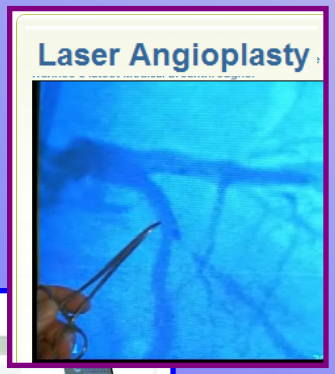
Healing Humanity One Spark at a Time: Medical Applications of LIBS

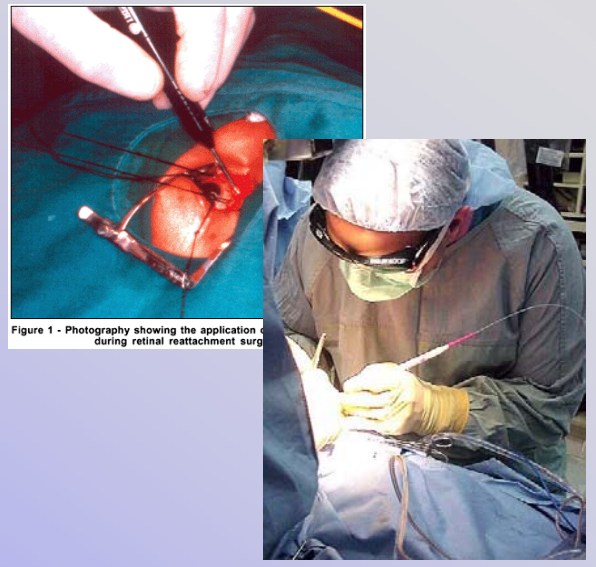


Steven J. Rehse
Univ. of Windsor
Windsor, Ontario, Canada

Lasers in Medicine

Now commonly used for:

- photocoagulation
- photoablation
- selective excitation / absorption



laser-induced breakdown spectroscopy

Can we utilize laser light and all the advantages of LIBS to add the ability to provide real-time diagnostic information to the medical professional?

- either during a procedure
- on a removed specimen for rapid analysis

Medicine

Prospects for laser-induced breakdown spectroscopy for biomedical applications: a review

Vivek Kumar Singh · Awadhesh Kumar Rai

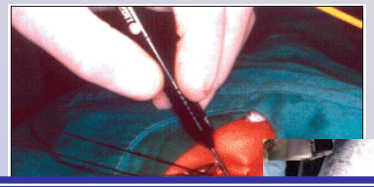
Lasers Med Sci (2011) 26:673–687

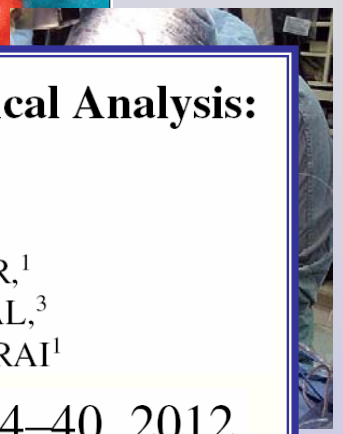
for:

– photocoagulati

– photoablation

– selective excita





Assessment of LIBS for Spectrochemical Analysis: A Review

ASHOK KUMAR PATHAK,[1] ROHIT KUMAR,[1]
VIVEK KUMAR SINGH,[2] RAHUL AGRAWAL,[3]
SHIKHA RAI,[1] AND AWADHESH KUMAR RAI[1]

Applied Spectroscopy Reviews, 47:14–40, 2012

opy

dd the
ical

REVIEW

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

S. J. Rehse*,[1], H. Salimnia[2] and A. W. Miziolek[3]

Journal of Medical Engineering & Technology, 2012; 36(2): 77–89

Journal of Medical Engineering & Technology, 2012; 36(2): 77–89

ISSN 0309-1902 print/ISSN 1464-522X online
DOI: 10.3109/03091902.2011.645946

http://www.uwindsor.ca/rehse/9/papers

REVIEW

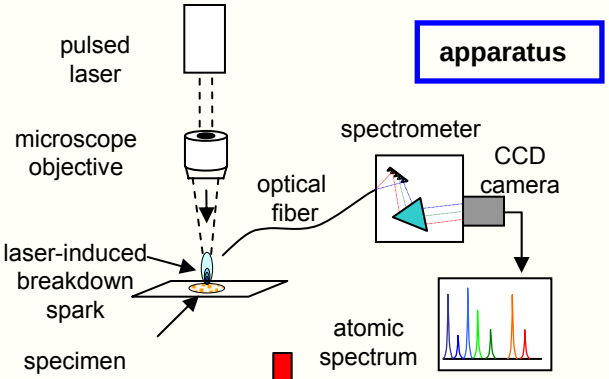
Laser-induced breakdown spectroscopy (LIBS): an overview of recent progress and future potential for biomedical applications

S. J. Rehse*,[1], H. Salimnia[2] and A. W. Miziolek[3]

"The usefulness of LIBS for characterizing or identifying human soft tissues for disease diagnosis is exemplified by the **small amount of sample required** and **the potential ability to perform a LIBS analysis *in vivo* by delivering the excitation laser pulse and collecting the plasma emission through a single optical fiber probe** [1995]. This has already been demonstrated, even when the fiber and LIBS plasma are submerged underwater [2002]. Therefore the ability to perform these analyses *in vivo* is theoretically possible.

