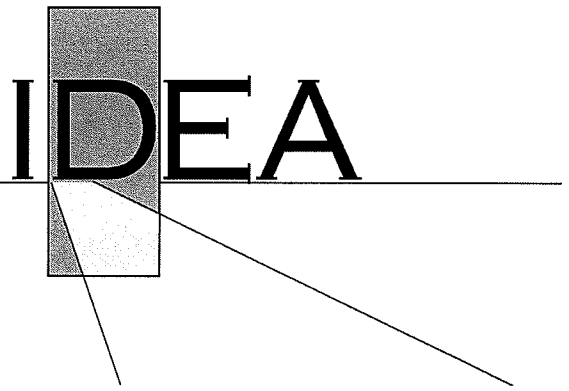

Innovations Deserving
Exploratory Analysis Programs

Transit IDEA Program

Innovative Bioterrorism Detection Technology for Transit Security

Final Report for Transit IDEA Project 35

Prepared by:
Douglas B. Rivers, Ph.D.
Science Applications International Corporation
Huntsville, AL

August 2005

TRANSPORTATION RESEARCH BOARD
OF THE NATIONAL ACADEMIES

Innovations Deserving Exploratory Analysis (IDEA) Programs Managed by the Transportation Research Board

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The Transit IDEA Program is one of four IDEA programs managed by TRB. The other IDEA programs are listed below.

- NCHRP Highway IDEA Program, which focuses on advances in the design, construction, safety, and maintenance of highway systems, is part of the National Cooperative Highway Research Program.
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IDEA Programs
Transportation Research Board
500 Fifth Street, NW
Washington, DC 20001

INNOVATIVE BIOTERRORISM DETECTION TECHNOLOGY FOR TRANSIT SECURITY

Final Report

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Prepared for

Transit IDEA Program

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EXECUTIVE SUMMARY

This Transit IDEA project investigated the detection and identification of potential biological warfare agents in both a benign laboratory environment and in a simulated subway environment, using environmental samples from a typical subway station. A potential terrorist threat could involve the possible release of biological agents in a transit environment. For this project, the transit system considered is an urban subway system. New York City Transit (NYCT) participated in the project by assisting investigators in identifying a sample site and in collecting the environmental samples of particulate matter from a typical subway station. NYCT also participated by reviewing and commenting on the technical progress of the project work. The technology used in this project successfully detected certain biological species and differentiated them from the background environmental sample from a NYCT subway station

The detection technology identified by Science Applications International Corporation (SAIC) is based on a proprietary laser spectroscopy system. This system uses advanced optics to assist in spectral data processing rather than relying on electronics to match patterns after the signal is spread in the frequency domain. This technology allows the use of more frequency bands to detail the resulting spectral profiles. With this system, the potential for a highly accurate, real-time detection device becomes a reality. This technique can be used for the analysis of organic substances found in composite surfaces or bulk material. The target material is bombarded with monochromatic radiation that results in a phenomenon known as Raman scattering. In a typical Raman[1] system[2] tens of bands are possible, but with the advanced optical processing system used in FastMetrix technology, 300 bands have already been demonstrated and more are believed possible. Increasing the number of bands can increase range and probability of detection, and lower power requirements, while improving the probability of false alarm and background rejection.

Conventional Raman spectroscopy technology, while being able to provide faster analysis times, has had on-going problems with material identification, due to the required number of spectral lines needed for signal pattern matching and noise rejection. Additionally, numerous photo-detectors are commonly required to analyze the entire spectrum of the signal. The additive effects of the background "noise" inherent to each detector within the system severely limit the sensitivity that can be achieved. As a result of this deficiency, conventional Raman spectroscopy must use signal averaging to increase the signal-to-noise ratio (SNR) to a level where a biological material might be recognized from the background. Previous attempts to use this common type of technology as part of its configuration have not been successful. As a consequence, the research team investigated the use of adjunct polymerase chain reaction (PCR) techniques to supplement the system.

During Stage 1 of the two-stage project, SAIC encountered and solved a number of unexpected technical challenges in addition to those of generating initial spectral signals specific to bacterial endospores. The pre-testing challenges, which resulted in more time required to carry out Stage 1, focused on the changes in regulations surrounding the use of biological select agents and obtaining the required gamma-killed sample of anthrax spores. The second set of challenges was presented by the testing itself. After considerable effort, it was determined that bacterial spores are probably the most difficult case we could have chosen as a proof-of-concept case. They have little in the way of observable surface characteristics, thus we made adjustments to our development plan to use additional methods to further amplify the Raman signals. Using Surface-Enhanced Raman spectroscopy, we were able to achieve the desired goal of showing that Raman spectra for anthrax and a closely related *Bacillus* species, were noticeably different. The spectra were then further enhanced by using an etching process to expose more spore surface characteristics.

During Stage 2 of this project, SAIC developed spectra for two additional closely related *Bacillus* species, collected background samples from a New York City Transit subway station, developed the spectra for the background environmental air particle samples to serve as the negative filter component,

differentiated the spectra of anthrax spores from the spores of the three closely related *Bacillus* species, and generated initial correlation data. The conclusion of this project work is that the technology developed in this project can successfully detect and identify spores of *Bacillus anthracis* and they can be differentiated from the spores of three closely related species (*Bacillus globigii, Bacillus thuringiensis, Bacillus cereus*) and the background environmental air particle sample of a NYCT subway station. Based upon the data and conclusions in this project, this technology warrants further development of a prototype for testing in a controlled transit environment first, and then in an operating transit environment.

1. IDEA PRODUCT, CONCEPT, AND INNOVATION

This report describes the product, concept and innovation developed in this Transit IDEA project. Conclusions, recommendations and a discussion of the investigation are also included.

1.1. IDEA PRODUCT

The objective of the overall project was to demonstrate that biological agents can be detected and characterized in a transit environment. This project was carried out in two stages. Specific objectives of Stage 1 of this project were to identify challenges faced by current biological agent detection systems, recommend an approach to meet these challenges in a transit environment, and integrate the proprietary advanced laser technology into a demonstration system to prove the principle of biological agent detection and characterization in a laboratory environment.

The critical issues are sensitivity of the spectral correlator technique (see description below) and its ability to discriminate between different biological compounds in a real-world environment and to eliminate "cross-talk" and interference generated by other chemicals. This performance is, in turn, very closely related to the design of the mask (spatial filter) in the spectral correlator. The design of this mask requires detailed knowledge of the Raman spectra of the chemicals of interest. We will measure the spectra of any additional chemical samples for which spectra are required and not available. The effort that was carried out in this project had several technological objectives and hardware development objectives.

The technological objectives that were addressed in this project were:

1. To experimentally verify that a spectral correlator can discriminate between the Raman spectra generated by anthrax agents and simulants
2. To experimentally verify that a spectral correlator is more efficient in collecting and detecting the Raman spectra than simple narrow band filtering
3. To estimate signal to noise figures

The hardware development objectives that were addressed in this project were:

1. To modify existing laboratory hardware to collect signatures from Biological agents
2. To put together a benchtop correlator and use it in Stage 2 development and to provide the ability to differentiate anthrax spores from the environmental air particle sample and three closely related spores.

The ultimate working product that may be realized is an optical/ chemical hybrid system for detecting anthrax in a public transportation system. Capabilities developed during this effort include developing a method to expose the Raman spectra of anthrax and three taxonomically closely related species onto photographic film. This film can be placed into an optical detection system to yield nearly instantaneous results. Film used for this endeavor would be exposed prior to instrument operation removing some of the extensive time is takes to capture these spectra onto the film media. Ultimately, the spectra captured on film will be translated to digital filters for a fielded system that is expected to provide capacity to detect and identify multiple targets. Recording the Raman spectrum of any biological agent optically is a fairly new and rapidly developing area of research. The largest challenge for transportation or any practical working system is to create enough signal amplification to justify the expense of obtaining the system. Independently, SAIC has developed a technology to capture and concentrate agents from the air and present them for analysis. Together, these two technologies provide a viable working system that could be tied through data systems into existing or planned transit security systems.

1.2. CONCEPT AND INNOVATION: ADVANCED LASER SPECTROSCOPY BACKGROUND

The advanced laser spectroscopy system being tested in this development program was originally invented at FastMetrix, Inc. by Mr. Gary Kamerman and is the subject of pending patents. The system is unique in that it focuses on optical signal processing to solve technical problems of conventional Raman spectroscopy. The results are increased sensitivity, signal to noise ratio (SNR), and background rejection. It also offers reduced thermal noise and device complexity. FastMetrix, Inc. was a subcontractor to SAIC on this project.

Review of Spectral Correlator Concept

During Stage 1 of this project, the performance and design requirements of a Raman Light Detection and Ranging (LIDAR) with a spectral correlation receiver were examined to detect biological agents in near real time. LIDAR consists of a transmitter (a laser emitting monochromatic ultraviolet, visible or infrared light), a receiver (such as a spectrometer) that is coupled to a detector that provides a mechanism to recognize target materials. Raman signatures are obtained by illuminating the sample with monochromatic light. This light stimulates the sample that radiates a spectrum of colors with a wavelength slightly longer than the wavelength of the transmitted laser. The wavelength offset of the radiated colors (i.e., the Raman spectrum) is a characteristic of the molecular structure of the sample of interest.

The spectral correlator uses optical processing to detect the target (biological agent) of interest from the Raman spectrum. This method is more sensitive than conventional Raman receiver techniques since it permits faster signal processing, and a finer range resolution of the Raman data. Correlation is a measure of similarity between two functions. The similarity of the received Raman spectrum with the Raman spectrum of a known sample is a measure of the concentration of that species. In this technique, the correlation calculation is performed directly on the received light before it is detected by a photodiode (i.e., an analog calculation in the optical domain). The photosensitive element in the receiver no longer detects the spectra of the incoming light. Instead, it detects the result of the calculation that compares the incoming light to the predetermined reference spectrum.

In a conventional Raman receiver, the weakest line that is computationally significant in the Raman spectrum determines the detection limit. Furthermore, since all lines from the Raman spectrum from a single sample are focused onto a single detector, the amplitude of the light to be detected is larger than it would have been even for the strongest line in the Raman spectrum by itself. These two effects can increase the detection sensitivity by 1000 or more. Ideally, the mask or filter used to identify any particular sample is orthogonal to the filters used to recognize any other biological species. This is non-trivial as the spectra of many important chemicals are similar (i.e., they are non-orthogonal functions).

Problem and Background

The need to detect biological weapons to protect public transportation venues has been well recognized. Emergency response units have very similar requirements when dealing with accidents involving hazardous materials. Recent events have illustrated the need to detect chemical or biological weapons in a civilian environment by conventional law enforcement and civil defense. The problem of biological agent monitoring is massive and critical.

The most common techniques for remote detection of airborne toxins are Differential Absorption LIDAR (DIAL), Infrared Fourier Transform Spectroscopy (IR FTS), and Raman LIDAR. Incoherent aerosol backscatter LIDAR has been successfully tested recently to track the dispersal pattern of chemical or biological agents,[3] but this system lacks any analytical capability to identify or quantify the threat. Each system has its own advantages and disadvantages and no single system currently satisfies all requirements. The choice of measurement system is site and application specific.

