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Begin Performance Period: 07-Sep-2021 **End Performance Period:** 06-Jan-2023
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Major Goals: As stated in the original proposal, the major objective of the small project was to use the grant funds to initiate collaboration between partners at the Army Research Laboratories in order to mutually support the application of REBS instrumentation for investigation of atmospheric aerosols. Because of the short-term, exploratory nature of the project, it was stated that specific goals would evolve with the project. Objectives are grouped in three areas, and accomplishments/findings are listed below under each objective.

Major Goal/Objective #1: REBS instrument maintenance, upgrade, and operation
Major Goal/Objective #2: Begin characterizing REBS instrument in the lab against standard particle types
Major Goal/Objective #3: Perform initial field application of REBS instrument

Accomplishments: As a result of major and unexpected maintenance work required for the instrument, a large portion of the project timing and funding was re-directed (with approval) toward rebuilding and upgrading the REBS instrument. Additional major work and accomplishments are highlighted here.

Objective #1: REBS instrument maintenance, upgrade, and operation
The central goal of the proposed project, as stated in the original proposal, was to collaborate with partners at "the Army Research Laboratories to mutually support the application of REBS instrumentation (Resource Effective Bio-Identification System) for investigation of atmospheric aerosols."

Early in the project period, it was determined that the REBS instrument was suffering from a computer-board failure based on faulty engineering by the manufacturer. After discussing back-and-forth with the manufacturer, Battelle, and ARL collaborators, it was determined that the best use of a large portion of the small project budget was to fix the instrument. As a result, the major goals of the project shifted significantly toward this first as primary goal. The first goal thus became the maintenance, upgrade, and renewed operation of the REBS instrument. Specific outcomes/findings from this component of the project are itemized here.

Finding 1a: REBS instrument computer board was replaced and instrument was returned to operability.
The process to initiate instrument maintenance took several months even to begin, because financial and legal terms between Battelle and the University of Denver required negotiation before work could begin. The maintenance required purchase and replacement of a new main computer board and a number of paid phone calls with Battelle. In following, the instrument laser and detector required tuning and an instrument acquisition software upgrade, which required more costly consultation with Battelle. After a months-long proces, the REBS instrument was up and running as of October 2022, only ~2.5 months before the end of the project period.

Finding 1b: The problem limiting REBS analysis time to 1-hour was solved.
Early in the project phase, we realized that the instrument had a severe limitation caused by the original engineering of the computer board, which limited the instrument from being operated for more than 60 minutes at a time. After that time limit, the instrument would re-start, even during an individual experiment. This flaw proved to be a fundamental limitation, because sample acquisition and analysis could not proceed in any detail until the instrument could be operated for longer periods of time. Following the computer board replacement, the computer can now operate continuously, as designed.

Finding 1c: REBS software was upgraded to be able to change laser power and change number of image replicates acquired.
Following instrument maintenance, the REBS can now operate as originally intended and we have the new capability to tune the laser power and change the number of replicate images acquired. The ability to tune laser power is important for sample analysis, because different types of samples can absorb different amounts of laser energy before charring or ablation begins. As a result, different laser power settings are required to optimize promotion of Raman scattering from different samples.

Further, the ability to change the number of image replicates acquired is a significant technical step forward, because it may enable us to begin looking at the point at which Raman spectra can be acquired, when photon-initiated charring begins, and when photobleaching begins.

Finding 1d: Desktop computer for data analysis was acquired, which improves analysis speed and efficiency.
The REBS instrument acquires .png images as the raw data, and those images are processed sequentially through two layers of analysis software. Up to this point acquired images had been analyzed using the lead-student's personal computer, and the process was slow.

Through project funds we acquired a desktop computer (Dell Optiplex 7000, i9-12900 with 32 GB RAM) to enable REBS data analysis. This significant computer upgrade makes analysis of both laboratory experiments and field data much easier and faster to accomplish. For example, the analysis process previously required multiple days of computing time to process a single batch of images, and now multiple sets of images can easily be processed in the same work day.

Objective #2: Begin characterizing REBS instrument in the lab against standard particle types.
Finding 2a: Raman shift calibration of REBS instrument was measured and is acceptable.
An important step towards applying the instrument more broadly for particle analysis has been to calibrate the Raman shift (cm-1) against a spectral standard. One of the first steps following the instrument work was to calibrate the Raman shift using a polystyrene wafer with known Raman band locations.