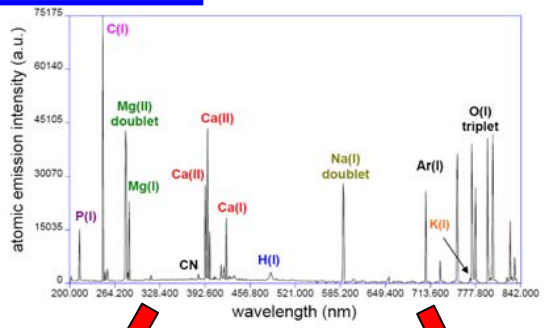
To date this type of analysis has not been performed. Still, this particular utilization would make the LIBS approach a recognizable technology to clinicians already familiar with laser-based endoscopic tools."

C.M. Davies, H.H. Telle, D.J. Montgomery, R.E. Corbett, *Spectrochimica Acta B* **50**, 1059 (1995).
D.C.S. Beddows, O. Samek, M. Liska, H.H. Telle, *Spectrochimica Acta B* **57**, 1461 (2002).



apparatus

LIBS spectrum

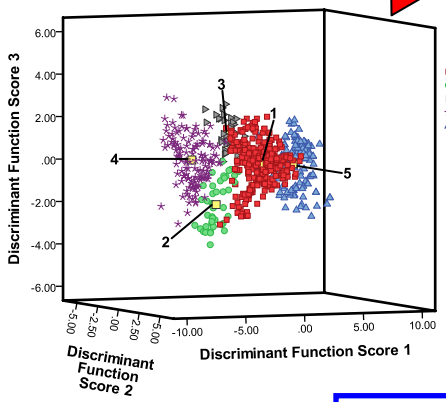


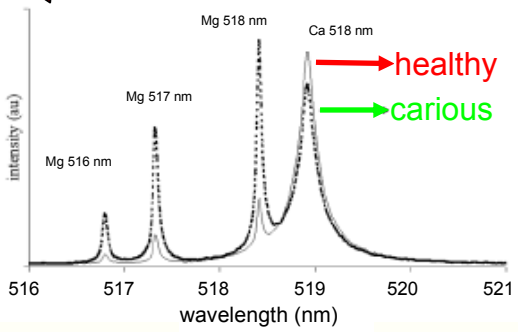
computerized chemometric analysis

analysis of element concentration

1

2





diagnosis of disease state or classification of specimen, either autonomously or by practitioner

ANALYSIS OF HARD/CALCIFIED TISSUES
- *Calcified tissues*
- *Dental studies*
- *Stones and calculi*
- *Fingernails*

ANALYSIS OF SOFT TISSUES
- *Organs*
- *Cancerous/malignant tissues*
- *Hair/skin*

ANALYSIS OF BIOMEDICAL SPECIMENS
- *Blood*
- *Proteins*

ANALYSIS OF MICROORGANISMS CAUSING HUMAN DISEASE
- *Bacterial pathogens*
- *Viral pathogens*
- *Molds, pollens, amoeba*

LIBS-GUIDED SURGERY
- *Laser-guided surgery*

(1) the LIBS spectrum is autonomously matched against a pre-compiled computerized library - provides identification / classification of unknowns targets (takes about one second)

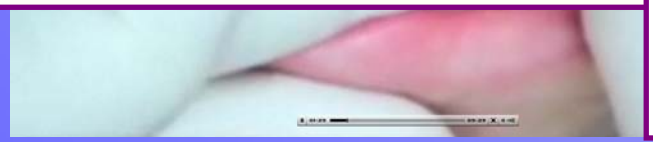
(2) specific lines in the LIBS spectrum are used to quantify the amount or concentration of some specific target element in the tissue or to monitor a change in tissue condition

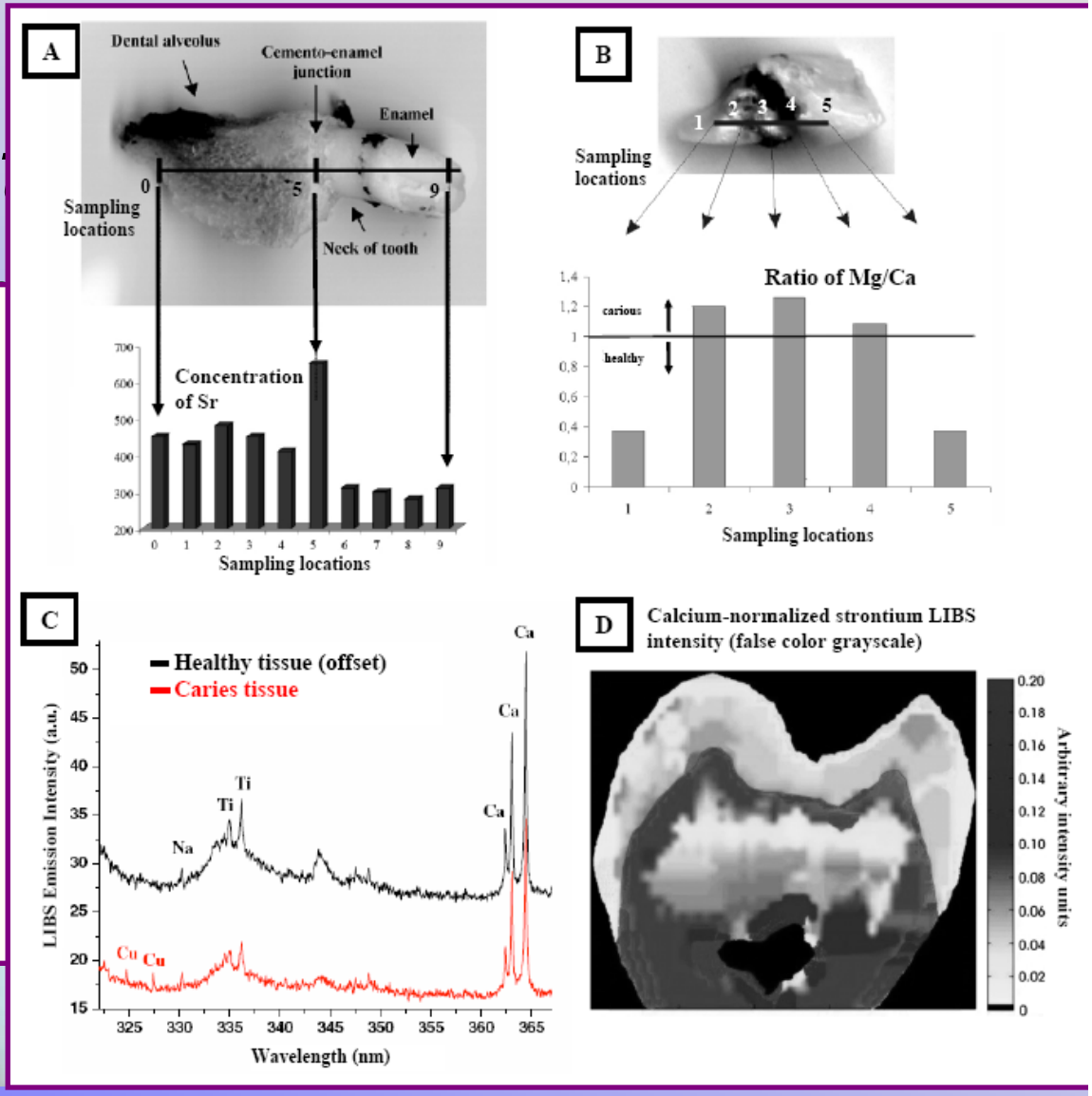
LIBS…on dental/mineralized tissue?

