Optical characterization of individual bio-aerosols

V. Sivaprakasam[1], J.W. Lou[2], M. Currie[1], J. Czege[2], and J. D. Eversole[*,1]

[1]*Naval Research Laboratory, 4555 Overlook Ave. S.W., Washington, DC 20375, USA.*
[2]*Global Defense Technology & Systems Inc., (at Naval Research Laboratory) 4555 Overlook Ave. S.W., Washington, DC 20375 USA.*

Our group at the US Naval Research Laboratory has experimentally investigated the optical properties of aerosol particles of biological composition, both ambient and laboratory-generated, for several years. This paper provides an update on recent laser-induced fluorescence results as well as a description of a new experiment to study angle-dependent Mueller matrix scattering elements starting this fall.

INTRODUCTION

The measurement method that has provided the most significant discrimination capability so far has been laser-induced fluorescence [1-6]. We are currently near completion of a 3-year program to develop mode-locked laser excitation, having pulse widths in the 0.5 ps range, and to evaluate its application to bio-aerosol classification. Comparison has been made of a custom-designed mode-locked fiber laser to commercially available Q-switched lasers in terms of the fluorescence and scattering cross sections for a variety of different aerosol particle compositions. The result shows that saturation and/or photo-bleaching effects are negligible for the fluence levels used for either laser type. Reasonable agreement of the two laser excitation sources was obtained. A potentially more significant result arises from the fact that such extremely short pulses create sufficiently high photon densities to make 2-photon transition pumping feasible. We have observed 2-photon excitation of UV emission with visible wavelength excitation. Measurements were made on different biological materials, each over a range of relevant particle sizes that yield quantitative 2-photon excitation/emission cross sections for bacterial samples for the first time.

In continuing efforts to develop optical measurement techniques that are applicable to a broader range of particle compositions than only those that exhibit fluorescence, we have recently gained approval to investigate feasibility of measuring selected Mueller matrix elements from single aerosol particles. If polarimetric scattering signatures of individual biological aerosol particles as a function of both scattering angles and incident wavelength could be obtained, it may be possible to correlate these signature patterns to the structure and composition of the particles, providing a basis for classification, and discrimination among different particle types.

* Corresponding author: Jay D. Eversole (jay.eversole@nrl.navy.mil)

MODE-LOCKED LASER 2-PHOTON EMISSION RESULTS

A previously fabricated apparatus for measuring fluorescent emission in multiple discrete spectral bands using commercially available Nd:YAG lasers frequency summed to the UV wavelengths of 266 and 355 nm was modified to accommodate a newly developed Yb-doped fiber laser. By switching between these two alternative sources, data could be efficiently acquired on the same aerosol sample flowing through the interrogation chamber, and comparison of the Nd:YAG and Yb-fiber excitation data could be evaluated. Two experiments were performed in which: (1) the mode-locked fiber laser was frequency-quadrupled to 262 nm, and (2) the same laser fundamental was frequency-doubled to 524 nm. Comparison of the two types of UV laser sources in the first case showed that there was reasonable agreement in the fluorescence intensity obtained from a variety of aerosol sample materials once differences in the incident excitation intensity was taken into account. For the second arrangement, using the 524 nm laser operating at 41 MHz, the aerosol particles transit the focal thickness of the beam (55 micron) in about 18 microseconds and receive an effective fluence of 463 mJ/cm^2. The PMT gate width was set to sufficient time to integrate the entire signal. Figure 1, below, shows a scatter plot of fluorescent emission from particles of two different compositions: (a) *Bacillus atrophaeous* spore (BG) and (b) *Yersinia rohdei* (YR) and for two distinct particles sizes for each sample, as labeled. The data points are values of individual particles, and the x and y axes are the emission intensities in 80 nm-wide spectral bands centered at 350 and 435 nm respectively. Particles of kaolin (green dots), a non-fluorescent material, were included in both plots as a reference. One can see that the emission from the biological particles can be as much as an order of magnitude higher than the kaolin particles of similar size. Scatter of the data for the biological particles results predominantly from spatial variation of the particle trajectories in the excitation beam.

