Forensic Science International 301 (2019) e55–e58

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Rapid Communication

Non-contact and non-destructive detection and identification of *Bacillus anthracis* inside paper envelopes

Shai Kendler[a,e,*], Ran Aharoni[a], Shay Cohen[a], Raviv Raich[c], Shay Weiss[d], Haim Levy[d], Ziv Mano[e], Barak Fishbain[e], Izhar Ron[b]

[a] Environmental Physics Department, Israel Institute for Biological Research, Israel
[b] Physical Chemistry Department, Israel Institute for Biological Research, Israel
[c] School of Electrical Engineering and Computer Science, Oregon State University, United States
[d] Infectious Diseases Department, Israel Institute for Biological Research, Israel
[e] Department of Environmental, Water and Agricultural Engineering Faculty of Civil & Environmental Engineering the Technion, Israeli Institute of Technology, Israel

ARTICLE INFO

Article history:
Received 4 December 2018
Received in revised form 9 April 2019
Accepted 2 May 2019
Available online 9 May 2019

Keywords:
Bacillus anthracis
Hyperspectral imaging (HSI)
Short-wave infrared (SWIR)
Algorithm
Bio-terror

ABSTRACT

Efficient and safe detection of *Bacillus anthracis* spores (BAS) is a challenging task especially in bio-terror scenarios where the agent is concealed. We provide a proof-of-concept for the identification of concealed BAS inside mail envelopes using short-wave infrared hyperspectral imaging (SWIR-HSI). The spores and two other benign materials are identified according to their typical absorption spectrum. The identification process is based on the removal of the envelope signal using a new automatic new algorithm. This method may serve as a fast screening tool prior to using classical bioanalytical techniques.

1. Introduction

Bacillus anthracis spores (BAS) were used in the past as a bio-terror weapon [1,2].While the reported number of bio-attacks is low, there is a flow of hoax using non-toxic white powders, concealed in envelopes. Whether it is a hoax or not, extreme precautions need to be taken, and only trained personnel in registered laboratories should handle these cases. Irenge and Gala reviewed several means and methods aimed to identify BAS in various bio-terror scenarios [3]. Keys et al. described high-throughput identification of microbes using mass spectral imaging (matrix-assisted laser desorption ionization mass-spectrometry, MALDI-MS) techniques [4]. Such fine bioanalytical techniques provide an accurate answer but at a cost of long analysis time and highly trained operators. There is a need for safe, non-contact, non-destructive automatic analysis of powders concealed within various containers, such as envelopes, plastic bags, for fast and efficient screening of suspected items before fine bioanalytical tests in certified labs. Thus, the need arises for an efficient and reliable detection technique for this type of substances.

One of the most promising tools is Hyper-Spectral imagers (HSI). To this end, several groups have proposed spectroscopic techniques, as a mean for detection of concealed explosives, drugs and other harmful materials. Johnson et al. developed a HSI that uses active broadband illuminator for the detection of concealed materials [5]. Kemp et al. provided a thorough description of the utilization of THz radiation for the identification of concealed materials [6]. Luggar et al. developed a statistical method to interpret X-ray diffraction data in order to detect concealed explosive materials [7] Despite these efforts and many others, to this end there is no single technique that can provide accurate identification of all possible scenarios in which a harmful material is concealed. Furthermore, none of these studies have addressed the difficult problem of detecting concealed bacteria. Concealment reduces the chances for detection as the target material's spectral signature is mixed with the signal of the background. A similar situation occurs in the food industry where the target material is mixed with other components of the food product. Nevertheless, some groups proved that this difficult situation may be resolved

* Corresponding author at: Environmental Physics Department, Israel Institute for Biological Research, Israel.
E-mail addresses: ikendler@gmail.com, shaik@iibr.gov.il (S. Kendler), tenzoran@gmail.com (R. Aharoni), shayco17@gmail.com (S. Cohen), raich@eecs.oregonstate.edu (R. Raich), shayw@iibr.gov.il (S. Weiss), haiml@iibr.gov.il (H. Levy), ziv10005@gmail.com (Z. Mano), fishbain@technion.ac.il (B. Fishbain), izharr@iibr.gov.il (I. Ron).