- Lasers (e.g. erbium) already used for dental caries removal
  - no anesthesia required
  - sterilizes as it drills

LIBS...on den

It has been shown that the light emitted by the ablation event can be used to provide real-time information to the dentist as to when all the carious tissue has been removed and healthy tooth is being "drilled"





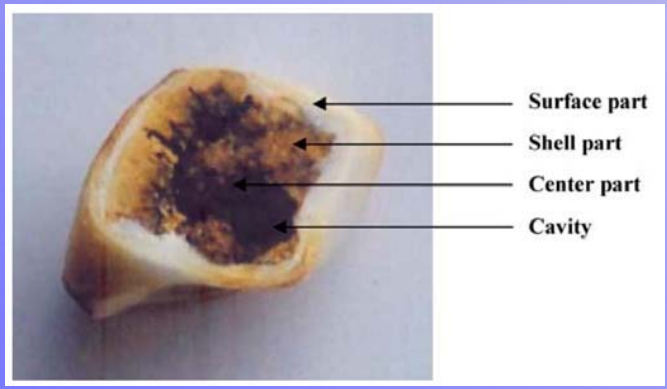
O. Samek, H.H. Telle, D.C.S. Beddows, BMC Oral Health **1**, 1 (2001)

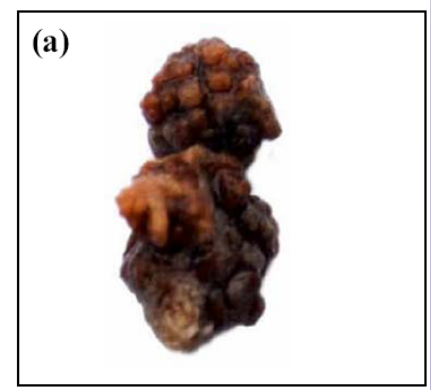
V.K. Singh, A.K. Rai, Lasers Med. Sci. **26**, 307 (2011)

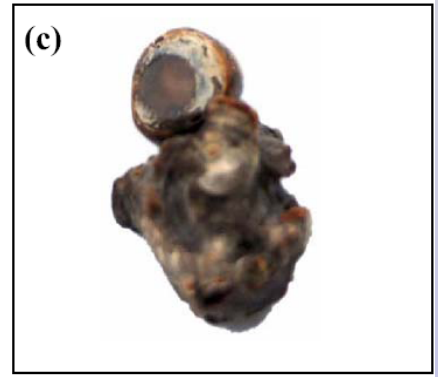
F.C. Alvira, F. Ramirez Rozzi, G.M. Bilmes, Appl. Spectrosc. **64**, 313 (2010)

LIBS…on dental/mineralized tissue?

LIBS has been utilized to elementally characterize removed gallstones [2008,2009], kidney stones [2009], and other urinary calculi (stones) [2005].



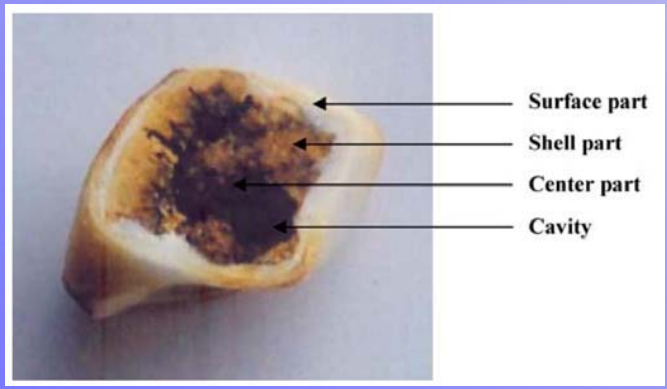




All of these stones occur in a *wide variety of elemental concentrations* and compositions, each uniquely identifiable via LIBS analysis.