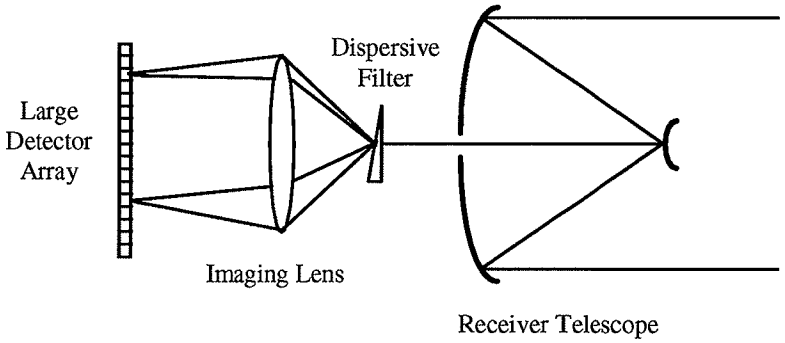
Raman LIDAR

Raman LIDAR is promising due to an inherent capability for positive identification of trace samples, insensitivity to atmospheric attenuation, and the ability to map concentrations in three dimensions by virtue of range gating. However, low signal cross-sections of Raman scattering, and trace sample concentration make effective field operations of the technique prohibitive.

By 1972, advances in technology allowed success in making field observations of Raman scattering by part per million (ppm)-level pollutants in stack plumes by several investigators.[4,5] However, later research into remote Raman detection concluded that small cross sections made application of Raman LIDAR techniques doubtful for Chemical and Biological (CB) warfare agent detection.[6,7] Although the Raman cross section has only been carefully measured for a few CB agents and simulants, detailed studies of the ultraviolet (UV) absorption of additional agents and simulants suggests that the Raman cross section of these agents is similar.[8]

Since all sample materials are exposed to the transmitted light simultaneously, all re-radiate their characteristic Raman spectra simultaneously. Thus, multiple chemical species can be detected in parallel.[9] The disadvantage of Raman LIDAR is that, historically, it has been less sensitive than Differential Absorption LIDAR (DIAL). This lower sensitivity is partially the result of receiver architectures that have been used in previous Raman LIDARs.

Figure 1. Conventional Raman LIDAR Architecture



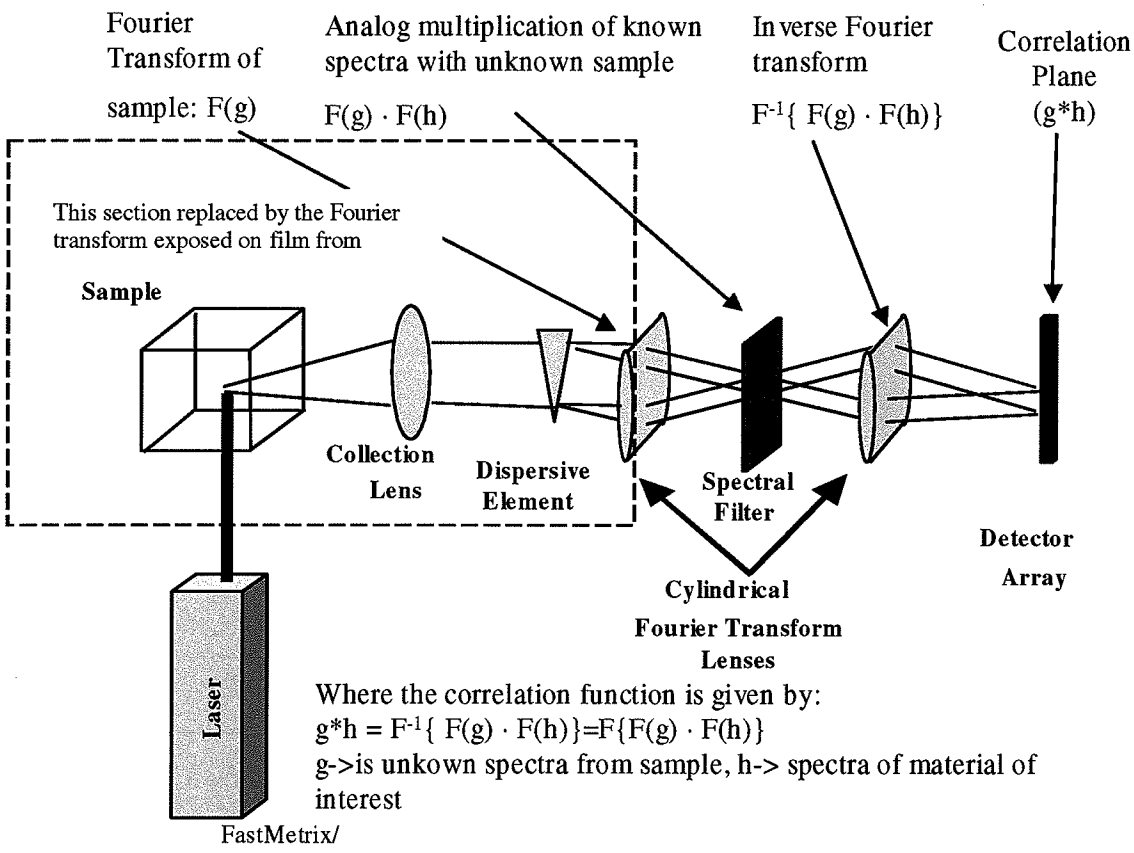
One implementation of a conventional Raman LIDAR receiver is shown in Figure 1. Received light is separated into its component colors by a dispersive filter (e.g., a grating or a prism). The separated light is then imaged onto a linear detector array. The spectral resolution of the linear array is determined by the grating, the f-number of the imaging lens and the size of the detector. The spectral range of the linear array is simply the resolution of a single detector multiplied by the number of elements in the array. The very close spacing of some Raman lines often dictates high spectral resolution. The wide spectral separation of other lines simultaneously requires a large spectral range. Satisfying both requirements necessitates a large number of detectors. Furthermore, the sensitivity of the system is defined by the ability to detect the weakest (critical) line in the Raman spectra.

Spectral Correlator Concept

The spectral correlator compares an unknown, received spectrum with a known, reference signal by optically calculating the correlation of the two spectra. Many conventional Raman LIDARs use this signal processing approach, but implement it with digital electronics after the components of the Raman spectra have been detected individually. However, in this technique, the calculation is performed directly on the received light before it is detected by a photodiode as an analog calculation in the optical domain. This calculation is acquired by inserting a specialized filter into the correlator optical train. The photosensitive element in the receiver detects the result of the calculation, which compares the incoming light to the stored reference.

The spectral correlator technical approach makes use of two properties from Fourier analysis. First, the Fourier transform is its own inverse. Second, the correlation of two functions is equal to the inverse Fourier transform of the product of the Fourier transforms of the two functions, i.e.,

received spectra with the Fourier transform of the known spectra. The optical product is then focused onto a detector by a second imaging lens. This lens performs an inverse Fourier transform on the product, resulting in a correlation function. Visually, a positive detection looks like a bright spot in the image plane where the amplitude is indicated by the intensity on the detector. A single detector may be used to detect the brightness of this spot, and no additional processing is required. The basic concept of the proposed effort is illustrated in Figure 4 below.



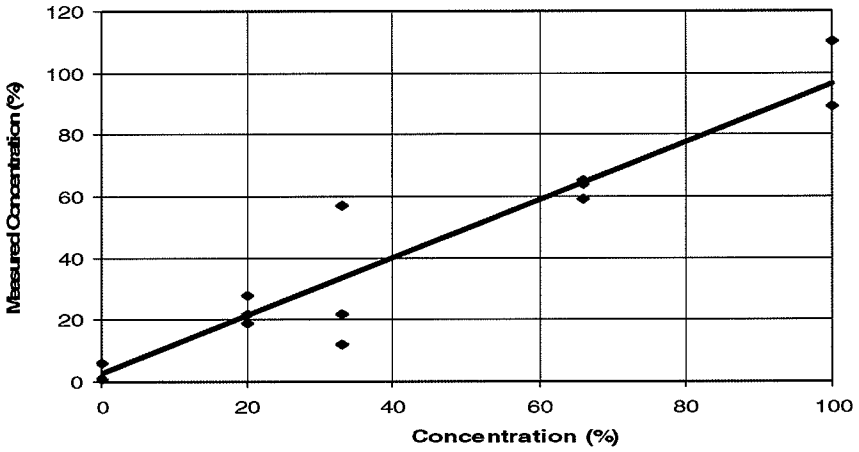
FastMetrix/

Figure 4. Spectral Correlator Approach

One filter is required for each simultaneous spectral correlation calculated. Only one detector is required for each simultaneous substance to be detected. One filter and detector should be reserved for nitrogen. The concentration of nitrogen in the atmosphere is fairly constant, and the amplitude of the Raman spectra from nitrogen as a function of time (and therefore, range) is a good measure of the atmospheric attenuation. It serves as a reference to evaluated concentration measurements.

Based on previous measurements acquired by FastMetrix, we can extrapolate a fairly linear response as a function of concentration. As the concentration (and therefore the immediate threat) decreases so will the probability that the sensor will trigger an alarm. Figure 5 demonstrates the detection probability defined by chemical concentration only. The lower detection limit will be a function of the particular BWA permissible exposure level (PEL). This can be preset with a detector-induced threshold. If the Raman LIDAR emits a pulse of laser light, the variation in the intensity of light reaching the detector over time is a direct measure of the target's concentration as a function of range. A solid state, 200mW ND:YAG laser provides the radiation required. Design trade offs will determine which harmonic is ultimately chosen; currently a visible frequency doubled ND:YAG is used.

