6-Months USDA-ARS Project Update Report
(June 1 to November 30, 2011)

BARDOT Project
Arun Bhunia, J. Paul Robinson, Euiwon Bae, Bartek Rajwa

Center for Food Safety Engineering
Purdue University
USDA Progress Report

Project Title: Improved Detection Techniques for Foodborne Pathogens
National Program: NP 108 – Food Safety
Objective 2: Develop, evaluate and adopt novel technologies for detection, identification, and quantification of viable and non-viable target microorganisms. Research areas to be addressed are biochip based technology, optical light scattering technology, fluorescence resonance energy transfer spatial technology, and fourier-transform infrared spectroscopy technology.

Subobjective 2B: Develop the BActerial Rapid Detection using Optical light scattering Technology (BARDOT) system to rapidly detect and identify pathogenic bacteria based on unique morphologies generated by concentrated colonies

Introduction

The optical light scattering sensor, BARDOT (BActerial Rapid Detection using Optical light scattering Technology (BARDOT) is a noninvasive label-free detection system which allows identification of bacterial colonies in real-time, and the speed of detection is unparallel to any existing detection device. In this semi-automated system, Petri-dish containing bacterial colonies is placed in the plate holder and the colony map of the plate is acquired. Incident laser beam sequentially runs through each preselected colony and generate scatter signature which is collected and compared to the image library for identification. BARDOT was also demonstrated to detect pathogens from inoculated food samples validating the system's ability to detect pathogens from food. During this reporting period, improvement in BARDOT instrument was made to detect smaller sized colonies. In addition, experiments were done to generate scatter signature library for serovars of *Salmonella* and Shiga-toxin producing *E. coli*, and *Staphylococcus*.

Reporting on Project Milestones

1) Automation of the BARDOT system: Milestone fully met
2) Capture of scattering images from different bacterial species and expansion of the database: Milestone substantially met
3) Begin studies with the Immunomagnetic separation (IMS) and Pathogen enrichment detection device (PEDD): Milestone substantially met.
4) Integration of IMS and PEDD to BARDOT system: Milestone fully met
5) Capture of scattering images from *E. coli*, *Salmonella* and *L. monocytogenes* grown in meat samples: Milestone fully met
6) Improvements in rapid detection by scattering small (<1 mm) bacterial colonies and identification to the genus, species and strain level: Milestone partially met
7) To improve and automate the image processing and analysis software: Milestone fully met

8) Integration of image analysis software into the packaged BARDOT system: Milestone fully met
9) Prototype will be sent to validation by other laboratories: Milestone substantially met

Progress Report:

Previously, we showed that BARDOT can detect and identify colonies of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* and *Vibrio*. During this reporting period, we show that BARDOT is able to differentiate and classify 26 different serovars of *Salmonella* when cultured on selective xylose-lysine deoxycholate (XLD) agar plate (**Manuscript in preparation**). Similarly, BARDOT was evaluated for its ability to distinguish seven common Shiga-toxin producing *E. coli* (STEC) serovars cultured on 4 commonly used chromogenic/selective media. BARDOT efficiently differentiated all seven serotypes when grown on SMAC (Sorbitol MacConkey) and Rainbow media suggesting it could possibly be used for initial typing and screening of suspect *Salmonella* and *E. coli* colonies (**Manuscript in preparation**). BARDOT was sucessfully used with samples that were either prepared by IMS or through PEDD device (**published in early 2011**). Furthermore, to reduce BARDOT-based total detection time for food samples; approach to detect micro-colony was initiated. Data show that BARDOT could differentiate micro-colonies (100 – 150 µm) of *Salmonella*, *E. coli*, *Staphylococcus*, and *Listeria* after 8-12 h of growth (**one manuscript published and one submitted**). A prototype of portable mini-BARDOT system is now under construction for use onsite for pathogen detection. BARDOT units have been delivered to Dr. John Thomas at West Virginia University, Prof. Dan Hirleman at University of California, Merced: and Battelle Laboratories, Columbus, Ohio.

Project accomplishments

Detection of bacterial micro-colonies using elastic light scattering

Forward light scattering patterns from bacterial colonies have been successfully used for bacterial rapid detection. To understand the biophysics behind this phenomenon, the scalar diffraction theory was employed: a bacterial colony is considered as a biological spatial light modulator with amplitude and phase modulation to the incoming light, which continues to propagate to the far-field to form a distinct scattering 'fingerprint'. An integrated colony morphology analyzer (Fig 1) was built to explore the amount of phase and amplitude modulation of micro-colonies (100~500

