The BIOMAS Project: Bacteria Identification by Optical, Molecular, and Atomic Spectroscopy

University of Western Ontario

Feb. 21st, 2008

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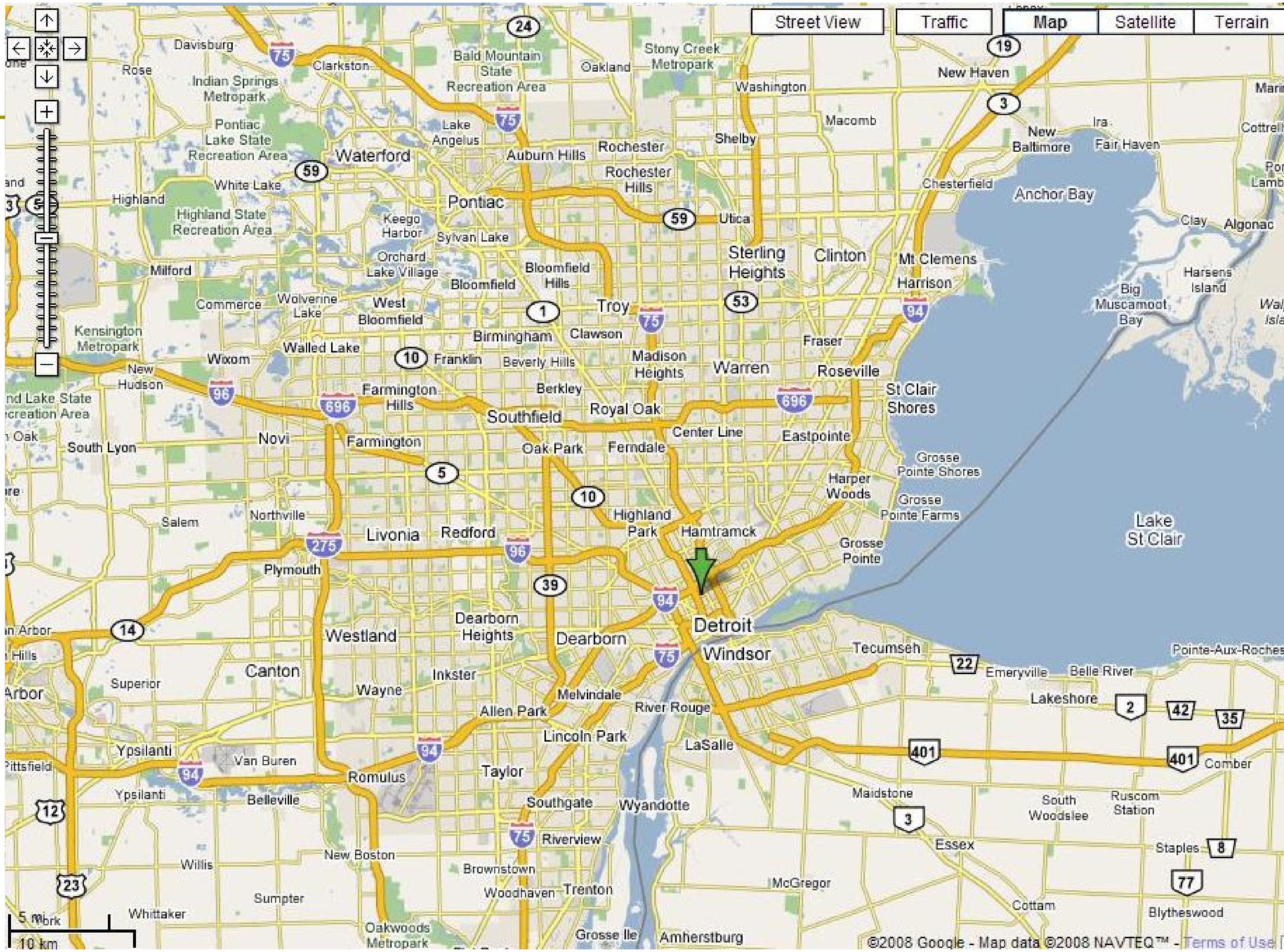
Street View
Traffic
Map
Satellite
Terrain
Davisburg
Rose
Clarkston
Indian Springs Metropark
Pontiac Lake State Recreation Area
Waterford
Bald Mountain State Recreation Area
Lake Angelus
Auburn Hills
Oakland
Stony Creek Metropark
Washington
Rochester
Rochester Hills
Shelby
Macomb
New Haven
New Baltimore
Ira
Fair Haven
Marine
Cottrellville
Port Lambton
Chesterfield
Anchor Bay
Clay
Algonac
White Lake
Highland
Highland State Recreation Area
Keego Harbor
Pontiac
Sylvan Lake
Utica
Sterling Heights
Clinton
Mt Clemens
Harrison
Harsens Island
Big Muscamoot Bay
Orchard Lake Village
Bloomfield
Bloomfield Hills
Milford
Commerce
Wolverine Lake
West Bloomfield
Troy
Kensington Metropark
Walled Lake
Birmingham
Clawson
Fraser
Wixom
Franklin
Beverly Hills
Madison Heights
Warren
Roseville
New Hudson
Farmington Hills
Berkley
Royal Oak
St Clair Shores
Southfield
Center Line
Eastpointe
Novi
South Lyon
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Oak Park
Ferndale
Grosse Pointe Shores
Harper Woods
Grosse Pointe Farms
Salem
Northville
Highland Park
Hamtramck
Livonia
Redford
Grosse Pointe
Lake St Clair
Plymouth
Dearborn Heights
Westland
Dearborn
Detroit
Windsor
Tecumseh
Pointe-Aux-Roches
Canton
Superior
Inkster
Wayne
Emeryville
Belle River
Melvindale
River Rouge
Lakeshore
Allen Park
Lincoln Park
Ypsilanti
LaSalle
Van Buren
Romulus
Taylor
Comber
Belleville
Southgate
Wyandotte
Maidstone
South Woodslee
Ruscom Station
Riverview
Essex
Staples
New Boston
Willis
Brownstown
Woodhaven
Trenton
McGregor
Sumpter
Whittaker
Cottam
Blytheswood
Oakwoods Metropark
Grosse Ile
Amherstburg
5 mi
10 km
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Our Department

- 29 faculty
- 53 grad students
- 30 undergrad students

My work:

Experimental atomic physics

- laser-induced breakdown spectroscopy (LIBS)
- laboratory astrophysics (continuation of work done at UWO with Holt/Rosner)

Outline

1. Why physics and bacteria?
2. What is LIBS? Why is it useful?
3. What have we done with it so far?

Bacteria in the news…

- Contaminated food
  - September 2006, *Escherichia coli* (*E. coli* strain O157:H7) bacteria found in uncooked spinach in 26 U.S. states.
  - By October 06, 2006, 199 people had been infected, including three people who died and 31 who suffered a type of kidney failure called hemolytic uremic syndrome.

- Contaminated water
  - 2000, the fresh drinking water supply of Walkerton, Ontario, is contaminated with this same highly dangerous strain of *E. coli* O157:H7, from farm runoff into an adjacent well.
  - Starting May 15, 2000, many residents of the town of about 5,000 began to simultaneously experience bloody diarrhea and other symptoms of *E. coli* infection.
  - As a result of this contamination and the subsequent lag in positive pathogen detection, seven people died and about 2,500 (more than 40% of the population at the time) became ill.

- Bioterrorism
  - Late September and early-October of 2001, two separate waves of bioterrorism attacks were conducted in the United States. Spore forms of the lethal bacterium *Bacillus anthracis* were mailed to U.S. news organizations and offices in the U.S. Congress, killing five people and infecting 17 others.

MP-LIBS A full laboratory High-Resolution Broadband LIBS system in a portable backpack.

What we need is a widget…

Other technologies…

- Evanescent-wave fiber-optic biosensor

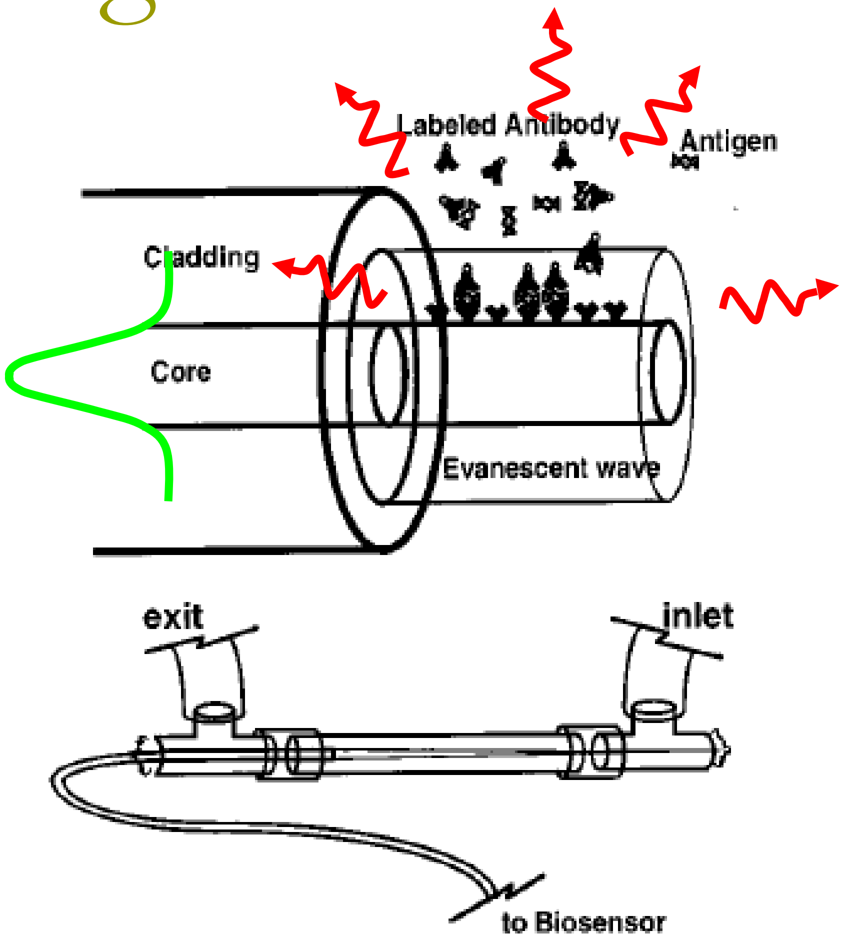
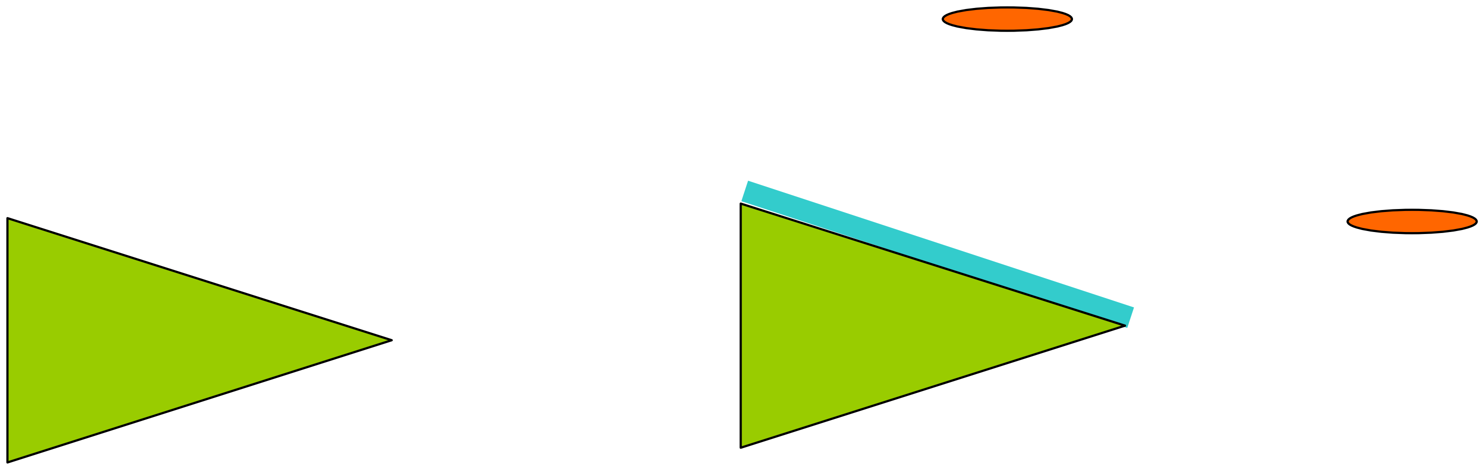


FIG. 1. Schematic representations of the sandwich fluoroimmunoassay and the fiber probe assay chamber.

Detection of *Yersinia pestis* Fraction 1 Antigen with a Fiber Optic Biosensor, JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1995, p. 336–341 Vol. 33, No. 2

Other technologies…

- MEMS cantilever resonance



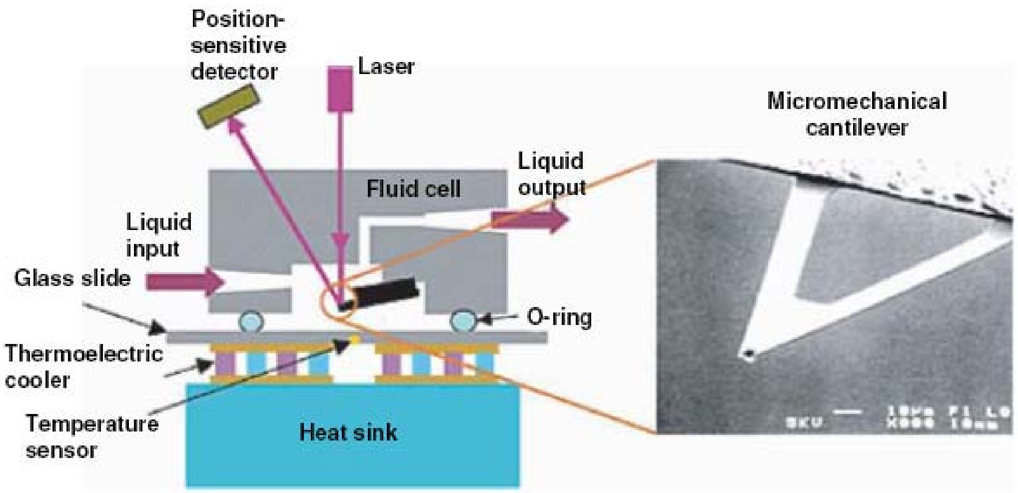


FIGURE 2.2. Schematic diagram of the experimental setup used by Wu *et al.* [35]. A cantilever was mounted in a fluid cell which allows liquid exchange through I/O ports. A laser was reflected off the cantilever and focused onto a PSD.

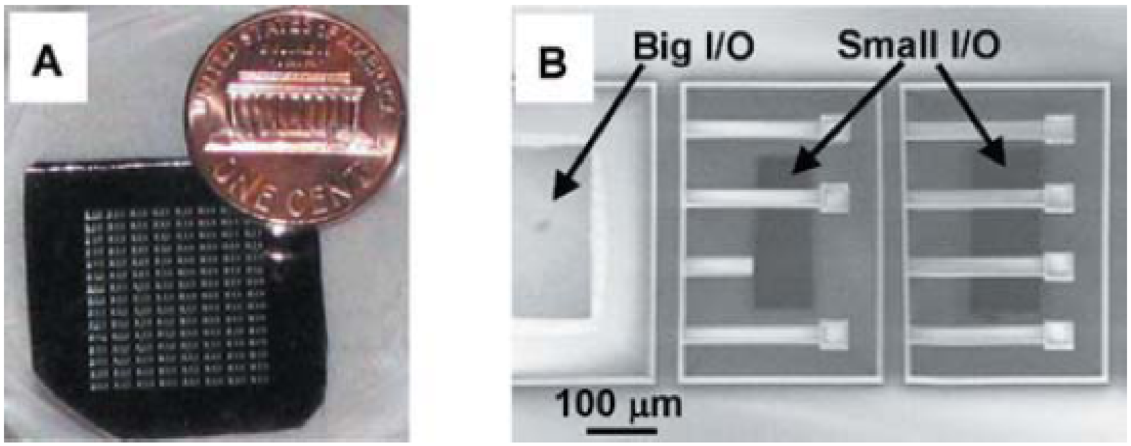
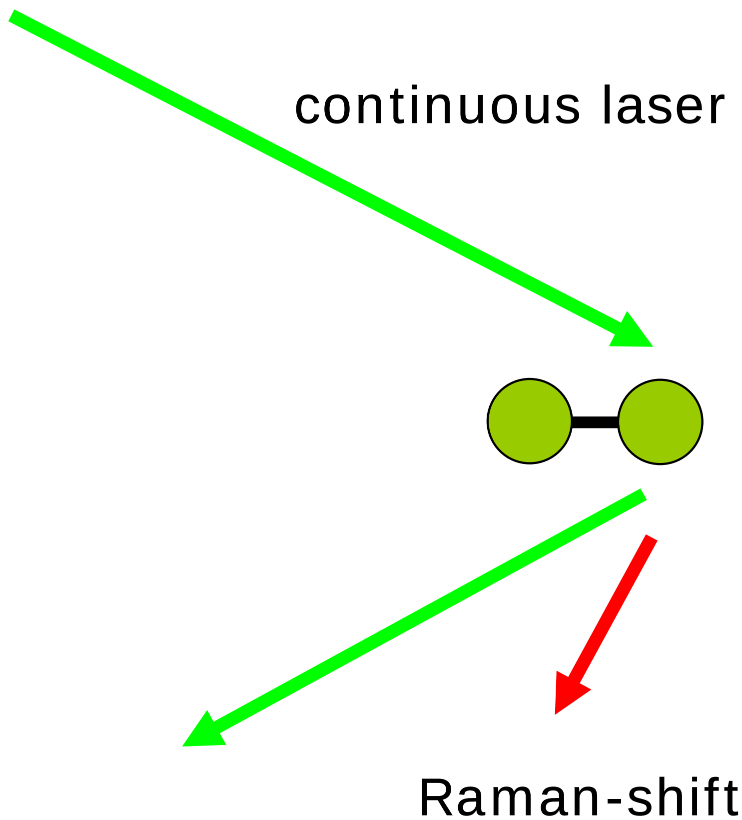


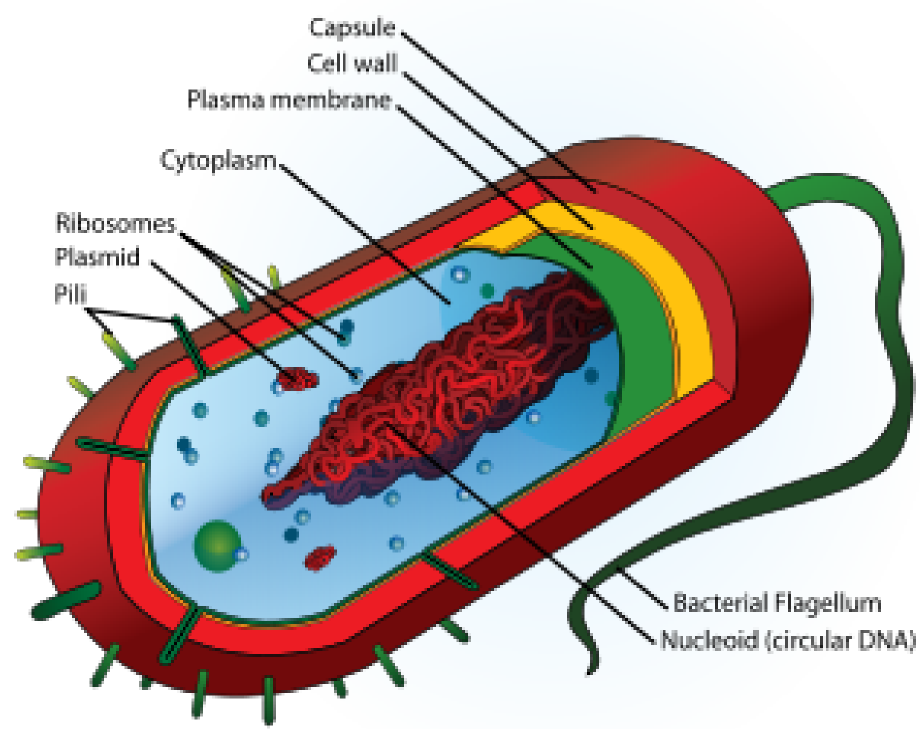
FIGURE 2.7. (A) A cantilever array chip containing a 2-D array of reaction wells, each well containing multiple cantilevers. The array is roughly the size of a penny; (B) Electron micrograph of a single reaction well showing 7 cantilever beams, a big inlet/outlet (I/O) port and two small I/O ports.

