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……..an interdisciplinary approach

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Euiwon Bae
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Dr. Nebeker
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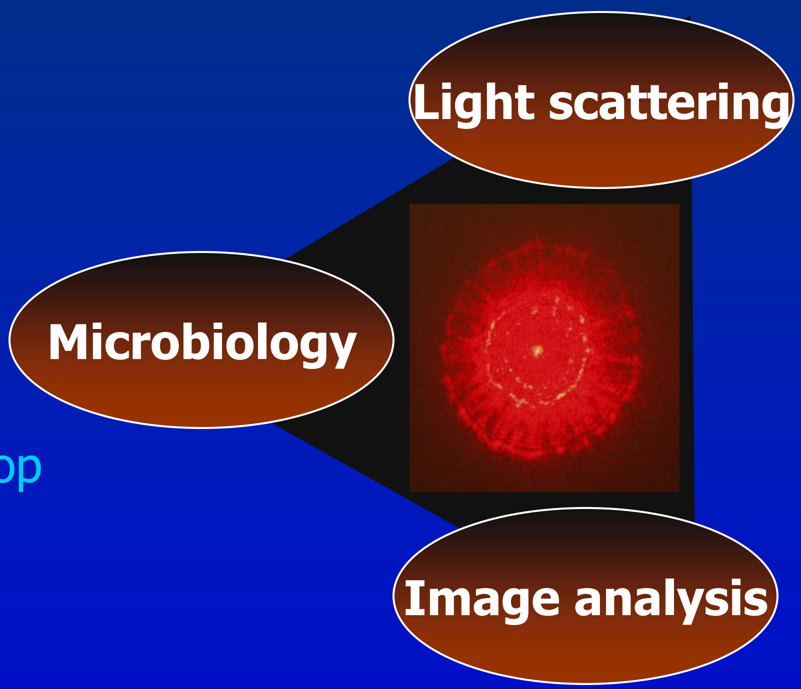
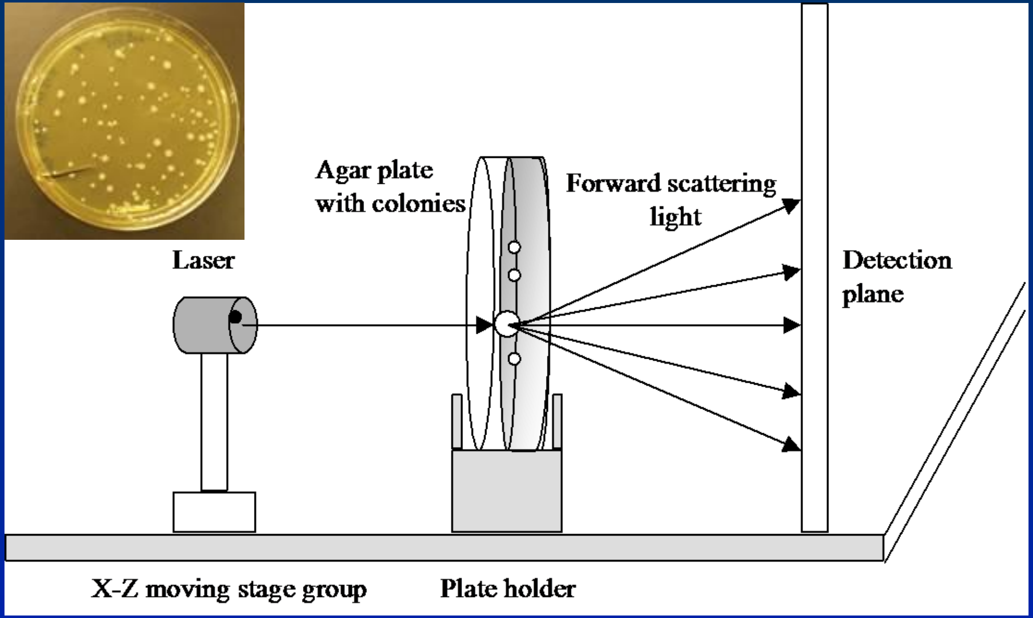
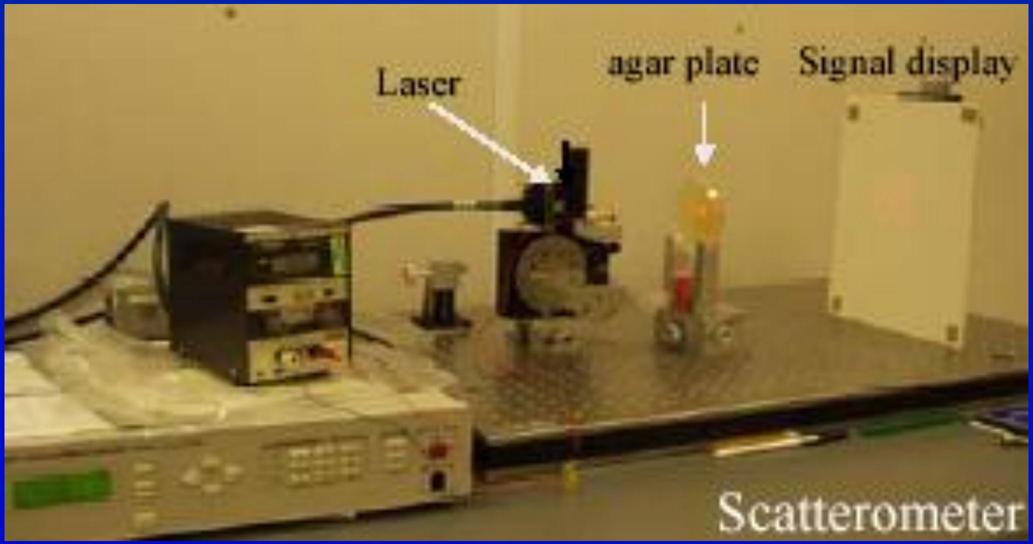