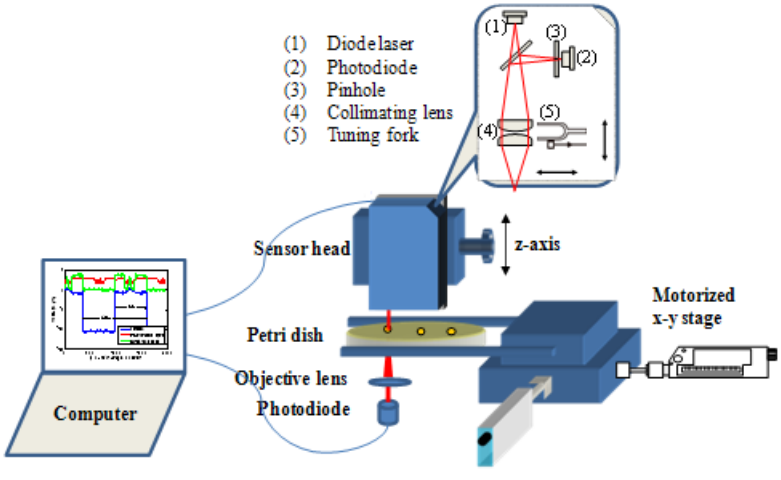

Figure 1. The schematic diagram of the optical analyzer with the laser confocal displacement sensor head at the top and the photodiode circuit at the bottom for transmittance measurement.

μm) due to their homogeneity. The instrument consists of a confocal displacement meter for profile measurements of the bacterial colonies, and a photodiode for simultaneous transmittance measurement. The integrated system was calibrated and tested on two bacterial species: *Escherichia coli* O157:H7 EDL933 (*E. coli* O157:H7) and *Staphylococcus aureus* ATCC 25923 (*S. aureus*). The developed system was able to clearly distinguish the selected bacteria species based on the profile and transmittance measurement (Fig 2), indicating their distinctive scattering patterns. Moreover, growth rates of the two colonies within the observation period were estimated from the experimental data to help us to understand the different growing dynamics of the two species. As colonies keep growing, complex phase structures are formed within. Phase modulation cannot be obtained through profile measurement only. Therefore, a quantitative phase imaging technique particularly with non-interferometric nature was explored. Two solvers based on finite element method and Fast Fourier Transform were developed. An optical fiber with known refractive index profile was used for calibrating phase measurement. The next step is to apply this technique for bacterial colonies phase imaging.

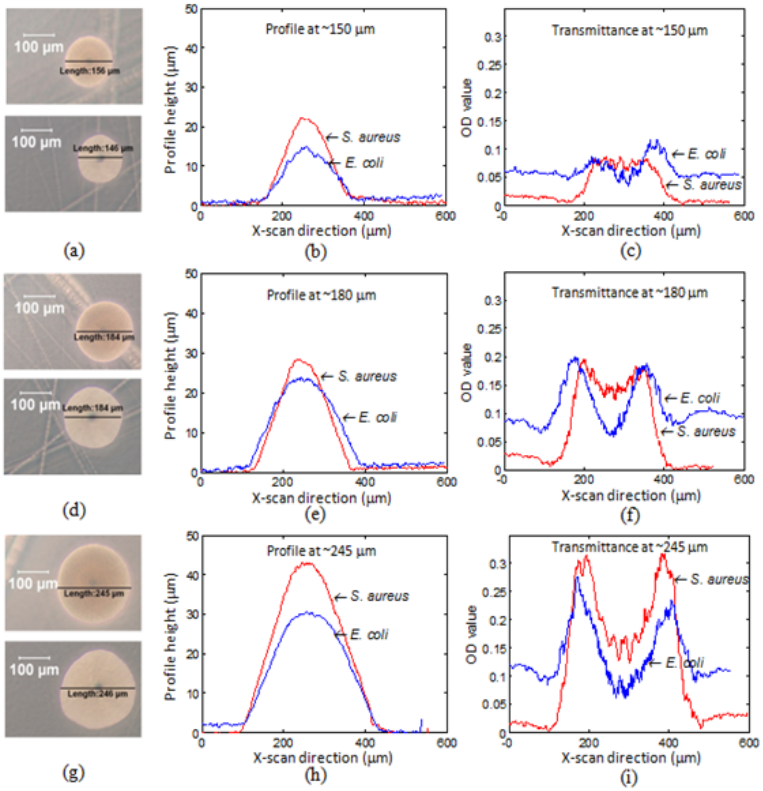

Figure 2. (a) Microscopic images of *S. aureus* (top) and *E. coli* O157:H7(bottom) at ~150 μm; (b) profile comparison of *S. aureus* and *E. coli* O157:H7 at ~150 μm; (c) transmittance comparison of *S. aureus* and *E. coli* O157:H7 at ~150 μm; (d) microscopic images of *S. aureus* (top) and *E. coli* O157:H7 (bottom) at ~180 μm; (e) profile comparison of *S. aureus* and *E. coli* O157:H7 at ~180 μm; (f) transmittance comparison of *S. aureus* and *E. coli* O157:H7 at ~180 μm; (g) microscopic images of *S. aureus* (top) and *E. coli* O157:H7 (bottom) at ~245 μm; (h) profile comparison of *S. aureus* and *E. coli* O157:H7 at ~245 μm; (i) transmittance comparison of *S. aureus* and *E. coli* O157:H7 at ~245 μm.

