Automated aerosol Raman spectrometer for semi-continuous sampling of atmospheric aerosol

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Abstract

Raman spectroscopy (RS) is useful in characterizing atmospheric aerosol. It is not commonly used in studying ambient particles partly because automated instrumentation for aerosol RS has not been available. Battelle (Columbus, Ohio, USA) has developed the Resource Effective Bioidentification System (REBS) for automated detection of airborne bioagents based on RS. We use a version of the REBS that measures Raman spectra of one set of particles while the next set of particles is collected from air, then moves the newly collected particles to the analysis region and repeats. Here we investigate the use of the REBS as the core of a general-purpose automated Aerosol Raman Spectrometer (ARS) for atmospheric applications. This REBS-based ARS can be operated as a line-scanning Raman imaging spectrometer. Spectra measured by this ARS for single particles made of polystyrene, black carbon, and several other materials are clearly distinguishable. Raman spectra from a 15 min ambient sample (approximately 35–50 particles, 158 spectra) were analyzed using a hierarchical clustering method to find that the cluster spectra are consistent with soot, inorganic aerosol, and other organic compounds. The ARS ran unattended, collecting atmospheric aerosol and measuring spectra for a 7 hr period at 15-min intervals. A total of 32,718 spectra were measured; 5892 exceeded a threshold and were clustered during this time. The number of particles exhibiting the D-G bands of amorphous carbon plotted vs time (at 15-min intervals) increases during the morning commute, then decreases. This data illustrates the potential of the ARS to measure thousands of time resolved aerosol Raman spectra in the ambient atmosphere over the course of several hours. The capability of this ARS for automated measurements of Raman spectra should lead to more extensive RS-based studies of atmospheric aerosols.

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1. Introduction

1.1. Importance of atmospheric aerosol particles and their size and composition

Atmospheric aerosols can cause diseases, degrade visibility, and affect the weather and global climate. Atmospheric aerosols are associated with increased cardiovascular disease, chronic obstructive pulmonary disease, and other illnesses [1]. Many harmful components of aerosols are known, e.g., carcinogens such as benzo[a]pyrene and other polycyclic aromatic hydrocarbons in soot, or heavy metals such as mercury in emissions from coal-fired power plants. However, there are many unknowns regarding the compositions and sizes of particles that contribute most to the harmful effects of aerosols on health [2–4]. Some diseases of humans (e.g., [5]), other animals (e.g. [6]) and many plant pathogens...
are transmitted through the air by fungal spores, bacteria, and viruses. Aerosols affect climate and weather by scattering, absorbing, and reemitting radiation, and acting as cloud \[8\] and ice \[9\] condensation nuclei. Bioaerosols such as bacteria and fungal spores may be significant contributors to ice nucleation under certain conditions \[10,11\]. There are large uncertainties regarding the effects of biological and other aerosols on climate \[12\]. As a result, improved techniques for characterizing atmospheric aerosol size and composition are needed for a better understanding of the effects of aerosols on health, agriculture, visibility, climate, and weather.

To address these needs, improved instruments that can run continuously and provide information on the composition and size of individual aerosol particles are needed. Single-particle measurements are important for quantifying minority species in a population, determining the mixing state of aerosols \[13,14\] and its relation to climate forcing \[15\], and understanding the processing of particles in the atmosphere. Examples of single-particle instruments include aerosol mass spectrometers \[16\], aerosol fluorescence spectrometers \[17,18\], and instruments which measure Raman spectra \[19\] or electron-beam excited x-rays \[20\] from collected particles. The topic of this paper is Raman spectroscopy (RS) for deriving information about single particles in the atmosphere using an automated measurement method.

1.2. Raman spectroscopy for characterization of atmospheric aerosols

Because the intended audience for this paper includes researchers who may have little familiarity with RS, we state here some of the features that make RS appealing for single-particle, and even semi-continuous, characterization of atmospheric aerosol:

a) Raman spectra have a relatively high information content because, like infrared (IR) absorption spectra, they are dependent on the vibrational and, when applicable, rotational frequencies of the molecules in a sample. Typically, many or most of the vibrational bands in IR spectra also appear in Raman spectra. In RS, an electron, in a ground state with frequency \(\omega_0\), is excited by a laser with photon energy \(h\omega\), where \(h\) is the Planck constant and \(c\) is the speed of light, to an intermediate transient state. The excited electron almost immediately drops to an energy level with vibrational frequency \(\omega_v\). To conserve energy, a photon is emitted having an energy \(h\omega = h\omega_0 - hc\Delta\omega\), where \(\Delta\omega = \omega_v - \omega_0\). Vibrations that are very weak in IR spectra may be strong in RS and vice versa. For many pure materials with molecular weights less than a few hundred daltons, Raman and/or IR spectra can serve as fingerprints. RS can differentiate between many different bacteria \[21\], fungal spores \[22\], and pollens \[23\]. RS may also be useful for differentiating types of soot \[24\], a feature important in studies of climate and the health effects of aerosols. The large majority of materials have readily measured Raman spectra. Although pure alkali halides (e.g., NaCl, KF) have negligible Raman spectra, sea spray aerosols, which are primarily NaCl, contain other inorganic salts and biological and other organic materials that appear in Raman spectra \[25\]. The fluorescence of most pure materials, on the other hand, is negligible or very weak. There are limits on the information that can be obtained for complex mixtures using RS because weaker lines of primary compounds may overlap the strong lines of secondary compounds \[19\]. Similar limits apply when studying complex mixtures using other high-information content techniques such as mass spectrometry (MS), IR spectroscopy, or nuclear magnetic resonance spectroscopy.

b) Raman spectra can be generated using one laser and one detector array, where the photon energies of the laser and the detected photons are far from the energies of photons at the vibrational frequencies of interest. A single laser in the visible or near IR can generate a Raman spectrum from 200 to 4000 cm\(^{-1}\). If, for example, the Raman spectrum is generated using light at 640 nm, the detector must only be sensitive to light at wavelengths from approximately 640 to 860 nm in order to measure the Raman spectrum from 0 to 4000 cm\(^{-1}\). Laser sources in the visible and near IR are relatively inexpensive. Charge-coupled device (CCD) detectors throughout the visible and near IR can be sensitive with low noise.

