BIOLOGICAL AEROSOL STANDOFF DETECTION FROM SPECTRALLY RESOLVED LASER INDUCED FLUORESCENCE

Sylvie Buteau[1], Jean-Robert Simard[1], Christian Laflamme[1] and Pierre Lahaie[1].

[1] Defence R&D Canada (DRDC) – Valcartier, 2459, boul. Pie-XI nord, Québec, QC, Canada, G3J 1X5

SUMMARY: SINBAHD, a lidar sensor based on spectrometric UV laser-induced fluorescence (LIF) developed by Defence R&D Canada (DRDC) at the end of the 90's, was invited to participate in the JBSDS (Joint Biological Standoff Detection System) increment II Tech Demo III trial. This international trial, which took place at Dugway Proving Ground (DPG), Utah, in August 2007, intended to challenge different sensor technologies to various biological simulants and interferents. In total, 91 releases were performed in the Joint Ambient Breeze Tunnel (JABT) and 15 in open-air during the two-week trial. The LIF from each disseminated material was spectrally characterized and the extracted signature was added to the library. Most of the tested biological agent simulants showed distinct spectral features and significant LIF signal levels. Correlation assessment between the SINBAHD stand-off sensor and the Aerodynamic Particle Sizer (APS) point sensor used as reference was done allowing an estimation of SINBAHD's sensitivity in particles per litre (ppl). The extracted sensitivity for BG and EH for a 20-m cloud at a range of 1.26 km is around 1.7 kppl and less than 12 ppl, respectively. Additionally, the extracted slope of the standoff metric versus the concentration data is proportional to the material fluorescence cross-section. This later relation allows comparison of the different materials in terms of LIF signal levels. Results show that the preparation and dissemination methodologies have different degree of impact on the spectral characteristic and on the generated fluorescence signal level.

INTRODUCTION: Most biological detection technologies currently deployed in operations are point detection systems, which need to be located within the biological cloud to detect it, hence a detect-to-threat capability. An efficient standoff biological warfare detection system providing a detect-to-warn capability would be an important asset for both defense and security communities. DRDC developed, at the end of the 90s, a stand-off bioaerosol sensor prototype based on spectrometric detection of LIF. This project, called SINBAHD, demonstrated the capability of using spectral LIF to detect and characterize bioaerosols from a standoff position. In August 2007, SINBAHD participated to the JBSDS increment II Tech Demo III trial. This trial was held at Dugway proving Ground, where tunnel and open-air releases of biological agent simulants and obscurants were performed.

SINBAHD: The SINBAHD sensor includes a UV laser source emitting about 120 mJ per pulse at 351 nm and at a pulse repetition frequency of 125 Hz, a beam expander, a folding mirror to obtain a co-axial beam with the collecting optical axis, and an elliptical steering mirror to select the starring LIDAR line of sight. The returned signal is collected via a 30 cm diameter Newtonian telescope which focuses it at the entrance slit of an imaging spectrometer. Simultaneously, a photo-multiplier tube connected to a transient recorder samples the elastic scatter returns as a function of range. The 300 line/mm grating, in combination with the 200 µm wide spectrometer entrance slit, confers a spectral resolution of 4.8 nm and a span of 230 nm. An intensified CCD camera, binned vertically, detects the dispersed fluorescence at the exit window of the spectrometer. The intensifier gate is synchronized with each fired laser pulse with a delay defining the range of the probed atmospheric volume.

SIGNATURE MEASUREMENTS: The bioaerosol clouds generated in the JABT were characterized in ppl by APSs located 70 m away from JABT front end. SINBAHD was located at 1.2 km from the front end of the tunnel. Various biological agent simulants were tested: Bacillus subtilis (BG), Erwinia

herbicola (EH), male-specific bacteriophage type 2 (MS2), Bacillus thuringiensis (BT), ovalbumin (OV), killed Bacillus anthracis (BA), killed Yersinia pestis (YP), killed francisella tularensis (FT) and ricin toxoid (RT) in addition to different common interferents. SINBAHD measured the LIF spectral characteristics of all the 91 tunnel releases. The extracted spectral signatures showed specificity and robustness in most cases. Figure 1 presents the signature extracted from all the simulant releases. Ricin and YP show significant spectral similarities and resemble the BA signature. MS2 and FT also show some resemblances but are characterized by more robust highly repeatable signatures. Even though BG signatures have a lower signal-to-noise ratio (SNR) than other simulants, they demonstrate repeatability from release to release for a given preparation and dissemination methodology. Indeed, it was observed that different aspects such as spore growth methodology, growth media type, sample preparation methodology, gamma-irradiation of the spore, wet versus dry release impact the LIF spectral signature. The growth methodology of bacterial spores appears to have the greatest impact as seen on Figure 1 when comparing New BG (dark pink) to BG (light pink) signatures. A weaker impact of washing or not the bacterial spores from their growth media can also be observed in the case of BA samples on Figure 1. The dissemination methodology, such as wet versus dry releases, has also shown some spectral signature variations in the case of BG samples. In the case of the bacterial vegetative cell EH, the different signatures showed the largest variability even though the preparation and dissemination followed a unique methodology. This spectral variability was attributed to the various microbial physiological states of the different samples, which is exacerbated in the case of live vegetative cells. The tested interferents demonstrated high variability in the LIF signal intensity. All extracted signatures were used to populate our LIF spectral library for subsequent data processing and analysis.