Improvements in rapid detection by scattering small (<1 mm) bacterial colonies

The primary objective of this component is to make design changes that will allow the robust measurement of smaller colonies, and thus reduce the time to identification. Our long term goal

would be to measure a 100 µm colony. The current colony size is approximately 1 mm and is robust. To reduce colony size there are 3 fundamental modifications that must be made:

1. Reduce the laser diameter to accommodate smaller colonies
2. Increase the resolution of the imaging camera so that colony location can be identified
3. Decrease the imaging distance to the scatter camera to accommodate the significantly larger scatter patters of small colonies.

We have built a prototype now with a 5 megapixel camera capable of identifying the smaller colonies but there are still a number of issued that must be dealt with. Additional funding from our NIH grant was used for most of the hardware prototyping. First, the software must be modified to accept the higher resolution image, and all of the calculations must be altered due to the significantly larger amount of data (doubling the resolution results in a four-fold increase in pixel number). The second issue is linking a smaller laser profile and this can be done by using a different lens, however, we cannot currently have a system that can measure both small and large colonies as this would require an automated lens system that we have designed but could only implement with future funding. Finally, the analytical engine must be changed as we are now dealing with significantly more data (slower speed) and larger data storage. For example, 50 colonies with a 5 megapixel camera now require over 300 megabytes per plate as opposed to around 50 megabytes with the previous camera. Regardless, we have now confidence that we can measure colonies around 300 µm which means a time reduction of 4-6 h over the previous 15-24 h of the first generation instruments. Our goal will be to approach 100 µm during the next round of funding which will mean much faster colony identification and make this system as effective as any current technology since most tools require sample preparation of a few hours regardless of the tools used.

Identification of serovars of Salmonella

The present investigation reports the application potential of laser optical sensor for detection and identification of *Salmonella* serovars, which relies on a non-invasive biophysical principle. Xylose lysine desoxycholate (XLD) agar was found to generate more discriminatory optical scatter patterns for *Salmonella* colonies in comparison to Brain heart infusion (BHI) agar medium based on DOTBAR analysis (Fig 3). Total 26 different *Salmonella* serovars comprising of 64 strains were used to build the scattering image library on XLD agar medium, which was further used to identify contaminating *Salmonella* isolates. Based on the recent *Salmonella* outbreaks as reported by CDC, eight *Salmonella* serovars (*Salmonella* Agona, *Salmonella* Enteritidis PT 21, *Salmonella* Hadar, *Salmonella* Heidelberg, *Salmonella* Montevideo, *Salmonella* Newport, *Salmonella* Typhi and *Salmonella* Typhimurium var. Copenhagen) were selected for detailed study. Scatter image and ribopattern based dendrogram revealed similar grouping of eight *Salmonella* serovars, which underscores and validate the discriminatory potential of the present technique. Spiked experiment with *Salmonella* Typhimurium var. Copenhagen in peanut butter did not reveal significant variation in scattering pattern over a period of 28 days. Laser optical biosensor revealed its application potential as screening tool for *Salmonella* contaminations in pork, turkey and chicken food samples, which was later confirmed by *Salmonella* serovar specific primers and ribotyping. The present laser optical sensor provides a non-destructive and simple tool for large scale screening of food contamination, which can be further confirmed, if necessary by molecular tools.

