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## Visualizing the cerebral distribution of chemical elements: a challenge met with LIBS elemental imaging

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## Abstract

Biological tissues contain various metals and metalloids ions with central role in the regulation of several pathophysiological functions. In parallel, the development and the evaluation of novel nanocompounds for biomedicine require the monitoring of their biodistribution in tissues of interest. Therefore, researchers need to use reliable and accessible techniques to detect and quantify major and trace elements in space-resolved manner. In this communication, we report how Laser-Induced Breakdown Spectroscopy (LIBS) can be used to image the distribution of chemical elements in brain tissues.

## 1 Introduction

The space-resolved detection of major and trace elements in brain sections can be performed using different analytical techniques including Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)<sup>1,2</sup>, Scanning Electron Microscopy-Energy Dispersive X-ray Spectrometry (SEM-EDS)<sup>3</sup>, Synchrotron-based chemical imaging<sup>4</sup>, Electron Microprobe Analysis or microprobe (EPMA)<sup>5</sup>, Auger Electron Spectroscopy (AES)<sup>6</sup>, X-ray Photoelectron Spectroscopy (XPS)<sup>7</sup>, Secondary Ion Mass Spectrometry (SIMS)<sup>5</sup> and Time of Flight (ToF-)SIMS<sup>6,8</sup>.

In addition to these techniques, Laser-Induced Breakdown Spectroscopy (LIBS) is an optical analytical method working at ambient conditions (atmospheric pressure and room temperature). With this experimental approach, laser pulses are focused at the surface of the biological specimen (tissue cryosection, tissue section or entire bulk of tissue embedded in paraffin or epoxy resin)<sup>9-12</sup> to ablate and excite a few nanograms of matter, generating a plasma sufficiently hot to excite atoms and ions<sup>13</sup>. From this plasma, the emitted light is collected and analyzed with adapted spectrometers and the spectroscopic data are further converted into images *i.e.* elemental maps<sup>14</sup>. Working at frequency of 100 Hz (as LA-ICP-MS does), this strategy for imaging chemical elements is fast, and advantageously complementary to the conventional histological investigations performed with optical microscopy for biomedical purposes. The spatial resolution varies with the nature of the sample and its hardness from 8-10 (hard samples) to 20  $\mu\text{m}$  (for paraffin-embedded samples), while achieving an in-depth resolution of 10  $\mu\text{m}$ . Additionally, almost all the elements from the periodic table can be detected with LIBS, including light elements<sup>15-17</sup> which may contrast with most of the above-mentioned competitive methods. In this paper, we will highlight the interest of using this analytical method to investigate the distribution of endogenous and exogenous elements in the brain.

## 2 Materials and Methods

2.1 *LIBS elemental imaging.* LIBS was performed on tissue cryosections analyzed at room temperature, using a previously described setup<sup>17,18</sup>. The system is an optical-based setup equipped with a Nd:YAG 1064 nm (8 ns pulses, 100 Hz) as laser injection line and a 3D motorized platform for sample positioning with accurate control of the focal distance between the objective and the sample. Images were obtained using single shot mode, under 1 L.min<sup>-1</sup> argon gas. Optical fibers were used for the collection of the light emitted by the plasma and were connected to three Czerny-Turner spectrometers combined with ICCD camera (Shamrock 505, Shamrock 303, and iStar, Andor Technology). The spectrum was acquired with a delay of 700 ns after the laser pulse, a gate of 5  $\mu\text{s}$  and a gain of 1500. The acquisition and data analysis were performed using a custom-developed LabVIEW software.

2.2 *Specimens collection.* Tissue specimens were obtained in accordance with European Guidelines and the French Ministry of Agriculture. Fetal mouse brains were sampled as described in Hasna et al<sup>19</sup>, and fixed in paraformaldehyde 4% before embedding in epoxy resin and sectioned for LIBS analysis. Brains from orthotopic tumor-bearing rats (F98 glioma model) were obtained as reported by Bulin et al.<sup>20</sup> The rats had previously received LaF<sub>3</sub>:Ce nanoparticles through an intratumoral injection performed by convection enhanced delivery (CED) and a subsequent synchrotron X-ray radiation therapy. The survival of the animals was determined and the brains were collected either after euthanasia when the

animal reached a humane endpoint or at day-120 after treatment when the long-term surviving rats were sacrificed. Brains were fixed and cryo-sectioned before LIBS imaging.

