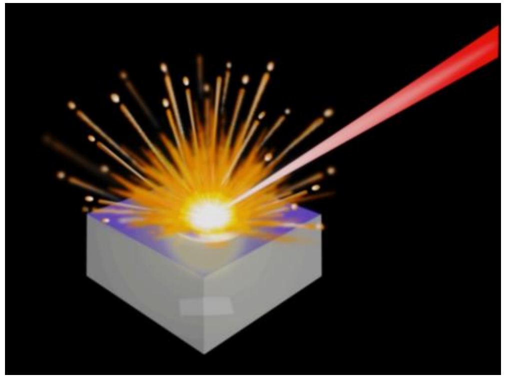
21^{st} Century Medicine, One Spark at a Time:

Biomedical Applications of Laser-Induced Breakdown Spectroscopy

Professor Steven J. Rehse

University of Windsor, Department of Physics



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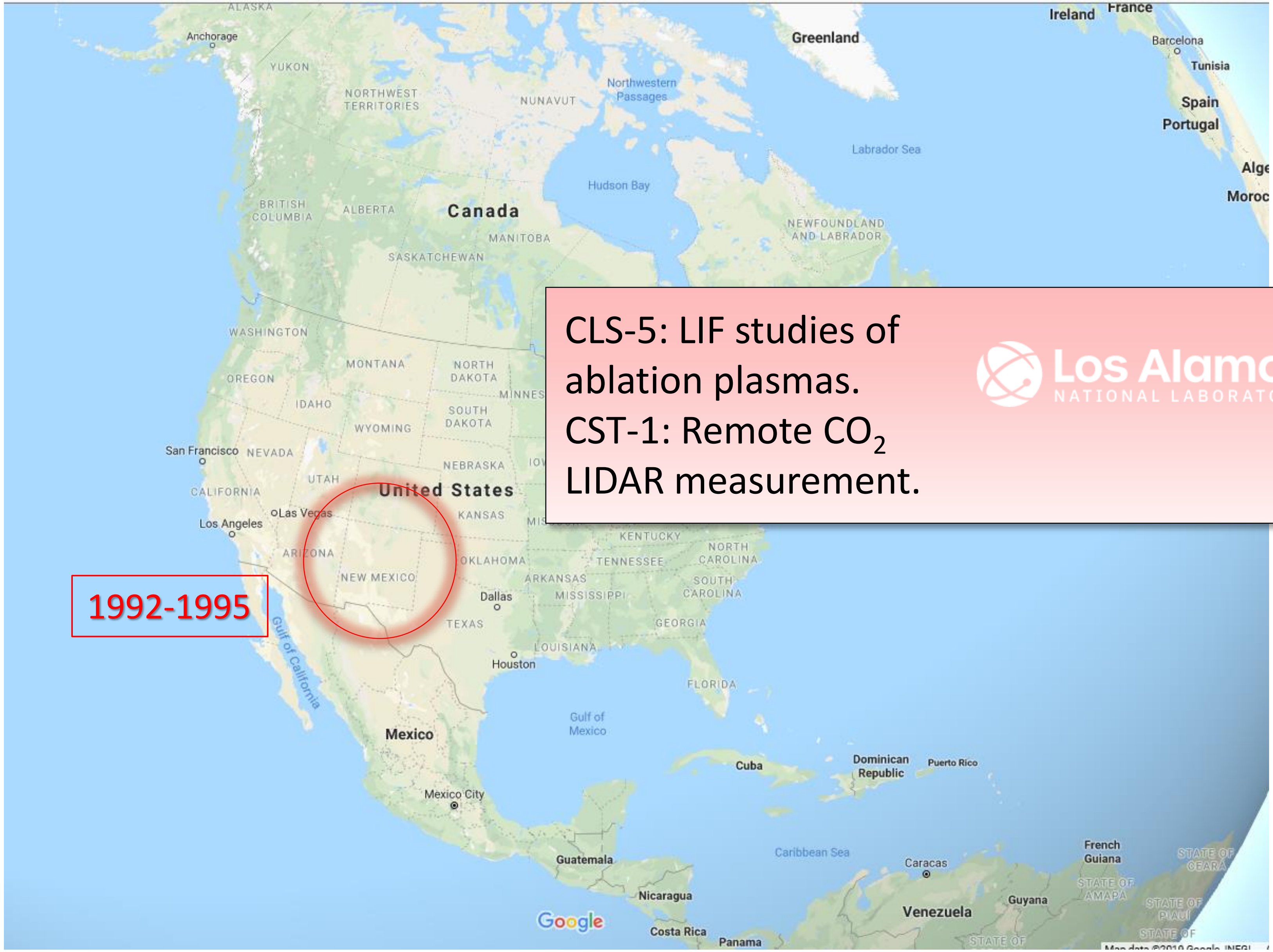
About Me...

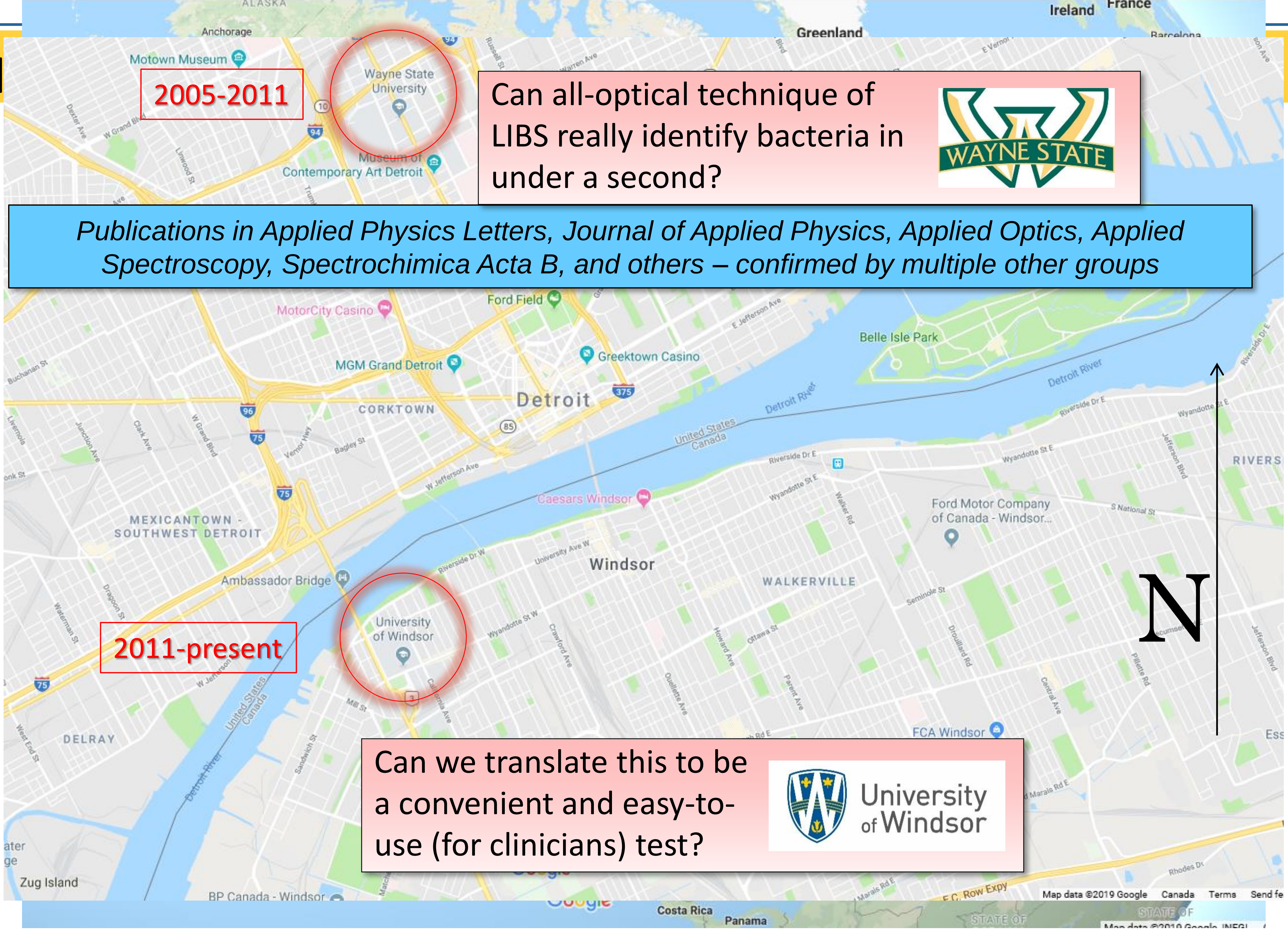


1992-1995

CLS-5: LIF studies of ablation plasmas.
CST-1: Remote CO_2 LIDAR measurement.

Los Alamos NATIONAL LABORATORY

Abou



Can all-optical technique of LIBS really identify bacteria in under a second?

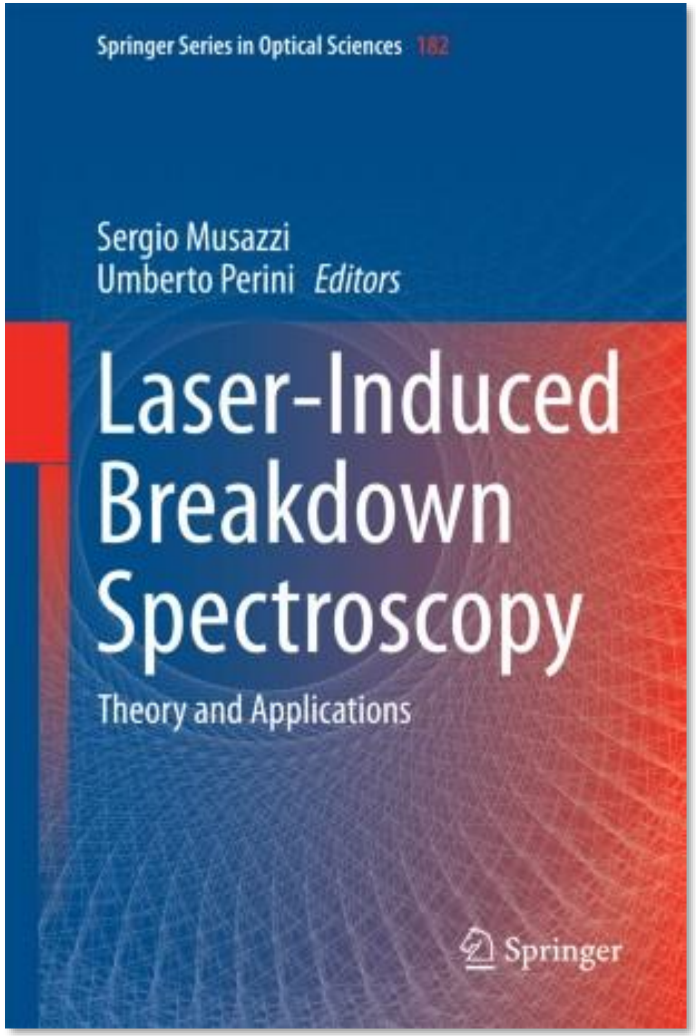
WAYNE STATE

Publications in Applied Physics Letters, Journal of Applied Physics, Applied Optics, Applied Spectroscopy, Spectrochimica Acta B, and others – confirmed by multiple other groups

Can we translate this to be a convenient and easy-to-use (for clinicians) test?

University of Windsor

About Me...



2014

Chapter 17
Biomedical Applications of LIBS

Steven J. Rehse

Abstract The use of laser-induced breakdown spectroscopy (LIBS) as a biomedical diagnostic tool is rapidly gathering significant attention due to successful demonstrations of its utility in a surprisingly broad range of applications. Broadly speaking, these applications can be divided into two categories: those which aim to quantify or monitor elemental concentrations in medical or biomedical specimens and those that use unique elemental compositions to rapidly identify or classify specimens. In this chapter, we will review recent progress in the application of LIBS in several broad classes of biomedical diagnostics, including the analysis of hard/calcified tissues; the analysis of soft tissues; the analysis of biomedical specimens; the identification/classification of agents causing human disease; and laser-guided surgery.

17.1 Introduction

17.1.1 Motivation

Lasers are one of the most important tools available in modern medicine. The applications of lasers in medicine are extremely disparate and exploit all of the various properties intrinsic to laser light, such as monochromaticity, focusability, high power density or fluence, and the ability to deliver energy in ultrashort pulses. Most of these applications involve the interaction of the laser's electromagnetic radiation with cells or tissues in some way. In the medical field it is common to define three different regimes of interactions depending on the energy density of the delivered laser light and the time duration over which the energy is deposited within the tissue. These three regimes are loosely defined as: photocoagulation, photovaporization (or photodisruption), and photoablation [1, 2].

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S. Musazzi and U. Perini (eds.), *Laser-Induced Breakdown Spectroscopy*, 457
Springer Series in Optical Sciences 182, DOI: 10.1007/978-3-642-45085-3_17,

Outline

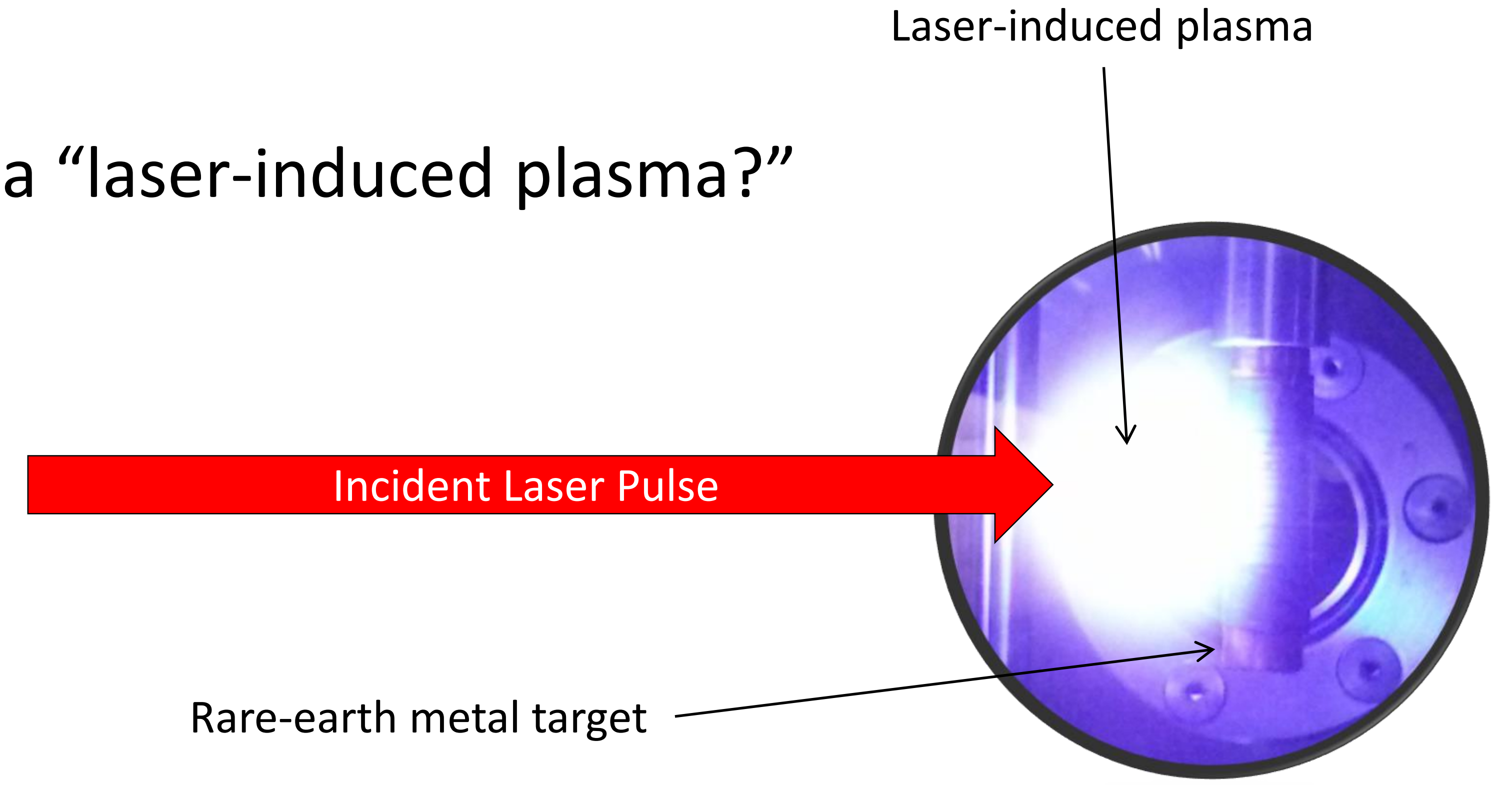
21st Century Medicine, One Spark at a Time:

Biomedical Applications of Laser-Induced Breakdown Spectroscopy

1. What is LIBS?
2. Principles of LIBS
   a. Basics / theory
   b. Apparatus
3. Advantages over other techniques
4. Specific medical/biomedical applications of LIBS

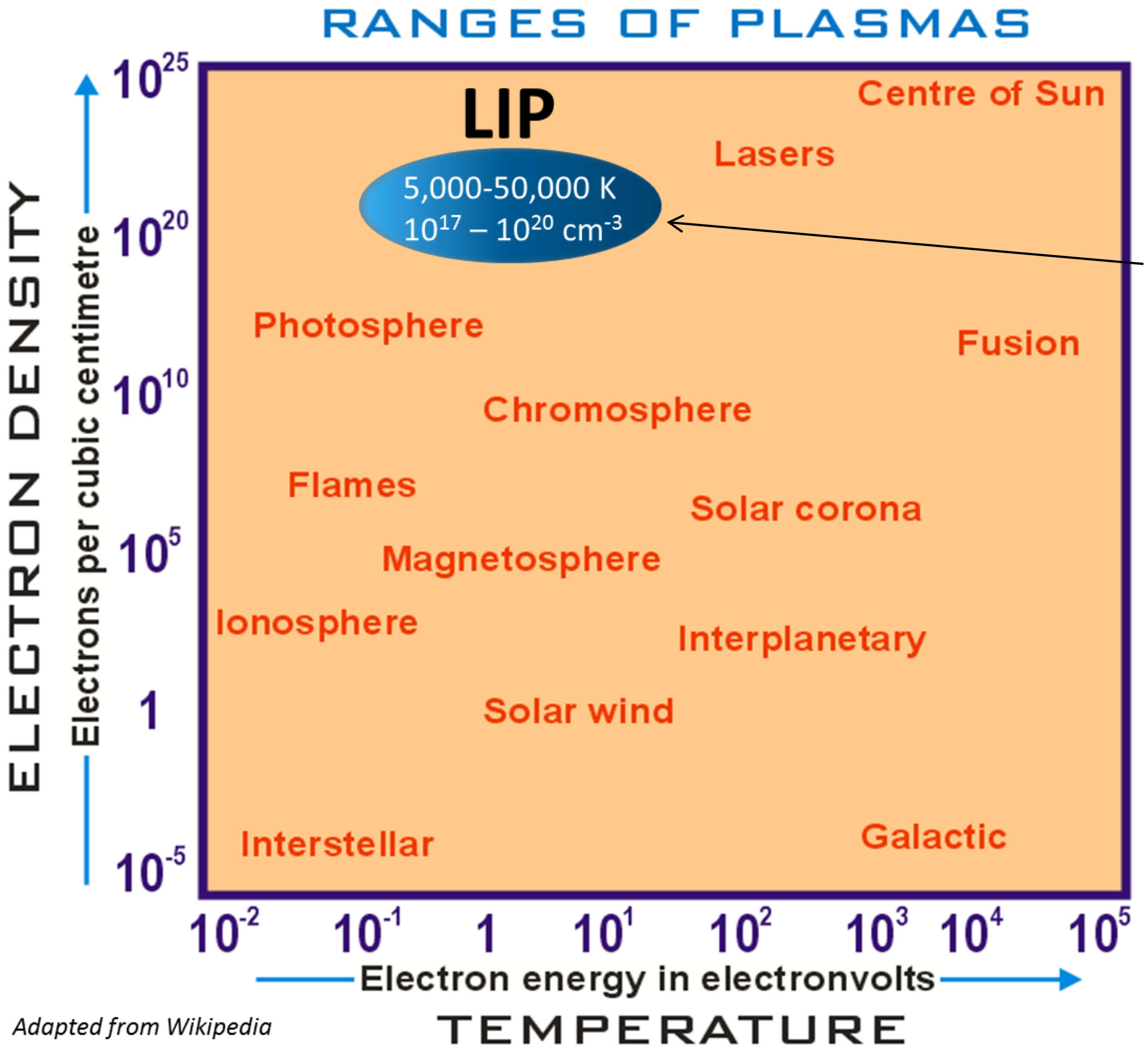
Spectroscopy of Laser-Induced Plasmas

- What is a “laser-induced plasma?”