LIBS...on den

LIBS has been utilized t
gallstones [2008,2009
urinary calculi (stones

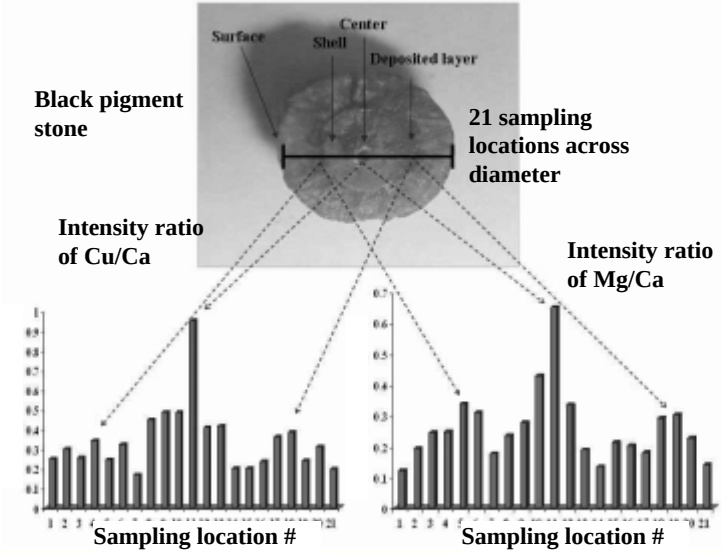


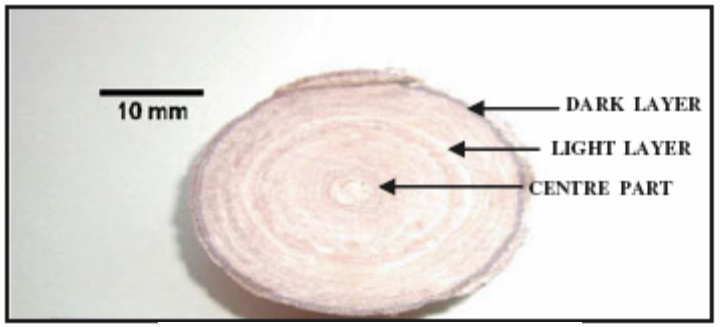
X. Fang, S.R. Ahmad, M. Mayo, S. Iqbal, Lasers Med. Sci. **20**, 132 (2005)

and compositions, each u

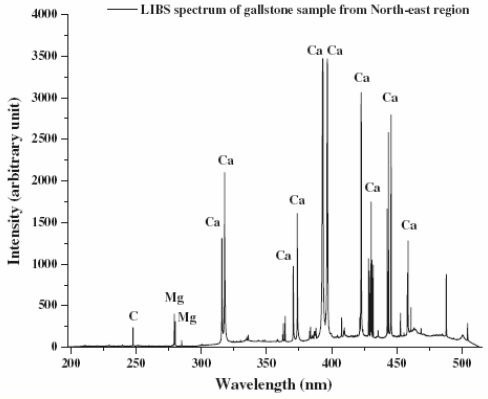
V.K. Singh, V. Singh, A.K. Rai, S.N. Thakur, P.K. Rai, J.P. Singh, Appl. Opt. **47**, G38 (2008)

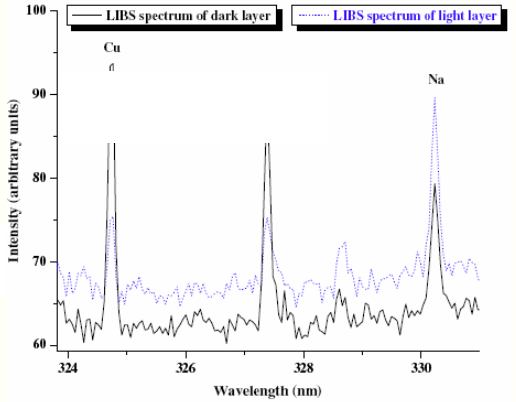
A.K. Pathak, V.K. Singh, N.K. Rai, A.K. Rai, P.K. Rai, P.K. Rai, S. Rai, G.D. Baruah, Lasers Med. Sci. **26**, 531 (2011)





Cross-section of a gallstone.





LIBS…on dental/mineralized tissue?

LIBS has also been used to sample fingernails for spectra consistent with alcoholism, drug doping, hyperthyroidism, fungal infection, and Wilson's disease (Cu/C ratios.)

Thus LIBS could be an important tool for clinical laboratories.

Such a spectro-chemical analysis of bone or other calcified tissue are also relevant in the fields of *forensic science* and *archaeology*

LIBS…on soft tissue?

Yueh et al. [2009] performed LIBS on **tissue specimens**

- *brain*, *lung*, *spleen*, *liver*, *kidney,* and *skeletal muscle*
- 20 normal six-week old chickens

→ LIBS can discriminate between tissues

can assist in the identification of organ/tissue type

Cancer…?

LIBS has been used to effectively:

- **distinguish normal and malignant tumor cells** from histological sections [2004] and to
- characterize several types of human malignancies [2010].

Table 3
PLS-2 results of the external validation tissue samples.