Finding 2b: D/G bands of soot are routinely detectable by the REBS instrument.
A second finding under this objective was that the D and G bands associated with carbon soot are routinely acquired in both laboratory experiments involving carbonaceous particles and via ambient sampling. During laboratory experiment where carbonaceous particles like fungal spores or other bioaerosol are analyzed, observation of soot particles is a good indication that the instrument laser power is too intense and that the organic material is being charred to form soot.

Finding 2c: Fungal spores aerosolized in lab experiments are detectable via Raman spectra.
Several species of fungi were grown on agar plates in the laboratory, aerosolized using a modified spore emission chamber, and analyzed with the REBS. A wideband integrated bioaerosol sensor (WIBS) was used in parallel to the REBS to monitor aerosolized spore (or mineral dust particle) concentration in real-time.

Only one species (Aspergillus versicolor) consistently provided sufficient numbers of aerosolized spores for analysis during the initial experiments, and so comparison across species was challenged. Given that the growth plates require several weeks to mature to the point of efficient spore aerosolization, further rounds of growth were not possible before the project period concluded. Fungal spore spectra collected and analyzed match broadly with bands to be expected from complex biological particles.

Finding 2d: Mineral dust aerosolized in laboratory experiments are detectable via Raman spectra.
Kaolinite, a silicate clay mineral was used as an example mineral dust particle aerosolized and interrogated by the REBS. The analyzed particles match well with literature spectra of kaolinite. It is noteworthy that the key peaks in

the REBS spectrum of kaolinite match with the literature spectrum, but the REBS spectrum is considerably noisier. This is to be expected, given the bulk nature of the literature spectrum (black curve) and the fact that each REBS spectrum is achieved from a single ~1-5 μm particle.

Objective #3: Perform initial field application of REBS instrument

Finding 3: Raman spectra of particles sampled from ambient outside air in Denver were collected, and spectra of carbonaceous soot are frequently observed.
As initial work, the REBS was deployed on a grassy lawn outside the University of Denver laboratory building for several rounds of ambient particle analysis, including several individual sampling experiments. By modulating the laser power between experiments, a change in the relative proportion of soot particles observed was observed, indicating that the degree of charring can be increased or decreased accordingly. For example, during two sets of ambient experiments, the relative proportion of particles classified as soot (e.g. see Finding 2b, above) decreased from 35% to 17% when relative laser power was reduced by increasing laser power attenuation from 0.33 to 0.45.

Training Opportunities: Doctoral student Maxwell Freeman was trained to use the Resource Effective Bio-Identification System, REBS, by a former undergraduate student. This training began immediately before the project period and extended through the first phase of the project. The funded project provided the opportunity for the student to learn instrument operation through working directly with the instrument manufacturer Battelle as he fixed and modified the instrument.

Results Dissemination: Nothing to Report

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: Graduate Student (research assistant)
Participant: Maxwell Freeman
Person Months Worked: 5.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: PD/PI
Participant: Alex Huffman
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Partners

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I certify that the information in the report is complete and accurate:
Signature: J. Alex Huffman
Signature Date: 4/25/23 11:59AM

Abstract

Naturally emitted atmospheric aerosol particles are ubiquitous in the ambient outdoor atmosphere and within built environments of all kinds. They contribute to serious human health effects, climate radiative forcing, visibility reduction, acid and nutrient deposition to the Earth surface, changes in the hydrological cycle, and can act as agents of biowarfare. Yet for many of these processes, uncertainties in the aerosol properties often dominate the total level of scientific understanding or the ability to confidently detect the particles at all types. Raman spectroscopy of individual aerosol particles can offer a powerful ability to interrogate particles with a high degree of chemical identification in order to rapidly differentiate between benign and potentially dangerous species or to observe chemical changes that take places in the atmosphere that can alter their detection signatures. The Resource Effective Bio-Identification System (REBS) can interrogate individual aerosol particles via Raman spectroscopy on a field-portable platform with the aim of characterizing the composition, and even source, of the particles.

