Anal. Chem. **2004,** *76,* 599−603

Reagentless Identification of Single Bacterial Spores in Aqueous Solution by Confocal Laser Tweezers Raman Spectroscopy

J. W. Chan,*[,†,§] A. P. Esposito,[‡,§] C. E. Talley,[‡,§] C. W. Hollars,[‡,§] S. M. Lane,[†,§] and T. Huser[†,‡,§]

Physics and Advanced Technologies Directorate, Chemistry and Materials Science Directorate, Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, California 94550, and NSF Center for Biophotonics Science & Technology, University of California, Davis, 4800 Second Avenue, Suite 2600, Sacramento, California 95817

We demonstrate that optical trapping combined with confocal Raman spectroscopy using a single laser source is a powerful tool for the rapid identification of micrometer-sized particles in an aqueous environment. Optical trapping immobilizes the particle while maintaining it in the center of the laser beam path and within the laser focus, thus maximizing the collection of its Raman signals. The single particle is completely isolated from other particles and substrate surfaces, therefore eliminating any unwanted background signals and ensuring that information is collected only from the selected, individual particle. In this work, an inverted confocal Raman microscope is combined with optical trapping to probe and analyze bacterial spores in solution. Rapid, reagentless detection and identification of bacterial spores with no false positives from a complex mixed sample containing polystyrene and silica beads in aqueous suspension is demonstrated. In addition, the technique is used to analyze the relative concentration of each type of particle in the mixture. Our results show the feasibility for incorporating this technique in combination with a flow cytometric-type scheme in which the intrinsic Raman signatures of the particles are used instead of or in addition to fluorescent labels to identify cells, bacteria, and particles in a wide range of applications.

Confocal Raman microscopy has become a widely accepted analytical tool for studying the molecular structure and chemical composition at localized volumes within a sample.[1−9] This technique involves collecting spectral signals from only the focal region of the objective while rejecting background signals that are out of focus through the use of a confocal pinhole. The high spatial resolution provided by confocal Raman microscopy enables detection of spectral features, even from single micrometer-sized particles. By combining confocal Raman microscopy with optical trapping techniques, there is potential for the use of micro-Raman spectroscopy as a noninvasive, nondestructive technique for the rapid detection and discrimination of particles based on their unique Raman signatures (i.e., chemical structures).

Since Ashkin's early work[10−12] on optical trapping of micrometer-sized particles, "optical tweezers" have become an invaluable tool for the micromanipulation of a variety of particles (e.g., viruses,[10] bacteria,[10,13] cells,[12−16] beads,[11,17,18] and water droplets[19]) in either gaseous or liquid media. The trap arises as a result of a balance of scattering and intensity gradient forces of radiation pressure, the equilibrium point being the focus of the laser beam. Typically, a strongly focused laser beam with a Gaussian transverse profile is sufficient to produce a single beam optical trap for particles with a refractive index higher than that of the surrounding medium. A variety of spectroscopic techniques have been incorporated with optical tweezers.[20,21] Most recently, optical trapping has been combined with micro-Raman spectroscopy to enable acquisition of Raman spectra of individual, isolated particles in

† Physics and Advanced Technologies Directorate, Lawrence Livermore National Laboratory.

‡ Chemistry and Materials Science Directorate, Lawrence Livermore National Laboratory.

§ University of California, Davis.

