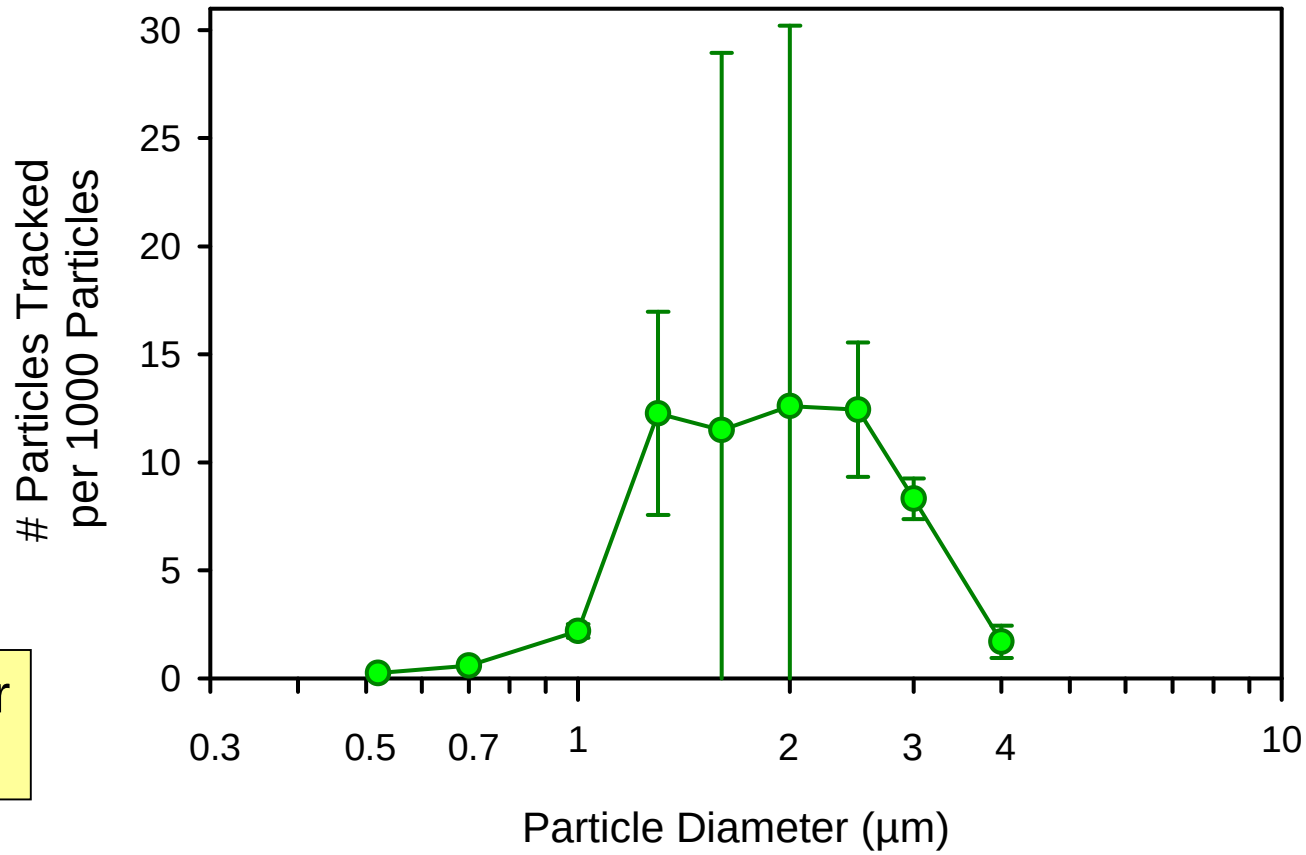


(1) Number of particles that are successfully tracked out of every 1000 particles sampled into particle inlet.

(2) This number includes transmission losses through the nozzle and skimmers, and particle beam divergence up to the second scattering laser.

