LIBS for Bacterial Discrimination and Detection and the Observation of Nutrient-Induced Biochemical Membrane Alteration

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STATE
06/04/2008

Introduction

Motivating Questions:

1. Can bacteria be identified from its atomic spectrum alone (using LIBS)?

   Yes! (since about 2003)

2. Can the atomic spectrum be used to do interesting science?

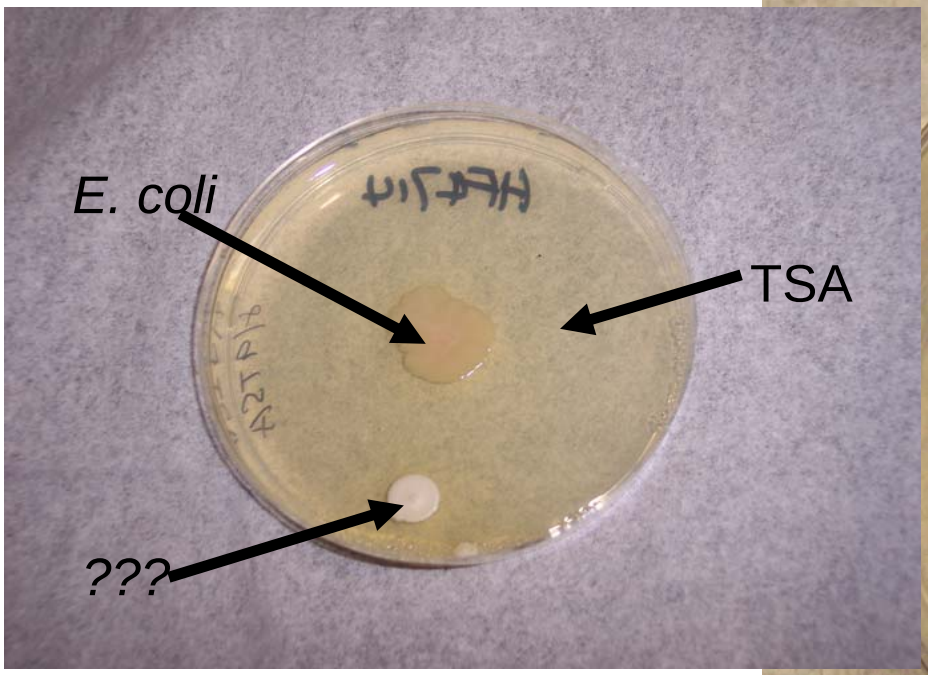
   Yes! (since ???)

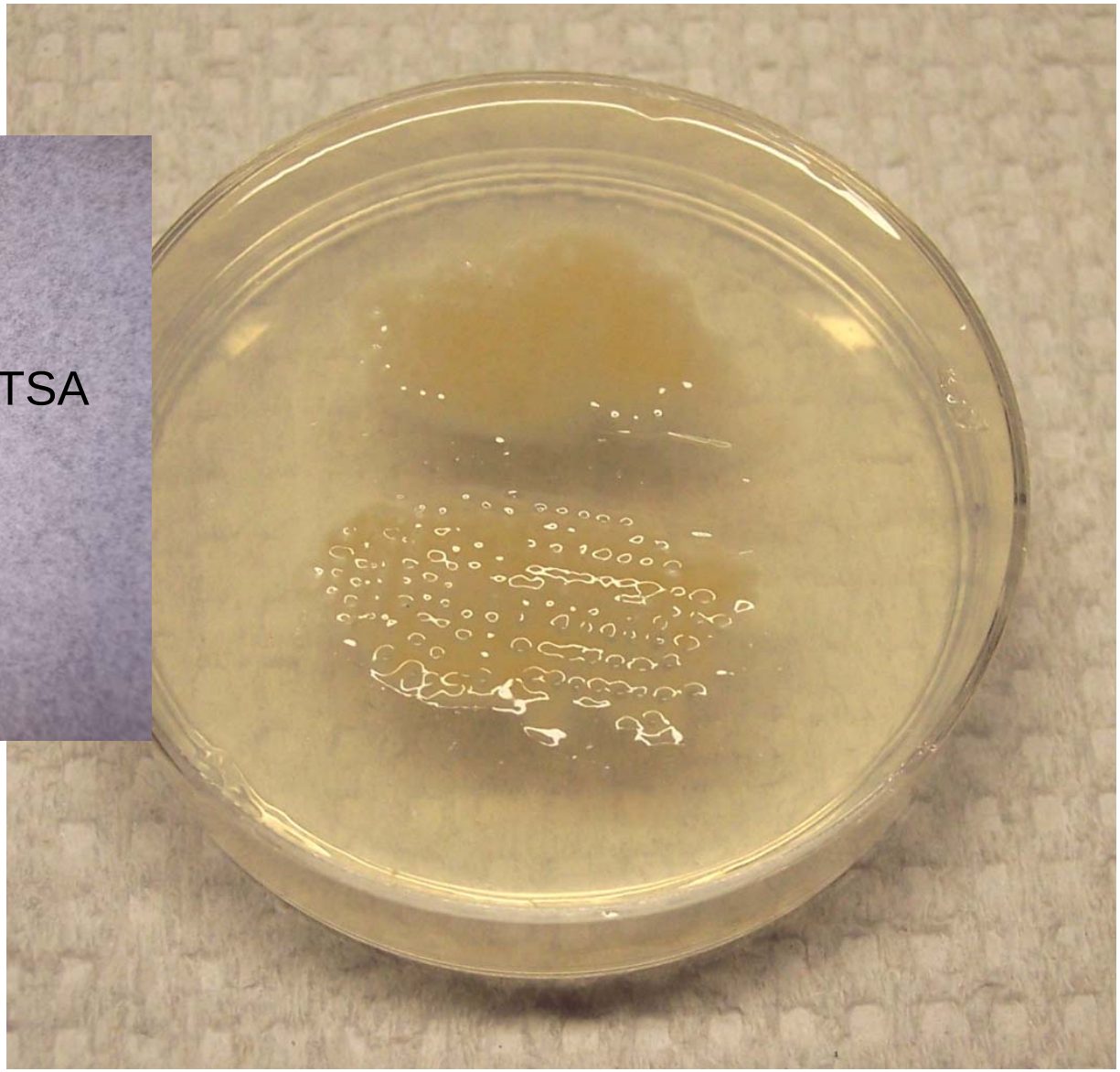
Motivation

- To characterize medically relevant bacteria using the LIBS technique.
  - To allow a clinical diagnosis.
  - To use as a research tool.

- To exploit the particular advantages of a LIBS-based diagnosis to “compete with” or “complement” other technologies.
  - PCR
  - FISH

