ELEMENTAL IMAGING WITH LASER SPECTROSCOPY IS ENTERING THE CLINIC AS A NEW DIAGNOSTIC TOOL

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1. INTRODUCTION

The physiological and pathological roles of exogenous metals, but also endogenous metallic and organic ions are of major interest for the biomedical community [Austin 2013, Wogelius 2011]. Elemental imaging of biological tissues is currently a technological challenge requiring complex instruments (such as synchrotron) with restricted accesses. We recently developed an all-optical method, fully compatible with standard microscopy systems, for multi-elemental imaging of biological tissues. Our instrument is based on Laser Induced Breakdown Spectroscopy (LIBS) and allows the in situ imaging and quantification of the elements of the periodic table within biological tissues, with ppm-scale sensitivity and lateral resolution at the micrometer scale [Sancey 2014, Gimenez 2016].

In LIBS, the laser-induced plasma generated by focusing laser pulses on the surface of the sample of interest allows a specific optical response to be elicited from the elements constituting the sample. This specific response, resulting from the electronic relaxation of atoms and ions excited by the high plasma temperature, is collected and analyzed using an optical spectrometer. The elemental “signal” (atomic and ionic emission lines) is then extracted from the recorded spectrum, and elemental maps can be obtained in a pixel-by-pixel manner by scanning the sample surface over the region of interest (**Figure 1.**). The implementation of LIBS is simple because a single laser pulse can simultaneously sample the material by laser ablation and excite the vaporized elements by heating the plasma plume. Hence, the acquisition speed for LIBS is mainly governed by the laser frequency rate. The apparent simplicity of the setup endows LIBS with a series of advantages over other elemental imaging methods; these benefits include an all-optical design, operation in ambient atmosphere, and fast acquisition.

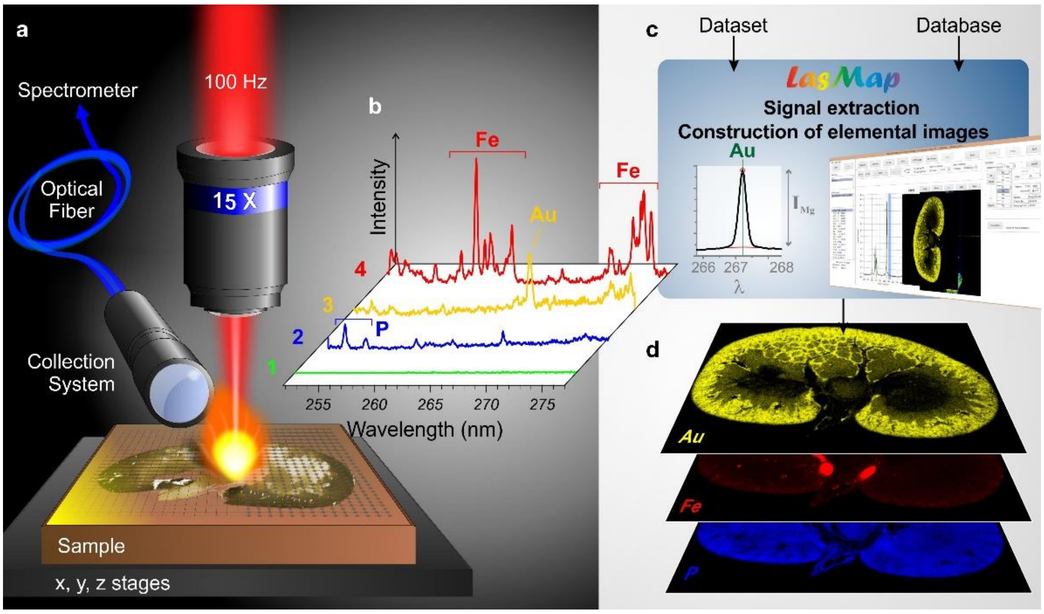

Figure 1. General protocol for LIBS imaging. (a) Schematic view of the LIBS instrument showing the major components: the microscope objective used to focus the laser pulse, the motorized platform supporting the sample and the optical detection system connected to the spectrometer via an optical fiber. (b) Example of single-shot emission spectra recorded in different regions of a rat kidney with the characteristic emission lines of iron (Fe), phosphorous (P), and gold (Au). (c) Methodology for signal extraction and image construction. (d) Example of relative abundance images of Au (yellow), Fe (red) and P (blue) represented in a false color scale.

The proof-of-concept was obtained by studying the bio-distribution of gadolinium or gold-nanoparticles in tumors or organs (kidneys/liver) after i.v. administration in mice [Kunjachan 2015, Sancey 2015, Le Guével 2018]. These experiments helped to describe and understand the kinetics of several metal-based (Gd, Au, Ag, Pt) nanoparticles in vivo. We recently upgraded our instrument to work faster and to image the elements contained in paraffin-embedded samples, which are the most frequent form of archived clinical specimens (surgical resections or biopsies) [Moncayo 2017, Busser 2017]. In the presentation to be held, we will present our latest results relating to the clinical applications of LIBS multi-elemental imaging, most of which constitute novel findings, both for LIBS and medical domains.

