BActerial Rapid Detection using Optical Scattering Technology (BARDOT)  

.........an interdisciplinary approach  

Nov 3, 2010

Professor A. Bhunia
Dr. A.K. Singh
Yanjie Tang
Dr. Xiulan Sun
Dept of Food Science

Professor J. P. Robinson
Dr. B. Rajwa
Valery Patsekin
Dept of Basic Medical Sciences, Bindley Bioscience Center

Prof. D. Hirleman
Dr. Euiwon Bae
Nan Bai
School of Mechanical Engineering

USDA-ARS Support/collaboration

USDA Collaborators: Drs. Gary Richards, George Paoli, Andrew Gehring, Yiping He, Shu-I Tu

Light scattering

Microbiology

Data analysis and processing
BARDOT – Reporting (2010)

- Effect of genetic mutations and storage conditions on bacterial scatter signatures (Bhunia)
- Effect of media formulations in scatter signatures of Shiga-toxin producing and nonproducing *E. coli* strains (Bhunia)
- Generate libraries for *Salmonella* and *E. coli* serovars: (Bhunia).
- Scatter signatures of micro-colonies (100-200 um) (Hirleman)
- Engineering BARDOT design to incorporate built-in incubator towards system’s automation (Hirleman)
- Improvement in image analysis algorithm and software design for previously unclassified bacterial colony identification (Robinson)
Evolution of BARDOT

2003

Laser
Petri dish
CCD camera

2005

2006

2009
First Generation Scatterometer : 2003
Objective 1

Effect of genetic mutations and food storage conditions on bacterial scatter signatures
Scatter patterns of *Listeria* mutants

<table>
<thead>
<tr>
<th><em>Listeria</em></th>
<th>Colony (BHI)</th>
<th>BHI</th>
<th>LB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lm 10403S (1/2a) WT</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>Lm 10403S ΔSecA2</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Lm F4244 (4b) WT</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td>Lm F4244 ΔSecA2</td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>Linn F4248 WT</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
</tr>
<tr>
<td>Linn F4248 ΔSecA2</td>
<td><img src="image16.png" alt="Image" /></td>
<td><img src="image17.png" alt="Image" /></td>
<td><img src="image18.png" alt="Image" /></td>
</tr>
</tbody>
</table>

SecA2: Protein secretion system: multiple proteins

(37°C; 22 h)

Bettasso (unpub)
**L. mono F4244** (wild type)  
**L. mono KB208** (lap deletion)  
**L. mono CKB208** (lap compl)  
**L. mono ΔinlA** (inlA deletion)  
**L. mono AKB103** (secA2 deletion)

**L. mono EGD** (wild type)  
**L. mono BUG8** (inlAB deletion)  
**L. mono M1** (hlyA deletion)  
**L. mono LUT12** (ActA deletion)  
**L. mono OD43** (PrfA deletion)

KB208 grown at 42°C  
Rest at 37°C, BHI, 24 h  
Bettasso (unpub)
Application of BARDOT for detection/identification of *Salmonella, E. coli* and *Listeria* from spiked and stored food

- Peanut butter/ground beef/milk/hotdog samples were inoculated with 100 CFU/25g or 25 ml
- Analyzed on different days
- Enriched for 0 or 12h
- Spread plated on selective and non-selective
- Incubated at 37°C for variable times
- Screened with BARDOT

Bettasso et al. Unpub
**Salmonella detection from peanut butter**

<table>
<thead>
<tr>
<th>Day</th>
<th>0h enr</th>
<th>XLD 12h enr</th>
<th>BHI 12h enr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 7</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 14</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 21</td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 28</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
</tr>
</tbody>
</table>