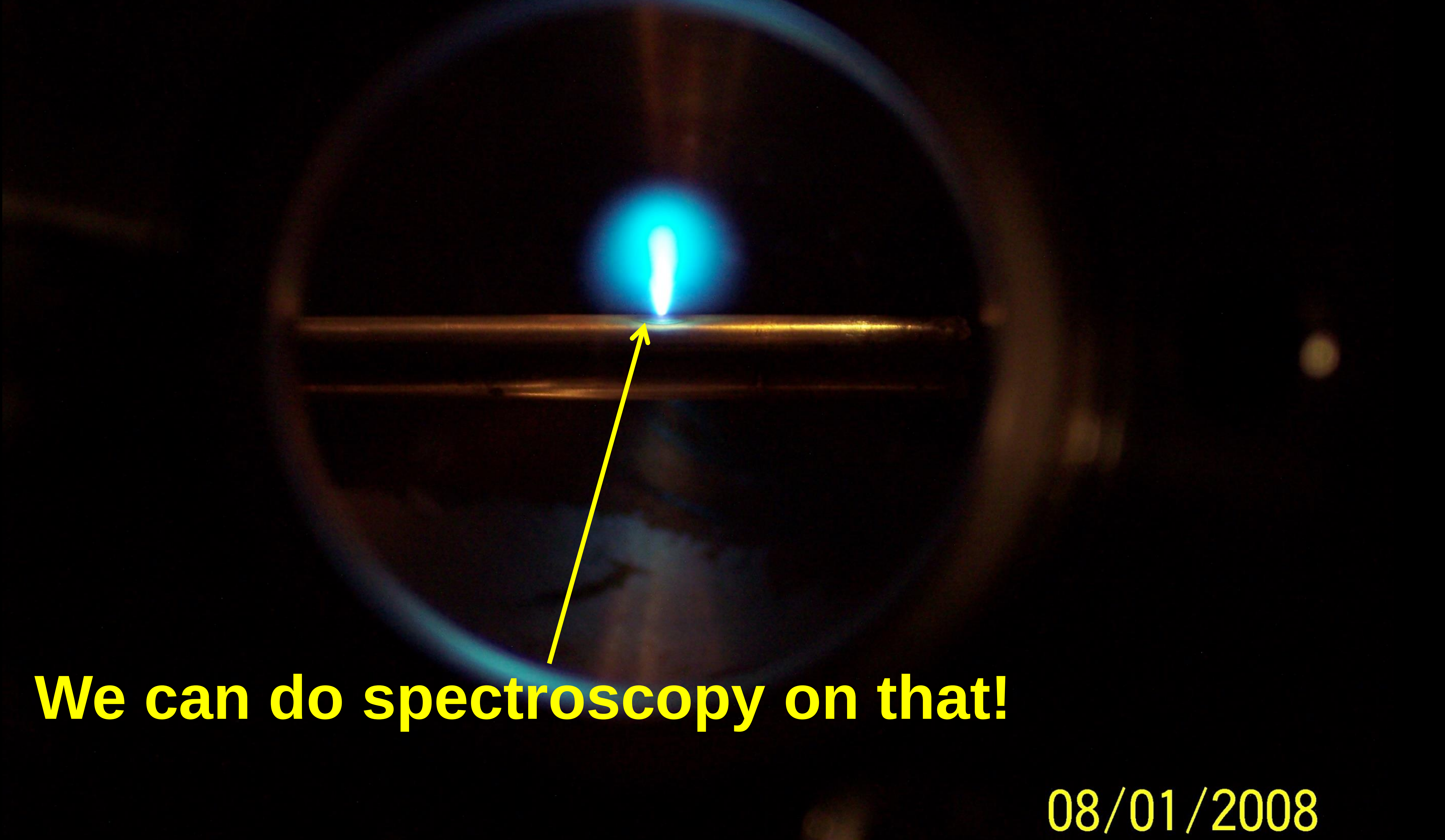
- Can be done with ns, ps, or fs lasers
- Threshold irradiance: $10^{10} - 10^{11}\ {}^{W}/_{m^2}$

1. What is LIBS? Definition



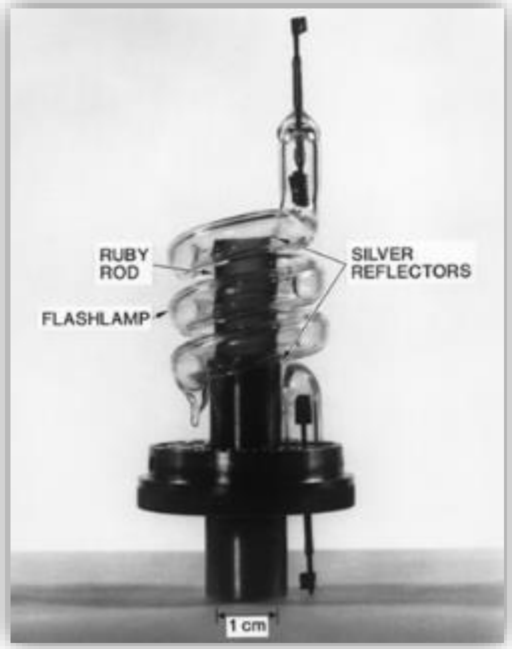
Adapted from Wikipedia

These are typically categorized as "weakly ionized" plasmas with approximately 10% ionization


We can do spectroscopy on that!
08/01/2008

1. What is LIBS? History

1960
Maiman: first ruby laser



1962
Brech, Cross: Birth of LIBS: detection of spectrum from ruby laser-induced plasma

Spectrochemical analysis using a pulsed laser source

(*Received* 12 *July* 1963)

INTRODUCTION

SINCE the discovery of the optical maser, or laser, announced some three years ago, considerable scientific thought and effort have been expended toward making it a useful tool. In 1962, BRECH [1] used a ruby laser to produce vapors which were excited by an auxiliary spark source to analyze metallic and nonmetallic materials through their emission spark spectra. Early in 1963, we observed atomic emission spectra produced by the coincident vaporization and excitation of metals and nonmetals by means of a giant-pulse ruby laser. Now it can be shown that spectra produced solely by laser excitation exhibit fairly reproducible quantitative relationships among the various elemental constituents of the sample. And, for the first time, spectroscopists have a means of directly exciting solid materials without having to supply electrical power to the sample material. The sample need not be an electrical conductor, and it can be situated in an environment hostile to more conventional analytical techniques: for example, within a furnace or a radioactive environment.

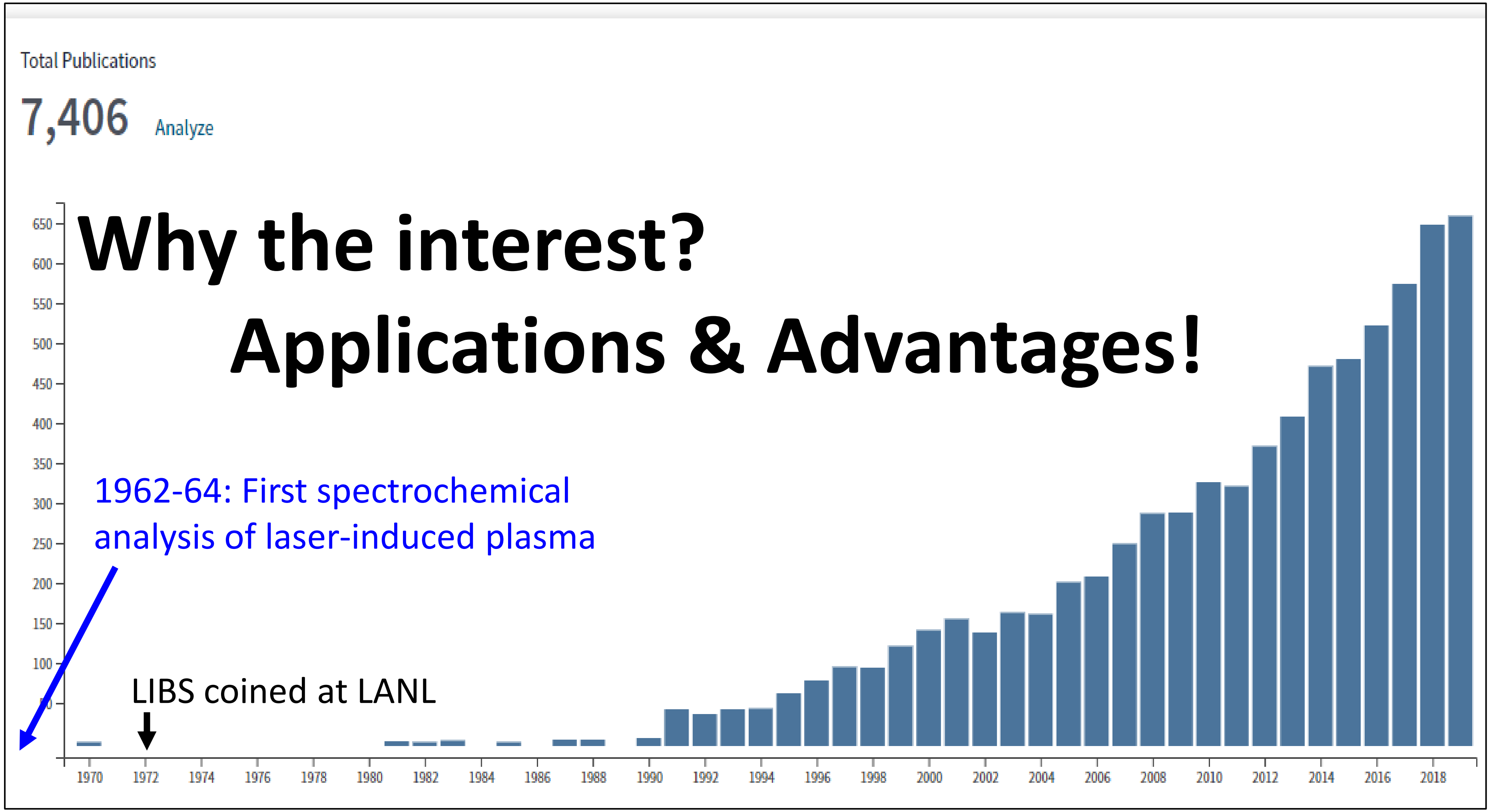
The experiments to be described were designed to test this quantitative nature of pure laser excitation. Neither the details of the apparatus nor the type of sample is of great significance in itself. The precision of the data obtainable is the object of the experiment.

1964
Runger et al.: First direct spectro-chemical analysis by LIBS

1965
Zel'dovich, Raizer: First theoretical model for laser breakdown of a gas

1. What is LIBS? History

“laser-induced breakdown spectroscopy” or “laser-induced plasma spectroscopy”
@ Web of Science (Thomson Reuters)



1. What is LIBS? Applications

- 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
  - glass / soil evidence matching

- art conservation / archeology
  - identifying pigments in paintings
  - dating/cleaning ancient marble

- geology
  - gold prospecting
  - conflict gem/mineral provenance
  - planetary exploration

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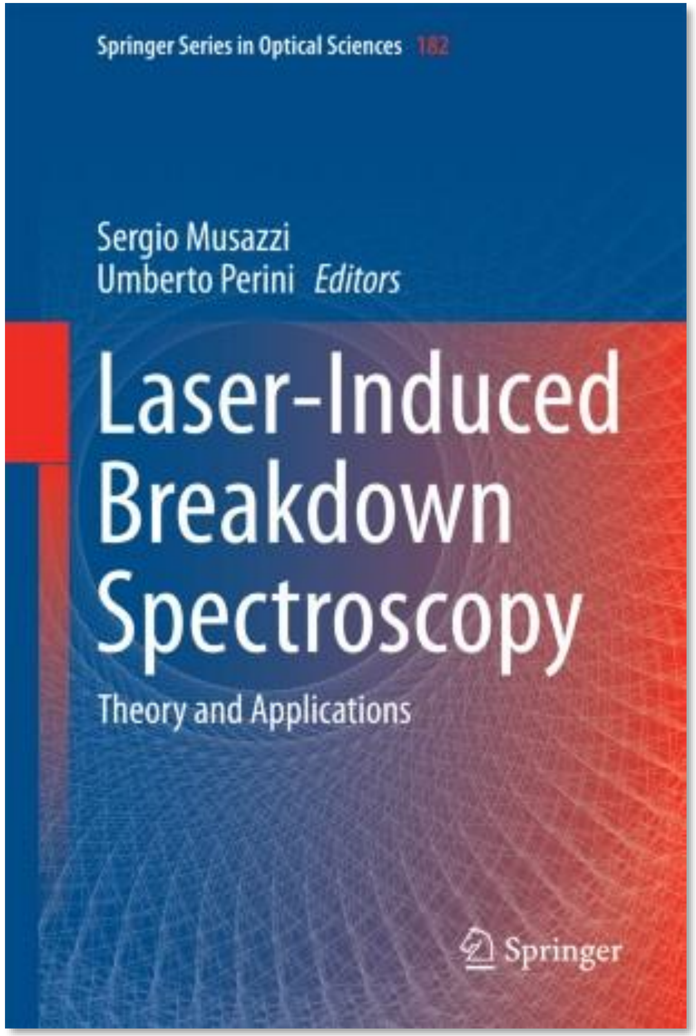
2. Principles of LIBS   Basics/Theory

The following brief primer will explain the fundamental physics of ***nanosecond LIBS*** (ns-LIBS). Femtosecond LIBS (fs-LIBS) is a different phenomenon.

For a complete explanation of the physics, plasma diagnostics, and applications, please consult the following references…



Laser-Induced Breakdown Spectroscopy (LIBS): Theory and Applications

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Ricerca sul Sistema Energetico - RSE , Italy

Umberto Perini
T&D Technology Department
Ricerca sul Sistema Energetico - RSE , Italy

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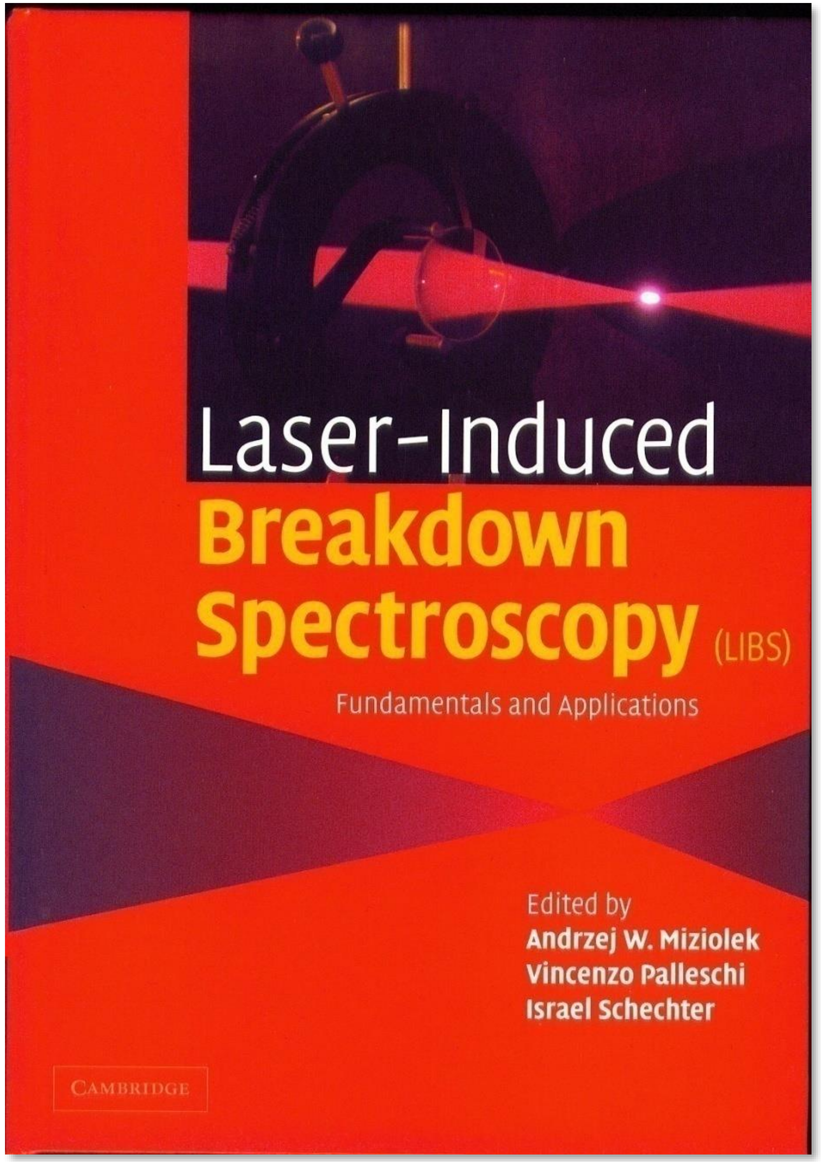
Springer Series in Optical Sciences

2. Principles of LIBS Basics/Theory



Laser-Induced Breakdown Spectroscopy (LIBS): Fundamentals and Applications

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Hardback (ISBN-13: 9780521852746 | ISBN-10: 0521852749)