https://doi.org/10.1016/j.forsciint.2019.05.007
0379-0738/

using HSI in combination with chemometric techniques. For example, Siripatrawan et al. described the detection of *Escherichia coli* in fresh spinach using HSI in the visible spectral range [8]. Feng et al. described the detection of *Enterobacteriaceae* in chicken filet using HSI in the near infrared range [9]. These applications and many others concerning food safety are summarized by Feng and Sun [10]. These recent achievements show that HSI is an effective technique for the detection of bacteria in realistic environments in which the target material's spectral signature is mixed with the spectral signature of other materials surrounding it.

In this work, we present the detection of the *B. anthracis* (BA) bacteria inside mail envelopes using Short Wave Infra-Red Hyper Spectral Imager (SWIR-HSI). This development is a step forward from previous works in which HSI was used to identify various bacteria inside food products. To this end, this short communication presents results of a preliminary work, aimed to develop the capabilities to detect BAS inside sealed envelopes using HSI in the SWIR (1000–2500 nm). Since in this application the variety of envelopes is large and unknown in advance, the computational model, presented here, is automatic and does not rely on any prior knowledge regarding the envelope [11].

2. Materials and methods

2.1. SWIR-HSI instrumentation

A line-scanner type HSI (SWIR-CL-400-N25E from Specim) having a spectral range 1000–2500 nm, spectral sampling/resolution 5.6 nm/12 nm with a 56 mm F/2 lens was used. Typical exposure time and frame rate were 10 ms and 30 fps, respectively, with a 1500 W halogen lamp as a light source. Prior to each measurement, the HSI automatically measures the dark current signal and the recorded data is corrected using this measurement. The HSI is equipped with a 384×288 pixels mercury cadmium telluride (MCT) focal plane array (FPA). Light enters the sensor through a 30-μm slit and is dispersed by the spectrometer into 288 wavelengths ("channels"). Rotating the spectrometer n times during the measurement creates data cubes of $384 \times 288 \times n$ data points. Typical data cubes in our experiments were $384 \times 288 \times 500$ from which the area of interest was cropped for the analysis. Reference spectra were measured using a non-imaging spectrometer (FieldSpec4^{TM} from ASD). Sampling/resolution was 1.1 nm/10 nm within the range of $\lambda = 1000$–2500 nm. Light emitted from the lamp ($I_0(\lambda)$) crosses the sample and the envelope, and the spectrum from a specific pixel 'i' ($I_i(\lambda)$) is recorded by the HSI. The incident light is affected both by the envelope ($B_i(\lambda)$) and the sample ($T(\lambda)$). The effect of the envelope on the measured signal and the analysis algorithm are described in the next section. A scheme of the experimental setup is shown in Fig. 1.

2.2. Sample preparation

Samples (100 mg) of polystyrene were deposited on paper; similarly, a silicone-based glue was placed on paper. After curing, the samples were inserted into paper envelopes. These materials are used to test the suggested method and algorithm. A suspension of non-toxigenic BAS (Vollum ΔpXO1ΔpXO2) [12], was placed on a Teflon filter. After 72 hours at ~30 °C, the water evaporated leaving a deposit of 4×10^8 spores on the filter that was then packed in a transparent plastic bag and inserted into a paper envelope.

2.3. Safety considerations

It is important to note that BAS is a dangerous agent and appropriate safety measures should be taken during research and implementation of any analytical technique dealing with this agent. The experiment should be carried out in a secured and adapted environment.