(1) Puppels, G. J.; de Mul, F. F. M.; Otto, C.; Greve, J.; Robert-Nicoud, M.; Arndt-Jovin, D. J.; Jovin, T. M. *Nature* **1990**, *347*, 301−303.
(2) Puppels, G. J.; Otto, C.; Greve, J. *TrAC, Trends Anal. Chem.* **1991**, *10*, 249−253.
(3) Schuster, K. C.; Reese, I.; Urlaub, E.; Gapes, J. R.; Lendl, B. *Anal. Chem.* **2000**, *72*, 5529−5534.
(4) Schuster, K. C.; Urlaub, E.; Gapes, J. R. *J. Microbiol. Methods* **2000**, *42*, 29−38.
(5) Peticolas, W. L.; Patapoff, T. W.; Thomas, G. A.; Postlewait, J.; Powell, J. W. *J. Raman Spectros.* **1996**, *27*, 571−578.
(6) Uzunbajakava, N.; Lenferink, A.; Kraan, Y.; Volokhina, E.; Vrensen, G.; Greve, J.; Otto, C. *Biophys. J.* **2003**, *84*, 3968−3981.
(7) Uzunbajakava, N.; Lenferink, A.; Kraan, Y.; Willekens, B.; Vrensen, G.; Greve, J.; Otto, C. *Biopolymers* **2003**, *72*, 1−9.
(8) Chadha, S.; Nelson, W. H.; Sperry, J. F. *Rev. Sci. Instrum.* **1993**, *64*, 3088−3093.
(9) Huang, Y. S.; Karashima, T.; Yamamoto, M.; Hamaguchii, H. *J. Raman Spectrosc.* **2003**, *34*, 1−3.
(10) Ashkin, A.; Dziedzic, J. M. *Science* **1987**, *235*, 1517−1520.
(11) Ashkin, A.; Dziedzic, J. M.; Bjorkholm, J. E.; Chu, S. *Opt. Lett.* **1986**, *11*, 288−290.
(12) Ashkin, A.; Dziedzic, J. M.; Yamane, T. *Nature* **1987**, *330*, 769−771.
(13) Ericsson, M.; Hanstorp, D.; Hagberg, P.; Enger, J.; Nystrom, T. *J. Bacteriol.* **2000**, *182*, 5551−5555.
(14) Xie, C. G.; Dinno, M. A.; Li, Y. Q. *Opt. Lett.* **2002**, *27*, 249−251.
(15) Xie, C. G.; Li, Y. Q. *J. Appl. Phys.* **2003**, *93*, 2982−2986.
(16) Guck, J.; Ananthakrishnan, R.; Mahmood, H.; Moon, T. J.; Cunningham, C. C.; Kas, J. *Biophys. J.* **2001**, *81*, 767−784.
(17) Ajito, K.; Torimitsu, K. *Appl. Spectrosc.* **2002**, *56*, 541−544.
(18) Xie, C. G.; Li, Y. Q. *Appl. Phys. Lett.* **2002**, *81*, 951−953.
(19) Magome, N.; Kohira, M. I.; Hayata, E.; Mukai, S.; Yoshikawa, K. *J. Phys. Chem. B* **2003**, *107*, 3988−3990.
(20) Kitamura, N.; Kim, H. B.; Habuchi, S.; Chiba, M. *TrAC, Trends Anal. Chem.* **1999**, *18*, 675−684.
(21) Liu, Y.; Sonek, G. J.; Berns, M. W.; Konig, K.; Tromberg, B. J. *Opt. Lett.* **1995**, *20*, 2246−2248.

10.1021/ac0350155 CCC: $27.50
Published on Web 12/18/2003

aqueous media. The tight focus required by both methods makes combining these techniques straightforward. Whereas confocal microscopy usually requires particle samples to be immobilized on a substrate, optical tweezers can be used to hold a particle, which would otherwise drift due to Brownian motion, in place in an aqueous environment. Since the particle is already in the focus, the same laser beam that is used for the trapping also provides the excitation for Raman scattering.

A number of recent studies have trapped a variety of particles, such as polystyrene beads,[14,22,23] organic microdroplets in water,[24] nanoparticles,[17] high-index crystals (silicon, germanium, $KNbO_3$),[18] aerosol particles,[25] and live cells (RBC, yeast),[14] and acquired their spectroscopic signatures using this laser tweezer Raman spectroscopy (LTRS) technique. This technique has also been used to study chemical processes of single particles in aqueous suspension.[26]

Here, we demonstrate the optical trapping of single bacterial spores in solution and the simultaneous acquisition of Raman spectra from the isolated spores. Previous Raman studies[27−30] on *Bacillus* spores involved concentrated spore samples. Our group has recently used micro-Raman spectroscopy to show that chemical information from individual spores can be obtained and has demonstrated the potential of the technique to investigate variations from spore to spore.[31] In that work, however, micro-Raman spectroscopy of single spores required the deposition of the spores on a CaF_2 substrate and imaging them in a dry state. The immobilized spore had to be located by fluorescence/Raman imaging prior to performing the spectroscopy experiment. Here, we demonstrate the ability to dramatically decrease the preparation time by optically trapping the spores in solution. In addition, optical trapping allows us to be confident that the signals are coming from a single spore trapped in the laser focus. This technique enables us to perform rapid analysis of single spores in solution to test for differences in spore spectra due to exposure to different environmental conditions and to induce and monitor chemical processes in a suspended, single spore.

