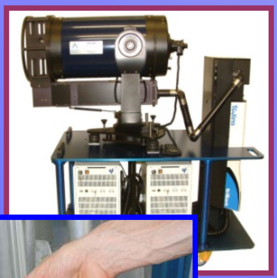
Recent advances in the use of laser-induced breakdown spectroscopy (LIBS) as a rapid point-of-care pathogen diagnostic

presented at the 2012 SPIE Defense, Security, and Sensing Conference.

Global Health I: Telemedicine and Point-of-Care Diagnostics
Monday, April 23rd, 2012

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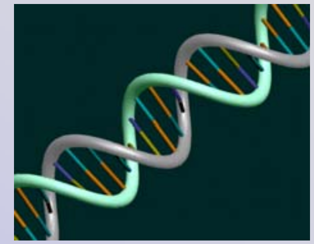
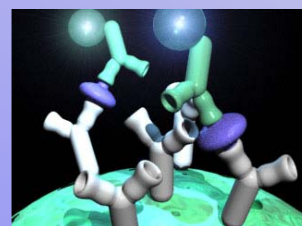
Infectious Pathogen Diagnosis

microbiological

genetic



serological



compositional

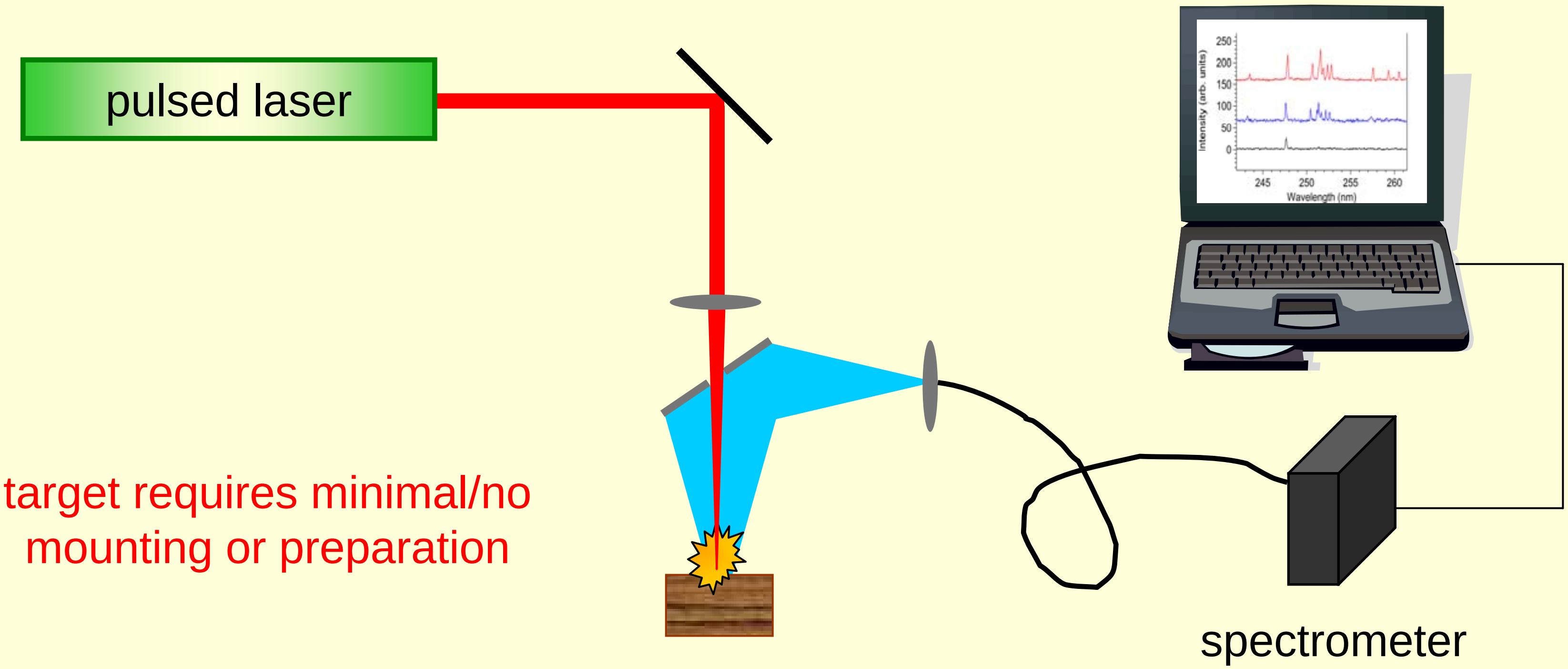
spectroscopic/spectrometric

Raman spectroscopy

