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14. ABSTRACT
We have developed new techniques for characterization of atmospheric biological particles using confocal Raman spectroscopy and optical trapping. Achievements include: (1) developed optical pulling technique for single light-absorbing particles and smut spores in air over a meter-scale distance using a single collimated laser beam based on negative photophoretic force; (2) characterized optical trapping and rotation of airborne absorbing particles with a single focused laser beam; (3) developed ultralow frequency Stokes and anti-Stokes Raman spectroscopy for the

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Report Title

Final Report: Characterization of Atmospheric Biological Particles Using Confocal Raman Spectroscopy and Optical Trapping

ABSTRACT

We have developed new techniques for characterization of atmospheric biological particles using confocal Raman spectroscopy and optical trapping. Achievements include: (1) developed optical pulling technique for single light-absorbing particles and smut spores in air over a meter-scale distance using a single collimated laser beam based on negative photophoretic force; (2) characterized optical trapping and rotation of airborne absorbing particles with a single focused laser beam; (3) developed ultralow frequency Stokes and anti-Stokes Raman spectroscopy for the analysis of single living cells and biomolecules; (4) measured Raman spectra of single airborne absorbing particles trapped by a single laser beam; (5) discovered memory of germinant stimuli in bacterial spores; (6) observed the dynamic germination of single bacterial spores using rapid Raman imaging; (7) characterized uptake of and resistance to the antibiotic berberine by individual dormant, germinating and outgrowing Bacillus spores as monitored by laser tweezers Raman spectroscopy; (8) studied uptake and levels of the antibiotic berberine in individual dormant and germinating Clostridium difficile and Bacillus cereus spores as measured by laser tweezers Raman spectroscopy; and (9) characterized cold atmospheric plasma inactivation of individual bacterial spores using Raman spectroscopy and phase contrast microscopy. These results help the characterization of atmospheric biological particles.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
01/25/2017	21	Shiwei Wang, Christopher J. Doona, Peter Setlow, Yong-qing Li. Characterization of cold atmospheric plasma inactivation of individual bacterial spores using Raman spectroscopy and phase contrast microscopy, Appl. Environ. Microbiol., ( ): 5775. doi:
01/25/2017	26	L. del Carmen Huesca-Espitia, M. Suvira, K. Rosenbeck, G. Korza, B. Setlow, W. Li, S.W. Wang, Yong-Qing. Li, P. Setlow. Effects of steam autoclave treatment on Geobacillus stearothermophilus spores, J. Appl. Microbiol., ( ): 1300. doi:
01/25/2017	27	C. Doona, F. Feeherry, B. Setlow, S.W. Wang, W. Li, F. C. Nichols, P. K. Talukda, M. R. Sarker, Y.-Q. Li, A. Shen, P. Setlow. Effects of High-Pressure Treatment on Spores of Clostridium Species, Appl. Environ. Microbiol., ( ): 5287. doi:
03/30/2016	19	Barbara Setlow, Peter Setlow, Yong-qing Li, Shiwei Wang. Uptake and levels of the antibiotic berberine in individual dormantand germinating Clostridium difficile and Bacillus cereus sporesas measured by laser tweezers Raman spectroscopy, J Antimicrob Chemth, (02 2016): 0. doi:
03/30/2016	20	Shiwei Wang, Jing Yu, Milomir Suvira, Peter Setlow, Yong-qing Li. Uptake of and resistance to the antibiotic berberine by individual dormant, germinating and outgrowing Bacillus spores as monitored by laser tweezers Raman spectroscopy, PLoS ONE, (12 2015): 144183. doi:
03/30/2016	18	Shiwei Wang, James R. Faeder , Peter Setlow , Yong-qing Li. Memory of Germinant Stimuli in Bacterial Spores, mBio, (11 2015): 1859. doi:
TOTAL:	**6**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 3.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper
01/25/2017	25 Yong-qing Li, Shiwei Wang, and Peter Setlow. Single-cell analysis of cellular heterogeneity and cellular memory in bacterial spore germination, ASM Conference on the individual microbe: single-cell analysis and agent-based modeling. 19-MAR-16, Wanshington DC, USA. : ,
01/25/2017	22 Adam Hart, Joshua Mangum, YONG-QING LI. Optical Pulling of Single Aerosol Particles over a Meter-Long Distance Using a Single Laser Beam, AAAR 35th Annual Meeting. 17-OCT-16, Portland, Oregon, USA. : ,
01/25/2017	23 A. G. Hart, J. Mangum, J. Lin, and Y. Q. Li. Optical pulling of airborne absorbing particles and biological aerosols in air over a meter-scale distance using a single laser beam, International Conference on the Frontiers in Atomic, Molecular, and Optical Physics. 23-MAY-16, Shanghai, China. : ,
01/25/2017	24 A. G. Hart, J. Mangum, J. Lin, and Y. Q. Li. Optical pulling of airborne absorbing particles and biological aerosols in air over a meter-scale distance using a single laser beam, International Conference on the Frontiers in Atomic, Molecular, and Optical Physics. 23-MAY-16, Shanghai, China. : ,

