Laser-Induced Breakdown Spectroscopy (LIBS): A Future Super Star of Atomic Spectrometry and Its Application to Rapid Bacteria Identification

Colorado State University, Feb. 11th, 2008

Steven J. Rehse
Department of Physics and Astronomy

WAYNE STATE UNIVERSITY

…or…

"what would happen if they held a conference and nobody came…?"

(DAMOP 2007)

Saturday, June 9, 2007 8:00AM – 9:24AM –
Session W6 Optical Diagnostics and Characterization TELUS Convention Centre Macleod BC

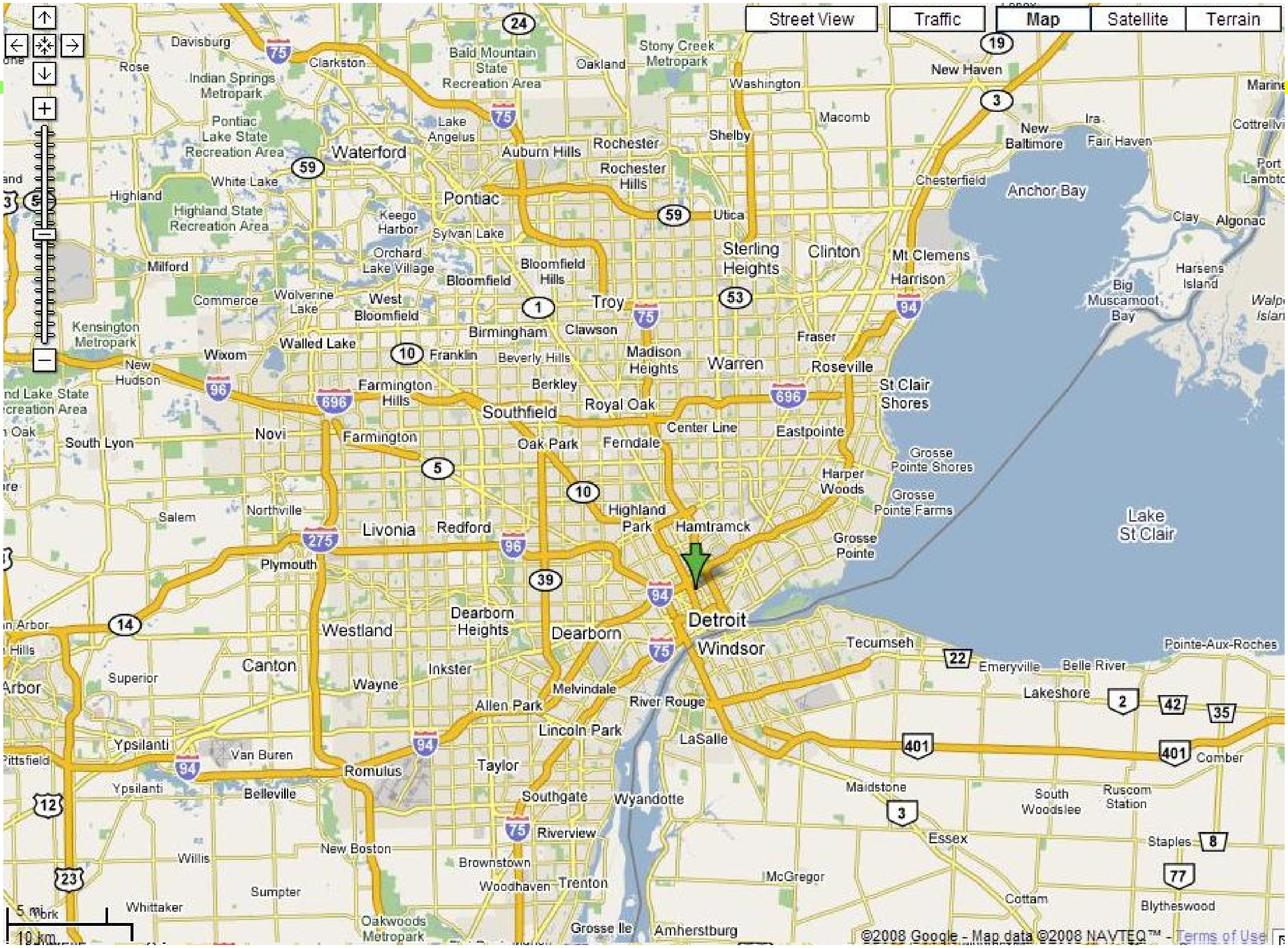
8:00AM W6.00001 Optical Absorption in Artificial Atoms, YIMING MI, School of Materials Engineering, Shanghai University of Engineering Science, YUICHI IWATA, RACE, The University of Tokyo, RACE, THE UNIVERSITY OF TOKYO COLLABORATION — Optical transitions in an artificial atom (AA) interacting with longitudinal optical phonons are studied theoretically, which can be solved exactly under the condition of finite number of carrier levels in the system. The linear optical properties are calculated, and the obtained results are compared with the ones of other theoretical model. Perhaps, the acquired theoretical results are of great interest and would get precise validations experimentally in near future.

8:12AM W6.00002 Energy relaxation and dephasing in CARS, SVETLANA MALINOVSKAYA, Department of Physics and Engineering Physics, Stevens Institute of Technology, Hoboken, NJ 07030 — We use the adiabatic passage control scheme developed for the CARS spectroscopy to investigate the energy and phase relaxation as factors in the optical polarization decay. We show that in strong fields the dynamics of induced polarization via adiabatic passage is dependent on phase relaxation and to less extent on energy relaxation for characteristic times close to pulse duration. We justify our conclusions with the dressed state dynamics analysis.

8:24AM W6.00003 Continuous Bremsstrahlung in Trojan atoms and molecules, MATT KALINSKI, Utah State University — We present a fully relativistic approach to the electron radiation in Trojan atoms, atoms of hydrogen in circularly polarized electromagnetic field. Unlike for the normal scattering event the Bremsstrahlung is the continuous process and the cyclotronic radiation due to circular pseudo-scattering, when the electron is internally excited. Depending on the electromagnetic coupling order and the relativistic v/c order the corrections can be interpreted as the native spontaneous emission and the Unruh-Davies effect. All contributions have the classical correspondence in equivalent parts of Lienard-Wiechert potentials.

8:36AM W6.00004 *Escherichia coli* identification and strain discrimination using nanosecond laser-induced breakdown spectroscopy, STEVEN REHSE, JONATHAN DIEDRICH, Wayne State University, Department of Physics and Astronomy, SUNIL PALCHAUDHURI, Wayne State University, Department of Immunology and Microbiology — Three strains of *Escherichia coli*, one strain of black mold and one strain of *Candida albicans* yeast have been analyzed by laser-induced breakdown spectroscopy (LIBS) using nanosecond laser pulses. All microorganisms were analyzed while still alive and with no sample preparation. Nineteen atomic and ionic emission lines have been identified in the spectrum, which is dominated by calcium, magnesium and sodium. A discriminant function analysis (DFA) has been used to discriminate between the bio-types and *E. coli* strains. This is the first demonstration of the ability of the LIBS technique to differentiate between different strains of a single species.

8:48AM W6.00005 Recombination fluorescence in ultracold plasmas, SCOTT BERGESON, Brigham Young University, FRANCIS ROBICHEAUX, Auburn University — The expansion dynamics of ultracold neutral plasmas are determined by electron physics. Three-body recombination and electron-Rydberg scattering heat the plasma electrons at early times and drive the expansion. The details of these processes are well understood in weakly-coupled plasmas. However, these processes may proceed differently in strongly-coupled neutral systems. We present a study of recombination fluorescence in ultracold plasmas. At low densities, we find good agreement between theory and experiment. At higher densities, theory and experiment diverge.


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

Outline

1. Introduction to LIBS

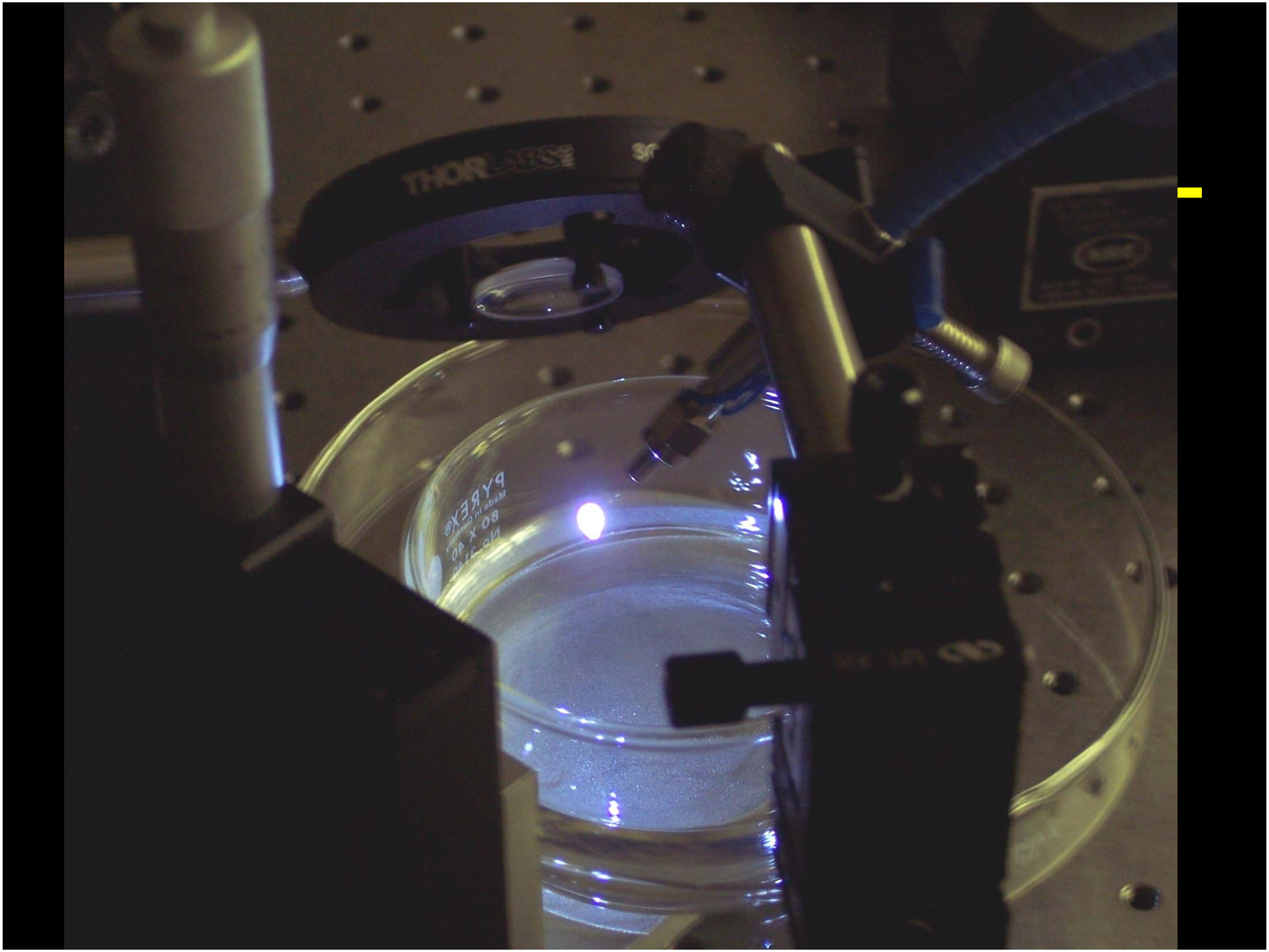
2. Potential Applications

3. My Applications
   - trace contaminants in simulated tissue
   - <u>identification/discrimination of bacteria</u>

LIBS Defined

One sentence?

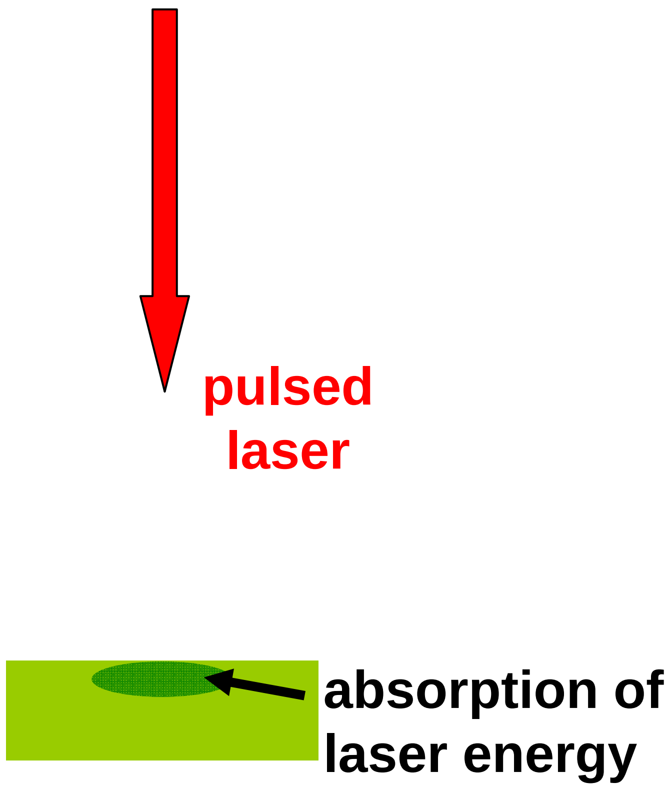
A spectrochemical technique which utilizes an intense laser pulse to determine the atomic/elemental composition of a sample via generation of a high-temperature micro-plasma followed by time-resolved optical spectroscopy.


THORLABS

The LIBS Process

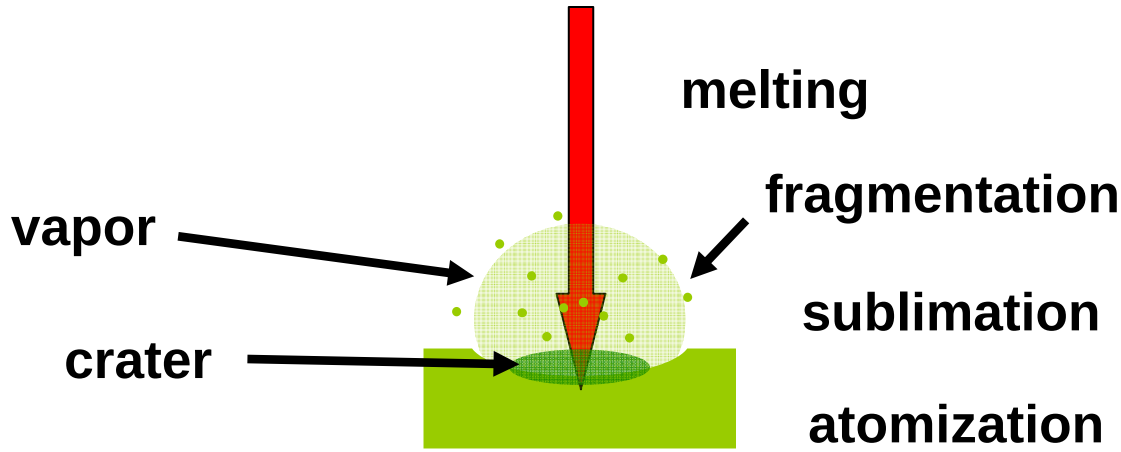
1. laser interaction with the target
2. removal of samples mass (ablation)
3. plasma formation (breakdown)
4. element specific emission

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.

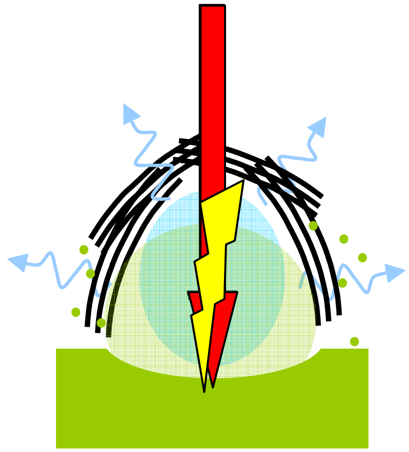
2) removal of samples mass (ablation)



- 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.

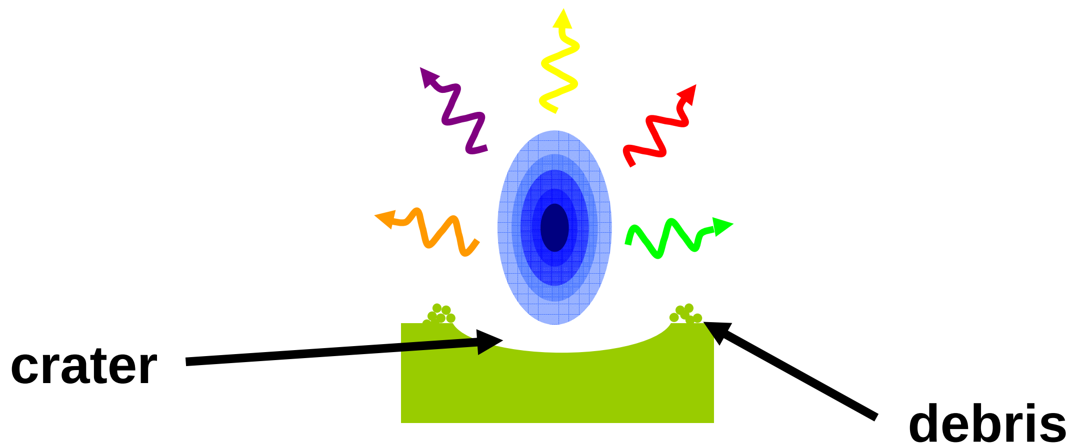
3) plasma formation (breakdown)



- 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.