Laser-induced breakdown spectroscopy LIBS

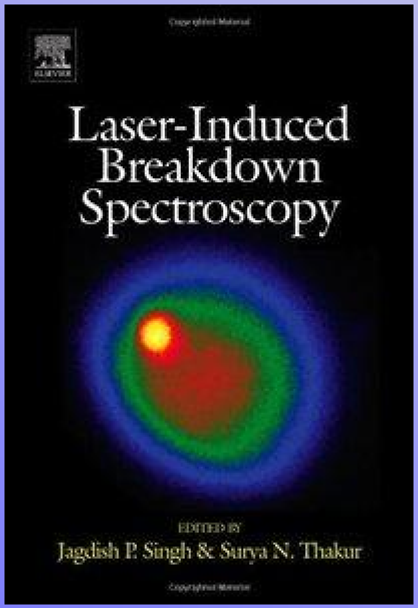
MALDI-TOF

Laser-Induced Breakdown Spectroscopy



LIBS Spectrum is like a Bar Code: Unique for Each Sample

Entire procedure can take under one second!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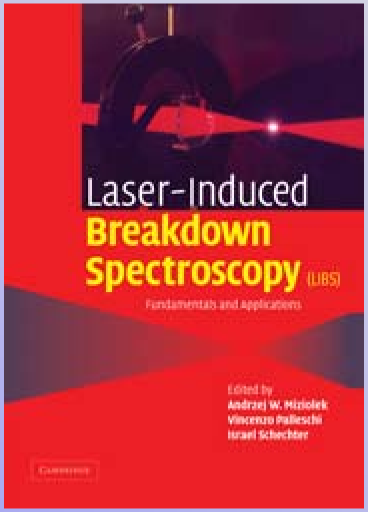
Laser-Induced
Breakdown
Spectroscopy (LIBS)
Fundamentals and Applications
Edited by
Andrzej W. Miziolek
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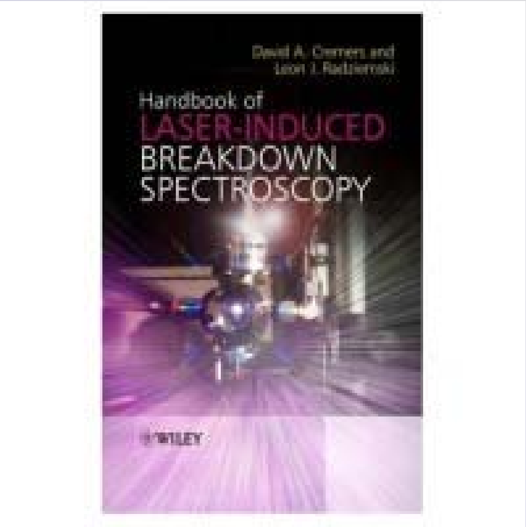
Israel Schechter


David A. Cremers and
Leon J. Radziemski
Handbook of
LASER-INDUCED
BREAKDOWN
SPECTROSCOPY
WILEY

LIBS…on bacteria?