Prof. Robinson
Dr. Rajwa
Bulent Bayraktar

Scatterometer





Scatter patterns of 108 *Listeria* species were analyzed

69 – *L. monocytogenes*

16- *L. innocua*

12- *L. ivanovii*

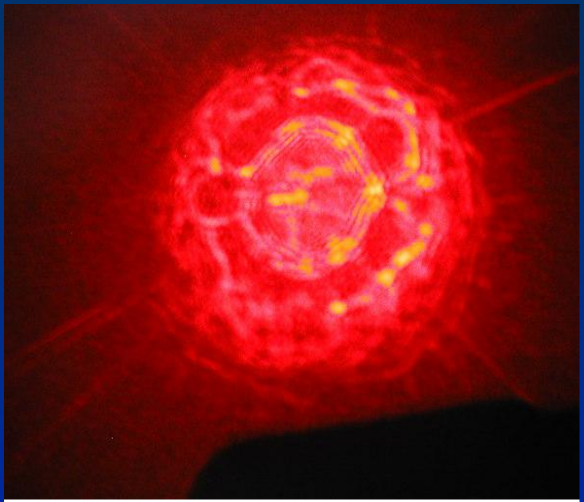
5- *L. seeligeri*

3- *L. welshimeri*

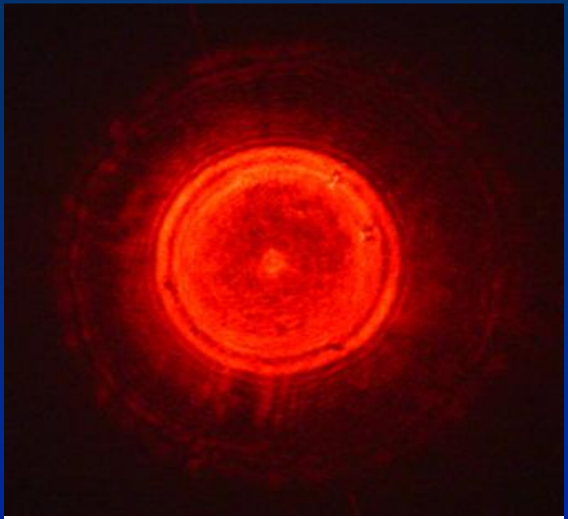
3- *L. grayi*

Scatter-images of representative *Listeria* species

L. monocytogenes ATCC19113

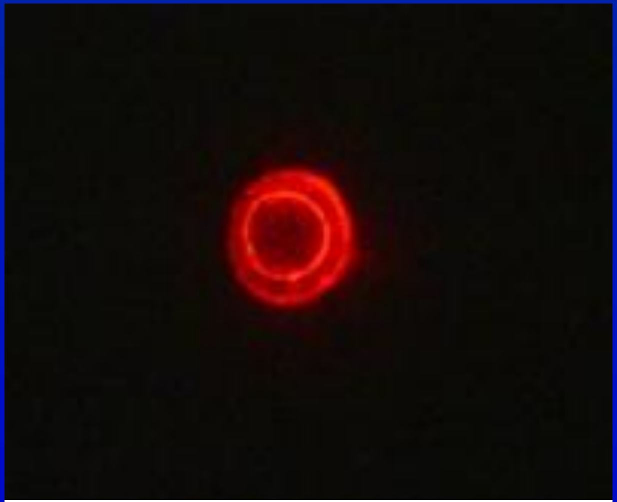


L. ivanovii ATCC 19119

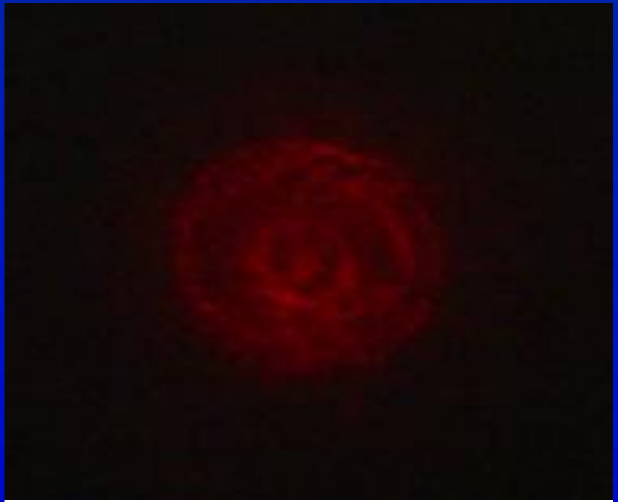


L. innocua F4248

L. seeligeri LA 15



L. welshimeri ATCC 35897



L. grayi LM37

Image analysis

I[st] step: **Feature Extraction and Moments**

Features are scalar properties of objects in images (e.g., area, perimeter) Feature extraction is a key step in pattern recognition applications.

By applying **Zernike Moments,** features were extracted

$$Z_{nm} = \frac{n+1}{\pi}\sum_x\sum_y f(x,y)\cdot V_{nm}^*(x,y) = \frac{n+1}{\pi}\sum_r\sum_\theta f(r\cos\theta, r\sin\theta)\cdot R_{nm}(r)\cdot \exp(jm\theta), \quad r\le 1$$

$$V_{nm}(x,y) = V_{nm}(r\cos\theta, r\sin\theta) = R_{nm}(r)\cdot\exp(jm\theta)$$

$$R_{nm}(r) = \sum_{s=0}^{(n-|m|)/2} (-1)^s \frac{(n-s)!}{s!\times(\frac{n+|m|}{2}-s)!\times(\frac{n-|m|}{2}-s)!} r^{n-2s}$$

2[nd] step: **Principal component analysis**

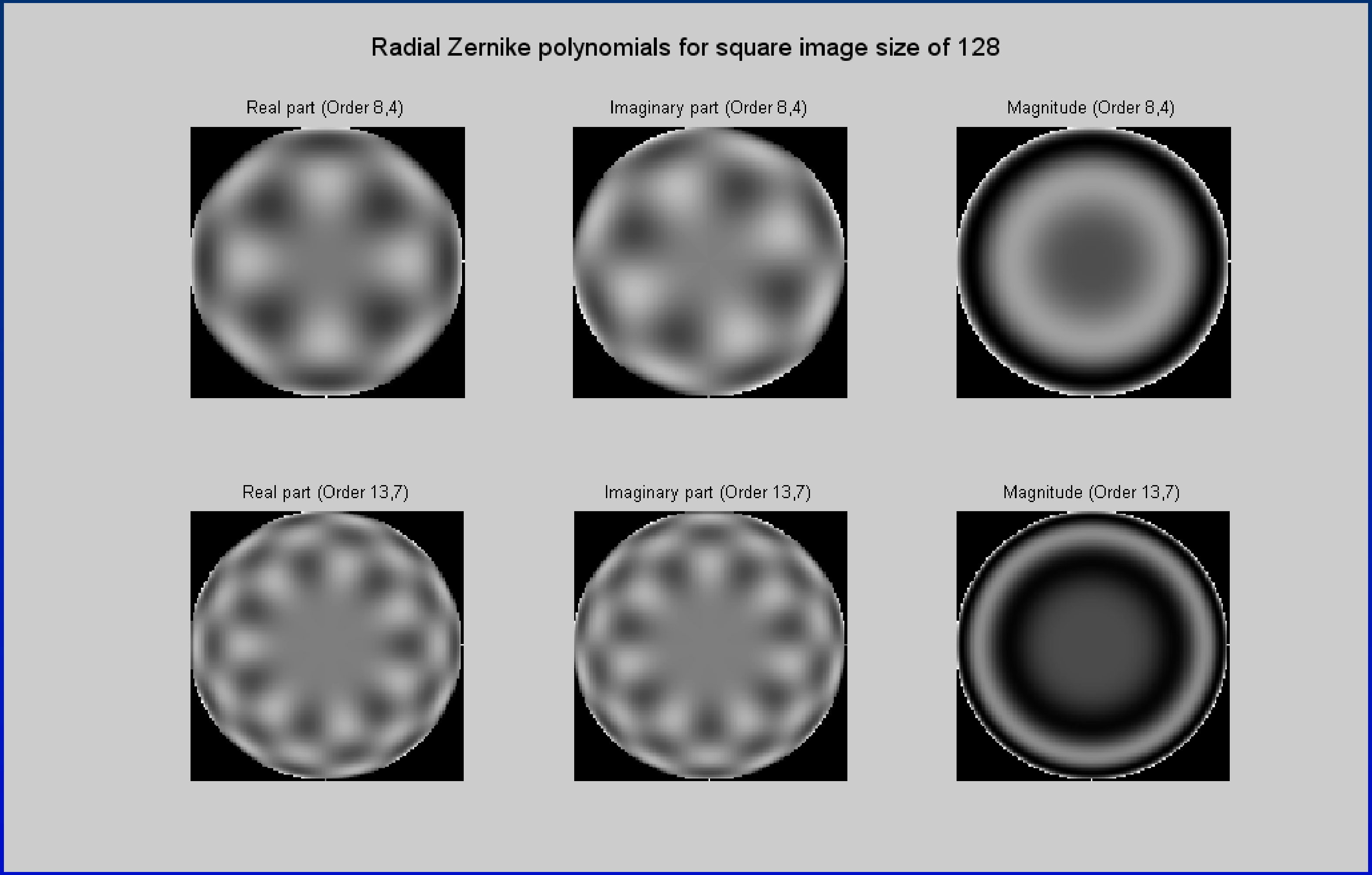
The main use of PCA is to reduce the dimensionality of a data set while retaining as much information as possible

It computes a compact and optimal description of the data set

3[rd] step: **K-Means clustering**

The simplest unsupervised clustering algorithms in image processing.
It uses the Euclidean distance to group data points