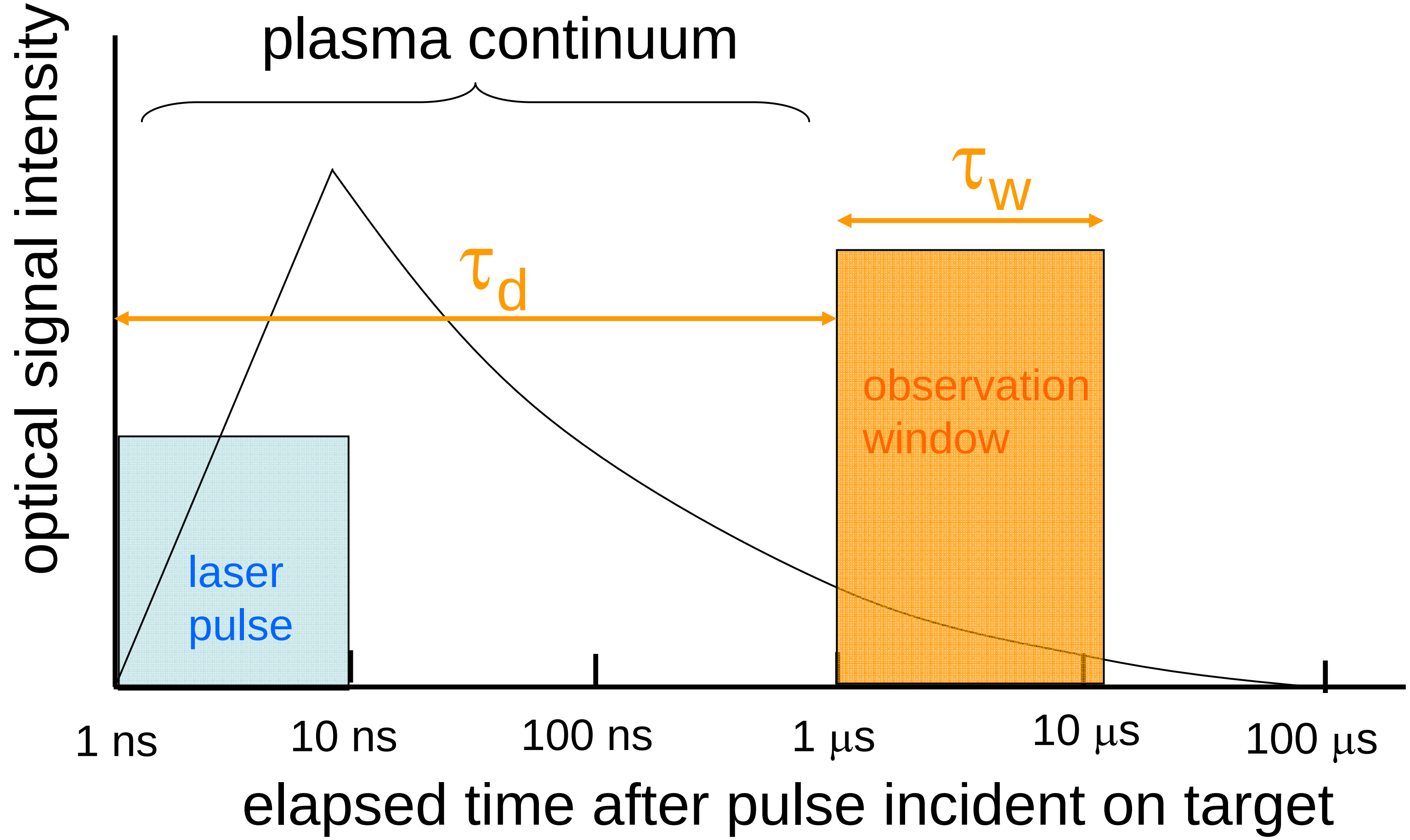
4) element specific emission (atomic or ionic)

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.

Temporal History of a LIBS Plasma



3 Current “Super-Stars” of Atomic Spectroscopy

1. electrothermal atomization-atomic absorption spectrometry (ETA-AAS)

2. inductively couple plasma-atomic emission spectrometry (ICP-AES)

3. inductively coupled plasma-mass spectrometry (ICP-MS)

Advantages of LIBS

1) extremely fast analysis compared to competing technologies

2) multi-elemental analysis, light from all constituents collected without bias

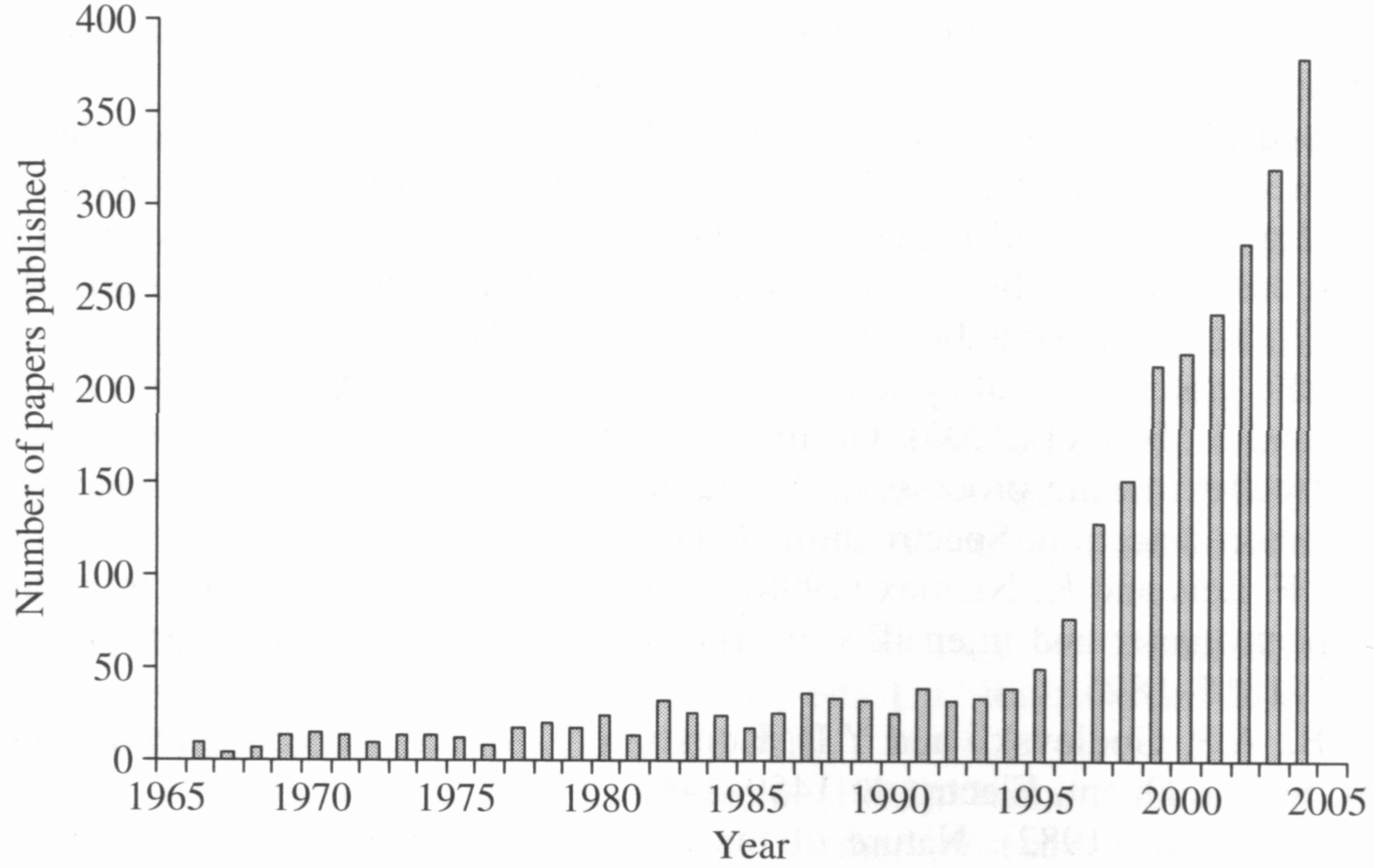
3) analysis can be performed at standoff distances

4) technique is applicable to all substrates (gas, solid, and liquid)

5) requires minimal or no sample prep

6) exquisite spatial resolution, ~1 μm

LIBS Publications



What's Driving the Interest in LIBS?

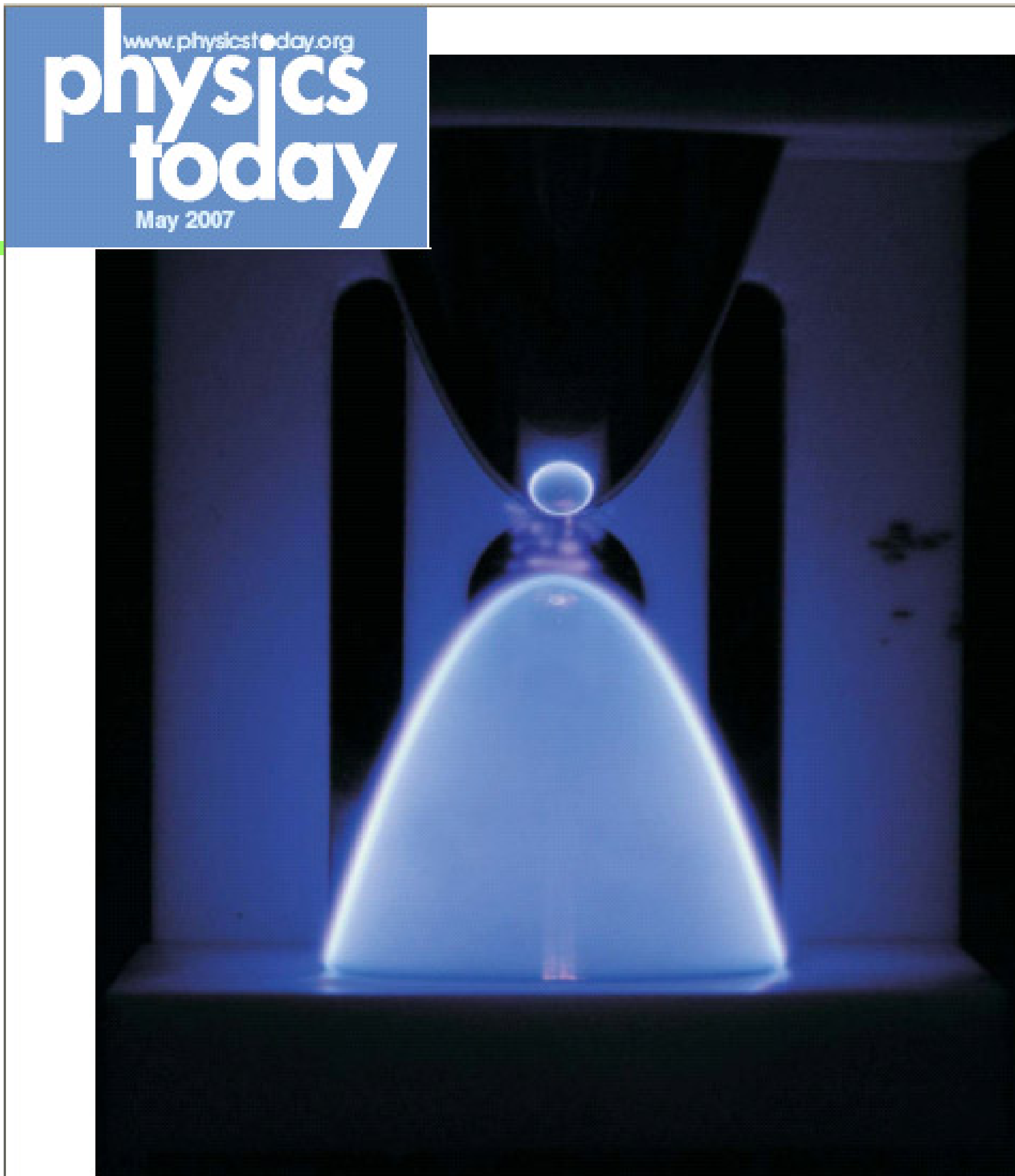
- mid-80's: reliable, small, inexpensive lasers
- mid-80's: intensified charge-coupled devices (ICCD)
- 90's – 00's: femtosecond pulsed lasers
- 90's – 00's: broadband spectrometers and Echelle spectrometers
- 00's: microchip lasers

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”)



A Stark look at
plasma 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

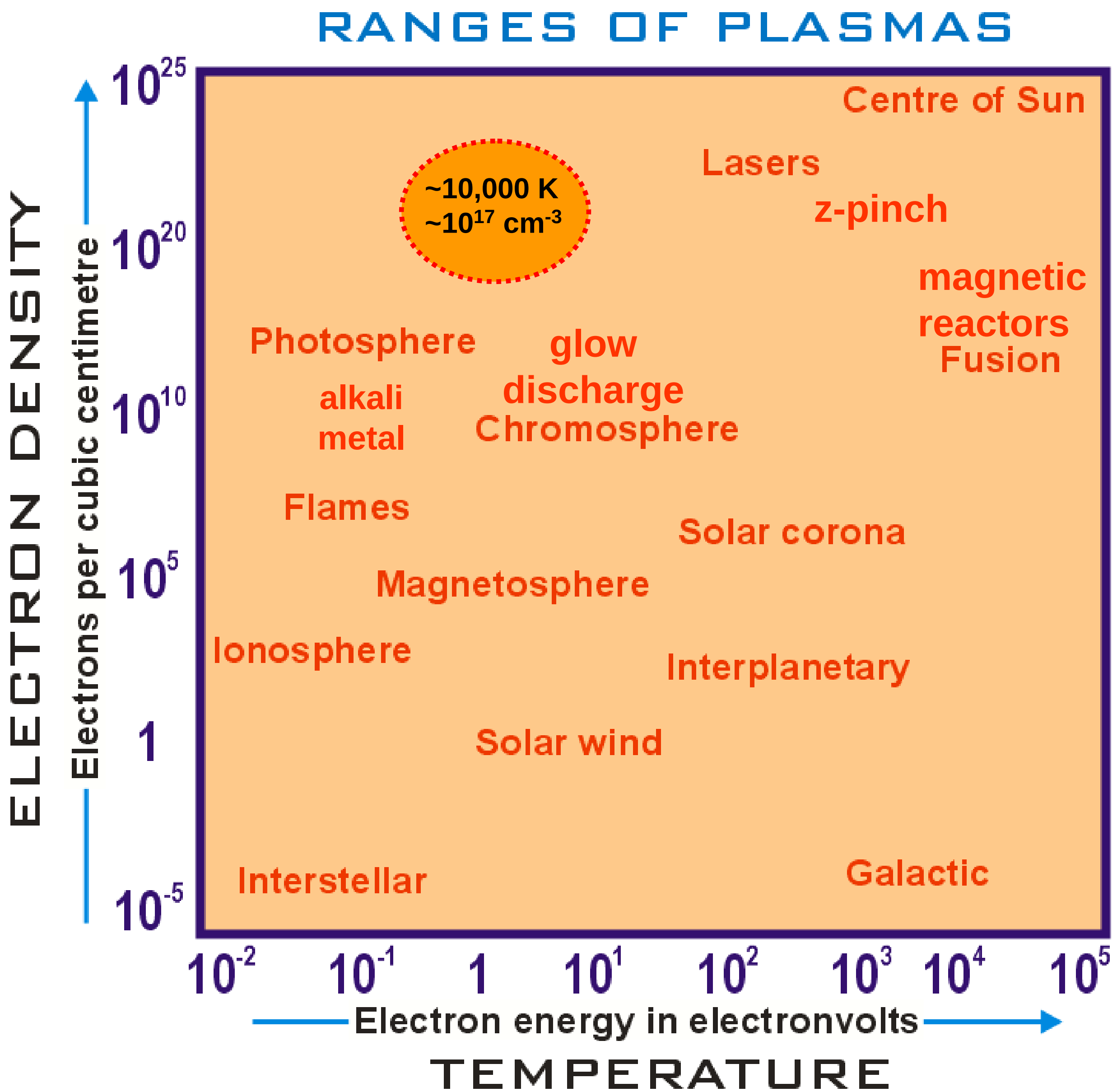
Physics of Plasma Formation: breakdown

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

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


RANGES OF PLASMAS
ELECTRON DENSITY
Electrons per cubic centimetre
10^25
10^20
10^10
10^5
1
10^-5
~10,000 K
~10^17 cm^-3
Centre of Sun
Lasers
z-pinch
magnetic
reactors
Fusion
Photosphere
glow
discharge
alkali
metal
Chromosphere
Flames
Solar corona
Magnetosphere
Ionosphere
Interplanetary
Solar wind
Interstellar
Galactic
10^-2
10^-1
1
10^1
10^2
10^3
10^4
10^5
Electron energy in electronvolts
TEMPERATURE