TOTAL: **4**

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received		Paper
08/20/2014	5.00	Linbo Kong, Yong-qing Li, Jinda Lin. Monitoring and analysis of single cell dynamics using confocal Raman imaging and ultralow frequency Raman micro-spectroscopy, XXIV International Conference on Raman Spectroscopy, Jena, Germany.. 10-AUG-14, . : ,
08/22/2014	8.00	Adam G, Hart, Jinda Lin, Yong-qing Li. Optical pulling, trapping and identification of single airborne absorbing particles and bioaerosols using negative photophoretic forces, The 6th International Symposium on Cold Atom Physics. 14-JUN-14, . : ,

TOTAL: **2**

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

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FTE Equivalent:	
Total Number:	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 0.00

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Names of Personnel receiving masters degrees

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Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment

Technology Transfer

Final Report

Title: Characterization of atmospheric biological particles using confocal Raman spectroscopy and optical trapping

Funding number: W911NF-12-2-0024

Author: Yong-qing Li (East Carolina University, Department of Physics, Greenville, NC 27858)

Abstract (200 words):

We have developed new techniques for characterization of atmospheric biological particles using confocal Raman spectroscopy and optical trapping. Achievements include: (1) developed optical pulling technique for single light-absorbing particles and smut spores in air over a meter-scale distance using a single collimated laser beam based on negative photophoretic force; (2) characterized optical trapping and rotation of airborne absorbing particles with a single focused laser beam; (3) developed ultralow frequency Stokes and anti-Stokes Raman spectroscopy for the analysis of single living cells and biomolecules; (4) measured Raman spectra of single airborne absorbing particles trapped by a single laser beam; (5) discovered memory of germinant stimuli in bacterial spores; (6) observed the dynamic germination of single bacterial spores using rapid Raman imaging; (7) characterized uptake of and resistance to the antibiotic berberine by individual dormant, germinating and outgrowing Bacillus spores as monitored by laser tweezers Raman spectroscopy; (8) studied uptake and levels of the antibiotic berberine in individual dormant and germinating *Clostridium difficile* and *Bacillus cereus* spores as measured by laser tweezers Raman spectroscopy; and (9) characterized cold atmospheric plasma inactivation of individual bacterial spores using Raman spectroscopy and phase contrast microscopy. These results help the characterization of atmospheric biological particles.

Scientific Progress and Accomplishments:

The goal of this proposal is to develop novel biosensor techniques for rapid characterization and measurement of single airborne biological particles based on combination of Raman spectroscopy, optical trapping, and fluorescence microscopy. Specific objectives include: (1) development of novel instruments (solid-phase Raman cytometry and microfluidic Raman tweezers) for Raman spectra collection of single bioaerosols; (2) characterization and discrimination of airborne microorganisms based on Raman spectra; (3) study of optical trapping and identification of single aerosols in air environments.