The small, exploratory project proposed was to utilize the REBS technology as an opportunity to enhance collaboration between the University of Denver and Army Research Laboratory by providing support to investigate Raman spectra of particles in both lab and field settings. The major goals/objectives of the project were originally stated to stoke collaboration, with specific project goals to be adapted as the project evolved. Specifically, it was proposed for work to perform: (a) basic instrument characterization with respect to operational parameters and lab-generated particles from both biological and non-biological sources, (b) preliminary field application, and possibly, (c) initial experiments on respiratory aerosols. Early in the project period the basic structure of those objectives was fundamentally altered, however, because the REBS instrument need substantial work to be fixed from issues arising from a manufacturer-related engineering problem. As a result, a superseding objective became to rebuild and upgrade the instrument according to manufacturer specifications. Following the instrument maintenance and upgrade, initial work was performed to characterize the instrument in the lab with respect to instrument operation, Raman spectral calibration, fungal spore and mineral dust particles, and as a part of initial outdoor field application. The project produced significant amounts of preliminary data in each of these areas to help produce momentum and seeds from which future collaborative work alongside ARL colleagues can continue to grow.

Objectives/Major Goals:

As stated in the original proposal, the major objective of the small project was to use the grant funds to initiate collaboration between partners at the Army Research Laboratories in order to mutually support the application of REBS instrumentation for investigation of atmospheric aerosols. Because of the short-term, exploratory nature of the project, it was stated that specific goals would evolve with the project. Objectives are grouped in three areas, and accomplishments/findings are listed below under each objective.

Objective #1: REBS instrument maintenance, upgrade, and operation
Objective #2: Begin characterizing REBS instrument in the lab against standard particle types
Objective #3: Perform initial field application of REBS instrument

Findings/Accomplishments:

As a result of major and unexpected maintenance work required for the instrument, a large portion of the project timing and funding was re-directed (with approval) toward rebuilding and upgrading the REBS instrument. For this reason, the depth of work and collaboration was less than anticipated, but major work and accomplishments are highlighted here.

Objective #1: REBS instrument maintenance, upgrade, and operation

The central goal of the proposed project, as stated in the original proposal, was to collaborate with partners at "the Army Research Laboratories to mutually support the application of REBS instrumentation (Resource Effective Bio-Identification System) for investigation of atmospheric aerosols." It was further stated that specific goals would "evolve with the project."

Early in the project period, it was determined that the REBS instrument was suffering from a computer-board failure based on faulty engineering by the manufacturer. After discussing back-and-forth with the manufacturer, Battelle, and ARL collaborators, it was determined that the best use of a large portion of the small project budget was to fix the instrument. As a result, the major goals of the project shifted significantly toward this first as primary goal. The first goal thus became the maintenance, upgrade, and renewed operation of the REBS instrument. Specific outcomes/findings from this component of the project are itemized here.

Finding 1a: REBS instrument computer board was replaced and instrument was returned to operability.

The process to initiate instrument maintenance took several months even to begin, because financial and legal terms between Battelle and the University of Denver required negotiation before work could begin. The maintenance required purchase and replacement of a new main computer board and a number of paid phone calls with Battelle. In following, the instrument laser and detector required tuning and an instrument acquisition software upgrade, which required more costly consultation with Battelle. After a many-month long process that was originally intended to be only a few weeks, the REBS instrument was up and running as of October 2022, only ~2.5 months before the end of the project period.

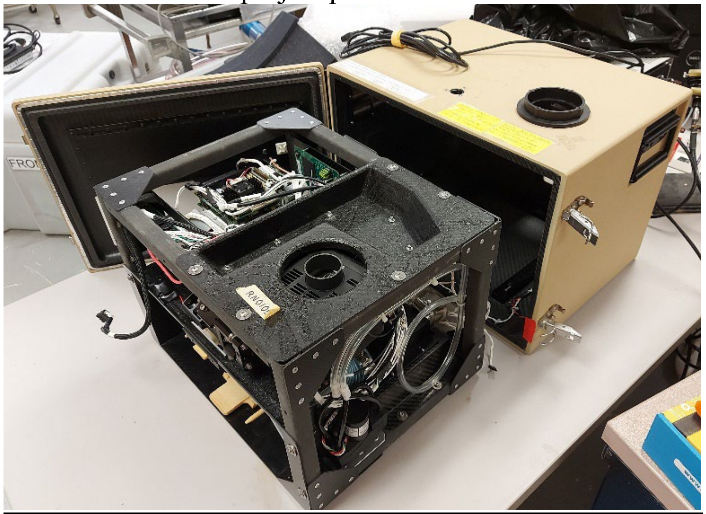

Figure 1: Image of REBS instrument during repair process.

Finding 1b: The problem limiting REBS analysis time to 1-hour was solved.

