MidInfrared Sensors for Pathogen Detection

**PIs:** Dr. L. Mauer, Dr. B. Reuhs, Dr. J. Irudayaraj

**Co-PIs:** Dr. M. Cousin, Dr. C. DebRoy, Dr. S. Krishnan, Dr. J. Gore, Dr. J. Bouldin

**Research Associates:** Dr. Reeta Davis, Dr. Sol Kim, A. Deering, Y. Burgula, D. Khali, J. Stratton, P.R. Sandeep
Fourier-transform infrared spectroscopy (FT-IR)

• Measures absorbance of infrared light
  – Beer’s Law: $A_\lambda = l \varepsilon_\lambda c$
  – Sensitive to functional groups
  – Specific bands in fingerprint region
• Non-destructive technique
• High speed
• High sensitivity
Basic components of an FT-IR spectrometer
FT-IR Analysis of Bacteria

- Measures molecular bond vibration of compounds, excited by frequency of infrared light.
- FT-IR spectra display a structural and biochemical fingerprint of the bacteria (protein, lipid, carbohydrates, DNA).
FT-IR Analysis

Representative FT-IR spectra of *L. monocytogenes* collected by Continuum IR Microscope
FT-IR Method Development: Sample Preparation Techniques

FT-IR main bench: filtration, magnetic nanoparticles

FT-IR microscope: filtration, immunomagnetic separation, cellular components
Filtration - the apparatus

Isolated colony → Culture → Membrane Filter

Food → Stomacher bag sample preparation
FT-IR and Filtration

Multi-bounce flat plate ATR (MATR)

Contact ATR on IR-microscope (Filtration)

- Contact ATR Objective
- Pressure Sensitive Stage
- Membrane Filter
- Contact Indicator Panel

Infrared spectra
FT-IR microscope

- Immunomagnetic separation
- Cellular components (LPS, OMP)

Continuum IR-microscope (ThermoElectron)
Applications of FT-IR Methods in Food Microbiology

1. Detection of bacteria
2. Discrimination of viable, injured, and dead bacteria
3. Taxonomic classification of bacteria
FT-IR methods for Pathogen Detection in Foods

Detection of *E. coli* O157:H7 and *Salmonella typhimurium* successful in:

- Fruit juices
- Skim milk
- Ground beef
- Chicken rinse water
- Medium aged Cheddar cheese
- Spinach
Cheddar cheese surface treated with *E. coli* O157:H7, *Salmonella* Typhimurium, and *E. coli* K12 using CVA of FT-IR spectra (1800-1000 cm⁻¹)
Ground Beef (IMS-FT-IR)
FT-IR Assay Sensitivity

- Detection Limit = ~40 cells in IR beam area

\[
\text{No. of cells} = \left( \text{Infrared beam area} \right) \left( \frac{\text{CFU} / \text{mL}}{\text{volume}} \right) \left( \frac{\text{filtration area}}{1000^2 * 35^2 / 4} \right)
\]

\[
\text{No. of cells} = \left( \frac{10^7 \text{CFU} / \text{mL} \times 100 \text{mL}}{\pi * 1000^2 * 35^2 / 4} \right) = 36.4
\]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Filtration (FT-IR)</th>
<th>Dynabeads (FT-IR)</th>
<th>Magnetic Nanoparticles (FT-IR)</th>
<th>Flow cytometry</th>
<th>ELISA (Eg. VIDAS)</th>
<th>PCR (Eg. BAX)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material cost</td>
<td>Filters</td>
<td>Antibodies</td>
<td>Antibodies</td>
<td>Dye</td>
<td>Enzymes</td>
<td>Primers</td>
</tr>
<tr>
<td>Time*</td>
<td>&lt; 7 h</td>
<td>&lt; 10 h</td>
<td>???</td>
<td>8 h</td>
<td>8 – 24 h</td>
<td>8 - 24 h</td>
</tr>
<tr>
<td>Actual assay time</td>
<td>&lt; 1 h</td>
<td>&lt; 4 h</td>
<td>30 min</td>
<td>0.5-2 h</td>
<td>1-4 h</td>
<td>3-4 h</td>
</tr>
<tr>
<td>Sensitivity at time of measurement</td>
<td>$10^{4-6}$ CFU/mL</td>
<td>$10^4$ CFU/mL</td>
<td>$10^6-10^7$ CFU/mL</td>
<td>$10^4-10^6$ CFU/mL</td>
<td>$10^7-10^9$ CFU/mL</td>
<td>$10^7-10^9$ CFU/mL</td>
</tr>
</tbody>
</table>
FT-IR Analysis of Structural Components of Bacteria