We also show how LTRS can be used to rapidly analyze the composition and relative concentration of complex, mixed samples noninvasively and without reagents. Our experiments investigated the use of optical trapping to rapidly identify spores and distinguish them from other particles (i.e., silica, polystyrene) in a mixture of similarly sized particles. On the basis of sampling of 100 particles, we were able to determine the relative concentration of each species in the mixture.

(22) Ajito, K.; Torimitsu, K. *TrAC, Trends Anal. Chem.* **2001**, *20*, 255−262.
(23) Crawford, K. D.; Hughes, K. D. *J. Phys. Chem. B* **1998**, *102*, 2325−2328.
(24) Ajito, K. *Appl. Spectrosc.* **1998**, *52*, 339−342.
(25) Lubben, J. F.; Mund, C.; Schrader, B.; Zellner, R. *J. Mol. Struct.* **1999**, *481*, 311−316.
(26) Houlne, M. P.; Sjostrom, C. M.; Uibel, R. H.; Kleimeyer, J. A.; Harris, J. M. *Anal. Chem.* **2002**, *74*, 4311−4319.
(27) Shibata, H.; Yamashita, S.; Ohe, M.; Tani, I. *Microbiol. Immunol.* **1986**, *30*, 307−313.
(28) Ghiamati, E.; Manoharan, R.; Nelson, W. H.; Sperry, J. F. *Appl. Spectrosc.* **1992**, *46*, 357−364.
(29) Nelson, W. H.; Manoharan, R.; Sperry, J. F. *Appl. Spectrosc. Rev.* **1992**, *27*, 67−124.
(30) Woodruff, W. H.; Spiro, T. G.; Gilvarg, C. *Biochem. Biophys. Res. Comm.* **1974**, *58*, 197.
(31) Esposito, A. P.; Talley, C. E.; Huser, T.; Hollars, C. W.; Schaldach, C. M.; Lane, S. M. *Appl. Spectrosc.* **2003**, *57*, 868−871.

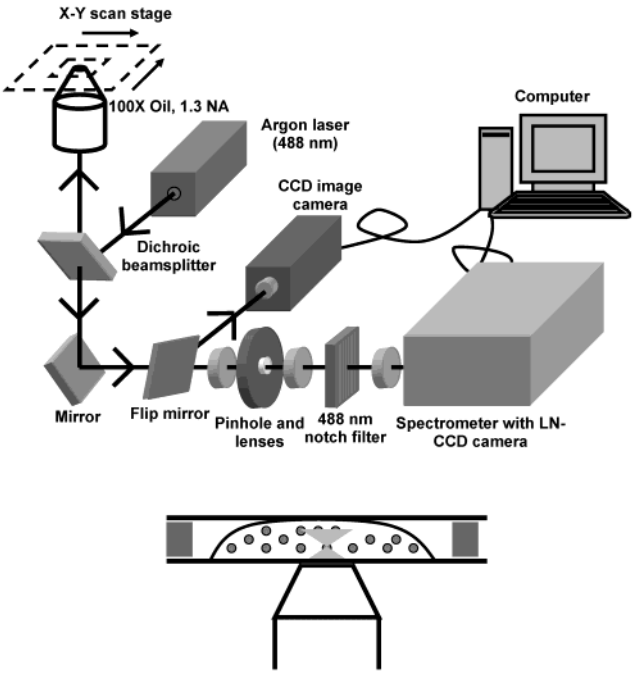

Figure 1. Schematics of the experimental setup for the laser-tweezers optical Raman spectrometer used in this study. A low-power (2−3 mW) 488-nm argon laser beam was used to both optically trap individual particles and to provide excitation for Raman spectroscopy.