2.4. Methodology

2.4.1. Signal characteristics

Manolakis and Shaw [13] described state-of-the-art algorithms for analyzing HSI data. The analysis is possible for cases where the signal arises purely from the target material and also for cases where the target material's signal is linearly mixed with the background. Such mixing occurs when the image of the target material covers only a portion of the pixel, e.g., – sub-pixel. Cases in which the light crosses both the target material and the background, result in non-linear mixing, sometimes referred to as "intimate mixing". Non-linear mixing situations have received less attention, and algorithms addressing such cases often rely on prior knowledge or assumptions regarding the scene [14]. The situation addressed in this work is one of intimate mixing since the light crosses both the envelope and the target material as described in Eq. (1).

$$I_i(\lambda) = I_0(\lambda) \times (B_i(\lambda) \times T(\lambda)) \quad (1)$$

Using Eq. (1), one can extract $T(\lambda)$, given $B_i(\lambda)$. Comparing $T(\lambda)$ to a known reference spectrum enables identification of the target material. While envelopes have some similarities in their major chemical components, differences in color, thickness, and other materials in the envelopes make it impractical to measure $B_i(\lambda)$, or even a set of $B_i(\lambda)$, in advance and use it later for analysis. This may be overcome by assuming that the sample is not

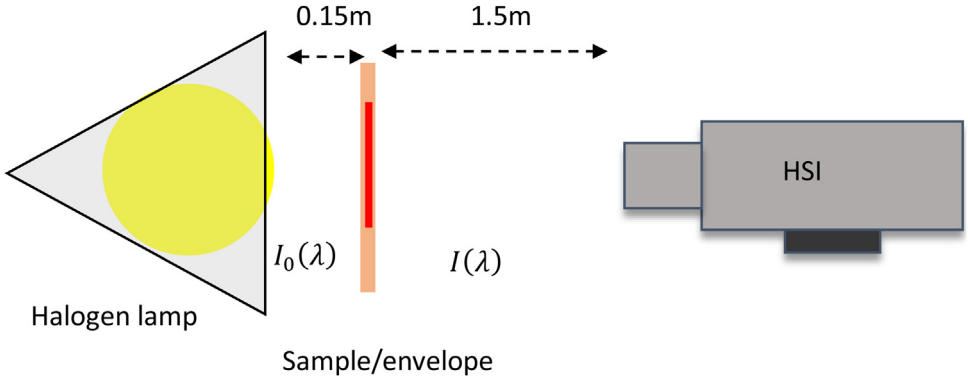

Fig. 1. A scheme of the experimental setup. See text for more details.

found in the entire scene, and some clean pixels may be located and used as a clean reference.

2.4.2. Background removal algorithm

Recently we described several algorithms that rely on this assumption [22]. For example the global automatic background removal — GABR that follows the following scheme: a data cube containing n*m pixels *l spectral channels, is analyzed in n*m cycles. In each cycle, the algorithm picks one of the pixels as a possible clean background and using Eq. (1) removes the background's contribution from the entire cube by division. At the second stage, the background-reduced cube (BRcube) is analyzed to locate the presence of the target material, by computing the Pearson-correlation between the obtained $T(\lambda)$ for each pixel in each one of the BRcubes and the reference spectra of the target materials that were recorded separately. The correlation is computed separately in two regions, SWIR1 = 1111–1388 nm and SWIR2 = 1658–2056 nm. A positive hit is recorded if the correlation in both SWIR1 and in SWIR2 crosses predetermined thresholds (ThSWIR1 and ThSWIR2). These threshold values are chosen by computing true positive and false alarm rates at different threshold values when a cube of a scene containing a known set of targets is analyzed. Using different thresholds for SWIR1 and SWIR2 is required since the signal-to-noise ratio in SWIR1 is about one order of magnitude higher than that in SWIR2. These thresholds are a product of a training phase in which true and false positive rates at different threshold values are calculated for a data cube of a scene containing a known set of targets. This process is repeated m*n times until all pixels are used as possible clean backgrounds. Computationally, this means that a single cube is analyzed m*n times. A variant of the GABR method is the focused Background Removal (FBR) which acts in a similar way, aside from the fact that only a portion of the sample, i.e., a predefined set of pixels, is used as a possible background. FBR requires predefinition of a portion of the sample as a possible background and results in a more accurate identification.