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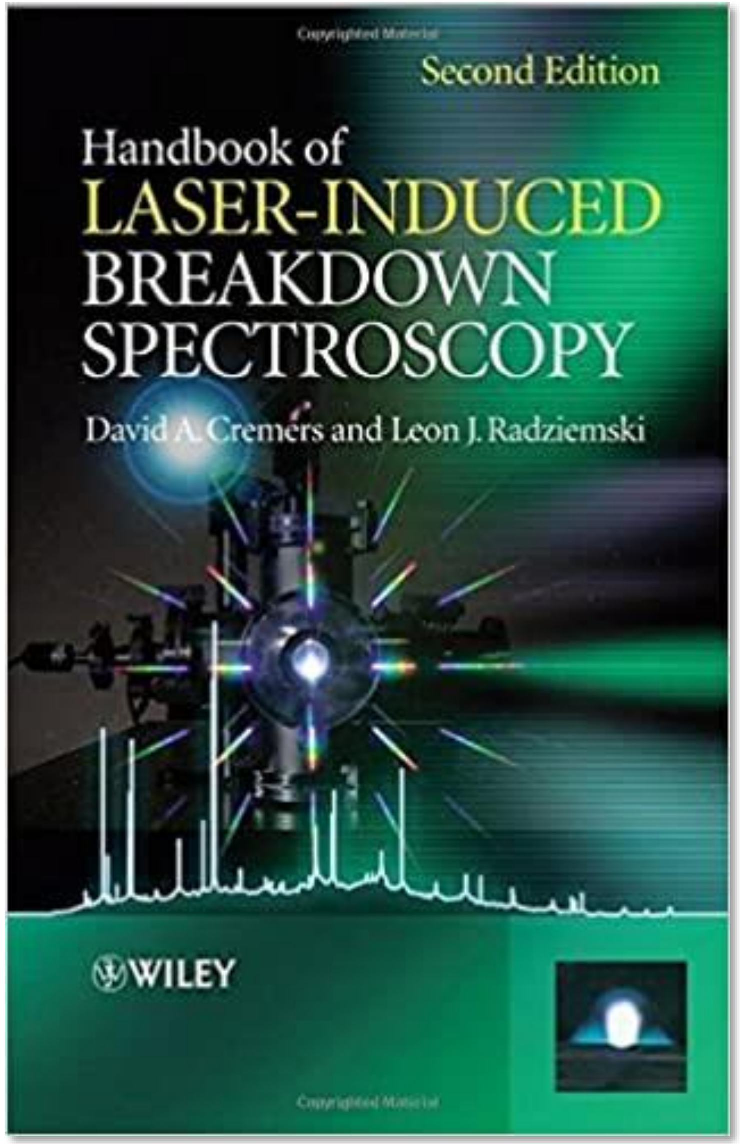
Cambridge University Press



Handbook of Laser-Induced Breakdown Spectroscopy

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David A. Cremers
Applied Research Associates, Inc., Albuquerque, NM

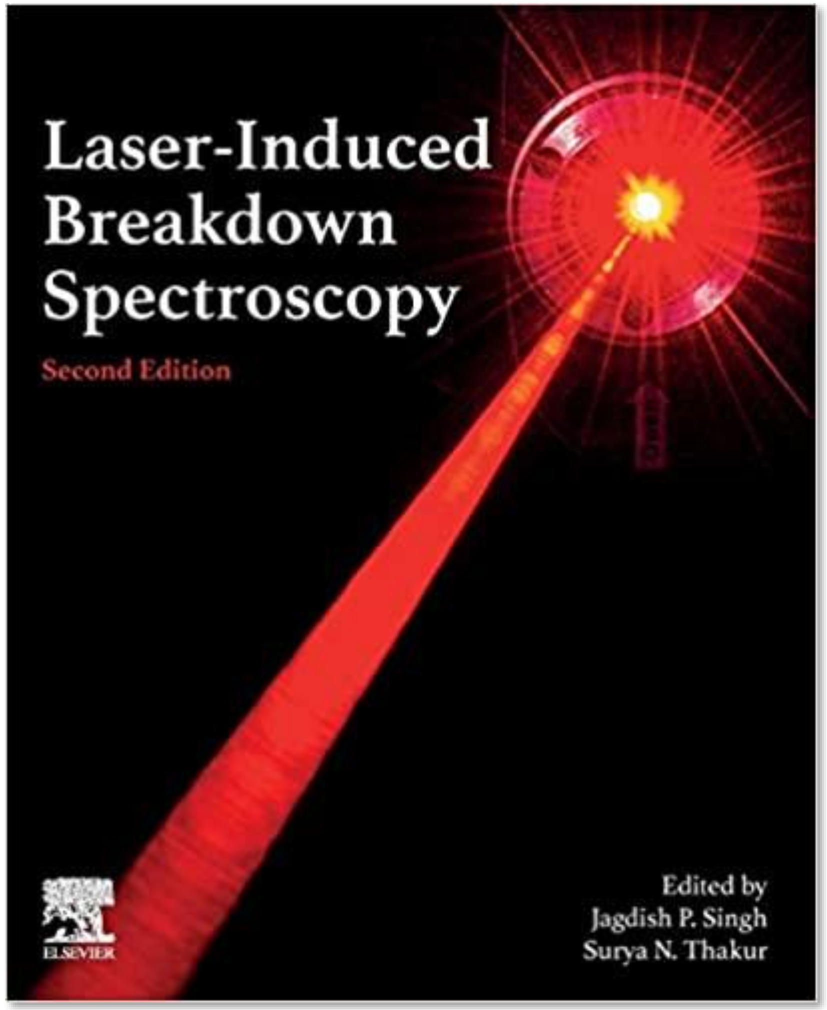
Leon J. Radziemski
Research Corporation, Tucson, AZ

Hardback (ISBN-13: 978-1119971122 | ISBN-10: 1119971128)

Published May 2013 | 432 pages |

John Wiley & Sons, Ltd

2. Principles of LIBS Basics/Theory



Laser-Induced Breakdown Spectroscopy

Edited by-

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Banaras Hindu University, Varanasi, India

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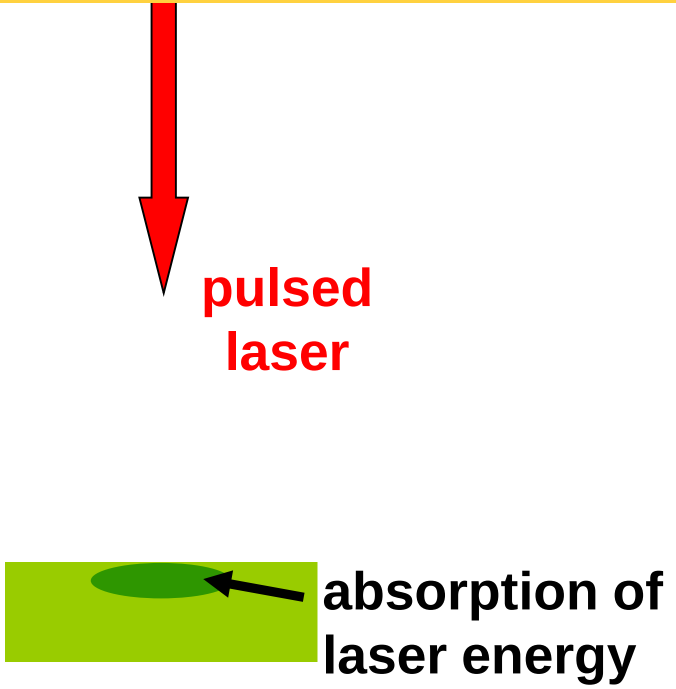
Published June 2020 | *620 pages* |

Elsevier B.V.

2. Principles of LIBS Basics/Theory

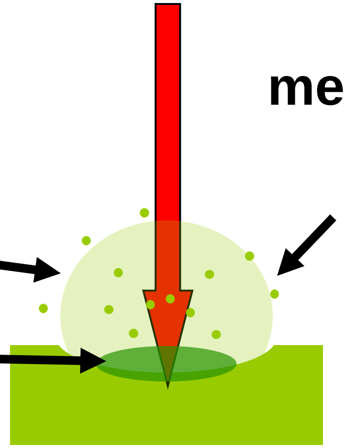
1) laser interaction with the target



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

2. Principles of LIBS   Basics/Theory

2) removal of samples mass (ablation)



melting

fragmentation

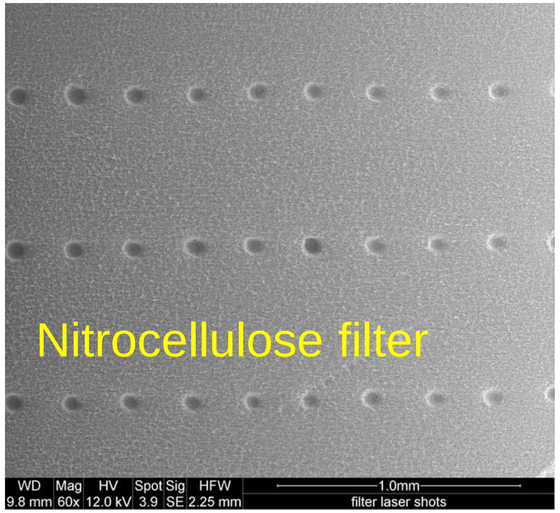
sublimation

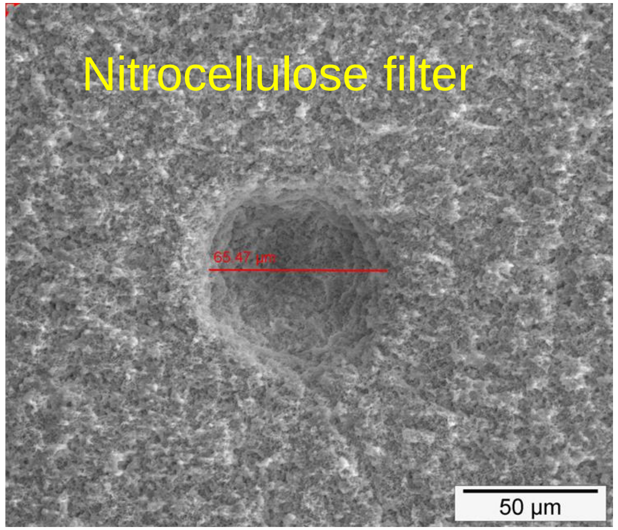
atomization

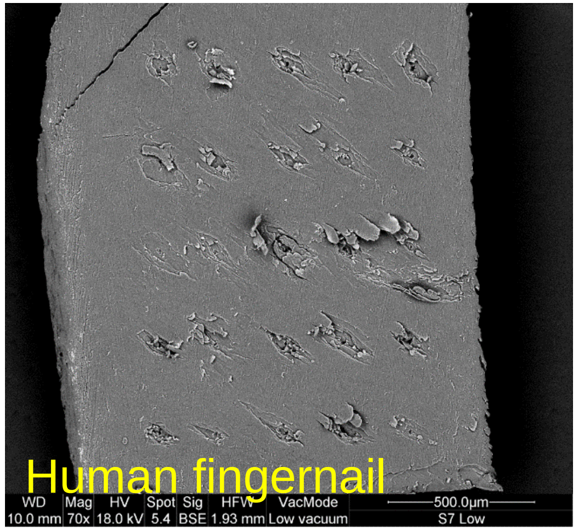
- absorbed energy is rapidly converted into heating, resulting in vaporization of the sample (*ablation*) when the temperature reaches the boiling point of the material.

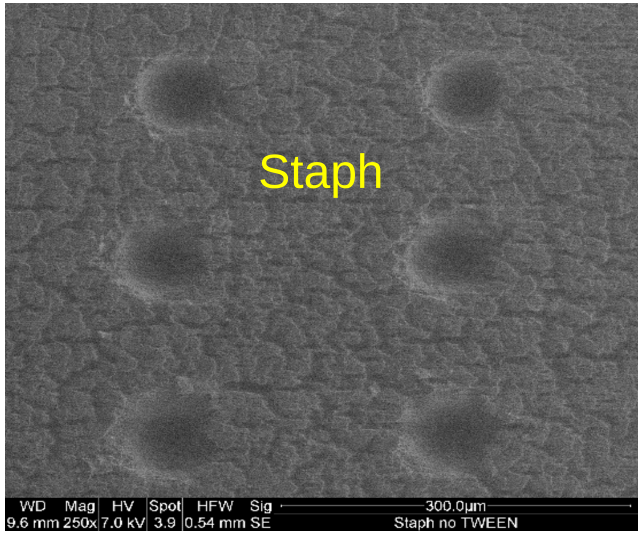
- removal of particulate matter from the surface leads to the formation of a vapor above the surface.

2) removal of samples mass (ablation)



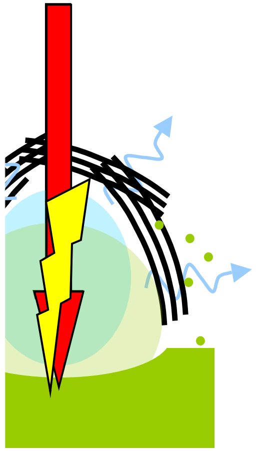






2. Principles of LIBS Basics/Theory

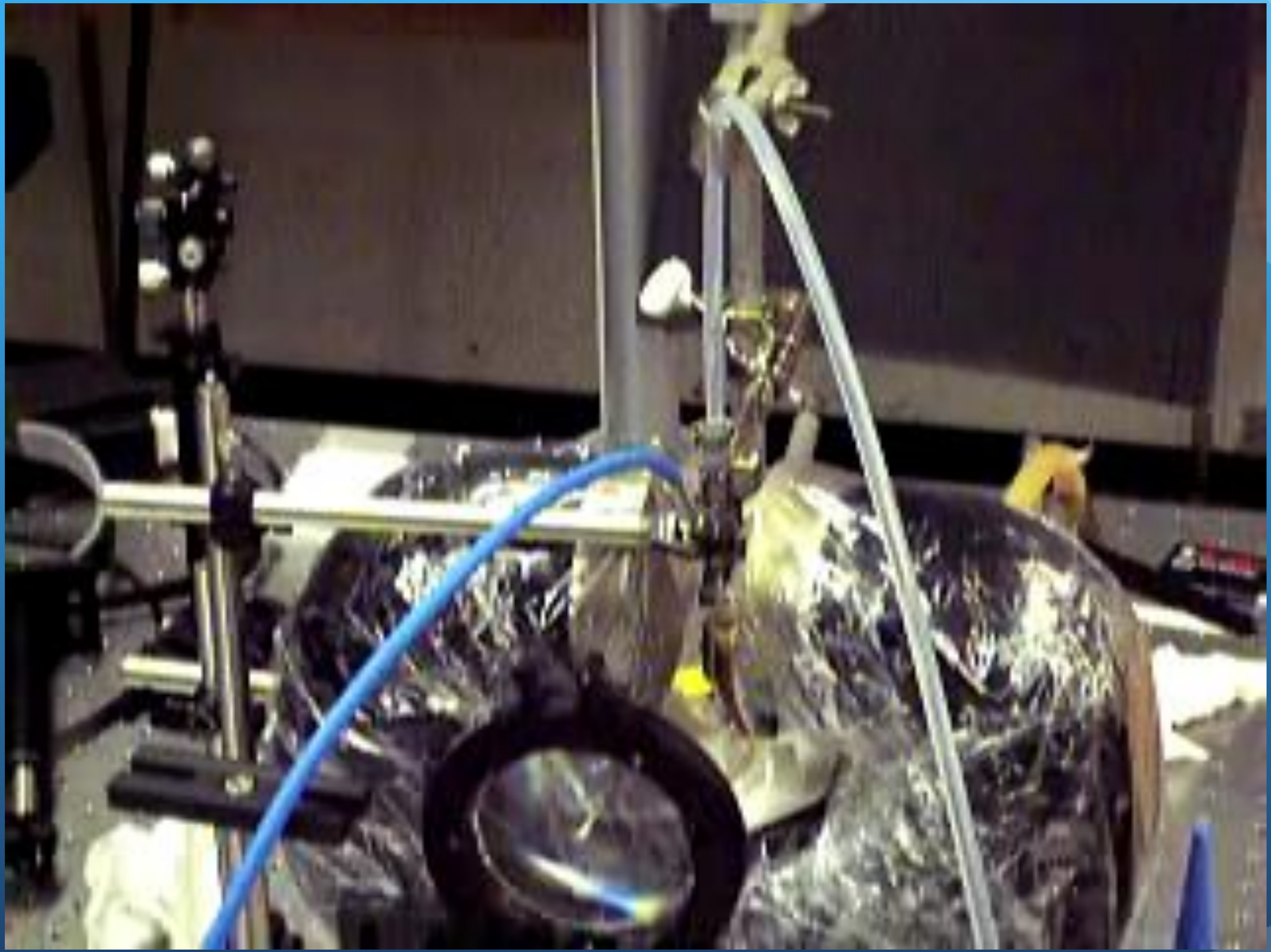
3) plasma formation (breakdown)



absorption of the laser
radiation by the vapor
continuum emission
electrical breakdown
and plasma formation
shock wave
bremsstrahlung

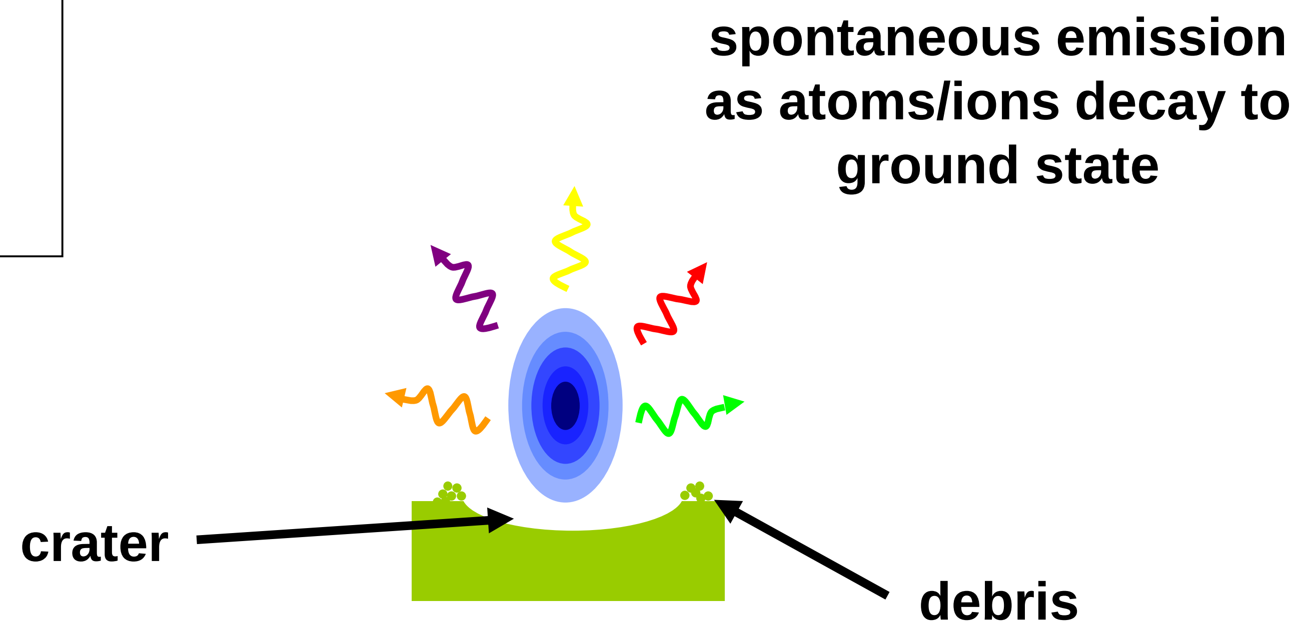
; to illuminate the vapor plume.

