The Quest for Detection and Identification of Bio-aerosols

Richard K. Chang[1], **Gustavo E. Fernandes**[1], **Yong-Le Pan**[1], **Kevin Aptowicz**[2] **and Ronald G. Pinnick**[3]

[1]Department of Applied Physics and Center for Laser Diagnostics, Yale University
New Haven, CT 06520-8284, USA
[2]Department of Physics, West Chester University, West Chester, PA 19383, USA
[3]US Army Research Laboratory, Adelphi, MD 20783, USA

Abstract— A brief review is made of the status of fluorescence techniques to detect and partially identify bio-aerosols. The potential and frustrations in extracting morphology information from the angularly-resolved elastic scattering pattern is summarized. The latest advancements in the measurement of angularly-resolved elastic-light scattering for single aerosol particles on-the-fly are surveyed. Special emphasis is placed on our more recent efforts to simultaneously measure the scattering patterns of aerosol particles in both the forward and backward hemispheres.

In recent years, the quest for bio-aerosol detection and identification has been an active field for research and development. The detected fluorescence spectra, either at two-bands or at 32-bands, and excitation either at one wavelength or two wavelengths, has provided the ability to classify whether an aerosol particle (in the 1–10 μm range) is bio- or not bio- [1]. A number of ambient aerosols have similar or even identical fluorescence spectra of BW particles. Hence, false positive identification occurs, leading to false alarms. There is a real need for additional identification diagnostic, hopefully all optical, techniques. Alternatively, there are biochemical techniques such as specific binding of antibody with antigens or RNA gene sequencing using μ-fluidic cells or lab-on-a-chip.

Among the optical techniques, FTIR and Raman scattering (resonance Raman, surface enhanced Raman, and ordinary Raman) are two hopeful candidates for more specific identification of a suspect particle. However, both these techniques require special substrates to be covered with aerosol samples. Because of their possible interference effect, it is desirable to have only biological samples devoid of non-biological samples such as Arizona road dust or organic soot particles. In this regard, we have developed a sorting technique that enriches the biological aerosol sample to deposit on the surface of the substrate. Cued by the pre-determined fluorescence spectra of a particle as it travels downstream, a puffer sends out a short-duration pulse of air to deflect that particle from the main stream of particles. In order to localize the deflected particle into a small area on the substrate, a pulse aerodynamic localizer (PAL) concentrates the deflected particles. Using a biochemical technique, we could aim the PAL to inject deflected bio-aerosols into the reservoir well of a μ-fluidic cell [2].

It has been frustrating to know that elastic scattering, i. e., angularly resolved elastic scattering intensity pattern, has the strongest signal in any optical technique [1]. Elastic scattering (at some angle averaged over a small range of angles) is mainly used for measuring the aerodynamic equivalent sphere size of any particle regardless of whether the particle is spherical or not. The pioneering work of Paul Kaye and associates [3, 4] made use of the different angular patches of the angular scattering pattern to determine the degree of symmetry, the size, and to some extent the shape. Specially shaped photo detectors were used to provide quasi-real time data on the angularly integrated intensity in different discrete angles. Therefore, Kaye has combined the power of angularly resolved intensity patterns with image processing techniques when he used different shapes of detectors. He progressed further by using an ellipsoidal mirror, thereby capturing the angularly resolved intensity pattern (over a large fraction of 4π solid angle) on a CCD camera.

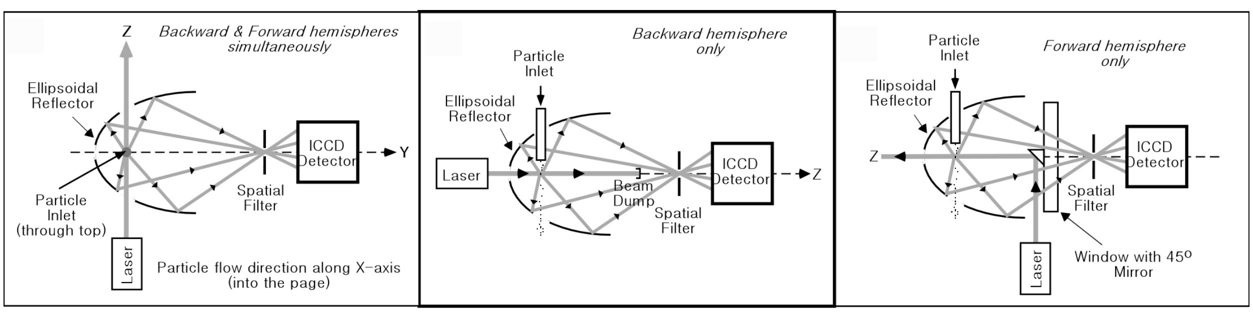
We went the next step and converted the XY coordinates of the CCD camera to angles θ and ϕ. In this paper we will go into depth on the angularly resolved intensities in the forward and backward hemispheres. However, this cannot be done in real time because it takes too long to read the CCD information into a standard computer as the inversion process is at best tedious and can give rise to particle size restrictions. Compromising on the large number of CCD pixels, a multi-anode photomultiplier (8×8 anodes) may be used to provide 64 channels of angularly integrated intensity patterns. Therefore, we need to give up resolution for speed. Theoretical or computational backing from the image processing and electromagnetic computation communities are essential for the future development of this technique.