Figure 5. Correlation of Concentration



The spectral correlator approach uses two Fourier analysis properties. First, the Fourier transform is its own inverse. Second, the correlation of two functions is equal to the inverse Fourier transform of the *product* of the Fourier transforms of the two functions:

$$g * h = \mathcal{F}^{-1}\{\mathcal{F}(g) \cdot \mathcal{F}(h)\}$$

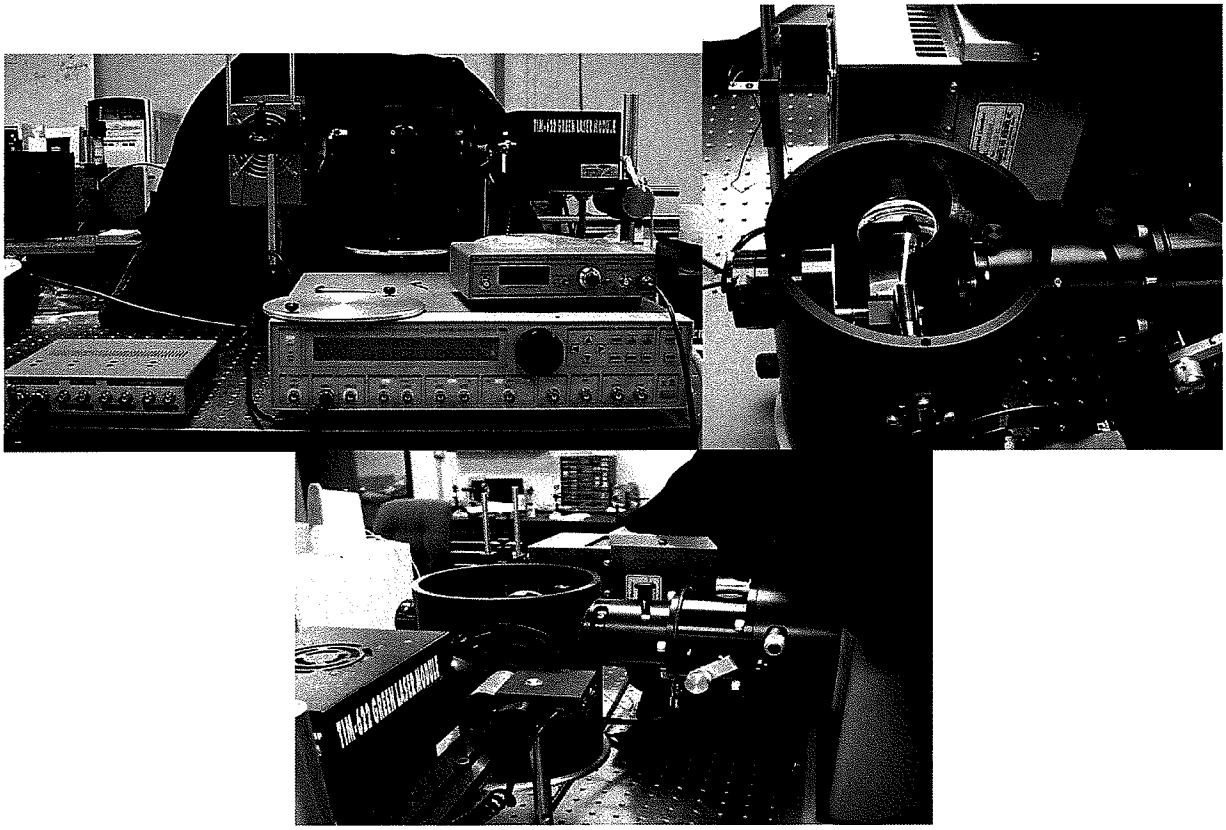
where $\mathcal{F}$ is the Fourier transform, $\mathcal{F}^{-1}$ is the inverse Fourier transform, and g and h are arbitrary functions. The multiplication of the known spectrum and sample spectrum occurs at the spectral filter plane. The filter operates to transmit spectra matching Raman characteristics. Spectral detection of Ba or any Biological Warfare Agent requires the recognition of the unique elements of one spectrum from all others.

1.3. EXPERIMENTAL APPROACH TO THE PROBLEM

Approach and Methods

Initially the approach was to use a traditional Raman architecture in order to acquire distinguishable spectra appropriate for creating correlation filters. To accomplish this task for the low signal levels expected, we used a Photon counting setup with a PMT detector and a Raman sample chamber. The sample chamber is designed to collect scattering at 90^0 and 180^0 with a 90^0 illumination angle. The incoming beam is focused at the sample and re-imaged onto the input slit of a commercial f/4.7 research-grade monochromator. The monochromator employs a diffraction grating to divide the incoming signal into individual wavelengths comprising the Raman spectrum. The equipment used for these measurements is shown in the images below.

FIGURE 6. Initial configuration with 90^0 illumination angle



1.3.1. Equipment Setup

Equipment setup was conducted by FastMetrix and is described and shown above.

1.3.2. Test Procedures

Preparation of challenge spores. Cultures of *Bacillus thuringiensis*, *Bacillus subtilis var. globigii*, and *Bacillus cereus* were obtained from the American Type Culture Collection, Rockville, MD. They were then grown in nutrient broth using an incubator shaker. Following growth, the resulting spores were centrifuged and washed three times in deionized water. The resulting pellets of spores were used for testing the three individual spore types and compared to gamma-killed anthrax spores.

Preparation of anthrax spores. *Bacillus anthracis* var. Ames spores, were obtained from the Armed Forces Institute of Pathology (AFIP), Washington, DC. The spores were produced by growth on an enriched medium followed by centrifugation and washing. The washed cells were then exposed to gamma irradiation to render them non-viable. The spores were certified killed by the U.S. Army Research Institute of Infectious Diseases (USAMRIID) on behalf of the Armed Forces Institute of Pathology (AFIP).

Test Matrix

The original text matrix for the complete program is shown below.

Test	Replicates	Purpose	Comments
1-Setup	2	Background	Empty well-slides
2-Setup*	3	Background	Millipore material
3-Target	2	Target Spectra	Concentration 1
4-Target	2	Target Spectra	Concentration 2
5-Interferent*	2	Environmental Background	Sample 1
6-Interferent*	2	Environmental Background	Sample 2
7-Mixed 1*	2	Sample 1	Concentration 1
8-Mixed 2*	2	Sample 2	Concentration 2
9-Blanks	2	Blank Samples	Blank samples for double-blind analysis
Two of the following three:			
10-*B. cereus* vs. *B. anthracis*	2	Interferent Separation	Morphologically similar to target
11-*B. thuringiensis* vs. *B. anthracis*		Interferent Separation	Morphologically similar to target
12- *B. subtilis var.globigii* vs. *B. anthracis*	2	Interferent Separation	Morphologically similar to target
Total test runs:	23		* Stage 2 testing

Sample a NYCT Subway Station

A subway station was selected based upon the desire to obtain as complex a sample as possible over a 24-hour period. To do so, NYCT personnel, led by Dr. Chuck Burrus, recommended the 72nd Street NYCT Station at Central Park West. This is an older, mid-level station with an entry and surface venting at Central Park. As a result, we expect to get plant/animal material in the sample mix, in addition to normal human and train traffic. The samples were taken using a lab vacuum pump to pull environmental air through 0.2μm filters. Samples were collected at 6, 18, and 24 hours. A control was taken at 0 time, and a long-term composite sample swab was also taken. All samples were subjected to etching with dodecylamine in ethanol and deposition on a SERS surface prior to spectral analysis.

2.0 INVESTIGATION

2.1. Stage 1 Results

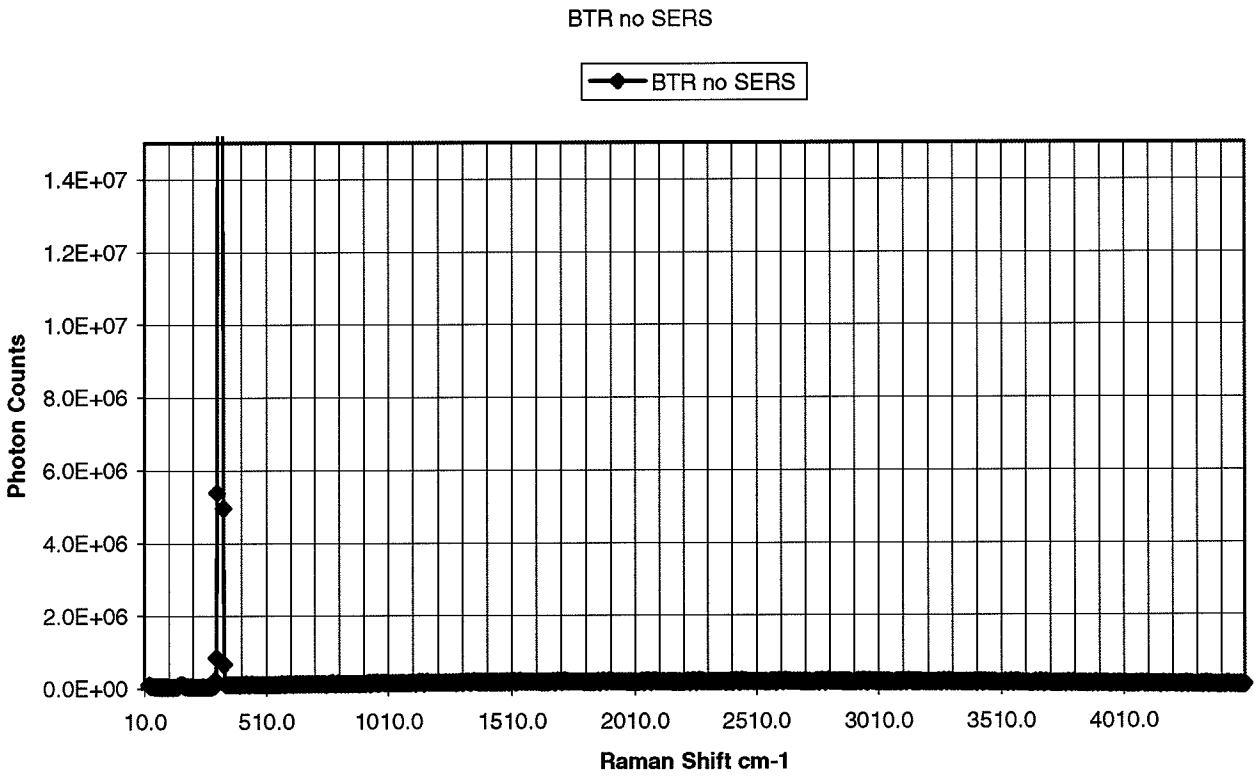
Literature Review

The Literature Review was submitted under separate cover in November 2003. The Review concluded that there were no detection systems currently available that can meet the needs of providing a highly accurate and near real time detection capability of biological agents in a transit environment.