ɔ sub-micrometer droplets that
attering of the laser beam,
ɔnization, and plasma formation.



2. Principles of LIBS Basics/Theory

4) expansion and element specific emission (atomic or ionic)



- The dynamic 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.

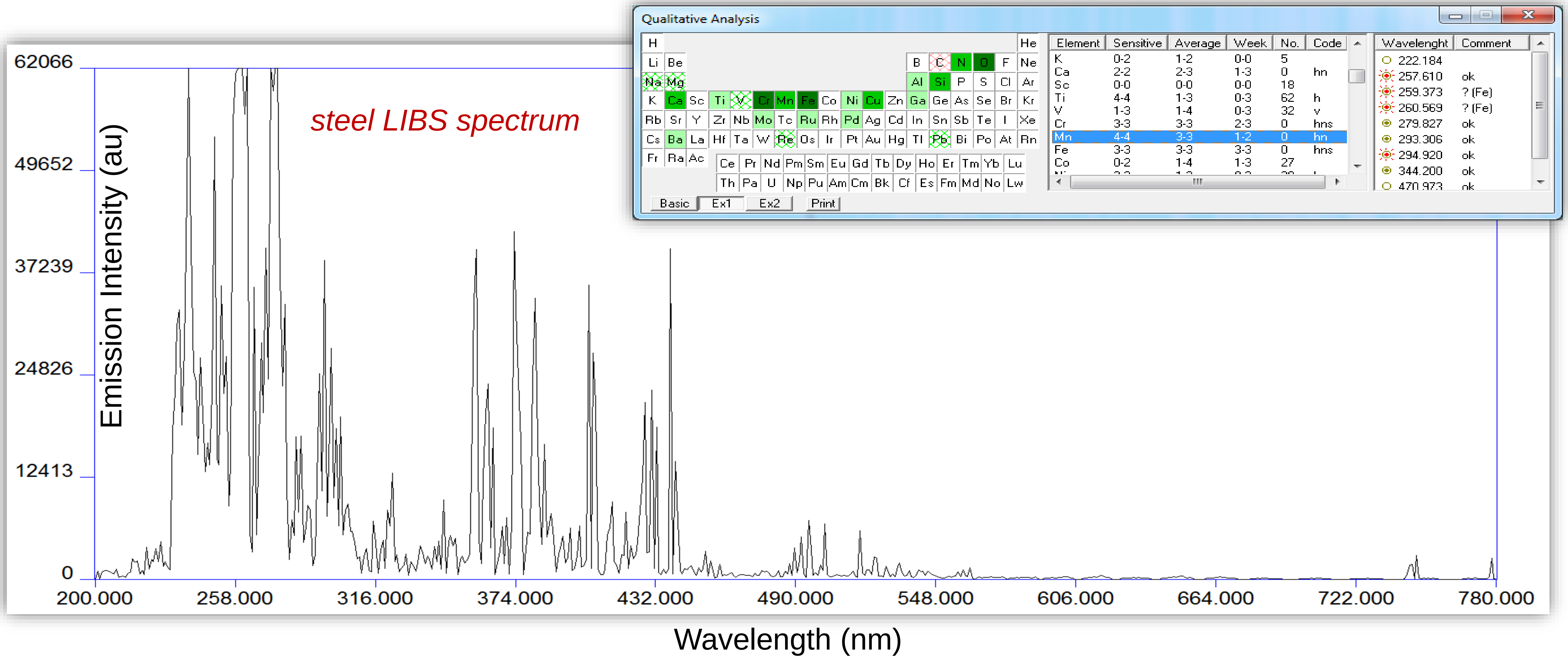
The Goal of LIBS Plasma Creation

to create an optically thin plasma which is in thermodynamic equilibrium (or LTE) and whose elemental composition is the same as that of the target/sample

- if achieved, **atomic emission spectral line intensities** can be related to **relative concentrations** of elements in the target/sample (sometimes absolute concentrations)

- typically these conditions are only met *approximately*

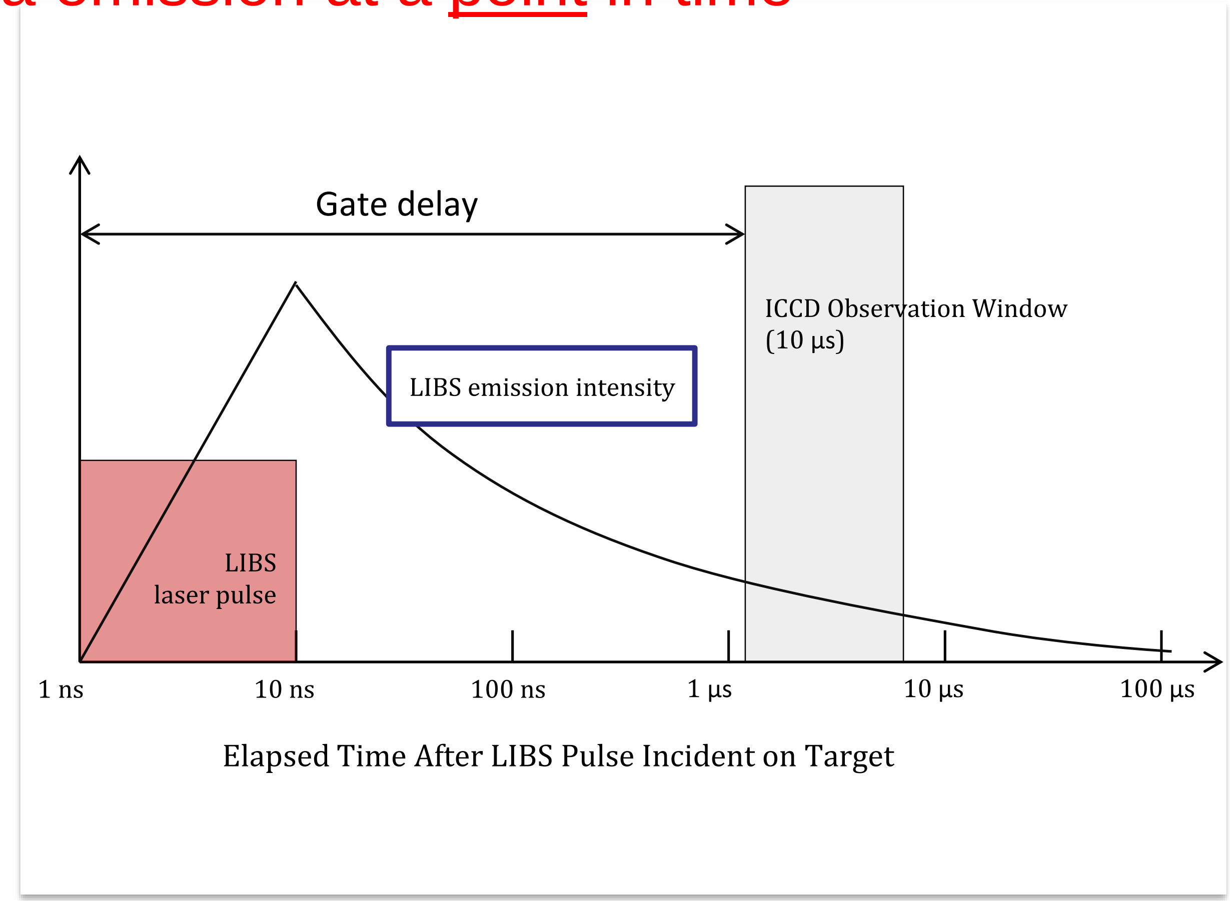
The Goal of LIBS Plasma Creation



2. Principles of LIBS Basics/Theory

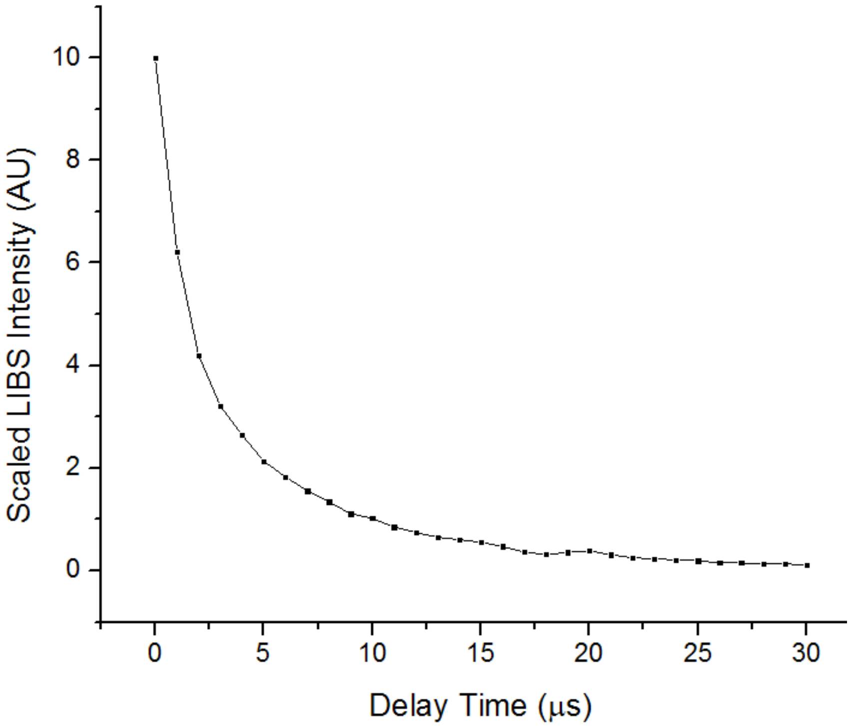
Time gating provides a "snapshot" of the plasma emission at a point in time

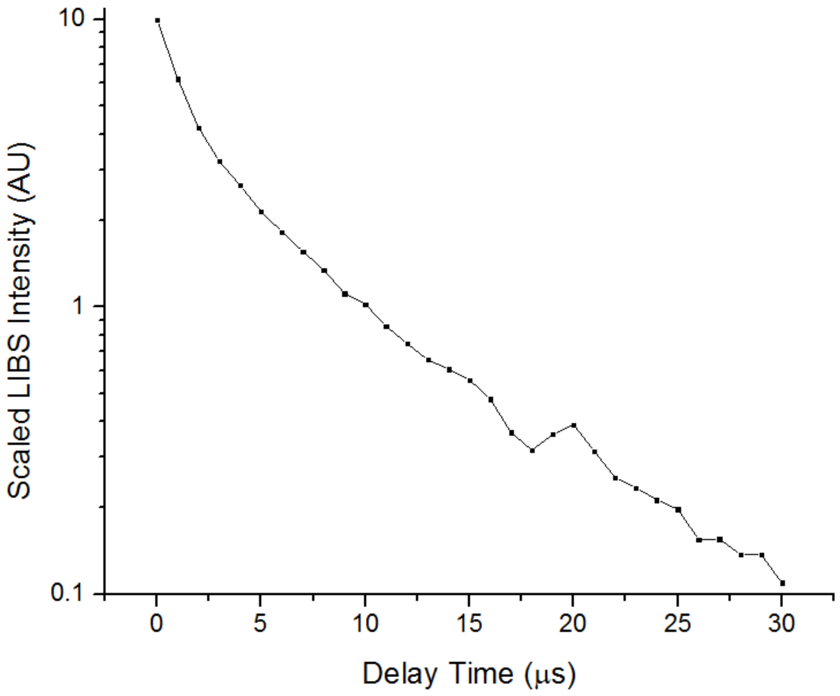
LIBS is traditionally a time-resolved spectroscopy of the rapidly evolving plasma



A finite gate window provides a time "averaged" spectrum over that observation time.

2. Principles of LIBS Basics/Theory





A scaled plot of the integrated emission intensity of Nd as a function of gate delay, using single pulse LIBS, 1064 nm incident pulse, 50 mJ/pulse, argon environment.

For all of our bacterial work, canonical parameters are:

τ_d = 2 μs

τ_w = 20 μs

Choice of time observation parameters should be determined experimentally to:

- maximize signal
- minimize noise
- reduce background
- highlight ions of interest
- reduce linewidth
- reduce line overlap
- observe molecules

Outline

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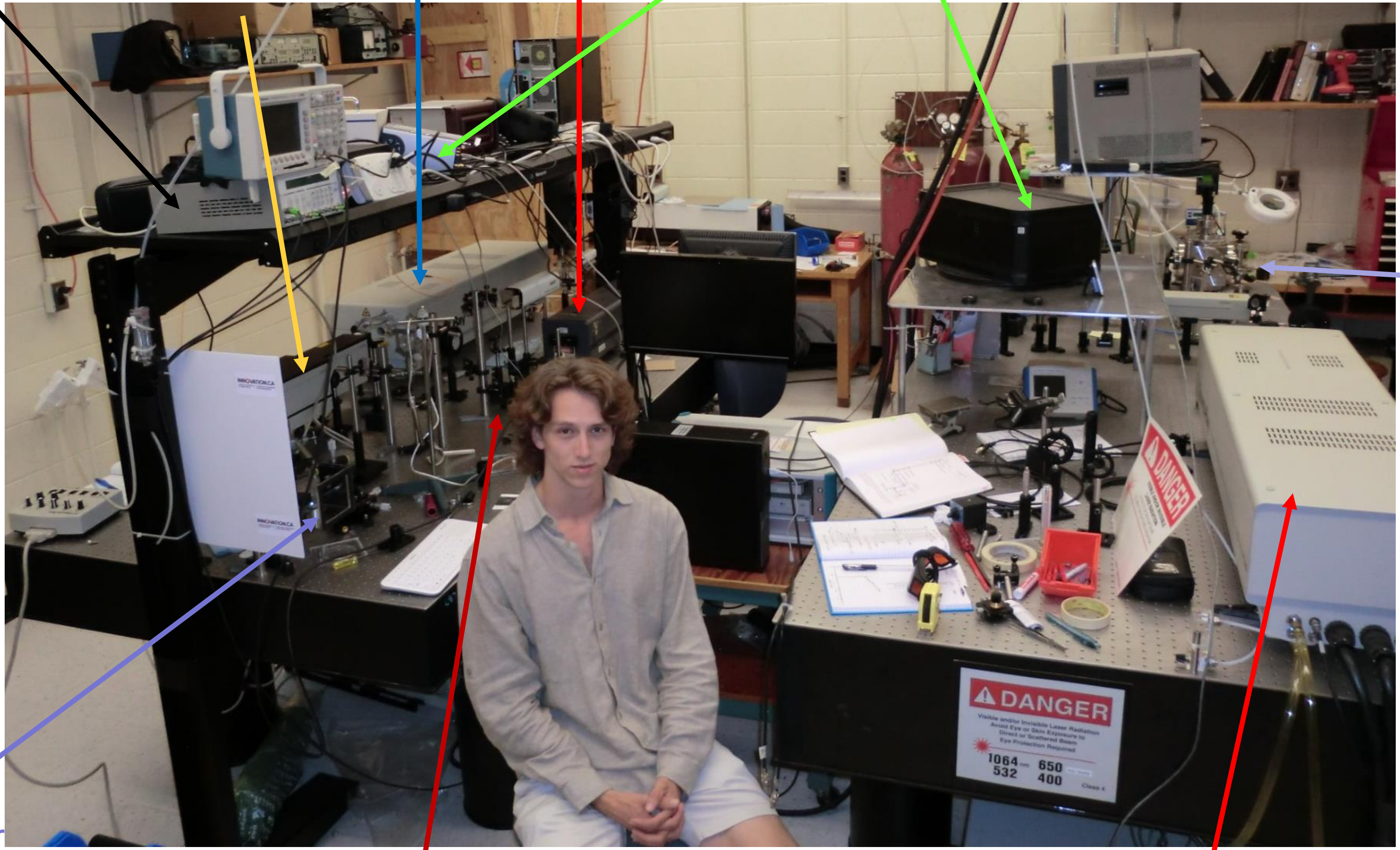
2. Principles of LIBS Apparatus

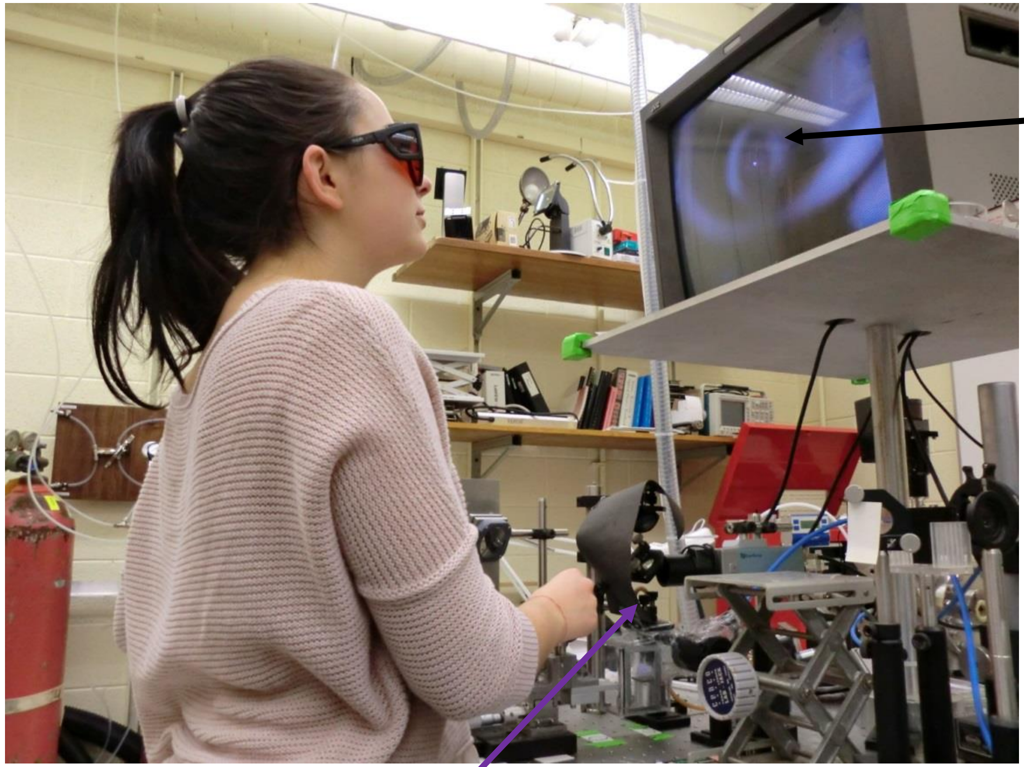


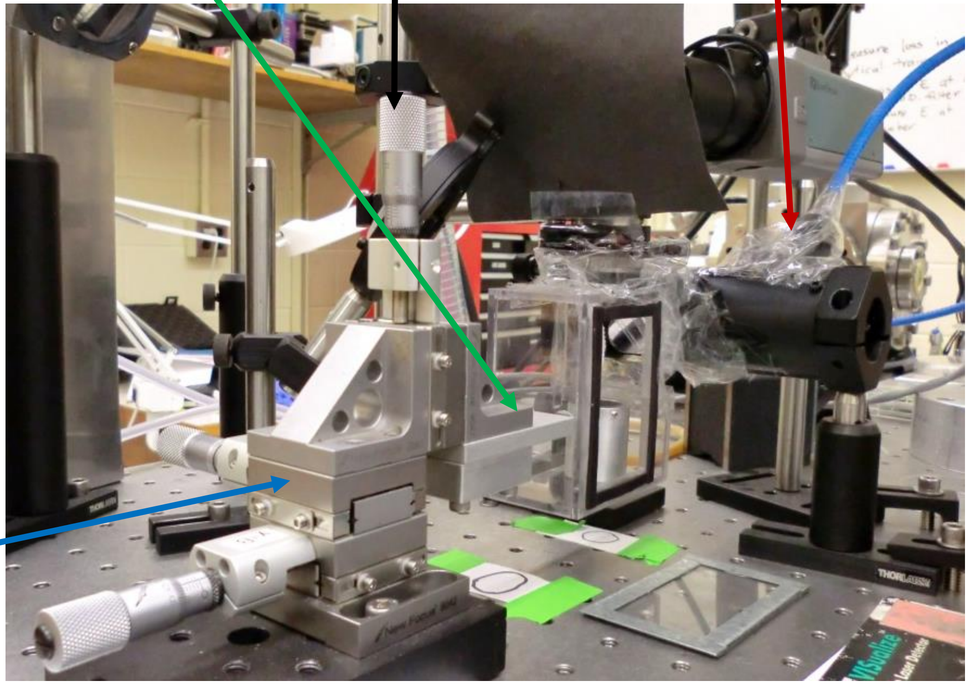
2. Principles of LIBS   Apparatus

Required:
pulsed laser

- ✓ ns, ps, fs all used
- ✓ in general "more power" is better (but μJ LIBS has been done)
  - in general 10 of mJ's wanted
- ✓ all wavelengths have been used
  - fundamental and harmonics of Nd:YAG dominate (355, 532, 1064 nm)
  - match to application, price, or availability
- ✓ fiber lasers?