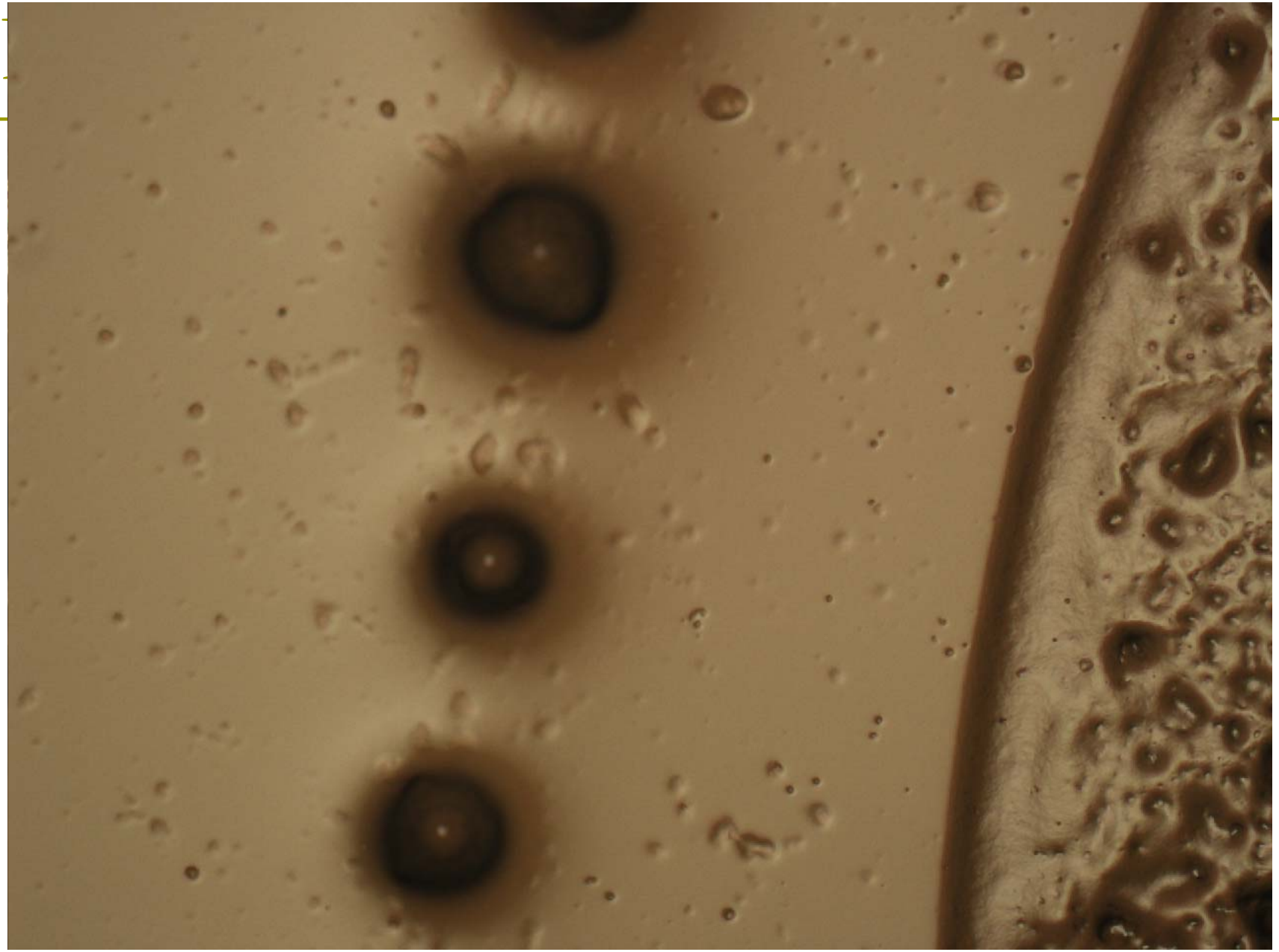
Ablated *E. coli* on Agar (a year ago)

