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RPPR Final Report
as of 24-Apr-2019

Agency Code:

Proposal Number: 68429CHRIP **Agreement Number: W911NF-16-1-0365**
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Report Date: 19-Sep-2017 Date Received: 07-Jan-2019
Final Report for Period Beginning 20-Jun-2016 and Ending 19-Jun-2017
Title: A Raman microspectroscopic system to transform analytical capability for detecting and characterizing aerosol particles
Begin Performance Period: 20-Jun-2016 **End Performance Period:** 19-Jun-2017
Report Term: 0-Other
Submitted By: John Huffman Email: alex.huffman@du.edu
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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 1 **STEM Participants:** 4

Major Goals: The proposed goal of the project was to purchase and acquire the Resource Effective Bio-Identification System (REBS) and the first round of associated consumable supplies through the Defense University Research Instrumentation Program (DURIP). We proposed several field and instrument development applications that the REBS could be applied to, depending on how funding became available for specific opportunities. Additionally, adding the REBS instrument for Raman spectroscopy of aerosol particles promised to add to the educational infrastructure of the Department of Chemistry and Biochemistry by adding the only Raman spectrometer to the list of department resources.

Accomplishments: Funded by the DURIP project, the REBS instrument was purchased and delivered on 4/19/2017. The PI and a group of four students were trained by manufacturer staff on basic operational skills to use the instrument during instrument delivery. The initial use of the instrument during the project period (06/2016 – 06/2017) was led by junior-level undergraduate student Jacqueline Merle. She was able to get the instrument running and aerosolized several types of fungal spores in the laboratory to analyze using the REBS. Via collaboration with Steven Hill and David Doughty at the US Army Research Labs, Jacqueline was able to begin extracting spectra from raw data. Jacqueline presented a poster on her preliminary results at the national conference of the American Association of Aerosol Research (AAAR) in Raleigh, NC. A copy of this poster presentation has been uploaded here.

Training Opportunities: PI Dr. Huffman and 4 students were trained by instrument manufacturer (Battelle) during delivery of REBS instrument. Battelle staff continued consultation and remote training for several months as instrument was led to be more functional.

The project provided general scientific research training for 4 students: 2 graduate students and 2 undergraduate students. In particular, effort was led by a junior-level undergraduate student who took responsibility for getting the instrument working and for all initial tests and data analysis. This opportunity provided the student with important early career opportunities to learn about scientific research.

Results Dissemination: One poster was presented at the 36th Annual Meeting of the American Association of Aerosol Research (AAAR) in Raleigh, NC on October 19, 2017. The poster was created and presented by undergraduate student Jacqueline Merle. The citation is below, and a copy of the poster was uploaded for reference.

Merle, J., Savage, N., Doughty, D., Hill, S., Huffman, J. A., "Raman Spectra of Individual Bioaerosol Particles in the Laboratory Using the Resource Effective Bio-Identification System (REBS)," 36th Annual Meeting of the American Association of Aerosol Research (AAAR), Raleigh, NC. (October 19, 2017).

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: John Huffman
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Jacqueline Merle
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Benjamin Swanson
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Samuel Scherer
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)

RPPR Final Report
as of 24-Apr-2019

Participant: Nicole Savage
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
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International Travel:
National Academy Member: N
Other Collaborators:

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Date Received: 07-Jan-2019 Conference Date: 19-Oct-2017 Date Published:
Conference Location: Raleigh, NC
Paper Title: Raman Spectra of Individual Bioaerosol Particles in the Laboratory Using the Resource Effective Bio-Identification System (REBS)
Authors: Jacqueline Merle, Nicole Savage, David Doughty, Steven Hill, John Huffman
Acknowledged Federal Support: **Y**

Raleigh, NC
36th Annual AAAR Conference

- University of Denver, Department of Chemistry and Biochemistry, Denver, USA
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1. Motivation

- Conduct primary research to gain understanding of REBS operations
- Demonstrate that Raman spectra and functional groups from that spectra can be obtained and identified

2. Experimental Setup

- 6 species of Fungi were grown including:
  - *Aspergillus niger*
  - *Saccharomyces cerevisiae* (yeast)
  - *Alternaria alternata*
  - *Cladosporium*
  - *Rhizopus stolonifer*
  - *Aspergillus brasiliensis*
- Each species was plated on growth media
- All showed significant growth except every plate of the *Alternaria alternata*
- All plates were kept in a closed environment, with temperature, moisture and humidity were monitored
- Each plate grew for 3 weeks before being tested
- Fungi species were aerosolized in the chamber

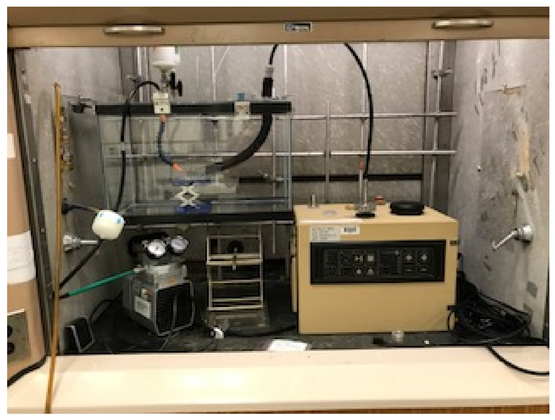

Figure 1. Chamber used to aerosolize fungi species and REBS

- Each species had a 30 minute particle collection period & a 30 minute CCD image collection period
- An example of the process of analysis is seen below