Early in the project phase, we realized that the instrument had a severe limitation caused by the original engineering of the computer board, which limited the instrument from being operated for more than 60 minutes at a time. After that time limit, the instrument would re-start, even during an individual experiment. This flaw proved to be a fundamental limitation, because sample acquisition and analysis could not proceed in any detail until the instrument could be operated for longer periods of time.

Following the computer board replacement, the computer can now operate continuously, as designed.

Finding 1c: REBS software was upgraded to be able to change laser power and change number of image replicates acquired.

Following instrument maintenance, the REBS can now operate as originally intended and we have the new capability to tune the laser power and change the number of replicate images acquired. The ability to tune laser power is important for sample analysis, because different types of samples can absorb different amounts of laser energy before charring or ablation begins. As a result, different laser power settings are likely required to optimize promotion of Raman scattering from different samples.

Further, the ability to change the number of image replicates acquired is a significant technical step forward, because it may enable us to begin looking at the point at which Raman spectra can be acquired, when photon-initiated charring begins, and when photobleaching begins. The earlier instrument settings were fixed to require the acquisition of four image replicates, but this value can now be increased to investigate detailed physico-chemical processes more fully.

Finding 1d: Desktop computer for data analysis was acquired, which improves analysis speed and efficiency.

The REBS instrument acquires .png images as the raw data, and those images are processed sequentially through two layers of analysis software. Up to this point acquired images had been analyzed using the lead-student's personal computer, and the process was slow.

Through project funds we acquired a desktop computer (Dell Optiplex 7000, i9-12900 with 32 GB RAM) to enable REBS data analysis. This significant computer upgrade makes analysis of both laboratory experiments and field data much easier and faster to accomplish. For example, the analysis process previously required multiple days of computing time to process a single batch of images, and now multiple sets of images can easily be processed in the same work day.

Objective #2: Begin characterizing REBS instrument in the lab against standard particle types.

The extent of the instrument maintenance and upgrades required (Objective #1) meant that relatively less time remained during the project for comprehensive analyses toward other aims, however several important steps were achieved.

Finding 2a: Raman shift calibration of REBS instrument was measured and is acceptable.

An important step towards applying the instrument more broadly for particle analysis has been to calibrate the Raman shift (cm^{-1}) against a spectral standard. One of the first steps following the instrument work was to calibrate the Raman shift using a polystyrene wafer with known Raman band locations. An example calibration spectrum (Fig. 2, below) shows the major Raman bands acquired in calibration to match expected bands (figure inset).

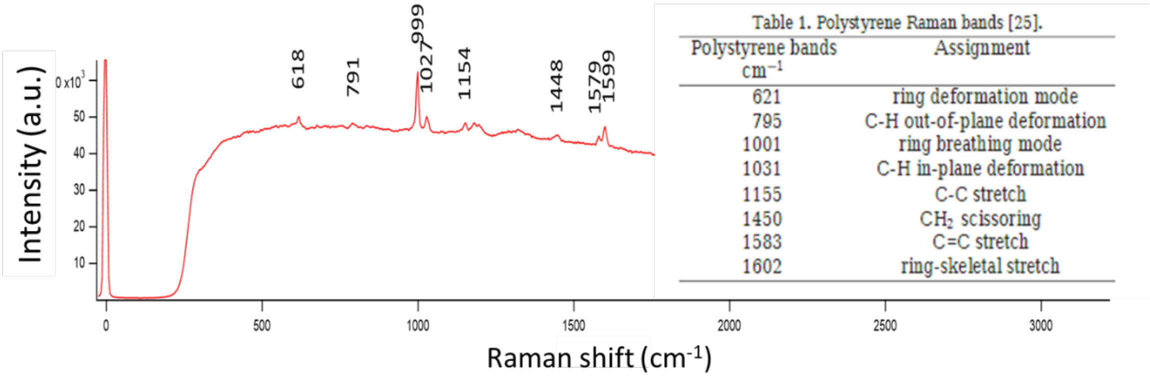

Table 1. Polystyrene Raman bands [25].

Polystyrene bands cm^{-1}	Assignment
621	ring deformation mode
795	C-H out-of-plane deformation
1001	ring breathing mode
1031	C-H in-plane deformation
1155	C-C stretch
1450	CH_2 scissoring
1583	C=C stretch
1602	ring-skeletal stretch

Figure 2: Raman spectrum of polystyrene acquired with REBS, including inset of table listing key Raman bands for polystyrene. Table of Raman bands adapted from Mazilu *et al.*, 2010.