*Bettasso (unpub)*

*Salmonella Typhimurium on XLD*
**E. coli O157:H7 in Ground Beef**

<table>
<thead>
<tr>
<th></th>
<th>CT-SMAC</th>
<th>BHI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 0</strong></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>(0h Enrich)</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Day 0</strong></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>(12h Enrich)</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Day 4</strong></td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
<tr>
<td>(0h Enrich)</td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Day 4</strong></td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
</tr>
<tr>
<td>(12h Enrich)</td>
<td><img src="image15.png" alt="Image" /></td>
<td><img src="image16.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Day 8</strong></td>
<td><img src="image17.png" alt="Image" /></td>
<td><img src="image18.png" alt="Image" /></td>
</tr>
<tr>
<td>(0h Enrich)</td>
<td><img src="image19.png" alt="Image" /></td>
<td><img src="image20.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Day 8</strong></td>
<td><img src="image21.png" alt="Image" /></td>
<td><img src="image22.png" alt="Image" /></td>
</tr>
<tr>
<td>(12h Enrich)</td>
<td><img src="image23.png" alt="Image" /></td>
<td><img src="image24.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Bettasso (unpub)
**L. monocytogenes in Hotdog**

**Images:**
- L. mono
- L. wels
- L. inno

**Results:**
- iap gene
  - Lm control: 1.05 kb
  - Linn control: 0.87 kb
  - Lwels control: 0.66 kb
  - Lm suspect: 1.05 kb
  - Linn suspect: 0.87 kb
  - Lwels suspect: 0.66 kb

Bettasso (unpub)
## L. monocytogenes in Milk

<table>
<thead>
<tr>
<th>Day</th>
<th>0 h Enrich</th>
<th>6 h Enrich</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td><img src="day_0_0h_enrich" alt="Image" /></td>
<td><img src="day_0_6h_enrich" alt="Image" /></td>
</tr>
<tr>
<td>Day 3</td>
<td><img src="day_3_0h_enrich" alt="Image" /></td>
<td><img src="day_3_6h_enrich" alt="Image" /></td>
</tr>
<tr>
<td>Day 7</td>
<td><img src="day_7_0h_enrich" alt="Image" /></td>
<td><img src="day_7_6h_enrich" alt="Image" /></td>
</tr>
</tbody>
</table>

Pos. Control

Day 0 NE  Day 0 E  Day 3 NE  Day 3 E  Day 7 NE  Day 7 E

660bp iap gene

Bettasso (unpub)
Objective 2

The Effect of Media Formulations in Scatter Signatures of Shiga-toxin Producing and Nonproducing *E. coli* Strains

Yanjie Tang (unpub)
Approach

- **Test pathogens**: Shiga toxin-producing *Escherichia coli* (STEC) and non-STEC
  - O157:H7
  - Non-O157 STEC (O26, O45, O103, O111, O145)
  - Others

- **Media examined**: BHI, SMAC, CT-SMAC, Rainbow-O157
Serovar: O-antigenic patterns

- Part of lipopolysaccharide (LPS) in gram-negative bacteria (L lipid A, Core polysaccharide, and O-polysaccharide)

- O-antigen specific chains determine specificity
Typical *E. coli* O157:H7 colonies on selective agars

- TC-SMAC
- Rainbow® Agar O157
- R&F® *E. coli* O157:H7

Plate Photo from FDA-BAM (~30 h)