Spectral Detection

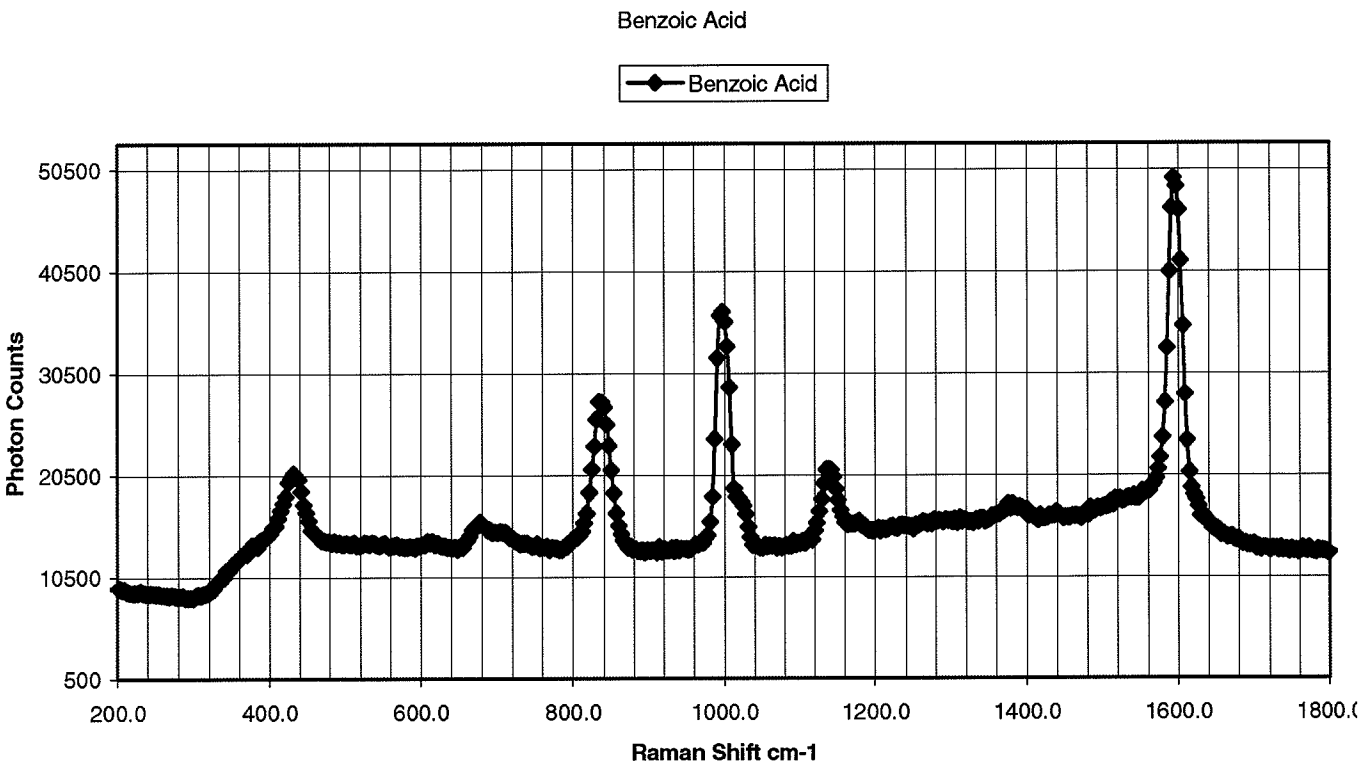
With the set up shown in Figure 6, all three of the anthrax simulants were measured. These include *Bacillus subtilis* var. niger (Bs), *Bacillus thuringiensis* (Bt), and *Bacillus cereus* (Bc). Each sample was integrated over a 24-hour period from 0 to 4000 wave numbers and illuminated with a frequency doubled YAG laser centered at 532nm. The output power of this laser was 50mW. The PMT detector signal was amplified prior to collection by the photon counter. However, no discriminating features were observable in any of the data. A characteristic spectrum from data collected in the traditional Raman configuration is shown in Figure 7.

FIGURE 7. Spectrum of *Bacillus thuringiensis* collected with traditional Raman methods using a photon counting setup



During the course of this work, we discovered that to detect low-level signatures typical for biological agents a SERS (Surface Enhanced Raman Scattering) approach was superior for these measurements. The SERS substrates selected for this effort have a thin layer of silver that provides a rough surface resulting in a signature increase of 10^7 typically. Prior to acquiring the enhanced substrates, no discriminating spectral features were evident as shown in Figure 7 above. Once the SERS substrates were received a control spectrum was collected using a sample provided by the substrate manufacturer. The sample was benzoic acid and was integrated for 22 seconds at each wavelength. This resulted in a change of scan time from 24 hours to approximately 8 hours. The control spectrum is shown below in Figure 8. Additionally a 200mW laser was purchased to increase the input signal level. It is considered a Class 3b laser.

Figure 8. Benzoic acid spectrum with SERS Substrate



An issue created by changing the sample media was the SERS substrates purchased for this project performs best when 180^0 backscatter is collected. This requires that the collector and emitter be co-located. To compensate for this requirement, the focusing lens was moved to the rear port of the sample chamber in place of one of the mirrors. This provided scattering through both walls of the SERS vial. Although transmission was still not optimized, it was an improvement over the 90^0 sample configuration shown in the pictures above. The modified setup is shown in Figure 9 below.

Figure 9. New Setup to accommodate SERS substrates

Figure 10. SERS vial

Data collected from theses samples show a clear improvement over the initial collections. These data are particularly important since the construction and fabrication of the correlation filters depends on collecting a clear target spectrum and clear interferant spectra. Once this process has been completed the gain of the spectral correlator can be realized. Once the filter is in place the detection time can be reduced from 22 seconds per wavelength to near real time. Only the gamma killed anthrax (Ba) and thuringiensis (Bt) were scheduled for SERS measurements in Stage 1.

As mentioned previously, Bt and Ba were selected for measurements in the SERS vials. From these two spectra it is evident there are discriminating features between *Bacillus anthracis* and a common simulant *Bacillus thuringiensis.* The first two spectra shown were taken from the spore samples (Figures 11 and 12). In the second two figures the samples were partially digested, or etched, to remove some of the spore surface (Figures 13 and 14). Etching was achieved by using detergent and hot ethanol for 1 minute. This allowed an enhanced look at the material composition of the spores. The etched samples provided a more accurate spectrum; however, the data collected without etching does show some distinguishable differences in the 3000 to 3700 wave number region. The results appear to be consistent with data collected by the Army Research Laboratory published in 2003[12]. This reinforces the theory that an optical mask could discriminate between the two spectra. In Stage 2, these spectra will be used as the basis to create optical masks.

Figure 11. SERS Spectrum for *Bacillus thuringiensis* spores in distilled water

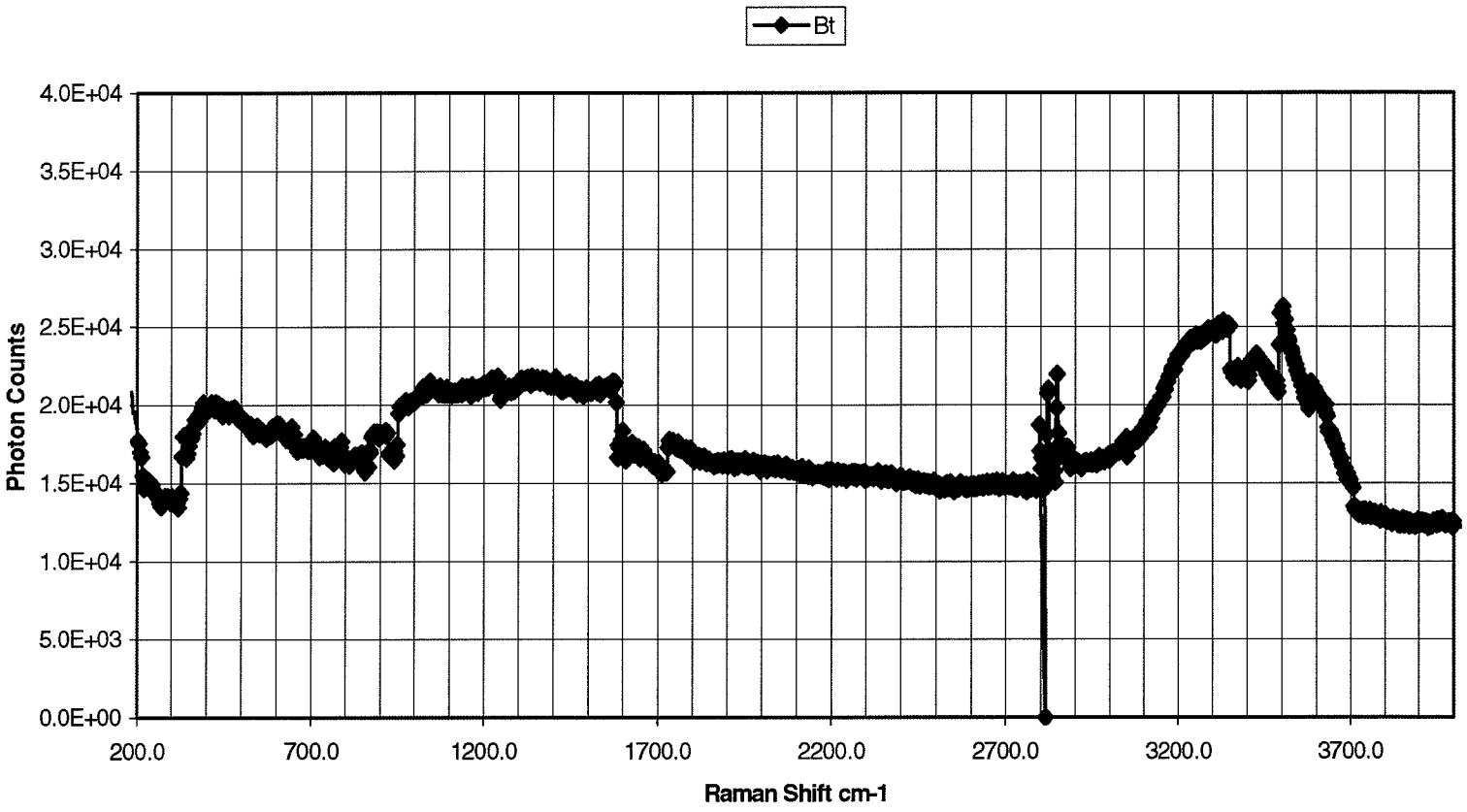


Figure 12. SERS Spectrum for *Bacillus anthracis* spores in distilled water

Figure 13. SERS Spectrum for *Bacillus thuringiensis* spores etched with Dodecylamine and Ethanol