EXPERIMENTAL SECTION

Confocal Raman Microscope. A diagram of the confocal Raman microscope is illustrated in Figure 1. The excitation source is a 488-nm argon ion laser (JDS Uniphase) capable of delivering a maximum of 50 mW. The beam passes through a quarter wave plate and a narrow 488-nm band-pass filter to remove residual laser lines and is then directed toward the rear entrance port of an inverted microscope (Zeiss Axiovert 200). A 488-nm dichroic beam splitter delivers the beam upward through a 100×, 1.3 NA oil immersion objective (Zeiss Plan-Neofluar), which focuses the beam to a spot size of roughly 0.5 μm, producing the single beam optical trap. Raman signals from an optically trapped particle are collected with the same objective and passed through the dichroic beam splitter. A 50-μm pinhole functions as the aperture that rejects any out-of-focus background signal, and a 488-nm long-pass filter is used to reject residual excitation light. The remaining signals are sent into a spectrometer (Jobin Yvon Spex Triax 320) equipped with a grating with 1200 grooves/mm (λ_b = 500 nm) to spectrally disperse the signal, which is then projected onto a liquid-nitrogen-cooled CCD camera (LN-CCD, Princeton Instruments). In addition, images of the trapped particles can be obtained by diverting the optical beam toward a second CCD camera designated for video capture.

Sample Preparation. Spore samples of *Bacillus cereus* were used in our experiments. The samples were grown in 10 mL of 1/4× TY media at 32 °C. During mid log phase, the vegetative cells were diluted 1:25 in 75 mL of the 1/4× TY media. After 7 days in a shaker incubator at 32 °C, the cells were removed and Gram and spore stains were performed to confirm the presence of >99% refractile bacterial spores. The spores were centrifuged at 8000*g* for 12 min, and supernatant was decanted. The spores were reconstituted in 75 mL of sterile double-distilled deionized water and then centrifuged to remove residual sporulation media.

The washing procedure was repeated twice, and the spores were reconstituted in 5 mL of sterile double-distilled water. Size analysis of the spores using atomic force microscopy and scanning electron microscopy showed a narrow size distribution centered around 1 μm. The polystyrene beads (1.07 μm) and silica microspheres (1 μm) used in our experiments were obtained from Duke Scientific (Catalog Nos. 5100A and 8100, respectively). Stock solutions of each particle type were prepared in double-distilled deionized water.

Optical Trapping of Spores. To create the optical trap, roughly 2 mW of 488-nm light delivered through the 100× objective was found to be sufficient to trap all three types of particles. The beam was focused through a microscope cover glass (Fisher Scientific), on top of which a drop of solution (10 μL) containing the bacterial spores was placed. Another microscope cover glass was then placed on top of the sample drop. A membrane of 375-μm thickness was used as a spacer between the two cover glass slips (see Figure 1 inset). A spore was chosen by first imaging on the CCD camera and moving a manual $x-y$ scanning stage until the spore was near the laser focus, at which point the particle was drawn toward the focus and held in the optical trap. The optical trap suspended the spore roughly 10−15 μm above the lower cover glass surface. Scanning the stage and changing the focus depth allowed manipulation of the trapped particle in all three dimensions and isolation of the particle from other particles if desired. Raman spectra were taken of the trapped particle using the same laser power. An integration time as short as 5 s was sufficient to obtain Raman spectra with a good signal-to-noise ratio in order to identify the characteristic Raman bands of the particles.

Rapid Differentiation of Spores in Mixtures of Particles. The LTRS technique was used to analyze the composition of a mixture of three particles (spores, silica beads, polystyrene beads). Portions (100 μL each) of each particle stock solution were combined to yield a heterogeneous mixture, of which 20 μL was pipetted onto the microscope cover glass. A particle was randomly chosen by moving the stage until the particle was near the laser focus, as imaged on the CCD camera. Individual particles were trapped, and Raman spectra were taken with a 5-s integration time. This process was repeated for 100 random particles, and the entire experiment was repeated three times.

