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## Infrared Sensors for Rapid Detection of Select Microbial Foodborne Contaminants


**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

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## Fourier-transform infrared spectroscopy (FT-IR)

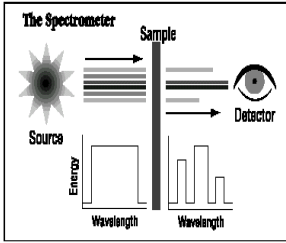
- Measures absorbance of infrared light
  - Beer's Law:  $A_{\lambda} = \epsilon_{\lambda} c$
  - Sensitive to functional groups
  - Specific bands in fingerprint region
- Non-destructive technique
- High speed
- High sensitivity



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## IR Spectroscopy Basics

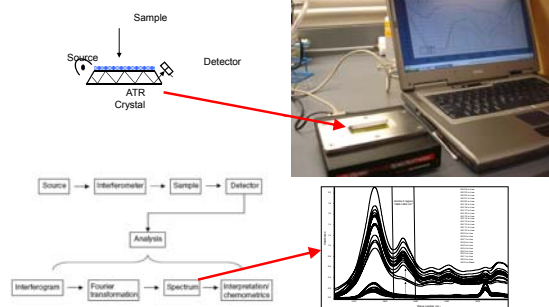
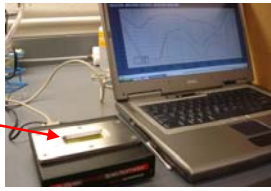
- IR radiation is sent through the sample
- Some of the radiation is absorbed and the rest passes through (transmitted)
- This creates a "fingerprint" or spectra unique to the sample
- No two structures have the same "fingerprint"



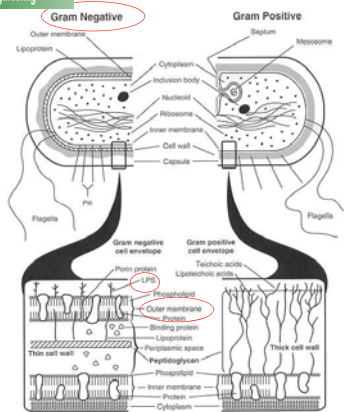
Thermo Nicolet Manual

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## FT-IR Sensor Technology

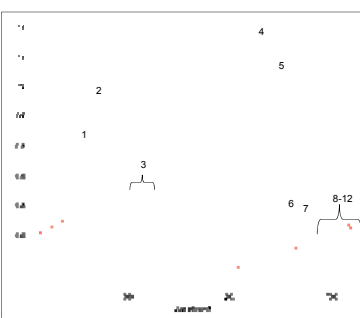



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## Major mid-IR peaks for bacteria



Peak #	Wavenumber (cm <sup>-1</sup> )	Assignment
1	3500	O-H stretching
2	3200	N-H stretching (amide A) of proteins
3	2959-2852	C-H, C-H <sub>2</sub> , C-H <sub>3</sub> stretching in fatty acids
4	1655-1637	Amide I of $\alpha$ -helical & $\beta$ -pleated structures
5	1548	Amide II band
6	1515	Tyrosine band
7	1468	CH <sub>2</sub> bending of methylene
8	1318-1240	Amide III band (components of proteins)
9	1250-1220, 1084-1088	POC stretching of phospholipids
10	1200-900	C-O-C, C-O of various polysaccharides
11	720	CH <sub>2</sub> rocking of methylene
12	900-600	"Fingerprint Region"

(Filtered *Salmonella* Typhimurium spectrum)

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## FT-IR Assay Sensitivity

- Detection Limit = ~40 cells in IR beam area

Improving delivery of cells to beam area will decrease overall number of cells needed in starting sample

$$\text{No. of cells} = (\text{Infrared beam area}) \left( \frac{\text{CFU/mL} \times \text{volume}}{\text{filtration area}} \right)$$