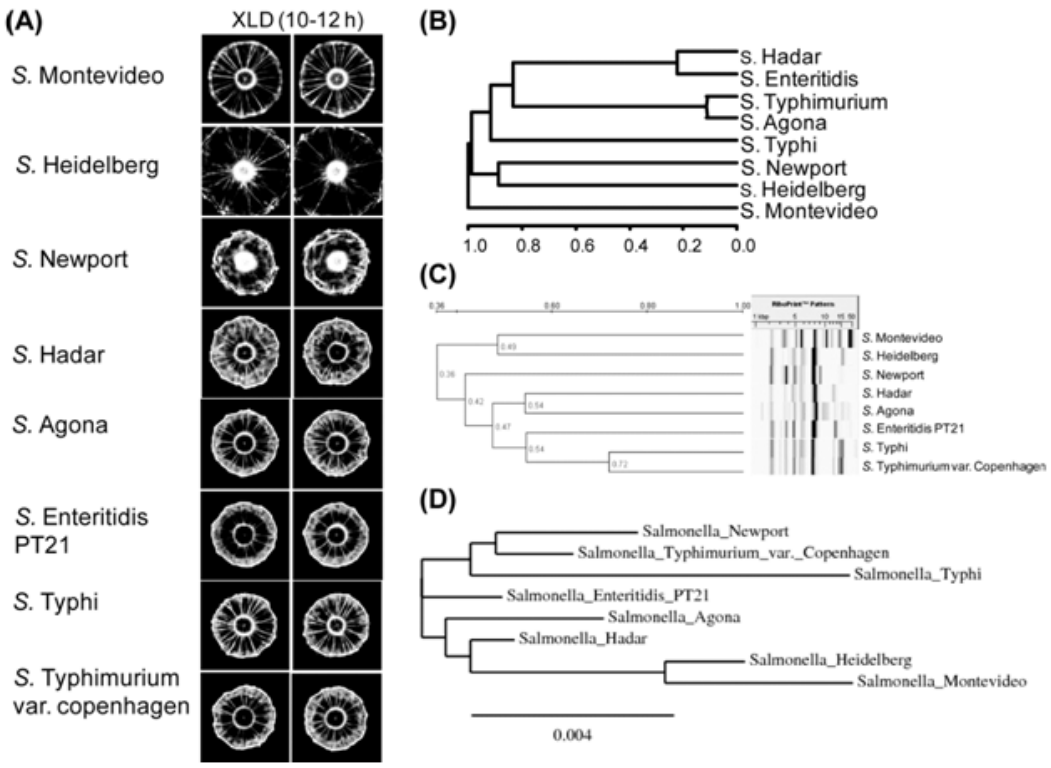

Fig. 3. Comparative analysis of scatter patterns of 8 serovars of *Salmonella* with genomic fingerprint based dendrograms. **(A)** Scatter patterns of select *Salmonella* serovars, **(B)** Dendrogram drawn on the basis of scatter pattern using DOTBAR program. **(C)** Dendrogram drawn on the basis of ribopatterns. **(D)** Dendrogram drawn on the basis of 16S rRNA gene sequence (~1350 bp) based on neighbor-joining algorithm (www.phylogeny.fr).

Identification of Shiga-toxin producing Escherichia coli (STEC)

Shiga-toxin producing *Escherichia coli* (STEC) strains are recognized as serious food-borne pathogens. STEC comprises of a diverse group of O-antigenic organisms, among which O157, O26, O111, O103, O121, O45, and O145 are most frequently implicated in cases of human diseases. Therefore, a sensitive and specific differentiation method for these serovars is in demand. Current culture-based isolation methodology followed by biochemical characterization is relatively time-consuming and may fail to correctly identify non-O157 STEC. Here we evaluated a novel light scattering technology for its ability to instantly differentiate colonies of various *E. coli* serovars on agar plates without compromising the colony integrity. A total of 20 *E. coli* strains of 7 different serotypes were grown on agar plates including Brain Heart Infusion (BHI) agar, Sorbitol MacConkey (SMAC) agar, Rainbow® Agar O157, BBL™ CHROMagar O157, and R&F® *E. coli* O157:H7 medium at 37°C until colony size reached to 1.1 ± 0.1 mm in diameter. The scatter patterns of the colonies were collected using BARDOT and serovar specific images were grouped using image analysis software. Optical microscopy was used to record colony morphology. Colonies of *E. coli* serovars produced distinct scattering patterns on each medium. Of all the media tested, both SMAC and Rainbow® Agar provided the best differential results allowing multi-class classification of all serotypes with average accuracy of 90% in 11-12 h, even though the colony morphology or colors were indistinguishable at that early stage of the growth