From: BioMEMS and Biomedical Nanotechnology, Volume IV: Biomolecular Sensing, Processing and Analysis "Cantilever Arrays: A Universal Platform for Multiplexed Label-Free Bioassays"

Other technologies…

- Raman

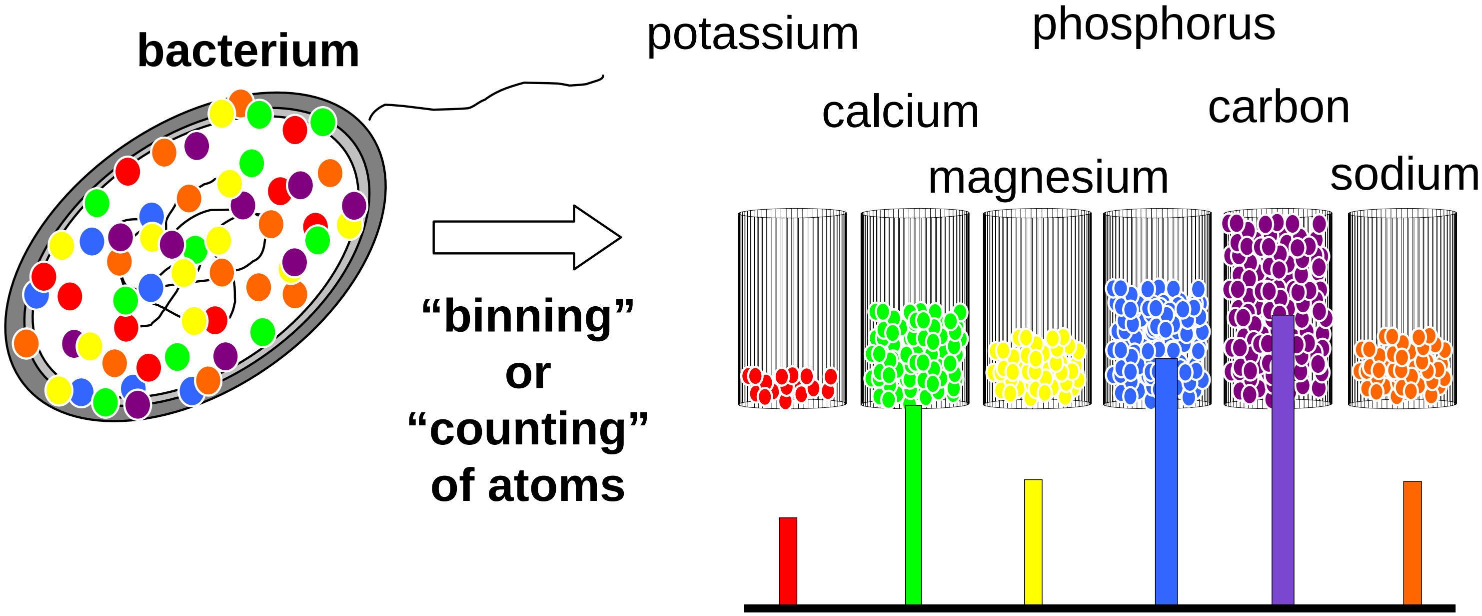




Gold standard

- PCR
  - polymerase chain reaction
  - takes times
  - requires amplification
  - laboratory technique

Our Idea…(not my idea)

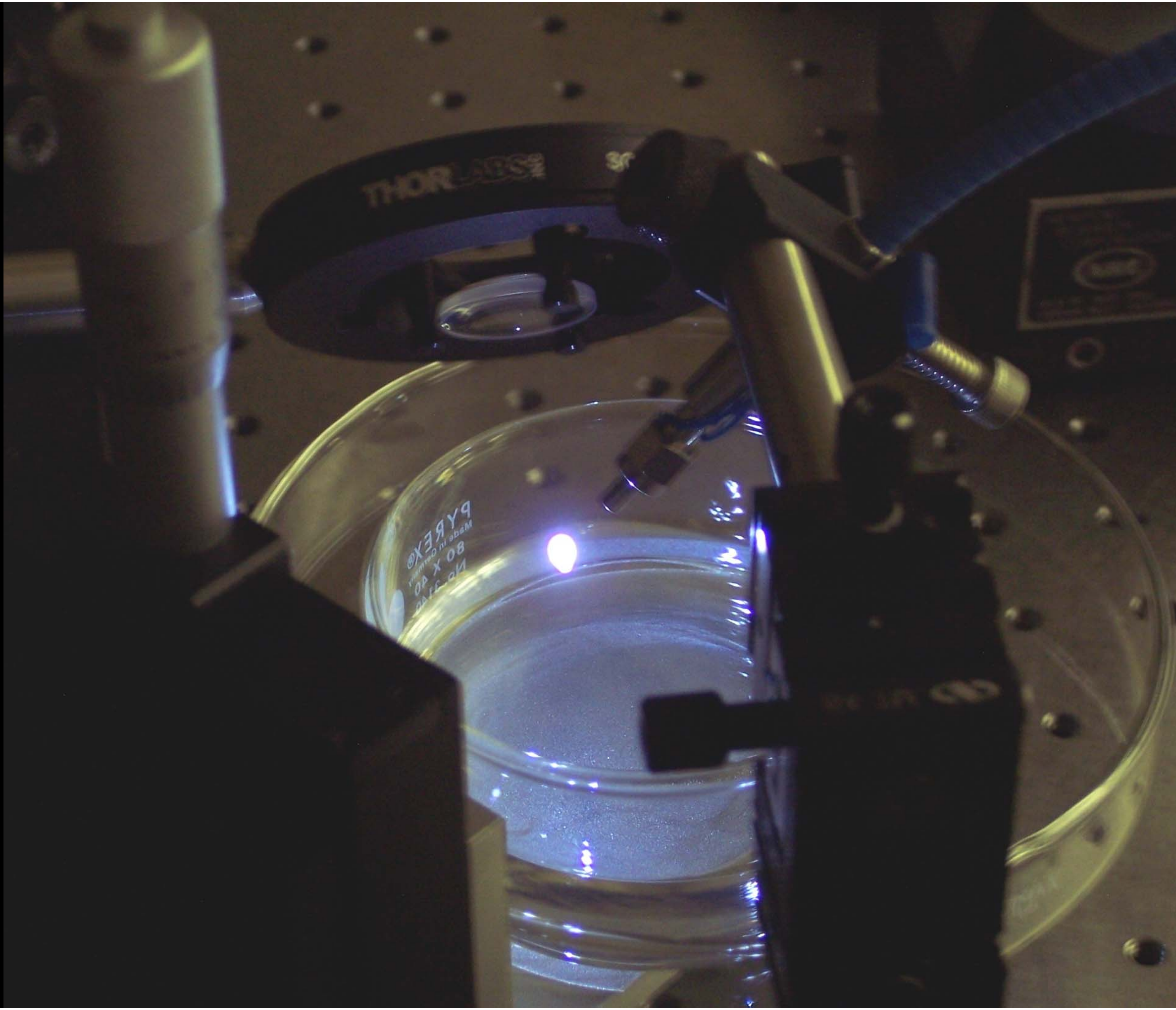


- A "spectral fingerprint" is created by determining the elemental composition of the bacterium and measuring the quantity of that element.
- Trace elements present at the ppm level in the bacterium are measured in this technique. The unique ratios of the quantities allow bacterial identification.

LIBS Defined

One sentence?

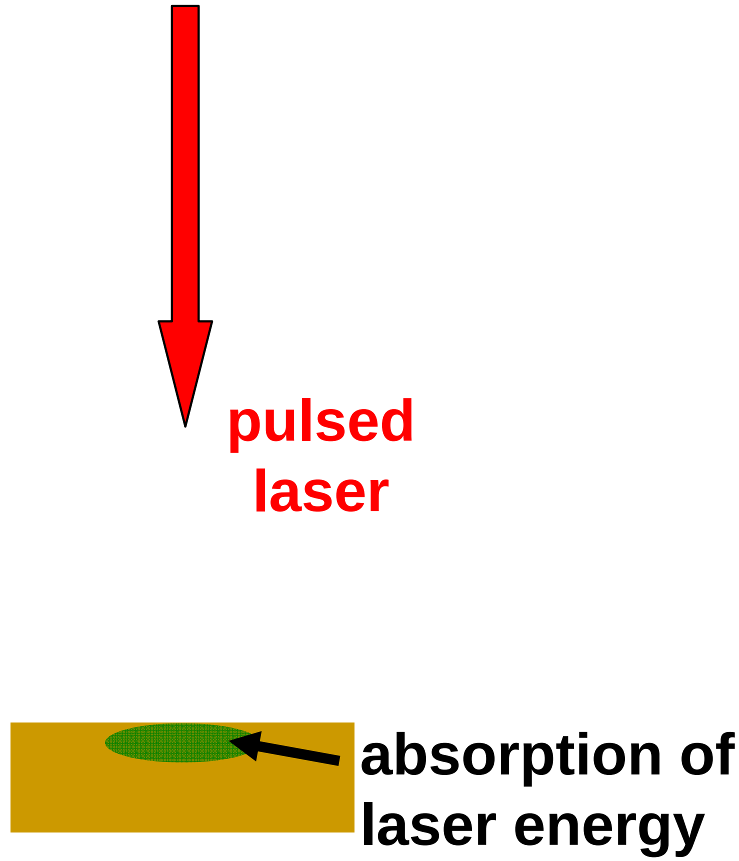
A spectrochemical technique which utilizes an intense laser pulse to determine the atomic/elemental composition of a sample via generation of a high-temperature micro-plasma followed by time-resolved optical spectroscopy.


THORLABS

The LIBS Process

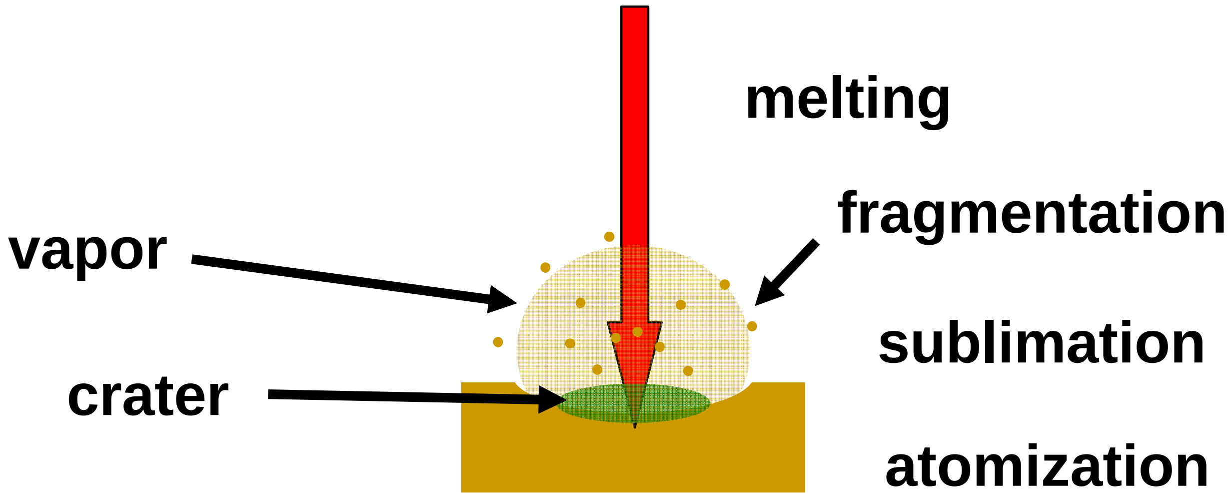
1. laser interaction with the target
2. removal of samples mass (ablation)
3. plasma formation (breakdown)
4. element specific emission

1) laser interaction with the target



- initiated by absorption of energy by the target from a pulsed radiation field.
- pulse durations are on the order of nanoseconds, but LIBS has been performed with pico- and femto-second laser pulses.

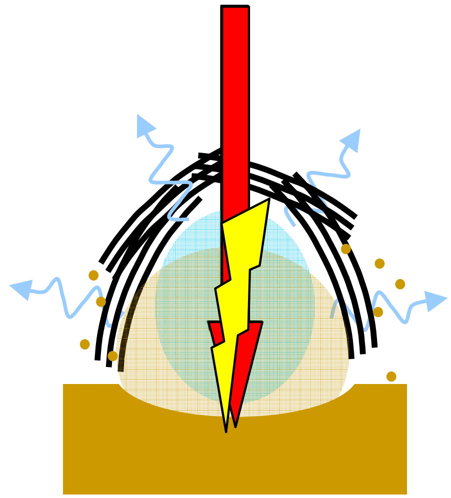
2) removal of samples mass (ablation)



- absorbed energy is rapidly converted into heating, resulting in vaporization of the sample (ablation) when the temperature reaches the boiling point of the material.

- removal of particulate matter from the surface leads to the formation of a vapor above the surface.

3) plasma formation (breakdown)



absorption of the laser radiation by the vapor

continuum emission

electrical breakdown and plasma formation

shock wave

bremsstrahlung

- The laser pulse continues to illuminate the vapor plume.
- The vapor condenses into sub-micrometer droplets that lead to absorption and scattering of the laser beam, inducing strong heating, ionization, and plasma formation.

Breakdown

"breakdown" is arbitrarily defined

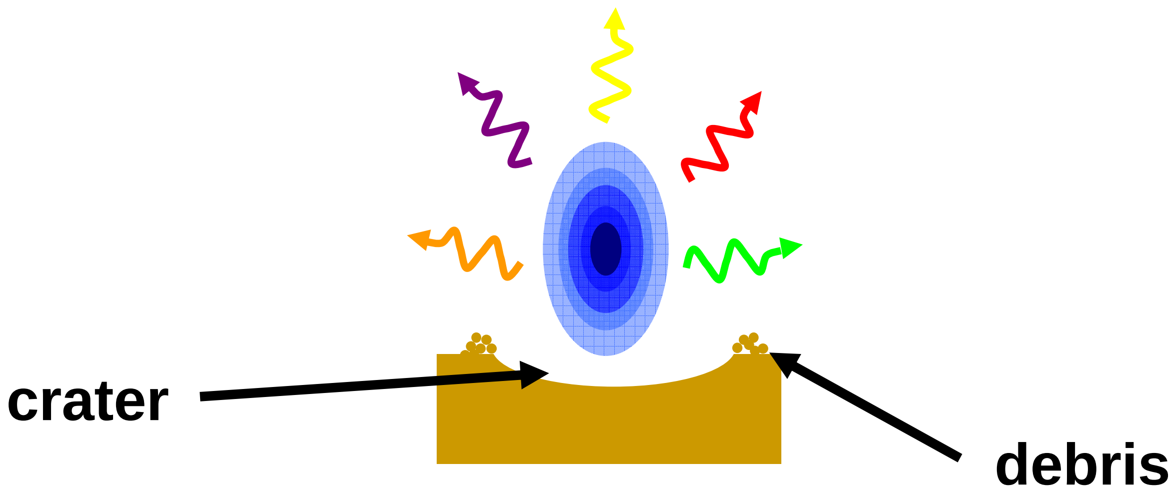
$n_e \sim 10^{13}$ cm^{-3} or degree of ionization of 10^{-3}

permits significant absorption and scattering of incident laser beam leads very fast to a fully developed plasma and shockwave

$$10^{13}\ \text{cm}^{-3} \rightarrow 10^{17}\text{-}10^{20}\ \text{cm}^{-3}$$

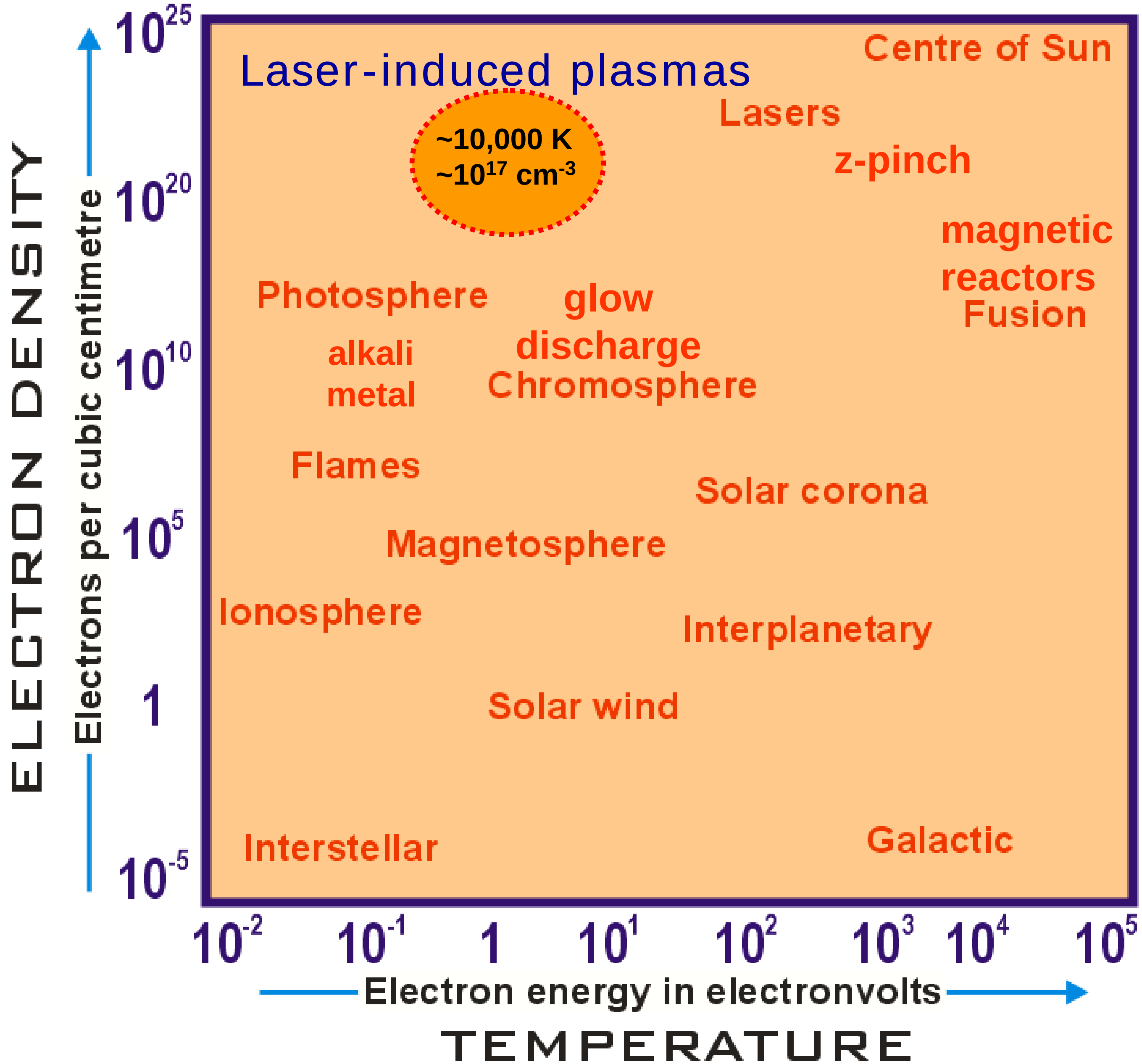
4) element specific emission (atomic or ionic)

spontaneous emission as atoms/ions decay to ground state

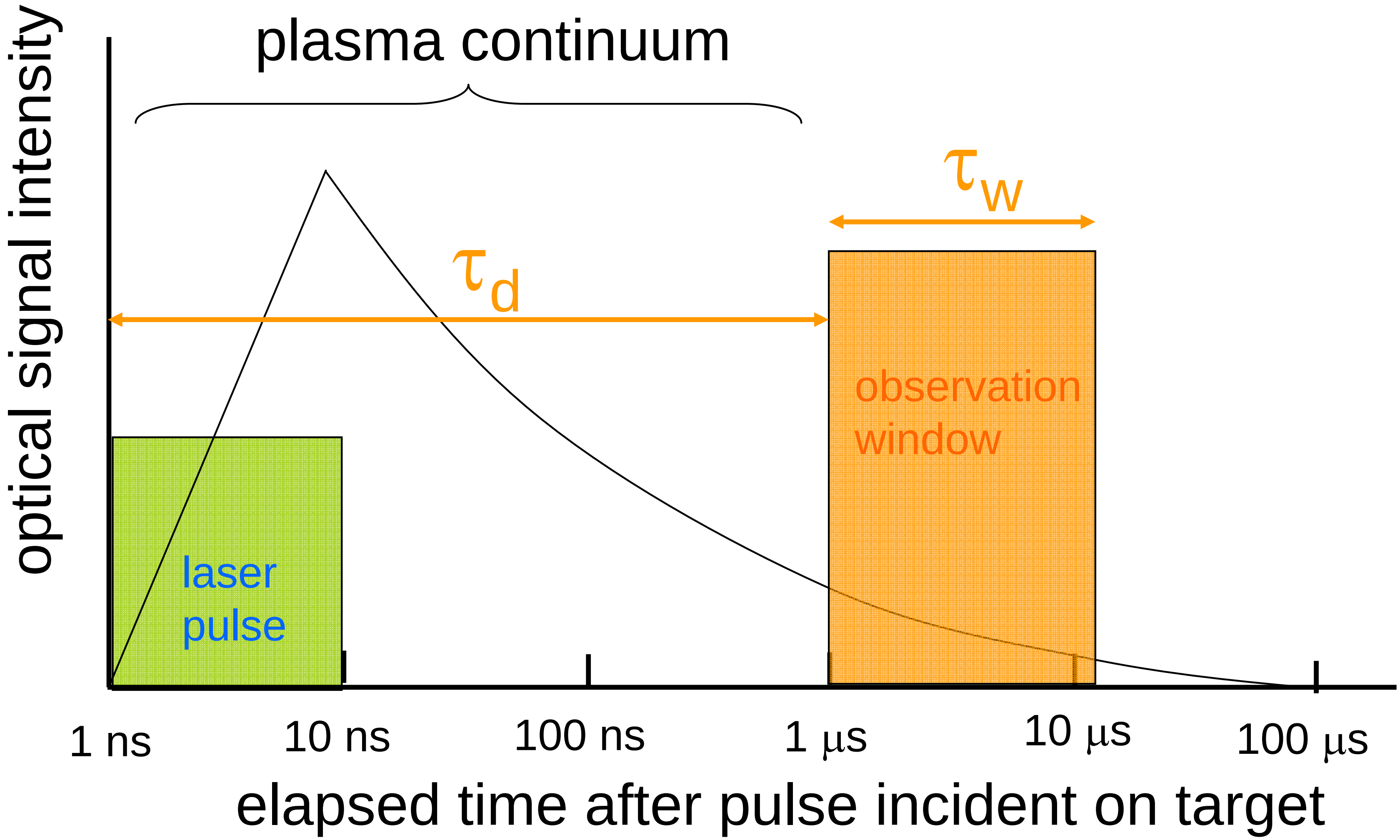


- The dynamical evolution of the plasma plume is then characterized by a fast expansion and subsequent cooling.
- Approximately 1 microsecond after the ablation pulse, spectroscopically narrow atomic/ionic emissions may be identified in the spectrum.

RANGES OF PLASMAS



Temporal History of a LIBS Plasma



Advantages of LIBS

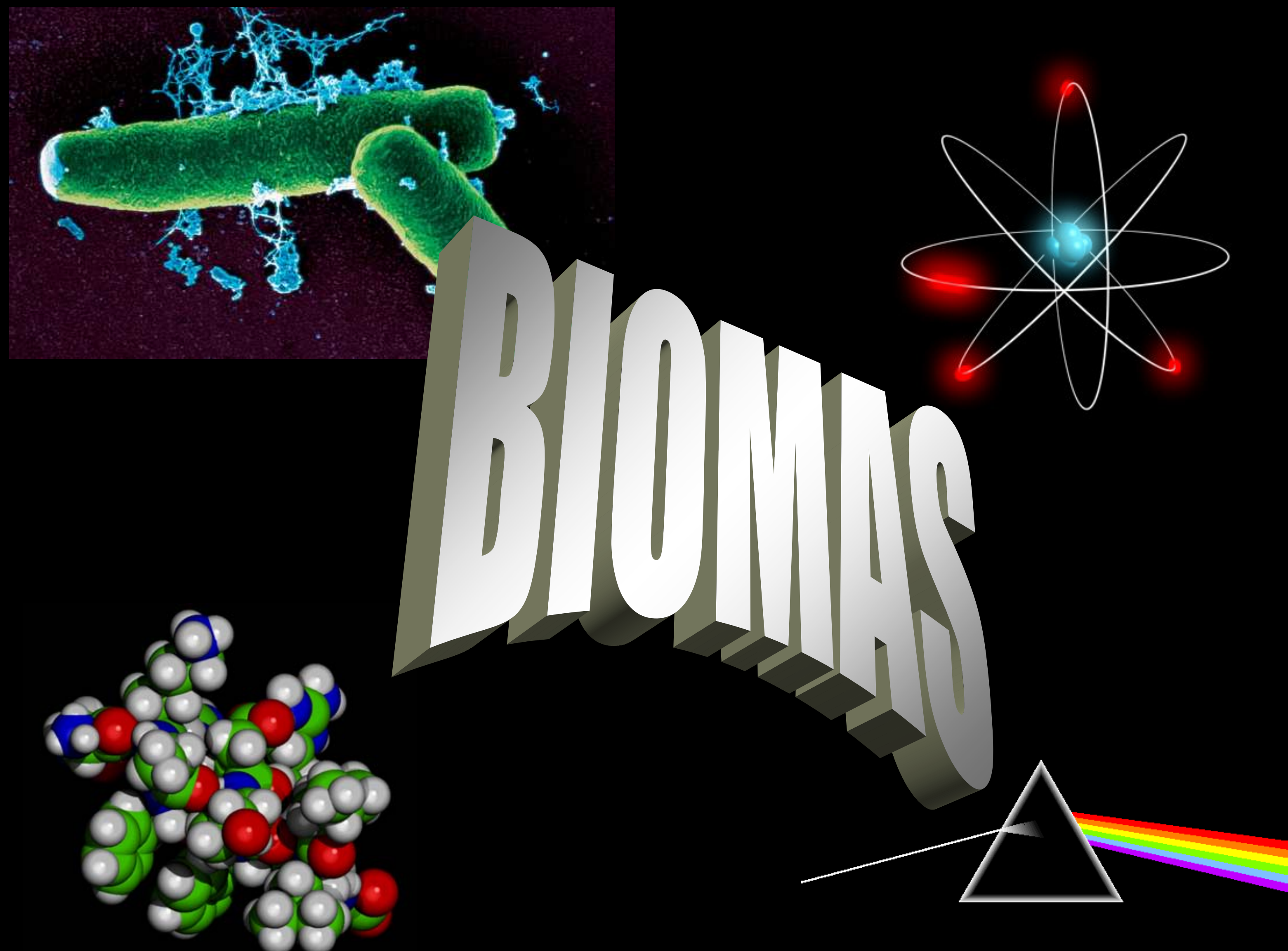
1) extremely fast analysis compared to competing technologies
2) multi-elemental analysis, light from all constituents collected without bias
3) analysis can be performed at standoff distances
4) technique is applicable to all substrates (gas, solid, and liquid)
5) requires minimal or no sample prep
6) exquisite spatial resolution, ~1 μm

The Goal of LIBS Plasma Creation

- to create an optically thin plasma which is in thermodynamic equilibrium and whose elemental composition is the same as that of the sample
  - if achieved, spectral line intensities can be connected to relative concentrations of elements
  - typically these conditions are only met *approximately*.