Tissue sample	Muscle		Lung		Liver		Kidney		Spleen		Brain	
	No.[a]	Pred.[b]	No.[a]	Pred.[b]	No.[a]	Pred.[b]	No.[a]	Pred.[b]	No.[a]	Pred.[b]	No.[a]	Pred.[b]
External validation samples	482	Muscle	672	Lung	776	Fail	843	Brain	163	Fail	717	Fail
	658	Fail	383	Lung	415	Brain	690	Kidney	185	Fail	744	Brain
	14	Fail	838	Lung	486	Liver	74	Kidney	27	Fail	162	Brain
	16	Muscle	192	Lung	788	Liver	63					
	340	Muscle	50	Lung	563	Liver	83					
	341	Fail										
	572	Liver										
	94	Muscle										
	97	Muscle							969	Spleen	318	Brain
	968	Muscle							652	Spleen		
Correct identification rate	6/10		5/5		3/5		3/5		3/10		4/9	
Fail statistics test rate	3/10		0/5		1/5		0/5		6/10		5/9	
Incorrect identification rate	1/10		0/5		1/5		2/5		1/10		1/9	

F.Y. Yueh, H. Zheng, J.P. Singh, S. Burgess, Spectrochim. Acta B **64**, 1059 (2009)

Table 2. Intensity Ratio from LIBS Spectra of Normal and Malignant Tissue

Analyte Line (nm)	Intensity Ratio: Normal Tissue	Intensity Ratio: Malignant Tissue
Al 396.153/K 766.491	0.5919	0.49
Ca 396.847/K 766.491	12.77	31.42
Cu 327.39/K 766.491	1.45	0.07
Fe 430.791/K 766.491	0.73	1.03
Mg 279.551/K 766.491	3.37	3.62
Na 588.995/K 766.491	1.07	3.84

A. Kumar, F.Y. Yueh, J.P. Singh, S. Burgess, Appl. Opt. **43**, 5399 (2004)

ns

tification of organ/tissue type

ancer…?

ffectively:

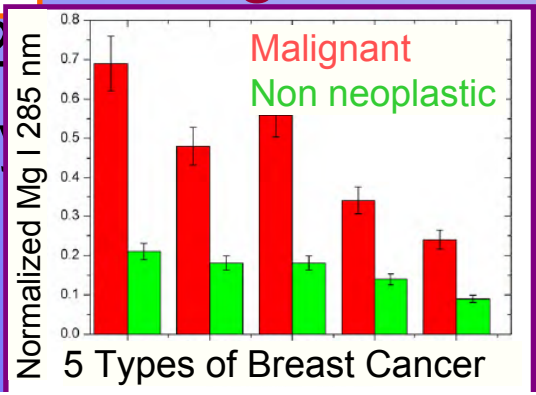
nd malignant tumor cells from

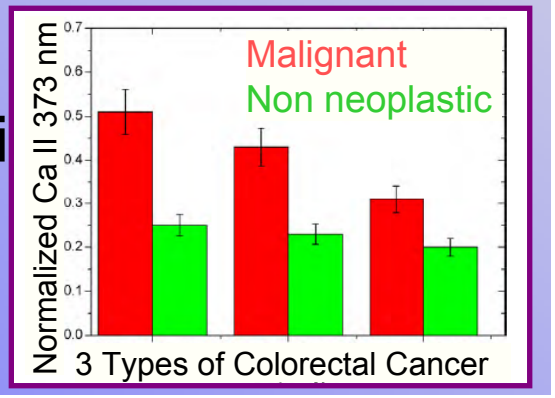
histological sections [2

• characterize several t

i

A. El-Hussein, A.K. Kassem, H. Ismail, M.A. Harith, Talanta **82**, 495 (2010)





LIBS…on soft tissue?

LIBS has been used to determine the **trace metal concentrations** in human hair and skin samples.

For example, the elemental composition of liver, kidney, muscle, and hepatopancreas tissues [2008].

D. Santos Jr., R.E. Samad, L.C. Trevizan, A.Z. deFreitas, N.D. Vieira Jr., F.J. Krug, Appl. Spectrosc. **62**, 1137 (2008)

It can be used to track nanoparticle distribution in histological specimens.

Thus LIBS could be an important tool for clinical laboratories.

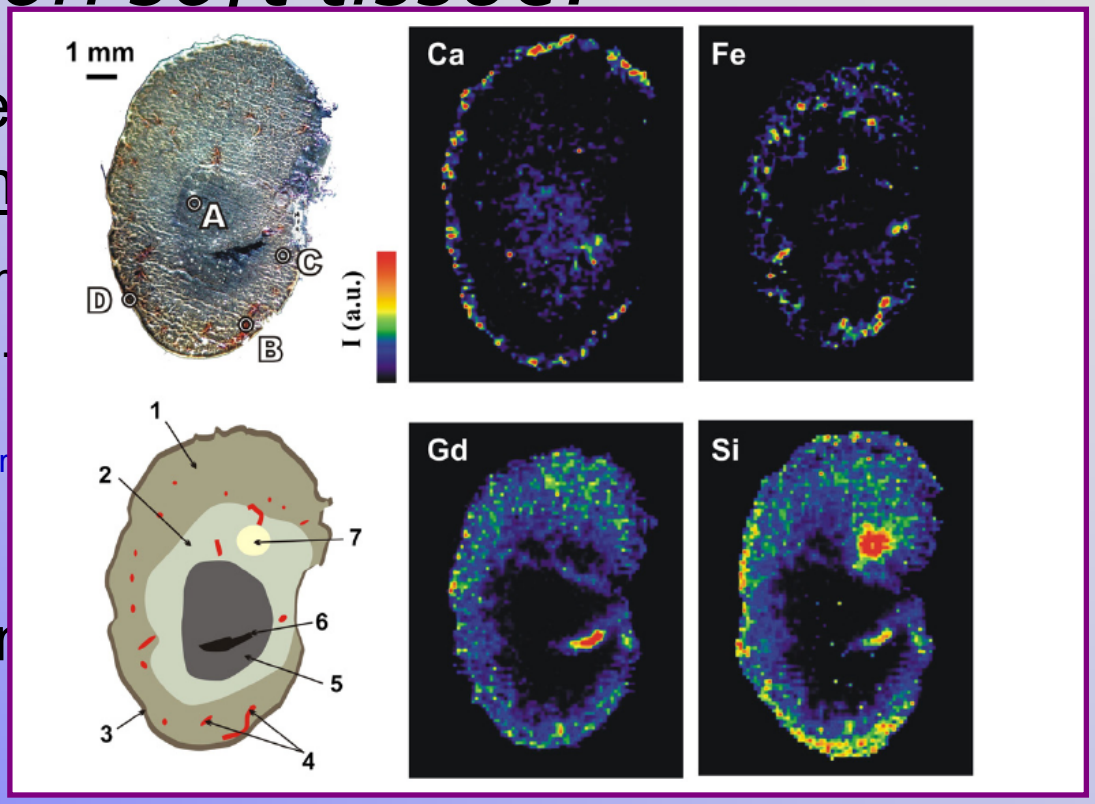
LIBS…on soft tissue?