To determine the theoretical composition of the mixture, a laser particle analyzer (Liquid Sampler LS-200 Particle Measuring Systems, Inc., Boulder, CO) was used to analyze diluted samples of the stock solutions. The absolute concentrations of the stock solutions were 6.2×10^6 polystyrene beads/mL, 1.9×10^6 silica spheres/mL, and 1.1×10^7 spores/mL, yielding a theoretical composition of 33% polystyrene beads, 11% silica spheres, and 56% spores for the mixture. The error margins of laser particle analyzer measurements are relatively large and difficult to define because the instrument returns a wide range of particle sizes and a background of $\sim 10^3$ particles, even for pure double-distilled deionized water, clearly demonstrating the need for novel, more powerful analysis techniques.

As an alternative check, the stock solutions were also analyzed with a hemacytometer. A 10-μL portion was taken from each stock solution for analysis. The large distance between the glass plates of the hemacytometer (20 μm) relative to the 1-μm particles also makes it difficult to accurately count the total number of particles in the entire volume, since a large number of particles are out of focus. Instead, the particles in one chosen focal plane (and within five marked squares) were counted for each solution, and relative concentrations were determined. The counting was repeated twice for each stock solution, and an average value was obtained. The percentage of polystyrene beads, silica particles, and spores in the mixture was determined to be 25, 17, and 58 (%), respectively.

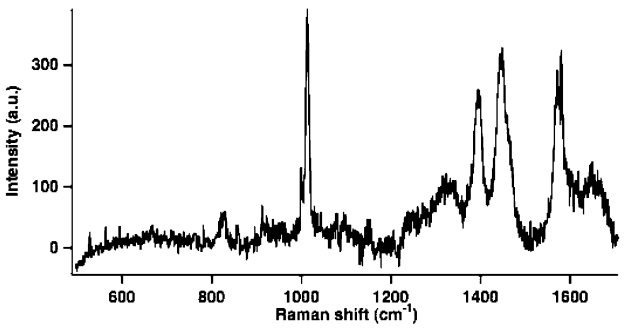

Figure 2. Background-corrected Raman spectrum of an individual, optically trapped *Bacillus cereus* spore. The probe power was 3 mW at 488-nm excitation, and the spectrum acquisition time was 20 s.

RESULTS AND DISCUSSION

Single Spore Identification. Figure 2 shows the Raman spectrum of a 1-μm-diameter optically trapped *Bacillus* spore in water, obtained with 488-nm excitation with an acquisition time of 20 s. A broad background signal due to autofluorescence was subtracted by fitting the spore Raman spectrum with a third-order polynomial baseline to obtain the final spectrum shown in Figure 2. The Raman signals are similar to that of a previous study on micro-Raman spectroscopy of bacterial spores immobilized on a CaF_2 substrate.[31] Strong Raman bands at 1013, 1395, 1445, and 1572 cm^{-1} and weaker vibrations at 822 and 658 cm^{-1} are assigned to calcium dipicolinate (CaDPA) and are in good agreement with previous studies.[27−29,31] Bands at 1245 and 1655 cm^{-1} are assigned to protein amide III and I vibrations, whereas the weak band at 1001 cm^{-1}, next to the strong 1013 cm^{-1} CaDPA peak, is assigned to phenylalanine. Individual spores tend to vary slightly in their Raman peak intensity ratios due to slight differences in their chemical concentrations; however, it is the location of the frequency shifts that is important in this case for spore identification. For exposure times longer than 20 s, a laser-induced modification to the bacterial spore was observed, and all Raman signals of CaDPA disappeared, while the protein-bands remained unchanged. We hypothesize that this change is a result of laser heating and damage of the spore followed by the release of DPA. Similar spectroscopic changes have been previously observed[32] for spores autoclaved at over 100 °C. Other mechanisms, such as photoinduced activation of the spore (release of DPA) followed by a transition to a vegetative cell over much longer times, are also plausible. Additional experiments are currently under way to elucidate the laser-activated changes and will be reported in a future publication.

Rapid Identification of Particles in Solution. For rapid identification of the particles, an integration time as short as 5 s was found to be sufficient to obtain Raman signals with clear

(32) Grow, A. E.; Wood, L. L.; Claycomb, J. L.; Thompson, P. A. *J. Microbiol. Methods* **2003**, *53*, 221−233.