We have successfully accomplished the goals of this project in the past year. Specific achievements include the followings:

1. Optical pulling of airborne absorbing particles and smut spores over a meter-scale distance with negative photophoretic force. We demonstrated optical pulling of single light-absorbing particles and smut spores in air over a meter-scale distance using a single collimated laser beam based on negative photophoretic force. The micron-sized particles are pulled with a collimated Gaussian beam back to the light source at a constant pulling speed of 1-10 cm/s while undergoing transverse rotation motion at a high speed of 0.2-10 kHz. The pulling speed of individual particles is dependent upon the laser intensity, gas pressure, as well as the size and shape of particles. The pulled particles can be precisely manipulated and positioned on the entrance window by steering the laser beam and their chemical compositions can then be characterized with micro-Raman spectroscopy. A variety of light-absorbing particles including carbon nano-clusters, biological smut spores, iron filings, and copper oxide powders were demonstrated to be pulled in this optical pipeline. Optical pulling over large distances with lasers in combination with Raman spectroscopy opens up potential applications for the collection and identification of atmospheric particles in low-pressure environments. [***Appl. Phys. Lett.*** **106, 171906 (2015)**].

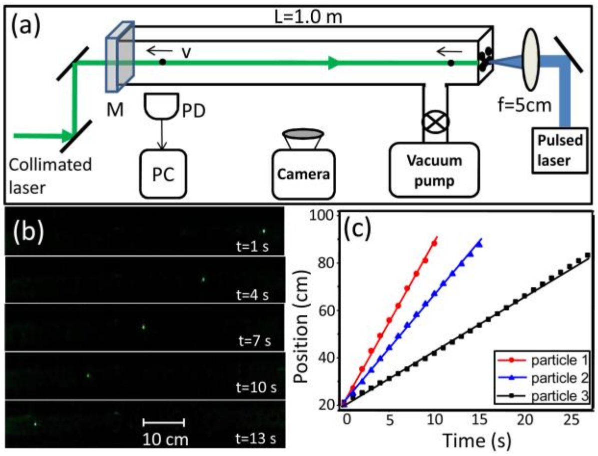

Fig. 1 (a) Schematic setup for pulling a particle with a collimated Gaussian beam in a low-pressure gas medium. The absorbing particles were erupted into the gas medium with a focused pulsed laser and pulled towards the entrance window with a continuous-wave collimated laser beam at velocity v. (b) images of the scattered light of a moving particle recorded with a video camera. The scale bar is 10 cm. (c) Position three individual particles pulled by a laser beam with an intensity of 30W/cm2 at air pressure of 110 torr.

2. Optical trapping and rotation of airborne absorbing particles with a single focused laser beam. We measure the periodic circular motion of single absorbing aerosol particles that are optically trapped with a single focused Gaussian beam and rotate around the laser propagation direction. The scattered light from the trapped particle is observed to be directional and change periodically at 0.4–20 kHz. The instantaneous positions of the moving particle within a rotation period are measured by a high-speed imaging technique using a charge coupled device camera and a repetitively pulsed light-emitting diode illumination. The centripetal acceleration of the trapped particle as high as ~20 times the gravitational acceleration is observed and is attributed to the photophoretic forces. [***Appl. Phys. Lett. 104*, 101909 (2014)**].