### 3 LIBS elemental imaging for endogenous elements

The cortex is a unique brain structure exclusively found in mammals that coordinates and regulates sequences of cell proliferation, migration, morphological differentiation and synaptogenesis.<sup>21</sup> Understanding the formation of the brain cortex is of prime importance, because any cortical malformations are associated with developmental neuropathologies and neurological disorders<sup>22</sup>.

With this objective, we studied the distribution and the time course regulation of zinc (Zn) during the brain development to assess the central role of Zn in the physiological formation of the brain as well as in the functioning of neural cells<sup>19</sup>.

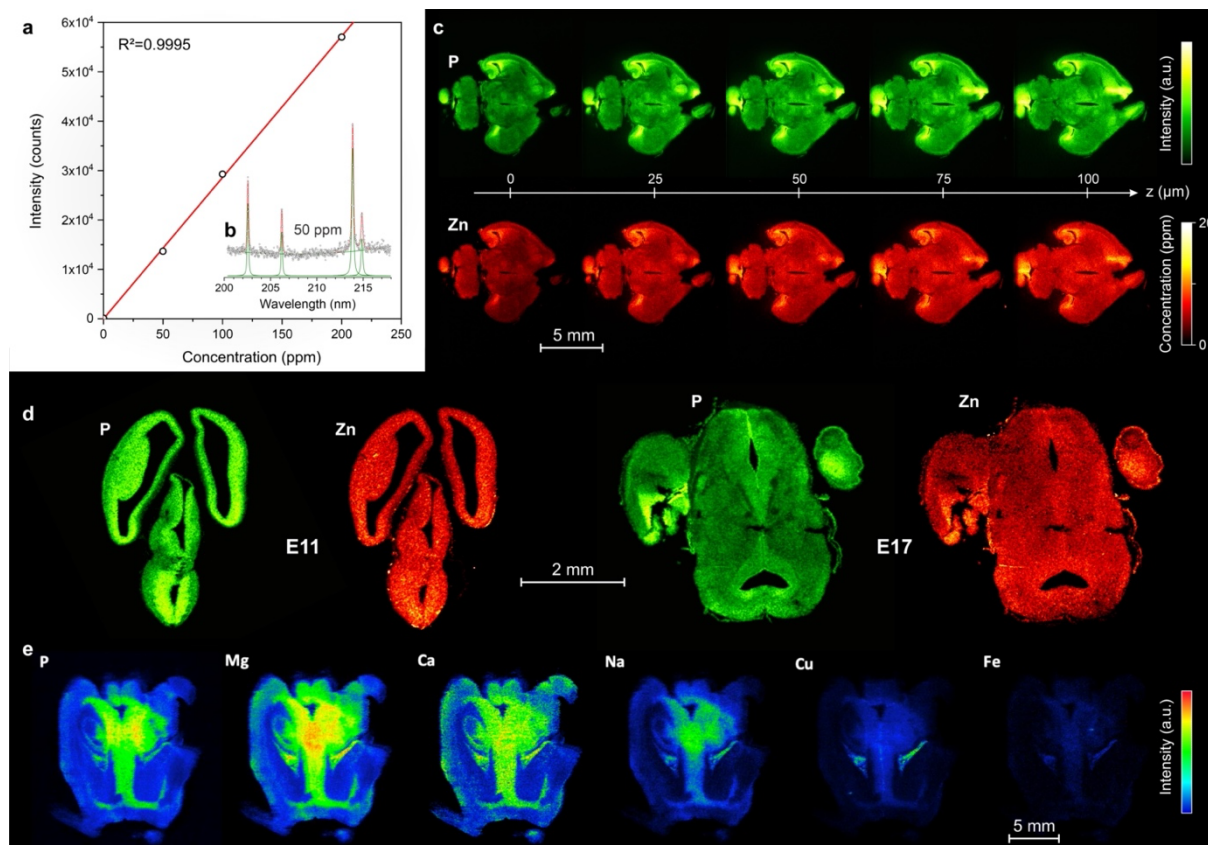
To obtain the distribution of Zn in tissues, we sampled the mouse brains collected at different fetal stages (i.e., embryonic day 11 (E11) and embryonic day 17 (E17) are reported in the Figure 1), and used an optimized LIBS system working at high frequency (100 Hz) to scan selected regions of interest. In emission spectroscopy, each element has its own specific optical signal, and Zn is an atom emitting in spectral ranges that are not easily accessible by conventional detection systems (around 200 nm, thus in the UV). However, with a system optimized for its detection, we characterized the spatial and temporal pattern of distribution for Zn in different brain sections, as shown in Figure 1.