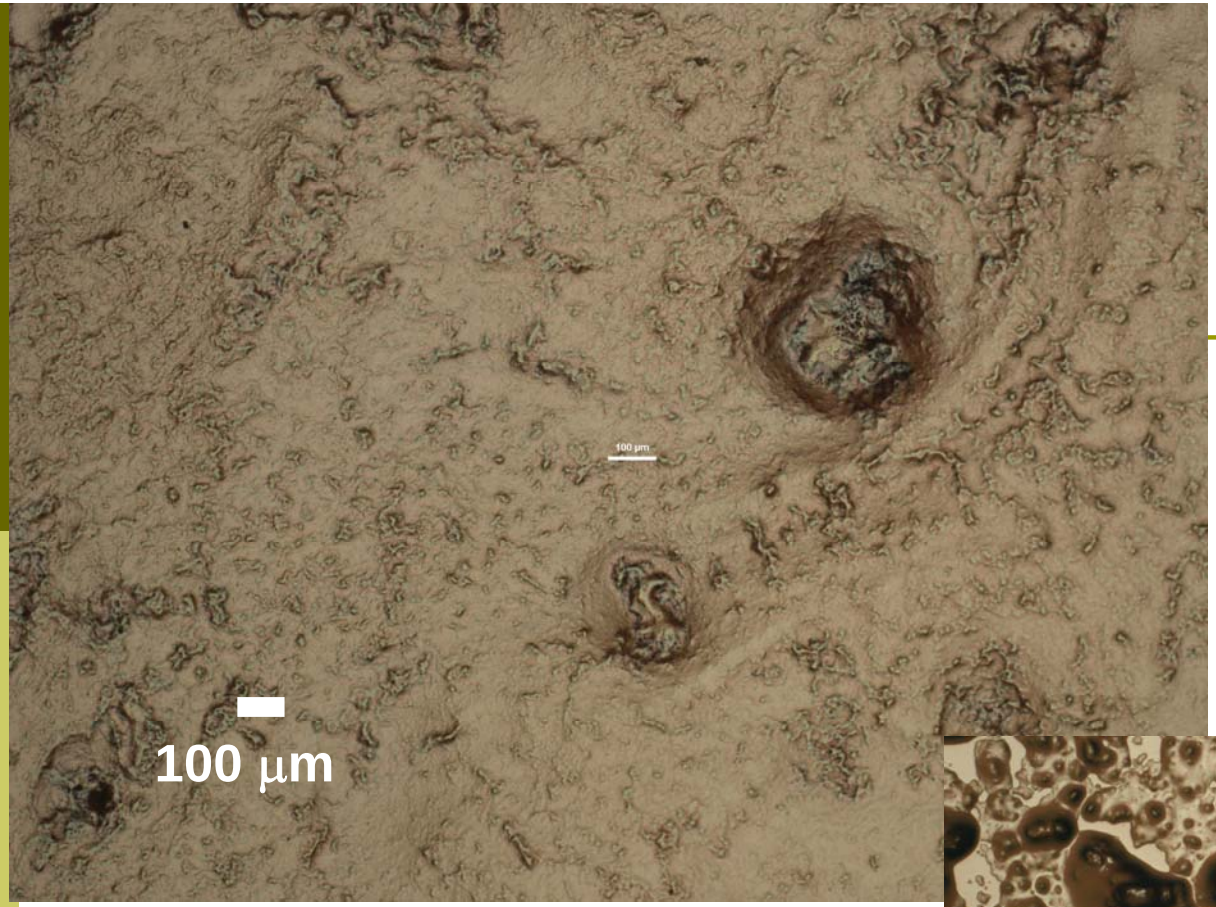




Now…

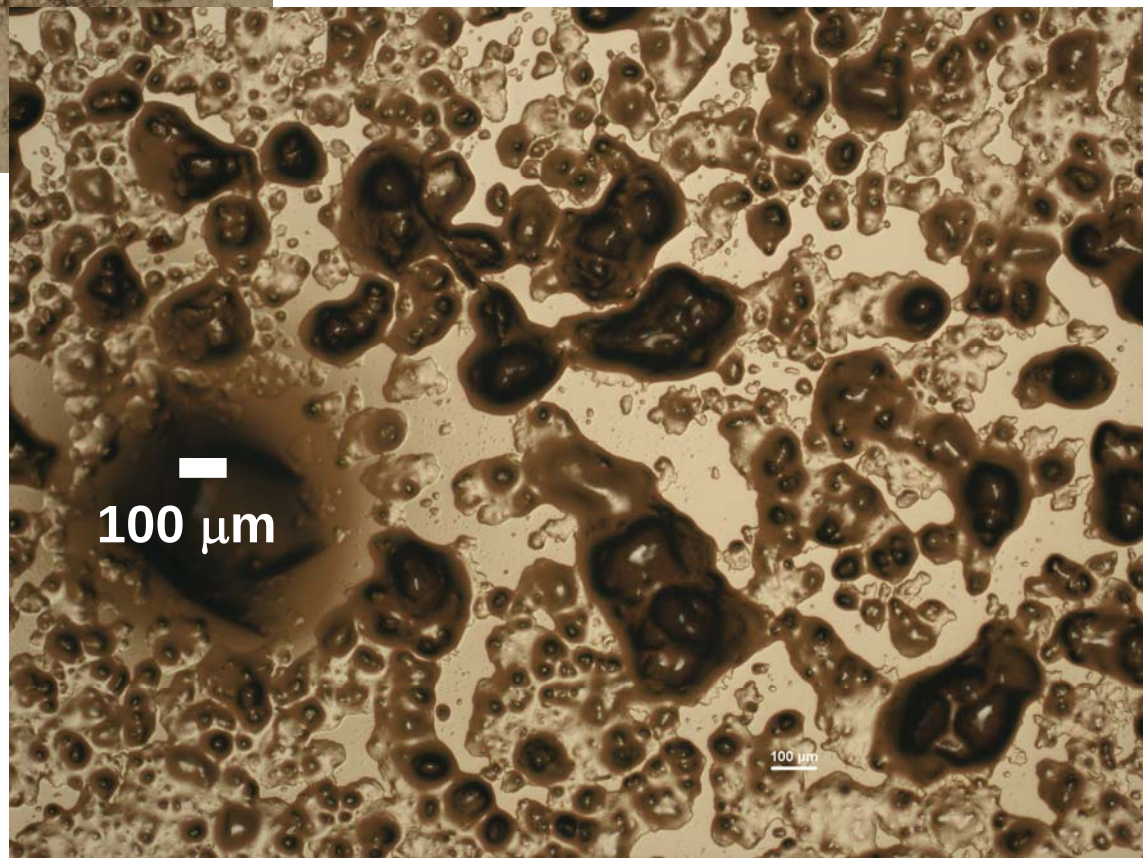

01/01/2004

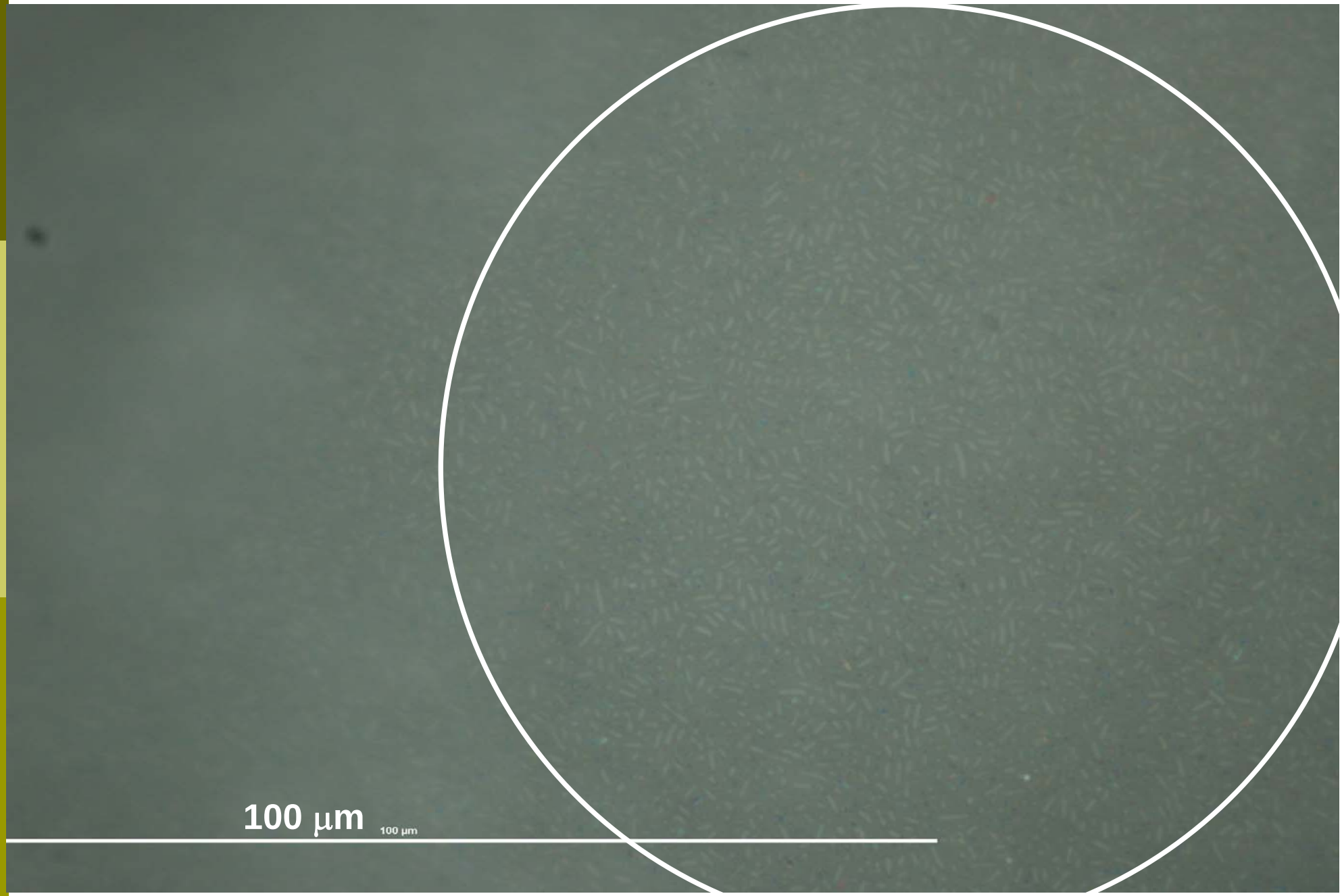


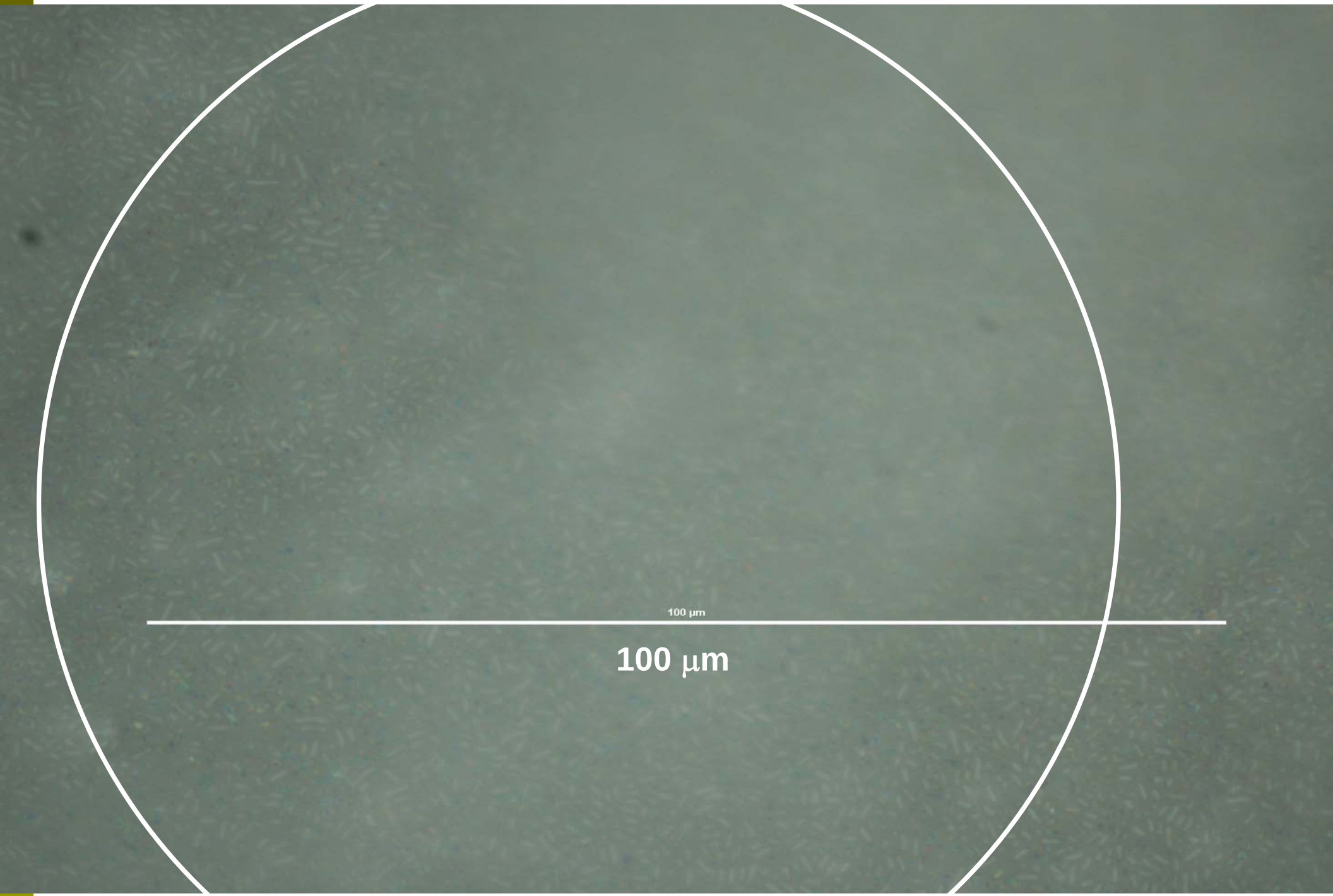


good mounting