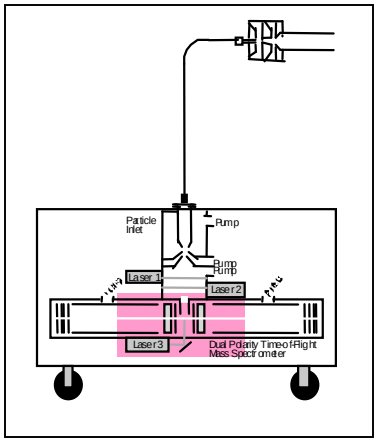
(3) Determined experimentally by measuring sampled aerosol using an APS (assuming 50% APS sampling efficiency).

Improvements needed:
Particle focusing over wide size range and tracking system.



(1) particle counter
(2) particle sizer

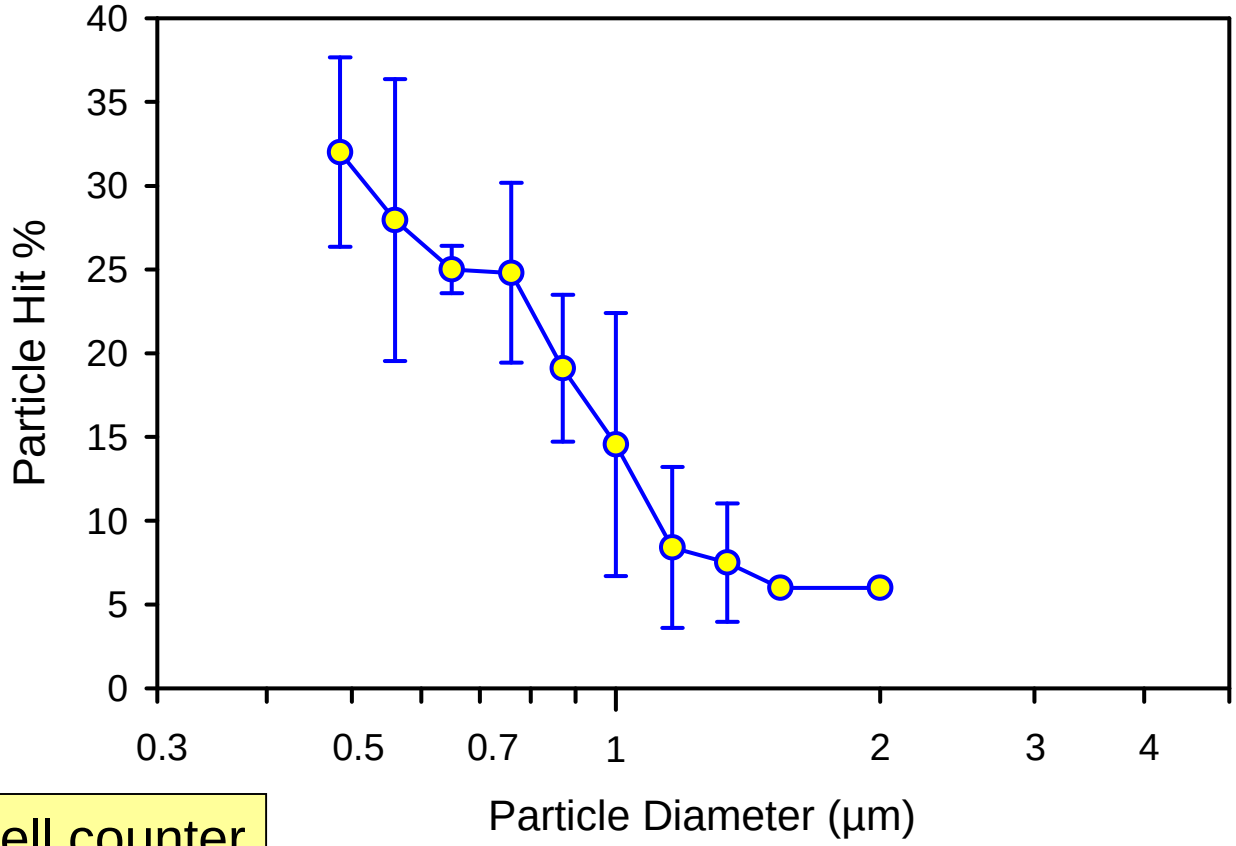
BAMS 1.0 Aerosol Chemical Analysis Rate (Desorption/Ionization-Hit %)



(1) Percentage of tracked particles/cells that are successfully desorbed, ionized, and produce BAMS mass spectra.