Finding 2b: D/G bands of soot are routinely detectable by the REBS instrument.

A second finding under this objective was that the D and G bands associated with carbon soot are routinely acquired in both laboratory experiments involving carbonaceous particles and via ambient sampling. The characteristic bands are shown in an example spectrum below (Fig. 3). During laboratory experiment where carbonaceous particles like fungal spores or other bioaerosol are analyzed, observation of soot particles is a good indication that the instrument laser power is too intense and that the organic material is being charred to form soot (Doughty and Hill, 2020).

This observation is both confirming, given the consistency of the acquisition of these spectra, and a source for future work. Now that the instrument has the capability to modulate the laser intensity and to collect more frequent images with shorter duration of exposure, it should be possible to optimize spectral acquisition for different particle types (i.e. biological aerosol versus mineral dust versus diesel soot). This next phase of experimentation will need to extend beyond this project.

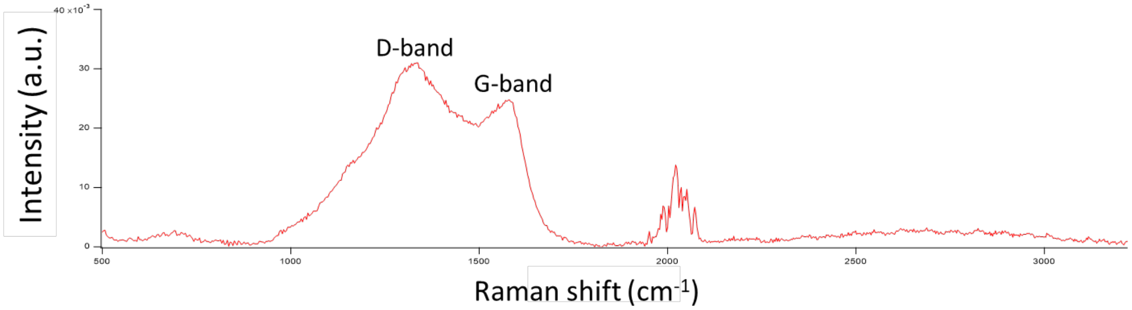

Figure 3: Raman spectrum showing D and G bands indicating carbonaceous soot.

Finding 2c: Fungal spores aerosolized in lab experiments are detectable via Raman spectra.

Several species of fungi (Aspergillus niger, Aspergillus versicolor, Cladosporium spp., and Penicillium chrysogenum) were grown on agar plates in the laboratory, aerosolized using a modified spore emission chamber, and analyzed with the REBS (Savage *et al.*, 2017). A wideband integrated bioaerosol sensor (WIBS) was used in parallel to the REBS to monitor aerosolized spore (or mineral dust particle) concentration in real-time.

Only one species (*Aspergillus versicolor*) consistently provided sufficient numbers of aerosolized spores for analysis during the initial experiments, and so comparison across species was challenged. Given that the growth plates require several weeks to mature to the point of efficient spore aerosolization, further rounds of growth were not possible before the project period concluded.

Fungal spore spectra collected and analyzed match broadly with bands to be expected from complex biological particles (Fig. 4). A continued phase of the project following the funding period will aim to explore differences in Raman spectra between fungal species in order to test the ability of machine-learning-based approaches to differentiate and classify species. This approach has been successful to classify pollen species through the use of fluorescence spectroscopy with a single-particle instrument developed in our laboratory (Swanson and Huffman, 2020), and we hope to extend that methodology in the future to Raman spectra collected with the REBS.

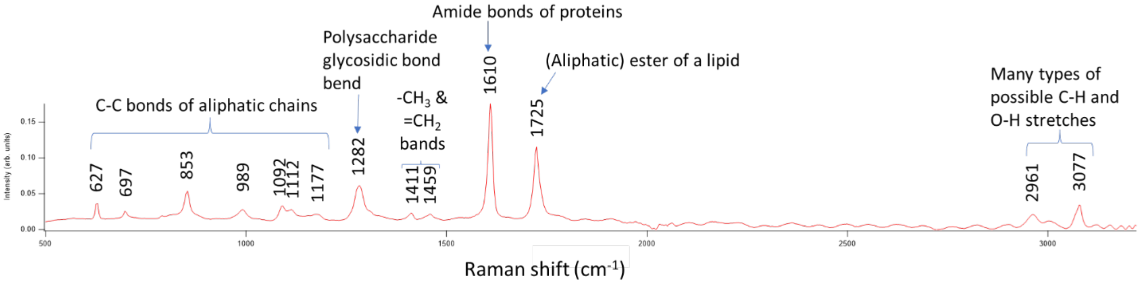

Figure 4: Raman spectrum of a spore from Aspergillus versicolor, shown as an example, with assignment of matching Raman bands.

