

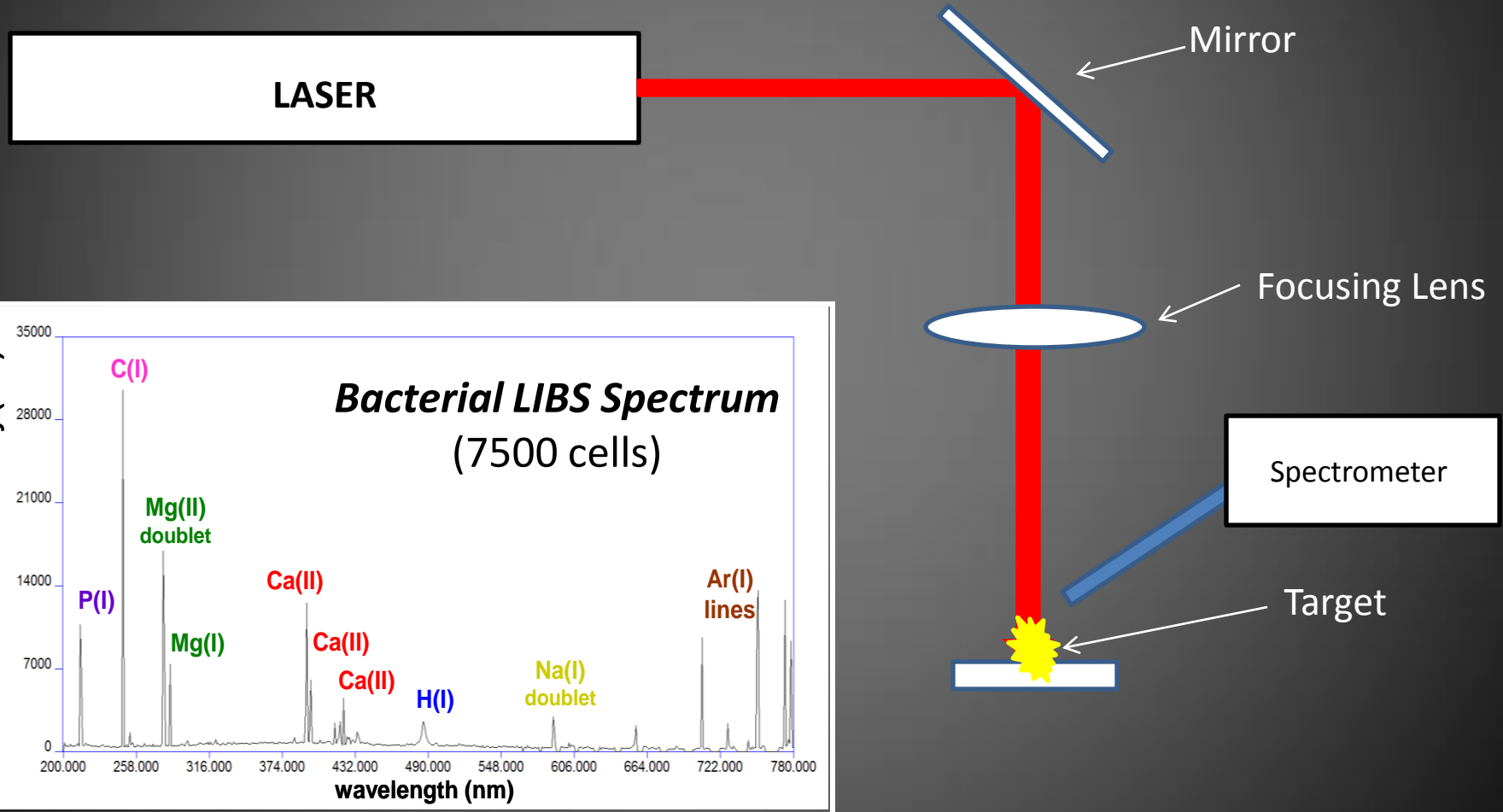
Spectral Analysis for Discrimination of Bacterial Fingerprints