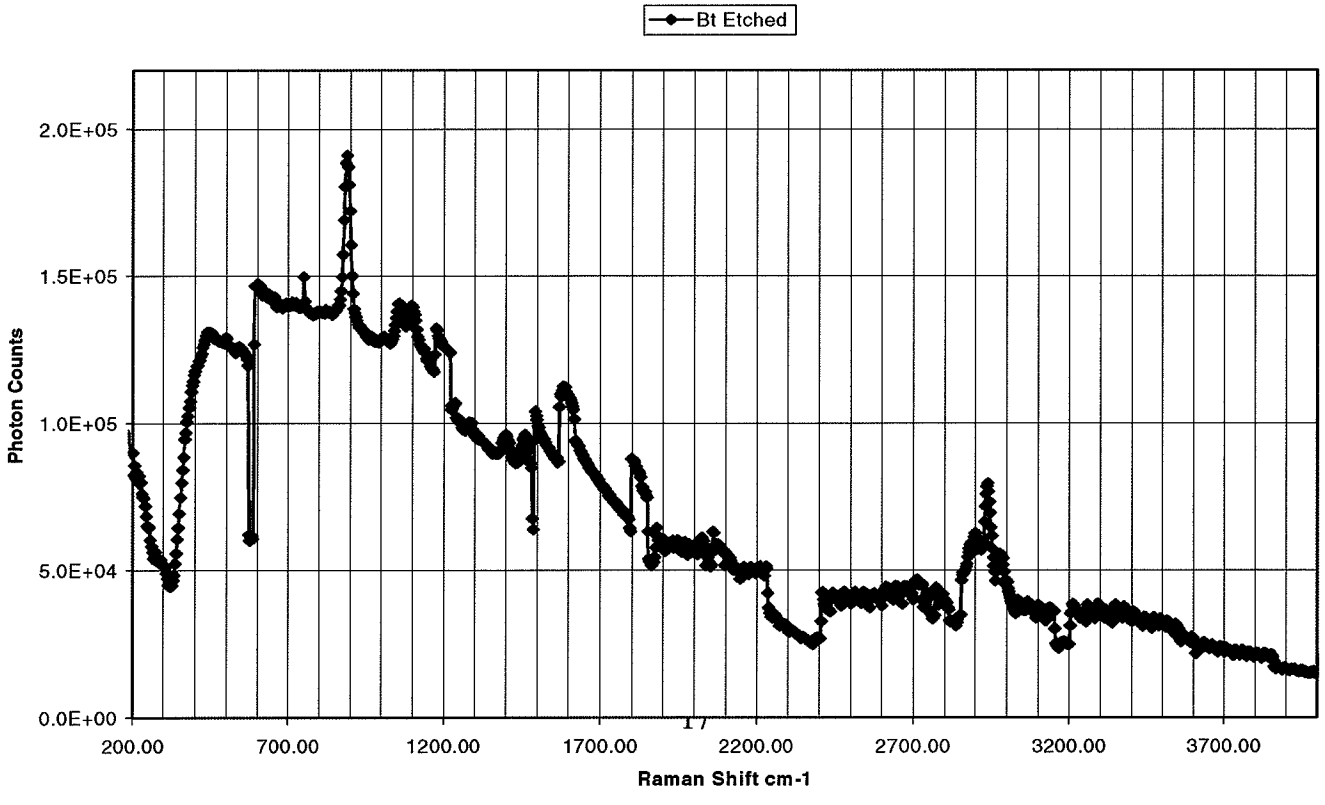
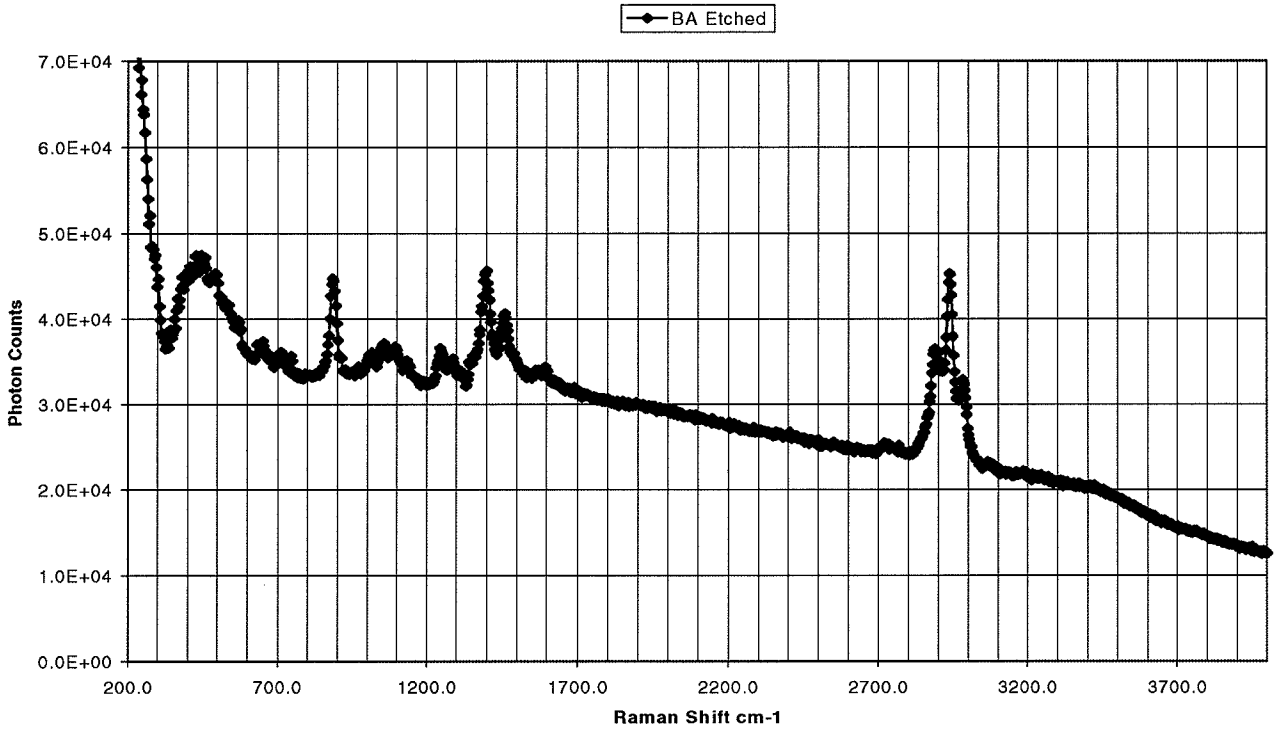


Figure 14. SERS Spectrum for *Bacillus anthracis* spores etched with Dodecylamine and Ethanol



2.2 Stage 2 Results

In Stage 2, SAIC built from what was learned in Stage 1. We proceeded to complete the spectra for two additional species that are considered closely related to *Bacillus anthracis*. The spectra for *Bacillus globigii*, the most commonly used simulant for anthrax, and *Bacillus cereus* were completed using the same methods as developed in Stage 1 for untreated in distilled water and after etching spores in a solution of dodecylamine in ethanol followed by application to a SERS surface for spectral analysis. The spectra for *Bacillus globigii* and *Bacillus cereus* are shown below in Figures 15 to18 below. The spectral comparisons show that untreated spores continued to show no discernable spectrum and that treatment by etching with dodecylamine in ethanol does result in a discernable spectrum for each of the two additional species tested in Stage 2. A comparison also shows that the spectra of these two species are distinct from either *Bacillus anthracis* or *Bacillus thuringiensis* spores tested earlier in Stage 1.

Figure 15. Spectrum for *Bacillus globigii* spores in distilled water

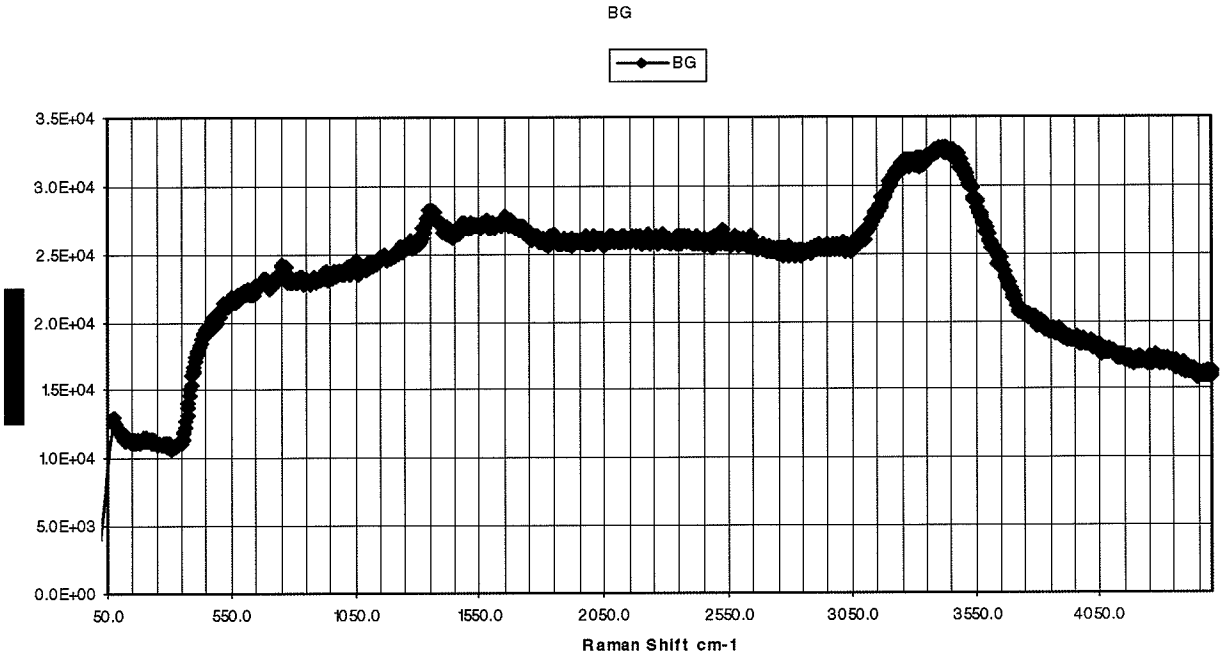


Figure 16. SERS Spectrum for *Bacillus globigii* spores etched with Dodecylamine and Ethanol