2. Principles of LIBS Apparatus





Laser spot alignment control and laser focus control

Emission collection optics

Environmental purge chamber

Laser focusing optics

Position adjustment

2. Principles of LIBS Apparatus

Required:
Focusing optics

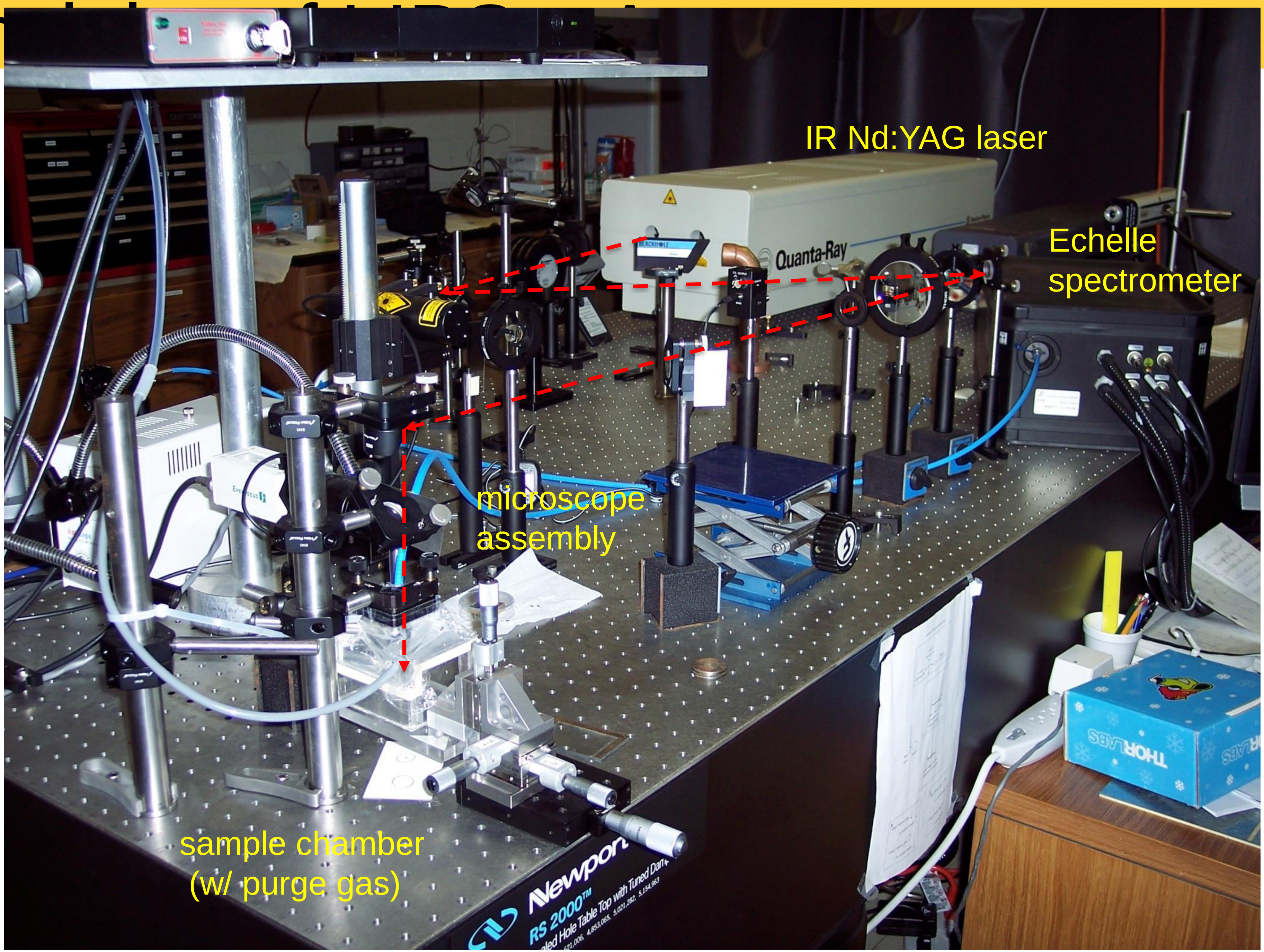
Target translation

Light collection

- ✓ lenses, microscope objective, telescope
  - in general, modest requirements
- ✓ the laser craters target, usually want fresh surface
  - alternately, raster the laser spot
- ✓ collect light and transmit it to spectrometer
  - lenses
  - mirrors
  - optical fibers
  - telescopes

Be careful about wavelength-dependent losses!

2. Prin


IR Nd:YAG laser
Echelle spectrometer
microscope assembly
sample chamber (w/ purge gas)
Quanta-Ray
Newport
RS 2000™

2. Principles of LIBS Apparatus

<u>Optional:</u>
Gas purge chamber

- ✓ not needed, but enhances signal
- ✓ noble gases (*argon*) most often used
- ✓ some users flow gas across surface to remove debris, remnants of previous ablation

2. Principles of LIBS   Apparatus

Required:
Spectrometer

- ✓ Echelle spectrometer
  - very broad bandwidth
  - high-throughput
  - $$$
- ✓ Czerny-Turner spectrometer
  - bandwidth not nearly as good
  - frequently ganged together to get complete spectral coverage
  - compact
  - rugged

2. Principles of LIBS Apparatus

Required:
Camera

✓ ICCD
- high sensitivity
- excellent bandwidth
- integrated time gating
- cooled chip reduces dark noise
- $$$

✓ CCD
- not as sensitive
- less prone to damage
- not intrinsically gated, but some users don't gate at all anymore
- much cheaper
- compact

2. Principles of LIBS   Apparatus

First responder CBRNE prototypes have been built…

ad's-up display

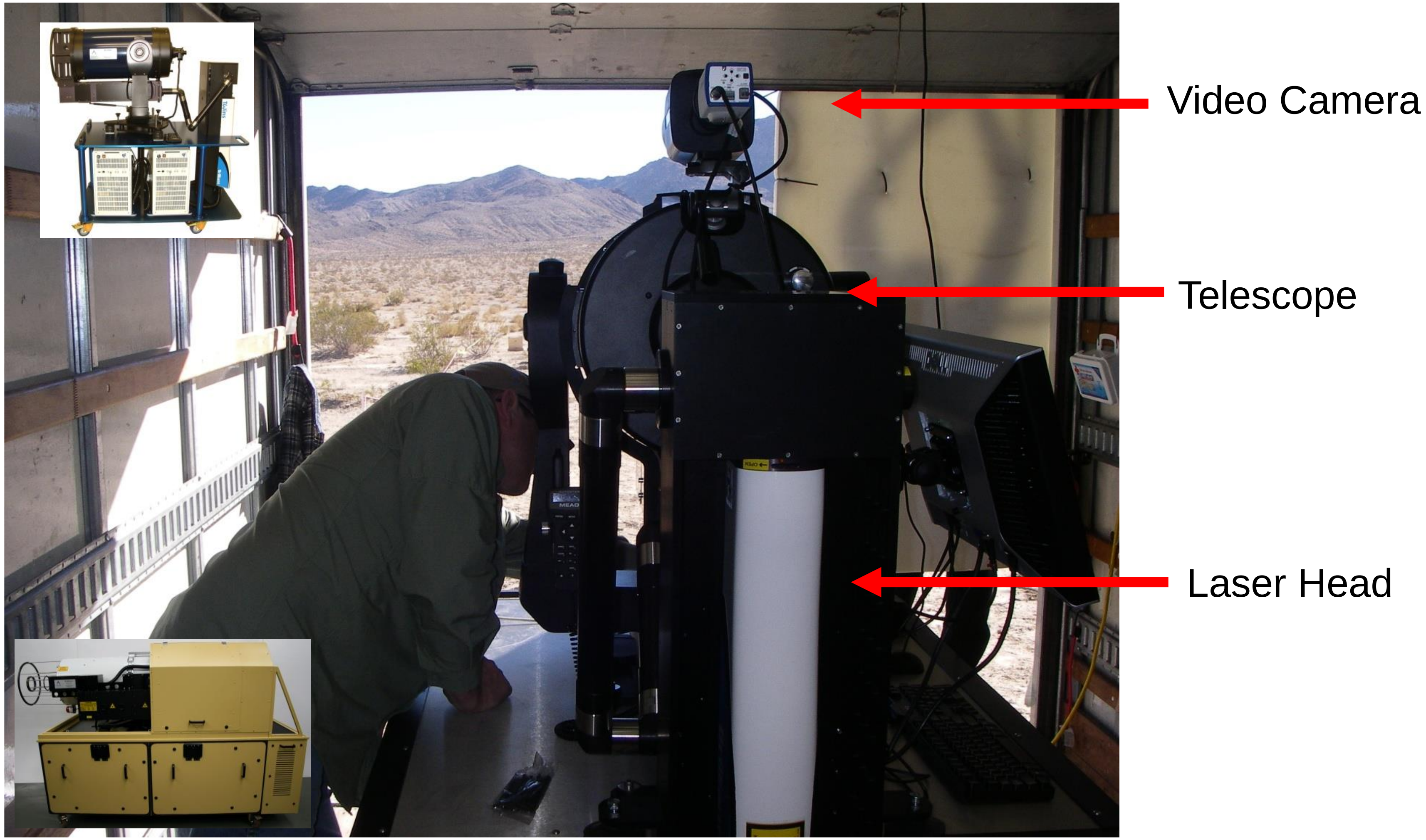
Backpack contai
broadband high-
resolution
spectrometer, las
power supply,
computer, and ba

nd-held probe contains
er, joystick for control,
l focus optics

Microplasma/
LIBS Event

courtesy of Ocean Optics.

High-energy remote systems have been built…



Courtesy of A.J. Miziolek, A. Whitehouse

Commercial benchtop systems have been built…

Coriosity Laser Imager - Elemission

J200 – Applied Spectra

ChemReveal LIBS Desktop Elemental Analyzer – TSO

Hand-held systems have been built…

mPulse – Oxford Instruments

LIBZ – SciApps, Inc

NanoLIBS – B&WTek

ChemLite- TSI, Inc

EOS500 - Bruker

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3. Advantages over other techniques

multi-element sensitivity

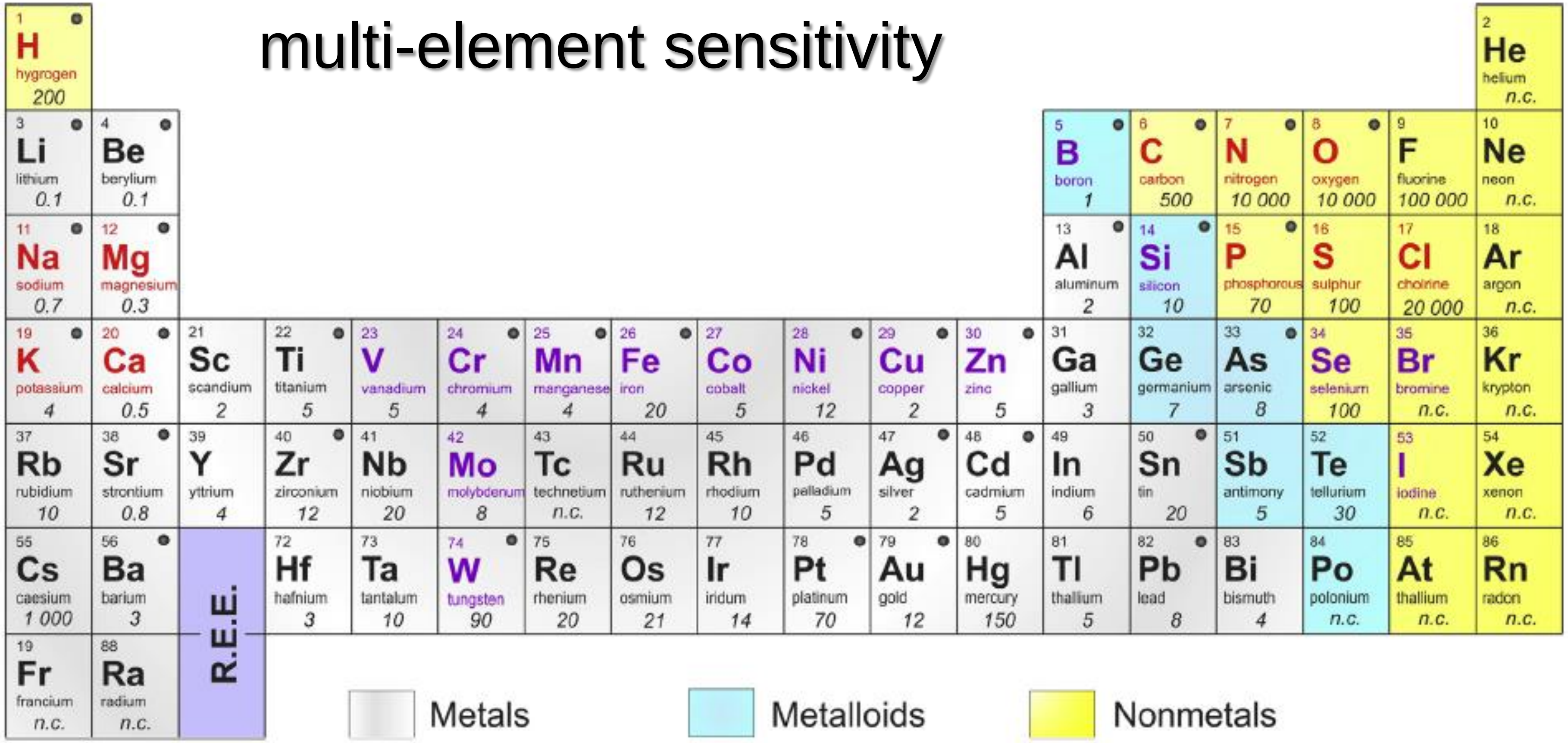


Fig. 1. Periodic table of the elements and LIBS analysis. Almost all elements, including metals, are detectable within biological tissues via LIBS. The essential chemical elements for most living organisms are displayed as follows: bulk biological elements are in red and essential trace inorganic elements for plants or animals are in purple, according to [25]. Endogenous and exogenous elements already detected in tissues via LIBS in previous biological studies are marked with a black dot. The theoretical LOD is given in parts per million and is indicated by the number in italics under the chemical name of the element. R.E.E.: rare earth elements.