Figure 2 presents a two-dimension result from a Principal Component Analysis (PCA) performed on the signatures extracted from different simulant signatures (Figure 1). This later result gives an appreciation of the possibilities of the spectral LIF technique for bioaerosol cloud classification.

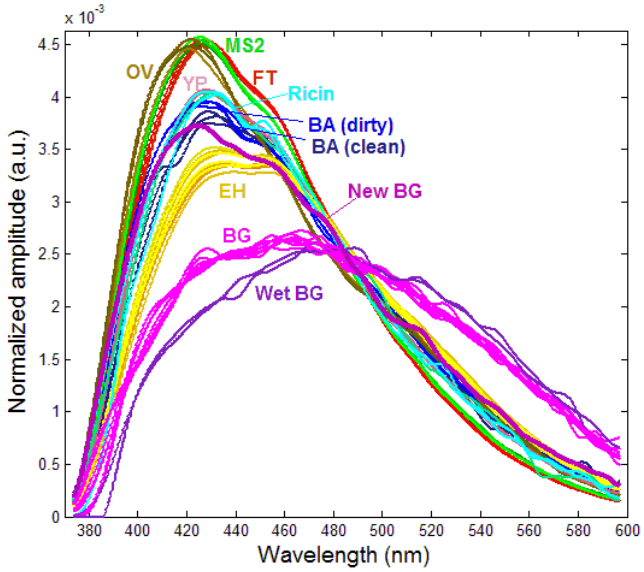

Figure 1. LIF signatures of biological agent simulants acquired by SINBAHD during the JBSDS II Tech Demo III trial, DPG, USA, August 2007.

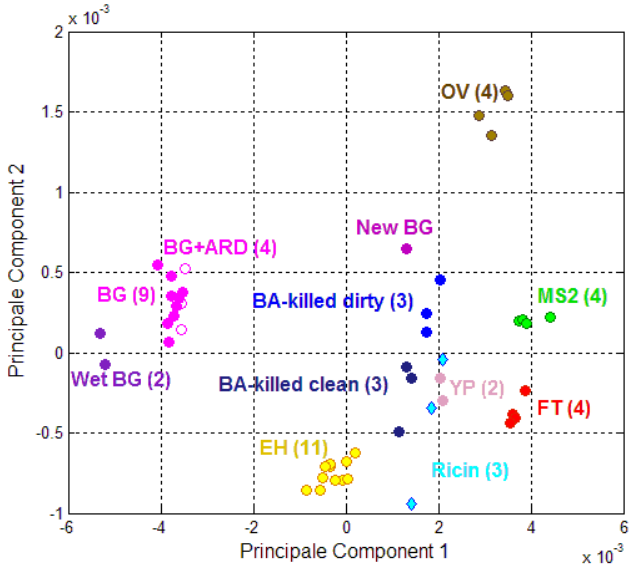

Figure 2. Two first components from a Principal Component Analysis (PCA) performed on the simulants LIF signatures.

DATA ANALYSIS: The acquired data was analyzed using a Multivariate Analysis (MA) technique, which extracts the energetic contributions of the different contributing inelastic scatterers from the acquired fluorescent signal. It is based on the best fitting of the collected inelastic spectra using a linear combination of normalized spectral signatures. This MA is a first generation of spectral data processing

developed for SINBAHD. A more sophisticated technique is actually under development under the BioSense technical demonstration Project [1].