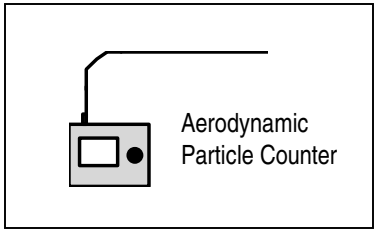
(2) 266nm Nd:YAG laser power = 0.66 mJ/pulse.

Improvements needed:
Particle tracking system and laser D/I scheme.



(1) live and dead cell counter
(2) cell identifier

Aerodynamic Particle Sizer (APS)

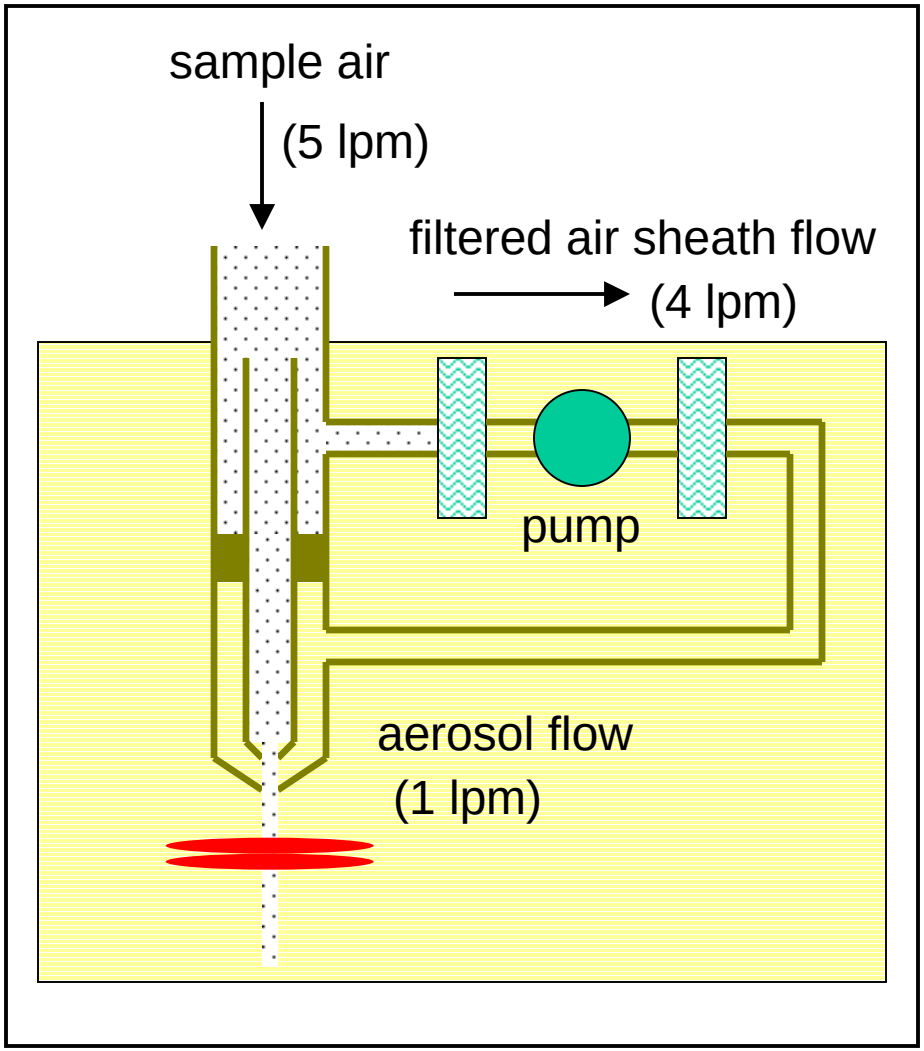


1) Commerically available (TSI 3321).