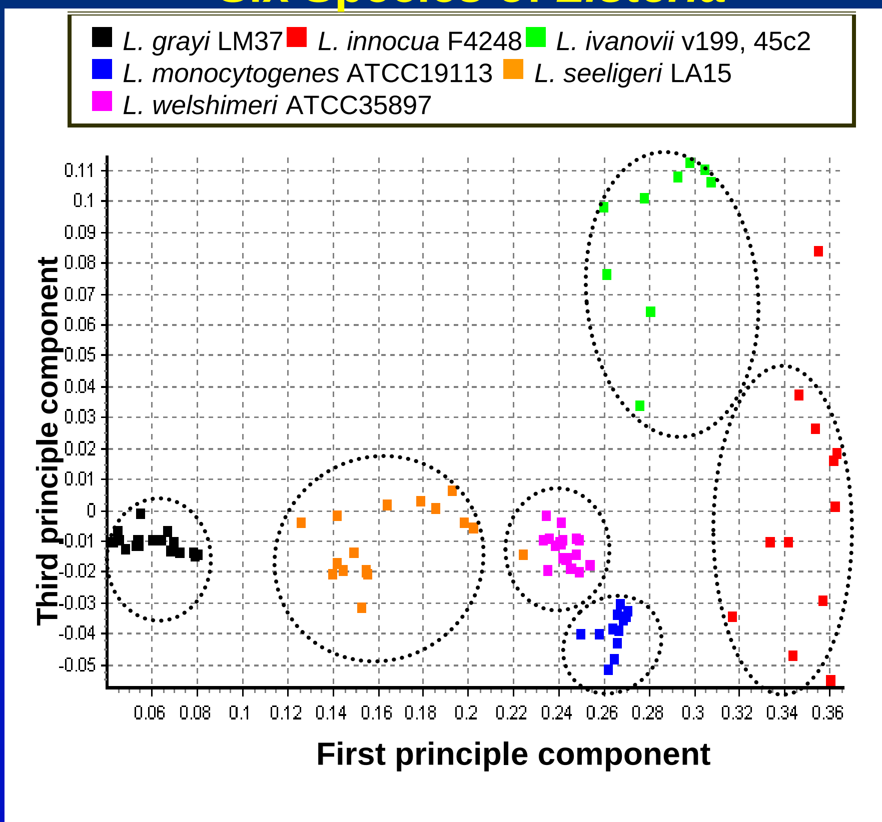
2D Radial Zernike polynomials



Magnitude images depict the circular symmetry where the rotational invariance of Zernike moments comes from

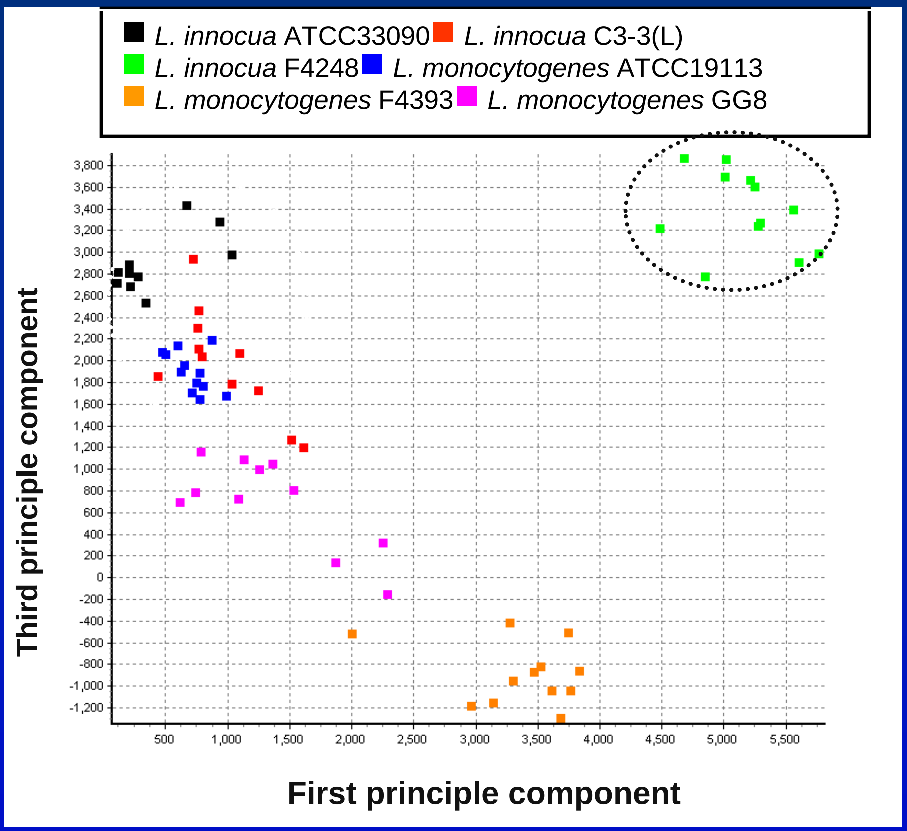
Principal Component Analysis (PCA) of images using cross-products of all Zernike moment invariant features