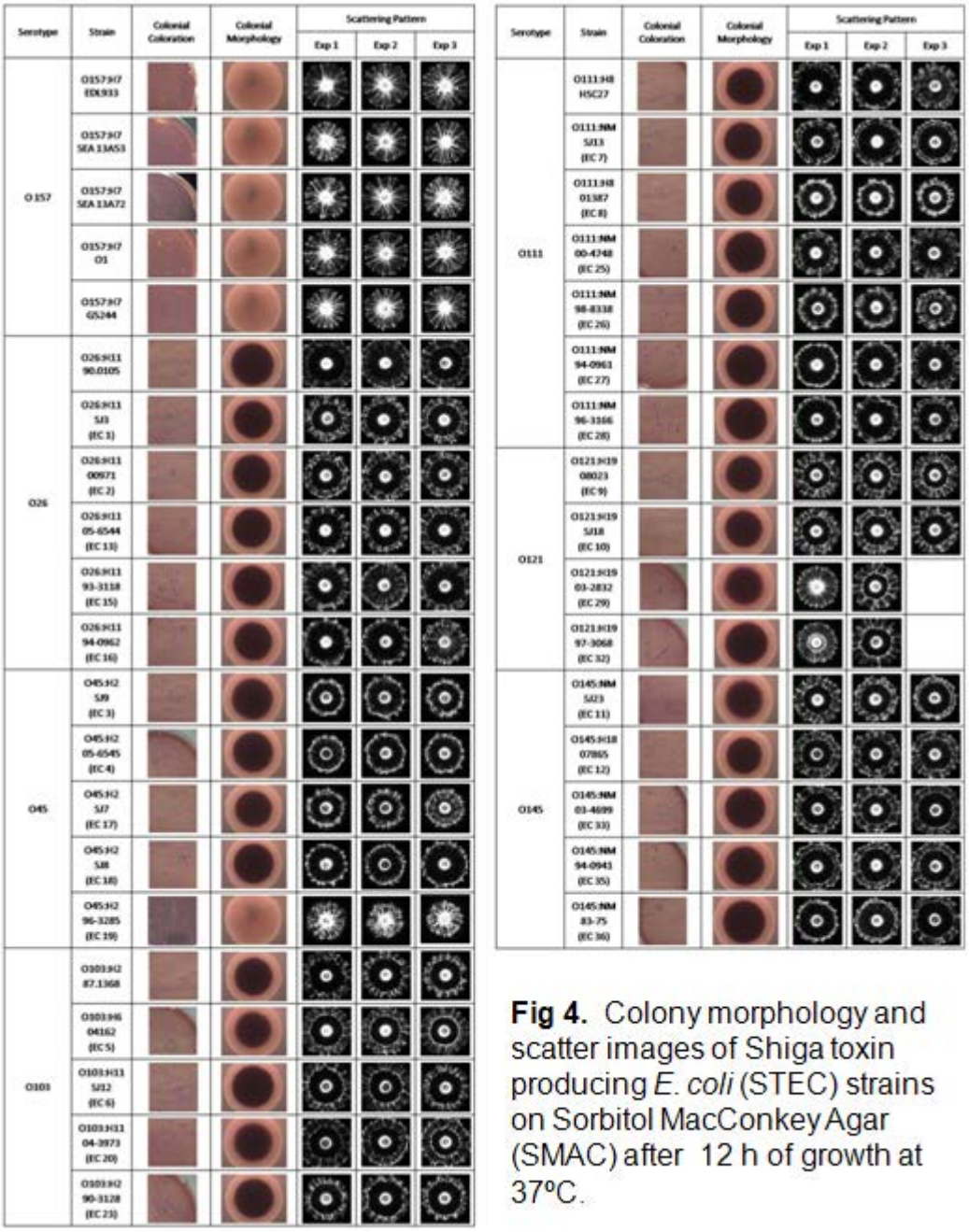

Fig 4. Colony morphology and scatter images of Shiga toxin producing *E. coli* (STEC) strains on Sorbitol MacConkey Agar (SMAC) after 12 h of growth at 37ºC.

(Fig 4). Performance of CHROMagar and R&F® Medium were unsatisfactory with BARDOT-based differentiation because of the relatively low classification accuracy and the poor growth of several non-O157 serotypes. Colonies of STEC were also confirmed by multiplex PCR (Fig 5). These data demonstrated that BARDOT could potentially be used as a prescreening tool for rapid differentiation of the most important STEC groups as they grow on selective agar plate during initial isolation from test samples.

Identification of Shiga-toxin producing Escherichia coli (STEC) using non-exhaustive learning (novelty detection) – study performed in collaboration with M. Murat Dundar (IUPUI)

The forward light scattering technology relies on statistical machine learning to perform recognition and classification. However, the currently used methods utilize traditional supervised techniques that assume completeness of training libraries. In other words, it is assumed that the classifier has an access to all the possible forms of the pathogens of interest. This restrictive assumption is known to be often false for real-life conditions, as we recently learned from the E. coli outbreak in Germany. Therefore, using additional funding from NIH (grant AI085531-01A1) we pursue a study demonstrating that detection and classification of unknown pathogens is indeed possible using the light-scatter based system.

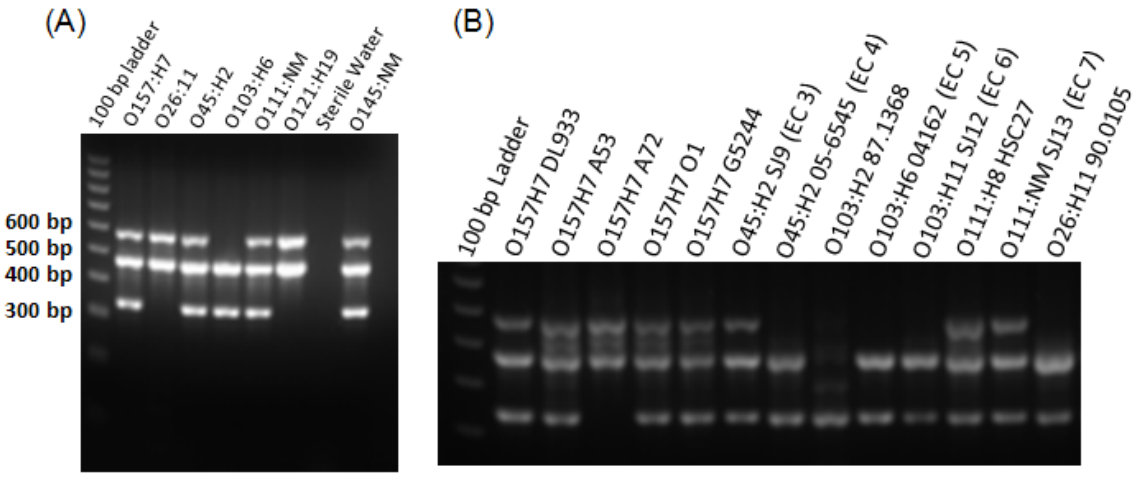

Fig 5. (A and B) Agarose gels showing results of the multiplex PCR assays targeting the *stx2*, *stx1*, and *eaeA* genes from different STEC strains