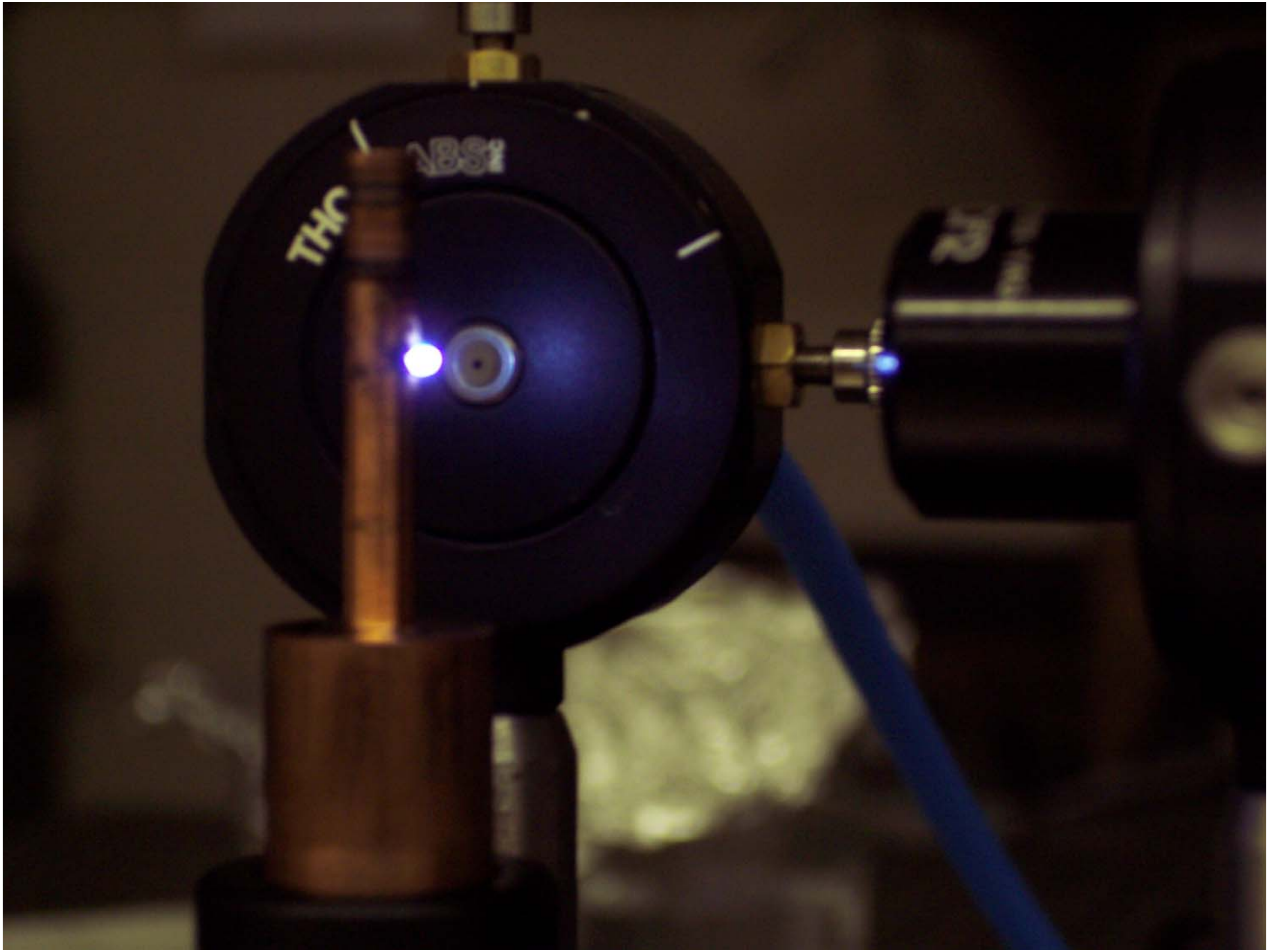
Physics of Plasma Formation: breakdown

3. "breakdown" is arbitrarily defined

n_e~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

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



The Goal of LIBS Plasma Creation

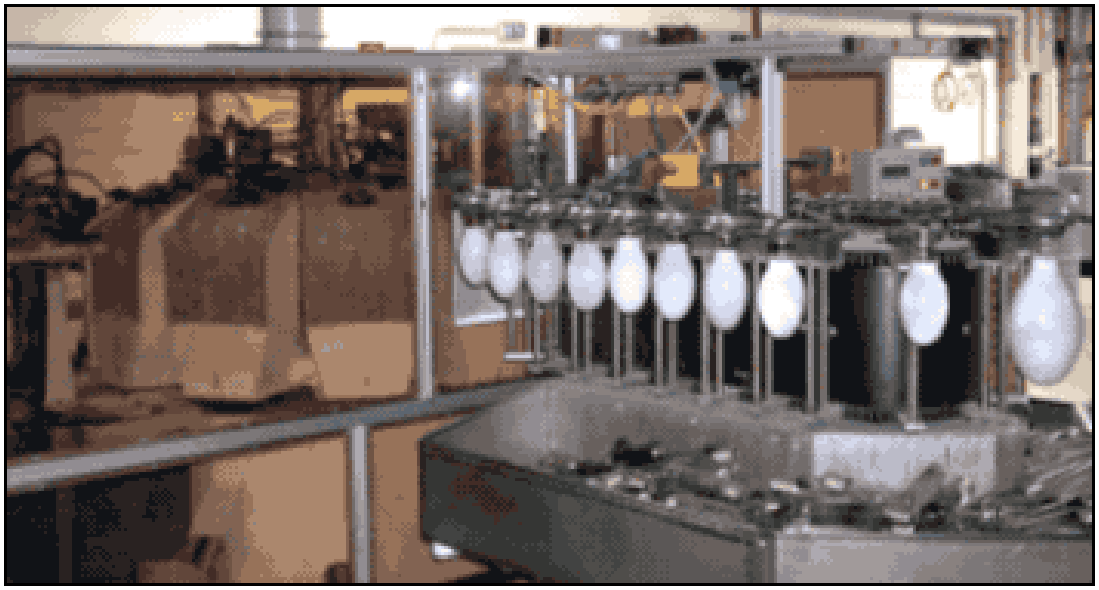
- to create an optically thin plasma which is in thermodynamic equilibrium and whose elemental composition is the same as that of the sample
  - if achieved, spectral line intensities can be connected to relative concentrations of elements
  - typically these conditions are only met *approximately*.

The Uses of LIBS

- industrial processes
  - analysis of steam generator tubes in nuclear power stations
  - grading of powered pellets for glass melts
  - analysis of treated wood in recycling centers
  - grading of iron-ore slurry prior to pelletizing
- environmental analysis
  - quantification of heavy metal content in soils, sand, and sludge
  - measurement of lead content in paint
  - waster quality assessments
  - hazardous waste remediation
  - atmospheric sampling
- biology
  - hair and tissue mineral analysis
  - identification of trace metals in teeth
  - spectral fingerprinting of bacterial strains
  - identification of bacterial spores, molds, pollens and proteins
- defense/homeland security
  - detection of uranium in material,
  - high sensitivity detection of chemical and biological agents
  - *in situ* detection of land mines
- forensic science
  - identifying gunshot residue on hands
  - pen ink characterization
- art conservation
  - identifying pigments in paintings
  - dating/cleaning ancient marble

Pharma-LIBS
for pharmaceutical slurry
analysis

on-line iron ore slurry additive
measurement



recycling of lamp glasses at WEREC GmbH

courtesy of LLA Instrumetns GmbH

courtesy of Applied Photonics Ltd, U.K.

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

Backpack contains broadband high-resolution spectrometer, laser power supply, computer, and battery

Head's-up display

Hand-held probe contains laser, joystick for control, and focus optics

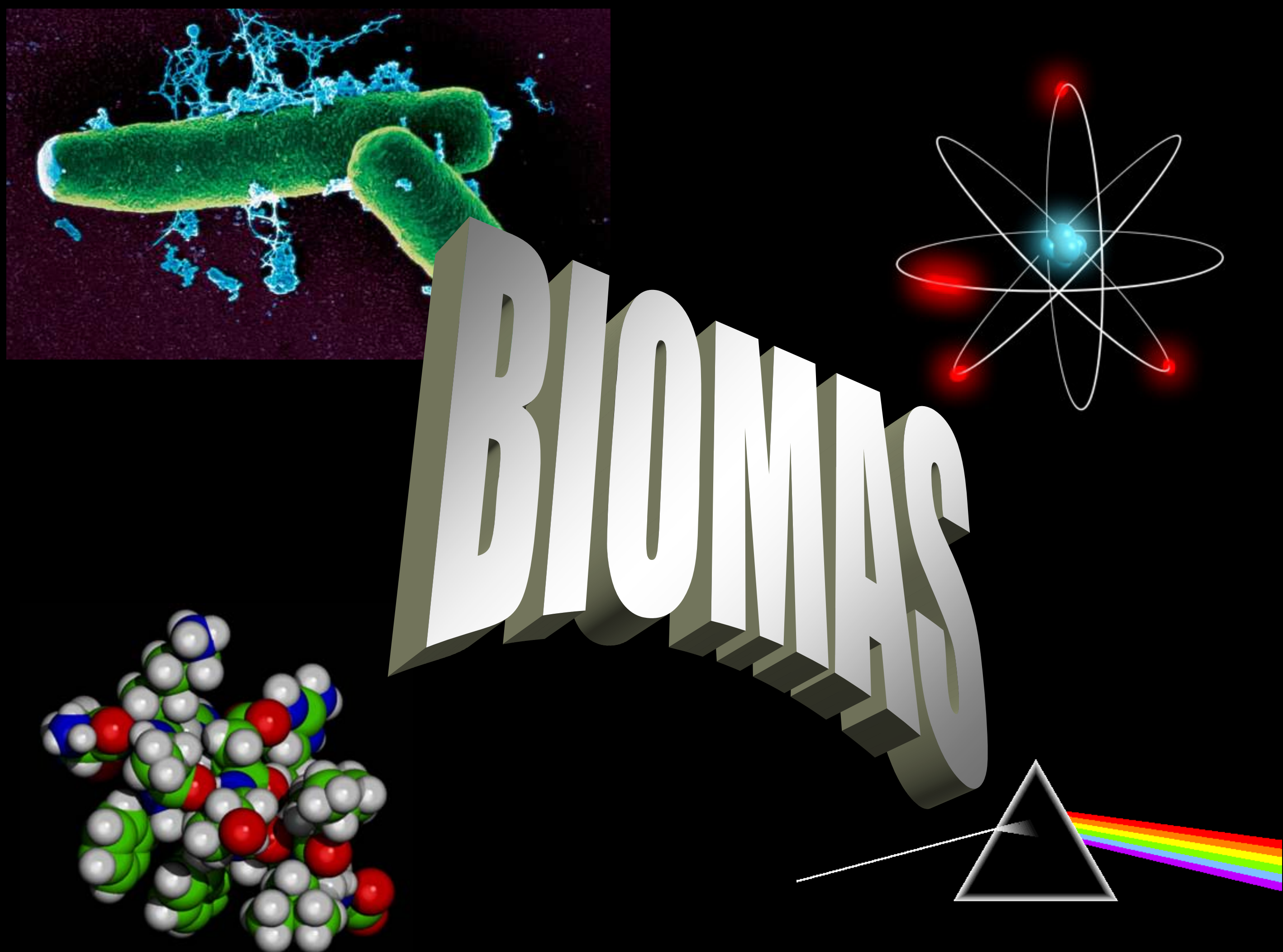
Microplasma/ LIBS Event

courtesy of Ocean Optics.

Detection and Discrimination of Bacteria Strains

The BIOMAS Project:

Bacteria Identification by Optical, Molecular, and Atomic Spectroscopy

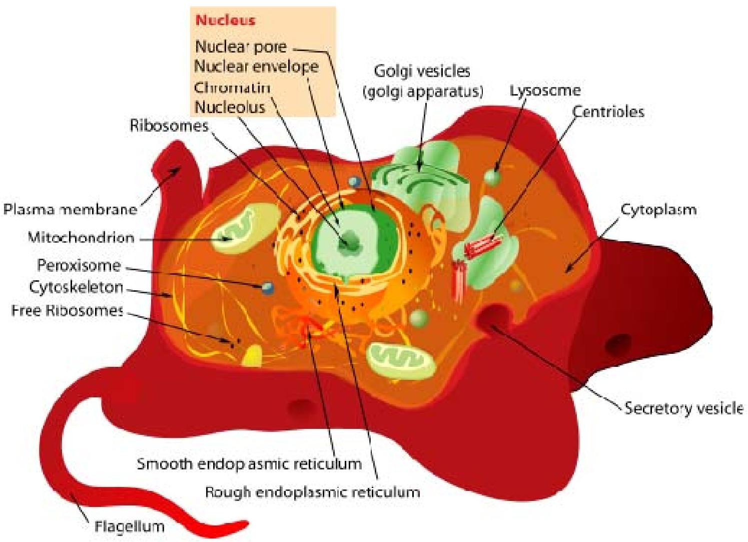


Motivation

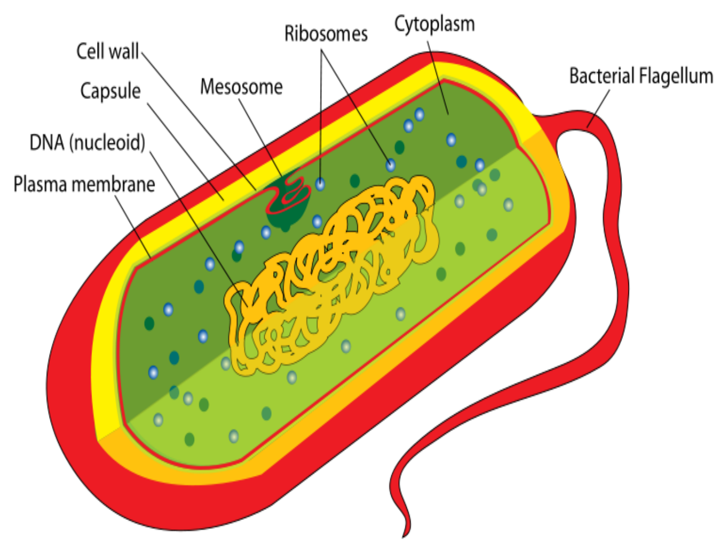
- Require a real-time early-warning detection technology for bio-agents (bacteriological)
  - other applications: EH&S, food inspection, clinical
- Downside of competing technologies:
  - speed
  - target-specific (shelf-life?)
  - expertise required