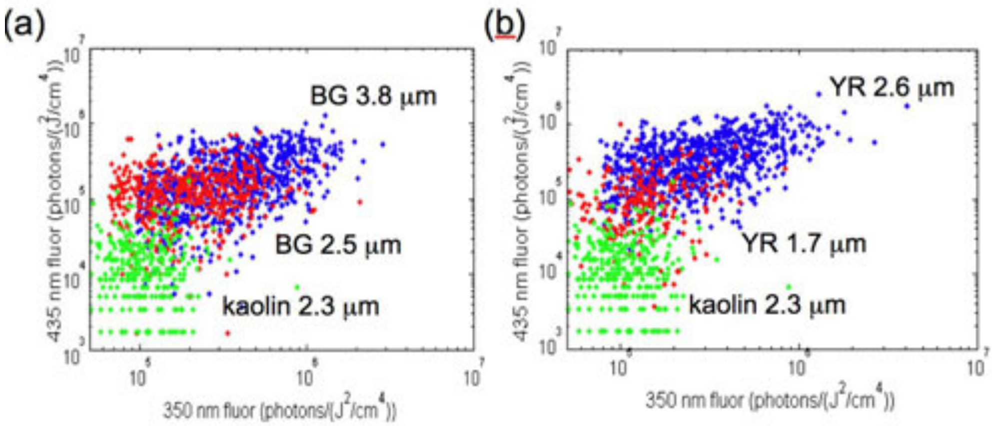

Figure 1. Shows a scatter-plot of 524 nm laser-induced emission from (a) *Bacillus* (BG) spore, and (b) *Yersinia rohdei* (YR) particles for a smaller (red) and larger (blue) particle size for each sample.

APPROACH FOR SINGLE PARTICLE POLARIMETRIC MEASUREMENT

A novel, highly parallel, detection scheme has been proposed that is a radical departure from previous, conventional approaches to the measurement of polarized scattering data. Successful demonstration of this new, rapid measurement approach will enable, for the first time, polarimetric characterization of individual free-flowing aerosol particles. Combining this experimental capability with state-of-the-art computational scattering models also creates, for the first time, an opportunity to use modern multivariate data analysis and pattern recognition methods to establish correlations between scattering data features and basic particle morphology and composition. Such quasi-empirical analytical approaches could lead to the use of scattering measurements to rapidly classify bioaerosol particles of interest.

Conventional methods for the measurement of polarized scattering involve using a single wavelength with a single detector scanned over many discrete angles. These traditional techniques have confirmed the potential utility of polarized scattering measurements for discriminating particles based on their size, shape and/or composition differences [7-8]. However, practical implementation of polarimetric scattering techniques for analysis of aerosol particles is not possible with such labor-intensive and time-consuming approaches.

A recently developed approach [9] in polarized scattering permits very rapid, multiple-angle and multiple wavelength elastic scatter measurements to be performed on ensembles of particles (organisms) suspended in solution. The approach is based on using an array of optical fibers, an imaging spectrometer and a focal plane array to instantaneously create a two-dimensional snapshot of scattering data as a function of both wavelength and scattering angle. In principle, all the parameters needed for inversion analysis of the particles' size, shape and composition can be measured simultaneously. Our plan to modify this multiple angle, multiple wavelength experimental method to be able to interrogate individual aerosol particles has been approved, and this effort will begin this fall. Preliminary calculations predict that single-particle measurements are feasible by modifying the new approach to incorporate currently available supercontinuum light sources, and intensified CCD arrays.