O157:H7 EDL933

K12

12 h
### E. coli on SMAC and BHI

<table>
<thead>
<tr>
<th></th>
<th>SMAC</th>
<th>BHI</th>
<th>SMAC</th>
<th>BHI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EHEC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O157:H7 (EDL933)</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td>O157:H7 (SEA13A53)</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
</tr>
<tr>
<td>O111:H11</td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
</tr>
<tr>
<td>O111:H8*</td>
<td><img src="image13" alt="Image" /></td>
<td><img src="image14" alt="Image" /></td>
<td><img src="image15" alt="Image" /></td>
<td><img src="image16" alt="Image" /></td>
</tr>
<tr>
<td>O26:H11*</td>
<td><img src="image17" alt="Image" /></td>
<td><img src="image18" alt="Image" /></td>
<td><img src="image19" alt="Image" /></td>
<td><img src="image20" alt="Image" /></td>
</tr>
<tr>
<td><strong>EPEC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O127:H6</td>
<td><img src="image21" alt="Image" /></td>
<td><img src="image22" alt="Image" /></td>
<td><img src="image23" alt="Image" /></td>
<td><img src="image24" alt="Image" /></td>
</tr>
<tr>
<td>O142:H6</td>
<td><img src="image25" alt="Image" /></td>
<td><img src="image26" alt="Image" /></td>
<td><img src="image27" alt="Image" /></td>
<td><img src="image28" alt="Image" /></td>
</tr>
<tr>
<td><strong>ETEC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O25:K98 NM</td>
<td><img src="image29" alt="Image" /></td>
<td><img src="image30" alt="Image" /></td>
<td><img src="image31" alt="Image" /></td>
<td><img src="image32" alt="Image" /></td>
</tr>
<tr>
<td>O78:H11</td>
<td><img src="image33" alt="Image" /></td>
<td><img src="image34" alt="Image" /></td>
<td><img src="image35" alt="Image" /></td>
<td><img src="image36" alt="Image" /></td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td><img src="image37" alt="Image" /></td>
<td><img src="image38" alt="Image" /></td>
<td><img src="image39" alt="Image" /></td>
<td><img src="image40" alt="Image" /></td>
</tr>
<tr>
<td>O46:H38</td>
<td><img src="image41" alt="Image" /></td>
<td><img src="image42" alt="Image" /></td>
<td><img src="image43" alt="Image" /></td>
<td><img src="image44" alt="Image" /></td>
</tr>
<tr>
<td>O91:H21</td>
<td><img src="image45" alt="Image" /></td>
<td><img src="image46" alt="Image" /></td>
<td><img src="image47" alt="Image" /></td>
<td><img src="image48" alt="Image" /></td>
</tr>
<tr>
<td>O22:H8</td>
<td><img src="image49" alt="Image" /></td>
<td><img src="image50" alt="Image" /></td>
<td><img src="image51" alt="Image" /></td>
<td><img src="image52" alt="Image" /></td>
</tr>
<tr>
<td>O29:NM</td>
<td><img src="image53" alt="Image" /></td>
<td><img src="image54" alt="Image" /></td>
<td><img src="image55" alt="Image" /></td>
<td><img src="image56" alt="Image" /></td>
</tr>
<tr>
<td>O103:H2</td>
<td><img src="image57" alt="Image" /></td>
<td><img src="image58" alt="Image" /></td>
<td><img src="image59" alt="Image" /></td>
<td><img src="image60" alt="Image" /></td>
</tr>
<tr>
<td>O113:H21</td>
<td><img src="image61" alt="Image" /></td>
<td><img src="image62" alt="Image" /></td>
<td><img src="image63" alt="Image" /></td>
<td><img src="image64" alt="Image" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>SMAC</th>
<th>BHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>O5 NM</td>
<td><img src="image65" alt="Image" /></td>
<td><img src="image66" alt="Image" /></td>
</tr>
<tr>
<td>O111:H11</td>
<td><img src="image67" alt="Image" /></td>
<td><img src="image68" alt="Image" /></td>
</tr>
<tr>
<td>O111:H8*</td>
<td><img src="image69" alt="Image" /></td>
<td><img src="image70" alt="Image" /></td>
</tr>
<tr>
<td>O26:H11*</td>
<td><img src="image71" alt="Image" /></td>
<td><img src="image72" alt="Image" /></td>
</tr>
</tbody>
</table>
## E. coli O157:H7 on CT-SMAC