Review

Elemental imaging using laser-induced breakdown spectroscopy: A new and promising approach for biological and medical applications

Benoit Busser[a,b,c,*], Samuel Moncayo[b], Jean-Luc Coll[a], Lucie Sancey[a,1], Vincent Motto-Ros[b,1]

Coordination Chemistry Reviews 358 (2018) 70–79

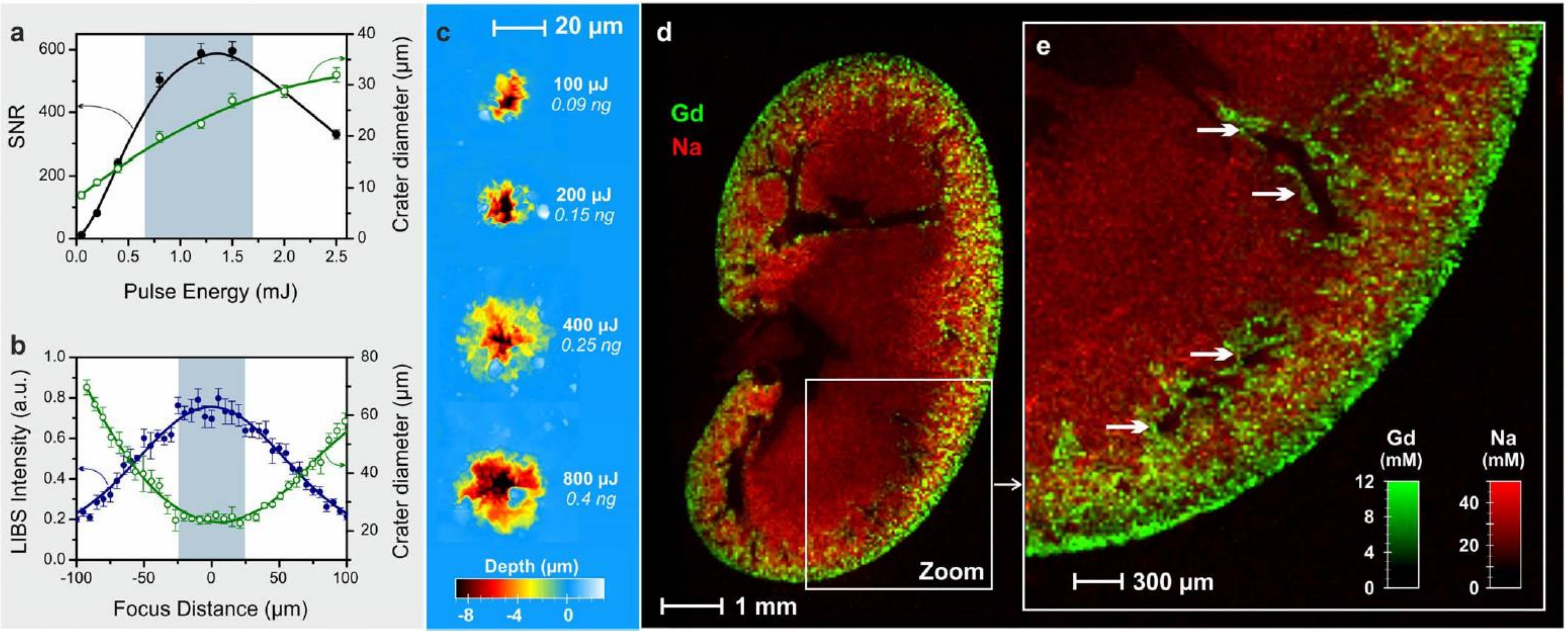
3. Advantages over other techniques

spatial resolution

Laser allows point sampling (1-100 micron)

Elemental “surface maps” can then be created

Gd nanoparticles in mouse kidney



Laser spectrometry for multi-elemental imaging of biological tissues

L. Sancey*, V. Motto-Ros*, B. Busser, S. Kotb, J. M. Benoit, A. Piednoir, F. Lux, O. Tillement, G. Panczer & J. Yu

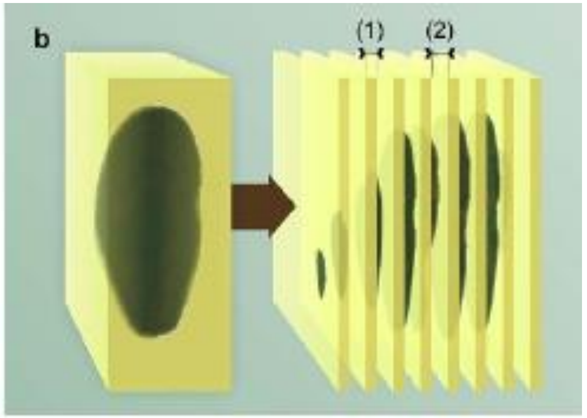
SCIENTIFIC REPORTS | 4 : 6065 | DOI: 10.1038/srep06065

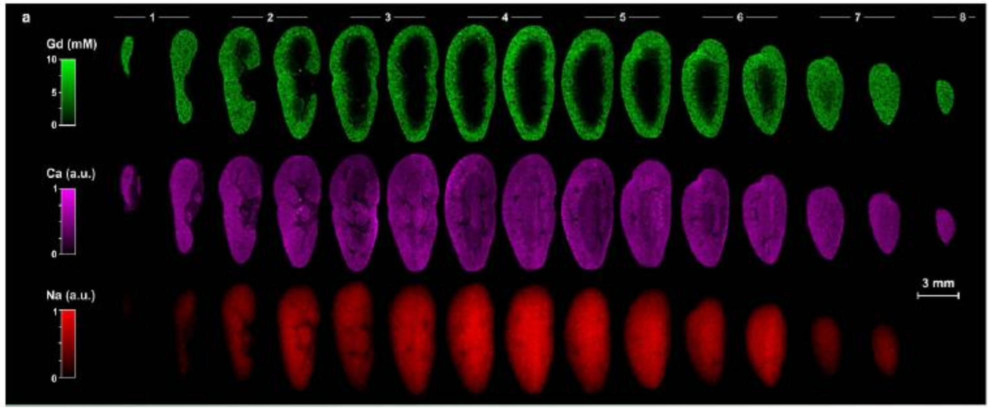
3. Advantages over other techniques

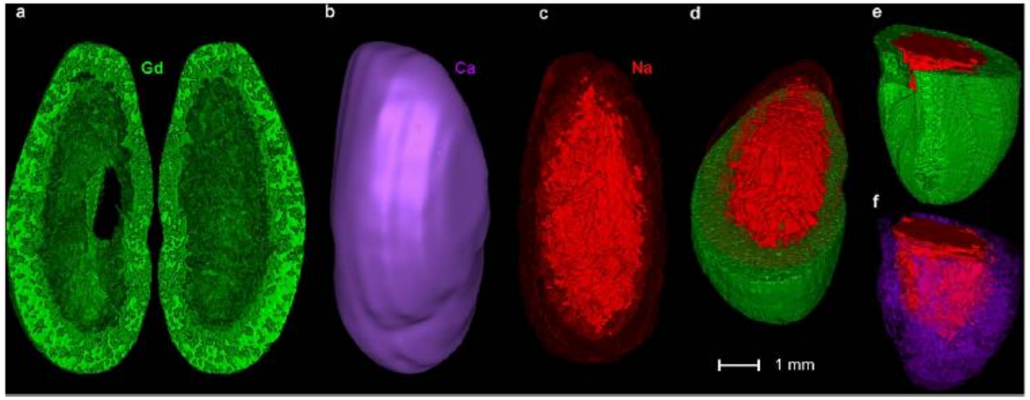
depth profiling

Because laser only removes μg to ng of material, ablation crater only microns deep

Subsequent shots thus sample progressively deeper layers







3D Imaging of Nanoparticle Distribution in Biological Tissue by Laser-Induced Breakdown Spectroscopy

Y. Gimenez[1], B. Busser[1], F. Trichard[1], A. Kulesza[1], J. M. Laurent[2], V. Zaun[3], F. Lux[1], J. M. Benoit[1], G. Panczer[1], P. Dugourd[1], O. Tillement[1], F. Pelascini[3], L. Sancey[1] & V. Motto-Ros[1]

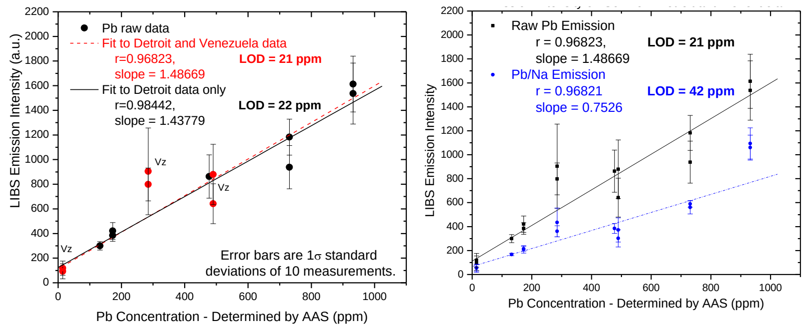
SCIENTIFIC REPORTS | 6:29936 | DOI: 10.1038/srep29936

3. Advantages over other techniques

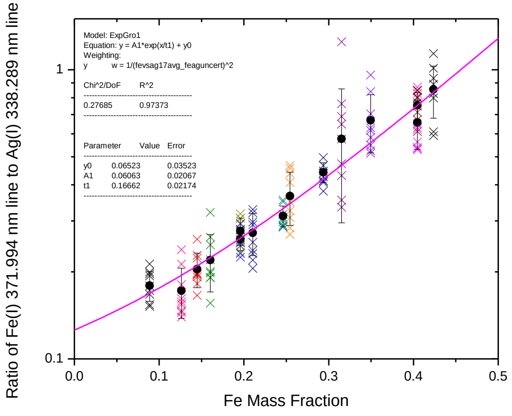
sensitivity & speed

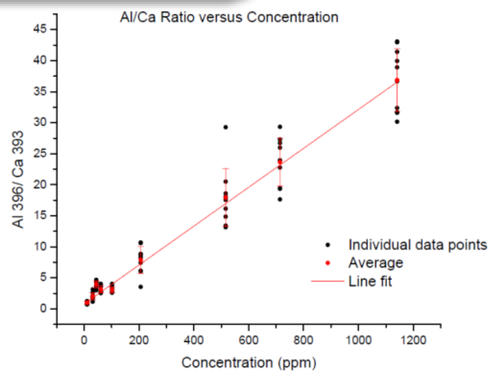
Concentrations of 1-100 ppm usually detectable in seconds using a standard LIBS apparatus

Lead in Soil (Detroit and Venezuela)



Fe in Biocompatible alginates



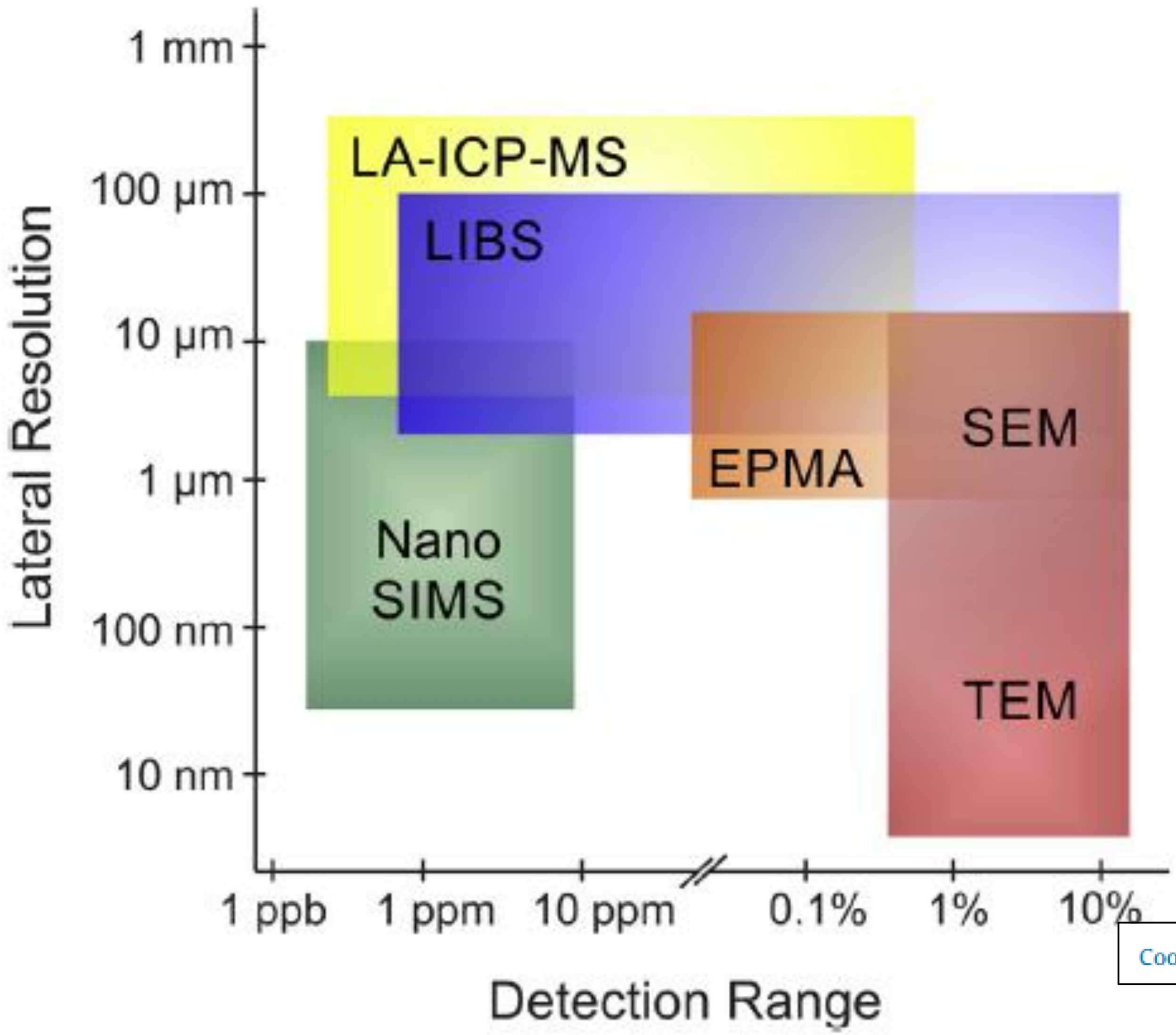


$R^2 = 0.89$

s = 0.0313

3. Advantages over other techniques

sensitivity & spatial resolution vs. other methods



Review

Elemental imaging using laser-induced breakdown spectroscopy: A new and promising approach for biological and medical applications

Benoit Busser[a,b,c,*], Samuel Moncayo[b], Jean-Luc Coll[a], Lucie Sancey[a,1], Vincent Motto-Ros[b,1]

Coordination Chemistry Reviews 358 (2018) 70–79

3. Advantages over other techniques

portability and stand-off potential

Outline

21st Century Medicine, One Spark at a Time:

Biomedical Applications of Laser-Induced Breakdown Spectroscopy

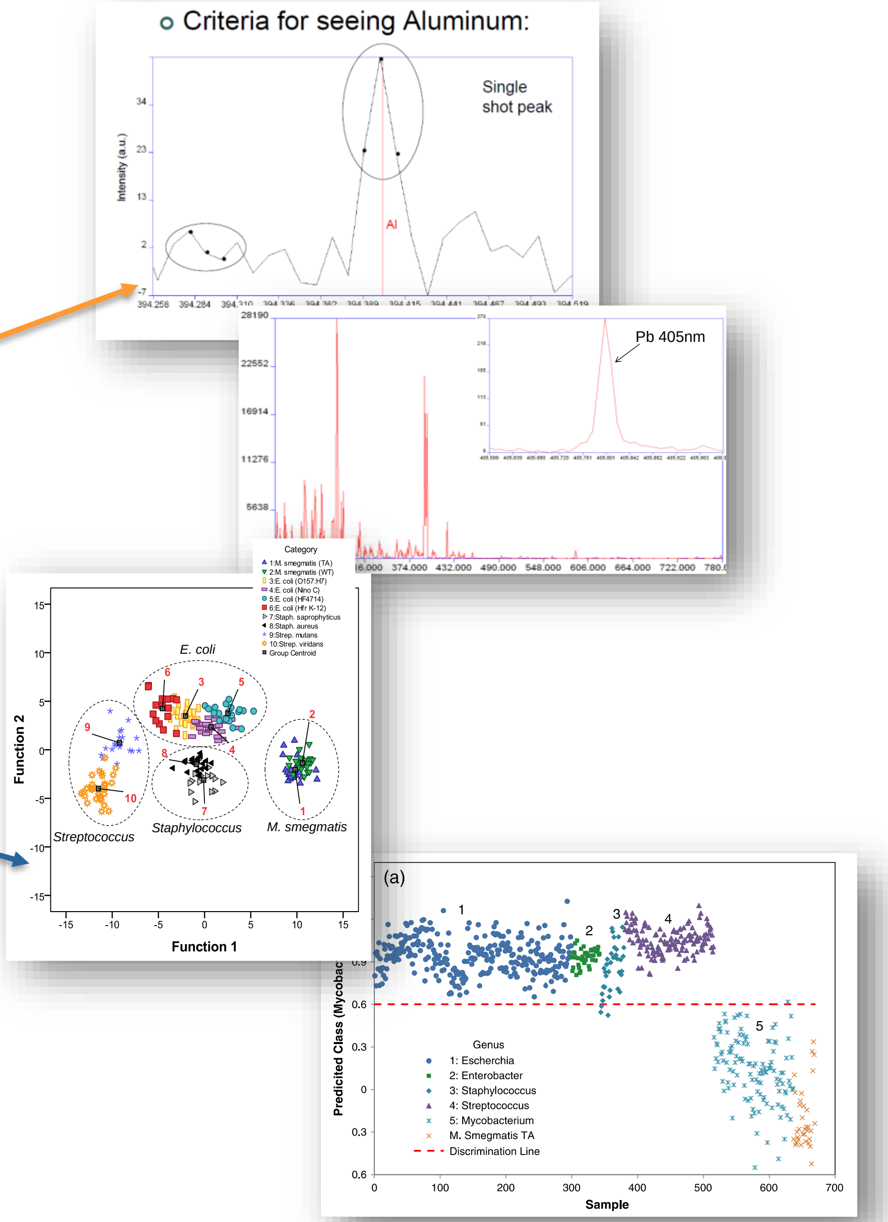
1. What is LIBS?
2. Principles of LIBS
   a. Basics / theory
   b. Apparatus
3. Advantages over other techniques
4. Specific medical/biomedical applications of LIBS

4. Specific medical/biomedical applications

No matter what your application is,
<u>you will be doing one of two things:</u>

1. Attempting to quantify the amount/concentration of some element by analyzing peak intensities

2. Attempting to identify/classify a target based on its unique elemental composition by analyzing the presence and intensity of all/many lines



4. Specific medical/biomedical applications

Spectrochimica Acta Part B 152 (2019) 123–148

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journal homepage: www.elsevier.com/locate/sab

Invited review

Laser-induced breakdown spectroscopy for human and animal health: A review

Rosalba Gaudiuso[a,b], Noureddine Melikechi[a,*], Zienab A. Abdel-Salam[c], Mohamed A. Harith[c], Vincenzo Palleschi[d], Vincent Motto-Ros[e], Benoit Busser[e,f,g]

[a] *University of Massachusetts Lowell, USA*
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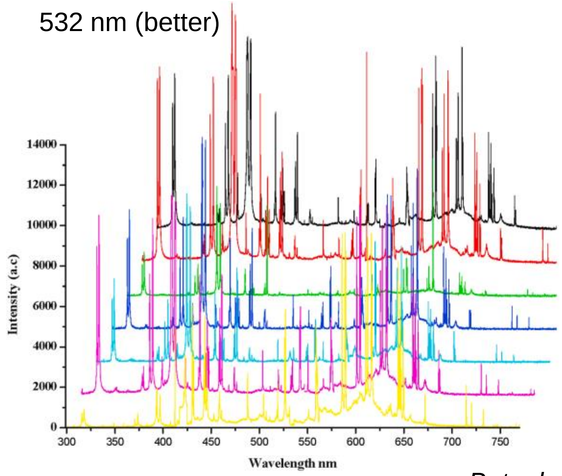
[e] *Institut Lumière Matière UMR 5306, Université Lyon 1 - CNRS, Villeurbanne, France*
[f] *Grenoble University Hospital, Grenoble, France*
[g] *Université Grenoble Alpes, Institute of Advanced Biosciences, Grenoble, France*

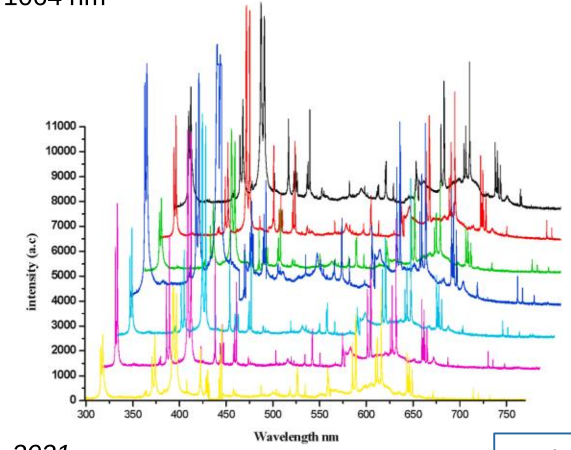
4. Specific medical/biomedical applications

Hard Tissues: Teeth

532 nm (better)



1064 nm



Batool et al., 2021

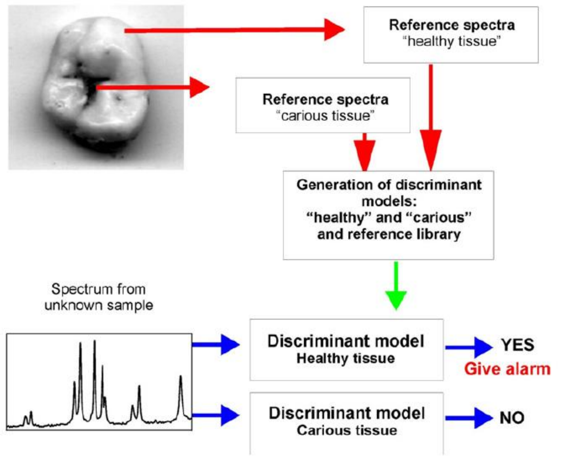
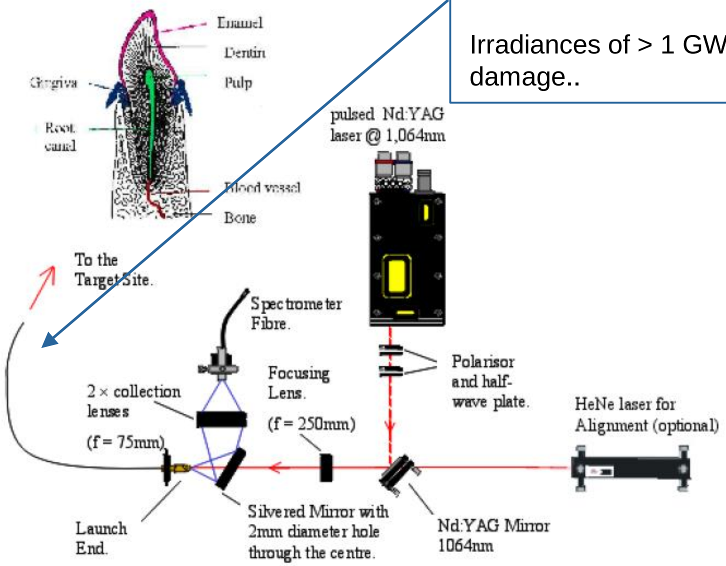


Figure 2
Principle of sample identification / screening applications based on discriminant analysis, here for warning when healthy tooth material is targeted during laser drilling.