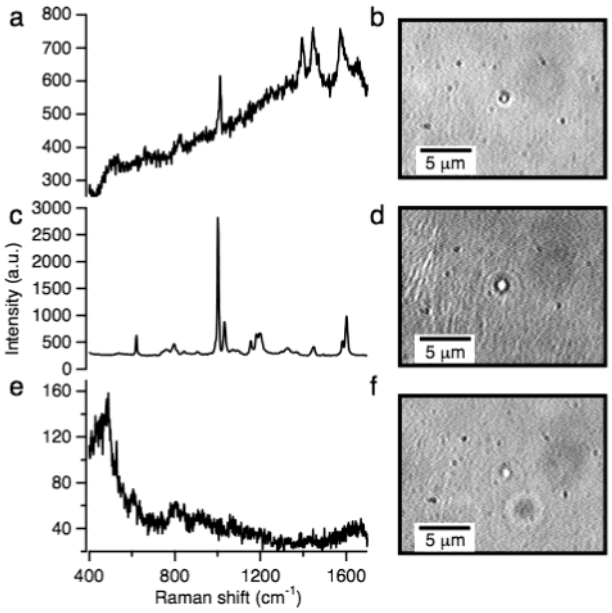

Figure 3. Raman spectra of a mixed sample of optically trapped (a) *Bacillus cereus* spores, (c) polystyrene microspheres, and (e) glass microspheres shown with their corresponding white light micrographs in (b), (d), and (f), respectively. All spectra were acquired with 3-mW laser power at 488 nm within a 5-s acquisition time.

features to identify the probed particles at 3-mW laser power. Figure 3 shows the raw Raman spectra taken with 5-s exposure time of a bacterial spore (Figure 3a), a polystyrene bead (Figure 3c), and a silica microsphere (Figure 3e), as well as accompanying white light images of the optically trapped particles (Figure 3b,d,f, respectively). Spectra for all three particles are consistent with previous Raman spectroscopy studies of these materials. For the bacterial spore, the spectrum shows the Raman signals as discussed above upon a broad autofluorescence background. The autofluorescence alone could be sufficient to discriminate the biological particle from the silica and polystyrene beads, but only the Raman bands provide enough information to accurately identify the composition of each particle without doubt. The polystyrene bead provides the strongest signal. The intense peaks at 621, 1001, 1155, 1450, and 1602 cm^{-1} are consistent with previous studies[14,22] on optically trapped polystyrene spheres. Although the silica beads yield the weakest Raman spectra, many characteristic Raman bands are still identifiable. The 490 and 605 cm^{-1} peaks are due to breathing modes of ring defects, and the broad 450 and 800 cm^{-1} bands are attributable to vibrations of the glass network.[33,34]

Concentration Analysis of Particles. Upon demonstrating the ability to distinguish similarly sized particles on the basis of their Raman signatures using LTRS, we then evaluated the use of the LTRS technique to analyze a mixture of unknown particles and rapidly determine their relative concentrations. Figure 4 shows the experimentally determined values of the mixture's composition for three independent experimental runs. Also shown in the figure are the absolute compositions of the mixture as independently determined by using a hemacytometer and laser particle analyzer to analyze the stock solutions before they were mixed to produce the sample. The average number of particles identified from sampling 100 randomly chosen particles in all three separate runs were 27 ± 1 polystyrene beads, 17 ± 3 silica spheres, and 56 ± 3 bacterial spores. The variation is only due to the statistical sampling process, and all numbers are well within their statistical limits. These relative concentrations are very close to those obtained from the theoretical analysis of the stock solutions. These experimental data show that the LTRS method is a precise technique for achieving reproducible results and accurately determining the composition of an unknown mixture. This technique is very useful even for the analysis of samples with a wide range of different concentrations, because ultimately, the positive analysis of even a single particle (e.g., bacterial spore) is enough to determine its presence in the mixture.