The comparison of experimental and theoretical results is frustrating because experimentalists tend to work in larger size parameter domain while theorists tend to work in smaller size parameter domain due to computer time restrictions Therefore, computation on multiple scattering and particle interactions cannot be fully verified by experiments. The inability to control and to know the orientation of the non-spherical particles with respect to laser direction and the cluster geometry makes it nearly impossible to compare theory with experiments. How soon will experiment converge with theory?

In spite of some of the weaknesses and complications of elastic scattering techniques, there is still hope that the angularly resolved scattering intensity patterns can provide some information on a particle's morphology. This additional information gleaned from elastic scattering may prevent many of the causes for false alarms in the present detection instruments. For example, soot and BW agents have similar fluorescence spectra, but very different morphology. We will describe in some detail the experimental information that could be obtained thus far in the hope that theorists will take notice and take interest in this rich informative data set covering almost all 4π solid angle.

Two-dimensional $I_s(\theta, \phi)$ Angular Optical Scattering (TAOS), has been investigated as a possible diagnostic tool for the characterization of aerosols in the respirable range (1–10 μm in diameter) [4, 5]. TAOS has the potential of being useful in rapid characterization of biowarfare and other aerosols, because it is sensitive to a particle's size, morphology and complex index of refraction. Thus TAOS should provide complementary information to that extracted from other real-time aerosol measurement techniques, e. g., single-particle fluorescence, or laser induced breakdown spectroscopy (LIBS). In addition, TAOS can be measured with a CCD camera in quasi real-time even for submicrometer-diameter particles that are illuminated by laser diodes, thus opening the possibility for compact, low-cost particle detection and characterization systems.

Previous studies [3, 5, 6] measured TAOS patterns for a variety of aerosols, including micro fibers, salt and ice crystals, fluid droplets, aggregates of polystyrene-latex (PSL) spheres, bioaerosol particles, and ambient atmospheric aerosols. In these studies, TAOS was measured either in the forward hemisphere alone (Fig. 1(b)) or in the backward hemisphere alone (Fig. 1(c)). It was previously noted [5] that the scattering pattern of large aggregates of aerosols such as BG spores and PSL spheres contained a larger number of high frequency peaks and valleys termed *islands* or *speckles*.



(a) (b) (c)

Figure 1: (a) Top view of the setup used to simultaneously collect the scattered radiation in the TAOS patterns of single aerosols on-the-fly in the forward and backward hemispheres. A single 30 ns laser pulse at 0.532 μm illuminates the particle. (b) and (c) Side view of the setup used in previous experiments that collected TAOS patterns in a single (*backward only* or *forward only*) hemisphere.

In a more recent experiment [7], we measured TAOS patterns of various single aerosols collected *simultaneously* in both the forward and backward hemispheres. A new configuration was used based on the illumination geometry shown in Fig. 1(a). The particle travels along the y-axis and is irradiated by a laser pulse propagating along the z-axis. Unlike previous experiments, the symmetry axis of the ellipsoidal reflector (Opti-Forms), oriented along the x-axis, is *perpendicular* to the direction of laser propagation. The scattering event occurs at the first focal point of the truncated-ellipsoidal reflector. A large portion (63% of the 4π sr) of the light that is scattered by the particle is intercepted by the reflector and projected onto the intensified CCD detector (ICCD)-1024×1024 pixels, Andor iStar. Half of the ICCD detector detects the forward-scattering pattern and the other half detects the backward-scattering pattern.

TAOS is measured for the scattering angles in the range $15° < \theta < 165°$ and for azimuthal angles covering as much as 360° in the near-forward and near-backward scattering. The full range of azimuthal angles ($0° < \phi < 360°$) is collected for all θ except where the reflector has parts removed by truncation of the ellipsoid and five holes drilled through the reflector. The particles enter through the top hole at $\theta = 90°$, $\phi = 270°$, and exit through the bottom hole at $\theta = 90°$, $\phi = 90°$. The laser beam enters through a side hole centered at $\theta = 180°$ and exits through a side hole at $\theta = 0°$. Lastly, there is the fifth hole in the back ($\theta = 90°$, $\phi = 180°$) used for the passage of the trigger laser diode beam.

The TAOS recorded on the ICCD were transformed into $I_s(\theta, \phi)$ in the spherical coordinates θ and ϕ by means of a ray-tracing computer code. As always, the z-axis was defined as the direction of the laser beam as in Fig. 1(a). The Log of the measured intensity $\mathrm{Log}_{10}(I_s(\theta, \phi))$ was plotted into two separate parts, corresponding to the forward $15° < \theta < 90°$, and the backward $90° < \theta < 165°$ hemispheres, as shown in Fig. 2. On the top row of Fig. 2, the recordable angle range of the TAOS patterns is marked for the spherical coordinates. The shaded areas represent the areas that are lost due to open space and the 5 holes.