In our recent publications[a] we demonstrated that classification of *Salmonella* phenotypes not only can be successfully performed with access to exhaustive training libraries, but can also be implemented in a nonexhaustive regimen, i.e., without assuming that access to the complete training set is always available. Although this study demonstrated clearly the feasibility of our approach, it also promoted speculation that a more complex distribution of phenotypes may lead to classification problems that ultimately would not be solvable with the help of parametric methods, i.e., techniques assuming some level of knowledge about the nature of the distributions.

We successfully completed a preliminary study showing an application of nonexhaustive learning technique to the problem of detecting non-O157 STEC. We showed promising preliminary results for models assuming both exhaustive and nonexhaustive training libraries, and we concluded that the complex nature of non-O157 STEC phenotype distribution indeed may require a new generation of statistical machine learning methods to cope with the extraordinary diversity of *E. coli.* Table 1 summarizes the results. It can be seen that even for the two most difficult cases (O103:H11, O103:H12) our system was able to detect presence of previously unseen classes, and correctly separate them from noise and background (with probability ~0.79).

[a] Akova, F., M. Dundar, V. J. Davisson, E. D. Hirleman, A. K. Bhunia, J. P. Robinson, and B. Rajwa. 2010. "A machine-learning approach to detecting unknown bacterial serovars." *Statistical Analysis and Data Mining* 3 (5): 289-301; Rajwa, Bartek, M. Murat Dundar, Ferit Akova, Amanda Bettasso, Valery Patsekin, E. Dan Hirleman, Arun K. Bhunia, and J. Paul Robinson. 2010. "Discovering the unknown: Detection of emerging pathogens using a label-free light-scattering system." *Cytometry Part A* 77A (12): 1103-1112.

Detected class	AUC	SD
4-class model		
O103	0.871	0.009
O111	0.943	0.006
O26	0.829	0.009
7-class model		
O103:H11	0.797	0.020
O103:H2	0.797	0.012
O103:H6	0.873	0.008
O111:H8	0.918	0.011
O111:NM	0.949	0.006
O26:H11	0.852	0.015

Table 1. AUC values illustrating performance of the emerging pathogen detection system for 4-class and 7-class models. The O157 serotype was assumed to be always known. The models used in the study did not assume access to complete information regarding the classes. It was expected that any new instance submitted for classification might in fact belong to a new class for which the current system had no examples. This type of classification reflected a real-life situation in which a laboratory would not have information available about an emerging pathogen that might be encountered in tested samples at the outset of an outbreak.

Prototype development of mini-BARDOT

With the successful implementation of the BARDOT system, there is a great need of miniaturizing the system to a shoe-box size such that the system can be easily carried with the first-responders to the outbreak scenes rather than shipping the samples to the main laboratory. To enable this, a portable bacterial identification system called mini-BARDOT is currently being developed (Fig 6) with additional support from NIH grant # 1R56AI089511-01 ("A distributed clinical & biodefense national network for rapid organism identification").

The overall foot print of mini-BARDOT is just 6"x 12"x 10.5" inches and only weighs **9** pounds. Since the main focus of the design was portability and accessibility, all the hardware connections to the computer have been upgraded to USB such that a simple laptop can be used to operate the system. In the next phase, integrating the output image with the regular BARDOT system will be implemented. Since the data captured via mini-BARDOT from remote location can be wirelessly transmitted to the main database system, it is crucial to test the image compatibility and classification results using the main database system against the mini-BARDOT images. These field tests are presently being performed

