Optical forward scattering for colony identification and differentiation of bacterial species

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Optical forward scattering for rapid identification and differentiation of bacterial species

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

an interdisciplinary approach

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Light scattering sensor for bacterial detection/identification

Schematic representation of the laser scatterometer used to perform analysis of bacterial colonies. A - 635-nm diode laser, B - Petri dish containing bacterial colonies, C - CCD camera, D - Petri-dish holder, and E - detection screen.

Scatter-images of representative Listeria species

L. innocua F4248
L. monocytogenes ATCC19113
L. seeligeri LA 15
L. ivanovii ATCC 19119
L. grayi FA248
L. welshimeri ATCC 35897
L. heilmannii ATCC 15887
L. greyi LM027

Banada et al. 2006. Biosensors and Bioelectronics, Published online [Sept 2006]

Modified approach in light scattering project

Objectives

- Improve BARDOT (BAceteria Rapid Detection using Optical Scattering Technology) design (Hirleman)
- Generate scatter images of bacterial colonies of different genera: stress, food testing (Bhunia)
- Image analysis (Robinson)

Bacteria Rapid Detection using Optical Scattering Technology (BARDOT)

Laser
Scatter image
Petri dish with colonies
CCD chip
Computer
**Bacteria Rapid Detection using Optical Scattering Technology (BARDOT)**

Generate scatter images of pure cultures of different bacteria (Genus) to create image libraries:
- Listeria
- Escherichia
- Salmonella
- Staphylococcus
- Bacillus
- Vibrio
- Miscellaneous

- Liquid cultures diluted, spread on BHI agar (15 ml) plate to obtain ~100 colonies.
- Colony size 1.5-2 mm
- Incubation temp (30-37°C) and time (12-48 h) variable

**Escherichia coli**

- Nonpathogenic
- Pattern I
- Pattern II
- Pattern III

**Salmonella**

- Salmonella Typhimurium
- Salmonella Enteritidis
- Salmonella Agona

**BARDOT automation**

- Integrating mechanical part (XY stage) and detector part (sensor) with algorithm
Staphylococcus

- S. aureus ATCC 25923
- S. aureus isolate 103
- S. aureus 5-41
- S. aureus ATCC 13301
- S. epidermidis ATCC 33547
- S. epidermidis isolate 101
- S. epidermidis isolate 302
- S. haemolyticus T6346
- S. xylosus 18 V042A
- S. lentus 4 V025B

Vibrio

- Vibrio cinchenticins
- Vibrio alginolyticus
- Vibrio campbellii
- Vibrio fluvialis
- Vibrio mimicus
- Vibrio para-haemolyticus
- Vibrio anguillarum
- Vibrio hollisae
- Vibrio orientalis
- Vibrio metchnikovii

Vibrio vulnificus

- Opaque (pathogenic)
- Transparent (nonpathogenic)

- MLT 352
- MLT 362
- MLT 1003
- MLT 1009
- LL 728

- V. vulnificus 406
- V. vulnificus 367
- V. vulnificus 404

Resuscitation after VBNC state

Control

Light scattering image of stress-exposed bacteria

*BARDOT analysis of viable but not culturable Vibrio after resuscitation

Osmotic stress

- Osmotic stress on Vibrio cincinnati

- Control Lm V7
- Heat stress recovered Lm V7

- L. monocytogenes F4244 grown in BHI broth with 5.5% NaCl for 24h then plated on BHI agar

Heat and acid stress

- Heat stress: Lm F4244 grown in BHI broth at 42°C for 3h
- Acid stress: Lm F4244 grown in BHI broth with pH of 4.0
Food testing with BARDOT

25 g meat spiked with $10^3$ cfu of bacteria

225 ml of enrichment broth (UVM for Listeria; mEC for E. coli O157:H7) incubated for 24 h

BHI agar:
- Incubate - 30-36 h for Listeria
- 16-18 h for E. coli O157:H7

BARDOT

BARDOT analysis with spiked meat sample

Isolate 1                    Isolate 2                    Isolate 3                   Isolate 4
L. monocytogenes F4244 CONTROL
Ground beef spiked with L. monocytogenes F4244

Huff et al 2006 (unpublished)

E. coli O157:H7 EDL 933 Control
Ground beef spiked with E. coli O157:H7 EDL 933

Huff et al 2006 (unpublished)