- Since 80's LIBS has been known as a fast, sensitive, and robust spectroscopic technique for rapid elemental analysis (on-line, in situ, portable)

- Not enough people outside the LIBS community realize that it is currently being used for
  - molecular analysis (explosive residues, nerve agents)
  - analysis of complex biological systems (bacteria, proteins, viruses)

EMMA:
Elemental Multivariate Microbiological Analysis

The History of EMMA: a LIBS-Based Pathogen Identification

2003-2004 **early days** **feasibility; proof of concept**

Samuels, DeLucia, Jr., Morel, Leone, Amoroux, Miziolek, Harmon, Hybl, Buckley

2005-2008 **advanced days**

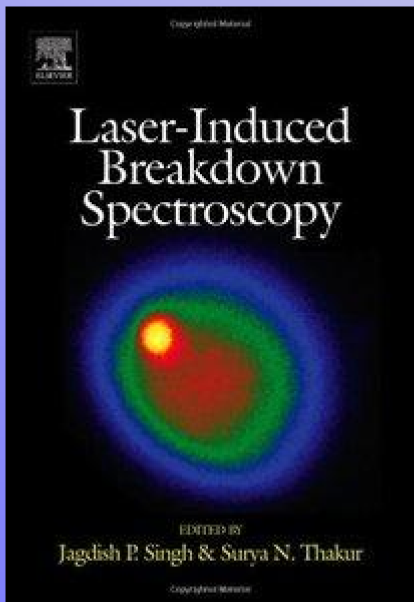
Baudelet, Wolf, Laloi, Gottfried, Dixon, Hahn

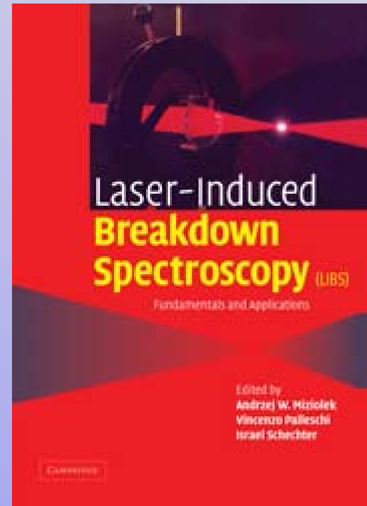
advanced chemometrics; single particle/bioaerosals; double pulse; femtosecond; use of molecules; stand-off; man-portable

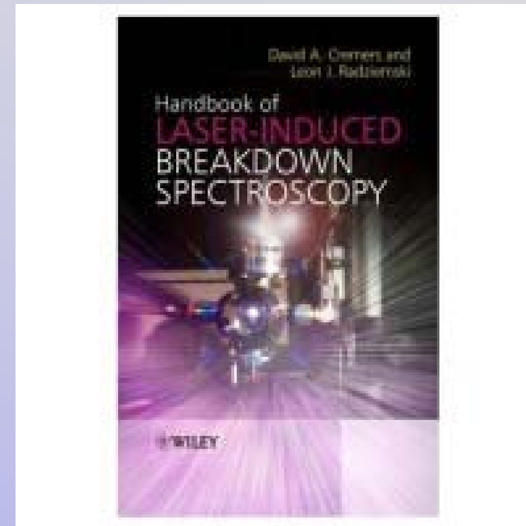
2008-2012 **current days**

Multari, Cremers, Caceres, Marcos-Martinez, Rehse, Mohaidat, Diedrich

discrimination of strains; microbiological diversity to simulate clinical specimens; realistic tests; chemometrics.







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REVIEW

Laser-induced breakdown spectroscopy (LIBS): an overview of recent progress and future potential for biomedical applications

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EMMA, a LIBS-Based Pathogen Identification: 1

(2007-2012) The bacterial LIBS spectrum for a given species **is stable and does not change with time** (experiments conducted on the same *E. coli* strain over the course of multiple years).