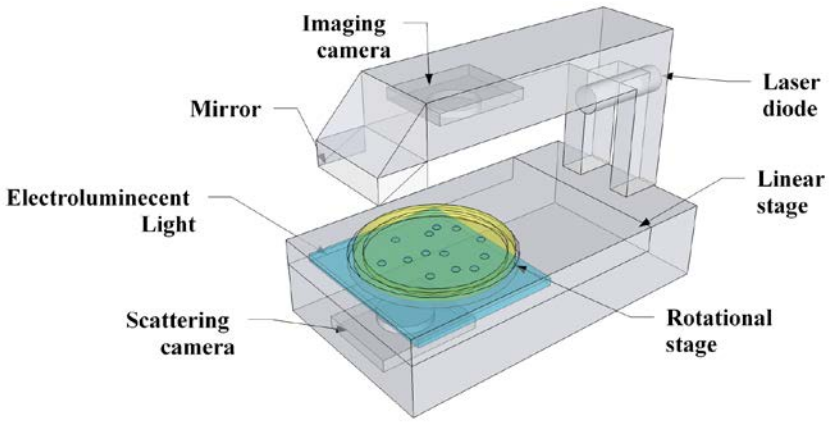

Fig. 6. *Schematic diagram of the portable bacterial identification system using elastic light scattering patterns. The system consists of a linear stage, a rotational stage, and two camera (imaging and scattering), laser diode of 635 nm, and electroluminescent light for back-illumination. Approximate foot print* ***6"x 12"x 10.5"***

Technology Transfer

1. BARDOT is currently being licensed by a startup company, Advanced Bioimaging Systems, LLC., West Lafayette, Indiana for commercial use by the microbiologists and food scientists for evaluation of food samples for possible contamination with foodborne pathogens.

International Cooperation/Collaboration

1. Three visiting scientists; one from Federal University at Pelatos, Brazil, from Jiangnan University, China and from France worked on BARDOT and evaluated its application on detection and identification of *Bacillus* and *Listeria* at Purdue University.
2. Arun Bhunia also visited Denmark Technical University (DTU) at Copenhagen and discussed BARDOT related research activities with the scientists at that institution
3. West Virginia University: A BARDOT instrument has been installed in the microbiology department and initial data are encouraging.
4. University of California, Merced: Professor Dan Hirleman has now received a BARDOT instrument and is establishing programs to collaborate.
5. Battelle Laboratories, Columbus Ohio: An instrument was delivered to Battelle collaborators and initial studies have just begun.

Popular press articles

1. "9/11 spawned big changes on campus" Los Angeles Times, by Scott Gold. Aug 31st, 2011.
2. "9/111 changes curricula on U.S. campuses" The Seattle Times, by Scott Gold. Sept 1, 2011

Publications and Presentations

1. Jaradat, ZW., Rashdan, A.M., Ababneh, Q.O., Jaradat, S.A., and Bhunia, A.K. 2011. Characterization of surface proteins of *Cronobacter muytjensii* using monoclonal antibodies and MALDI-TOF Mass spectrometry. BMC Microbiol 11:148.
2. Bae E., Bai, N., and Hirleman, E.D. 2011 Application of sampling criterion on numerical diffraction from bacterial colonies. Appl Opt 50:2228.
3. Robinson, J.P., Rajwa, B.P., Bae, E., Patsekin, V., Roumani, A.M., Bhunia, A.K., Dietz, J.E., Davidson, V.J., Dunder, M.M., Thomas, J., Hirleman, E.D. 2011. Using scattering to identify bacterial pathogens. Optics and Photonics News. 22(10): 21-27.
4. Bai, N., Tang, Y., Bhunia, A.K., King, G.B., Hirleman, E.D., and Bae, E. 2011. An integrated optical analyzer for dynamic characterization of bacterial micro-colonies (submitted to Journal of Biophotonics)
5. Bae, E., Patsekin, V., Rajwa, B., Bhunia, A.K., Hirleman, E.D., Holdman, C., and Robinson, J.P. 2011. Development of a microbial high-throughput screening instrument based on elastic light scatter patterns" Review of Scientific Instruments (submitted Nov 29, 2011).

6. Sun, X., Bae, E., Bai, N., Tang, Y., and Bhunia, A.K. Rapid detection and identification of *Bacillus* species based on label-free light scattering sensor (to be submitted to BMC Microbiology)
7. Tang, Y., Bae, E., Singh, A.K., Rajwa, B., Robinson,J.P., Hirleman, E.D., and Bhunia, A.K. Differentiation of Shiga toxin producing *Escherichia coli* serogroups by Laser light scatterometer. (in preparation for BMC Microbiology)
8. Bettasso, A., Singh, A.K., Rajwa, B., Bae, E., Robinson, J.P., Hirleman, E.D., and Bhunia, A.K. A laser optical sensor based detection and classification of *Salmonella enterica* serovars. (In preparation)

Book chapter

1. Bhunia, A.K., Bae, E., Rajwa, B., Robinson, J.P. and Hirleman, E.D. 2011. Utilization of optical forward scatter image biological database: food-borne pathogen colony differentiation and detection. *In* Omics Technologies and Microbial Modeling in Food-borne Pathogens Study. Editors: Yan, X., Juneja, V., Huang, L. DEStech Publications, Inc. Lancaster, PA. pp 599-624.
2. Bae, E. and A. K. Bhunia. 2011. Nano Optical Sensors for Food Safety and Security, In Optochemical Nanosensors, Editor, M. Giordano, Wiley Interscience (submitted July 12, 2011)