The BIOMAS Project:

Bacteria Identification by Optical, Molecular, and Atomic Spectroscopy



Motivation

- Require a real-time early-warning detection technology for bio-agents (bacteriological)
  - other applications: EH&S, food inspection, clinical
- Downside of competing technologies:
  - speed
  - target-specific (shelf-life?)
  - expertise required

Escherichia coli

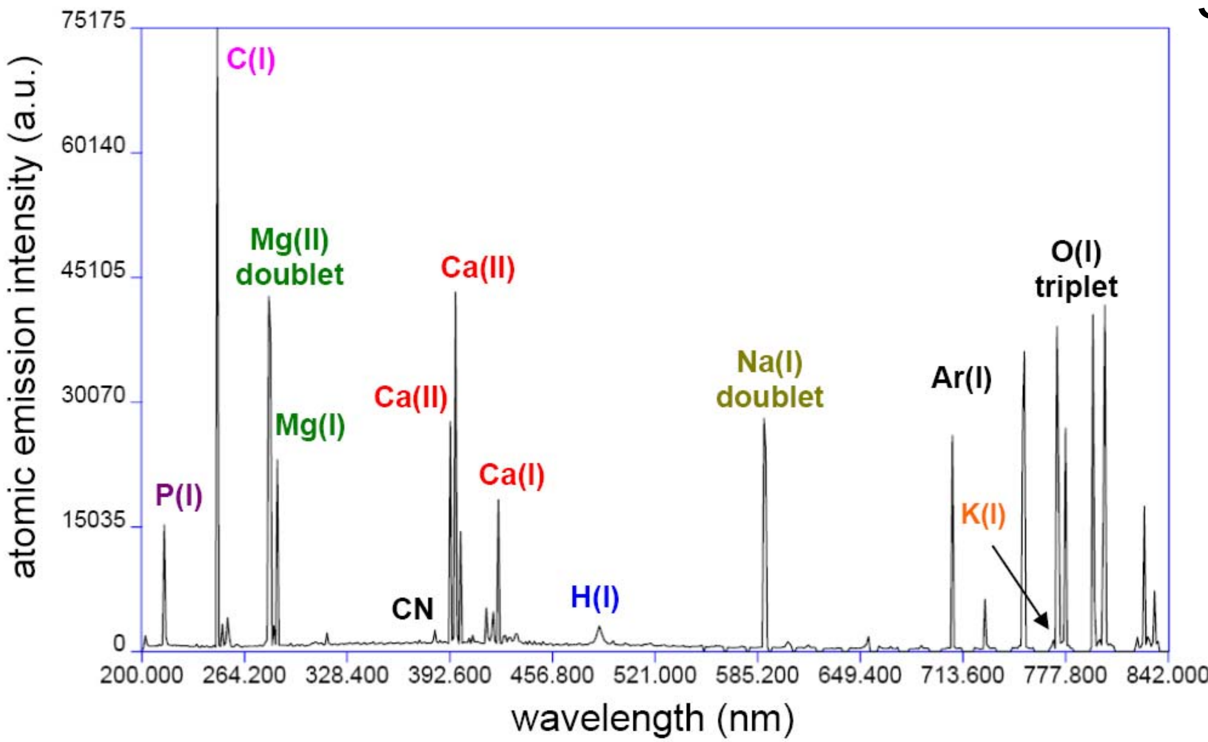
- Very common laboratory micro-organism
- Has many strains, most harmless, some pathogenic
- EHEC or *E. coli* 0157:H7 causes kidney failure in children (hemolytic uremic syndrome)

Inorganic Composition of *E. coli*

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

Composition



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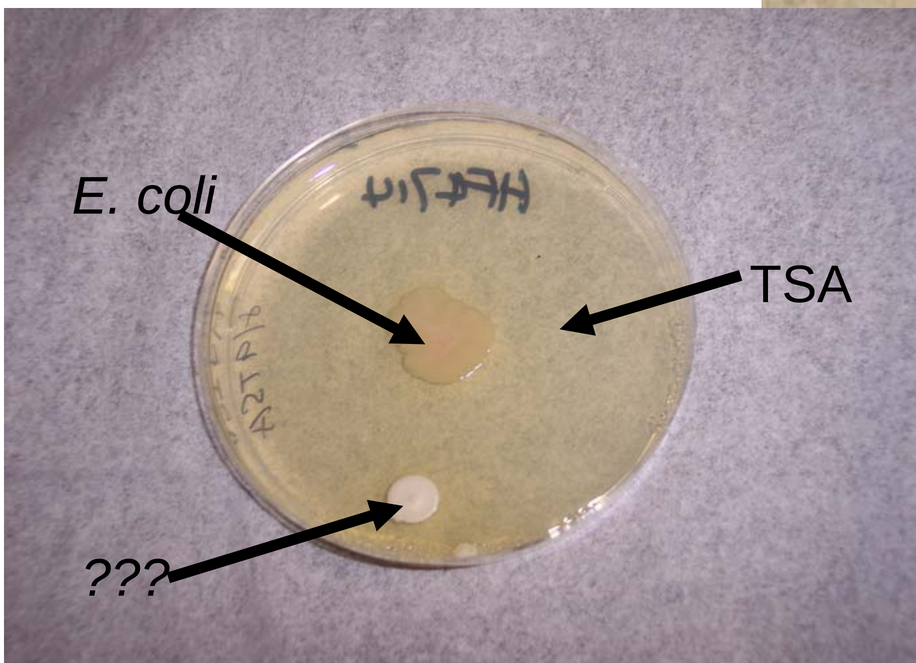
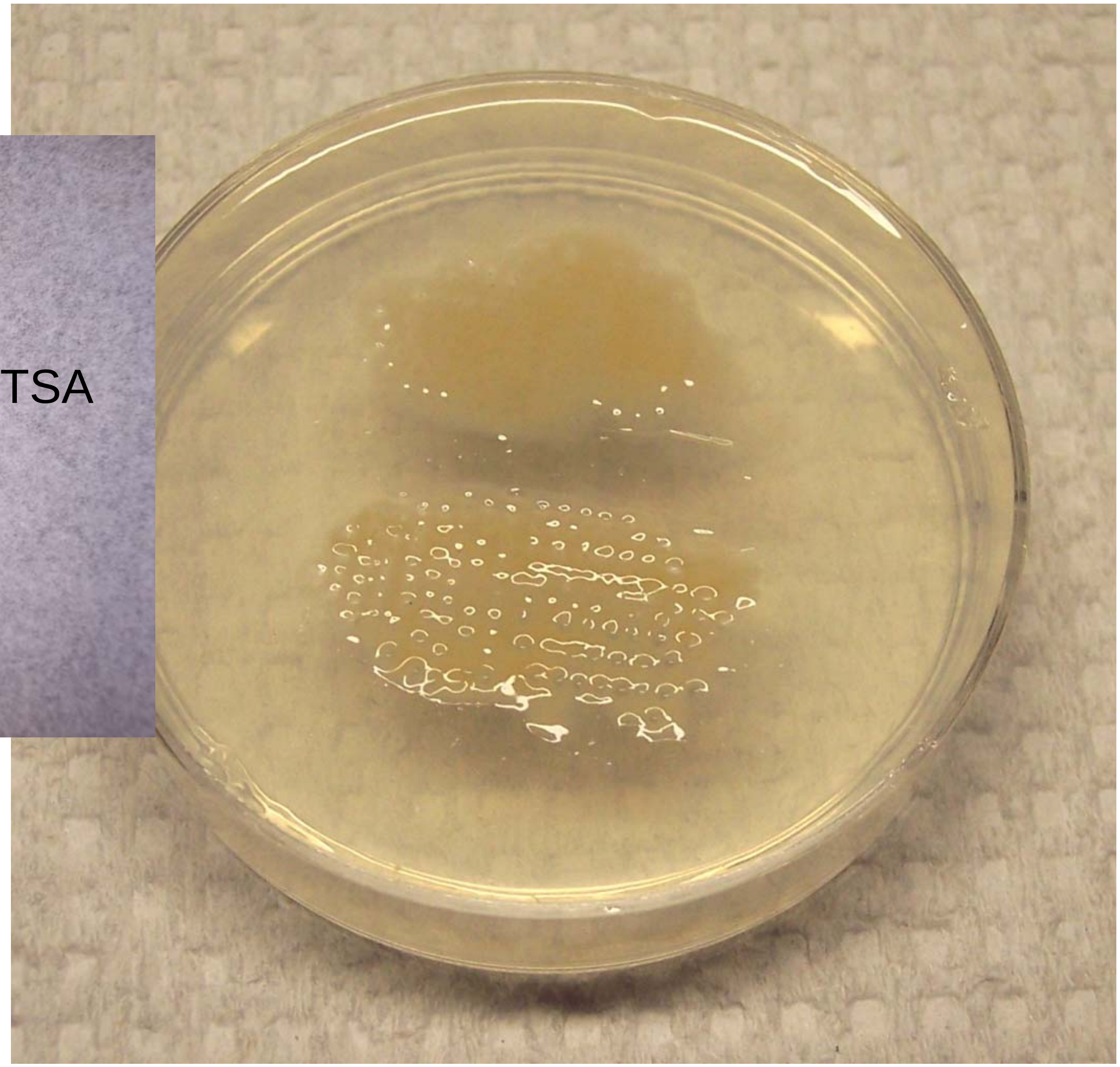
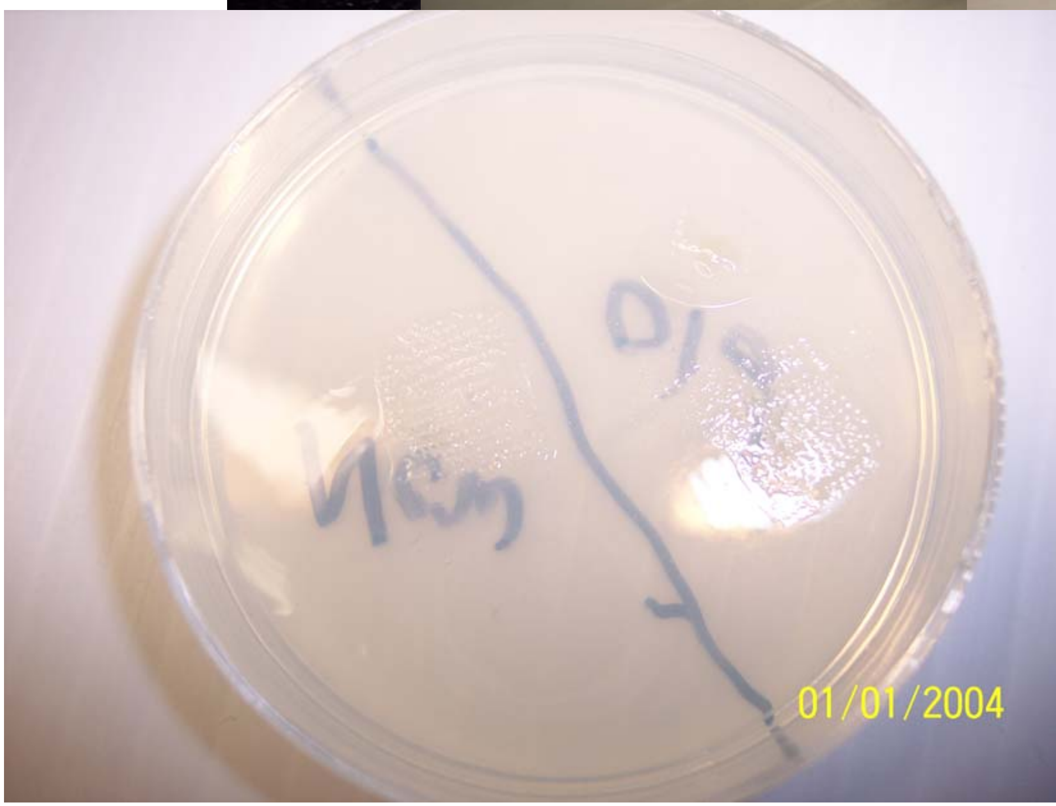
Ablated *E. coli* on Agar (a year ago)

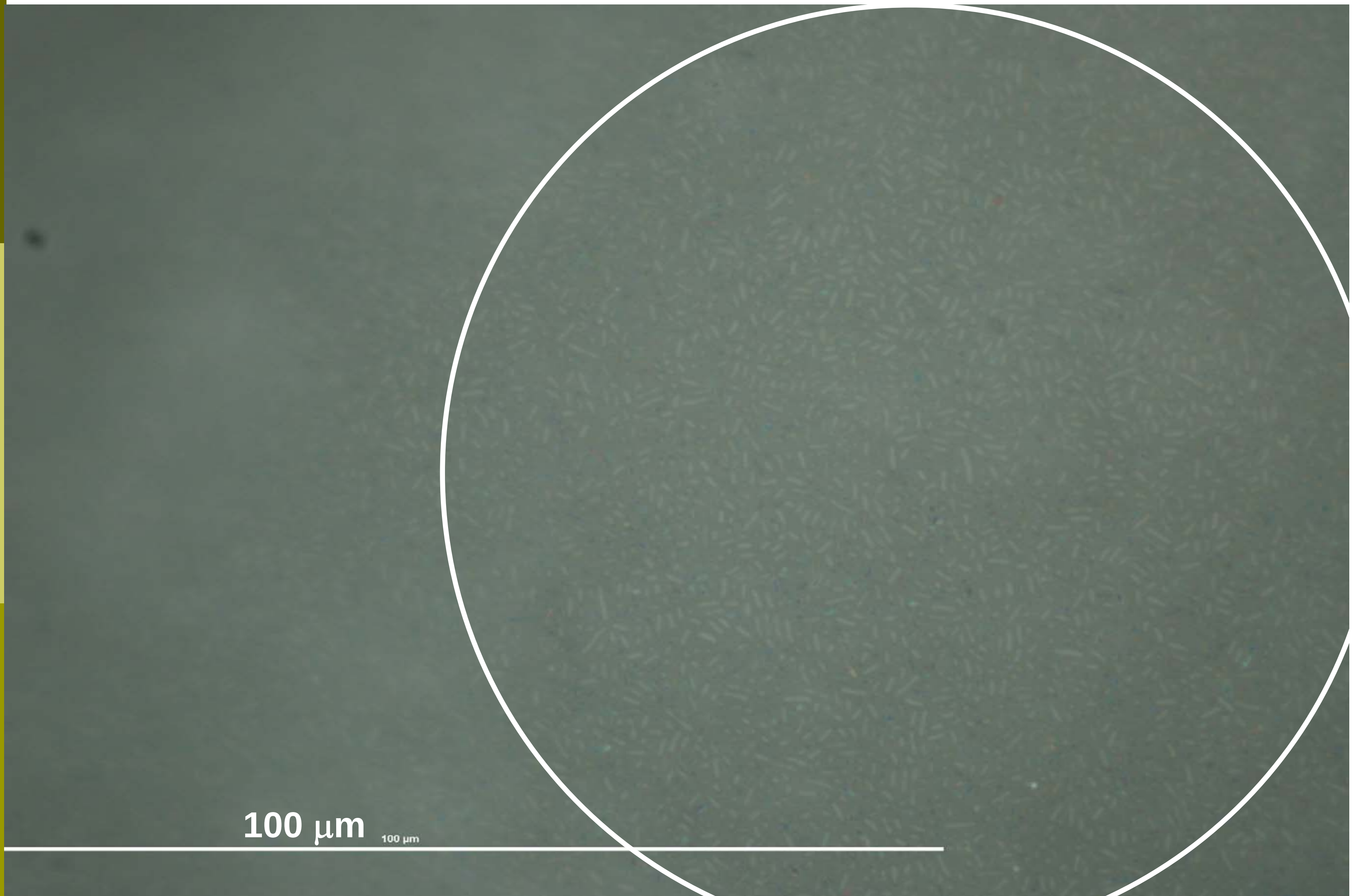




Now…




01/01/2004


100 μm
100 µm

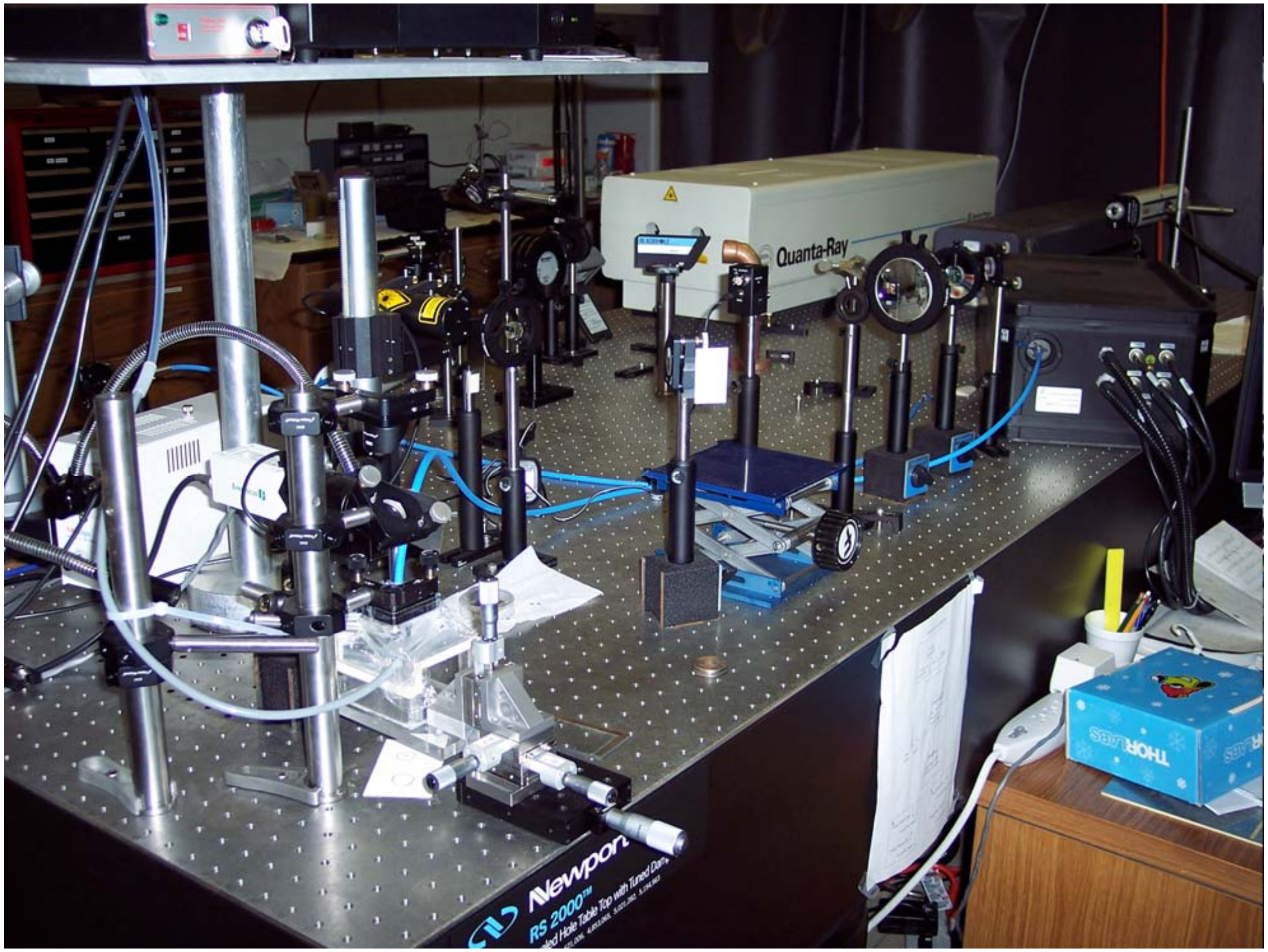
Our Apparatus

argon purge
gas chamber

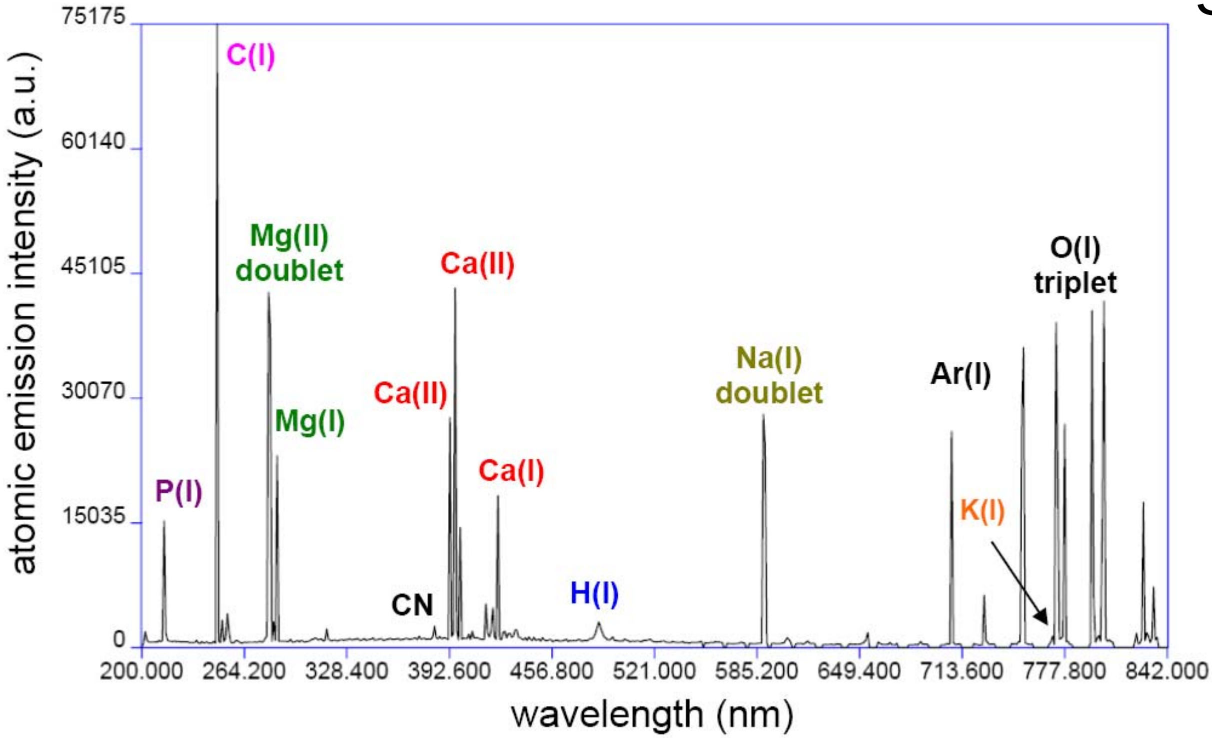
single-pulse
1064 nm

fiber collect
(UV)