Russell A. Putnam

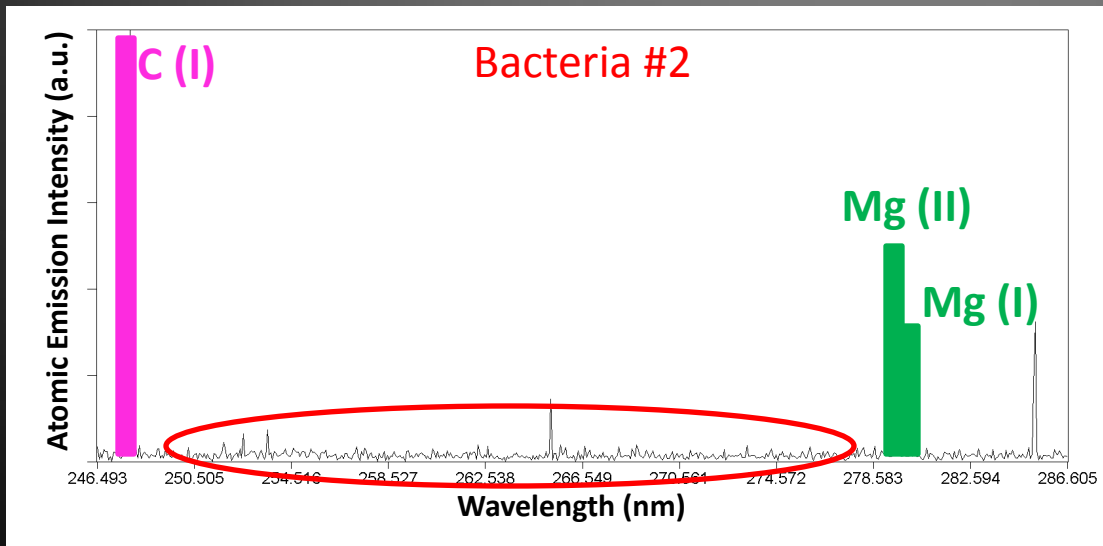
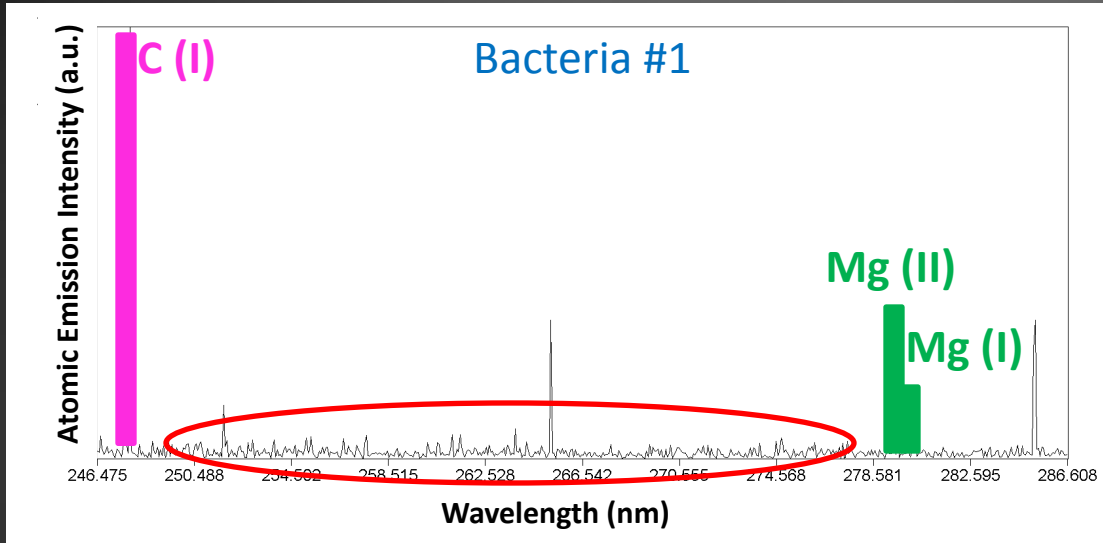
Rehse Group

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Laser-based identification of bacteria