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

Parameter	Filtration (FT-IR)	Dynabeads (FT-IR)	Magnetic Nanoparticles (FT-IR)	Flow cytometry	ELISA (Eg. VIDAS)	PCR (Eg. BAX)
Material cost	Filters	Antibodies	Antibodies	Dye	Enzymes	Primers
Time*	< 7 h	< 10 h	???	8 h	8 – 24 h	8 -24 h
Actual assay time	< 1 h	< 4 h	30 min	0.5-2 h	1-4 h	3-4 h
Sensitivity	10 <sup>6</sup> -10 <sup>7</sup> CFU/mL	10 <sup>6</sup> CFU/mL	10 <sup>6</sup> -10 <sup>7</sup> CFU/mL	10 <sup>4</sup> -10 <sup>6</sup> CFU/mL	10 <sup>7</sup> -10 <sup>9</sup> CFU/mL	10 <sup>7</sup> -10 <sup>9</sup> CFU/mL
Reference	Mauer group	Mauer group	Mauer & Irudayaraj	Givan, 2001	Fung, 2002	Hochberg et al., 2000

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## FT-IR Method Development Sample Preparation Techniques

FT-IR main bench: filtration, magnetic nanoparticles

FT-IR microscope: filtration, immunomagnetic separation, cellular components

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## Filtration- the apparatus

Labels: Filtration Apparatus, Membrane Filter, Vacuum

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## FT-IR and Filtration

Multi-bounce trough plate ATR

Multi-bounce flat plate ATR (MATR)

Contact ATR on IR-microscope (Filtration)

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## FT-IR microscope

- Immunomagnetic separation - Dynabeads
  - Dynabeads with EC
  - Antibodies
- Cellular components (LPS, OMP)

Continuum IR-microscope (ThermoElectron)

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## Library of cell wall components and whole cells

- Escherichia coli*
  - *E. coli* DH5, *E. coli* K12, *E. coli* O157:H12, *E. coli* O157:H19, *E. coli* O26, *E. coli* O111, *E. coli* O103, *E. coli* O157:H16, *E. coli* O157:H5, *E. coli* O157:H19, *E. coli* O157:H7
  - Acc No: 5.2262, 99.0874, 0.1292, 99.0894, 0.0027, 0.1288, 0.1304, 7.3853, 7.3860
- Salmonella*
  - *Salmonella enterica* serovars *S. typhimurium*, *S. enteritidis*, *S. thomasville*, *S. brandenburg*, *S. hadar*, *S. seftenberg*
  - ~160 LPS isolates from J. Bouldin (USDA-ARS)
- Campylobacter*
  - *C. jejuni*, *C. lari*, *C. coli*
- Listeria*
  - *L. monocytogenes*, *L. innocua*
- Shigella flexneri*, *Staphylococcus aureus*

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## FT-IR method development for cell identification and quantification in water, cultural media, and foods

*E. coli* O157:H7  
*Salmonella typhimurium*

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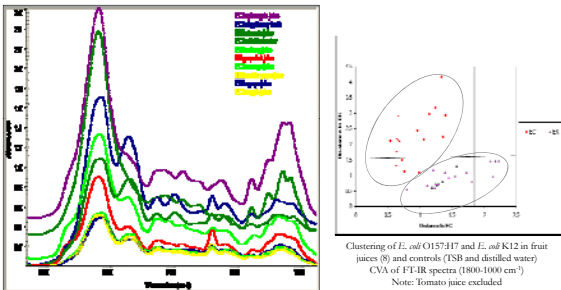
## FT-IR Sensor Potential Applications

- Pathogen detection in foods
  - To date, detection of *E. coli* O157:H7 and *Salmonella typhimurium* successful in:
    - Fruit juices
    - Skim milk
    - Ground beef
    - Chicken rinse water
    - Shredded medium aged Cheddar cheese
    - Medium aged Cheddar cheese
- Differentiation of live and dead cells
  - Quantification of live cells in the presence of dead cells
  - Differentiation of cells based on treatment (heat, salt, UV, antibiotic, alcohol)

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## Fruit juices (Filtration-FT-IR)

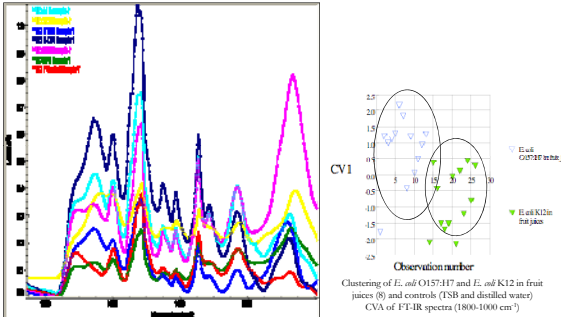


Clustering of *E. coli* O157:H7 and *E. coli* K12 in fruit juices (8) and controls (TSB and distilled water) CVA of FT-IR spectra (1800-1000 cm<sup>-1</sup>)  
Note: Tomato juice excluded

Burgula, Reuhs, and Mauer. 2008. Rapid FT-IR methods for detection of Escherichia coli O157:H7 in fruit juices. *World of Food Science*. Volume 3: Food Safety.

