Detecting anthrax in the mail by coherent Raman microscopy

Rajan Arora,a Georgi I. Petrov,b Vladislav V. Yakovlev,a,b and Marlan O. Scullyc,d,1

aDepartment of Physics, University of Wisconsin, Milwaukee, WI 53211; bDepartment of Biomedical Engineering and cColleges of Science and Engineering, Texas A&M University, College Station, TX 77840; and dCollege of Engineering and Applied Sciences, Baylor University, Waco, TX 76798

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In this report, we show the collection of spatial information through a turbid medium by coherent Raman microspectroscopic imaging. In particular, the technique is capable of identifying anthrax endospores inside a sealed paper envelope.

CARS spectroscopy is often limited by a strong so-called nonresonant background, which originates from a nonspecific four-wave mixing, and the line shapes in CARS spectra are rather complicated. Conventional methods of suppressing the nonresonant background [e.g., using time-delayed probing and/or polarization properties of the nonresonant CARS signal (7) and interferometric detection to heterodyne the CARS signal (8, 9)] do not work in a turbid medium. Hence, to extract the signal, we assume that the nonresonant tensor component, $X_{NR}$, is real and frequency-independent, and we use a maximum entropy technique (10) to extract the imaginary part of the resonant tensor component, $X_{R}$, which is directly proportional to the Raman signal. The resultant Raman spectrum does not rely on any other assumptions and can be used successfully in both transparent and a highly scattering media (11); we call it the retrieved Raman spectrum.

In these experiments, we used a powder of a dipicolinic acid (DPA), whose chemical structure is shown in Fig. 1b, because it is a major chemical component of bacterial endospores (Fig. 1b). The comparative Raman spectra of different bacteria endospores have the common Raman lines (see ref. 12), corresponding to the Raman lines of DPA (Fig. 1c), and as was shown earlier, it can serve as a chemical marker to detect endospores by means of coherent Raman spectroscopy (2, 3). Using these vibrational lines as an indicator for the presence of the DPA powder, we image its distribution through a regular envelope in the presence of other lookalike powders [e.g., chalk or calcium carbonate (CaCO3)].

Our experimental setup is very similar to the one described elsewhere (2, 13) and is shown schematically in Fig. 2. In brief, a megahertz-rate home-built picosecond Nd:YVO4 laser was amplified by a diode-pumped Nd:YVO4 amplifier to achieve 5 μJ/pulse at a repetition rate of 1 MHz and a pulse duration of about 10 ps. Approximately 2 μJ energy were sent into a large-mode diameter single-mode fiber, which was doped with GeO2 to promote efficient stimulated Raman scattering, leading to a broadband continuum generation in the spectral region from 1,000 to 1,500 nm (13). This supercontinuum radiation served as a broadband Stokes pulse, whereas the rest of the fundamental radiation at 1,064 nm was used as a narrow band (<5 cm⁻¹) pump pulse to achieve a simultaneous excitation of all Raman modes in the spectral region of interest (200–3,000 cm⁻¹). The two beams were combined together and collinearly focused onto the sample using an achromatic aspherical lens (ThorLabs, Inc.), which was placed on a computer-controlled translational stage. The signal was collected in a transmission geometry using an achromatic objective (N.A. = 0.55; Mitutoyo) and directed through an anti-Stokes Raman filter (Omega Optical) into a 0.5-m

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1To whom correspondence should be addressed. E-mail: scully@tamu.edu.

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spectrometer (Horiba) with the attached CCD camera (Andor). All of the collected CARS spectra were normalized to the CARS spectra of fused silica, which does not have strong Raman lines in the spectral region of interest. Normalization procedures allowed us to avoid most of the problems caused by a number of spectral features of the supercontinuum, spectral transmission function of all optical components, etc.

Typical CARS, retrieved Raman, and experimentally collected Raman spectra for DPA and calcium carbonate are shown in Fig. 3. Raman spectra were collected using a home-built confocal Raman microscope, which uses an intracavity frequency-doubled Nd:YVO₄ laser (λ = 532 nm) as the excitation source. Some minor discrepancies of the retrieved Raman and experimentally measured Raman spectra are attributed to a large difference in the excitation wavelength. However, all of the spectral features are essentially identical.

Small amounts of powders of DPA, chalk, and a mixture of the two substances were placed inside the envelope. Then, CARS images of the powder were collected when the envelope was inspected by a transmission optical microscope.

As expected, the amplitude of the CARS signal dramatically decreased because of the reduced light intensity as a result of light scattering. However, all of the spectral features of the DPA and chalk were clearly pronounced. This definition allows a hyperspectral imaging to be performed at a spatial resolution of about 2 μm, which was roughly the size of the focal spot on transmission through the first layer of paper. To do this procedure, we first focused on an upper paper surface and injected 500 μm into the sample to position the focal spot between the paper layers of the envelope. The 2D scanning was performed in the plane perpendicular to the beam propagation.

A nonspecific CARS image is shown in Fig. 4A, where the intensity of CARS signal is evaluated at around 2,000 cm⁻¹, because there are no specific vibrations, and signal is caused by a nonresonant background. After we retrieved Raman spectra from CARS spectra and plotted the same area to display the intensity of Raman intensity at around 1,150 cm⁻¹, where Raman spectrum is dominated by the Raman spectrum of paper, we get a distribution of paper material in the scanned area, which is shown in Fig. 4B. Repeating the same procedure for 1,000 (a sharp vibrational line of Ca-DPA), and 1,090 cm⁻¹ (a characteristic vibration of calcium carbonate), we get the distribution of DPA and chalk, which are shown in Fig. 4C and D, respectively. Finally, we can combine all these images into one image (Fig. 4E), where each color represents the concentration of different chemicals in the scanned area (red, DPA; blue, chalk; green, paper).

In summary, we have shown the unique capability of CARS microspectroscopy to provide spatially selective, chemically specific imaging of molecular species in a turbid medium. At this point, the limiting parameter for high-speed imaging is the amplitude of the signal. It takes less than 1 ms to attain a good-
quality CARS spectrum in an optically transparent system, whereas it takes 100 ms or more to achieve the same quality spectrum from a powder species hidden beneath a scattering medium, like envelope paper. One way to improve the signal strength is to increase the light intensity on the sample, while simultaneously increasing the beam spot size on the sample. This improvement would require a substantial increasing of the incident power, but given the recent spectacular progress of ultrafast fiber technology, it is feasible to use laser beams 10 times more powerful, leading to three orders of magnitude signal enhancement (14). Beam multiplexing through a multifoci focusing mechanism (15) will also speed up the data acquisition. With the above-mentioned upgrades, we anticipate at least four orders of magnitude improvement in image clarity and the time required for the data acquisition. Furthermore, because the suspicious area inside an envelope can be easily identified in a regular transmission optical microscope, there is no need to scan the whole envelope area, which will also reduce the inspector’s time requirements.

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Supporting Information

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The following references provided important methodological information for this paper.