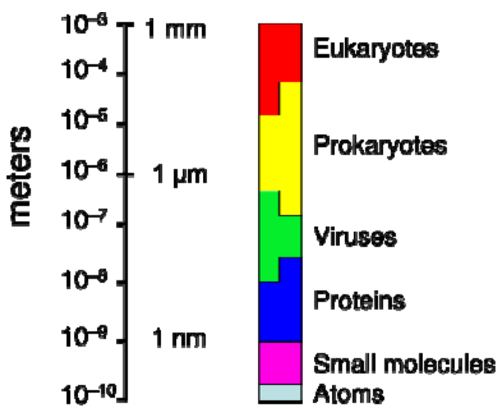
Types of Cells

Eukaryote



Prokaryote





www.en.wikipedia.org

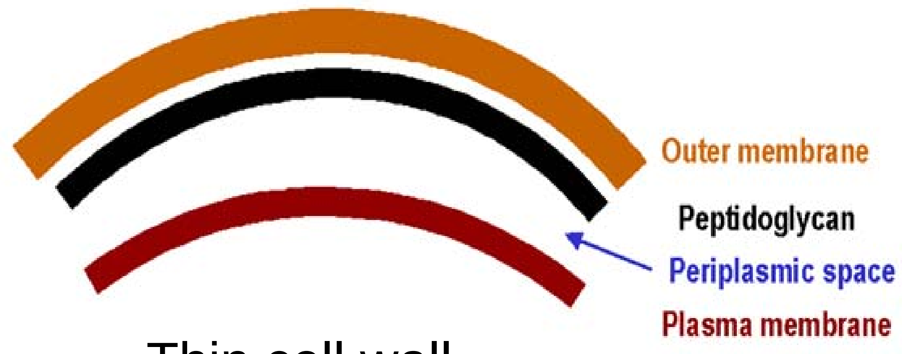
Bacteria

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

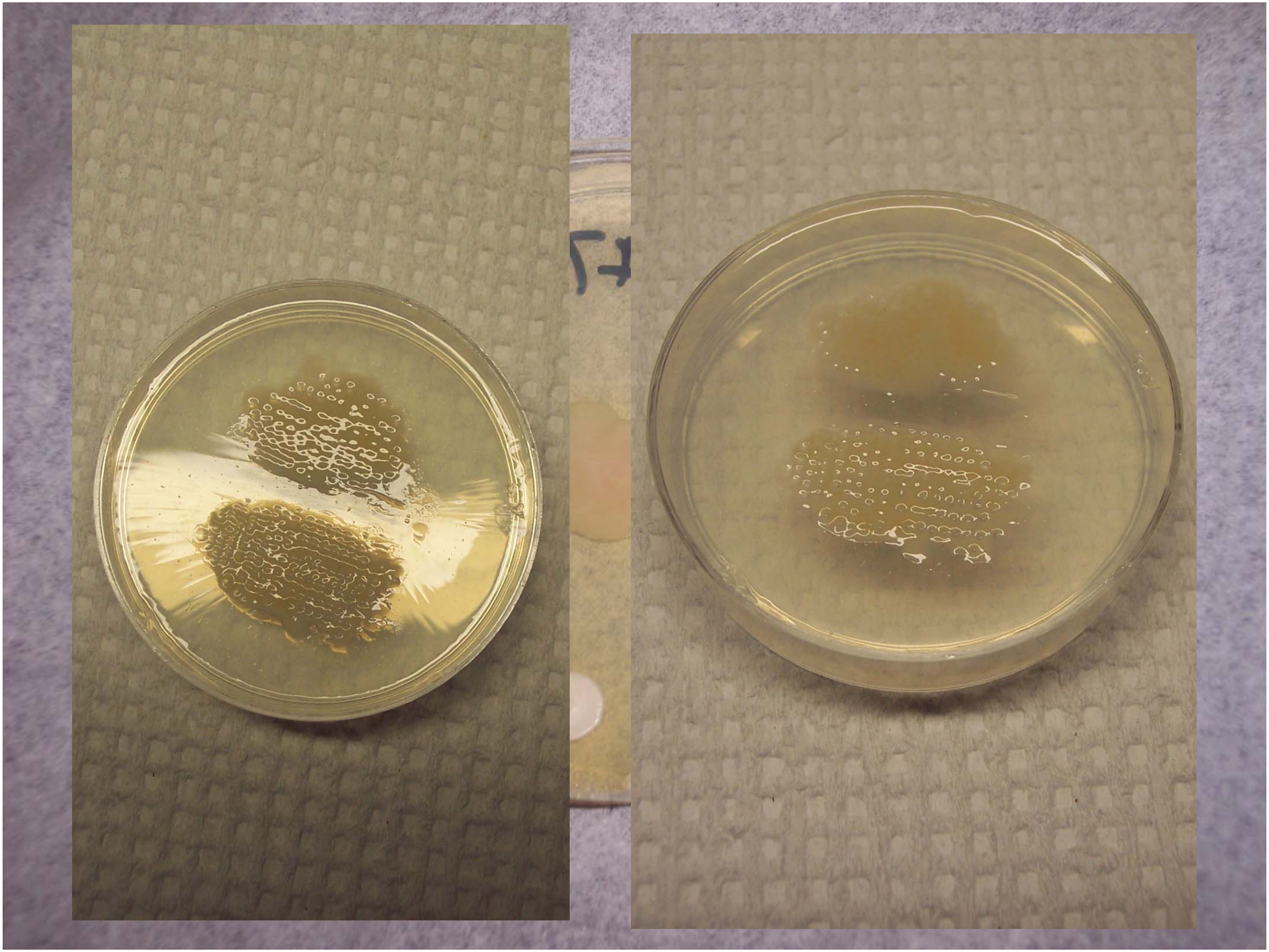
Escherichia coli

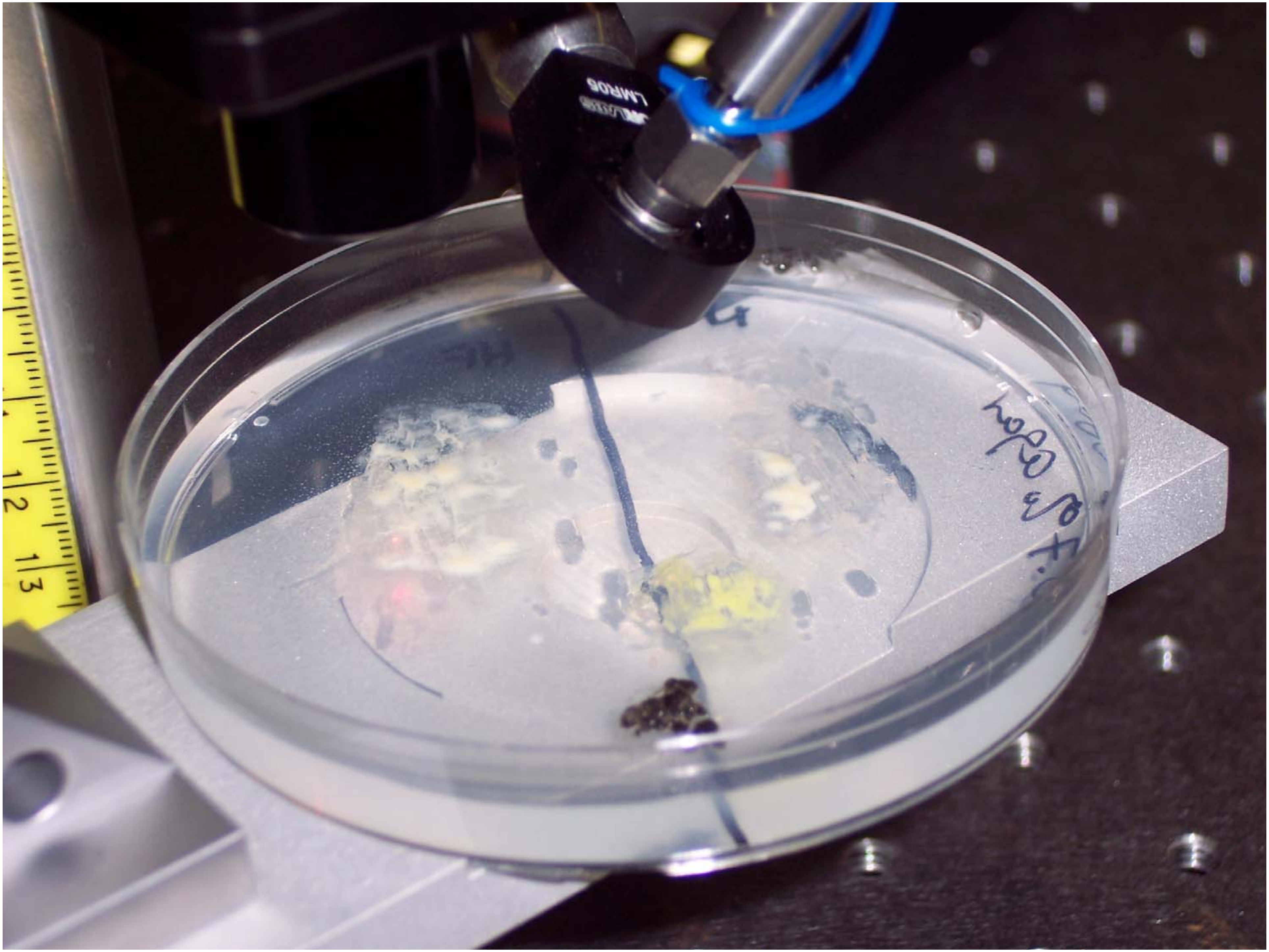
- Very common laboratory micro-organism
- Has many strains, most harmless, some pathogenic
- EHEC or *E. coli* 0157:H7 causes kidney failure in children (hemolytic uremic syndrome)

Inorganic Composition of *E. coli*

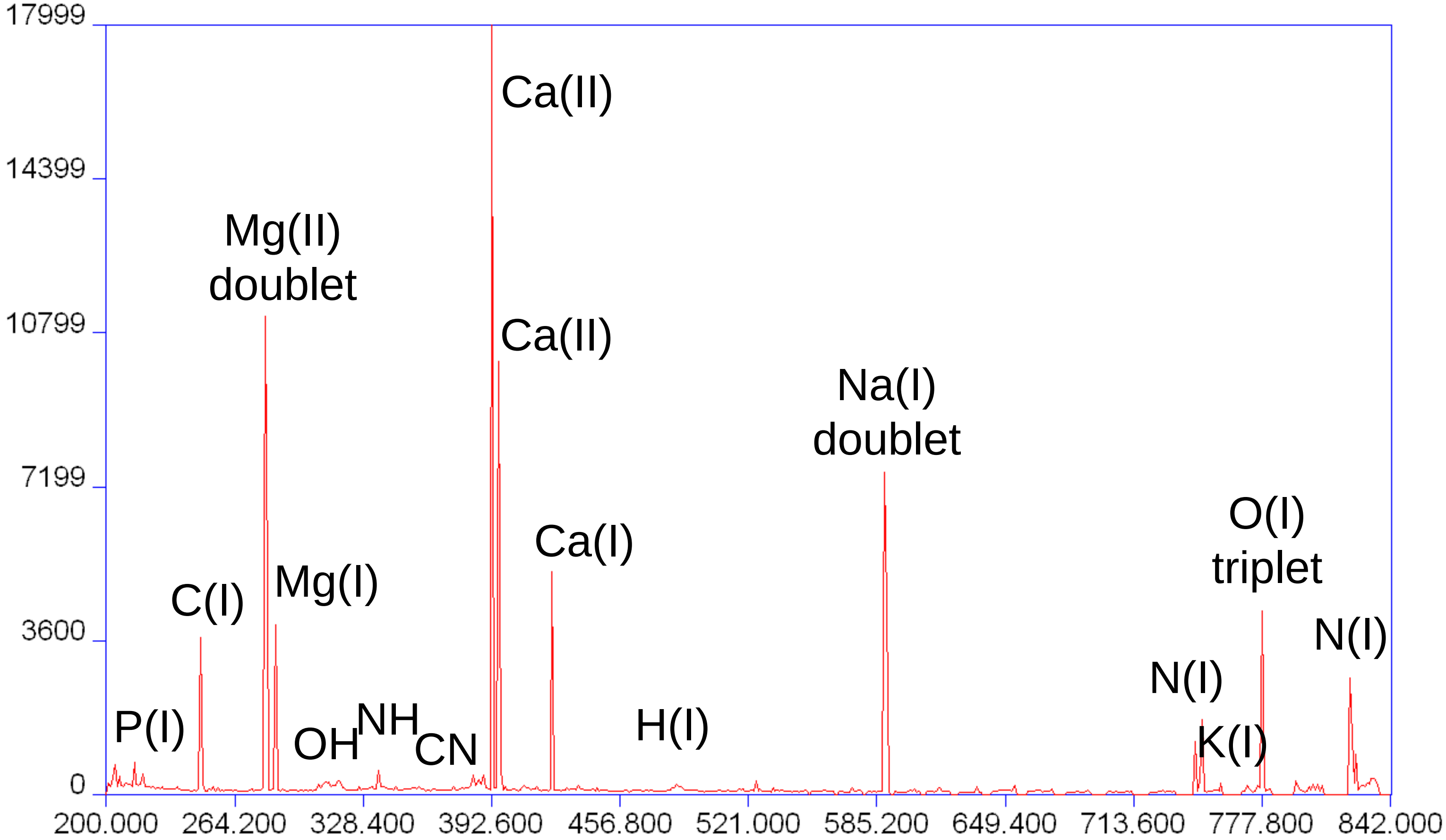
from “*The Bacteria: A Treatise on Structure and Function*” I.C. Gunsalus and R.Y. Stanier, eds

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




LMR05

E. coli Spectrum



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)

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

one entire LIBS spectrum reduced to this

Canonical Discriminant Functions

- DFA constructs *N*-1 “Canonical Discriminant Functions”
  - essentially the eigenvectors of the system
  - use the eigenvalues to rate the importance of the canonical discriminant functions

$$DF^{1} = \left[b_1^1 b_2^1 b_3^1 \ldots b_{19}^1 \right]$$

$$DF^{N-1} = \left[b_1^{N-1} b_2^{N-1} b_3^{N-1} \ldots b_{19}^{N-1} \right]$$

decreasing importance to the overall discrimination.

Discriminant Functions Scores

- Using the *N*-1 Canonical Discriminant Functions, *discriminant function scores* are constructed

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

discriminant function (eigenvector)

experimental data

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

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

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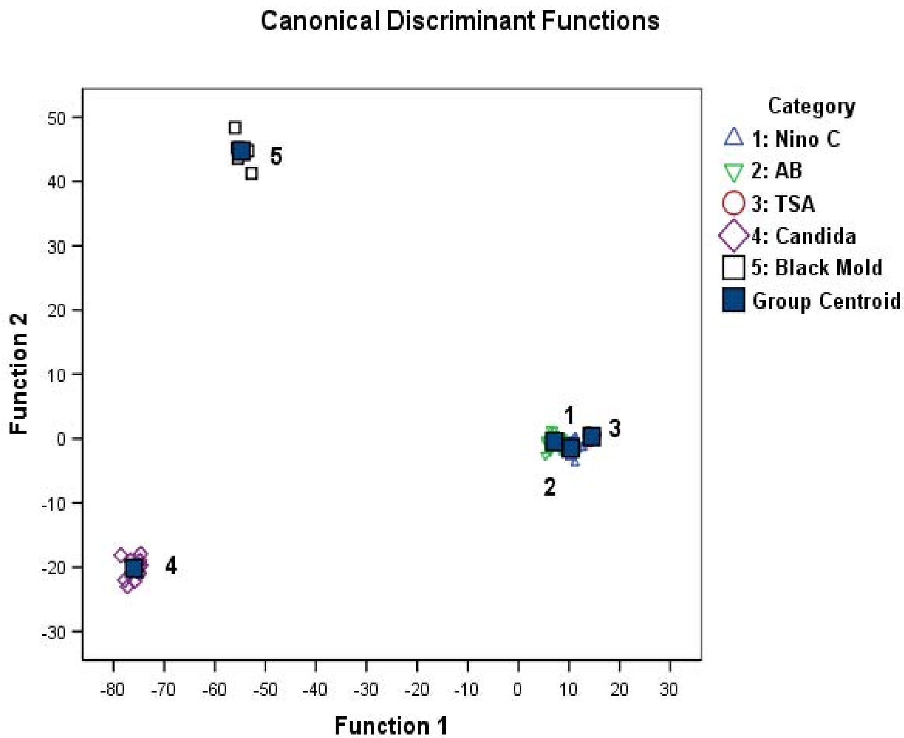
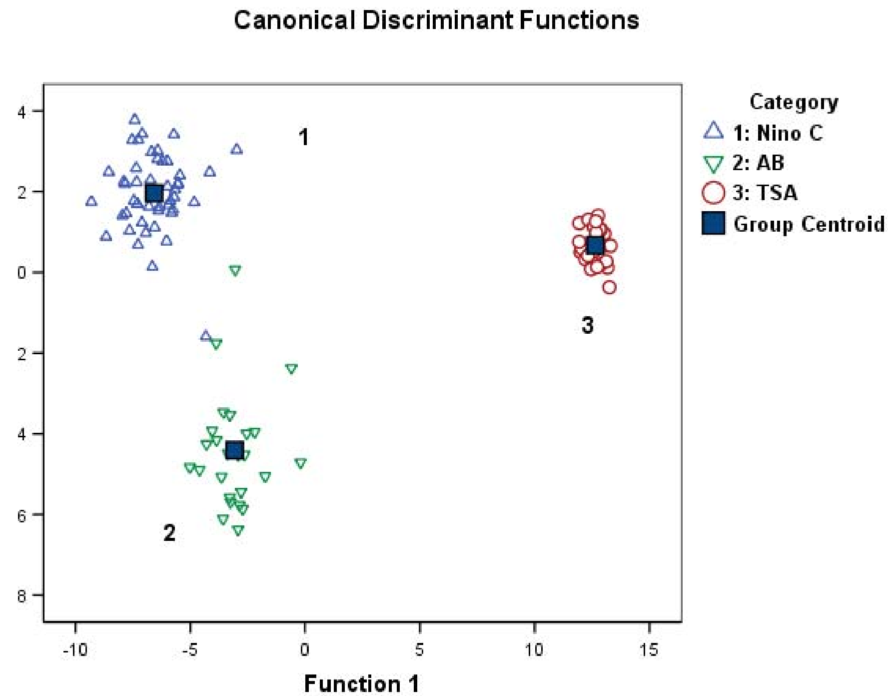
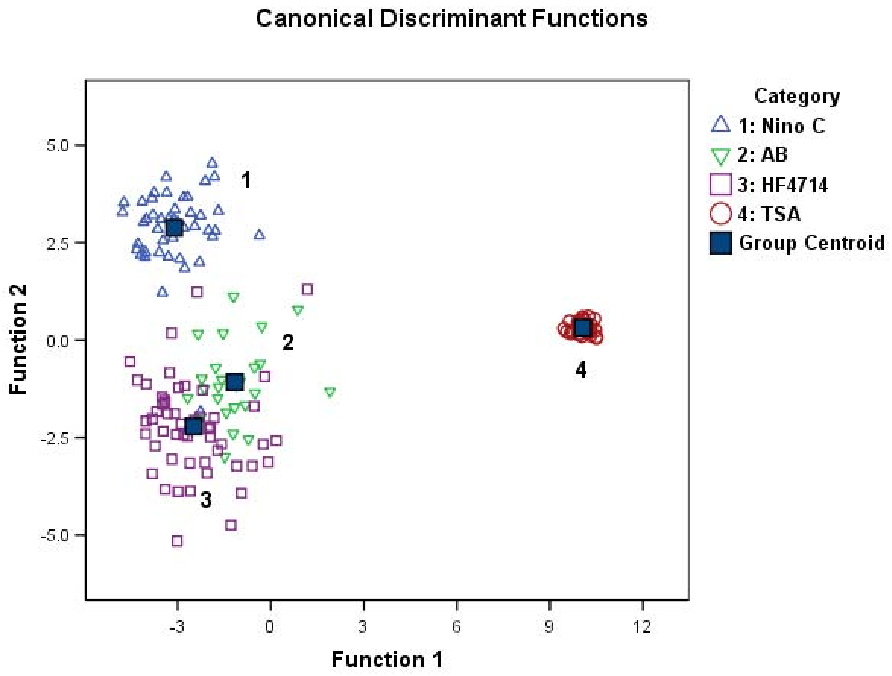
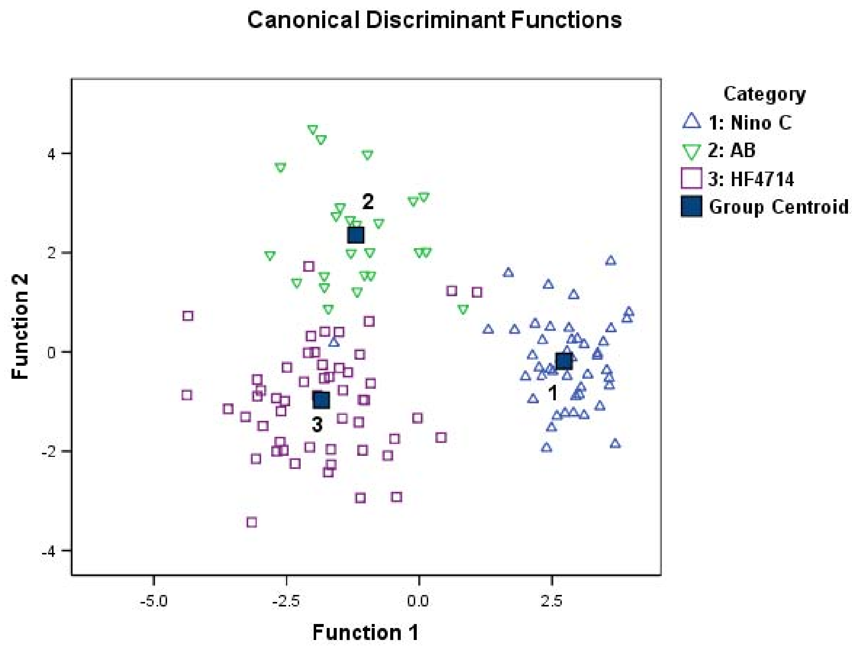