Proceedings

1. Bai, N., Y. Tang, A. K. Bhunia, E. D. Hirleman, E. Bae. 2011. Characterization of optical properties of bacterial micro-colonies via the comprehensive morphology analyzer. *Proceedings of SPIE*. 8027: 80270C
2. Rajwa, B., Dundar, M.M., Akova, F., Patsekin, V., Bae, E., Tang, Y., Dietz, J.E., Hirleman, E.D., Robinson, J.P., and Bhunia, A.K. 2011. Digital microbiology: detection and classification of unknown bacterial pathogens using a label-free laser light scatter-sensing system. *Proceedings of SPIE*. Vol. 8029.
3. Slone, W.L., Waters, C.L., Corum, L., Robinson, J.P., Bae, E., and Thomas, J.G. 2011. Integrating optical scatter technology (BARDOT) to define microbial pathogens in wounds; Society of Acute Wound Care, Las Vegas, Oct 13 -16, 2011
4. Slone, W.L., Waters, C.L., Corum, L., Thomas, J.G., Robinson, J.P., and Motlagh, H.M. 2011. Application of Optical Scatter Technology (BARDOT) to rapidly unmask and map resistant profiles in wound surveillance, Society of Acute Wound Care Las Vegas, Oct 13 -16, 2011

Presentations

1. Bhunia, A.K. 2011. High throughput screening of foods for pathogens using biosensor" Workshop on Novel sampling and Sensing for Improving Food Safety" Georgia Tech, Atlanta, GA June 16-17, 2011
2. Bhunia, A.K., Bae, E., Bai, N., Singh, A., and Tang, Y. Harnessing light scattering for label-free identification of food-borne pathogens. ASABE meeting, Louisville, KY, Aug 8, 2011.
3. Bae, E., Bai, N., Singh, A., and Tang, Y., Bhunia, A.K., Light scattering sensor: Biophysics of colony-light interactions. ASABE Annual Meeting, Louisville, KY, Aug 8, 2011.

4. Bhunia, A.K. 2011. Novel Biosensor Tools and Pathogenic Mechanism: Complimentary Approaches to Food Safety. Dept of Food Science and Human Nutrition, Iowa State University, Ames, IA, Sept 7, 2011
5. Bhunia, A.K. 2011. Novel biosensor technologies for high throughput screening of pathogens and toxins. 6th International Conference for Food Safety and Quality, Chicago, Nov 8-9, 2011
6. Bhunia, A.K. 2011. Novel optical biosensors for detection of viable pathogens and toxins, The Sixth International Forum on Food Safety and 2011 Annual Meeting of MOST-USDA Joint Research Center for Food Safety. Shanghai, China. Sept 27-29, 2011.
7. Bhunia, A.K. 2011. Biosensor Technologies for Pathogen and Toxin Detection, Jiangnan University, Wuxi, China, Sept 30, 2011.
8. Bhunia, A.K. 2011. Advanced pathogen detection systems, 5th International Symposium on Recent Advances in Food Analysis (RAFA 2011). Prague, Czech Republic, Nov 1-4, 2011
9. Bhunia, A.K. 2011. Novel Biosensor Tools and Bacterial Pathogenesis: Complimentary Approaches to Food Safety. Technical University of Denmark (DTU), Copenhagen, Denmark, Nov 2, 2011.
10. Bae, E, 2011. Current efforts in harnessing elastic light scattering in multi-scale problem, Korea Advanced Institute of Science and Technology (KAIST), Daejon, Korea, Jul 13, 2011
11. Bae, E, 2011. Applying elastic light scattering in bacterial identification-From laboratory to a national surveliance network, Korea Institute of Science and Technology (KIST), Seoul, Korea, Jul 11, 2011

Thesis/Dissertation

Hyochin Kim. Listeria adhesion protein mediated *Listeria monocytogenes* translocation and pathogenesis in cell culture model. PhD Thesis. 2011. Purdue University. 177 p.

Members of BARDOT team

1. Arun Bhunia: PI
2. J. Paul Robinson: Co-PI
3. Euiwon Bae: Co-PI
4. Bartek Rajwa: Co-PI
5. Atul K. Singh: Postdoc
6. Mary-Ann Roshni Amalaradjou: Postdoc
7. Valery Patsekin: Technical Associate
8. Yanjie Tang: Grad student: Strategies to scatterotype Shiga-toxin producing Escherichia co li (STEC) using scatterometer
9. Nan Bai: Grad student: Engineering of BARDOT to detect bacterial micro-colonies
10. Huisung Kim: Grad student: Engineering of BARDOT to detect bacterial micro-colonies
11. Dawei Ying: Grad student: Robust image analysis system for closely related bacterial sero vars

Plans for collaboration with USDA-ARS

A second generation BARDOT unit was delivered to Dr. Shu-I Tu and Dr. George Paoli about 3 years back. Now, Advanced Bioimaging Systesms, LLC is in the process of delivering the newest model of BARDOT to USDA in early January (1212).