(2007) A rapid discrimination of live bacteria on the basis of LIBS signature alone is possible, as well as discrimination from other biotypes such as yeast or mold.[18]

(2007) Discrimination of the pathogenic enterohemmorhagic *E. coli* O157:H7 strain **from other non-pathogenic *E. coli* strains** has been shown.[19]

(2007&2011) Bacterial identification appears to be **independent of the growth condition and culture medium** in which the bacteria were grown (a nutrient rich tryptic soy agar, broth, or blood agar medium).[20] This result has been confirmed by Marcos-Martinez et al. on three similar growth media *(2011)*.[21]

(2008) Detection and discrimination of the biological warfare agent anthrax surrogate *Bacillus subtilis* var. niger and ricin surrogate ovalbumin has been demonstrated with 0% false negatives and 1% false positives at 20 meters using a standoff system.[22]

(2009) Bacterial LIBS signatures are correlated with bacterial membrane composition (for Gram-negative bacteria).[14]

EMMA, a LIBS-Based Pathogen Identification: 2

(2010) Discrimination is possible **between three** clonal methicillin-resistant *Staphylococcus aureus* **(MRSA) strains** and one unrelated MRSA strain.[24]

(2010) Intensity of the LIBS spectrum is linearly dependent on cell number, but the specificity is not dependent on cell number. **1500 cells provides adequate signal-to-noise**.[25]

(2011) Bacterial LIBS spectra **do not change** with time as the bacterial culture ages **on an abiotic surface** (necessary for accurate identification/detection of surface contamination with swipes).[26]

(2011) Bacterial LIBS spectra can be obtained from **killed** (via autoclaving) or **inactivated (via UV light)** specimens, and such treatment (which renders the specimen completely safe for handling) does not decrease identification specificity and does not decrease LIBS spectral intensity.[26]

(2011) Bacterial LIBS spectra can **identify** *Salmonella enterica* serovar Typhimuriumin at various concentrations **in various liquids** such as milk, chicken broth, and brain heart infusion. Titers of 10^5 and 10^6 cfu/mL provide adequate sensitivity for such testing.[28]

EMMA, a LIBS-Based Pathogen Identification: 3

(2011&2012) Bacteria in **mixed samples are identifiable**. The dominant or majority bacterial component of a two-component bacterial mixture is reliably identified provided it comprises 70% of the mixture or more. **Trace mixture or contamination is insignificant**.[25,15]

(2012) Bacteria can be identified with high sensitivity and specificity when specimens are **obtained from clinical samples (e.g. sterile urine** containing organic and inorganic solutes) without the need to remove other compounds present in the sample.[15]