c) Raman spectra can be measured from particles with volumes of less than 1 \(\mu\text{m}^3\), depending upon the material and the excitation and collection optics, because it is an inelastic scattering measurement. Raman microprobes, RS instruments with confocal, epi-illumination geometries, have been used for over 40 years \[26\]. IR absorption spectroscopy, in contrast, typically requires larger sample volumes. Attempts to develop techniques to measure the IR absorption by measuring and analyzing the elastic scattering patterns of individual particles have been made \[27\].

d) RS has relatively simple instrumentation requirements that are relatively inexpensive. RS only requires a narrowband laser, a good CCD, a spectrometer, imaging optics and optical filters, and a way to collect the aerosol and move the sample into the focal plane under the excitation-collection optics. It does not require sample preparation or high vacuum.

e) Raman spectra include contributions from the molecular vibrations of molecules throughout the particles, at least for particles with little or no black carbon or other particles that are not too large, e.g., greater than 30 \(\mu\)m, depending upon the excitation wavelengths. This ability to include information about molecules throughout the particle (when not too large or absorbing) arises because Raman spectra can typically be measured with excitation and emission wavelengths where most of the light is not absorbed by the particle.

f) The measured Raman spectrum is the sum of the Raman and fluorescence emission and therefore can also provide information about molecules that are fluorescent at the excitation wavelength. Although fluorescence spectra are broad and lack the informational content of Raman, we suggest that their combination with RS can be useful, especially regarding bacteria, pollens, and
fungal spores. It can also be used to help determine regions to investigate further using RS. Because the fluorescence peaks are typically much broader than Raman peaks, they can often be estimated and subtracted from the Raman spectrum. The Raman signal relative to the fluorescence depends upon the particle’s composition and the excitation wavelength. The fluorescence can obscure the Raman spectral features if it is too much larger than the Raman spectrum and does not photobleach sufficiently within the time available for the measurement.

g) RS is a mostly non-destructive technique, as long as the laser intensity is kept below a threshold to avoid damaging the sample and the laser wavelength is not too short [28]. Thus, after the Raman spectra are measured, it is possible to further analyze the particles using, e.g., antibodies, DNA hybridization, MS, or other techniques. However, many fluorophores are sensitive to light and may be photobleached by the laser (Appendix A).

Because of the advantages listed above, RS has been used to determine the composition of atmospheric aerosols [19,28–36] including particles containing black carbon [28,33,34] and/or organic carbon and/or inorganic materials [19,32]. Raman spectra can also aid in characterizing atmospheric fungal spores [22], pollens [37], and bacteria [21,38,39]. RS has been used to help understand the composition of desert dust [13,40], sea spray [25], and oceanic aerosols [32], as well as chemical reactions in aerosol droplets. Raman spectra obtained in these studies have been used to assign particles to categories such as bacteria, black carbon, etc. (e.g. [31]). Single-particle Raman spectra have been used to analyze the properties of carbonaceous aerosols in Europe [34] and ambient volcanic ash particles [35], and identify chemical agent simulants [41]. Methods to assign measured Raman spectra to specific materials have been developed [42,21].

A main difficulty in using RS for studies of atmospheric aerosol is that Raman emission is typically several orders of magnitude weaker than fluorescence from the same quantity of highly fluorescent molecules. RS is approximately $10^{13}$ to $10^{14}$ times weaker than elastic scattering from micrometer-sized particles of the same volume and of magnitude weaker than elastic scattering. Therefore, a very sensitive Raman spectrometer is required to collect (or trap) particles from air, either onto a surface or into a trap that holds the particle in air [36,44], and then, if necessary, move it to the focal region of the measurement-collection optics [30,34]. Battelle (Columbus, Ohio, USA) has been developing instruments they term the “Resource Effective Biodentification System” (REBS). Some of these, but not the one described in the only open-literature publication [21], collect particles from air, measure their Raman spectrum, and then determine whether specific biological or chemical agents are in the particles.

1.3. This paper

The main objectives of this paper are to describe a REBS-based ARS and to suggest and illustrate how it appears to be useful for characterizing size-composition distributions of atmospheric aerosols. We describe the ARS we use and briefly discuss some of the Battelle’s software and some of our own. We illustrate the use of the ARS, initially with uniformly sized polystyrene latex (PSL) spheres. We illustrate the hyperspectral data-cube and spectra that are obtained using the instrument with carbohydrate, tryptophan, inorganic materials, soot-like particles, and some ambient aerosols. We analyze some ambient spectra measured with the ARS in two ways: unstructured hierarchical cluster analysis for a 15 min period; and the number of soot-like spectra in each 15 min period for a duration of 7 h. We also briefly discuss issues such as aerosol sample rate, analysis methodology, laser charring, and the ability to control the instrument in detail.

2. Experimental

2.1. The REBS-based ARS and its operation

The core of the ARS we use, designed and manufactured by Battelle, collects particles from air onto a metalized tape and then measures the Raman spectra of those particles while the next set of particles is collected. Battelle terms this instrument the Resource Effective Biodentification System (REBS), but also uses “REBS” for several other systems. A newer REBS is the Rapid Enumerative Biodentification System. In a chapter by Ronningen et al. [21], the only open literature scientific publication we know of describing a REBS, there was no mention of collecting particles from air or transporting them into a collection region on tape. Instead, samples were pipetted onto an aluminized glass slide which was placed under the automated microscope system. That chapter emphasized the spectral analysis methods used to discriminate between specific biogents and other aerosols [21]. Because there have been multiple variants of the REBS over the past 10 years, and because we do not use the “bioidentification system,” but instead use software we have written for characterizing atmospheric aerosols, we call our REBS-based automated Aerosol Raman Spectrometer an ARS.