2) Tubing losses are < 5%.[1]

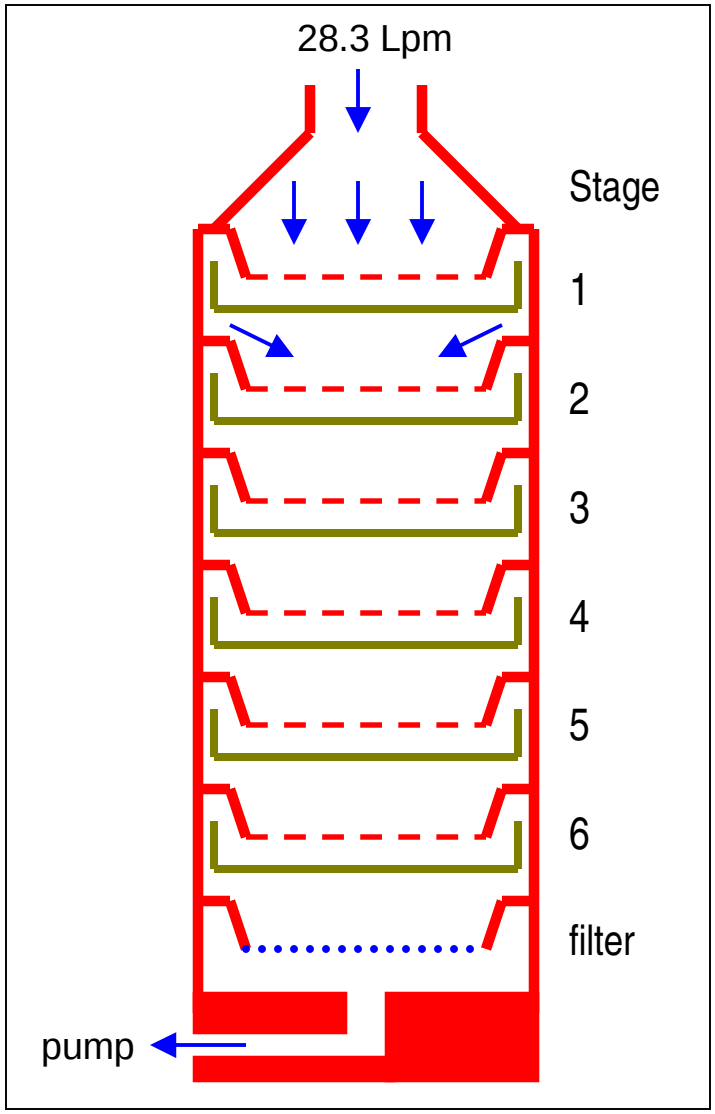
3) ~50% particle sampling efficiency.[2]

[1]Experimental measurements.
[2]Peters, T.; Leith, D. *Aerosol Sci. Tech*. 34, (2003), 627-634.



(1) particle counter
(2) particle sizer

Andersen 6-Stage Impactor



Count colony forming units (CFUs) directly from each plate.

Stage	50% cutoff (µm)
1	7.0
2	4.7
3	3.3
4	2.1
5	1.1
6	0.6

(1) live cell counter
(2) live cell sizer

Filter Cassette: SYBR® Green Assay

- Filter cassette sampler- 37mm, 1.0 µm pore size, collection at 3.7 L/min.
- SYBR® Green- highly sensitive fluorescent stain that binds to nucleic acids.
- Direct cell count (live and dead).

