C3D Technology: Grand Prize Winner of U.S. Food and Drug Administration's first Food Safety Challenge!
The Center for Food Safety Engineering (CFSE) at Purdue University was established in 2000 as a partnership with the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Eastern Regional Research Center (ERRC). The mission of the CFSE is to develop new knowledge, technologies, and systems for detection and prevention of chemical and microbial contamination of foods while training the next generation of food safety scientists and engineers. Our researchers are developing systems that use advanced engineering principles coupled with microbiological techniques. These systems include:

1. C3D: Effective food sampling protocols and filtration techniques to maximize microbial cell separation and concentration in an automated instrument.
2. Biochip: Biochip systems, using immunobiology and electrochemistry, for detecting viable cells of *Salmonella enterica* serovars, Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* in food.
3. BARDOT: The Bacterial Rapid Detection using Optical scattering Technology (BARDOT) system for microcolony detection and identification of bacteria, including pathogenic *L. monocytogenes*, select *Salmonella* serovars, and STEC.
5. Raman Sensor: A Raman biosensing platform for detecting single pathogenic cells.
6. And many others, such as a lateral flow immunochromatography system, next-generation metagenomic sequencing methods, immunocytochemistry techniques, and immunobiosensors.

As the collaboration between the Center for Food Safety Engineering (CFSE) at Purdue University and the USDA, Agricultural Research Service (ARS) Eastern Regional Research Center (ERRC) continues, I am pleased to witness the growth, maturation and impact of this partnership. The partnership is considered an important part of USDA-ARS efforts in food safety research, addressing high priority ARS research goals focused on foodborne pathogens and pathogen detection technologies.

There were many highlights and banner accomplishments during 2014-2015. The winner of the FDA's first Food Safety Challenge was our very own C3D technology. Our biochip technology was also among the top 5 finalists in this challenge. CFSE members circled the globe, participating in important food safety conferences and activities from our home base to China, Taiwan, Afghanistan, Brazil, Denmark, and beyond. Dr. Stephen On, a prominent food safety researcher from New Zealand visited the CFSE in May. This newsletter contains only an overview of the many activities and accomplishments of the CFSE. If you are interested in learning more about the CFSE, please visit our Web site at www.cfse.purdue.edu or contact me directly. Together, we can work to ensure the safety of the global food supply.

Director
Dr. Lisa Mauer
Professor, Purdue University Department of Food Science

As the ARS principal investigator and collaborative participant, I look forward to the continued growth and maturation of the CFSE technologies and the ERRC-CFSE partnership.

Dr. George C. Paoli
USDA, ARS Principal Investigator

The outstanding research and the growth of the CFSE technologies were evident by the quality and variety of the presentations made by CFSE scientists at the ERRC-CFSE Annual Meeting held at the ERRC in January of this year. In addition to this annual meeting, USDA-ARS and CFSE scientists were invited to travel to China to participate in scientific meetings of two collaborative research centers established between the USDA and The China Ministry of Science and Technology (MOST): The USDA-MOST Virtual Food Safety Research Center at Shanghai Jiao Tong University and The China Joint Research Center for Food Quality and Safety Control at Nanjing Agricultural University; providing CFSE scientists an international forum in which to present their research accomplishments.

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Dr. George C. Paoli
USDA, ARS Principal Investigator
The 2014 FDA Food Safety Challenge announced on September 23, 2014, is a call to scientists, academics, entrepreneurs, and innovators from all disciplines to submit concepts applying novel and/or advanced methodologies to foster revolutionary improvements in foodborne pathogen detection. Specifically, concepts should apply cutting-edge techniques to achieve significant improvements in the speed of the FDA’s detection methods for Salmonella with identification to the subtype/serovar level in minimally processed fresh produce. FDA is most interested in concepts that explore the acceleration or elimination of sample preparation and/or enrichment in the testing process, and/or those that employ novel or revolutionary techniques to achieve pathogen detection. As FDAs food safety program incorporates preventive control measures through the implementation of the FDA Food Safety Modernization Act, quicker detection of these harmful bacteria will help to prevent foodborne illnesses.