One should note that a quantitative measurement of Zn abundance has been performed using several external references made with similar matrices containing known Zn concentration, using a previously reported procedure<sup>17</sup>. Briefly, Zn resin standards of known concentration from 0 to 200 ppm have been produced. Each standard was scanned in the same experimental conditions as the brain specimens. Zn standard signal for each concentration is the result of 1,000 average spectra, each spectrum corresponding to a single laser shot. As the signal increases linearly with the concentration (Figure 1a), quantitative Zn maps were generated for different brain sections from the calibration curve obtained from the Zn intensity maps ( $R^2$  of 0.9995). An example of LIBS mono-elemental spectrum obtained from a resin containing 50 ppm (part per million) of Zn is shown in Figure 1b. From this spectrum, we estimated the limit of detection for Zn to  $\sim 2$  ppm.

Matrix effect is an issue that affects all techniques based on laser ablation, and if the matrix of the calibrators is not strictly similar to the matrix of the specimens, it is recommended not to attempt a quantitative measurement. In our experiment, both calibrators and brains were embedded in the same epoxy resin, and despite being as close as possible in terms of hardness, brain tissue was not present in the calibrators and we cannot rule out the presence of matrix effects. However, we considered these calibrators as proxies sufficiently similar to the specimens for performing quantitative measurements and associated quantitative images that are shown in this work, with cerebral Zn concentrations that are in accordance with the literature<sup>23,24</sup>.

At embryonic day 17 (E17), the Zn was mainly found in cerebral regions with high cell density, as indicated with the strong colocalization of P and Zn in the consecutive sections (Figure 1c). However, a slight decrease of the amount of Zn was observed with time, between E11 and E17 (Figure 1d).

The evaluation of the main genes participating in the cellular transport, storage, and release of Zn during corticogenesis completed the study<sup>19</sup>. Altogether, there is a tight temporal and spatial regulation of Zn homeostasis during fetal brain development and LIBS imaging could allow the observation of the distribution of Zn element in brains collected at different fetal stages, at the entire organ scale, both in 2D and in 3D. During these acquisitions, other elements of interest can be observed simultaneously, such as Mg, Ca, Na or others (see Figure 1e).