The ARSS we describe here were made approximately 5 years ago. We are not sure of the ways they differ from presently available REBSs. An ARS instrument used in this study is illustrated in Fig. 1. The dimensions are $53 \times 36 \times 36$ cm. The instrument weighs 20 kg. The outer covering is rainproof. We successfully operated the ARS in rain and high winds.
In the collection operation, the particles are drawn into the ARS, where they are given a charge by a unipolar corona charger. The particles are drawn by electrostatic forces to a grounded metallized tape (13 mm width) on which they are collected. The collection flow rate is nominally 8 lpm and is determined by the pump, which draws the sample air through the ARS. The inlet tubing for the ARS is 1 inch in diameter. The particles are collected onto the tape in a spot of Δx~1 cm by Δy~1.6 mm.

For the spectral measurements, the ARS used here employs an epi-illumination image-preserving spectrograph. The excitation laser has a wavelength of approximately 640 nm and maximum laser power of ~50 mW. This laser is focused to a line with dimensions on the tape of Δx~1 μm and Δy~40 μm. The elastic and Raman scattering are collected by the same 100× objective used to focus the laser onto the particles. The spectrally dispersed emission is recorded by a 128×1024 pixel MITY CCD H7031 1007-DS-LE that is thermoelectrically cooled, typically to ~5 °C. The ~40-μm line parallel to the y-dimension on the tape is spread over approximately 40 of the 128 pixels in the y-direction of the CCD. Once the tape has been advanced (x-direction) from the collection region to the spectral measurement region, a vacuum pump holds the tape against a block at the top of a motorized x-z stage. Because the tape is flexible, the short segment of tape held to the stage can then be positioned in the x and/or z direction, while the segment of tape held at the collector does not move. The stage is then adjusted in the z-direction until the laser line is focused onto the tape as indicated by the laser intensity at the peak. If desired, a particle of interest could be studied further by stepping through several focal planes to obtain a z-scan. The first spectrum is then recorded. Then the tape, held by the x-z stage, is stepped (typically 2 μm) in the x direction and held fixed while the next set of (typically 40) spectra are acquired by the CCD. This procedure of step-tape-then-acquire-spectra is repeated until the (typically) 15-min time period is finished. If, for example, three 10-s spectra are taken at each of the 2-μm steps, the total time is approximately 30 s per step, and so in the 15 min time period, approximately 15/0.5 = 30 sets of spectra are obtained, and the laser was stepped over approximately 60 μm in the x direction.

In this paper, two REBS-based ARSs are used. One instrument (REBS serial number 13) is used for the laboratory studies discussed in this paper, including the calibration statistics in Section 3. The other instrument (REBS serial number 14) is used for the ambient studies.

2.2. Data handling and analysis procedures

The background spectrum is estimated in two ways. First, it is measured for a clean section of tape, before collecting any particles. Second, a time varying background is also calculated because the background can vary, possibly because the CCD is not sufficiently cooled when the ARS is operated in direct sunlight on a hot day. For each 15 min period, the background is estimated by taking the lowest tenth percentile of intensities measured in that
period at each wavelength.

Bad pixels in the CCD are discovered by comparing background spectra and spectra measured for broadly fluorescent materials, looking for positive or negative spikes, and then keeping track of the locations of these spikes. The intensities at these bad pixels are replaced by the average of the pixels on either side. Because multiple spectra are measured at each position of the laser, cosmic rays are removed by comparing the second derivative of each spectrum. In cases where the second derivative at any pixel is above a threshold in one spectrum but not the other, those locations are removed, along with one pixel on either side of those locations. This value is replaced by a linear fit between the values on either side of the removed pixels.

In this paper, fluorescence is only removed for the cluster analysis. All other spectra shown in this paper do not have fluorescence removed. Fluorescence backgrounds were also estimated using an Asymmetric Least Squares (ALS) algorithm [45]. ALS is similar to a least squares fit, but weights valleys more than peaks and uses an adjustable parameter to change the curvature of the fit.

Cluster analysis is done using R [46]. Spectra from the preprocessing are analyzed using the hyperSpec package [47]. Because large fluorescence values can cause features which may be artificial in the Raman spectra, we removed all spectra with fluorescence above 2.87 (the 75th percentile value of summed fluorescence), with a positive ratio of $<0.00075$ of maximum value after fluorescence removal to summed fluorescence. The goal is to remove spectra with high fluorescence values, which have low intensity peaks after the fluorescence has been removed. After cluster analysis, these spectra are added back in an additional cluster ‘Fluorescent’. The remaining spectra are only clustered if the maximum value is above 1% of the saturation intensity so that noisy spectra are not analyzed. Each spectrum to be clustered is normalized to its mean value. Distance was assessed using $R^2$ values between each of the spectra. The hclust routine in R is used with Ward’s method [48,49].

### 2.3. Air samples, test particles, and methods of aerosolizing particles

The primary location sampled was an unused asphalt road at the edge of a lawn and approximately 14 m from a two-lane road with often heavy traffic, 0.8 km from the Washington, DC beltway with 4 or 5 lanes of traffic in each direction and 1.1 km from the intersection of interstate highway I-95 with the Washington, DC beltway.

Samples were introduced into the inlet in three ways: i) for ambient sampling, the factory inlet with a black inverted cylinder with a rain shield (Fig. 1A) was used; ii) for dry materials, small particles were generated and suspended in air by agitating bulk particulates in a container, typically using a coffee grinder, then removing the lid of the container near the inlet to the ARS, and allowing the aerosols to be drawn into the ARS; and iii) for aqueous suspensions, e.g., PSL spheres, the suspension was nebulized and dried using a Royco nebulizer system. In some cases, the ARS also drew room air into the inlet throughout much of the collection period, but the number of test aerosol particles greatly exceeded the numbers of background aerosols that were also collected. For analysis of bulk samples, the tape was removed from the microcontroller stage and the sample was either placed on a microscope-slide cover or in a small plastic cup, either of which was then placed on the stage. The bulk samples placed on the slide or in the cup were sufficiently thick that spectral features of the plastic or glass could not be observed in the measured spectra.