bad mounting




100 µm
100 µm

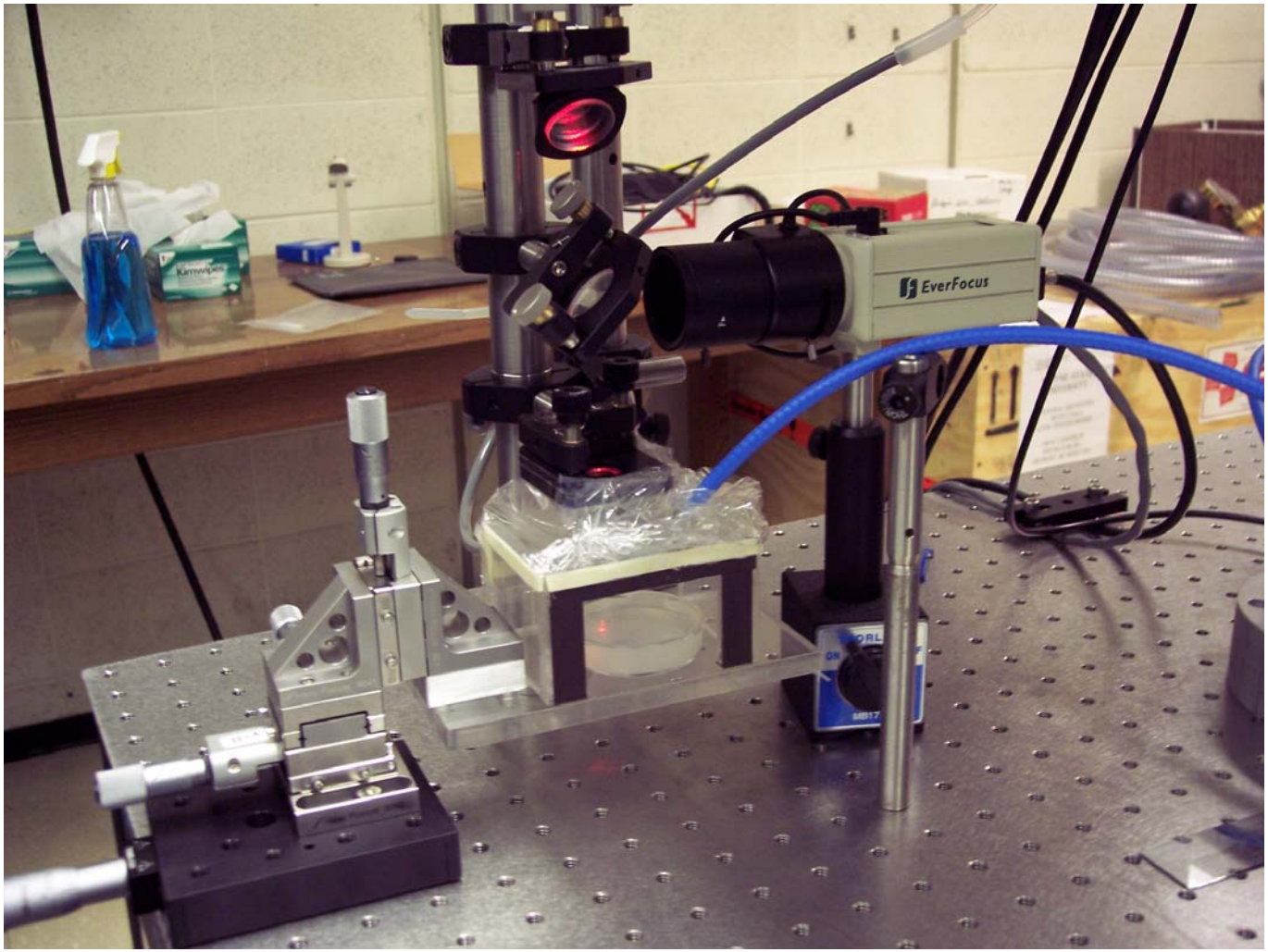

100 µm
100 µm

periscope / box

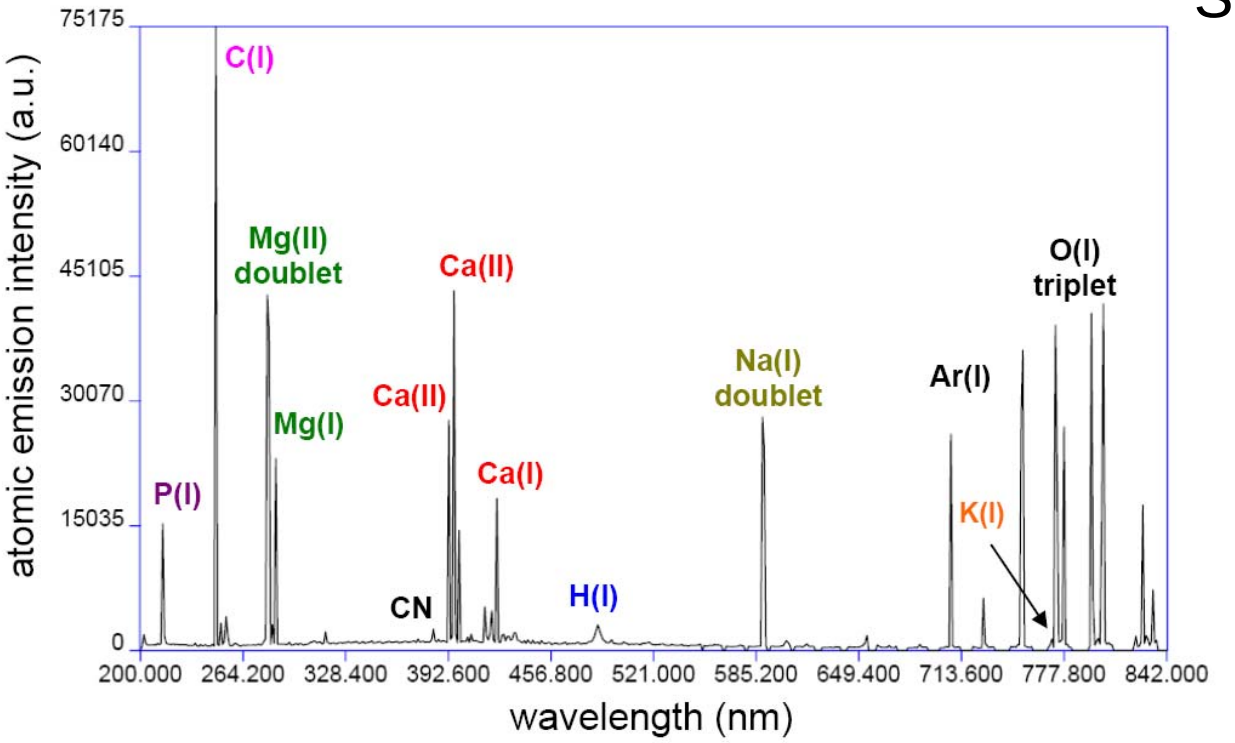
argon purge gas
(poster AP6)

single-pulse 1064 nm

fiber collect (UV)