Six Species of *Listeria*

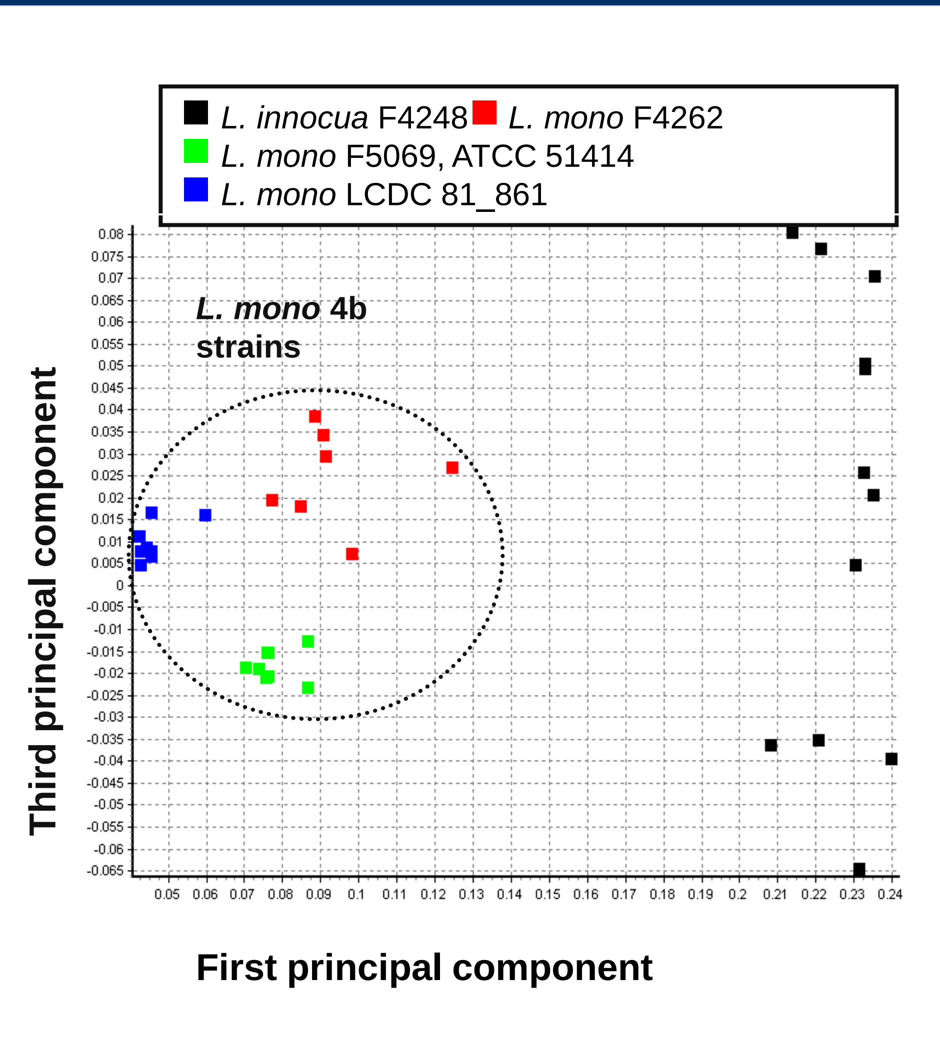


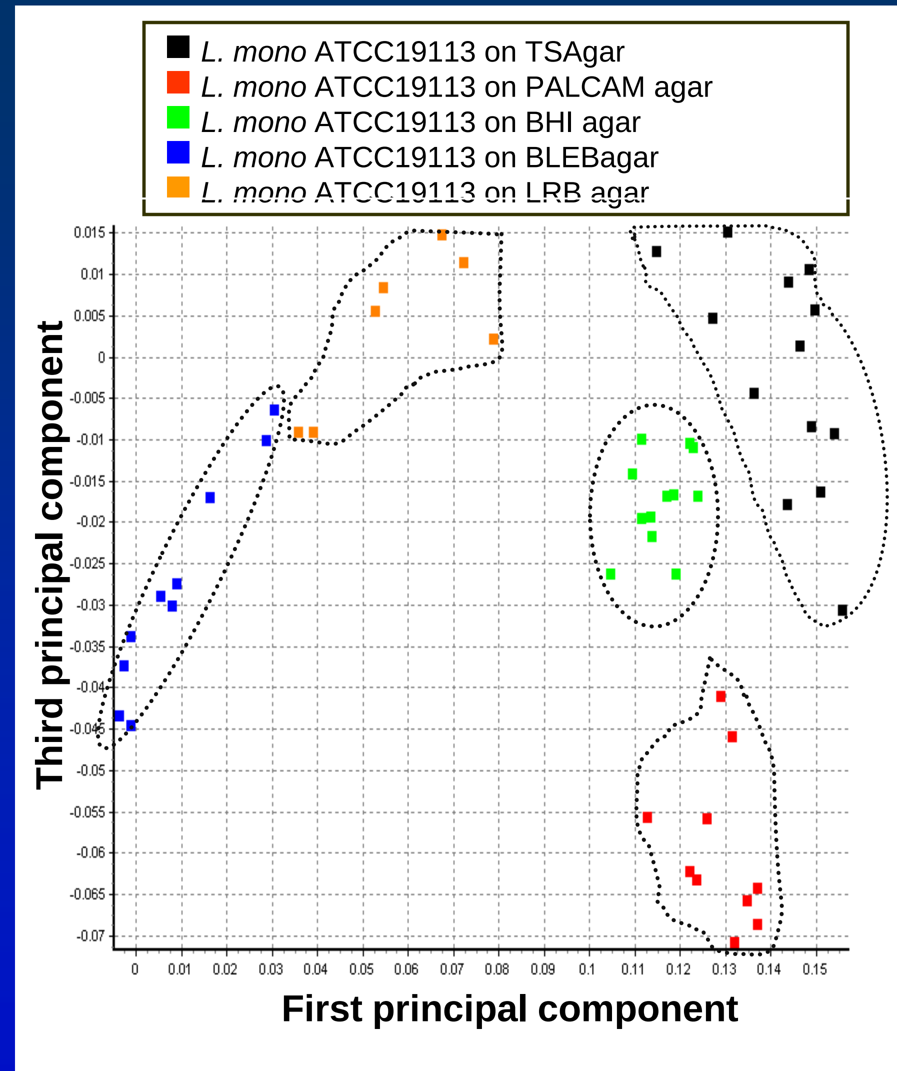
Data for a total of 103 images is shown and about 15-18 images for each strain.

L. monocytogenes and L. innocua



Data for a total of 68 images are shown (2 species and 6 strains)

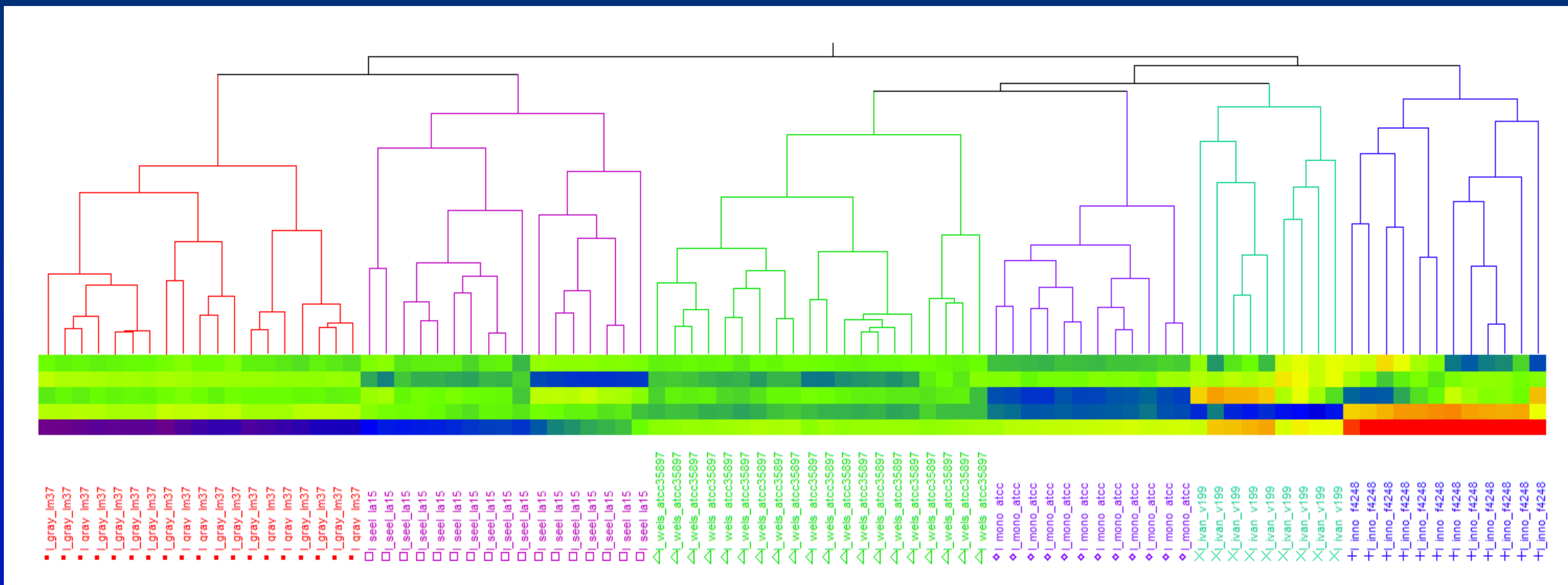




L. monocytogenes* 4b serotypes and *L. innocua

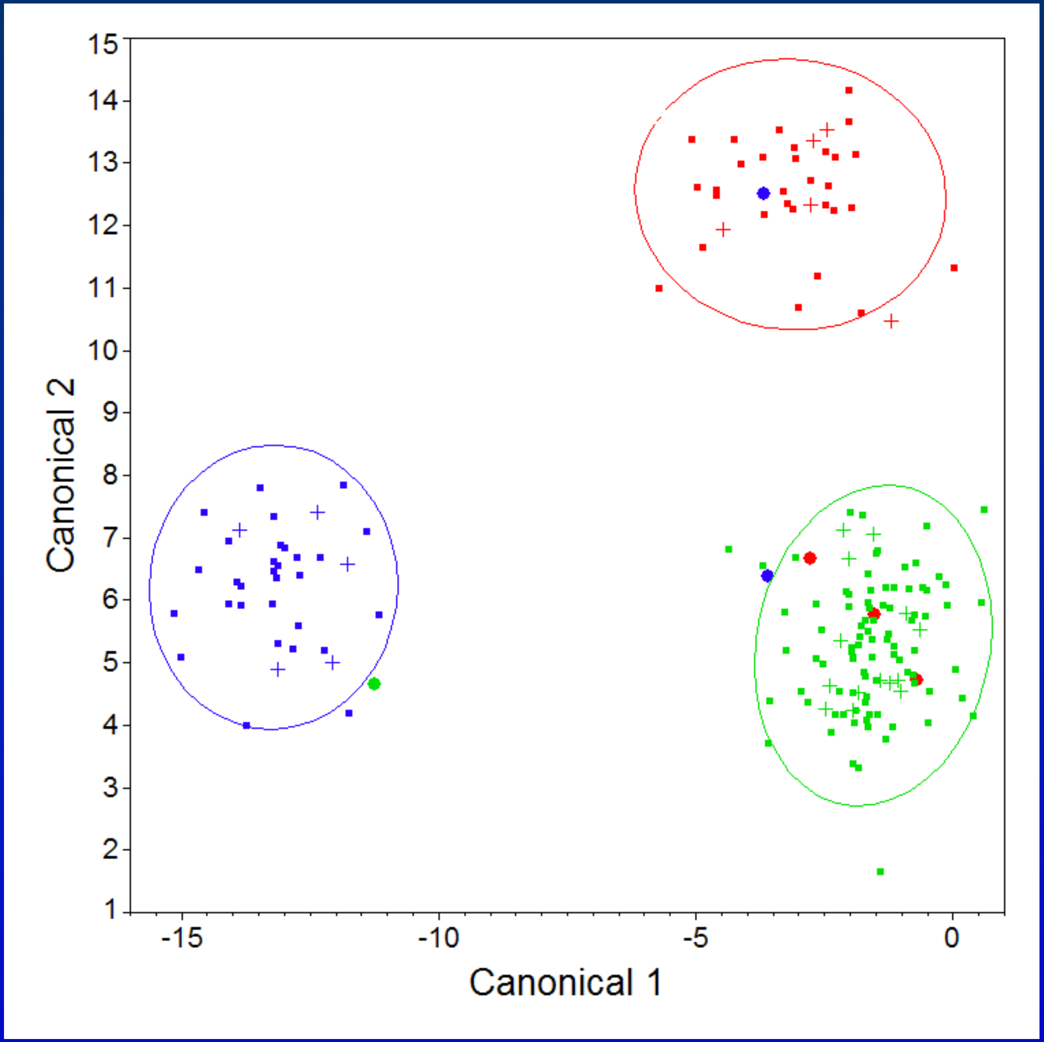
***L. monocytogenes* on various selective and non-selective media**