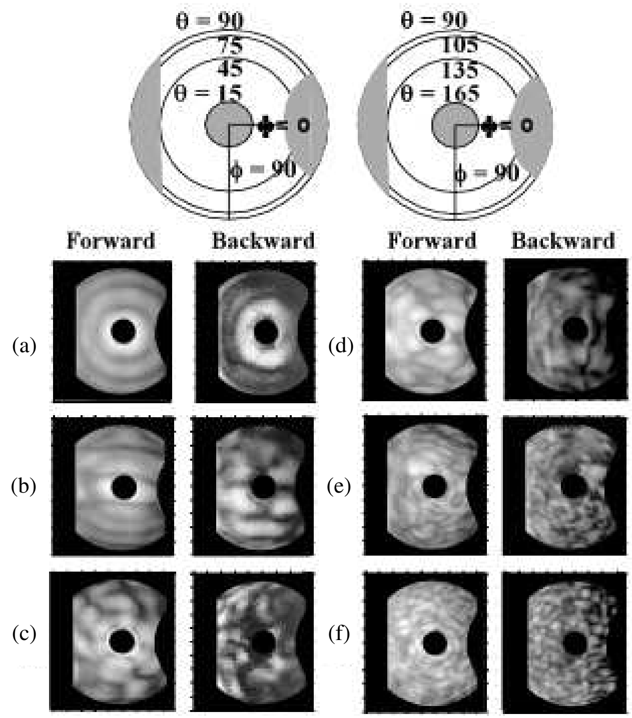


Figure 2: Simultaneously measured forward and backward TAOS patterns for randomly oriented clusters of PSL spheres. The clusters were illuminated by a 30 ns pulse of the second harmonic of an Nd-YAG laser at 0.532 µm. The black circle in the center of each pattern corresponds to holes on the ellipsoidal reflector used for the passage of the illuminating laser beam.

Figure 2 shows the forward and backward TAOS patterns for particles generated by putting 1.44-µm-diameter PSL spheres in the inkjet aerosol generator. Each pair of patterns labeled (a) through (f) correspond to the simultaneously measured forward (left) and backward (right) hemisphere scattering patterns. By varying the concentration of the solutions in the particle generator, the mean number of spheres per cluster was varied from 1 (row a) to 6 particles (row f). The TAOS patterns were generated for random orientations of the particle symmetry axis relative to the beam axis. Thus several different patterns were obtained from a given concentration of PSL spheres placed in the aerosol generator. The patterns displayed here were chosen among the many different patterns obtained in each data sample. An increasing density of islands in the scattering patterns is observed here [5] as the mean number of particles per cluster is increased in the scattering patterns shown in Figs. 2 (a)–(f).

Figure 3 shows the scattering patterns obtained for single *B. subtilis* spores. Because the BG spore has an elongated shape, resembling a small cylinder 1 m in height and 0.5 µm in diameter, its orientation relative to the illuminating laser beam axis affects the scattering pattern. Shown in

Figs. 3 (a)–(d) are measurements of the scattering pattern obtained simultaneously in the forward and backward hemispheres for four random orientations of the BG spore relative to the illuminating laser beam.

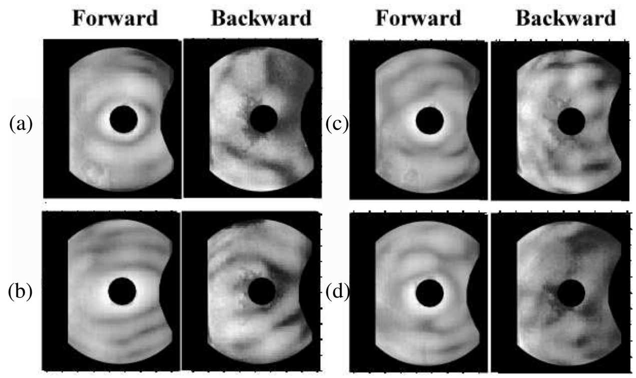


Figure 3: Simultaneously measured forward and backward TAOS patterns from single, randomly oriented, *B. subtilis* spores. The spores were illuminated by a 30 ns pulse of the second harmonic (at 0.532 μm) of a Nd-YAG laser.

The most recent efforts in measuring the angularly-resolved elastic-light scattering (TAOS) pattern of single aerosol particles on-the-fly were surveyed. A new configuration was presented that allows the measurement of TAOS patterns simultaneously in both the forward and the backward hemispheres for aerosols on-the-fly. Results were presented, for single and clusters of polystyrene latex spheres and single *B. subtilis* spores. We expect that this new configuration presented here will lead to a more complete characterization of individual aerosol particles than that could be obtained with TAOS measured over a single hemisphere.

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