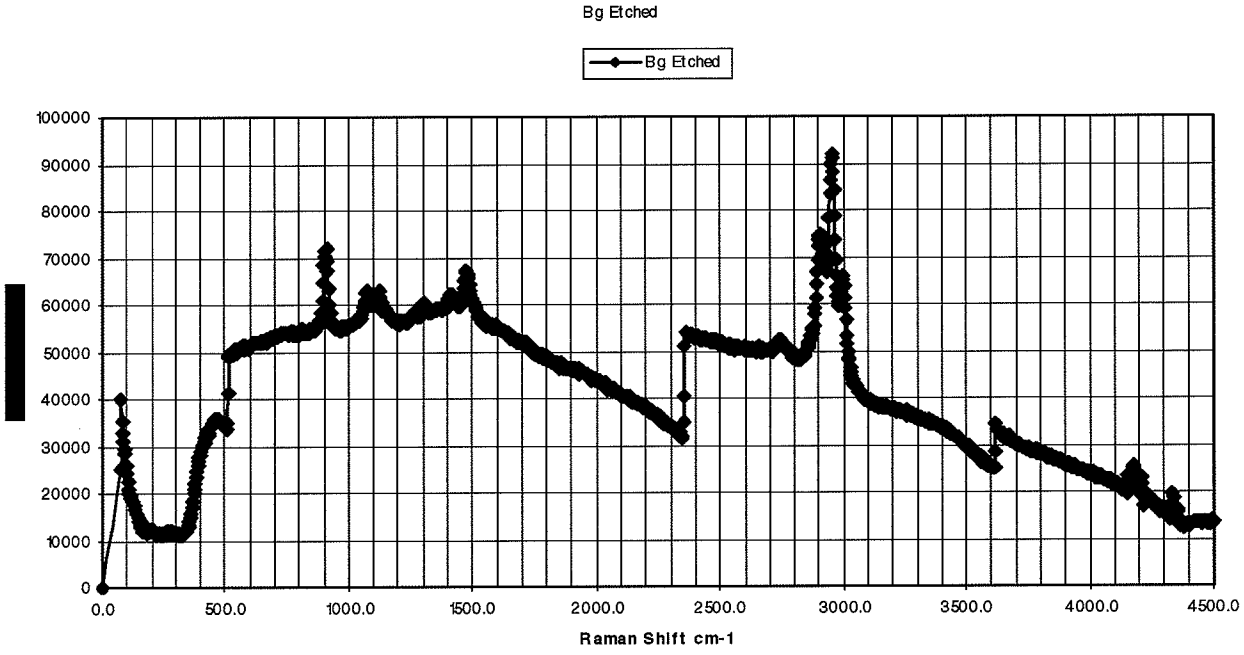


Figure 17. Spectrum for *Bacillus cereus* spores in distilled water

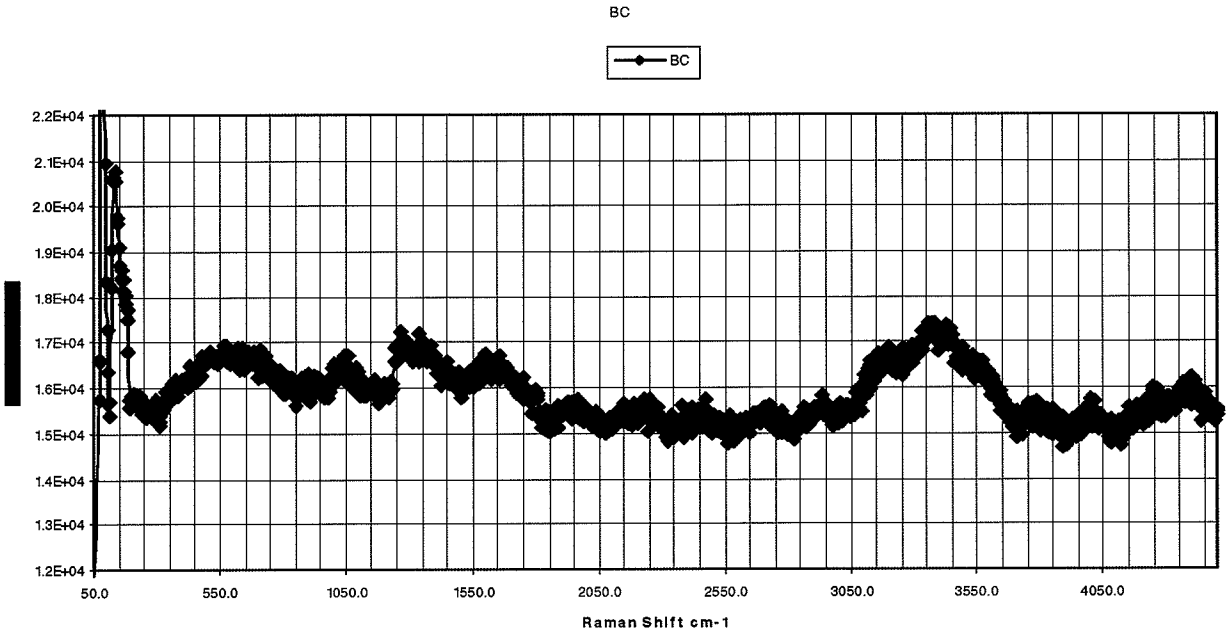
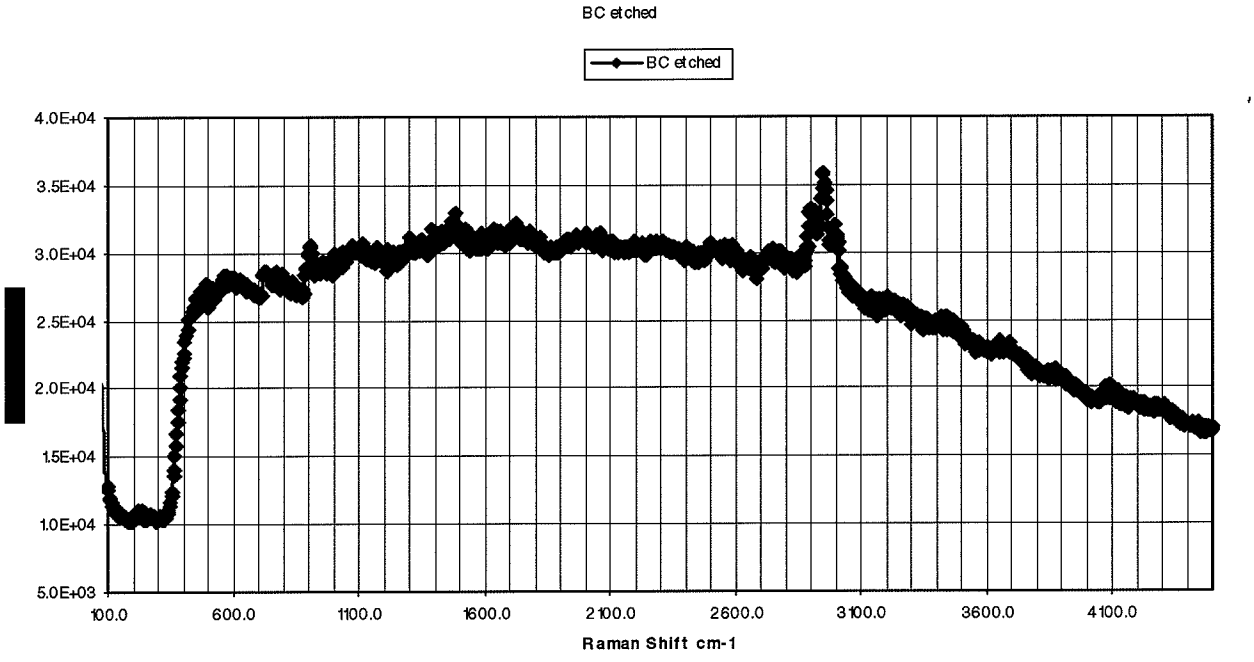


Figure 18. SERS Spectrum for *Bacillus cereus* spores etched with Dodecylamine and Ethanol



For the spectra shown above and below, Raman scattering begins at the reflection point of the sample under test being illuminated by the laser reflected to the input of the monochromator. An optical filter is inserted

between these two components to "remove" the high intensity laser line at 532 nm. What is passed into the monochromator is the scattering light from the sample under test. The scattering is passed inside the monochromator and impinges a high precision optical grating. The grating disperses the light according to wavelength and the intensity pattern is observed at the video camera and photomultiplier tube (PMT) detector with photon counting capability. The two-axis display is seen on the video monitor from a scanned intensity plot controlled by the computer. The computer controls the scan motor of the monochromator and records the intensity results for each wavelength. This 2-dimensional plot is actually a "finger print" of the sample under test. The general shape of this plot is repeatable and determines very accurately the nature of the sample. From lessons learned in Phase 1, it was determined that a surface enhanced substrate is required to measure the spores of *B. anthracis* and spores of three closely related species, *B. cereus*, *B. globigii*, and *B. thuringiensis*. Also since the surface of the spore contains little molecular information the samples were chemically etched. It is our conclusion that to measure spores some combination of this chemical/ optical technique will be required. The SERS substrates used were silver deposited onto the inner wall of a glass vial. The samples were mixed with a warm solution of ethanol and dodecylamine used as a mild detergent.

All charts are in photon counts versus wave number and have a similar vertical scale. Bacillus thuringiensis has a much higher signal than its counter parts. This may be due in part to the SERS substrates finite life cycle. The measurements of *B. thuringiensis* were collected first after the samples were prepared. Also, the concentrations of each sample were largely unquantified, which directly affects the Raman cross-section or signal strength.

2.2.1. Collect Samples in a NYCT Transit Station

Ideally, the background sample(s) used to develop spectra for use that the basis for a negative filter. In this case, we felt that an older station would have the potential to provide a background with more diverse character. We also felt that a station located close to Central Park might add additional character with added potential for pollen and other plant/animal-related material. Dr. Chuck Burrus, Director of NYCT System Safety, identified the 72nd Street Station at Central Park as a good candidate. This station is across the street from Central Park and on the southwest corner of 72nd Street at Central Park West on the B and C lines. It is a mid level station, indicating that there are service levels above and below the station. Pictures from the sample site are shown below in Figure 19.

Figure 19a.Entry to station from Central Park. **Figure 19b.Entry looking toward Central Park.**

Figure 19c. B and C Lines toward Downtown. **Figure 19d. A mid level station – stairs down.**

Figure19e.Train:passengers loading/unloading. **Figure19f.Street level venting at Central Park.**

Sampling was conducted in a stairwell that had been unused for a number of years as shown below in Figure 20. The sample system was set up on the landing in the stair well at the base of the stairs that led to a closed street-level entrance. The landing was gated to eliminate access when the entry was

closed. It was approximated 12 feet from the tracks. The sample system consisted of a lab vacuum pump connected to a series of 0.2μm membrane filters by Tygon tubing and was powered by battery. The system ran for 24 hours. Samples were taken at 0, 6, 18, and 24 hours and stored in plastic sample bags at room temperature. Samples were also taken from the residue that had built up in the stairwell as a long-term sample and stored in the same manner.

Figure 20. Sampling device shown through barred gate restricting access to the close stair well. Device was housed in a plywood container with vacuum pump in front left corner and filters across back and right sides. Batteries behind box.

Spectra for the subway samples were developed in the same manner as for spores. They were etched with dodecylamine in ethanol and placed on a SERS surface to spectral analysis. Figure 19 shows the 5 samples collected. The Background 0 (0 hr/control) shows a flat spectrum except in the 2900 range. This represents the material of the filters used to collect samples and provides us a baseline control since it was not exposed to the environment. Background samples 1 (6 hr), 2 (18 hr), and 3 (24 hr) also show little spectral character except in the same 2900 range and at the same intensity. The 18-hour sample shows a peak at about 2250, and we are unsure as to what may have cause the peak; since, the 24- hour sample would have been expected to show the same peak. The Background 4 sample that represents a composite of material deposited in the stairwell over a number of years also shows a peak at 2900, which we have attributed to the filter medium used to collect sample. The large shoulder beginning at about 350 may be representative of a longterm composite that would be expected to represent relatively fresh sample and a larger conglomeration of various compounds and their breakdown products that have accumulated and broken down over time. The important observation is that these areas do not interfere with what we believe to be the primary anthrax signature that occurs between 2850 – 3000 (Figure 21). The samples collected in the subway demonstrate a definite overlap with the biological samples. In fact, the difference in Raman shifted spectra between *B. globigii*, *B. anthracis*, and background from the subway is 0.5nm. This yields a resolution requirement not previously quantified. Any instrument used to determine the spectral

differences in anthrax and a closely related species or background must have at least a 0.5nm resolution.