Example: Hierarchical clustering



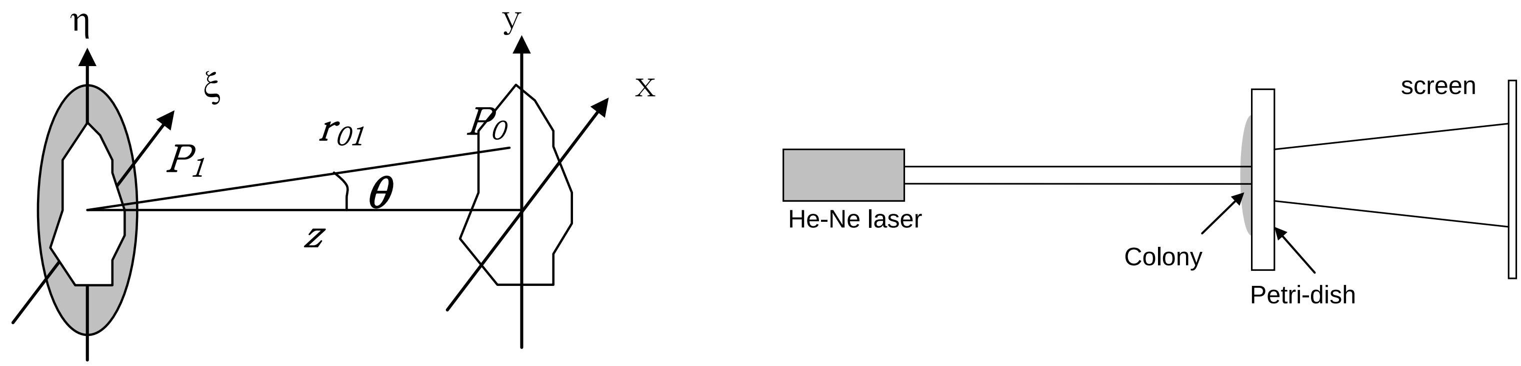
Hierarchical clustering results from our analysis of *Listeria* species and strains using Zernike moment feature extraction. Each color represents distinct *Listeria* strains that are accurately classified.

Example: Classification by Linear discriminant analysis (LDA) or neural network system (JMP Statistical Discovery 5.1 package)



- Would reduce the "curse of dimensionality
- More accurate grouping of features increasing specificity
- Would help in automation

Modeling or simulation of scatter patterns using diffraction theory



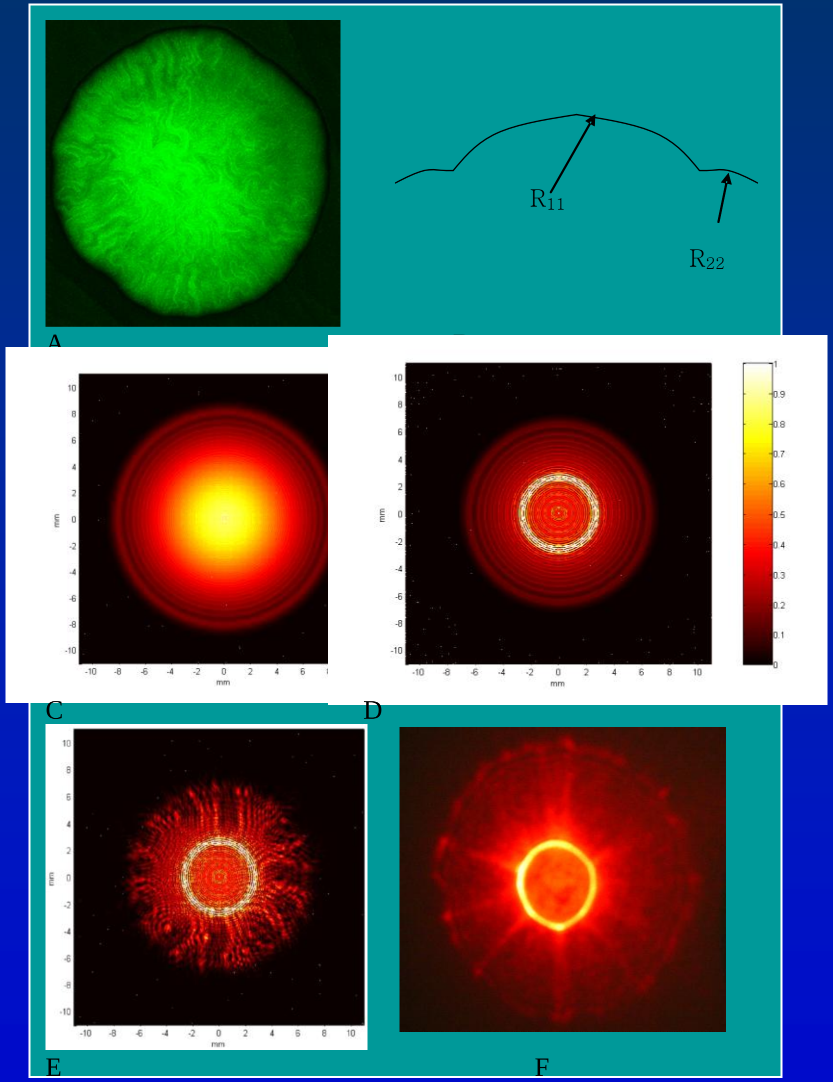
$$U(x,y)=\frac{e^{ikz}}{i\lambda z}e^{j\frac{k}{2z}(x^2+y^2)}\iint_{\Sigma}U'(\xi,\eta)e^{i\frac{k}{2z}(\xi^2+\eta^2)}e^{-i\frac{2\pi}{\lambda z}(x\xi+y\eta)}d\xi d\eta$$

(A) Coordinates for object plane and image plane and; (B) Gaussian beam incident on types of region

η and ξ: **Object planes; P1**: Point on the object plane; **P0**: Object on the image plane;

r01 - distance from the object to image plane; z: Distance from object plane to image plane; θ: Angle between r**0 and z; x** and **y**: Coordinates for image plane