Composition

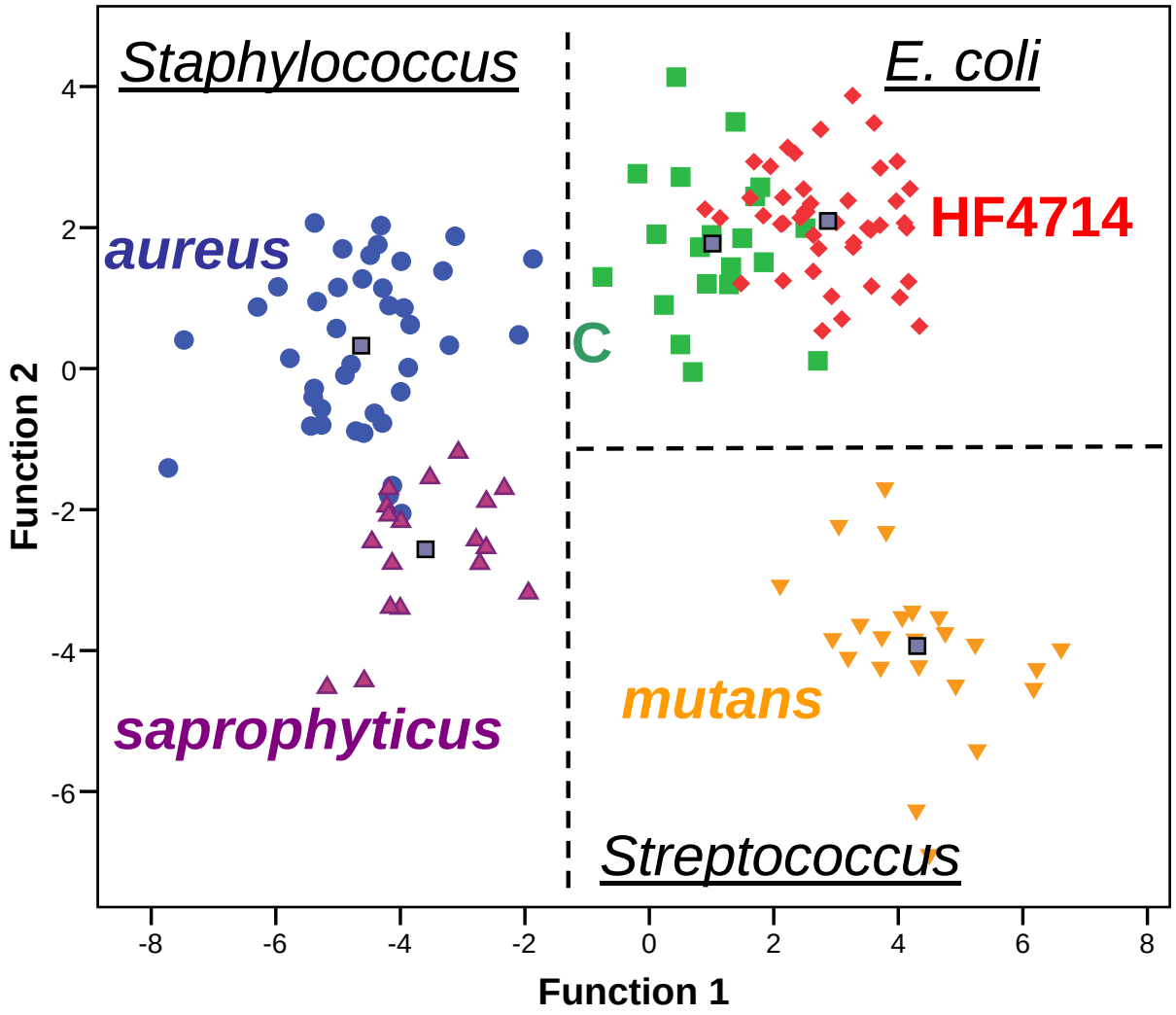


from "*The Bacteria: A Treatise on Structure and Function*" I.C. Gunsalus and R.Y. Stanier, eds

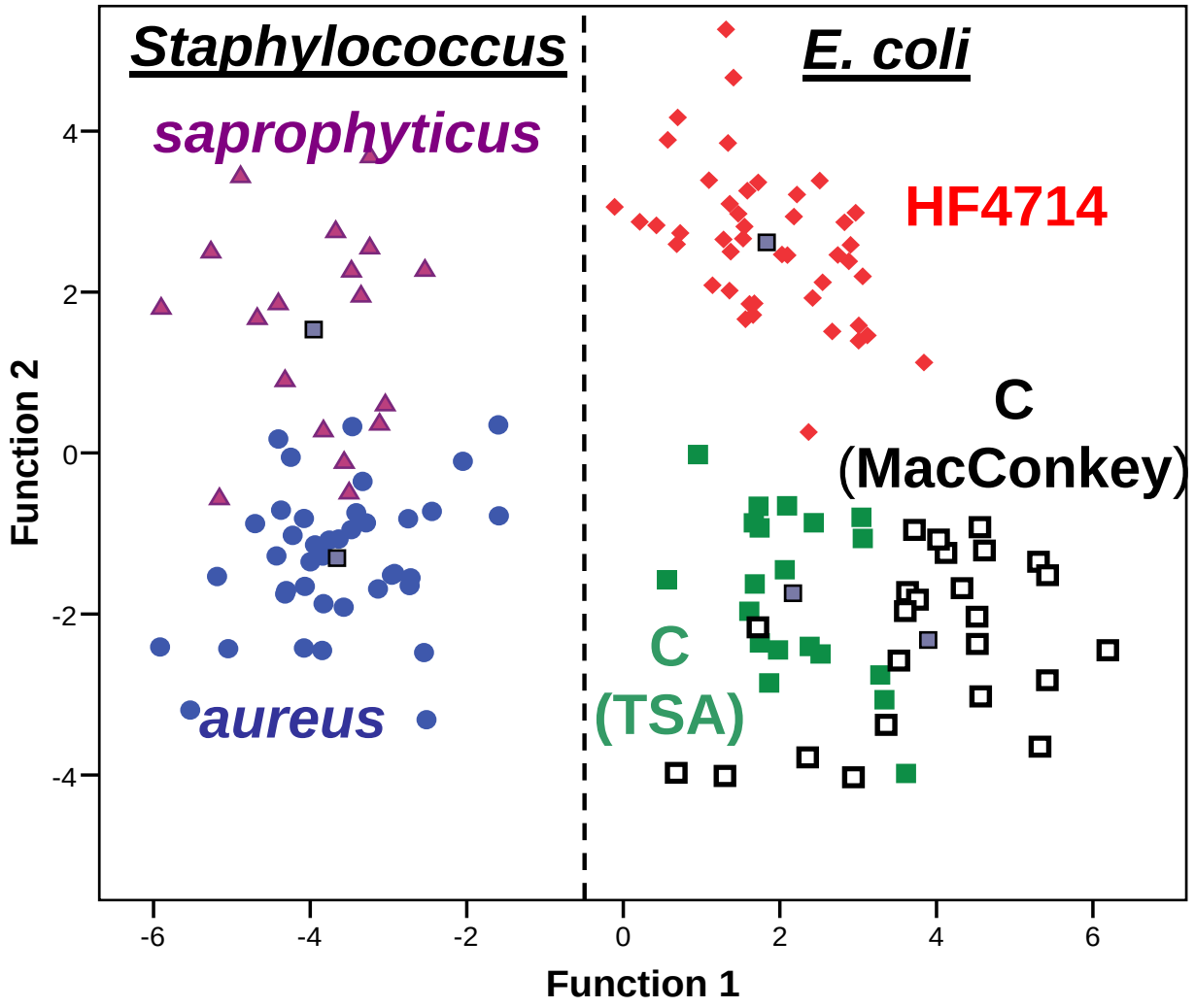
Element	% of fixed salt fraction
Sodium	2.6
Potassium	12.9
Calcium	9.1
Magnesium	5.9
Phosphorus	45.8
Sulfur	1.8
Iron	3.4