LIBS has been used to de

"Mapping was performed for metallic elements such as Gd, Si, Ca and Fe, with a resolution of 100 μm on kidney slices sampled from a mouse 24 h after intravenous injection of a solution of gadolinium-based nanoparticles."

It can be used to track nar
specimens.

mouse kidney



V. Motto-Ros, L. Sancey, X.C. Wang, Q.L. Ma, F. Lux, X.S. Bai, G. Panczer, O. Tillement, J. Yua, Spectrochimica Acta B **87,** 168 (2013).

Thus LIBS could be an important tool for clinical laboratories.

LIBS…on biomedical specimens (blood, proteins, etc.)?

Preliminary tests conducted on specimens of **whole blood** determined the LIBS emission spectrum – significant C and Fe - no quantitative results.

N. Melikechi, H. Ding, S. Rock, A. Marcano O., D. Connolly, Proc. of SPIE **6863**, 68630O (2008)

Residues of **amino acids on swipes** (high-purity powdered L-asparagine and L-leucine) showed differentiable LIBS spectra with suitable chemometrics.

R. Chinni, D.A. Cremers, R. Multari, Appl. Opt. **49**, C143 (2010)

Multi-element coded particle assay for the detection of analytes.

Rather than quantifying differences in elemental concentrations, perform a sensitive detection of an analyte (**the ovarian cancer biomarker CA 125**) by utilizing an elemental "tag."

Immuno-conjugated silicon micro-particles (1.5 μm) with agarose beads carrying CA 125 molecules allows a LIBS-based detection of silicon in the emission spectrum indicating the presence of the ovarian cancer biomarker.

Y. Markushin, N. Melikechi, in *Ovarian Cancer - Basic Science Perspective*, ed. by S.A Farghaly (InTech, Rijecka, 2012)

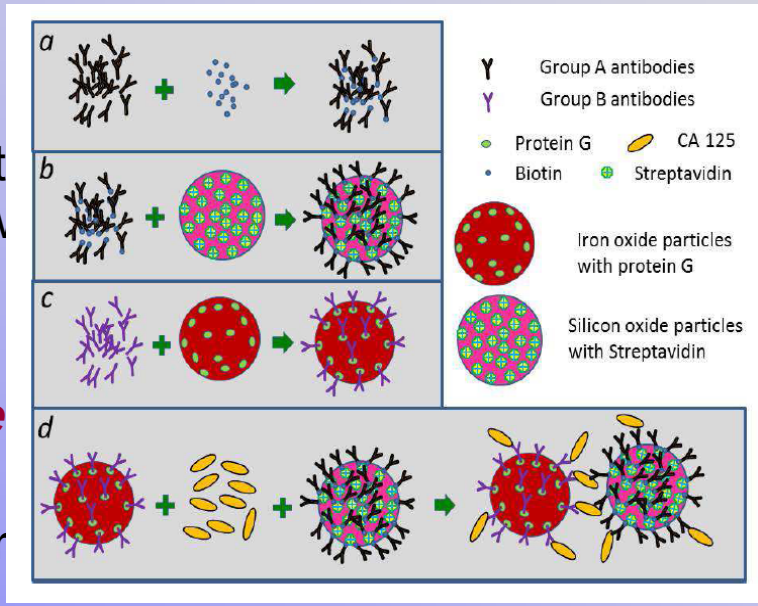
LIBS…on biomedical specimens (blood, proteins, etc.)?

Preliminary tests conducted on specimens of **whole blood** determined the LIBS emission spectrum – significant C and Fe - no quantitative results.

N. Melikechi, H. Ding, S. Rock, A. Marcano O., D. Connolly, Proc. of SPIE **6863**, 68630O (2008)

Limits of detection

- approximately 1 U (international units)/ml for CA 125
- 11 µg/ml for the ovarian cancer biomarker Leptin (*comparable to current existing enzyme-linked immunosorbent assays (ELISA)*)
- 30 ppb for the model protein avidin (in a similar experiment performed with an Fe micro-particle assay)



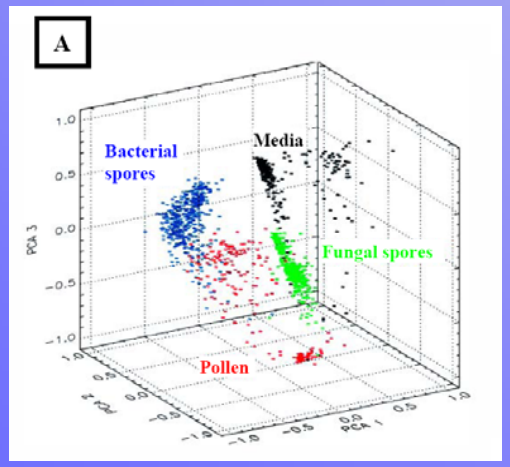
Immuno-conjugated silicon micro-particles (1.5 µm) with agarose beads carrying CA 125 molecules allows a LIBS-based detection of silicon in the emission spectrum indicating the presence of the ovarian cancer biomarker.