### 3. Results with test particles

#### 3.1. Acquisition of Raman spectra and hyperspectral images

PSL spheres with a 2-μm diameter ($2.013 \pm 0.025 \, \mu m$) were used in our initial illustration of the ARS’s capabilities. Such spheres have been used extensively in characterizing aerosol instruments. The PSL spheres were aerosolized using a Royco nebulizer, collected on the tape by the ARS, and their Raman spectra were measured. Fig. 2a illustrates the Raman intensity vs. spectral shift for 38 pixels in the y-direction on the tape. The y direction is parallel to the axis of the focused laser line on the tape. It is also perpendicular to the stepping direction for building a hyperspectral image. The pixel size in the y direction (vertical in Fig. 2b) is approximately 1 μm. The Raman shift is approximately 3 cm$^{-1}$ per pixel. Only 38 of the 128 pixels at each shifted frequency are shown because the intensity of the illuminating laser line (and hence rows where Raman spectra can be measured) is small or negligible for the other pixels.

The strongest Raman line in Fig. 2a occurs at approximately 1000 cm$^{-1}$ and corresponds to the phenyl ring breathing mode [50]. This line is seen for two particles: one with a strong signal centered on line 26 and another with a weaker Raman intensity centered on line 3. The particle with the strong Raman peak at 1000 cm$^{-1}$ exhibits additional peaks associated with aliphatic and aromatic C–H stretch frequencies. Fig. 2b shows the sum of all of the Raman shift intensities for a given position on the laser line. The particle with highest Raman intensity can be seen with the intensity maximum at line 26. As indicated by the arrows in Fig. 2b, the full-width at half of the maximum value is 2.3 pixels, which is consistent with 2-μm particles and suggests that the two-dimensional cross-section of a particle can be estimated from the peak widths, and is consistent with the vertical bins having a resolution of approximately 1 μm/bin. Two-dimensional cross sections of some test particles and atmospheric particles are illustrated in Fig. 3 and in Section 4, respectively. A key point of this section is that the ARS, within a 10-s imaging time, measures clear Raman spectra of individual 2-micron-sized aerosol particles made of polystyrene.

To obtain the surface plot in Fig. 3, the laser line is stepped in the x-direction across the sample, taking a set of spectra, such as the one shown in Fig. 2a, at each position. In this paper, the length of the step of the laser is approximately 2 μm. Because the tape is stepped under the excitation/collection optics, acquiring approximately 40 Raman spectra at each step, an image can be built for each wave number band. Fig. 3 illustrates an image from
the collection period when Fig. 2 was taken, focusing on the 1000 cm\(^{-1}\) band. The interpolated sum of intensities between 995 and 1005 cm\(^{-1}\) is the value plotted at each \(x-y\) position. Each spectrometer image corresponds to a line of values along the ordinate of a graph. As the spectrometer steps in space, these line values are accumulated to produce a 2-dimensional array of values over this wavenumber band, as illustrated in Fig. 3. This surface plot in Fig. 3 suggests that the cross-sectional size and shape of particles could be estimated from ARS data for particles larger than approximately 1.5 to 2 \(\mu\)m, for example using the full-width at half-maximum (see Fig. 2b). Because the Raman intensity for particles of a given material should be approximately proportional to particle volume, for particles that absorb only little of the illuminating intensity, the relative uniformity of the peak heights, or the total intensity summed over the image of each particle, should also be useful for estimating particle size. Further work is needed to quantify how accurately the particle cross-sectional size can be determined from the images, and how this accuracy depends upon particle size, shape and composition (including Raman cross sections, inhomogeneity of the materials composing the particle, and the polarization-sensitivity of different Raman bands of the material). Although that work is beyond the scope of this paper, aspects of these problems and the problem of estimating particle volume are briefly discussed in Section 5.2.

Fig. 4 is our attempt to illustrate the high-intensity parts of the hyperspectral image, which includes the full set of the data. In Fig. 4 there is a whole spectrum for each illuminated point on the tape. Here, the image from Fig. 2a has been rotated 90°, so that the y-axis is parallel to the axis of the laser line-source. Also, the x-axis in Fig. 2a is now the vertical axis (labeled ‘Raman Shift (cm\(^{-1}\) )’). Then the other spectra similar to those in Fig. 2a, but obtained for different steps of the line source and collection optics position on the tape, are aligned parallel to this initial plane. Surfaces of constant intensity (arbitrary units) are indicated by blue, green, and red colors as intensity increases. Thus, as the laser steps along the tape, a 3-D matrix of values is assembled. This matrix has two dimensions in physical space, and the third dimension is the Raman shift. The phenyl ring breathing mode can be seen at the bottom of Fig. 4 for three PSL particles. The aromatic and aliphatic C–H stretches show up in red at the top of the graph. The particles have the appearance of ‘tubes’ that change diameter and shape as Raman shift
varies because of fluorescence, which spans many wavelengths. For inorganic particles with no fluorescence, these tubes are replaced by disks at the Raman emission lines.