Echelle
spectrometer



Composition



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Spectral Fingerprint

The intensities of 19 spectral lines from 6 elements provides a *spectral fingerprint*

wavelength (nm)	line identification	Fraction of total spectral power	Wilks' Lambda
213.618	P I	0.034	.619
214.914	P I	0.040	.492
247.856	C I	0.099	.521
253.56	P I	0.007	.771
279.553	Mg II	0.202	.040
280.271	Mg II	0.113	.061
285.213	Mg I	0.109	.037
373.69	Ca II	0.002	.909
383.231	Mg I	0.015	.782
383.829	Mg I	0.005	.588
393.366	Ca II	0.099	.034
396.847	Ca II	0.037	.060
422.673	Ca II	0.033	.062
430.253	Ca I	0.002	.803
518.361	Mg I	0.004	.773
585.745	Ca I	0.000	.920
588.995	Na I	0.124	.020
589.593	Na I	0.067	.022
769.896	K I	0.012	.931

Discriminant Function Analysis

- The relative strengths of the 19 emission lines forms the basis of an identification
- A statistical analysis called Discriminant Function Analysis (DFA) looks for similarities and differences in spectra from different strains

Discriminant Function Analysis

- We want to see the difference between *N* groups (*N* strains), each group composed of spectra containing 19 independent variables (predictor variables)

$$X = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ \ldots \\ x_{19} \end{bmatrix}$$

← one entire LIBS spectrum reduced to this

Discriminant Functions Scores

- DFA constructs N-1 "Canonical Discriminant Functions", from these, *discriminant function scores* are constructed

$$DF^j = b_0^j + \sum_{k=1}^{19} b_k^j x_k = b_0^j + \left[b_1^j b_2^j \ldots b_{19}^j \right] \begin{bmatrix} x_1 \\ x_2 \\ \ldots \\ x_{19} \end{bmatrix}$$

j_{th} discriminant function score

discriminant function (eigenvector)

experimental data

APPLIED PHYSICS LETTERS **90**, 163901 (2007)

E. c

Escherichia coli identification and strain discrimination using nanosecond laser-induced breakdown spectroscopy

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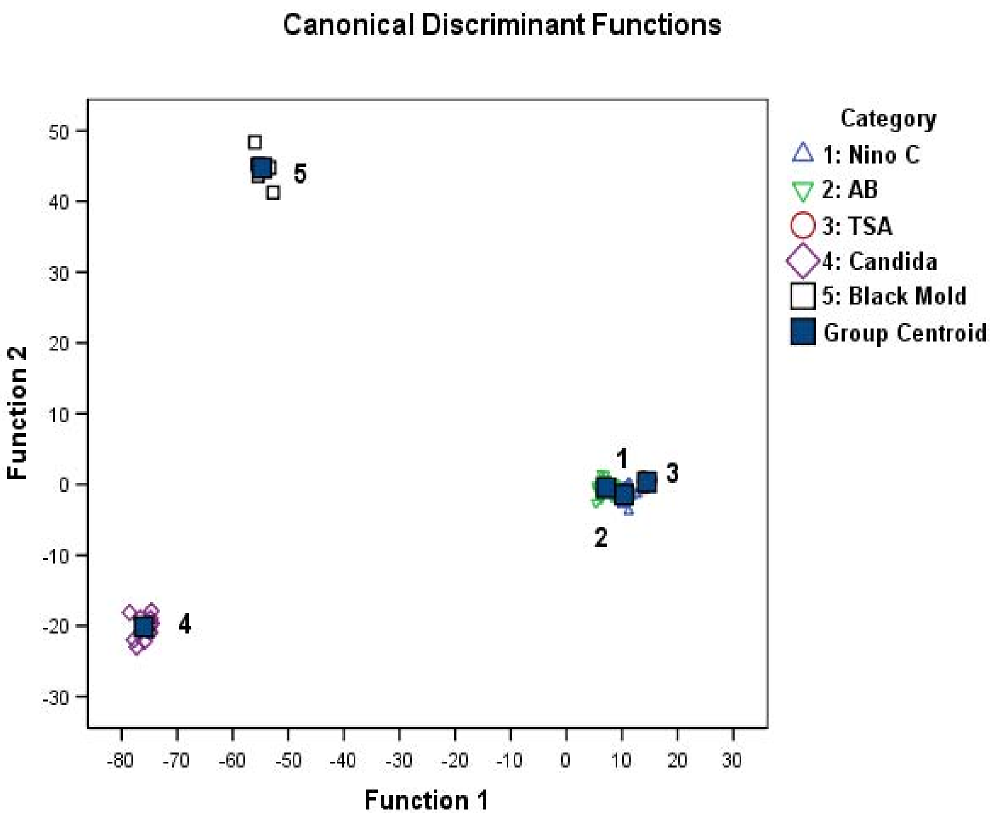
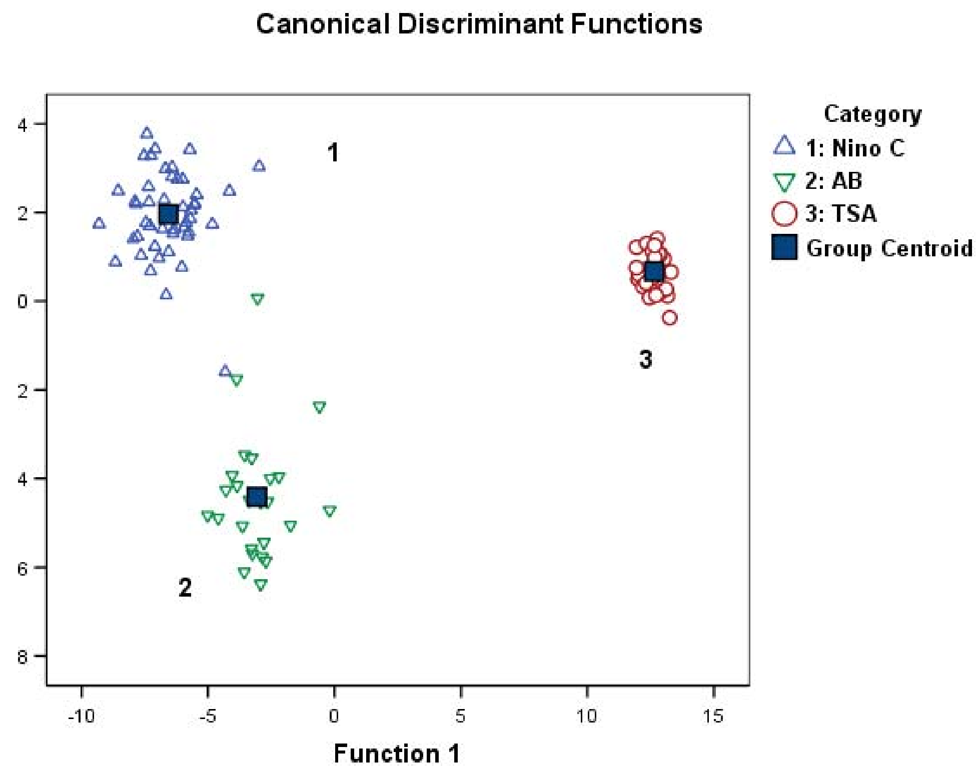
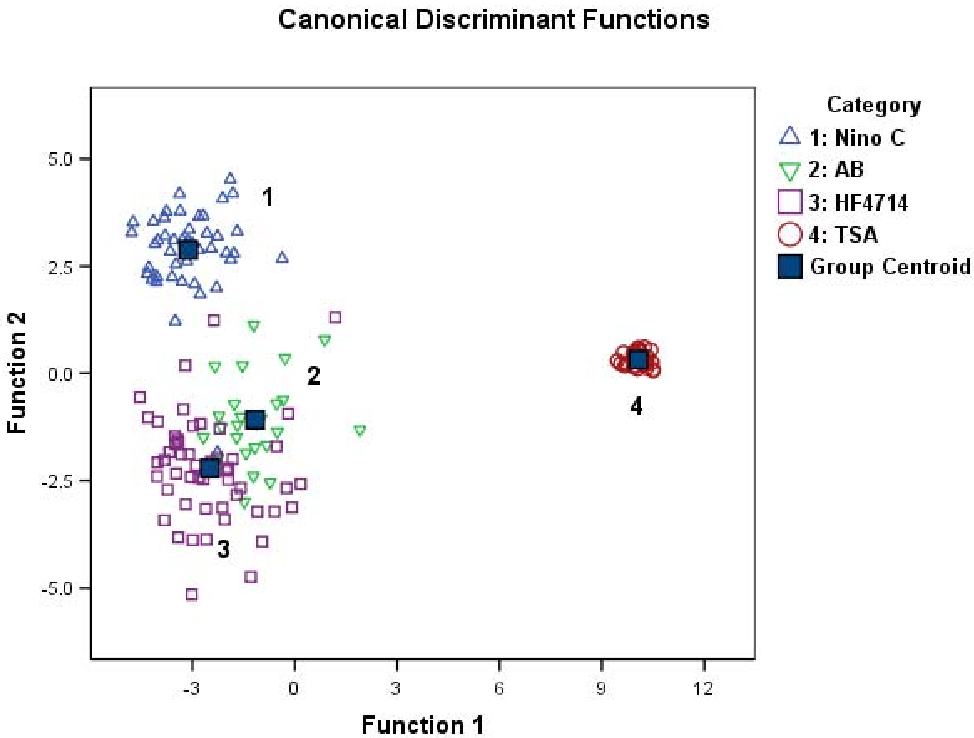
Department of Physics and Astronomy, Wayne State University, Detroit, Michigan 48201

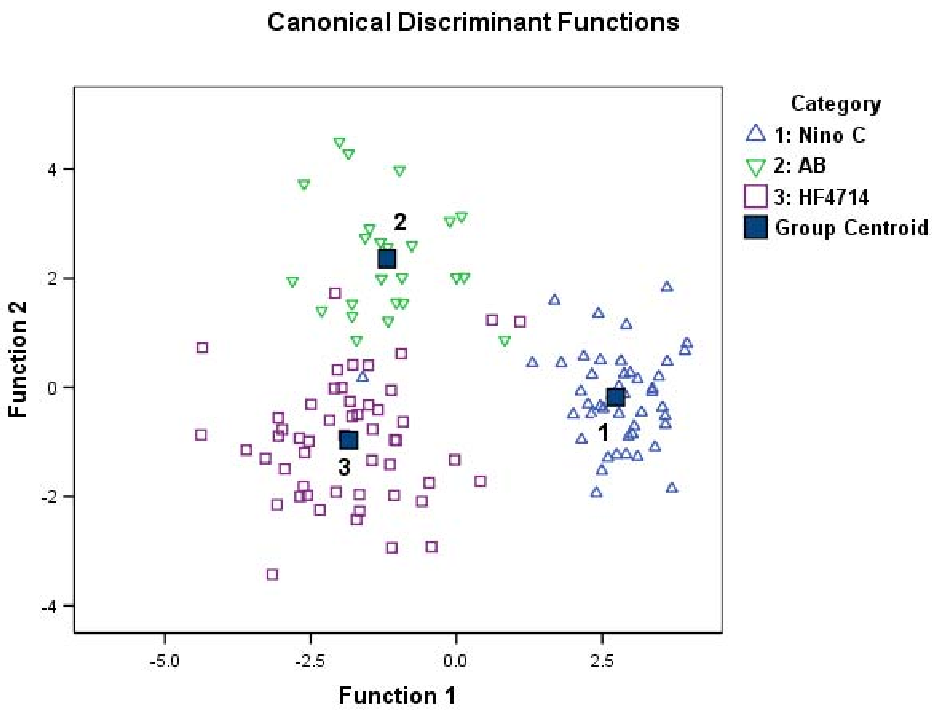
Sunil Palchaudhuri
Department of Immunology and Microbiology, Wayne State University, Detroit, Michigan 48201





E. coli Results





JOURNAL OF APPLIED PHYSICS **102**, 014702 (2007)

Pathogenic *Escherichia coli* strain discrimination using laser-induced breakdown spectroscopy

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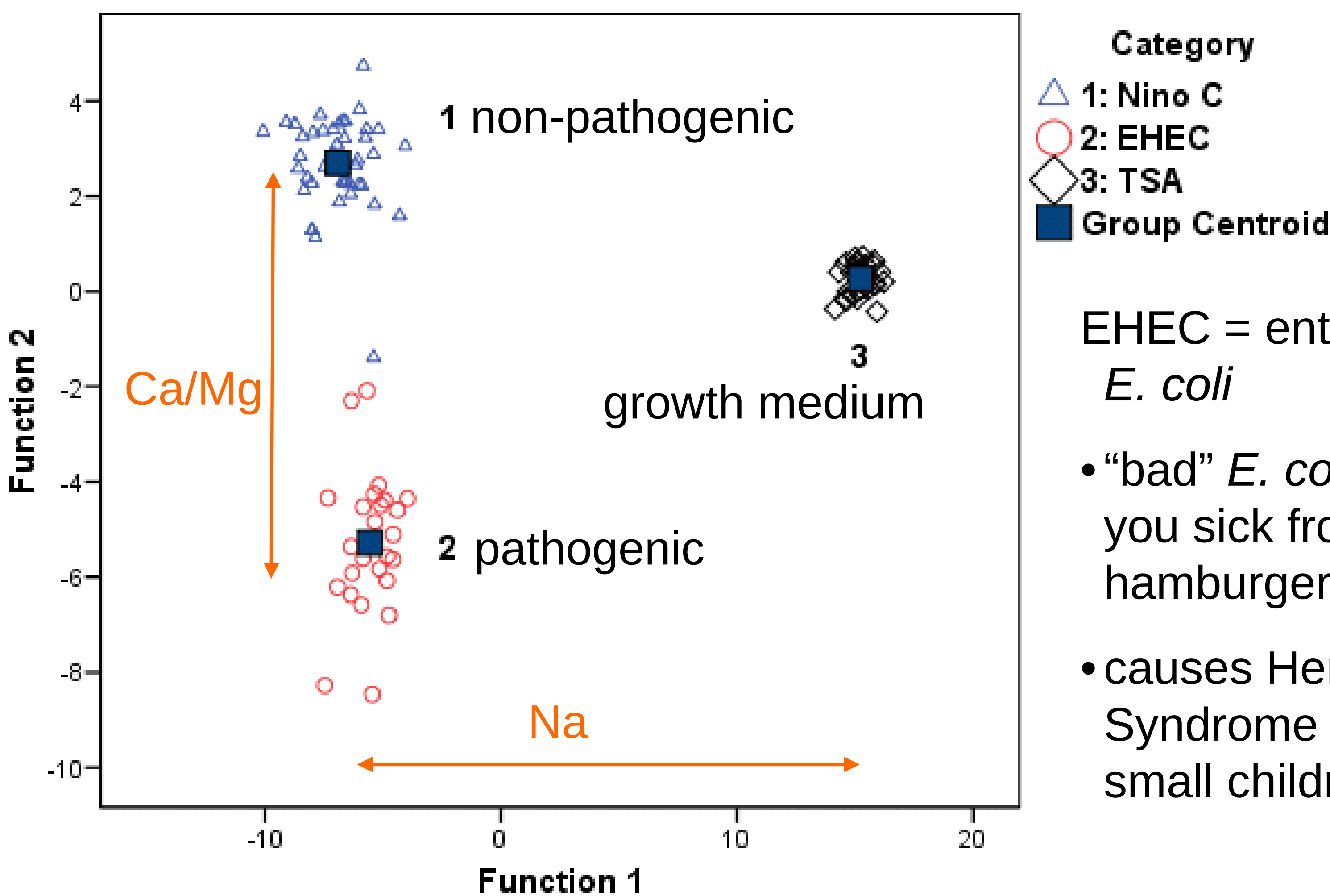
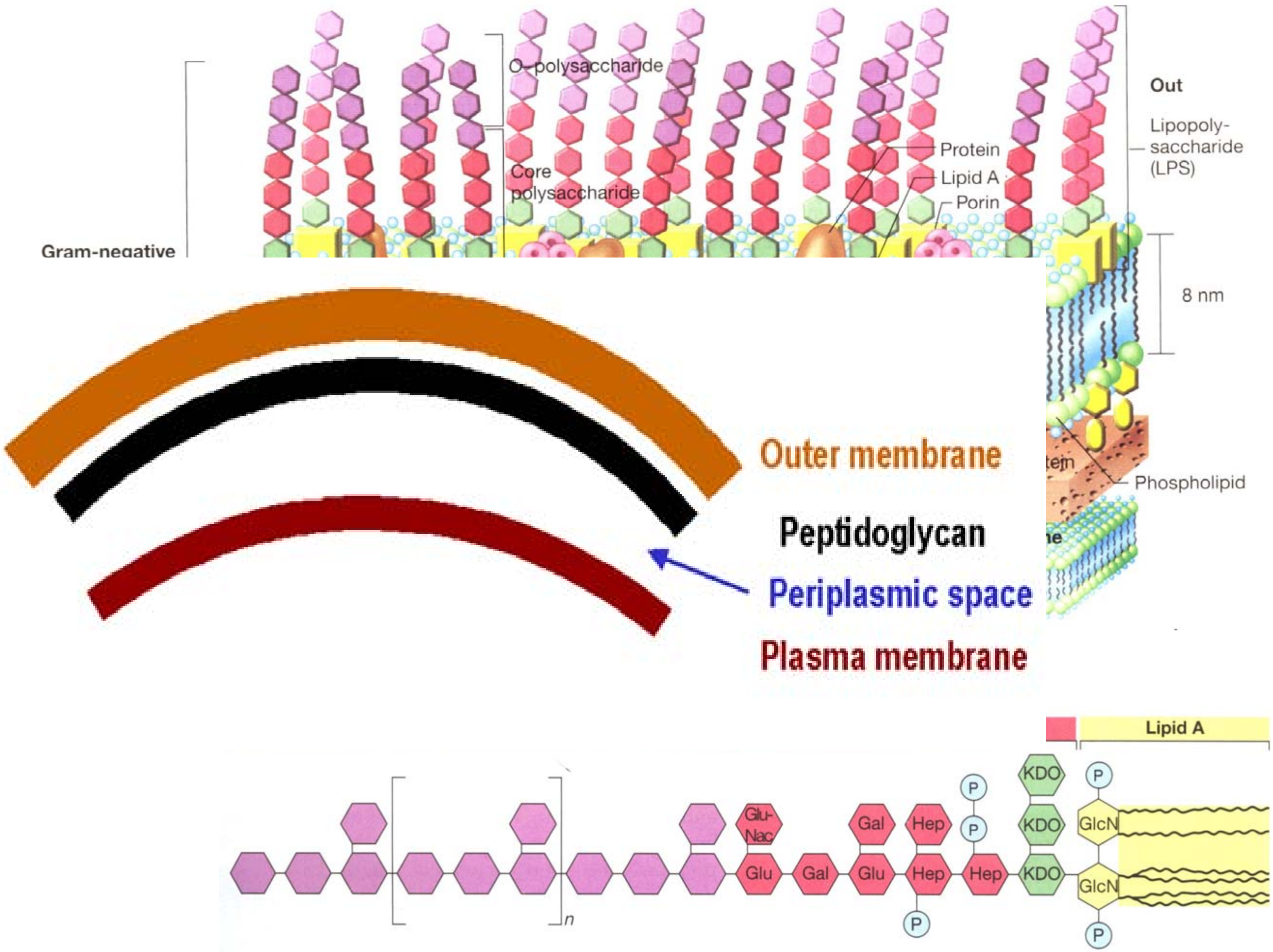
(Received 7 February 2007; accepted 28 May 2007; published online 5 July 2007)



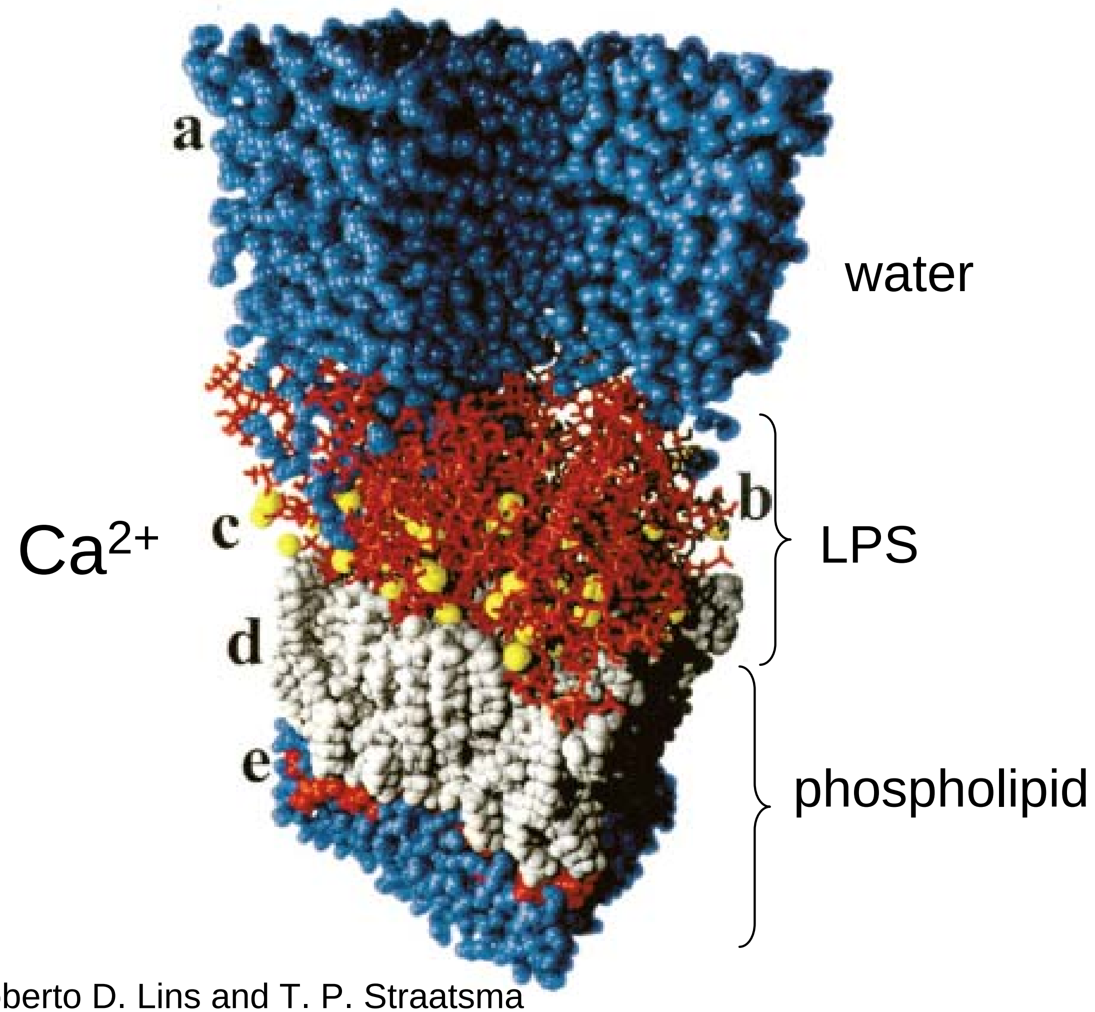
EHEC = enterohemorrhagic *E. coli*

- "bad" *E. coli* which makes you sick from eating raw hamburger.
- causes Hemolytic Uremic Syndrome (HUS) fatal to small children

Why Ca? Why Mg?