Finding 2d: Mineral dust aerosolized in laboratory experiments are detectable via Raman spectra.

Kaolinite, a silicate clay mineral was used as an example mineral dust particle aerosolized and interrogated by the REBS. The analyzed particles match well with literature spectra of kaolinite, as shown in Figure 5 (Samyn *et al.*, 2015). It is noteworthy that the key peaks in the REBS spectrum of kaolinite match with the literature spectrum, but the REBS spectrum is considerably noisier. This is to be expected, given the bulk nature of the literature spectrum (black curve) and the fact that each REBS spectrum is achieved from a single ~1-5 µm particle.

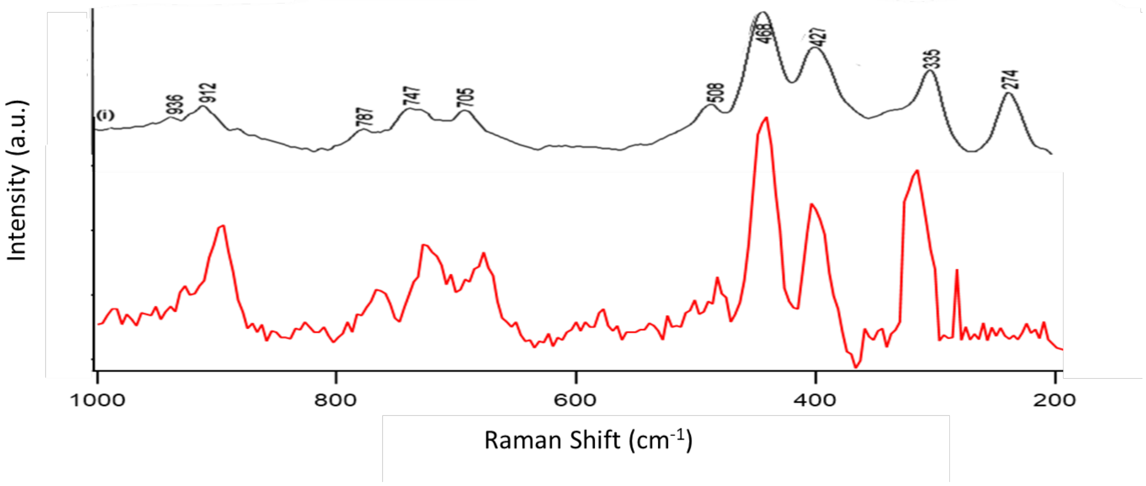

Figure 5: Raman spectra of kaolinite particle. REBS: bottom, red curve. Literature spectrum: top, black curve; reproduced from Samyn *et al.*, 2015. Note that the Raman shift axis is reversed with respect to the other figures shown here.

Objective #3: Perform initial field application of REBS instrument

Finding 3: Raman spectra of particles sampled from ambient outside air in Denver were collected, and spectra of carbonaceous soot are frequently observed.

As initial work, the REBS was deployed on a grassy lawn outside the University of Denver laboratory building for several rounds of ambient particle analysis, including several individual sampling experiments. By modulating the laser power between experiments, a change in the relative proportion of soot particles observed was observed, indicating that the degree of charring can be increased or decreased accordingly. For example, during two sets of ambient experiments, the relative proportion of particles classified as soot (e.g. see Finding 2b, above) decreased from 35% to 17% when relative laser power was reduced by increasing laser power attenuation from 0.33 to 0.45. These results are only preliminary, however, and further ambient experiments will be required to provide a quantitative level of confidence to the observed changes. Further, results from ambient experiments using the REBS need to be interpreted carefully, because the number of particles collected is relatively small, and so the statistics are poor in all cases (Doughty and Hill, 2020). Additionally, soot can be present in the ambient atmosphere, originating from combustion sources like diesel soot or wood-burning (Doughty and Hill, 2017), but carbonaceous particles can also be converted to soot during laser-interrogation within the REBS. It is likely that fine differences between the types of soot spectra collected under these broadly different categories are different and may eventually be differentiable, but this will require follow-up experimentation.

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