3.2. Spectra of aerosols from test particles

Spectra of sucrose (table sugar), an example carbohydrate, are shown in Fig. 5. Dry, powdered sucrose was aerosolized near the instrument, and 12-s Raman spectra of the collected aerosol particles were recorded. Since the particle shown in Fig. 5 only appeared in two bins in the collected aerosol particles were recorded. Since the particle shown in Fig. 5 only appeared in two bins in the y direction, and on no other steps of the tape, we surmise that this particle was probably less than 2 μm in diameter. The example spectrum shown in Fig. 5a is dominated by a broad fluorescence, with many smaller Raman peaks superimposed. The fluorescence, estimated using an asymmetric least squares fitting algorithm, is shown in the smooth line in Fig. 5a. This fluorescence was subtracted from the total signal to obtain the Raman spectrum illustrated (green line) in Fig. 5b, which was rescaled to be of comparable magnitude to the 1064-nm excited reference sucrose spectrum (also in Fig. 5b) from the SPECARB database (http://www.models.life.ku.dk/~specarb/). The C-H stretch in the aliphatic region at approximately 2912 cm⁻¹ in the ARS spectra also occurs in the SPECARB (2913) spectrum and the sucrose spectrum in [51]. The strong peak near 850 cm⁻¹ is also observed in our spectrum (847 cm⁻¹) as are many other peaks [51]. Raman shifts from 15 peaks in Fig. 5 differ by −0.2 to 5 cm⁻¹ (mean=2.67 cm⁻¹) except for the low peak near 943 cm⁻¹ which had a difference of 6.6 cm⁻¹. The differences might be caused by impurities in the non-reagent grade sugar used in this experiment. The SPECARB resolution is listed as 4 cm⁻¹. The ARS has a resolution of approximately 3 cm⁻¹.

Light absorbing carbonaceous particles containing, e.g., soot, humic-like substances (HULIS), and some humic substances, exhibit two broad peaks centered around 1350 and 1600 cm⁻¹, in bands labeled D and G. These broad peaks are a combination of multiple narrower peaks including the graphite peak and several defect peaks [34]. Fig. 6a shows an example of a Raman spectrum dominated by these D and G peaks in an ambient sample taken downwind of a running gasoline-powered vehicle. Fig. 6b and c illustrate spectra of inorganic particles in Arizona Test Dust (ATD), (Powder Technology, Inc., Arden Hills, MN) that was aerosolized and then measured with the ARS. The spectrum in Fig. 6b exhibits peaks at 481, 510, and 1067 cm⁻¹ and is probably a particle with a combination of NaNO₃ (1067 cm⁻¹) and albite (479 and 507 cm⁻¹). The spectrum in Fig. 6c has peaks at 714 and 1087 cm⁻¹ which are consistent with calcite.

Spectra with D and G peaks are common in atmospheric samples [24,26]. Also, however, charring of organic particles by lasers has been observed [28]. Ault et al. [25] assumed that particles with D and G peaks in their sample of marine particles had been charred by the laser used to generate the Raman spectra. Such charring is more likely to occur with excitation wavelengths shorter than 540 nm instead of the 640 nm laser used here. The concerns of Ault et al. [25] and the presence of D and G peaks in spectra of atmospheric samples obtained with the ARS, raise the question that some HULIS/Soot type spectra may occur because of charring by the ARS. To look for evidence of charring of samples by the laser in the ARS, we tested the laser at full power on bulk samples of tryptophan, leaf litter, and Cladosporium herbarum, and did not see evidence of charring. See Appendix A which illustrates results for tryptophan. A concern that the wavelength calibration of the ARS might vary with time is investigated in Appendix B, where the variation is seen to be small over a period examined, i.e., 4 months.

4. Example results with atmospheric aerosol

4.1. Raman spectra of ambient aerosol particles with hierarchical cluster analysis

Figs. 7 and 8 illustrate Raman spectra of particles collected from air during a 15-min collection period
beginning at 1 a.m. (EDT) on May 8, 2015. This period was selected for analysis because it contained both inorganic and HULIS/soot spectra, as well as spectra with a peak at the C–H stretch region, indicating organic materials. Two images were taken at each location (10 and 15 s). Of the 1178 spectra obtained in this 15-min period, 158 spectra were clustered using a hierarchical cluster analysis. Thirty-two spectra were classified as ‘Fluorescent’ and were not clustered. The mean and standard deviations of the Raman spectra for each individual cluster are shown in Fig. 7.

The cluster in Fig. 7a (118 spectra) is named HULIS/Soot because spectra of soot and some HULIS have similar peaks. These appear to be the D and G bands of amorphous carbon. The spectra of the cluster in Fig. 7b (10 spectra) have a clear peak in the C–H stretch region, consistent with organic carbon. The cluster in Fig. 7c (30 spectra) has spectra with no peaks in the C–H stretch region and weak or no peaks in the D/G region, but has strong peaks near 1000 cm\(^{-1}\), and so appears to be inorganic. Some of these inorganic spectra also exhibit relatively weak D/G peaks. These classes of spectra are similar to those observed in [19], but without the TiO\(_2\) category.

Fig. 7 illustrates several individual spectra selected from the clusters found. The two Raman spectra in Fig. 8a illustrate differing relative heights of the D and G peaks. The spectrum in blue has been increased by a factor of three to facilitate comparisons of the spectral shapes of the two spectra. The spectrum indicated by the yellow line in Fig. 8c has been increased by a factor of three to facilitate comparison with the other spectrum. The two dominant peaks in Fig. 8c (which overlap in Figs. 7c and 8c because of the thickness of the lines), near ~1006 cm\(^{-1}\) and 1017 cm\(^{-1}\), are likely gypsum and anhydrite, respectively. It can be difficult to extract the exact composition of organic and inorganic species in marine aerosols from the aerosol Raman spectrum, especially when the composition is mixed [19]. However, Potgieter-Vermaak and Van Grieken in discussing their study of atmospheric aerosol stated that, “the molecular identification of most inorganic particles is trivial” [52].

Fig. 9 shows a map of the pixels which had Raman spectra which were clustered or classified as fluorescent. The pixels are colored according to which cluster-type is dominant in the spectrum. Also in Fig. 9 are the contour lines for the interpolated total signal intensity (summed peaks).
between 166 and 3185 cm\(^{-1}\). As in Fig. 3, Fig. 9 suggests that for particles larger than approximately 1.5 to 2 \(\mu m\), the cross-sectional size and shape of particles could be estimated from the Raman signal and/or fluorescence.

The interpolated total signal, represented by the shaded contours, yields information about the shapes and numbers of particles present on the tape, at least in terms of the Raman + fluorescence intensity. The fact that most of the clustered areas are centered on regions of high total signal suggests that our method for selecting spectra was appropriate. We did not interpolate clusters, so Fig. 9 is plotted with a 1-\(\mu m\) gap separating each pair of lines with clustered spectra.

