Optical forward scattering for colony identification and differentiation of bacterial species

E. D. Hirleman (ME/ECE), Arun K. Bhunia (FS), J.P. Robinson (BME/BMS) and B. Rajwa (BMS)
Center for Food Safety and Engineering
Purdue University
CFSE Annual Review
Nov 2, 2005

Optical forward scattering for rapid identification and differentiation of bacterial species......an interdisciplinary approach

Scatterometer

>1500 scatter patterns from cultures of 108 Listeria strains were measured and analyzed
69 - L. monocytogenes
16 - L. innocua
12 - L. ivanovii
5 - L. seeligeri
3 - L. welshimeri
3 - L. grayi

Scattergraphs of representative Listeria species

Understanding the Physics:
Modeling and simulation of forward scatter patterns using diffraction theory

U(x,y) - Field on image plane (the scattergraph)
U'(ζ,η) - Field just behind the colony + substrate, a function of:
  • Incident Gaussian beam properties
  • Scattering and attenuation by sample + substrate

Cryo-NanoSEM of Listeria colonies
Modeling and Simulations

A. Maximum Intensity Projection
Confocal microscope image of Listeria monocytogenes

B. Colony Surface Model using two different radii of curvature R11 and R22 representing 2-stage curvature

C. Simulated image with single-stage curvature (focal length of 20 mm)

D. Simulated image with 2-stage curvature (focal length of 20 mm and 70 mm)

E. Simulated image with 2-stage curvature including phase modulation

F. Scatter image of colony

Image analysis using 2D radial Zernike polynomials

The Zernike polynomials are a set of orthogonal polynomials that arise in the expansion of a wavefront function for optical systems with circular pupils. They were introduced by F. Zernike in 1934: Zernike, F. “Beugungstheorie des Schneidenverfahrens und seiner verbesserten Form, der Phasenkontrastmethode.” Physica 1, 689-704, 1934.

Automation of the Classification Process

Image analysis of bacterial scatter patterns (developed by Bayraktar & Rajwa, 2005)

Discriminated clusters visualized on a 2-D principal components plot and a canonical plot

Hierarchical clustering based on Zernike moment invariants

Automated classification

<table>
<thead>
<tr>
<th></th>
<th>Human pathogen</th>
<th>Animal pathogen</th>
<th>Nonpathogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classified by PLS</td>
<td>66.67%</td>
<td>2.78%</td>
<td>30.56%</td>
</tr>
<tr>
<td>Classified by NN</td>
<td>75.00%</td>
<td>5.56%</td>
<td>19.44%</td>
</tr>
<tr>
<td>Classified by LDA</td>
<td>66.67%</td>
<td>8.33%</td>
<td>25.00%</td>
</tr>
</tbody>
</table>

Example: automated classification using artificial neural network system

Thirty scatter patterns belonging to 15 different strains representing three groups of bacteria (pathogenic for humans (red, top-right corner of the plot), for animals (blue, left side of the plot), and non-pathogenic (green, bottom-right corner of the plot)) were classified.

The data points are visualized in a canonical plot. The classified patterns are shown as crosses while the patterns belonging to the training set are shown as filled squares. 80% of patterns are classified correctly in the example presented. Misclassified patterns are shown as filled colored circles.

Publications


Disclosures on light scattering


New light scattering project

(Start date Feb 01, 2005 for two years)

Objectives

- Improve BARDOT (Bacteria Rapid Detection using Optical Scattering Technology) design (Hirleman group)
- Broaden the library of scatter images for additional bacterial colonies (Bhunia group)
- Automated Scattergraph Analysis (Robinson group)
Scatter images of *Listeria* species using BARDOT system

- *L. innocua* ATCC 19113
- *L. monocytogenes* V7
- *L. monocytogenes* F4248
- *L. ivanovii* ATCC 19119
- *L. ivanovii* V199 45C2

Conclusions

- Scattering is simple, robust, and powerful
- Modeling of light scattering physics using diffraction theory and colony micro/macro morphology captures dominant features of scattergraphs
- Media composition affects scatter patterns
- Image analysis (quasi physics-based) utilizes scattergraph features for characterization and differentiation of closely related species/strains with 75-99% accuracy

Conclusions (cont’d)

- The BARDOT system (Gen 2 Scatterometer) is an improvement
- Scatter patterns of *Salmonella, E. coli, Listeria, Bacillus, Enterococcus, and Enterobacter* appear to be distinct – EXCITING!!!
- Genetic analysis and fingerprinting confirmed culture identities
- Opportunity for improvements in physics-based automated analysis for better differentiation, classification and clustering

Immediate future focus

- Continue acquiring more images of different bacteria
- Improvements in the Scatterometer set up and automation
- Development of a new set of orthogonal features, for better representation and grouping of the scatter patterns
- Application of more sophisticated classification tools, including quadratic discriminant analysis, and backpropagated neural networks for automation
- Ultimately, develop a fully-automated device for scatter data collection, feature analysis, classification and identification