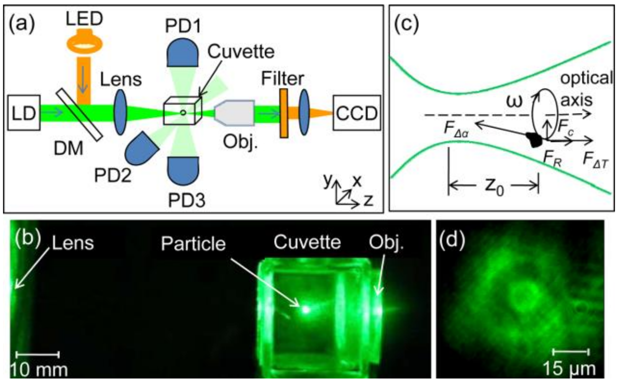

Fig.2 Realization of trapping a rotating absorbing particle. (a) Schematic setup for direct observation of single particles trapped by a single Gaussian beam. (b) Trapping of an absorbing particle in air. (c) Illustration of a particle motion inside a Gaussian beam. (d) Image of the position trajectory of the rotating trapped particle (viewed from facing the laser beam).

<u>3. Development of ultralow frequency Stokes and anti-Stokes Raman spectroscopy of single living cells and microparticles using a hot rubidium vapor filter.</u> We have developed ultralow frequency Stokes and anti-Stokes Raman spectroscopy of single living cells and microsized particles in an aqueous medium with a frequency shift down to 10 cm^{-1} by the combination of a hot rubidium (Rb) vapor filter, a confocal pinhole, and optical trapping. A single frequency-stabilized diode laser beam at 780.2 nm is used to optically trap and excite a single living cell or microparticle, and the Rayleigh scattering light from the particle is effectively blocked with a Rb vapor cell and a confocal pinhole. Ultralow frequency Raman spectra of the trapped cells or microparticles in both Stokes and anti-Stokes regions are then measured with a single-stage CCD spectrograph. [***Opt. Lett. 39, 108*** **(2014)**].

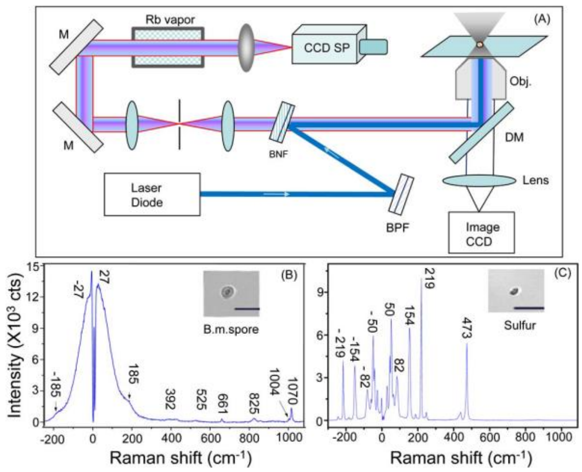

Fig. 3 (A) The schematic of the experimental setup. BPF, Bragg bandpass filter; BNF, Bragg notch filter; Obj., objective; M, mirror; DM, dichroic mirror; SP, spectrograph. (B) Raman spectrum of an optically trapped B. megaterium spore in distilled water. (C) Raman spectrum of a sulfur particle in the trap. The inset is the bright-field image of the particle with a scale bar of 4 μm.

4. Measurement of Raman spectra of single airborne absorbing particles trapped by a single laser beam. We demonstrate a method for optical trapping and Raman spectroscopy of micron-sized, airborne absorbing particles using a single focused laser beam. A single Gaussian beam at 532 nm is used to trap and precisely manipulate absorbing airborne particles. The fluctuation of the position of the trapped particles is substantially reduced by controlling the power of the laser beam with a position-sensitive detector and a locking circuit. Raman spectra of the position-stabilized particles or clusters are then measured with an objective and CCD spectrograph [***Optics Letters*, 38, 416-418 (2013)**].

5. Discovery of memory of germinant stimuli in bacterial spores. Cellular memory is defined as a sustained response to a transient environmental stimulus, and yet its generation and storage have not been described in bacterial spores. We demonstrated that bacterial spores of multiple species retain memory of transient exposures to germinant stimuli that can result in altered responses to subsequent exposure. Memory was induced by activation of germinant receptors (GRs) or by GR-independent germinants and was accessed by both GR-dependent and GR-independent germinants. Analysis of effects on memory of exposure to GR-dependent and GR-independent germinants as well as in spores lacking various germination proteins suggests a model in which memory is stored primarily in metastable states of SpoVA proteins which comprise a channel for release of spore dipicolinic acid. Spore memory can also significantly reduce the concentration of nutrient germinants necessary to trigger germination, and this may be used to respond to low levels of nutrient germinants [***mBio*, 6(6), e01859-15 (2015)**].