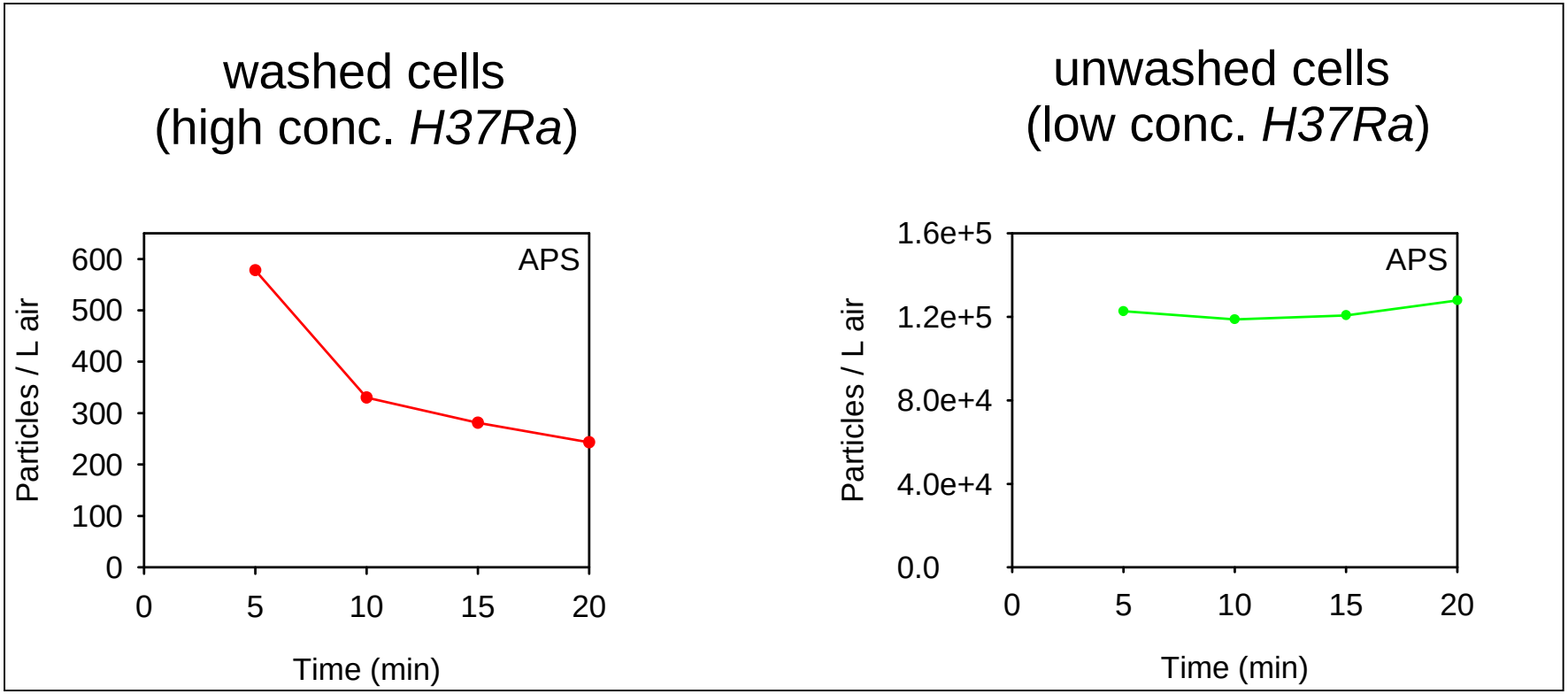
(1) live and dead cell counter

Filter Cassette: Quantitative PCR

- Filter cassette sampler- same as above.
- PCR-amplified DNA- highly conserved mycobacteria 16S rRNA genetic region common to species of mycobacteria in the genus.
- Awaiting results.