E. coli Results





JOURNAL OF APPLIED PHYSICS 102, 014702 (2007)

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

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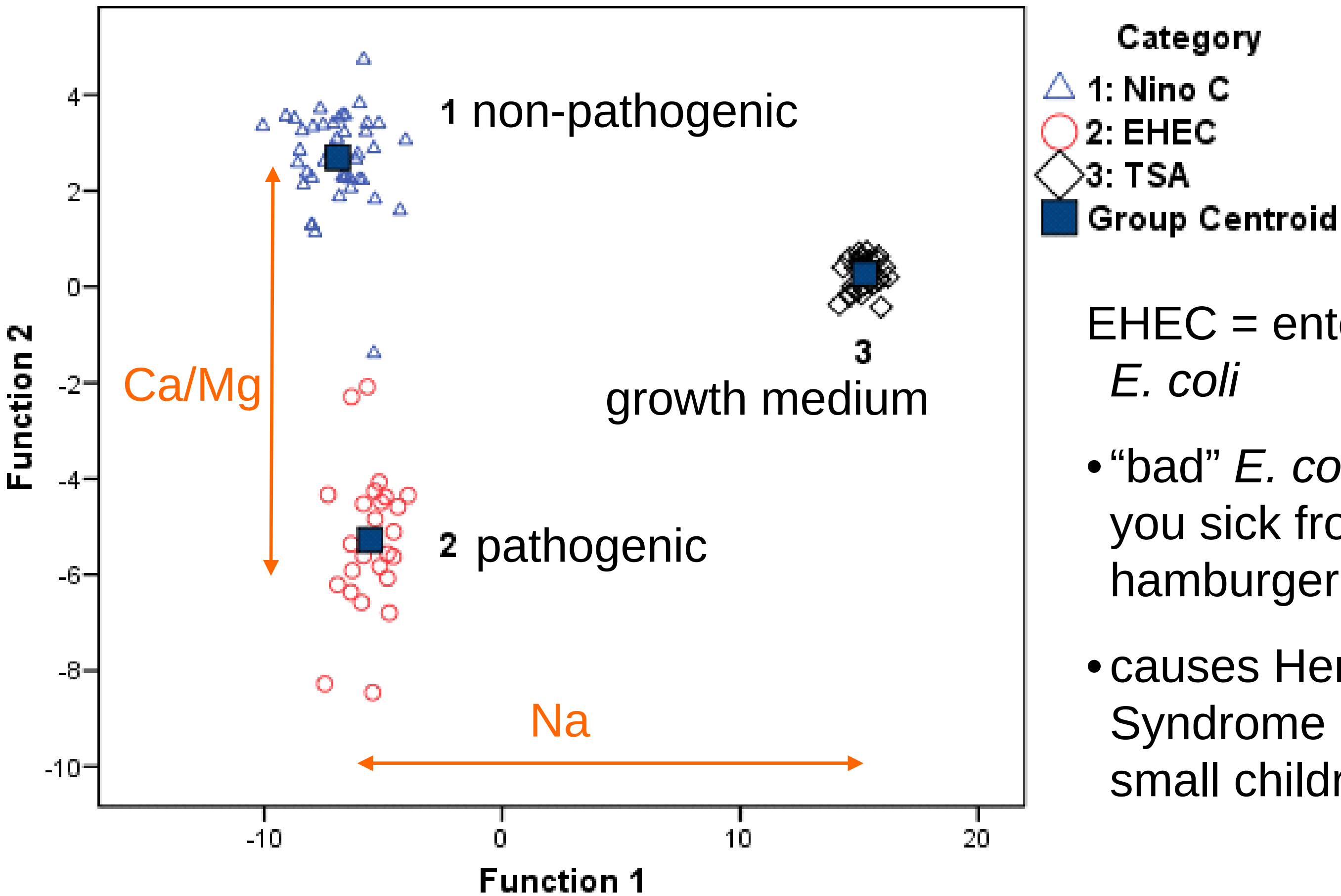
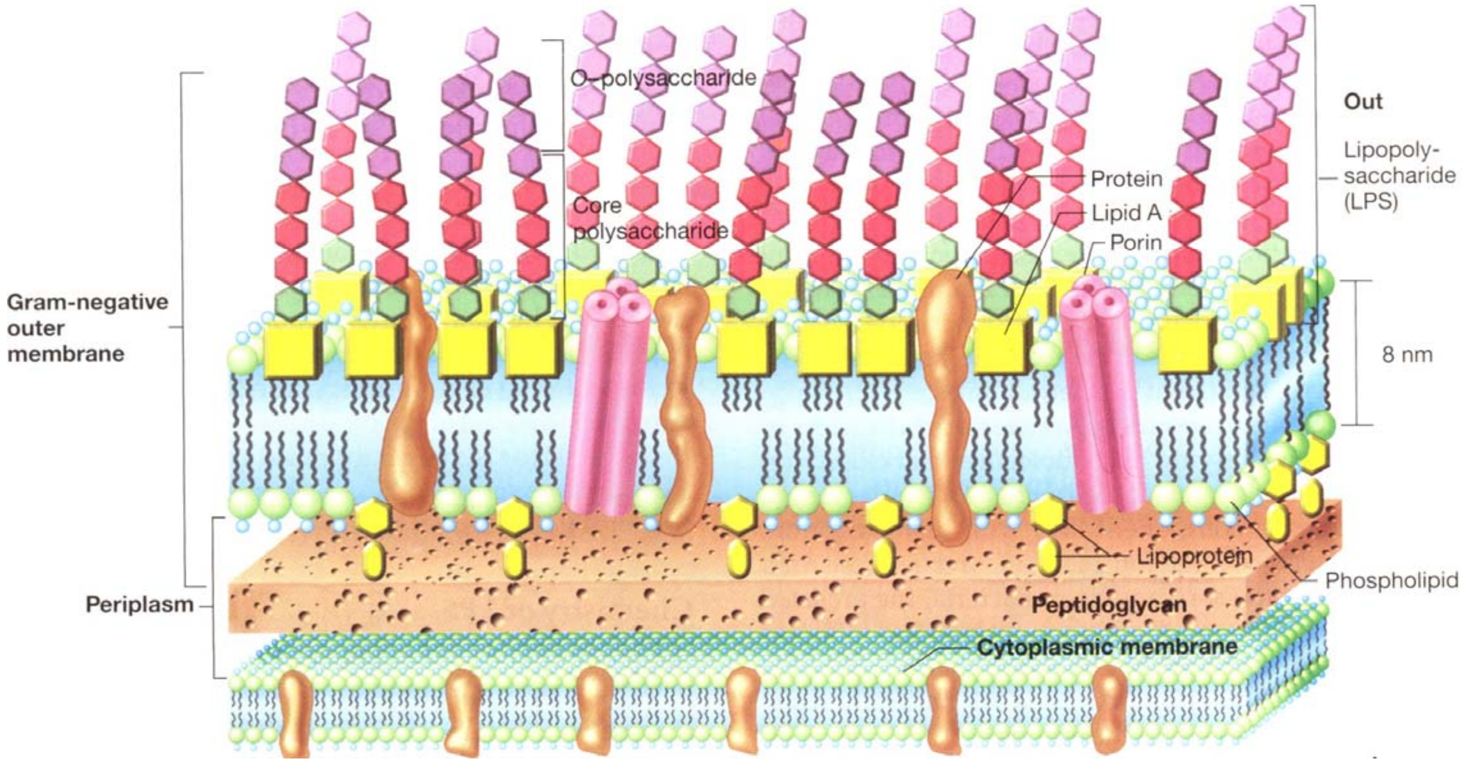
(Received 7 February 2007; accepted 28 May 2007; published online 5 July 2007)

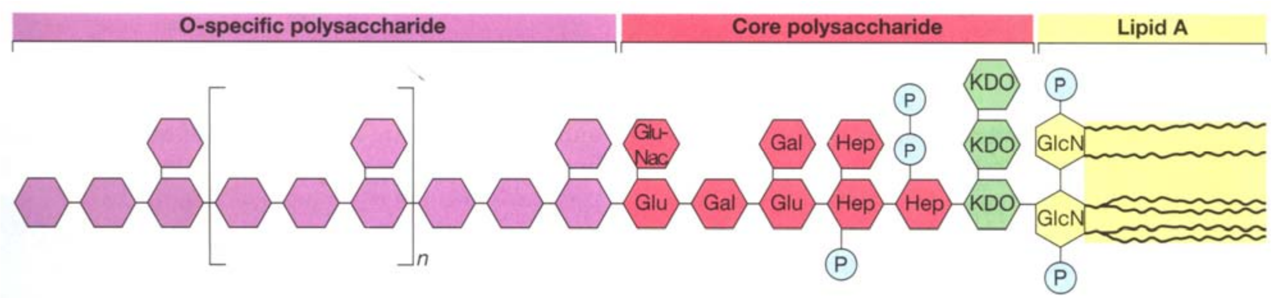


EHEC = enterohemorrhagic *E. coli*

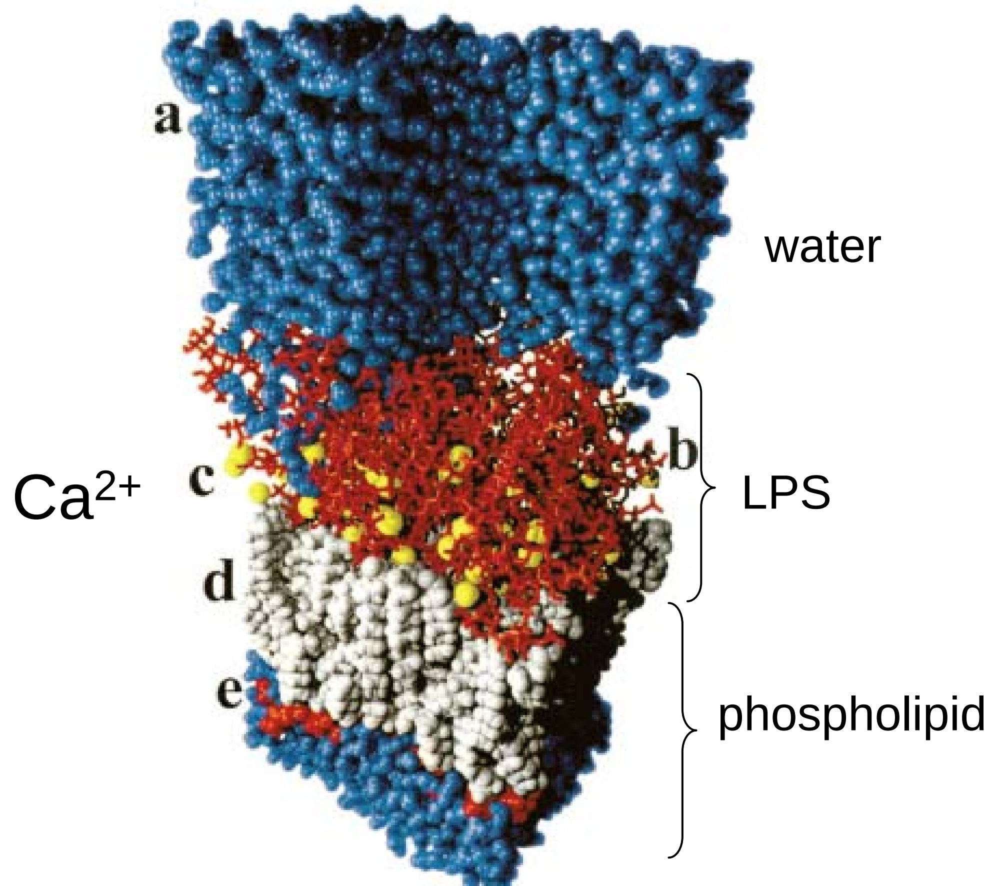
- "bad" *E. coli* which makes you sick from eating raw hamburger.
- causes Hemolytic Uremic Syndrome (HUS) fatal to small children

Why Ca? Why Mg?


O-polysaccharide
Core polysaccharide
Out
Lipopoly-saccharide (LPS)
Protein
Lipid A
Porin
8 nm
Gram-negative outer membrane
Lipoprotein
Phospholipid
Periplasm
Peptidoglycan
Cytoplasmic membrane


O-specific polysaccharide
Core polysaccharide
Lipid A
n
Glu-Nac
Glu
Gal
Gal
Glu
Hep
Hep
Hep
P
KDO
KDO
KDO
GlcN
GlcN

Divalent Cations Regulate Membrane Permeability



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

Cation Bio-chemistry

- Increasing concentrations of divalent Ca^{2+} and Mg^{2+} reduced the antimicrobial effect of a standard peptide (protamine).

- Addition of divalent cations to LPS suspensions modified the in-plane packing of LPS molecules from hexagonal to a nonhexagonal lattice (as confirmed by X-ray diffraction).