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## Fruit juices (IMS-FT-IR)

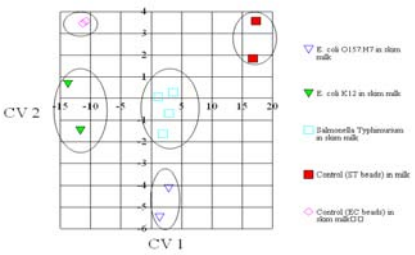


Clustering of *E. coli* O157:H7 and *E. coli* K12 in fruit juices (8) and controls (TSB and distilled water) CVA of FT-IR spectra (1800-1000 cm<sup>-1</sup>)

Burgula, Reuhs, and Mauer. 2008. Rapid FT-IR methods for detection of Escherichia coli O157:H7 in fruit juices. *World of Food Science*. Volume 3: Food Safety.

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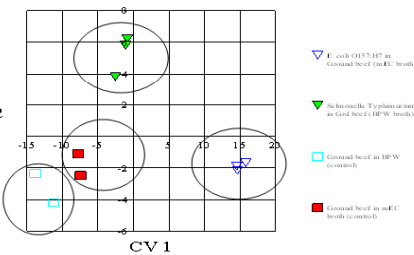
## Skim milk (IMS-FT-IR)



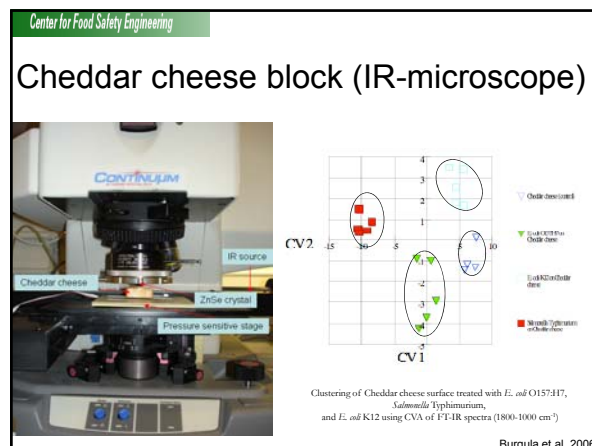
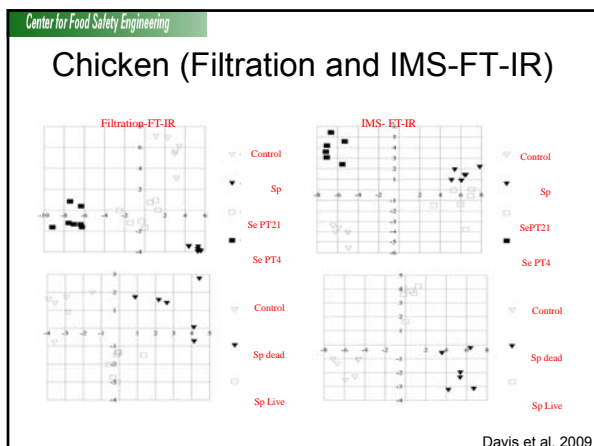
Clustering of milk samples containing *E. coli* O157:H7, *Salmonella typhimurium*, and *E. coli* K12 using CVA of FT-IR spectra (1800-1000 cm<sup>-1</sup>). Plate counts of EC, EK, and ST were 8.6, 8.7, and 8.5 log CFU/mL on TSA  
Burgula et al. 2006

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## Ground beef (Filtration)