O-polysaccharide
Core polysaccharide
Protein
Lipid A
Porin
Out
Lipopoly-saccharide (LPS)
Gram-negative
8 nm
Phospholipid
Outer membrane
Peptidoglycan
Periplasmic space
Plasma membrane
Lipid A
Glu-Nac
Glu
Gal
Hep
KDO
GlcN
P
n

Divalent Cations Regulate Membrane Permeability



Roberto D. Lins and T. P. Straatsma
Biophysical Journal ***81,*** 1037–1046 (2001)

ELSEVIER

Spectrochimica Acta Part B 62 (2007) 1169 – 1176

www.elsevier.com/locate/sab

Identification and discrimination of *Pseudomonas aeruginosa* bacteria grown in blood and bile by laser-induced breakdown spectroscopy

Steven J. Rehse [a,*], Jonathan Diedrich [a,1], Sunil Palchaudhuri [b,2]

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[b] *Department of Immunology and Microbiology, Wayne State University, Detroit, MI 48201, USA*

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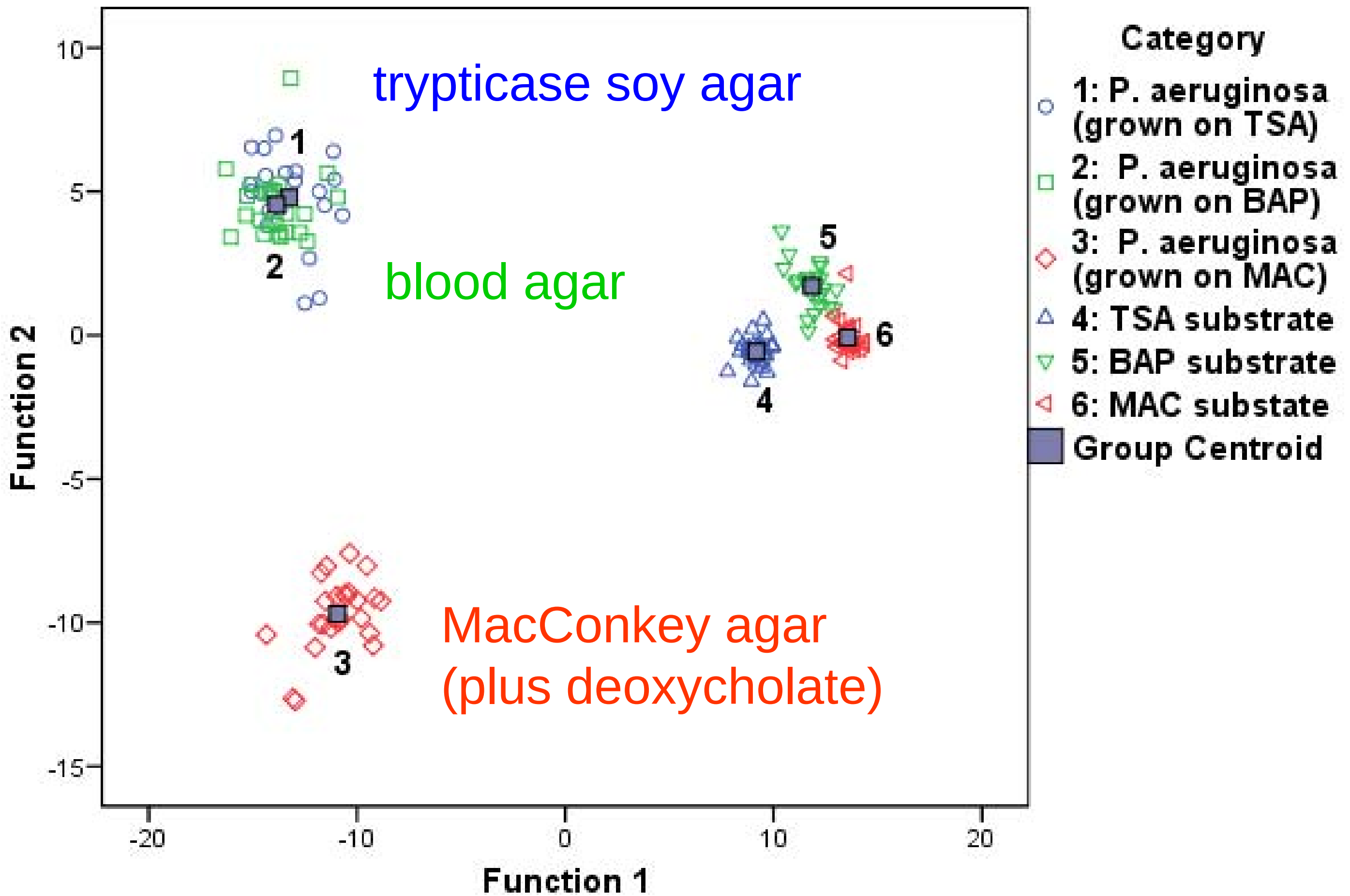
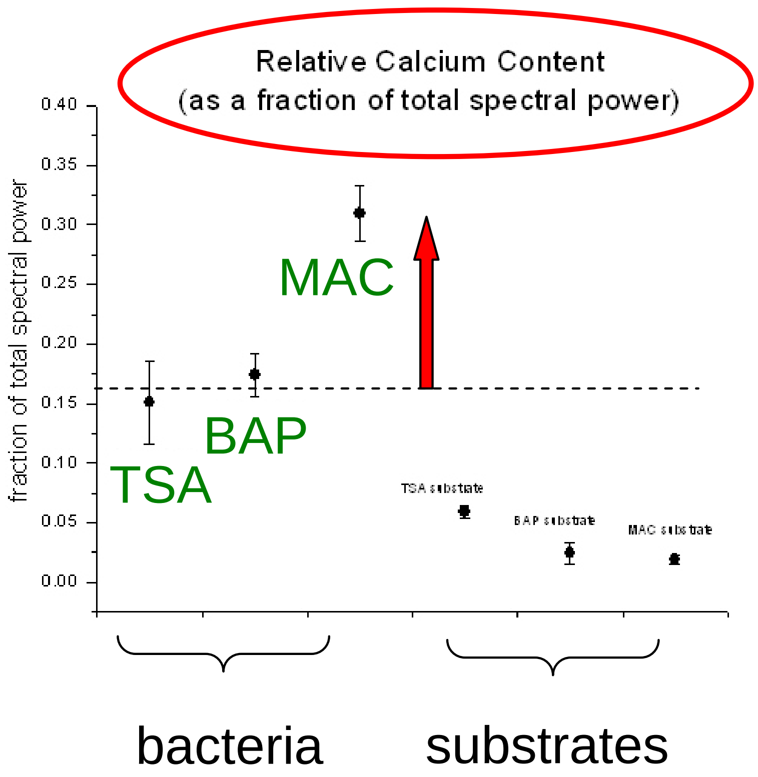
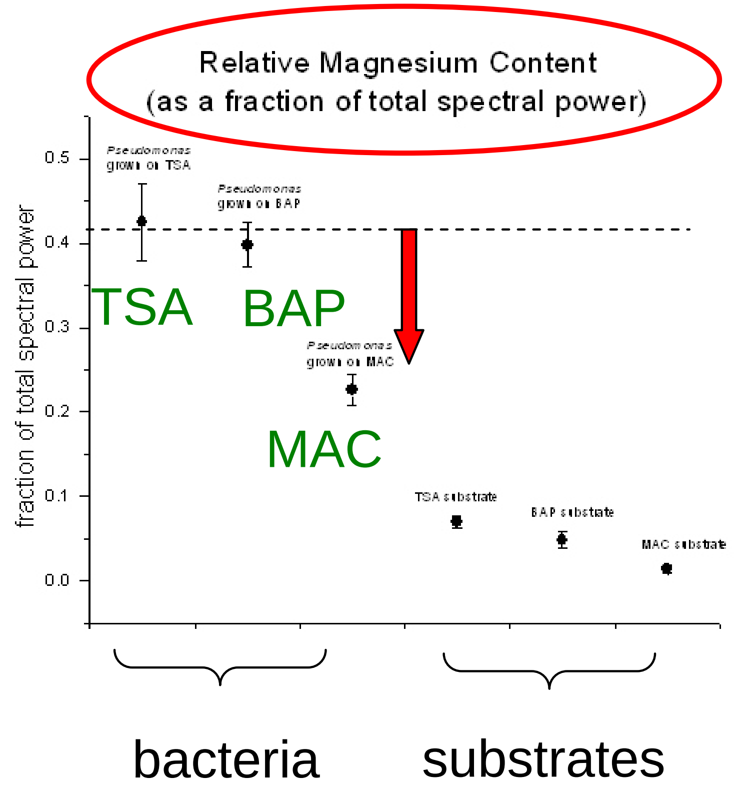
Available online 1 August 2007