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

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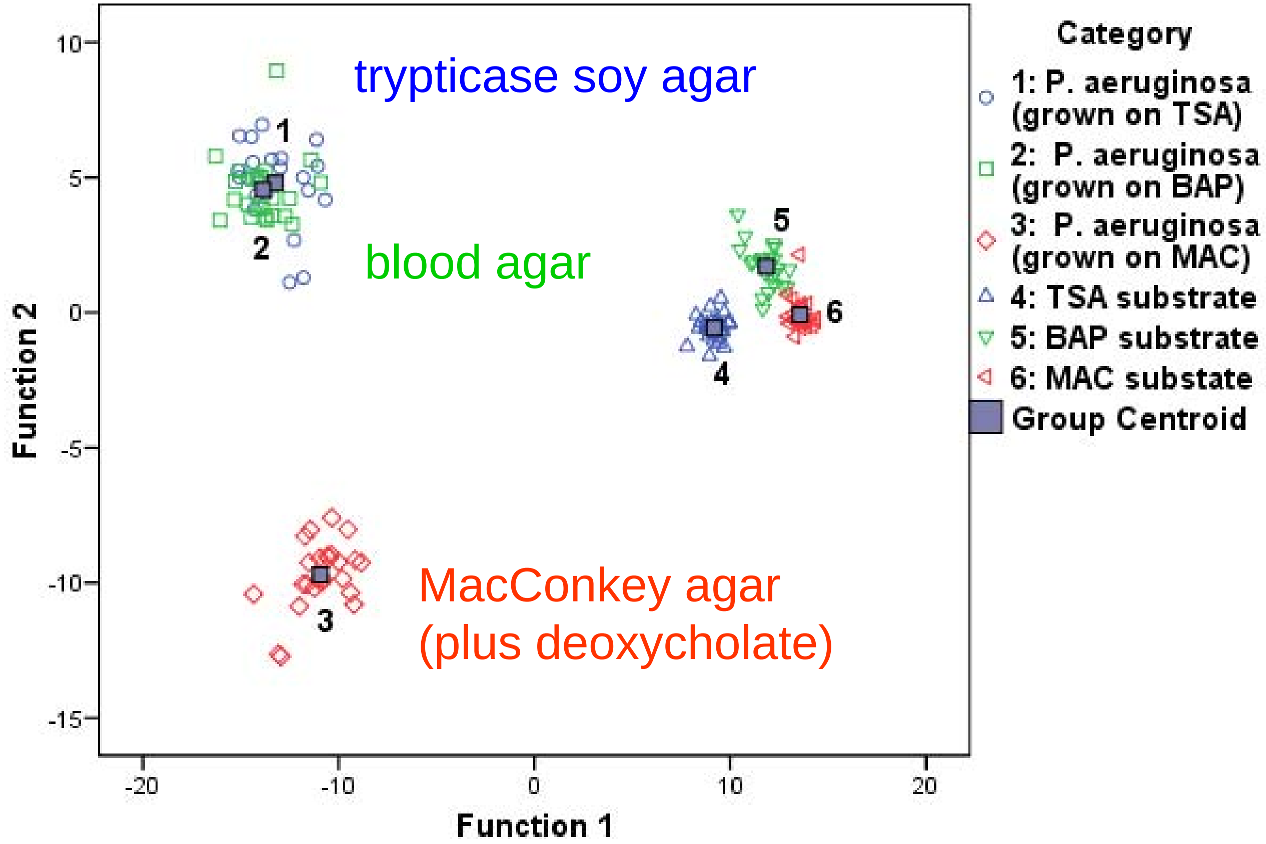
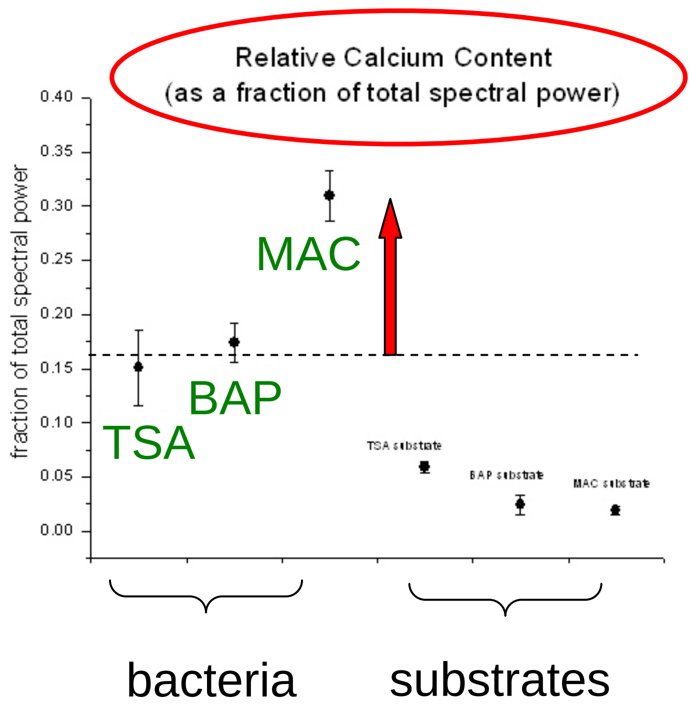
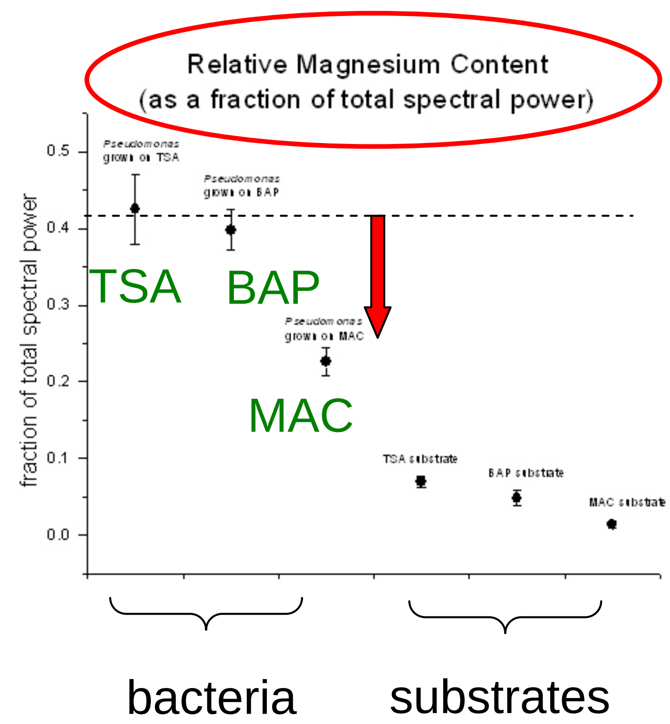
Received 23 May 2007; accepted 23 July 2007
Available online 1 August 2007

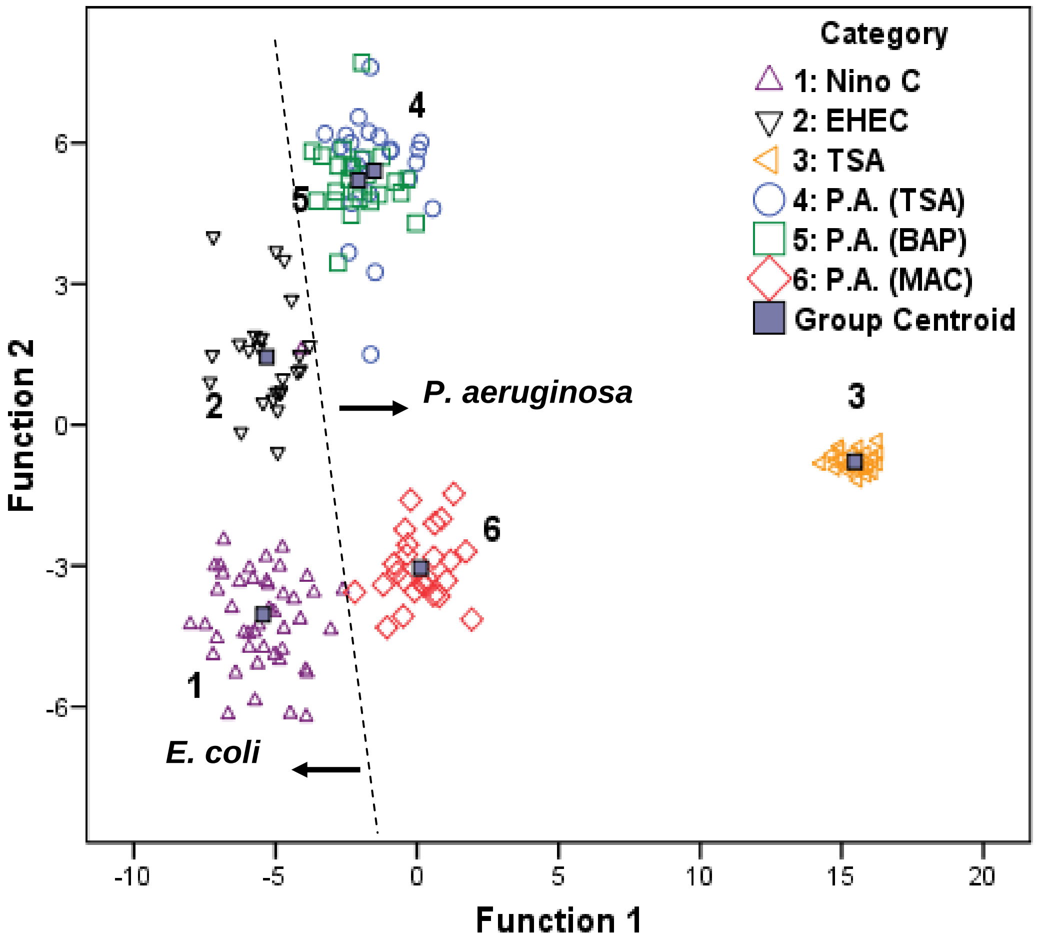


Divalent Cations (Ca^{2+}, Mg^{2+}) Concentrations Are Altered by Environment

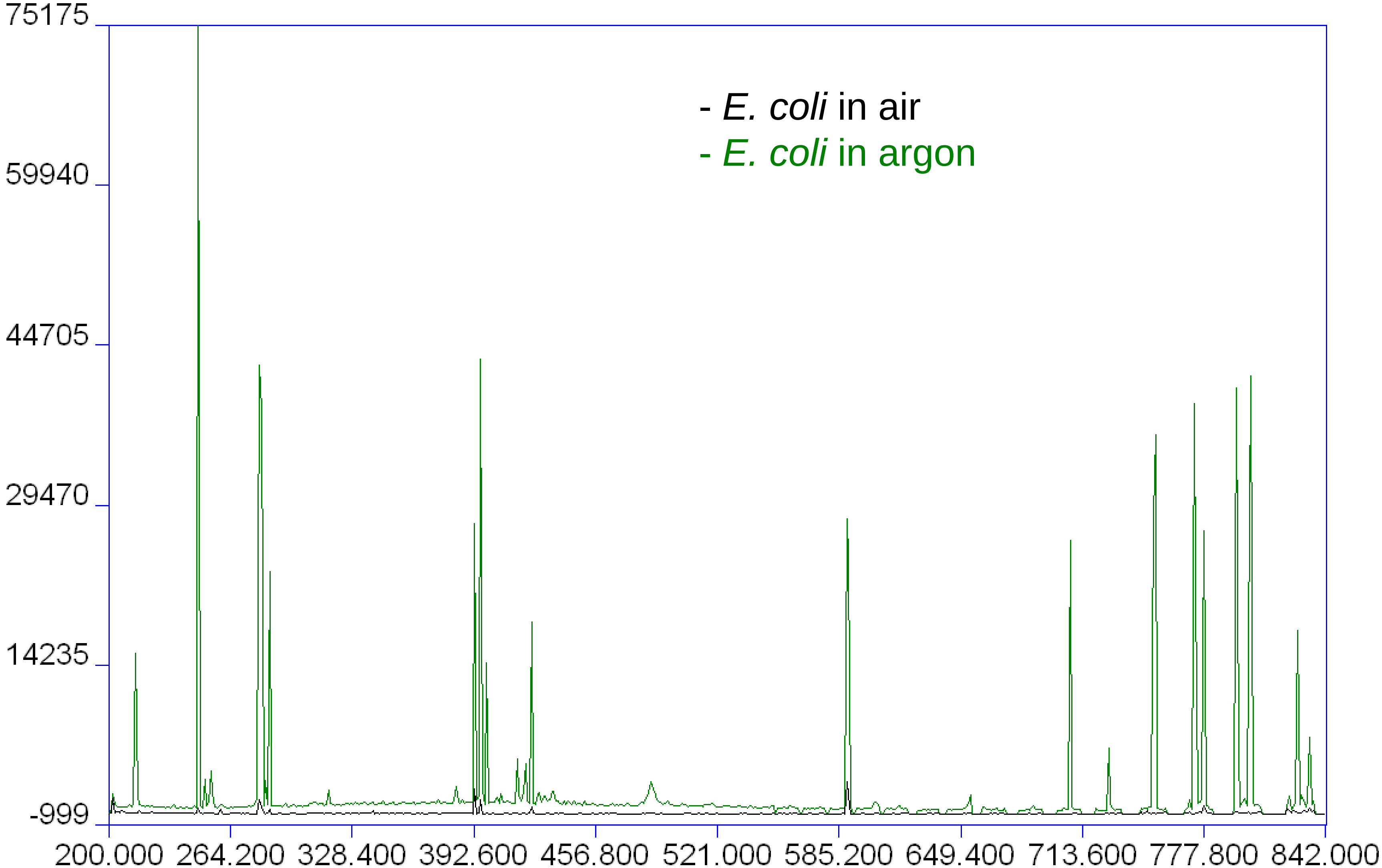


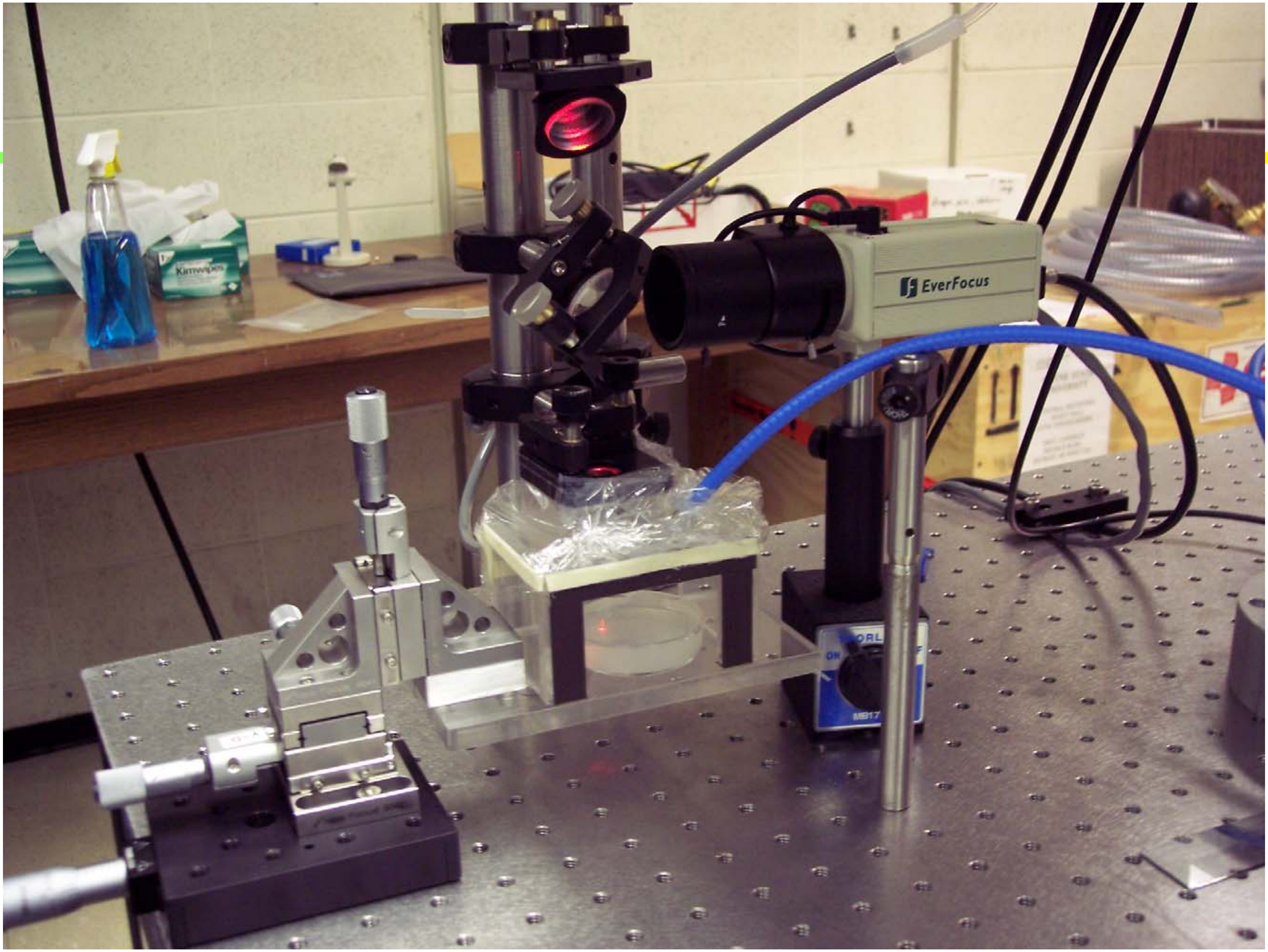


E. coli and *P. aeruginosa*



Improvements




EverFocus
Kimwipes

Improvements

- noble gases (Ar, He)
  - dual-gas environments
- liquid cultures (not colonies)
- different chemometric analysis (PCA)
- Gram-positive bacteria
- Raman spectroscopy

Conclusions

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

Thank you for your attention!

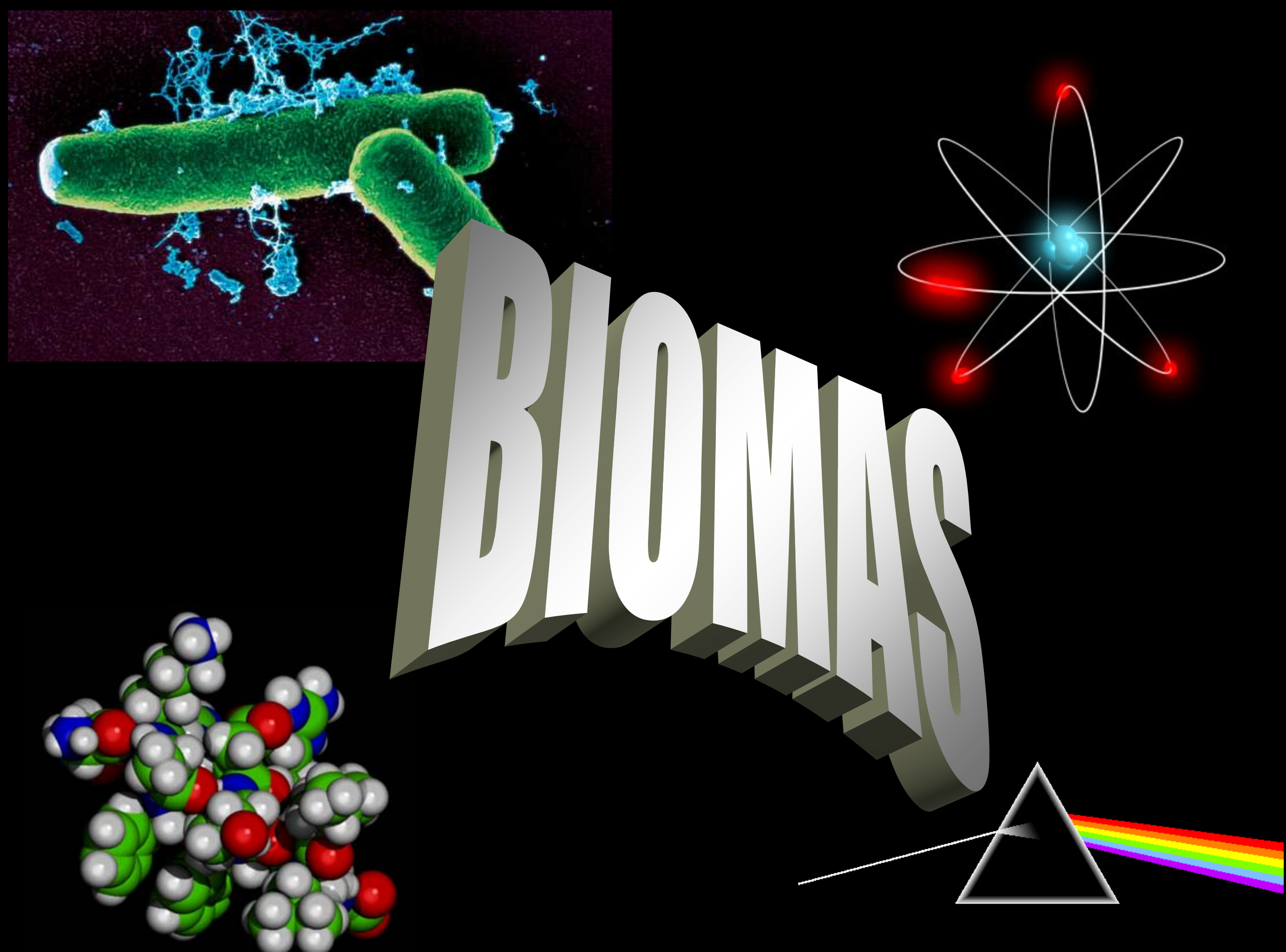
<u>Graduate Students</u>

- Jon Diedrich, M.S.
- Narmatha Jeyasingham, M.S.
- Arathi Padhmanabhan
- Caleb Ryder
- Qassem Mohaidat
- Khozima Hamasha