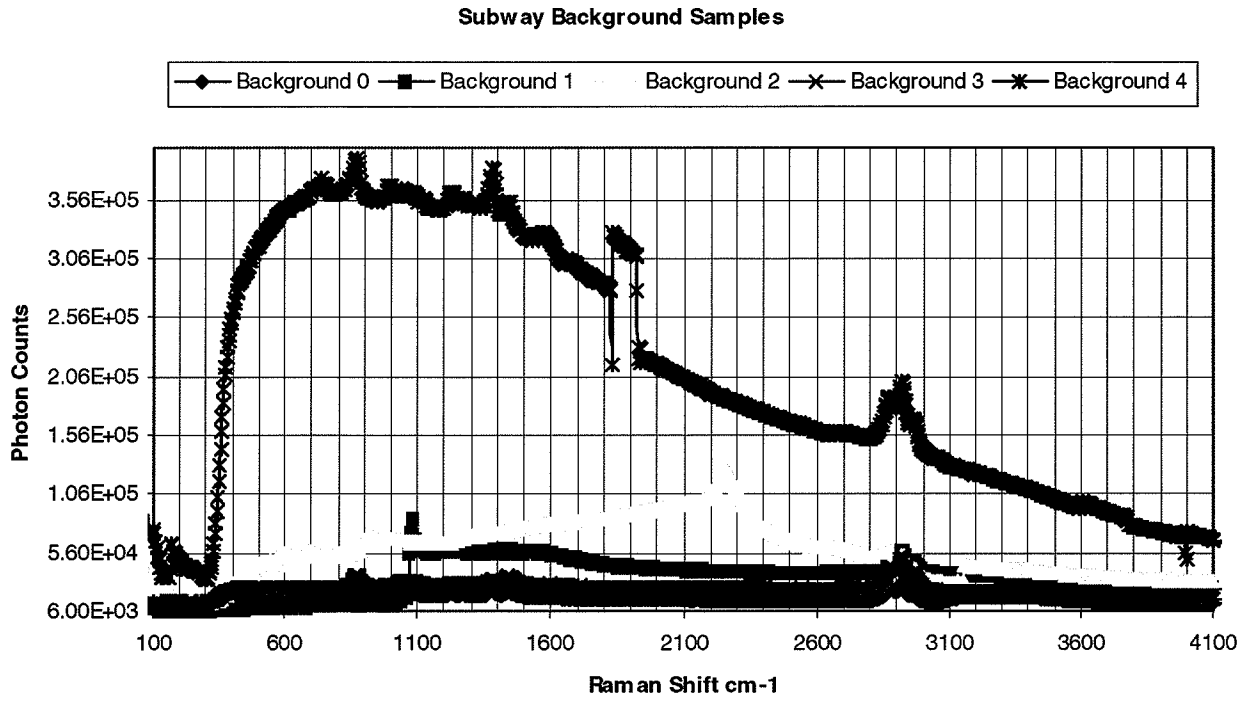


Figure 21. NYCT Background Samples (Background 0 = 0 hr, Background 1 = 6 hr, Background 2 = 18 hr, Background 3 = 24 hr, Background 4 = Stairwell Swab)

Resolution Requirements

The following set of charts demonstrates the resolution required to discriminate between simulant, BWA, and background. These charts show signal (photon counts) as a function of wavelength. The offset between each spectrum is approximately .5nm. While this is a fairly tight restriction it is a positive result to have a slightly different Raman shift for these materials in the spectral region of interest[1]. It is also important to not that the signal amplitude is different for each measurement. This may be used to create threshold levels when filtering background information in the correlator.

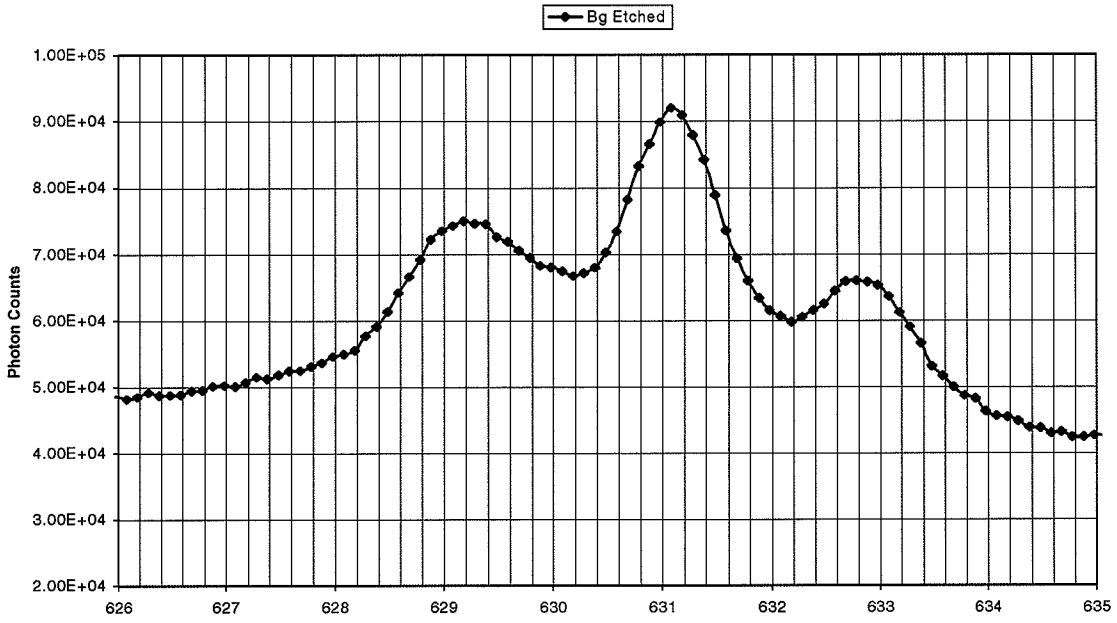


Figure 22. Raman spectrum of NY in region of interest demonstrating Raman shifted comparison.

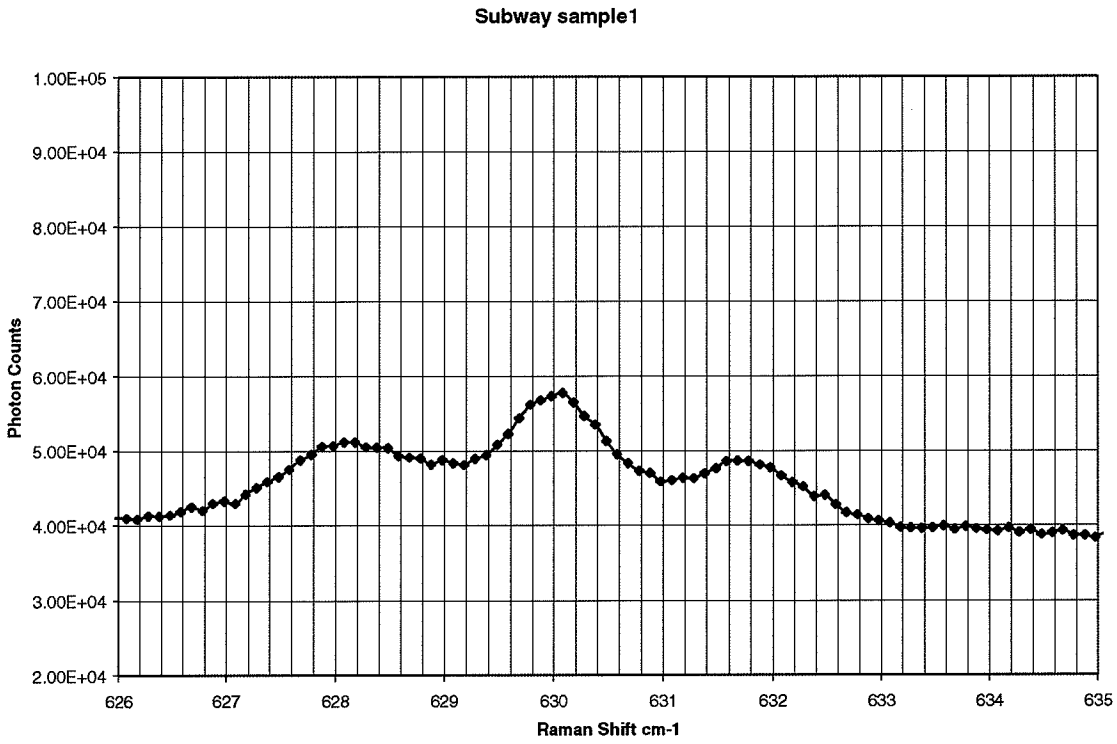


Figure 23. Raman spectrum *Bacillus subtillus* var. *globigii* in region of interest demonstrating Raman shifted comparison.

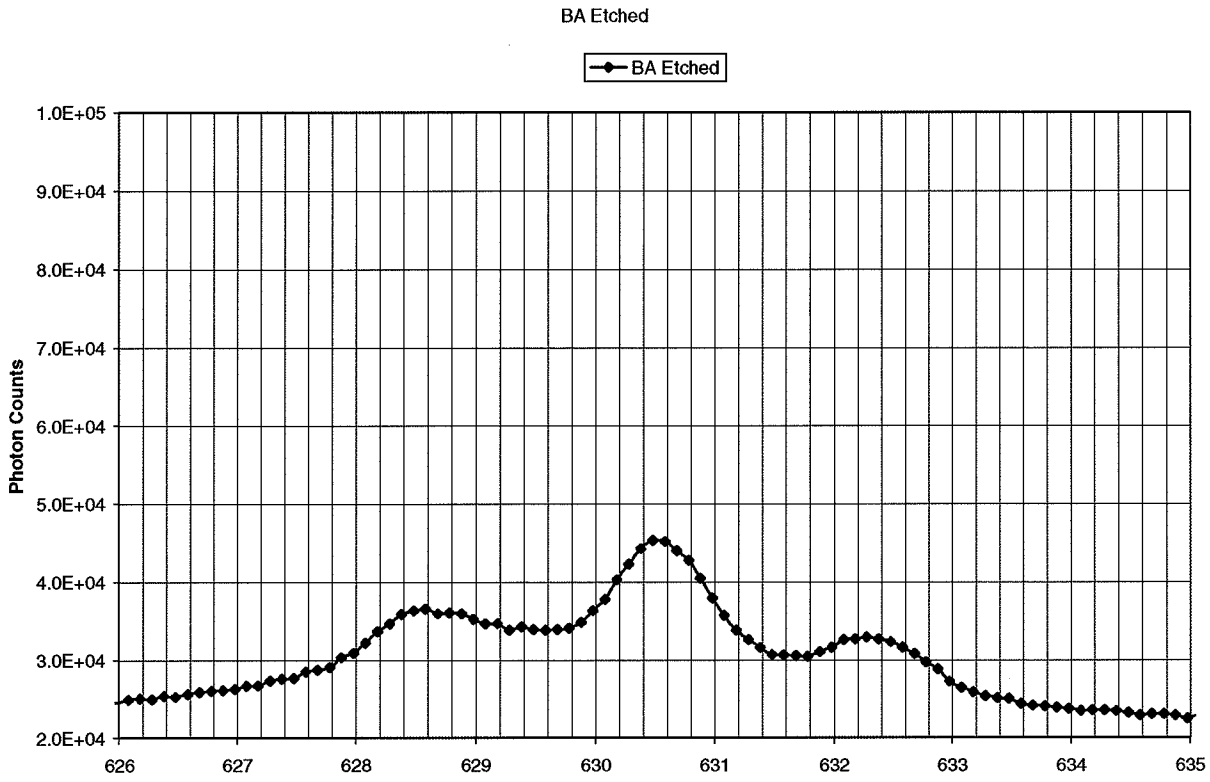


Figure 24. Raman spectrum *Bacillus anthracis* (anthrax) in region of interest demonstrating Raman shifted comparison.

3.0 PLANS FOR IMPLEMENTATION

Implementation of this concept involves more extensive filter development in order to discriminate between several different spectra simultaneously. The approach is explained below.

If the measured spectra are treated as large dimensional vectors (i.e., one by approximately 2000 element matrices), then the concept of uniqueness is equivalent to vector orthogonality. The component of a vector, A, which is orthogonal to another vector, B may be expressed as $D_{A,B}$=A-(A·B)B. This orthogonal component is the linear discriminant that permits the recognition of A from B. If the parallel component of other vectors such as the spectra of C, D, etc. are subtracted from $D_{A,B,}$ then the resultant, $D_{A,BCDE}$, will also be orthogonal to these other vectors.