### Highly reproducible
Differentiation of *E. coli* serotypes on SMAC agar

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Plate (12h)</th>
<th>Microscopic Colony Morphology (12h)</th>
<th>SMAC (12h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157:H7 (EDL933)</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>O26:H11</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>O103:H2</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td>O111:H8</td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>K12</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td>Rainbow (12h)</td>
<td>Colony (12h)</td>
<td>Plate (12h)</td>
</tr>
<tr>
<td>------</td>
<td>---------------</td>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>O157:H7</strong></td>
<td><img src="Image" alt="Rainbow agar O157" /></td>
<td><img src="Image" alt="Image" /></td>
<td><img src="Image" alt="Image" /></td>
</tr>
<tr>
<td><strong>O26</strong></td>
<td><img src="Image" alt="Rainbow agar O157" /></td>
<td><img src="Image" alt="Image" /></td>
<td><img src="Image" alt="Image" /></td>
</tr>
<tr>
<td><strong>O103</strong></td>
<td><img src="Image" alt="Rainbow agar O157" /></td>
<td><img src="Image" alt="Image" /></td>
<td><img src="Image" alt="Image" /></td>
</tr>
<tr>
<td><strong>O111</strong></td>
<td><img src="Image" alt="Rainbow agar O157" /></td>
<td><img src="Image" alt="Image" /></td>
<td><img src="Image" alt="Image" /></td>
</tr>
<tr>
<td><strong>K12</strong></td>
<td><img src="Image" alt="Rainbow agar O157" /></td>
<td><img src="Image" alt="Image" /></td>
<td><img src="Image" alt="Image" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>EDL933</th>
<th>O111</th>
<th>K12</th>
<th>O103</th>
<th>O26</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDL933</td>
<td>98.9</td>
<td>0</td>
<td>1</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>O111</td>
<td>0</td>
<td>95.5</td>
<td>0</td>
<td>3.1</td>
<td>1.3</td>
</tr>
<tr>
<td>K12</td>
<td>0.1</td>
<td>0</td>
<td>98.6</td>
<td>0</td>
<td>1.2</td>
</tr>
<tr>
<td>O103</td>
<td>2.1</td>
<td>5.7</td>
<td>0</td>
<td>90</td>
<td>2.2</td>
</tr>
<tr>
<td>O26</td>
<td>5.6</td>
<td>9.7</td>
<td>0</td>
<td>3.1</td>
<td>81.7</td>
</tr>
</tbody>
</table>

Rainbow agar O157
Next

- Media to be examined: SMAC, Rainbow, CHROM agar, R&F agar
- Custom made media
- Creative libraries for: O26, O45, O103, O111, O145; O157
Objective 3

Expand scatter signature libraries for *Salmonella* and *E. coli* serovars
Salmonella serovars tested

1. S. Agona (1) 
2. S. Anatum (1) 
3. S. Arizona (1) 
4. S. Berta (1) 
5. S. Choleraesuis (1) 
6. S. Enteritidis (14) 
7. S. Gallinarum (1) 
8. S. Hadar (1) 
9. S. Heidelberg (3) 
10. S. Indiana (1) 
11. S. Infantis (1) 
12. S. Kentucky (1) 
13. S. Litchfield (1) 
14. S. Marscescens (1) 
15. S. Montevideo (2) 
16. S. Newport (1) 
17. S. Poona (1) 
18. S. Schottmuelleri (1) 
19. S. Schwarzengrund (1) 
20. S. Seftenberg (2) 
21. S. Stanley (1) 
22. S. Tennessee (1) 
23. S. Thompson (1) 
24. S. Typhi (1) 
25. S. Typhimurium (21)
DotBar

BHI (12h) | XLD (12h)
---|---
S. Berta
S. Anatum
S. Enteritidis 13a
S. Hadar
S. Stanley
S. Thompson

Singh (unpub)
S. Enteritidis PT7
S. Enteritidis PT8
S. Enteritidis 13a
S. Typhimurium NOS11
S. Typhimurium NOS12
S. Typhimurium NOS13
S. Typhimurium NOS23
S. Typhimurium NOS25
S. Enteritidis PT4
S. Enteritidis PT8
S. Newport
S. Montevideo