2. EXPERIMENTAL

2.1. Experimental setup

The instrumental setup was based on a homemade optical microscope. The LIBS experiment used Nd:YAG laser pulses of 1064 nm, focused onto the sample by a 15x magnification objective (LMM-15X-P01, Thorlabs). The pulse duration was 8 ns, and the repetition rate was 100 Hz. During the experiments, the sample could be translated along the 3 axes by an XYZ motorized stages. All the measurements were performed at room temperature and under ambient conditions. The light emitted by the plume was collected by two quartz lens – optical fiber systems connected to two different Czerny-Turner spectrometers both equipped with an ICCD camera (Shamrock and iStar, Andor Technology). The simultaneous used of these two spectrometers allows to detect a large number of elements such as Fe, Ca, Na, P, Mg, Zn, Al, Mn, Co, Si, Cr, Ti and Cu.

2.2. Samples

All the studied samples consisted in human biopsies embedded in paraffin. The LIBS results obtained with these specimens of human origin were systematically gathered in elemental pathology reports and subsequently sent back to medical doctors in charge of the patients. The clinicians involved in this research are experts in pulmonology and occupational medicine. They obtained signed informed consent from their patients to perform LIBS imaging analyses with their biopsies. Such a procedure is mandatory, and we believe we are pioneering in the exploration of LIBS imaging as a diagnostic tool, following the standards of clinical research.

3. RESULTS AND DISCUSSION

Because the impact of mineral and metallic particles in lung diseases is probably underestimated, we started to analyze the elemental content of human lung tissues with LIBS. Clinicians selected lung biopsies from patients with various lung diseases with potential occupational/environmental origin. We identified several exogenous elements (including carcinogens) in the lungs of the patients (i.e Be, Ti, Si, Al, Cr), in different lung areas and with very different concentration ranges. Importantly, LIBS produced valuable clinical data for most of the patients and elemental findings were in complete accordance with patients' personal past exposures. Based on these important positive preliminary findings, we initiated the first multicenter retro- and prospective clinical trial aiming at evaluating the feasibility of implementing LIBS imaging as a routine diagnostic test for respiratory diseases (100 patients) [Sancey 2014]. We will present the most relevant results from this ongoing study.

In this presentation we will present some of the most relevant results from this ongoing study with the idea to highlight the potential LIBS-based imaging as a tool useful for pathologists and clinicians in their daily work and management of patients:

1) A large-sized lung specimen (~2 cm^2) with several positive pixels for beryllium (Be). This result was indeed consistent with the diagnosis of chronic beryllium disease, first case to be solved with a space-resolved configuration.
2) A lung biopsy in which more than 4000 pixels were positive for at least one of the following exogenous elements: Si, Al and Ti (**Figure 2.**). This result was in

accordance with the history of environmental and occupational exposures of the patient, suffering from a rare lung disease with no known origin (idiopathic). However, none of the usual elemental techniques used by the clinicians could elucidate the aetiology.

3) A lung specimen with important levels of Si, actually not detected by classical elemental analysis (SEM-EDX). This LIBS finding was the key to convince experts to re-classify the idiopathic disease of the patient as an occupational disease. Additionally, to personal recognition, the patient will therefore benefit from a significant financial compensation.

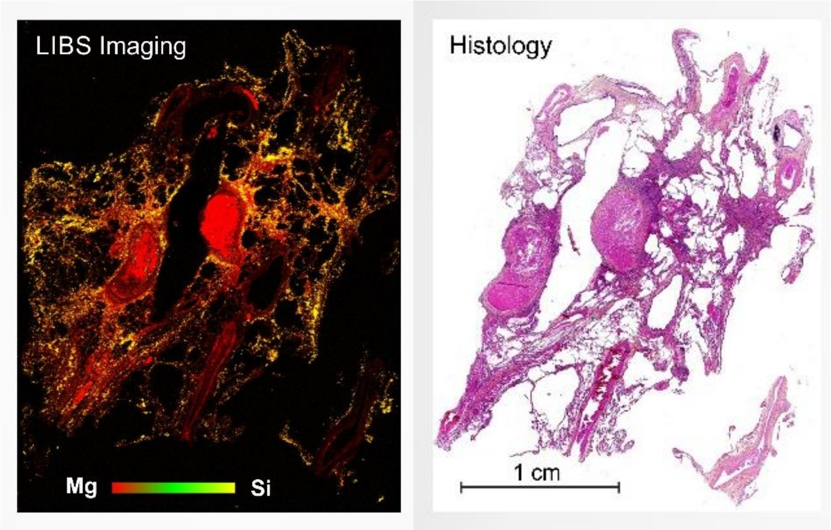

Figure 2. Images of human lung tissue with conventional histological staining (HES, left) and complementary LIBS elemental image (right) representing Magnesium (red) and Silicon (green). These results reveal important levels of Si, Al and Ti (not shown) in the lung tissue from an occupationally exposed patient with idiopathic lung disease.

4. CONCLUSION

This work demonstrates that LIBS allowed the in situ identification of several chemical agents related to patient's exposure (Si, Al, Cr, Zr, etc.), which may help clinicians in assessing the occupational/environmental origin of various lung diseases. As a routine diagnostic tool, LIBS could be indeed an invaluable source of information to help clinicians to better understand the origin and pathogenesis of a number of respiratory diseases.

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