A single core fiber, φ = 550 μm, length = 5 m to deliver laser AND collect plasma emission.

Irradiances of > 1 GW/cm^2 possible without damage..



Samek et al., 2001

Researchers have measured a dramatic variation in the relative concentrations of Ca, Sr, Na, Ti, and Cu in <u>carious</u> tooth tissue relative to <u>healthy</u> tissue.

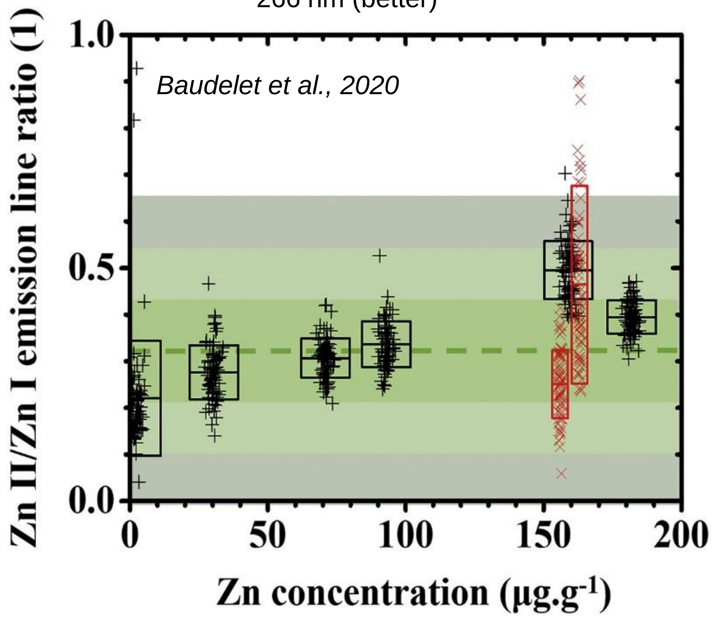
LIBS has the potential to become a useful tool for *in vivo / in vitro* caries identification during a drilling or cleaning process

Spatial resolution on the order of 100–200 μm and a depth resolution of approximately 10 μm.

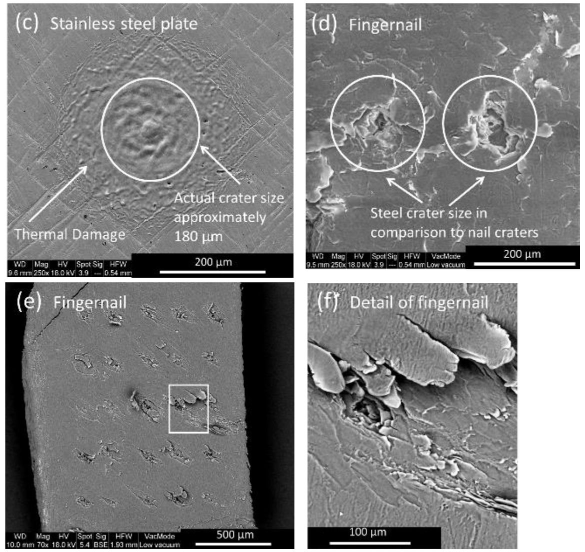
4. Specific medical/biomedical applications

Nails

266 nm (better)



1064 nm



Riberdy et al., 2017

Nails are mostly <u>keratin</u>.

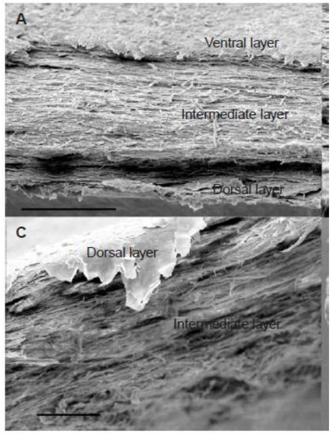
Due to their structure, ablation has shot to shot repeatability issues.

Keratin "standards" not effective.

Mostly used to monitor trace metals reflective of human health:

- zinc (deficiency)
- vitamin D deficiency
- hyperthyroidism and hypothyroidism
- archaeology
- opium addiction

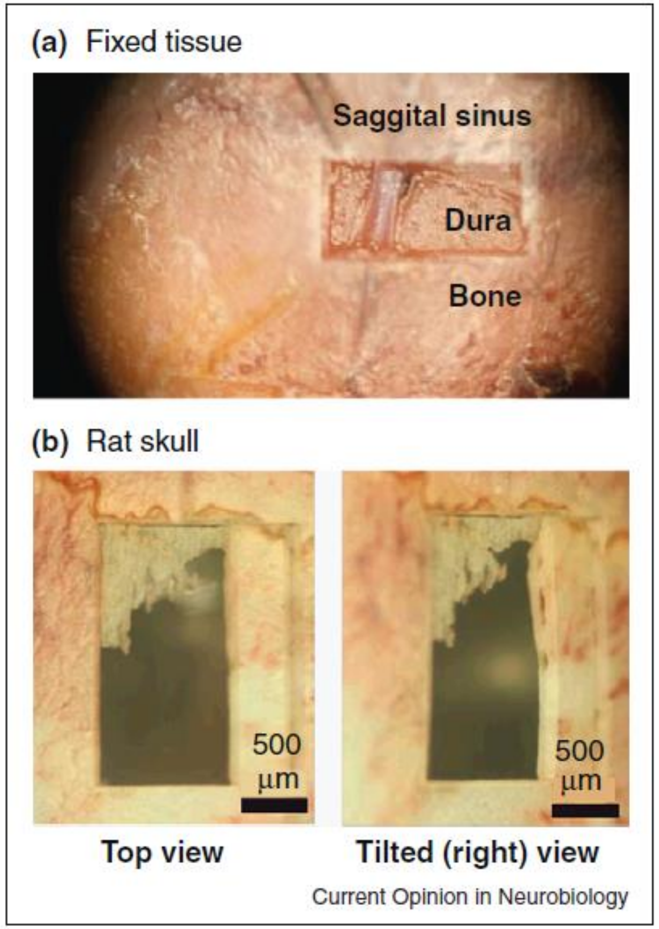
Point sampling and the fact that it can be done *in vivo* allows a monitoring of the progression of pathology with time



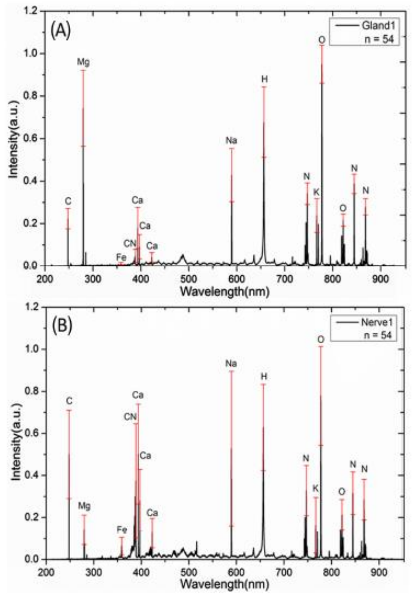
Farren et al., 2004

4. Specific medical/biomedical applications

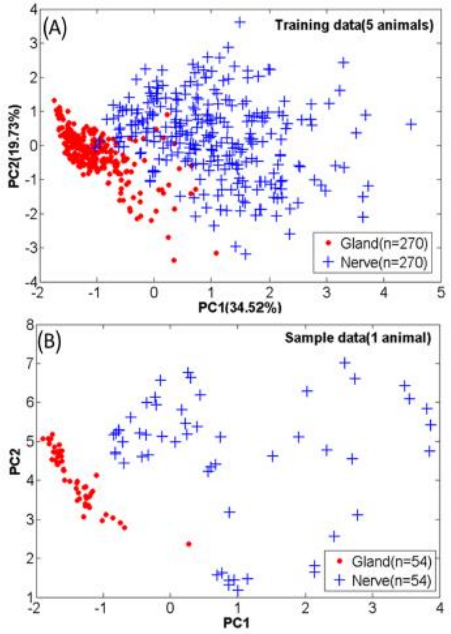
Laser Guided Surgery



Jeong et al., 2012

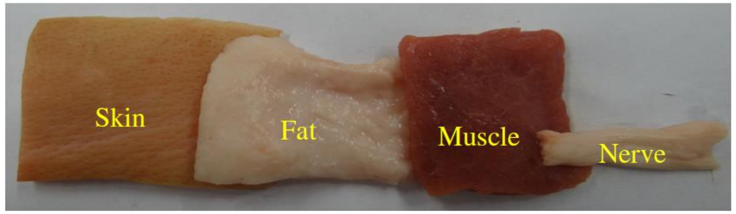


2 Mean LIBS spectra of Gland (A) and Nerve tissue of animal #1



Mehari et al., 2016

Kanawade et al., 2013



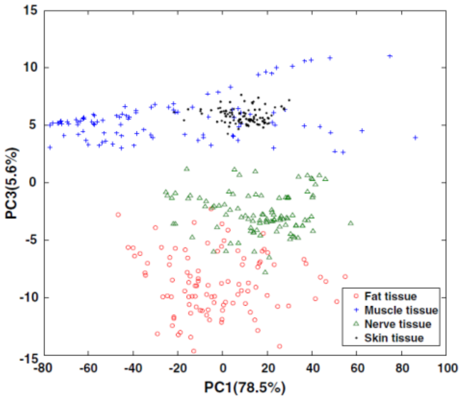


Fig. 4. PCA scores along PC1 and PC3 of four tissue types of Pig 2.

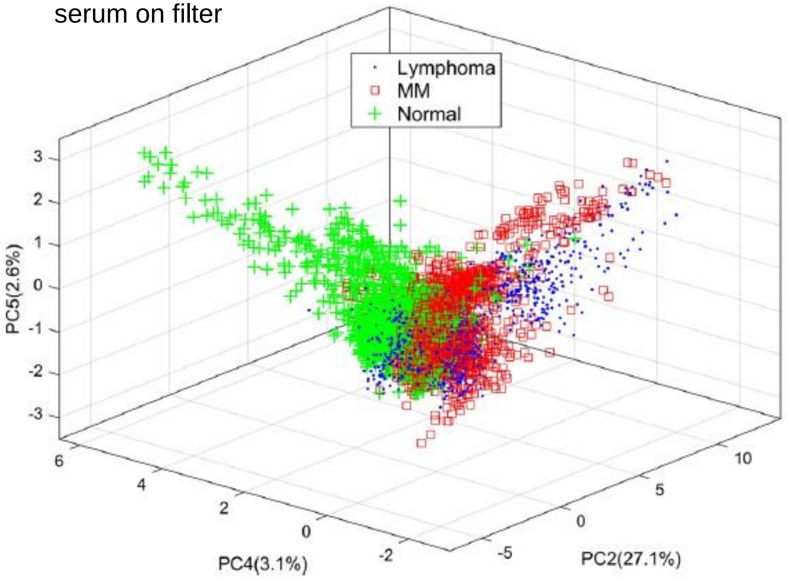
Used to differentiate tissue types for surgical removal (margins)

- Skull vs. brain/spinal cord
- Micromachining during surgery (precision craniotomy)
- Gland vs. nerve
- Malignant vs. benign tissues

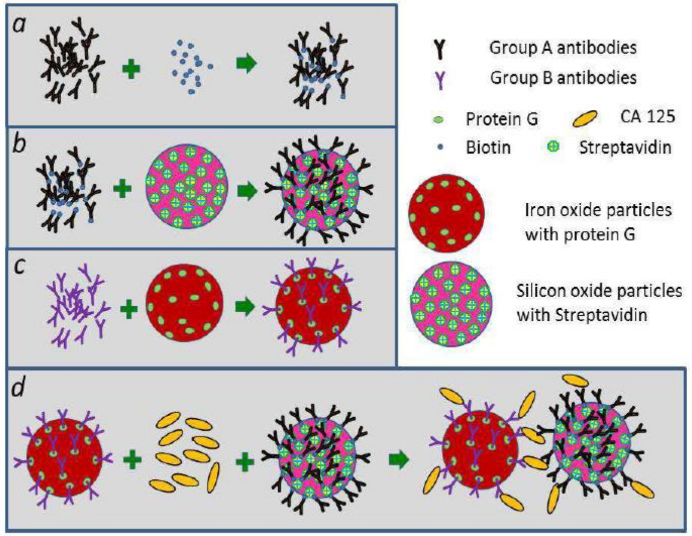
4. Specific medical/biomedical applications

Malignant Tissue

serum on filter



Chen et al., 2018



Markushin et al., 2012

CA125 ovarian cancer biomarker detected at the level of 10 U/mL (estimated LOD ~ 1 U/mL)

Tissues

Higher concentrations of major and trace elements such as Mg, Fe, Ca, Na, and K in the neoplastic tissues.

Unfortunately a lot of work done *ex vivo* and in less-than-realistic experiments (caution)!

Biomarkers in fluid specimens

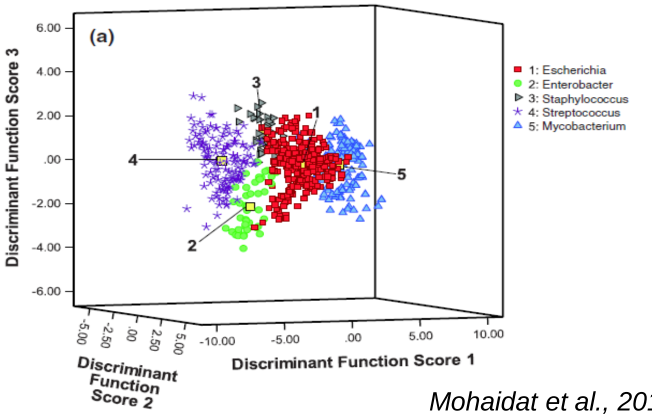
With a combination of appropriate substrate and algorithm, melanoma biomarkers in blood showed discrimination between healthy and diseased mice with accuracy up to 96%, but *direct analysis of LIBS spectra did not provide any conclusive results*. (*Gaudiuso et al. 2018)*

Multi-element micro- and nanoparticles labelling approach an attractive alternative (*Markushin et al. 2012*)

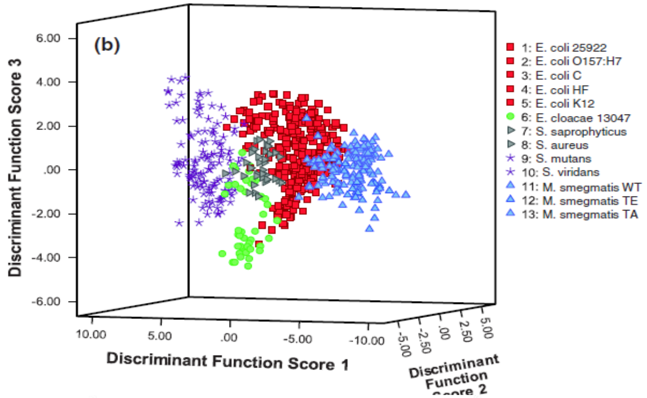
1 U or IU is defined as the enzyme quantity that liberates 1 microgram or micromole of product per ml of the reaction mixture per minute

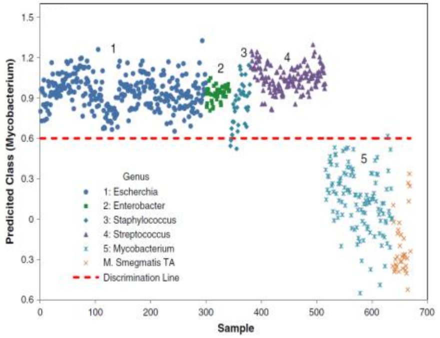
4. Specific medical/biomedical applications

Bacteria



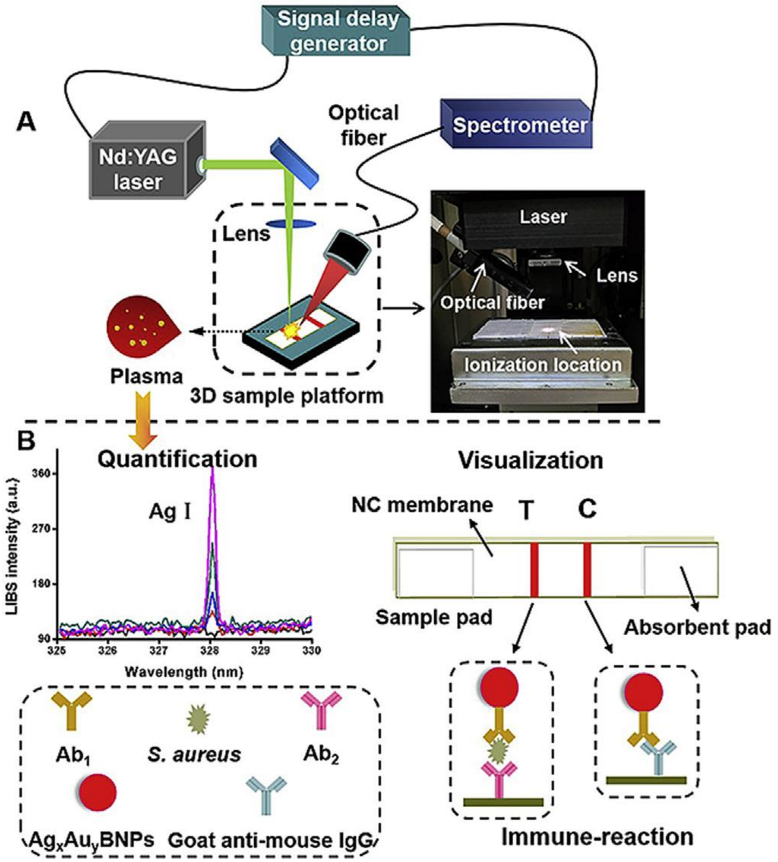
Mohaidat et al., 2012





Putnam et al., 2013

Wu et al., 2019



Differentiation provided by trace elements.