6. Observation of the dynamic germination of single bacterial spores using rapid Raman imaging. We developed a methodology that uses a fast Raman imaging system in combination with real-time phase contrast microscopy to observe the dynamics of bacterial spore germination. By using a multifocus scan scheme, the spontaneous Raman-scattering imaging acquisition speed was increased to ~30 s per frame while maintaining diffraction limited resolution, which allowed monitoring of the dynamics of spore germination on a time scale of tens of seconds to a few minutes. This allowed simultaneous gathering of rich spatial distribution information on different cellular components including time-lapse molecular images of Ca-dipicolinic acid, protein, and nucleic acid during germination of single bacterial spores for the periods of 30 to 60 min. [***J. Biomed. Opt.* 19, 011003 (2014)**].

7. Characterization of uptake of and resistance to the antibiotic berberine by individual dormant, germinating and outgrowing Bacillus spores as monitored by laser tweezers Raman spectroscopy. The therapeutic effect of berberine has been attributed to its interaction with nucleic acids and blocking cell division. However, levels of berberine entering individual microbial cells minimal for growth inhibition and its effects on bacterial spores have not been determined. We measured the kinetics and levels of berberine accumulation by individual dormant and germinated spores were measured by laser tweezers Raman spectroscopy and differential interference and fluorescence microscopy, and effects of berberine on spore germination and outgrowth and spore and growing cell viability were determined [***Plos One*, 10 (12), e0144183 (2015)**].

8. Study of uptake and levels of the antibiotic berberine in individual dormant and germinating *Clostridium difficile* and *Bacillus cereus* spores as measured by laser tweezers Raman spectroscopy. Spores of Clostridium difficile and Bacillus cereus are major causes of nosocomial diarrhoea and foodborne disease. Our aim was to measure the dynamics of the uptake of the antibiotic berberine by individual germinating spores and the levels of berberine accumulated in germinated spores. Laser tweezers Raman spectroscopy (LTRS) and differential interference contrast microscopy were used to measure levels of berberine accumulated in single germinating spores and to monitor berberine uptake and germination of individual C. difficile and B. cereus spores. We found that high levels of berberine can enter spores of C. difficile and B. cereus soon after germination is initiated, thus inhibiting spore outgrowth and minimizing hazards posed by germinated spores [***J. Antimicrob. Chemoth.* 71(6):1540-6 (2016)**].

9. Characterization of cold atmospheric plasma inactivation of individual bacterial spores using Raman spectroscopy and phase contrast microscopy. Raman spectroscopy and phase-contrast microscopy were used to examine calcium dipicolinate (CaDPA) levels and rates of nutrient and non-nutrient germination of multiple individual *Bacillus subtilis* spores treated with cold atmospheric plasma (CAP). Major results for this work are: 1) >5 logs of spores deposited on glass surfaces were inactivated by CAP treatment for 3 min, while deposited spores placed inside an impermeable plastic bag were inactivated only ~2 logs in 30 min. 2) >80% of the spores treated for 1-3 min with CAP were non-culturable and retained CaDPA in their core, while >95% of spores treated with CAP for 5-10 min lost all CaDPA. These results suggest that exposure to the present CAP configuration severely damages spore's inner membrane and key germination proteins, such that treated spores either lose CaDPA or can neither initiate nor complete germination with nutrients or CaDPA. Analysis of the various CAP components indicated that UV photons contributed minimally to spore inactivation, while charged particles and reactive oxygen species contributed significantly [***Appl. Environ. Microbiol*. 82, 5775-5784 (2016)**].

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