Our results indicate that LTRS provides a feasible method for the rapid and reagentless analysis of both the composition and component concentrations of an unknown mixed sample. The entire LTRS procedure for analyzing and identifying 100 particles took ~10 min (5 s for Raman acquisition from each particle and a few seconds to release and trap the particles). This time can be greatly reduced by automating the setup and by using higher laser powers, which would reduce the spectral acquisition time. For example, increasing the laser power by a factor of 10 would decrease the acquisition time for each spectrum to the order of hundreds of milliseconds. The Raman spectrum taken of a particle serves as a molecular fingerprint that, when compared with a database of Raman spectra, can be used to positively identify the particle. Sampling of an adequate number of particles in a mixture allows the composition to be reconstructed. Examples of other comparable techniques that allow rapid identification of individual particles and estimating concentrations without sample preparation are mass spectrometry and fluorescence spectroscopy, but both techniques have their own shortcomings. Mass spectrometry is a destructive technique, and not all particles can be analyzed using this technique because more robust particles, such as silica spheres, will not easily disintegrate. Fluorescence spectroscopy provides limited information about the analyzed material because its broad spectrum does not contain sufficient details to make unequivocal identification. Commercially available fluorescence particle analyzers can distinguish the presence of biological particles from inanimate materials on the basis of their autofluorescence, but it cannot distinguish between inanimate materials or determine exactly what biological particles are present. Other techniques, such as fluorescence flow cytometry, require prior knowledge of the biological sample so that it can be fluorescently tagged or also depend on autofluorescence. The LTRS technique described in the present article eliminates the need for specific tagging.

A potential application of this technique involves the instantaneous, reagentless detection of bacterial spores. The main techniques for spore identification involve microbiological methods which require time-consuming sample preparation involving the use of a variety of reagents.[35,36] Currently, the "gold standard" for bacterial spore identification is the growth of cell cultures of such samples, which takes several days. An example of a novel rapid technique requiring no reagents or sample preparation has recently been demonstrated with mass spectrometry for the

(33) Pasquarello, A.; Car, R. *Phys. Rev. Lett.* **1998**, *80*, 5145−5147.
(34) Geissberger, A. E.; Galeener, F. L. *Phys. Rev. B* **1983**, *28*, 3266−3271.
(35) Delehanty, J. B.; Ligler, F. S. *Anal. Chem.* **2002**, *74*, 5681−5687.
(36) Stratis-Cullum, D. N.; Griffin, G. D.; Mobley, J.; Vass, A. A.; Vo-Dinh, T. *Anal. Chem.* **2003**, *75*, 275−280.

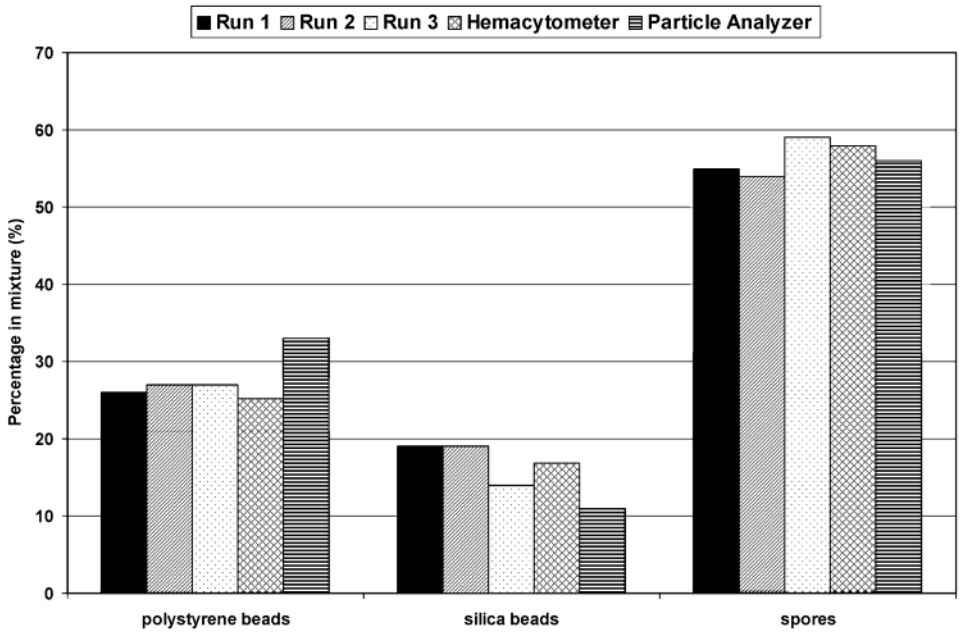

Figure 4. Relative number of particles in the mixed sample as determined by their Raman spectra in three separate, independent runs. Also shown are the theoretical compositions based on analysis of the stock solutions using a hemacytometer and a laser particle analyzer.