In Fig. 9, particles that appear to be clusters do not appear to be homogeneously mixed. Most of the particles do not appear to be relatively homogeneous. In the lower left of Fig. 9, a relatively large particle with a spectra classified as organic (with a few spectra classified as ‘fluorescent’) has one pixel with a spectrum of HULIS/Soot on its left-most edge. It might have been internally mixed in the atmosphere or might have been formed by two particles landing on the tape at different times.

The particle in the lower right hand corner of Fig. 9a appears to have spectra clustered as ‘inorganic’ in the center, and spectra clustered as ‘HULIS/Soot’ or ‘fluorescent’ around the outside. Fig. 10 shows this particle in more detail, showing spectra from vertical and horizontal slices across this particle. The spectra clustered as HULIS/Soot (labeled 11 and 2 in Fig. 10) appear to only have the D/G features. Of the spectra clustered as ‘inorganic’, some have the D/G features (especially spectrum 12), in addition to peaks associated with inorganic material primarily. Although the spectra on three edges of this particle (11, 13, 2) exhibit D/G features, spectra 8 and 9 at the ‘top’ edge of the particle do not have clear D/G peaks. This suggests that this is not an inorganic particle uniformly coated with soot or humic acid. Overall, there are many small and large particles with Raman dominated by HULIS/Soot; one large organic particle; one or two inorganic particles; several fluorescent particles; and some particles with the spectra indicating a mixed structure. Most of the particles in Fig. 9 either have Raman spectra dominated by HULIS/Soot or have Raman spectra dominated by HULIS/Soot.

**Fig. 8.** Example individual Raman spectra of ambient particles in each of the first three clusters: (a) HULIS/soot (note that the blue spectrum has been scaled larger by a factor of 3 to have a similar magnitude to the other spectrum); (b) Organic; (c) inorganic particles (note that the yellow spectrum has been scaled larger by a factor of 3 to have similar magnitude to the other spectrum).

**Fig. 9.** Map of particles by cluster category (bar on upper right) and by overall intensity (bar on lower right). This atmospheric sample was collected in a 15-min period at approximately 1:00 a.m. on 8 May 2015. The red box indicates the region covered by Fig. 10a. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
soot signatures, or are surrounded by material with a HULIS/Soot Raman signature.

This observation that soot appears more at the surface is consistent with the results in [14]. The prevalence of spectra with the D and G peaks of amorphous carbon raises the question of whether small soot particles are agglomerating with other particles or whether the laser is charring organic materials in the majority of aerosols. However, we note, again, that we could not find evidence of charring in tests with different laser powers with tryptophan, table sugar, and dried leaves (see Appendix A). The most prevalent spectral category in Figs. 8 and 9, as shown by the number of spectra falling into each cluster, is HULIS/Soot. The large number of spectra does not mean that the particle mass is primarily HULIS/Soot. Materials with extensive conjugation of π electrons tend to have Raman cross sections that are many times larger than molecules with no or little conjugation. For example, the 514.5-nm-excited Raman cross sections (10^30 cm^2 molecule sr^-1), for selected peaks, increase from 5.6 for glucose, to 28.6 for benzene, to 540 for anthracene, to 6200 for 1,4-bis(2-methylstyryl)benzene, and to 1.1 x 10^7 for β-carotene (see pp. 26–27 [53]). The conjugation of π electrons in soot is extensive [54,55]. Also, Bladt et al. [56] showed that the Raman spectra of soot particles containing 0 to 42% CaSO_4 were essentially indistinguishable, and none exhibited Raman peaks of CaSO_4. Similarly, the signals from CeO, CeSO_4 and Na_2SO_4 in soot were very weak compared to the D/G peaks. In another example illustrating the strength of the Raman signal from soot, Blaha et al. [28] collected a NaNO_3 particle from the atmosphere and measured its Raman spectrum before and after illumination with 514.5-nm light at approximately 3.2 mW/μm^2 (an intensity approximately 2.7 times higher than the 640-nm, lower photon energy, light used here). After illumination, Blaha et al. [28], observed strong D and G peaks in the Raman spectrum. However, under an optical microscope they observed “no changes in the morphology or birefringent character of the particle” [28]. The particle size was unchanged (2.5 × 3.5 μm). They concluded that the 3.2 mW/μm^2 light at 514.5-nm converted some organic material on the NaNO_3 particle into amorphous, soot-like carbon. Blaha et al. [28], also coated an approximately 4.5 μm diameter anhydrite particle with a 20 nm coat of vitreous carbon, which has extensive sp^2 graphic type bonding [28]. Even though the ratio of the volume of vitreous carbon to anhydrite was only 0.013, the area under the D and G peaks of the Raman spectra exceeded that under the Raman peaks of the anhydrite. The data of McCreery [53], Bladt et al. [56], and Blaha et al. [28] suggest that a large number of D and G peaks should not be assumed to indicate that there is more mass of soot, HULIS or other black carbon than inorganic and non-aromatic species in a sample.

Researchers have calibrated their Raman microprobe systems using different types of black carbon (see [57] and references cited therein). However, a further complication in relating the Raman intensity of HULIS/Soot to the particle mass is that the penetration depth of 640 nm light into a uniform sheet of black carbon is substantially less than 1 μm, and typically closer to 0.1 μm. As a result, for solid BC particles smaller than approximately 0.1 μm, the intensities of the D and G peaks will be more proportional to cross sectional area. To make the inverse problem even worse, the soot may be fractal, and of an unknown, maybe low density. A conclusion is that estimating the mass of soot and HULIS from the amplitudes of the D and G peaks is non-trivial.