3. Results and discussion

Prior to the measurement of the concealed materials inside paper envelopes, the transmission spectra of the target materials were measured. As depicted in Fig. 2, every material has a typical spectral signature that, potentially, can be used for its identification. The basic requirement for a successful material identification using SWIR-HSI is having some light transmission through the sample and the envelope. Fig. 3a presents a color image of a paper envelope with a sample of BAS. The image was created using, for each pixel, three (1490, 2117 and 2280 nm) channels, out of the 288 channels. It shows that the envelope and the sample attenuate the light and only a portion of the light reaches the sensor. The sample inside the envelope contributes to the signal and can be, potentially, identified using its spectral signature. Fig. 3b shows the identification results of BAS (blue), polystyrene (red) and silicone glue (green) inside paper envelopes and on paper. It also shows a single false positive pixel for BA. Such accuracy in real-time (few seconds) detection of concealed materials is a very promising result. However, even a single false-positive alarm can have undesired consequences. Hence, our research, aimed at improving the data analysis algorithm, is ongoing. One possible direction is to use the FBR method and using the sample area as a possible clean background. Fig. 3c presents an example for such application of the FBR method. It shows that BAS may be identified accurately without any false positive alarms.

This set of results is a proof-of-concept regarding the possibility to identify concealed target materials. Fig. 3c shows that only a single pixel out of 24,000 pixels was falsely identified, while all three materials were correctly identified. Further development of this method as a forensic method requires careful estimation of false positive and false negative alarm rates at various scenarios. Admittedly, classical bioanalytical methods [3,4], such as MALDI-MS will outperform SWIR-HSI in terms of sensitivity and selectivity but lack the possibility for high throughput, non-destructive operation. Modern scanners based on X-ray diffraction or millimeter-wave imaging [15], may be fast but lack the specificity of SWIR-HSI. Scanners based on THz radiation may be specific as they rely on the absorption spectrum of the tested material, but a considerable effort in hardware development is still required [12]. Hence, HSI-SWIR may be an attractive option for fast screening purposes combining speed and specificity with mature hardware.

4. Conclusions

Identification of BAS concealed inside paper envelopes using SWIR-HSI is presented. The critical issue, in this case, is the ability to separate the spectral contribution of the target material from that of the envelope. This work describes the proof-of-concept for the data analysis procedure for automatically removing the clean envelope contribution. Non-contact, non-destructive fast analysis

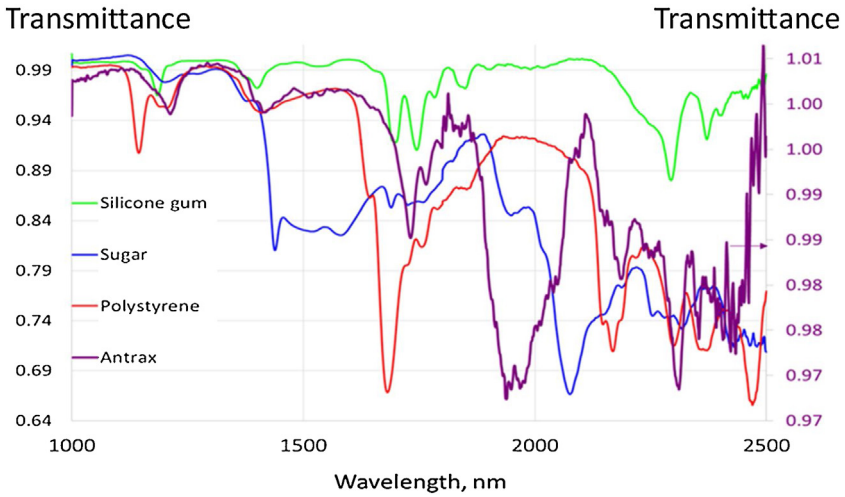

Fig. 2. Transmission spectra of the target materials measured without the envelope interference. These spectra are used as reference signatures for the identification algorithm. Note, the spectra of anthrax (purple trace) is related to the right 'Y' axis.