Single cells detectable (in aerosol or via levitation).

Strain differentiation demonstrated.

LOD can be improved with the “multi-element label” technique.

4. Specific medical/biomedical applications

Bacteria

Spectrochimica Acta Part B 154 (2019) 50–69

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Invited Review

A review of the use of laser-induced breakdown spectroscopy for bacterial classification, quantification, and identification

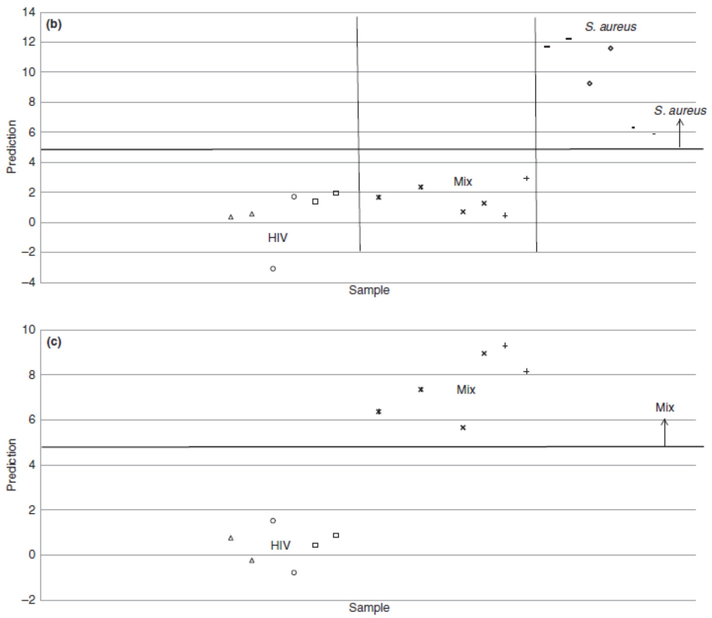
Check for updates

Steven J. Rehse*

University of Windsor, Department of Physics, Windsor, Ontario N9B 3P4, Canada

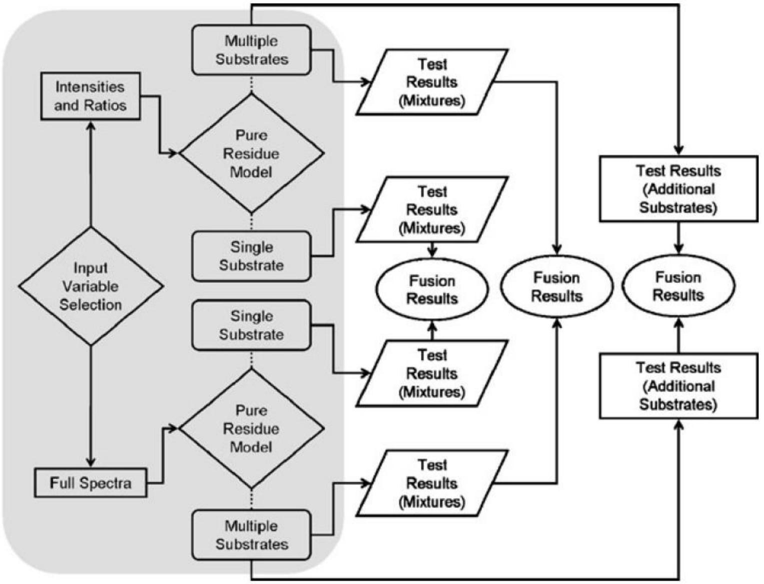
4. Specific medical/biomedical applications

Viruses



Multari et al., 2019

Discrimination of blood with HIV from blood with *S. aureus*



Gottfried et al., 2011

The ability of LIBS to detect the presences of an MS-2 bacteriophage

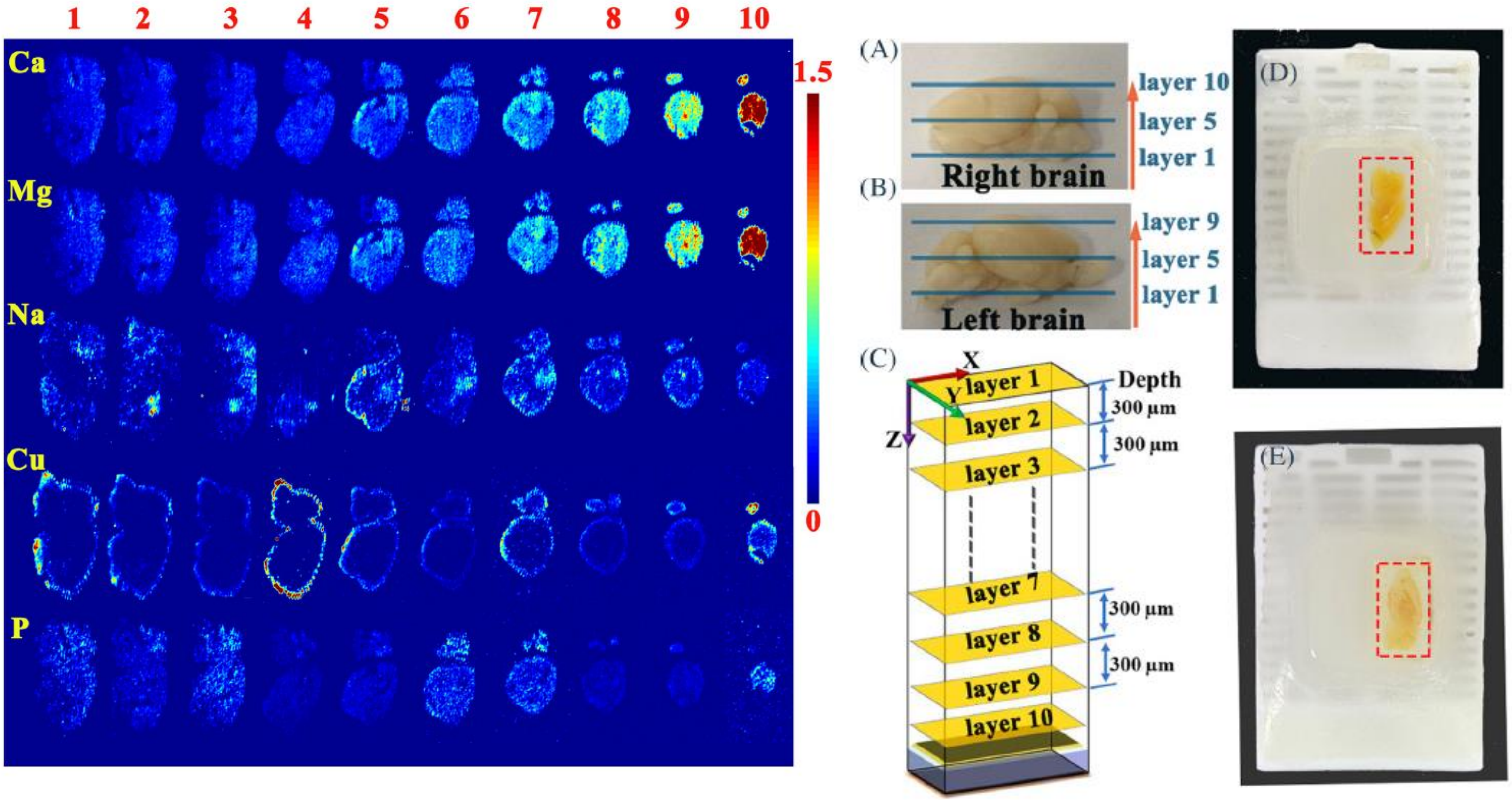
<u>Some early work..</u>

the mass of a virus particle is approximately 10^9 times less than a bacterial cell due to their vast difference in size.

Lack of any trace inorganic or metal atoms (e.g. Ca, Mg, Na, or K) in the virus.

Differentiation of four strains of live *hantavirus* responsible for numerous infections (*Multari, 2012*).

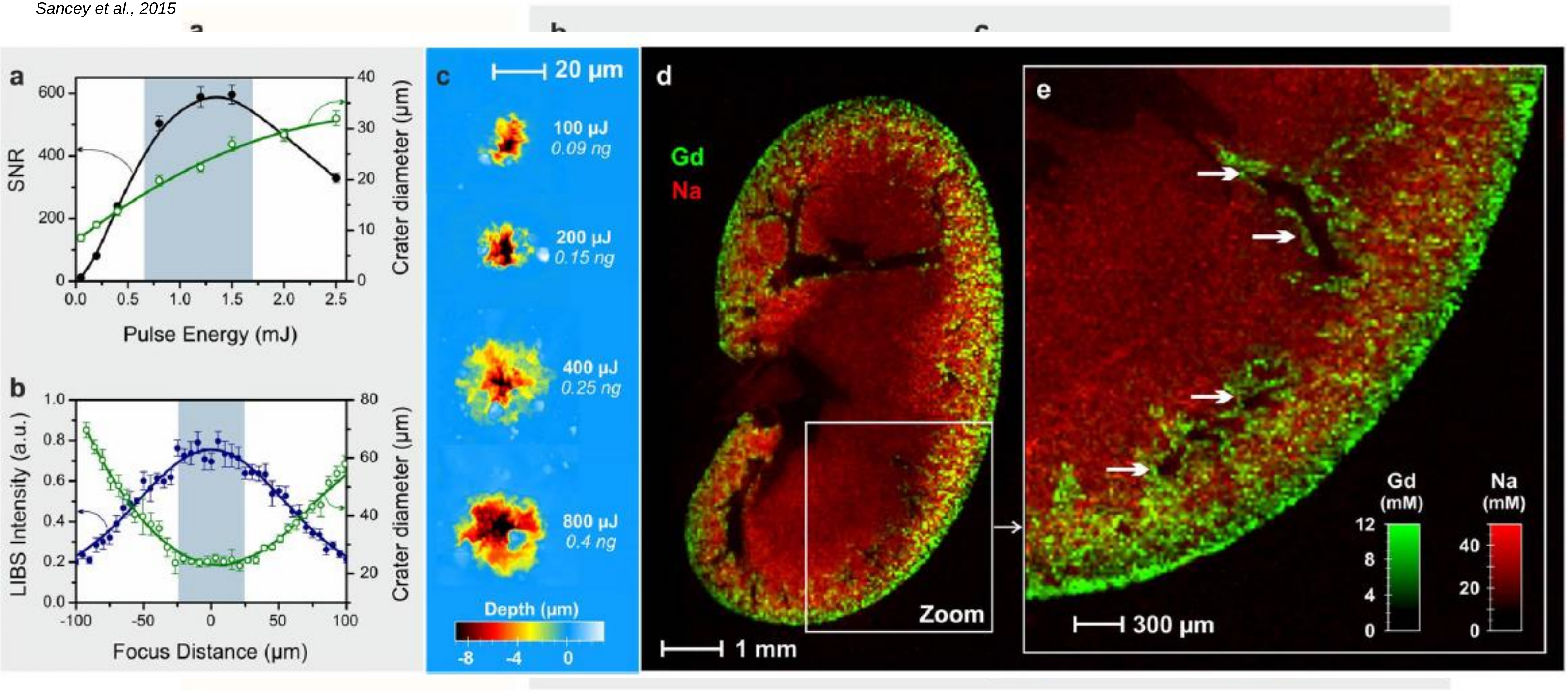
4. Specific medical/biomedical applications



4. Specific medical/biomedical applications

Tissue Mapping

Sancey et al., 2015



4. Specific medical/biomedical applications

Tissue Mapping

Moon et al., 2018

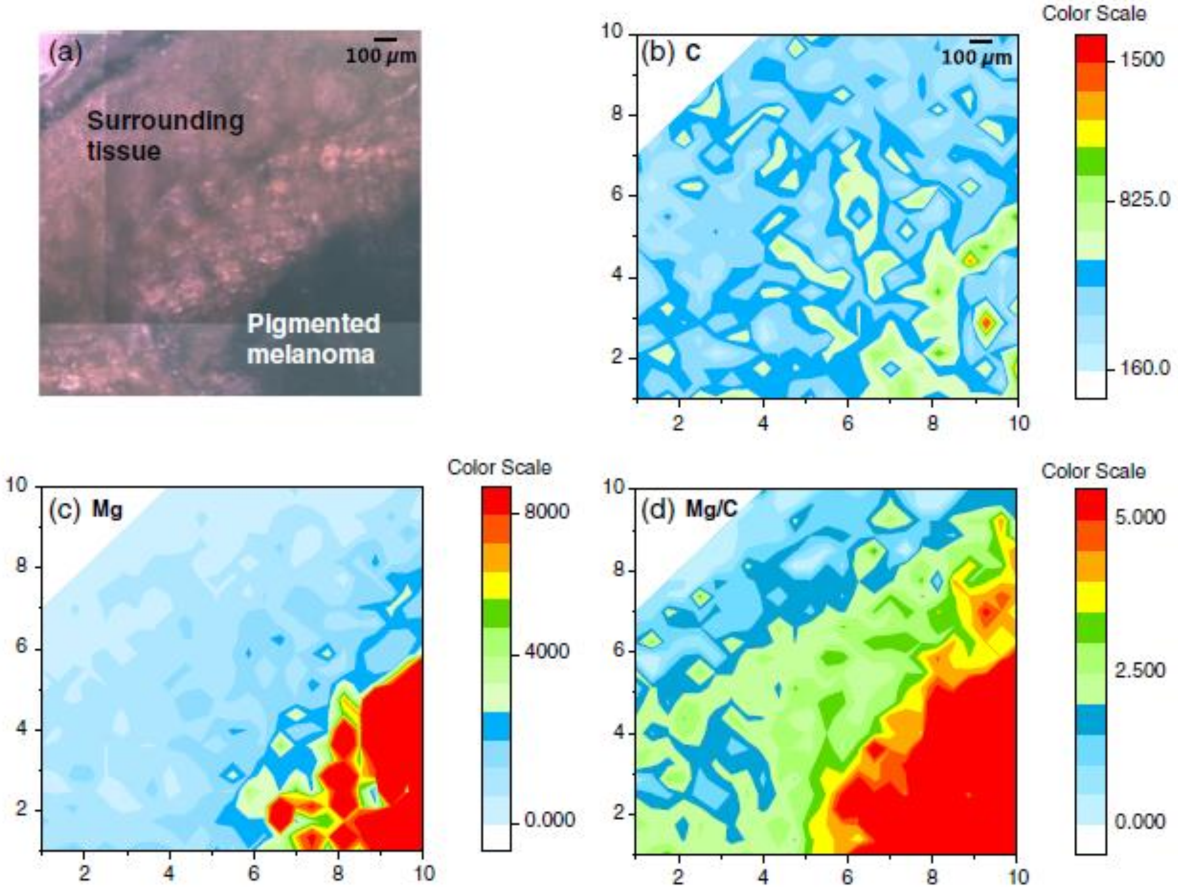


Fig. 4 (a) CCD image of the melanoma tissue section on silicon wafer before ablation, and the LIBS intensity maps of (b) C(I) 247.856 nm and (c) Mg(II) 279.553 + 280.270 nm lines, and (d) the map of Mg(II)/C(I) intensity ratio.

fs-LIBS for melanoma differentiation (frozen tissue sections)

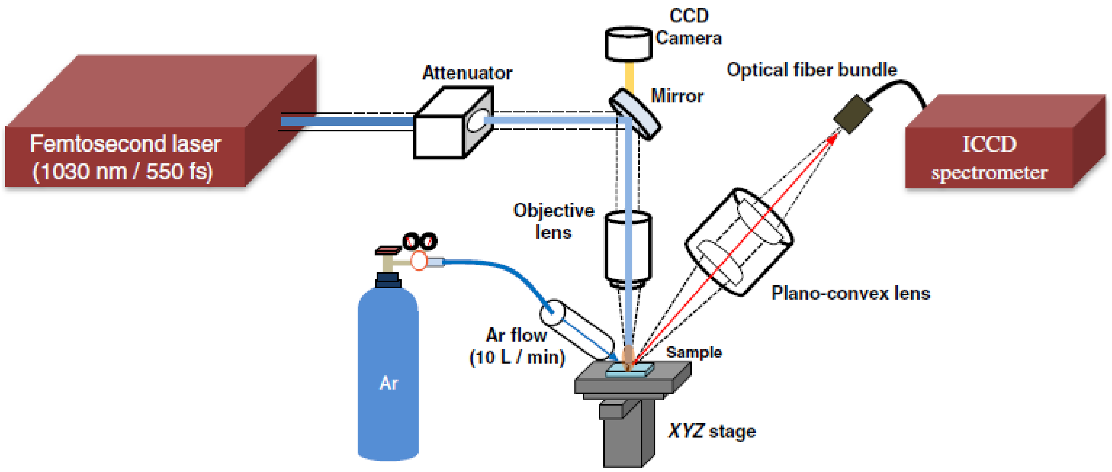


Fig. 2 Schematic diagram of the LIBS system.

Concluding thoughts

21st Century Medicine, One Spark at a Time:

Biomedical Applications of Laser-Induced Breakdown Spectroscopy

- LIBS has tremendous potential to be useful tool in the clinic, clinical laboratory, operating suite, mobile hospital, etc.
- Not yet adopted for true clinical use yet.
  - regulatory hurdles?
  - simple enough for non-professionals to use?
  - use of chemometric algorithms?

Concluding thoughts

21st Century Medicine, One Spark at a Time:

Biomedical Applications of Laser-Induced Breakdown Spectroscopy

- LIBS is far from the only spectroscopic modality in this situation…
- Achieve early-adoption in clinical laboratories first?
  - (i.e. MALDI-TOF)

For Anyone Interested in Graduate Studies With Us…

Go to YouTube, “UWindsor physics research”

For Anyone Interested in My Group…

Go to YouTube, "UWindsor physics rehse"

THANK YOU!

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And thank you to all my students over the years who have allowed me to study these things…