# Optical forward scattering for bacterial colony differentiation and identification

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## Project Rationale

The CDC estimates that 76 million people get sick, more than 300,000 are hospitalized, and 5,000 Americans die each year from foodborne pathogen infections. Preventing foodborne illnesses remains a major public health challenge. *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* are three major foodborne pathogens of concern in the U.S. There has been an increase in foodborne illnesses, multiple outbreaks, product recalls, and loss of lives resulting from the association of pathogens in raw and processed, ready-to-eat food products. Bacterial contamination in products not only places the public at risk, but it is also costly to companies due to the loss of production time, product recalls and liability.

For detecting and evaluating foods contaminated with *L. monocytogenes* or *E. coli*, USDA/FSIS recommends initial enrichment and subsequent plating on a selective agar media, which is often followed by further identification procedures. These procedures are often time consuming and lengthy, since they take as much as 5 to 7 days. The present industrial demand is to increase the speed of the detection and to decrease economical losses and the chance of public health concerns. Our main objective was to develop a simple light scattering instrument now known as BARDOT. We introduced a model for optical forward scattering by bacterial colony based on scalar diffraction theory. The model treats the colony as an amplitude/phase modulator and suggests macroscopic factors that cause the distinctive features shown in forward scattering signatures of the three types of *Listeria* species. We used phase contrast and confocal microscopy to provide independent information on the structure and morphology of the colonies that are fixed parameters on the scattering model. We validated the experimental system using a chrome mask reference sample with known diffraction properties. Distinctive scattering patterns measured for three important species of *Listeria* were found to show good agreement with the model predictions. The results provide a physical explanation for the unique and distinctive scattering signatures produced by colonies of closely related *Listeria* species and support the efficacy of forward scattering for rapid detection and classification of pathogens without the use of labeling molecules. This improvement has provided the groundwork for developing the portable, stand-alone type of bacterial characterization instrument now known as BARDOT.

## Project Objectives

- Analyze bacterial colonies of different foodborne bacteria on non-selective and selective agar media.
- Validate the technology by using inherently contaminated food samples and samples that have been inoculated with selected pathogens.
- Analyze cellular composition, cell arrangement, refractive index and colony contents using electron microscopy, FT-IR or GC-MS.
- Analyze the scatter signal images using “standard feature extraction” and “moments of shape analysis” methods.

## Project Highlights

The most significant accomplishment this year was the design of an automated BARDOT system and related algorithm. We introduced a model for optical forward scattering by bacterial colony based on scalar diffraction theory. The model treats the colony as an amplitude/phase modulator and suggests macroscopic factors that cause the distinctive features shown in forward scattering signatures of the three types of *Listeria* species. We used phase contrast and confocal microscopy to provide independent information on the structure and morphology of the colonies that are fixed parameters on the scattering model. We validated the experimental system using a chrome mask reference sample with known diffraction properties. Distinctive scattering patterns measured for three important species of *Listeria* were found to show good agreement with the model predictions. The results provide a physical explanation for the unique and distinctive scattering signatures produced by colonies of closely related *Listeria* species and support the efficacy of forward scattering for rapid detection and classification of pathogens without the use of labeling molecules. This improvement has provided the groundwork for developing the portable, stand-alone type of bacterial characterization instrument now known as BARDOT.

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