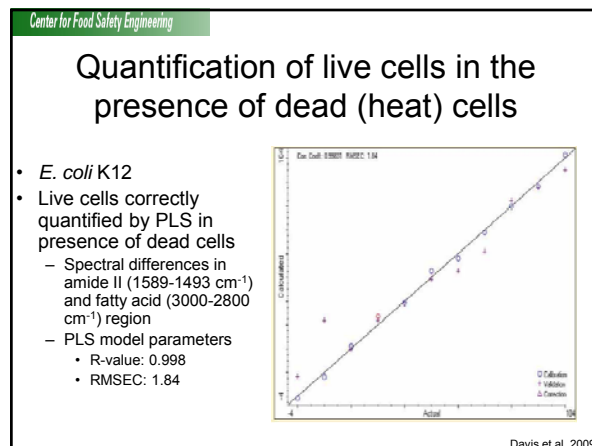
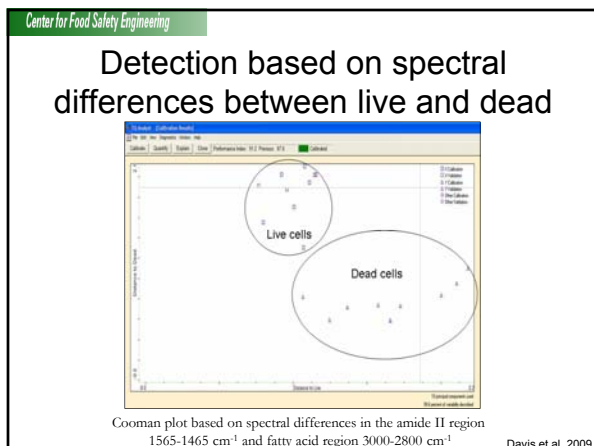
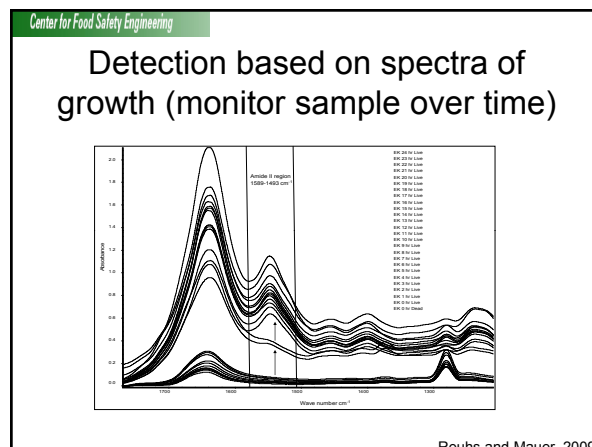
Davis et al. 2009

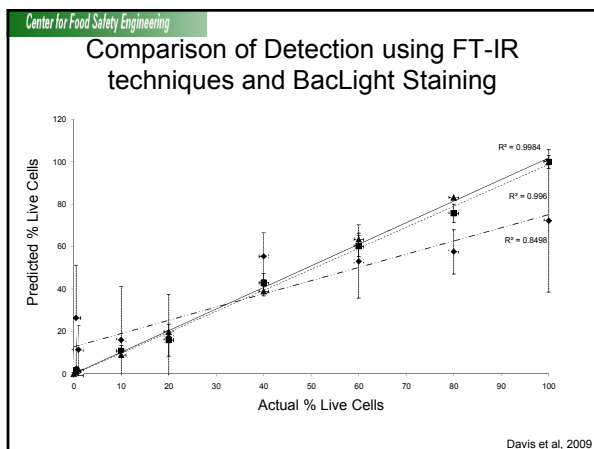
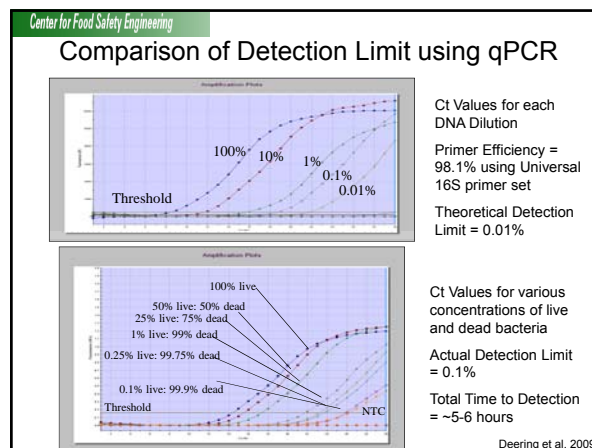
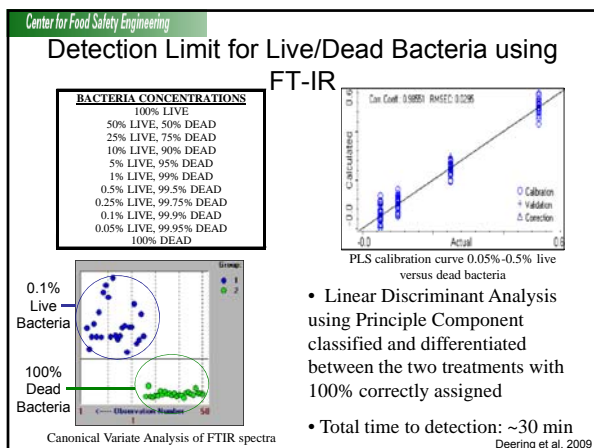