Singh (unpub)
DotBar Analysis

18 *Salmonella* serovars (100 ± 20 images) from XLD (12 h)
Analysis 1

Test sample: *Salmonella* Enteritidis

100 ± 20 images of each 7 strains of *Salmonella* Enteritidis serovar
100 ± 20 images of each 10 strains of *Salmonella* Typhimurium serovar

XLD (12h)
Analysis 2

Test sample: *Salmonella* Typhimurium

100 ± 20 images of each 7 strains of *Salmonella* Enteritidis serovar
100 ± 20 images of each 10 strains of *Salmonella* Typhimurium serovar

XLD (12h)

First Principal component

67 (99%) *Salmonella Typhimurium*
1 (1%) *Salmonella Enteritidis*
68 Total
Objective 4

Early identification of microcolonies via forward scattering technology
Tasks

- Analysis of scatter signatures of micro-colonies of *E. coli*, *Listeria* and *Salmonella* with the goal of reducing bacterial identification time

- Correlation between the forward scattering patterns and the phenotypical features of bacterial colonies

- Microcolony detection (80~160 mm): Characterization, Prediction of scattering patterns; Compare the predicted scattering patterns with experimental results

- Engineering BARDOT design to incorporate built-in incubator towards system’s automation
Theoretical Modeling

- A diffraction based model for forward light scattering (FLS) from bacterial colonies
- Numerical simulation analysis
- Correlations between colony morphology and its forward scattering pattern (diffraction ring count & maximum diffraction angle)
Height Variation vs. Diffraction Pattern

- $H_0$ increased D constant, the maximum diffraction angle and the total number of rings increased

<table>
<thead>
<tr>
<th>Condition</th>
<th>Diffraction Angle</th>
<th>Total Rings</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D=1.024,\text{mm}$, $H_0=30,\mu\text{m}$</td>
<td>$2.13$</td>
<td>$28$</td>
</tr>
<tr>
<td>$D=1.024,\text{mm}$, $H_0=70,\mu\text{m}$</td>
<td>$5.71$</td>
<td>$65$</td>
</tr>
<tr>
<td>$D=1.024,\text{mm}$, $H_0=90,\mu\text{m}$</td>
<td>$5.71$</td>
<td>$65$</td>
</tr>
</tbody>
</table>
Diameter Variation vs. Diffraction Pattern

- $H_0$ constant, $D$ increased, the maximum diffraction angle decreased and the total number of rings remained constant.

- $D=0.384\text{mm}$, $H_0=50\mu\text{m}$, Angle=7.676

- $D=0.48\text{mm}$, $H_0=50\mu\text{m}$

- $D=0.704\text{mm}$, $H_0=50\mu\text{m}$

- $D=0.96\text{mm}$, $H_0=50\mu\text{m}$, Angle=3.676
Number of diffraction ring was proportional to the colony center height (for fixed diameter) but not colony diameter (for fixed height)
Modeling (II)

Optical-field-induced refractive-index -> diffraction rings change*

Optical path length = refractive index \times distance

L. monocytogenes F4244, d=157 µm^+

\[ \sim 100-200 \, \mu m \]

\[ \sim 15 \, \mu m \]

Diffraction Angle and Ring Counts

<table>
<thead>
<tr>
<th>Simulation</th>
<th>$\theta / 2 \max$</th>
<th>$\frac{1}{k} \left( \frac{d\Delta \Phi}{dr} \right)_{\max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.13</td>
<td>1.91</td>
<td></td>
</tr>
<tr>
<td>3.21</td>
<td>3.08</td>
<td></td>
</tr>
<tr>
<td>4.42</td>
<td>4.25</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Simulation Mean Value</th>
<th>$N_{\text{ring}} \approx \frac{\Delta \Phi}{2\pi}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.0</td>
<td>28</td>
</tr>
<tr>
<td>39.8</td>
<td>40</td>
</tr>
<tr>
<td>50.9</td>
<td>52</td>
</tr>
</tbody>
</table>

