



AEROSOL FLOURESCENCE IN THE ACTIVE STANDOFF CHAMBER

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Introduction

The Active Standoff Chamber (ASC) at Dugway Proving Ground (DPG) was initially developed to provide static aerosol challenges for testing light detection and ranging (LiDAR) systems to be used as standoff biological agent detectors. The chamber is a large stainless-steel box with openings on each end large enough to allow standoff interrogation of simulant clouds. Air curtains at each opening prevent the escape of particles, effectively creating a controlled outdoor environment in which static clouds can be maintained, characterized, and left to decay.

The annual Technology, Experimentation, and Characterization Field Trials (TECFT) were conducted in June 2023 at DPG. A portion of the TECFT event was executed in the ASC, using four bio-simulants [*Bacillus atrophaeus* (BG), *Bacillus thuringiensis* variant *kurstaki* (Btk), *Erwinia herbicola* (EH), ovalbumin (OV)] and one interferent [Arizona road dust (ARD)] as challenge materials. Realistic aerosol concentrations and particle size distributions were generated in the chamber, during which particle data was recorded with BioTesting Division (BTD) referee systems [Aerodynamic Particle Sizer (APS) and Wideband Integrated Bioaerosol Sensor (WIBS)]. An analysis of the data produced by the APS and WIBS is summarized and compared in this study.

Methods

The WIBS can potentially discriminate biological from non-biological particles, using three fluorescence channels to broadly facilitate this separation. The fluorescence channels can be expressed as **A** (excitation 280nm, emission 310 – 400nm), **B** (excitation 280nm, emission 420 – 650nm) and **C** (excitation 370nm, emission 420 – 650nm). Biological particles usually show high fluorescence emission intensity in all three fluorescence channels, varying by species and chemistry.

The BTD test team prepared powder material (BG, BTK, OV and ARD) and EH slurry to generate aerosols in the ASC. Images of the ASC are shown in **Figure 1**. During trials, aerosol particle size and concentration were measured by multiple APS and WIBS units. Mixing fans were used to homogenize aerosol concentration throughout the chamber. This was confirmed in real time by referencing the APS at three locations within the ASC. The Open Architecture Data Management System (OADMS) (**Figure 2**) was used to monitor testing and retrieve referee data in real time. Data from a single APS and WIBS at one referee station were analyzed and presented in this study.



Figure 1. **left.** The APS and WIBS are being configured inside the chamber; **right.** ASC Outside View from the West.



Figure 2. Screenshot of Trial 23 playback in OADMS. Three APS and WIBS units collect particle data while West Desert LIDAR interrogates the cloud.

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Results

Particle counts in each of the 52 APS size bins were analyzed to obtain particle number and mass distribution vs. time and peak number, as well as mass distribution vs. particle diameter. The MATLAB function Pview (written by Francis D'Amico, DEVCOM CBC, Sensors, Signatures & Aerosol Technology Branch) was used to reduce raw WIBS data (H5 files) to a size binned text file format emulating APS data. Number concentration with fluorescence fraction vs number and particle size distribution at number peak of each trial were also generated using Pview. The Igor Pro function WIBS Toolkit_v1.33 (written by Droplet Measurement Technologies) was used to generate number, mass, and surface area concentrations vs. time for each trial from the raw WIBS H5 files. The summarized analysis data [number median diameter (**NMD**), mass median diameter (**MMD**), particle number, and mass concentrations at the release peaks of dissemination] of the APS and WIBS are shown in **Table 1**. Data relating particle number, cross sectional area, and mass to fluorescence is found in **Table 2**. The total particle counts and total counts for each of the three WIBS fluorescence channels (A, B, C) in all 52 size bins were analyzed to obtain peak number, cross section area, and mass distribution vs. particle diameter (**Figure 3**) and particle number, mass, and cross section area distribution vs. time (**Figure 4**).

Trial	Simulant	Peak Time MDT (hh:mm:ss)		NMD (µm)	MMD (µm)	N (ppl)	M (ng/L)
		Number	Mass				
APS							
20	ARD	23:22:31	23:22:42	0.90	5.83	219600	2461
21	EH	17:27:45	17:28:05	1.11	5.83	17070	164
22	OV	20:26:45	20:26:45	0.90	4.07	49188	171
23	BTK	20:43:05	20:43:35	0.72	3.79	22350	33
24	BG	20:54:35	20:53:55	0.90	2.64	127896	124
WIBS							
20	ARD	23:22:09	23:21:47	0.72	4.07	97220	885
21	EH	17:27:04	17:27:15	0.84	8.98	9820	88
22	OV	20:26:45	20:27:35	0.72	13.82	22180	158
23	BTK	20:42:55	20:43:05	0.72	9.40	9860	102
24	BG	20:53:55	20:53:45	0.67	6.26	84840	196

Table 1. Summary of **NMD**, **MMD**, Number Concentration (**N**), Mass Concentration (**M**) at the Release Peaks of Dissemination Measured by one APS and WIBS in the ASC; **NMD**: Peak Number Median Diameter; **MMD**: Peak Mass Median Diameter; **N**: Peak Aerosol Number Concentrations; **M**: Peak Aerosol Mass Concentrations.