BARDOT Specifications/Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beam diameter</td>
<td>50 mm/0.01 mm</td>
</tr>
<tr>
<td>XY stage</td>
<td>0.635 nm</td>
</tr>
<tr>
<td>Unit pixel</td>
<td>1 µm</td>
</tr>
<tr>
<td>Stage holder</td>
<td>11” – 44”</td>
</tr>
</tbody>
</table>

Modeling the Colony

Phase Contrast (12 hr)

Confocal Microscope

Modeling I

Applying Rayleigh Sommerfeld diffraction theorem, we model the diffraction using amplitude and phase modulation

\[
E_r(t_x,t_y) = C \int \int [\int [h(x,t_z) \exp \left(\frac{-2\pi}{\lambda} (x^2 + y^2) \right) \exp(i \phi(x,t_z))]] dx \, dy \, dt_z
\]

\[
= \exp[-2\pi (f_x t_x + f_y t_y)] \delta W \delta t_r
\]
Modeling II

- Phase Contrast Micrograph of *Listeria ivanovii* showing microstructure

Modeling III

*Listeria ivanovii*, z= 40 mm

Model Prediction

Modeling IV

*Listeria innocua*, z= 40 mm

Model Prediction

Ensemble of features

- The feature selection step can be used to choose a subset of features which gave performance equivalent to the entire set of candidate features, while utilizing less computational resources.

- Feature selection can be also used to boost the performance of the classifier.

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 Phasekontrastmethode." *Physica* 1, 697-704, 1934.

New set of features – Haralick texture descriptors

In many images, structures are discernible visually based on textural rather than brightness differences. Texture operators are able to enhance and quantify these differences.

- Graphical representation of radial Zernike polynomials: $Z_{n,m}$, n≥0, m≤n (image size 128 x 128 pixels), and their magnitudes: A – real part $Z_{20}$; B – imaginary part $Z_{20}$; C – magnitude of $Z_{20}$; D – real part $Z_{30}$; E – imaginary part $Z_{30}$; F – magnitude of $Z_{30}$. The larger the $n - |m|$ difference, the more oscillations are present in the shape. Features used in this study are the magnitudes of Zernike polynomials. One may note that the values of the magnitudes do not change when arbitrary rotations are applied.

- Scatter image of *L. Ivanovii* processed with three different Haralick operators.
Method: Automated classification using support vector machines

- The SVM algorithm creates a hyperplane that separates the data into two classes with the maximum-margin. The SVM idea was proposed by Vladimir Vapnik in 1963.
- For categorical variables a dummy variable is created with case values as either 0 or 1. Thus, a categorical dependent variable consisting of three levels, say (A, B, C), is represented by a set of three dummy variables: A: [1, 0, 0], B: [0, 1, 0], C: [0, 0, 1].

Classification success rate for *Listeria spp.*

<table>
<thead>
<tr>
<th>Species</th>
<th>L. welshimeri ATCC 35897</th>
<th>L. innocua V58</th>
<th>L. ivanovi ATCC 19119</th>
<th>L. ivanovi SE98</th>
<th>L. monocytogenes ATCC 19113</th>
<th>L. monocytogenes V7</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. welshimeri ATCC 35897</td>
<td>92.88%</td>
<td>6.10%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.68%</td>
<td>0.34%</td>
</tr>
<tr>
<td>L. innocua V58</td>
<td>90.61%</td>
<td>4.24%</td>
<td>0.30%</td>
<td>0.61%</td>
<td>2.73%</td>
<td>1.52%</td>
</tr>
<tr>
<td>L. ivanovi ATCC 19119</td>
<td>100.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>L. ivanovi SE98</td>
<td>93.62%</td>
<td>0.00%</td>
<td>6.38%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>L. monocytogenes ATCC 19113</td>
<td>97.65%</td>
<td>0.24%</td>
<td>1.18%</td>
<td>0.47%</td>
<td>0.00%</td>
<td>0.47%</td>
</tr>
<tr>
<td>L. monocytogenes V7</td>
<td>97.76%</td>
<td>0.61%</td>
<td>0.41%</td>
<td>1.22%</td>
<td>0.00%</td>
<td>97.76%</td>
</tr>
</tbody>
</table>

Confusion matrix showing classification success for mixture of colonies belonging to four species (six strains) of *Listeria*. Classification rates have been established using 5x2 crossvalidation.

Failure of clustering – we need supervised machine learning
Visualization of the classification results

Classification example – mixture of species

Overview of the classification system (in development)

Summary - BARDOT

Summary - BARDOT

Thank You