Two CFSE technologies were among the top 5 finalists announced on May 11, 2015: the portable system for multiplexed detection of foodborne pathogens in microfluidic biochips through isothermal DNA amplification and electrical detection; and the physical method for concentrating Salmonella to detectable levels using automated microfiltration. All finalists moved to the field accelerator phase of the challenge, in which their concepts were improved with coaching and mentorship from experts in food safety and pathogen testing. At an in-person boot camp with FDA experts, the finalists focused on design, innovation, and the FDAs testing process to help iterate and strengthen their concepts. The finalists presented their concepts at Demo Day in Washington, DC, before a live audience of stakeholders in food and food safety from government and the private sector.

As reported by U.S. News and World Report:

“During the competition, five finalists offered solutions aimed at making Salmonella detection not only easier, but portable. The idea is to give every key player in the food processing chain – from harvest and packaging to distribution and retail – the ability to conduct food safety tests. The goal is to make “farm to fork” a risk-free journey for consumers.”

In his remarks, Captain Palmer Orlandi, Acting Chief Science Officer and Research Director in the FDAs Office of Foods and Veterinary Medicine, spoke to the importance of the program for FDA:

“We have some fantastic ideas. That was the whole concept of our challenge,” Capt. Orlandi said. “There are so many needs for food safety. [This competition] is not a means to an end, but to building a relationship moving forward.”

The Purdue University team’s winning submission, announced on July 22, is the physical method for concentrating Salmonella to detectable levels using automated microfiltration, which could decrease sample preparation time from 24-28 hours to a range of two to three hours. Congratulations to the winning team, led by Michael Ladisch and including Eduardo Ximenes, Kirk Foster, Seockmo Ku, Amanda Deering, and Thomas Kreke.

Read more at: www.foodsafetychallenge.com
Review Article Featuring CFSE Technologies

Joseph Irudayaraj pulled the CFSE team together to write a review article on foodborne pathogen detection technologies. The article, “Nano/Micro and Spectroscopic Approaches to Food Pathogen Detection”, is featured in Volume 7 of the Annual Review of Analytical Chemistry and can be accessed at doi:10.1146/annurev-anchem-071213-020249.

Annual USDA-CFSE Research Planning Meeting

USDA scientists traveled to Purdue to participate in the annual research planning meeting in November, 2014. In addition to research updates and planning of collaborative studies, the pathogen detection technologies were demonstrated in the Purdue laboratories, and a poster session featured ongoing work and student accomplishments.

Scientists in the Center for Food Safety Engineering at Purdue University are teaching the next generation about microorganisms in food. CFSE faculty and students showed youngsters how to plate spinach and tests for microorganisms during Spring Fest, an annual event that draws over 30,000 people to campus to learn about science and technology.
Dr. Bruce Applegate

**Project Title**  
**Phage**: Development of bacteriophages for the detection of *E. coli* O157:H7 and other pathogenic bacteria in food

**Project Description**  
Bacteriophages are viruses that are only able to infect bacteria. The goal of this project is to have the bacteriophages produce and emit light when they have infected a target bacterium. The light can then be detected to rapidly identify if a harmful pathogenic bacteria, such as *E. coli* O157:H7, was present in a food sample.

**Project Highlight**  
The developed technology platform exploiting the modified bacteriophage ΦV10lux can be integrated with current FDA protocols for detection of *E. coli* O157:H7 in leafy greens without protocol modification.

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Dr. Rashid Bashir

**Project Title**  
**BIOCHIP**: Microfabrication of biochips able to concentrate, quantify, and detect pathogenic bacteria from food using PCR

**Project Description**  
Biochips are miniature laboratories, the size of a postage stamp, that are able to perform many simultaneous functions to rapidly screen numerous samples. Polymerase chain reaction (PCR) is a commonly used laboratory technique in which a little DNA from a target bacterium is used to generate thousands to millions of copies of the DNA sequence. These copies can then be detected using the biochips. Coupling PCR with the biochip technology results in a rapid detection method for identifying pathogenic bacteria in a food sample.