Wavefront from the colony

$$\Delta \Phi \cong \frac{1}{k} \left( \frac{d\Delta \Phi}{dr} \right)_{\max}$$

$$N_{\text{ring}} \approx \frac{\Delta \Phi}{2\pi}$$

Graph showing phase vs. $X_i$ (mm) for different values of $k$.
Experimental Apparatus

- Phase contrast microscope (PCM)
- Laser confocal displacement meter (CDM)

Applying ‘confocal principle‘ in triangulation sensor enables Profile measurement from Translucent samples
Aspect Ratio Comparison

(a) Graph showing the comparison of Height (µm) vs. X (µm) for E.coli DH5α, L.monocytogenes F4244, and Salmonella Montevideo.

(b) Graph showing the comparison of Height (µm) vs. colony diameter (µm) for E.coli DH5α, L.monocytogenes F4244, and Salmonella Montevideo.

(c) Heatmaps for E.coli DH5α, L.monocytogenes F4244, and Salmonella Montevideo.
Time-Resolved Scattering Patterns

Salmonella
Montevideo

- 96 µm
- 111 µm
- 119 µm
- 124 µm
- 145 µm

Listeria
Monocytogenes
F4244

- 94 µm
- 98 µm
- 107 µm
- 134 µm
- 157 µm

E.coli
DH5α

- 79 µm
- 104 µm
- 111 µm
- 129 µm
- 136 µm
Comparison of colony morphology and scattering patterns of *Salmonella* Montevideo and *EcoliDH5α*.

<table>
<thead>
<tr>
<th>Bacteria Species</th>
<th>Diameter (um)</th>
<th>Height (um)</th>
<th>Maximum Diffraction Angle</th>
<th>No. of Diffraction Rings</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>EcoliDH5α</em></td>
<td>139</td>
<td>9.94</td>
<td>7.03</td>
<td>6</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>137</td>
<td>14.16</td>
<td>7.78</td>
<td>8</td>
</tr>
</tbody>
</table>
Incubator Design Specifications

- Temperature control: 37 (±1) Celsius degree
- Temperature measurement:
  - Thermal coupler (economical but not so accurate)
  - IR imaging camera (very expensive)
- Heating sources
  - Heating coils/pads supplied by electrical power
  - Heat exchange with water pipes
  - Heat exchange with warm air
- Dimension of Petri-dish
  - Diameter: 9cm
  - Height: 1.5cm
- Shape of chamber
- Material requirements
  - Transparent
  - Heat sustainable
  - Economical
  - Good insulation for efficiency
- Other design concerns:
  - Moisture level
  - Air ventilation
Schematic Diagram of the Final Design
Objective 5

Improvement in image analysis
algorithm and software design for
previously unclassified bacterial colony
identification
Exhaustive and non-exhaustive training library

- Traditional supervised learning - exhaustive training library
  - The number of classes is known
  - All the classes are represented in the training library
  - No new (emerging) classes are expected

- Supervised learning with novelty detection - **blind detection**
  - Some classes are known; however, the total number of classes in unknown
  - Some classes are not represented in the training library

- New BARDOT detection/classification algorithm operates under the **assumption of non-exhaustiveness**
Progress in development of the new machine learning approach II

- Bayesian approach based on Wishart priors for detecting samples of emerging pathogens
  - Tested with over 400 samples representing seven common serotypes of *Salmonella*.
- The feasibility studies have been published:
Example: finding *Salmonella*

- Blind detection capability was tested for each of the serotypes by building a library which **did not contain** any examples of that serotype.
- The detection accuracy is expressed by AUC (area under receiver operating curve).
- AUC can be interpreted as the probability that the detection test result from a randomly chosen colony representing unknown pathogen is more indicative of presence of an unknown pathogen than a result from a randomly chosen known colony.