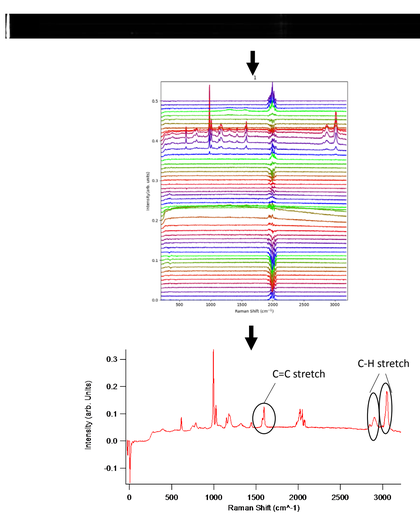

Figure 2. process of raw data analysis

- The laser line illuminates the width of the tape and provides a raw cropped CCD image ~51 µm wide
- The tape then moves and a new CCD image is collected
- The CCD images are then converted into Raman spectra images in which single lines of spectra can be extracted

3. REBS Instrument

- The Resource Effective Bio-Identification System (REBS) uses Raman micro-spectroscopy
- This allows rapid chemical and size characterization of individual aerosol particles
- The REBS provides detailed information about particle composition than other measurements i.e. Laser-induced fluorescence (LIF).
- The instrument offers the ability to differentiate particle types much more finely than LIF instruments
- Bioaerosol particles are reported by the manufacturer (Battelle) to be identifiable at the biological species level
- The REBS is made up of 2 systems within itself including the air inlet system the Raman spectroscopy system

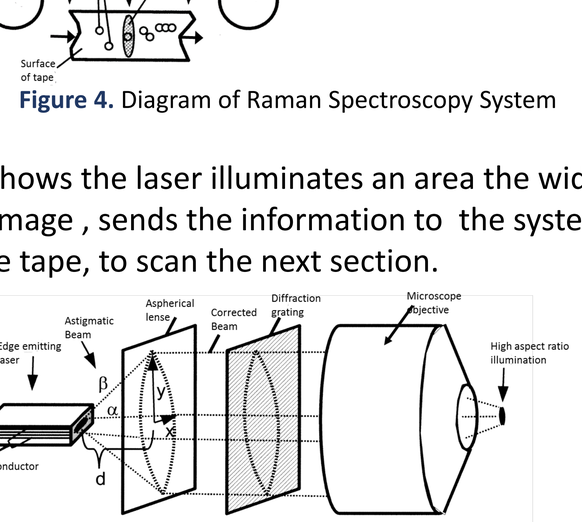

Figure 3. Diagram of air inlet system

- Particles are impacted onto aluminum coated Mylar tape. The impaction is controlled by electrode in the electrostatic collector as seen in Figure 3
- Collection flow rate: 7 meters per second

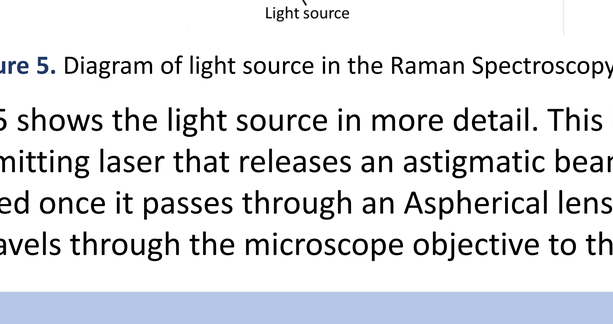

Figure 4. Diagram of Raman Spectroscopy System

- Figure 4 shows the laser illuminates an area the width of the tape, takes an image , sends the information to the system control then moves the tape, to scan the next section.

Figure 5. Diagram of light source in the Raman Spectroscopy System

- Figure 5 shows the light source in more detail. This includes an edge emitting laser that releases an astigmatic beam that is corrected once it passes through an Aspherical lense. The beam then travels through the microscope objective to the tape

4. Individual Fungal

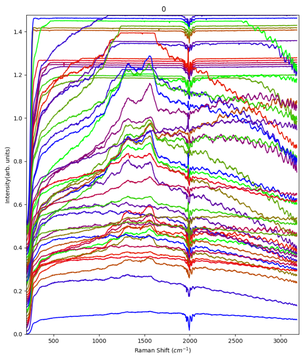

Figure 6. Spectral Image from Saccharomyces cerevisiae

Figure 7. S
Rhizo

- Spectra was collected of all fungi spe
show the very preliminary results via

Issues:

- Spectra present very strong fluoresc
seen in the wavy lines due to the Eta
- Yeast: broad peaks at 1350 cm^{-1} an
indicates that particle burning from
- The detector is also saturating, whic
spectra as solely saturates and not i

Possible solutions:

- Image for longer time
- Take last few replicates and combine
- First bleach to get usable Raman spe
- Throw out first 10 seconds of imagin
the worst of the fluorescence
- Test to see if fungi species are actua
dumping raw replicates, if peaks at
1600 cm^{-1} grow than it is burning

5. Summary/next

- REBS was able to characterize function
- Spectra was obtained from several diff
- Run time and aerosolization method n
evaluated to produce cleaner spectra
- Create sizing calibration

6. Acknowledgments &

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