Gram-positive / Gram-negative

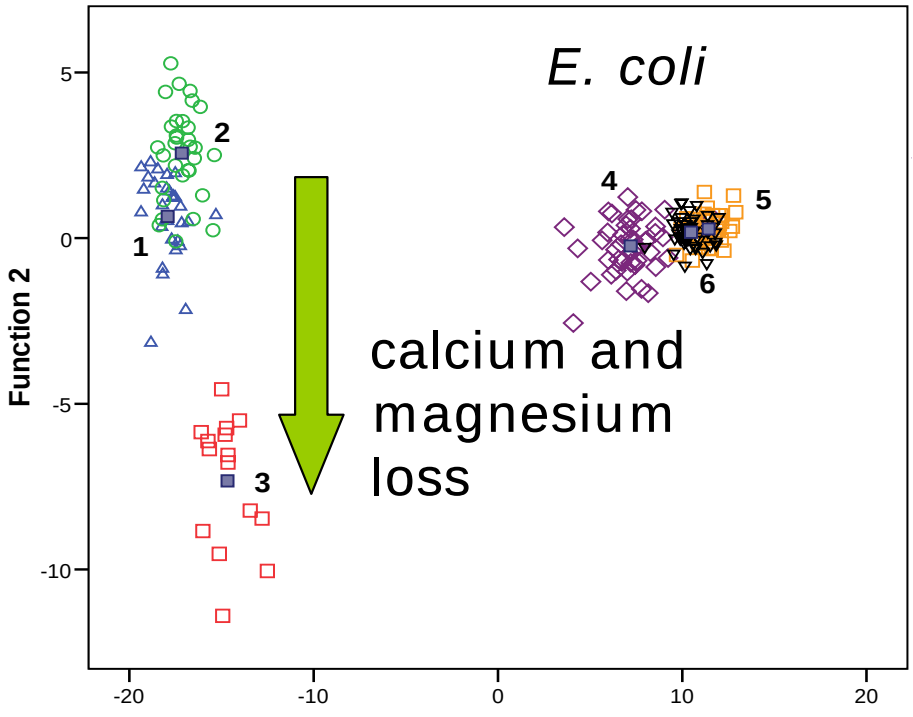
Intensity of 13 lines used in the DFA



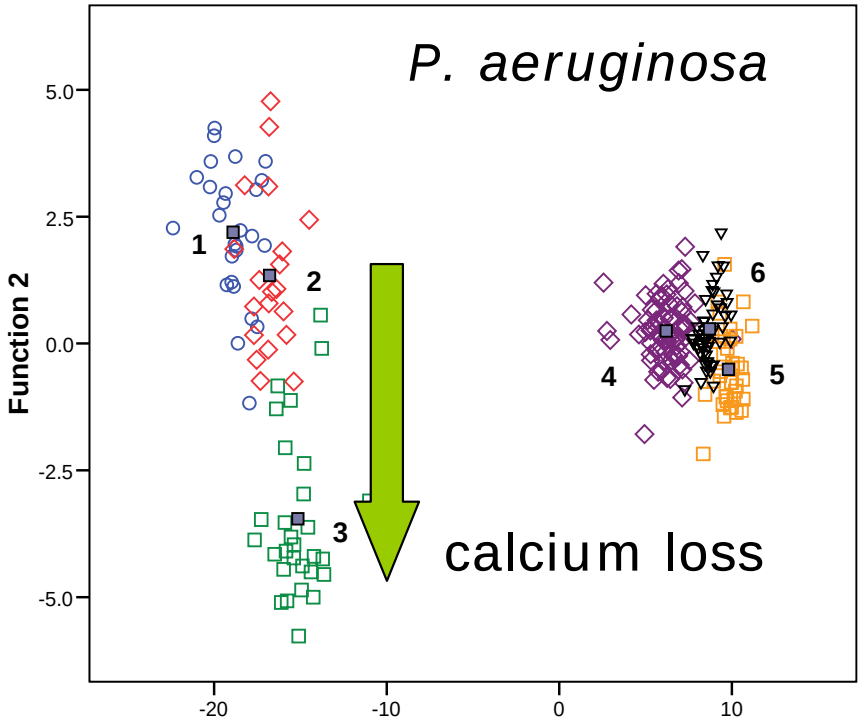
Intentional Membrane Alteration



Intentional Membrane Alteration

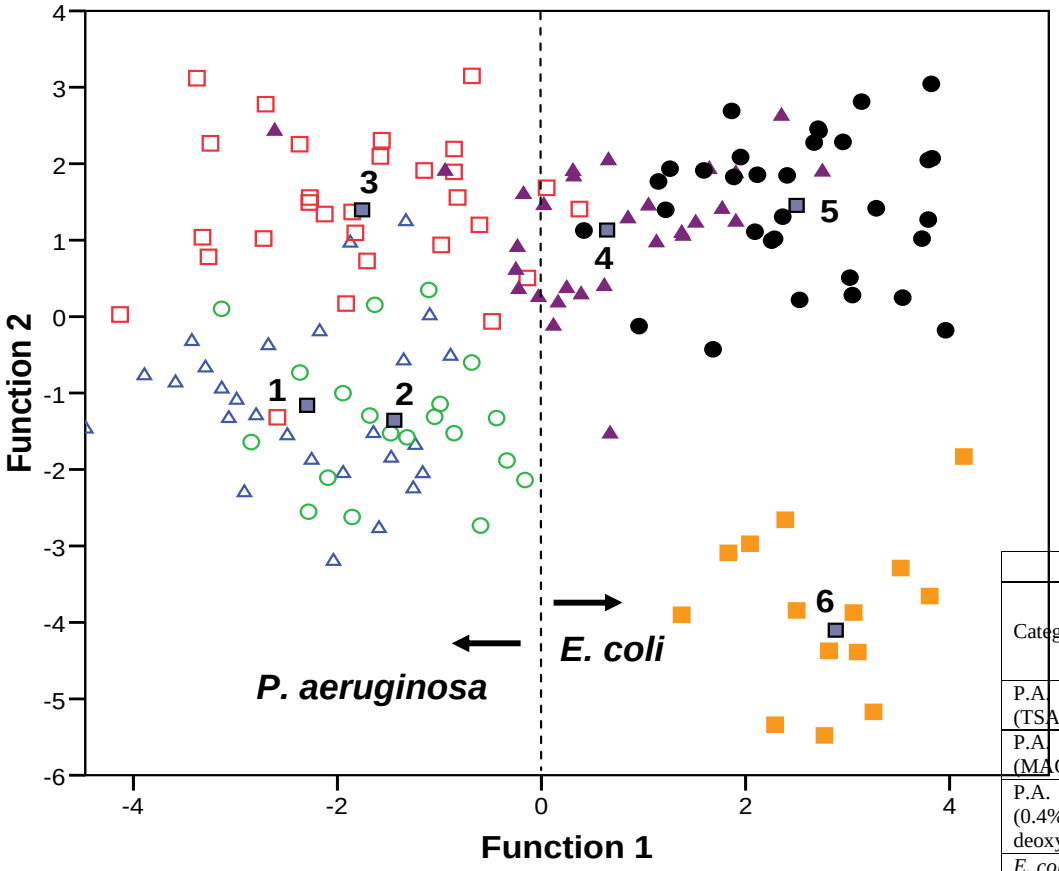


Category
1: E. coli (TSA)
2: E. coli (MAC)
3: E. coli (0.4% deoxy)
4: TSA
5: MAC
6: 0.4% deoxy
Group Centroid