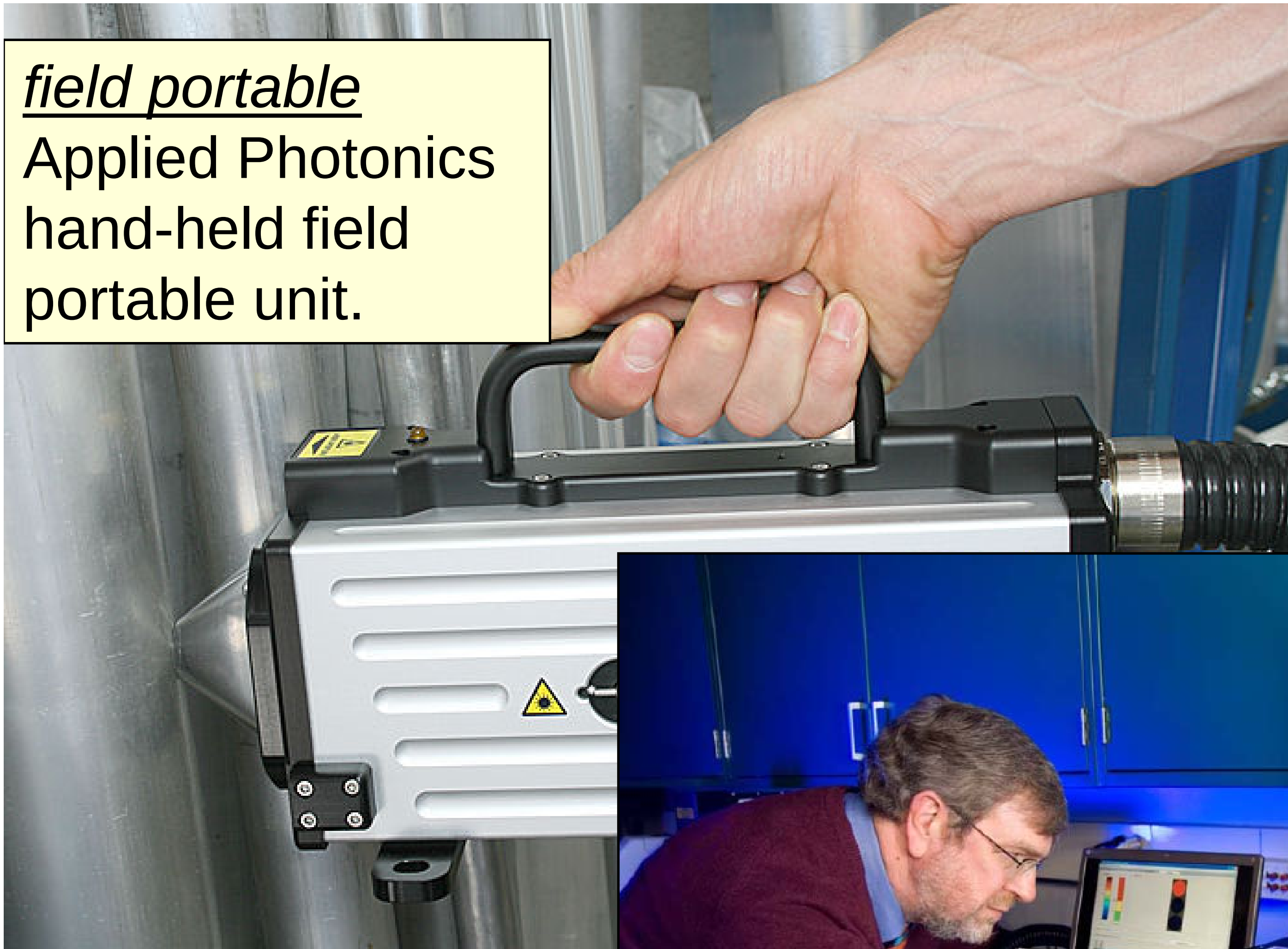
(2012) Bacterial classification of a “spectral library” composed of spectra from **five bacterial genera and 13 distinct taxonomic groups** showed sensitivities of approximately 85% and specificities above 95% when classified in a five-genus model. Positive predictive values (PPV) of 95%, 60%, 92%, and 96% were shown for the genera: *Escherichia*, *Staphylococcus*, *Streptococcus*, and *Mycobacterium*.[15]