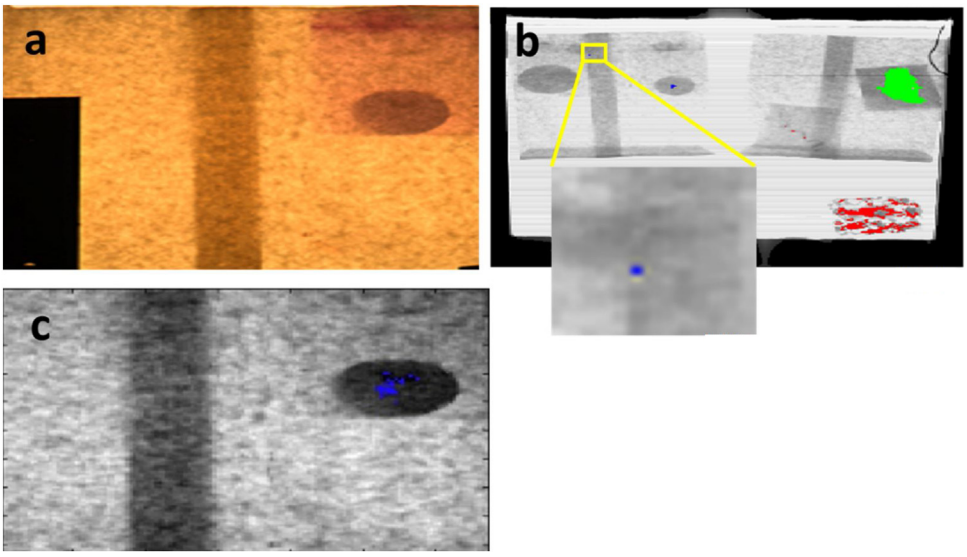

Fig. 3. Images produced by HSI in several cases. (a) a false-color image of an envelope containing BAS deposit on a filter, (b) analysis results using the GABR method for a scene containing polystyrene (red), silicone (green) and BAS (blue), the inset shows a single false positive hit for BA. (c) Analysis results of an envelope containing BAS (same as in (b)), using the FBR method taking the sample area as a possible background.

of chemical/biological agents inside envelopes is a highly complicated analytical challenge. System capabilities still have to be carefully tested and verified using a large set of measurements to account for possible variations that occur in realistic conditions. Such tests are part of our ongoing efforts in this direction. One of the most valuable outputs of these tests is the system's reliability, i.e., low false alarm and high true-positive rates in realistic conditions. Data analysis algorithm and measurement techniques need to be adjusted to suit operational requirements. Such mail-scanner can be a trigger for the operation of certified laboratories in relevant cases.

CRediT authorship contribution statement

Shai Kendler: Conceptualization, Formal analysis, Supervision, Writing - original draft, Writing - review & editing. **Ran Aharoni:** Writing - review & editing. **Shay Cohen:** Data curation, Project administration. **Raviv Raich:** Data curation, Writing - review & editing. **Shay Weiss:** Writing - original draft. **Haim Levy:** Writing - original draft. **Ziv Mano:** Data curation, Validation. **Barak Fishbain:** Data curation, Validation, Writing - original draft, Writing - review & editing. **Izhar Ron:** Writing - original draft, Writing - review & editing.