Category
1: P.a. (TSA)
2: P.a. (MAC)
3: P.a. (0.4% deoxy)
4: TSA
5: MAC
6: 0.4% deoxy
Group Centroid

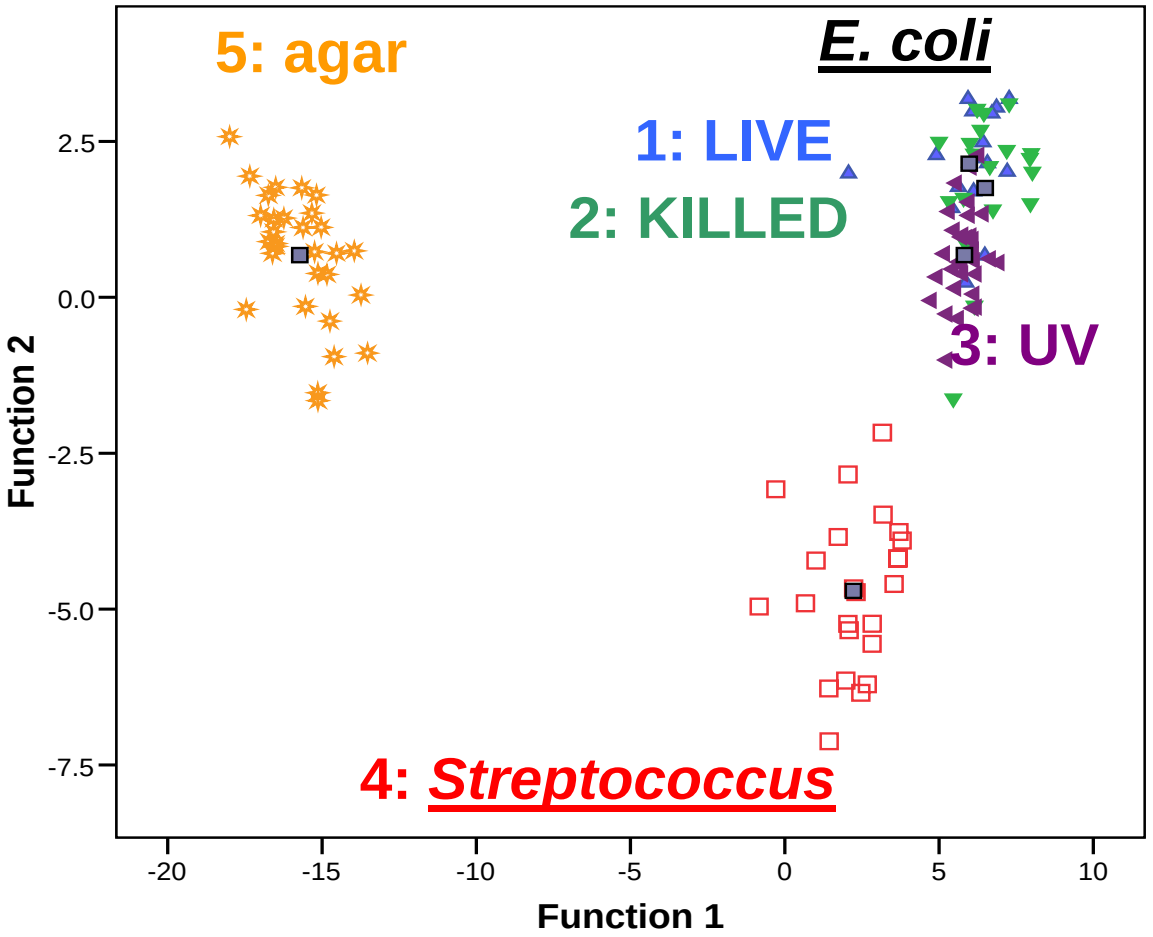
Membrane Disruption Does not Destroy Identification



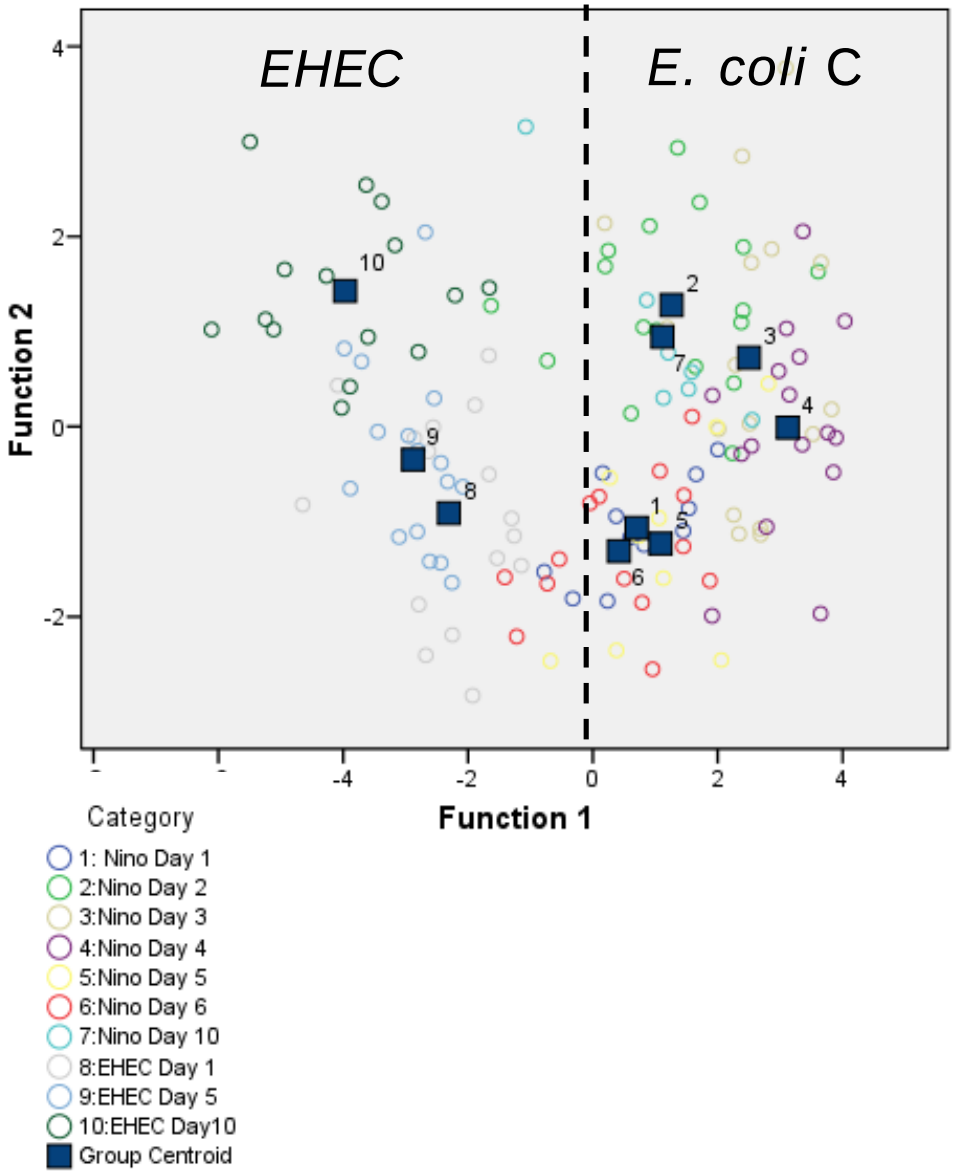
Category
- 1: P.a. (TSA)
- 2: P.a. (MAC)
- 3: P.a. (deoxy)
- 4: E. coli (TSA)
- 5: E. coli (MAC)
- 6: E. coli (deoxy)
- Group Centroid