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### Development of analytical methods to discriminate between Live and Dead cells

Based on spectra of growth  
Based on spectral differences





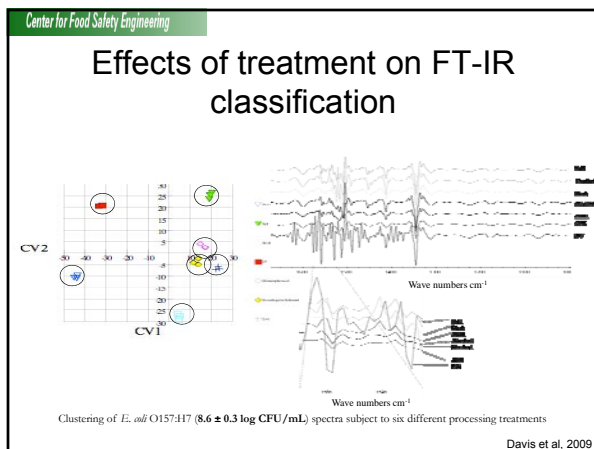
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### Comparison of Current Detection Limits for Live/Dead Bacteria

Method	Detection Limit	Time to Detection
• FT-IR	0.1% = $10^4$ CFU/ml	~30 min
• EMA-PCR	0.1% = $10^4$ CFU/ml	5-6 hours
• PMA <sup>1</sup> -PCR <sup>2</sup>	$10^4$ CFU/ml	5-6 hours
• BacLight Staining kit + flow cytometry <sup>3</sup>	10% ~ $10^6$ CFU/ml	~1-2 hours

<sup>1</sup> PMA = Propidium monoazide <sup>2</sup> Source: Nocker et al. 2009 <sup>3</sup> Source: Swarts et al. 1998

Deering et al., 2009



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### Melamine adulteration detection

Table 2. Partial Least Squares Analysis of FTIR and NIR Spectra for Quantifying Melamine in Infant Formula Powder\*

method	spectrum range ( $cm^{-1}$ )	relative error (% w/w)	no. of PLS factors	calibration		validation			
				R <sup>2</sup>	RMSEEC	R <sup>2</sup>	RMSECV		
FTIR-ATR	3300-2900 1321-882.5	50-0.0001	10	0.996	0.80	14.1	0.9011	0.90	12
FTIR-DRIFT	3695-1969 1655-1319	40-0.0001	6	0.993	0.80	10.9	0.9016	0.89	12.3
NIR	12407-4098 5400-4248.5	50-0.0001	6	0.996	0.475	46	0.9902	0.816	35

\*RMSEEC, root mean square error of estimation; RMSECV, root mean square error of prediction; RPD, residual prediction deviation.

Table 3. Melamine Detection Limits and Times to Detection from Infant Formula Powder for FTIR and NIR Methods

method of melamine detection	assay time (min)	total time to detection (min)	detection limit ( $\mu g/ml$ )	confidence level (%)	selectivity
FTIR-ATR	3	4	1	99.99	2.09
FTIR-DRIFT	3	4	1	99.99	2.44
NIR	1	2	1	99.99	2.4

Mauer et al., 2009

# QUESTIONS?

PI: Dr. Lisa Mauer    Email: [mauer@purdue.edu](mailto:mauer@purdue.edu)