Similar Light Fingerprints

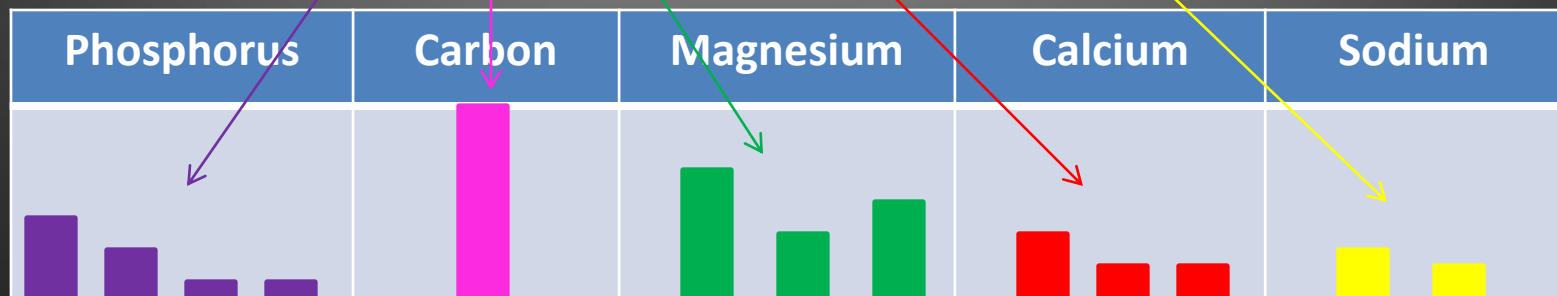
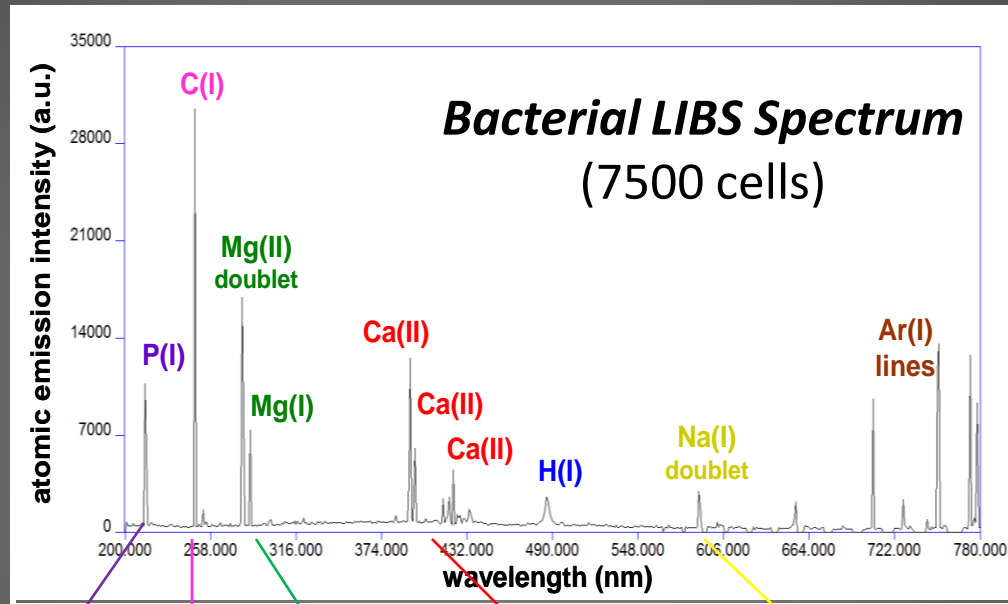


- Fingerprints consist of the SAME lines but in varying ratios

- i.e. # 2 has slightly more Mg in comparison to C than # 1

- What is the important information?

Down-selection of variables

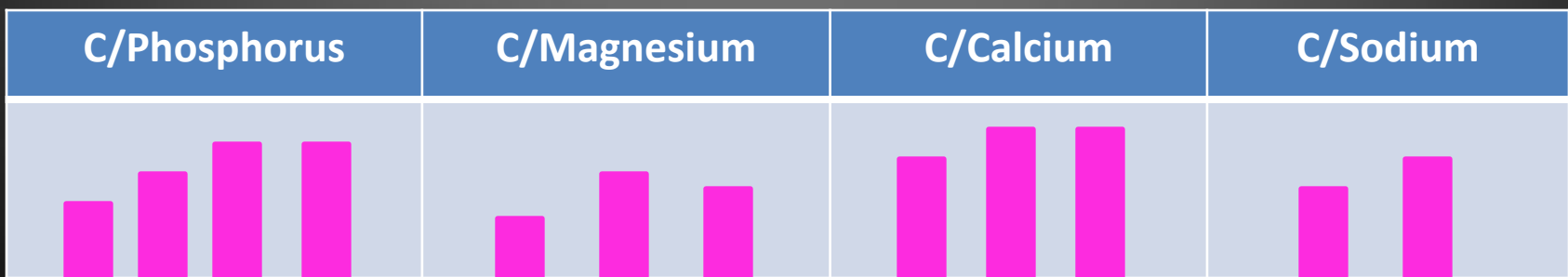


Down-selection of variables

More in-depth explanation of the specific ratios used can be read in:

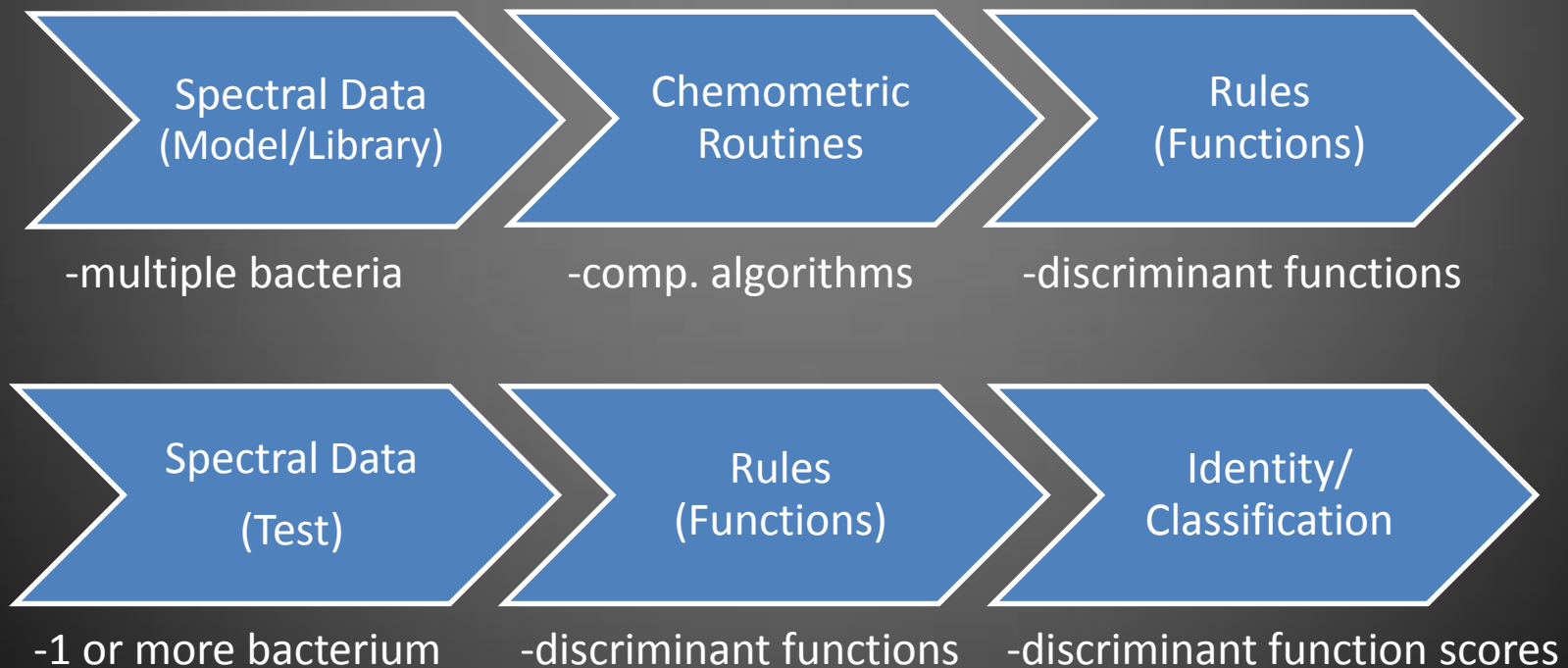
“A comparison of Multivariate Analysis Techniques and Variable Selection Strategies in a Laser-Induced Breakdown Spectroscopy Classification”, R. A. Putnam, A. Daabous, and S. J. Rehse.

lines... Use the ratios as the variables!