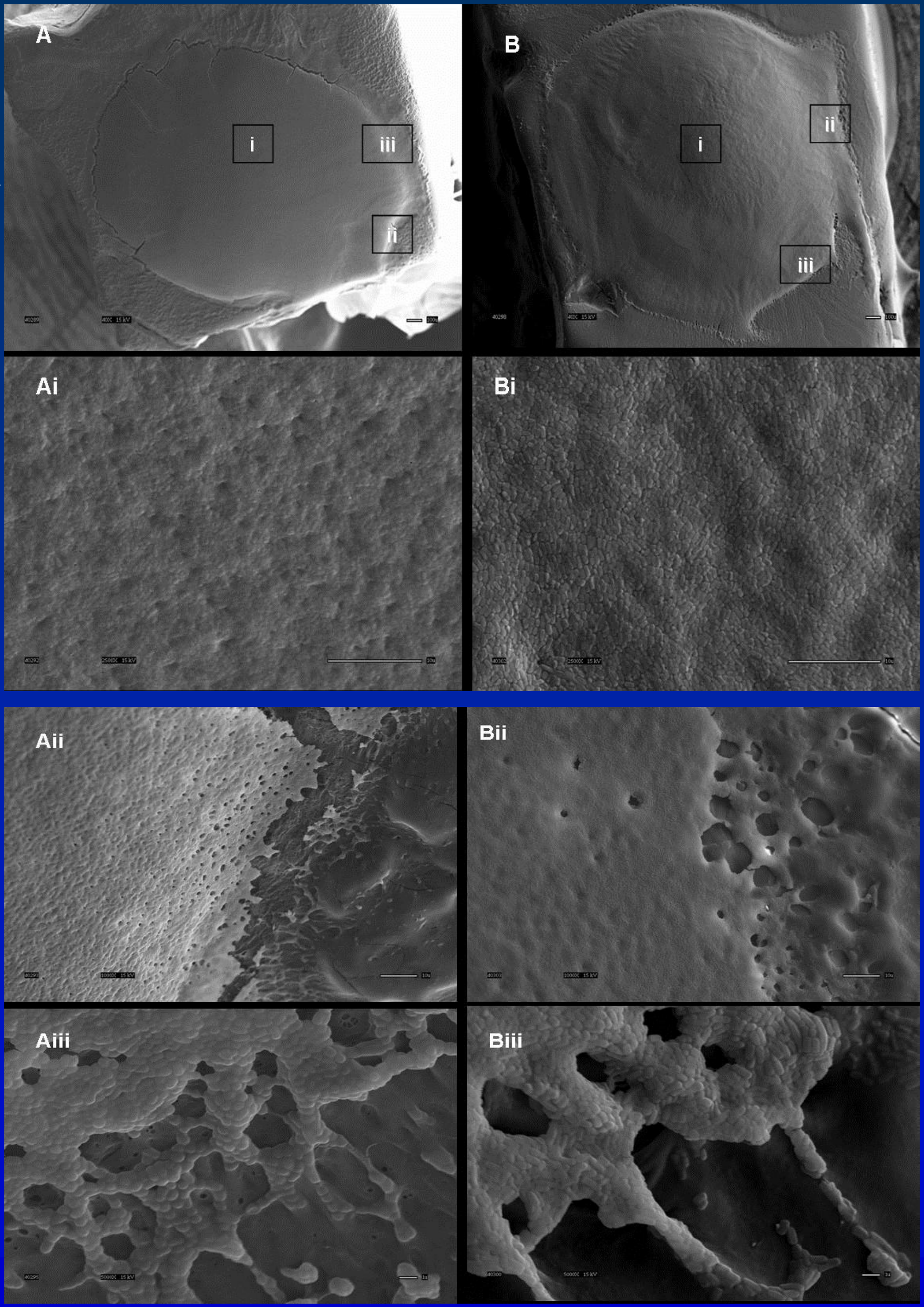
Comparison of the scattering patterns from experiment and simulation



A. Confocal microscopy image of *Listeria monocytogenes*
B. Modeling using two different radius of curvature R11 and R22 explaining the 2-stage curvature on the surface of the colony
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

L. monocytogenes

L. innocua



Cryo-NanoSEM of *Listeria* colonies

Publications

- Banada et al 2005. Optical forward scattering for identification and differentiation of *Listeria* species. Proc. Nat. Acad. Sci. (submitted Oct 2005)
- Bayraktar et al. 2005. Bacterial colony differentiation using computational image analysis and pattern recognition tools. Bioinformatics (submitted Oct 2005)

Disclosures on light scattering

❑ **Guo, S., E.D. Hirleman, and A.K. Bhunia. 2004. Method for rapid detection and characterization of bacteria colonies using forward light scattering.**

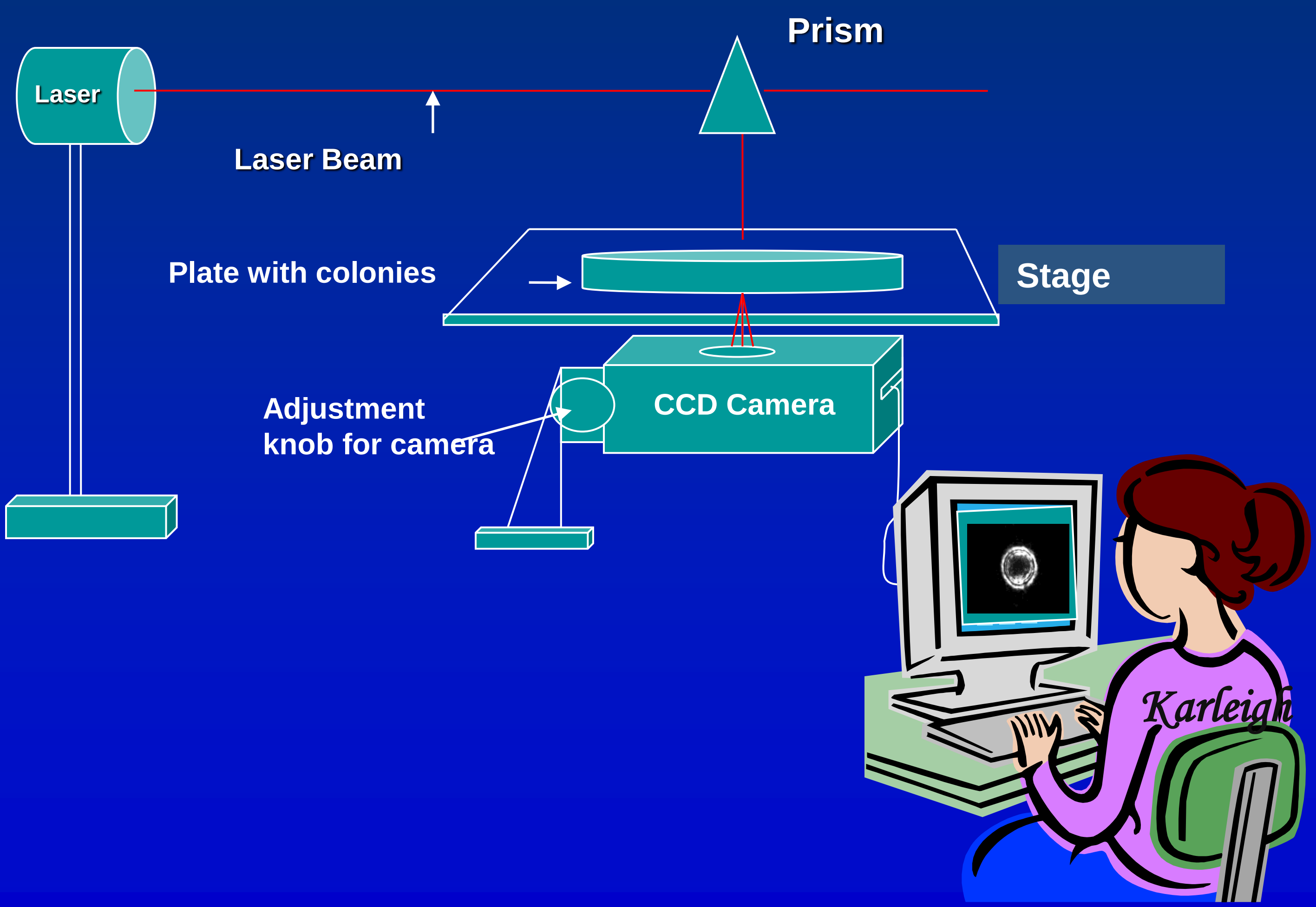
❑ **Bayraktar, S., B. Rajwa, J.P. Robinson, A. Bhunia, and E.D. Hirleman. 2005. Identification of bacterial phenotypes using orthogonal complex moment invariants.**

New Light scattering project
(Start date Feb 01, 2005 for two years)