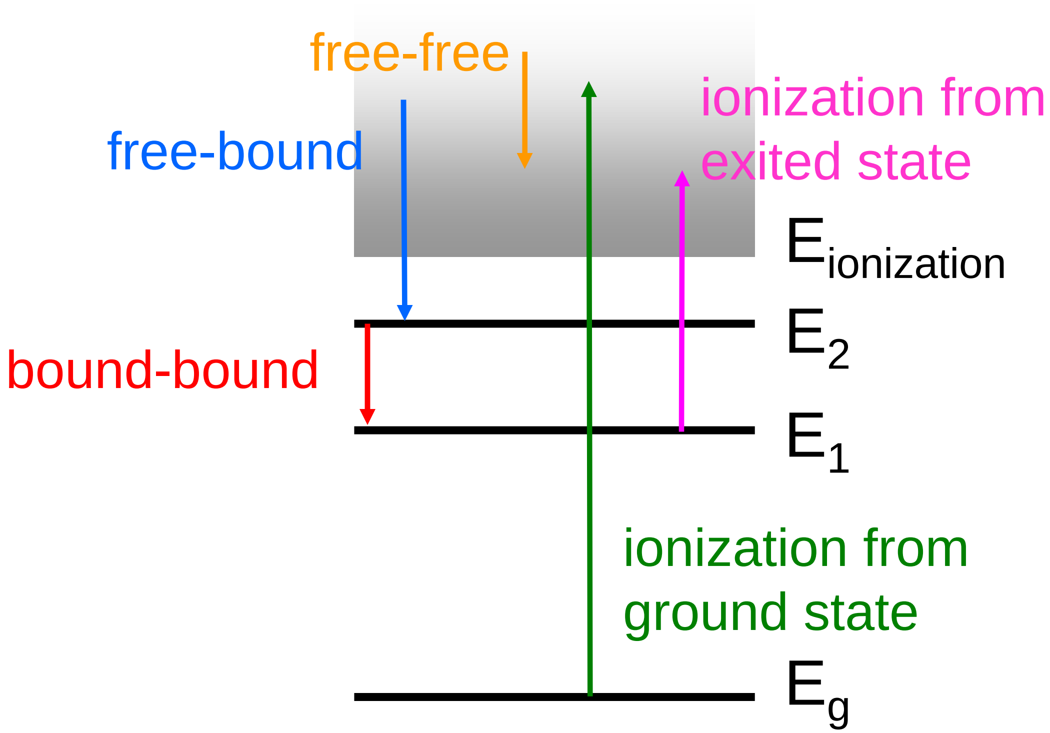
<u>Undergraduate Students</u>

- Marian Adamson
- Emmett Brown
- Garrett Godfrey

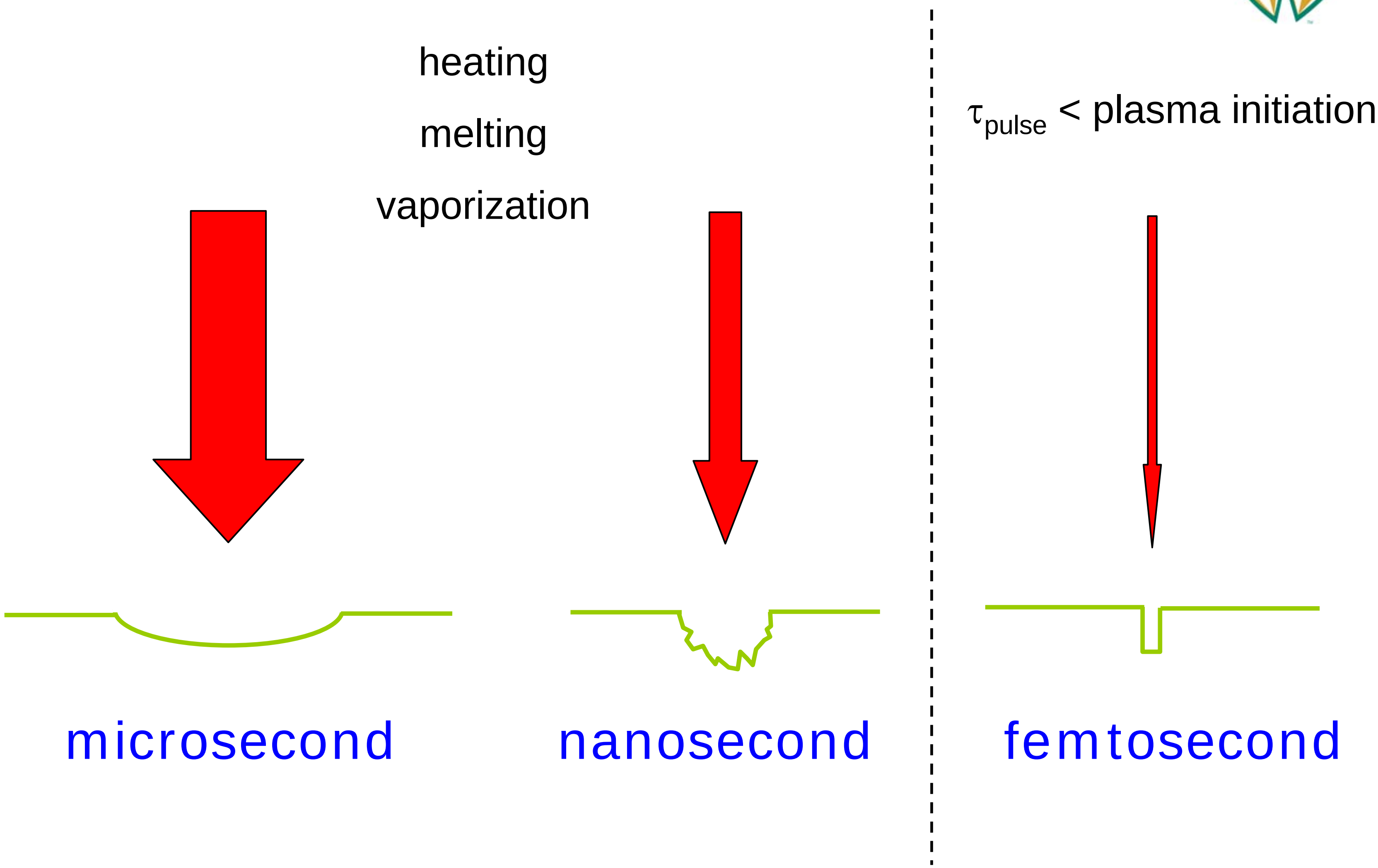
The BIOMAS Project:
Bacteria Identification by Optical, Molecular, and Atomic Spectroscopy



Transitions in an Atom or Ion



Physics of Plasma Formation: ablation



Physics of Plasma Formation: ablation

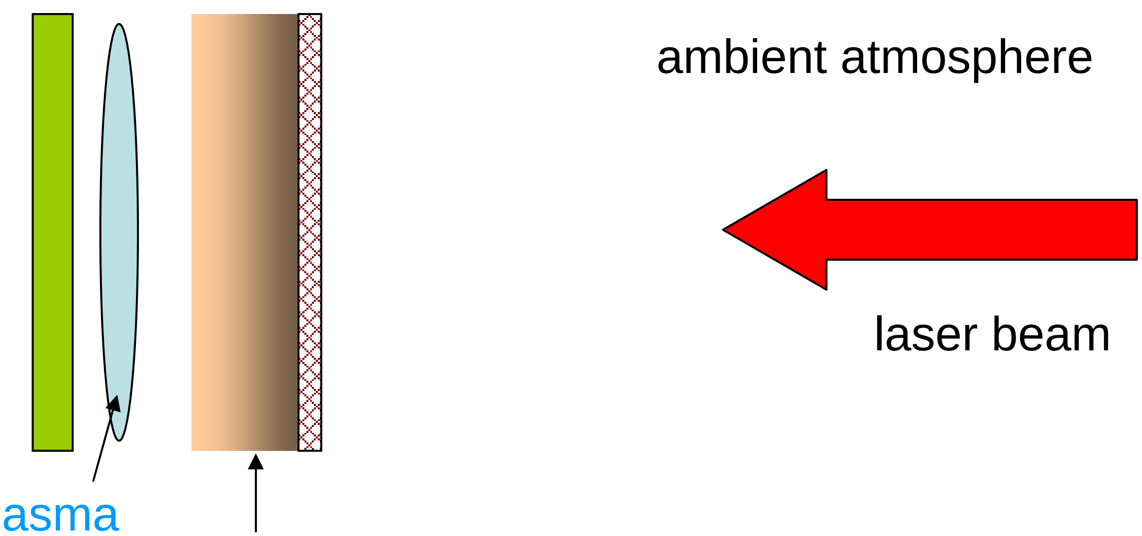
$$I_{\min} = \frac{\rho L_V \kappa^{1/2}}{\Delta t^{1/2}} (\mathrm{W/cm^2})$$

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

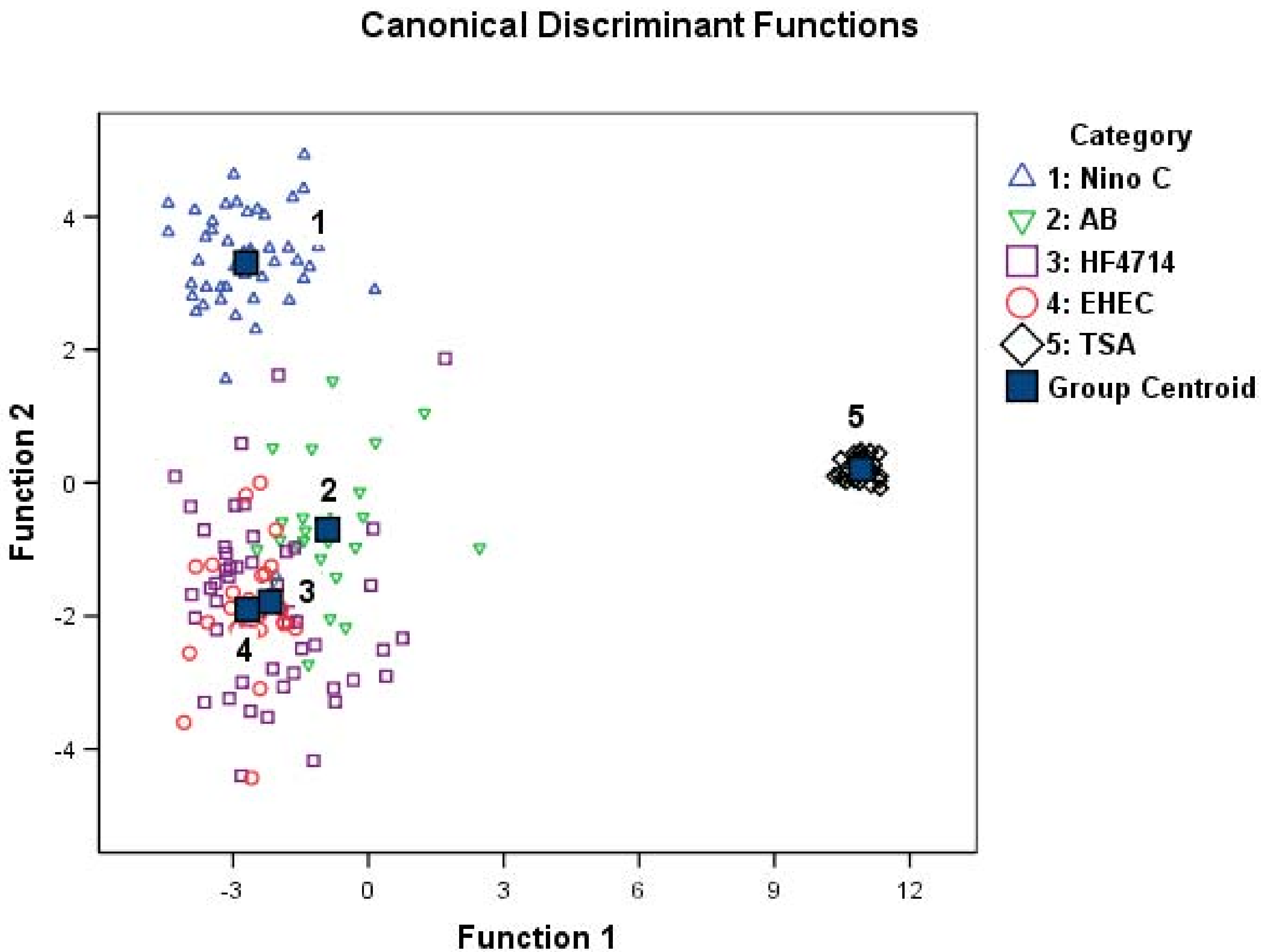
- $I_{\min}$ Al = 1.75×10^8 W/cm^2
  - for a 10 ns pulse, focused to a 100 μm spot: ~130 μJ

Physics of Plasma Formation: laser detonation wave

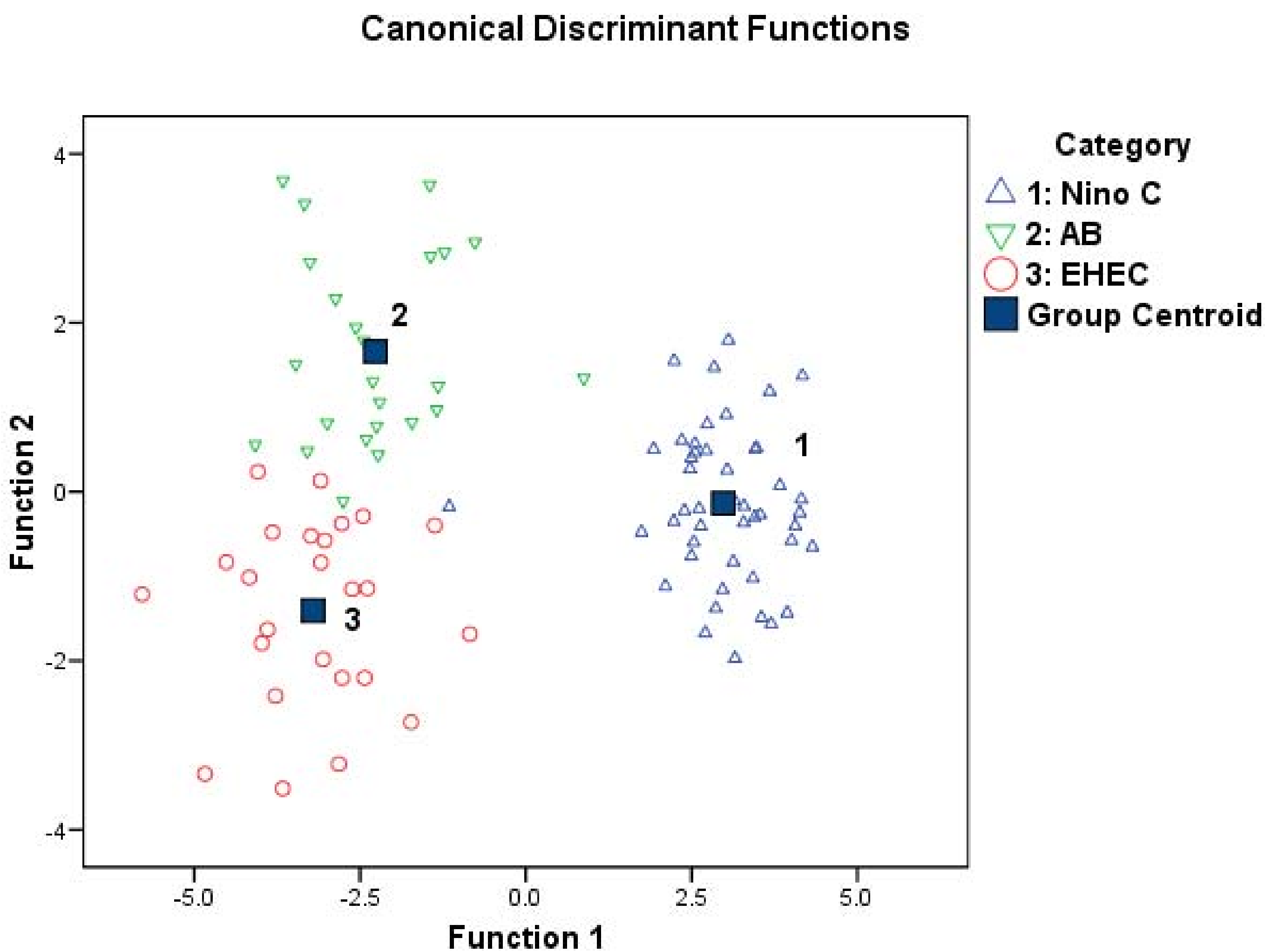
laser-supported detonation wave (LSD or LDW) with a supersonic, rapidly expanding shock-wave front