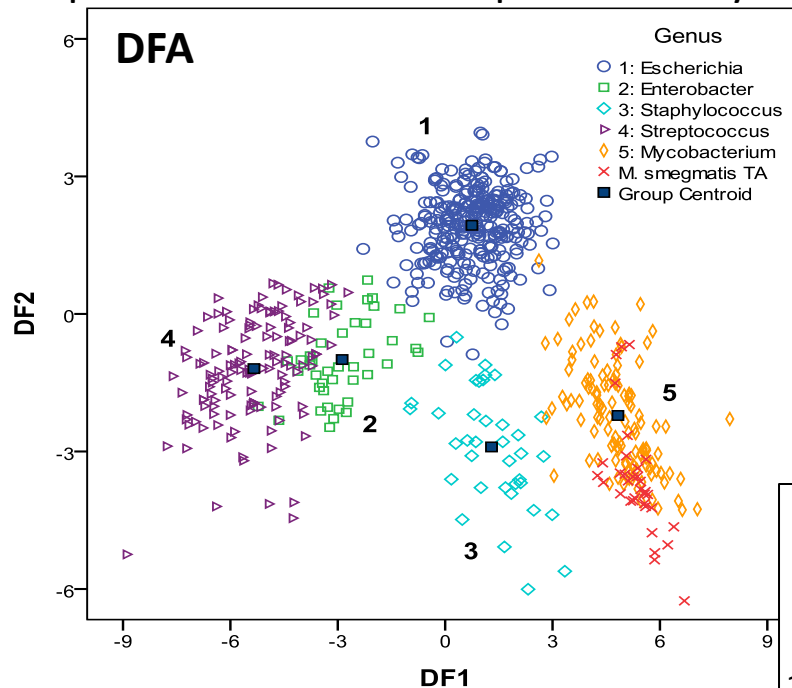
Multivariate Chemometric analysis

- Extract information by analyzing small differences in bacterial fingerprints – small differences in down-selected variables



Discriminant Function Analysis and Principle Least-Squares Discriminant Analysis

All spectral information represented by 2 scalars



- Emission Spectrum
- 45,000 Channels

- Emission Line Intensities
- 13 variables

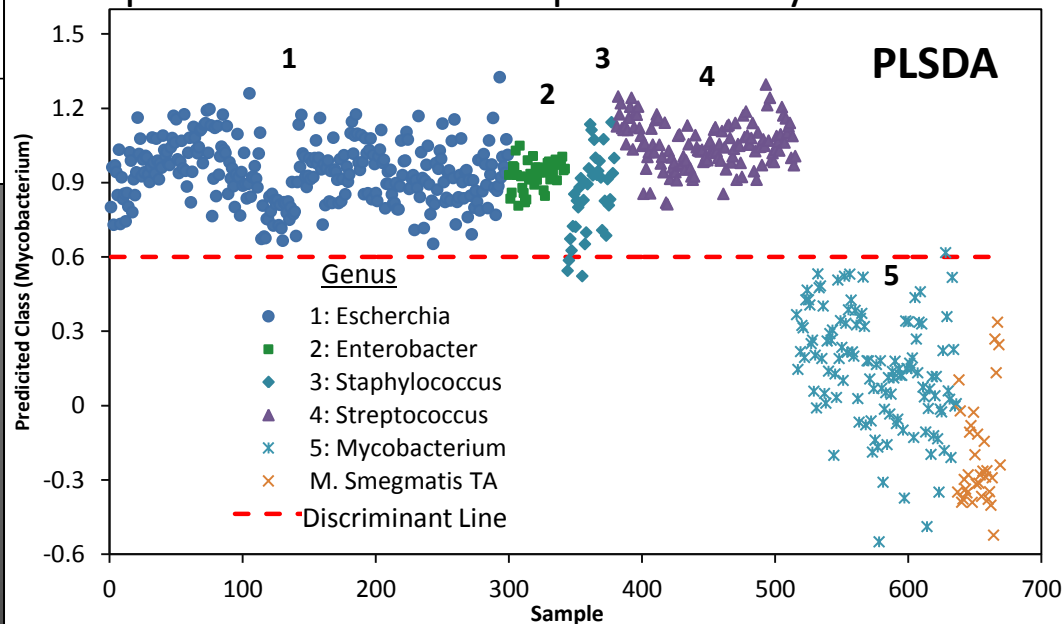
- Discriminant Function Scores
- 2 scalar quantities

- Emission Spectrum
- 45,000 Channels

- Emission Line Intensities
- 13 variables

- Predictor Score
- 1 scalar quantity

All spectral information represented by 1 scalar



Testing: External vs. Internal

- Internal validation - the model and test group contain spectra from the same day and same cultures OR even contains the test spectra
- External validation: model and test groups are exclusive.
- The model fingerprints are taken on different days and from different cultures of bacteria than the test fingerprints
- External validation is a rigorous test to see if the model is isolating true differences in bacterial fingerprints

Conveying Results: Truth Tables

WAS IT CORRECT?