Objectives

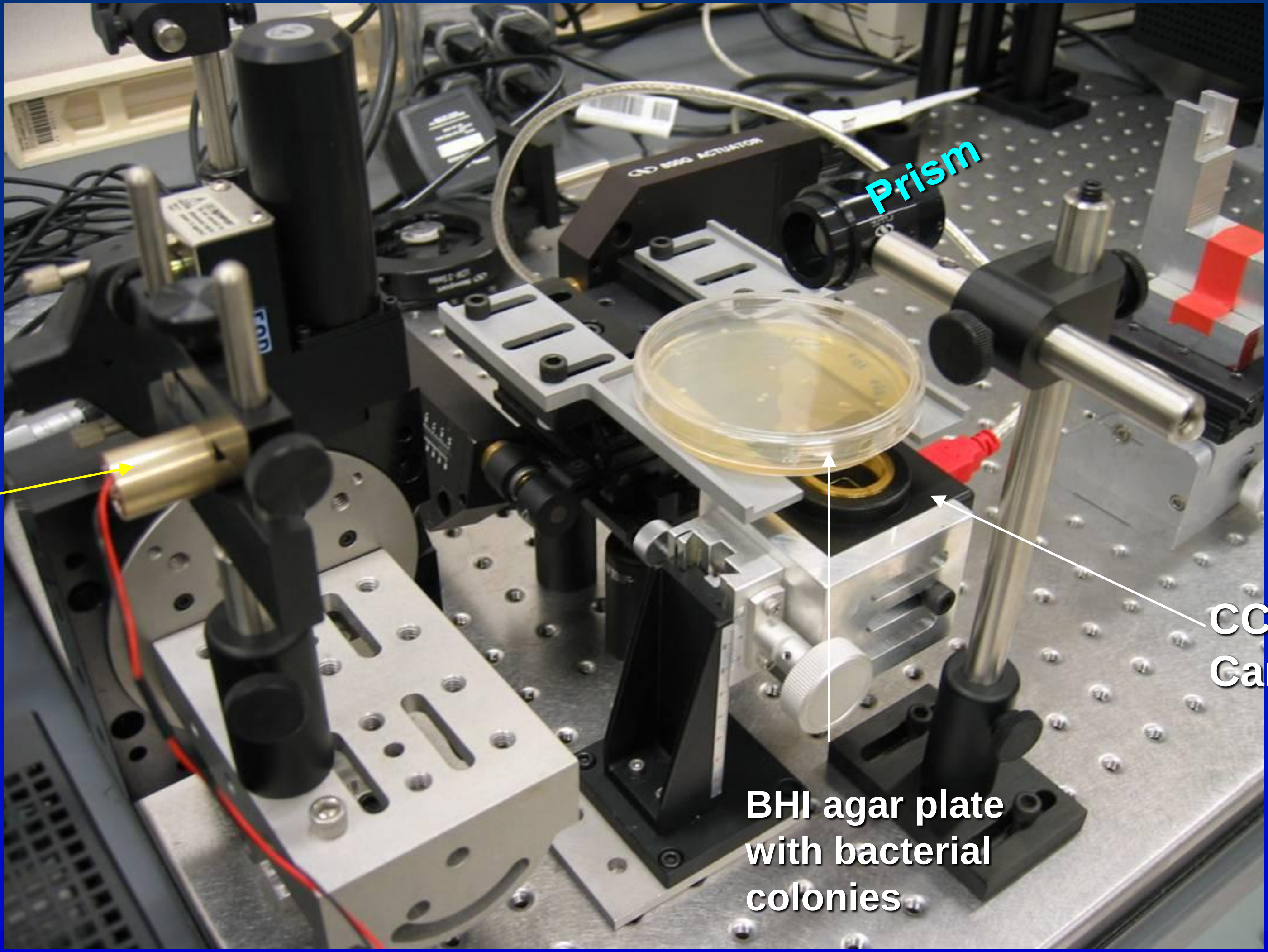
- Improve BARDOT (Bacteria Rapid Detection using Optical Scattering Technology) design. (Hirleman)
- Generate scatter images of different bacterial colonies (Bhunia)
- Image analysis (Robinson)

Bacteria Rapid Detection using Optical Scattering Technology (BARDOT)

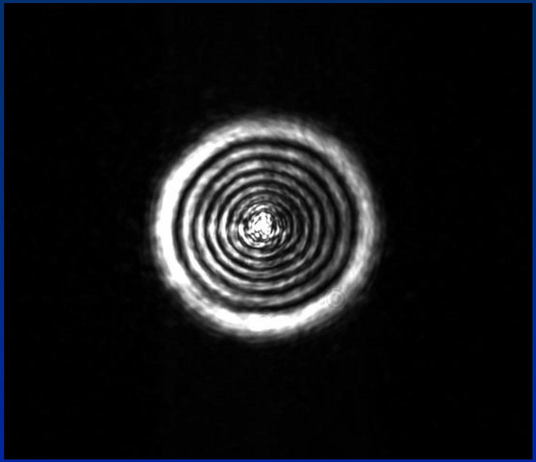


BARDOT

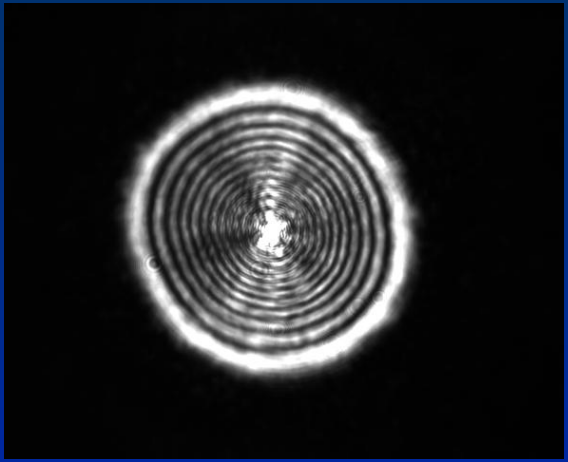
Laser



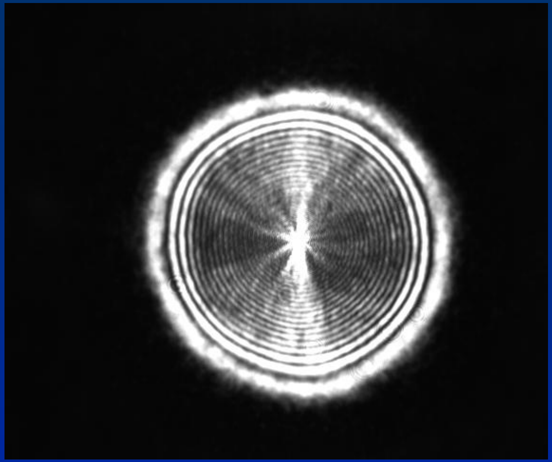
Scatter images of *Listeria* species using BARDOT system