**Project Highlight**  
Our group is at the forefront of the development of field effect transistor technology having designed methods for sample preparation and a solid platform for multiplexed electrical detection with one million sensors working in parallel. This project has the potential to yield a novel detection system that quickly interrogates the presence of multiple pathogens in an inexpensive, easy-to-use, portable device.
Drs. Arun Bhunia, J. Paul Robinson, Euiwon Bae, and Bartek Rajwa

**Project Title**
**BARDOT: Development of light scattering technologies for the identification of pathogenic bacteria**

**Project Description**
A laser sensor is used to instantly screen bacterial colonies on a Petri-dish for early pathogen detection. The sensor, designated BARDOT (Bacterial Rapid Detection using Optical light scattering Technology), is a noninvasive label-free detection and identification system that works by passing a laser beam through each bacterial colony present on a Petri-dish. This generates a light scatter signature that is specific to each bacterium (analogous to a human fingerprint) and enables the identification of bacterial pathogens in food samples. The BARDOT instrument has been miniaturized into a portable device that is similar in size to a sewing machine.

**Project Highlight**
BARDOT is a user-friendly high throughput detection device that can rapidly screen food samples for the presence of harmful bacterial pathogens to enhance food safety, reduce foodborne outbreaks, and save lives.


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**Dr. Amanda Deering**

**Project Title**
**Plant-Pathogen Interactions**

**Project Description**
Fresh produce has been implicated in numerous outbreaks in recent years. This project aims to better understand the interactions between human pathogenic bacteria and plant tissues, using classical microbiological techniques and developing immunocytochemical methods to assess the ability of pathogens to persist, grow, and localize on and within plants. Understanding these interactions will help growers understand where pathogens can be introduce during growing to help develop methods of prevention that will aid in providing safe fresh produce to consumers.

**Project Highlight**
By better understanding how human pathogenic bacteria interact with plants, prevention methods can be developed to aid in providing safe fresh produce to consumers.
Dr. Joseph Irudayaraj

**Project Title**

**Raman Sensor:** Development of a highly sensitive enhanced Raman spectrosensor for the identification of foodborne pathogens

**Project Description**

Surface enhanced Raman spectroscopy (SERS) can be used to detect bacteria in foods by identifying different spectra that are unique to each bacterium, much like fingerprint analysis. The SERS signal enhancement enables the detection of low concentrations of pathogenic bacteria in food samples. Sensitive fluorescent assays were also developed to quickly detect low numbers of pathogenic bacteria in foods.

**Project Highlight**

Two sensitive biosensor techniques, using Raman spectroscopy and fluorescent immunoassays, are portable and useful for rapid onsite detection of low concentrations of foodborne pathogens.

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Dr. Michael Ladisch

**Project Title**

**C3D:** Concentration of pathogenic bacteria from food samples

**Project Description**

An important first step in pathogen detection is the ability to quickly separate the microorganisms from large food samples without harming them. The complexity of food samples makes this a particular challenge. The C3D technique combines mechanical shearing and enzyme treatments with rapid microfiltration through special membranes. After passing through the C3D, the small volume of concentrated sample contains the bacteria that were present in the initial food sample. This is important when the final detection method is only capable of analyzing small volumes, as is the case for the PCR and biochip techniques.

**Project Highlight**

We could think of C3D as a quick way to find a few needles in a haystack, and then be able to test the needles to find out what kind they are. Our cell concentration unit can be followed by USDA and FDA approved methods to speed the time to pathogen detection.
Dr. Haley Oliver

**Project Title**
Virulence Capacity

**Project Description**
The long-term goal of this effort is to assess genetic variation and virulence capacity within *Salmonella* Heidelberg and *Listeria monocytogenes*. This work will provide insight into characteristics that make certain strains more likely to cause human disease and may help determine which should be considered higher priority for regulation and detection.

**Project Highlight**
Over the last year, we found that *Listeria monocytogenes* isolates from retail deli environments have a higher likelihood to potentially cause disease when compared to isolates from retail foods, suggesting that cross-contamination from the deli environment to foods poses a significant public health risk. We have also found that some *S. Heidelberg* isolates related to a foodborne outbreak have enhanced heat resistance compared to non-outbreak strains, which may partially explain the large scope of the outbreak and the severity of disease associated with these strains.

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Dr. Robert Pruitt

**Project Title**
Bacterial Community Analysis

**Project Description**
Our project uses molecular methods to validate both classical selective enrichment methods and more modern laser scatter pattern detection methods for their ability to accurately identify specific human pathogenic bacteria. These techniques are being used to characterize the complete bacterial community found on fresh produce that has been associated with foodborne illness outbreaks.