		WAS IT CORRECT?	
		TRUE	FALSE
COMPUTER I.D.	<i>Bacteria # 1</i>		
	POSITIVE	Identified as Bact. #1 when the specimen <u>was</u> Bact. #1 (good – correct)	Identified as Bact. #1 when the specimen <u>was not</u> Bact. #1 (bad – incorrect)
	NEGATIVE	<u>Not</u> identified as Bact. #1 when the specimen <u>was not</u> Bact. #1 (good – correct)	<u>Not</u> identified as Bact. #1 when the specimen <u>was</u> Bact. #1 (bad – incorrect)

Sensitivity	True Positives	Specificity	100% – False Positives
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Results

- Ratio Model 2: 80 down-selected variables consisting of the 13 elemental lines with various ratios of emission lines to sums of lines

PLSDA

<i>E. COLI</i>	True	False
Positive	95.65%	9.17%
Negative	90.83%	4.35%
<i>STAPHYLOCOCCUS</i>	True	False
Positive	54.05%	0.51%
Negative	99.49%	45.95%
<i>STREPTOCOCCUS</i>	True	False
Positive	95.59%	1.02%
Negative	98.98%	4.41%
<i>MYCOBACTERIUM</i>	True	False
Positive	88.31%	1.06%
Negative	98.94%	11.69%

DFA

<i>E. COLI</i>	True	False
Positive	89.63%	15.95%
Negative	84.05%	10.37%
<i>STAPHYLOCOCCUS</i>	True	False
Positive	86.49%	5.85%
Negative	94.15%	13.51%
<i>STREPTOCOCCUS</i>	True	False
Positive	99.26%	13.32%
Negative	88.68%	0.74%
<i>MYCOBACTERIUM</i>	True	False
Positive	96.10%	4.08%
Negative	95.92%	3.90%

DFA: Sensitivity: 91.37 ± 16.39 %

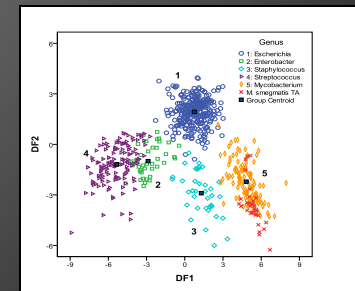
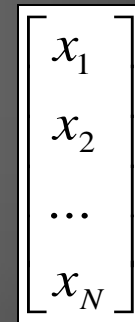
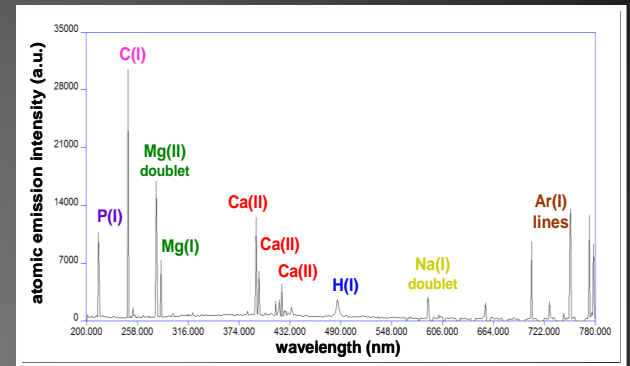
Specificity: 97.46 ± 9.35 %

PLSDA: Sensitivity: 93.13 ± 10.25 %

Specificity: 90.60 ± 21.33 %

In Summary

- Bacterial spectra are down-selected to useful information
- The useful information is placed into a chemometric analysis to produce a set of rules
- The rules are then used to compute the useful information into few scalars to discriminate between bacteria



Thank you for your attention!

Questions?

Thank you to Andrew Daabous and
my supervisor Dr. Steven J. Rehse