The correlation between the SINBAHD metric, the energetic contribution of the released material, and the reference point sensor results, was evaluated for each release performed in the JABT. In most cases, the correlation could be extracted automatically and if not, a visual correlation was tentatively estimated. This was particularly applicable to low signal levels, mixtures and double trend correlations, for which the visual correlation was more representative than the one obtained from the correlation sub-routine. The correlation between SINBAHD measured energetic contribution of a given material and the APS result is used to determine SINBAHD sensitivity in concentration unit (ppl). The sensor sensitivity is defined as four times the standard deviation of the detection metric when no cloud is present. The 4 σ sensitivity, which represents the detection criterion, is commonly used in standoff biological threat detection.

The correlation process is limited in accuracy due to the volume difference of the cloud probed by the standoff sensor and by the reference sensor. Ideally, those measurements would be performed within a homogeneous cloud, which is not always the case even for releases performed in a tunnel. To minimise the variability related to the cloud homogeneity, the SINBAHD probed volume was set to 20 meters centered on the APS range rather than probing the entire 80 m tunnel volume. A 12 sec-time convolution was performed on the APS data to be compatible with the SINBAHD sampling of about 10 seconds. Figure 3 presents the visual correlation between the 12-second convoluted APS reference data (blue line) and the SINBAHD metric, consisting of the energetic contribution of the released material (pink line) for a BG release in the JABT (trial 02). Figure 4 presents the automatic correlation evaluation result performed on the same data set.

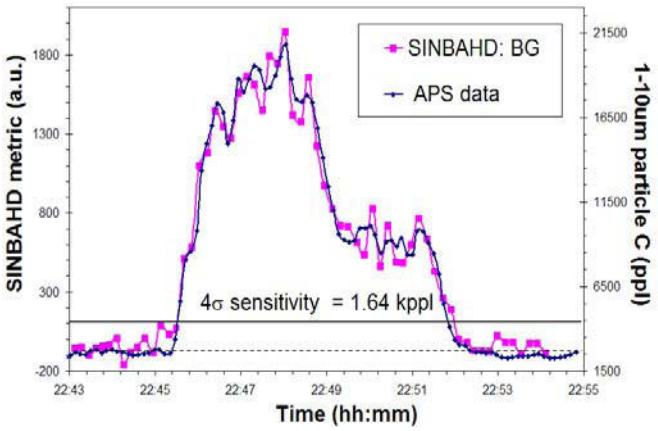

Figure 3. SINBAHD metric: BG signature amplitude (pink) and APS data (blue) for release T02 of BG with a 20-m gate at a range of 1.26 km.

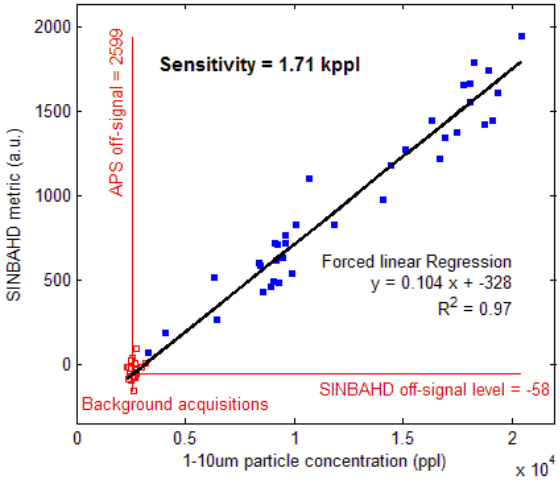

Figure 4. Automatic correlation evaluation between SINBAHD and APS data during background (red) and material release (blue) for a 20-m cloud of BG at 1.26 km (T02)

In some cases, the correlation evaluation cannot be performed due to incompatibility between the detection modes of the two sensors. Indeed, SINBAHD is measuring a LIF signal, while the APS is counting particles. The correlation evaluation between these two sensors must then be done with this limiting factor in mind. For example, in the case of inorganic material such as calcium carbonate, Arizona road dust, Kuwait mixture and silica beads, the APS detected a lot of particle while the SINBAHD LIF signal was very weak to inexistent. This result indicates that those interferents do not produce significant fluorescence and will not interfere with the bioaerosol detection.

The slope of the extracte d correlation of the SIN BAHD metric and the concentration data is directl y proportional to the probed bioaerosol fluorescent cross-section. This relation is used to com pare different type of sam ples in ter ms of their cross-sections. Fi gure 5 presents an exa mple of this conc ept for the releases of d ifferent BT p reparations. It shows that the BT sa mples grown in the Cas ein Acid Digest (CAD) media (blue results) have the same intensity of LIF whether they were washed (dark blue) or un-washed (light blue). In this particular case, this c learly dem onstrates that t he LIF signa l originates effectively from the ba cterial spore rat her than th e growth media. This figure also de monstrates the significant impact of the choice of growth media, whether the samples are washed (pink versus dark blue) or not (green versus light blue), on the intensity level of LIF. Another interesting observation from Figure 5 concerns the greater slo pe, hence cro ss-section amplitude, of the killed BT sam ples (red lines), when compared with their live counterparts (y ellow). T his difference could possibl y be attributed to the significantly higher pr oportion of gamma-irradiated bacterial spores having compromised mem brane (permeabilized) compared to un-irradiated ones [2].