References

[1] P. Keim, K.L. Smith, C. Keys, H. Takahashi, T. Kurata, A. Kaufmann, Molecular investigation of the Aum Shinrikyo anthrax release in Kameido, Japan, J. Clin. Microbiol. 39 (2001) 4566–4567.
[2] D.B. Jernigan, P.L. Raghunathan, B.P. Bell, R. Brechner, E.A. Bresnitz, J.C. Butler, M. Cetron, M. Cohen, T. Doyle, M. Fischer, C. Greene, K.S. Griffith, J. Guarner, J.L. Hadler, J.A. Hayslett, R. Meyer, L.R. Petersen, M. Phillips, R. Pinner, T. Popovic, C. P. Quinn, J. Reefhuis, D. Reissman, N. Rosenstein, A. Schuchat, W.J. Shieh, L. Siegal, D.L. Swerdlow, F.C. Tenover, M. Traeger, J.W. Ward, I. Weisfuse, S. Wiersma, K. Yeskey, S. Zaki, D.A. Ashford, B.A. Perkins, S. Ostroff, J. Hughes, D. Fleming, J.P. Koplan, J.L. Gerberding, Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings, Emerg. Infect. Dis. 8 (2002) 1019–1028.
[3] L.M. Irenge, J.L. Gala, Rapid detection methods for *Bacillus anthracis* in environmental samples: a review, Appl. Microbiol. Biotechnol. 93 (2012) 1411–1422.
[4] C.J. Keys, D.J. Dare, H. Sutton, G. Wells, M. Lunt, T. McKenna, M. McDowall, H.N. Shah, Compilation of a MALDI-TOF mass spectral database for the rapid screening and characterization of bacteria implicated in human infectious diseases, Infect. Genet. Evol. 4 (2004) 221–242.
[5] B. Johnson, R. Joseph, M. Nischan, A. Newbury, J. Kerekes, H. Barclay, B. Willard, J. Zayhowski, A compact, active hyperspectral imaging system for the detection of concealed targets, Proc. SPIE 3710 (1999) 144–153.
[6] a M.C. Kemp, P.F. Taday, B.E. Cole, J.A. Cluff, A.J. Fitzgerald, W.R. Tribe, Security applications of terahertz technology, Proc. SPIE 507044 (2003) 44–52;
b C. Corsi, F. Sizov, THz and Security Applications Detectors, Sources and Associated Electronics for THz Applications Proceedings of the NATO Advanced Research Workshop on THz and Security Applications-Detectors, Sources and Associated Electronics for THz Applications, Kiev, Ukraine 26–29 May, 2013.
[7] R.D. Luggar, M.J. Farquharson, J.A. Horrocks, R.J. Lacey, Multivariate analysis of statistically poor EDXRD spectra for the detection of concealed explosives, X-Ray Spectrom. 27 (1998) 87–94.
[8] U. Siripatrawan, Y. Makino, Y. Kawagoe, S. Oshita, Rapid detection of *Escherichia coli* contamination in packaged fresh spinach using hyperspectral imaging, Talanta 85 (2011) 276–281.
[9] Y.Z. Feng, G. Elmasry, D.W. Sun, A.G.M. Scannell, D. Walsh, N. Morcy, Near-infrared hyperspectral imaging and partial least squares regression for rapid and reagentless determination of Enterobacteriaceae on chicken fillets, Food Chem. 138 (2013) 1829–1836.
[10] Y.Z. Feng, D.W. Sun, Application of hyperspectral imaging in food safety inspection and control: a review, Crit. Rev. Food Sci. Nutr. 52 (2012) 1039–1058.
[11] S. Kendler, I. Ron, S. Cohen, R. Raich, Z. Mano, B. Fishbain, Detection and identification of sub-millimeter films of organic compounds on environmental surfaces using short-wave infrared hyperspectral imaging: algorithm development using a synthetic set of targets, IEEE Sens. J. 19 (2019) 2657–2664.
[12] S. Weiss, H. Levy, M. Fisher, D. Kobiler, Z. Altboum, Involvement of TLR2 in innate response to *Bacillus anthracis* infection, Innate Immun. 15 (2009) 43–51.
[13] D. Manolakis, G. Shaw, Detection algorithms for hyperspectral imaging applications, IEEE Signal Process. Mag. 19 (2002) 29–43.
[14] J.M. Bioucas-Dias, A. Plaza, N. Dobigeon, M. Parente, Q. Du, P. Gader, J. Chanussot, Hyperspectral unmixing overview: geometrical, statistical, and sparse regression-based approaches, IEEE J. Sel. Top. Appl. Earth Obs. Remote Sens. 5 (2012) 354–379.
[15] D.M. Sheen, D.L. McMakin, T.E. Hall, Three-dimensional millimeter-wave imaging for concealed weapon detection, IEEE Trans. Microw. Theory Tech. 49 (2001) 1581–1592.