(1) live and dead cell counter
(2) live and dead cell identifier

Nebulized Aerosol Concentration Stability



Mycobacteria clump when cells are washed

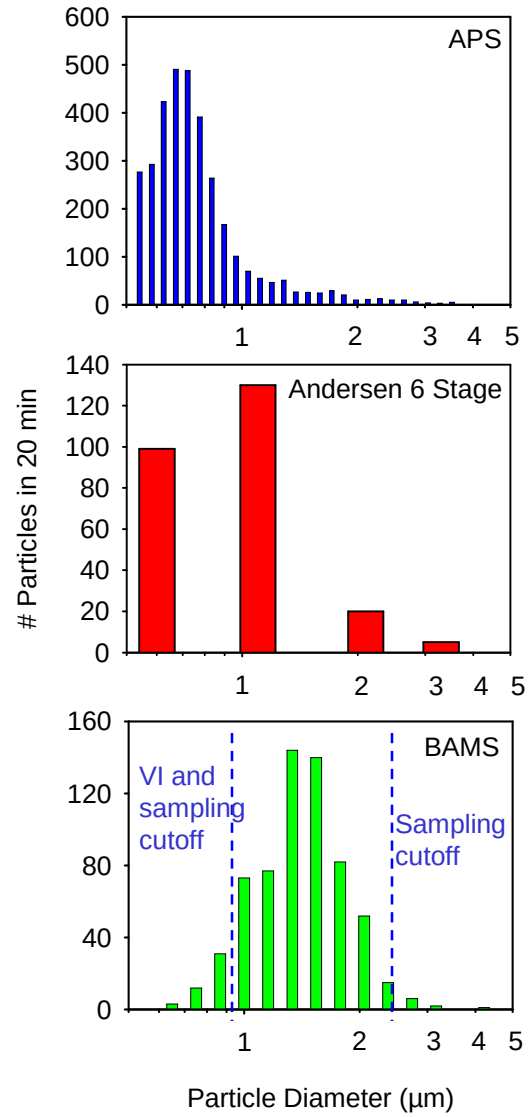
Measured Particle Size Distributions for H37Ra

(washed cells)

APS →

Andersen 6 Stage Impactor →

BAMS 1.0 →



All data plotted are uncorrected for any sampling biases.

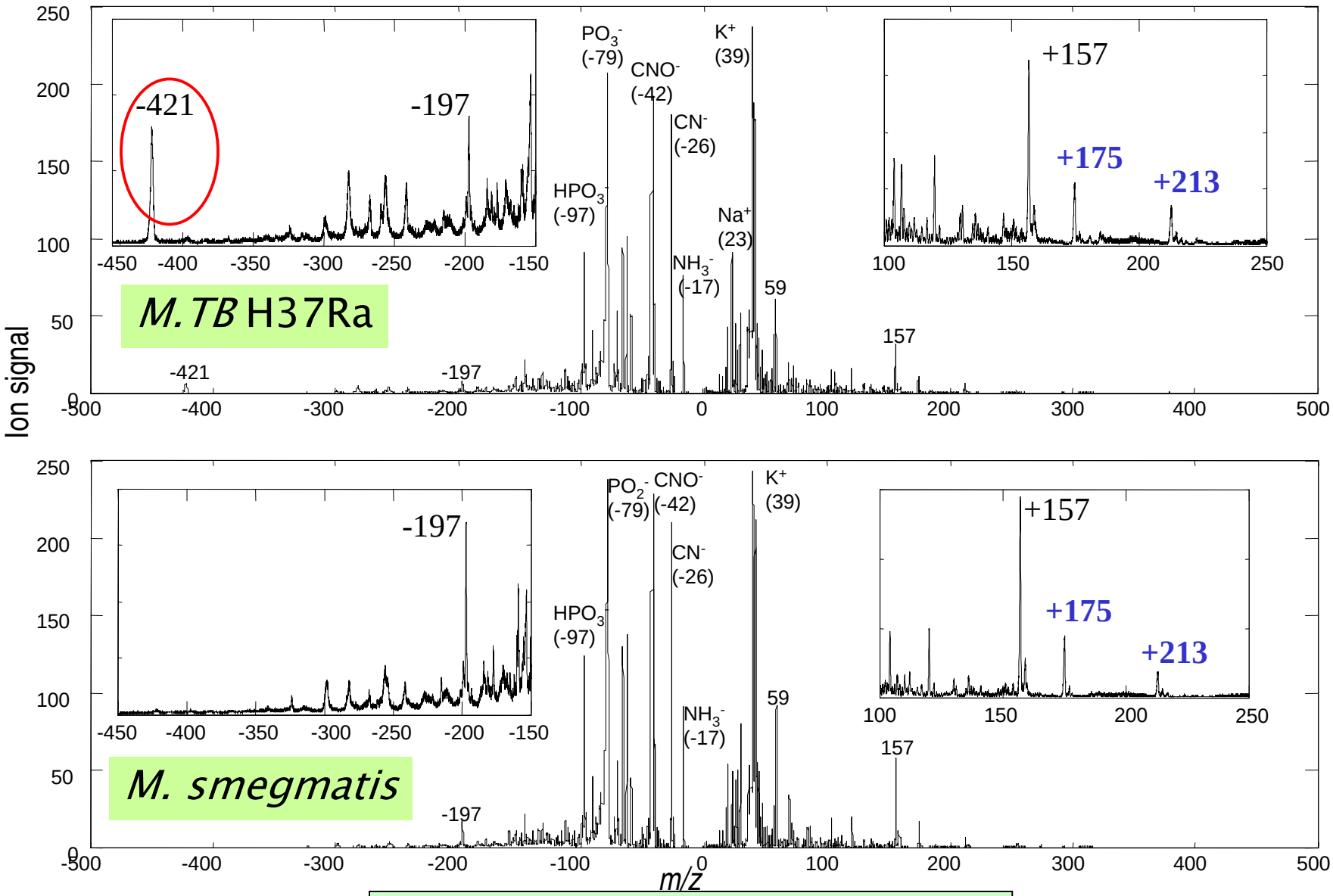
BAMS Experimental Validation (20 min samples of washed *M. Tuberculosis H37Ra* cells)