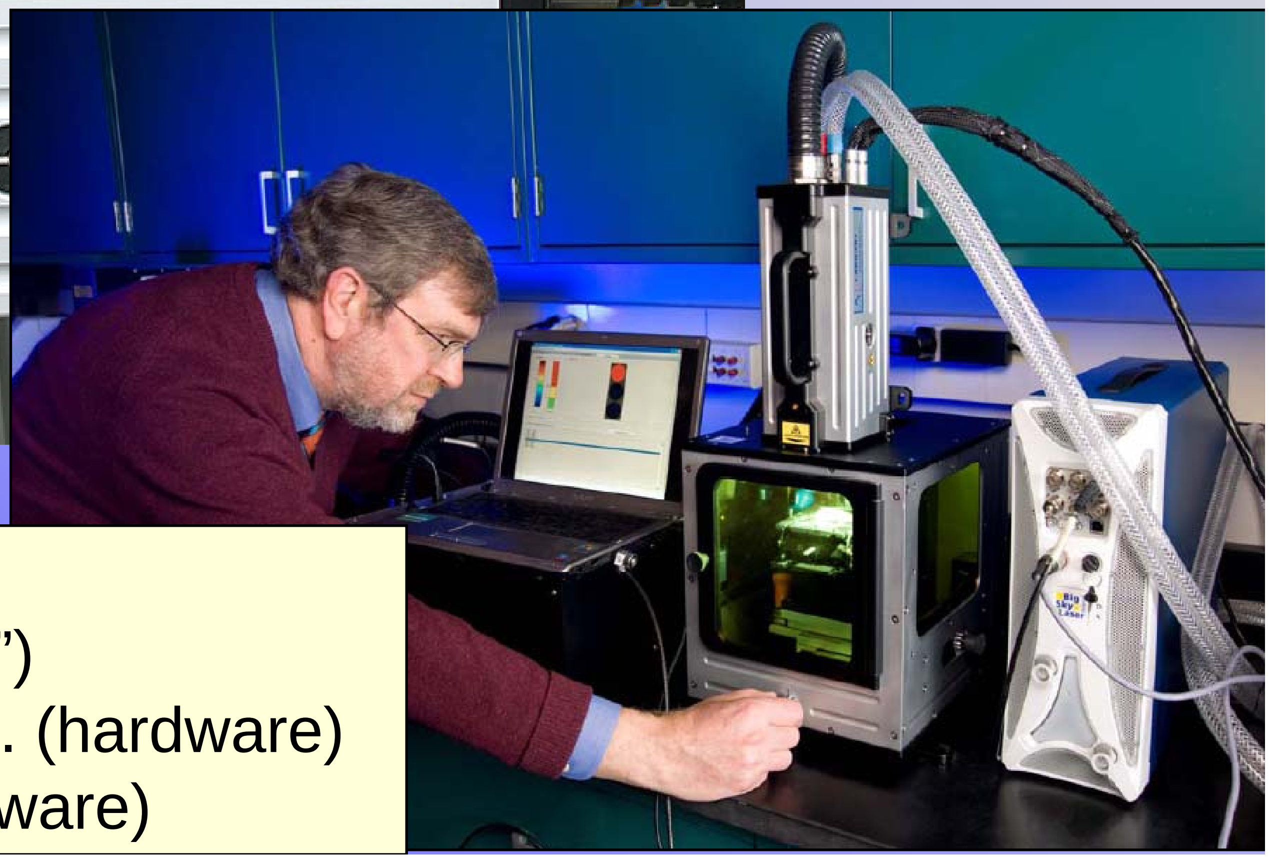
(2012) Live pathogenic *Bacillus anthracis* Sterne strain and *Francisella tularensis* can be differentiated **regardless of mounting protocol** (as lawn and/or colonies on agar, dilutions on agar, and dilutions on glass slides.)[29]

trated

en

<u>field portable</u>
Applied Photonics hand-held field portable unit.





bench-top
ARL (LIBS “know how”)
Applied Photonics, Inc. (hardware)
New Folder, Inc. (software)