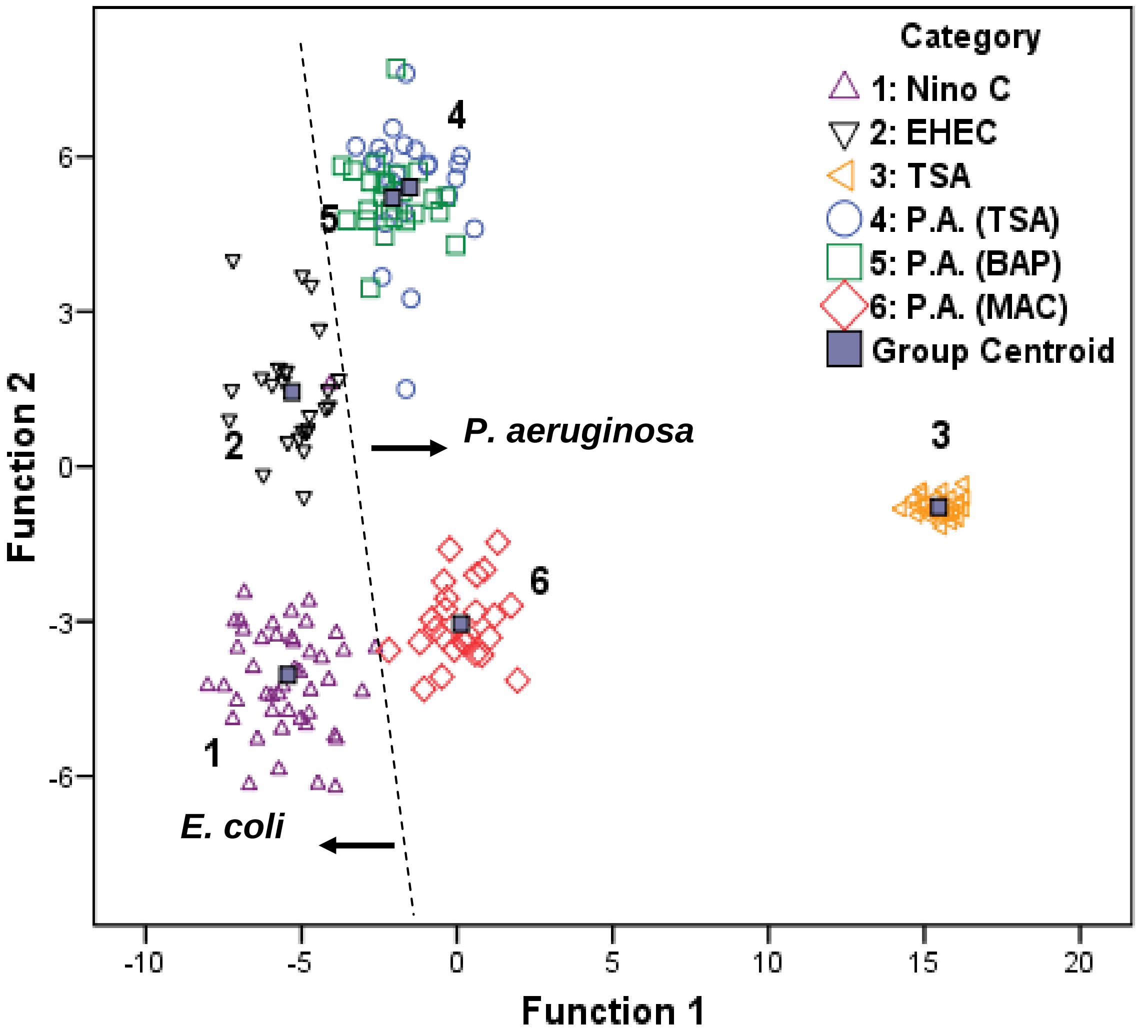


Divalent Cations (Ca^{2+}, Mg^{2+}) Concentrations Are Altered by Environment



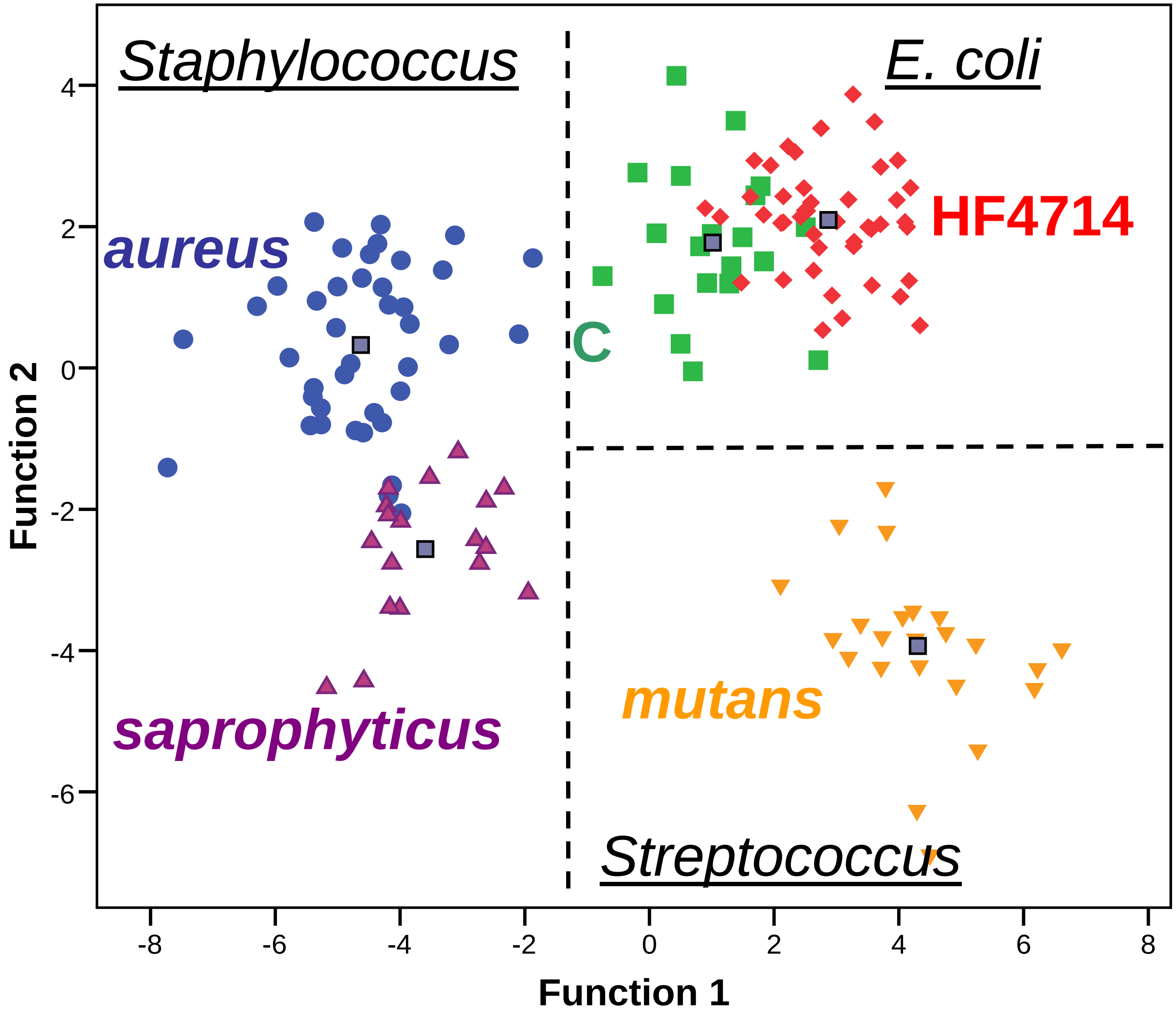


E. coli and *P. aeruginosa*

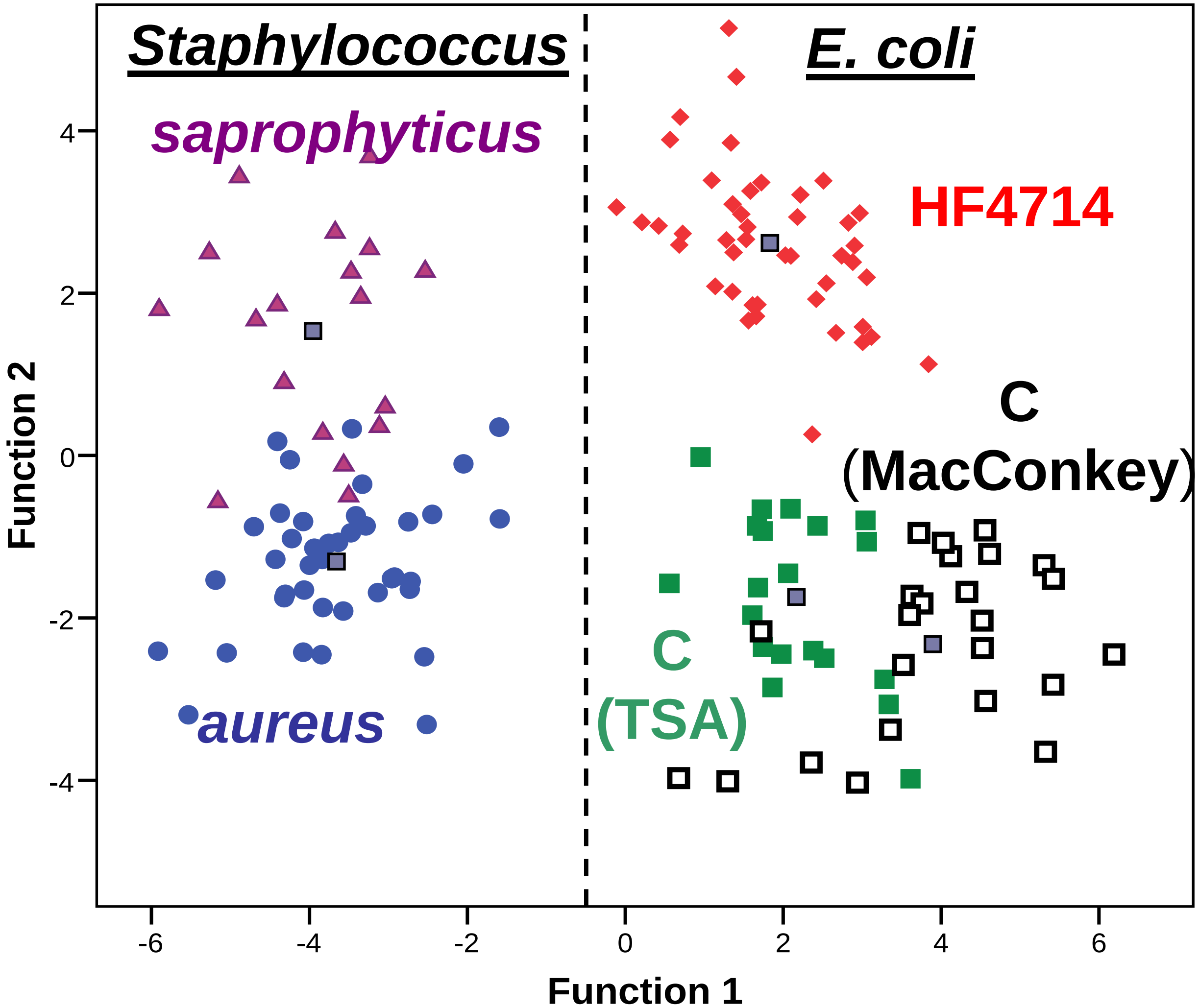


Gram-positive / Gram-negative

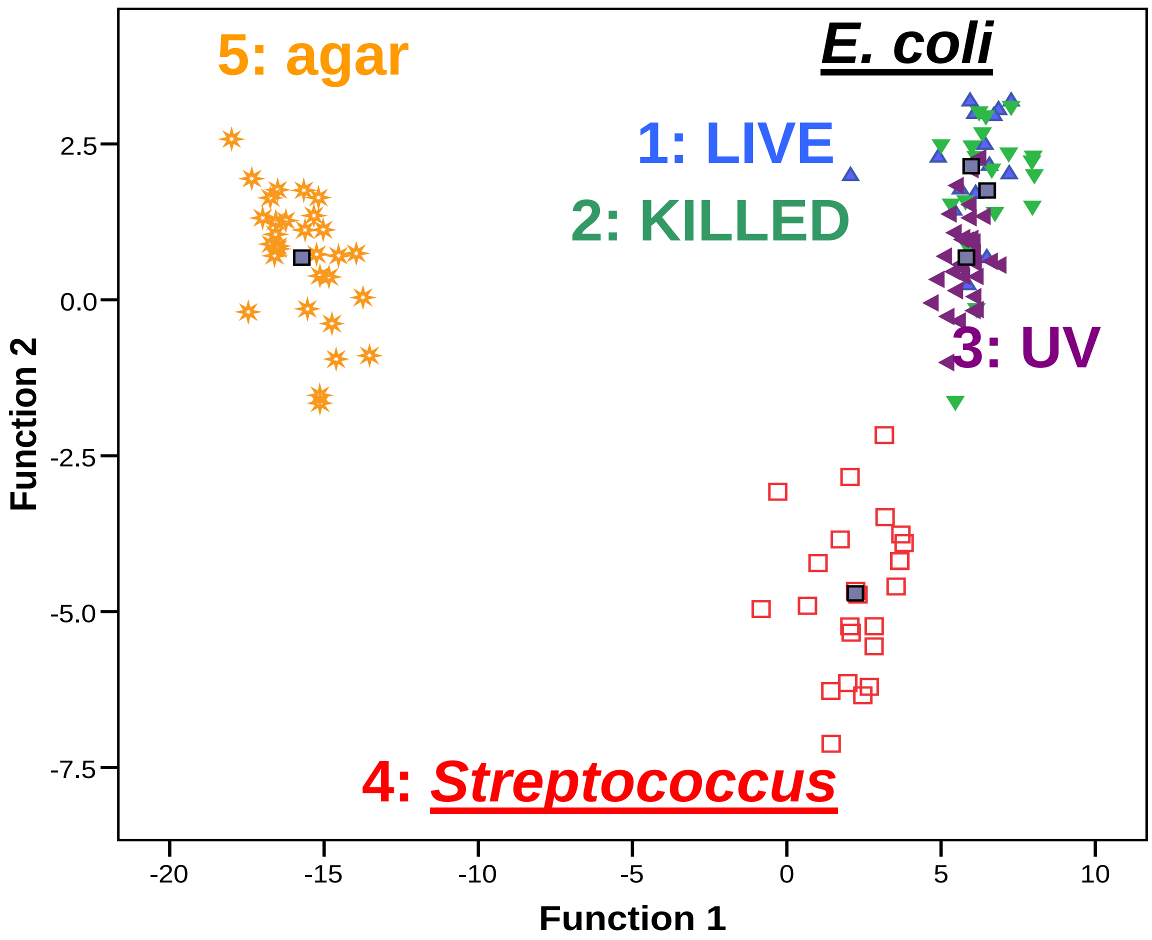
Intensity of 13 lines used in the DFA



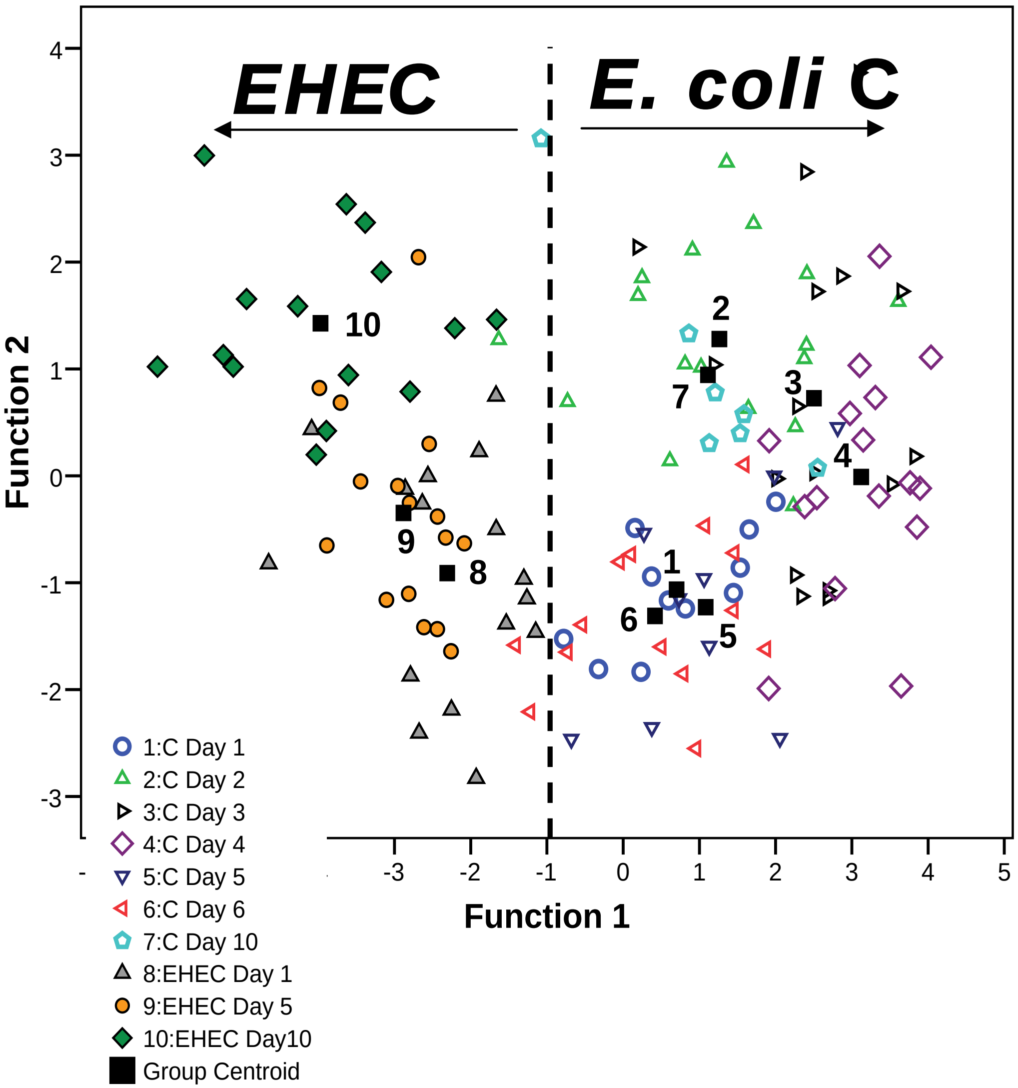
Intentional Membrane Alteration

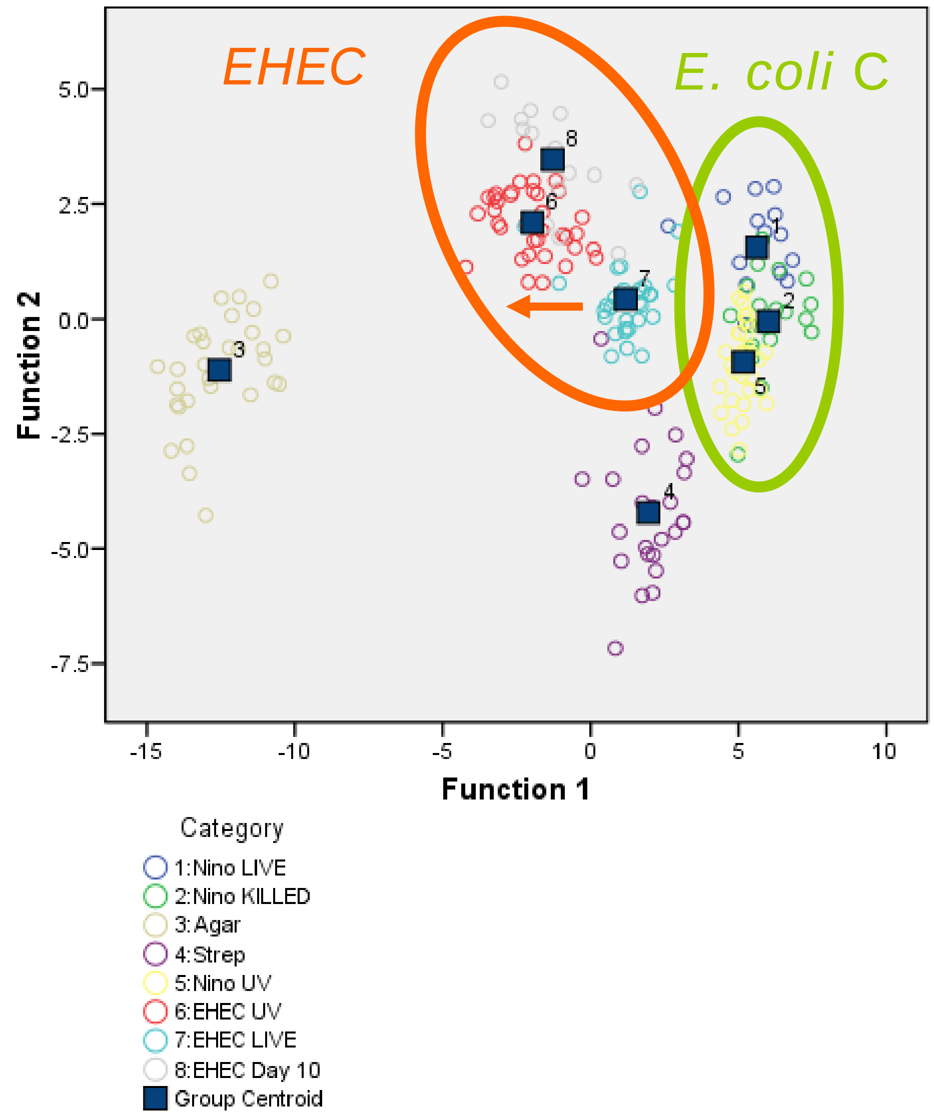


LIBS Strengths!
Live/killed/UV exposed



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





Conclusions

- LIBS a versatile, extremely useful technology with application in microbiology
- 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 possible
  - Testing on “starved” bacteria seems possible

Thank you for your attention!

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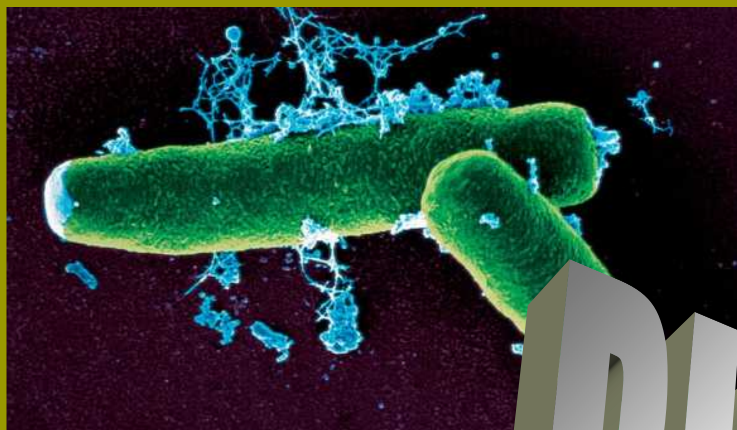
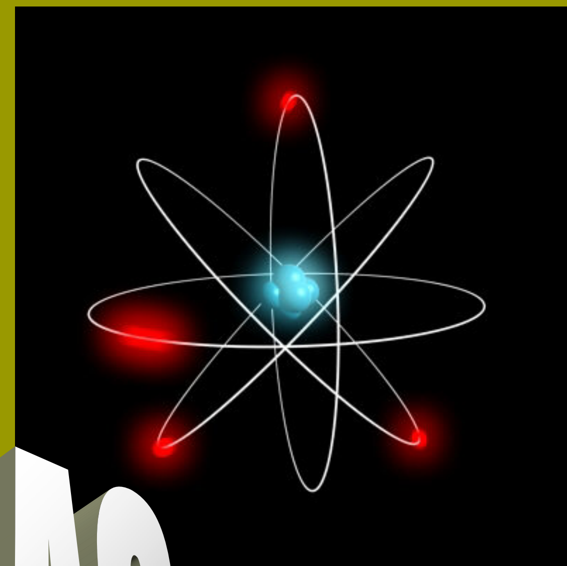
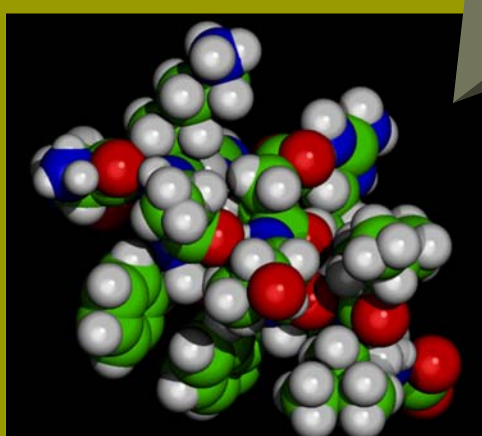
The BIOMAS Project:

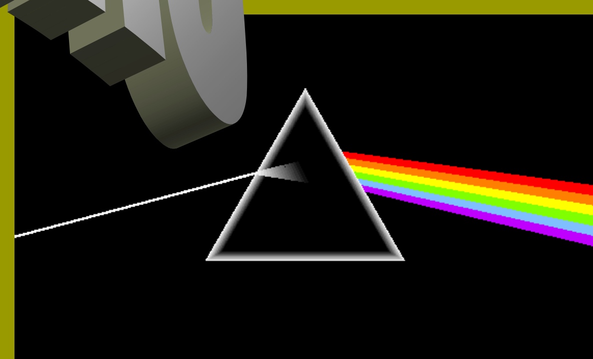
Bacteria Identification by Optical, Molecular, and Atomic Spectroscopy