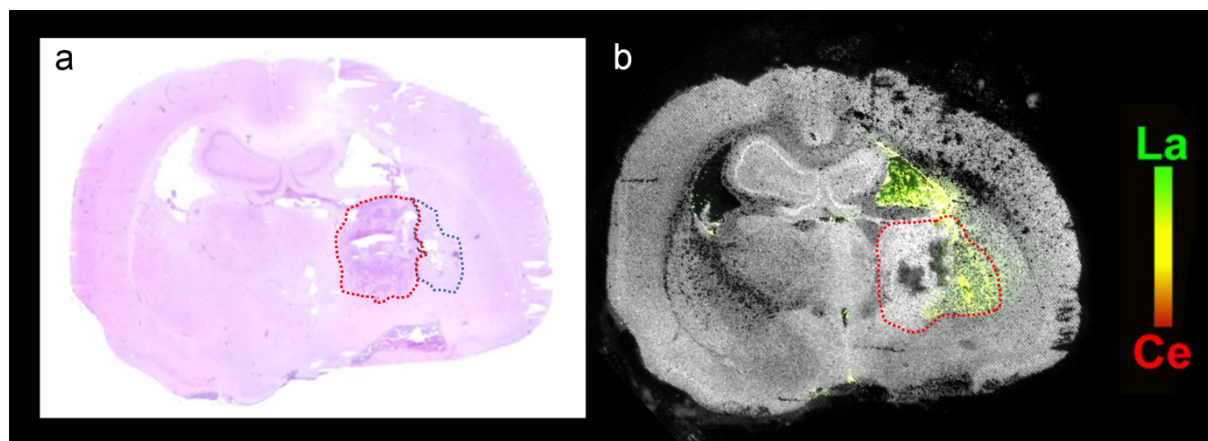


**Figure 1:** (a) Zinc calibration experiment, with Zn resin standards. (b) Example of a LIBS mono-elemental spectrum obtained from a resin containing 50 ppm of Zn. (c) Adjacent sections of the brain of a fetal mouse (Embryonic day 17, E17) representing P and Zn distribution obtained on 5 successive ablation sequences (depth resolution 25  $\mu\text{m}$  / sequence). (d) Spatio-temporal comparison between E11 and E17 for P and Zn elements. (e) Example of various elemental images in a selected section of the brain of a mouse.

#### 4 LIBS elemental imaging for exogenous elements, including metal nanoparticles

In addition to endogenous ones, exogenous chemical elements can also be evidenced and mapped in biological tissues using LIBS. This is of prime importance in the context of drug and nanoparticle development, but also for exposure-related studies. For oncology purposes, we investigated the therapeutic value of rare-earth composite nanoscintillators for radiation dose-enhancement purposes<sup>20</sup>. In this perspective, we administered  $\text{LaF}_3:\text{Ce}$  nanoparticles to rat-bearing glioblastoma before treating them with synchrotron X-ray radiation therapy. For such treatment modalities, the optimization of the injection-to-irradiation delay is crucial for i) achieving the highest nanoparticle tumor uptake to maximize the efficacy of the radiotherapy, and for ii) inducing the lowest accumulation in the healthy surrounding tissues to reduce potential side effects. We evidenced that the local administration of the  $\text{LaF}_3:\text{Ce}$  nanoparticles by convection-enhanced delivery (CED) induced a local retention of the nanoparticles within the tumor site, for a prolonged time (Figure 2). After synchrotron-based radiation therapy, the survival of animals was monitored, and the brains were collected for further analyses, by histology and LIBS. LIBS allowed to document the presence of the 2 main elements of the nanoparticles, *i.e.* La (lanthanum) and Ce (cerium), locally at the tumor site<sup>20</sup>. The colocalization of these 2 principal chemical components *in situ* was in accordance with the stability of the nano-formulation *in vivo*. We were also able to demonstrate the positive correlation between the presence of the nanoparticles at the tumor site and the long-term survival of animals. On the contrary, the non-responding animals presented an abnormal distribution of the nanoparticles, with an unwanted accumulation in the healthy tissue and a weak or partial tumor accumulation. We selected a relevant example (Figure 2) in which we observed a strong local control of the tumor growth in areas where the nanoparticles were accumulated before the

radiotherapy (Figure 2a, blue area, and Figure 2b). Some tumor cells remained present in the region with no or low amounts of nanoparticles before radiotherapy (Figure 2a, red area, and Figure 2b).



**Figure 2:** Example of long-term surviving rat brain after CED administration of  $\text{LaF}_3\text{:Ce}$  nanoparticles and radiotherapy. (a) Hematoxylin/Eosin section of the brain, evidencing a region with remaining tumor cells (red) and a cured part of the brain (blue). (b) Rat brain after CED administration of  $\text{LaF}_3\text{:Ce}$  nanoparticles evidencing a part of the metal nanoscintillators at the tumor site (red area) and in the ventricle.

#### 4 Conclusion

The brain is a very complex and well-structured organ. The identification of the distribution of chemical elements in tissues could help to collect chemical images for fundamental and applied sciences, as exemplified in this short communication. To obtain fast and reliable information, LIBS elemental imaging could be used in addition to conventional histology on adjacent tissue sections. LIBS enables performing large-sized tissue elemental mapping, in-depth investigations (3D)<sup>25, 26</sup> or 2D analysis of regions of interest with very minimal sample preparation. In parallel to preclinical investigations, LIBS is currently entering the clinic as a diagnostic method to evaluate environmental and professional exposures<sup>27, 28</sup>.

Credit Author Statement: Conceptualization, A-L.B., H.E., A.B., V.M-R., L.S., Investigation and data analysis, all authors, writing—original draft preparation, L.S.; writing—review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest: none

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