<table>
<thead>
<tr>
<th>REMOVED SEROTYPE</th>
<th>AUC</th>
<th>AUC SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella agona</em></td>
<td>1.0</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Salmonella cholerasuis</em></td>
<td>0.85</td>
<td>0.02</td>
</tr>
<tr>
<td><em>Salmonella indiana</em></td>
<td>1.0</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Salmonella kentucky</em></td>
<td>0.88</td>
<td>0.03</td>
</tr>
<tr>
<td><em>Salmonella schottmuelleri</em></td>
<td>0.94</td>
<td>0.02</td>
</tr>
<tr>
<td><em>Salmonella tennessee</em></td>
<td>0.99</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Salmonella typhimurium (Copenhagen)</em></td>
<td>0.97</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Very high detection probability!
Classification of *Salmonella* (example)

- After successful detection of an unknown serotype, the classifier can be retrained.
- With the sufficient training data (automatically detected), the classifier can achieve very high sensitivity and specificity.
- Results for the tested example:

<table>
<thead>
<tr>
<th>CLASSIFIED SEROTYPE</th>
<th>NO. OF TRAINING INSTANCES</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
<th>ACCURACY</th>
<th>AUC</th>
<th>AUC SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella agona</td>
<td>76</td>
<td>0.9868</td>
<td>0.9972</td>
<td>0.9951</td>
<td>0.9997</td>
<td>0.000958</td>
</tr>
<tr>
<td>Salmonella cholerasuis</td>
<td>49</td>
<td>0.8775</td>
<td>0.9843</td>
<td>0.9715</td>
<td>0.9950</td>
<td>0.008322</td>
</tr>
<tr>
<td>Salmonella indiana</td>
<td>33</td>
<td>0.9697</td>
<td>1</td>
<td>0.9976</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Salmonella kentucky</td>
<td>41</td>
<td>0.8780</td>
<td>0.9872</td>
<td>0.9761</td>
<td>0.9793</td>
<td>0.046659</td>
</tr>
<tr>
<td>Salmonella schottmuelleri</td>
<td>48</td>
<td>0.9167</td>
<td>0.9948</td>
<td>0.9855</td>
<td>0.9933</td>
<td>0.015494</td>
</tr>
<tr>
<td>Salmonella tennessee</td>
<td>94</td>
<td>1</td>
<td>0.9881</td>
<td>0.9903</td>
<td>0.9994</td>
<td>0.001241</td>
</tr>
<tr>
<td>Salmonella typhimurium (Copenhagen)</td>
<td>90</td>
<td>0.9444</td>
<td>0.9883</td>
<td>0.9785</td>
<td>0.9959</td>
<td>0.007536</td>
</tr>
</tbody>
</table>
Conclusions and future focus

- Mutation in genes with regulatory or global function affect scatter signatures. More strains with different mutations to be tested.
- Scatter signatures of bacteria present in food for extended period generally are not affected by food.
- Serovar classification of *Salmonella* and *E. coli* looks promising.
- Additional selective or chromogenic media will be evaluated for serovar classification especially for STEC.
Scalar diffraction was introduced for explaining the forward scattering pattern features with the paraxial approximation.

Correlation between colony morphology and its forward scattering pattern was established through computational simulation and experiment.

Experiments were conducted to measure the colony morphology with focus on microcolonies to identify their phenotypic differences.

New Machine Learning approach for classification of unclassified/unknown bacteria.


Funding
Food Safety Engineering Center Grant supported by USDA-ARS Special Grant Program, NIH

Special thanks to Jim Lindsay and Rich Linton

Padmapriya Banada
Ewiwon Bae
Amornrat Aroonual
Nan Bai
Karleigh Huff
Amanda Bettasso

Songlin Guo, Xiulan Sun; Valery Patsekin, Bartek Rajwa; Bulent Bayraktar

Atul Singh
Yanjie Tang

Dan Hirleman
J. Paul Robinson
Thank you