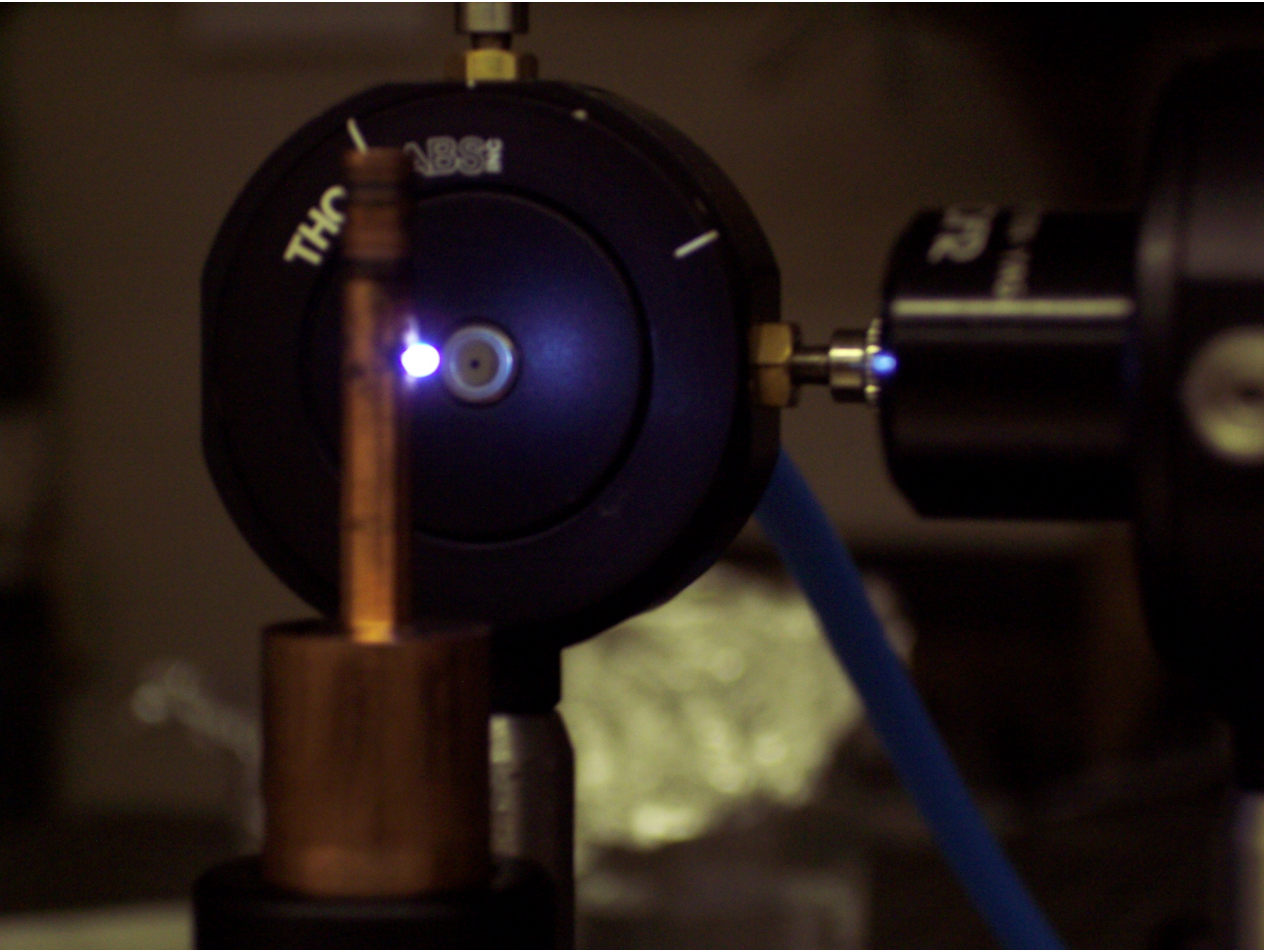




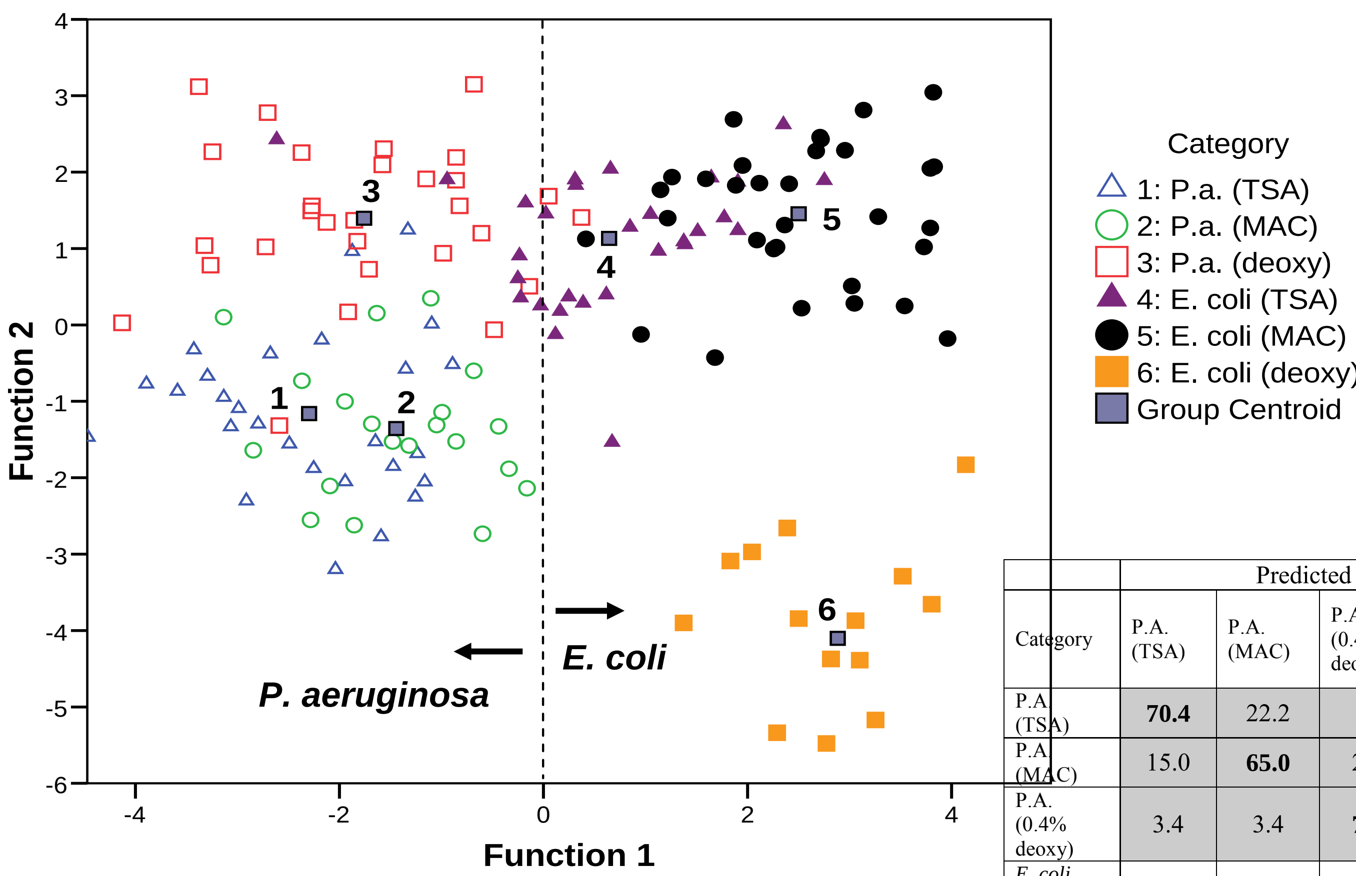
BIOMAS





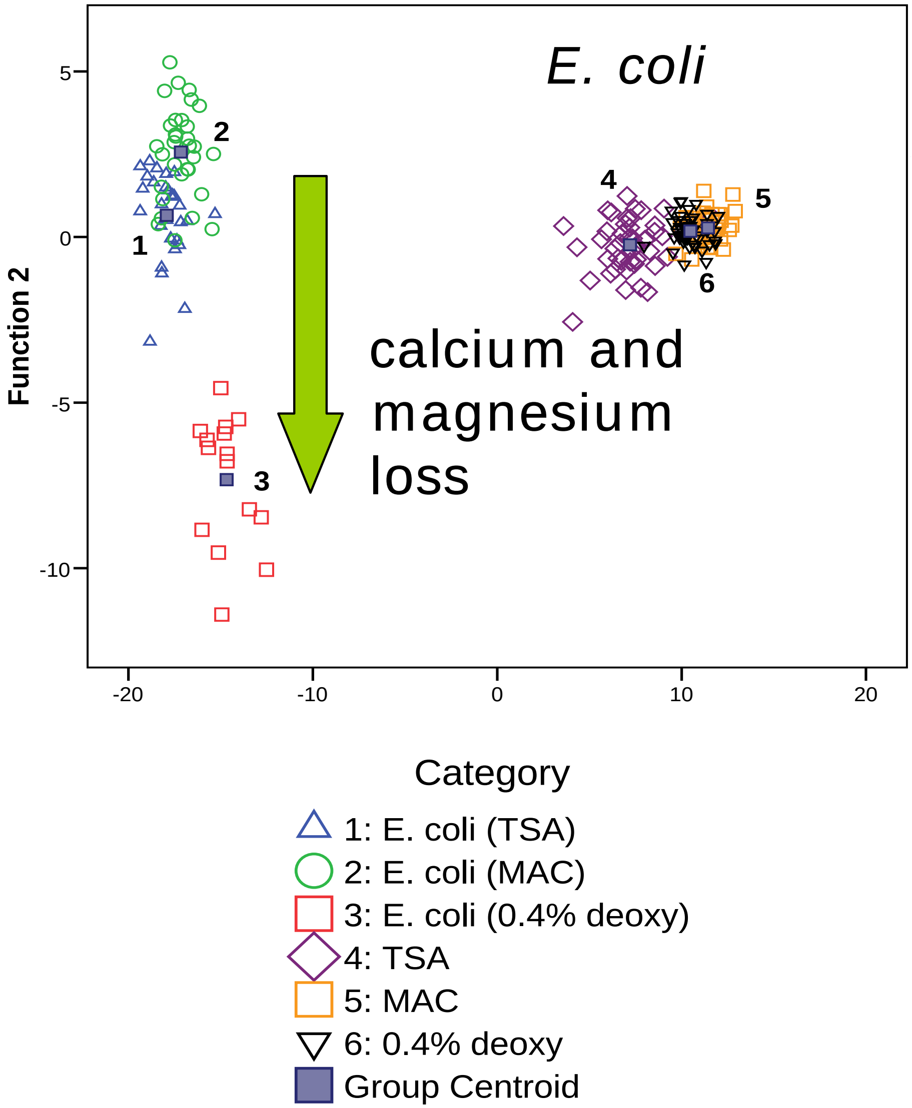


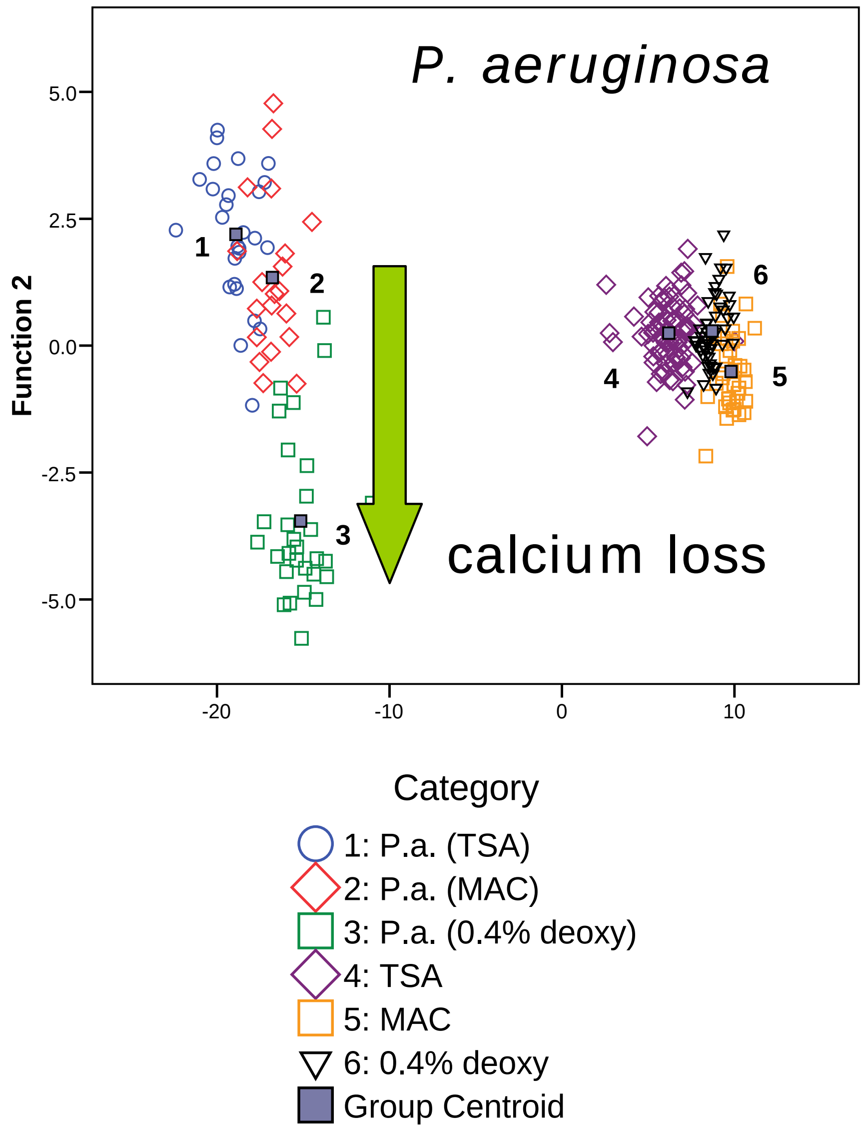
Membrane Disruption Does not Destroy Identification



	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

Intentional Membrane Alteration





Conclusions

- LIBS a versatile, extremely useful technology
- Many applications in biological systems (and elsewhere)
- Physicists can make valuable contributions in the biological sciences.

Physics of Plasma Formation: breakdown

Problem: how do photons of relatively low energy, 1-2 eV, (compared to ionization threshold of common gases) generate a breakdown?

Three distinct but overlapping stages:

1. plasma ignition
2. plasma growth (electron avalanche or cascade) and interaction with laser pulse
3. plasma development accompanied by shock wave generation and propagation ("breakdown")

Physics of Plasma Formation: breakdown

1. cascade or avalanche requires an initial electron
   - multiphoton absorption/ionization

$$M + mh\nu \rightarrow M^{+} + \mathrm{e}^{-}$$

   - local radioactivity
   - cosmic rays

Physics of Plasma Formation: breakdown

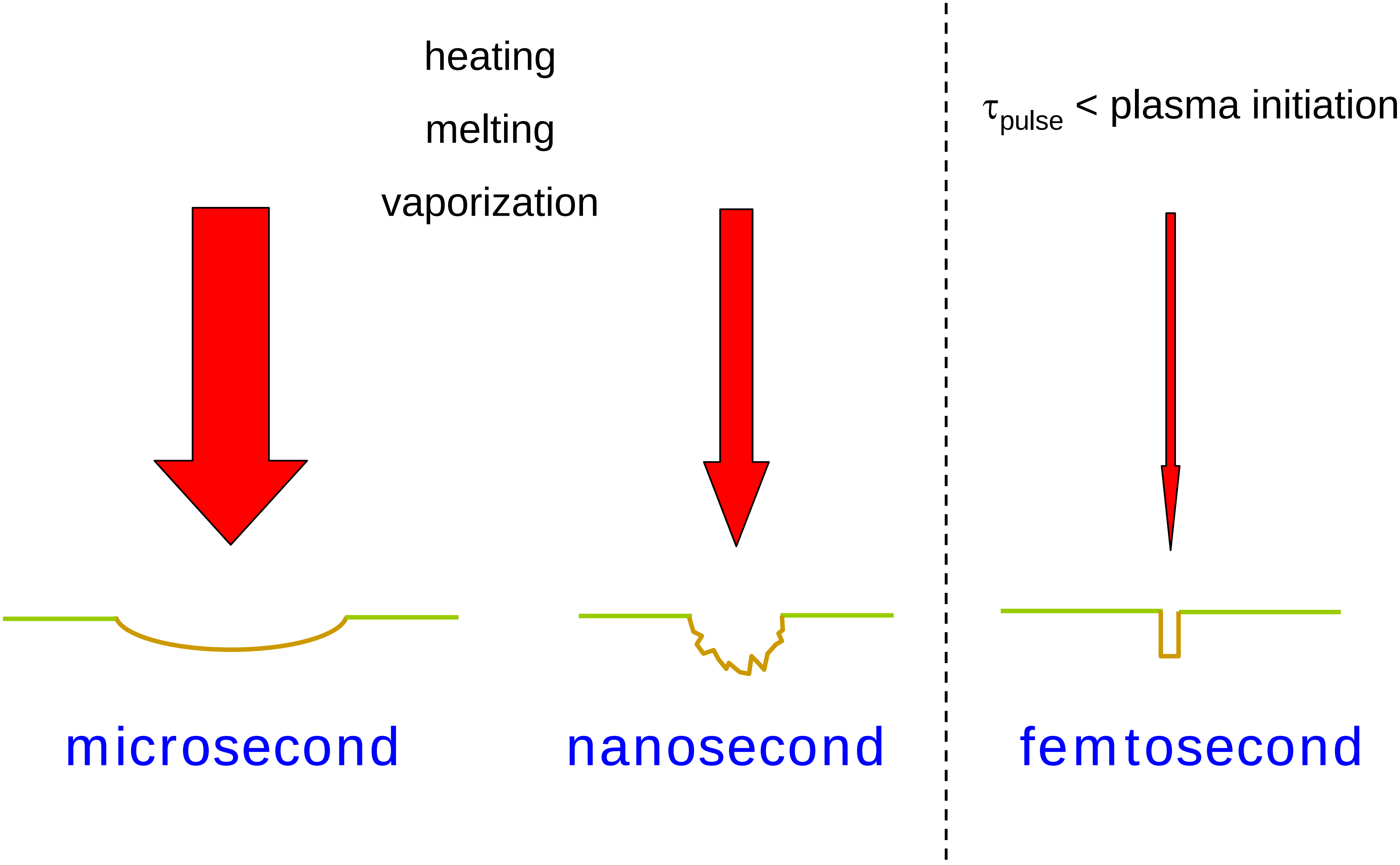
2. electron cascade or avalanche occurs by inverse bremsstrahlung (free-free absorption)

$$e^-(\text{slow}) + h\nu \rightarrow e^-(\text{fast})$$

- electrons absorb photons from laser field (in the presence of gas) for momentum transfer between collisions with neutral species
- acquire sufficient energy for collisional ionization of gas atoms
- electron density increases exponentially via cascade

$$n_e \sim 1\text{-}10\ \text{cm}^{-3} \rightarrow 10^{17}\text{-}10^{20}\ \text{cm}^{-3}$$

Physics of Plasma Formation: ablation



Physics of Plasma Formation: ablation

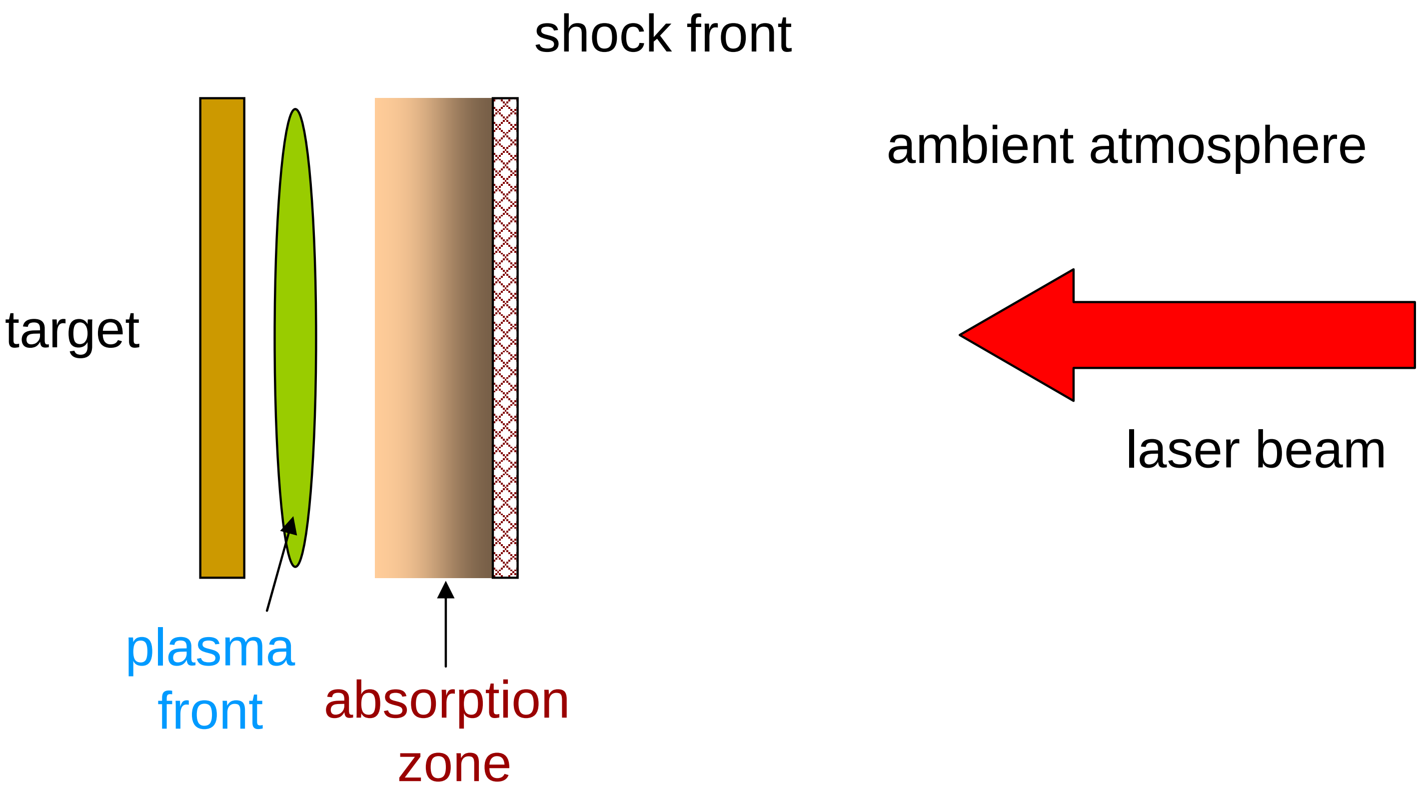
$$I_{\min} = \frac{\rho L_V \kappa^{1/2}}{\Delta t^{1/2}} (\mathrm{W/cm^2})$$

- ρ = density
- L_V = latent heat of vaporization
- κ = thermal diffusivity
- Δt = laser pulse length

- $I_{\min}$ Al = 1.75 x 10^8 W/cm^2
  - for a 10 ns pulse, focused to a 100 μm spot: ~130 μJ

Physics of Plasma Formation: laser detonation wave

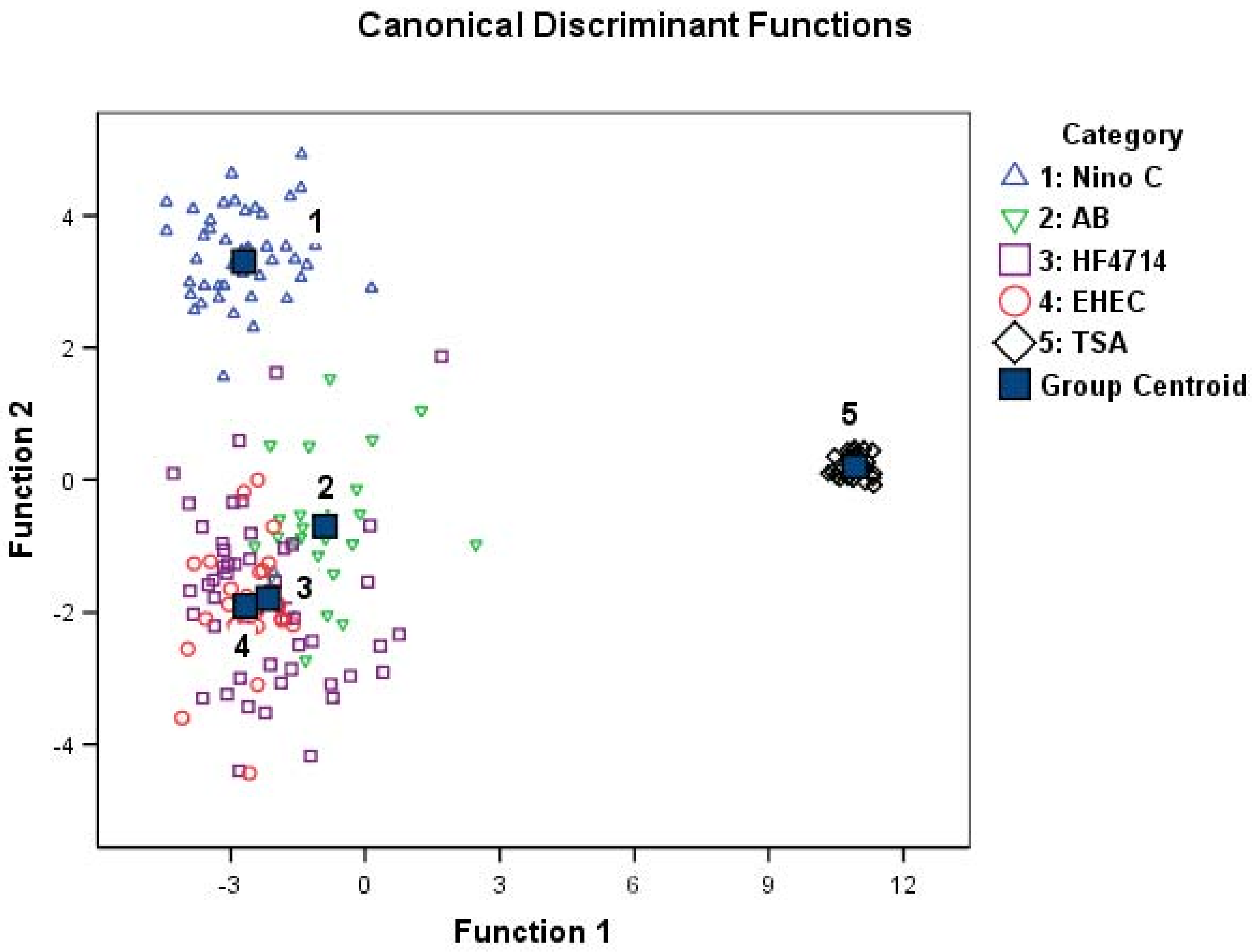
laser-supported detonation wave (LSD or LDW) with a supersonic, rapidly expanding shock-wave front



