Optical forward scattering for bacterial colony differentiation and identification

Project Rationale
We are in the process of refining our BActeria Rapid Detection using Optical Technology (BARDOT) system to locate, capture, and classify foodborne pathogenic bacteria. The system requires a human operator to position the bacterial colony to the incident laser beam and adjust it such that the forward-scattering patterns are rotationally symmetric. This task is an iterative process and requires constant operator attention. By automating the instrument operation, we will increase its efficiency and reduce the time required for bacterial identification. In this reporting period, we concentrated our efforts on developing a fully automated detection and identification system which integrates the hardware and software of the forward-light scatterometer with the in-house classification software package. In addition, we tested the BARDOT system’s ability to detect microcolonies at the 100 to 200 μm diameter range (which, if successful, would substantially reduce the time required for bacterial growth and, therefore, time-to-result for test samples), and to detect pathogens in cooked and raw food samples.

Project Objectives
- Experiment with bacterial colony scattering at colony diameters of less than 500 μm.
- Acquire scatter images of colonies of select foodborne bacteria on non-selective and selective agar media.
- Test the BARDOT system’s ability to detect foodborne pathogens from artificially contaminated meat, vegetables, and seafood samples.

Project Highlights
We demonstrated that BARDOT can identify and differentiate microcolonies (at approximately 173 micrometerμm in diameter) of Listeria innocua and E. coli after only eight hours of growth on an agar plate. Furthermore, laser triangulation sensors provided some profilometric data that show differences in colony morphology for different bacterial species.

In addition, we have generated scatter signatures for 14 serovars of Salmonella, 100 serotypes of Escherichia coli O157:H7 and other serotypes, and several other bacterial cultures to expand our scatter image library. Finally, we have shown that BARDOT can be used for detecting Salmonella from inoculated peanut butter and tomato, E. coli O157:H7 from ground beef and spinach, and Listeria monocytogenes from inoculated hotdog and milk samples, even after a prolonged storage at very low concentrations.

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