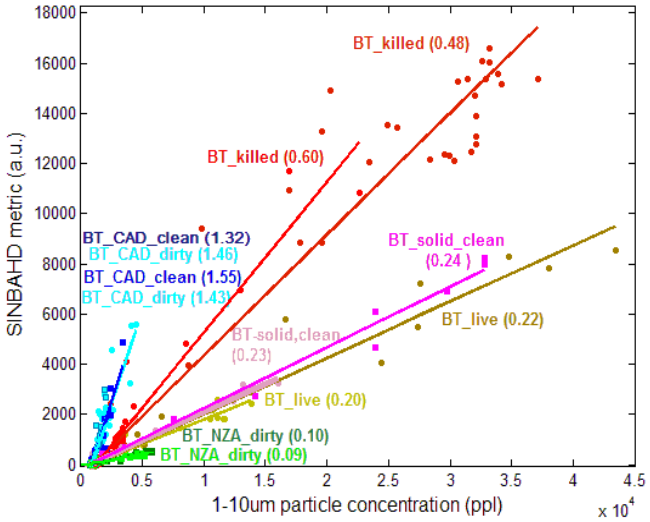

Figure 5. Correlation evaluation between SINBAHD metric and APS data for different BT releases (20-m cloud at 1.26 km).

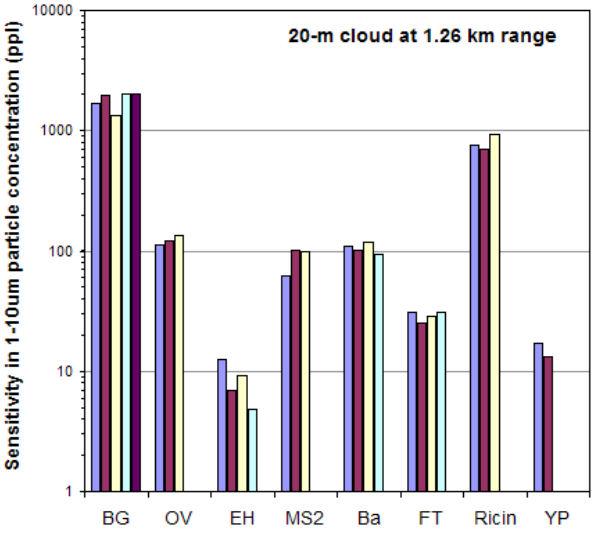

Figure 6. SINBAHD sen sitivities for various simulant releases obtained in the JABT during the Tech Demo III (20-m cloud at 1.26 km).

The correlation slopes are then used with the standard deviation of the SINBAHD metric when no cloud is present (si gma) to evaluate the 4 σ sensitivity of the SINBAHD senso r. Figure 6 presents extracted sensitivities for 1 to 10 µm parti cle concentration, ppl, for va rious si mulants relea ses. It must be emphasized that these extracted sensiti vities are depe ndent on the particle size distribution of each release. For a 20-m cloud at a range of 1.26 km, the obtained sensitivities for BG and EH are around 1.7 kppl and less than 1 2 ppl, respectively . The sensitivities extracted for the other sim ulants take different values in between these two extremes with increasing sensitivities: dry BG, Ricin, wet BG, BA, OV, MS2, FT, YP and EH.

CONCLUSION: SIN BAHD suc cessfully acquired numerous si gnature measurements at t o the JBSDS trial. MA of SINBAHD data have demonstrated inte resting correlations with the reference point sensor with sensitivities ranging from 1.7 kppl and less than 12 ppl for the tested simulants.

[1] Lahaie, P., Simard, J.R. McFee J., Buteau S., Ho J., Math ieu P., Roy G., Larochelle V. *Spectral processing of laser-induced f luorescence f rom t hreatening bi ological aer osols*, International Jo urnal of Hi gh Speed Electronics and Systems, Vol. 18, no 2, pp. 429-423, 2008.

[2] Laflamme, C., Lavi gne, S ., Ho, J. and Duchaine, C ., *Assessement of bact erial e ndospore vi ability w ith fluorescent dyes*, Journal of Applied Microbiology, 96, pp. 684-692, 2004.