First responder CBRNE prototypes have been built…

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

ad's-up display

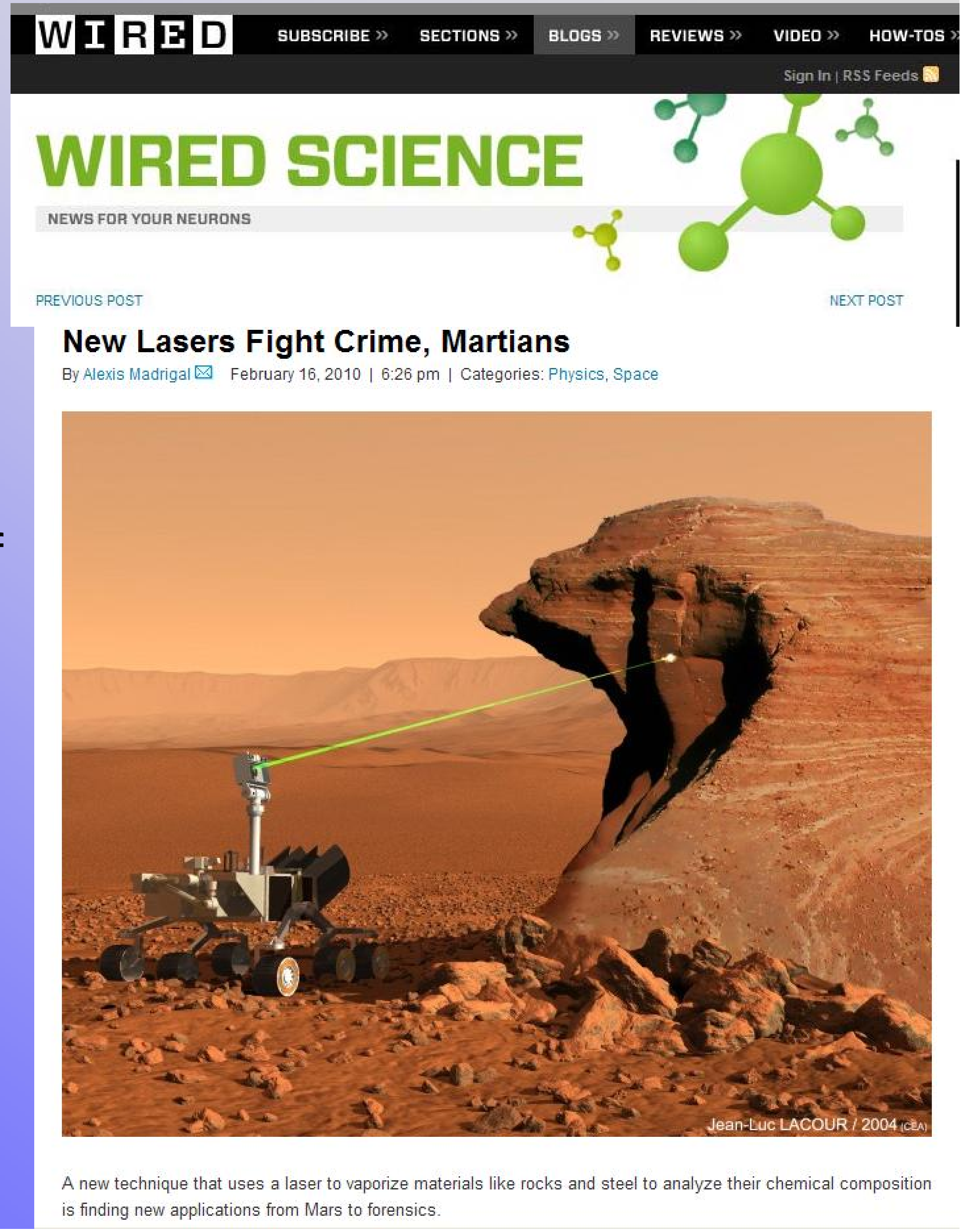
nd-held probe contains
er, joystick for control,
l focus optics

Microplasma/
LIBS Event

courtesy of Ocean Optics.

the new “Mars Science Laboratory” (MSL), Mars Rover “Curiosity”, blasted off for Mars on Nov. 25th, 2011

http://mars.jpl.nasa.gov/msl/



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New Lasers Fight Crime, Martians

By Alexis Madrigal February 16, 2010 | 6:26 pm | Categories: Physics, Space

Jean-Luc LACOUR / 2004 (CEA)

A new technique that uses a laser to vaporize materials like rocks and steel to analyze their chemical composition is finding new applications from Mars to forensics.

<u>*Conclusions*</u>

- "It is well-known…" Urgent need for non-PCR technology
  - current NIAID funding priorities: to develop the "*next-generation of novel or emerging rapid and innovative clinical diagnostic technologies that do not involve nucleic acid amplification methods to detect NIAID Category A, B, or C Priority Pathogens*."
- Thus, a serological / surface antigen approach or <u>compositional approach</u> are your best options.