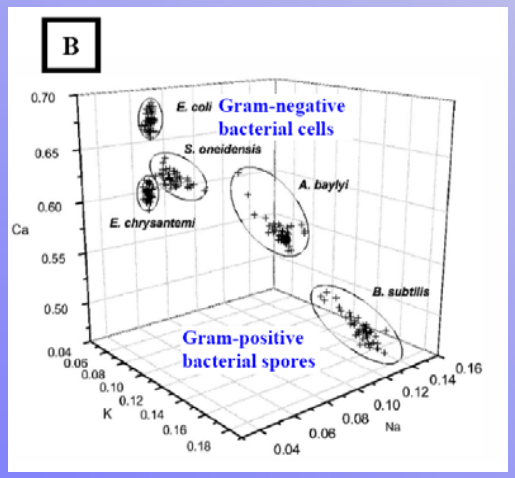
Y. Markushin, N. Melikechi, in *Ovarian Cancer - Basic Science Perspective*, ed. by S.A Farghaly (InTech, Rijecka, 2012)

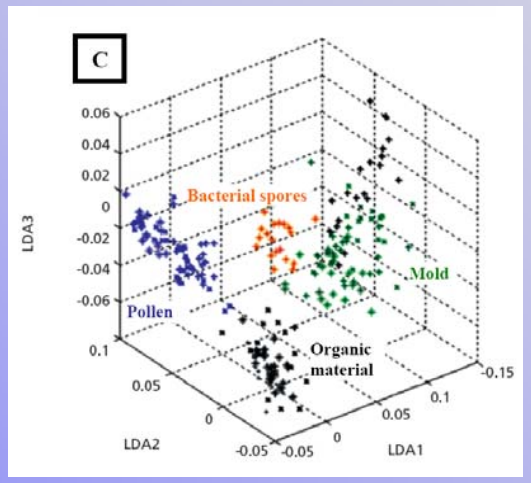
LIBS…for pathogen diagnosis (bacterial, fungal, viral, etc.)?

Since 2003, >40 papers on LIBS for identification of bacteria, molds, pollens, yeast, fungal spores, and viruses.

Bacteria are the most promising targets, and are easily differentiated from background interferents or similar biological targets (using the appropriate chemometric algorithm.)







A. J.D. Hybl, G.A. Lithgow, S.G. Buckley, Appl. Spect. **57**, 1207 (2003)

B. M. Baudelet, J. Yu, M. Bossu, J. Jovelet, J.-P. Wolf, T. Amodeo, E. Frejafon, P. Laloi, Appl. Phys. Lett. **89**, 163903 (2006)

C. D.W. Merdes, J.M. Suhan, J.M. Keay, D.M. Hadka, W.R. Bradley, Spectroscopy **22**, 28 (2007)

LIBS…for pathogen diagnosis?

Important Bacterial Results:

(*2007 & 2011*) Identification appears to be independent of the growth condition and culture medium in which the bacteria were grown, including milk, chicken broth, and brain heart infusion

D. Marcos-Martinez, J.A. Ayala, R.C. Izquierdo-Hornillos, F.J. Manuel de Villena, J.O. Caceres, Talanta **84**, 730 (2011)

C. Barnett, C. Bell, K. Vig, C.A. Akpovo, L. Johnson, S. Pillai, S. Singh, Anal. Bioanal. Chem. **400**, 3323 (2011)

(2007 & 2010) Discrimination is possible at the strain level. 4 *E. coli* strains and 3 clonal methicillin-resistant *Staphylococcus aureus* (MRSA) strains and one unrelated MRSA strain

R. Multari, D.A. Cremers, J.M. Dupre, J.E. Gustafson, Appl. Spectrosc. **64**, 750 (2010)

(2011) Bacterial LIBS spectra do not change with time as the bacteria age on an abiotic surface (necessary for accurate identification/detection of surface contamination with swipes)

(2012) Bacteria can be identified 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

(2012) 4 strains of UV-killed hantavirus that had been diluted in liquid iodixanol and plated on glass slides were discriminated (no determination of the number of viruses tested)

R. Multari, D.A. Cremers, M.L. Bostian, Appl. Opt. **51**, B57 (2012)

LIBS…for laser-guided surgery?

Despite common uses of lasers in surgery, it is known that,
"*…the surgeon gets no feedback during laser ablation. There is no depth sensation and no tissue specificity with a laser incision…Future prospects may solve these problems by means of an optical feedback mechanism that provides a tissue-specific laser ablation*."