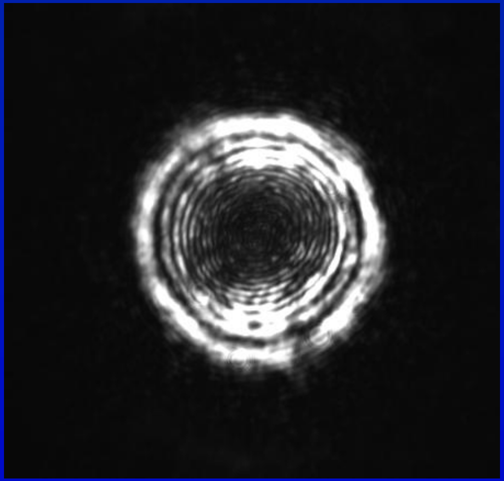
L. monocytogenes ATCC 19113



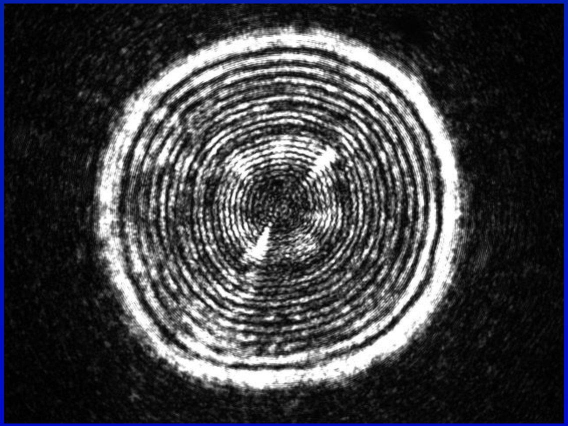
L. monocytogenes V7



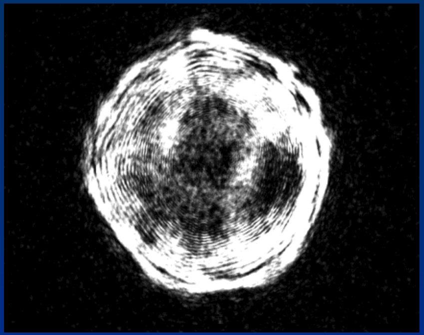
L. innocua F4248



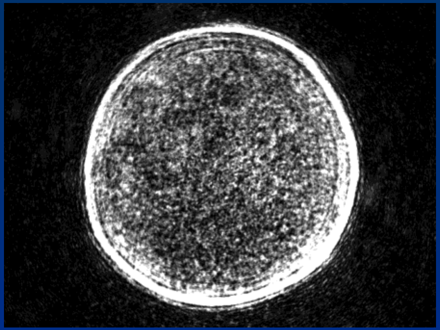
L. ivanovii ATCC 19119



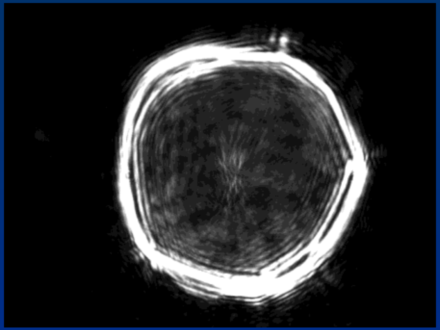
L. ivanovii V199 45C2



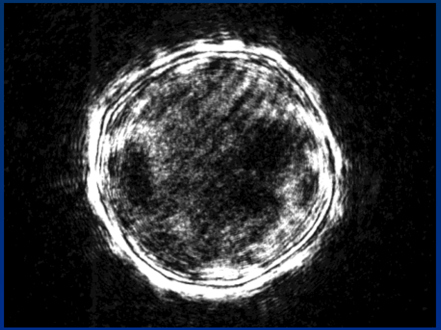
Sal. Enteritidis ATCC 13096



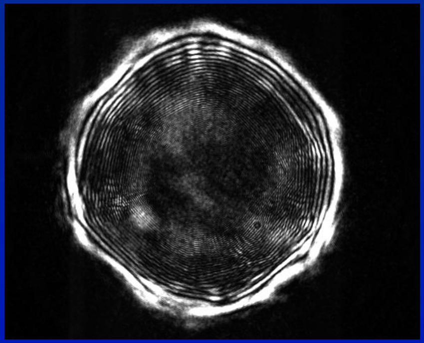
Sal. Typhimurium

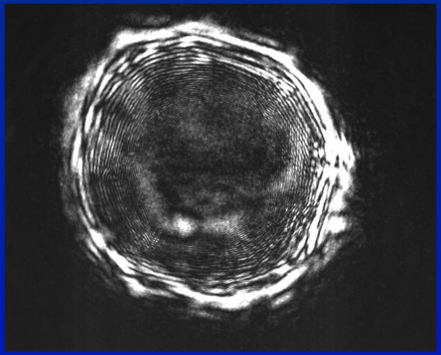


Sal. Typhimurium (Copenhagen)

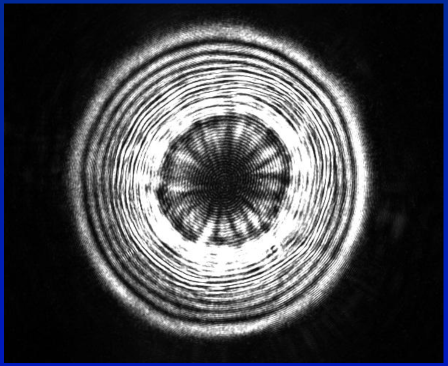


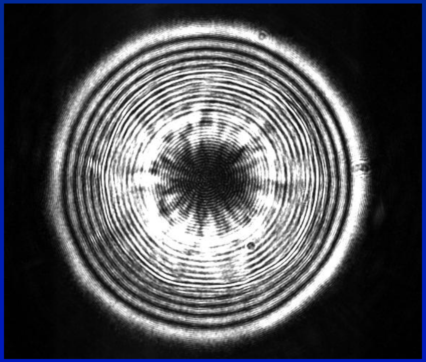
Sal. Typhimurium Kentucky



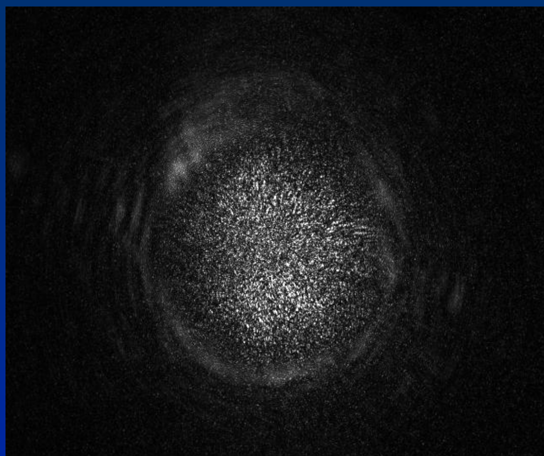


Escherichia coli K12

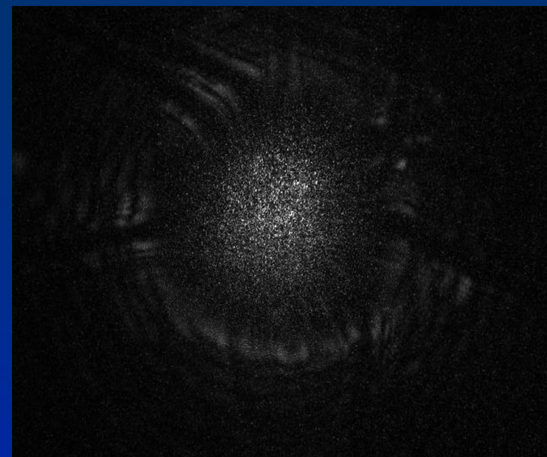




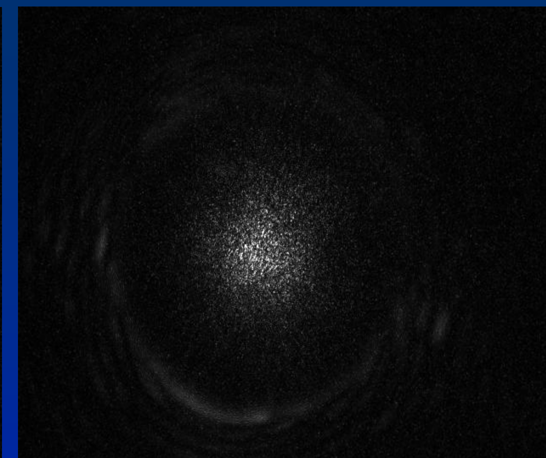
Enterobacter aerogenes



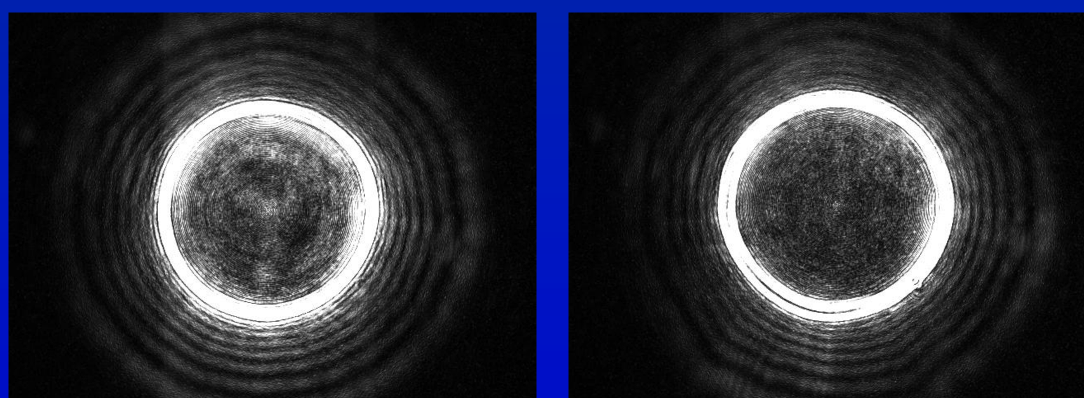
Bacillus cereus MS1-9



Bacillus megaterium ATCC9885



Bacillus polymyxa 719-X



Enterococcus faecalis CG110

Conclusions

- The Scatterometer system is simple yet powerful and robust and its potential is not fully explored yet.
- Image analysis utilizes simple features like area, perimeter, shape, diffusion, etc to develop alogarithm for characterization and differentiation of closely related species/strains with 91-99% accuracy.
- Media composition affects scatter patterns
- The properties behind these scatter patterns could be modeled using diffraction theory applying the confocal images.
- The “Aperture effect” revealed the appearance of circular patterns and the center spot and “fuzzy waves” on the colony proved the spikes.

Conclusions

- **The BARDOT system proved to be 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.**
- **More features could be extracted from these patterns for better differentiation, classification and clustering.**

Immediate future focus

- We will 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.