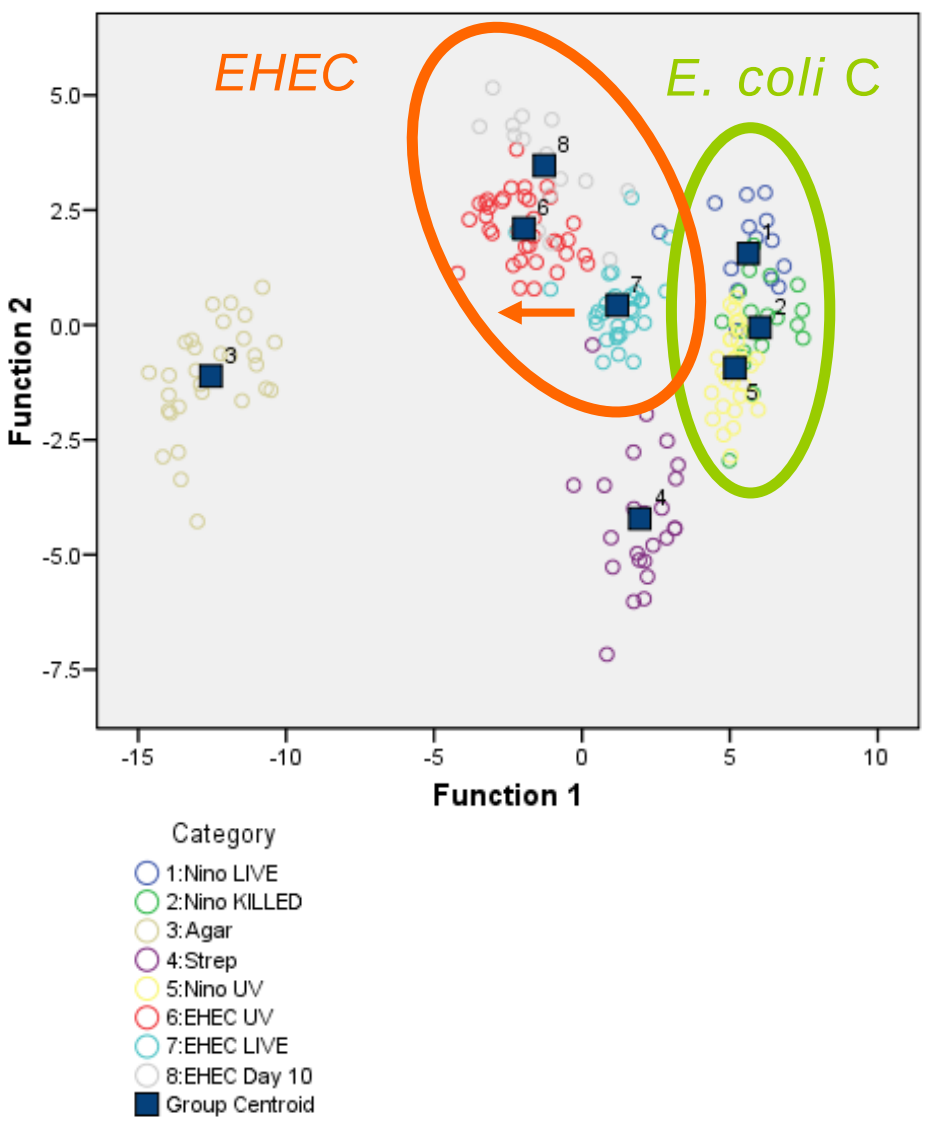
	Predicted Group Membership (%)							
Category	P.A. (TSA)	P.A. (MAC)	P.A. (0.4% deoxy)	*E. coli* Nino C (TSA)	*E. coli* Nino C (MAC)	*E. coli* Nino C (0.4% deoxy)	Correct Genus ID'd	Total
P.A. (TSA)	**70.4**	22.2	3.7	3.7	0.0	0.0	96.3	100.0
P.A. (MAC)	15.0	**65.0**	20.0	0.0	0.0	0.0	100.0	100.0
P.A. (0.4% deoxy)	3.4	3.4	**79.3**	13.8	0.0	0.0	86.2	100.0
E. coli Nino C (TSA)	0.0	6.9	6.9	**72.4**	10.3	3.4	86.2	100.0
E. coli Nino C (MAC)	6.3	0.0	0.0	3.1	**90.6**	0.0	93.8	100.0
E. coli Nino C (0.4% deoxy)	0.0	13.3	0.0	6.7	6.7	**73.3**	86.7	100.0

LIBS Strengths!
Live/killed/UV exposed



Starvation of Lysogenic/ Non-lysogenic *E. coli*





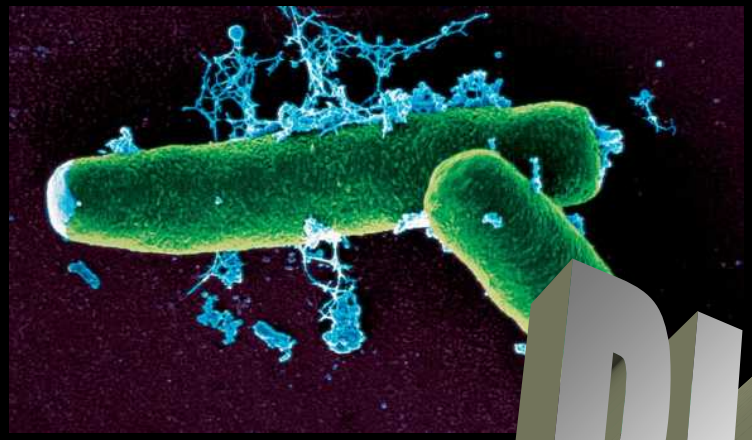
Conclusions

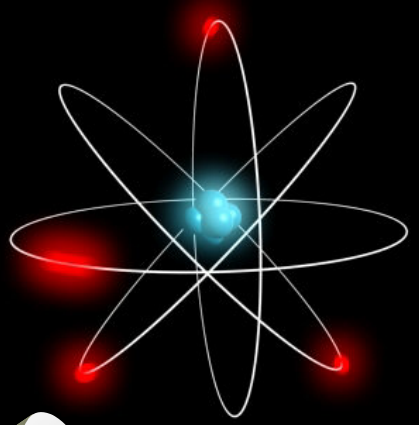
- Some of LIBS signal is definitely membrane related
- Membrane alteration (leading to lyses) is detectable
- Membrane alteration does not destroy identification
- Good discrimination amongst a variety of organisms
- LIBS has some real advantages:
  - Testing on killed specimens seems viable
  - Testing on “viable, but non-culturable” states seems viable

Thank you!

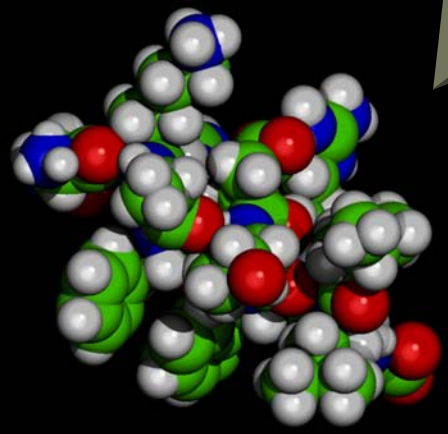
The BIOMAS Project at Wayne State:

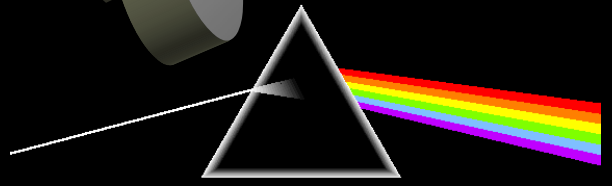
Bacteria Identification by Optical, Molecular, and Atomic Spectroscopy





BIOMAS





Thank you!