Trial	Simulant	Total Number (ppL)	Number Channel A (ppL)	Number Channel B (ppL)	Number Channel C (ppL)
20	ARD	97220	4220 (4%)*	3480 (4%)*	3280 (3%)*
21	EH	9820	6060 (62%)*	6000 (61%)*	6020 (61%)*
22	OV	22180	2220 (10%)*	2320 (10%)*	2280 (10%)*
23	BTK	9860	3400 (34%)*	3360 (34%)*	2900 (29%)*
24	BG	84840	14660 (17%)*	9760 (12%)*	8580 (10%)*
Trial	Simulant	Total Surface Area (µm ² /L)	Surface Area Channel A (µm ² /L)	Surface Area Channel B (µm ² /L)	Surface Area Channel C (µm ² /L)
20	ARD	148395	8560 (6%)*	10582 (7%)*	11413 (8%)*
21	EH	24516	18370 (75%)*	18433 (75%)*	18431 (75%)*
22	OV	38682	10767 (28%)*	12477 (32%)*	13289 (34%)*
23	BTK	20814	8056 (39%)*	8215 (39%)*	7845 (38%)*
24	BG	84344	15935 (19%)*	14675 (17%)*	14293 (17%)*
Trial	Simulant	Total Mass (ng/L)	Mass Channel A (ng/L)	Mass Channel B (ng/L)	Mass Channel C (ng/L)
20	ARD	885	69 (8%)*	90 (10%)*	81 (9%)*
21	EH	88	71 (81%)*	71 (81%)*	71 (81%)*
22	OV	158	120 (76%)*	122 (77%)*	123 (78%)*
23	BTK	102	96 (94%)*	97 (95%)*	97 (95%)*
24	BG	196	26 (13%)*	25 (13%)*	25 (13%)*

Table 2. Summary of Fluorescence Signal in three Fluorescence Channels (**A, B, C**) relating to Total Number, Total Cross Section Area and Total Mass; (%) *: Percentage of concentration in each fluorescence signal channel e.g., (**Number Channel A /Total Number**) × 100%.

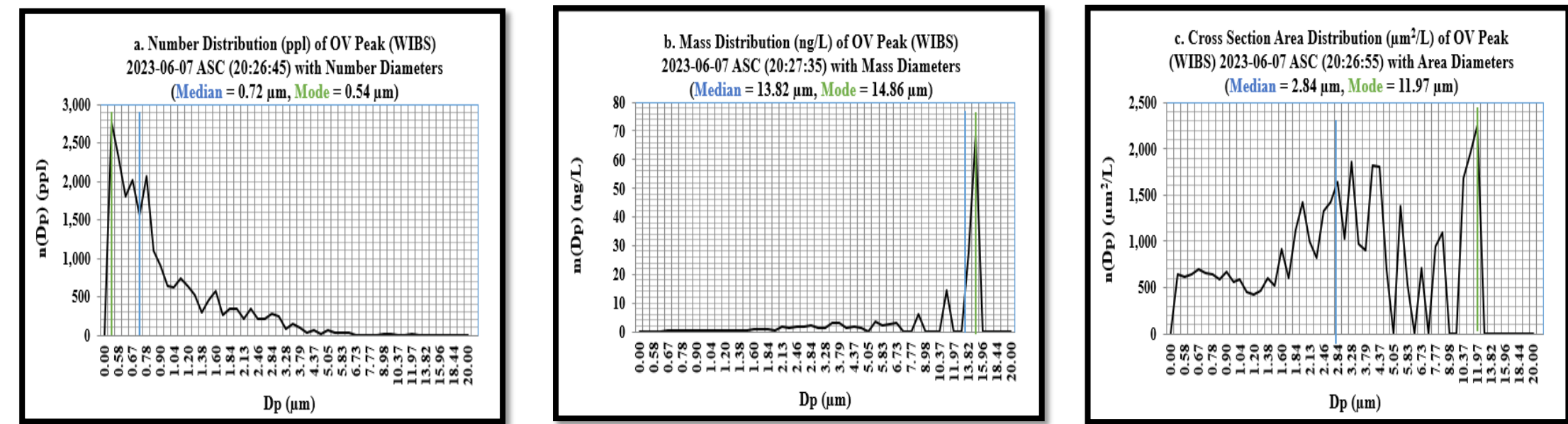


Figure 3. **a.** Number Distribution, **b.** Mass Concentration (ng/L) Distribution, **c.** Cross Section Area Distribution, at Peak of OV Trial TFA-22, Measured by Wideband Integrated Bio-aerosol Sensor (WIBS).