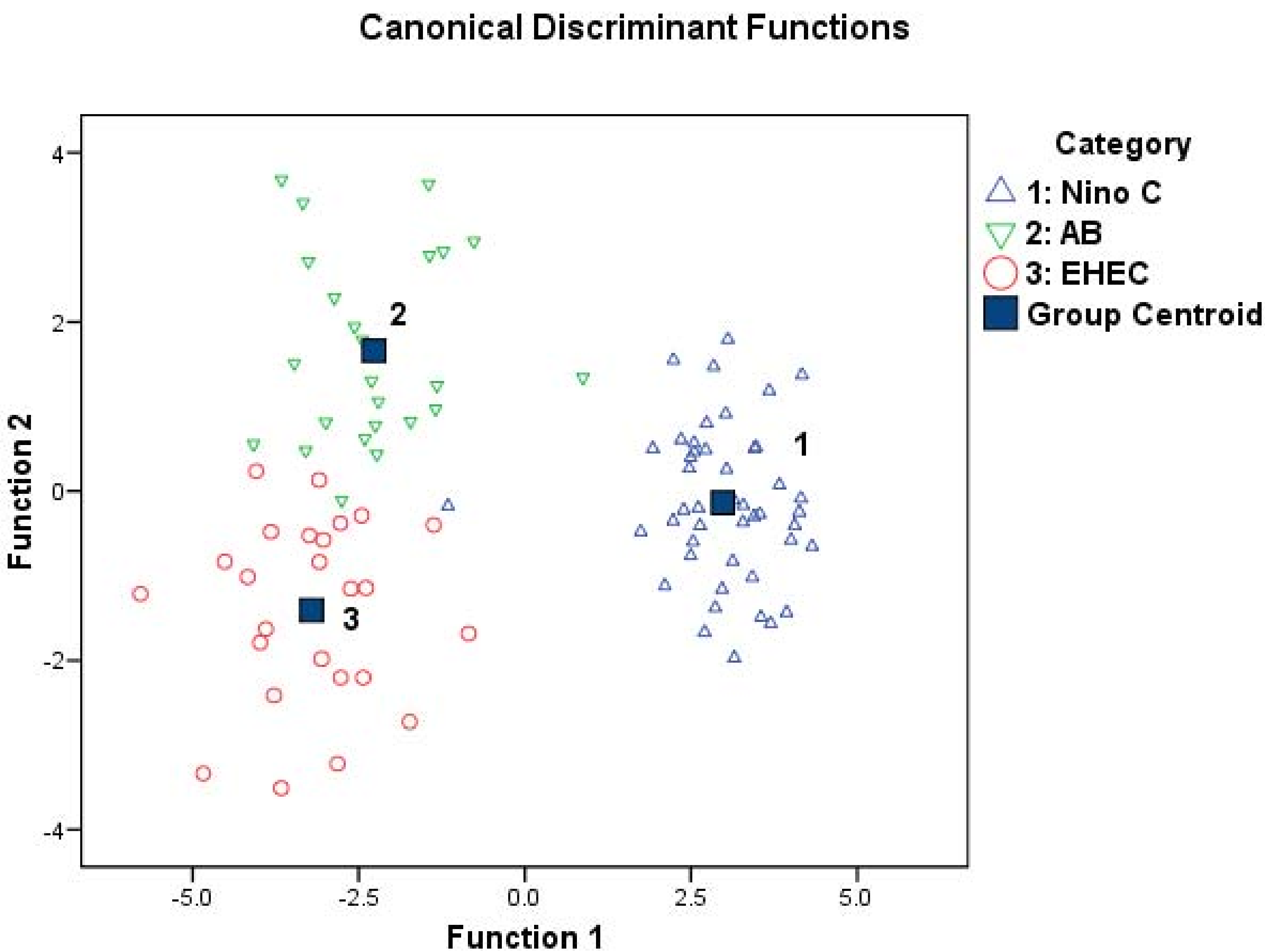
target

plasma
front

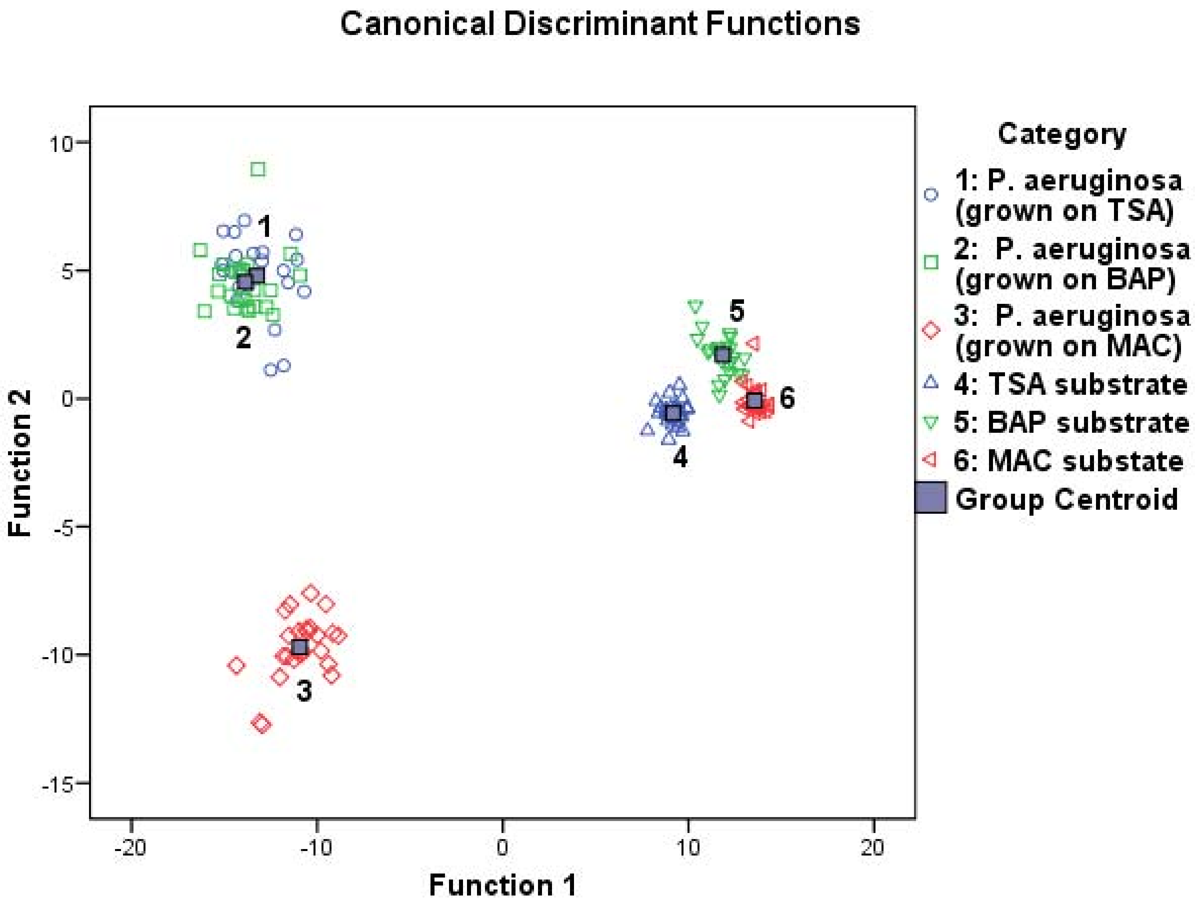
EHEC Results



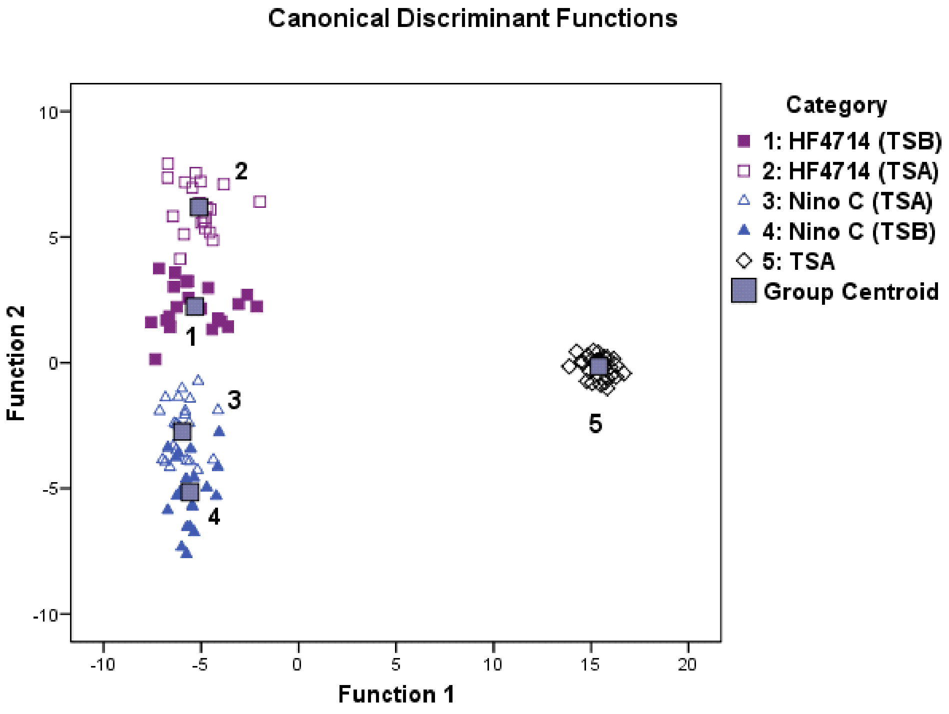
EHEC Results

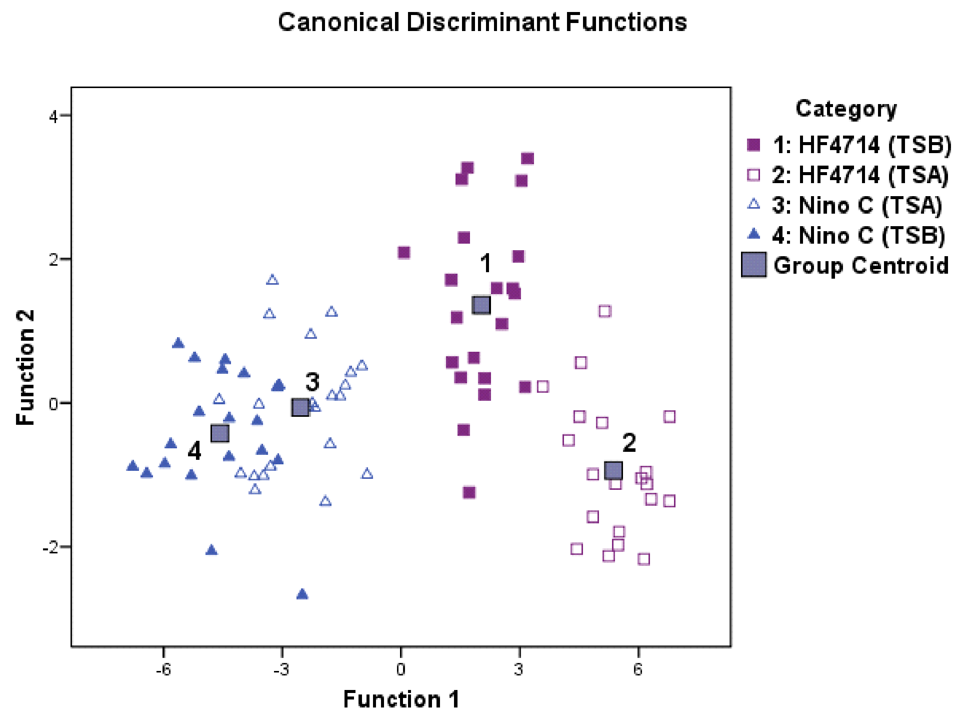


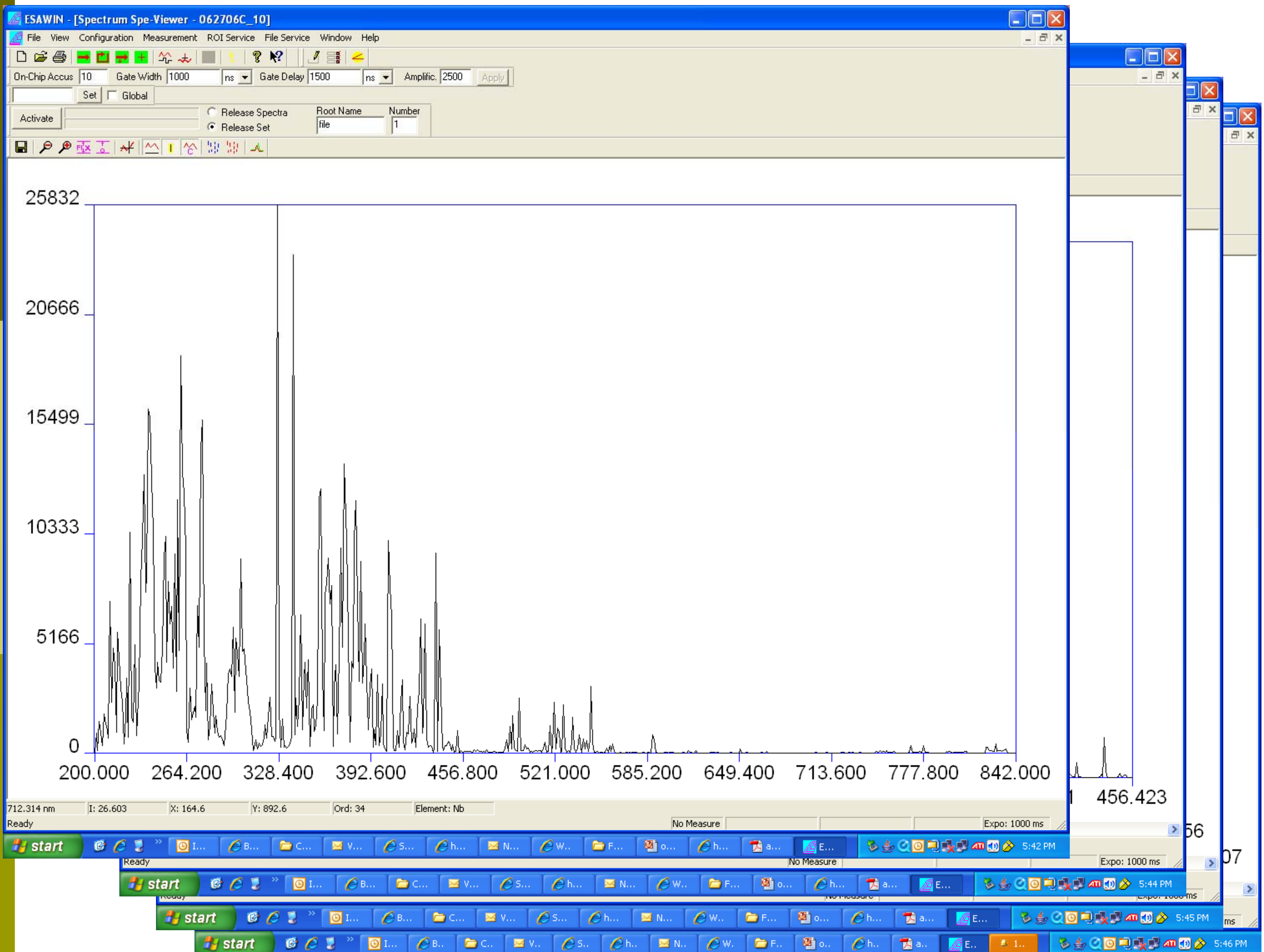
Effect of Growth Environment on *P. aeruginosa*



Effect of Growth Environment on *E. coli*



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On-Chip Accus 10 Gate Width 1000 ns Gate Delay 1500 ns Amplific. 2500 Apply
Set Global
Activate
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Root Name file
Number 1
25832
20666
15499
10333
5166
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I: 26.603
X: 164.6
Y: 892.6
Ord: 34
Element: Nb
Ready
No Measure
Expo: 1000 ms
start
5:42 PM
456.423
Expo: 1000 ms
5:44 PM
5:45 PM
5:46 PM

Spectral Line Radiant Intensity

$$I = {}^{h\nu gAN}\!\Big/_{4\pi} = \left({}^{hcN_0 gA}\!\Big/_{4\pi\lambda Z} \right) \exp\left({}^{-E}\!\Big/_{kT} \right)$$

I = intensity (given in units of W/sr)
g = statistical weight of level
A = Einstein A coefficient
N_0 = total species population
Z = partition function (statistical weight of ground state)
E = Energy of upper state of transition

Temperature

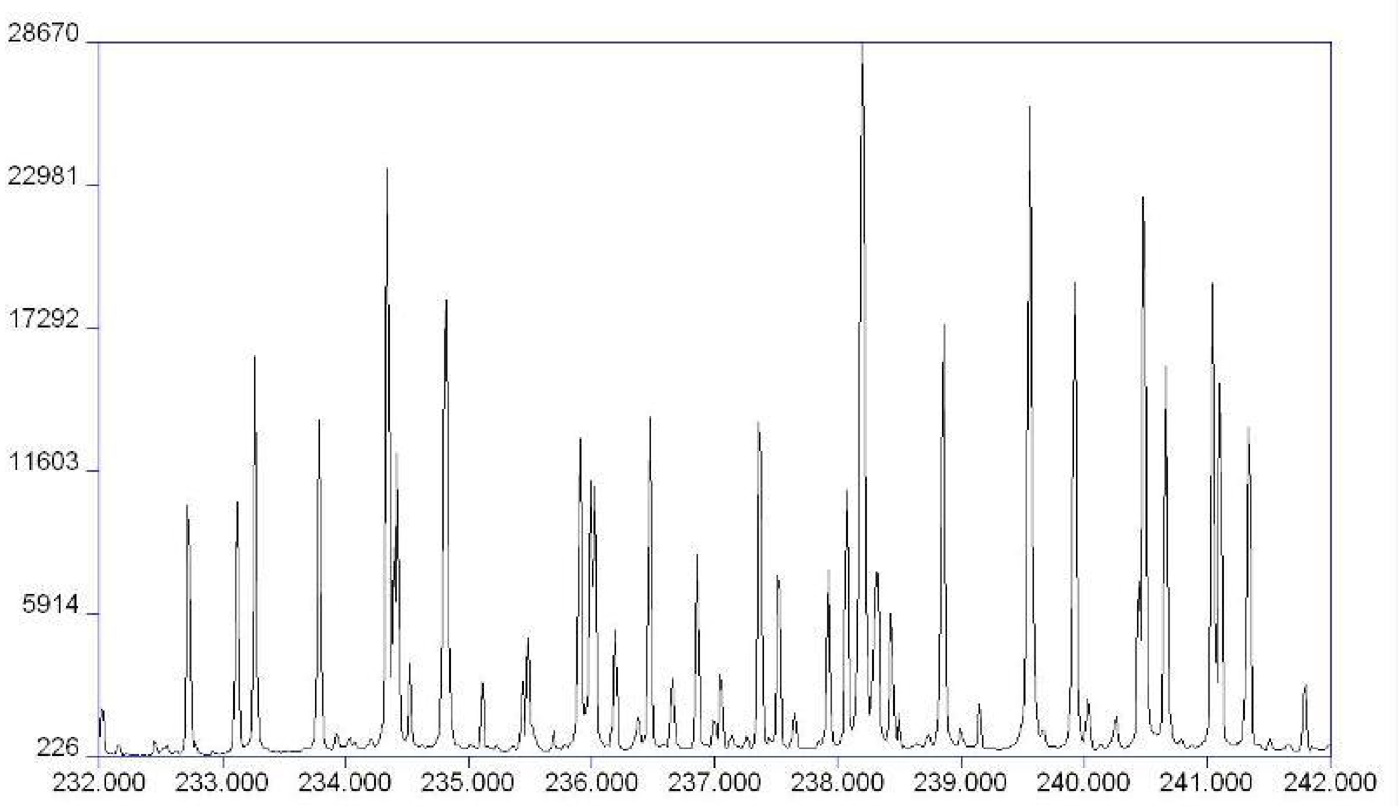
- confusing! better to write...

$$\ln\left(\frac{I\lambda}{gA}\right) = -{E}/{kT} - \ln\left(\frac{4\pi Z}{hcN_0}\right)$$

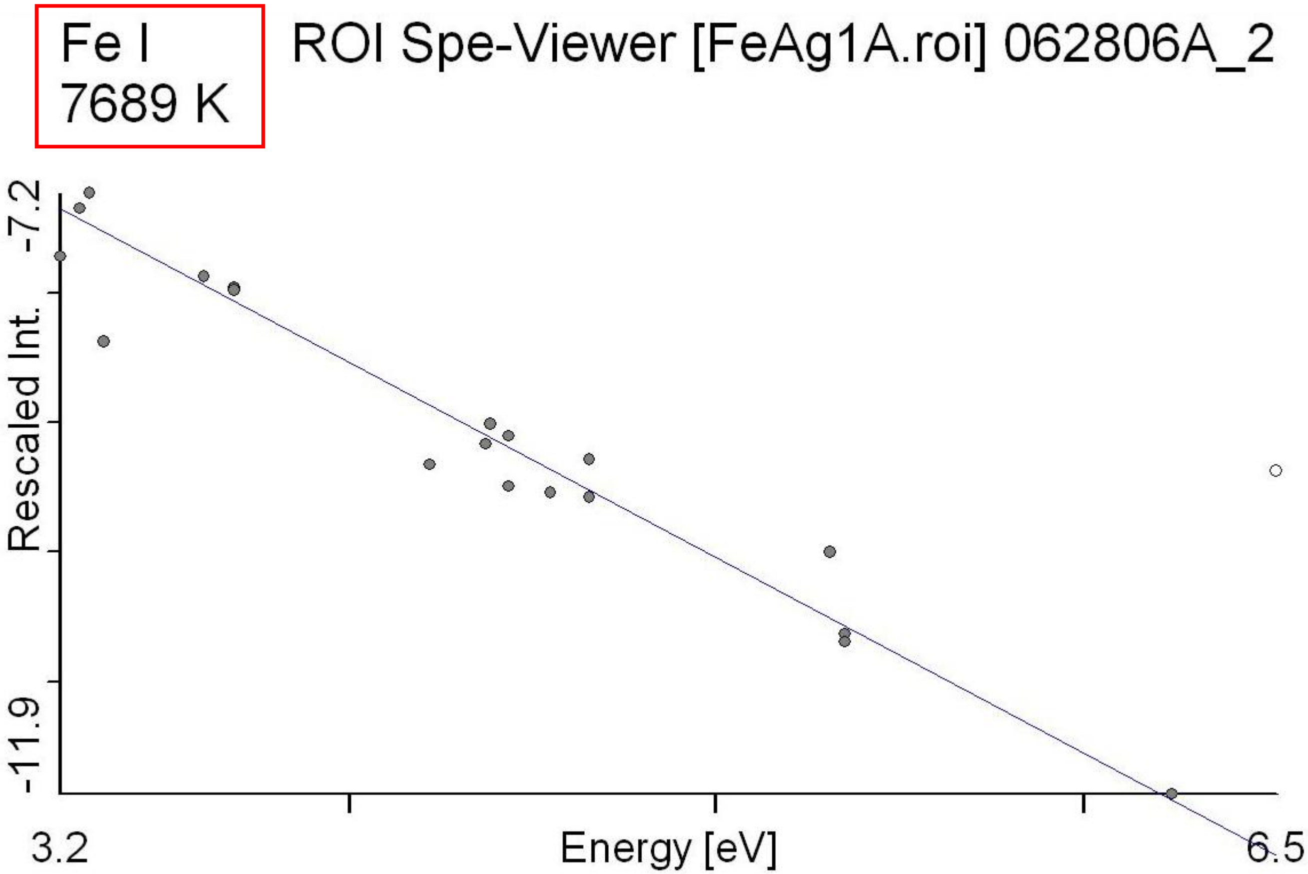
- This is a straight line with slope of $-1/kT$!

- So if we plot the adjusted measured line intensity vs. the upper state energy of transitions we can measure T of our plasma.

Fe_2O_3 / Ag Mixture



Fe Temperature



Boltzmann plot for 22 Fe transitions

Plasma Diagnostics

Temperature

plasma on water surface

Temperatures calculated from H_β / H_γ intensity ratio using Boltzmann equation:

$$\frac{I_1}{I_2} = \frac{g_1 A_1}{g_2 A_2} \frac{\lambda_2}{\lambda_1} \exp\left(- \frac{|E_1 - E_2|}{kT_e} \right)$$

Plasma Diagnostics

electron density

FWHM of Stark-broadened lines used to calculate electron density N_e

$$N_e = C\left(N_e, T\right)\Delta\lambda_{FWHM}^{3/2}$$

- N_e must be > $N_{e,crit}$

Physics of Plasma Formation: plasma shielding

eventually, the plasma becomes opaque to the laser beam and the target is shielded

occurs when plasma frequency becomes greater than the laser frequency

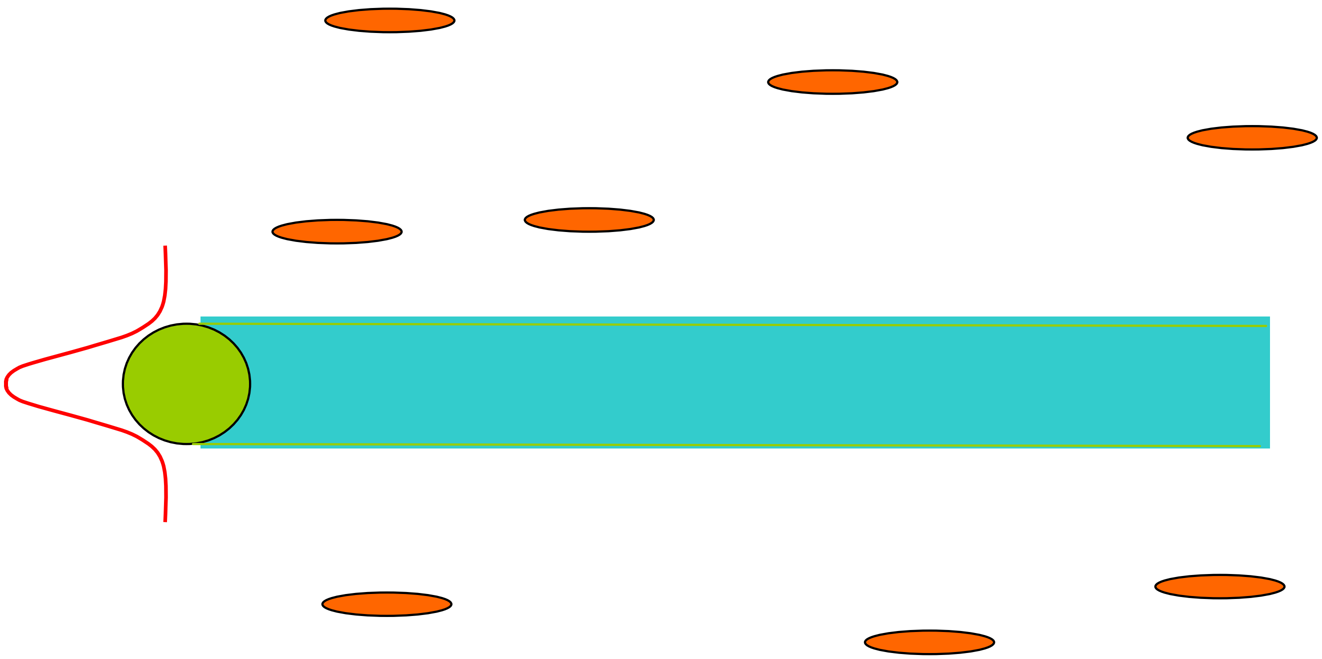
$$\omega_p \approx \omega$$

or when

$$n_e \sim \left(10^{21}/\lambda^2\right)\mathrm{cm}^{-3}$$

Other technologies…

- Evanescent wave fiber optic biosensor



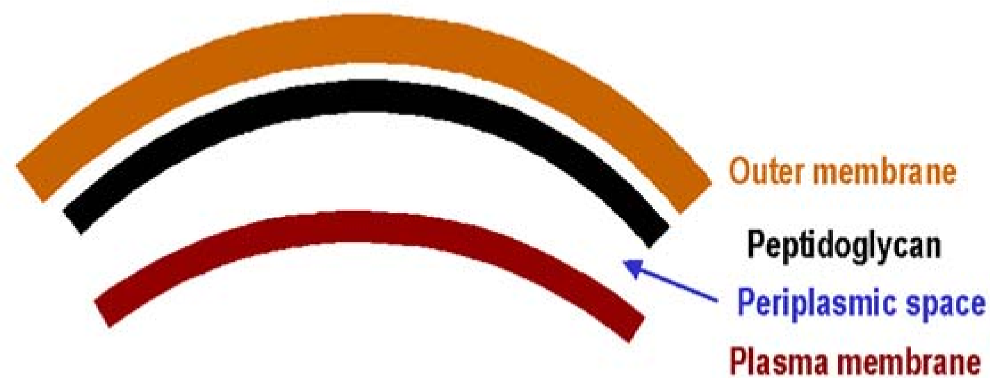
Bacteria

Prokaryote (no nucleus)

Gram-positive

- Thick cell wall
- No outer membrane
- No periplasm

Gram-negative



- Thin cell wall
- Outer membrane
- Periplasm

Example:

•*Escherichia coli* (Nino C, HF 4714, AB)
•*Pseudomonas aeruginosa*