A critical element of this concept is the design and production of a filter that demonstrates the performance and utility of this technique. To accomplish this we developed a pair of differential filters which would identify a concentration of ethanol in an ethanol/methanol mixture in the presence of broadband noise sources. These filters will have graded transmission as a function of optical frequency. As a simplified example, the Raman spectrum of methanol overlaps the spectra of ethanol very strongly in the 2900 cm-1 band and acts as an interferant. To reject broadband noise, the differential filters must have equal average optical transmittance so that the difference generated by the noise approaches zero (within the shot noise limit). Since the monochromator used in this example has much higher resolution than is expected for an operation instrument, the spectral band from 2825 cm-1 to 2975 cm-1 wave numbers was divided into only 4 separate bands for the spatial filter.

To calculate the transmittance in each of the spatial filter bands, we first define:

$E = [e_1\ e_2\ e_3\ e_4]$ and

$M = [m_1\ m_2\ m_3\ m_4]$,

with e_i, m_i being the spectral intensities of ethanol and methanol, respectively, in segment i.

We are looking for:

$E = [t_1\ t_2\ t_3\ t_4]$ such that:

$E \cdot E = 1$

$E \cdot M = 0$

E is thus a unit vector that is orthogonal to M. In general E will take the form

$E = [t_1\ t_2\ t_3\ t_4] = [p_1\ p_2\ p_3\ p_4] + [n_1\ n_2\ n_3\ n_4]$

with $[p_1\ p_2\ p_3\ p_4]$ containing the transmittances of the positive mask, and $[n_1\ n_2\ n_3\ n_4]$ containing the transmittances of the negative mask.

Let:

$E = w_{EE}\, E + w_{EM}\, M$

Then we can write:

$E \cdot E = w_{EE}\, EE + w_{EM}\, ME = 1$

$E \cdot M = w_{EE}\, EM + w_{EM}\, MM = 0$

Using the experimental values for e_i, m_i , we solved for w_{EE} and w_{EM} and obtained:

$w_{EE} = -7.839$

$w_{EM} = 9.122$

We substitute in Equation (1) and get:

$E = [0.9259, 2.6060, 2.935, 2.655] + [-2.675, -0.766, -1.540, -1.319]$

Next we normalize and separate the positive and negative terms to obtain:

$E = [-0.95, 1, 0.76, 0.73] = [0, 1, 0.76, 0.73] + [-0.95, 0, 0, 0]$

as the sum of a positive and negative masks.

These coefficients are the transmittances in each of the spectral regions of the positive and negative filters used in the experiment. Instead of grading the transmittance over all of the area of the spatial filter in these bands, it was decided to take a binary approach. Specifically, a portion of the spatial filter would be made opaque and the remainder left highly transparent. The ratio of the opaque to the transparent regions would be transmittances calculated above.

Figure 23. is a plot of the ethanol and methanol spectra in the analytical region, showing the four segments used in the mask calculation. The transmittances of the positive mask (pink) and the negative mask (blue) are overlaid on the spectra.

Diagrams (approximately twice actual size) of the masks are shown in Figure 7.

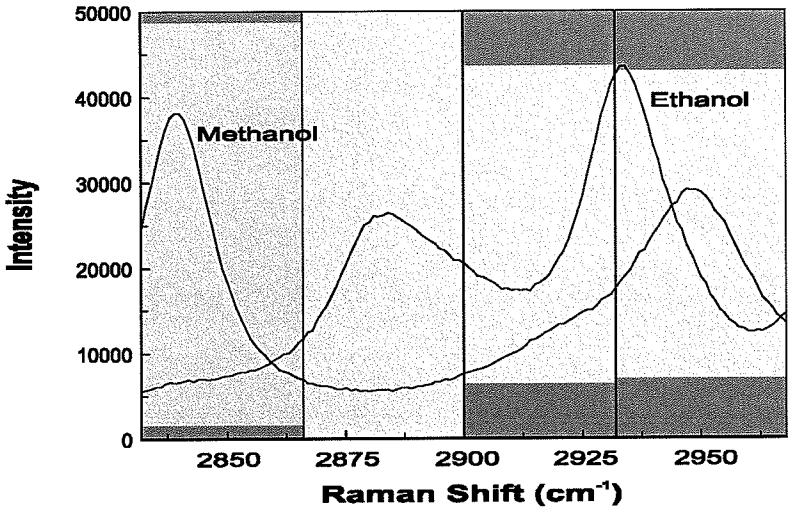


Figure 23. Raman spectra of ethanol and methanol showing four spectral segments and transmittances of positive (pink) and negative (blue) masks.

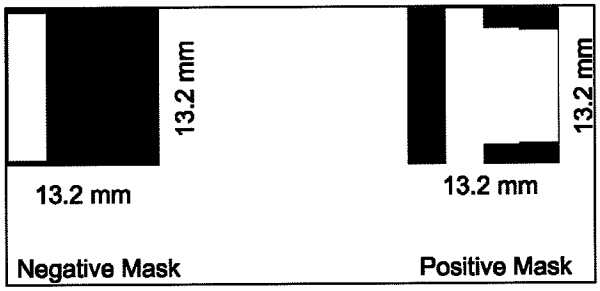


Figure 24. Negative and positive masks resulting from calculations in the text.

The resulting device is expected to be self-contained, automated, and able to function unattended for extended periods of time. Filters containing multiple spectra, each having the Fourier transform of the Raman spectrum of different substances of interest, may be installed in the 4-f correlator in order to simultaneously compare the received spectra to multiple known spectra in the training set. This arrangement is possible when the characteristic spectra do not overlap and cause a resemblance to interferant spectra. Additional filters may be added to cover the large range of BWAs without creating potential for false alarms.

3.1 Anomalies

There have been two primary anomalies. Both occurred in Stage 1. They are listed below.

First, the events of 9/11 triggered a complete revision of the regulations whereby laboratories are allowed to work with select agents, even if they are killed (dead) when received, and after the award of this contract. This caused a significant delay in receipt of lab certification to work with gamma-killed anthrax. This further delayed our ability to request the killed anthrax from the Armed Forced Institute of Pathology (AFIP). Requests for agents were not allowed under the new regulations until after certification was received. Furthermore, AFIP was then required to request permission to transmit the killed spores, but only after they had been produced and killed. We finally received the spores in mid-April.

Second, initial spectra were non-descript as, described above. The lack of discernable spectra was in multiple ways. First, a stronger laser was purchased to improve instrument performance. When this failed to produce the desired results, surface enhanced Raman spectroscopy (SERS) was identified as a potential alternative approach. This did produce discernable spectra. We were still not satisfied; however, and then chemically etched spores were developed by a newly published method, which produced further improved results, as reported above.

3.2 Plans for Implementation

SAIC plans to pursue multiple opportunities to advance this technology through the remainder of development and on to field implementation. At this time, we plan to prepare a Phase 2 proposal to be submitted to the Transit IDEA Program. We have also received and responded to an RFI from the MTA, New York City, NY, and se plan to respond to the RFP when released. We will also explore other public agency opportunities and other target environments for implementation that may open other sources of development and fielding funding. Assuming that the development path continues to be successful, SAIC, along with FastMetrix, plans to pursue design and production of a field item that can be used to detect bioagents in a transit environment.

4.0 CONCLUSIONS

First, SAIC conducted a review of the literature to show the published state-of-the-art for detection of biological materials. The literature review concluded that there are ongoing improvements to existing and new technologies, but currently there are no available technologies that meet the demands of subway transit systems. The literature review was previously delivered November 2003 and presented to the expert review panel at that time. The review is not included as part of the Final Report since its length would extend the length of the Final Report.

Next, SAIC and FastMetrix completed preliminary work indicating that gamma-killed spores from *Bacillus anthracis,* Ames strain, did produce a distinct Raman spectrum signature. However, a distinct signature using the base detection system originally described in our proposal was not observed. Apparently, bacterial spores have very little characteristic surface detail that is readily picked up in Raman. Therefore, SERS was used to enhance the signal. A definable signature spectrum was observed. Further enhancement based upon chemical etching of the spore surface was also tested. This produced an even more enhanced signature.

Additional work has shown that the FastMetrix Raman spectroscopic technology can produce a defined signature for anthrax (gamma-killed). We also showed that that signature is different from that of 3 closely related spores, (*Bacillus thuringiensis, Bacillus globigii, Bacillus cereus*). In addition, 4 samples collected from the 72nd Street NYCT subway station at Central Park West that could be used as the basis for a negative filter in the detection system. The samples collected had some spectral definition, particularly the sample that represented a long term (multi-year) composite. However, there was no overlap with the spectra generated for anthrax spores. Therefore, we conclude that anthrax spores are detectable and differentiatable from the subway background and the three closely related spores tested using the subject technology

Based upon these data and conclusions, this technology warrants further development toward a prototype for testing in a controlled transit environment first, and then in an operating transit environment.

5.0 INVESTIGATOR PROFILE

This final report was authored by Dr. Douglas B. Rivers of SAIC who served as the principal investigator of the project and Ms. Elizabeth A. Tanner of FastMetrix, Inc. All questions regarding the content of this document should be addressed to Dr. Rivers.

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Dr. Rivers has a Ph.D. in fermentation and microbial physiology. He has 28 years of academic and commercial experience identifying, developing, and taking new technologies to the field. He has over 10 years experience related to microbial detection in the environment.

6.0 REFERENCES

1. Raman spectroscopy is an analytical technique that can be used for the analysis of organic substances found in composite surfaces or bulk material. A material is bombarded with monochromatic radiation that results in a phenomenon known as Raman scattering. The frequencies of the Raman spectra are, like infrared absorption bands, characteristics of the molecule and the various groups and linkages in the molecule.

2. The Raman effect arises when the incident light excites molecules in the sample that subsequently scatter the light. While most of this scattered light is at the same wavelength as the incident light, some is scattered at a different wavelength. This inelastically scattered light is called Raman scatter. It results from the molecule changing it's molecular motions. The energy difference between the incident light (Ei) and the Raman scattered light (Es) is equal to the energy involved in changing the molecule's vibrational state (i.e. getting the molecule to vibrate, Ev). This energy difference is called the Raman shift. Several different Raman shifted signals will often be observed; each being associated with different vibrational or rotational motions of molecules in the sample. The particular molecule and it's environment will determine what Raman signals will be observed. A plot of Raman intensity vs. Raman shift is a Raman spectrum.

3. The XM-95 developed by Los Alamos National Laboratory for the Edgewood Engineering and Development Center, Aberdeen Proving Ground, Maryland has demonstrated the ability to track aerosol clouds at ranges over 15 kilometers. It is deployed as the primary payload in a Blackhawk helicopter specifically outfitted for this mission. The XM-95 transmits 0.5 Joule, 1.06 micrometer laser pulse and detects the aerosol reflection with a 20 inch diameter telescope. The system weighs 1500 pounds.

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