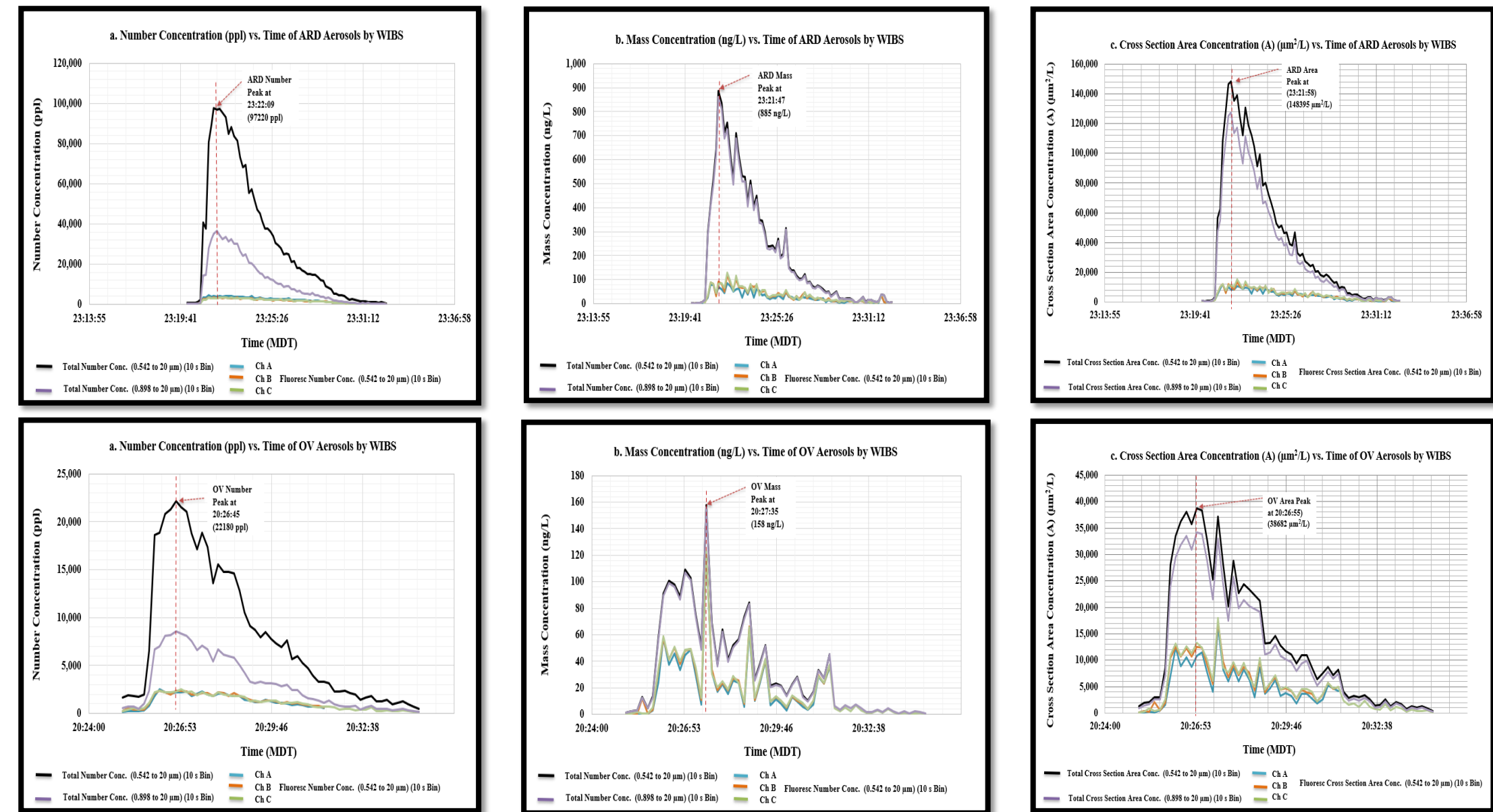


Figure 4. **Top row.** ARD Trial TFA-20; **Bottom row.** OV Trial TFA-22; **a.** Number Concentration (ppL) vs. Time, **b.** Mass Concentration (ng/L) vs. Time, **c.** Cross Section Area Concentration (µm²/L) vs. Time Measured by Wideband Integrated Bio-aerosol Sensor (WIBS).

Conclusion

Fluorescence signal analysis using particle number concentration exhibited a lower percentage of fluorescent signal in each channel, as displayed in **Table 2** and **Figure 2**. This was especially true for OV, where it was impossible to discriminate a known biologically fluorescent simulant signal from the ARD signal. On the other hand, a higher proportion of fluorescence was noted when analyzing fluorescent signal as a function of particle cross sectional area or mass. In both cases, a higher fluorescent signal could be related to particles with higher cross-sectional areas and more mass. Therefore, it can be stated that particles with more cross-sectional area and mass values fluoresce more intensely than those with smaller values. Conversely, when calculating fluorescent fraction by number concentration, the smaller, less fluorescent particles weigh down the percentage of fluorescence intensity.

Given the above data, it is strongly recommended to study fluorescence signals relating to cross section area and mass concentrations of aerosol particles, especially in attempts at biological aerosol classification and identification. Since fluorescence intensity varies strongly with size, it is probably necessary to consider cross section area concentration and mass concentration besides the usual focus on number concentration.

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