Planned initial studies will be conducted on known (nonspherical) particles in suspension and validated with models based on T-matrix, discrete dipole approximation (DDA) or other established computational scattering methods for ensembles of particles, averaged over all orientations. These selected samples will be studied to determine which scattering matrix parameters contribute the greatest degree of discrimination, as well as establish a predictive computational model framework for conducting sensitivity analyses.

The next step will be to extend the technique to interrogate individual aerosol particles. We will start with engineered particles of known geometry and composition, and validate orientation-dependent models using experimental measurements. To date, we are aware of only one prior, but recent, study of polarimetric scattering from a homogeneous aerosol sample (incorporating ensemble orientational averaging) [10]. If successful, our proposed study will collect polarized scattering measurements from individual, freely-suspended aerosol particles. Collecting data on different matrix parameters for both polar and azimuthal angles simultaneously will hopefully lead to discovery of appropriate combinations of measured parameters that will decouple the influence of the particle orientation, and permit particle classification on the basis of its general shape and composition into broad categories.

Recent modeling studies have shown that the depolarization ratio at near backscattering angles are strongly dependent on the particles' aspect ratio, while the periodicity of the backscatter is a strong function of the incident wavelength [11]. This feature has not yet been exploited experimentally, and may be especially relevant for biological particles such as *Bacillus* spores.

Ultimately, a basic understanding of the connection between particle size, shape and composition, and its polarimetric two-dimensional angular scattering profile as a function of wavelength could lead to enhanced capabilities for real-time bioaerosol agent detection.

REFERENCES

[1] R.G. Pinnick, S.C. Hill, P. Nachman, J.D. Pendleton, G.L. Fernandez, M.W. Mayo, and J.G. Bruno. Fluorescent particle counter for detecting airborne bacteria and other biological particles. Aerosol Sci. Tech. **23**(4) (1995).

[2] M. Seaver, J.D. Eversole, J.J. Hardgrove, W.K. Cary Jr., and D.C. Roselle. Size and fluorescence measurements for field detection of biological aerosols. Aerosol Sci. Tech. **30**(2) (1999).

[3] F.L. Reyes, T.H. Jeys, N. R. Newbury, C.A. Primmerman, G.S. Rowe, and A. Sanchez. Bio-aerosol fluorescence sensor. Field Anal. Chem. and Technol. **3**(4-5) (1999).

[4] Y-L Pan, J. Hartings, R.G. Pinnick, S.C. Hills, J. Halverson, and R.K. Chang. Single particle fluorescence spectrometer for ambient aerosols. Aerosol Sci. Tech. **37**(8) (2003).

[5] P.H. Kaye, J.E. Barton, E. Hirst, and J.M. Clark. Simultaneous light scattering and intrinsic fluorescence measurement for the classification of airborne particles. Appl. Opt. **39**(21) (2000).

[6] V. Sivaprakasam, A. Huston, C. Scotto, and J. Eversole. Multiple UV wavelength excitation of bioaerosols. Optics Express **12**(19) (2004).

[7] B.V. Bronk, S.D. Druger, Jozsef Czege, and W.P. Van DeMerwe. Measuring Diameters of Rod Shaped Bacteria in Vivo with Polarized Light Scattering. Biophysical J. **69** (1995).

[8] W.S. Bickel, J.F. Davidson, D.R. Huffman, and R. Kilkson. Application of polarization effects in light scattering: A new biophysical tool. In: *Proceedings of the National Academy of Sciences of the Unites States of America* **73**(2) (1976).

[9] Czeke and B.V. Bronk. Process and apparatus for measurements of Mueller matrix parameters of polarized light scattering. International Patent application PCT/US2006/03846 (2006).

[10] J.M. Richardson and J.C. Aldridge. The standoff aerosol active signature testbed (SAAST) at MIT Lincoln Laboratory. In: *Chemical and Biological Standoff Detection III (Proc. SPIE 5995)* (2005).

[11] D.D. Duncan and M.E. Thomas. Particle shape as revealed by spectral depolarization. Applied Optics **46**(24) (2007).