- LPS extract
- OMP or LPS extract dried on a gold slide
- FTIR measurement
- IR spectra of LPS extract from six *S. enterica* serotypes
- Classification of *S. enterica* serotypes based on LPS spectra
Development of analytical methods to discriminate between Live and Dead cells

Based on spectra of growth
Based on spectral differences
E. coli K12 growth curve-Filtration

Detection based on spectral differences between live and dead

![Graph showing spectral differences between live and dead samples]
Detection based on spectral differences between live and dead

Cooman plot based on spectral differences in the amide II region 1565-1465 cm\(^{-1}\) and fatty acid region 3000-2800 cm\(^{-1}\).
Raw FT-IR spectra of Live and dead *E. coli* O157:H7

Treatment: 4 min in 99 °C waterbath

First derivative FT-IR spectra of Live and dead *E. coli* O157:H7

Treatment: 4 min in 99 °C waterbath
First derivative FT-IR spectra of 100% Live, 100% dead, and 99.95% dead: 0.05% live of *E. coli* O157:H7.

Treatment: 4 min in 99 °C waterbath.
Cooman plot Showing the differentiation of 100% live, 100% dead, and 0.05% live : 99.95% dead E. coli O157:H7

Heat treatment: 4 min in a 99 °C Water bath
Region selected: 1800-750 cm⁻¹
Preprocessing: Baseline correction and Normalization
Quantification of live cells in the presence of dead (heat-treated) cells

- Live cells correctly quantified by PLS in presence of dead cells
PLS analysis for the quantification different ratios of live and dead *E. coli* O157:H7

Heat treatment: 4 min in a 99 °C Water bath
Region selected: 1800-850 cm⁻¹,
Preprocessing: first derivative, normalization
Effects of treatment on FT-IR classification

Clustering of *E. coli* O157:H7 (8.6 ± 0.3 log CFU/mL) spectra subject to six different processing treatments
Cooman Plots showing the differentiation of *E. coli* O157:H7 at different time intervals during heat treatment.

Region selected: 1800-850 cm\(^{-1}\)
Preprocessing: Area normalization
No of PC scores: 6
4 min

Dead

1% Live

Live

8 min

Dead

1% Live

Live

16 min

Dead

1% Live

Live

24 min

3500-850 cm\(^{-1}\),
Area normalize ,
6 PCs
FT-IR Methods for the Taxonomic Classification of Bacteria
Taxonomic Classification and FT-IR microscope

Isolated colonies  →  14 h culture in TSB  →  10ml culture, centrifuge, wash with saline and suspend in 10ml 0.09% NaCl  →  20μl suspension dried on gold coated glass slide

Chemometrics/ Hierarchical Cluster analysis Dendrogram  →  Infrared spectra  →  FT-IR reflectance microspectroscopy

256 scans at 4 cm\(^{-1}\) resolution, 4000-650 cm\(^{-1}\)
Serotype Classification of $L.\ monocytogenes$

First derivative of absorbance vs. Wavenumber $\text{cm}^{-1}$

- Blue line: $4b$
- Pink line: $1/2b$
- Red line: $1/2a$

Scatter plot in CV (Canonical Variate) space for:
- $4b$
- $4c$
- $1/2a$
- $1/2b$
E. coli O157:H7 Strain Identification

First derivative of absorbance vs. Wavenumber cm⁻¹
FT-IR techniques coupled with different chemometrics approaches offer a wide range of applications for:

– Detection,
– Differentiation,
– Quantification, and
– Taxonomic level classification of bacteria from culture broth or food matrices.

Thank you!