Conclusions

Of the two, LIBS' advantages are:

1. autonomous, computer-enabled statistical ID made in under one second, unambiguously conveyed to operator
2. no consumables, no shelf-life
3. not pathogen specific
4. insensitive to mutations
5. robust ID
6. contamination insensitive, bacteria in mixtures
7. bacteria alive or dead (non-culturable)

Conclusions

The time is ripe to leverage the investment in hardware and successful proof-of-concept experiments to develop the instrumentation and protocols to translate LIBS diagnostics (EMMA) into the:

– emergency room, clinic, doctor's office
– front-line aid station
– telemedicine / robotic platform
– first responder / haz-mat arsenal

Thank you for your attention.

Questions?

Table 1. Demonstrated LIBS capabilities with relevance to medical applications and specific potential future medical applications.

Demonstrated LIBS capability….	…can lead to this capability….	…which could be applied in these medical applications
delivery of laser pulses & collection of plasma emission through optical fibre spectra obtained underwater (via fibre coupling) and in high-pressure environments differentiation of malignant / healthy tissues & classification of different malignancies elemental analysis of calcified tissues ("stones") elemental analysis of bone/tooth tissue; discrimination of dental caries from healthy tissue sensitivity to all heavy metals (e.g. lead, chromium) and sensitive detection of metals in human tissue and surrogates	LIBS *in vivo* analysis of tissue for real-time analysis	*in vivo* or *in vitro* "optical biopsies" (discrimination of cancerous / malignant / pre-cancerous tissues) *in vivo* identification of ulcerated tissue *in vivo* stone analysis real-time (during procedure) identification of dental caries tissue *in vivo* measurement of heavy metal concentrations in tissues with high-spatial resolution (i.e. in different parts of bone, in joints, in different regions of liver, etc.)
rapid bacterial identification based on elemental composition rapid discrimination of closely-related bacterial strains enhancement of specificity/sensitivity using LIBS/ Raman fusion enhancement of LIBS specificity by multi-element tagging of macromolecules	real-time diagnosis of pathogen presence in human fluids (blood, urine, CSF, sputum)	autonomous (no expertise required) identification of bacteria in human fluid specimen rapid screening for MRSA infections in hospital real-time meningitis test rapid strain-classification for epidemic control in hospitals/other on-line sensing of water for purity/contamination monitoring screening of asymptomatic persons via swab or saliva contribution for early infection detection (e.g. airport screening) monitoring of surface contamination for hygiene compliance office based UTI test remote operation (i.e. on a medical robot) for real-time patient analysis in hostile / battlefield environments

courtesy of Applied Photonics Ltd, U.K.

ST-LIBS Gen 4 at Hidden Valley, NTC, Fort Irwin, California
(Published with permission of U.S. Army, National Training Center, Fort Irwin)
Image 13 of 23
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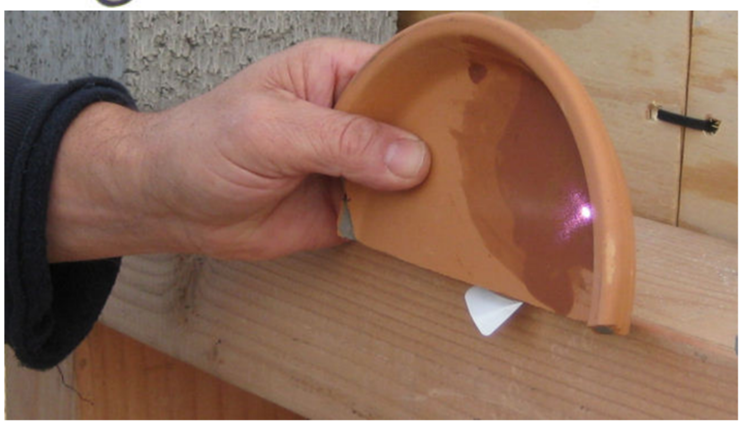

Laser plasma on clay target at 50 metres range (Hidden Valley, NTC, December 2007)
(Published with permission of U.S. Army, National Training Center, Fort Irwin)
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