**Project Highlight**
By using modern DNA sequencing technology to better understand the communities of bacteria that naturally associate with plants and how those communities can sometimes be infiltrated by human pathogens, we can better understand situations that result in outbreaks of foodborne illness.
Dr. Manpreet Singh

Project Title
Poultry Food Safety

Project Description
Contamination of poultry may occur throughout the production chain, and important risk factors for contamination at each stage of the process need to be identified. An efficient and cost-effective risk-based control program needs to be developed to reduce the horizontal spread of *Salmonella* and *Campylobacter* at various stages of the production chain. The overall goal of this project is to evaluate rapid detection methods as an alternative to the current USDA-FSIS methods of *Salmonella* detection on chilled poultry carcasses.

Project Highlight
In collaboration with Dr. Bhunia’s laboratory, we are working on decreasing the time to determine prevalence of *Salmonella* on poultry carcasses. Validation of the BARDOT system with carcass rinse samples has been performed and compared to the traditional cultural methods followed by the USDA-FSIS. Validating the accuracy of the BARDOT with field samples is critical for the poultry industry in addition to the ability of detection methods to identify pathogens that are present in a mixed microflora.

Demonstration of a whole carcass rinse for determining *Salmonella* and *Campylobacter* contamination as per the USDA-FSIS guideline.
JOURNAL ARTICLES:

1) Bhunia, A.K. 2014. One day to one hour: how quickly can foodborne pathogens be detected? Future Microbiology. 9(8):935-946.


BOOK CHAPTERS:


BOOK:


PROCEEDINGS:


Dissertation/Thesis:

30) Bach, C.E. 2015. Influence and characterization of microbial contaminants associated with the FDA BAM method used to detect Listeria monocytogenes from Romaine lettuce. [M.S. Thesis]. West Lafayette, IN: Purdue University.


Work Presented at the Following:

- International Association of Food Protection Annual Meeting – Indianapolis, IN
- Izmir Institute of Technology – Izmir (Urla), Republic of Turkey
- National Health Research Institute – Zhunan, Taiwan
- NNEAFF Farm to Fork Symposium, University of Illinois – Urbana-Champaign, IL
- North Central Regional Center for Rural Development (NCRCRD) at Michigan State University - East Lansing, MI
- Plant Science Poster Networking Session, Purdue University – West Lafayette, IN
- Purdue University Undergraduate Research Symposium – West Lafayette, IN
- Rapid Detection for Food Safety. Knowledge Foundation – Bethesda, MD
- Qualcomm Innovation Fellowship Finals – San Diego, CA
- Quantum DX – New Castle, England
- Sichuan University – Chengdu, China
- Southwest University for Nationalities – Chengdu, China
- Taiwan Semiconductor Manufacturing Company – Hsinchu, Taiwan
- Third Annual Indiana Small Farm Conference – Danville, IN

2014-2015 Center for Food Safety Engineering Key Scientists

Dr. Bruce Applegate
765-496-7920
applegate@purdue.edu

Dr. Arun Bhunia
765-494-5443
bhunia@ecn.purdue.edu

Dr. Michael Ladisch
765-494-7022
ladisch@ecn.purdue.edu

Dr. Bartek Rajwa
765-496-1153
brajwa@purdue.edu

Dr. Euiwon Bae
765-494-4762
baee@ecn.purdue.edu

Dr. Amanda Deering
765-494-0512
adeering@ecn.purdue.edu

Dr. J. Paul Robinson
765-494-6449
wombat@purdue.edu

Dr. Rashid Bashir
217-333-3097
rbashir@illinois.edu

Dr. Joseph Irudayaraj
765-494-0388
josephi@ecn.purdue.edu

Dr. Haley Oliver
765-494-3913
hfoliver@ecn.purdue.edu

Dr. Robert Pruitt
765-496-6794
rep@ecn.purdue.edu

Dr. Manpreet Singh
765-494-0823
manpreet@ecn.purdue.edu
INFORMATION CONTACTS

Dr. Lisa Mauer  
Professor and Director  
amuer@purdue.edu  
765-494-9111

Dr. Amanda Deering  
Operations Manager  
adeering@purdue.edu  
765-494-0512

Or visit our website:  
www.cfse.purdue.edu