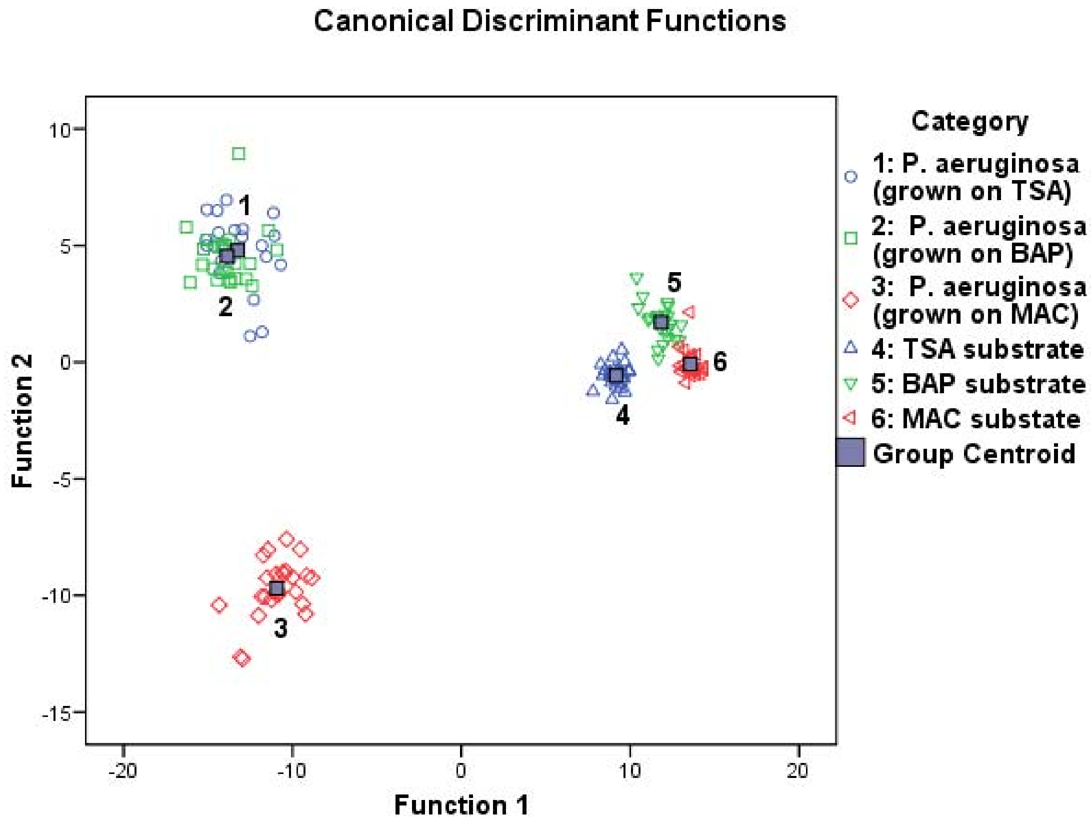
EHEC Results



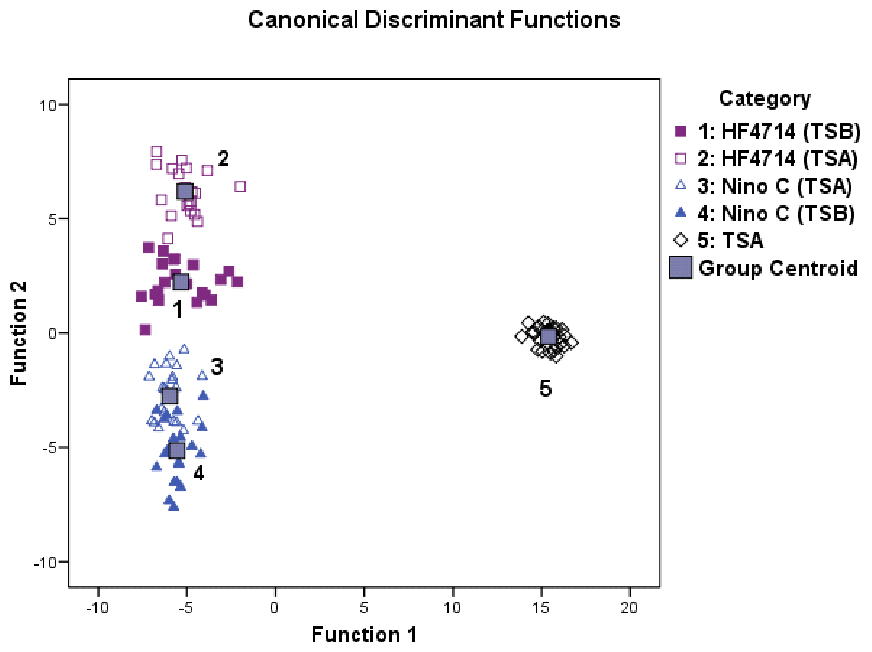
EHEC Results

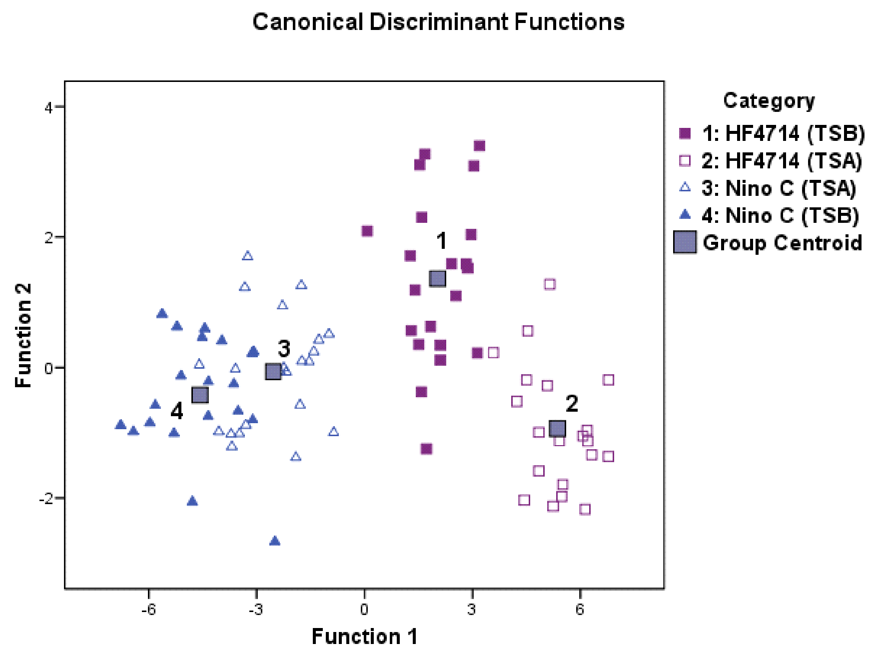


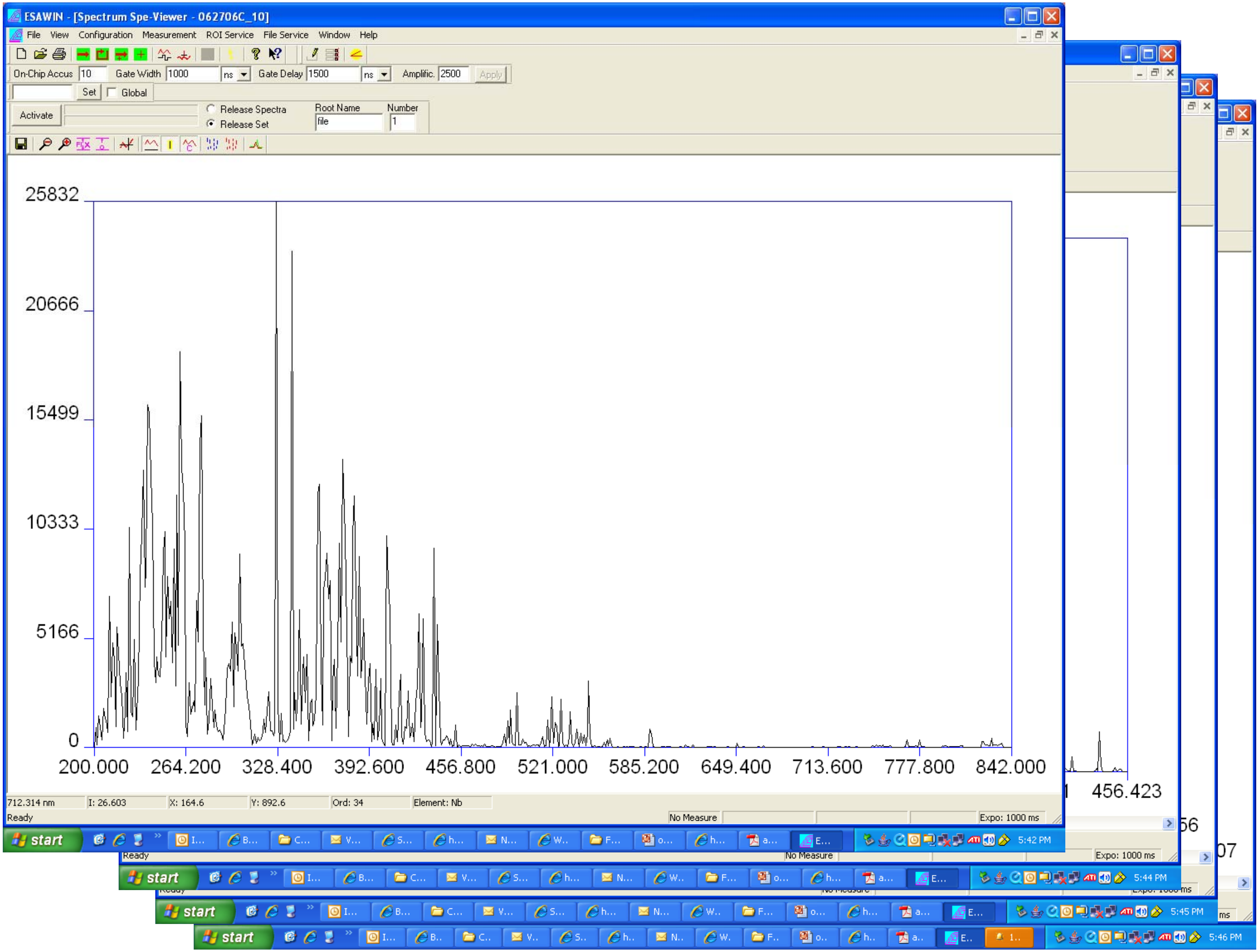
Effect of Growth Environment on *P. aeruginosa*



Effect of Growth Environment on *E. coli*







ESAWIN - [Spectrum Spe-Viewer - 062706C_10]
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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

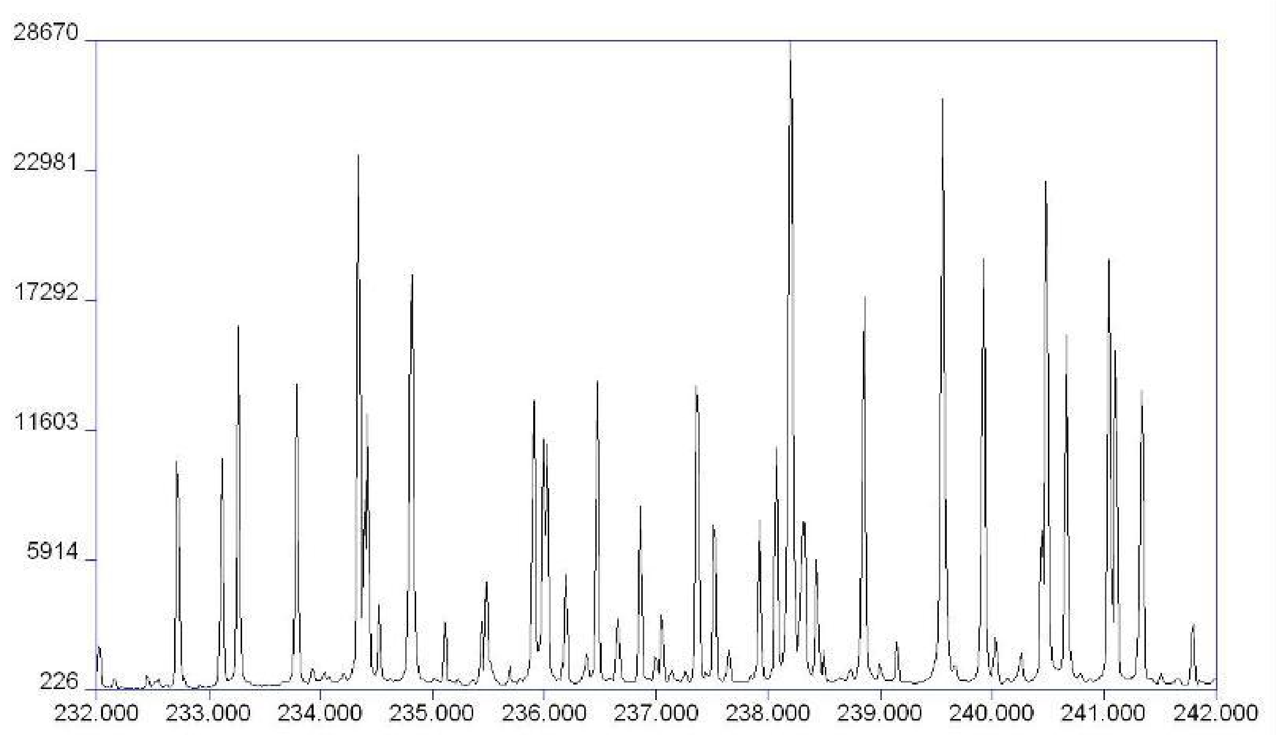
- confusing! better to write…

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

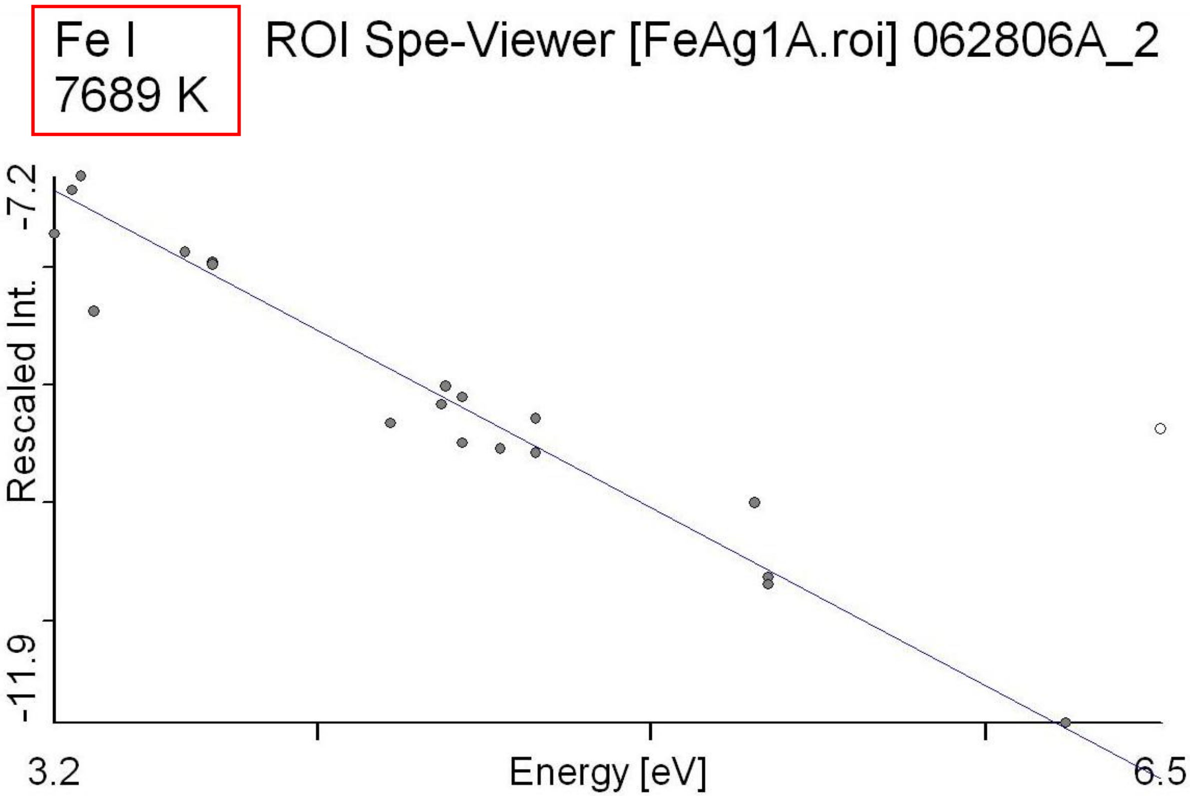
- This is a straight line with slope of $-1/kT$!

- 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

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