Fig. 10. (a) Expanded view of the small region delimited by the red box in Fig. 9. Colors represent cluster type, HULIS/Soot (gray), Inorganic (green), Fluorescent (blue). The spectra are numbered so they can be linked to their spectra (shown in 10b); (b) Raman spectra of the numbered pixels in 10a, where the number for each spectrum is shown on the far right side of 10b. The colors of these numbers (right side) indicate the cluster type as in 10a. The colors of the spectra are stepped in sequence to aid in discriminating between the different lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
4.2. Clusters in ambient aerosols: 7 hr total

A seven-hour period (5:26 a.m. to 12:30 p.m.) during the morning commute on May 8, 2015 was selected for analysis. During this time, 32,718 spectra were measured. The spectra were clustered using the hierarchical method described in Sections 2.2 and 4.1, except that a higher threshold for signal was used (0.025), seven clusters were analyzed, and total summed fluorescence intensity required for a spectrum to be ‘fluorescent’ was increased. A total of 5892 spectra were clustered; also 2752 spectra were considered to be fluorescent. Cluster 1 included HULIS/Soot (n=5627). Cluster 2 (n=18) is organic with a CH stretch. Clusters 3, 4, 5, and 7 have no CH stretch and are considered to be ‘inorganic.’ The spectrum of cluster 4 has 6 peaks (406, 492, 617, 667, 1004, 1132 cm\(^{-1}\)) close to those of CaSO\(_4\)·H\(_2\)O. The spectrum of cluster 7 has 3 peaks (721, 1065, and 1382 cm\(^{-1}\)) close to those of NaNO\(_3\). Clusters 3 and 5, and to a lesser extent, 4, have weak D/G peaks. Cluster 6 (n=41) is the Raman spectrum of the tape (which appears when there are defects in the metallization of the tape). Cluster 8 is not shown because it includes the fluorescent particles.

4.3. Time-series of numbers in clusters of ambient aerosols: 7 hr total, 15-min intervals

Fig. 12 shows a time-series of the Fluorescent, HULIS/Soot, and inorganic clusters during the measurement period. Fig. 12 illustrates that the number of spectra in Cluster 1 increases to a maximum around 9:00, then decreases, but with a smaller spike at the imaging time starting at 10:56. The increase and decrease of numbers of spectra in cluster 1 is consistent with the expected behavior for a morning commute in an urban location, e.g. [58]. In Ref. [58] the measured quantity is light absorption by the sample, which is primarily useful for measurement of soot and HULIS. In contrast, here: i) Raman spectra are used to estimate the numbers of spectra dominated by the D/G peaks of HULIS/Soot, and ii) the analysis illustrated barely begins to address the many questions that can be asked using a dataset with 5892 spectra above threshold, which should be useful for discriminating between various bioparticles, organic and inorganic materials, and soot/HULIS. The spectra in cluster 4, 5, and 7 do not have any discernable trend due to the low numbers in that category. These low numbers may be due to the high threshold used, which may have excluded spectra with weak inorganic signal. Fig. 12 illustrates that the ARS is able to obtain Raman spectra which provide information about the composition of the atmosphere and how that composition changes with a time resolution of 15 min.

5. Discussion

5.1. Estimates of maximum sample rates

Figs. 3 and 9 illustrate maps of particles collected within a 15-min period and measured within the next 15 min. In each of these figures, the y direction has approximately 40 points, each separated by approximately 1 \(\mu\)m. In Fig. 3 the map has 63 points in the y direction, each separated by 2 \(\mu\)m, and so has approximately 2520 pixels.

In Fig. 3 all the 19 particles detected are 2-\(\mu\)m PSL. It is not difficult to imagine that four or five times as many 2-\(\mu\)m PSL spheres could be detected. However, with higher particle densities there would be more aggregates of particles, where it is not possible to determine whether the particle was an aggregate in the atmosphere or formed when a particle was collected onto a particle already on the tape. If we decide that a density of particles 2.5 times that in Fig. 3 is the largest density acceptable, then the maximum number of particles measured in a 15-min period is 50. That suggests a maximum of 200 particles per hour, or 4800 particles per day, can be measured. The map in Fig. 9 with 31 points in the x direction has approximately 1240 pixels. If the number of particles measured in Fig. 9 is 35 and collections of similar density were collected all day, then the maximum number of particles counted in a day would be 3360. In cases where sampling more particles is a higher priority than is imaging or obtaining multiple spectra of the same particle, the line source could be stepped more than 2 \(\mu\)m after each spectrum in order to reduce the probability that any particle is sampled more than once.

5.2. Effects of polarization on the ability to estimate particle composition and size

Many crystals illuminated by linearly polarized light have Raman spectra that depend upon the orientation of the crystal relative to the polarization of the illumination beam and on the polarization-dependence of the collection optics and spectrograph. Because the laser illuminating the particles in the ARS is linearly polarized, and some atmospheric aerosol have orientation-dependent Raman spectra, the effect of polarization and particle orientation on the estimations of composition and particle size must be considered. First, soot, humic and humic-like substances, biological materials and most other organic particles likely to be in the atmosphere, are not likely to have their molecules sufficiently aligned to exhibit large polarization-dependent effects. Also some of the primary inorganic species in the atmosphere, e.g., ammonium sulfate, ammonium nitrate, as well as other minerals [59], often do not exhibit strong effects of polarization, because they are agglomerates of differently-oriented crystals or they crystalize with other materials. Second, most polarization sensitive bands are still apparent in all or almost all orientations. The method used to assign material type(s) to the particles can be selected to emphasize the peak locations not peak height. Not all lines in a Raman spectrum of a pure crystal are polarization dependent in the same way.
If the crystal is sufficiently large and pure so that it is possible to identify it from its Raman spectrum, then it should be possible to estimate the orientation of the crystal, once the needed information is programmed into the analysis code. This orientation should be of use in estimating the relative contributions of different materials to the measured spectra. Third, the cross-sectional sizes and shapes of the particles are primarily estimated from the numbers of pixels and their positions. Raman intensity is used to help estimate the mass of the component or components of the particle at that pixel. From the mass and the density for that material, the particle volume can be estimated if the Raman cross sections for the materials are known. The problem of estimating mass from the Raman spectrum is not simple, in part because of the variation of illumination intensity throughout particles of different sizes, and variations in collection efficiency from different regions of particles. Fourth, for applications where the polarization effects are unacceptable, the problem can largely be circumvented by circularly polarizing the laser beam and then modifying as necessary the optics used to block the elastic scattering from reaching the spectrometer.