detection of individual airborne bacillus spores.[37] Additionally, fluorescence spectroscopy of bioaerosols has been demonstrated[38,39] as a reagentless technique, but differentiating pathogens from other background materials on the basis of their fluorescence spectra is difficult if not impossible. The optical tweezers micro-Raman spectroscopy method discussed in this article provides an optical method for reagentless identification of spores, offering the ability to rapidly identify completely unknown samples as bacterial spores or background particles in native environments. Furthermore, there is a zero false positive rate for distinguishing spores from background materials due to the uniqueness of the Raman spectral features to the particular materials. The LTRS technique for reagentless identification of spores and other pathogens could be automated and acquisition time drastically reduced by combining it with an apparatus analogous to a flow cytometer. One can envision a stop-flow scheme in a microchannel in which flowing particles are trapped by a high-powered laser. When a particle is detected via laser scattering, flow would be turned off as the laser traps and spectra are obtained of the particle. Within milliseconds, flow is turned back on to push the particle out of the trap, and this process is repeated until sufficient data are collected. High signal-to-noise measurements can still be obtained despite the low spectral acquisition times by increasing the laser power and improving the detection capabilities of the system. In addition, changing the laser wavelength to the near-infrared would minimize laser damage to optically trapped biological samples.[14]

CONCLUSION

Optical trapping combined with micro-Raman spectroscopy has been used to rapidly obtain spectral information from single bacterial spores in aqueous environment. The technique eliminates the time-consuming sample preparation of fixing spores onto substrates. The technique has been demonstrated as a new method for particle analysis, using the inherent Raman signatures of materials to identify particles in solution. The concentration of a mixture of polystyrene beads, silica spheres, and spores has been determined using this technique and compared to theoretical values. Our work has demonstrated the feasibility of using this technique to rapidly identify different particles in solution, which has potential applications in a flow cytometric-type analysis. Whereas flow cytometry relies on sample particles with autofluorescence, fluorescent dyes, or fluorescently tagged antibodies, we demonstrate in our work the use of the unique Raman signatures of particles as the intrinsic label by which different particles can be identified and sorted, thus eliminating the need for prior sample preparation. Our results show promise for the development of a Raman flow cytometry technique incorporating Raman optical tweezers with a flow cytometric-type system for direct applications in particle identification and concentration analysis or rapid cancer cell screening.

(37) Ullom, J. N.; Frank, M.; Gard, E. E.; Horn, J. M.; Labov, S. E.; Langry, K.; Magnotta, F.; Stanion, K. A.; Hack, C. A.; Benner, W. H. *Anal. Chem.* **2001**, *73*, 2331−2337.

(38) Hill, S. C.; Pinnick, R. G.; Nachman, P.; Chen, G.; Chang, R. K.; Mayo, M. W.; Fernandez, G. L. *Appl. Opt.* **1995**, *34*, 7149−7155.

(39) Chen, G.; Nachman, P.; Pinnick, R. G.; Hill, S. C.; Chang, R. K. *Opt. Lett.* **1996**, *21*, 1307−1309.

ACKNOWLEDGMENT

We acknowledge Maurice Pitesky for providing us with the *Bacillus* spores and for the use of the laser particle analyzer. This work was performed under the auspices of the U.S. DOE by UC, LLNL Contract No. W-7405-Eng-48. This work was also supported in parts by funding from the National Science Foundation through the Center for Biophotonics Science and Technology. The Center for Biophotonics, an NSF Science and Technology Center, is managed by the University of California, Davis, under Cooperative Agreement No. PHY 0120999.

Received for review August 28, 2003. Accepted November 12, 2003.

AC0350155