F.W. Neukam, F. Stelzle, Physics Procedia **5**, 91 (2010)

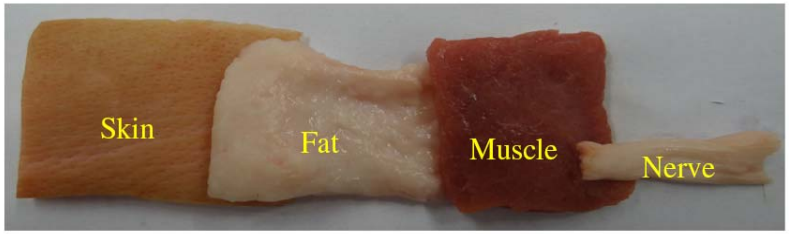


Fig. 1. Tissue samples used for the LIBS experiment.

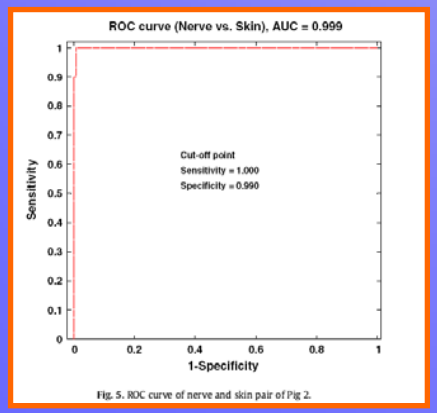
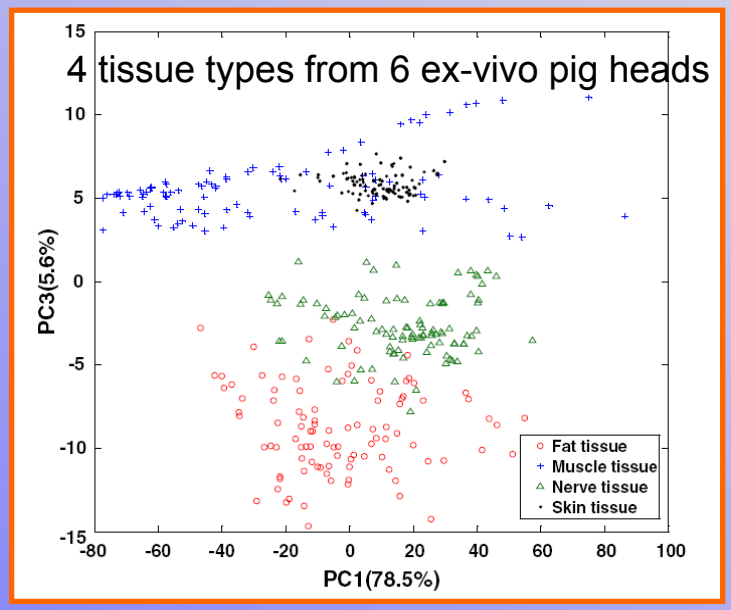


Fig. 5. ROC curve of nerve and skin pair of Pig 2.



R. Kanawade, F. Mehari, C. Knipferd, M. Rohde, K. Tangermann-Gerk, M. Schmidt, F. Stelzle, Spectrochim. Acta B **87**, 175 (2013).

LIBS…for laser-guided surgery?

Despite common uses of lasers in surgery, it is known that,
"*…the surgeon gets no feedback during laser ablation. There is no depth sensation and no tissue specificity with a laser incision…Future prospects may solve these problems by means of an optical feedback mechanism that provides a tissue-specific laser ablation*."

F.W. Neukam, F. Stelzle, Physics Procedia **5**, 91 (2010)

"*No device currently exists that combines the cutting capability of plasma-mediated ablation, using ultra-short laser pulses to ensure negligible collateral tissue damage, with feedback control of the cutting process.*"
D.C. Jeong, P.S. Tsai, D. Kleinfeld, Curr. Opin. Neurobiol. **22**, 24 (2012)

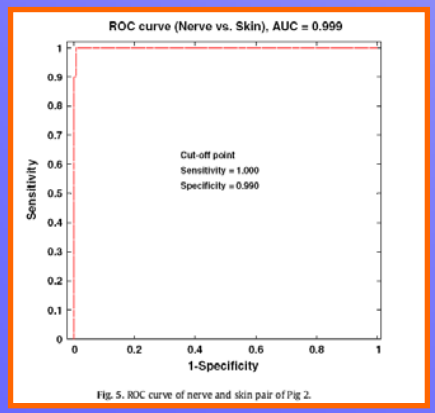
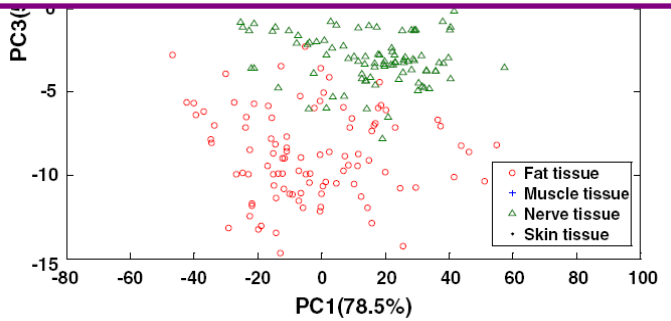


Fig. 5. ROC curve of nerve and skin pair of Fig 2.



R. Kanawade, F. Mehari, C. Knipferd, M. Rohde, K. Tangermann-Gerk, M. Schmidt, F. Stelzle, Spectrochim. Acta B **87**, 175 (2013).

Conclusions

Trends in modern medicine are toward increased

- non- or less-invasive procedures
- “smarter” procedures
- quicker/cheaper procedures

The real-time diagnostic information that LIBS provides could offer an attractive tool for the health-care professional.

Conclusions

Although we are not there yet, the time is ripe to leverage our

- investment in hardware and
- successful proof-of-concept experiments

to develop the instrumentation and protocols to translate LIBS diagnostics 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?