5.3. Advantages of the REBS for an ARS

The ARS can increase the sampling rate compared with aerosol trapping techniques [36, 44] because, as an image-preserving Raman spectrograph, it can obtain many Raman spectra simultaneously. Theoretically, particles could be sampled from ambient air, held in a linear quadrupole trap [60, 61], and their Raman spectra measured using an image preserving Raman spectrograph. However, as far as we know, such measurements have not been reported. The REBS at the core of our ARS allows the user to vary many parameters, e.g., sample times, spectral acquisition times, and number of spectra to record at each laser position. The ARS can be operated with different parameters most suitable for different particle sizes. Also, with it, users have access to the raw data. Because the user can write scripts that rapidly use the raw spectra to classify particles, these spectra and partial classifications can be used to make real-time decisions about when to take additional spectra, how far to step the tape, etc. The imaging capabilities allow users to more clearly determine if a particle is homogeneous or an agglomerate, if a particle is layered, or if the spatial distribution of the Raman spectrum from an inhomogeneous particle (e.g., a pollen) is consistent with known values of that distribution.

6. Final comments

Investigations of the REBS-based ARS described here suggest the potential of this ARS for multiple applications.
We demonstrated the ability of the ARS to measure Raman spectra of 2-μm PSL, sucrose, tryptophan, CaSO₄ and other particles, as well as Arizona test dust and HULIS/Soot. Raman peaks for sucrose were on average within 2.67 cm⁻¹ of standard values. Cluster analysis of spectra from one 15-min period indicated the following types of materials: HULIS/Soot, organic, and fluorescent particle types. Raman spectra near a road were measured and analyzed for a 7 h period (15 min increments). The time-series of several spectral clusters showed that the HULIS/Soot category increased and decreased in a manner consistent with a morning commute. Neither the inorganic nor the fluorescent categories exhibited this temporal pattern. Even though additional work is needed to develop the processes to extract information from the Raman spectra, we think that with additional work, ARSs should be useful in characterizing time-resolved size and chemical composition of atmospheric and laboratory-generated aerosols, studying aerosol processing in the atmosphere, and in obtaining data needed to determine which particle compositions are related to the various health effects correlated with atmospheric aerosol [1–3].

Acknowledgments

This work was supported by US Army Research Laboratory mission funds as part of the Atmospheric Science Center and the Meteorological Sensor Array. Two anonymous reviewers provided feedback which significantly helped improve the quality of this manuscript.

Appendix A. Are particles charred by the laser in the ARS?

If the laser intensity is too high and/or the laser wavelength is too short, the illumination to excite Raman can convert carbonaceous materials to black carbon as evidenced by D and G peaks in the Raman spectra [28]. To investigate whether the laser used here generates materials with D and G peaks we tested it with several samples. In each case the sample was illuminated by the laser for more than six minutes. Fig. 13 shows the spectra from four 2-s measurements of the Raman spectrum of bulk tryptophan over the course of 6 min. The laser was on continuously over the time period (6 min and 2 s). The lowest (dark blue) line in Fig. 7 shows the difference between when the laser was first turned on and ~6 min later. No discernable amorphous carbon peaks appear in the Raman spectra of this sample of tryptophan. Therefore, the spectra do not indicate the generation of amorphous carbon in this bulk sample after 6 min of illumination at full power. The same test was done with leaf litter and with C. herbarum. Again, there was no evidence of generation of amorphous carbon. Therefore, we suspect that the possibility for the lasers in the ARS to char aerosol samples is low. However, we plan to continue to look for evidence of charring and look for materials that may occur in atmospheric particles which may be especially prone to charring when illuminated 640 nm.

Blaha et al. [28] observed evidence of D and G peaks in the Raman spectrum of a mostly NaNO₂ particle collected from the atmosphere. Spectra were measured before and after illumination with 514.5-nm light at approximately 3.2 mW/μm², an intensity approximately 2.7 times higher than the 640-nm light we use here. Also, the energy per photon is 187 kJ/mole at 640 nm (ARS) and 232.5 kJ/mole with the 514.5 nm light used by Blaha et al. [28].

Appendix B. Stability of the calibration of emission wavelengths

The ARS was calibrated by Battelle using the atomic emission lines from a neon lamp, as well as Raman shifts from PSL block and a silicon (Si) wafer. We verified the calibration using only a PSL block and Si wafer, using a third-order polynomial to fit the Raman shifts to spectrometer bins. The PSL peaks used are at 620.9, 785.8, 1001.4, 1031.8, 1155.3, 1450.5, 1602.3, 2852.4, 2904.5, and 3054.3 cm⁻¹, as found in the ASTM standard spectra for polystyrene. A peak-finding algorithm was used to determine the peaks from our ARS spectra. First, the second derivative of the Raman spectrum was used to determine approximate peak location. Then, for all peaks meeting specified criteria for the absolute peak intensity and the slope of the second-derivative, a Gaussian function was fit to the spectrum in the vicinity of the peak to extract a more accurate peak location. Between 500 cm⁻¹ and the upper limit of the Raman shift, the spectrometer varied less than 4 cm⁻¹ from the factory calibration. Using similar calibration methods (but different pieces of Si and PSL), the calibration varied less than 1 cm⁻¹ between 9 July and 19 November 2015, even though the instrument was transported to several different locations.

Fig. 14 shows the difference between the factory calibration and two of our calibrations. As a check for the validity of Raman spectra, both in the field or in the laboratory, PSL spheres are periodically tested in the instrument.
Fig. 14. Difference in Raman shift between the factory calibration (20 Apr 2015) and our calibrations on 9 July 2015 (blue) and 19 Nov 2015 (orange).

The difference between our 9 July and 19 Nov calibrations is in green.

References