M. tuberculosis H37Ra concentration measured using plate counts (# cells/L air)	*M. tuberculosis* H37Ra concentration as measured by BAMS 1.0 (# cells/L air)
0.9	2.7 ± 0.9
1.4	23.9 ± 22.0
13	15.4 ± 4.3

Performance Model Prediction of # cells identified (1.4µm) by BAMS 1.0	# of *M. tuberculosis* H37Ra cells identified (1.4µm) by BAMS 1.0
1.7	4
2.6	10
31	25

BAMS can detect *M. tuberculosis* H37Ra particles at concentrations > 50 particles/L air in 1 min. (using the metric 5 particle identifications = a detection)

BAMS Spectra for *M. tuberculosis* H37Ra and *M. smegmatis*



Further work needed for peak identification

Conclusions

BAMS 1.0

-Durable and transportable system for field work and remote site applications.

-Feasibility as tool for public health applications merits further investigation.

Tuberculosis detector: promising but more work needed.

BAMS 1.x (future systems)

-Improvements required in

1. Preconcentration
2. Sampling (Particle Focusing),
3. Tracking (Particle Pre-selection)
4. Cell signatures (D/I, Mass spectrometry)

Acknowledgements

Bio-Aerosol Mass Spectrometry Team

- Keith Coffee
- James Birch
- Paul Steele
- Abneesh Shrivastava
- Nir Dover
- Shuba Ramani
- Coby Adamczyk
- Laura Ludvigson
- Prof. Carlito Lebrilla
- Scott Russell
- Gregg Czerwieniec
- Norm Madden
- Vincent Riot
- Bruce Woods
- Darryl Spurlock

Funding:

- Technical Support Working Group, Department of Defense
- LLNL Lab Directed Research and Development Program (02-ERD-002)
- LLNL Chemical and Materials Sciences Postdoctoral Program
- Defense Advanced Research Projects Agency

This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

LLNL/UCD-BAMS Related Posters at ASMS

MPA 006 Abneesh Srivastava Coupling Two Step Laser Desorption-Ionization with Bio-Aerosol Mass Spectrometry for Analysis of Individual Bacterial Cells.

MPA 014 Gregg A Czerwieniec Novel Online MALDI Techniques for Improved Signal of Biomarkers in an Aerosol Mass Spectrometer.

MPA 017 Paul T. Steele Laser Effects on Mass Signatures from Individual Bacillus Spores and Biological Particles in Bio-Aerosol Mass Spectrometry.

ThPI 154 Abneesh Srivastava Study of single micron sized phospholipid aerosol particles by aerosol mass spectrometry: Influence of laser fluence and matrix addition.

ThPM 242 Scott C Russell Improvements in Sensitivity and Mass Range by Increased Ion Transmission Efficiency in an Aerosol Time of Flight Mass Spectrometer.